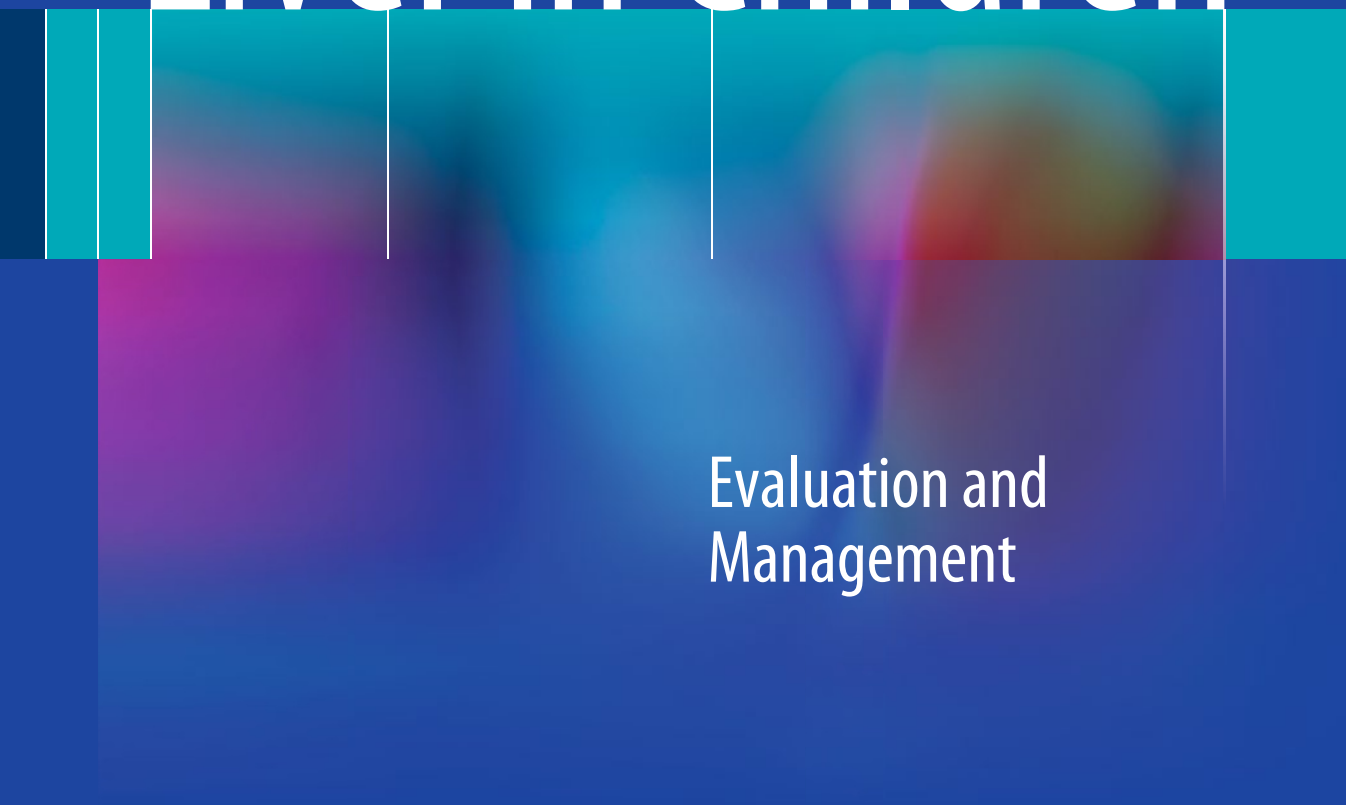
Karen F. Murray
Simon Horslen
Editors

Diseases of the Liver in Children

Evaluation and Management



Springer

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To my husband Bernie and children Michael and Katrina for their love, understanding, patience, and ongoing encouragement and support.

Karen F. Murray

To Sarah, my greatest support, thank you. To Joss and Leah – never a dull moment!

Simon Horslen

Preface to the First Edition

Pediatric liver disease presents to the primary care provider via incidental laboratory detection, during investigation of persistent signs and symptoms, or after dramatic clinical presentation. Over the last decades, our understanding of the normal embryology, cellular pathogenesis of disease, and genetic basis of these conditions has permitted clearer clinical diagnoses and the ability to prognosticate and offer therapies.

This is the first text to focus entirely on pediatric liver diseases aimed at providing the tools necessary for the primary care provider to do an initial assessment and appropriately refer the patients for specialty care. We have brought together experts in pediatric liver diseases. The book provides a clear, in-depth, and well-illustrated understanding of normal liver biology, decodes the interpretation of liver-related laboratory assessments and provides a context in which these tests are valuable, discusses the most common of the pediatric liver diseases, and describes the potential complications and their treatments. Additionally, the text reviews the laboratory, radiological, and pathological findings important in diagnosis.

This book's purpose is to serve as a reference for primary care providers and specialists in training and practice encountering and caring for children with liver diseases.

Seattle, WA, USA — Karen F. Murray, MD
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Part I

General Concepts

Anatomy and Development of the Liver

1

Mark D. Stringer

Introduction

The anatomy of the liver has been studied since ancient times, but it was Francis Glisson's *Anatomia Hepatis* published in 1654 that probably marked the dawn of a new era in the understanding of this topic. In the late 1880s, after the introduction of anaesthesia and antisepsis, the first liver resections were attempted. Further advances in hepatic anatomy were made at the turn of the nineteenth century, but it was not until the late 1950s that the segmental structure of the liver was fully recognised [1]. This discovery, coupled with general advances in surgery and anaesthesia, precipitated a quantum leap forward in hepatic surgery that witnessed the development of increasingly sophisticated segmental liver resections [2]. The introduction of liver transplantation in 1967 and the advent of cross-sectional imaging in the 1970s ushered in further advances in the understanding of liver anatomy, ultimately leading to techniques such as reduced and split-liver transplantation, living-related donation, and even more ambitious hepatic resections. This chapter focuses on the anatomy and development of the liver.

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Anatomy

Shape and Surfaces

The liver is the largest abdominal organ, occupying most of the right upper quadrant of the abdomen. It is normally a reddish-brown colour but may appear pale yellow when infiltrated by fat. It is a solid wedge-shaped organ (Fig. 1.1) with the thin end of the wedge lying in the left upper quadrant. Its anterior, posterior, superior, and right lateral surfaces are in contact with the diaphragm and mostly convex, while its inferior (visceral) surface has an irregular contour. The diaphragmatic and visceral surfaces meet anteriorly at a sharp inferior border. The right lateral surface of the liver extends between the 7th and 11th ribs in the midaxillary line, separated from the right lung and pleura only by the diaphragm as it sweeps down to the costophrenic angle. The posterior surface has several notable landmarks including the inferior vena cava, which occupies a deep groove between the bare area and caudate lobe, and the fissure for the ligamentum venosum, which contains the obliterated ductus venosus that once connected the left portal vein to the left hepatic vein within the fetal liver (see below) (Fig. 1.2).

The inferior surface of the liver faces downwards and backwards. The inferior surface of the right lobe is related to the hepatic flexure of the colon, right kidney, and right suprarenal gland, while that of the left lobe lies anterior to the fundus of the stomach. On the inferior surface of

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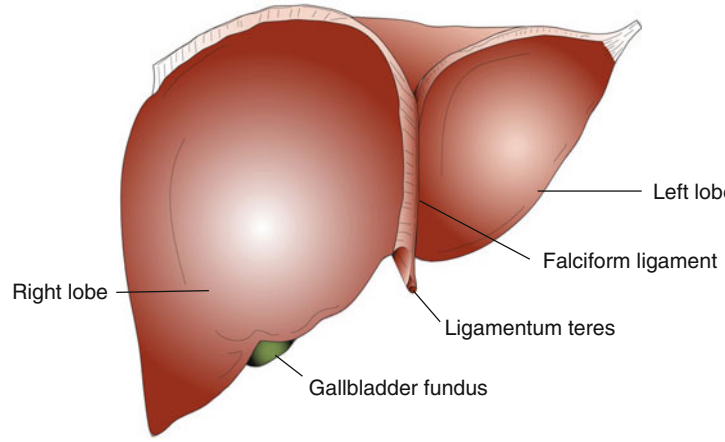

Fig. 1.1 Anterior surface of the liver (©RMcP/MDS 2013)

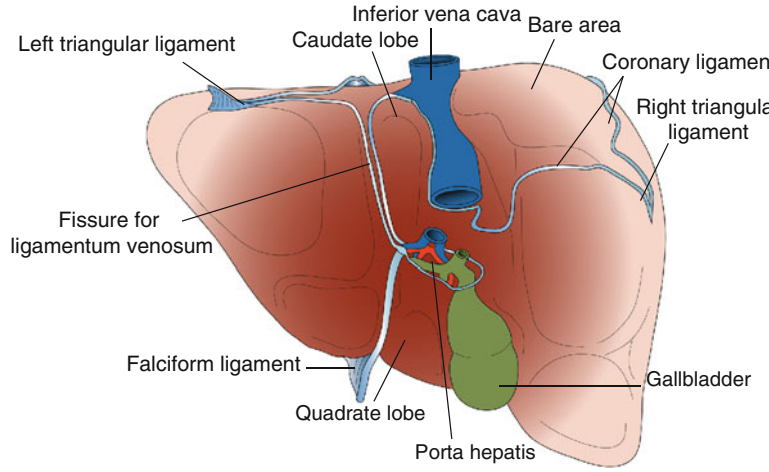

Fig. 1.2 The posterior and inferior surfaces of the liver viewed from behind (©RMcP/MDS 2013)

the liver, between the left lobe and quadrate lobe and in continuity with the free inferior border of the falciform ligament, is the fissure for the ligamentum teres (also known as the umbilical fissure). This contains the remnants of the left umbilical vein that transported oxygenated blood to the left portal vein in the fetus. The gallbladder is attached to the undersurface of the liver between the quadrate lobe and right lobe and embedded within the liver parenchyma to a variable degree. The porta hepatis is a transverse cleft on the inferior surface of the liver wedged between the caudate process behind and the quadrate lobe in front (Fig. 1.2). In this region the portal vein divides and enters the liver behind the right and left hepatic ducts with branches of the hepatic artery between. These structures are enveloped in a sheath of fibrous tissue that also contains lymphatics and autonomic nerves and extends into the liver around the branches of the portal triad.

Weight and Size

The median weight of the normal adult liver is about 1700 g in men and 1400 g in women [3, 4], but the range is wide and related not only to sex but to age, body mass index, ethnicity, and the presence and degree of steatosis. The ratio of liver to body weight decreases from about 4 % at birth (liver weight approximately 130 g) to 3 % by 1 year of age and the adult value of 2 % by 16 years of age [5, 6]. The average vertical dimension of the liver in the midclavicular

line increases from about 6–8 cm in infancy [7] to approximately 14 cm in adults but varies with body mass index [8]. Liver volume increases from a mean value of about 180 cm^3 in infants to approximately 1,100 cm^3 in young adults [9] and is closely correlated with body surface area [10]. The liver changes relatively rapidly during infancy and then more slowly until maturity.

In adults, the sharp inferior border of the liver is often palpable at or just below the right costal margin during inspiration. In infants, the inferior border of the liver is routinely palpable 1–2 cm below the costal margin because the infant has a proportionally larger liver, a flatter diaphragm, more horizontal ribs, and a smaller pelvic cavity [11].

Anatomical Lobes and Ligaments

Viewed from the front, the intact liver has been divided historically into a large right and small left lobe separated by the falciform ligament (Fig. 1.1). Viewed from below, the right lobe can be seen to include two other surface lobes, the quadrate and caudate lobes, separated by the porta hepatis (Fig. 1.2). The caudate lobe forms part of the anterior wall of the lesser sac.

Most of the liver is invested in peritoneum. Where this is reflected on to the diaphragm, abdominal wall, and adjacent viscera, it forms peritoneal ligaments. These ligaments help to stabilise the liver, but the organ is mostly supported by the hepatic veins. The falciform ligament attaches the anterior and superior surfaces of the liver to the anterior abdominal wall above the umbilicus; running in its free inferior margin is the ligamentum teres. The left triangular ligament connects the left lobe of the liver to the undersurface of the diaphragm. There is a corresponding right triangular ligament behind the right lobe, but it is short and the two layers of peritoneum that form it, the upper and lower layers of the coronary ligament, diverge as they pass medially around the bare area of the liver. The bare area of the liver has no covering peritoneum and is in contact with the diaphragm and right adrenal gland (Fig. 1.2).

The lesser omentum is a double fold of peritoneum that connects the undersurface of the liver to the lesser curvature of the stomach and first part of the duodenum. It is attached to the liver along the line of the ligamentum venosum and to the porta hepatis and contains a variable amount of fat. The lesser omentum has a free right margin extending between the liver and first part of the duodenum (also called the hepatoduodenal ligament) in which the three components of the portal triad can be found: the bile duct anteriorly on the right, the hepatic artery anteriorly on the left, and the portal vein posteriorly (Fig. 1.3). This free border of the lesser omentum forms the anterior boundary of the epiploic foramen, a narrow slitlike passageway leading from the peritoneal cavity to the lesser sac behind the stomach.

Internal Anatomy

The anatomical lobes of the liver are demarcated by its external appearance, but the functional divisions of the organ require an understanding of its internal anatomy. Based on the divisions of the portal vein, the liver is composed of eight functional segments, each with its own branch of the portal vein, hepatic artery, and bile duct (Fig. 1.4). The hepatic veins run *between* the liver segments. The segments were originally assigned Roman numerals, but Arabic numerals have since been recommended [12]. The segmental nature of the liver allows it to be subdivided as follows:

1. Right and left *hemilivers* are divided by a midplane that runs from the medial margin of the gallbladder fossa to the middle of the inferior vena cava. The middle hepatic vein runs in this plane.
2. Each hemiliver is divided into two *sectors* according to the divisions of the portal vein. Thus, the right hemiliver has anterior (segments 5 and 8) and posterior (segments 6 and 7) sectors and the left hemiliver has medial (segments 3 and 4) and lateral (segment 2) sectors. The three main hepatic veins run between the sectors (Fig. 1.4). There is an alternative nomenclature that uses the word sections rather than sectors [13]. Liver sections

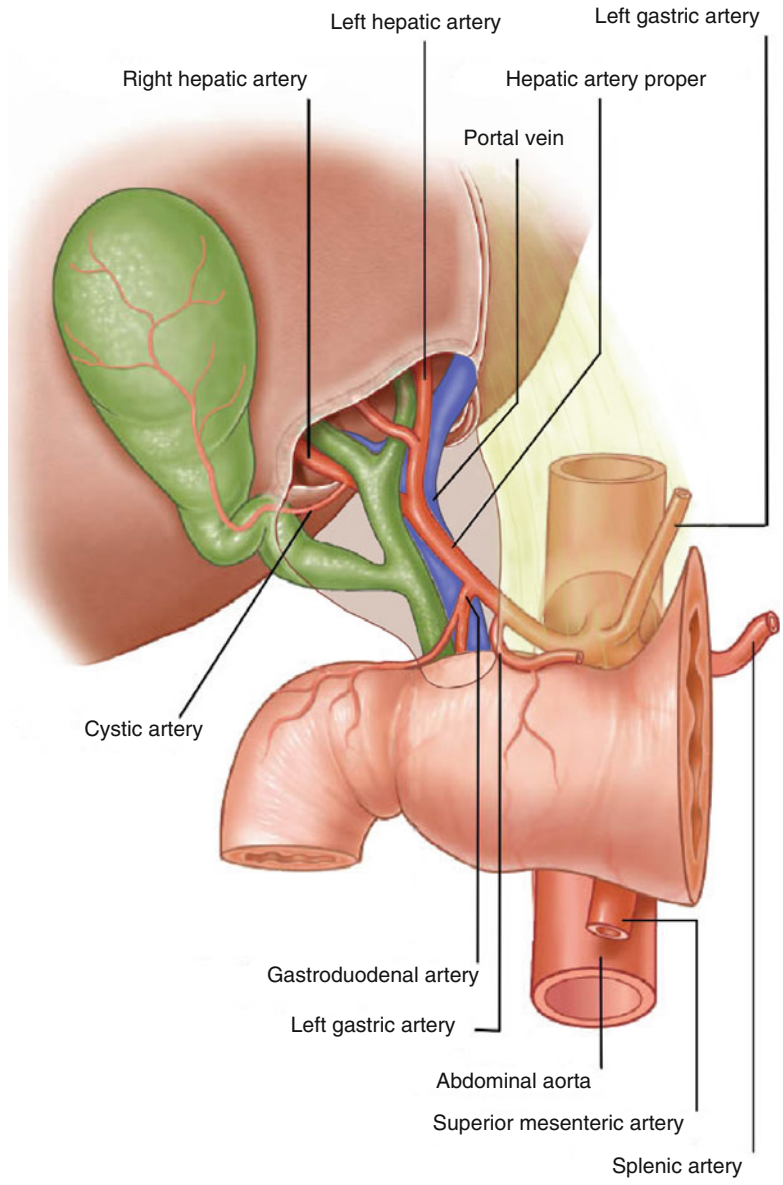

Fig. 1.3 Diagram to illustrate the portal triad within the opened free border of the lesser omentum (©RMcP/MDS 2013)

are configured on the basis of biliary and hepatic artery subdivisions and are equivalent to sectors in the right hemiliver but slightly different in the left hemiliver.

3. The caudate lobe (segment 1) is functionally separate from both hemilivers. It usually receives small vessels from the right and left branches of the portal vein and hepatic artery, often communicates with both right and left hepatic ducts, and drains by several short veins directly into the inferior vena cava.

Blood Supply and Lymphatic Drainage

Hepatic Artery

The common hepatic artery normally arises from the celiac trunk. After giving off the gastroduodenal artery, it becomes the hepatic artery proper, which ascends in the free edge of the lesser omentum anterior to the portal vein and medial to the bile duct (Fig. 1.3). It divides at a variable level into a right and left branch which supply their respective

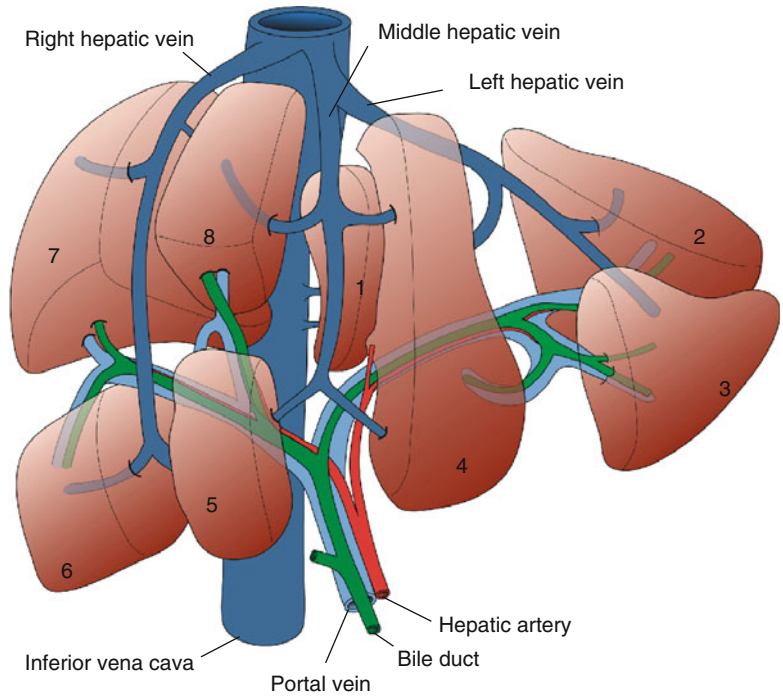

Fig. 1.4 The segmental anatomy of the liver. The numbers 1–8 refer to the numbered segments of the liver. (©RMcP/MDS 2013)

hemilivers. An artery that supplies a liver lobe in addition to the usual artery supplying that lobe is called an *accessory* hepatic artery, while an artery arising from an unusual origin and providing the sole supply to a liver lobe is termed a *replaced* hepatic artery. Accessory or replaced hepatic arteries are common, occurring in up to 30 % of individuals [14–16]. They are particularly important in liver surgery, transplantation, and interventional vascular procedures. The commonest is an accessory or replaced right hepatic artery arising from the superior mesenteric artery or an accessory or replaced left hepatic artery arising from the left gastric artery. The former usually runs immediately behind the portal vein in the free edge of the lesser omentum, while the latter runs within the lesser omentum between the lesser curvature of the stomach and the umbilical fissure. The hepatic artery/arteries contribute about 30 % of hepatic blood supply, while the portal vein accounts for about 70 %.

Portal Vein

The portal vein conveys venous blood from most of the gastrointestinal tract, pancreas, and spleen to the liver. It is a muscular valveless vein. It is formed by the confluence of the superior mesenteric vein and splenic vein behind the neck of the pancreas from where it ascends in the free edge of the lesser omentum before dividing into a right and left branch at the porta hepatis. As it ascends, it lies behind the first part of the duodenum in front of the inferior vena cava surrounded by small nerves and lymphatics. The portal vein has a few tributaries, the most important of which is the left gastric (coronary) vein (Fig. 1.5) which becomes markedly dilated in the presence of portal hypertension.

The left branch of the portal vein runs laterally towards segment 2 but angles forward in the umbilical fissure to give branches to segments 3 and 4 (Fig. 1.4). At this site in the fetus, it received the left umbilical vein (which becomes the ligamentum teres after birth). The right branch of the portal vein has a short course outside the liver and usually divides into anterior and posterior sectoral branches soon after entering the liver substance. Variations in the normal branching pattern of the portal vein occur. For example, it may trifurcate at the liver hilum giving one left and two right portal vein branches, or it may bifurcate within the substance of the liver rather than at the hilum.

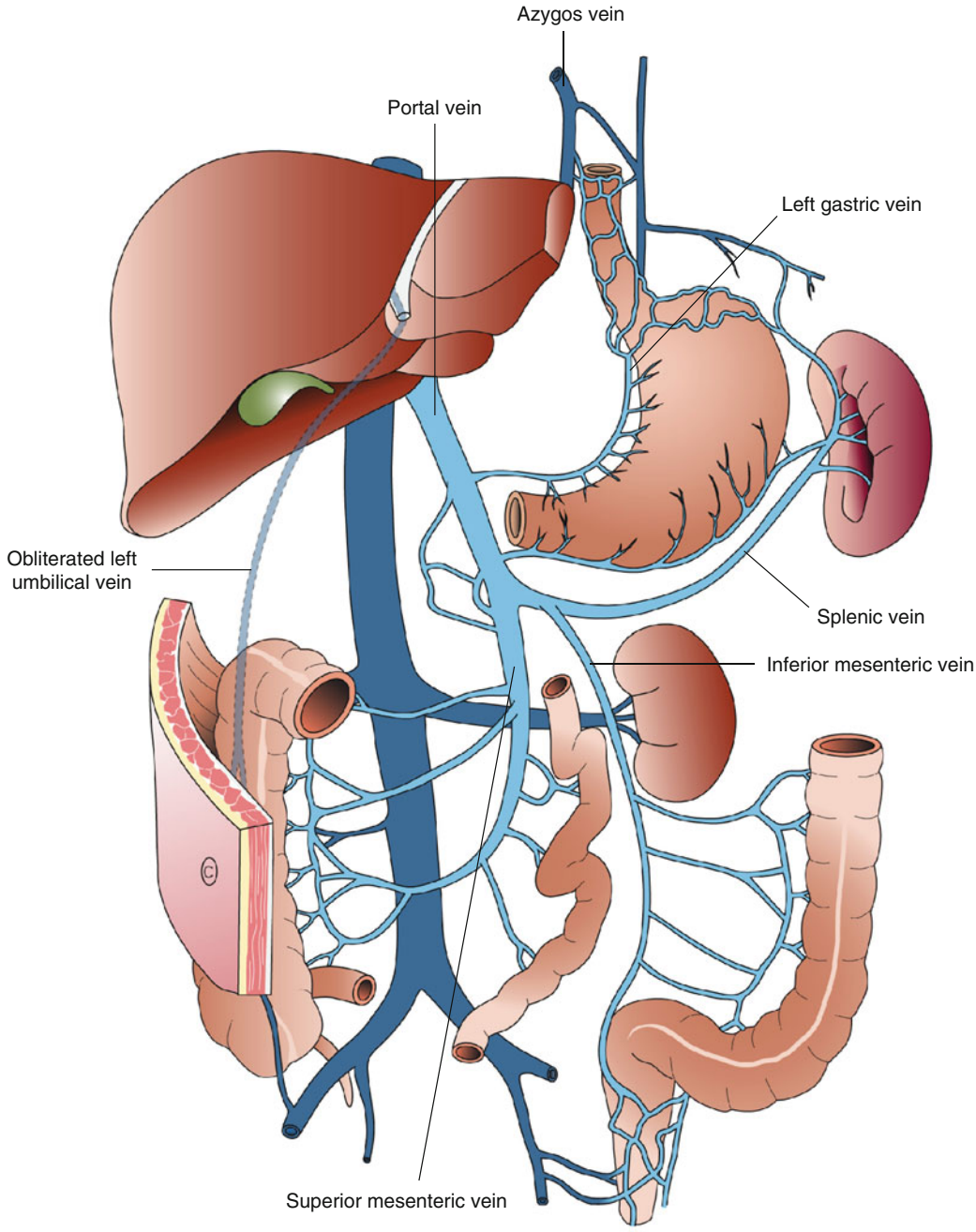

Fig. 1.5 The portal venous system. Portosystemic anastomoses exist around the lower oesophagus, anorectum, and periumbilical region and in the retroperitoneum (©RMcP/MDS 2013)

Hepatic Veins

There are three main hepatic veins, right, middle, and left, which run between the four sectors of the liver (Figs. 1.4, 1.5, and 1.6). The middle hepatic vein occupies the midplane of the liver. The hepatic veins drain venous blood from the liver directly into the inferior vena cava just below the diaphragm. The left and middle veins usually join to form a short common trunk before entering the inferior vena cava [17]. Almost 20 %

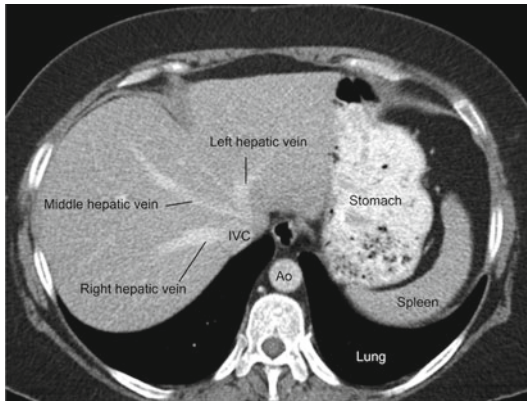

Fig. 1.6 Axial abdominal CT scan showing the three main hepatic veins. *Ao* aorta, *IVC* inferior vena cava (©MDS 2013)

of adults have an additional right inferior hepatic vein (>5 mm) which is important in right lobe liver transplantation.

In addition to the three main hepatic veins, numerous small veins, mostly from the caudate lobe, drain directly into the retrohepatic inferior vena cava. If the three main hepatic veins are blocked (Budd-Chiari syndrome), the caudate lobe is able to continue draining independently and undergoes hypertrophy to compensate for deteriorating liver function in the other congested segments of the liver.

Lymphatic Drainage

Superficial lymphatics on the surface of the liver drain to lymph nodes at the porta hepatis, and around the inferior vena cava and celiac trunk; some drain directly to the thoracic duct. Lymphatics within the liver parenchyma accompany hepatic veins to paracaval lymph nodes or drain to lymph nodes at the porta hepatis.

Innervation

The liver is supplied by somatic and autonomic nerves. The liver capsule is innervated by lower intercostal nerves. Stretching or tearing the capsule causes localised pain in the right upper quadrant of the abdomen. The autonomic nerves comprise parasympathetic fibres conveyed via hepatic branches of the vagus nerve and sympathetic fibres travelling via the celiac plexus. These autonomic nerves supply the gallbladder and extrahepatic bile ducts before entering the liver at the porta hepatis to run with intrahepatic bile ducts and blood vessels. Visceral pain from within the liver is poorly localised and usually referred to the epigastrium.

Intrahepatic Bile Ducts

Intrahepatic bile duct branching typically follows the same segmental pattern as the portal vein and hepatic artery. The right and left hepatic ducts are formed at the hilum of the liver and usually drain their corresponding hemilivers, although anatomical variants are recognised [18]. The caudate lobe (segment 1) drains bile from several small ducts in the region of the confluence of the right and left hepatic ducts. The left hepatic duct has a longer and more horizontal course than the right, a feature that can be beneficial in biliary bypass surgery and operations for congenital choledochal dilatation [19].

The anatomy of the gallbladder and extrahepatic bile ducts is beyond the remit of this chapter on the liver. Figure 1.7 shows a normal cholangiogram demonstrated by endoscopic retrograde cholangiography to illustrate the basic normal anatomy of the biliary tree.

Cross-Sectional Imaging of the Liver: Anatomical Aspects

The interpretation of cross-sectional images of the liver requires an understanding of normal and abnormal anatomy and merits some further comment. The anatomy of the liver and its vascular and biliary elements are well seen using ultrasound and colour Doppler imaging (Fig. 1.8). Intrahepatic bile ducts are not usually visible in healthy infants using ultrasound [20]. If there is obstructing bile duct pathology such as congenital choledochal dilatation, intrahepatic duct dilatation is often evident [21]. However, despite the obstructive nature of the disease, it is very rarely observed in the common type of biliary atresia (type 3) because the intrahepatic ducts are abnormal and there is liver fibrosis.

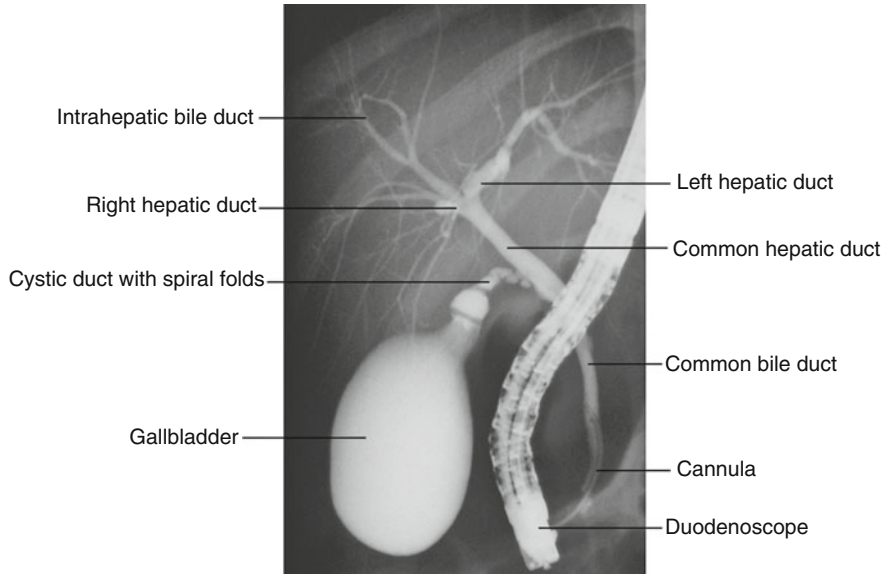

Fig. 1.7 An endoscopic retrograde cholangiogram demonstrating normal biliary tract anatomy (©MDS 2013)

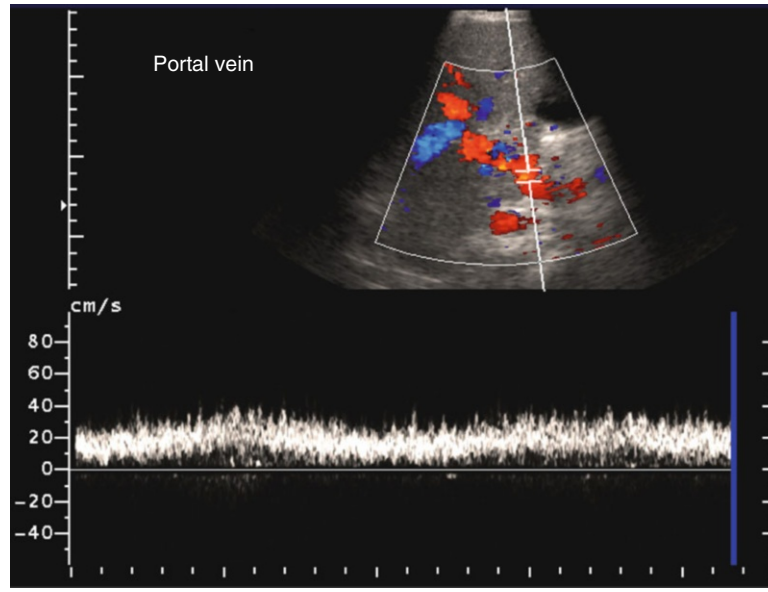

Fig. 1.8 The portal vein and its blood flow are well visualised using ultrasound with colour Doppler (©MDS 2013)

Despite the absence of dilated intrahepatic bile ducts in type 3 biliary atresia, a range of anatomical ultrasound features have been reported which, when assessed collectively by an expert sonographer, can prove highly accurate in diagnosis [22]. The diameter of the common bile duct varies with age, normal values being up to 2 mm in infancy, 4 mm in childhood, and 7 mm in adolescence [20].

Computed tomography (CT) with intravenous contrast enhancement, magnetic resonance imaging (MRI), and gadolinium-enhanced magnetic resonance angiography (MRA) provide invaluable detail on the anatomy of the liver and its vessels prior to surgical resection. MR cholangiography offers the ability to noninvasively assess the biliary tree, but its resolution is a limiting factor in infancy [23].

Liver Resection: Anatomical Aspects

One of the commonest indications for liver surgery is a liver tumour. The goals of surgery are complete excision of the mass with appropriate margins leaving the residual liver with a sealed cut surface, adequate arterial and portal inflow, intact biliary drainage, and satisfactory venous outflow. During a liver resection, it may be necessary to temporarily compress the portal triad in the free edge of the lesser omentum (known as the Pringle manoeuvre) to occlude vascular inflow. This helps to reduce blood loss during transection of the liver parenchyma [24]. An ischemic period of up to 1 h is tolerated well by the normal liver and even longer periods are possible if the portal triad is clamped intermittently [25] or after ischemic preconditioning [26]. The underlying mechanism may be downregulation of apoptotic pathways in hepatocytes. Venous congestion of the gut can be a problem with temporary portal vein occlusion, especially in patients without preexisting portal hypertension.

Maintenance of a low central venous pressure (CVP, 0–4 cm H_2O) during the phase of parenchymal transection helps to minimise blood loss and is facilitated by a slight head down tilt [27]. The CVP should be maintained above 0 cm H_2O to avoid the risk of air embolism when hepatic veins are opened.

Standard liver resections are based on segmental hepatic anatomy [1, 2]. The terminology that has been used to describe these resections is confusing, but attempts have been made to standardise the nomenclature [12, 13, 28]. It is estimated that up to 85 % of the normal liver may be successfully resected in a child provided that the remaining parenchyma is healthy and the arterial and portal inflow and hepatic venous outflow are protected [29, 30]. Major resections are better tolerated if the residual healthy liver has undergone compensatory hypertrophy as a result of the disease process. Nonanatomic hepatic resections, i.e. those not involving standard anatomical planes, can be performed for excision of small tumours and repeat resections and in liver trauma.

Following advances in anatomy, surgery, chemotherapy, imaging, and anaesthesia, the limits of successful liver tumour excision in children have been extended using a variety of techniques. Thus, large central tumours and those involving the hepatic vein confluence or inferior vena cava can now be treated with curative intent. Such techniques include preoperative chemotherapy to shrink the tumour [31], selective preoperative portal vein embolisation to encourage growth of an anticipated liver remnant [32], partial excision and/or reconstruction of the inferior vena cava or hepatic vein confluence [33], and liver transplantation (the ultimate hepatic resection for an otherwise unresectable liver tumour with no evidence of persistent extrahepatic disease) [34].

Paediatric Liver Transplantation: Anatomical Aspects

A relative shortage of size-matched donors in paediatric liver transplantation forced the development of a variety of techniques to create a liver graft suitable for a child from an adult donor. Preparation of these grafts is dependent on a sound understanding of liver anatomy. The various graft options from a brain dead adult donor (Fig. 1.9) include:

- Whole liver graft.
- Reduced-size graft in which a single smaller liver graft is prepared from a liver donor by excision of part of the liver [35].
- Split-liver graft in which the donor liver is divided into a larger right and smaller left liver graft to enable transplantation of two recipients [36, 37]. When an anatomical left lobe of liver is transplanted (segments 2 and 3), this is often referred to confusingly as a ‘left lateral segment graft’.
- Monosegment graft. Segment 2 *or* 3 can be prepared from a paediatric donor for transplant into a small infant weighing as little as 3 kg [38]. A variant of this is a reduced ‘left lateral segment’ graft [39].

A further option is a liver graft procured from a living donor, usually a close relative. The ‘left lateral segment’ living donor graft was introduced first [40, 41], but adult-to-adult right lobe liver transplantation [42] has since become increasingly popular.

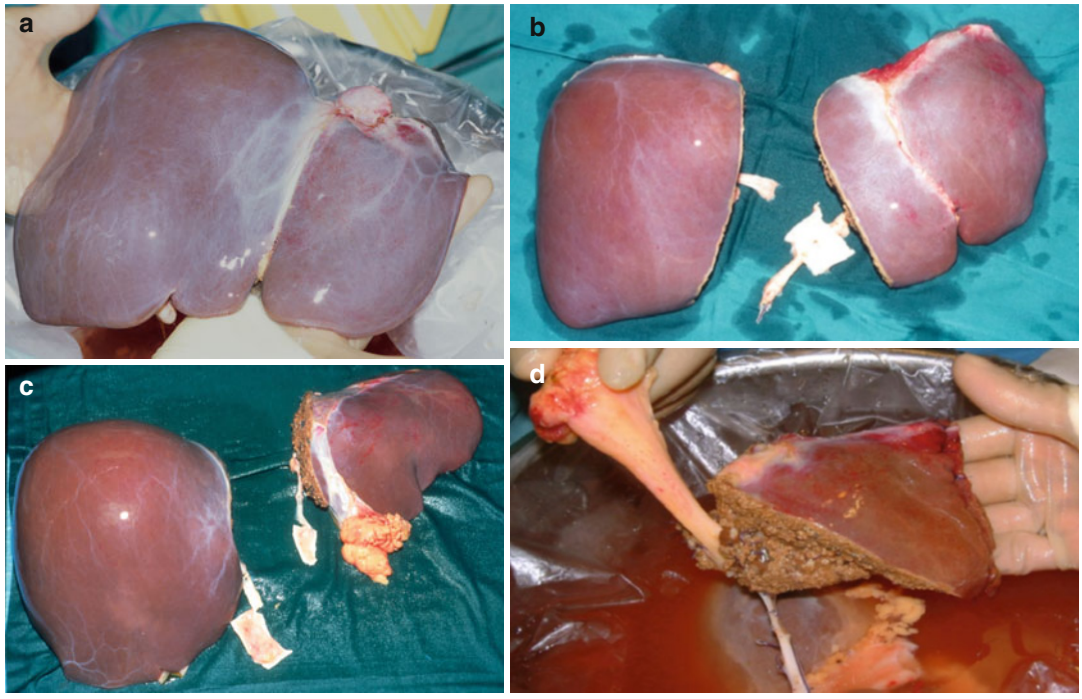

Fig. 1.9 Types of liver grafts from deceased adult donors. (**a**) Whole liver graft. (**b**) Split-liver grafts: right and left hemilivers. (**c**) Split-liver grafts: right and left lobe grafts. (**d**) Reduced left lateral segment graft (similar to a monosegment) (©MDS 2013)

Beyond infancy, the ratio of a child's liver weight to their body weight is about 2.5 %. When transplanting a liver graft from a living donor, the aim is to obtain a minimum graft-to-recipient body weight ratio of 1 % [43]. Thus, the minimum graft weight for a 20 kg child would be about 200 g (a greater liver mass is desirable from a brain dead donor). The function of the transplanted liver graft not only depends on this critical ratio but also on a range of other graft and host factors. Small-for-size grafts (<0.8 % graft-to-recipient body weight ratio) are associated with reduced metabolic and synthetic capacity and poorer graft survival.

Large-for-size grafts are also associated with increased morbidity and a greater risk of graft failure. Broad guidelines about adult donor-to-paediatric recipient weight mismatches are available to reduce the risk of transplanting excessively large-for-size grafts. With whole graft and right lobe liver transplantation, the donor-to-recipient weight mismatch can be up to 1.5:1 (sometimes more if the recipient has marked hepatomegaly or ascites); with left lobe grafts the ratio is up to 3:1; with 'left lateral segment' grafts, up to 10:1; and with a monosegment, up to 15:1 (occasionally greater). In adults, segments 2 and 3 combined (the so-called left lateral segment) comprise approximately 20–25 % of total liver weight and segments 2, 3, and 4 (the left hemiliver) about 40 %.

Portal Hypertension: Anatomical Aspects

The portal vein drains the spleen, pancreas and most of the gut via the splenic and mesenteric veins. The portal venous system communicates with systemic veins at multiple sites. In the presence of sustained portal hypertension, when portal venous pressure persistently exceeds the normal value of 7–12 mmHg, these portosystemic anastomoses dilate and may form varices. The classical sites at which varices become

apparent are the distal oesophagus (oesophageal varices), the anorectum (anorectal varices), and around the umbilicus (ligamentum teres and anterior abdominal wall) (Fig. 1.5). Submucosal varices in the distal 5 cm of the oesophagus are particularly prone to rupture.

The treatment of portal hypertension is dictated by the underlying cause and anticipated prognosis of the condition [44]. A detailed understanding of portal venous anatomy is essential for surgical management which may include:

- Portosystemic shunt surgery – fashioning a shunt between a large portomesenteric vein such as the superior mesenteric or splenic vein and a large systemic vein such as the inferior vena cava or renal vein
- Mesenterico-portal bypass – bypassing an occluded portal vein with an interposition graft between the superior mesenteric vein and the left portal vein within the liver (suitable for portal vein occlusion in the absence of liver disease) [45]
- Liver transplantation – for severe portal hypertension complicating end-stage liver disease

Transjugular intrahepatic portosystemic stent shunt (TIPS) is a less invasive but less durable alternative to portosystemic shunt surgery. It may nevertheless be a useful option in selected cases. It involves creating an intrahepatic portosystemic shunt by percutaneously inserting an expandable metal stent between the hepatic and portal veins in the liver [46].

Liver Microstructure

Most of the surface of the liver is covered by peritoneum. Beneath this, and enveloping the whole liver, is a thin fibrous capsule that is loosely connected to connective tissue trabeculae within the liver and continuous with the connective tissue sheath surrounding the branches of the portal triad ramifying within the liver. Each portal tract contains a branch of the portal vein and one or more hepatic arterial branches and terminal bile ducts, together with lymphatics and autonomic nerves. The intrahepatic bile ducts are supplied by hepatic arterial branches. Both intra- and extrahepatic bile ducts have peribiliary mucous glands in their walls.

The structural unit of the liver is the *lobule*, while the functional unit is the *acinus* (Fig. 1.10). A lobule has a poorly defined connective tissue framework and is characterised by a central vein (a terminal hepatic venule) and plates of epithelial cells (hepatocytes) radiating out to portal triads at the periphery of the lobule (Fig. 1.11). Hepatocyte plates are two cells thick in infants and young children but only one cell thick in adults. They are separated by intercommunicating hepatic sinusoids. The acinus, in contrast, is an ovoid mass of tissue centred on a terminal branch of an hepatic arteriole and portal venule; the hepatic veins are peripheral. In the acinus, hepatocytes can be divided into three zones: zone 1 (periportal) nearest the terminal branches of the hepatic arteriole and portal venule, zone 2 which is intermediate, and zone 3 (perivenular) closest to the central vein. Hepatocytes in each of these zones are adapted to different metabolic functions. For example, zone 1 hepatocytes are better adapted to oxidative activities such as gluconeogenesis, β-oxidation of fatty acids, and bile acid secretion, whereas zone 3 hepatocytes are concerned more with glycolysis and drug metabolism.

Hepatic sinusoids are lined by endothelial cells and supplied by arterial and portal venous blood (Fig. 1.10). Outside the sinusoidal endothelium is the narrow perisinusoidal space (of Disse) which communicates with intrahepatic lymphatics. Blood flow is towards the central veins and thence to hepatic veins. Bile is secreted by hepatocytes into biliary canaliculi which eventually join to form bile ductules. Bile flow is towards the periphery of the lobule.

The liver contains a variety of cell populations (Table 1.1; Fig. 1.12). Bile drains into small canaliculi between adjacent hepatocytes and these empty into a trough-like structure called a canal of Hering [48]. The canals of Hering are lined by both cholangiocytes and hepatocytes and drain into biliary ductules which, in turn, open into terminal bile ducts within the portal tracts; biliary ductules and ducts are lined by cholangiocytes. Chloride transport by cholangiocytes is dependent on the cystic fibrosis transmembrane

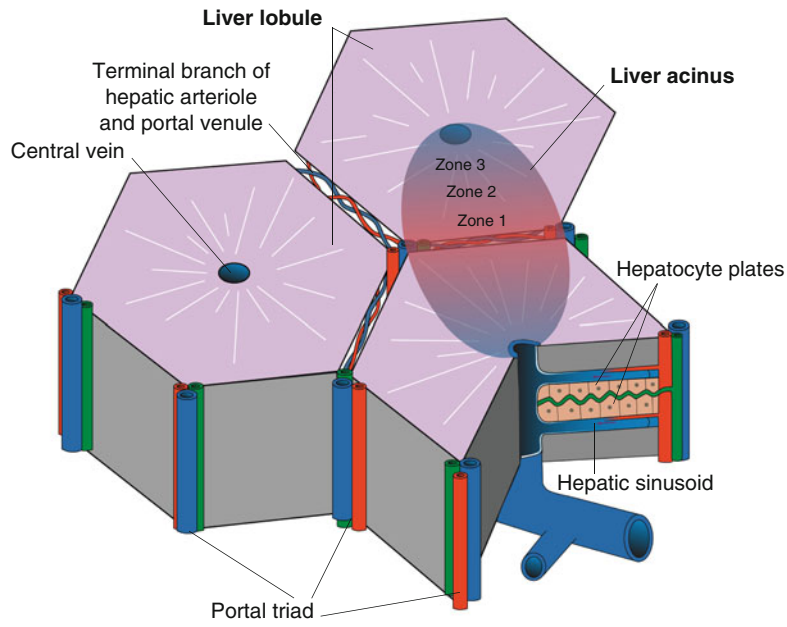

Fig. 1.10 Schematic diagram of liver histoarchitecture (© RMcP/MDS 2013)

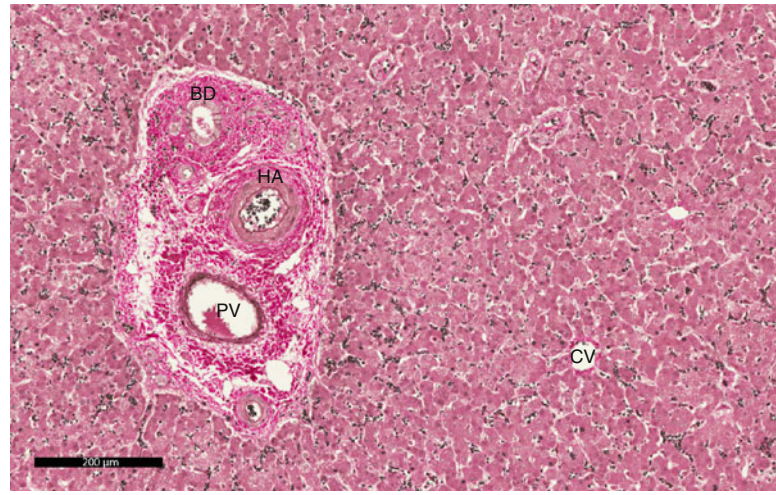

Fig. 1.11 Photomicrograph of normal liver (haematoxylin and eosin). Note the portal triad containing a bile duct (*BD*), hepatic artery (*HA*), and portal vein (*PV*) and the irregular plates of hepatocytes radiating out from the central venule (*CV*) of each lobule (©MDS 2013)

conductance regulator protein encoded by the CFTR gene and is an important determinant of normal bile composition and flow.

The existence of intrahepatic interstitial cells of Cajal (ICCs) is controversial. These cells are found in close proximity to smooth muscle throughout the gut and are involved in smooth muscle motility, providing electrical coupling between nerve fibre terminals and smooth muscle fibres [49]. Although one study reported c-kit positive cells with ICC-like morphology within intrahepatic portal triads [50], mast cells can have a similar appearance and, using rigorous immunohistochemical techniques, another study found evidence of ICCs only in extrahepatic bile ducts [49].

Table 1.1 Cell types within the liver

Cell type	Features	Functions
Hepatocytes	Commonest cell type, ~20 μm across. Microvilli on sinusoidal surfaces and biliary canaliculus between adjacent lateral surfaces. Most have single nucleus but polyploidy common. Prominent intracellular organelles, glycogen granules, lipid vacuoles, peroxisomes, iron storage vacuoles, etc. (Fig. 1.12)	Metabolic functions of the liver, bile secretion
Sinusoidal endothelial cells	Highly fenestrated with minimal basement membrane. Fenestrations are larger in periportal zone and can change in size in response to pressure, toxins, drugs, etc. Fenestrations are clustered forming a sieve	Allow direct communication between sinusoids and hepatocytes
Hepatic stellate cells (Ito cells)	Found within perisinusoidal space of Disse. Contain lipid droplets	Store vitamin A and growth factors and synthesise collagen. Can transform into myofibroblast-like cells which are involved in hepatic fibrosis
Macrophages (Kupffer cells)	Macrophages residing in the sinusoids and attached to sinusoidal endothelium	Phagocytic removal of microbes and effete red cells and secretion of cytokines
Hepatic stem cells	Found in ductal plates in fetal livers and in canals of Hering in adult livers	Pluripotent precursors of hepatoblasts and therefore hepatocytes and biliary epithelium [47]
Cholangiocytes	Line bile ductules	Transport of bile from biliary canaliculi via canals of Hering
Haemopoietic cells	Present in the normal human fetal liver	In the fetus, the liver is a major site of haemopoiesis
Fibroblasts	Found in loose connective tissue framework of liver	Collagen synthesis
Lymphocytes	Mostly T cells in portal tracts and sinusoids	Able to neutralise tumour cells and viruses

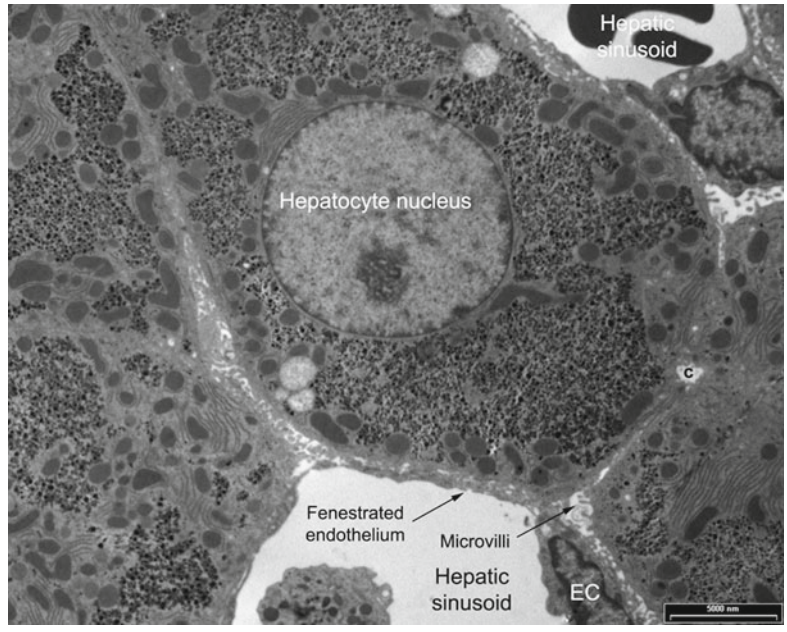

Fig. 1.12 Transmission electron micrograph of a rat hepatocyte showing prominent intracellular organelles, a microvillus sinusoidal border, and a biliary canaliculus (*c*) with adjacent tight junctions. The glycogen granules have been preserved and appear black. *EC* endothelial cell (Kindly provided by Allan Mitchell, Otago Centre for Electron Microscopy)

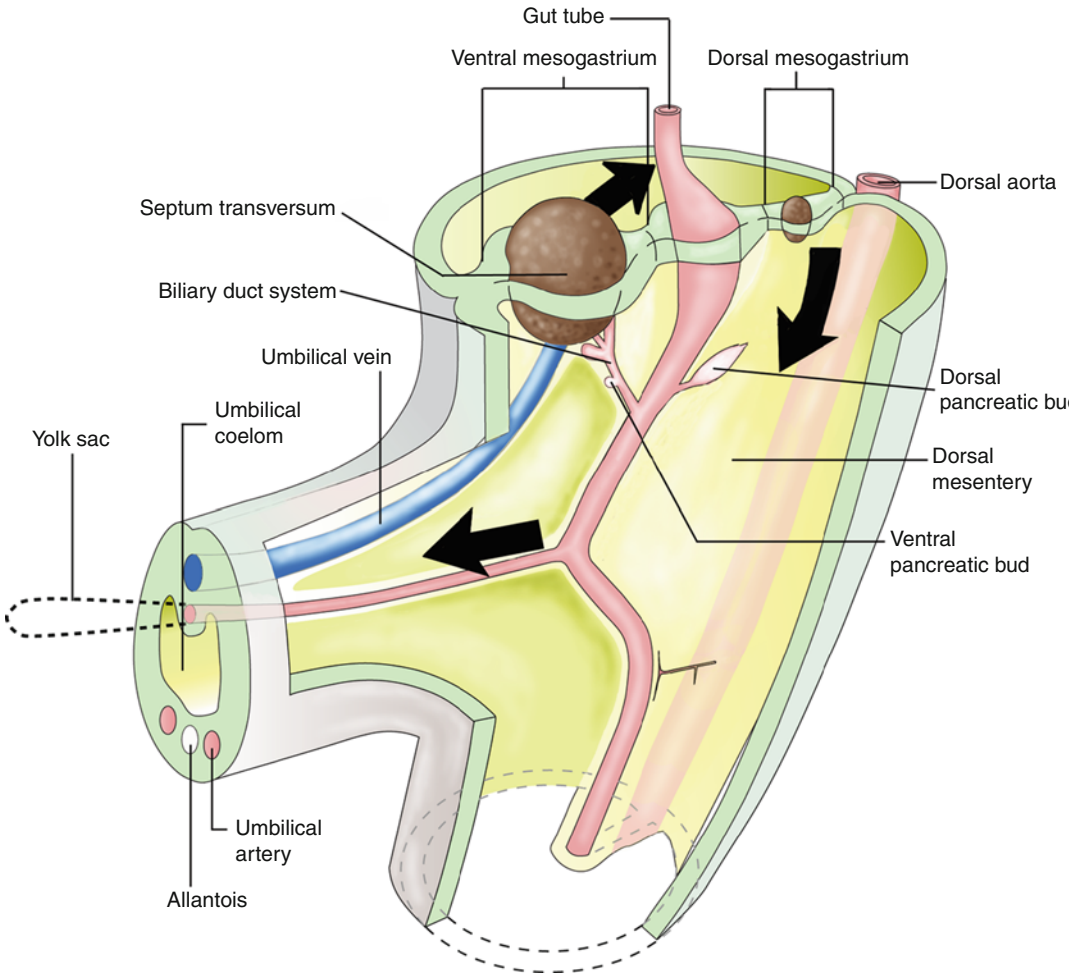

Fig. 1.13 Schematic diagram of the developing liver in the embryo. The liver forms in the ventral mesogastrium and the spleen in the dorsal mesogastrium. The upper two *thick black arrows* indicate the subsequent direction of rotation of the mesogastrium and its associated viscera. The *lower arrow* represents the direction of temporary midgut herniation into the umbilicus (©RMcP/MDS 2013)

Development

Liver

The liver develops within a double fold of peritoneum, the ventral mesogastrium that connects the stomach to the anterior abdominal wall (Fig. 1.13). The liver is derived from two components: (1) an endodermal outgrowth from the foregut that grows into (2) the septum transversum, a mass of mesenchymal tissue caudal to the heart. This endodermal foregut diverticulum is first recognisable at about 4 weeks' gestation. Its caudal part forms the gallbladder and the extrahepatic bile ducts with the exception of the right and left hepatic ducts which develop from its cranial part [51]. The cranial part of the diverticulum also gives rise to epithelial cells that migrate into the septum transversum and develop into primitive hepatocytes that later differentiate into mature hepatocytes or bile duct epithelium [52]. These epithelial cells stimulate the surrounding mesenchyme to form endothelial cells and rudimentary sinusoids that become connected to the paired vitelline veins within the liver (the precursors of the portal and hepatic veins). Between about 5 and 20 weeks' gestation, the

sinusoidal endothelium changes from a continuous to a fenestrated type [53].

The liver is proportionally large in the embryo and fetus and occupies much of the abdominal cavity at about 3 months' gestation. It is a major site of haemopoiesis from about 4–6 weeks of gestation [54] until mid-gestation when the bone marrow and spleen take over this function.

Intrahepatic Bile Ducts

The development of intrahepatic portal triads begins with the formation of a portal vein branch which induces the formation of a bile duct and then an artery. The primitive hepatocytes surrounding a portal vein branch initially form a sleeve of cells called the ductal plate [51, 52]. Through a process of remodelling, the ductal plate forms intrahepatic bile ducts, while the adjacent mesenchyme gives rise to arterial branches that connect with the hepatic artery. The formation of intrahepatic bile ducts advances from the hilum of the developing liver to its periphery; these ducts normally remain patent throughout development [55]. Bile pigments can be detected within hepatocytes and ductal plate epithelial cells from the 12th week of gestation [56]. About one-third of infants with biliary atresia, and a greater proportion of others with some other types of congenital disorders of the liver, show evidence of ductal plate malformation characterised by the persistence of embryonic bile duct structures within portal tracts [57].

Extrahepatic Bile Ducts

The gallbladder and all of the extrahepatic bile ducts except for the right and left hepatic ducts are formed from the caudal part of the endodermal hepatic diverticulum. The common bile duct retains its connection to the foregut at the major duodenal papilla, which opens into the second part of the duodenum. In this region, the terminal common bile duct unites with the terminal pancreatic duct to form a short common channel. An abnormal union of the ducts is often associated with an abnormally long common channel, which predisposes to admixture of pancreatic juice and bile within the ductal system. This abnormality is often a feature of congenital choledochal dilatation and predisposes to recurrent acute pancreatitis in the short term and gallbladder cancer in the long term [58]. A normal common channel is up to 3 mm long in infants, 5 mm in older children [59], and no more than 5–10 mm in adults.

Portal Vein

In the embryo, the right and left vitelline veins emerge from the yolk sac, cross the septum transversum, and drain into the sinus venosus. In the 4-week embryo, cross communications develop between the vitelline veins (Fig. 1.14a). The three principal ones are the (1) subhepatic ventral duodenal, in the region of the porta hepatis; (2) intermediate dorsal duodenal; and (3) caudal ventral duodenal, distal to the entry of the common bile duct [60]. These cross-communicating veins anastomose with each other forming a figure of 8 around the developing duodenum. Selective involution yields the final configuration of the portal vein during the embryonic period of gestation (weeks 3–10) (Fig. 1.14b). The intrahepatic branches of the portal vein are derived from an anastomosing plexus of paired vitelline veins within the developing liver in the septum transversum. Abnormalities in development of the portal vein may lead to congenital portal vein obstruction [44] or, more rarely, a congenital portosystemic venous shunt [61].

Ductus Venosus

The subhepatic ventral duodenal anastomosis connects to the subdiaphragmatic anastomosis by a median longitudinal channel, the primitive ductus venosus, lying dorsal to the expanding hepatic primordium (Fig. 1.14a). In the 5–6-week embryo, the right umbilical vein regresses completely so that blood from the placenta subsequently enters the liver exclusively via the left umbilical vein. As much as 30 % of this inflow

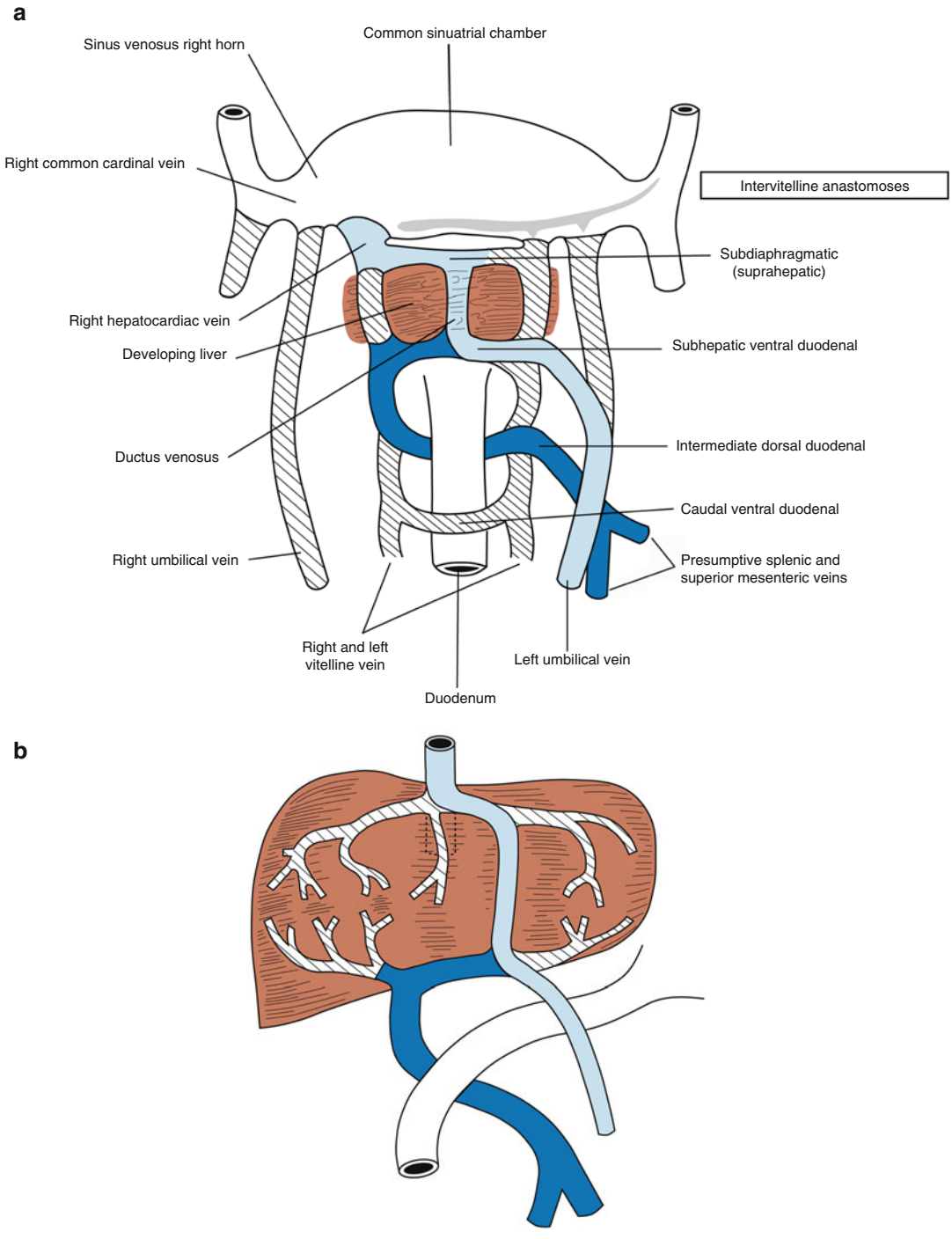

Fig. 1.14 (**a**) Diagram illustrating the development of the portal vein in the embryo. The future portal vein is in dark blue and the crosshatched areas represent veins that normally regress or become small venous channels. (**b**) The normal fetal arrangement of portal and hepatic venous anatomy (Adapted from Stringer [61] ©RMcP/MDS 2013)

short-circuits the liver through the ductus venosus [62] which arises in the fetus from the posterior aspect of the left branch of the portal vein opposite the opening of the left umbilical vein within the umbilical recess of the liver. The ductus venosus passes cranially and laterally to join the left hepatic vein near its entry into the inferior vena cava. Spontaneous closure of the ductus venosus begins immediately after birth [63], and functional closure is normally complete by about 17 days of age [64, 65]. The residual ligamentum venosum runs within the fissure between the anatomic left and right lobes of the liver. A persistent patent ductus venosus is rare and may be associated with hypoplasia of the intrahepatic portal veins and be complicated by hepatic encephalopathy and/or the development of hepatopulmonary syndrome [66–69].

The complex molecular mechanisms underlying liver and bile duct development are beyond the scope of this chapter but have been reviewed in the literature [70, 71].

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References

1. Couinaud C. Controlled hepatectomies and exposure of the intrahepatic bile ducts. Anatomical and technical study. Paris: C. Couinaud; 1981.
2. Bismuth H, Houssin D, Dastaing D. Major and minor segmentectomies “reglees” in liver surgery. World J Surg. 1982;6:10–24.
3. de la Grandmaison GL, Clairand I, Durigon M. Organ weight in 684 adult autopsies: new tables for a Caucasoid population. Forensic Sci Int. 2001;119:149–54.
4. Molina DK, DiMaio VJ. Normal organ weights in men: part ii-the brain, lungs, liver, spleen, and kidneys. Am J Forensic Med Pathol. 2012;33:368–72.
5. Schulz DM, Giordano DA, Schulz DH. Weights of organs of fetuses and infants. Arch Pathol. 1962;74:244–50.
6. Archie JG, Collins JS, Lebel RR. Quantitative standards for fetal and neonatal autopsy. Am J Clin Pathol. 2006;126:256–65.
7. Konuş OL, Ozdemir A, Akkaya A, Erbaş G, Celik H, Işik S. Normal liver, spleen, and kidney dimensions in neonates, infants, and children: evaluation with sonography. AJR Am J Roentgenol. 1998;171:1693–8.
8. Kratzer W, Fritz V, Mason RA, Haenle MM, Kaechele V, Roemerstein Study Group. Factors affecting liver size: a sonographic survey of 2080 subjects. J Ultrasound Med. 2003;22:1155–61.
9. Noda T, Todani T, Watanabe Y, Yamamoto S. Liver volume in children measured by computed tomography. Pediatr Radiol. 1997;27:250–2.
10. Urata K, Kawasaki S, Matsunami H, Hashikura Y, Ikegami T, Ishizone S, Momose Y, Komiyama A, Makuuchi M. Calculation of child and adult standard liver volume for liver transplantation. Hepatology. 1995;21:1317–21.
11. Stringer MD. Clinical anatomy of the newborn. In: Puri P, editor. Newborn surgery. 3rd ed. London: Hodder Arnold; 2011. p. 29–38.
12. Celinski SA, Gamblin TC. Hepatic resection nomenclature and techniques. Surg Clin North Am. 2010;90:737–48.
13. The Terminology Committee of the IHPBA. The Brisbane 2000 terminology of hepatic anatomy and resections. HPB. 2000;2:333–9.
14. Hiatt JR, Gabbay J, Busuttil RW. Surgical anatomy of the hepatic arteries in 1000 cases. Ann Surg. 1994;220:50–2.
15. Gruttadauria S, Foglieni CS, Doria C, et al. The hepatic artery in liver transplantation and surgery: vascular anomalies in 701 cases. Clin Transplant. 2001;15:359–63.
16. López-Andújar R, Moya A, Montalvá E, et al. Lessons learned from anatomic variants of the hepatic artery in 1,081 transplanted livers. Liver Transpl. 2007;13:1401–4.
17. Nakamura S, Tsuzuki T. Surgical anatomy of hepatic veins and inferior vena cava. Surg Gynecol Obstet. 1981;152:43–50.
18. Cucchetti A, Peri E, Cescon M, et al. Anatomic variations of intrahepatic bile ducts in a European series and meta-analysis of the literature. J Gastrointest Surg. 2011;15:623–30.
19. Stringer MD. Wide hilar hepatico-jejunostomy: the optimum method of reconstruction after choledochal cyst excision. Pediatr Surg Int. 2007;23:529–32.
20. Siegel MJ. Liver and biliary tract. In: Siegel MJ, editor. Pediatric sonography. New York: Raven; 1995. p. 171.
21. Lee HC, Yeung CY, Chang PY, Sheu JC, Wang NL. Dilatation of the biliary tree in children: sonographic diagnosis and its clinical significance. J Ultrasound Med. 2000;19:177–82.
22. Humphrey TM, Stringer MD. Biliary atresia: US diagnosis. Radiology. 2007;244:845–51.
23. Chan YL, Yeung CK, Lam WW, Fok TF, Metreweli C. Magnetic resonance cholangiography – feasibility and application in the paediatric population. Pediatr Radiol. 1998;28:307–11.
24. Man K, Fan ST, Ng IO, et al. Prospective evaluation of Pringle maneuver in hepatectomy for liver tumors by a randomized study. Ann Surg. 1997;226:704–13.
25. Belghiti J, Noun R, Malafosse R, et al. Continuous versus intermittent portal triad clamping for liver resection. A controlled study. Ann Surg. 1999;229:369–75.

26. Clavien PA, Yadav S, Sindram D, Bentley RC. Protective effects of ischemic preconditioning for liver resection performed under inflow occlusion in humans. Ann Surg. 2000;232:155–62.
27. Rees M, Plant G, Wells J, Bygrave S. One hundred and fifty hepatic resections: evolution of technique towards bloodless surgery. Br J Surg. 1996;83: 1526–9.
28. Strasberg SM, Phillips C. Use and dissemination of the Brisbane 2000 nomenclature of liver anatomy and resections. Ann Surg. 2013;257:377–82.
29. Starzl TE, Putnam CW, Groth CG, et al. Alopecia, ascites, and incomplete regeneration after 85 to 90 per cent liver resection. Am J Surg. 1975;129:587–90.
30. Filler RM. Liver resections. In: Spitz L, Coran AG, editors. Pediatric surgery. London: Chapman and Hall Medical; 1995. p. 579–89.
31. Ortega JA, Krailo MD, Haas JE, et al. Effective treatment of unresectable or metastatic hepatoblastoma with cisplatin and continuous infusion doxorubicin chemotherapy: a report from the Childrens Cancer Study Group. J Clin Oncol. 1991;9:2167–76.
32. Kaneko K, Ando H, Watanabe Y, et al. Aggressive preoperative management and extended surgery for inflammatory pseudotumor involving the hepatic hilum in a child. Surgery. 2001;129:757–60.
33. Superina RA, Bambini D, Filler RM, Almond PS, Geissler G. A new technique for resecting 'unresectable' liver tumors. J Pediatr Surg. 2000;35:1294–9.
34. Stringer MD. The role of liver transplantation in the management of paediatric liver tumours. Ann Roy Coll Surg Engl. 2007;89:12–21.
35. Bismuth H, Houssin D. Reduced-sized orthotopic liver graft in hepatic transplantation in children. Surgery. 1984;95:367–70.
36. Pichlmayr R, Ringe B, Gubernatis G, Hauss J, Bunzendahl H. Transplantation of a donor liver to 2 recipients (splitting transplantation) – a new method in the further development of segmental liver transplantation. Langenbecks Arch Chir. 1988;373:127–30.
37. Bismuth H, Morino M, Castaing D, et al. Emergency orthotopic liver transplantation in two patients using one donor liver. Br J Surg. 1989;76:722–4.
38. Srinivasan P, Vilca-Melendez H, Muiesan P, et al. Liver transplantation with monosegments. Surgery. 1999;126:10–2.
39. Attia MS, Stringer MD, McClean P, Prasad KR. The reduced left lateral segment in pediatric liver transplantation: an alternative to the monosegment graft. Pediatr Transplant. 2008;12:696–700.
40. Strong R, Ong TH, Pillay P, Wall D, Balderson G, Lynch S. A new method of segmental orthotopic liver transplantation in children. Surgery. 1988;104:104–7.
41. Broelsch CE, Whitington PF, Emond JC, et al. Liver transplantation in children from living related donors. Surgical techniques and results. Ann Surg. 1991;214:428–37.
42. Lo CM, Fan ST, Liu CL, et al. Adult-to-adult living donor liver transplantation using extended right lobe grafts. Ann Surg. 1997;226:261–9.
43. Kiuchi T, Kasahara M, Uryuhara K, et al. Impact of graft size mismatching on graft prognosis in liver transplantation from living donors. Transplantation. 1999;67:321–7.
44. Stringer MD. Portal hypertension. In: Puri P, Hollwarth M, editors. Pediatric surgery. Diagnosis and management. Berlin: Springer; 2009. p. 561–70.
45. Stringer MD. Improved body mass index after mesenterico-portal bypass. Pediatr Surg Int. 2007;23: 539–43.
46. Uflacker R, Reichert P, D'Albuquerque LC, et al. Liver anatomy applied to the placement of transjugular intrahepatic portosystemic shunts. Radiology. 1994;191:705–12.
47. Saxena R, Theise N. Canals of Hering: recent insights and current knowledge. Semin Liver Dis. 2004;24: 43–8.
48. Schmelzer E, Zhang L, Bruce A, et al. Human hepatic stem cells from fetal and postnatal donors. J Exp Med. 2007;204:1973–87.
49. Ahmadi O, Nicholson ML, Gould ML, Mitchell A, Stringer MD. Interstitial cells of Cajal are present in human extrahepatic bile ducts. J Gastroenterol Hepatol. 2010;25:277–85.
50. Rusu MC, Pop F, Hostiuc S, Curcă GC, Streinu-Cercel A. Extrahepatic and intrahepatic human portal interstitial Cajal cells. Anat Rec. 2011;294:1382–92.
51. Nakanuma Y, Hoso M, Sanzen T, Sasaki M. Microstructure and development of the normal and pathologic biliary tract in humans, including blood supply. Microsc Res Tech. 1997;38:552–70.
52. Shiojiri N. Development and differentiation of bile ducts in the mammalian liver. Microsc Res Tech. 1997;39:328–35.
53. Couvelard A, Scoazec JY, Dauge MC, et al. Structural and functional differentiation of sinusoidal endothelial cells during liver organogenesis in humans. Blood. 1996;87:4568–80.
54. Tavian M, Hallais MF, Péault B. Emergence of intraembryonic hematopoietic precursors in the pre-liver human embryo. Development. 1999;126:793–803.
55. Vijayan V, Tan CE. Development of the human intrahepatic biliary system. Ann Acad Med Singapore. 1999;28:105–8.
56. Tan CE, Moscoso GJ. The developing human biliary system at the porta hepatis level between 11 and 25 weeks of gestation: a way to understanding biliary atresia. Part 2. Pathol Int. 1994;44:600–10.
57. Awasthi A, Das A, Srinivasan R, Joshi K. Morphological and immunohistochemical analysis of ductal plate malformation: correlation with fetal liver. Histopathology. 2004;45:260–7.
58. Stringer MD, Dhawan A, Davenport M, Mieli-Vergani G, Mowat AP, Howard ER. Choledochal cysts: lessons from a 20 year experience. Arch Dis Child. 1995;73:528–31.
59. Guelrud M, Morera C, Rodriguez M, Prados JG, Jaén D. Normal and anomalous pancreaticobiliary union in children and adolescents. Gastrointest Endosc. 1999;50:189–93.

60. Standring S. Gray's anatomy: the anatomical basis of clinical practice. 40th ed. Elsevier: Churchill Livingstone; 2008. p. 1219–20.
61. Stringer MD. The clinical anatomy of congenital portosystemic venous shunts. Clin Anat. 2008;21: 147–57.
62. Kiserud T, Rasmussen S, Skulstad S. Blood flow and the degree of shunting through the ductus venosus in the human fetus. Am J Obstet Gynecol. 2000;182: 147–53.
63. Meyer WW, Lind J. The ductus venosus and the mechanism of its closure. Arch Dis Child. 1966;41: 597–605.
64. Loberant N, Barak M, Gaitini D, Herskovits M, Ben-Elisha M, Roguin N. Closure of the ductus venosus in neonates: findings on real-time gray-scale, color-flow Doppler, and duplex Doppler sonography. Am J Roentgenol. 1992;159:1083–5.
65. Fugelseth D, Lindemann R, Liestol K, Kiserud T, Langslet A. Ultrasonographic study of ductus venosus in healthy neonates. Arch Dis Child. 1997;77: F131–4.
66. Matsubara T, Sumazaki R, Saitoh H, et al. Patent ductus venosus associated with tumor-like lesions of the liver in a young girl. J Pediatr Gastroenterol Nutr. 1996;22:107–11.
67. Uchino T, Endo F, Ikeda S, Shiraki K, Sera Y, Matsuda I. Three brothers with progressive hepatic dysfunction and severe steatosis due to a patent ductus venosus. Gastroenterology. 1996;110:1964–8.
68. Orii T, Ohkohchi N, Kato H, et al. Liver transplantation for severe hypoxemia caused by patent ductus venosus. J Pediatr Surg. 1997;32:1795–7.
69. Yoshimoto Y, Shimizu R, Saeki T, et al. Patent ductus venosus in children: a case report and review of the literature. J Pediatr Surg. 2004;39:E1–5.
70. Spear BT, Jin L, Ramasamy S, Dobierzewska A. Transcriptional control in the mammalian liver: liver development, perinatal repression, and zonal gene regulation. Cell Mol Life Sci. 2006;63: 2922–38.
71. Zong Y, Stanger BZ. Molecular mechanisms of liver and bile duct development. WIREs Dev Biol. 2012;1:643–55.

Normal Functional Biology of the Liver

2

Anne M. Larson and Matthew Hauswald

Introduction

The liver is a complex multifaceted organ that plays a fundamental role in many processes crucial to bodily function. To accomplish this, the liver is populated with multiple cell types, including hepatocytes, cholangiocytes, stellate cells, endothelial cells, and cells of the immune system (i.e., Kupffer cells). Each cell type performs unique functions essential to the overall performance of the liver. A full treatise of each cell and function is beyond the scope of this chapter.

This chapter will present an overview of the normal physiologic function of the liver. Subsequent chapters will address the disturbances in these functions which lead to the clinical manifestations of liver disease.

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Liver Cell Function

Hepatocytes

The hepatocytes are the most numerous cells within the liver, constituting approximately 80 % of the total liver volume [1, 2]. These powerhouse cells perform numerous functions that are essential to life (Table 2.1). The hepatocyte is the only cell in the body that manufactures albumin, fibrinogen, and the prothrombin group of clotting factors. It is the predominant site for the synthesis of lipoproteins, ceruloplasmin, transferrin, and glycoproteins. To complete these tasks, the hepatocytes contain an extraordinarily well-developed system of organelles and membranes which support their extensive roles in energy production, protein synthesis, and metabolism/detoxification.

The hepatocyte has multiple membrane compartments – the plasma membrane, the rough and smooth endoplasmic reticulum (rER; sER), the Golgi apparatus, peroxisomes, lysosomes, mitochondria, and vesicles involved in transport. Mitochondria constitute about 20 % of the volume of the hepatocyte and are responsible for cellular respiration [3]. They are the site of the tricarboxylic acid (TCA) cycle, fatty acid oxidation, and oxidative phosphorylation [4]. Additionally, they participate in the urea cycle, fatty acid synthesis, gluconeogenesis, regulation of intracellular calcium, and heme biosynthesis and play a key role in apoptosis [5]. The hepatocyte has both exocytotic and endocytotic capabilities, which are crucial to the intracellular trafficking, export and

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Table 2.1 Hepatocyte function

Function	Product examples
Protein synthesis	Albumin
	Carrier/transport proteins
	Coagulation factors
	Hormonal and growth factors
	Acute-phase proteins
Bile synthesis	Bile acids
Production of bile carriers	Cholesterol
	Lecithin
	Phospholipids
Nutrient regulation	Glucose
	Glycogen
	Lipids
	Cholesterol
	Amino acids
Endogenous and exogenous	Bilirubin
Lipophilic compound metabolism	Drugs
	Toxins
	Cations

import of molecules [6–10]. Endocytosis leads to the import of extracellular macromolecules via several different mechanisms, including pinocytosis (nonselective bulk-phase uptake), phagocytosis (ingestion of particles and regions of the cell surface), receptor-mediated endocytosis (uptake of specific molecules), and caveolar internalization (budding off of caveolin-altered plasma membranes – plasmalemmal vesicles) [11, 12].

The endoplasmic reticulum constitutes the largest membrane compartment within the cell, about 15 % of total cell volume, with an extremely large surface area [13]. The rER and the free ribosomes are the predominant site of active protein synthesis, processing, and folding. The newly formed proteins are then transported via complex cellular machinery through the rough and smooth ER [14, 15]. The sER and Golgi are then involved in the posttranslational modification, intracellular transport, and secretion of the proteins. Within the Golgi, the proteins are sorted to different destinations, including the plasma membrane, lysosomes, and endosomes [16–22]. The sER also participates in lipid biosynthesis and metabolism, detoxification, and regulation of ion gradients (i.e., calcium). Lysosomes and peroxisomes participate in protein processing and transport. Peroxisomes and other lysosomal vesicles mediate and participate in chemical detoxification.

The plasma membrane separates the intracellular space from the extracellular space and has both barrier and transport functions. Hepatocytes are structurally and functionally polarized [23]. There are numerous ion channels, carrier protein transporters, and pumps on the hepatocyte plasma membranes responsible for the transport of substances between the blood and the hepatocytes and between the hepatocytes and the other liver cells [24]. The plasma membrane has three domains – lateral (contiguous), basolateral (sinusoidal), and apical (canalicular).

The contiguous or lateral surface constitutes 20 % of the total hepatocyte surface. It both separates adjacent hepatocytes and binds them together. The membrane is responsible for intercellular communication, carried out via gap junctions, tight junctions, and desmosomes. The gap junctions occupy about 3 % of the hepatocyte surface, indicating the importance of intercellular communication [25, 26].

The basolateral (sinusoidal) membrane covers over 70 % of the hepatocyte surface [24]. This semipermeable hydrophobic phospholipid membrane is extensively covered with microvilli which face the space of Disse and the sinusoids. The basolateral membrane Na^+/K^+-ATPase generates a plasma-to-cytosol gradient within the hepatocyte which is crucial to maintaining the cellular electrochemical gradient. The basolateral membrane also participates in both passive and active uptake of nutrients, proteins, and other molecules. Transport is either sodium dependent or sodium independent. Organic anions, such as bilirubin and glutathione, and cations are taken up by groups of membrane-bound transport proteins (Fig. 2.1). Examples of these transport proteins include the Na^+/taurocholate cotransporting polypeptide (NTCP), the organic anion-transporting polypeptides (OATP), and the organic anion and organic cation transporters (OATs and OCTs). These molecules are then further modified within the hepatocyte by conjugation and excreted into the bile via specific

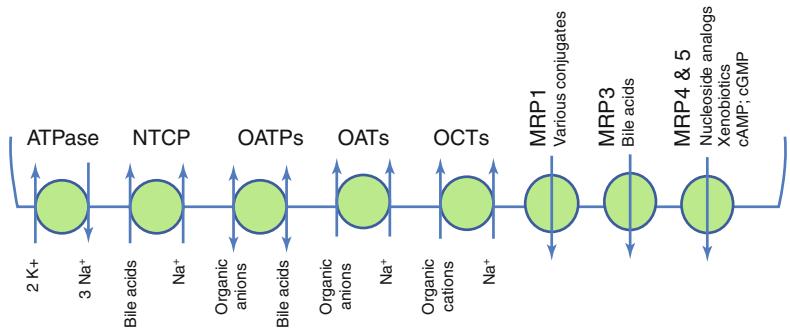

Fig. 2.1 The basolateral membrane transporters. The basolateral membrane is responsible for uptake of organic anions and cations into the hepatocyte. Additionally, it is responsible for secretion of ions. *NTCP* Na^+/taurocholate cotransporting polypeptide, *OATPs* organic anion-transporting polypeptides, *OATs* organic anion transporters, *OCTs* organic cation transporters, *MRP* multidrug resistance-associated proteins, Na^+ sodium ion, K^+ potassium ion. *ATP*ase adenosine triphosphatase, *cAMP* cyclic adenosine monophosphate, *cGMP* cyclic guanosine monophosphate

transporters within the canalicular membrane. The basolateral membrane is also responsible for the secretion of ions, via the family of multidrug resistance-associated proteins (MRPs), which contains 6 members (MRP1–6). These proteins are all located on the basolateral membrane except for MRP2, which is located on the canalicular membrane. The membrane also participates in both endocytic and pinocytic activity.

The apical or canalicular surface of the hepatocyte forms the canaliculi, about 10–15 % of the hepatocyte surface, through which bile and conjugated waste products are secreted. These tiny ducts are formed by the membranes of two adjacent hepatocytes and are sealed by tight junctions that separate the canalicular bile from the hepatic lymph [27]. The bile formed within the hepatocyte and excreted into the canaliculi flows into the bile ductules where it is further modified on its way to the intestine. The apical membrane transporters are predominantly ATP-dependent membrane proteins belonging to the ABC transporter family (Fig. 2.2). These include the multidrug resistance (MDR) P-glycoproteins and the bile salt export pump (BSEP). In addition, the MRP2 and the lipid flippases are located on the apical membrane.

The hepatocyte is the main source of glucose and has the major responsibility for maintaining a balanced plasma glucose concentration. The hepatocyte forms fatty acids from carbohydrates and synthesizes triglycerides and phospholipids from fatty acids and glycerol. They also synthesize apoproteins, which can assemble with lipids for export as lipoproteins, such as very-low-density lipoprotein (VLDL) and high-density lipoprotein (HDL). The liver also metabolizes remnants of chylomicrons from low-density lipoprotein (LDL) received from the systemic circulation. It synthesizes cholesterol from acetate and converts it into bile salts, a major pathway for the removal of cholesterol [28].

The Acinus

The hepatocytes are anatomically organized into lobules – portal areas at the periphery and terminal hepatic (central) veins within the center of the lobule. However, from a *functional* standpoint, it is the "acinus," not the classical lobule, that defines the functional unit of the liver [29]. The acinus is the smallest functional unit in the liver. Blood flows down the sinusoids from the portal vein and the hepatic artery to the central vein, with the hepatocytes progressively removing oxygen and nutrients. Bile flows in the opposite direction. This results in concentration gradients along the sinusoids, dividing the acinus into three different zones: zone 1 (periportal), zone 2, and zone 3 (centrilobular). The cells in zone 1 are the closest to the portal vein and hepatic artery. Hepatocytes located in this zone see the highest oxygen concentration and levels of substances coming in from the portal circulation. Therefore,

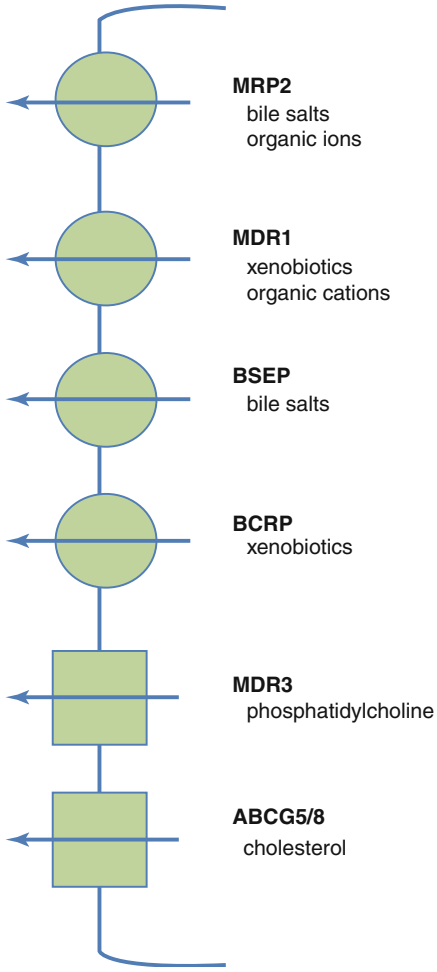

Fig. 2.2 The canalicular membrane transporters. The apical membrane transporters are predominantly ATP-dependent membrane proteins belonging to the ABC transporter. *MRP2* multidrug resistance-associated protein 2, *MDR1* multidrug resistance P-glycoprotein 1, *BSEP* bile salt export pump, *BCRP* breast cancer resistance protein, *MDR3* multidrug resistance P-glycoprotein 3 (an ATP-dependent phospholipid – translocase/flippase), *ABC* ATP-binding cassette

hepatocytes are successively less well oxygenated as blood flows from zone 1 to zone 3, closest to the central vein. The functional capabilities of the hepatocytes (i.e., the repertoire and contents of the enzymes and subcellular organelles) also exhibit a gradient down the acinus [30, 31]. Cells in zone 1 are the first to receive nutrients and to be exposed to absorbed toxins. They are also the first cells to show changes following bile duct injury or occlusion. Because they are the most well oxygenated, they are the last cells to die and first to regenerate. Zone 3 cells are the farthest from the portal triad – the first to show ischemic injury and the last to be injured by biliary processes.

Sinusoidal Endothelial Cells

The sinusoidal endothelial cells make up about 20 % of the total liver cells and act as a barrier (endothelium) against direct contact of the blood with the hepatocytes [32]. They create the space of Disse between the endothelial cells and the hepatocytes. Unlike endothelial cells in the peripheral capillaries, however, sinusoidal endothelial cells lack a basement membrane and have numerous fenestrations [33]. This provides free access of sinusoidal plasma to the basolateral surface of the hepatocyte via the intervening space of Disse. This arrangement allows the plasma proteins to come into direct contact with the numerous microvilli on the basolateral surfaces of the hepatocyte [34–36]. There are differences in fenestra diameter along the differing zones of the acinus, and their diameter is controlled in response to chemical changes within the sinusoidal plasma [37]. This system facilitates the efficient exchange of protein-bound molecules between the plasma and the hepatocyte.

The cells also have a well-developed endocytic capability and secrete a variety of proteins, cytokines (tumor necrosis factor-a, interleukin-1, endothelin), and prostaglandins [38]. It has additionally been suggested that these cells may be able to function as antigen-presenting cells, but this remains unsettled [39].

Hepatic Stellate Cells

Hepatic stellate cells (HSCs) represent about 5–8 % of the liver cells and have been known by many names, including Ito cells, perisinusoidal cells, vitamin A-storing cells, lipocytes, or fat-storing cells [40]. They have unique features within the liver [41]. In the normal liver, these small spindle-shaped mesenchymal cells are quiescent and located in the subendothelial space

between the parenchymal cells and the sinusoidal endothelial cells. They function as pericytes with long processes surrounding the sinusoids regulating sinusoidal diameter and controlling blood flow throughout the sinusoids [41]. They help to maintain homeostasis within the hepatic sinusoid via paracrine, autocrine, and chemoattractant factors. They secrete apolipoprotein E, prostaglandins, cytokines (i.e., TGF-beta, interleukin-10), and many other proteins [42–44]. The HSC is the major cell type involved in the production of extracellular matrix [44, 45]. Resting HSC secretes non-fibril-forming type IV and VI collagens which create a matrix similar to a basement membrane. It has been suggested that the HSC, while quiescent, may also function as antigen-presenting cells [46, 47].

HSC also function as a major site for storage of vitamin A. It is here that over 90 % of the total vitamin A in the body is stored. Vitamin A is esterified within the intestinal epithelial cells and transported to the liver via chylomicron remnants. It is taken up by the hepatocytes by receptor-mediated endocytosis and hydrolyzed to retinol. The majority of the retinol is then transported from the hepatocyte to the HSC where it is again esterified and stored primarily in the form of retinyl esters (predominantly retinyl palmitate) [48, 49]. The HSCs are packed with cytoplasmic fat vacuoles. The retinyl esters are stored within these vacuoles, which also contain small amounts of phospholipids, triglycerides, cholesterol, and free fatty acids [49]. HSCs are also able to take up small amounts of retinoids from the plasma which are bound to retinal-binding protein.

HSCs are activated in response to both acute and chronic liver injury through the phagocytosis of apoptotic bodies and cellular debris [50]. It is this activation which is the central event in the formation of hepatic fibrosis and cirrhosis [51, 52]. There appear to be at least two different cell populations involved – HSC and bone marrow-derived fibroblasts – although the fibroblasts play a more minor role [53]. The HSCs undergo transdifferentiation (activation) into proliferative myofibroblasts. The activated HSC gradually loses its cytoplasmic lipid droplets (containing the vitamin A), enlarges in size, and develops a more prominent protein secretory apparatus (rER, Golgi), and its processes become longer [54, 55]. Its myofibroblast-like phenotype is characterized by proliferation, contractility, chemotaxis, release of leukocyte chemoattractants and cytokines, overproduction of extracellular matrix proteins, and matrix degradation (via metalloproteinases) [41, 56]. The activated HSC first secretes fibronectin and types III and IV collagen. As hepatocellular injury progresses, a matrix rich in fibrillar type I and IV collagen, elastin, proteoglycans, and glycoproteins is laid down, and the cell becomes imbedded within this fibrous scar [44, 45, 53]. HSCs are also involved in the degradation of hepatic fibrosis [57, 58].

Carbohydrate Metabolism

The liver plays a fundamental role in the maintenance of plasma carbohydrate levels. Carbohydrates are consumed predominantly in the form of hexose polymers (glucose, galactose, and fructose). In the oral cavity, the oligosaccharides are broken down via salivary amylase, dextrinase and glucoamylase to disaccharides. Further digestion of the disaccharides occurs by the action of pancreatic amylase within the small intestine. The transport proteins present in the small intestinal mucosa absorb monosaccharides via the sodium-dependent glucose transporters, solvent drag, and facilitated diffusion, which is independent of saline gradients. The monosaccharides, particularly glucose and fructose, are then delivered to the liver via the portal venous blood.

Glucose

Glucose is the most important and plentiful of the monosaccharides – it is the major fuel for the tissues of the body. The liver produces 85–90 % of the body's endogenous glucose, with the remainder being produced by the kidneys [59]. Glucose is converted within the hepatocyte to glucose-6-phosphate which is the backbone molecule for the synthesis of glycogen or fatty

acids. The liver acts as a "glucose monitor" and maintains blood glucose concentrations within a narrow margin.

Uptake and Release

The presence of portal venous glucose enhances net glucose uptake into the hepatocyte [60]. During periods of intestinal absorption, over 50 % of the portal venous glucose is taken up by the hepatocytes. It is then converted to glycogen (5 %) or used to generate fatty acids (30–40 %). The remaining glucose is passed on to the skeletal muscle and the tissues of the body for use as an energy source [61]. If portal venous glucose is low, as in periods of fasting, hepatic glucose is released back into the circulation – two thirds from glycogenolysis and one third from gluconeogenesis. Glucose uptake and release across the sinusoidal membrane is bidirectional and occurs by the action of the glucose transport proteins [61].

These transporters move glucose across the cellular membrane via facilitated diffusion – driven by the glucose concentration gradient and independent of insulin. Glucose transporter 2 (GLUT2) is the predominant transport protein in the hepatocyte and has a high capacity for glucose uptake. Glucokinase activity within the hepatocyte determines the rate of glucose uptake. Glucokinase rapidly phosphorylates glucose to glucose-6-phosphate (G6P). The phosphorylation of glucose removes free glucose from the intracellular milieu, maintaining the glucose concentration gradient across the sinusoidal membrane [59]. Glucose-6-phosphate is a key molecule in glucose metabolism, and it is involved in multiple metabolic pathways. An additional glucose transporter, GLUT1, exists on hepatocytes facing the systemic venous system. This transporter has a high affinity for glucose, but a low capacity for its transport. Glucose release from the hepatocytes is facilitated by glucose-6-phosphatase, located on the luminal side of the sER, which dephosphorylates G6P. Within the hepatocytes, glucose participates in five primary metabolic pathways: glycogen synthesis, glycogenolysis, glycolysis, gluconeogenesis, and the pentose-phosphate pathway.

Glycogen Synthesis (Glycogenesis)

Glycogen is the storage form of glucose and is synthesized when blood glucose levels are high. It is found predominantly within the liver (one third of the body's glycogen) and skeletal muscle [62]. It is a large branched polymer of glucose, containing up to 50,000 carbohydrate molecules [63]. Glycogen is stored as a reservoir. In the liver, it is used to maintain a consistent carbohydrate level in the circulation; however, the liver contains only enough glycogen stores to sustain the blood glucose level for ~24 h. Once the glycogen stores are consumed, substrates from skeletal muscle amino acids (i.e., alanine) and adipose tissue (glycerol) must be used to generate glucose (gluconeogenesis).

As noted, hepatocellular glucose is converted to G6P via glucokinase [64]. G6P is then transformed by phosphoglucomutase via the intermediate of glucose-1,6-bisphosphate into glucose-1-phosphate (G1P). Further conversion of G1P requires energy, which is obtained by the hydrolysis of uridine-5′-triphosphate (UTP). Uridyl transferases (UDP-glucose pyrophosphorylase) combine G1P with UTP the product of which is rapidly hydrolyzed by inorganic pyrophosphatase to form uridine diphosphate (UDP-) glucose and liberating two molecules of inorganic phosphate. This activated glucose subunit is the launch point of glycogen synthesis. De novo glycogen synthesis requires a primer molecule, glycogenin, and uses UDP-glucose as a substrate that autocatalyzes the addition of an α1γ4-linked glucose oligosaccharide to a tyrosine residue [65]. Once the chain is four residues or longer, the glucose polymer is elongated by the action of glycogen synthase which adds glucose molecules to the growing chain. Branching is performed by a branching enzyme (amylo-α(1:4)γ(1:6) transglycosylase).

Glycogenolysis

When serum glucose levels are low, storage glycogen is converted back into glucose for use as an energy source. The action of glycogen

phosphorylase is the predominant enzyme in the breakdown of glycogen and requires a covalently bound pyridoxal-5′-phosphate (vitamin B6) cofactor [66]. This enzyme catalyzes the cleavage of a terminal glycogen and yields one glucose 1-phosphate molecule per reaction. When glycogen phosphorylase is within four glucose molecules of a chain branch point, it can no longer cleave glycogen residues. At this point, oligo-(α1:4 → 1:4)-glucantransferase transfers three residues to an adjacent glycogen chain, leaving a single molecule at the branch point. This leaves another linear glucose chain for glycogen phosphorylase to again begin breaking down the molecule until the next branch point is reached. The debranching enzyme, amylo-1,6-glucosidase, then hydrolyzes the remaining residue at the α1:6 linkage branch point, resulting in a free glucose molecule.

G1P then enters glycolysis and is converted to G6P via phosphoglucomutase. During periods of low serum blood glucose concentration, the G6P is hydrolyzed to glucose (glucose-6-phosphatase) and exported into the bloodstream.

Glycolysis

Glycolysis is the metabolic pathway by which either glucose or glucose-6-phosphate is transformed to pyruvate and/or lactate. Before glucose can enter into the glycolysis, it must first be phosphorylated via glucokinase to form G6P, consuming an ATP in the process. In an energy-neutral step, glucose phosphate isomerase then converts G6P to fructose-6-phosphate. This molecule is then further phosphorylated utilizing an additional ATP via phosphofructokinase to fructose-1,6-biphosphate. This step is irreversible and a regulatory point in the process of glycolysis [67].

Fructose-1,6-biphosphate is converted to two 1,3-bisphosphoglycerate molecules via two intermediates – dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. This process generates nicotinamide adenine dinucleotide (NADH) and H+. Phosphoglycerate kinase transfers a phosphate group from 1,3-bisphosphoglycerate to ADP, generating ATP and 3-phosphoglycerate. Phosphoglycerate kinase is an x-linked enzyme which is a key enzyme for ATP generation in the glycolytic pathway [68]. This is another regulatory point in glycolytic pathway, and phosphoglycerate kinase is regulated by the level of cellular energy – it is upregulated in the setting of low cellular ATP and downregulated in the presence of elevated ATP levels. 3-phosphoglycerate is converted to 2-phosphoglycerate via phosphoglycerate mutase and then to phosphoenolpyruvate by enolase. Pyruvate kinase then removes a phosphate molecule from phosphoenolpyruvate generating pyruvate and ATP. This is the third regulatory step in the glycolysis pathway.

Under physiologic (aerobic) conditions, pyruvate then enters the mitochondria and is oxidized within the citric acid (Krebs) cycle to yield carbon dioxide and water. Energy is generated by this process and is used to form ATP and NADH. The NADH is oxidized in the electron transport chain, regenerating the NAD^+ used by the glycolysis pathway. Under anaerobic conditions, pyruvate is converted by lactate dehydrogenase to lactate – a much less efficient system with fewer ATP molecules generated.

Gluconeogenesis

Gluconeogenesis is an energy consuming process which converts noncarbohydrate compounds into glucose. The main substrates are (1) lactate from the skeletal muscle and erythrocytes, (2) glucogenic amino acids from skeletal muscle or absorbed from the intestinal tract, and (3) glycerol generated in the adipose tissue. The liver provides 85–90 % of the body's gluconeogenesis, generating up to 250 g of glucose daily. The renal tubular epithelial cells are responsible for the remaining 10–15 % of gluconeogenesis [69]. Gluconeogenesis involves multiple cellular compartments, including the mitochondria, endoplasmic reticulum, and the cytosol. Key enzymes involved in the process are regulation points – pyruvate carboxylase, phosphoenolpyruvate (PEP) carboxylase, fructose-1,6-biphosphatase, and glucose-6-phosphatase.

Glucogenic amino acids are those which can be transformed into glucose. One mechanism is the generation of pyruvate from amino acids which are metabolized within the cytosol. The most prominent of these amino acids is alanine. The pyruvate molecule then crosses the mitochondrial membrane. Pyruvate is then carboxylated by pyruvate carboxylase to oxalacetate. Amino acids other than alanine are degraded within the mitochondrial citric acid cycle, generating either pyruvate or oxalacetate. Oxalacetate is then reduced to malate. Malate is transported via specific transport systems back into the cytosol and is reconverted to oxalacetate. This is then converted via PEP carboxylase into phosphoenolpyruvate which is ultimately transformed via a fructose-1,6-biphosphate intermediate to G6P. Within the sER, G6P is converted (glucose-6-phosphatase) to glucose which is released into the bloodstream.

Lactate entering the cytosol is converted to pyruvate, which follows the same pathway of glucose generation as the glucogenic amino acids. Glycerol conversion, however, does not involve the mitochondria. It is converted within the cytosol to glycerone-3-phosphate, then fructose-1,6-biphosphate, and ultimately to G6P. G6P generated by these two mechanisms is converted within the sER to glucose.

The Pentose-Phosphate Pathway

The pentose-phosphate pathway is an alternate pathway for glucose oxidation. This pathway generates $NADPH + H^+$ – reducing equivalents needed for glycolysis and for the synthesis of fatty acids and isoprenoids. In addition, the pathway generates ribose-5-phosphate, which is a precursor necessary for nucleotide synthesis. ATP is not generated within this pathway.

Regulation

Glucose metabolism is regulated at three branch points – glucokinase, phosphofructokinase, and pyruvate kinase. Each of these enzymes catalyzes an irreversible reaction. Multiple hormones work to regulate the serum level of blood sugars. Increased plasma glucose triggers insulin release which then stimulates peripheral glucose uptake (via glucokinase) and suppresses its endogenous production [69]. This facilitates glucose conversion to either glycogen or fat formation. The presence of excess adenosine monophosphate (AMP) suppresses glucose uptake. Phosphofructokinase is a central control point for both glycolysis and gluconeogenesis. Insulin, glucose, AMP, and fructose-2,6-biphosphate all induce the enzyme, leading to increased endogenous production of glucose. ATP inhibits it. Pyruvate kinase is also induced by insulin and glucose, but inhibited by both glucagon and epinephrine.

Glycogen metabolism is also highly regulated. Control of its production and utilization involves predominantly posttranslational modification (covalent phosphorylation) and allosteric ligand binding [62]. For example, glycogen phosphorylase is activated by phosphorylation, whereas glycogen synthase is inhibited by it. Additionally, metabolic proteins translocate between the glycogen particle and other cellular structures [64, 70]. Lastly, the glycogen particle is spatially and temporally regulated. Insulin induces the expression of hepatocyte glycogen synthase, leading to an increase in glycogen synthesis and a decrease in gluconeogenesis. Epinephrine and glucagon inhibit glycogen synthesis and stimulate glycogenolysis [71]. Epinephrine activates glycogen phosphorylase and inhibits glycogen synthase. Additionally, fructose-1,6-biphosphatase is repressed by the action of AMP and fructose-2,6-biphosphate. The four predominant enzymes in the process are induced by glucocorticoids, glucagon, and epinephrine. Cyclic AMP (cAMP) also induces pyruvate carboxylase and PEP carboxylase. Other hormones also participate in glucose regulation, predominantly via effects on insulin and glucagon [69, 72]. These include amylin, GIP (glucose-dependent insulinotropic polypeptide), and GLP-1 (glucagon-like peptide-1).

Fructose

Fructose is a sugar which is found predominantly in fruits and vegetables. The liver is the predominant site of fructose metabolism. Hepatocyte

fructokinase converts (phosphorylates) ingested fructose into fructose-1-phosphate. This molecule is then converted into dihydroxyacetone phosphate and glyceraldehyde. Glyceraldehyde is ultimately metabolized by the glycolytic pathway. A small fraction of fructose may be reduced to glycerol.

Galactose

Galactose is found mainly in dairy products, and as is the case with fructose, its metabolism is primarily hepatic. It is phosphorylated and epimerized to glucose-1-phosphate and further metabolized by glycolysis.

Lipid Metabolism

Multiple forms of lipids exist, including fatty acids, triglycerides, phospholipids, and steroids (e.g., cholesterol, bile acids, steroid hormones) [73]. These molecules are generally insoluble within the plasma and circulate bound to albumin or esterified with glycerol to form triglycerides (triacylglycerol [TAG]) and packaged within the lipoprotein complexes – spherical particles with a hydrophobic core which is surrounded by a single layer of amphipathic molecules.

Fatty Acids

Most of the fatty acids are obtained from the diet. During the fasting period, they are a major fuel source for oxidative metabolism, particularly in the cardiac and skeletal muscles. The naturally occurring fatty acids are made up of an unbranched carbon backbone with an even number of carbon branches. They may be either saturated or unsaturated. Three fatty acids are essential, cannot be synthesized within the liver, and must be obtained from the diet – linoleic acid, linolenic acid, and arachidonic acid. These three fatty acids are essential precursors for synthesis of the eicosanoids. The hepatocyte must esterify or "activate" the fatty acids to acyl-CoA esters (fatty acyl-CoA) before they can be used to form triglycerides, phospholipids, or cholesterol esters.

Lipogenesis (Fatty Acid Synthesis)

Endogenous fatty acid synthesis occurs in the liver where the fatty acids are synthesized de novo [74]. The most important source of carbon for this process is glucose. In the setting of glucose excess, fatty acids are generated. Key enzymes in this process are acetyl-CoA carboxylase and fatty acid synthase [75]. Glucose is converted to pyruvate which, via the mitochondrial citric acid (Krebs) cycle, generates citrate. Citrate is transferred to the cytosol, and it is converted to acetyl-CoA and oxaloacetate by ATP citrate lyase [76]. The oxaloacetate is then reduced to malate (malate dehydrogenase) and transported back to the mitochondria for use within the citric acid cycle [77]. Acetyl-CoA is then carboxylated in the sER via acetyl-CoA carboxylase 1 (ACC1) to form malonyl-CoA. ACC1 is the key regulatory enzyme in this process. It is activated by the presence of citrate and inhibited by palmitoyl-CoA (the final product of fatty acid synthesis). Hepatocyte fatty acid synthase is a multienzyme complex which then creates saturated long-chain fatty acids (up to 16 carbons) from acetyl-CoA, malonyl-CoA, and NADPH [78]. Hepatocytes also take up circulating chylomicrons and very-low-density lipoprotein TAG via lipoprotein lipase, which is located on the capillary endothelium. These fatty acids are derived predominantly from the adipose tissue following triglyceride hydrolysis (lipolysis). A smaller amount comes from intestinal absorption of fatty acids and systemically circulating triglyceride-rich lipoproteins.

Lipolysis (Fatty Acid Breakdown)

TAGs are an excellent energy source with twice as many calories per gram as glycogen [79]. When TAGs are hydrolyzed, they release up to 3 fatty acids and one glycerol into the serum. This occurs primarily within the white adipose tissue and is catalyzed by the lipases – adipose

triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), and monoglyceride lipase (MGL) [80–82]. The enzymes of lipolysis are regulated by both lipolytic (i.e., PPARγ, β-adrenergic stimulation) and lipogenic (i.e., insulin, hypoglycemia) signals [79]. Insulin is the predominant lipogenic hormone, leading to transcriptionally mediated lipogenesis via sterol regulatory element-binding protein-1c (SREBP-1c) [83]. Lipogenesis is also transcriptionally mediated by carbohydrate response element-binding protein (ChREBP). Glucose stimulates ChREBP, ultimately leading to pyruvate which enters the Krebs cycle as noted above, leading to the formation of fatty acids [76]. The lipolytic hormones, which stimulate adipose tissue lipolysis, include epinephrine, norepinephrine, ghrelin, growth hormone, and possibly cortisol [75].

Uptake of the free fatty acids by hepatocytes and other cells is regulated predominantly by their serum concentration. Under normal conditions, TAGs are found within the chylomicrons or very-low-density lipoproteins. They are cleaved by endothelial lipases, releasing non-esterified fatty acids. During periods of fasting, albumin-bound fatty acids interact with a hepatic Na+− dependent cotransporter (membrane fatty acid transport protein). Upon release into the cytosol, they are bound to fatty acid-binding proteins. Within the hepatocyte, they may be utilized for either lipid synthesis or β-oxidized for energy. The fatty acids are first acetylated (activated) by fatty acyl-CoA synthetase/fatty acid thiokinase to long-chain acyl-CoA. This molecule may be utilized for other processes such as formation of cholesterol esters, bile acids, steroid hormones, triglycerides or phospholipids. Alternatively, it may be converted to carnitine esters and transferred via carnitine palmitoyltransferase 1 (CPT1) to the mitochondria for oxidation. This is the rate-limiting step, and the enzyme is inhibited by malonyl-CoA, a principle intermediate in de novo lipogenesis [75, 84].

The major degradative pathway of the fatty acyl-CoA is β-oxidation. Each step generates acetyl-CoA by the sequential removal of two carbon fragments from the fatty acyl-CoA. Acetyl-CoA enters the citric acid cycle and is further oxidized to NADH, $FADH_2$, and ATP. The β-oxidation of fatty acids generates cellular energy – a completely oxidized 6-carbon fatty acid will yield 44 molecules of ATP [73].

Ketogenesis

Ketogenesis occurs in several settings. Under physiologic conditions, only small amounts of ketones are formed. During starvation or fasting, when blood glucose and insulin levels are low, fatty acids are used to generate energy, leading to ketone formation. In the setting of excess glucose levels (i.e., diabetes), the surplus glucose is stored by creating a surfeit of fatty acids. β-oxidation of the fatty acids leads to the production of excess acetyl-CoA and subsequently the formation of ketones [85]. Additionally, diets rich in fats and low in carbohydrates will lead to ketone formation. The ketones formed are acetoacetate, D-3-β-hydroxybutyrate, and acetone.

During starvation or fasting, fatty acids are released during adipose lipolysis (see above). Within the mitochondria, β-oxidation of the fatty acids results in high levels of ATP and NADH. These inhibit isocitrate dehydrogenase in the citric acid cycle, leading to the development of excess acetyl-CoA. Thiolase catalyzes the condensation of two acetyl-CoA molecules to form acetoacetyl-CoA – the first molecule in the ketogenesis pathway. An additional molecule of acetyl-CoA is added via the highly regulated 3-hydroxy-3-methylglutaryl-CoA synthase leading to the formation of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA). HMG-CoA lyase cleaves an acetyl-CoA molecule from HMG-CoA to form free acetoacetate [86]. Acetoacetate is the pivotal ketone from which the others are derived. It is spontaneously decarboxylated to form acetone, which cannot be converted back into acetoacetate and diffuses into the circulation. Additionally, acetoacetate is reversibly reduced to form D-3-β-hydroxybutyrate via the action of D-β-hydroxybutyrate dehydrogenase [87].

Acetone is a waste product that is excreted in the urine or exhaled. However, both acetoacetate and D-3-β-hydroxybutyrate can be utilized as

energy sources. In the extrahepatic tissues, they are converted back to acetoacetate by succinyl-CoA:3-ketoacid CoA transferase (SCOT). They supply up to 50 % of the energy requirements for the tissues, such as the heart and kidney, and up to 70 % of the brain's requirements [87].

The process of ketogenesis is highly regulated at three points in fatty acid metabolism: (1) the mobilization of free fatty acids from adipose tissue, (2) fatty acid entry into the mitochondria via CPT1 (inhibited by malonyl coenzyme A), and (3) control of 3-hydroxy-3-methylglutaryl-CoA synthase [88]. Elevated plasma levels of insulin suppress each of these processes, while glucagon and cortisol promote ketogenesis.

Triglycerides

Triglycerides (triacylglycerol [TAG]) are esters derived from glycerol and three fatty acids. Their formation begins with esterification of fatty acyl-CoA molecules to glycerol-3-phosphate via acyl transferases, forming phosphatidic acid (PA). This is the branch point in the synthesis of triacylglycerol (TAG), phosphatidylglycerol (PG), phosphatidylinositol (PI), and cardiolipin (CL) [79, 89]. PA is hydrolyzed by PA phosphohydrolase (lipin or PAP) to form diacylglycerol (DAG). DAG acyltransferases (DGAT-1 and DGAT-2) within the ER perform the final step in of esterification of PA to TAG [79, 90]. PAP is highly regulated by both substrate supply and hormones. Triglycerides are then either metabolized by the hepatocyte (lipolysis via lipases) or packaged into lipoproteins for export.

Phospholipids

Phospholipids are the predominant component of all cell membranes. They contain a hydrophilic head and a hydrophobic chain. As noted, phosphatidic acid is hydrolyzed by PA phosphatase to form diacylglycerol. This is the precursor of phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine. Phosphatidylcholine (lecithin) makes up about 98 % of the phospholipids, with the remainder being phosphatidylethanolamine (cephalin), phosphatidylserine, and phosphatidylinositol.

Cholesterol

Cholesterol is an essential component of all cell membranes and the backbone of both bile acids and steroid hormones [91]. It is derived equally from intestinal absorption and endogenous synthesis. It consists of a system of steroid rings and an aliphatic side chain. Although it can be synthesized by most cells, it is within the hepatocyte sER that the vast majority (80–90 %) of cholesterol is formed. The majority of cellular cholesterol (70–80 %) is esterified with fatty acids. Cholesterol absorption and synthesis are determined by genetic factors and by the metabolic state [92].

The process of cholesterol synthesis is extremely complex [93]. Acetyl-CoA is converted to mevalonate via the intermediate 3-hydroxy-3-methylglutaryl-CoA (3-HMG-CoA) by HMG-CoA reductase. This is the first and rate-limiting step of cholesterol formation. It is controlled by negative feedback inhibition from free cholesterol and glucagon, while insulin and thyroxin stimulate it. Phosphorylation and decarboxylation of HMG-CoA form isopentenyl pyrophosphate molecules, several of which condense to form squalene. Further processing leads to cholesterol formation.

Intestinally absorbed cholesterol is combined with apolipoprotein B48 (ApoB48) and integrated into chylomicrons. Via the lymph system, they reach the circulation and ultimately reach the tissues. They are recognized and bind to lipoprotein lipase on the capillary endothelial cells of the adipose tissue and skeletal muscle. Lipoprotein lipase hydrolyzes triglycerides from the core of the chylomicron, releasing free fatty acids. The majority of these fatty acids are taken up by the tissues and used for energy (skeletal muscle) or storage (adipose tissue). Some of the free fatty acids bind to albumin and are taken to the liver. In addition, the cholesterol-rich chylomicron remnants formed are also transported to

the liver, where they are taken up via the LDL receptor (LDL-R) and LDL receptor-related protein (LDL-RP).

Once internalized, within the hepatocyte, a cholesterol vesicle forms which then fuses with the lysosomes. Lysosomal lipases split the cholesterol ester. The free cholesterol formed migrates into the cytoplasm where it acts to inhibit HMG-CoA reductase. In addition, it inhibits the synthesis of the LDL receptors and stimulates esterification with fatty acids via ACAT (acyl-CoA cholesterol acyltransferase).

Both absorbed and newly synthesized cholesterol esters then follow one of several pathways. They can be packaged with lipoproteins (VLDL) and secreted into the bloodstream. The cholesterol can be converted to bile acids (see below) and disposed of through the biliary system. Finally, cholesterol esters can be stored in lipid droplets or cellular membranes. It can be converted back to free cholesterol as needed by cholesterol ester hydrolase.

Lipoproteins

The lipoproteins are spherical particles with a core of nonpolar lipids (i.e., triglycerides, cholesterol esters) and an envelope membrane of polar lipids (phospholipids) and proteins (i.e., apolipoproteins). The lipoproteins are classified into chylomicrons, very-low-density lipoprotein (VLDL), intermediate-density lipoproteins (IDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL). The apolipoproteins (Apo) are required for the assembly of the lipoproteins, activate enzymes involved in lipoprotein metabolism, and are recognized by cellular membrane receptors that mediate the uptake of the lipoproteins.

The hepatocyte has a very complex role in lipoprotein metabolism and is crucial to the uptake of chylomicron remnants and LDL. It also synthesizes and secretes high-density lipoproteins (HDLs), very-low-density lipoproteins (VLDLs), and apoproteins. HDL is secreted in particles which contain apolipoproteins ApoAI and ApoAII, both of which are also synthesized by the hepatocytes. HDL transports surplus cholesterol to the liver where it is metabolized and excreted in the bile. HDL is further metabolized in the plasma by lecithin-cholesterol acyltransferase (LCAT) which is synthesized in and secreted from the hepatocyte. LCAT forms cholesterol esters from cholesterol and phosphatidylcholine. VLDL, assembled in the endoplasmic reticulum and Golgi, is triglyceride rich and contains ApoB100, ApoC, and ApoE. VLDL is the major form by which triglyceride is secreted from the hepatocyte [94]. The hepatocyte is also key in the removal of chylomicron remnants and low-density lipoprotein (LDL) from the bloodstream. These particles interact via their surface apoproteins (ApoB100) with specific hepatocellular receptors (LDL-R). The LDLs are the major form of cholesterol transport to the tissues. Cholesterol derived from the LDL inhibits HMG-CoA reductase and stimulates LCAT.

Protein Metabolism

The liver plays a major role in the metabolism of both proteins and amino acids – neither of which can be stored. Approximately 400 g of protein are metabolized daily – 100 g transformed to glucose and 300 g degraded and newly synthesized. Ingested proteins are hydrolyzed within the gut to form free amino acids, di- and tripeptides which reach the liver via the portal venous system. Amino acids are taken up across the basolateral membrane via group-specific transport systems. Although hepatocytes are unable to take up dipeptides, aminopeptidases within the cell membrane may be able to cleave alanine-containing polypeptides. Of the proteins synthesized within the liver, about 50 % are used as hepatocyte structural proteins and enzymes, while the remaining 50 % are secreted [95, 96].

Plasma Proteins

Approximately 90 % of the plasma proteins, except the immunoglobulins, are synthesized by the hepatocytes [97]. These newly formed

proteins are transferred from the cytoplasmic ribosomes into the rER [98]. There, they undergo further modifications and are transported along specific secretory pathways via the Golgi apparatus [99]. They are transported to the surface in secretory vesicles and released into the circulation across the sinusoidal membrane [100]. Protein synthesis is regulated by multiple factors, including nutritional status, hormones (insulin, glucagon), and tissue injury/inflammation (acute-phase reactants) [101]. The predominant proteins synthesized in and secreted from the liver include transport proteins, protease inhibitors, complement proteins, coagulation/fibrinolysis proteins, and acute-phase proteins [102].

Albumin is synthesized solely by the liver and is the most abundant protein secreted by it. This plasma protein is crucial for the maintenance of plasma oncotic pressure and as a major transport protein, making up 55–60 % of all plasma proteins [103]. Other transport proteins manufactured by the liver include transferrin, transcortin, ceruloplasmin, vitamin D-binding proteins, thyroid hormone-binding proteins, sex hormone-binding proteins, lipoproteins, hemopexin, and α_1-fetoprotein. The retinol-binding proteins are produced in the stellate cells. The liver is the only site of manufacture of the proteins and enzymes of the coagulation cascade. The exception to this is factor VIII, which is synthesized in the vascular endothelium, and von Willebrand factor, which is synthesized in the sinusoidal endothelial cells. The majority of the procoagulation factors are serine proteases (fibrinogen, prothrombin, factor VII, factors IX–XII). Factors V and VIII are glycoproteins, while factor XIII is a transglutaminase. Factors produced in the liver which are involved in fibrinolysis include protein C, protein S, plasminogen, and antithrombin III.

Important protease inhibitors produced in the hepatocytes include α_1-antitrypsin, α_1-antichymotrypsin, α_2-macroglobulin, α_2-antiplasmin, antithrombin III, and C1 inhibitor. Kupffer cells also produce a very small amount of α_1-antitrypsin. The majority of the proteins involved in the complement system are synthesized by hepatocytes (C1q,r,s, C2–C9, Factor B, Factor D); however, these proteins are also produced in macrophages [104]. Acute-phase proteins are produced and released in response to local inflammatory activity. These include C-reactive protein, fibrinogen, haptoglobin, ceruloplasmin, α_1-macroglobulin, and α_1-antitrypsin. Apoproteins (VLDL, HDL) and prohormones (angiotensinogen, kininogen) are also produced in the hepatocytes.

The hepatocyte is the site of clearance of the plasma proteins as well. Protein uptake is by receptor-mediated endocytosis. Within the serum, the terminal *N*-acetylneuraminic acid is cleaved exposing the galactose units of the proteins. The asialoglycoprotein receptor (ASGPR) located within the hepatocyte membrane has a high affinity for the galactose units, and most of the serum proteins are taken up by this receptor. Once internalized, the proteins are metabolized to amino acids, replenishing the amino acid pool. Protein breakdown occurs both within the cytosol and the lysosomes. Cytosolic catabolism is ATP dependent and requires ubiquitin. Ubiquitin is a small 76-amino-acid regulatory protein which covalently binds to proteins and directs them through the endocytotic process [105]. The lysosomal degradation pathway is ATP independent and relies on lysosomal proteases [106].

Protein metabolism is hormonally regulated, a process which is modulated depending upon the hydration state of the hepatocyte. High concentrations of serum amino acids and insulin stimulate protein synthesis and inhibit protein catabolism. Alternatively, low amino acid concentrations and glucagon stimulate protein degradation. Albumin synthesis is also stimulated by corticosteroids, growth hormone, and thyroid hormone.

Amino Acids

In humans, about 20 different amino acids are utilized for protein synthesis (Table 2.2). The essential amino acids must be acquired through the diet as they cannot be synthesized de novo. The remaining amino acids are nonessential, although some of these may become essential during periods of illness or stress. Additionally, some of the "essential" amino acids can be synthesized from

Table 2.2 Human amino acids

Essential	Nonessential
Histidine	Alanine
Isoleucine	Arginine[a]
Leucine	Aspartate
Lysine	Asparagine
Methionine	Cysteine[a]
Phenylalanine	Glutamate
Threonine	Glutamine[a]
Tryptophan	Glycine[a]
Valine	Ornithine[a]
	Proline[a]
	Serine[a]
	Tyrosine[a]

[a]Conditional amino acids – not essential except in times of illness or stress

structurally similar precursors (i.e., homocysteine can be converted into methionine). Amino acids are enzymatically hydrolyzed and absorbed in the small intestine, reaching the liver via the portal venous blood. They are then removed by the hepatocytes for use in both protein synthesis and as a source of energy via gluconeogenesis. Under normal physiologic conditions, the majority of the plasma amino acids are derived from intestinal absorption.

The liver plays a key role in the metabolism of all amino acids. The liver is unable to process the branched chain essential amino acids (leucine, valine, and isoleucine). These are minimally taken up and pass through to the systemic circulation where they are extracted and used by skeletal muscle [107]. The liver is the chief site of synthesis and modification of the nonessential amino acids, and the reamination of most of the essential amino acids. The liver reserves some amino acids for production of plasma protein, ATP, and fatty acids. It also releases amino acids into the peripheral circulation to be used by other cells for protein synthesis [108]. Excess amino acids are not stored within the liver, but are degraded generating ammonia and urea.

Most amino acids move on from the liver unencumbered to be used by other cells for protein synthesis, but this metabolism is hormonally controlled via insulin, corticosteroid, and thyroid hormone stimulation. The level of hydration also determines the metabolism of proteins in the liver. Hepatocyte expansion from differing hydration states and enzyme activity result in inhibition of proteolysis and protein accumulation in hepatocytes, while at the same time activating protein synthesis.

Ammonia and Urea

Ammonia is generated predominantly by amino acid catabolism but also from purines and pyrimidines, in the skeletal muscle, in the kidneys, and to a lesser extent by intestinal bacteria. Ammonia is highly toxic, and the liver is responsible for the removal of the majority of the ammonia produced. More than 90 % of excess nitrogen is cleared by conversion of ammonia to urea in the periportal hepatocytes via the hepatic urea cycle. Within the mitochondria, carbamoyl phosphate synthetase catalyzes the ATP-dependent synthesis of carbamoyl phosphate from ammonia and bicarbonate [109]; the carbamoyl phosphate molecule initiates the urea cycle. The remaining 10 % is converted to glutamine by glutamine synthetase predominantly within the perivenular hepatocytes [110].

Xenobiotic Metabolism

The liver is responsible for concentrating, metabolizing, and eliminating the majority of drugs, toxins, and xenobiotics ("substances") which are introduced into the body. Such metabolism and excretion of miscellaneous substances is required to protect against ingested toxins that are absorbed from the intestine and reach the liver via the portal venous blood. There are a variety of soluble and membrane-bound enzymes, particularly related to the endoplasmic reticulum, which process these substances. Each substance has a specific disposal pathway involving one or more of these enzymes systems. The majority of absorbed substances from the gastrointestinal tract are lipophilic and water-insoluble. Hepatic metabolism renders them water-soluble and more easily excreted, predominantly through the kidneys or in the bile. Exogenous substances

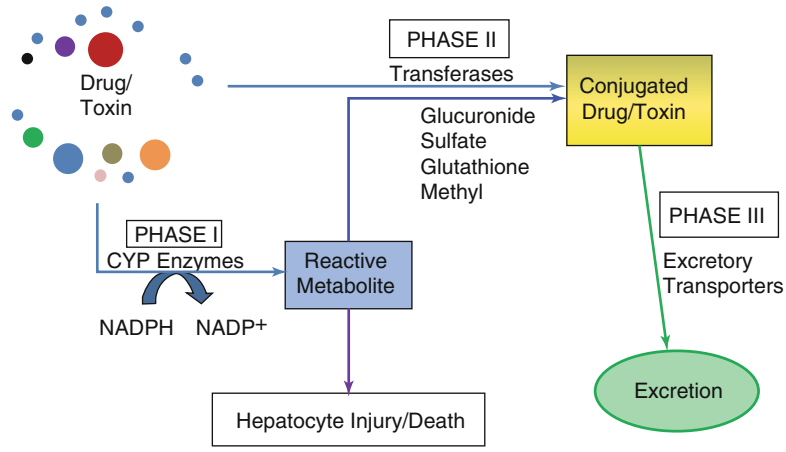

Fig. 2.3 Xenobiotic metabolism. *CYP* cytochrome P450, *NADPH* nicotinamide adenine dinucleotide phosphate

are metabolized predominantly by means of two mechanisms: phase I and phase II reactions or a combination of both (Fig. 2.3) [111–113]. The final products are then moved across the canalicular or sinusoidal membranes via excretory transporters (phase III reactions) [114].

Phase I Reactions

Phase I reactions transform lipophilic molecules into more polar, hydrophilic molecules via oxidation, reduction, or hydrolysis. These particular reactions are catalyzed by the cytochrome P450 (CYP) superfamily of mixed function oxidases [115–119]. The P450 molecules work in conjunction with NADPH and are membrane-bound hemoproteins composed of an apoprotein and a heme prosthetic group (the oxidizing center).

There are over 50 proteins in the CYP group which are organized into 18 families (e.g., CYP2) and 43 subfamilies (e.g., CYP2E1) [120]. Most of these enzymes are located on the cytoplasmic side of the smooth ER membrane (microsomal type) or the mitochondria (mitochondrial type) [120]. Phase I drug metabolism is performed predominantly by the microsomal type. There is also zonal heterogeneity in the expression of these enzymes within the liver. Some are more prominent in the perivenular (zone 3) hepatocytes (i.e., P450 2E1), while others are clustered within other acinar zones.

The majority of the P450 enzymes are specific for the metabolism of endogenous substances and are not inducible [120, 121]. Hepatic metabolism of exogenous drugs and toxins is performed mainly by the CYP1, CYP2, and CYP3 families, with a smaller contribution from CYP4 [120, 122–127]. The most important drug-metabolizing member within the liver is CYP3A4, which comprises about 60 % of all hepatic cytochromes and catalyzes the biotransformation of over 50 % of commonly used drugs [120, 128]. Free radicals and electrophilic compounds can be produced during this process.

CYP Activity

Cytochrome activity varies substantially depending upon the concentration of the enzymes within the cell. In addition, both endogenous and exogenous factors may alter the activity of a particular enzyme. This can increase the toxicity of a compound (either by reducing its conversion to nontoxic metabolites or by increasing its conversion to toxic metabolites) or decrease its therapeutic effectiveness (e.g., by increasing the rate of metabolism of active drug) (see Chapter 4) [129]. Alternate detoxification routes may become overloaded leading to hepatotoxicity, such as the case of acetaminophen, which is not toxic in normal therapeutic doses but is toxic when increased amounts are ingested.

Several factors may alter the activity of any of these drug-metabolizing reactions and influence metabolism [130]. Many genetic polymorphisms

in the CYP isoenzymes have been identified [117, 121, 131–138]. There is a strong association between certain HLA haplotypes and the development of hepatotoxicity from medications such as flucloxacillin, ximelagatran, and amoxicillin-clavulanate [135, 139–142]. These genetic alterations may lead to diminished metabolism, lack of metabolism, or excessive metabolism of a xenobiotic substance [143]. The best-studied examples are in the alcohol-metabolizing CYP2E1 subfamily and in the CYP2D6 subfamily (responsible for the metabolism of drugs such as metoprolol, quinidine, and desipramine) [144, 145]. This genetic variability may explain some of the individual hypersensitivity reactions to specific xenobiotics.

Phase II Reactions

Phase II reactions occur either directly with a parent compound or with a metabolite formed by a phase I reaction which is still not adequately hydrophilic for excretion. Phase II reactions conjugate these products to highly polar ligands, such as glucuronic acid, sulfate, acetate, glycine, glutathione, or a methyl group (Fig. 2.3). These reactions result in the formation of water-soluble, readily excreted, nontoxic substances [111, 146]. Phase II reactions take place primarily within the hepatocyte cytoplasm via the UDP-glucuronyl transferases (UGT1 and UGT2), sulfotransferases, and glutathione-S-transferases [113]. The effect of conjugation ordinarily leads to a decrease in pharmacologic activity (detoxification) with enhanced clearance of the lipophilic compound (e.g., acetaminophen, furosemide, and bilirubin). The nontoxic products are usually then ready for excretion. Phase II enzymes are rarely responsible for toxic metabolite formation; however, exceptions do occur. Impaired or reduced activity of the glucuronidation of morphine leads to increased analgesic potency. Impaired glucuronidation of the anticancer drug irinotecan leads to increased drug side effects [147–149]. And impaired sulfation of minoxidil, required for its antihypertensive effect, leads to ineffective blood pressure control [150].

Phase III Reactions

Phase III reactions lead to the transport of compounds into the bile and are mediated by ATP-dependent transporters located in the bile canaliculi. These transporters are members of the adenosine triphosphate (ATP)-binding cassette (ABC) superfamily. Their predominant role is that of regulation of bile formation and the excretion of xenobiotics [151]. Hepatotoxicity can arise if the activity of these transporters is altered. Once xenobiotics or their inactive conjugates are secreted into the bile, they may be deconjugated and/or reduced by intestinal bacteria. Deconjugation regenerates the oxidized, often toxic, metabolites from the phase I reactions. These may be partially reabsorbed in the intestine via enterohepatic circulation to reach the liver, where they may contribute to hepatotoxicity or carcinoma. The unabsorbed metabolites that reach the colon may have oncogenic effects on the colonic epithelium. The deconjugated metabolites may also be reduced by gut flora to the original xenobiotics, which may likewise undergo enterohepatic circulation, prolonging their half-life in the body.

Genetic polymorphisms exist in the phase II and the phase III enzymes also, leading to either decreased or increased activity. This is seen in glutathione-S-transferases, *N*-acetyltransferase 2, and UDP-glucuronosyltransferases [152–157]. Genetic differences in the hepatobiliary transporters may also predispose to xenobiotic-induced cholestasis or injury [158–160]. For example, variable pharmacokinetics have been reported with digoxin and cyclosporine, depending upon which genetic variant of the phase III hepatobiliary enzyme was present [161].

Bilirubin and Bile Acid Metabolism

One of the major functions of the liver is the removal of organic anions (such as bilirubin), cations, and bile acids from the circulation. Most of these products are lipophilic and are bound to serum albumin. These compounds are taken up into the hepatocyte by both sodium-independent

and sodium-dependant mechanisms. Bile salt and anionic steroidal compound uptake is mediated by the basal Na^+/taurocholate cotransporting polypeptide (NTCP), which is driven by the cellular sodium gradient. Organic anions such as bilirubin and cations are taken across the basolateral membrane in a sodium-independent manner by groups of transport proteins with overlapping specificity [162]. Organic cation uptake is mediated by the organic cation transporter (OCTs) family. Organic anion uptake is mediated by families of organic anion-transporting polypeptides (OATPs) and organic anion transporters (OATs) [162, 163]. It is at this step that they are dissociated from albumin, which is returned to the serum [164–167].

Bilirubin Formation

Bilirubin is the toxic byproduct resulting from the breakdown of heme-containing compounds such as hemoglobin, myoglobin, and the cytochromes [168]. Up to 75 % of bilirubin formed comes from erythrocyte hemoglobin degradation. Under normal circumstances, circulating free bilirubin is present in the bloodstream in only a trace amount. About 300 mg of bilirubin is produced daily in the normal adult, and it is synthesized predominantly in reticuloendothelial system of the spleen, followed by the bone marrow, kidneys, and liver [169]. Heme is first degraded in the endoplasmic reticulum to biliverdin, a linear tetrapyrrole, by microsomal heme oxidase. Biliverdin reductase then catalyzes the biliverdin to IX α-bilirubin.

Hepatocytes

The liver is responsible for the disposal of hydrophobic bilirubin [168]. Lipophilic bilirubin is carried to the liver tightly bound to albumin, where it is rapidly and efficiently removed by the hepatocytes. Its transport is Na+ independent. It remains uncertain which hepatocellular transporter is the most important and efficient in the uptake of bilirubin. Bilitranslocase is a carrier protein in the basolateral membrane which has shown specificity for bilirubin and is believed to participate in its uptake by the hepatocyte [170].

Bilirubin Metabolism

Once internalized, bilirubin is bound to cytosolic carrier proteins (Y- and Z-protein) which prevent reflux back into the plasma. The Y-protein, glutathione-S-transferase B, is the more important of the two. Z-protein is only activated in settings of high bilirubin concentrations. The lipid-soluble, ligandin-bound bilirubin is then converted (detoxified) within the endoplasmic reticulum by conjugation with uridine diphosphate (UDP)-xylose, UDP-glucose, and UDP-glucuronic acid. This results in hydrophilic bilirubin monoglucuronide (20–40 %) and diglucuronide (60–80 %) forms which are then ready for excretion into the bile [168, 171]. Diglucuronide forms of conjugated bilirubin are the predominant type which is excreted. In humans, the most important form of bilirubin conjugation is glucuronidation via the microsomal UDP-glucuronosyltransferases (UGTs).

The human UGTs are divided into two families – UTG1 and UGT2 [168, 172, 173]. The UGT2 family is subdivided into two subfamilies (UGT2A, UGT2B) [174]. The major UGT involved in bilirubin metabolism is UGT1A1 and, in humans, is found only within the liver [113, 175]. The UGT1A family is encoded by a single gene and codes for nine functional UGT1a enzymes and four pseudogenes [176]. These isoforms are highly expressed in the liver, small intestine, colon, and kidney [177]. If the activity of this enzyme is either impaired or reduced, it leads to an increase of unconjugated bilirubin (Gilbert syndrome, Crigler-Najjar syndrome) [173, 178].

The hydrophilic bilirubin conjugates are then ready to be moved across the apical (canalicular) membrane for secretion into the bile. The apical transporters are all ABC (ATP-binding cassette) proteins which are ATP dependent and actively export their substrates into the bile

using energy derived from the hydrolysis of ATP. The concentration of bilirubin in the bile is 100-fold higher that in the hepatocyte. Bilirubin is secreted by MRP2, the ATP-dependent multidrug resistance protein (previously known as multispecific canalicular organic anion transporter [cMOAT]) [179]. It is a member of the ABC transporter subfamily C of multidrug resistance-associated proteins [179–181]. Less than 5 % of the bilirubin is excreted by exocytosis. The organic cations which are taken up into the hepatocyte by the basolateral OCT transporters are secreted into bile by MRP1.

Bile Acid Metabolism and Transport

Bile Acid Formation

Bile acids are highly conserved proteins which aid in the maintenance of cholesterol homeostasis, stimulate bile flow, and emulsify dietary lipids to improve their intestinal absorption [1]. The liver plays a crucial role in bile acid metabolism, the hepatocytes being the only cell that can convert cholesterol into bile acids [182]. They are the end products of cholesterol metabolism and serve to rid the body of excess cholesterol. The hepatocytes also efficiently remove bile acids from the portal venous and hepatic arterial blood. Following uptake, the hepatocytes then secrete the bile acids into the canaliculus. Secretion of bile salts, phospholipids, and cholesterol into bile is mediated by several ATP-dependent, canalicular transporters.

The basic bile acid molecular skeleton is that of cholesterol – a steroid nucleus and an aliphatic side chain. Bile salts are termed *cholanoids* and consist predominantly of taurine or glycine conjugates of bile acids [183]. The bile acids formed from cholesterol that are called the *primary* bile acids are cholic acid and chenodeoxycholic acid. This nomenclature is used to differentiate them from the bile acids formed by intestinal bacterial transformation of the primary bile acids – the *secondary* bile acids (deoxycholic acid and lithocholic acid). The *tertiary* bile acids are formed from the secondary bile acid after they have been reabsorbed.

Bile acid synthesis from cholesterol is a complex multistep process, involving at a minimum of five steps for the nucleus and five steps for the side chain [183]. The end result is a saturated nucleus and a conjugated side chain. There are two pathways of synthesis. The neutral pathway occurs via the microsomal cholesterol 7α-hydroxycholesterol and accounts for approximately 90–95 % of bile acid synthesis. The alternative acidic pathway via 27-hydroxylase (CYP27A1) is located within the mitochondria.

The steroid nucleus of cholesterol is first modified by reduction and epimerization of the 3-hydroxyl group to become 7α-hydroxycholesterol. This step occurs in the endoplasmic reticulum and is facilitated by the cytochrome P450-dependent microsomal enzyme cholesterol 7α-hydroxylase (CYP7A1), which can increase its activity by 10-fold depending upon the need. CYP7A1 is regulated via negative feedback by the level of bile acids returning to the liver via the enterohepatic circulation [182, 184]. It is the rate-limiting step, and its regulation has been shown to be facilitated by bile acid binding to the nuclear receptor, farnesoid X receptor (FXR), and via bile acid stimulation of ileal release of fibroblast growth factor 19 (FGF19) [185, 186]. Cholic acid is the strongest activator of FXR. 7α-hydroxycholesterol is then further enzymatically modified via an isomerase and a reductase to choles-7α-hydroxy-Δ^4-3-one. This product is further oxidized and reduced and the side chain modified to form the primary bile acids. Over 99 % of the bile acids are then conjugated via bile acid-CoA synthetase or N-acyltransferase prior to being secreted by the hepatocytes [1]. Bile acids are most commonly conjugated to glycine or taurine, with glucuronidation and sulphation occurring to a much lesser extent unless cholestasis is present. Conjugation creates strong acids which become ionized (lipophilic) at both biliary and intestinal pH, improving lipid digestion and diffusion across cell membranes [187].

Secretion, Bile Flow, and Enterohepatic Circulation

The secreted bile salts and lecithin form mixed micelles, which act as a sink for further lipids

and lipophilic substances within the biliary tree. A large osmotic effect is generated by the excreted bile leading to an influx of water into the canaliculus [188]. Canalicular contractions, mediated via pericanalicular actin filaments, calcium, and ATP, then move the fluid down the canaliculus and into the biliary ductules (bile acid-dependent flow). The conjugated bile acids pass down the biliary tree and are either secreted into the intestine or stored within the gallbladder.

Once reaching the duodenum, the bile acids enhance the absorption of lipids and fat-soluble vitamins. Most bile salts undergo very little absorption until reaching the lower third of the small intestine. In the ileum, approximately 95 % of the bile salts are actively absorbed via carrier-mediated transport (ileal bile acid transporters) [189, 190]. They are then moved into the portal venous blood via the ileal basolateral transport system, consisting of the organic solute transporters α and β (OST-α, OST-β). The bile acids then return to the liver via the portal blood (enterohepatic circulation) where they are actively taken up by the hepatocytes [191] [163, 180, 192, 193].

These efficient processes clear the portal venous blood of more than 60–80 % of the bile salts within a single passage through the liver. The remaining bile acids are returned to the liver in the arterial and portal circulation and further extracted [194]. These deconjugated bile acids are then reconjugated, and the process begins again. Bile acids which are not reabsorbed by the ileum are processed by the colonic bacteria into the secondary bile acids, deoxycholic acid, and lithocholic acid, a quarter of which are reabsorbed from the colon. Less than 5 % are excreted into the feces.

Bile Acid Transporters

Bile acids within the plasma are predominantly albumin bound, with a small fraction (<10 %) bound to lipoproteins [195]. Bile acids are actively taken up by the hepatocyte basolateral (sinusoidal) membrane by both sodium-dependent and

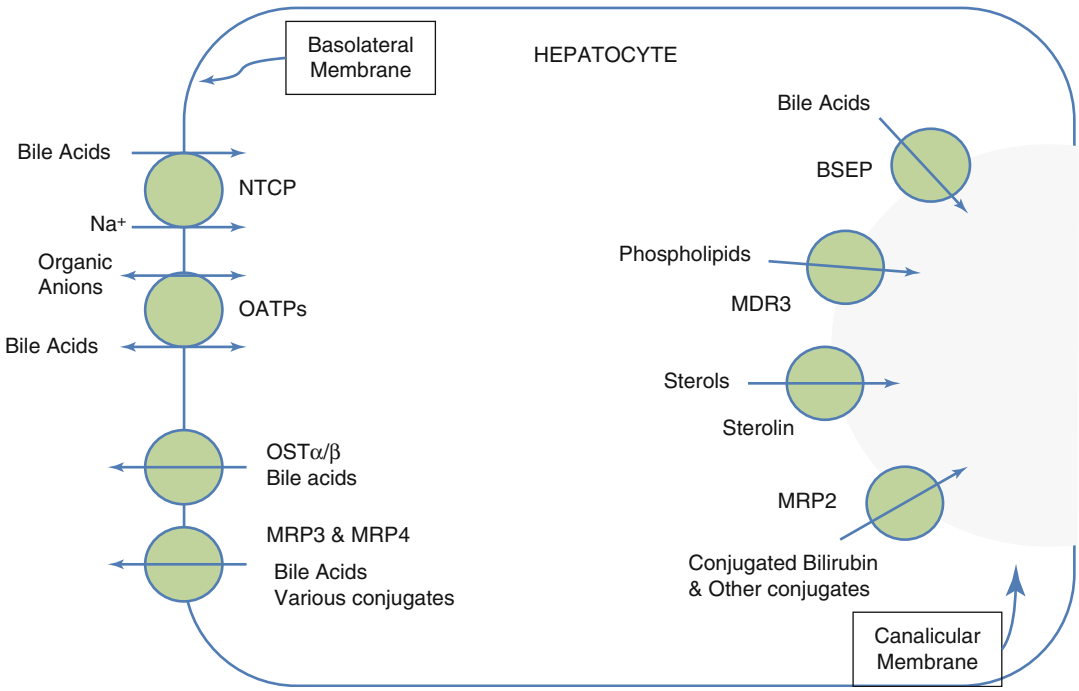

Fig. 2.4 Bile acid secretion. *NTCP* Na^+/taurocholate cotransporting polypeptide, *OATPs* organic anion-transporting polypeptides. *OST-α* and *OST-β* organic solute transporters α and β, *MRP* multidrug resistance-associated proteins, *BSEP* bile salt export pump, *MDR3* multidrug resistance P-glycoprotein 3 (an ATP-dependent phospholipid – translocase/flippase), *MRP2* multidrug resistance-associated protein 2

sodium-independent systems (Fig. 2.4). Sodium-dependent uptake is mediated by the basolateral sodium-taurocholate cotransporting polypeptide (NTCP) and sodium-independent uptake via the superfamily of organic anion-transporting proteins (OATPs) [163, 180, 192, 193]. Approximately 80 % of the conjugated bile acid uptake is performed by NTCP, while it accounts for <50 % of the unconjugated bile salt uptake. The OATPs function as anion exchangers. The rate of hepatocyte uptake of the bile acids is influenced by the activity of the transport systems, the presence of albumin, the chemical structure of the bile acid, and the intracellular concentration of bile acids [183, 189]. It is unclear how they are transported across the hepatocyte.

The newly conjugated bile acids, and those removed from the blood, are then secreted into the bile across a concentration gradient via the apical (canalicular) bile salt export pump (BSEP) [180, 196–198]. This is the rate-limiting step in the overall transport of the bile salts, phospholipid, and sterols into the bile [199, 200]. BSEP is a P-glycoprotein belonging to class B of the ABC transporter superfamily (ABCB11). In addition, sodium-independent carrier-mediated transport is driven by the negative potential difference between the hepatocyte and the canaliculus.

Multiple other transporters exist in the apical membrane. Non-ATP-dependent chloride bicarbonate exchangers secrete bicarbonate into the bile, stimulating bile flow and controlling the volume of the bile. Chloride channels and sodium-dependent purine-specific nucleoside cotransporters aid in the conservation of nucleosides and amino acids. At least 4 ATP-dependent transporters have been identified which participate in the transport of organic cations, xenobiotics, phospholipids, and cytokines [179, 201]. Transport of cytokines is mediated by MDR1 (multidrug resistance protein 1 or P-glycoprotein 170) [202]. Organic ions such as bilirubin and glutathione conjugates, as well as conjugated xenobiotics, are transported via MRP2 (multidrug resistance-associated protein 2) [201, 203]. The BSEP, as noted above, is crucial to the transport of bile acids. In addition, bile acids induce hepatocyte secretion of phospholipids, predominantly phosphatidylcholine (via the phospholipid flippase MDR3), and cholesterol from the canalicular membrane [204–206]. MDR3 accounts for about 80 % of the MDR family in the bile canaliculus.

Also located within the basolateral membrane are biliary exporters, which play a major role in removing accumulated substances from the hepatocyte if they are unable to be excreted into the bile. These include OST-α, OST-β, MRP3, and MRP4. These exporters explain the elevations in serum bilirubin and bile acid levels in the setting of biliary obstruction or cholestasis.

Cholangiocytes

The cholangiocytes are the epithelial cells lining the biliary tree and constitute about 3–5 % of the liver cells [207, 208]. Bile secretion depends on the function of the transport systems within both the hepatocyte and the cholangiocyte. Biliary canaliculi are tubular structures formed by adjacent hepatocytes which, by osmotic gradient, favor the formation and secretion of bile. The cholangiocytes then form a complex network of interconnecting tubular structures which are conduits for delivering bile to the intestine. These tubules gradually increase in size from ductules to large ducts emptying into the small intestine. In addition to their structural function, the cholangiocytes are highly active, modifying canalicular bile via secretory and reabsorptive processes [208–212]. They are responsible for about 30 % of the bile volume [207].

Like hepatocytes, these cells are highly polarized, consisting of a well-defined apical (lumenal) surface and a basolateral surface. The apical surface provides an increased surface area via numerous microvilli and has cilia which aid in bile flow [213]. Tight junctions are located within close proximity to the hepatocyte apical surface, and cholangiocytes communicate via gap junctions [214]. Cholangiocytes have a well-organized network of organelles, including abundant mitochondria [215, 216]. The cholangiocyte expresses proteins, some of which play a role in cholangiocyte function and others whose role has yet to be defined. Cholangiocytes express both secretin and somatostatin receptors. Secretin stimulates cholangiocyte secretion via an increase

in cellular cyclic AMP (cAMP) levels. This leads to the phosphorylation of the CFTR chloride channels and activation of the apical Cl^-/HCO_3^- exchanger, leading to secretion of bicarbonate into the ductal bile [217, 218]. Somatostatin inhibits bile flow and secretin-induced choleresis [219, 220]. Gamma-glutamyl transferase is membrane bound and is involved in the metabolism of glutathione and glutathione-S-conjugated electrophiles [208]. Carcinoembryonic antigen (CEA) is expressed by cholangiocytes, but its significance is still uncertain. Cholangiocytes have also been reported to possess both phase I and phase II enzymes used in the detoxification of xenobiotics. These enzymes, however, are differentially expressed, and phase II enzymes predominate [221].

Multiple transporters have been identified on both the apical and basolateral membrane of the cholangiocyte [24]. These transporters are responsible for the transfer of substances between the hepatocytes and the bile. Several processes, including bile and glucose uptake, are regulated by pairs of transport proteins. The uptake of conjugated bile acids is performed at the luminal surface by a sodium-dependent bile salt transporter (ABAT). At the basolateral surface is a sodium-independent truncated form of this protein (ASBT) which mediates the efflux of bile acids from the cell [24]. Similarly, glucose reabsorption from the bile is mediated by a sodium-dependent glucose transporter (SGLT1) in the apical membrane and a facilitative glucose transporter within the basolateral membrane. In addition, the cells regulate transport of water, chloride ions, calcium, and electrolytes. These cells are high regulated and appear to be responsive to gastrointestinal hormones, such as secretin and somatostatin [222, 223]. Moreover, cholangiocytes play a role in the regulation of extracellular matrix composition.

Immune Function

Kupffer Cells

Kupffer cells are specialized tissue macrophages. They are a part of the reticuloendothelial system and make up 80–90 % of the total fixed macrophage population within the body. Their development begins within the bone marrow, moving into the peripheral blood as monocytes and then to the liver where they differentiate into Kupffer cells [224, 225]. Kupffer cells are one of the first lines of defense against intestinally derived foreign material. They are responsible for the degradation of gut-derived antigens and products (i.e., endotoxin), initiation of immunological responses, and induction of tolerance to antigens absorbed from the gut [226–228].

These phagocytic cells are located on the luminal surface of the hepatic endothelium and are highly active in removing toxic or foreign substances which reach the liver via the portal system [39, 229]. They are filled with lysosomes, reflecting their role in degrading substances. The process of phagocytosis is energy-dependent and leads to the production of multiple reactive molecules, such as superoxide anion, hydrogen peroxide, and hydroxyl radicals which aid in intracellular degeneration of foreign antigens [230]. Kupffer cells also actively secrete vasoactive toxic mediators and increase in number and activity in response to chemical, infectious, or immunologic liver injury [39, 231]. These secreted free radicals lead to lipid peroxidation and injury to the surrounding hepatocytes [232]. Kupffer cells produce prostaglandins (PGD, PDE, PDF, thromboxane, prostacyclin) and eicosanoids. When activated by endotoxin, they synthesize interleukin 1, interleukin 6, and tumor necrosis factor alpha. These products act as mediators of the acute-phase response [101, 233, 234].

Kupffer cells express major histocompatibility (MHC) class I and II antigens [235]. They are capable of acting as antigen-presenting cells, a feature modulated by innate signals [235, 236]. While capable of presenting antigen to T cells, they are less efficient than macrophages at other sites in the body [237].

Natural Killer Cells

The liver also contains a natural killer (NK) cell population. These cells were initially called Pit cells because of their electron-dense cytoplasmic

granules seen in electron microscopy [238, 239]. They constitute a large portion of the hepatic lymphocyte population. They migrate from the blood to the liver and further differentiate into a unique subpopulation. NK cells are embedded in the sinusoids anchored to the sinusoidal endothelial cells by villous extensions and often located near the Kupffer cells [238]. In keeping with other cells within the liver, these cells have marked polarity and face the bloodstream directly [238].

NK cells play a central role in regulating the nonadaptive immune response as well as shaping the adaptive immune responses [50]. They function via cell-to-cell interaction and cytokine secretion [1, 50]. They continuously sense their environment [50]. They are able to kill certain malignant cells as well as virus-infected cells without being sensitized [240, 241]. Additionally, these cells may play a role in the regulation of growth and differentiation of liver cells [242]. There are also T cells present which coexpress NK markers (NT cells) which are thought to be important in innate immune responses to pathogens [39, 227, 238].

NK cells are also involved in hepatic fibrosis remodeling [50, 57] NK cells appear to induce activated HSC death and reduce the severity of fibrosis formation. Activated HSC are more prone to NK-induced cell death due to upregulation of proapoptotic molecules. Upregulation of the TNF-related apoptosis-inducing ligand (TRAIL) on the HSC is likely to be one of the most crucial events in NK cytotoxicity [243]. In addition, activated HSC show upregulation of retinoic acid early inducible-1, a ligand for the NKG2D receptor [50].

References

1. Roy-Chowdhury N, Roy-Chowdhury J. Liver physiology and energy metabolism. In: Feldman M, Friedman LS, Brandt LJ, editors. Sleisenger and Fordtran's gastrointestinal and liver disease – pathophysiology/diagnosis/management. 9th ed. Philadelphia: Saunders/Elsevier; 2010. p. 1207–26.
2. Blouin A, Bolender RP, Weibel ER. Distribution of organelles and membranes between hepatocytes and nonhepatocytes in the rat liver parenchyma. A stereological study. J Cell Biol. 1977;72(2):441–55.
3. Srere PA, Sumegi B. Processivity and fatty acid oxidation. Biochem Soc Trans. 1994;22(2):446–50.
4. Racker E, Horstman LL. Partial resolution of the enzymes catalyzing oxidative phosphorylation. 13. Structure and function of submitochondrial particles completely resolved with respect to coupling factor. J Biol Chem. 1967;242(10):2547–51.
5. Newmeyer DD, Ferguson-Miller S. Mitochondria: releasing power for life and unleashing the machineries of death. Cell. 2003;112(4):481–90.
6. Dean RT, Barrett AJ. Lysosomes. Essays Biochem. 1976;12:1–40.
7. Yin XM, Ding WX, Gao W. Autophagy in the liver. Hepatology. 2008;47(5):1773–85.
8. Uchiyama Y, Shibata M, Koike M, Yoshimura K, Sasaki M. Autophagy-physiology and pathophysiology. Histochem Cell Biol. 2008;129(4):407–20.
9. Roma MG, Crocenzi FA, Mottino AD. Dynamic localization of hepatocellular transporters in health and disease. World J Gastroenterol. 2008;14(44):6786–801.
10. Ihrke G, Hubbard AL. Control of vesicle traffic in hepatocytes. Prog Liver Dis. 1995;13:63–99.
11. Conner SD, Schmid SL. Regulated portals of entry into the cell. Nature. 2003;422(6927):37–44.
12. Crowther RA, Pearse BM. Assembly and packing of clathrin into coats. J Cell Biol. 1981;91(3 Pt 1):790–7.
13. DePierre JW, Dallner G. Structural aspects of the membrane of the endoplasmic reticulum. Biochim Biophys Acta. 1975;415(4):411–72.
14. Walter P, Lingappa VR. Mechanism of protein translocation across the endoplasmic reticulum membrane. Annu Rev Cell Biol. 1986;2:499–516.
15. Hurtley SM, Helenius A. Protein oligomerization in the endoplasmic reticulum. Annu Rev Cell Biol. 1989;5:277–307.
16. de Duve C, Wattiaux R. Functions of lysosomes. Annu Rev Physiol. 1966;28:435–92.
17. Inui A, Fujisawa T, Sogo T, Komatsu H, Isozaki A, Sekine I. Different outcomes of vertical transmission of hepatitis C virus in a twin pregnancy. J Gastroenterol Hepatol. 2002;17(5):617–9.
18. Komatsu M. Liver autophagy: physiology and pathology. J Biochem. 2012;152(1):5–15.
19. Mayor S, Presley JF, Maxfield FR. Sorting of membrane components from endosomes and subsequent recycling to the cell surface occurs by a bulk flow process. J Cell Biol. 1993;121(6):1257–69.
20. Luzio JP, Mullock BM, Pryor PR, Lindsay MR, James DE, Piper RC. Relationship between endosomes and lysosomes. Biochem Soc Trans. 2001;29(Pt 4):476–80.
21. Pieuchot L, Jedd G. Peroxisome assembly and functional diversity in eukaryotic microorganisms. Annu Rev Microbiol. 2012;66:237–63.
22. Waterham HR, Wanders RJ. Metabolic functions and biogenesis of peroxisomes in health and disease. Biochim Biophys Acta. 2012;1822(9):1325.
23. Zegers MM, Hoekstra D. Mechanisms and functional features of polarized membrane traffic in epithelial and hepatic cells. Biochem J. 1998;336(Pt 2):257–69.

24. Esteller A. Physiology of bile secretion. World J Gastroenterol. 2008;14(37):5641–9.
25. Yee AG, Revel JP. Loss and reappearance of gap junctions in regenerating liver. J Cell Biol. 1978;78(2):554–64.
26. Yancey SB, Easter D, Revel JP. Cytological changes in gap junctions during liver regeneration. J Ultrastruct Res. 1979;67(3):229–42.
27. Jones AL, Schmucker DL, Renston RH, Murakami T. The architecture of bile secretion. A morphological perspective of physiology. Dig Dis Sci. 1980;25(8):609–29.
28. Forker EL. Two sites of bile formation as determined by mannitol and erythritol clearance in the guinea pig. J Clin Invest. 1967;46(7):1189–95.
29. Rappaport AM. The microcirculatory acinar concept of normal and pathological hepatic structure. Beitr Pathol. 1976;157(3):215–43.
30. Katz NR. Metabolic heterogeneity of hepatocytes across the liver acinus. J Nutr. 1992;122(3 Suppl):843–9.
31. Gonzalez J, Esteller A. Heterogeneity of rabbit hepatocytes for bile secretion after acinar zone 3 damage induced by bromobenzene. Effect of bilirubin and bile salt infusions. Biochem Pharmacol. 1985;34(4):507–14.
32. Smedsrod B, Le CD, Ikejima K, Jaeschke H, Kawada N, Naito M, et al. Hepatic sinusoidal cells in health and disease: update from the 14th International Symposium. Liver Int. 2009;29(4):490–501.
33. Yokomori H, Oda M, Yoshimura K, Hibi T. Recent advances in liver sinusoidal endothelial ultrastructure and fine structure immunocytochemistry. Micron. 2012;43(2–3):129–34.
34. Goresky CA. A linear method for determining liver sinusoidal and extravascular volumes. Am J Physiol. 1963;204:626–40.
35. Grisham JW, Nopanitaya W, Compagno J. Scanning electron microscopy of the liver: a review of methods and results. Prog Liver Dis. 1976;5:1–23.
36. Fraser R, Dobbs BR, Rogers GW. Lipoproteins and the liver sieve: the role of the fenestrated sinusoidal endothelium in lipoprotein metabolism, atherosclerosis, and cirrhosis. Hepatology. 1995;21(3):863–74.
37. Arias IM. The biology of hepatic endothelial cell fenestrae. Prog Liver Dis. 1990;9:11–26.
38. Smedsrod B, De Bleser PJ, Braet F, Lovisetti P, Vanderkerken K, Wisse E, et al. Cell biology of liver endothelial and Kupffer cells. Gut. 1994;35(11):1509–16.
39. Crispe IN. Liver antigen-presenting cells. J Hepatol. 2011;54(2):357–65.
40. Geerts A. History, heterogeneity, developmental biology, and functions of quiescent hepatic stellate cells. Semin Liver Dis. 2001;21(3):311–35.
41. Sato M, Suzuki S, Senoo H. Hepatic stellate cells: unique characteristics in cell biology and phenotype. Cell Struct Funct. 2003;28(2):105–12.
42. Friedman G, Liu LM, Friedman SL, Boyles JK. Apolipoprotein E is secreted by cultured lipocytes of the rat liver. J Lipid Res. 1991;32(1):107–14.
43. Athari A, Hanecke K, Jungermann K. Prostaglandin F2 alpha and D2 release from primary Ito cell cultures after stimulation with noradrenaline and ATP but not adenosine. Hepatology. 1994;20(1 Pt 1):142–8.
44. Hernandez-Gea V, Friedman SL. Pathogenesis of liver fibrosis. Annu Rev Pathol. 2011;6:425–56.
45. Friedman SL. Hepatic fibrosis – overview. Toxicology. 2008;254(3):120–9.
46. Unanue ER. Ito cells, stellate cells, and myofibroblasts: new actors in antigen presentation. Immunity. 2007;26(1):9–10.
47. Winau F, Hegasy G, Weiskirchen R, Weber S, Cassan C, Sieling PA, et al. Ito cells are liver-resident antigen-presenting cells for activating T cell responses. Immunity. 2007;26(1):117–29.
48. Senoo H, Kojima N, Sato M. Vitamin A-storing cells (stellate cells). Vitam Horm. 2007;75:131–59.
49. Senoo H, Yoshikawa K, Morii M, Miura M, Imai K, Mezaki Y. Hepatic stellate cell (vitamin A-storing cell) and its relative – past, present and future. Cell Biol Int. 2010;34(12):1247–72.
50. Krueger PD, Lassen MG, Qiao H, Hahn YS. Regulation of NK cell repertoire and function in the liver. Crit Rev Immunol. 2011;31(1):43–52.
51. Moreira RK. Hepatic stellate cells and liver fibrosis. Arch Pathol Lab Med. 2007;131(11):1728–34.
52. Hui AY, Friedman SL. Molecular basis of hepatic fibrosis. Expert Rev Mol Med. 2003;5(5):1–23.
53. Brenner DA, Kisseleva T, Scholten D, Paik YH, Iwaisako K, Inokuchi S, et al. Origin of myofibroblasts in liver fibrosis. Fibrogenesis Tissue Repair. 2012;5 Suppl 1:S17.
54. Stanciu A, Cotutiu C, Amalinei C. New data about ITO cells. Rev Med Chir Soc Med Nat Iasi. 2002;107(2):235–9.
55. Eyden B. The myofibroblast: phenotypic characterization as a prerequisite to understanding its functions in translational medicine. J Cell Mol Med. 2008;12(1):22–37.
56. Kobold D, Grundmann A, Piscaglia F, Eisenbach C, Neubauer K, Steffgen J, et al. Expression of reelin in hepatic stellate cells and during hepatic tissue repair: a novel marker for the differentiation of HSC from other liver myofibroblasts. J Hepatol. 2002;36(5):607–13.
57. Pellicoro A, Ramachandran P, Iredale JP. Reversibility of liver fibrosis. Fibrogenesis Tissue Repair. 2012;5 Suppl 1:S26.
58. Friedman SL. Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver. Physiol Rev. 2008;88(1):125–72.
59. DeFronzo RA. Pathogenesis of type 2 diabetes mellitus. Med Clin North Am. 2004;88(4):787–835, ix.
60. Moore MC, Coate KC, Winnick JJ, An Z, Cherrington AD. Regulation of hepatic glucose uptake and storage in vivo. Adv Nutr. 2012;3(3):286–94.
61. Bryant NJ, Govers R, James DE. Regulated transport of the glucose transporter GLUT4. Nat Rev Mol Cell Biol. 2002;3(4):267–77.
62. Roach PJ. Glycogen and its metabolism. Curr Mol Med. 2002;2(2):101–20.

63. Ryu JH, Drain J, Kim JH, McGee S, Gray-Weale A, Waddington L, et al. Comparative structural analyses of purified glycogen particles from rat liver, human skeletal muscle and commercial preparations. Int J Biol Macromol. 2009;45(5):478–82.
64. Ferrer JC, Favre C, Gomis RR, Fernandez-Novell JM, Garcia-Rocha M, de la Iglesia N, et al. Control of glycogen deposition. FEBS Lett. 2003;546(1):127–32.
65. Lomako J, Lomako WM, Whelan WJ. Glycogenin: the primer for mammalian and yeast glycogen synthesis. Biochim Biophys Acta. 2004;1673(1–2):45–55.
66. Livanova NB, Chebotareva NA, Eronina TB, Kurganov BI. Pyridoxal 5'-phosphate as a catalytic and conformational cofactor of muscle glycogen phosphorylase B. Biochemistry (Mosc). 2002;67(10):1089–98.
67. Wegener G, Krause U. Different modes of activating phosphofructokinase, a key regulatory enzyme of glycolysis, in working vertebrate muscle. Biochem Soc Trans. 2002;30(2):264–70.
68. Yoshida A, Watanabe S. Human phosphoglycerate kinase. I. Crystallization and characterization of normal enzyme. J Biol Chem. 1972;247(2):440–5.
69. Triplitt CL. Understanding the kidneys' role in blood glucose regulation. Am J Manag Care. 2012;18(1 Suppl):S11–6.
70. Prats C, Cadefau JA, Cusso R, Qvortrup K, Nielsen JN, Wojtaszewski JF, et al. Phosphorylation-dependent translocation of glycogen synthase to a novel structure during glycogen resynthesis. J Biol Chem. 2005;280(24):23165–72.
71. Rodgers RL. Glucagon and cyclic AMP: time to turn the page? Curr Diabetes Rev. 2012;8(5):362–81.
72. Drucker DJ, Nauck MA. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. Lancet. 2006;368(9548):1696–705.
73. Dancygier H, Merle U, Stremmel W, Niederau C. Hepatic metabolism. In: Dancygier H, editor. Clinical hepatology – principles and practice of hepatobiliary diseases. New York: Springer; 2010. p. 75–102.
74. Hellerstein MK. De novo lipogenesis in humans: metabolic and regulatory aspects. Eur J Clin Nutr. 1999;53 Suppl 1:S53–65.
75. Macfarlane DP, Forbes S, Walker BR. Glucocorticoids and fatty acid metabolism in humans: fuelling fat redistribution in the metabolic syndrome. J Endocrinol. 2008;197(2):189–204.
76. Browning JD, Horton JD. Molecular mediators of hepatic steatosis and liver injury. J Clin Invest. 2004;114(2):147–52.
77. Ferre P, Foufelle F. SREBP-1c transcription factor and lipid homeostasis: clinical perspective. Horm Res. 2007;68(2):72–82.
78. Wakil SJ, Abu-Elheiga LA. Fatty acid metabolism: target for metabolic syndrome. J Lipid Res. 2009;50(Suppl):S138–43.
79. Coleman RA, Mashek DG. Mammalian triacylglycerol metabolism: synthesis, lipolysis, and signaling. Chem Rev. 2011;111(10):6359–86.
80. Zechner R, Kienesberger PC, Haemmerle G, Zimmermann R, Lass A. Adipose triglyceride lipase and the lipolytic catabolism of cellular fat stores. J Lipid Res. 2009;50(1):3–21.
81. Saario SM, Laitinen JT. Monoglyceride lipase as an enzyme hydrolyzing 2-arachidonoylglycerol. Chem Biodivers. 2007;4(8):1903–13.
82. Yeaman SJ. Hormone-sensitive lipase – new roles for an old enzyme. Biochem J. 2004;379(Pt 1):11–22.
83. Brown MS, Goldstein JL. The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell. 1997;89(3):331–40.
84. Zammit VA. Carnitine palmitoyltransferase 1: central to cell function. IUBMB Life. 2008;60(5):347–54.
85. McPherson PA, McEneny J. The biochemistry of ketogenesis and its role in weight management, neurological disease and oxidative stress. J Physiol Biochem. 2012;68(1):141–51.
86. Fu Z, Runquist JA, Montgomery C, Miziorko HM, Kim JJ. Functional insights into human HMG-CoA lyase from structures of Acyl-CoA-containing ternary complexes. J Biol Chem. 2010;285(34):26341–9.
87. White H, Venkatesh B. Clinical review: ketones and brain injury. Crit Care. 2011;15(2):219.
88. Fukao T, Lopaschuk GD, Mitchell GA. Pathways and control of ketone body metabolism: on the fringe of lipid biochemistry. Prostaglandins Leukot Essent Fatty Acids. 2004;70(3):243–51.
89. Carman GM, Han GS. Roles of phosphatidate phosphatase enzymes in lipid metabolism. Trends Biochem Sci. 2006;31(12):694–9.
90. Stone SJ, Levin MC, Zhou P, Han J, Walther TC, Farese Jr RV. The endoplasmic reticulum enzyme DGAT2 is found in mitochondria-associated membranes and has a mitochondrial targeting signal that promotes its association with mitochondria. J Biol Chem. 2009;284(8):5352–61.
91. Maxfield FR, van Meer G. Cholesterol, the central lipid of mammalian cells. Curr Opin Cell Biol. 2010;22(4):422–9.
92. Silbernagel G, Lutjohann D, Machann J, Meichsner S, Kantartzis K, Schick F, et al. Cholesterol synthesis is associated with hepatic lipid content and dependent on fructose/glucose intake in healthy humans. Exp Diabetes Res. 2012;2012:361863.
93. van der Wulp MY, Verkade HJ, Groen AK. Regulation of cholesterol homeostasis. Mol Cell Endocrinol. 2013;368(1–2):1–16.
94. Raabe M, Veniant MM, Sullivan MA, Zlot CH, Bjorkegren J, Nielsen LB, et al. Analysis of the role of microsomal triglyceride transfer protein in the liver of tissue-specific knockout mice. J Clin Invest. 1999;103(9):1287–98.
95. Christensen HN. Role of amino acid transport and countertransport in nutrition and metabolism. Physiol Rev. 1990;70(1):43–77.
96. Feldmann G, Scoazec JY, Racine L, Bernuau D. Functional hepatocellular heterogeneity for the production of plasma proteins. Enzyme. 1992; 46(1–3):139–54.
97. Rothschild MA, Oratz M, Schreiber SS. Serum albumin. Hepatology. 1988;8(2):385–401.

98. Benham AM. Protein secretion and the endoplasmic reticulum. Cold Spring Harb Perspect Biol. 2012;4(8):a012872.
99. Braakman I, Bulleid NJ. Protein folding and modification in the mammalian endoplasmic reticulum. Annu Rev Biochem. 2011;80:71–99.
100. Chung KN, Walter P, Aponte GW, Moore HP. Molecular sorting in the secretory pathway. Science. 1989;243(4888):192–7.
101. Andus T, Bauer J, Gerok W. Effects of cytokines on the liver. Hepatology. 1991;13(2):364–75.
102. Burt AD, Day CP. Pathophysiology of the liver. In: MacSween RNM, Burt AD, Portmann BC, Ishak KG, Scheuer PJ, Anthony PP, editors. Pathology of the liver. 4th ed. Philadelphia: Churchill Livingstone; 2002. p. 67–106.
103. Fanali G, di MA, Trezza V, Marino M, Fasano M, Ascenzi P. Human serum albumin: from bench to bedside. Mol Aspects Med. 2012;33(3):209–90.
104. Johnson E, Hetland G. Mononuclear phagocytes have the potential to synthesize the complete functional complement system. Scand J Immunol. 1988;27(5):489–93.
105. Haglund K, Dikic I. The role of ubiquitylation in receptor endocytosis and endosomal sorting. J Cell Sci. 2012;125(Pt 2):265–75.
106. Mortimore GE, Poso AR. Intracellular protein catabolism and its control during nutrient deprivation and supply. Annu Rev Nutr. 1987;7:539–64.
107. Harper AE, Miller RH, Block KP. Branched-chain amino acid metabolism. Annu Rev Nutr. 1984;4:409–54.
108. Christensen HN. Interorgan amino acid nutrition. Physiol Rev. 1982;62(4 Pt 1):1193–233.
109. Simmer JP, Kelly RE, Rinker Jr AG, Scully JL, Evans DR. Mammalian carbamyl phosphate synthetase (CPS). DNA sequence and evolution of the CPS domain of the Syrian hamster multifunctional protein CAD. J Biol Chem. 1990;265(18):10395–402.
110. Adeva MM, Souto G, Blanco N, Donapetry C. Ammonium metabolism in humans. Metabolism. 2012;61(11):1495–511.
111. Park BK, Pirmohamed M, Kitteringham NR. The role of cytochrome P450 enzymes in hepatic and extrahepatic human drug toxicity. Pharmacol Ther. 1995;68(3):385–424.
112. Park K, Williams DP, Naisbitt DJ, Kitteringham NR, Pirmohamed M. Investigation of toxic metabolites during drug development. Toxicol Appl Pharmacol. 2005;207(2 Suppl):425–34.
113. Sugatani J. Function, genetic polymorphism, and transcriptional regulation of human UDP-glucuronosyltransferase (UGT) 1A1. Drug Metab Pharmacokinet. 2013;28(2):83–92.
114. Gunawan BK, Kaplowitz N. Mechanisms of drug-induced liver disease. Clin Liver Dis. 2007;11(3):459–75, v.
115. Nelson DR, Koymans L, Kamataki T, Stegeman JJ, Feyereisen R, Waxman DJ, et al. P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. Pharmacogenetics. 1996;6(1):1–42.
116. Peterson JA, Graham SE. A close family resemblance: the importance of structure in understanding cytochromes P450. Structure. 1998;6(9):1079–85.
117. Smith G, Stubbins MJ, Harries LW, Wolf CR. Molecular genetics of the human cytochrome P450 monooxygenase superfamily. Xenobiotica. 1998;28(12):1129–65.
118. Werck-Reichhart D, Feyereisen R. Cytochromes P450: a success story. Genome Biol. 2000;1(6): REVIEWS3003.
119. Gonzalez FJ. Molecular genetics of the P-450 superfamily. Pharmacol Ther. 1990;45(1):1–38.
120. Danielson PB. The cytochrome P450 superfamily: biochemistry, evolution and drug metabolism in humans. Curr Drug Metab. 2002;3(6):561–97.
121. DeLeve LD, Kaplowitz N. Mechanisms of drug-induced liver disease. Gastroenterol Clin North Am. 1995;24(4):787–810.
122. Ehrenpreis ED, Ehrenpreis S. Cytochrome P450: role in drug-induced hepatotoxicity. Clin Liver Dis. 1998;2:457.
123. Watkins PB. Drug metabolism by cytochromes P450 in the liver and small bowel. Gastroenterol Clin North Am. 1992;21(3):511–26.
124. Wrighton SA, Vandenbranden M, Ring BJ. The human drug metabolizing cytochromes P450. J Pharmacokinet Biopharm. 1996;24(5):461–73.
125. Wilkinson GR. Cytochrome P4503A (CYP3A) metabolism: prediction of in vivo activity in humans. J Pharmacokinet Biopharm. 1996;24(5):475–90.
126. Ketter TA, Flockhart DA, Post RM, Denicoff K, Pazzaglia PJ, Marangell LB, et al. The emerging role of cytochrome P450 3A in psychopharmacology. J Clin Psychopharmacol. 1995;15(6):387–98.
127. Lehmann DE. Enzymatic shunting: resolving the acetaminophen-warfarin controversy. Pharmacotherapy. 2000;20(12):1464–8.
128. Waxman DJ. P450 gene induction by structurally diverse xenochemicals: central role of nuclear receptors CAR, PXR, and PPAR. Arch Biochem Biophys. 1999;369(1):11–23.
129. Walgren JL, Mitchell MD, Thompson DC. Role of metabolism in drug-induced idiosyncratic hepatotoxicity. Crit Rev Toxicol. 2005;35(4):325–61.
130. Chalasani N, Bjornsson E. Risk factors for idiosyncratic drug-induced liver injury. Gastroenterology. 2010;138(7):2246–59.
131. Dorne JL, Walton K, Renwick AG. Human variability in xenobiotic metabolism and pathway-related uncertainty factors for chemical risk assessment: a review. Food Chem Toxicol. 2005; 43(2):203–16.
132. Tanaka E. Update: genetic polymorphism of drug metabolizing enzymes in humans. J Clin Pharm Ther. 1999;24(5):323–9.
133. Hasler JA. Pharmacogenetics of cytochromes P450. Mol Aspects Med. 1999;20(1–2):12–137.
134. Pachkoria K, Lucena MI, Molokhia M, Cueto R, Carballo AS, Carvajal A, et al. Genetic and

molecular factors in drug-induced liver injury: a review. Curr Drug Saf. 2007;2(2):97–112.
135. Daly AK, Day CP. Genetic association studies in drug-induced liver injury. Semin Liver Dis. 2009;29(4):400–11.
136. Vuilleumier N, Rossier MF, Chiappe A, Degoumois F, Dayer P, Mermillod B, et al. CYP2E1 genotype and isoniazid-induced hepatotoxicity in patients treated for latent tuberculosis. Eur J Clin Pharmacol. 2006;62(6):423–9.
137. Huang YS, Su WJ, Huang YH, Chen CY, Chang FY, Lin HC, et al. Genetic polymorphisms of manganese superoxide dismutase, NAD(P)H:quinone oxidoreductase, glutathione S-transferase M1 and T1, and the susceptibility to drug-induced liver injury. J Hepatol. 2007;47(1):128–34.
138. Aithal GP, Ramsay L, Daly AK, Sonchit N, Leathart JB, Alexander G, et al. Hepatic adducts, circulating antibodies, and cytokine polymorphisms in patients with diclofenac hepatotoxicity. Hepatology. 2004;39(5):1430–40.
139. Daly AK, Donaldson PT, Bhatnagar P, Shen Y, Pe'er I, Floratos A, et al. HLA-B*5701 genotype is a major determinant of drug-induced liver injury due to flucloxacillin. Nat Genet. 2009;41(7):816–9.
140. Kindmark A, Jawaid A, Harbron CG, Barratt BJ, Bengtsson OF, Andersson TB, et al. Genome-wide pharmacogenetic investigation of a hepatic adverse event without clinical signs of immunopathology suggests an underlying immune pathogenesis. Pharmacogenomics J. 2008;8(3):186–95.
141. O'Donohue J, Oien KA, Donaldson P, Underhill J, Clare M, MacSween RN, et al. Co-amoxiclav jaundice: clinical and histological features and HLA class II association. Gut. 2000;47(5):717–20.
142. Andrade RJ, Lucena MI, Alonso A, Garcia-Cortes M, Garcia-Ruiz E, Benitez R, et al. HLA class II genotype influences the type of liver injury in drug-induced idiosyncratic liver disease. Hepatology. 2004;39(6):1603–12.
143. Ueshima Y, Tsutsumi M, Takase S, Matsuda Y, Kawahara H. Acetaminophen metabolism in patients with different cytochrome P-4502E1 genotypes. Alcohol Clin Exp Res. 1996;20(1 Suppl):25A–8.
144. Lee WM. Drug-induced hepatotoxicity. N Engl J Med. 1995;333(17):1118–27 [see comments].
145. Marez D, Legrand M, Sabbagh N, Lo Guidice JM, Spire C, Lafitte JJ, et al. Polymorphism of the cytochrome P450 CYP2D6 gene in a European population: characterization of 48 mutations and 53 alleles, their frequencies and evolution. Pharmacogenetics. 1997;7(3):193–202.
146. Murray M. Mechanisms and significance of inhibitory drug interactions involving cytochrome P450 enzymes (review). Int J Mol Med. 1999;3(3):227–38.
147. Takahara N, Nakai Y, Isayama H, Sasaki T, Satoh Y, Takai D, et al. Uridine diphosphate glucuronosyl transferase 1 family polypeptide A1 gene (UGT1A1) polymorphisms are associated with toxicity and efficacy in irinotecan monotherapy for refractory pancreatic cancer. Cancer Chemother Pharmacol. 2013;71(1):85–92.
148. Sim SC, Kacevska M, Ingelman-Sundberg M. Pharmacogenomics of drug-metabolizing enzymes: a recent update on clinical implications and endogenous effects. Pharmacogenomics J. 2013;13(1):1–11.
149. Tukey RH, Strassburg CP, Mackenzie PI. Pharmacogenomics of human UDP-glucuronosyltransferases and irinotecan toxicity. Mol Pharmacol. 2002;62(3):446–50.
150. Anderson RJ, Kudlacek PE, Clemens DL. Sulfation of minoxidil by multiple human cytosolic sulfotransferases. Chem Biol Interact. 1998;109(1–3):53–67.
151. Pauli-Magnus C, Meier PJ. Hepatobiliary transporters and drug-induced cholestasis. Hepatology. 2006;44(4):778–87.
152. Ohno M, Yamaguchi I, Yamamoto I, Fukuda T, Yokota S, Maekura R, et al. Slow N-acetyltransferase 2 genotype affects the incidence of isoniazid and rifampicin-induced hepatotoxicity. Int J Tuberc Lung Dis. 2000;4(3):256–61.
153. Cho HJ, Koh WJ, Ryu YJ, Ki CS, Nam MH, Kim JW, et al. Genetic polymorphisms of NAT2 and CYP2E1 associated with antituberculosis drug-induced hepatotoxicity in Korean patients with pulmonary tuberculosis. Tuberculosis (Edinb). 2007;87(6):551–6.
154. Possuelo LG, Castelan JA, de Brito TC, Ribeiro AW, Cafrune PI, Picon PD, et al. Association of slow N-acetyltransferase 2 profile and anti-TB drug-induced hepatotoxicity in patients from Southern Brazil. Eur J Clin Pharmacol. 2008;64(7):673–81.
155. Roy B, Chowdhury A, Kundu S, Santra A, Dey B, Chakraborty M, et al. Increased risk of antituberculosis drug-induced hepatotoxicity in individuals with glutathione S-transferase M1 'null' mutation. J Gastroenterol Hepatol. 2001;16(9):1033–7.
156. Leiro V, Fernandez-Villar A, Valverde D, Constenla L, Vazquez R, Pineiro L, et al. Influence of glutathione S-transferase M1 and T1 homozygous null mutations on the risk of antituberculosis drug-induced hepatotoxicity in a Caucasian population. Liver Int. 2008;28(6):835–9.
157. Lucena MI, Andrade RJ, Martinez C, Ulzurrun E, Garcia-Martin E, Borraz Y, et al. Glutathione S-transferase m1 and t1 null genotypes increase susceptibility to idiosyncratic drug-induced liver injury. Hepatology. 2008;48(2):588–96.
158. Daly AK, Aithal GP, Leathart JB, Swainsbury RA, Dang TS, Day CP. Genetic susceptibility to diclofenac-induced hepatotoxicity: contribution of UGT2B7, CYP2C8, and ABCC2 genotypes. Gastroenterology. 2007;132(1):272–81.
159. Choi JH, Ahn BM, Yi J, Lee JH, Lee JH, Nam SW, et al. MRP2 haplotypes confer differential susceptibility to toxic liver injury. Pharmacogenet Genomics. 2007;17(6):403–15.
160. Lang C, Meier Y, Stieger B, Beuers U, Lang T, Kerb R, et al. Mutations and polymorphisms in the

bile salt export pump and the multidrug resistance protein 3 associated with drug-induced liver injury. Pharmacogenet Genomics. 2007;17(1):47–60.
161. Zhao WH, Ma ZM, Zhou XR, Feng YZ, Fang BS. Prediction of recurrence and prognosis in patients with hepatocellular carcinoma after resection by use of CLIP score. World J Gastroenterol. 2002;8(2):237–42.
162. Kimoto E, Yoshida K, Balogh LM, Bi YA, Maeda K, El-Kattan AF, et al. Characterization of organic anion transporting polypeptide (OATP) expression and its functional contribution to the uptake of substrates in human hepatocytes. Mol Pharm. 2012; 9(12):3535–42.
163. Iusuf D, van de Steeg E, Schinkel AH. Functions of OATP1A and 1B transporters in vivo: insights from mouse models. Trends Pharmacol Sci. 2012;33(2):100–8.
164. Weisiger RA, Pond S, Bass L. Hepatic uptake of protein-bound ligands: extended sinusoidal perfusion model. Am J Physiol. 1991;261(5 Pt 1):G872–84.
165. Weisiger RA, Gollan JL, Ockner RK. The role of albumin in hepatic uptake processes. Prog Liver Dis. 1982;7:71–85.
166. Stollman YR, Gartner U, Theilmann L, Ohmi N, Wolkoff AW. Hepatic bilirubin uptake in the isolated perfused rat liver is not facilitated by albumin binding. J Clin Invest. 1983;72(2):718–23.
167. Inoue M, Hirata E, Morino Y, Nagase S, Chowdhury JR, Chowdhury NR, et al. The role of albumin in the hepatic transport of bilirubin: studies in mutant analbuminemic rats. J Biochem. 1985;97(3):737–43.
168. Ostrow JD, Murphy NH. Isolation and properties of conjugated bilirubin from bile. Biochem J. 1970;120(2):311–27.
169. Robinson SH. Formation of bilirubin from erythroid and nonerythroid sources. Semin Hematol. 1972;9(1):43–53.
170. Passamonti S, Terdoslavich M, Margon A, Cocolo A, Medic N, Micali F, et al. Uptake of bilirubin into HepG2 cells assayed by thermal lens spectroscopy. Function of bilitranslocase. FEBS J. 2005;272(21):5522–35.
171. Radominska-Pandya A, Czernik PJ, Little JM, Battaglia E, Mackenzie PI. Structural and functional studies of UDP-glucuronosyltransferases. Drug Metab Rev. 1999;31(4):817–99.
172. Tukey RH, Strassburg CP. Human UDP-glucuronosyltransferases: metabolism, expression, and disease. Annu Rev Pharmacol Toxicol. 2000;40:581–616.
173. Mackenzie PI, Owens IS, Burchell B, Bock KW, Bairoch A, Belanger A, et al. The UDP glycosyltransferase gene superfamily: recommended nomenclature update based on evolutionary divergence. Pharmacogenetics. 1997;7(4):255–69.
174. Turgeon D, Carrier JS, Levesque E, Beatty BG, Belanger A, Hum DW. Isolation and characterization of the human UGT2B15 gene, localized within a cluster of UGT2B genes and pseudogenes on chromosome 4. J Mol Biol. 2000;295(3):489–504.
175. Strassburg CP, Manns MP. Jaundice, genes and promoters. J Hepatol. 2000;33(3):476–9.
176. Gong QH, Cho JW, Huang T, Potter C, Gholami N, Basu NK, et al. Thirteen UDPglucuronosyltransferase genes are encoded at the human UGT1 gene complex locus. Pharmacogenetics. 2001;11(4):357–68.
177. Ohno S, Nakajin S. Determination of mRNA expression of human UDP-glucuronosyltransferases and application for localization in various human tissues by real-time reverse transcriptase-polymerase chain reaction. Drug Metab Dispos. 2009;37(1):32–40.
178. Crigler Jr JF, Najjar VA. Congenital familial nonhemolytic jaundice with kernicterus. Pediatrics. 1952;10(2):169–80.
179. Keppler D. Cholestasis and the role of basolateral efflux pumps. Z Gastroenterol. 2011;49(12):1553–7.
180. Jansen PL, Muller M. The molecular genetics of familial intrahepatic cholestasis. Gut. 2000;47(1):1–5.
181. Slot AJ, Molinski SV, Cole SP. Mammalian multidrug-resistance proteins (MRPs). Essays Biochem. 2011;50(1):179–207.
182. Vlahcevic ZR, Heuman DM, Hylemon PB. Regulation of bile acid synthesis. Hepatology. 1991;13(3):590–600.
183. Hofmann AF. Bile acids. In: Arias IM, Boyer JL, Chisari FV, Fausto N, Schachter D, Shafritz DA, editors. The liver – biology an pathobiology. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 361–72.
184. Russell DW. The enzymes, regulation, and genetics of bile acid synthesis. Annu Rev Biochem. 2003;72:137–74.
185. Vlahcevic ZR, Pandak WM, Stravitz RT. Regulation of bile acid biosynthesis. Gastroenterol Clin North Am. 1999;28(1):1–25, v.
186. Hofmann AF. Bile acids: trying to understand their chemistry and biology with the hope of helping patients. Hepatology. 2009;49(5):1403–18.
187. Kamp F, Hamilton JA, Kamp F, Westerhoff HV, Hamilton JA. Movement of fatty acids, fatty acid analogues, and bile acids across phospholipid bilayers. Biochemistry. 1993;32(41):11074–86.
188. Nathanson MH, Boyer JL. Mechanisms and regulation of bile secretion. Hepatology. 1991;14(3):551–66.
189. Dawson PA, Lan T, Rao A. Bile acid transporters. J Lipid Res. 2009;50(12):2340–57.
190. Ung KA, Olofsson G, Fae A, Kilander A, Ohlsson C, Jonsson O. In vitro determination of active bile acid absorption in small biopsy specimens obtained endoscopically or surgically from the human intestine. Eur J Clin Invest. 2002;32(2):115–21.
191. Weinberg SL, Burckhardt G, Wilson FA. Taurocholate transport by rat intestinal basolateral membrane vesicles. Evidence for the presence of an anion exchange transport system. J Clin Invest. 1986;78(1):44–50.
192. Caflisch C, Zimmerli B, Reichen J, Meier PJ. Cholate uptake in basolateral rat liver plasma membrane vesicles and in liposomes. Biochim Biophys Acta. 1990;1021(1):70–6.

193. Svoboda M, Riha J, Wlcek K, Jaeger W, Thalhammer T. Organic anion transporting polypeptides (OATPs): regulation of expression and function. Curr Drug Metab. 2011;12(2):139–53.
194. Carey MC, Duane WC. Enterohepatic circulation. In: Arias IM, Boyer JL, Chisari FV, Fausto N, Schachter D, Shafritz DA, editors. The liver – biology and pathobiology. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 361–72.
195. Hedenborg G, Norman A, Ritzen A. Lipoprotein-bound bile acids in serum from healthy men, postprandially and during fasting. Scand J Clin Lab Invest. 1988;48(3):241–5.
196. Kubitz R, Droge C, Stindt J, Weissenberger K, Haussinger D. The bile salt export pump (BSEP) in health and disease. Clin Res Hepatol Gastroenterol. 2012;36(6):536–53.
197. Jansen PL, Sturm E. Genetic cholestasis, causes and consequences for hepatobiliary transport. Liver Int. 2003;23(5):315–22.
198. Weinman SA, Graf J, Boyer JL. Voltage-driven, taurocholate-dependent secretion in isolated hepatocyte couplets. Am J Physiol. 1989;256(5 Pt 1):G826–32.
199. Poupon R, Poupon R, Dumont M, Erlinger S. Hepatic storage and biliary transport maximum of taurocholate and taurochenodeoxycholate in the dog. Eur J Clin Invest. 1976;6(6):431–7.
200. Stapelbroek JM, van Erpecum KJ, Klomp LW, Houwen RH. Liver disease associated with canalicular transport defects: current and future therapies. J Hepatol. 2010;52(2):258–71.
201. Keppler D. Multidrug resistance proteins (MRPs, ABCCs): importance for pathophysiology and drug therapy. Handb Exp Pharmacol. 2011;201:299–323.
202. He SM, Li R, Kanwar JR, Zhou SF. Structural and functional properties of human multidrug resistance protein 1 (MRP1/ABCC1). Curr Med Chem. 2011;18(3):439–81.
203. Franke RM, Gardner ER, Sparreboom A. Pharmacogenetics of drug transporters. Curr Pharm Des. 2010;16(2):220–30.
204. Oude Elferink RP, Paulusma CC. Function and pathophysiological importance of ABCB4 (MDR3 P-glycoprotein). Pflugers Arch. 2007;453(5):601–10.
205. Schmitz G, Langmann T. Structure, function and regulation of the ABC1 gene product. Curr Opin Lipidol. 2001;12(2):129–40.
206. Borst P, Zelcer N, van Helvoort A. ABC transporters in lipid transport. Biochim Biophys Acta. 2000;1486(1):128–44.
207. Tavoloni N. The intrahepatic biliary epithelium: an area of growing interest in hepatology. Semin Liver Dis. 1987;7(4):280–92.
208. Alpini G, Phillips JO, LaRusso NF. The biology of biliary epithelia. In: Arias IM, Boyer JL, Chisari FV, Fausto N, Schachter D, Shafritz DA, editors. The liver – biology and pathobiology. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 421–36.
209. Strazzabosco M, Fabris L, Spirli C. Pathophysiology of cholangiopathies. J Clin Gastroenterol. 2005;39(4 Suppl 2):S90–102.
210. Strazzabosco M, Fabris L. Functional anatomy of normal bile ducts. Anat Rec (Hoboken). 2008;291(6):653–60.
211. Alpini G, McGill JM, LaRusso NF. The pathobiology of biliary epithelia. Hepatology. 2002;35(5):1256–68.
212. Banales JM, Prieto J, Medina JF. Cholangiocyte anion exchange and biliary bicarbonate excretion. World J Gastroenterol. 2006;12(22):3496–511.
213. LaRusso NF, Masyuk TV. The role of cilia in the regulation of bile flow. Dig Dis. 2011;29(1):6–12.
214. LaRusso NF. Morphology, physiology, and biochemistry of biliary epithelia. Toxicol Pathol. 1996;24(1):84–9.
215. Ishii M, Vroman B, LaRusso NF. Isolation and morphologic characterization of bile duct epithelial cells from normal rat liver. Gastroenterology. 1989;97(5):1236–47.
216. LaRusso NF, Ishii M, Vroman BT. The ins and outs of membrane movement in biliary epithelia. Trans Am Clin Climatol Assoc. 1991;102:245–58.
217. Alvaro D, Alpini G, Jezequel AM, Bassotti C, Francia C, Fraioli F, et al. Role and mechanisms of action of acetylcholine in the regulation of rat cholangiocyte secretory functions. J Clin Invest. 1997;100(6):1349–62.
218. McGill JM, Basavappa S, Gettys TW, Fitz JG. Secretin activates Cl– channels in bile duct epithelial cells through a cAMP-dependent mechanism. Am J Physiol. 1994;266(4 Pt 1):G731–6.
219. Tietz PS, Alpini G, Pham LD, LaRusso NF. Somatostatin inhibits secretin-induced ductal hypercholeresis and exocytosis by cholangiocytes. Am J Physiol. 1995;269(1 Pt 1):G110–8.
220. Kato A, Gores GJ, LaRusso NF. Secretin stimulates exocytosis in isolated bile duct epithelial cells by a cyclic AMP-mediated mechanism. J Biol Chem. 1992;267(22):15523–9.
221. Schrenk D, Eisenmann-Tappe I, Gebhardt R, Mayer D, el Mouelhi M, Rohrdanz E, et al. Drug metabolizing enzyme activities in rat liver epithelial cell lines, hepatocytes and bile duct cells. Biochem Pharmacol. 1991;41(11):1751–7.
222. Marzioni M, Fava G, Alvaro D, Alpini G, Benedetti A. Control of cholangiocyte adaptive responses by visceral hormones and neuropeptides. Clin Rev Allergy Immunol. 2009;36(1):13–22.
223. Alvaro D, Cho WK, Mennone A, Boyer JL. Effect of secretion on intracellular pH regulation in isolated rat bile duct epithelial cells. J Clin Invest. 1993;92(3):1314–25.
224. Naito M, Hasegawa G, Ebe Y, Yamamoto T. Differentiation and function of Kupffer cells. Med Electron Microsc. 2004;37(1):16–28.
225. Naito M. Macrophage differentiation and function in health and disease. Pathol Int. 2008;58(3):143–55.
226. Van Bossuyt H, Desmaretz C, Wisse E. The fate of lipopolysaccharide in cultured rat Kupffer cells.

Virchows Arch B Cell Pathol Incl Mol Pathol. 1989;58(1):89–93.
227. Seki S, Habu Y, Kawamura T, Takeda K, Dobashi H, Ohkawa T, et al. The liver as a crucial organ in the first line of host defense: the roles of Kupffer cells, natural killer (NK) cells and NK1.1 Ag+ T cells in T helper 1 immune responses. Immunol Rev. 2000;174:35–46.
228. Thomas HC, McSween RN, White RG. Role of the liver in controlling the immunogenicity of commensal bacteria in the gut. Lancet. 1973;1(7815):1288–91.
229. Wake K, Decker K, Kirn A, Knook DL, McCuskey RS, Bouwens L, et al. Cell biology and kinetics of Kupffer cells in the liver. Int Rev Cytol. 1989;118:173–229.
230. Arthur MJ, Kowalski-Saunders P, Wright R. Effect of endotoxin on release of reactive oxygen intermediates by rat hepatic macrophages. Gastroenterology. 1988;95(6):1588–94.
231. Rogoff TM, Lipsky PE. Role of the Kupffer cells in local and systemic immune responses. Gastroenterology. 1981;80(4):854–60.
232. Arthur MJ. Reactive oxygen intermediates and liver injury. J Hepatol. 1988;6(1):125–31.
233. Thurman II RG. Alcoholic liver injury involves activation of Kupffer cells by endotoxin. Am J Physiol. 1998;275(4 Pt 1):G605–11.
234. Brouwer A, Hendriks HF, Knook DL. The role of eicosanoids in the acute phase response. J Hepatol. 1990;11(3):283–6.
235. You Q, Cheng L, Kedl RM, Ju C. Mechanism of T cell tolerance induction by murine hepatic Kupffer cells. Hepatology. 2008;48(3):978–90.
236. Maemura K, Zheng Q, Wada T, Ozaki M, Takao S, Aikou T, et al. Reactive oxygen species are essential mediators in antigen presentation by Kupffer cells. Immunol Cell Biol. 2005;83(4):336–43.
237. Rubinstein D, Roska AK, Lipsky PE. Liver sinusoidal lining cells express class II major histocompatibility antigens but are poor stimulators of fresh allogeneic T lymphocytes. J Immunol. 1986;137(6):1803–10.
238. Doherty DG, O'Farrelly C. Innate and adaptive lymphoid cells in the human liver. Immunol Rev. 2000;174:5–20.
239. Tu Z, Bozorgzadeh A, Crispe IN, Orloff MS. The activation state of human intrahepatic lymphocytes. Clin Exp Immunol. 2007;149(1):186–93.
240. Trinchieri G. Biology of natural killer cells. Adv Immunol. 1989;47:187–376.
241. Shi FD, Ljunggren HG, La CA, Van KL. Organ-specific features of natural killer cells. Nat Rev Immunol. 2011;11(10):658–71.
242. Wisse E, Luo D, Vermijlen D, Kanellopoulou C, De ZR, Braet F. On the function of pit cells, the liver-specific natural killer cells. Semin Liver Dis. 1997;17(4):265–86.
243. Mehal W, Imaeda A. Cell death and fibrogenesis. Semin Liver Dis. 2010;30(3):226–31.

Laboratory Assessment of Hepatic Injury and Function

3

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The approach to the child with liver disease should be based on an accurate clinical history and a thorough physical examination. Investigating the liver relies on a multidisciplinary approach involving clinical chemistry, hematology, immunology, imaging studies, endoscopy, histopathology, and microbiology. Mutational analyses for many genetic liver diseases are now available. This chapter will outline the basic laboratory assessment of the liver and main disease categories and summarize specialized laboratory investigations which identify the underlying diagnosis.

Baseline Investigations: Biochemical Liver Function Tests

The main functions of the liver include synthesis (albumin, coagulation factors, bile acids), metabolism (carbohydrate, lipid, protein), degradation/detoxification, and excretion (Table 3.1). Biochemical liver function tests (Table 3.2) reflect the severity of hepatic dysfunction but rarely provide diagnostic information on individual diseases.

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Bilirubin: Conjugated bilirubin is nearly always elevated in liver disease [1]. The presence of bilirubin is always abnormal if detected in a fresh urine specimen.

Aminotransferases are intracellular enzymes, which are present in liver, heart, and skeletal muscles. Increases in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) indicate hepatic necrosis irrespective of etiology (Table 3.2). ALT is more liver specific than AST but has a longer plasma half-life (approximately 24 h). A rise in AST is an early indication of liver damage and is a useful marker of rejection post-liver transplant. Elevated aminotransferases are often the first indication of the development of nonalcoholic fatty liver disease (NAFLD) in an obese child. Elevated aspartate and/or alanine aminotransferases are also found in muscular dystrophy and this diagnosis should be considered if there are no other signs of liver disease. These enzymes, however, may be normal in compensated cirrhosis.

Alkaline phosphatase is found in the liver, kidney, bone, placenta, and intestine. In pediatric liver disease, a raised alkaline phosphatase indicates biliary epithelial damage, malignant infiltration, cirrhosis, rejection, or osteopenia secondary to vitamin D deficiency. In a growing child, however, the potential contribution from bone makes alkaline phosphatase measurement less specific for liver pathology.

Gamma-glutamyl transpeptidase (*GGT*) is present in biliary epithelia and hepatocytes and also in the cell membrane of many other human

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Table 3.1 Functions of the liver

Function	Effect of dysfunction	Assessment
Metabolism/storage		
Carbohydrate/glycogen	Loss of glucose homeostasis	Hypoglycemia in fasting/stress
Lipid	Lipid accumulation in hepatocytes	High/low cholesterol
	↓Oxidation of fatty acids	↑Lactate, ↑ratio FFA:BOH
		↑Acylcarnitine, organic aciduria
Protein	↑Catabolism	Low BCAA, urea
		↑Ammonia, ↑tyrosine, phenylalanine, methionine
Synthesis		
Albumin	Loss of muscle mass	Low albumin
	protein energy malnutrition	
Factors II, VII, IX, X	Coagulopathy	Prolonged PT/PTT
Degradation		
Drugs	Prolonged drug effect, e.g., sedation	Clinical
Estrogens	Telangiectasia, gynecomastia	Clinical
Toxic products	Encephalopathy	Abnormal EEG/clinical signs
Bile synthesis and excretion	Cholestasis	↑Conjugated bilirubin
	Fat malabsorption	↑GGT ↑ALP ↑cholesterol
	Fat-soluble vitamin deficiency	Anthropometry
	Pruritus, malnutrition	

ALP alkaline phosphatase, *BOH* β-hydroxybutyrate, *BCAA* branched-chain amino acids, *EEG* electroencephalogram, *FFA* free fatty acids, *GGT* gamma-glutamyl transpeptidase, *PT* prothrombin time, *PTT* partial thromboplastin time

Table 3.2 Baseline and initial investigations for the evaluation of liver function

Reference range of test	Liver-associated abnormality
Conjugated bilirubin <20 μmol/l	Elevated: hepatocyte dysfunction or biliary obstruction
Aminotransferases Aspartate (AST) <50 U/l Alanine (ALT) <40 U/l	Elevated: hepatocyte inflammation/damage
Alkaline phosphatase (ALP) <600 U/l (age dependent)	Elevated: biliary inflammation/obstruction, nonspecific
Gamma-glutamyl transpeptidase (GGT) <30 U/l (age dependent)	Elevated: biliary inflammation/obstruction, nonspecific
Albumin 35–50 g/l	Reduced: chronic liver disease
Prothrombin time (PT) 12–15 s	Prolonged: (i) hepatocellular dysfunction, (ii) vitamin K deficiency
Partial thromboplastin time (PTT) 33–37 s	
Ammonia <50 mmol/l	Elevated: abnormal protein catabolism/urea cycle defect/other inherited metabolic disease
Glucose >4 mmol/l	Reduced in: acute or chronic liver failure/metabolic disease/ hypopituitarism

organs, including kidney, pancreas, spleen, brain, breast, and small intestine. An elevated GGT is not specific for liver disease. In addition, the reference range is age related, with higher levels in neonates (up to 385 IU/l). It is elevated in many forms of liver damage. However, GGT does not increase in the serum of patients with bone disease or children with active bone growth, thus helpful in confirming the liver origin of a raised alkaline phosphatase. It may be normal in certain forms of intrahepatic cholestasis (progressive familial intrahepatic cholestasis 1 and 2; PFIC 1 & 2) [2].

Table 3.3 Laboratory assessment in chronic liver disease

Second-line investigations
Bacterial culture of blood, urine, +/– cerebrospinal fluid
Serology for hepatitis A, B, C, E
α1-Antitrypsin level and phenotype
Abdominal ultrasound
Metabolic investigations
Immunoreactive trypsin
Plasma lactate, BOH, FFA, ammonia
Acylcarnitine
Serum iron and ferritin
Plasma amino acids
Cholesterol, triglyceride
α-Fetoprotein
Parathyroid hormone, wrist X-ray for bone age/rickets
Urine: reducing sugars, organic acids, amino acids, succinylacetone, bile salts

BOH β-hydroxybutyrate, *FFA* free fatty acids

Table 3.4 Age-specific investigations in chronic liver disease

Neonate	TORCHES screen
	Galactose 1-phosphate uridyltransferase
	Free T4, TSH, AM cortisol
	Targeted DNA mutational analysis
	Sweat test (>4 weeks)
Older child (>2 years)	Cu, ceruloplasmin, urinary Cu
	C3, C4, ANA, SMA, LKM, immunoglobulins
	EBV
If indicated	Liver biopsy for: histology, electron microscopy, enzyme analysis, immunohistochemistry, culture, copper concentration
	Skin biopsy, ophthalmology, cardiology, bone marrow aspirate
	Endoscopy, ERCP

ANA antinuclear antibodies, *C3*, *C4* complement components 3 and 4, *Cu* copper, *DNA* deoxyribonucleic acid, *EBV* Epstein–Barr virus, *ERCP* endoscopic retrograde cholangiopancreatography, *LKM* liver–kidney microsomal antibodies, *SMA* smooth muscle antibodies, *T4* thyroxine, *TORCHES* toxoplasmosis, rubella, cytomegalovirus, herpes simplex, syphilis, *TSH* thyroid-stimulating hormone

The most useful tests of liver "function" are *plasma albumin* concentration and *coagulation time*. In the absence of excessive urinary or gastrointestinal loss or prolonged starvation, a low serum albumin, which has a half-life of 20 days, indicates chronicity of liver disease. Abnormal coagulation, especially prothrombin time (PT) after vitamin K deficiency is ruled out, indicates significant hepatic dysfunction, either acute or chronic. *Fasting hypoglycemia* in the absence of other causes (e.g., hypopituitarism or hyperinsulinism) indicates poor hepatic function and is a guide to prognosis in acute liver failure. If these baseline investigations suggest hepatic dysfunction, then more specific investigations for metabolic disease are appropriate to consider [3–5] (Table 3.3).

Second-Line Investigations

Hepatic dysfunction may be secondary to sepsis, particularly urinary sepsis, inborn errors of metabolism, or endocrine disorders. It is usual to exclude sepsis by performing bacterial culture of the urine and/or blood and cerebrospinal fluid cultures if appropriate (Tables 3.3 and 3.4).

In neonates, hypopituitarism may be difficult to exclude as thyroid function tests may be equivocal or in the low normal range. It is useful to perform a 09.00 h cortisol level at the same time as measuring free thyroxine and thyroid-stimulating hormone (TSH) [6].

If the infant is unwell, or has evidence of acute liver failure, galactosemia and tyrosinemia should be excluded (see below). Urea cycle defects should also be considered particularly if the serum ammonia is raised.

Alpha-1-antitrypsin deficiency is the most common inherited metabolic liver disease and should always be excluded, regardless of age. As α-1-antitrypsin is an acute-phase protein, it is necessary to measure both concentration and phenotype in order to differentiate between normal and an acute-phase response in the setting of homozygous or heterozygous deficiency.

Although cystic fibrosis is a rare cause of liver disease in the neonatal period, it should be considered in the differential diagnosis of neonatal liver disease and excluded by performing an immunoreactive trypsin test, a sweat test, and mutational analysis if either is positive.

Wilson disease rarely presents before the age of 3 years but may mimic any form of liver disease and should always be excluded in older children [7]. An autoimmune screen and immunoglobulin levels should detect 75 % of children with autoimmune hepatitis.

Serum cholesterol is usually elevated in children with severe cholestasis (e.g., Alagille syndrome, biliary atresia). In contrast, low or normal cholesterol is characteristic of bile acid transport disorders or terminal liver disease.

Plasma ammonia and amino acids (particularly phenylalanine, tyrosine, and methionine) may be raised in either acute or chronic liver failure and are nonspecific indications of hepatic dysfunction. Primitive hepatic cells synthesize α-fetoprotein. The levels are highest in the newborn (>1,000 mg/l) and fall in the first few months of life. It may be a useful screening test in the diagnosis of tyrosinemia type I and hepatoblastoma or for detection of hepatocellular carcinoma in chronic carriers of hepatitis B and C. The α-fetoprotein level can be as high as 100,000 mg/l in hepatoblastoma [8].

Neonatal Liver Disease

Most infants with liver disease present in the neonatal period with persistent jaundice. Although physiologic jaundice is common in neonates, infants who develop severe or persistent jaundice should be investigated to exclude hemolysis, sepsis, or underlying liver disease. Neonatal jaundice that persists beyond 14 days in term infants and 21 days in preterm infants should always be investigated, even in breast-fed babies [1]. It is also necessary to establish whether the jaundice is due to an increase in conjugated or unconjugated hyperbilirubinemia.

Unconjugated hyperbilirubinemia: Common causes include ABO and rhesus incompatibilities, breast-milk jaundice, sepsis, Gilbert syndrome, and rarely, Crigler–Najjar type I or II (Table 3.5).

Table 3.5 Common and uncommon causes of unconjugated hyperbilirubinemia in infancy

Conditions	Frequency	Features/comments
Physiologic jaundice	Very common	Usually benign, ~ 8–20 % of infants with physiologic jaundice may have serum bilirubin >200 μmol/l
Hemolytic jaundice	Common	Causes include ABO and Rh incompatibilities, glucose-6-phosphate dehydrogenase deficiency, red cell membrane defects
Breast-milk jaundice	Common	May overlap with physiologic jaundice and may last till 1–2 months
Sepsis	Common	Sick infants; blood, urine, cerebrospinal fluid cultures, chest X-ray
Hypothyroidism	Common	Thyroid function tests show high thyroid-stimulating hormone and low T4
Gilbert syndrome	Common	Important cause of unconjugated hyperbilirubinemia of unknown cause, benign, polymorphism of the 5′ end of the promoter of the UGT1A1 gene homozygous insertion of the TA pair – genotype UGT1A1*28/*28
Crigler–Najjar syndrome type 1	Rare	Autosomal recessive, mutations in the UGT1A1 gene resulting in either truncated nonfunctional enzyme or nonrecognition of the substrate Bilirubin; rapid rise in conjugated bilirubin early in life may lead to kernicterus
Crigler–Najjar syndrome type 2	Rare	Autosomal recessive, mutations have been reported in exon 1A1 of the UGT1A1 gene, clinically less severe than type 1 disease and responsive to phenobarbital therapy

Table 3.6 Laboratory assessment of the cholestatic infant

Blood	
Liver function tests	Baseline liver investigation (see Tables 3.2 and 3.3)
Electrolytes	Sodium, potassium, urea, creatinine, calcium, phosphate, bicarbonate, glucose
Hematology	Full blood count with differential and reticulocyte count; clotting screen including fibrinogen; blood group and Coombs test
Serology	TORCHES, adenovirus antibody, consider herpes simplex PCR, hepatitis B serology
Metabolic	Fasting bloods for cortisol, glucose, lactate, β-hydroxybutyrate, free fatty acids and amino acids; cholesterol and triglycerides (see also Table 3.11)
Specific metabolic tests	Galactosemia and tyrosinemia screen; thyroid function tests, parathyroid hormone; chitotriosidase; immunoreactive trypsin; α-1-antitrypsin level and phenotype
Vitamin levels	Vitamins A, D, and E
Urine	
General	Urine pH, glucose, ketones, and protein. Protein-to-creatinine ratio if protein is present, culture
Clinical chemistry	Amino acids; reducing substances; bile acids (if possible); tubular reabsorption of phosphate
Virology	CMV culture
Microbiology	Urine microscopy and culture

Note: see also Tables 3.2, 3.3, and 3.11

Conjugated hyperbilirubinemia: A rise in conjugated bilirubin always signifies an underlying liver condition and warrants further assessment (Table 3.6) [1]. It is important to exclude surgical disorders such as biliary atresia in infants with neonatal cholestasis as early surgery is associated with a better outcome (Table 3.7) [9, 10]. Similarly, bacterial infections and metabolic conditions have improved outcomes with early identification and treatment and hence warrant rapid investigation. Although not usually posing a diagnostic dilemma, the successful management of preterm infants as young as 25 weeks' gestation has increased the number of children treated with parenteral nutrition (PN) and a consequential rise in referrals of these infants with persistent jaundice [11]. Other conditions to be considered that can present as neonatal cholestasis are listed in Table 3.8 [1, 12].

Table 3.7 Assessments of infants (2 weeks–6 weeks of age) suspected of biliary atresia

Assessment	Remarks/results
General	Generally well and thriving
Stool color	Progresses to persistently pale (Fig. 3.1)
Liver	Enlarged and usually firm in consistency
Spleen	May be enlarged
Ascites	Rare
Clinical biochemistry	Conjugated hyperbilirubinemia; raised ALT, AST, ALP, and GGT
Abdominal ultrasound	Absent or contracted gall bladder common; presence of triangular cord (a fibrous cone of tissue at the bifurcation of the portal vein); exclude choledochal cyst
Hepatobiliary scintigraphy	Absence of biliary excretion of radioisotope
Histopathology	Preservation of overall hepatic architecture, prominent bile ductular proliferation, canalicular and cellular bile stasis, portal fibrosis

Liver Disease in Older Children

Liver disease in children older than 6 months may be acute or chronic. As in infancy, inherited disorders need to be excluded (Table 3.9), but jaundice may not be a prominent feature. Acute or chronic liver disease may be due to infection, autoimmune disease, drug-induced hepatitis, and metabolic diseases (Table 3.9).

Acute Liver Disease

Underlying causes and clinical presentation depends on the age, but the following clinical features are common: a prodrome of malaise, lethargy, and anorexia, and nausea, vomiting, or diarrhea. There may be weight loss, abdominal discomfort, tender hepatomegaly, splenomegaly, ascites (rarely, except for acute Budd–Chiari), rash, or joint pains. It is noteworthy that jaundice is not always present.

Fig. 3.1 A typical appearance of pale stool

Table 3.8 Differential diagnosis of infantile conjugated hyperbilirubinemia when biliary atresia has been excluded

Categories	Differential diagnosis	Investigations	Results
Infective	Congenital infection Bacteremia, urinary tract infection	Serology for toxoplasma, rubella, CMV buffy coat, herpes simplex, syphilis, PCR urine microscopy, urine and blood culture	Positive testing
Endocrine	Hypothyroidism	TFTs	Raised TSH, low T4
	Hypopituitarism	TFTs, cortisol, glucose	Low TSH, cortisol, hypoglycemia
Metabolic (see also Table 3.11)	Galactosemia	Urine reducing substances Plasma Gal-1-PUT	Positive reducing substances if on a galactose-containing diet Absent or reduced Gal-1-PUT detected
	Tyrosinemia	Urine succinyl acetone, DNA	High succinylacetone Mutations in *FAH*
	Storage disease, e.g., Niemann Pick C	Liver biopsy, bone marrow biopsy Filipin staining DNA	Storage cells on bone marrow and liver biopsy (can be difficult to see in young children), positive Filipin staining of fibroblasts Mutation in *NPC1 and 2*
	Bile salt synthesis disorders	Urinary bile salts (not accurate if on ursodeoxycholic acid), DNA	Abnormal peaks on urine mass spectroscopy Mutation in *AKR1D1*
	Peroxisomal disorders	Plasma very long-chain fatty acids DNA	High levels of very long-chain fatty acids Mutation in *PEX* genes
Genetic	α-1-Antitrypsin deficiency	α-1-Antitrypsin level and phenotype	Low α-1-antitrypsin level and PiZZ or ZS phenotype
	Arthrogryposis, renal dysfunction, cholestasis (ARC) syndrome	GGT DNA	Low GGT cholestasis Mutation in *VPS33B* or *VIPAR*
	Citrin deficiency	Plasma and urine amino acids DNA	Increased plasma and urine citrulline and arginine Mutation in *SLC25A13*
Toxic	Intestinal failure-associated liver disease	Liver biopsy	Cholestasis with hepatocellular necrosis, abundant lipofuscin, fatty infiltration, mild giant cell transformation, portal tract infiltration, bile duct reaction, ±portal fibrosis

Note: *GGT* gamma-glutamyl transpeptidase, *TFTs* thyroid function tests, *TSH* thyroid-stimulating hormone

Table 3.9 Causes of acute liver disease and failure in older children

Disease	Investigations	Comments
Infections		
Viruses:		
Hepatitis A	Anti-HAV IgM	
Hepatitis B	HBsAg, anti-HBc Ab	
Hepatitis C	Anti-hepatitis C Ab, HCV PCR	
Herpes viruses	Antibody	
Epstein–Barr virus	Antibody	
Bacterial	Leptospiral antibody	If clinically indicated
Drugs		
Acetaminophen overdose	Acetaminophen level	Level to be compared to the nomogram by Rumack and Matthews
Metabolic		
Wilson disease	Serum copper, ceruloplasmin 24-h urine copper	With penicillamine challenge when indicated
Others	Serum amino acid	See also Table 3.11
	Urine organic acid	
	Urine reducing sugars	
Autoimmune hepatitis	Autoantibodies	
	Antinuclear antibodies	Type 1 AIH anti-ANA and anti-SM are positive
	Anti-smooth muscle	
	Anti-liver–kidney microsomal antibody	Type 2 AIH
	Immunoglobulins	Raised IgG in both forms
	Liver biopsy	Interface hepatitis, bridging fibrosis, dense mononuclear and plasma cells infiltrate, hepatic regeneration with rosette formation

The differential diagnosis of acute hepatitis in older children includes (Table 3.9) [13, 14]: viral hepatitis A, B, C, and E, sero-negative hepatitis, autoimmune hepatitis, drug-induced hepatotoxicity, and metabolic liver disease especially Wilson disease.

Important causes of neonatal liver failure include viral infections, metabolic liver disease, and ischemic causes (Table 3.10) [15].

Chronic Liver Disease

- Chronic liver disease is frequently asymptomatic but detected through other analyses, such as incidental detection of abnormal liver enzymes or hepatomegaly
- Family screening for hepatitis B/C or metabolic disorders (Wilson disease)
- Transfusion recipient following diagnosis of donor infection
- Coexistent disease, e.g., inflammatory bowel disease and celiac disease
- Recipient of a known toxic agent, e.g., methotrexate

When symptomatic children may present with:

- Intermittent fatigue, anorexia, and weight loss
- Abdominal discomfort
- Variable or fluctuating jaundice with pruritus and pale stools
- Hematemesis or melena from variceal bleeding – especially with portal hypertension

Liver Biopsy and Histopathology

The diagnosis of most chronic liver diseases requires histological confirmation [16]. An aspiration technique, using a Menghini needle (or

Table 3.10 Causes of neonatal acute liver failure

Diseases	Investigations
Infections	
Herpes viruses	Tissue culture
	Direct immunofluorescence of swabs or tissue
	Molecular technique
	(Note: serology is of no value in perinatal infection due to presence of maternal IgG to herpes simplex virus)
Adenovirus	Immunoassay or PCR to detect virus in stool, blood, or liver tissue
Echovirus	Tissue culture
	Direct immunofluorescence of swabs or tissue
Hepatitis B	Hepatitis B surface antigen, anti-HBc antibody
Metabolic (*see also* Table 3.11)	
Galactosemia	Galatose-1-phosphate uridyltransferase
Tyrosinemia	Serum tyrosine, methionine, α-fetoprotein, urine succinylacetone
Gestational alloimmune liver disease (neonatal Hemochromatosis)	Serum ferritin, extrahepatic siderosis (magnetic resonance imaging, or tissue biopsy showing hemosiderosis; oral or buccal mucosa); liver biopsy: immunostaining of hepatocytes for the C5b-9 complex
Mitochondrial	See Table 3.11
Other metabolic conditions	See Table 3.11
Ischemic	
Congenital heart disease	Chest x-ray, ECG, and echocardiogram; cardiac enzymes (if myocarditis is suspected)

disposable variant), has a complication risk of 1:1,000 liver biopsies and may be performed under sedation with local anesthesia. In fibrotic or cirrhotic livers, the use of a Tru-Cut needle, with a cutting-edge beveled end, may be necessary. Transjugular liver biopsies, in which the liver is biopsied through a special catheter passed from the internal jugular vein into the hepatic veins, are now possible for children as small as 6 kg and are a safer way to perform a biopsy if coagulation times remain abnormal despite support (prothrombin time [PT] >5 s prolonged over control value) or for those with large ascites [17]. The complications of this potentially dangerous procedure (see below) are much reduced if performed in expert hands, in specialized units, under controlled conditions [18]. It is essential to be aware of the absolute and relative contraindications of liver biopsy. Biopsy specimens should be obtained for routine histopathology and can be analyzed for microbiology, electron microscopy, immunohistochemistry, and copper (if appropriate) and snap frozen in liquid nitrogen for enzymatic or metabolic investigations. As the interpretation of the histology may be difficult and requires considerable specialist expertise, and tissue preparation for other analyses requires special handling, coordination with the liver pathologist before tissue acquisition is advisable.

In experienced facilities and with careful patient selection, it is possible to carry out a liver biopsy as a day procedure [19, 20]

Complications of Percutaneous Liver Biopsy

Although uncommon, the main complication of percutaneous liver biopsies is bleeding. Subclinical bleeding (as evident on ultrasound imaging) is common and intrahepatic and subcapsular hematomas with no hemodynamic compromise are seen in up to 23 % of patients [21]. Significant nonfatal bleeding (as seen with evidence of active bleeding, shock, or a hemoglobin drop of 2.0 g/l) occurs more frequently in children than adults. In adults significant hemorrhage occurs in 0.3–0.5 % of cases, while bleeding requiring transfusion is seen in up to 2.8 % of children [22]. Evidence of persistent bleeding following liver biopsy despite medical support and blood transfusion warrants urgent hepatic angiography and embolization or surgery.

Other complications include:

- Pneumothorax or hemopneumothorax
- Infection (particularly if the biopsy is combined with another procedure, e.g., dental extraction)
- Perforation of the gall bladder or bile ducts leading to biliary peritonitis

Adequate monitoring of vital signs post biopsy is essential to detect complications such as hemorrhage or infection [22].

Metabolic Investigations (Table 3.11)

Many inborn errors of metabolism present with hepatomegaly and/or liver disease. It is essential to screen for these diseases as part of the investigation of liver disease in neonates and in older children

Bone Marrow Aspiration

Bone marrow aspiration may be useful in infants with undiagnosed neonatal hepatitis and hepatomegaly and splenomegaly, in order to exclude Niemann–Pick type C, or at any age if a storage disorder is suspected and genetic testing unavailable.

Skin Biopsy with Fibroblast Culture

This procedure can be useful in diagnosing inborn errors of metabolism (e.g., Niemann–Pick type A, B, or C or tyrosinemia type I) when genetic testing is not available (Table 3.11).

Genetic Tests (Chromosome and DNA)

With the rapid development of molecular techniques for diagnosis and detection of genetic diseases, samples for DNA analysis and/or chromosomes from both child and parent are essential and now possible for many genetic conditions affecting the liver.

Neurophysiology

Electroencephalography (EEG) is mostly used in the assessment of hepatic encephalopathy. It will identify abnormal rhythms secondary to encephalopathy due to either acute or chronic liver failure or drug toxicity such as posttransplant immunosuppression, but findings are frequently nonspecific. EEG may also be of value in verifying brain death as a flat EEG in the absence of sedation is an indication for withdrawal of therapy.

Ophthalmology (Table 3.12)

A number of inherited conditions have associated ophthalmic lesions (e.g., posterior embryotoxin in Alagille syndrome, Kayser–Fleischer rings in Wilson disease), and thus, ophthalmological examination should be part of the assessment process when these conditions are suspected. Additionally, children with Alagille syndrome have a higher-than-normal incidence of benign intracranial hypertension, and thus, annual fundoscopy for papilledema is essential [23].

Endoscopic Retrograde Cholangiopancreatography (ERCP)

This procedure is invaluable for the assessment of extrahepatic biliary disease in older children (e.g., choledochal cysts, primary sclerosing cholangitis) or for the assessment of chronic pancreatitis. It involves an endoscopic technique where a fiberoptic duodenoscope is passed into the first part of the duodenum, the ampulla of Vater is identified, the pancreatic and biliary ducts are cannulated, and radiological contrast is injected. The technique has an 80 % success rate in skilled hands. Although this technique should be of value in the differential diagnosis of neonatal cholestasis, technical difficulties in the cannulation of bile ducts in small infants may provide equivocal information. Recently, in some

Table 3.11 Specific investigations for metabolic liver diseases

Clinical presentation	Disorders	Enzymes defect/ mutations	Investigations	Results
Liver failure	Galactosemia	Galactose-1-phosphate uridyltransferase (*GALT*) gene, numerous mutations identified	(Serum, erythrocyte, dried blood spots) Galactose-1-phosphate Total galactose (Gal) GALT activity DNA mutation analysis	Increased Increased Reduced/absent enzyme activity More than 130 *GALT* gene (located at 9p13) mutations identified
	Tyrosinemia type 1	Fumarylacetoacetase deficiency	Plasma amino acids (AA) Urine organic acids (OA) (succinylacetone) Urine porphyrins Serum α-fetoprotein Mutational analysis	Increase in tyrosine, methionine Increased succinylacetone (diagnostic), 4-OH-phenyl derivatives Increase δ-aminolevulinic acid Increased serum Mutation in *FAH* gene (located at 15q25.1)
	Hereditary fructose intolerance	Common mutation A149P in aldolase B gene/ Aldolase B	Plasma lactate Urine DNA mutation analysis Enzyme analysis on liver biopsy	Increased Increased urine glucose, albumin, amino acids, reducing substance, positive effect of withdrawing fructose Mutation in *ALBOD* gene (located at 9q31.1) Reduced/absent
	Mitochondrial respiratory chain defects	Respiratory chain defects Mitochondrial DNA (mtDNA) defects	Plasma and CSF lactate Urine OA Plasma and CSF AA Glucose challenge CSF protein mtDNA analysis (blood) Muscle biopsy for DNA, histology, histochemistry, and enzyme analysis	Increased Increase in lactate, ketones Increase in alanine, threonine Markedly increased serum lactate (>20 %) Increased Mutation, depletion, heteroplasmy Ragged-red fibers
	Long-chain fatty acid oxidation defects (usually with associated hypoglycemia)	Long-chain acyl-CoA dehydrogenase (LCAD) deficiency	Urine OA Plasma/blood spot acylcarnitines DNA mutation analysis	Increase in C6—C14 decarboxylic acids Increase in C14:1, ratio C14:1/C12:1 mtDNA mutation
	α-1 antitrypsin (αAT) deficiency		Serum α1-antitrypsin and phenotype	ZZ genotype, decrease in αAT
Encephalopathy or "Reye-like" illness	Fatty acid oxidation defect	Acyl-CoA dehydrogenases deficiency/ abnormality	Plasma/serum Acylcarnitine profile (dried blood spot) Urine OA Fibroblast enzymes Liver biopsy DNA mutation analysis for MCAD and LCHAD deficiency	Markedly increase in plasma NH_3, lactate, liver enzymes, creatine kinase, myoglobin Markedly reduced glucose, ketones, free fatty acids Reduced total serum carnitine Increase in acylcarnitine/total carnitine ratio Increase in specific metabolites Increase in specific decarboxylic acids; specific acylglycines Reduced or absent enzyme activities, Fatty degeneration Mutation K329E in *ACADM* gene; mutation E510Q, *HADHA* gene
	Organic acidemias	Enzyme disorder involving complex metabolism of branched-chain amino acid	Plasma/serum Blood count Urine OA and plasma AA Acylcarnitine profile (dried blood spot) Carnitine	Increase in lactate, NH_3 Ketosis/ketoacidosis hypoglycemia; hypocalcemia Neutropenia, thrombocytopenia; pancytopenia Increase in specific metabolites Decreased

Table 3.11 (continued)

Clinical presentation	Disorders	Enzymes defect/ mutations	Investigations	Results
Cholestasis (neonatal or early childhood)	Peroxisomal disorders	Multiple (or all) peroxisomal enzymes	Plasma very long-chain fatty acids (VLCFA), phytanic/pristanic acid, plasmalogens Peroxisomal morphology in liver/fibroblasts	Raised VLCFA, phytanic acid Increase or decrease in pristanic acid Reduced plasmalogens Raised CSF protein Raised specific bile acids Complete absent/reduced/abnormal structure of peroxisomes
	CDG syndrome	Specific gene mutation for enzymes of protein glycosylation	Plasma transferrin isoforms in isoelectric focusing (IEF)	Specific IEF pattern
	Lysosomal storage disorders	Specific lysosomal enzymes gene mutation	Peripheral blood film Liver/bone marrow biopsy or skin fibroblasts Leukocytes/fibroblasts Urine glycosaminoglycans and oligosaccharides Chitotriosidase (serum, dried blood spot)	Vacuolated leukocytes Storage cells (vacuolated cells) Specific enzyme assay for enzyme activities Increased Markedly increased
	Niemann–Pick A and B disease	Sphingomyelinase deficiency	Bone marrow/liver	"Niemann–Pick," storage cells, reduced sphingomyelinase in white cells
	Neimann-Pick C	Defect of intracellular cholesterol transport	Filipin staining on skin fibroblasts Cholesterol esterification studies Mutational analysis	Positive Filipin staining of fibroblasts Unesterified cholesterol Mutation in *NPC1 and 2*
	Bile acid synthesis defects	Deficiency of: 3β-Hydroxy-Δ^5-C_{27}-sterol dehydrogenase Δ^4-3-Oxosterol 5β-reductase Oxysterol 7α-hydroxylase	Urine and plasma bile acids	Increase in conjugated bilirubin, transaminases, AP, PTT Normal γGT Decrease in calcium, cholesterol, vitamins A, D, E, K Increase in specific bile acids (bile, plasma, urine)
Isolated hepatomegaly or hepatosplenomegaly	Glycogen storage diseases type I, III, IV, VI, IX, XI	Specific enzyme gene mutation	Plasma Glucose challenge Urine oligosaccharides Urine organic acids Liver histology Enzyme analysis on liver/muscle/skin fibroblasts	Increase lactate, TGs, uric acid, transaminases; hypoglycemia Decrease in lactate Increased Increase in 2-oxoglutaric acid Distended hepatocytes with prominent glycogen and lipid vacuoles Reduced/absent enzyme activities
Lysosomal storage disorders	As above			

centers, the diagnostic value of ERCP has been superseded by magnetic resonance imaging (MRI) via performance of a magnetic resonance cholangiopancreatography (MRCP) which is noninvasive. Limitations of MRCP due to experiential lack of sensitivity mean ERCP is still valuable as a diagnostic tool, and ERCP also retains an important role in therapy [24].

Table 3.12 Ophthalmic lesions in liver disease

Disease	Lesions	Comments
Galactosemia	Cataracts	
Congenital infections	Chorioretinal scar/	Congenital toxoplasmosis, CMV, HSV, VZ infections
	Active chorioretinitis	Congenital toxoplasmosis, CMV, HSV, VZ infections
	Cataracts	Less specific than chorioretinitis, congenital rubella
Wilson disease	Kayser–Fleischer ring	Not specific for Wilson disease
Alagille syndrome	Posterior embryotoxin	Not specific for Alagille
	Optic disc drusen	
	Pigmentary retinopathy	
Niemann–Pick type A	Cherry-red spot	
Tay–Sachs disease	Cherry-red spot	

Molecular Biology

The development of molecular biology has revolutionized methodology for many complex diagnostic procedures, transforming many techniques into routine laboratory procedures [25], particularly in screening for rare neonatal diseases [26]. Progress in identifying specific genes and DNA sequencing has made possible the diagnosis of many inborn errors of metabolism and inherited disease (e.g., Alagille syndrome, Wilson disease, tyrosinemia type I) and led to the identification of specific mitochondrial disorders.

Advances in methodology for gene cloning and molecular cloning methods have been helpful in identifying viruses such as hepatitis C and G [27], while the polymerase chain reaction has been used to diagnose active infection and monitor patients with many different viral diseases, such as hepatitis C, cytomegalovirus (CMV), and Epstein–Barr virus (EBV). Diagnosis for autoimmune disorders has improved, with specific assays that use recombinant protein antigens (e.g., antinuclear antigens and liver–kidney microsomal antibodies). The rapid development of molecular techniques is certain to lead to further improvements in diagnostic methods and to a better understanding of pediatric liver disease.

References

1. Moyer V, Freese DK, Whitingtin PF, et al. Guideline for the evaluation of cholestatic jaundice in infants: Recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. J Pediatr Gastoenterol Nutri. 2004;39(2):115–28.
2. Davit-Spraul A, Gonzales E, Baussan C, Jacquemin E. Progressive familial intrahepatic cholestasis. Orphanet J Rare Dis. 2009;4:1.
3. Kelly DA, Green A. Investigation of paediatric liver disease. J Inherited Metab Dis. 1991;14:531.
4. Kelly DA, Hull J. Investigation of prolonged neonatal jaundice. Curr Paediatr. 1991;1:228–30.
5. McKiernan PJ. The infant with prolonged jaundice: investigation and management. Curr Paediatr. 2001;11:83–9.
6. Spray CH, McKiernan P, Waldron KE, Shaw N, Kirk J, Kelly DA. Investigation and outcome of neonatal hepatitis in infants with hypopituitarism. Acta Paediatr. 2000;89(8):951–4.
7. Roberts EA, Schilsky ML. Diagnosis and treatment of Wilson disease: an update. Hepatology. 2008;47(6):2089–111.
8. Davenport KP, Blanco FC, Sandler AD. Pediatric malignancies: neuroblastoma, Wilm's tumor, hepatoblastoma, rhabdomyosarcoma, and sacrococcygeal teratoma. Surg Clin North Am. 2012;92(3):745–67.
9. Chen SM, Chang MH, Du JC, et al. Screening for biliary atresia using an infant stool color card in Taiwan. Pediatrics. 2006;117(4):1147–54.
10. Lee WS, Chai PF. Clinical features differentiating biliary atresia from other causes of neonatal cholestasis. Ann Acad Med Singapore. 2010;39(8):648–54.
11. Rangel SJ, Calkins CM, Cowles RA, et al. Parenteral nutrition-associated cholestasis: an American Pediatric Surgical Association Outcomes and Clinical

Trials Committee systematic review. J Pediatr Surg. 2012;47(1):225–40.
12. Lee WS, Chai PF, Boey CCM, Looi LM. Aetiology and outcome of neonatal cholestasis in Malaysia. Singapore Med J. 2010;51(5):434–9.
13. Yeung LT, Roberts EA. Current issues in the management of paediatric viral hepatitis. Liver Int. 2010;30(1):5–18.
14. Lee WS, McKiernan PJ, Kelly DA. Etiology, outcome and prognostic indicators of childhood fulminant hepatic failure in the United Kingdom. J Pediatr Gastroenterol Nutri. 2005;40(5):575–81.
15. Devictor D, Tissieres P, Durand P, Chevret L, Debray D. Acute liver failure in neonates, infants and children. Expert Rev Gastroenterol Hepatol. 2011; 5(6):717–29.
16. Rockey DC, Caldwell SH, Goodman ZD, Nelson RC, Smith AD. American Association of the Study of the Liver Position Paper: Liver Biopsy. Hepatology. 2009;49(3):1017–44.
17. Furuya KN, Burrows PE, Phillips MJ, Roberts EA. Transjugular liver biopsy in children. Hepatology. 1992;15:1036–42.
18. Cohen MB, A-Kader HH, Lambers D, Heubi JE. Complications of percutaneous liver biopsy in children. Gastroenterology. 1992;102:629–32.
19. Vivas S, Palacio MA, Rodriguez M, et al. Ambulatory liver biopsy: complications and evolution in 264 cases. Revista Espanola de Enferedades Digestivas. 1998;90:175–82.
20. Gonzalez-Vallina R, Alonso EM, Rand E, Black DD, Whitington PJ. Outpatient percutaneous liver biopsy in children. J Pediatr Gastroenterol Nutri. 1993;17:370–5.
21. McGill DB, Rakela J, Zinsmeister AR, Ott BJ. A 21-year experience with major haemorrhage after percutaneous liver biopsy. Gastroenterology. 1990;99:1396–400.
22. Westheim BH, Ostensen AB, Aagenaes I, Sanengen T, Almaas R. Evaluation of risk factors for bleeding after liver biopsy in children. J Pediatr Gastroenterol Nutr. 2012;55:82–7.
23. Narula P, Gifford J, Steggall MA, Lloyd C, Van Mourik ID, McKiernan PJ, Wilsaw HE, Kelly D. Visual loss and idiopathic intracranial hypertension in children with Alagille Syndrome. J Pediatr Gastroenterol Nutr. 2006;43(3):348–52.
24. Tipnis NA, Werlin SL. The use of magnetic resonance cholangiopancreatography in children. Curr Gastroenterol Rep. 2007;9(3):225–9.
25. Worman HJ. Molecular biological methods in diagnosis and treatment of liver diseases. Clin Chem. 1997;43:1476–86.
26. Dhondt JL. Expanded neonatal screening: social and ethical issues. J Inherit Metab Dis. 2010;33 Suppl 2:S211–7.
27. Ploss A, Dubuisson J. New advances in the molecular biology of hepatitis C virus infection: towards the identification of new treatment targets. Gut. 2012;61 Suppl 1:25–35.

Mechanisms of Liver Injury

4

Kimberly J. Riehle and Nelson Fausto†

Introduction

A diverse range of pathophysiologic processes lead to liver injury in the pediatric population. This chapter discusses mechanisms of hepatocyte or other liver cellular injury, beginning with a brief discussion of liver functions and general mechanisms of cell death. Particular attention is paid to the role of apoptotic pathways and mitochondrial damage in liver diseases, including metabolic disorders, infections, toxin ingestions, and diseases that primarily affect the biliary tree.

General Structure and Function of the Liver

Hepatocytes are the liver's primary cell type and perform most of the functions that are attributed to the liver, including detoxification of material arriving from the GI tract via the portal circulation and regulation of glucose metabolism via management of glycogen stores [1]. They also synthesize the majority of serum proteins, regulate lipid metabolism, and are the only cell type that complete the urea cycle. Hepatocytes are arranged into lobules, which have a hexagonal pattern with portal vein, hepatic artery, and bile duct branches (portal triads) at the corners and draining hepatic vein branches in the center of the lobule. Other liver cell types include endothelial cells, which line the vessels mentioned above as well as fenestrated sinusoids; stellate cells, which normally function in vitamin A storage; biliary epithelial cells; and cells of the immune system, including lymphocytes and resident macrophages (Kupffer cells). Complex interactions between these cells maintain liver health in the face of a host of different injuries, and hepatocytes are well known for their remarkable ability to regenerate themselves after cell death occurs [2]. Periportal hepatocytes have metabolic functions that are different from those of pericentral hepatocytes, in that the former are involved in gluconeogenesis and lactate uptake, while the latter release lactate and support glycolysis [1]. Importantly, pericentral hepatocytes are more sensitive to hypoxia, given their relatively lower baseline oxygen tension.

Regeneration of the mammalian liver is an evolutionarily conserved process involving dozens of pathways organized into cytokine, growth factor, and metabolic networks [2, 3]. This compensatory hyperplastic process has been extensively studied in rodents; many of the key factors that regulate regeneration are shown in Fig. 4.1. Of increasing interest is the importance of non-parenchymal

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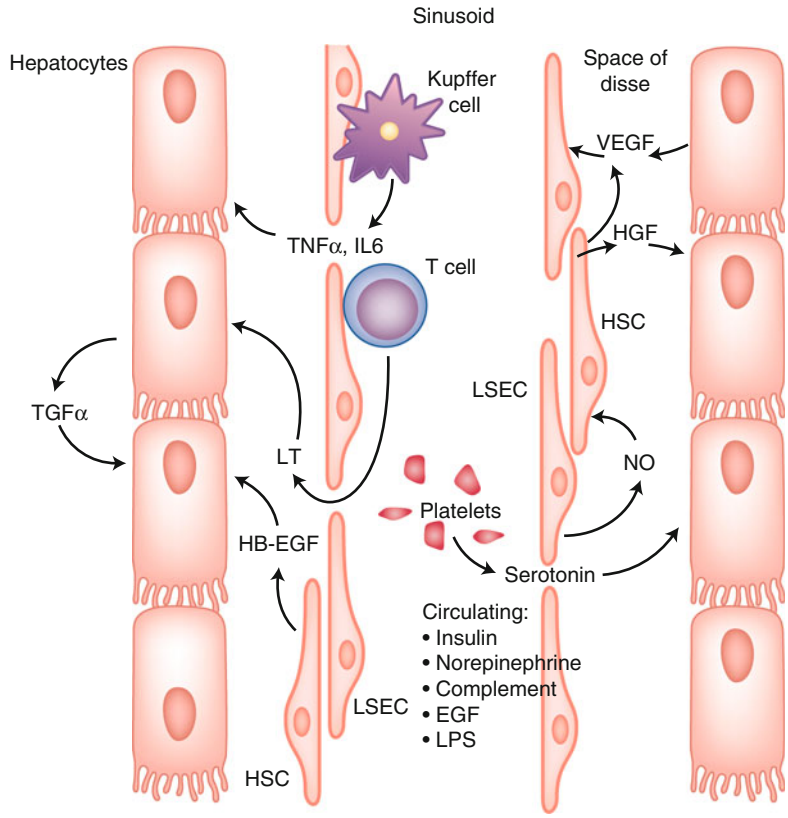

Fig. 4.1 Cytokines and growth factors critical to liver regeneration. *HSC* hepatic stellate cell, *LSEC* liver sinusoidal endothelial cell, *TNF* tumor necrosis factor, *IL6* interleukin 6, *VEGF* vascular endothelial growth factor, *HGF* hepatocyte growth factor, *NO* nitric oxide, *LT* lymphotoxin, *TGFα* transforming growth factor-α, *TGFβ* transforming growth factor-β, *HB-EGF* heparin-binding epidermal growth factor-like growth factor, *EGF* epidermal growth factor, *LPS* lipopolysaccharide

cells (NPCs) in maintaining the regenerative capacity of hepatocytes, as NPCs secrete multiple growth factors that drive hepatocyte proliferation. It has been demonstrated that if the NPC environment is normal, then hepatocytes have a nearly unlimited capacity to recover from injury. However, if NPCs are inhibited or otherwise abnormal, the response to hepatocyte injury will be collagen deposition and the development of fibrosis. Fibrosis is the common end point for liver diseases of diverse etiologies and involves the activation of stellate cells to myofibroblasts, a process that is probably driven by cytokines secreted by Kupffer cells and other inflammatory cells [4]. The histological pattern of fibrosis differs among diseases, however, and may facilitate making specific diagnoses [5, 6]. Interestingly, liver regeneration usually entails proliferation of fully mature, differentiated hepatocytes; progenitor cells only appear to be stimulated to proliferate in extreme circumstances, such as in patients with severe fibrosis or cirrhosis [7].

Mechanisms of Cell Death

It has long been proposed that there are two general mechanisms of cell death: necrosis, which is considered disorderly, and apoptosis, which is "programmed." More recently, it has been postulated that these two processes are really part of the same spectrum, in that necrosis may simply be massive apoptosis [8, 9]. Necrosis is due to cellular energy depletion and an inability to maintain ion gradients across the cell membrane, leading to cell swelling and eventual rupture with subsequent activation of an inflammatory response. Apoptosis conversely involves cell shrinkage, membrane blebbing, and phagocytosis by other cells without induction of significant inflammation. Apoptosis classically can be induced via two major pathways, the extrinsic and intrinsic pathways, though in many disease states the two pathways are tightly interconnected (see Fig. 4.2). The extrinsic pathway involves

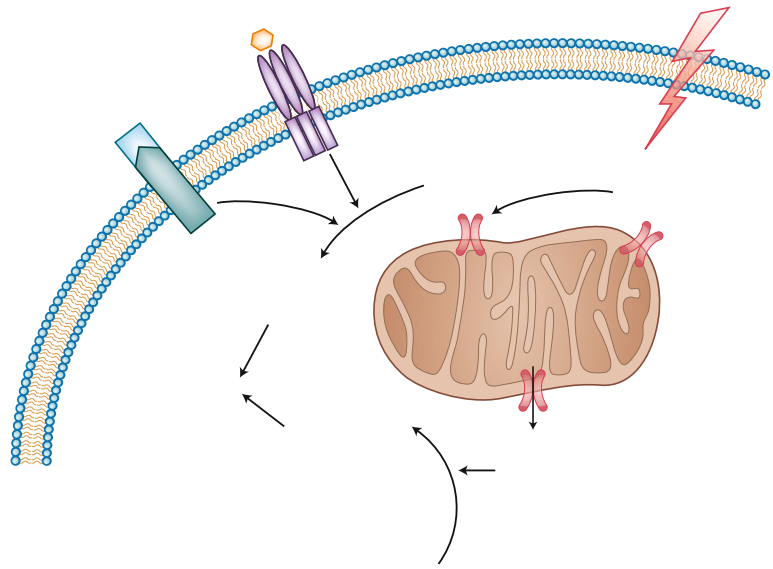

Fig. 4.2 Intrinsic and extrinsic pathways for initiation of apoptosis. *TNF* tumor necrosis factor

activation of intracellular apoptotic cascades by ligand binding of "death receptors" on the cell surface, including Fas, TNFR1, and TRAIL. Binding of these receptors leads to activation of caspase 8, cleavage of Bid, and subsequent activation of caspases 3, 6, and 7, which are called the "effector caspases." The intrinsic pathway involves more direct activation of mitochondrial apoptotic pathways and can be initiated via diverse mechanisms, including DNA damage, endoplasmic reticulum (ER) stress, and oxidative stress. The Bcl2 family of proteins regulates the mitochondrial phase of apoptosis. Liver homeostasis involves low-level apoptosis, in which aged hepatocytes express Fas, which is recognized by Fas ligand expressed by natural killer cells or T lymphocytes. Apoptosis then ensues, and the resultant hepatocytic apoptotic bodies are phagocytosed by either Kupffer cells or stellate cells. More significant hepatocytic apoptosis occurs in many liver disorders and can lead to injury, inflammation, and fibrosis.

As mentioned above, ER stress can be a major initiator of apoptosis and is key to the pathogenesis of several pediatric liver diseases. The ER is the largest membranous organelle in the cell, and under normal conditions, chaperone proteins in the ER control proper folding of newly translated proteins. ER stress can be due to accumulation of misfolded proteins, inhibition or alteration of glycosylation, UV irradiation, reactive oxygen species (ROS), or glucose deprivation [10]. All of these stresses lead to the unfolded protein response (UPR), in which misfolded proteins are degraded by the proteasome and apoptosis is induced. As mentioned above, mitochondria play vital roles in cell death, including production of electrons and resultant ROS, and in the initiation of apoptosis. Specifically, the space between the inner and outer membranes of mitochondria holds pro-apoptotic proteins (e.g., cytochrome c), and if the outer membrane becomes permeable, these proteins are released into the cytosol, where proteases are activated and apoptosis ensues. Mitochondrial damage is a central component of the pathophysiology of multiple etiologies of liver injury [11], including fatty liver [12], toxicity from bile acids [13], and the hepatotoxicity of several drugs [14]. Oxidative stress occurs as a byproduct of aerobic metabolism in mitochondria and generates oxidized DNA, nitrated proteins, and peroxidized lipids (see Fig. 4.3) [8, 15]. Hepatocytes generate numerous antioxidant defenses, but when these are overwhelmed, the mitochondrial permeability

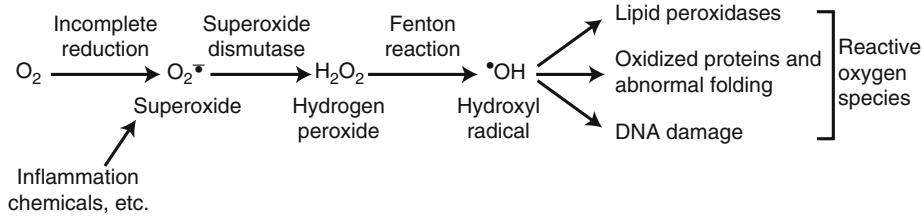

Fig. 4.3 Generation of reactive oxygen species

transition (MPT) occurs. MPT leads to loss of the electrochemical gradient across the inner mitochondrial membrane, uncoupling of oxidative phosphorylation, and an inability to synthesize ATP. MPT is also involved in ischemic necrosis, in which a lack of O_2 and nutrients leads to an inability of the cell to make ATP.

Mitochondrial Disorders

Mitochondrial liver disorders may be caused by mutations in the mitochondrial DNA molecule (mtDNA), mitochondrial DNA depletion syndrome (MDS), or by mutations in nuclear DNA (nDNA) that affect transcription or translation of mtDNA or affect the assembly of respiratory chain complexes and mitochondrial biogenesis [16]. Multiple toxins and agents also injure mitochondria, leading to pathology similar to that of the genetic disorders. Mitochondrial hepato-pathies have characteristic histologic features of glycogen depletion and microvesicular lipid accumulation, and can rapidly progress to liver failure in the neonatal period. The underlying pathophysiology likely involves stellate cell activation, triggering aberrant signaling via the transcription factor nuclear factor κB (NFκB), production of ROS, and impaired β-oxidation of lipids.

The proximity of the mtDNA molecule to the inner mitochondrial membrane, where ROS are produced and DNA repair mechanisms are incomplete, makes it very sensitive to oxidative damage [8]. Interestingly, patients with mtDNA mutations usually have a mixture of mutated and wild-type mitochondria within the same cell (heteroplasmy), and the proportion of mitochondria that are mutated vs. wild-type varies between cells. An increasing number of nDNA genes have been identified as being critical for proper transcription and translation of mtDNA, as well as for assembly of respiratory chain complexes and mitochondrial biogenesis [11]. Examples of these nDNA mutations include those in *BCS1L*, which lead to GRACILE syndrome (Fellman disease), resulting in steatosis, iron overload, severe lactic acidosis, and early death from energy depletion. Mutations in other nDNA genes lead to MDS, a generalized decrease in mtDNA content in the cell without mutations in mtDNA. The most common of these is Alpers syndrome, which is due to mutations in DNA-dependent polmerase γ. Another such mutation is in the deoxyguanosine kinase (*dGK*) gene, whose protein product maintains a balanced mitochondrial deoxyribonucleotide pool for mtDNA synthesis. Navaho neurohepatopathy is due to mutations in the nDNA gene *MPV17*, which encodes a mitochondrial membrane protein.

Disorders of mitochondrial respiratory chain complexes are not well understood. Defects in specific transport proteins, such as carnitine/acylcarnitine translocase, and in proteins involved in mitochondrial beta-oxidation of fatty acids result in episodic hypoglycemia and microvesicular steatosis but not cirrhosis. Interestingly, a defect in long chain 3-hydroxyacyl CoA dehydrogenase, one of the enzymes of the mitochondrial trifunctional protein, does cause liver failure. Citrin is the liver-specific isoform of the mitochondrial aspartate/glutamate carrier, and its deficiency can cause either neonatal intrahepatic cholestasis or adult onset type II citrullinemia [17]. The neonatal form is poorly recognized but is characterized by prolonged cholestasis, aminoacidemia, and galactosuria. Hepatocytes with citrin deficiency may have augmented oxidative stress responses,

but the mechanism of liver injury in these patients is largely unknown.

Inherited Metabolic Disorders

Disorders of Amino Acid Metabolism

Hereditary Tyrosinemia Type 1

Fumarylacetoacetase deficiency (hereditary tyrosinemia type 1, HT1) results in accumulation of upstream metabolites in the tyrosine catabolism pathway, including maleylacetoacetate (MAA) and fumarylacetoacetate (FAA). FAA initiates hepatocyte apoptosis via the mitochondrial pathway [17], and a byproduct of FAA is succinylacetone (SA), the detection of which in the urine is diagnostic of HT1. SA forms adducts with amino acids and probably inhibits DNA ligase activity, contributing to the persistence of damaged DNA in replicating hepatocytes. HT1 is notable in that infants who survive beyond infancy are at a high risk of developing hepatocellular carcinoma (HCC), likely related to chromosomal breakage and impaired DNA repair [18].

Disorders of Carbohydrate Metabolism

Galactosemia

A defect in galactose-1-phosphate uridyltransferase leads to galactosemia, which presents with progressive cholestasis, renal tubular acidosis, cataracts, and coagulopathy [19]. It is unknown why liver damage occurs in galactosemia, but good metabolic control prevents liver damage in these patients, and sepsis worsens their liver dysfunction [20].

Hereditary Fructose Intolerance

Hereditary fructose intolerance (HFI) is due to deficiency of aldolase B, which normally degrades fructose-1-phosphate to dihydroxyacetone phosphate and glyceraldehyde. Fructose administration to these patients leads to rapid accumulation of fructose-6-phosphate, with associated phosphate and ATP deficiency [8, 21]. Experimentally, ATP deficiency does not affect hepatocyte viability, but it has a significant effect on the hepatocyte apoptotic response to other insults, including TNFα. It is unclear whether a similar mechanism occurs in human livers, but fructose avoidance prevents liver damage in HFI.

Glycogen Storage Diseases

Patients with glycogen storage disease type 1a have defects in glucose-6-phosphatase-α and develop hepatomegaly due to an excessive accumulation of glycogen and fat [22]. For unknown reasons, these patients have a long-term risk of hepatocellular adenoma with the chance for malignant transformation. Deficient glycogen-branching enzyme activity leads to glycogen storage disease type IV (GSDIV). GSDIV leads to deposition of unbranched glycogen (amylopectin) in multiple organs, including the liver, and commonly presents in the first few months of life [23]. Unfortunately, no adequate treatment exists, and most patients die by 5 years of age from complications of cirrhosis (Table 4.1).

Carbohydrate Deficiency Glycoprotein Syndrome

A variety of enzyme deficiencies cause carbohydrate deficiency glycoprotein syndrome (CDG), which consists of abnormal *N*-glycan synthesis and subsequent hepatic fibrosis [24, 25]. The most common type of CDG is type 1A, phosphomannomutase deficiency, and CDG type 1B involves a defect in phosphomannose isomerase activity. The hallmark of these disorders is abnormal glycosylation of transferrin, and while type 1A is not treatable, type 1B is corrected by mannose therapy.

Disorders of Lipid or Cholesterol Metabolism/Lysosomal Storage Diseases

Lysosomes are membranous organelles that are filled with acidic enzymes and are involved in the breakdown of complex macromolecules. When there is a deficiency of an enzyme involved in one of these breakdown pathways, the

Table 4.1 Inborn metabolic disorders leading to liver injury

Disease	Enzyme deficiency	Liver phenotype
Type I hereditary tyrosinemia	Fumaryl acetoacetase	Hepatocyte apoptosis and DNA damage, risk of HCC
Galactosemia	Galactose-1-uridyl transferase	Cholestasis for unknown reasons
Hereditary fructose intolerance	Aldolase B	Severe phosphate and ATP deficiency
Glycogen storage disease type 1A	Glucose-6-phosphatase-α	Accumulation of glycogen and fat, risk of adenoma
Glycogen storage disease type IV	Glycogen-branching enzyme	Excess amylopectin deposition, cirrhosis
Carbohydrate deficiency glycoprotein syndrome	Multiple, e.g., phosphomannomutase	Abnormal N-glycan synthesis, fibrosis
Niemann-Pick type B	Acid sphingomyelinase	Infiltration by foamy histiocytes, ballooning of hepatocytes
Niemann-Pick type C	ATP-binding cassette transporter A1 (ABCA1)	Cholesterol accumulation in lysosomes of hepatocytes, cholestasis, risk of HCC
Cholesterol ester storage	Lysosomal acid lipase	Fibrosis
Gaucher disease type 1	Acid β-glucosidase	Glycosylceramide accumulation in lysosomes of Kupffer cells, secondary effects on hepatocytes, fibrosis, risk of HCC
Disorders of bile acid synthesis	Multiple, e.g., 3-β-hydroxy-steroid dehydrogenase	Cholestasis, hepatocellular injury from production of aberrant bile acids

partially degraded substrate accumulates within lysosomes, which may then interfere with other normal cellular functions. Additionally, abnormal intracellular components such as free fatty acids or bile salts can lead to lysosomal permeabilization and release of enzymes into the cytosol, activating the mitochondrial apoptotic pathway.

Gaucher Disease

Mutations in acid β-glucosidase lead to impaired catabolism of glycosphingolipids and Gaucher disease type 1, the most common lysosomal storage disorder [26]. In Gaucher disease, the enzyme deficiency results in accumulation of undegraded substrate (glycosylceramide) in the lysosomes of monocytes/macrophages. Given that the liver contains the largest organ bulk of tissue macrophages (Kupffer cells), patients with Gaucher disease get liver infiltration, fibrosis, and eventual cirrhosis, with subsequent portal hypertension, liver failure, and risk for HCC [27]. The precise explanation of why patients with Gaucher disease get such severe liver dysfunction is unclear [26]. It may be the effects of sphingolipid accumulation on the immune system that lead to liver inflammation, apoptosis, and changes in cytokine metabolism with secondary hepatocellular failure.

Niemann-Pick Disease

The rate-limiting step in the formation of high-density lipoproteins (HDL) is lipidation of apolipoprotein and other proteins by ATP-binding cassette transporter A1 (ABCA1). Regulation of ABCA1 is impaired in Niemann-Pick disease type C1, leading to decreased formation of HDL particles. The glycoprotein product of the gene mutated in Niemann-Pick type C2 is involved in regulating intracellular cholesterol homeostasis via directly binding free cholesterol. Niemann-Pick disease leads to cholesterol accumulation in lysosomes of hepatocytes, infantile cholestasis, and neurodevelopmental problems [28]. Niemann-Pick disease type B, which is due to mutations in acid sphingomyelinase, leads to development of hepatomegaly due to infiltration of the liver by foamy histiocytes and ballooning of hepatocytes [29].

Cholesterol Ester Storage Disease

Cholesterol ester storage disease is caused by a deficiency in lysosomal acid lipase (LAL) activity and presents with hepatic fibrosis, adrenal insufficiency, and malabsorption [30]. The most severe form of LAL deficiency is Wolman disease, in which there is no residual LAL activity. It was

recently discovered that LAL deficiency leads to impaired regulation of ABCA1, and that is why these patients have low HDL particle formation.

Acquired Metabolic Disorders

Nonalcoholic Fatty Liver Disease (NAFLD)

Steatosis (fat accumulation in hepatocytes) develops when free fatty acid (FFA) uptake and synthesis outweigh FFA oxidation and secretion. Steatosis can result from a variety of diseases, including obesity (nonalcoholic fatty liver disease or NAFLD), Wilson disease, parenteral nutrition-associated liver disease (PNALD), cystic fibrosis, chronic alcohol consumption, and hepatitis B and C [8]. Regardless of etiology, steatosis increases the susceptibility of the hepatocyte to additional insults, including hypoxia, oxidative stress, and sepsis. NAFLD is the most common pediatric liver disease in developed countries and is associated with insulin resistance and increased circulating FFA [31]. Excess FFA in hepatocytes leads to ER stress, upregulation of pro-apoptotic pathways (termed lipoapoptosis), and lysosomal permeabilization. Mitochondrial caspases are activated and likely drive the progression from steatosis to steatohepatitis (termed NASH), defined as inflammation and fibrosis in the setting of steatosis [12]. Insulin resistance independently reduces β-oxidation of FA in mitochondria, further increasing accumulation of triglycerides in hepatocytes, suggesting that steatosis may simply be the liver manifestation of the metabolic syndrome. Polymorphisms in multiple genes predispose to the development of NAFLD, including genes regulating lipid metabolism, oxidative stress, and metabolism.

Parenteral Nutrition-Associated Liver Disease

PNALD develops in infants and older children who are on parenteral nutrition for prolonged periods of time, and is most common in patients with prematurity, low birth weight, intestinal failure, and sepsis. The precise mechanism underlying the pathophysiology of PNALD is unclear but is likely multifactorial. Increasing data suggest that vegetable oil-based lipid formulations drive the development of PNALD (see Fig. 4.4). First, phytosterols present in these formulations induce the production of TNFα and IL6, and can accumulate in the serum and decrease bile acid secretion. Second, vegetable oil formulations contain ω-6 polyunsaturated FAs, which are cleared with less lipolysis and decreased release

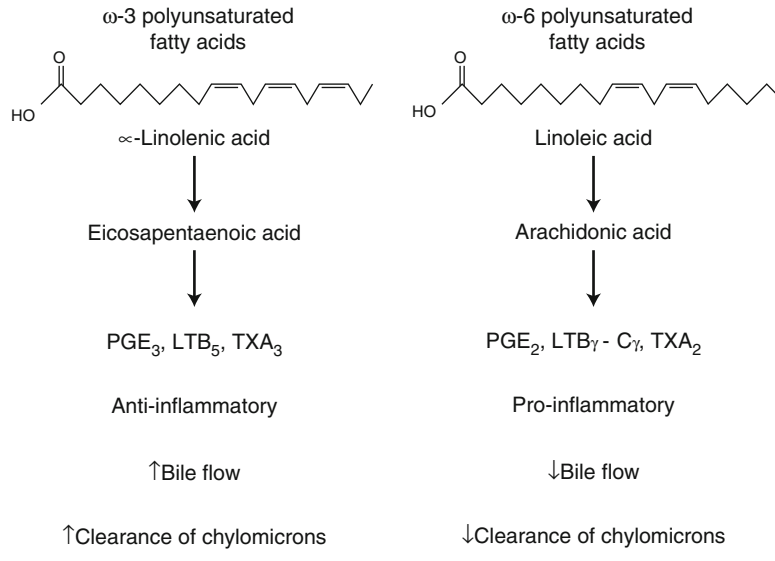

Fig. 4.4 Comparison between ω-3 and ω-6 fatty acids. *PG* prostaglandin, *LT* leukotriene, *TX* thromboxane

of FFA into chylomicrons compared to ω-3 FAs, which are found in fish oils [32]. Finally, arachidonic acid, which is also found in soybean oil, is the precursor for several proinflammatory molecules, including prostaglandin E, leukotrienes, and thromboxane A_2. Minimizing administration of ω-6 FAs or substituting the ω-3 FAs found in fish oil has shown significant clinical benefit in patients at risk for PNALD. ω-3 FAs have been shown experimentally to have anti-inflammatory properties, to decrease the production of free radicals, and to increase bile flow and clearance of chylomicrons and triglycerides. Thus, vegetable-based lipid formulations may cause liver damage both by cholestasis and by inducing inflammation, and minimizing vegetable-based lipids or substituting fish oil-based preparations may prevent or reverse these changes [33].

Disorders of Iron Metabolism

Under normal conditions, about 25 % of the body's iron is stored as ferritin or hemosiderin, and uptake of iron into the cells that store it is regulated by the iron responsive element-binding protein (IRE-BP). Additional iron is bound by circulating transferrin, but when transferrin is saturated, the remaining iron circulates in a labile form. This excess iron can lead to the generation of ROS (see Fig. 4.3) and resultant tissue damage [8]. As mentioned below, the disease previously known as neonatal hemochromatosis is now called gestational alloimmune liver disease (GALD) [34] and is unrelated to hereditary hemochromatosis. Premature infants who receive multiple blood transfusions do have evidence of hepatic iron overload, but this hepatic iron is almost exclusively in Kupffer cells and is not associated with liver damage. Mutations in the high Fe (HFE) gene, which cause hereditary hemochromatosis, are not seen in neonates with GALD or hepatic iron overload [35]. HFE mutations do not tend to cause liver disease in childhood, but an uncommon form of hereditary hemochromatosis that is more severe than the adult form is termed juvenile hemochromatosis, and is due to mutations in the genes encoding hemojuvelin or hepcidin.

Transport Defects

α_1-Antitrypsin Deficiency

α_1-Antitrypsin (A1AT) is a glycoprotein synthesized in the liver that usually undergoes glycosylation and folding in the ER, followed by secretion via the Golgi apparatus. The most common form of A1AT deficiency is caused by a mutation that leads to improper folding and subsequent polymerization and formation of insoluble aggregates in the ER of hepatocytes (see Fig. 4.5), leading to cell damage, inflammation, and ultimately fibrosis [36]. The insoluble intracellular aggregates are degraded via autophagy, a process by which a cell catabolizes its own components in the lysosome. Medications that enhance autophagy, such as carbemazepine, thus may slow the development of liver disease in these patients [37]. The formation of A1AT aggregates in the ER leads to a decrease in circulating A1AT, which normally functions in protecting alveoli by inhibiting neutrophil elastase; uninhibited elastase activity is responsible for the lung disease in these patients. Not all patients with A1AT deficiency get liver disease, but possible presentations include neonatal cholestasis, chronic liver disease, or elevated liver function tests without clinical evidence of liver disease. The other factors that make a particular individual with AIAT deficiency susceptible to liver injury are unknown.

Disorders of Copper Metabolism

Copper is reduced and actively transported through the basolateral membrane of hepatocytes by a protein called CTR1, and within the cell it is carried by a series of chaperone proteins (see Fig. 4.5). One of these, ATOX1, carries it to ATP7B in the trans-Golgi complex, where ATP7B mediates the incorporation of copper molecules into apoceruloplasmin. The resultant ceruloplasmin is then secreted into plasma. ATP7B is an ATPase that binds copper at its N-terminal domain, and also sequesters excess copper into cytoplasmic vesicles. Mutations in *ATP7B* lead to Wilson disease, with failure of adequate copper

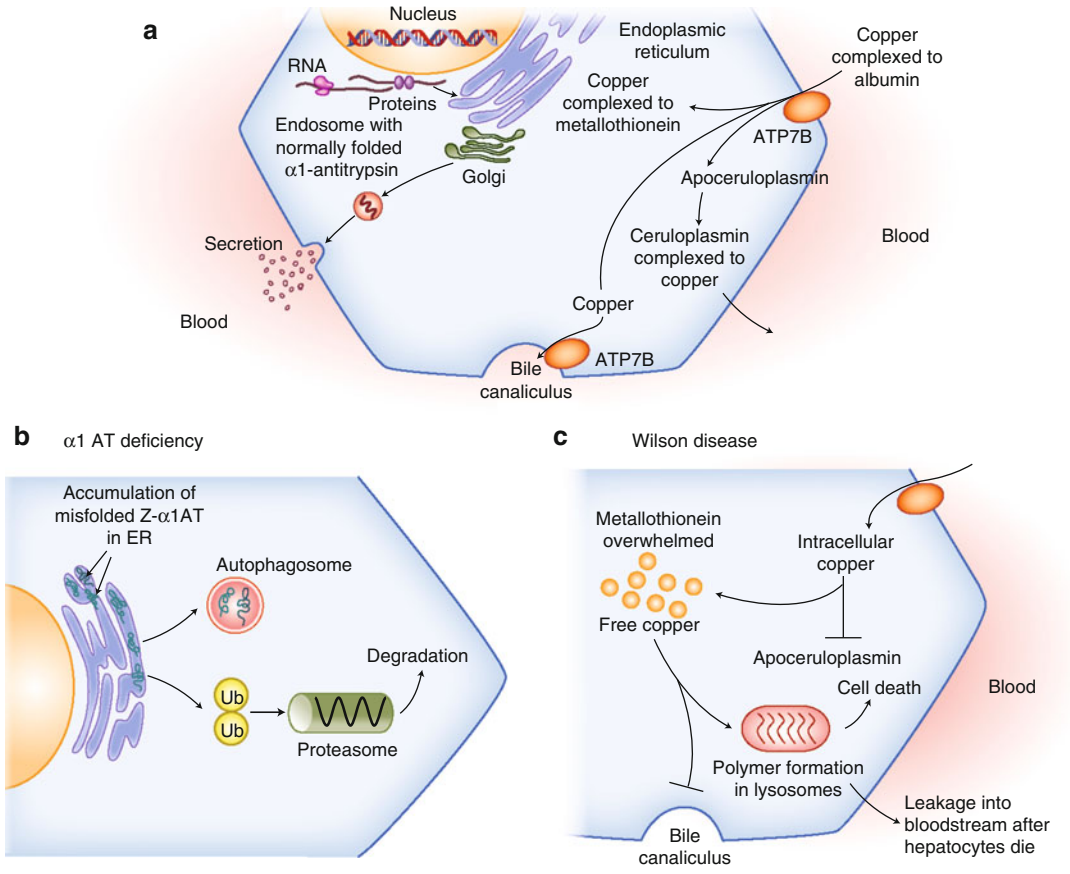

Fig. 4.5 Processing of α-1-antitrypsin and copper in normal hepatocytes (**a**) in α-1-AT (α1AT) deficiency (**b**) and in Wilson disease (**c**). *ER* endoplasmic reticulum, *ATP7B* ATPase Cu^{2+} transporting β polypeptide

transport into bile and subsequent accumulation of copper in hepatocytes [38, 39]. Excess hepatocytic free ionic copper is initially bound by glutathione, and the remainder is transferred to intracellular metallothionein [40]. Once metallothionein's capacity is exceeded, copper is deposited in lysosomes, where it is believed to form dense polymers that are hepatotoxic. The resulting hepatocyte damage is likely due to a combination of enhanced free radical production and hepatocyte apoptosis and necrosis. When copper-laden hepatocytes die, their copper content leaks into the bloodstream, leading to the other manifestations of Wilson disease in the brain and other tissues [41].

Interestingly, liver copper levels do not necessarily correlate with the degree of liver damage in Wilson disease or other disorders, and animal models of high copper ingestion do not develop cirrhosis. Furthermore, patients in whom ceruloplasmin is absent manifest tissue deposition of iron rather than copper, suggesting that a lack of ceruloplasmin does not cause the observed phenotype in Wilson disease. Other disorders of copper metabolism may cause earlier and more severe liver disease than Wilson disease, particularly those that are due to ingestion of large amounts of copper in infancy. One example is Indian childhood cirrhosis, which results from contamination of animal milk feeds with copper utensils [8]. These patients get ballooning necrosis with hyaline inclusions and dense intralobular pericellular fibrosis. Mutations in *ATP7A* lead to Menkes disease, which entails a failure of copper transport from the intestine and subsequent copper deficiency.

Infections

Hepatitis B Virus

Hepatitis B virus (HBV) is a DNA virus that incorporates a portion of its genome into the DNA of host hepatocytes. HBV has a unique replication strategy that allows its minichromosome to persist in hepatocytes long term, using host machinery to transcribe and translate viral proteins. Interestingly, HBV is not believed to be directly cytotoxic; the host immune response causes most of the hepatocellular damage in HBV infection [42]. Neonates who acquire HBV via postnatal exposure or vertical transmission do not typically develop acute clinical hepatitis, though incorporation into host DNA usually leads to chronic infection and a long-term risk of HCC. Most adults newly infected with HBV are able to limit disease progression by developing cytotoxic T lymphocytes (CTLs) against core antigens expressed on the hepatocyte cell surface, leading to cell death by necrosis and acute hepatitis. CTLs also cause indirect hepatocyte injury via production of IFNγ and TNFα, which elicit antiviral effects and perpetuate inflammation. Compensatory hepatocyte proliferation occurs in response to the hepatocellular necrosis induced by the immune response to HBV. Unexpectedly, hepatocyte regeneration may lead to a *reduction* in HBV DNA, since HBV DNA lacks centromeres to ensure correct migration during mitosis. There is significant experimental evidence that the strength of the initial innate immune response to HBV determines the outcome of both acute and chronic infection.

Hepatitis C Virus

Hepatitis C virus (HCV) is an enveloped single-stranded RNA virus whose genome is translated as a single polyprotein that is cleaved by host and viral proteases. Seventy percent of patients who get acute hepatitis from HCV develop chronic infection, and 5–10 % of infants born to mothers with chronic HCV will acquire the infection as well. Interestingly, only 50–60 % of infected infants will develop chronic hepatitis, and a significant percentage will clear the virus by 2–3 years of age. HCV-specific CD8+ CTLs are detectable in chronically infected patients, but they are inadequate to clear the infection, perhaps because they generate a polyclonal and multispecific response [43]. Viral clearance is more likely if the patient mounts a T helper type 1 profile rather than a T helper type 2 profile. Similar to HBV infection, liver damage in HCV infection is probably immune mediated, as in most model systems HCV is not cytolytic, and chronic viral infection may exist without liver damage. Additionally, immunosuppression transiently normalizes AST/ALT in these patients.

Infections Causing Neonatal Cholestasis

Bacterial sepsis is frequently associated with hepatobiliary dysfunction, particularly in neonates. One study demonstrated that >50 % of bacteremic preterm infants had elevated bilirubin, particularly those with gram-negative sepsis [44]. The pathogenesis of cholestasis in bacteremia is likely multifactorial, but studies in animals suggest a significant role of LPS. Circulating LPS is cleared by Kupffer cells, leading to release of cytokines, which bind receptors on hepatocytes and cholangiocytes and thus alter expression of bile transporters. Impaired bile formation and accumulation of bile acid and toxins are the end result. A wide range of other organisms also cause cholestasis in newborns, including toxoplasmosis, rubella, cytomegalovirus, herpes, syphilis, echovirus, adenovirus, and parvovirus B19 [45].

Immune-Mediated Liver Diseases

Gestational Alloimmune Liver Disease (Neonatal Hemochromatosis)

As mentioned above, GALD was formerly called neonatal hemochromatosis, since it typically presents with hepatic failure and accumulation of iron both in and outside of the liver [46]. Recently,

Table 4.2 Immune-mediated liver diseases

Disease	Diagnostic vs. pathogenic antibodies	Antigen/cellular target
Gestational alloimmune liver disease	Unknown maternal IgG	Unknown
Autoimmune hepatitis	Smooth muscle antigen, antinuclear, liver kidney microsomal antibody 1, asialoglycoprotein receptor	CYP2D6 or other p450 enzymes in hepatocytes
Primary biliary cirrhosis	Antimitochondrial antibodies	E2 subunit of pyruvate dehydrogenase and other members of oxidative phosphorylation in portal bile duct epithelial cells
Primary sclerosing cholangitis	Antinuclear, anti-smooth muscle	Unknown antigens on epithelial cells of medium-to-large bile ducts and colonic epithelial cells

it has been appreciated that iron deposition in this and other neonatal liver diseases is a result of liver injury rather than its cause, and that GALD can occur in the absence of iron deposition. GALD is caused by transplacental passage of specific maternal IgG that activate the complement cascade in the fetus, leading to formation of a membrane attack complex and fetal liver injury [34]. The fetal protein to which the maternal immune system is responding and developing antibodies has not yet been identified, but recurrence of the disease in subsequent pregnancies is around 90 %. For this reason, gestational therapy with IVIG has been attempted, with a great improvement in outcome for affected infants [47] (Table 4.2).

Autoimmune Hepatitis

Certain features are common to all autoimmune liver diseases: cytotoxic T lymphocyte-mediated injury, the presence of serum autoantibodies, loss of immune tolerance, and rapid progression to fibrosis. Autoimmune hepatitis (AIH) is believed to occur when a predisposed individual is exposed to environmental factors that trigger an immune response against hepatocyte antigens [48]. Some of the identified environmental triggers in autoimmune hepatitis (AIH) include the hepatitis viruses, Epstein Barr virus, measles, rubella, and a variety of pharmaceuticals. Drug-induced AIH is much less common in children than in adults, though when it does occur, the most common triggering medication is minocycline. Antibodies identified in patients with AIH include those against smooth muscle (SMA), antinuclear (ANA), liver kidney microsomal antibody 1 (LKM1), and asialoglycoprotein receptor (ASPGR), though these antibodies are likely markers for rather than mediators of disease. LKM1 in particular has been shown to target CYP2D6, a cytochrome p450 enzyme that metabolizes multiple medications. Interestingly, anti-LKM antibodies have also been found in patients with chronic hepatitis C, and patients with AIH who are LKM-1 antibody negative commonly have antibodies to other P450 enzymes. Natural killer and regulatory T cells are deficient in AIH patients, while CD4 and CD8+ T cells recognize antigens on the plasma membrane of hepatocytes, leading to immune-mediated hepatocellular death. Cytokines secondarily enhance the immune mediated attack. Classic histologic findings include a periportal inflammatory infiltrate of lymphocytes and plasma cells with associated piece-meal necrosis and extensive hepatocyte cell death, particularly in zone 1 of the acinus.

Primary Biliary Cirrhosis

Extensive overlap exists between AIH and other autoimmune liver disorders, including primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC). In PBC, T lymphocyte-mediated destruction of epithelial cells of portal bile ducts leads to cholestasis. Accumulation of bile acids leads to secondary hepatocyte damage, and oxidative stress and DNA damage lead

to further liver injury [49, 50]. The diagnostic hallmark of PBC is the presence of antimitochondrial antibodies against specific autoantigens that are members of the oxidative phosphorylation cascade. The most common of these autoantigens is the E2 subunit of pyruvate dehydrogenase, but as in AIH, the autoantibodies are considered to be markers of disease rather than the cause. Mutations leading to PBC susceptibility include those in HLA class II genes or in the genes for IL12 and its receptor. The environmental factors that precipitate the development of PBC are unknown, but candidates include certain toxins, as well as infection with retroviruses or one of several bacteria.

Primary Sclerosing Cholangitis

In primary sclerosing cholangitis (PSC), intrahepatic T cells attack the epithelial cells of medium-to-large bile ducts, leading to inflammation and progressive obliterative fibrosis [51]. Overlap between AIH and PSC occurs much more frequently in children than in adults. The precise underlying etiology of PSC is unknown, though autoimmunity is most likely. The most common autoantibodies found in these patients are antinuclear or anti-smooth muscle antibodies, but it is unlikely that they are pathogenic [49]. Additionally, the autoantigens to which these antibodies are generated in PSC are unknown, though they may cross-react with colonic epithelial cells. Other etiologic factors may include chronic entry of bacteria into the portal circulation or abnormal production of bile acids by colonic bacteria, either of which may in part explain the clinical association with ulcerative colitis. Mutations in the adenosine triphosphate-binding cassette B4 (ABCB4) gene likely cause a number of cases of small duct PSC in children but are rare in adults.

AIPGS

Autoimmune polyglandular syndrome type 1 (APS1) is an autosomal recessive disease that features AIH, Addison disease, hypoparathyroidism, and other endocrinopathies [8]. The mutated gene responsible for APS1 is *AIRE* (autoimmune regulator) and is located on chromosome 21. AIRE may function in the development of anergy of self-reactive thymocytes, so AIRE deficiency thus leads to failure of immune self-tolerance.

Drug-Induced Liver Injury

Drug-induced liver injury (DILI) is much less common in children than in adults but still can occur in response to multiple medications [14]. Hepatocytes express a number of specific enzymatic pathways that function in drug metabolism, and genetic variations in these pathways are increasingly recognized as crucial to the development of DILI. Drug metabolism can be broken down into three phases: phase I, catalyzed by cytochrome p450 (CYP) enzymes; phase II, driven by transferases that ensure water solubility of metabolites; and phase III reactions, which lead to excretion of metabolites across the canalicular membrane. Polymorphisms in genes encoding CYPs have been best described, though genetic variations in expression of transferases and transporters may also place certain patients at increased risk for DILI (Fig. 4.6).

Acetaminophen

Acetaminophen is the most common cause of drug-induced liver failure in children and is most likely to occur following ingestion of >150 mg/kg. At these doses, the normal conjugation pathways in hepatocytes are overwhelmed, so the drug must be metabolized through the CYP system, producing a highly reactive toxic metabolite that depletes glutathione. Pericentral hepatocytes are preferentially sensitive to this metabolite and are thus more likely to undergo necrosis with acetaminophen toxicity. Treatment with N-acetylcysteine repletes glutathione and thus minimizes damage from the reactive byproduct of CYP activity [52].

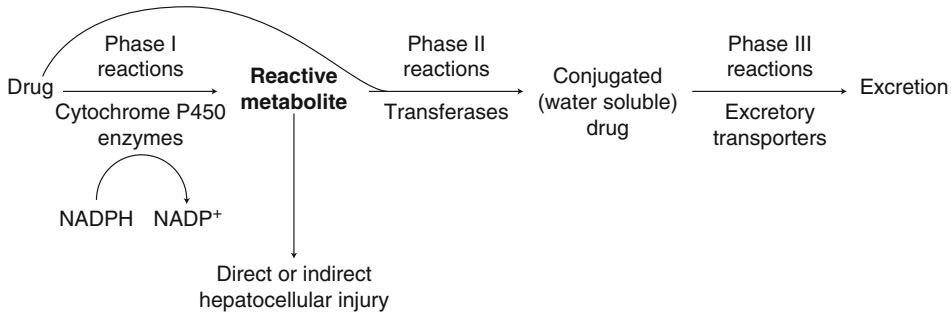

Fig. 4.6 Basic pathway of drug metabolism in hepatocytes. *NADPH* nicotinamide adenine dinucleotide phosphate

Antibiotics

Hepatotoxicity from antibiotics is relatively uncommon and often idiosyncratic. Amoxicillin-clavulanic acid is the most common cause of hepatotoxicity among penicillins, and while it tends to cause hepatocellular injury in children, bile duct damage and proliferation are seen in older adults. Hepatotoxicity from this antibiotic is associated with signs of a hypersensitivity reaction, suggesting an allergic component to this injury, and it is likely that the clavulanic acid is the cause of the liver damage, rather than the amoxicillin [53]. Tetracyclines can cause dose-related toxicity, particularly in pregnant women and in patients with renal disease. The mechanism of liver injury by tetracyclines is believed to involve increased concentration of FFAs in the liver due to inhibition of mitochondrial β-oxidation, leading to steatosis. Less common is vanishing bile duct syndrome in association with tetracycline or doxycycline or hypersensitivity hepatitis from minocycline use. Erythromycin-induced liver injury is probably immunoallergenic in nature, as evidenced by peripheral and liver eosinophilia, rash, and fever, though there are some in vitro data suggesting a direct hepatocellular toxic effect as well. Sulfonamide hepatotoxicity is related to breakdown of the drug by the CYP system, leading to the generation of toxic metabolites. Centrilobular cholestasis is the predominant pathology, though granulomatous hepatitis may also develop. Antituberculosis therapy frequently causes hepatotoxicity in adults but rarely does so in children. Toxic metabolites of isoniazid, including intermediates of monoacetyl hydrazine, are likely the cause of hepatocellular damage due to this drug and are generated by the CYP system.

Other Medications

Metabolism by the CYP system is also at the root of halothane-induced liver injury. Halothane may be metabolized either by reduction, which is not harmful, or by oxidation, which leads to the creation of an unstable intermediate, trifluoroacetyl chloride (TFA). TFA can cause direct hepatocellular damage and can form adducts with CYP2E and other liver proteins, inducing a pathologic immune response to cytochrome 2E1 specifically [54]. In fact, 70 % of patients with severe halothane hepatotoxicity have antibodies that recognize CPY2E1. Valproic acid is a synthesized branch chain fatty acid that in certain patients sequesters coenzyme A, depleting the intramitochondrial pool and thus inhibiting beta-oxidation of fatty acids. Patients with mitochondrial disease are particularly susceptible to valproate toxicity. Tienilic acid is a loop diuretic that leads to hepatitis associated with LKM2, targeted against CYP2C9, the microsomal mixed function oxidase, which metabolizes tienilic acid to a reactive sulfoxide. Current research efforts are being directed towards precise identification of the genetically determined variables that lead to these types of severe drug reactions.

Biliary Disorders

Disorders discussed in this section include those related to malformation of components of bile, such as bile acids, and disorders that alter the transport or flow of bile, e.g., cystic fibrosis and biliary obstruction. Accumulation of hydrophobic bile acids in hepatocytes initiates apoptosis, and certain metabolites generate ROS and caspase activation. In diseases involving biliary obstruction, periductular fibroblasts transdifferentiate into a myofibroblast phenotype, leading to bile duct proliferation, inflammation, and deposition of portal tract collagen, with eventual biliary cirrhosis [55].

Disorders of Bile Acid Synthesis

Bile acid synthetic disorders lead to accumulation of unusual bile acids and their intermediates, which can be hepatotoxic [56]. These synthesis defects lead to absence of cholic and chenodeoxycholic acid in plasma, and treatment with primary bile acids reduces synthesis of toxic intermediates by feedback inhibition of the native pathways. Defects causing this phenotype include those in 3-beta-hydroxysteroid dehydrogenase, 4-oxosteroid 5-beta reductase, 7-alpha-hydroxylase, sterol-27-hydroxylase, and alpha-methylacyl CoA racemase. These defects all lead to giant cell hepatitis in the neonatal period. The final steps of bile acid synthesis occur in peroxisomes, so defects in peroxisome biosynthesis (e.g., Zellweger disease) manifest with defective bile acid synthesis and more severe hepatic dysfunction with cholestasis, steatosis, and ultimately cirrhosis. Since peroxisomes contain multiple enzymes involved in lipid metabolism, their absence leads to extensive metabolic problems elsewhere in the body.

Progressive Familial Intrahepatic Cholestasis

Progressive familial intrahepatic cholestasis (PFIC) comprises a heterogeneous group of disorders characterized by defective secretion of bile acids or other components of bile. PFIC type 1 is due to a mutation in FIC1, which encodes a P-type ATPase that functions in aminophospholipid transport across the canalicular membrane of cholangiocytes [57, 58] (Fig. 4.7). These patients have increased bile salt uptake in the ileum and decreased bile salt secretion from the liver, but it is unclear exactly how FIC dysfunction leads to abnormal bile acid transport. The mutated gene in PFIC type 2 encodes the primary bile salt export pump (BSEP or ABCB11); these mutations may lead to impaired substrate binding, trafficking, or protein misfolding. Patients with PFIC2 have filamentous bile and rapidly develop cirrhosis and liver failure, in addition to an unexplained risk of HCC [59]. Defects in the multidrug resistance 3 gene (*MDR3* or ABCB4) lead to PFIC type 3. MDR3 is a flippase that replenishes phosphatidylcholine in the outer lipid membrane of the bile canaliculus from the inner

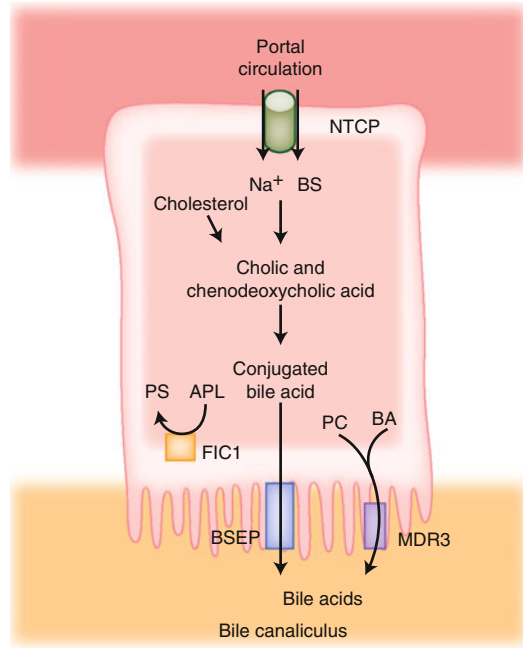

Fig. 4.7 Schematic of bile acid synthesis and transport, including genes mutated in PFIC1 (FIC1), 2 (SBEP), and 3 (MDR3). *NTCP* sodium taurocholate cotransporting polypeptide, *BS* bile salt, *BA* bile acid, *APL* aminophospholipids, *PS* phosphatidylserine, *BSEP* bile salt export pump, *PC* phosphatidylcholine, *MDR3* multidrug resistance gene 3

lipid layer [60]. Without phosphatidylcholine translocation, bile acids damage the canalicular membrane, causing progressive destruction of small bile ducts. Patients with PFIC3 tend to get liver disease later than those with PFIC 1 or 2, and portal fibrosis is a more prominent feature.

Cystic Fibrosis

The cystic fibrosis transmembrane regulator (CFTR) is expressed in biliary epithelial cells but not in hepatocytes. Due to the abnormalities in chloride transport in these cells in patients with CF, bile flow is sluggish, leading to accumulation of bile salts [61]. The thick tenacious bile in these patients leads to blockage of intrahepatic bile ducts, release of proinflammatory molecules, and the activation of stellate cells. The typical picture of a CF patient with liver disease is thus portal tract fibrosis with preserved hepatocellular function. Only a minority of CF patients develop liver disease, however, so other genetic and non-genetic factors must be involved as well [62].

Alagille Syndrome

Alagille syndrome is characterized by a paucity of interlobular bile ducts, leading to severe structural abnormalities of the biliary tree and a lack of bile flow [63]. This disorder is caused by mutations in the *Jagged1* gene in more than 90 % of patients and mutations in its receptor, *NOTCH2*, in the most of the remainder [64]. The Notch signaling pathway is critical to determining cell fate throughout the body during development, and in addition to chronic cholestasis, these patients have dysmorphic facies and cardiac and vertebral anomalies. A similar presentation and histologic pattern (paucity or absence of bile ducts) is seen in children who lack *Villin* expression in bile canaliculi. Villin binds actin and arranges actin fibers into bundles, leading to the generation of normal microvilli. Patients with abnormal *Villin* expression present with severe liver dysfunction in childhood and may be clinically mistaken to have biliary atresia (see below) [65].

Biliary Atresia

Biliary atresia is a progressive fibro-obliterative disease of the extrahepatic biliary tree that presents as direct hyperbilirubinemia in the neonatal period [66]. Inflammatory damage to bile ducts with subsequent sclerosis and eventual liver fibrosis and failure is characteristic of these patients, though the underlying driver of this inflammatory process is unknown. Fibrosis and eventual obstruction of the extrahepatic biliary tree lead to proliferation of intrahepatic bile ducts with edema, fibrosis, and eventual cirrhosis. Putative pathogenic mechanisms include immune dysregulation, as maternal lymphocytes have been found in the livers of neonates with biliary atresia, and infections with cytomegalovirus, reovirus, or rotavirus. Additionally, biliary atresia in lambs has been attributed to maternal toxin ingestion with a secondary inflammatory response, though this mechanism has not been demonstrated in humans [67]. A minority of patients with biliary atresia have associated anomalies, including lateralization defects. In these patients, it is suspected that a genetic cause is more likely, e.g., mutations in the gene for the cryptic protein.

Congenital Hepatic Fibrosis/Caroli Disease

Patients with autosomal recessive polycystic kidney disease (ARPKD) frequently develop congenital hepatic fibrosis (CHF) [68]. The gene for fibrocystin is mutated in ARPKD; this protein is found in primary cilia and apical membranes of renal tubular and bile duct epithelial cells and is required to maintain the 3D tubular architecture of the kidney and liver. Dysfunction of cholangiocyte cilia results in defective remodeling of the developing biliary system, resulting in ductal plate malformation (DPM). DPM manifests as the retention of excessive numbers of primitive bile duct remnants in their original ringlike position. Patients with fibrocystin mutations primarily present with portal hypertension, though a subset will develop cystic dilation of the bile

ducts, termed Caroli disease [69]. Caroli disease patients develop recurrent cholangitis and, for unclear reasons, are at an increased risk of developing cholangiocarcinoma. Cyst formation in Caroli disease requires epithelial cell proliferation, which is at least in part mediated by cyclic adenosine monophosphate (cAMP). Epithelial cells in these patients have an abnormal proliferative response to collagens and aberrant expression of matrix metalloproteinases, but a primary defect in localization of proteins seems critical to its pathogenesis.

References

1. Kmiec Z. Cooperation of liver cells in health and disease. Adv Anat Embryol Cell Biol. 2001;161:III–XIII, 1–151.
2. Fausto N, Campbell JS, Riehle KJ. Liver regeneration. Hepatology. 2006;43(2 Suppl 1):S45–53.
3. Fausto N, Campbell JS, Riehle KJ. Liver regeneration. J Hepatol. 2012;57(3):692–4.
4. Friedman SL. Mechanisms of hepatic fibrogenesis. Gastroenterology. 2008;134(6):1655–69.
5. Alisi A, et al. Liver fibrosis in paediatric liver diseases. Best Pract Res Clin Gastroenterol. 2011;25(2):259–68.
6. Goldschmidt I, Baumann U. Hepatic fibrosis in paediatric liver disease. Clin Res Hepatol Gastroenterol. 2012;36(3):268–70.
7. Riehle KJ, et al. New concepts in liver regeneration. J Gastroenterol Hepatol. 2011;26 Suppl 1:203–12.
8. Tanner MS. Mechanisms of liver injury relevant to pediatric hepatology. Crit Rev Clin Lab Sci. 2002;39(1):1–61.
9. Guicciardi ME, Gores GJ. Apoptosis as a mechanism for liver disease progression. Semin Liver Dis. 2010;30(4):402–10.
10. Malhi H, Kaufman RJ. Endoplasmic reticulum stress in liver disease. J Hepatol. 2011;54(4):795–809.
11. Sokol RJ, Treem WR. Mitochondria and childhood liver diseases. J Pediatr Gastroenterol Nutr. 1999;28(1):4.16.
12. Ibrahim SH, Kohli R, Gores GJ. Mechanisms of lipotoxicity in NAFLD and clinical implications. J Pediatr Gastroenterol Nutr. 2011;53(2):131–40.
13. Sokol RJ, et al. "Let there be bile"–understanding hepatic injury in cholestasis. J Pediatr Gastroenterol Nutr. 2006;43 Suppl 1:S4.9.
14. Murray KF, et al. Drug-related hepatotoxicity and acute liver failure. J Pediatr Gastroenterol Nutr. 2008;47(4):395–405.
15. Jaeschke H. Reactive oxygen and mechanisms of inflammatory liver injury: Present concepts. J Gastroenterol Hepatol. 2011;26 Suppl 1:173–9.
16. Fellman V, Kotarsky H. Mitochondrial hepatopathies in the newborn period. Semin Fetal Neonatal Med. 2011;16(4):222–8.
17. Dimmock D, et al. Citrin deficiency, a perplexing global disorder. Mol Genet Metab. 2009;96(1):44.9.
18. Couce ML, et al. Tyrosinemia type 1 in Spain: mutational analysis, treatment and long-term outcome. Pediatr Int. 2011;53(6):985–9.
19. Leslie ND. Insights into the pathogenesis of galactosemia. Annu Rev Nutr. 2003;23:59–80.
20. Devictor D, et al. Acute liver failure in neonates, infants and children. Expert Rev Gastroenterol Hepatol. 2011;5(6):717–29.
21. Bouteldja N, Timson DJ. The biochemical basis of hereditary fructose intolerance. J Inherit Metab Dis. 2010;33(2):105–12.
22. Kim SY, et al. Necrotic foci, elevated chemokines and infiltrating neutrophils in the liver of glycogen storage disease type Ia. J Hepatol. 2008;48(3):479–85.
23. Magoulas PL, et al. Diffuse reticuloendothelial system involvement in type IV glycogen storage disease with a novel GBE1 mutation: a case report and review. Hum Pathol. 2012;43(6):943–51.
24. Koscielak J. Carbohydrate-deficient glycoprotein syndromes. Acta Biochim Pol. 1999;46(3):727–38.
25. Grunewald S, Matthijs G, Jaeken J. Congenital disorders of glycosylation: a review. Pediatr Res. 2002;52(5):618–24.
26. vom Dahl S, Mengel E. Lysosomal storage diseases as differential diagnosis of hepatosplenomegaly. Best Pract Res Clin Gastroenterol. 2010;24(5):619–28.
27. Ayto RM, et al. Long-term outcomes of liver transplantation in type 1 Gaucher disease. Am J Transplant. 2010;10(8):1934.9.
28. Rutledge JC. Progressive neonatal liver failure due to type C Niemann-Pick disease. Pediatr Pathol. 1989;9(6):779–84.
29. Takahashi T, et al. Heterogeneity of liver disorder in type B Niemann-Pick disease. Hum Pathol. 1997;28(3):385–8.
30. Bowden KL, et al. Lysosomal acid lipase deficiency impairs regulation of ABCA1 gene and formation of high density lipoproteins in cholesteryl ester storage disease. J Biol Chem. 2011;286(35):30624.35.
31. Alisi A, et al. Pediatric nonalcoholic fatty liver disease: a multidisciplinary approach. Nat Rev Gastroenterol Hepatol. 2012;9(3):152–61.
32. de Meijer VE, et al. Parenteral fish oil monotherapy in the management of patients with parenteral nutrition-associated liver disease. Arch Surg. 2010;145(6):547–51.
33. Nehra D, Fallon EM, Puder M. The prevention and treatment of intestinal failure-associated liver disease in neonates and children. Surg Clin North Am. 2011;91(3):543–63.
34. Debray FG, et al. Neonatal liver cirrhosis without iron overload caused by gestational alloimmune liver disease. Pediatrics. 2012;129(4):e1076–9.
35. Bacon BR, et al. Diagnosis and management of hemochromatosis: 2011 practice guideline by the

American Association for the Study of Liver Diseases. Hepatology. 2011;54(1):328–43.
36. Nelson DR, et al. Diagnosis and management of patients with alpha1-antitrypsin (A1AT) deficiency. Clin Gastroenterol Hepatol. 2012;10(6):575–80.
37. Hidvegi T, et al. An autophagy-enhancing drug promotes degradation of mutant alpha1-antitrypsin Z and reduces hepatic fibrosis. Science. 2010;329(5988):229–32.
38. Petrukhin K, et al. Mapping, cloning and genetic characterization of the region containing the Wilson disease gene. Nat Genet. 1993;5(4):338–43.
39. Bull PC, et al. The Wilson disease gene is a putative copper transporting P-type ATPase similar to the Menkes gene. Nat Genet. 1993;5(4):327–37.
40. Vulpe CD, Packman S. Cellular copper transport. Annu Rev Nutr. 1995;15:293–322.
41. Pfeiffer RF. Wilson's disease. Semin Neurol. 2007;27(2):123–32.
42. Dandri M, Locarnini S. New insight in the pathobiology of hepatitis B virus infection. Gut. 2012;61 Suppl 1:i6–17.
43. Jhaveri R. Diagnosis and management of hepatitis C virus-infected children. Pediatr Infect Dis J. 2011;30(11):983–5.
44. Khalil S, et al. Prevalence and outcome of hepatobiliary dysfunction in neonatal septicaemia. J Pediatr Gastroenterol Nutr. 2012;54(2):218–22.
45. Epps RE, Pittelkow MR, Su WP. TORCH syndrome. Semin Dermatol. 1995;14(2):179–86.
46. Fellman V, et al. Iron-overload disease in infants involving fetal growth retardation, lactic acidosis, liver haemosiderosis, and aminoaciduria. Lancet. 1998;351(9101):490–3.
47. Vanden Eijnden S, et al. Iron overload in gestational alloimmune liver disease: still more questions than answers. Prenat Diagn. 2012;32(8):810–2.
48. Roberts EA. Autoimmune hepatitis from the paediatric perspective. Liver Int. 2011;31(10):1424.31.
49. Gupta A, Bowlus CL. Primary sclerosing cholangitis: etiopathogenesis and clinical management. Front Biosci (Elite Ed). 2012;4:1683–705.
50. Sorrentino P, et al. Oxidative stress and steatosis are cofactors of liver injury in primary biliary cirrhosis. J Gastroenterol. 2010;45(10):1053–62.
51. Shneider BL. Diagnostic and therapeutic challenges in pediatric primary sclerosing cholangitis. Liver Transpl. 2012;18(3):277–81.
52. Rivera-Penera T, et al. Outcome of acetaminophen overdose in pediatric patients and factors contributing to hepatotoxicity. J Pediatr. 1997;130(2):300–4.
53. Chawla A, et al. Rapidly progressive cholestasis: an unusual reaction to amoxicillin/clavulanic acid therapy in a child. J Pediatr. 2000;136(1):121–3.
54. Eliasson E, Kenna JG. Cytochrome P450 2E1 is a cell surface autoantigen in halothane hepatitis. Mol Pharmacol. 1996;50(3):573–82.
55. Kosters A, Karpen SJ. The role of inflammation in cholestasis: clinical and basic aspects. Semin Liver Dis. 2010;30(2):186–94.
56. Sundaram SS, et al. Mechanisms of disease: Inborn errors of bile acid synthesis. Nat Clin Pract Gastroenterol Hepatol. 2008;5(8):456–68.
57. Bull LN, et al. A gene encoding a P-type ATPase mutated in two forms of hereditary cholestasis. Nat Genet. 1998;18(3):219–24.
58. Strautnieks SS, et al. A gene encoding a liver-specific ABC transporter is mutated in progressive familial intrahepatic cholestasis. Nat Genet. 1998;20(3):233–8.
59. Suchy FJ, Ananthanarayanan M. Bile salt excretory pump: biology and pathobiology. J Pediatr Gastroenterol Nutr. 2006;43 Suppl 1:S10–6.
60. de Vree JM, et al. Mutations in the MDR3 gene cause progressive familial intrahepatic cholestasis. Proc Natl Acad Sci U S A. 1998;95(1):282–7.
61. Rowland M, Bourke B. Liver disease in cystic fibrosis. Curr Opin Pulm Med. 2011;17(6):461–6.
62. Bartlett JR, et al. Genetic modifiers of liver disease in cystic fibrosis. JAMA. 2009;302(10):1076–83.
63. Alagille D, et al. Syndromic paucity of interlobular bile ducts (Alagille syndrome or arteriohepatic dysplasia): review of 80 cases. J Pediatr. 1987;110(2):195–200.
64. Subramaniam P, et al. Diagnosis of Alagille syndrome-25 years of experience at King's College Hospital. J Pediatr Gastroenterol Nutr. 2011;52(1):84.9.
65. Phillips MJ, et al. Abnormalities in villin gene expression and canalicular microvillus structure in progressive cholestatic liver disease of childhood. Lancet. 2003;362(9390):1112–9.
66. Bates MD, et al. Biliary atresia: pathogenesis and treatment. Semin Liver Dis. 1998;18(3):281–93.
67. Harper P, Plant JW, Unger DB. Congenital biliary atresia and jaundice in lambs and calves. Aust Vet J. 1990;67(1):18–22.
68. Srinath A, Shneider BL. Congenital hepatic fibrosis and autosomal recessive polycystic kidney disease. J Pediatr Gastroenterol Nutr. 2012;54(5):580–7.
69. Nakanuma Y, et al. Recent progress in the etiopathogenesis of pediatric biliary disease, particularly Caroli's disease with congenital hepatic fibrosis and biliary atresia. Histol Histopathol. 2010;25(2):223–35.

Radiology of the Liver in Children

5

Nghia "Jack" Vo and Narendra Shet

Introduction

Imaging can play an integral role in the evaluation, diagnosis, and even treatment of children with new or chronic hepatobiliary disorders involving a native or transplanted liver. The primary imaging modalities that are routinely called upon to evaluate the pediatric liver include ultrasound (US), computed tomography (CT), magnetic resonance imaging (MRI), and nuclear scintigraphy. The detailed anatomical capabilities offered by US, CT, and MRI have essentially eliminated the need for conventional x-ray images.

The appropriate and efficient utilization of the diverse diagnostic imaging modalities available is of great interest in order to maximize the diagnostic yield while limiting the potential side effects or risk to the child [1]. Each imaging study has its limitations and frequently multiple imaging modalities are necessary. The information garnered from various imaging studies can be complementary not only to one another but also further serve to complement the clinical exam along with various laboratory tests.

Ultrasound (*US*) is the imaging modality most frequently called upon for the initial screening evaluation of children with liver disorders. There are several reasons why sonography is quite appealing in the evaluation of the pediatric population. First, US permits real-time and diverse multi-planar imaging capabilities. Furthermore, Doppler US techniques provide the ability to characterize vascular flow in real time which can be critical in circumstances such as liver transplants. Second, US does not expose the child to ionizing radiation. Children from both their younger age and longer lifetime expectancy are more vulnerable to radiation-induced cancers than adults [2]. US offers the opportunity to follow longitudinally various conditions that may affect the child's liver with sequential follow-up examinations. Finally, it is a readily available modality that is also portable. This permits its utilization when the patient cannot ideally be transported to the imaging department.

Despite US having very appealing characteristics, it does have limitations. Although the spatial resolutions can be quite exquisite, the acoustic windows needed for imaging can be limited by overlying bandaging, bowel gas, and patient body habitus. Probably the greatest limitation of US is that it is very much operator dependent. It is critical that the sonographer, radiologist, and pediatric specialist requesting the study effectively communicate the expectation and

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limitations of the US imaging studies to maximize the utility of imaging interpretation.

Computed tomography (*CT*) has a valuable role in the imaging evaluation of children with liver disease. The modern multi-detector CT scanner offers the ability to produce multi-planar cross-sectional imaging studies with exquisite anatomical spatial resolution. The technology is readily available; studies can be rapidly performed without the need for sedation or anesthesia and are reliably reproducible. These reasons may account for the increased utilization of CT in children [3]. Given that CT exposes a child to ionizing radiation, its use should be limited to appropriate clinical indications, as with any medical test or study. CT imaging protocols that set parameters to optimize image production quality (adequate resolution) weighed against radiation exposure dose to the child should be taken into account by the radiology service providers. This is of special importance when children are imaged by providers that also routinely image adult patients and thus should adjust to pediatric appropriate imaging settings [3, 4].

Magnetic resonance imaging (*MRI*) has the capability of providing comprehensive evaluation of the liver parenchyma, biliary system, and vasculature. The imaging protocols can be tailored to optimize the evaluation of the liver to address specific clinical goals and imaging needs. Therefore, it is critical that the referring specialist and radiologist communicate prior to image acquisition in order to appropriately protocol the imaging sequences to be performed and maximize the information that can be acquired. Hepatic tumors are especially well suited to be evaluated by MRI in children with the goal of tumor characterization and assess the appropriateness of resection and tumor staging. The imaging characterization of liver tumor compared to normal hepatic parenchyma both before and following contrast enhancement is able to provide greater tissue differentiation than available with CT or US [5]. The multi-planar capabilities permit anatomical localization similar to that of CT with the added advantage of not exposing the child to ionizing radiation. The relative lack of operator dependence is an additional advantage compared to that of US. The primary limitation of MRI is often the time required to complete a comprehensive contrast study, which can be upwards of 1 h. As a result young children frequently require sedation or even anesthesia.

Nuclear scintigraphy is useful for the physiologic imaging of the pediatric hepatobiliary system. Iminodiacetic acid (IDA) binds readily to Technetium-99m and is excreted through the biliary system and thus the physiologic excretion pathway can be imaged. Normal IDA scan studies will demonstrate homogenous uptake throughout the liver typically within 5 min with subsequent clearance and excretion into the biliary ductal system. The gallbladder will retain tracer which can be identified along with excretion into the duodenum. In circumstances of biliary obstruction, the normal flow of radiopharmaceutical will not occur along with delayed hepatic uptake and clearance. In cases of suspected biliary leak, the radiotracer will accumulate in an abnormal extrabiliary location and single positron emission computed tomography (SPECT) can often clarify the location of the radiotracer material in difficult cases. Conditions with underlying hepatocyte dysfunction or absence of normal hepatocytes will have delayed or absent radiotracer uptake to the affected region. Technetium-99m sulfur colloid is a radiotracer that is not excreted into the biliary system. Its uptake is related to the normal function of hepatocytes during phagocytosis. As a result the distribution reflects the function of the reticuloendothelial cells of the liver and hepatic perfusion distribution.

Pediatric Liver Masses

Benign Tumors

One-third of primary tumors affecting children are benign and of mesenchymal or epithelial origin [6]. In a large series from the Armed Forces Institute of Pathology of liver tumors in patients under 21 years of age, infantile hemangioendothelioma was the most common benign primary hepatic tumor followed by focal nodular

hyperplasia (FNH), mesenchymal hamartoma, regenerating nodules, then hepatocellular adenoma [7].

Infantile hemangioendothelioma (*IH*) (also known as infantile hemangioma) is a benign (biologically) vascular neoplasm of infancy. They are most frequently diagnosed in the first 6 months of life, and one third will be diagnosed within the first month and manifests as an asymptomatic abdominal mass. However, serious complications can arise as a result of the hypervascularity and shunting effects such as congestive heart failure, Kasabach-Merritt syndrome, hypothyroidism, and rarely tumor rupture into the peritoneum.

IH can be solitary or multifocal and US findings can be suggestive but are not typically diagnostic. The main role of US is in the initial detection and for follow-up. When multifocal in the liver, there may also be involvement of other organs to include the chest and brain. Multifocal lesions tend to be small and uniform in appearance by US. Large focal or solitary lesions with high flow by Doppler US due to the hypervascularity can demonstrate enlargement of the hepatic arteries and veins with associated downstream tapering of the aorta below the celiac trunk Fig. 5.1.

MRI can offer confident diagnosis with characteristic findings before and after intravenous contrast. Pre-contrast tumors are generally well delineated from adjacent normal liver parenchyma. Smaller lesions tend to have a homogenous appearance and larger masses can have heterogeneous signal intensity. The lack of ionizing radiation permits the opportunity to perform multiple imaging phases following IV contrast to identify the enhancement characteristics that are typical of IH. The enhancement pattern of a large tumor typically demonstrates intense peripheral enhancement during the arterial phase with progressive central enhancement of the tumor during the portal venous phase. Smaller multifocal tumors can enhance intensely uniformly Fig. 5.2.

Multiphase dynamic contrast-enhanced CT can also be diagnostic of IH, but the need for ionizing radiation should limit this modality's use in these circumstances. Pre-contrast images will show a hypodense well-circumscribed mass compared to normal liver. In large lesions, speckles of calcifications are not uncommon. As with MRI, following the administration of IV contrast, there will be peripheral nodular enhancement during the arterial phase with progressive filling on delayed imaging phases Fig. 5.3.

Catheter angiography is no longer routinely used for diagnosis of IH but rather for percutaneous endovascular interventions. Embolization of the feeder arteries can be performed to slow down or occlude the hyperdynamic flow pattern supplying the tumor in children that develop clinical complications. Angiographic findings will show an enlarged hepatic arterial supply and dilated early draining veins.

Mesenchymal hamartoma is the second most common benign liver tumor occurring in children. They have imaging features that differ from IH. Diagnosis is dependent on pathologic findings, which can range from a predominantly complex heterogeneous mass with internal septations to a solid-appearing mass. Doppler US will show little vascularity except in the septations. Imaging by CT and MRI will demonstrate enhancement of only the internal septa and solid portions. The cystic portions will have similar characteristics as other fluid-filled structures in the abdomen such as the gallbladder or bladder. If predominantly solid, it will have a hypodense (by CT) or hypointense (by MRI) enhancement pattern following contrast Fig. 5.4.

Focal nodular hyperplasia (*FNH*) is a benign epithelial liver tumor composed of hepatocytes, Kupffer cells, vascular structures, and biliary ducts. Although most commonly identified in adult women, it can occur in young children and adolescents. The imaging appearance reflects the pathologic composition, very similar to the normal liver. By US an FNH can appear as a homogenous, well-circumscribed mass that is isoechoic, hypoechoic, or even hyperechoic to that of adjacent normal liver parenchyma. The "central scar" will be hyperechoic with increased vascularity compared to the rest of the mass. On unenhanced CT an FNH is well circumscribed and can be isodense or slightly hypodense relative to normal liver. Following IV contrast, the mass can have uniform enhancement to that of adjacent normal

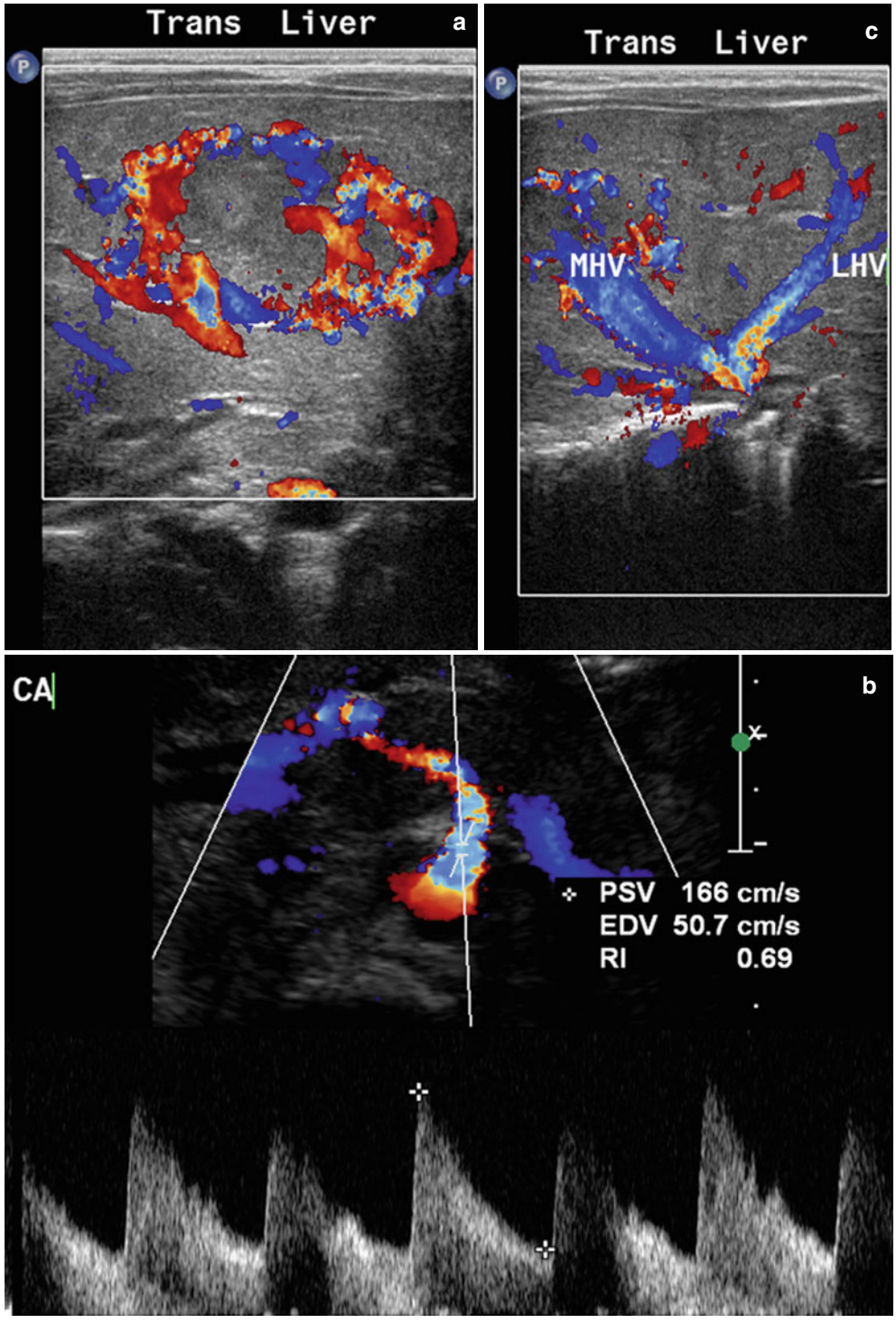


Fig. 5.1 Infantile hemangioendothelioma. Multiple ultrasound images demonstrate marked peripheral vascularity within the hemangioendothelioma (**a**). The feeding celiac artery (*CA*) is enlarged (**b**), as is the draining middle hepatic vein (*MHV*) (**c**)

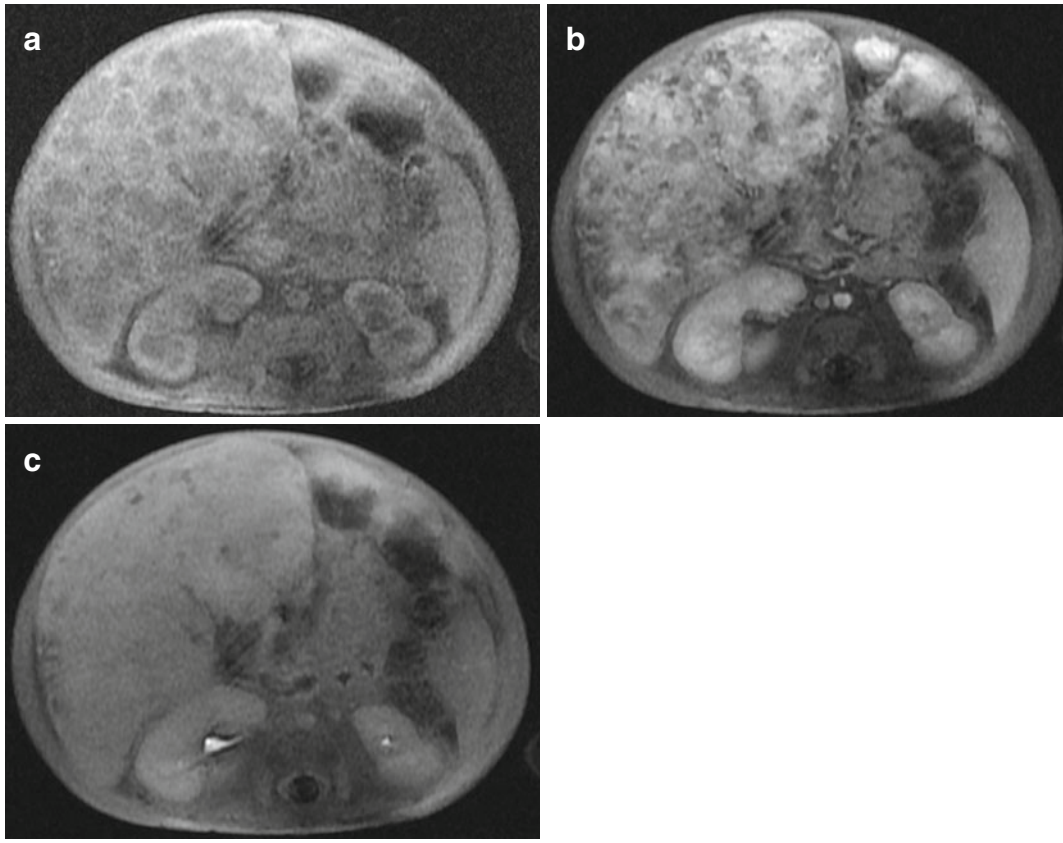


Fig. 5.2 Multiple hemangioendotheliomas on MRI. Precontrast axial T1 (**a**) demonstrates innumerable small hypointense lesions throughout the liver. Early postcontrast T1 (**b**) demonstrates heterogeneous enhancement within the liver, reflecting early peripheral enhancement. More delayed postcontrast T1 (**c**) demonstrates homogeneity of the liver, representative of progressive central enhancement of hemangioendotheliomas

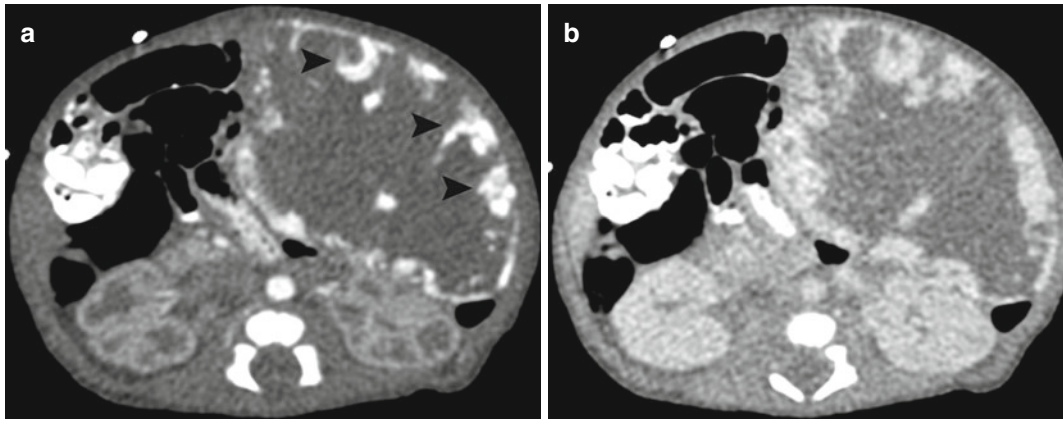


Fig. 5.3 Large hepatic hemangioma on CT. Arterial phase imaging (**a**) shows peripheral nodular discontinuous enhancement (*arrowheads*) characteristic of a hemangioma. Portal venous phase imaging (**b**) demonstrates progression of enhancement, which continues to proceed centrally as shown on delayed (excretory) phase imaging (**c**)

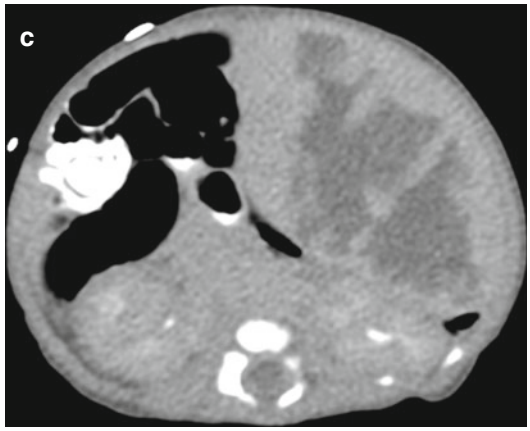


Fig. 5.3 (continued)

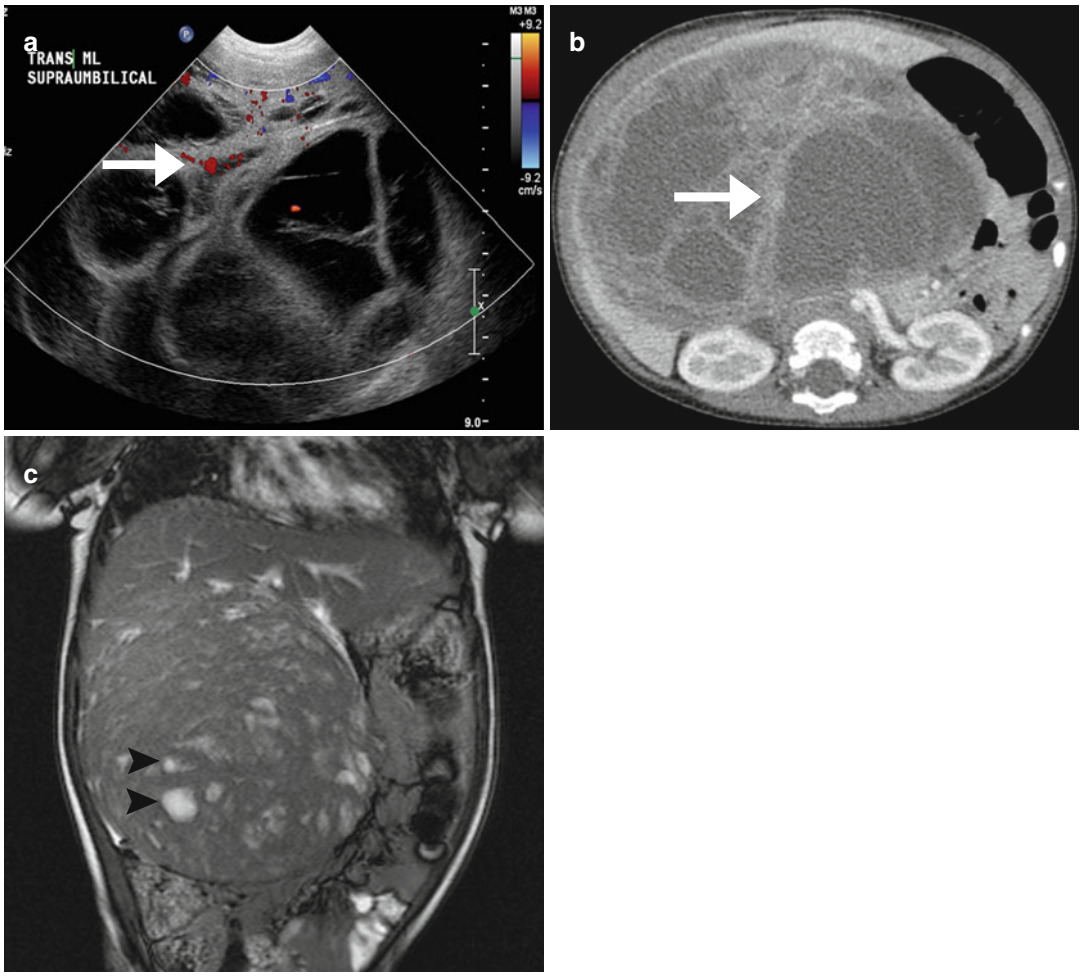


Fig. 5.4 Mesenchymal hamartoma, evaluation on multiple modalities. On ultrasound (**a**), mesenchymal hamartoma appears as a multicystic mass, with little internal vascularity except for within septations (*arrow*). The same mass was further evaluated on CT (**b**) and is seen as a well-circumscribed hypodense mass, with enhancing internal septa (*arrow*). On MRI (**c**), mesenchymal hamartoma appears as a well-circumscribed mass with internal cystic foci, which appear hyperintense (*arrowheads*) on this T2-weighted coronal image

liver and thus difficult to appreciate. The "central scar" is usually distinguishable by MRI compared to normal liver and will frequently demonstrate delayed enhancement Fig. 5.5. The vast majority of FNH will demonstrate normal uptake by nuclear scintigraphy using Technetium (Tc)-99m sulfur colloid due to the presence of normal Kupffer cells. Normal or increase uptake of sulfur colloid helps distinguish FNA from a hepatic adenoma or a malignant solid mass.

Hepatic adenoma is a benign neoplasm commonly associated with the use of oral contraceptives in young women. Most frequently it is asymptomatic but on rare occurrences can rupture leading to hemorrhage. Adenomas are typically homogenous in appearance by imaging but the presence of hemorrhagic or intracellular fat can produce some characteristic imaging features. By US adenomas can appear hypoechoic compared to adjacent liver tissue in the setting of diffuse fatty infiltration of the liver. The absence of central arterial wave pattern by Doppler US helps distinguish adenoma from FNH (which has a brisk arterial pattern from the central scar). A hepatic adenoma is typically well marginated from adjacent parenchyma by CT. Non-contrast CT will demonstrate the mass to be hypodense, hyperdense in the arterial phase, then isodense during the portal venous or delayed phase. The presence of intra-tumoral fat or hemorrhage can also produce a heterogeneous appearance Fig. 5.6. MRI of hepatic adenomas will show them to be well marginated from adjacent liver tissue and have high signal intensity on both T1- and T2-weighted imaging sequences due to the presence of fat. The enhancement pattern by MRI is similar to that of CT. Nuclear scintigraphy will demonstrate a photopenic defect by 99mTc sulfur colloid scan, but using a tracer excreted through the biliary system may show the mass to have

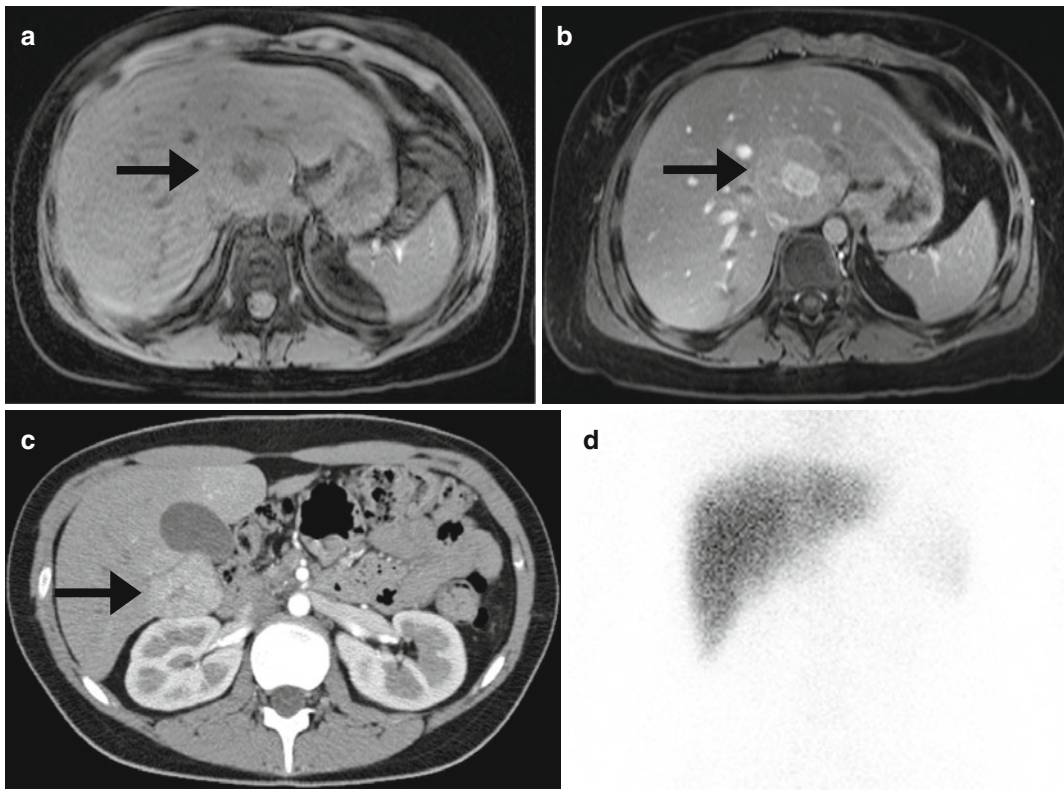


Fig. 5.5 Focal nodular hyperplasia (FNH). On early postcontrast T1-weighted MRI (**a**), FNH appears as an isointense mass with a central area of hypointensity representing the "central scar" (*arrow*). On more delayed postcontrast imaging (**b**), note that the central scar enhances, while the peripheral portion of the mass remains isointense to the liver (*arrow*). In a separate case, FNH appreciated on CT (**c**, *arrow*) demonstrates normal radiotracer uptake on a Technetium-99m sulfur colloid scan; no photopenic defect is appreciated (**d**)

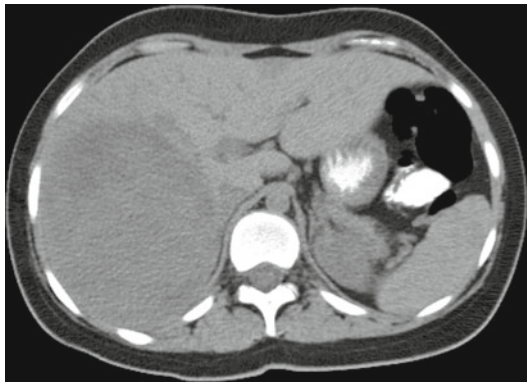


Fig. 5.6 Hepatic adenoma. Axial CT image obtained without the administration of intravenous contrast demonstrates a well-circumscribed mass which is hypodense, related to the presence of fat. In the setting of acute pain, imaging may be used to quickly assess for hemorrhage

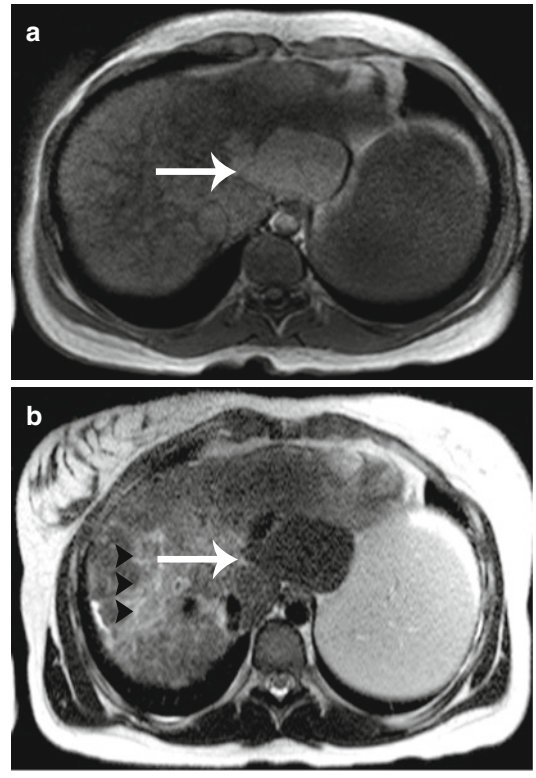


Fig. 5.7 Regenerative nodule on MRI. Axial T1-weighted imaging (**a**) obtained in a 17-year-old female with autoimmune hepatitis demonstrates a slightly hyperintense well-circumscribed mass within the posterior left hepatic lobe (*arrow*). On the corresponding T2-weighted image (**b**), the mass (*arrow*) is uniformly hypointense, compatible with a regenerative nodule. Also note the presence of T2 hyperintensity within the stroma (*arrowheads*) representing areas of fibrosis

increased radiotracer retention due to the lack of bile ducts for clearance from the mass.

Regenerating nodules can vary in size from a few millimeters to several centimeters. Given that they are composed of hepatocytes similar to surrounding parenchyma, they can be difficult to discern by imaging. Frequently there will be a nodular surface pattern of the liver indicating cirrhosis or findings related to portal hypertension can serve to suggest a history of chronic injury and regeneration. At US diffuse small regenerating nodules may simply appear as a heterogeneous liver with architectural distortion of the vascular or biliary structures. When visible by US, nodules typically will appear well circumscribed and hypoechoic. By CT the nodules are usually hypodense compared to adjacent liver on non-contrast imaging and can be isodense or hyperdense following contrast. MRI with contrast is a good imaging technique to demonstrate the extent of liver involvement and may be helpful in the assessment for malignant degeneration Fig. 5.7.

Hepatic cysts are frequent in the pediatric population. These can be seen as an incidental finding, or can be seen in the setting of polycystic disease, such as autosomal recessive polycystic kidney disease. On US, simple hepatic cysts exhibit several distinguishing characteristics, including thin walls, an anechoic internal structure, and posterior acoustic enhancement. On CT and MRI, cysts demonstrate a homogeneous appearance reflecting their water content, with no significant postcontrast enhancement. If large, hepatic cysts can cause mass effect or become symptomatic. In these circumstances percutaneous drainage and sclerotherapy can be considered Fig. 5.8.

Malignant Tumors

Similar to adults, the most common neoplasms involving the pediatric liver are related to metastatic disease associated with neuroblastoma, Wilms tumor, or lymphoma. In children, two

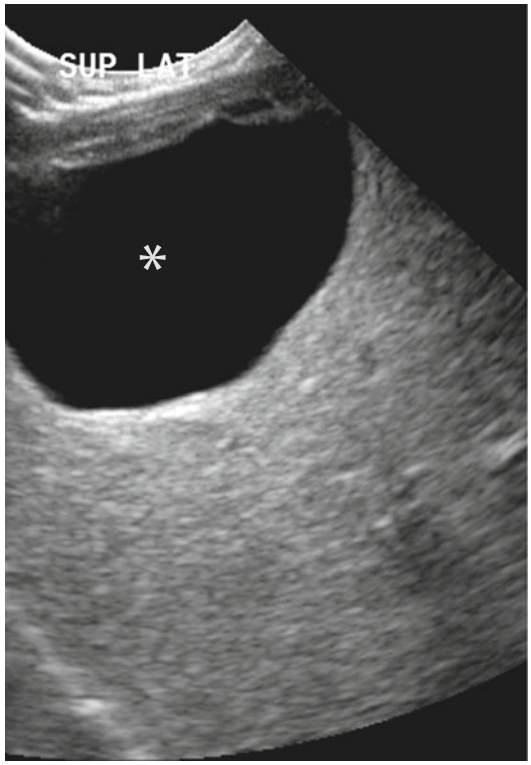


Fig. 5.8 Simple hepatic cyst on ultrasound. A lesion (*) within the right hepatic lobe meets the criteria for a simple cyst: (1) sharp, well-defined walls, (2) sonolucent, and (3) increased through sound transmission

thirds of primary liver tumors are malignant. The most frequent is hepatoblastoma followed by hepatocellular carcinoma (HCC), undifferentiated (embryonal) sarcoma, angiosarcoma, and rhabdomyosarcoma [8].

The role of imaging in assessing malignant tumors starts with defining the extent of the tumors involvement within the liver and determining if there is any extrahepatic disease [9]. US is frequently the initial screening modality to characterize the makeup of the tumor and the vascular structures of the liver. Additional cross-sectional imaging modalities (MRI and/or CT) serve as a complimentary technique to better characterize the extent of the mass, delineate anatomical borders and further assess for vascular invasion, as well as to evaluate for extrahepatic involvement.

The vast majority of *hepatoblastomas* (90 %) manifest before 5 years of age and a significant majority (68 %) present in the first 2 years. Furthermore, 80 % will present as a large mass greater than 12 cm, with the remainder as multiple hepatic masses [10]. By US most are well marginated from adjacent liver parenchyma with a heterogeneous and/or hyperechoic appearance. On CT, areas of calcification are a common finding, as these are seen in approximately 50 % of cases [10]. Following intravenous (IV) contrast for CT and MRI, hepatoblastomas will typically enhance heterogeneously and to a lesser extent than that of normal liver tissue Fig. 5.9. The critical role for imaging lies in the need to identify the extent of involvement and for the presence of vascular invasion preoperatively.

The age of presentation helps to distinguish *hepatocellular carcinoma* (*HCC*) from hepatoblastoma. HCC rarely occurs in children under 5 years of age. The US appearance is variable with typical larger lesions at the time of presentation being more heterogeneous. A hypoechoic halo around the tumor can be identified in those with a capsule [10]. Appreciating that HCC is predominantly supplied by the hepatic artery, the tumor will briskly enhance during the arterial phase by CT and MRI Fig. 5.10. During the portal venous phase, the tumor can have a similar enhancement appearance to that of normal liver or a variable appearance depending upon size, presence of intra-tumoral hemorrhage, or central tumor necrosis as a result of outgrowing arterial supply. As with hepatoblastoma, the critical role of imaging is to characterize the extent of tumor involvement within the segments of the liver, the presence of vascular invasion, and to identify extrahepatic extension. Unfortunately, due to extensive involvement of HCC, frequently the patient may not be a surgical or transplant candidate. Taking advantage of the predominantly arterial supply to the tumor, transarterial chemoembolization (TACE) for the treatment of HCC has been shown to be an effective palliative treatment option that can prolong life in adults affected with HCC [11]. Beyond palliation in children, there are descriptions of TACE serving as a treatment technique to downstage a tumor from unresectable to resectable or as a bridge until a transplant is available [12].

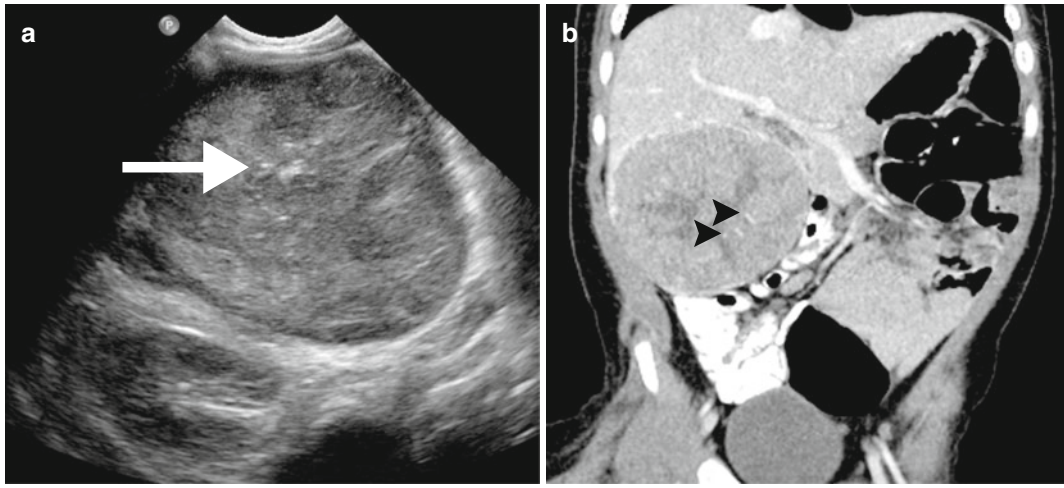


Fig. 5.9 Hepatoblastoma. On ultrasound (**a**), a heterogeneous hyperechoic mass is seen within the right hepatic lobe. Central echogenic foci (*arrow*) represent foci of calcification. Contrast-enhanced CT was subsequently performed (**b**), the coronal plane reformatted CT image similarly demonstrates a well-marginated heterogeneous mass, which enhances to a lesser degree than normal liver parenchyma. Hyperdense foci (*arrowheads*) correlate with the calcifications seen on ultrasound

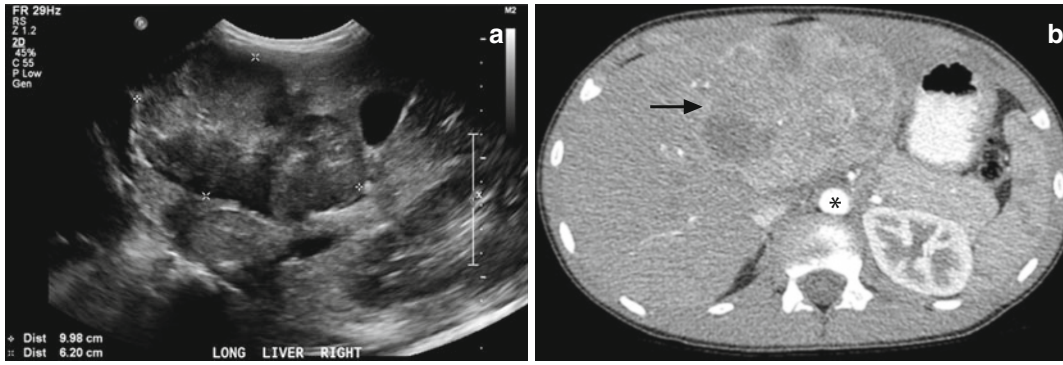


Fig. 5.10 Hepatocellular carcinoma. In this 13-year-old male, ultrasound (**a**) was performed to assess for appendicitis, but incidentally noted is a heterogeneous bilobed circumscribed mass (marked by *cursors*), with a uniform hypoechoic halo. On contrast-enhanced CT (**b**), arterial enhancement of the mass (*arrow*) is appreciated; clues indicating this is the arterial phase can be ascertained by the dense opacification of the aorta (*)

Undifferentiated sarcoma of the liver is an aggressive tumor of mesenchymal origin most frequently affecting children older than 5 years of age. The imaging features unique to undifferentiated sarcoma of the liver are a result of the myxoid component. As a result undifferentiated sarcomas appear to be a solid tumor by US but have a cystic appearance by MRI and/or CT Fig. 5.11.

Angiosarcomas and embryonal rhabdomyosarcoma are rare malignant tumor that can occur anywhere in the body including the liver in children. By imaging these tumors have nonspecific features of a heterogeneous solid liver tumor and are frequently invasive, extending beyond the margins of the liver.

Congenital Neonatal Cholestasis

Biliary atresia (*BA*) is an important cause of neonatal jaundice that must be distinguished from neonatal hepatitis to permit the opportunity for early surgical correction. It accounts for greater than 90 % of obstructive cholestasis cases [13].

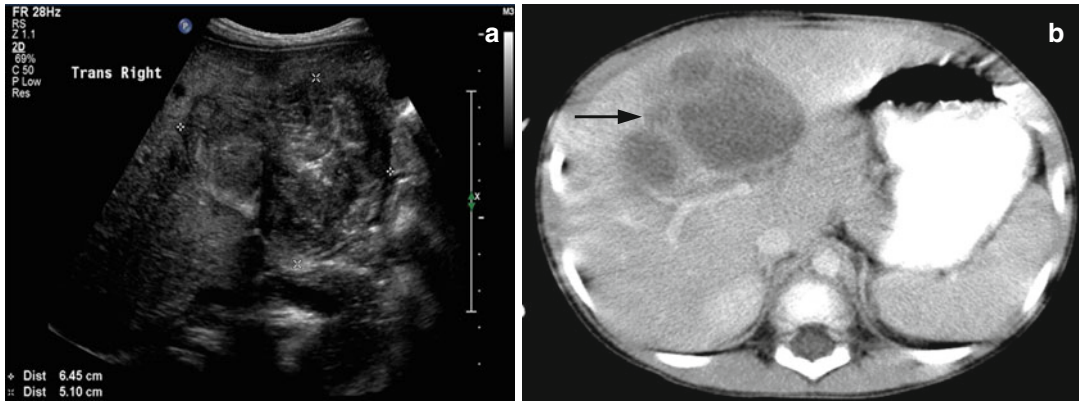


Fig. 5.11 Undifferentiated sarcoma. On ultrasound (**a**), the partially circumscribed mass (*cursors*) is heterogeneous and solid appearing. However, when evaluated on contrast-enhanced CT (**b**), the mass demonstrates low attenuation with intervening areas of density (*arrow*), suggesting it is cystic in nature

US and nuclear medicine studies are the imaging studies of choice to help differentiate these two conditions and are noninvasive. US visualization of a gallbladder favors the diagnosis of neonatal hepatitis, but 20 % of infants with BA may have a gallbladder. The *triangular cord sign* is a commonly accepted finding by US to diagnose biliary atresia [14]. It refers to an echogenic focus that represents the remnant of the obliterated biliary tract seen in the vicinity of the portal vein. Although the sensitivity of this sign is variable by reports, the specificity is regularly greater than 95 % [15]. Nuclear medicine hepatobiliary studies using a radiopharmaceutical that is excreted into the biliary system is a good physiologic imaging study to differentiate between BA and neonatal hepatitis. The excretion of the radiotracer into the bowel excludes the diagnosis of BA. Neonates with BA are able to extract the radiotracer into the liver parenchyma but fail to excrete and pass into the small bowel. An important element of the hepatobiliary scan to distinguish these two entities is that the optimal exam requires a 5-day preparatory course of phenobarbital. The purpose of this premedication is to prevent false positives. In neonatal hepatitis, liver function is impaired, and consequently, excretion into the bowel may be markedly delayed; administration of phenobarbital optimizes liver metabolism of radiotracer Fig. 5.12.

Choledochal cysts are congenital dilations of the common duct. The classic clinical presentation is an infant or young child who presents with jaundice, abdominal pain, and mass. US is the imaging technique of choice to diagnose issues of the biliary ducts and identify choledocal cysts. These will be cystic structures that are in direct communication with the biliary ducts. There are five types: *Type I* is the most common and involves dilation of the common duct Fig. 5.13. *Type II* are diverticular dilations arising off the common duct; *Type III* are choledochoceles; *Type IV* involves cystic dilation of intra- and extrahepatic biliary ducts; and *Type V* or Caroli disease involves only intrahepatic biliary ducts [16] Fig. 5.14.

Infections of the Liver

Viral hepatitis beyond the perinatal period (associated with jaundice) rarely requires imaging during the acute phase of the infection. When present any imaging modality is capable of confirming the hepatomegaly appreciated on physical exam. As a result, US should be the modality of choice for initial imaging screening. In the acute phase, no specific appearance has been described. In fact, the most common appearance is hepatomegaly [17]. Though classically the "starry sky" appearance of the liver has been described, referring to relative echogenicity of the portal triads relative to diffusely edematous liver parenchyma, this has not proven to be a sensitive finding [18]

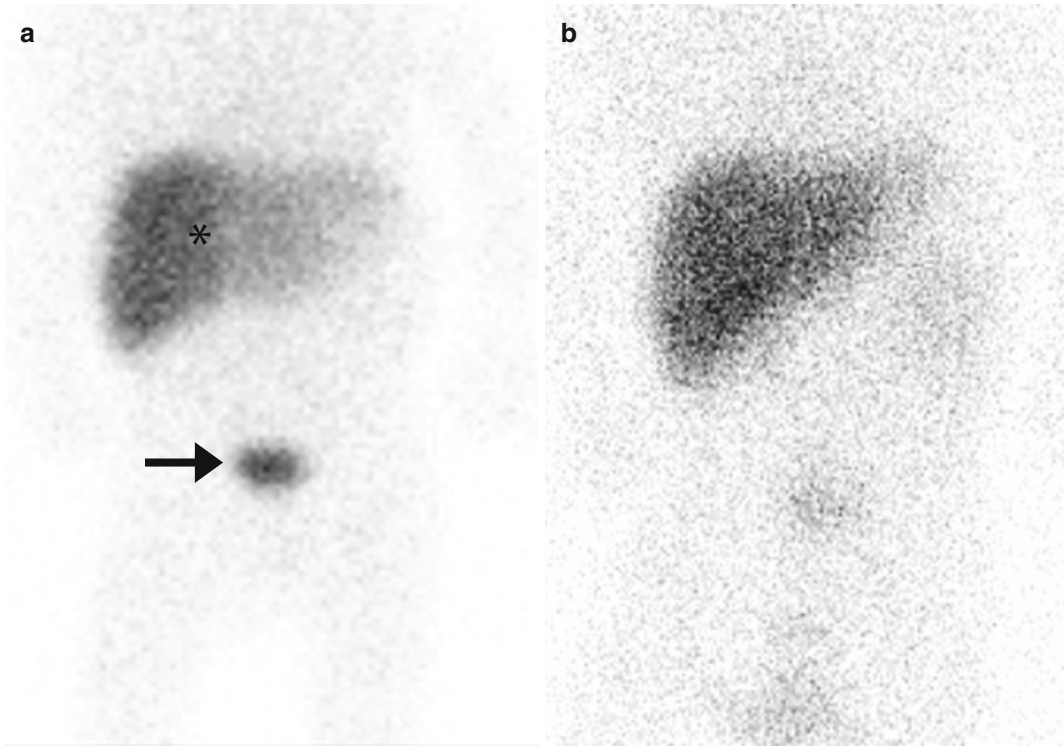


Fig. 5.12 Biliary atresia evaluation using hepatobiliary scan. (**a**) Initial planar image on the left obtained at 1 h post radiotracer injection demonstrates activity within the liver (*) as well as within the urinary bladder (*arrow*). (**b**) Planar image on the right obtained at 24 h demonstrates diffuse hepatic activity without evidence of bowel uptake, compatible with biliary atresia

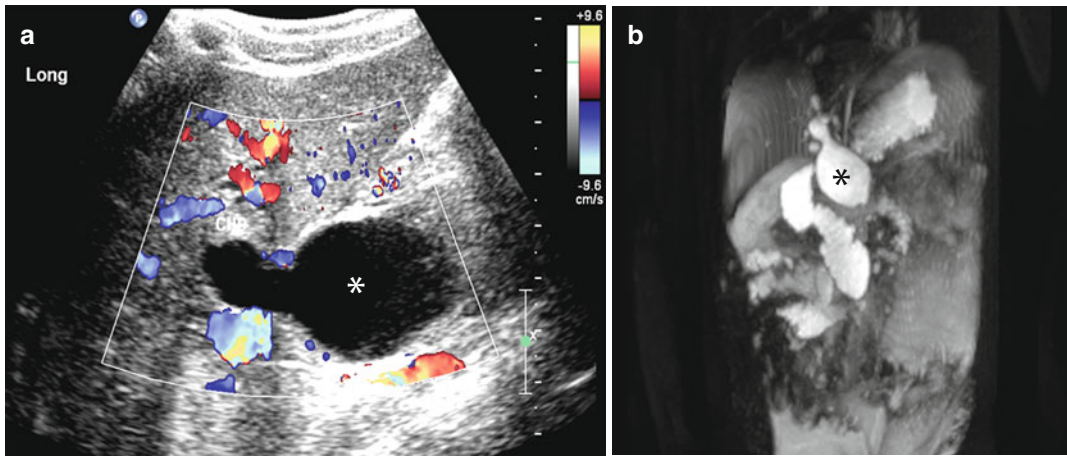
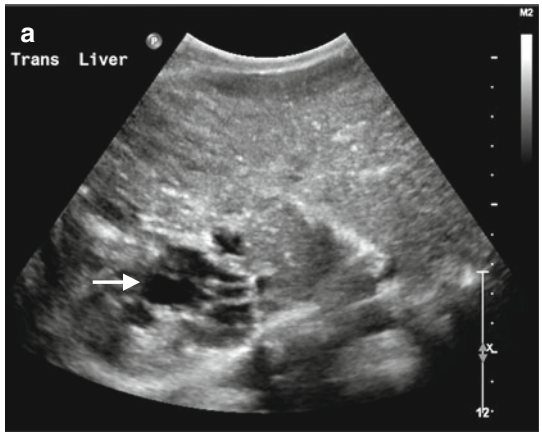


Fig. 5.13 Choledochal cyst, type I. Ultrasound image (**a**) demonstrates fusiform dilatation of the common bile duct (*). Color flow confirms that this is indeed a biliary structure. Coronal maximal intensity projection (MIP) reconstruction from a magnetic resonance cholangiopancreatogram (MRCP) (**b**) confirms this finding of a dilated common bile duct (*) and better demonstrates the anatomy



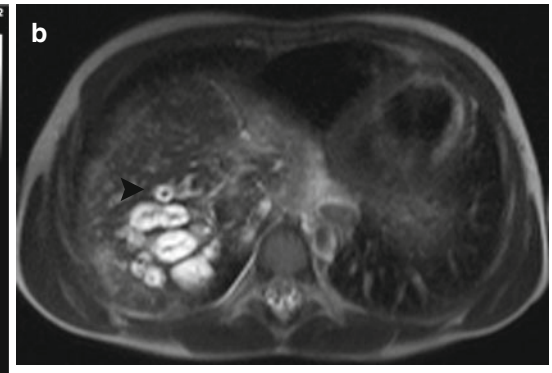


Fig. 5.14 Caroli disease (choledochal cyst, type V). On initial ultrasound evaluation (**a**), cystic foci are focally identified within the posterior right hepatic lobe (*arrow*). Subsequently MRI was performed (**b**) and on axial T2-weighted imaging, multiple dilated biliary ducts are identified in the posterior right hepatic lobe as areas of hyperintensity. Note the presence of central hypointensity (*arrowhead*), which represents the normally sized portal radicle

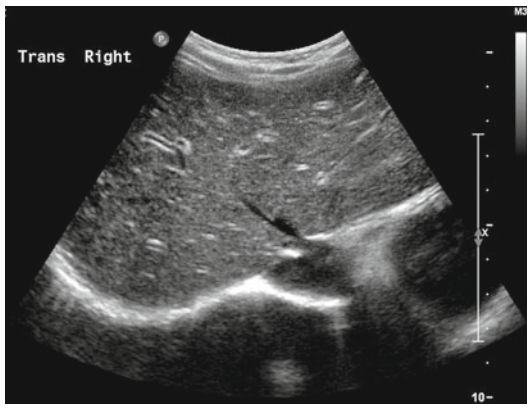


Fig. 5.15 Viral hepatitis related to hepatitis B. There is diffusely decreased echogenicity of the liver parenchyma, relating to diffuse edema, with echogenic foci representing normal portal triads, which are accentuated in this setting. This appearance, the so-called "starry sky" pattern, is not specific to viral hepatitis and can also be seen in heart failure or infiltrating malignancy

Fig. 5.15. In the chronic phase, changes of cirrhosis may develop. Gallbladder wall thickening can also be present as a result of edema.

Pyogenic abscesses are typically associated to children that are immunocompromised or immunosuppressed. Other children susceptible to the development of pyogenic abscess include those with chronic granulomatous diseases, inflammatory bowel disease, or those with other intra-abdominal infections. By US the dominant abscess will be predominantly hypoechoic internally and surrounded by a hypoechoic halo representing hepatic parenchyma edema. There is frequently debris within the abscess, and thus the collection is not simply an anechoic structure as would be a simple cyst. Contrast-enhanced CT and MRI will demonstrate enhancement of the abscess wall frequently surrounded by a halo of parenchymal that has less enhancement compared to normal liver as a result of edema Fig. 5.16. Internally the abscess will have little internal enhancement except for some internal septations, which helps differentiate these from solid tumors. Satellite microabscesses may also be present, which may be identified on cross-sectional imaging: on CT and MRI, these appear as areas of decreased enhancement, while on US, these demonstrate a hypoechoic appearance. There should not be uptake of radiotracer in the lesion by nuclear scintigraphy.

Fungal and parasitic infections can also affect the liver of children. Findings of fungal infections are typically nonspecific and when multiple small lesions are present will appear as hypoechoic lesions by US and hypodense following IV contrast by CT or MRI. Amebic abscesses appear similar to pyogenic abscesses. Echinococcal cysts often exhibit a dominant cyst with several smaller "daughter" cysts Fig. 5.17.

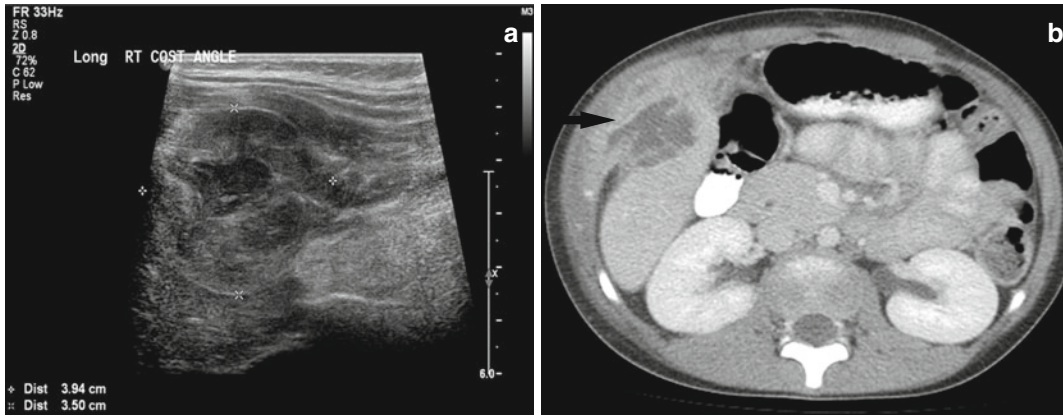


Fig. 5.16 Pyogenic abscess. On initial ultrasound evaluation for right upper quadrant pain (**a**), a heterogeneous mass is appreciated (*cursors*) with no appreciable internal vascularity (color flow image not shown). Abscess was clinically suspected and CT subsequently performed (**b**). On contrast-enhanced CT, a peripherally enhancing fluid collection extends beyond the liver capsule into the overlying anterior abdominal wall (*arrow*). Aspirate yielded methicillin-sensitive *Staphylococcus aureus*

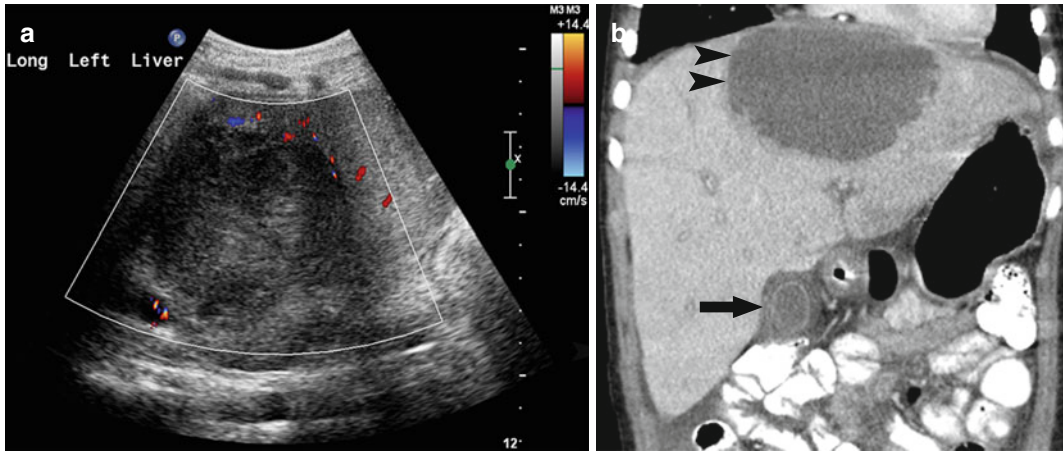


Fig. 5.17 Parasitic abscess. Ultrasound evaluation (**a**) in a child with right upper quadrant pain and a recent travel history to Mexico demonstrates a heterogeneous collection with peripheral vascularity, suspicious for abscess. Contrast-enhanced CT (**b**) performed to better define the extent of the collection demonstrates a low density collection with peripheral enhancement (*arrowheads*). Aspirate yielded *Entamoeba histolytica*. Note also the presence of gallbladder wall thickening (*arrow*), a nonspecific finding

Diffuse Hepatic Parenchymal Disease

Cirrhosis is a chronic condition that can be a result of numerous diseases (congenital or acquired) in children resulting in diffuse fibrotic replacement of the normal hepatic parenchyma. The key role of imaging is to confirm the clinical diagnosis, provide a means to follow the disease progression or response to therapy, and evaluate for complications associated with cirrhosis such as portal hypertension or the development of malignancies. Imaging features include a nodular surface pattern of the liver with heterogeneous appearance of the parenchyma. Typically the right lobe is small with compensatory hypertrophy of the caudate lobe and even the lateral segment of the left lobe. US is a good initial imaging study and can demonstrate diffuse increased echogenicity of the parenchyma Fig. 5.18. Contrast-enhanced CT and MRI can demonstrate

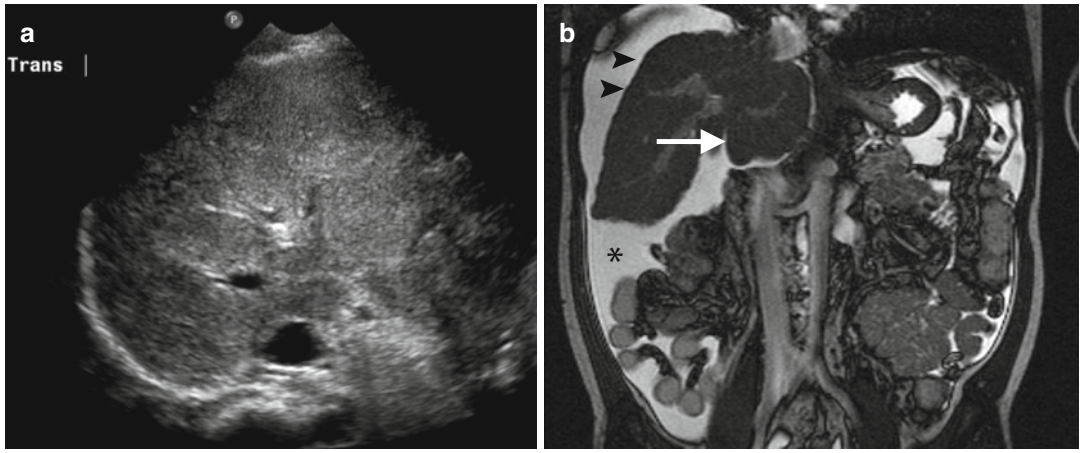


Fig. 5.18 Cirrhosis. On sonographic evaluation (**a**), there is marked heterogeneity of the liver parenchyma. T2-weighted coronal MRI (**b**) demonstrates a shrunken nodular contour to the right hepatic lobe (*arrowheads*). Moderate amount of ascites is appreciated (*). Finally, note compensatory hypertrophy of the caudate lobe (*arrow*)

the diffuse heterogeneity due to regenerating nodules in the background of fibrosis. It can be difficult to differentiate a regenerating nodule from hepatocellular carcinoma by any imaging modality. However, MRI may be more sensitive in distinguishing regenerating nodule versus tumor using various imaging sequences before and after contrast administration, as previously shown (Fig. 5.7).

Fatty infiltration of the liver can be seen in diseases beyond that of cirrhosis. US will show diffuse increased echogenicity of the liver, CT will demonstrate diffuse hypodensity, and MRI will reveal signal intensity on the various sequences that correspond to fat (such as fat suppression sequences) Figs. 5.19 and 5.20. With *hemochromatosis*, by contrast, excessive iron deposition results in diffusely increased hepatic density on CT and strikingly decreased signal intensity on MRI. US is often unrevealing in children with hemochromatosis.

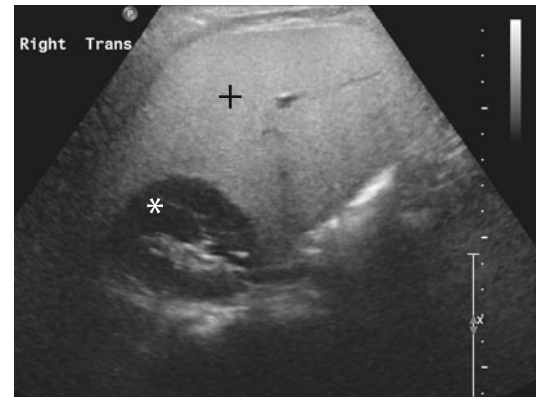


Fig. 5.19 Fatty infiltration of the liver, ultrasound evaluation. On this ultrasound image of the right hepatic lobe, the liver (+) demonstrates a markedly echogenic appearance relative to the right kidney (*). Normally, the liver is isoechoic to slightly echogenic relative to the kidney

Portal Hypertension

Imaging is critical in determining the anatomic level of vascular obstruction and can have great implications regarding the treatment options available for children with complications associated with portal hypertension. Although frequently associated with cirrhosis, *portal hypertension* can also occur as a result of extraparenchymal vascular conditions. Classically portal hypertension can be classified by the anatomic level of portal flow resistance: prehepatic, intrahepatic, or posthepatic [19]. Secondary imaging findings to support portal hypertension can include evidence of cirrhosis, ascites, splenomegaly, varices, an enlarged main portal vein, and a hepatofugal flow pattern Fig. 5.21. *Congenital arterial portal fistulas* can also result in neonatal portal hypertension due to high inflow.

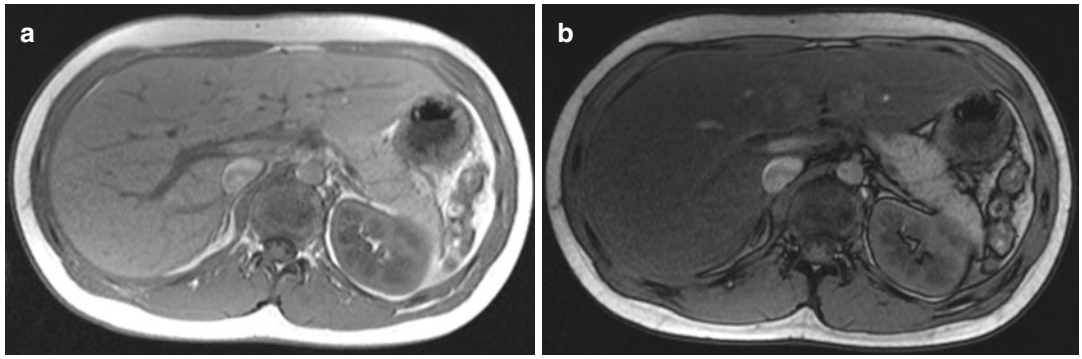


Fig. 5.20 Fatty infiltration of the liver, MRI. (**a**) Chemical shift imaging is used to demonstrate the presence of intracellular fat; (**b**) signal dropout within the liver on "out-of-phase" imaging, as seen on the image at right, reflects such fat deposition

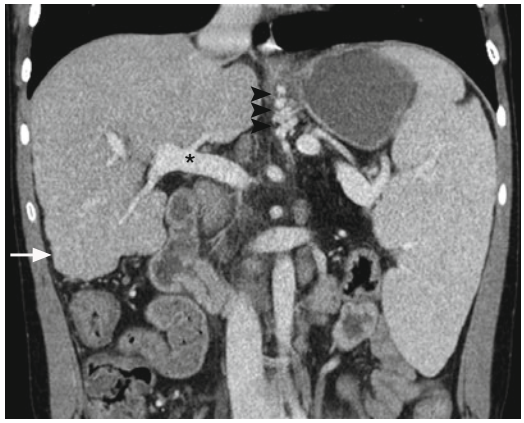


Fig. 5.21 Portal hypertension, secondary findings. On this coronal contrast-enhanced CT, note nodularity of the liver (*arrow*) seen in the setting of cirrhosis. Correspondingly, the main portal vein is enlarged (*), gastroesophageal varices are present (*arrowheads*), and marked splenomegaly is noted

Doppler and spectral waveform US offers a noninvasive method to assess and characterize in real time for hepatofugal flow, patency of the portal vein, varices, or for abnormal fistulous communications. MRI and CT are useful to give a global anatomical assessment of the liver parenchyma and hepatic vasculature, to clarify etiology of the portal hypertension, and assess patency of hepatic vascular structures including the portal vein, for planning of a surgical shunt or a transjugular intrahepatic portosystemic shunts (TIPS). TIPS, which will be discussed in detail later, is a percutaneous endovascular image-guided procedure that has been shown to be effective in treating variceal bleeding associated with portal hypertension in children and infants [20].

The pediatric interventional radiologist can be called upon to perform a percutaneous or transjugular route catheter portal venographic study to directly characterize portal flow, measure portal pressure to determine the portal systemic gradient, demonstrate, and embolize or sclerose varices.

US is a good screening tool for the evaluation of extrahepatic portal vein obstruction as the etiology for *prehepatic portal hypertension*. Chronic main portal vein occlusion will frequently demonstrate an extensive collateral network or *cavernous transformation* in the absence of an identifiable main portal vein by contrast-enhanced CT or MRI Fig. 5.22. Catheter arteriogram of the superior mesenteric artery with delayed angiographic imaging into the portal venous phase is useful to demonstrate the flow pattern of the portal supply and the patency of the intrahepatic portal structures. Determining the patency and location of the left intrahepatic portal vein is valuable in order to determine the feasibility for the construction of a surgical Rex shunt to relieve the portal hypertension. The Rex shunt joins the extrahepatic portal vein to the umbilical segment of the intrahepatic left portal vein [21].

Acute portal vein thrombosis is well demonstrated with US. Color Doppler US will show absence of flow in the portal vein, and grayscale

imaging can show the portal vein to be hyperechoic and even distended due to the clot. Contrast-enhanced MRI and CT may be required to determine if the mesenteric veins or splenic veins are also involved [22] Fig. 5.23.

Budd-Chiari syndrome resulting from obstruction of the hepatic veins or suprahepatic IVC can result in *posthepatic portal hypertension*. The outflow obstruction acutely can lead to hepatic enlargement and a heterogeneous echotexture by US. The major hepatic veins may not be identified by US. Contrast-enhanced CT and MRI will demonstrate an enlarged heterogeneous liver and the absence of identifiable major hepatic veins. There is also prolonged retention of contrast. Caudate lobe hypertrophy is present in 75 % of patients due to the separate venous drainage into the IVC [22].

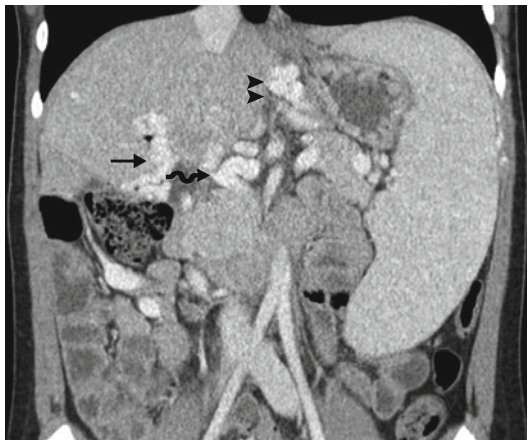


Fig. 5.22 Portal hypertension associated with cavernous transformation as a result of main portal vein occlusion. On this coronal contrast-enhanced CT, the main portal vein is not identified, and intrahepatic cavernous transformation is seen (*arrow*). Associated secondary findings of portal hypertension, including gastroesophageal varices (*arrowheads*) supplied by an engorged coronary vein (*curved arrow*), as well as marked splenomegaly is present

Liver Transplantation Imaging

Following an orthotopic liver transplant in a child, the clinical presentation of acute rejection is nonspecific and the imaging findings are often unrevealing. Therefore, a biopsy is required to diagnose acute rejection. The major role of imaging is to exclude complications that are potentially correctable. Imaging of post transplant complications can be separated into three major categories: (a) vascular complications, (b) biliary tract complications, and (c) perihepatic fluid collections [23]. Furthermore, imaging also offers the opportunity to potentially serve as a tool to perform percutaneous minimally invasive treatment that addresses the various potential complications.

Doppler sonography is the primary initial noninvasive imaging modality to screen for

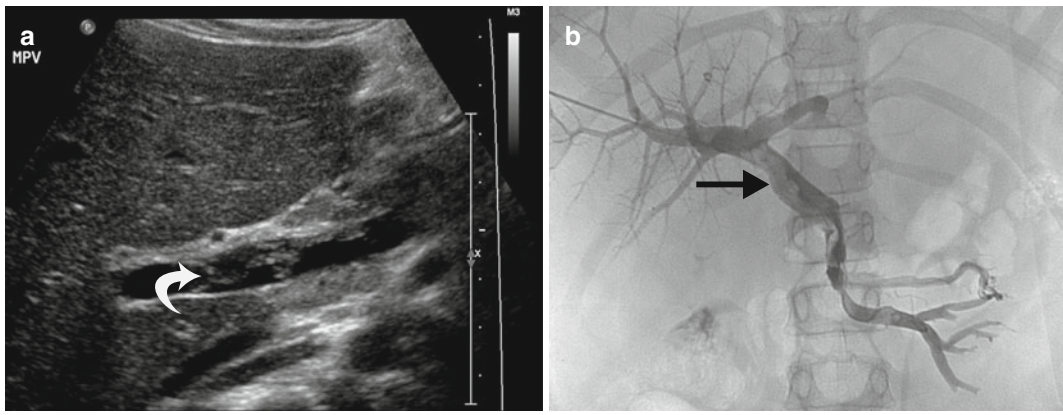


Fig. 5.23 Portal vein thrombosis. On grayscale ultrasound (**a**), note the presence of echogenic debris (*curved arrow*) within the main portal vein, representing thrombus. The patient was then referred for catheter-directed thrombolysis. (**b**) A transhepatic portal venogram performed prior to thrombolysis demonstrates the corresponding filling defect (*arrow*) representing thrombus within the main portal vein

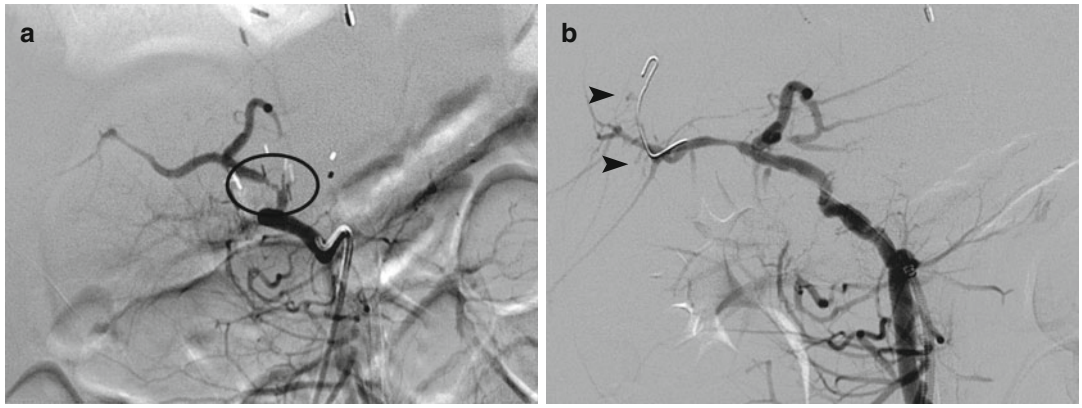


Fig. 5.24 Hepatic artery stenosis after liver transplant. Digitally subtracted angiographic image obtained in an 8-month-old child post transplant initially demonstrates high-grade stenosis of the hepatic artery (**a**, *circled*) with poor hepatic perfusion. Following balloon angioplasty, repeat angiogram was performed (**b**) and demonstrates improvement in caliber of the hepatic artery. Liver perfusion has improved, as signified by increased arborization of arterial vessels (*arrowheads*)

vascular complications. The most common vascular complication involves the hepatic artery followed by the portal vein. Less frequently, hepatic vein or IVC complications can be encountered. Hepatic artery thrombosis is identified as the absence of flow while hepatic artery stenosis will demonstrate increased velocity at the point of stenosis with associated diminished resistive index (RI) and a parvus-tardus waveform beyond the stenosis. An RI of less than 0.50 and a prolonged systolic acceleration time greater than 0.08 s is highly suggestive of a thrombosis or high-grade stenosis. In the acute period following transplant, surgical revision is often necessary. However, beyond 2 weeks it is not uncommon for an interventional radiologist to perform catheter-directed thrombolysis or angioplasty +/− stenting of the culprit vessel Fig. 5.24.

Similarly, US can be used to screen for portal vein or hepatic vein stenosis. CT and MRI although capable of identifying an anatomical narrowing cannot determine the hemodynamic significance of a narrowing. If an abnormality is identified by noninvasive imaging, then proceeding with a percutaneous catheter study for confirmation would be warranted. Although invasive, percutaneous interventions have several benefits. It can definitively confirm the diagnosis with anatomic and physiologic pressure gradient testing as well as offer the opportunity to treat a thrombosis or stenosis with angioplasty or stenting during the same session.

When biliary tract complications, such as bile duct stenosis/stricture with resultant obstruction, are clinically suspected, it needs to be aggressively evaluated. US is a good initial screening method and can demonstrate the dilated biliary ducts. However, it is not uncommon that despite a severe stenosis and clinical evidence for obstruction, biliary ductal dilation will not be present or identified by noninvasive imaging. Anastomotic strictures are frequently a result of fibrosis, whereas intrahepatic strictures are often associated with a history of hepatic arterial compromise leading to biliary ischemia [23]. As a result a *percutaneous transhepatic cholangiogram* (*PTC*) can be performed to directly visualize the biliary system and identify point(s) of stenosis Fig. 5.25. This also offers the opportunity to dilate the stricture(s) and position an internal-external biliary drain for decompression of the obstructed system. CT or MRI is useful to determine if there is an extrinsic compressing causing obstruction of the duct.

The final category in which imaging plays a primary role is for the identification of infected perihepatic fluid collections. These can arise as a

result of a bile leak or the development of an abscess (bacterial or fungal). Once again US is a good screening tool, but frequently CT or MRI are necessary to provide information regarding the full extent of involvement and to help determine if image-guided percutaneous drainage tube placement is feasible. Nuclear scintigraphy can be used to confirm that a fluid collection is a result of a bile leak Fig. 5.26.

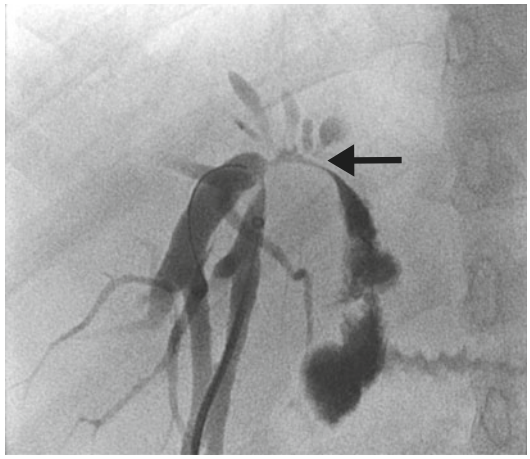


Fig. 5.25 Biliary stricture. Percutaneous transhepatic cholangiogram demonstrates a high-grade anastomotic stricture (*arrow*) in an 8-year-old girl with a split liver transplant

Image-Guided Hepatic Interventions

The utility of imaging has evolved beyond that merely of a diagnostic method in the evaluation of a child's liver. It can also serve as a tool to guide and direct a pediatric interventional radiologist during the performance for the successful execution of a wide variety of minimally invasive percutaneous procedures with either further diagnostic or even therapeutic intent.

In general percutaneous liver biopsies do not require image guidance. However, in the setting of uncorrectable coagulopathy and/or the need to maintain anticoagulation or antiplatelet therapy, a *transjugular liver biopsy* is a reliable alternative image-guided technique that can be used even in children with liver transplants [24]. In addition image guidance is useful when a discrete hepatic lesion needs to be directly sampled. US has the benefit of permitting real-time guidance as well as permitting more diverse off-angle capabilities during the performance of a directed *percutaneous liver biopsy*. US also is the imaging modality of choice for the positioning of a percutaneous *radiofrequency ablation* (*RFA*) probe in the treatment of children with discrete hepatic masses. RFA and other local regional

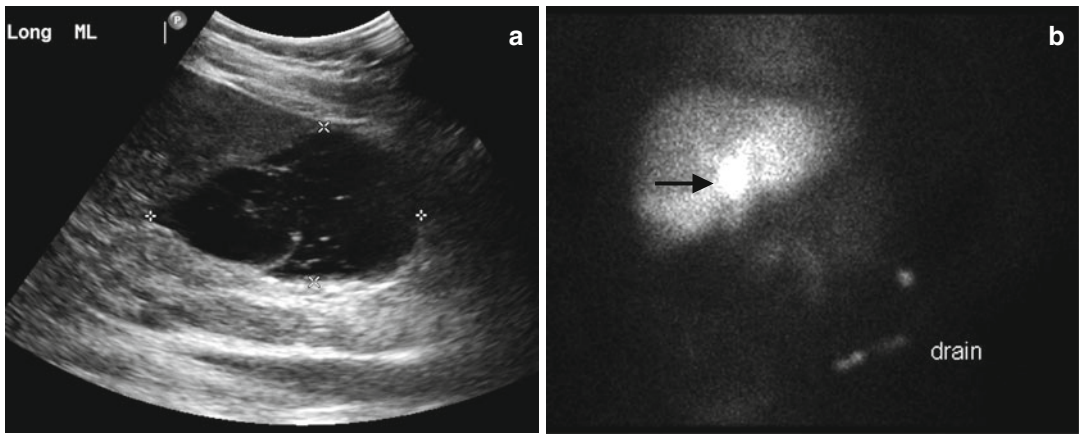


Fig. 5.26 Bile leak, status post transplant. On ultrasound (**a**), a perihepatic fluid collection was identified on initial postoperative imaging (*cursors*). Due to increasing size, bile leak was suspected, and hepatobiliary scan was performed (**b**). On 4 h delayed image, focal radiotracer activity was demonstrated in the right upper quadrant (*arrow*). In a patient status post cholecystectomy, this is representative of bile leak. Note also the presence of radiotracer at the level of an external drain

techniques such as *transarterial chemoembolization* (*TACE*) are increasingly becoming available to pediatric patients as a minimally invasive option for the treatment of localized malignant hepatic tumors. These local regional treatments can serve as an adjunct and/or potential bridge to transplant [25]. TACE takes advantage of the dual vascular supply supporting the liver, in which the majority of tumor supply is contributed from the hepatic arterial system. The hepatic artery can be selectively catheterized from a percutaneous femoral arterial access route to permit direct infusion of the chemotherapeutic agent directly into the arterial supply of the tumor while further increasing the dwell time of the agent by occluding the arterial supply with an embolic particle upon completion. This also leads to tumor devascularization. Frequently both TACE and RFA are performed to address a hepatic tumor and can be complementary in order to treat the entirety of a localized tumor.

Percutaneous *transjugular intrahepatic portal systemic shunt* (*TIPS*) procedure is a feasible alternative to surgical shunt procedures in children with medically uncontrollable complications associated with portal hypertension such as GI bleeding, refractory ascites, hepatic hydrothorax, or hepatorenal syndrome. The construction of a TIPS consists of deploying a stent (frequently a covered endograft) across the hepatic parenchymal tract to join the hepatic venous outflow directly to the portal venous inflow. This effectively forms a shunt that serves to relieve the portal hypertension and decompress varices. TIPS in children and infants has been shown to be clinically effective and durable with low complication rates and do not preclude the possibility to perform a liver transplant in the future [20] Fig. 5.27.

A transjugular approach similar to TIPS or a percutaneous transhepatic approach using US can be performed to gain direct access into an acutely thrombosed portal vein. Spontaneous clearance of acute portal vein thrombosis is unlikely [26]. *Catheter-directed thrombolysis* (*CDT*) permits the ability to directly infuse a thrombolytic agent via a catheter embedded into the clot. A thrombectomy procedure can be performed during the same session as CDT allowing for a lower dose requirement of a thrombolytic agent and speeds up time to achieve clearance compared to systemic intravenous infusion or indirect mesenteric arterial catheter infusion when addressing acute portal vein thrombosis [27].

Percutaneous image-guided *abscess drainage tube* placement procedures are well suited to address hepatic abscesses. US guidance is preferred in children to reduce the need to use ionizing radiation, but CT imaging may be required depending upon abscess location. The placement

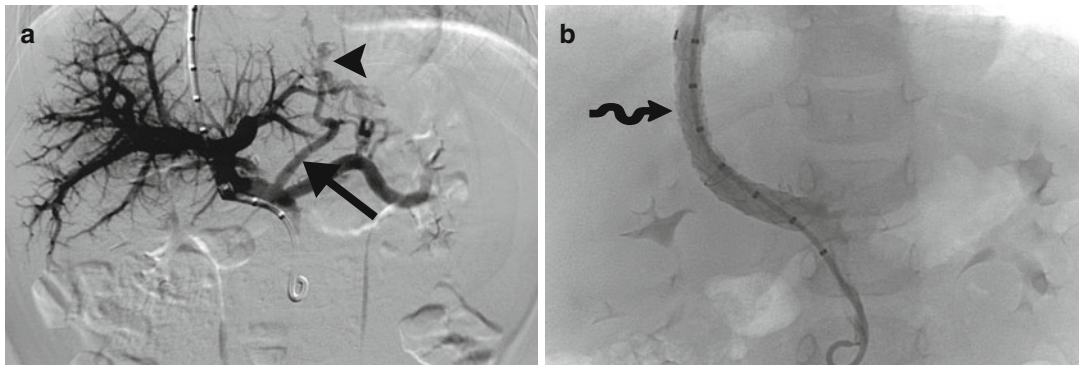


Fig. 5.27 Transjugular intrahepatic portosystemic shunt (TIPS). 17-month-old male with biliary atresia and portal hypertension presenting with recurrent upper GI bleeding. Digitally subtracted image of a portal venogram (**a**) demonstrates opacification of not only the main portal vein but also the coronary vein (*arrow*) and esophageal varices (*arrowhead*). TIPS was constructed, and follow-up (**b**) demonstrates contrast passage through the shunt (*curved arrow*); the coronary vein and varices are no longer opacified. Portal systemic gradient decreased from 14 mmHg pre-TIPS to 5 mmHg post-TIPS

of a drainage tube to drain the abscess is more effective than needle aspiration alone [28]. Percutaneous drainage has been shown to be effective and safe even in the presence of complex and multiple hepatic abscesses [29].

Complications of liver transplants that may benefit from image-guided intervention can be separated into three major categories as discussed under the section of transplant imaging. The needs of these children are best served in a multidisciplinary format with involvement of the pediatric transplant hepatologist, transplant surgeon, and the interventional radiologist.

References

1. Williams C, Frush D. Compendium of national guidelines for imaging of the pediatric patient. Pediatr Radiol. 2012;42:82–94.
2. Brenner D, Hall E. Computed tomography – an increasing source of radiation exposure. NEJM. 2007;357:2277–84.
3. Strauss K, Goske M, Kaste S, et al. Image gently: ten steps you can take to optimize image quality and lower CT dose for pediatric patients. AJR Am J Roentgenol. 2010;194:868–73.
4. Borders H, Barnes C, Parks D, et al. Use of a dedicated pediatric CT imaging service associated with decreased patient radiation dose. J Am Coll Radiol. 2012;9:340–3.
5. Vasanawal S. MRI of the liver – how to do it. Pediatr Radiol. 2010;40:431–7.
6. Chung E, Cube R, Lewis R, et al. Pediatric liver masses: radiologic-pathologic correlation part 1. Benign tumors. Radiographics. 2010;30:801–26.
7. Stocker J. Hepatic tumors in children. Clin Liver Dis. 2001;5(1):259–81.
8. Ishak K, Goodman Z, Stocker J. Tumors of the liver and intrahepatic bile ducts. Washington, DC: Armed Forces Institute of Pathology; 2001.
9. Roebuck D. Assessment of malignant liver tumors in children. Cancer Imaging. 2009;9:S98–103.
10. Chung E, Lattin G, Cube R, et al. Pediatric liver masses: radiologic-pathologic correlation part 2. Malignant tumors. Radiographics. 2011;31:483–507.
11. Llovet J, Real M, Montana X, et al. Arterial embolization or chemoembolization versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomized controlled trial. Lancet. 2002;359:1734–9.
12. Malogolowkin M, Stanley P, Steele D, et al. Feasibility and toxicity of chemoembolization for children with liver tumors. J Clin Oncol. 2000;18:1279–84.
13. Lee MS, Kim M, Lee MJ, et al. Biliary atresia: color Doppler US findings in neonates and infants. Radiology. 2009;252(1):282–9.
14. Park W, Choi S, Lee H. The ultrasonographic "triangular cord" coupled with gallbladder images in the diagnostic prediction of biliary atresia from infantile intrahepatic cholestasis. J Pediatr Surg. 1999;34:1706–10.
15. Mittal V, Saxena A, Sodhi K, et al. Role of abdominal sonography in the preoperative diagnosis of extrahepatic biliary atresia in infants younger than 90 days. AJR Am J Roentgenol. 2011;196:W438–45.
16. Kim O, Chung H, Choi B. Imaging of the choledochal cyst. Radiographics. 1995;15:69–88.
17. Tchelepi H, Ralls P, Radin R, Grant E. Sonography of diffuse liver disease. J Ultrasound Med. 2002;21(9):1023–32.
18. Giorgio F, Amoroso P, Fico P, et al. Ultrasound evaluation of uncomplicated and complicated acute viral hepatitis. J Clin Ultrasound. 1986;14(9):675–769.
19. Pariente D, Franchi-Abella S. Paediatric chronic liver diseases: how to investigate and follow up? Role of imaging in the diagnosis of fibrosis. Pediatr Radiol. 2010;40:906–19.
20. Vo N, Shivaram G, Andrews R, et al. Midterm follow-up of transjugular intrahepatic portosystemic shunts using polytetrafluoroethylene endografts in children. J Vasc Interv Radiol. 2012;23(7):919–24.
21. Bambini D, Superina R, Almond P, et al. Experience with the Rex shunt (mesenterico-left portal bypass) in children with extrahepatic portal hypertension. J Pediatr Surg. 2000;35:13–9.
22. DeLeve L, Valla D, Garcia-Tsao G. Vascular disorders of the liver. Hepatology. 2009;49:1729–64.
23. Singh A, Nachiappan A, Verma H, et al. Postoperative imaging in liver transplantation: what radiologists should know. Radiographics. 2010;30:339–51.
24. Miraglia M, Maruzzelli M, Spada M, et al. Transjugular liver biopsy in pediatric patients with left split liver transplantation and severe coagulation impairment. Pediatr Transplant. 2012;16:58–62.
25. Lencioni R, Crocetti L, Pina M, Cioni D. Percutaneous image-guided radiofrequency ablation of liver tumors. Abdom Imaging. 2009;34:547–56.
26. Hall T, Garcea G, Bilku D, Dnnison A. Management of acute non-cirrhotic and non-malignant portal vein thrombosis: a systematic review. World J Surg. 2011;35(11):2510–20.
27. Darcy M. Single-session treatment of portal vein thrombosis using combined pharmacomechanical thrombolysis. Seminars Interv Radiol. 2007;24(3):327–32.
28. Zerem E, Hadzic A. Sonographically guided percutaneous catheter drainage versus needle aspiration in the management of pyogenic liver abscess. AJR Am J Roentgenol. 2007;189(3):W138–42.
29. Liu C, Gervais D, Hahn P, et al. Percutaneous hepatic abscess drainage: do multiple abscesses or multiloculated abscesses preclude drainage or affect outcome? J Vasc Interv Radiol. 2009;20(8):1059–65.

Phenotypes of Liver Diseases in Infants, Children, and Adolescents

6

Simon Horslen

Introduction

Liver disease in childhood is rare and is frequently the cause of dismay as the medical care provider attempts to recall the myriad of diagnoses that they read about during their training but may never have encountered. In the minds of most, jaundice or elevated liver function tests equals liver disease, but many questions tend to surface: What is the etiology? Can referral wait until more diagnostic information is available? What are the appropriate tests to do and how urgently? What is the likely progression and are there potentially life-threatening consequences of delayed diagnosis and treatment? None of these questions can be answered without formulating a reliable differential diagnosis.

The aim of this chapter is to describe hepatic disease phenotypes based simply on age and primary manifestation of liver disease such as cholestasis, hepatomegaly, or acute liver failure (see Table 6.1) and to provide a reasonably comprehensive list of hepatic diseases that may present with these clinical phenotypes. The hope is to help primary medical providers determine the differential diagnosis and thus guide early studies and appropriate referral and pediatric gastroenterologists and trainees to determine a comprehensive differential diagnosis for their patients on which to base a rational work-up and management plan. This chapter is not intended to be a plan for the detailed investigation of a child with liver disease or a commentary on the probability of any given diagnosis when encountering a patient that fulfils a particular clinical phenotype, but to list the diagnoses that have been recognized, even if only very rarely. Any schema describing the investigations to work through a differential diagnosis is dependent on local conditions dictated by resource availability, priorities, and the probability of a specific diagnosis. For example, in the northwest of the USA, dengue fever would not be included in most differential diagnoses of a local patient, but in Southeast Asia, this would be a major priority.

Hepatology patients range from day-old premature infants to 18-year-old young adults and from seemingly healthy children in the outpatient clinic to profoundly sick infants in the intensive care unit (ICU) on life support. The best way to determine a diagnosis safely and efficiently is to develop a deep understanding of the pathophysiology of liver disease, but even for the most experienced, a checklist of diagnostic possibilities may be helpful to ensure no oversights.

Physicians have always suspected a patient of having disease of the liver from a relatively limited number of clinical signs or symptoms, namely, jaundice, a palpable liver mass or generalized hepatomegaly, splenomegaly, or ascites [1]. Within the last century, blood test abnormalities [2] or aberrant anatomical findings on imaging, commonly

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Table 6.1 List of clinical phenotypes in pediatric patients with liver involvement

The sick infant in NICU with other known disease (frequently a premature infant with respiratory distress syndrome or chronic lung disease of prematurity and often on parenteral feeding support)
(a) Congenital ascites with hepatomegaly or hepatosplenomegaly
(b) Hemorrhage (e.g., gastrointestinal or intracranial) with coagulopathy or low platelets
(c) Necrotizing enterocolitis or surgical resection for congenital bowel malformation
(d) Hepatomegaly or liver dysfunction associated with congenital heart disease or heart failure
(e) An abdominal mass
(f) Cholestatic jaundice or abnormal transaminases
The sick infant in the emergency room or transferred from another hospital
(a) Hepatitis – acute hepatitis or fulminant hepatic failure
(b) Metabolic decompensation – acidosis, hyperammonemia, or hypoglycemia
(c) Abdominal mass with heart failure or Kasabach-Merritt syndrome
(d) Hepatomegaly and abnormal liver function with systemic infection
The stable infant referred to clinic with liver disease
(a) Cholestasis
(b) Elevated liver function tests
(c) Hepatomegaly
(d) Abnormalities found on ultrasound
(e) Asymptomatic infant of mother with chronic viral hepatitis
(f) Asymptomatic sibling of child with known liver disease
Younger child with liver involvement (cholestasis, transaminitis, and/or hepatomegaly)
(a) Acute hepatitis
(b) Presentation of a chronic liver disease
(i) Jaundice
(ii) Ascites
(iii) Gastrointestinal bleeding
(c) Hepatomegaly or hepatosplenomegaly on routine exam
(d) Abdominal mass
(e) Elevated liver function tests on routine screening or tests drawn for other reasons
(f) Abnormal liver or spleen finding on abdominal ultrasound done for other reasons
Older child/adolescent with liver involvement (cholestasis, transaminitis, and/or hepatomegaly)
(a) Acute hepatitis
(b) Presentation of a chronic liver disease
(i) Jaundice
(ii) Ascites
(iii) Gastrointestinal bleeding
(c) Hepatomegaly or hepatosplenomegaly on routine exam
(d) Abdominal mass
(e) Elevated liver function tests on routine screening or tests drawn for other reasons
(f) Abnormal liver or spleen finding on abdominal ultrasound done for other reasons
Acute liver failure
(a) Acute hepatitis with coagulopathy and encephalopathy
Consult from other specialties with liver dysfunction in association with known disease
(a) Gastroenterology
(i) Abnormal liver function or hepatomegaly associated with known gastrointestinal disease, e.g., inflammatory bowel disease or celiac disease
(ii) Transaminitis or cholestasis in children on long-term parenteral nutrition for intestinal failure
(b) Cardiology
(i) Ascites (often chylous) with Fontan circulation
(ii) Hepatosplenomegaly, abnormal LFTs, or ascites-associated heart failure
(iii) Ischemic hepatitis (shock liver) and acute liver failure post-cardiac surgery
(iv) Alagille syndrome

Table 6.1 (continued)

(c) Hematology/oncology
(i) Liver dysfunction associated with liver tumor
(ii) Cholestasis, elevated transaminases, and hepatomegaly in child with hematologic disease, e.g., sickle-cell anemia
(iii) Hepatitis and/or cholestasis associated with chemotherapeutic agents
(iv) Hepatitis and/or cholestasis following bone marrow/stem cell transplantation
(d) Pulmonology
(i) Liver diseases in patients with cystic fibrosis
(ii) Hepatitis in patients with other pulmonary diseases, e.g., sarcoidosis, histoplasmosis, or tuberculosis
(e) Nephrology
(i) Hepatomegaly and splenomegaly associated with polycystic kidney disease
(ii) Cholestasis or abnormal liver function and tubulointerstitial nephritis/nephronophthisis
(iii) Portal hypertension associated with IgA nephropathy or membranoproliferative glomerulonephritis
(f) Endocrine
(i) Hyperlipidemic and obese patients with abnormal transaminases
(ii) Hepatomegaly and transaminitis in poorly controlled diabetics
(iii) Cirrhosis in patients with generalized lipodystrophy
(g) Immunology
(i) Hepatitis or cholestasis in children with defined immunodeficiency syndromes
(ii) Granulomatous hepatitis in chronic granulomatous disease
(h) Rheumatology
(i) Hepatitis associated with lupus, other generalized autoimmune conditions, juvenile idiopathic arthritis, and macrophage activation syndrome
(i) Genetics
(i) Liver abnormalities as part of a dysmorphic or multisystem syndrome
Others: Isolated splenomegaly, unconjugated jaundice, isolated ascites, itching, hemorrhage, or hypocalcemia and rickets

sonography, have suggested liver problems prior to the appearance of clinical signs. Patients commonly have other features of disease such as hypoglycemia, metabolic bone disease, or anemia, and these additional features do indeed constitute the patient's disease phenotype and aid with the refining of a differential diagnosis. However, one should be wary of attempting to define precise and detailed liver disease phenotypes because they may fail to take into account the great variability in how individual disease states may be manifest and consequently lead to a diagnosis being overlooked if not "classical" in its presentation.

Disclaimer

The goal of this chapter is to form the basis of an organized list of diseases of the pediatric liver. It is likely that the most significant gaps will relate to extremely rare hepatological diagnoses and multisystem genetic syndromes with occasional incidental hepatic associations.

Many diagnoses are the subject of detailed discussion in other chapters (and cross-reference will be included where appropriate); however, the more esoteric and unusual may require other resources for in-depth review.

Clinical Assessment: The Importance of a Careful History and an Expert Physical Examination

The value of careful history taking cannot be overemphasized. It is important to understand the timing and the evolution of the features of liver disease, as well as any symptoms originating outside of the liver and gastrointestinal tract. A full inquiry about birth and pregnancy history, previous medical history, family history, and social history is crucial and should include any exposure to drugs and dietary supplements; household, garden, or garage products; and complementary medicinal products. Similarly, a careful history of contact with infectious disease, foreign travel,

and occupational exposure should be obtained. It is important to examine the urine and stool color if possible or, at a very minimum, have the parents describe these colors; stool color charts have been designed for this purpose (see chapter 13). It is not adequate to ask them whether the urine and stools are normal.

Similarly a skilled physical examination will not only assist with making a prompt diagnosis but can save considerable cost to the care of the patient. Eliciting clinical signs on physical examination of a patient may not be easy, particularly with a squirming infant, a frightened toddler, a giggling child, or an overtly belligerent adolescent, but the skills must be well learned and then practiced patiently and conscientiously throughout one's career. It is not enough to expect the correct diagnosis to eventually reveal itself if a multitude of laboratory tests and imaging studies are ordered. In the absence of an expert physical examination, a child with ascites of cardiac origin may undergo multiple studies of the liver over many weeks before the correct etiology is recognized. Similarly the patient with pancytopenia, but in whom the modest splenomegaly is not recognized, may be investigated extensively (and expensively) in the hematology department before portal hypertension with hypersplenism is diagnosed.

In the clinical assessment of a patient with suspected liver disease, there are a number of general considerations to be taken into account when trying to formulate a differential diagnosis. The first of these is age at presentation, primarily because many diseases have a typical age (range) of onset. This may allow us to eliminate some diseases completely, for example, primary biliary cirrhosis has never been described in a young child, and to relegate the possibility of yet others, for example, hepatomegaly first encountered in an adolescent is unlikely to be due to glycogen storage disease. Evidence of liver disease present at birth may imply an intrauterine process such as defects of embryonic developmental genes, congenital infections, or isoimmune phenomena, whereas early postnatal disease may indicate an inborn error of metabolism or infection acquired perinatally.

The next general consideration is whether there is a predisposition to developing certain forms of liver disease, i.e., other medical conditions that may predispose to specific liver diseases such as primary sclerosing cholangitis in patients with preexisting ulcerative colitis or hepatoblastoma in a child with Beckwith-Wiedemann syndrome. Similarly the onset of liver disease during pregnancy such as HELLP (hypertension, elevated liver tests, and low platelets) syndrome or acute fatty liver of pregnancy may indicate metabolic disease in the fetus and usually carrier status in the woman. A detailed family history will help reveal potential familial predisposition such as parental consanguinity or a previous sibling with a single-gene defect. Occasionally there is manifestation of a heterozygous status in relatives such as cholelithiasis in the family of the child with MDR3 deficiency. In a patient suspected of having autoimmune hepatitis, a history in the extended family of other autoimmune conditions such as lupus or hypothyroidism is likely to be relevant.

Environmental exposure to infectious or toxic agents is another general consideration that may alter probabilities within a list of differential diagnoses. Infectious etiologies may gain priority due to an endemic risk at place of abode or recent travel to an at-risk area. A history of drinking or bathing in water from a local supply while traveling raises the possibility of a whole range of infectious agents capable of inducing liver injury. International travel is not, however, a prerequisite for unusual infections that may manifest as liver disease; for example, Histoplasma capsulatum, a cause of granulomatous hepatitis especially in the immunocompromised, is endemic in some central and southern US states [3], and baylisascariasis, a rare cause of hepatomegaly and meningoencephalitis, has been described in young children who have ingested soil contaminated with raccoon feces [4]. Knowledge that a mother has a chronic transmissible infection, particularly hepatitis B but also including hepatitis C, HIV, malaria, and Chagas disease [5], may simplify the diagnosis of an infant with liver disease. Exposure to industrial toxins, particularly from pollutant

spills, is usually reported, but particularly in the developing world, there may not be a full disclosure; therefore, direct questioning is essential. Also all drug exposures should be assessed including herbal and homeopathic remedies and dietary supplements. Ingestion of wild mushrooms is a particular risk. Amanita species are found in many parts of the world, while other foods collected straight from nature may be either directly toxic or chemically contaminated. Not all effects of environmental exposure occur immediately upon exposure; minocycline-induced autoimmune hepatitis takes a period of 12–20 months from initiation of treatment for teenage acne, for instance, before liver dysfunction appears [6].

Triggering factors are specific environmental exposures that do not cause disease but reveal its presence. Glycogen storage disease type 1 may become manifest only at weaning from breastfeeding or during an episode of poor intake due to intercurrent illness. Hereditary fructose intolerance may be revealed by the inadvertent administration of intravenous fructose or medicine containing sucrose or sorbitol [7] and the administration of valproate to an infant with seizures due to an unrecognized mitochondrial cytopathy may trigger acute liver failure [8].

Why Diagnosing Liver Disease May Be Difficult

Pediatric liver disease is not common in the general practitioners' experience, and therefore, the diagnoses and its diagnostic work-up may be relatively unfamiliar. Additionally, although established liver disease is rare, it is not uncommon to see mild abnormalities of liver function tests. In infants jaundice is frequently seen in the form of physiologic neonatal jaundice and breast milk jaundice. Although these causes manifest as unconjugated hyperbilirubinemia, it does mean that the mere presence of jaundice in a newborn infant does not necessarily raise the suspicion of liver disease. The difficulties of diagnosis are doubly increased when dealing with acute liver failure; not only are there severe constraints on the time available for a full diagnostic work-up but hepatic metabolic function is severely deranged, sometimes making the differentiation between primary and secondary metabolic abnormalities virtually impossible by biochemical means. Fortunately there are now much greater nucleic acid-based options for primary diagnosis of specific inborn errors of metabolism, but results can take time to become available. Another concern in the early detection of pediatric liver disease relates to the difficulty of maintaining basic clinical examination skills in an age of advanced imaging techniques. The ability of the physician to use hands and eyes to detect clinical signs such as hepatomegaly, ascites, and cutaneous features of liver disease needs to be carefully nurtured among medical students and junior medical staff. Finally, there are a number of terms that are commonly used in regard to liver disease that mean the diagnosis has not been identified such as "idiopathic" fulminant hepatic failure, "cryptogenic" cirrhosis, and "neonatal hepatitis." It is important to remember that these are not diagnoses but an admission that the diagnostic work-up has failed to identify the primary cause of liver disease. The acceptance of these terms as seemingly discreet diseases may be in some way responsible for incomplete diagnostic work-up. Narkewicz et al. describe how frequently a suboptimal diagnostic work-up is seen in children presenting with acute liver failure when given a diagnosis of idiopathic liver failure even in the context of a multicenter study [9].

Phenotypes

The Sick Newborn

Most consultations done for suspected liver disease in the newborn nursery or neonatal intensive care are on infants with other reasons to be there and have not yet been home. Less frequently their primary reason is because of liver dysfunction at or within days of birth. Liver dysfunction may be inherent from congenital infection or inborn error of metabolism or secondary to other peri- or postnatal events such as isch-

emia, necrotizing enterocolitis, congenital heart disease, abdominal surgery, or the need for parenteral feeding.

Clinical features which may indicate liver disease include ascites, hyperammonemia, hypoglycemia and coagulopathy, hepatomegaly with or without splenomegaly, cholestasis, and abnormal liver function tests. Congenital liver disease may result in early fetal loss, but the classical presentation is the hydropic infant with hepatomegaly, congenital ascites, hypoalbuminemia, coagulopathy, and very-early-onset cholestasis. These findings are not specific for primary congenital liver disease as congenital ascites is commonly due to severe fetal anemia, both isoimmune and nonimmune causes such as alpha thalassemia, or fetal heart failure, and is mostly seen in the setting of generalized hydrops fetalis (see Table 6.2 for a more complete list of causes). When primary liver disease is suspected, an important diagnosis to consider is "neonatal hemochromatosis." Studies by Whitington and colleagues have demonstrated that the majority of these cases are due to a maternal factor (presumably an IgG alloantibody) crossing the placenta and inducing complement-mediated hepatocellular injury, one result of which is excessive iron deposition [10]. This immune-mediated "neonatal hemochromatosis" condition has been renamed *gestational alloimmune liver disease* (GALD) (see chapter 10).

There are also infants who were seemingly healthy at birth but within a few hours to a few days develop features of acute liver failure (see Table 6.3) with coagulopathy and hyperammonemia. There is considerable overlap with

Table 6.2 Causes of congenital ascites

Immune	Isoimmune hemolytic disease of newborn
	Gestational alloimmune liver disease (neonatal hemochromatosis)
	Congenital lupus erythematosus
Nonimmune anemias	Other hemolytic disorders affecting fetus
	Disorders of red cell production, e.g., α-thalassemia
	Congenital leukemia
	Fetal hemorrhage
	Twin-to-twin transfusion
Infectious	Parvovirus B19
	Cytomegalovirus (CMV)
	Syphilis
	Herpes simplex
	Toxoplasmosis
	Hepatitis B
	Adenovirus
	Ureaplasma urealyticum
	Listeria monocytogenes
	Enterovirus
	Lymphocytic choriomeningitis virus (LCMV)
Chromosomal	Cri-du-chat syndrome (chromosomes 4 and 5)
	Trisomy 13
	Trisomy 18
	Trisomy 21 (Down syndrome)
	Turner syndrome
Genetic syndromes	Smith-Lemli-Opitz syndrome
	Beckwith-Wiedemann syndrome
	Klippel-Trenaunay-Weber syndrome
	Yellow nail syndrome
Metabolic	Glycogen storage disease, type IV
	Gaucher disease, type II
	Morquio disease (MPS IV)
	Hurler syndrome (MPS 1H)
	Sly syndrome (MPS VII)
	Farber disease
	G_{M1} gangliosidosis
	Sialidosis II
	I-cell disease
	Niemann-Pick type C
	Wolman
	Infantile sialic acid storage disorder
	Primary carnitine deficiency
	Congenital defects of glycosylation
Cardiovascular	Congenital heart disease
	Congenital arrhythmias
	Congenital myocarditis
Liver tumors	Hepatoblastoma
	Mesenchymal hamartoma
	Hemangioendothelioma
Miscellaneous	Intussusception
	Meconium peritonitis
	Lymphangiectasia
	Urinary tract malformations
	Placental abnormalities
	Teratoma
	Extra-abdominal tumors
	Hypothyroidism and hyperthyroidism

Table 6.3 Causes of acute liver failure in infancy

Infectious	
Viral	Herpes simplex
	Varicella zoster
	Cytomegalovirus
	Human herpes virus 6
	Adenovirus
	Enterovirus
	Hepatitis B
	Parvovirus B19
	Influenza
Bacterial	Septicemia
Protozoal	Malaria
Genetic	Tyrosinemia type 1
	Galactosemia
	Hereditary fructose intolerance
	Fructose 1,6-bisphosphatase deficiency
	Organic acidemias
	Urea cycle disorders
	Fatty acid oxidation defects
	Mitochondrial/respiratory chain defects
	Carnitine defects
	Niemann-Pick type C
	Glycogen storage disease type 1
Immune	Neonatal hemochromatosis
	Hemophagocytic lymphohistiocytosis
	Autoimmune hemolytic anemia with giant cell hepatitis
Vascular	Heart failure
	Cardiac surgery
	Ischemic hepatitis
	Budd-Chiari
	Congenital portal vein anomalies
Neoplastic	Infantile leukemia
	Hemangioendothelioma
Nutritional/toxic	Drugs/toxins
Other	Reye syndrome

the causes of congenital ascites although viral infection acquired at or around the time of birth is more likely, disseminated neonatal herpes simplex being a frequently encountered cause. Generalize septicemia and, in at-risk populations, congenital malaria may also present in this manner. Inborn errors of metabolism with infantile acute presentation may include lysosomal storage defects but are more likely to be defects of intermediate metabolism such as galactosemia, organic acidemias, and glycogen storage disease type I (see chapter 8). GALD may also present similarly although there is usually evidence of chronic liver disease. An important diagnosis to exclude, because liver transplantation is contraindicated, is hemophagocytic lymphohistiocytosis (HLH), but HLH characteristically has a more delayed onset with patients tending to present later in infancy (see chapters 12 and 29). Severe liver dysfunction may be seen with vascular compromise as well, such as thrombosis of the inferior vena cava or hepatic veins, and with congenital portal vein anomalies. Heart failure secondary to congenital heart disease may result in an ischemic hepatitis, and rarely congenital leukemia or myelodysplasia can present with acute liver failure.

Commonly, despite intensive work-up, no specific cause is found; however, as has been cautioned in the introduction, the diagnosis of "idiopathic" neonatal hepatitis should not be assumed until all recognized causes have been excluded (see chapter 12). Certainly the proportion of patients with this label of idiopathic neonatal hepatitis has fallen over the years with the discovery of novel conditions and disease mechanisms, and it is to be hoped that eventually the term will become unnecessary. Until then idiopathic neonatal hepatitis should be seen as a challenge to further diagnostic adventure rather than an end in itself.

Although not all infants in the neonatal unit with evidence of liver disease are as sick as patients with hydrops or acute liver failure, the differential diagnosis for hepatitis with or without cholestasis remains large (see Table 6.4). Certainly the diagnoses that were entertained for the sicker group of infants may be found manifest with less severe disease; thus, congenital infection, storage disorders, and GALD still appear in the differential diagnosis for infants with simple cholestasis and elevated transaminases, but a larger spectrum of etiologies also needs to be considered including genetic and chromosomal

Table 6.4 Causes of hepatitis and/or cholestasis in infancy

Infectious	
Viral	Herpes simplex
	Varicella zoster
	Cytomegalovirus
	Human herpes virus 6
	Adenovirus
	Enterovirus
	Hepatitis B
	Rubella
	Parvovirus B19
	Human immunodeficiency virus (HIV)
Bacterial	Syphilis
	Listeria
	Septicemia
	Urinary tract infection
	Acute cholecystitis
	Acute cholangitis
	Pyogenic liver abscess
Fungal	Hepatosplenic candidiasis
	Other systemic fungemias
Protozoal	Malaria
	Toxoplasmosis
	Congenital Chagas disease
Immune	Neonatal hemochromatosis
	Hemophagocytic lymphohistiocytosis
	Neonatal lupus
	Autoimmune hemolytic anemia with giant cell hepatitis
	Erythroblastosis fetalis
	Graft versus host syndrome
	Autoimmune hepatitis
	Kawasaki disease
	Myeloproliferative disorder
Nutritional/toxic	TPN/intestinal failure
	Drugs/toxins/herbals
	Breast milk jaundice
	Indian childhood cirrhosis
	Other copper toxicoses
Metabolic	Crigler-Najjar
	Dubin-Johnson syndrome
	Rotor syndrome
	Tyrosinemia type 1
	Galactosemia
	Glycogen storage diseases
	Organic acidemias
	Urea cycle disorders
	Citrin deficiency
	Fatty acid oxidation defects
	Cholesterol synthesis defects
	Mitochondrial/respiratory chain defects
	Congenital defects of glycosylation
	Bile acid synthesis defects
	Peroxisomal defects
	Niemann-Pick type C
	Lysosomal storage diseases
	Mucopolysaccharidoses
	Alpha 1-antitrypsin deficiency
	Cystic fibrosis
	Defects of biliary transport (PFIC)
	Familial cholanemias
	Erythropoietic protoporphyria
Syndromic	Trisomy 21
	Trisomy 18
	Trisomy 13
	Cat-eye syndrome
	Alagille syndrome
	ARC syndrome
	Aagenaes syndrome
	Donohue syndrome
	MODY 5
	Jeune syndrome
	COACH syndrome
	Joubert syndrome
	Bardet-Biedl syndrome
	Ivemark syndrome
	Beckwith-Wiedemann syndrome
	NISCH syndrome
	North American Indian childhood cirrhosis
	Cri-du-chat syndrome
	GRACILE syndrome
	McCune-Albright syndrome
	Septo-optic dysplasia
	Smith-Lemli-Opitz syndrome
Endocrine	Panhypopituitarism
	Hypothyroidism
	Hypoadrenalism
	Hyperinsulinism
Cardiovascular	Portal vein thrombosis
	Congenital anomalies of portal vein
	Ischemic hepatitis
	Heart failure
	Hepatic artery-portal vein fistula
	Sinusoidal obstruction syndrome
	Budd-Chiari
Neoplastic	Langerhans cell histiocytosis
	Hepatoblastoma
	Leukemia
	Other primary or metastatic liver tumors

Table 6.4 (continued)

Others	Choledochal cyst
	Choledocholithiasis
	Biliary atresia
	Inspissated bile
	Spontaneous perforation of bile duct
	UVC trauma and extravasation
	Neonatal sclerosing cholangitis
	Caroli disease

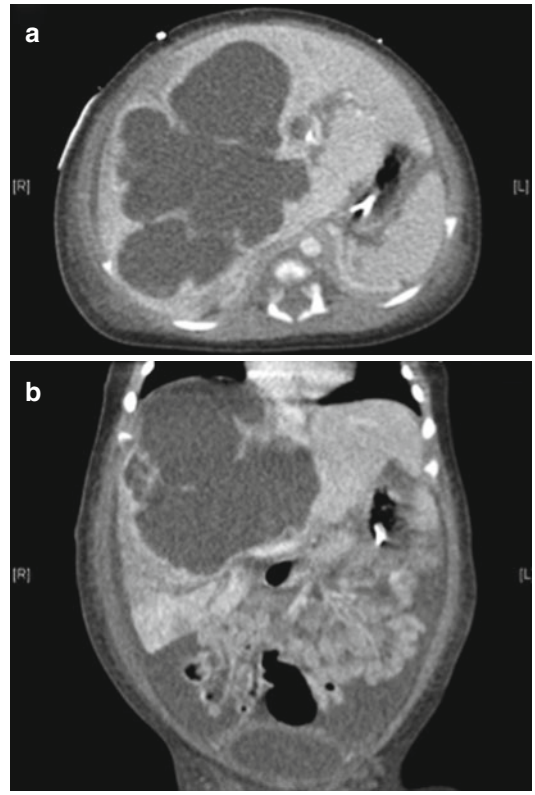

Fig. 6.1 Massive extravasation of PN solution from an umbilical venous catheter which migrated into the liver – (**a**) axial and (**b**) coronal CT views

syndromes that may be apparent from abnormal physical features, as well as vascular, endocrine, and neoplastic causes. Additionally, in those infants who have intestinal failure (usually related to surgical resection for necrotizing enterocolitis or congenital intestinal anomalies), cholestasis is related to enteral starvation and the need for intravenous feeding (see chapter 17). Less commonly iatrogenic causes of neonatal liver disease may be seen, such as portal vein thrombosis or extravasation of parenteral fluids into the liver substance (see Fig. 6.1) complicating umbilical venous catheterization [11].

Although hepatomegaly, abnormal transaminase, and even cholestasis may be present in multisystem diseases, these may not be the key features on which the diagnosis is based. For example, in many lysosomal storage conditions, abnormal facies, neurological findings, skeletal malformations, or other tissue involvement may point to the diagnosis (a list of metabolic defects that have been associated with liver disease is shown in Table 6.5). Certain syndromes such as ARC (arthrogryposis, renal anomalies, and cholestasis), Aagenaes, Donahue, or Beckwith-Wiedemann syndrome also have characteristic physical manifestations, and the presence of hepatomegaly or liver function abnormalities simply supports the diagnosis. Liver involvement in multisystem diseases may manifest in some infants with hepatomegaly alone and will be reliant upon a careful physical examination to identify the relevant finding. The request for a hepatological opinion in such circumstances is to determine if the findings are consistent with the primary diagnosis, if there is a second diagnosis responsible for the liver abnormalities observed, to estimate prognosis, and to advise on appropriate management of the liver dysfunction.

The Sick Infant

Infants discharged from the nursery presenting within days of birth with acute liver failure (see Table 6.3) (see chapter 23) have a large diagnostic overlap with those who have been seen as a consultation presenting in the newborn period. Inborn errors of metabolism, perinatally acquired infection, GALD, and other conditions may have an apparent period of health prior to postnatal decompensation.

The history collected from family must include preceding signs and symptoms, details of the pregnancy and delivery, a dietary history with

Table 6.5 Inborn errors for metabolism associated with hepatic manifestations

Enzyme defects	Specific disease	Manifestations
Defects of bilirubin conjugation	Gilbert	UH
	Crigler-Najjar	UH
Defects of carbohydrate metabolism	Galactosemia	NC, ALF, CLD
	Hereditary fructose intolerance	NC, ALF, CLD
	Fructose 1,6-bisphosphatase def.	NC, ALF
	PEPCKD	S, R
	GSD 1a	HM, T, adenoma
	GSD3	HM, T, CLD
	GSD4	NC, T, CLD
	GSD6	HM, T
	GSD9	HM, T
	Glycerol-3-phosphate dehydrogenase deficiency 1	HM, T
Defects of amino acid metabolism	Tyrosinemia type1	NC, ALF, CLD, HCC
	s-Adenosylhomocysteine hydrolase	NC, CLD
	Maple syrup urine disease	NC
	Methylmalonic acidemia	R, HM
	Propionic acidemia	R
	Isovaleric acidemia	R
	3-Methylcrotonylglycinuria	R
	3-OH-3-methylglutaryl-CoA lyase def.	R
	Holocarboxylase synthase def.	R
(Urea cycle disorders)	N-Acetylglutamate synthase def.	R
	Carbamoylphosphate synthase def.	R
	Ornithine transcarbamylase def.	R
	Citrullinemia	R
	Argininosuccinic aciduria	R, T
Defects of fatty acid oxidation	VLCAD def.	HM, R
	LCHAD def.	T, R, ALF
	MCAD def.	R
	3-Hydroxyacyl-CoA dehydrogenase def.	R, ALF
Defects of ketogenesis	3-HMG-CoA lyase	HM
Defects of carnitine metabolism	Primary carnitine deficiency	HM, R
Defects of mitochondrial metabolism	Mitochondrial DNA mutations	NC, T, ALF, R, CLD
	Mitochondrial DNA deletions	NC, T, ALF, R, CLD
	Mitochondrial DNA depletion	NC, T, ALF, R, CLD
	Respiratory chain defects	T, R, ALF, CLD
	Multiple Acyl-CoA dehydrogenase def.	HM, R
Peroxisomal defects	Zellweger syndrome	NC, T, HM, CLD
	Neonatal adrenoleukodystrophy	NC, T, HM, CLD
	Infantile Refsum	NC, T, HM, CLD
	Pipecolic acidemia	NC, T, HM, CLD
	Bifunctional protein def.	NC, T, HM, CLD
Defects of lipoprotein metabolism	Tangier disease	HM, SM
	Abetalipoproteinemia	HM, S
Defect of cholesterol synthesis	Cerebrotendinous xanthomatosis	NC
	Smith-Lemli-Opitz syndrome	NC
	Mevalonate kinase def.	NC

Table 6.5 (continued)

Enzyme defects	Specific disease	Manifestations
Lysosomal storage diseases	GM1 gangliosidosis	HM, SM
	GM2 (Sandhoff) gangliosidosis	HM, SM
	Niemann-Pick A and B	HM, SM
	Niemann-Pick C	NC, SM, CLD, HCC
	Gaucher	HM, SM
	Farber	HM, SM
	Lysosomal acid lipase def. (Wolman and CESD)	NC, T, S, HM, SM, CLD
	Mucopolysaccharidoses (I, II, III, IV, VII)	HM, SM
	Multiple sulfatase def.	HM, SM
	I-cell disease	HM, SM
	Pseudo-Hurler polydystrophy	HM, SM
	Aspartylglucosaminuria	HM, SM
	Fucosidosis	HM, SM
	α-Mannosidosis	HM, SM
	Sialidosis	HM, SM
	Galactosialidosis	HM, SM
	Infantile sialic acid storage disease	HM, SM
Congenital defects of glycosylation	Type I subtypes a, b, h	NC, T, HM, SM, CLD
	Type II subtypes b, e, h	NC, T, HM, SM, HSM
Porphyrias	Erythropoietic protoporphyria	T, CLD
Defects of bile acid metabolism	Cerebrotendinous xanthomatosis	NC
	3β-Hydroxy-Δ_5-C^{27}-steroid oxidoreductase def.	NC, T, CLD
	Oxysterol 7α-hydroxylase def.	NC, T, CLD
	2-Methylacyl-CoA racemase def.	NC, T, FSVM
	BAAT def. (bile acid CoA:amino acid N-acyltransferase)	NC, T, FSVM, P
	TJP2 def. (tight junction protein)	FSVM, P
	Epoxide hydrolase def.	FSVM, P
Endoplasmic reticulum storage diseases	α_1-Antitrypsin def.	NC, T, CLD
	Fibrinogen storage disease	T, CLD
	Hereditary amyloidosis	T, HM, CLD
Defects of metal metabolism	Wilson disease	T, HM, ALF, CLD
	Hemochromatosis	T, CLD, HCC
Transporter defects		
Defects of bilirubin transport	Dubin-Johnson syndrome	NC
	Rotor syndrome	NC
Defects of biliary transport	FIC1 def. (PFIC 1)	NC, T, CLD
	BSEP def. (PFIC 2)	NC, T, CLD, HCC
	MDR3 def. (PFIC 3)	NC, T, CLD
Defects of carbohydrate transport	GSD1b	HM, T, adenoma
	Fanconi-Bickel	HM,
Defects of amino acid transport	HHH syndrome	R
	Citrin def.	NC, T, S, R
	Lysinuric protein intolerance	R
Defects of ion transport	Cystic fibrosis	NC, T, CLD

(continued)

Table 6.5 (continued)

Enzyme defects	Specific disease	Manifestations
Defects of carnitine transport	Carnitine-acylcarnitine translocase deficiency	HM, S, CLD
	Carnitine palmitoyltransferase II deficiency	HM, S, R, CLD
	Carnitine palmitoyltransferase I deficiency	HM, S, R

Splenomegaly, fat-soluble vitamin malabsorption, and pruritus are only designated if the feature is present in the absences of fibrotic liver disease

NC neonatal cholestasis, *UH* unconjugated hyperbilirubinemia, *T* elevated hepatic transaminases, *S* steatosis, *HM* hepatomegaly, *SM* splenomegaly, *ALF* acute liver failure, *CLD* chronic fibrotic liver disease, *HCC* hepatocellular carcinoma, *FSVM* fat-soluble vitamin malabsorption, *P* pruritus

attention to weaning, fasting and recent feed changes, and consideration of possible precipitating exposures along with a family history, noting any consanguinity of the parents.

Infants have limited responses to severe illness, and the complaints from family may be nonspecific and include poor feeding, vomiting, lethargy, and seizures. There is little in the physical examination that will differentiate cause in such cases, but it is important to remember that many of the diagnoses may result in multisystem involvement and not just liver failure. Therefore, investigation should also be directed at detecting encephalitis, myocarditis, renal failure, adrenal and thyroid insufficiency, and almost any other tissue involvement. Significant encephalopathy in infants is more likely to be due to primary metabolic encephalopathy or infectious encephalitis, as hepatic encephalopathy tends to occur in infants only very late in the course of their liver disease, if at all.

While investigating the specific diagnosis is important, the most urgent need is to respond to the immediate threats to life. Empiric antibacterial and antiviral medications should be considered once appropriate cultures (blood, urine) and viral studies (herpes simplex) have been collected. Manage hypoglycemia, fluid and electrolyte imbalance, and acidosis if present. Intravenous fluids must supply adequate glucose to prevent catabolism, while defects of protein or lipid metabolism (or both as in glutaric aciduria type II) are being investigated – hepatic glucose release for healthy infants may be in the range of 12–14 mg/kg/min, and therefore, this should be the delivery rate to prevent hypoglycemia and endogenous protein catabolism. Coagulopathy may be very severe without outward signs, so it is imperative to check coagulation profile with initial suspicion of liver dysfunction. Occasionally biliary atresia or other cholestatic infantile conditions present acutely with hemorrhage or ecchymoses secondary to vitamin K deficiency.

For details on diagnostic workup and management, see chapter 23.

The Stable Infant with Liver Disease

The infant referred to clinic with jaundice in the first few weeks of life is another common scenario for the general pediatrician and pediatric gastroenterologist. The general pediatrician sees more children with unconjugated hyperbilirubinemia secondary to prolonged physiologic jaundice or breast milk jaundice than they do children with cholestatic jaundice. Infants with unconjugated hyperbilirubinemia have colorless urine and pigmented stools (yellow-green or brown) [12]. It is important to recognize that infants without conjugated hyperbilirubinemia do not pass yellow or amber urine because they drink at least 100 mL/kg/day and therefore pass dilute urine, unlike adults who frequently pass more concentrated yellow urine. Unconjugated jaundice still needs to be investigated if it is either very early in onset, prolonged beyond 10–14 days, very high levels, or of late onset. If breast milk jaundice is suspected and there is no evidence of hemolysis or infection, thyroid function is normal, and bilirubin levels are not progressively increasing,

breast-feeding does not need to be discontinued. However, if the bilirubin level continues to rise, defects of bilirubin conjugation (Crigler-Najjar syndrome) should be considered. The high frequency of unconjugated jaundice in the infant population is one factor that has been suggested for late referrals of infants with biliary atresia; the rare case of conjugated hyperbilirubinemia is like a proverbial needle in the haystack of infants with unconjugated jaundice! It is important for the general pediatrician to remain alert to the possibility that an infant's jaundice is cholestatic and request split bilirubin levels – total and direct or better still conjugated and unconjugated bilirubin levels (see chapter 3).

Referrals to the pediatric gastroenterologist or hepatologist are most commonly for conjugated hyperbilirubinemia, although the type of jaundice is not always characterized before referral. Due to the importance of a timely hepatoportoenterostomy to the prognosis of the affected infant, the diagnosis of biliary atresia needs to be ruled out promptly. There has been much discussion on the best combination of sonography, scintigraphy, liver biopsy, and cholangiography (see chapter 13), but efficiency of work-up demands a certain degree of experience in the team and institution caring for the infant. If it is not possible to make this diagnosis in a few days, the infant should be transferred to a center that has the required experience and where the appropriate surgical expertise exists to proceed to portoenterostomy. There is no justification for delaying transfer until the diagnosis is certain.

The investigations for other causes of infantile liver disease should be carried out concomitantly with the evaluation for biliary atresia. The priorities depend on the most likely causes relevant to the population served. Citrin deficiency, for example, is a relatively common cause of neonatal cholestasis in Japan and China but rare in Northern Europe, while for α_1-antitrypsin deficiency and cystic fibrosis, the relative regional prevalence is reversed. It is wise to have an established protocol for first-line investigations according to your particular population, followed by a second line of investigations for less frequently encountered conditions and finally the very rare causes investigated sequentially to make best use of both patient's and hospital's resources.

Quite frequently a healthy, asymptomatic infant with proven or suspected perinatally acquired hepatitis B or hepatitis C will be referred to clinic for confirmation of diagnosis, management, and parental counselling. The child may be accompanied by natural parents, but often the patient may be brought to clinic by a foster family and the child's social worker or by the adoptive parents in the case of an adoptee from a region of the world where HBV is endemic. See chapter 15 for an approach to these infants. Another group of asymptomatic infants seen in clinic with liver-related questions include those with siblings affected by an inheritable liver disease whose parents either wish this child to be tested or who is already known to carry the mutation(s) and are seeking advice (e.g., α_1-antitrypsin deficiency).

Rarely a family seeks medical attention because they have either identified a lump in the abdomen or notice abdominal distention. More commonly nonspecific features such as poor feeding or growth, vomiting, sweatiness, or tachycardia may first bring the infant to medical attention. Occasionally hepatomegaly with or without splenomegaly may be identified as one feature of multisystem genetic disorders such as a cilial dysfunction syndrome (see chapter 14) or a lysosomal storage disorder. In yet others, a completely asymptomatic mass may be identified on routine examination during a well-baby check. It is not always easy, especially in infants, to determine if an enlarge liver is due to diffuse enlargement of the liver or due to a circumscribed lesion or lesions.

The investigation of these infants depends largely on whether there is homogenous hepatic enlargement or the finding of a mass or masses arising from the liver (see Table 6.6). Ultrasound findings are the best guide to further diagnostic approach. Bland hepatomegaly would point to the possibilities of hepatitis, metabolic storage, outflow obstruction as in heart failure, or syndromic

Table 6.6 Infant with a liver mass

Benign tumor	Hemangioma	Infectious	Amebic abscess
	Infantile hemangioendothelioma		Pyogenic liver abscess
	Mesenchymal hamartoma		
	Adenoma	Traumatic	Hepatic hematoma
			Fluid extravasation from UVC
Malignant tumor	Hepatoblastoma	Other	Choledochal cyst
	Rhabdoid tumor		Mucocele of gall bladder
	Rhabdomyosarcoma		Simple hepatic cyst
	Neuroblastoma		Riedel's lobe
	Other hepatic malignancy		

organomegaly (e.g., Beckwith-Wiedemann syndrome). Discrete lesions on imaging imply tumor, cyst, or abscess. Undoubtedly, further imaging can help refine the diagnosis in these cases (see chapter 5), and some lesions may have highly characteristic findings on well-conducted studies. Delaying referral to an experience center is not usually beneficial and can result in multiple nonuseful investigations being carried out. Early referral to an experience center who can direct an appropriate diagnostic workup (chapter 22) is recommended. For those without diagnostic findings on imaging, the lesions may need histological evaluation, and therefore, biopsy is necessary.

Young Child with Liver Disease (1–4 Years)

Most patients with newly recognized liver disease (see Table 6.7) in this age range will present with hepatitis (inflammation of the liver) recognized by the primary provider because of elevated liver function tests. The blood tests are usually provoked by some combination of new onset of jaundice, fever, anorexia, vomiting, or malaise, although in a significant number of cases may be found on testing for nonspecific complaints of headache or abdominal pain or even on routine well-patient screening.

Causes of hepatitis include infectious, commonly viral, autoimmune, and some metabolic diseases which had escaped detection in ear-

Table 6.7 Causes of cholestasis in the younger child (1–4 years)

Infectious	
Viral	Hepatitis A
	Hepatitis B
	Hepatitis C
	Cytomegalovirus
	Epstein-Barr
	Adenovirus
	Parvovirus B19
Bacterial	Urinary tract infection
	Septicemia
	Acute cholangitis
	Pyogenic abscess
	Leptospirosis
Parasitic	Malaria
	Trematodes (liver fluke)
	Ascariasis
	Hydatid (Echinococcus)
	Amebic abscess
Immune	Autoimmune hepatitis
	Sclerosing cholangitis
	Kawasaki disease
	Graft versus host disease
	Immunodeficiencies
Metabolic	α_1-Antitrypsin deficiency
	Tyrosinemia
	FIC1 def. (PFIC1)
	BSEP def. (PFIC2)
	MDR3 def. (PFIC3)
	Peroxisomal defects
	Mitochondrial defects
	Cholesteryl ester storage disease
	Dubin-Johnson syndrome
	Rotor syndrome
	Erythropoietic protoporphyria

Table 6.7 (continued)

Syndromic	Alagille syndrome
	Ciliopathies (e.g., COACH, Bardet-Biedl)
	NISCH syndrome
	North American Indian childhood cirrhosis
	Mulibrey nanism
	Tubulointerstitial nephropathy with cholestasis
Toxic	Drugs/toxins/herbals
	Copper toxicosis
Vascular	Budd-Chiari
	Sinusoidal obstruction syndrome
	Constrictive pericarditis
	Heart failure
	Congenital heart disease
	Liver trauma
Neoplastic	Langerhans cell histiocytosis
	Tumors of liver or bile ducts
	Leukemia
Other	Choledochal cyst
	Caroli disease
	Choledocholithiasis
	Sickle-cell disease

lier life. Children with chronic viral hepatitis (hepatitis B and hepatitis C) contracted from the mother at birth may remain completely asymptomatic and be detected during investigation of other complaints, especially if the mother has not been previously diagnosed and had suboptimal antenatal care. This is particularly applicable to young children from high endemic risk areas of the world (recent immigrants and adoptees) and mother's with a history of at-risk behaviors such as intravenous substance abuse. Autoimmune hepatitis can occur at any age, but in this age group, liver-kidney-microsomal (LKM) antibody-positive disease constitutes a larger proportion of cases than is seen in older children (see chapter 16). Kawasaki disease, although rarely seen in GI clinic, very commonly has abnormal transaminases and bilirubin levels at presentation. Similarly, children with celiac disease may have abnormal transaminases, but these tend to resolve upon diagnosis and initiation of a gluten-free diet.

Chronic liver diseases with a gradual progression may be first revealed in the young child, as in some cases of α-1-antitrypsin deficiency (see chapter 9) and Alagille syndrome (see chapter 11). Despite there being no history of neonatal cholestasis, these diseases may progress silently to fibrotic liver disease, detected either by the astute primary doctor finding splenomegaly on routine examination or with the first onset of complications of cirrhosis or portal hypertension.

In the developed world, the infectious causes of liver disease are relatively limited especially in the essentially healthy child, but in the tropics, there are many more pathogens that may induce deranged liver function (see Table 6.8). On the other hand, obesity is now endemic (and some have said epidemic) in the developed world driven by excessive nutritional intake and an increasingly sedentary lifestyle. Nonalcoholic fatty liver disease is now regularly being diagnosed as early as second year of life (see chapter 18). Lastly, drug-induced or toxic hepatitis may result from inadvertent ingestion by the adventurous toddler or due to an idiosyncratic reaction to prescribed medication (see chapter 19) (see Table 6.9).

Non-hepatic causes of laboratory abnormalities and clinical signs and symptoms typically attributed to liver disease must also be considered. Although abnormal "liver" function tests equate in most practitioners' mind to liver disease, there are important exceptions. "Transaminitis" without jaundice is commonly seen in diseases of skeletal and cardiac muscle. The inclusion of γ-glutamyl transferase and creatine kinase (or other muscle-derived enzyme such as aldolase) can help differentiate the tissue source of elevated levels of AST and ALT. Duchenne muscular dystrophy is a particularly important diagnosis to consider. Another cause of elevated transaminase levels in the absence of liver disease is due to macroenzymes (especially macro-AST) [13]. Serum enzymes may complex with immunoglobulins resulting in a high molecular weight complex that has a prolonged half-life because of reduced plasma clearance. Although macroenzymes have been associated with acute, chronic,

Table 6.8 Infectious agents associated with hepatic involvement

Viral	*Bacterial*	*Fungal*
Hepatitis A	Urinary tract infection	Histoplasmosis
Hepatitis B	Septicemia	Hepatosplenic candidiasis
Hepatitis C	Acute cholangitis	Disseminated aspergillosis
Hepatitis D	Acute cholecystitis	Trichosporon cutaneum
Hepatitis E	Pyogenic abscess	Penicillium marneffei
Herpes simplex	Perihepatitis (gonorrhea, chlamydia)	Coccidioidomycosis
Varicella zoster	Toxic shock syndrome (staphylococcus)	Cryptococcosis
Cytomegalovirus	Scarlet fever	
Epstein-Barr virus	Salmonella typhi/paratyphi	*Protozoal*
Human herpes virus 6	Shigella	Toxoplasmosis
Human herpes virus 7	Yersinia	Malaria
Human herpes virus 8	Clostridium perfringens	Amebic abscess
Rubella	Brucellosis	Toxocariasis
Adenovirus	Listeriosis	Cryptosporidium
Enterovirus	Borrelia (Lyme borreliosis)	Chagas disease
Parvovirus B19	Leptospirosis	Leishmaniasis
Paramyxovirus	Syphilis	Babesiosis
Reoviruses (Colorado tick fever)	Bartonella (cat scratch, Carrion disease)	
Influenza/parainfluenza	Actinomycosis	*Parasitic*
Coronavirus (SARS)	Legionella	Clonorchiasis
Human immunodeficiency virus (HIV)	Tularemia	Fascioliasis
Yellow fever	Melioidosis	Opisthorchiasis
Dengue fever	Tuberculosis	Dicrocoelium dendriticum
Other flavivirus hemorrhagic fever	Leprosy	Paragonimiasis
Lassa fever	Rocky Mountain spotted fever	Schistosomiasis
Lymphocytic choriomeningitis virus	Scrub typhus	Echinococcus (hydatid disease)
Other arenavirus hemorrhagic fevers	Ehrlichiosis	Ascariasis
Filovirus fevers (Ebola, Marburg)	Q fever	Strongyloides
Hantavirus hemorrhagic fever		Trichinella
Other bunyavirus hemorrhagic fevers		Capillariasis
		Baylisascariasis

and malignant liver disease, most cases appear benign and self-limiting although the triggers for formation are not fully understood. Benign (or transient) hyperphosphatasia is frequently seen in young children possibly provoked by a mild intercurrent illness [14]. The alkaline phosphatase level can peak in the thousands and remains elevated for a few weeks to a few months before settling to the normal range. If the isoenzymes of alkaline phosphatase are assayed, the largest increase is seen in bone-derived isoenzyme although liver isoenzyme can be increased as well. In the absence of bone disease (e.g., rickets or fracture) and otherwise normal liver laboratory values, no further investigation is required.

Jaundice may be secondary to acute hemolysis; for example, a child with the glucose-6-phosphate dehydrogenase "favism" variant may be first exposed to broad (fava) beans as a young child and present with new-onset jaundice. Even massive ascites may not be what it seems; Fig. 6.2 shows the CT appearance of a young child with a giant omental cyst.

Hepatomegaly or a liver mass may turn out to be the anatomical variant, Riedel's lobe, an elongated right lobe. Incidental hepatomegaly is the likely presenting feature for the benign glycogen storage diseases due to phosphorylase and phosphorylase kinase deficiency (GSD VI and IX, respectively). Sizable liver masses, however, at

Table 6.9 Pharmaceuticals and toxins that have been reported to cause liver disease

Antibiotics	Anticonvulsants and CNS-acting drugs	Chemotherapeutics	Miscellaneous drugs	Wild plants/herbals	Dietary supplements and recreational drugs	Non-medicinal chemicals
Amoxicillin/clavulanate	Carbamazepine	Methotrexate	Acetaminophen	Amanita	Vitamin A	Carbon tetrachloride
Flucloxacillin	Lamotrigine	Azathioprine	Alcohol	Comfrey	Hydroxy Cut	Chlorobenzene
Sulfonamides	Phenobarbital	6-Mercaptopurine	Allopurinol	Pyrrolizidine alkaloids	Ecstasy	Vinyl chloride
Erythromycin	Phenytoin	Cyclophosphamide	Amiodarone	Senna glycosides	Cocaine	Other organic solvents
Azithromycin	Valproate	Propylthiouracil	Estrogens	Germander		Pyrrolizidine alkaloids
Cephalosporins	Felbamate	Nitrosoureas	Anabolic steroids	Chaparral		Yellow phosphorus
Minocycline	Atomoxetine	cis-Platinum	Aspirin	Kava-kava		2-Nitropropane
Nitrofurantoin	Chlorpromazine	Many others	Diclofenac	Jin bu huan		Copper
Isoniazid	Methyldopa	Rarely used singly	Ketoprofen	Ephedra (ma huang)		Iron
Rifampin	Fluoxetine		Ibuprofen			
Quinine/quinolones			Naproxen			
Azole antifungals			Halothane			
Antiretrovirals			Troglitazone			
			Cyclosporin			
			Pemoline			
			Cimetidine			

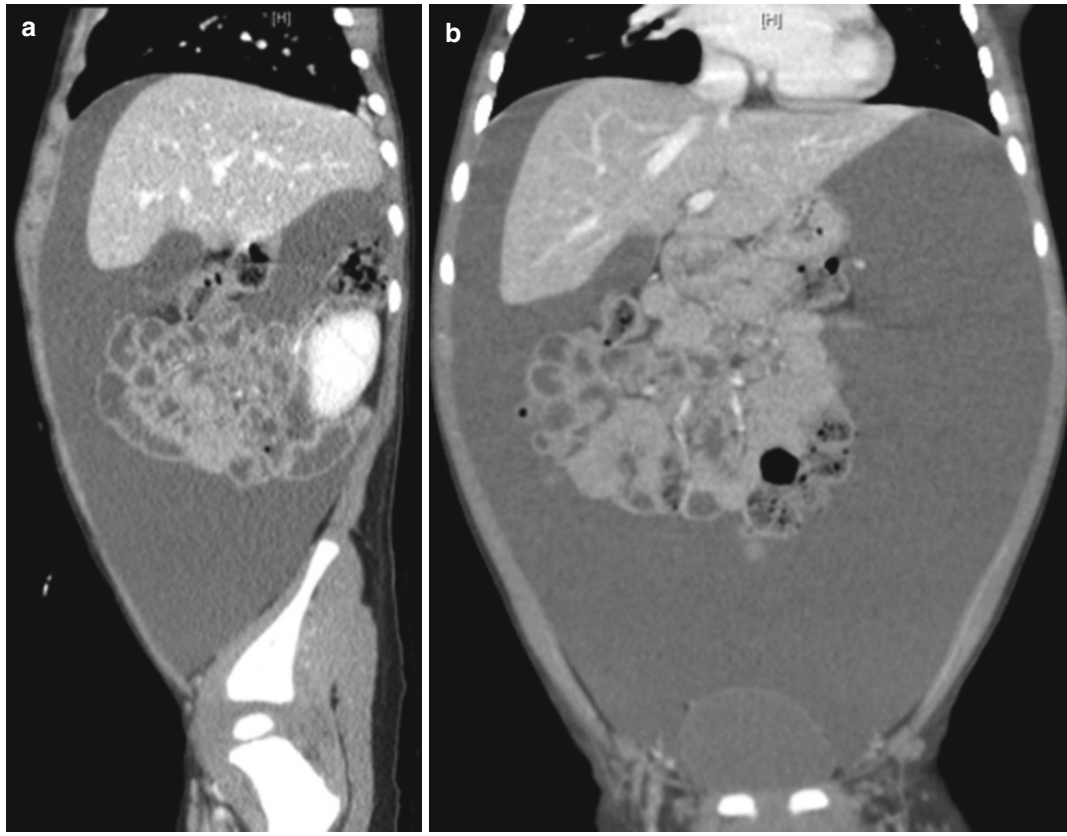

Fig. 6.2 Pseudo-ascites due to giant omental cyst – (**a**) sagittal and (**b**) coronal CT views

this age are likely to be primary hepatoblastoma, neuroblastoma, or other abdominal malignancy (see chapter 22). Hepatocellular carcinoma (HCC) occurs in association with advanced chronic liver disease and in conditions with a specific predisposition such as tyrosinemia and PFIC type 2 but is vanishingly rare as a de novo tumor in an otherwise healthy young child. Benign lesions are most commonly detected on abdominal imaging that was done for other indications and, as discussed for infants, early referral to an experienced center will expedite appropriate management.

Older Child/Adolescent with Liver Disease (>5 Years)

Just like in the younger children, suspicion of liver disease arises from the appearance of specific symptoms such as jaundice, chance pickup on routine physical examination, or investigation of nonspecific and possibly unrelated medical concerns (see Table 6.10). Acute hepatitis due to hepatitis A and Epstein-Barr virus (EBV) is relatively common and new infection with hepatitis B and hepatitis C becomes more common in adolescent populations. Hepatitis E in endemic areas rarely causes significant clinical disease in young children but is more likely to cause acute hepatitis as subjects' age and is particularly dangerous for the pregnant teenage girl (see chapter 15). Autoimmune hepatitis often presents with acute hepatitis and although more frequent in adolescent girls can occur in both sexes and at any age (see chapter 16). Most cases of Wilson disease diagnosed in childhood and adolescence manifest as acute, often fulminant, hepatitis, and although rare, an expedient diagnosis may prevent death or liver transplantation (see chapter 9).

Everyone involved in the care of children recognizes the obesity crisis that is so highly prevalent in the USA and is also sweeping the rest of the developed world. This single factor accounts for the majority of new referrals of children with elevated transaminases in the USA as a result of nonalcoholic fatty liver disease (NAFLD) (see chapter 18). This was an uncommon diagnosis 25 years ago, and despite multicenter collaborative efforts to design treatments for this condition, it is likely that the solution, if one is to be forthcoming, will be in the realm of public policy development to curtail obesity rather than single-patient medical management.

Gall stones and gall bladder disease can cause jaundice and elevated transaminases and increase as adulthood approaches (see chapter 20). Gilbert syndrome is an essentially benign condition manifest as an unconjugated jaundice with normal transaminases. The jaundice is frequently first noted around the time of puberty and these patients are often referred to liver clinics. As for younger children, elevated transaminases with no jaundice may be seen in many liver diseases, but muscle injury, myositis, muscular dystrophy, or cardiomyopathy should not be dismissed without a careful history, physical examination, and a creatine kinase level.

Chronic liver disease may present at any age (see chapters 25 and 26). As disease progresses jaundice may eventually appear. Abdominal distention from hepatomegaly or ascites may rarely lead to the request for medical attention. More commonly isolated splenomegaly is identified, and if portal hypertension is not considered, the child or teenager may go through detailed hematological investigations unnecessarily. Extrahepatic portal hypertension, due to portal vein thrombosis having occurred usually years

Table 6.10 Causes of cholestasis in older children and adolescents

Infectious	
Viral	Hepatitis A
	Hepatitis B
	Hepatitis C
	Hepatitis E
	Cytomegalovirus
	Epstein-Barr virus
	Adenovirus
Bacterial	Septicemia
	Acute cholangitis
	Pyogenic abscess
	Leptospirosis
	Rocky Mountain spotted fever
	Borrelia (Lyme's)
	Salmonella typhi/paratyphi
	Tuberculosis
Parasitic	Malaria
	Trematodes (fluke)
	Ascariasis
	Leishmaniasis
	Echinococcus (hydatid)
	Amebic abscess
	Cryptosporidium
Metabolic	Wilson disease
	Cholesteryl ester storage disease
	Benign recurrent intrahepatic cholestasis
	Juvenile hemochromatosis
	HELLP syndrome (pregnant teenage women)
Immune	Autoimmune hepatitis
	Sclerosing cholangitis
	Primary biliary cirrhosis
	Kawasaki disease
	Graft versus host disease
	Immunodeficiencies
	Systemic lupus erythematosus
	Sarcoidosis
Toxic	Drugs/toxins/herbals
Vascular	Budd-Chiari
	Sinusoidal obstruction syndrome
	Congenital heart disease
	Constrictive pericarditis
	Heart failure
	Liver trauma
	Hereditary hemorrhagic telangiectasia
Neoplastic	Langerhans cell histiocytosis
	Leukemia
	Lymphoma
	Hepatocellular carcinoma
	Cholangiocarcinoma
	Other tumors of liver or bile ducts
Other	Choledochal cyst
	Caroli disease
	Choledocholithiasis
	Sickle-cell disease
	Postnecrotic cirrhosis

Table 6.11 Causes of portal hypertension

Post-hepatic	Heart failure
	Cardiomyopathy
	Congenital heart disease
	Constrictive pericarditis
	Inferior vena caval thrombosis
	Congenital web in inferior vena cava
	Budd-Chiari syndrome
	Tumor
Intrahepatic	
Post-sinusoidal	Veno-occlusive disease
Sinusoidal	Cirrhosis
	Nodular regenerative hyperplasia
	Hypervitaminosis A
Pre-sinusoidal	Schistosomiasis
	Congenital hepatic fibrosis
	Sarcoidosis
	Portosclerosis
	Hepatic artery-portal vein fistula
Pre-hepatic	Portal vein thrombosis
	Portal vein stenosis
	Cavernous transformation of portal vein
	Congenital anomalies of portal vein
	Tumor
Sinistral (left sided)	Splenic vein thrombosis
	Pancreatitis
	Pancreatic pseudocyst
	Tumor
	Retroperitoneal fibrosis
	Retroperitoneal abscess

Table 6.12 Causes of hepatic masses beyond infancy - found either on physical examination or imaging

Neoplastic	
Malignant	Hepatoblastoma
	Hepatocellular carcinoma
	Neuroblastoma
	Neuroendocrine tumors
	Rhabdoid tumor
	Rhabdomyosarcoma
	Embryonal sarcoma
	Epithelioid hemangioendothelioma
	Cholangiocarcinoma
	Hepatic teratoma
	Other primary hepatic malignancy
	Metastatic malignancy in liver
Benign	Adenoma
	Mesenchymal hamartoma
	Fibronodular hyperplasia
	Hemangioma
Infectious	Amebic abscess
	Hydatid (Echinococcus)
	Pyogenic liver abscess
Other	Nodular regenerative hyperplasia
	Simple cyst
	Hematoma
	Mucocele of gallbladder
	Choledochal cyst
	Focal fatty infiltration
	Riedel's lobe

before frequently in infancy related to umbilical sepsis or catheterization, can present with splenomegaly. Alternatively, the detection of portal hypertension may be the first sign of chronic liver injury, for example, in autoimmune hepatitis or chronic viral hepatitis, resulting in cirrhosis, but with no history of previous ill health in the child (see Table 6.11). In these circumstances the liver is usually shrunken, and therefore not palpable, but on imaging is heterogenous and nodular and the biopsy specimen severely fibrotic.

With increasing age the incidence of embryonal tumors diminishes and the risk of HCC and metastatic liver tumors increases (see Table 6.12). Greatest risk for HCC is in association with chronic fibrotic liver disease, but the fibrolamellar variant can be encountered in the adolescent without preexisting liver disease. Benign lesions can get big enough to present as a mass or with abdominal discomfort and include nodular regenerative hyperplasia, focal nodular hyperplasia, and adenoma. Leukemias and lymphomas are the most common cancers in children and adolescents and may present with evidence of liver infiltration causing deranged liver function.

Although identification of asymptomatic chronic viral hepatitis via at-risk screening (immigration or infected family) is less common in this age group, they do occur. Adoptees and recent immigrants from HBV endemic regions may present with chronic HBV carriage for management advice. Additionally, family screening

because of hereditary hemochromatosis may identify an older child with biochemical evidence of iron overload but normal or mildly altered transaminases (see chapter 9). Similarly, a diagnosis of Wilson disease mandates the screening of all first-degree relatives, including other children, before signs of liver injury exist.

Acute Liver Disease and Failure

Acute liver disease is the recent onset of liver dysfunction in a patient with no history or investigational evidence of preexisting liver disease (see Table 6.13). This gets a little convoluted when one considers the acute onset of, say, Wilson disease, acute decompensation of chronic hepatitis B, or autoimmune liver disease, where the clinical picture is of a disease of no longer than a few weeks duration but in whom the liver biopsy shows established fibrosis. As is the convention for this chapter in general, we will limit the discussion to the clinical phenotype, i.e., those who have an apparent acute onset of disease with no history of preexisting liver disease.

Acute liver failure results from massive or submassive hepatocellular necrosis, but the syndrome is defined in terms of the duration of the illness (less than 8 weeks) and the presence of coagulopathy and hepatic encephalopathy (see chapter 23 for more details). One important consideration is to differentiate between the acute failure of liver functions manifest by coagulopathy, cholestasis, hyperammonemia, loss of glycemic control, encephalopathy, etc., and the criteria for entry into the PALF (Pediatric Acute Liver Failure) study. In an attempt to be inclusive and to observe the progression from early disease to liver failure, the criteria for entry into PALF were set relatively low, namely, coagulopathy secondary to acute liver disease with an INR of ≥1.5 with hepatic encephalopathy or ≥2.0 in the absence of encephalopathy. There was never an intention to liberalize the definition of acute liver failure, and yet more and more frequently, these study entry criteria are being quoted as the "definition" of acute liver failure which they patently are not.

Table 6.13 Causes of acute liver failure outside of infancy

Viral	Hepatitis A
	Hepatitis B
	Hepatitis D
	Hepatitis E
	Herpes simplex
	Epstein-Barr virus
	Varicella zoster
	Paramyxovirus
	Adenovirus
	Parvovirus B19
	SARS
	Hemorrhagic fever viruses
Bacterial	Septicemia
	Leptospirosis
	Salmonella typhi/paratyphi
	Bartonella
	Rocky Mountain spotted fever
Metabolic	Hereditary fructose intolerance
	Urea cycle disorders
	Organic acidemias
	Fatty acid oxidation defects
	Mitochondrial disorders
	Carnitine defects
	Wilson disease
	Tyrosinemia type 1
	Acute fatty liver of pregnancy
Immune	Autoimmune hepatitis
	Hemophagocytic lymphohistiocytosis
Toxic	Drugs/toxins/herbals
	Amanita phalloides
Vascular	Budd-Chiari
	Sinusoidal obstruction syndrome
	Ischemic hepatitis/shock liver
	Post-cardiac surgery
	Liver trauma
Neoplastic	Leukemia
	Lymphoma
	Hepatocellular carcinoma
Other	Reye syndrome
	Hypothermia
	Heat stroke
	Massive liver resection
	Sickle-cell anemia

Differentiating between the causes of acute liver failure can be challenging. Nonspecific

metabolic derangement adds another level of complication to identifying a liver based-metabolic disease. Similar challenges to acute diagnosis exist with many other causes, and great care is required to not miss a potentially treatable cause of liver disease such as autoimmune hepatitis or Wilson disease. Although the single largest group of acute liver failure patients in childhood is those in whom a specific etiology is not identified, it does not negate the need for a full and detailed workup. Sadly, even in the context of a multicenter study with recommendations made for appropriate investigation, inadequate investigation of cause is frequently encountered [9].

There may be clues such as a history of medication or herbal ingestion (see chapter 19), encephalopathy that is fluctuating or out of proportion to the liver dysfunction may point towards a mitochondrial or other metabolic disease, hemolysis can suggest Wilson disease, or foreign travel increasing the likelihood of an infectious cause. Unfortunately these features are neither specific nor sensitive, and as much as we may like to narrow our differential diagnosis to address the most likely or most serious diagnoses first, in the situation of acute hepatic failure, all causes are life-threateningly serious, and therefore, an inclusive panel of investigation is the safest and most efficient approach. Clearly, investigations, especially for infectious causes, will be tempered by knowledge of local occurrence; we in Seattle do not, for example, routinely check for the flavivirus that causes yellow fever. For more details on the approach to acute liver failure, see chapter 23.

Consult from Other Services with Liver Dysfunction in Association with Known Disease

When a consult comes in from another specialist service, there is always the possibility that the liver disease has nothing to do with the condition that the specialist has been managing. This is essentially a caution because the referral from cardiology of the child with Eisenmenger syndrome, abnormal transaminases, and mild splenomegaly may automatically become hepatic congestion and portal hypertension secondary to right heart failure, while their autoimmune hepatitis goes unrecognized and untreated! Therefore, no matter who the originator of the referral is, the approach to differential diagnosis should be based on the child themselves as outlined in the sections above. Having said this, there is probably a different weighting of the diagnostic possibilities in patients with non-liver diseases with known hepatic pathological associations. Table 6.14 lists some of the particular diagnostic considerations based on the specialty team requesting a hepatological consultation on one of their patients.

Other Phenotypes

Although singly or in combination jaundice, hepatitis, hepatomegaly, or liver mass account for the majority of new presentations of liver disease in childhood, occasionally none of these features are immediately obvious and in some cases absent completely (see Table 6.15). Pruritus in the absence of jaundice can rarely be due to cholestasis, although dermatological causes are far more prevalent. Gastrointestinal bleeding from esophageal varices or ascites usually occurs in the presence of recognized chronic liver disease but in some cases are the first overt signs of its presence. Variceal bleeding as a first presenting feature is seen not uncommonly in cavernous transformation of the portal vein secondary to temporally distant portal vein thrombosis. The child or teenager with isolate splenomegaly may be found to have developed cirrhosis with no past history of jaundice or other features of liver disease. Finally, a few infants and toddlers may manifest with hypocalcemic tetany or rickets and be found to have profound fat-soluble vitamin deficiency. If due to liver disease, other features almost always are

Table 6.14 Conditions in other organ systems associated with recognized patterns of liver involvement

(A) Gastroenterology
(i) Inflammatory bowel disease associated with sclerosing cholangitis or autoimmune hepatitis
(ii) Celiac disease associated with autoimmune hepatitis
(iii) Parenteral nutrition-associated liver disease in patients with intestinal failure
(iv) Shwachman-Diamond syndrome – pancreatic insufficiency and neutropenia often associated with hepatomegaly and transaminitis
(B) Cardiology
(i) Fontan circulation with ascites
(ii) Heart failure associated with hepatosplenomegaly, hepatic fibrosis, and sinusoidal dilation
(iii) Cardiac surgery leading to shock liver (ischemic hepatitis) and acute liver failure
(iv) Alagille syndrome
(v) Constrictive pericarditis
(vi) Cardiomyopathy
(C) Hematology/oncology
(i) Sinusoidal obstruction syndrome
(ii) Drug effect
(iii) Hemolysis leading to biliary inspissation/sludge/stones
(iv) Sickle liver
(v) Transfusion-related iron overload
(vi) Transfusion-related infectious hepatitis
(vii) Budd-Chiari
(a) Hypercoagulable states
(b) Myeloproliferative disorders
(c) Paroxysmal nocturnal hemoglobinuria
(viii) Leukemia and lymphoma
(ix) Langerhans cell histiocytosis
(x) Liver tumors
(xi) Stem cell transplantation
(a) Graft versus host disease
(b) Sinusoidal obstruction syndrome
(c) Opportunistic liver infections
(D) Pulmonology
(i) Cystic fibrosis liver disease
(ii) Sarcoidosis
(iii) Tuberculosis
(E) Nephrology
(i) Polycystic kidney disease, nephronophthisis
(ii) Alagille syndrome
(iii) Membranoproliferative glomerulonephritis associated with portal hypertension
(F) Endocrine
(i) Nonalcoholic steatohepatitis
(ii) Diabetic glycogen hepatopathy (Mauriac syndrome)
(iii) Hypopituitarism
(iv) Autoimmune polyendocrine syndrome type 1
(v) McCune-Albright syndrome
(vi) Generalized lipodystrophy (Berardinelli-Seip syndrome)
(G) Immunology
(i) Immunodeficiencies associated with sclerosing cholangitis or hepatic abscess
(ii) Granulomatous hepatitis in chronic granulomatous disease (CDG)

(continued)

Table 6.14 (continued)

(iii) Wegener's granulomatosis
(iv) Immunodysregulation, polyendocrinopathy, and enteropathy, X-linked (IPEX)
(v) Hyper-IgM syndrome (CD40 ligand deficiency)
(vi) HIV particularly congenitally acquired
(H) Rheumatology
(i) Systemic lupus erythematosus
(ii) Systemic juvenile idiopathic arthritis (JIA)
(iii) Juvenile dermatomyositis
(iv) Macrophage activation syndrome
(v) Secondary amyloidosis
(I) Genetics
(i) Liver abnormalities as part of multisystem syndromes

Table 6.15 Differential diagnosis for clinical signs that may suggest liver disease presenting without jaundice, hepatomegaly, or elevated transaminases

(a) Isolated splenomegaly
(i) Portal hypertension (causes of portal hypertension, see Table 6.15)
(ii) Anemia
(iii) Infection
(iv) Sequestration, e.g., sickle-cell disease
(v) Metabolic storage diseases
(vi) Cancer
(vii) Immune, e.g., Felty syndrome, macrophage activation
(b) Ascites
(i) Portal hypertension
(ii) Fontan circulation, heart failure, constrictive pericarditis
(iii) Infection – peritonitis, tuberculosis
(iv) Cancer
(v) Lymphatic dysplasia
(vi) Nephrotic syndrome
(vii) Trauma – lymphatic, urinary
(viii) Vasculitis, serositis
(ix) Kwashiorkor
(x) Pseudo-ascites, e.g., giant omental cyst (Fig. 6.2)
(c) Pruritus
(i) Anicteric cholestasis
(ii) Acute and chronic dermatological conditions
(iii) Cutaneous allergic reactions
(iv) Drug reaction
(v) Opiate withdrawal
(vi) Psychological
(vii) Renal failure
(d) Gastrointestinal bleeding
(i) Varices (usually esophageal or gastric)
(ii) Peptic ulcer

Table 6.15 (continued)

(iii) Mallory-Weiss tear
(iv) Hemorrhoids
(v) Inflammatory bowel disease
(vi) Meckel's diverticulum
(vii) Polyps
(viii) Cancer
(ix) Trauma/ingestion
(x) Intestinal vasculitis, e.g., Henoch-Schönlein purpura
(xi) Angiodysplasia
(e) Metabolic bone disease/hypocalcemia
(i) Nutritional vitamin/calcium/phosphorus deficiency
(ii) Fat-soluble vitamin malabsorption
1. Anicteric cholestasis
2. Bile acid deficiency
(a) Ingestion of bile acid binders
(b) External biliary drainage
(c) Bile acid synthesis and conjugation defects
3. Celiac disease
4. Pancreatic insufficiency
5. Lymphangiectasia
(iii) Hypoparathyroidism

present with the notable exception of defects of bile acid amidation (see chapter 8).

References

1. Reuben A. The liver has a body–a Cook's tour. Hepatology. 2005;41(2):408–15.
2. Reuben A. Never say dye. Hepatology. 2004;39(1):259–64.

3. Fredricks DN, Rojanasthien N, Jacobson MA. AIDS-related disseminated histoplasmosis in San Francisco, California. West J Med. 1997;167(5):315–21.
4. Gavin PJ, Kazacos KR, Shulman ST. Baylisascariasis. Clin Microbiol Rev. 2005;18(4):703–18.
5. Bern C, Martin DL, Gilman RH. Acute and congenital Chagas disease. Adv Parasitol. 2011;75: 19–47.
6. Teitelbaum JE et al. Minocycline-related autoimmune hepatitis: case series and literature review. Arch Pediatr Adolesc Med. 1998;152(11):1132–6.
7. Ali M, Rellos P, Cox TM. Hereditary fructose intolerance. J Med Genet. 1998;35(5):353–65.
8. Finsterer J, Zarrouk Mahjoub S. Mitochondrial toxicity of antiepileptic drugs and their tolerability in mitochondrial disorders. Expert Opin Drug Metab Toxicol. 2012;8(1):71–9.
9. Narkewicz MR et al. Pattern of diagnostic evaluation for the causes of pediatric acute liver failure: an opportunity for quality improvement. J Pediatr. 2009; 155(6):801–6.e1.
10. Pan X et al. Novel mechanism of fetal hepatocyte injury in congenital alloimmune hepatitis involves the terminal complement cascade. Hepatology. 2010; 51(6):2061–8.
11. Yigiter M, Arda IS, Hicsonmez A. Hepatic laceration because of malpositioning of the umbilical vein catheter: case report and literature review. J Pediatr Surg. 2008;43(5):E39–41.
12. Crofts DJ et al. Assessment of stool colour in community management of prolonged jaundice in infancy. Acta Paediatr. 1999;88(9):969–74.
13. Lee M, Vajro P, Keeffe EB. Isolated aspartate aminotransferase elevation: think macro-AST. Dig Dis Sci. 2011;56(2):311–3.
14. Carroll AJ, Coakley JC. Transient hyperphosphatasaemia: an important condition to recognize. J Paediatr Child Health. 2001;37(4):359–62.

7 Psychosocial, Cognitive, and Quality of Life Considerations in the Child with Liver Disease and Their Family

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Introduction

Chronic liver disease (CLD) encompasses a wide variety of pathologic conditions that may present in infancy such as biliary atresia (BA), progressive familial intrahepatic cholestasis (PFIC), and metabolic syndromes or present later in life such as Wilson disease (WD) and anywhere in between such as autoimmune hepatitis.

Children with liver disease frequently have chronic morbidity necessitating frequent hospitalizations and invasive medical procedures. Unexpected complications, complex medication regimens, unpalatable dietary requirements, and uncertainty regarding outcomes are sources of mounting stress for the child and their family. Other stressors include difficulty with schoolwork due to prolonged absences and cognitive deficits, trouble concentrating, and ridicule by peers. Liver transplantation (LT), although lifesaving, can also be a distressing and challenging experience for many families.

The study of the psychosocial, cognitive, and health-related quality of life (HRQOL) outcomes of chronic liver disease and liver transplantation in children and adolescents is relatively recent. The shift from research focused on reducing mortality to investigation of functional outcomes did not occur until survival rates improved in the mid-1990s. Research efforts have been limited by the small numbers of potential participants at individual medical centers, and only recently have research groups been able to organize and fund multicenter studies to explore outcomes. Several psychosocial and cognitive analyses have been performed in small, heterogeneous, single-center samples, often including patients with broad age ranges and varied disease presentations. Further, different measures are often needed for subjects of different ages within the same sample, making

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interpretation more difficult and pre- vs. post-transplant comparisons less useful.

Qualitative analysis of the perceptions of parents of children with CLD reveals a tremendous sense of guilt, frustration regarding loss of control, fear of the future, and anxiety related to uncertainty of the child's outcomes [1]. Children with CLD also perceive their illness as being out of their control [2]. These are important considerations as youth with a chronic illness are two to four times more likely than their healthy peers to have a psychiatric diagnosis at some time during their childhood or adolescence [3].

We will briefly review the psychosocial and cognitive development as well as HRQOL of patients with liver disease and discuss changes that may occur after transplantation. Psychosocial development includes mood, behavior, and social interactions and reflects the child's ability to adjust to difficult situations such as liver disease and transplantation. Cognitive development reflects the child's ability to think, learn, concentrate, problem-solve, and communicate. HRQOL is a broad multidimensional concept that reflects an individual's total well-being including the emotional, social, and physical aspects of their life. The current body of data is limited in its utility due to reliance primarily on small, single-center samples; however, these studies represent an important first step in furthering our knowledge.

Chronic Liver Disease

Chronic liver disease is caused by a heterogeneous group of disorders that can present at any age. The developmental problems faced by an infant with end-stage liver disease secondary to biliary atresia are quite distinct from those of an adolescent with compensated cirrhosis secondary to autoimmune hepatitis. However, there are some features that are shared including the impact of repeated hospitalizations and the potential for cognitive and motor dysfunction due to minimal hepatic encephalopathy, malnutrition, and other medical factors. We will first review some of these commonalities and then detail analyses that have been performed in single-disease cohorts.

Minimal Hepatic Encephalopathy (MHE)

Cognitive Outcomes

MHE may affect children with chronic liver disease of any etiology. The signs and symptoms that may reflect early encephalopathy in children such as crying, irritability, and inattention to task are also observed in children that are moderately ill from any cause, making the diagnosis of MHE in pediatric patients, especially those who are very young, much more challenging than in adults. The consequences of long-term MHE on the developing child's brain are largely unknown. A few studies in the USA [4] and India [5–7] have examined MHE in children using magnetic resonance spectroscopy, finding significant correlations between metabolic brain function and biochemical markers of encephalopathy (plasma ammonia levels and the ratio of branched-chain to aromatic amino acids [BCAA/AAA]). Additionally, correlations between mean diffusivity on diffusion tensor imaging (DTI), plasma ammonia, and brain glutamine/glutamate levels implicate ammonia as playing a key role in the development of low-grade cerebral edema in MHE in children, as in adults [5–7]. Increased pro-inflammatory cytokines have been found in patients with MHE relative to controls, suggesting that both hyperammonemia and pro-inflammatory cytokines play a role in the development of cerebral edema associated with MHE [7]. These studies provide important clues regarding the mechanism for development of hepatic encephalopathy (HE) and a role for imaging in the diagnosis of MHE in children. Also, greater deficits in visuomotor coordination, short-term memory, and visual perception were seen in patients with MHE, and these were associated with increased mean diffusivity, indicating subclinical edema [6].

Some studies suggest that cognitive impairment may improve, especially in fluid abilities, with interventions targeting MHE. Treatment of portal hypertension [8–10] in two patients with surgical repair of congenital portal systemic shunts (PSS) resulted in improvement in

learning/memory, stamina/energy, mood, fine motor speed, reading, and IQ [8]. Improvement was reported in all 4 patients who had cognitive deficits in a retrospective review of 10 patients who had surgical repair of congenital PSS [10]. A prospective study of 12 patients who had extrahepatic portal vein thrombosis and no overt HE also found improvement in fluid abilities (attention, mental speed, and verbal memory) and motor speed/dexterity following surgical repair [9]. However, fluid abilities (executive functions) have not been found to improve universally after treatment of liver disease. In a multisite, longitudinal study of children with hepatitis C virus [11, 12], patients showed worse executive function on a parental questionnaire compared with norms even after 24 weeks of pharmacological treatment. Executive deficits have also been found to persist at least 2 years after liver transplantation [13].

Psychosocial and HRQOL Outcomes

The emotional impact of MHE in children is unknown. Studies in adults with chronic liver disease have demonstrated that MHE through its effects on executive and psychomotor function leads to inability to perform complex tasks and even premature retirement from the workforce [14]. A study of Chinese patients with MHE revealed lower scores in all domains of the Short Form 36 (SF-36) when compared to patients with chronic liver disease without MHE [15]. However, a larger prospective study of 77 patients, 29 of whom had MHE, did not reveal significant differences in HRQOL using the SF-36 [16]. These studies used different criteria for diagnosing MHE which may explain the discrepancies in their findings. With these data, we may surmise that children who typically have fewer coping skills than adults may be at higher risk of developing emotional, behavioral, and social problems as well as lower HRQOL related to MHE. Older children with MHE, specifically those in demanding academic environments, may have difficulty keeping up with their peers, which will also affect their HRQOL.

Cirrhosis

Psychosocial and HRQOL Outcomes

Overall, most children with CLD are well adjusted compared to their peers but feel less in control due to their illness [2]. Children with cirrhosis have changes in their energy levels and appearance that may adversely affect regular social interactions. Reports from the late 1980s found that children with liver disease had moderate to severe deficits in social functioning prior to LT as measured by the Child Behavior Checklist (CBCL) and the Minnesota Child Development Inventory (MCDI) [17, 18]. In a more recent study, parents also reported social deficits in children with less advanced liver disease, suggesting that alterations in peer relationships may be an early feature. Disease severity in that study did not correlate with the level of social functioning. Instead, increased family cohesion, as measured by the Family Adaptability and Cohesion Evaluation Scales (FACES-III), was a marker for better social adaptation even though these children scored significantly lower when compared to a normative sample of healthy children [19].

Patients with chronic liver disease are expected to have lower quality of life as compared to a normative population, especially in the physical domain. Assessment of HRQOL in pediatric patients with cirrhosis has not kept pace with that of liver transplant recipients and studies that assess HRQOL before and after transplant are rare. An important obstacle in this area is that many CLD patients present in infancy or early childhood and the lack of HRQOL assessment scales for these age groups has made measuring functional status and improvements in quality of life difficult. Development of newer tools specifically designed for infants may improve our ability to target this population [20]. One study of infant transplant candidates measured HRQOL at listing for transplant and at 6- and 12-month follow-up after transplant, using the Infant Toddler Health Status Questionnaire (ITHQ). Scores were significantly improved after transplant across multiple domains, with the largest improvements seen in Global Health, Growth and Development, Discomfort and Pain, and Parental Emotional Impact [21].

Biliary Atresia

Cognitive Outcomes

In the few studies that have been performed in children with biliary atresia (BA) surviving with their native liver, IQ and developmental functioning ranges from borderline to average. Early disease onset (age 0–5), diminishing liver function, and growth failure (especially in younger children) have been highlighted as important correlates of intellectual deficits. In an early landmark study, overall cognitive functioning on the Bayley or Stanford-Binet L-M fell in the borderline range (infants M = 79.5; children M = 76.1) with extremely low motor skills (infants M = 69.7; children M = 56.9). Infants' mental and motor developments were associated with growth parameters, whereas children's development was more closely associated with liver function [22]. The most recent study of very young BA patients mirrored earlier findings of very significant delays especially in motor skills (M = 71.8) and expressive language (79.9) on the Mullen Scales associated with liver function, growth parameters, and age at Kasai procedure (the earlier the better) [23].

Similarly, in more heterogeneous samples of patients with end-stage liver disease (ESLD), those with early disease onset (<1 year) had lower IQ than those with later onset (early M = 85.0 vs. late M = 99.5) [24]. Worse outcomes were related to longer illness duration, poorer nutritional status, and vitamin E deficiency. In a subsequent study [25], lower IQ was also found in early- vs. late-onset patients, although late-onset patients scored lower than test norms only on verbal IQ on the Wechsler Intelligence Scale for Children-Revised (WISC-R). IQ was best predicted by liver function and duration of disease, suggesting that patients with the highest risk for poor cognitive outcomes are those with onset of liver disease in the first year of life.

Psychosocial and HRQOL Outcomes

There are likewise few studies focusing on the emotional well-being of non-transplanted survivors of biliary atresia. One report compared long-term survivors in the UK and Japan to healthy controls using the SF-36 and found that Japanese patients reported significantly lower scores in emotional and social functioning compared to their peers in the UK. However, the overall numbers were small (21 vs. 25 patients) [26]. Prior to LT, children are noted to be overly dependent and demanding, and as with all patients with cirrhosis, physical appearance and energy levels restrict physical activity and social interactions [18].

Inherited Cholestatic Diseases

Cognitive Outcomes

Original descriptions of patients with Alagille syndrome described cognitive delay as an important feature. However, it now appears more likely that cognitive delay in these early reports may have been related to prolonged hospitalization, malnutrition, and especially fat-soluble vitamin deficiencies [27, 28]. Improvements in nutritional management may have resulted in fewer reports of associated developmental delay. There is a single case report of a 16-year-old with progressive familial intrahepatic cholestasis (PFIC) whose symptoms included apathy, cognitive impairment, and extrapyramidal syndrome [29]. However, there are no studies that systematically address cognitive outcomes in children with PFIC prior to transplantation. Assessment of these outcomes in this and other rare forms of childhood liver disease has been a priority for the Childhood Liver Disease Research and Education Network (ChiLDREN http://childrennetwork.org), and thus these data should be forthcoming.

Psychosocial and HRQOL Outcomes

One report of HRQOL in 71 patients with Alagille syndrome revealed lower HRQOL for both psychosocial and physical function as compared to the general population; see Fig. 7.1. Cardiac catheterization or surgery, mental health diagnosis, and poor sleep were associated with lower HRQOL in this cohort [30].

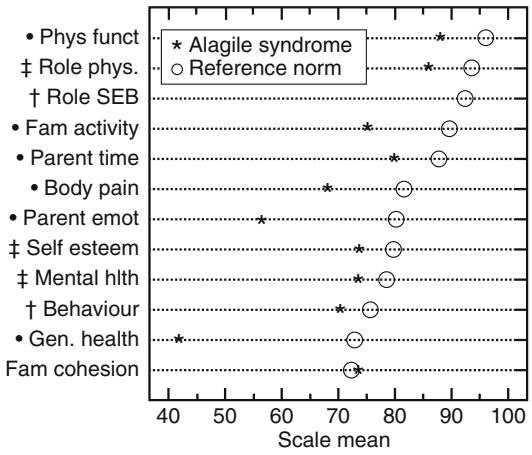

Fig. 7.1 Mean CHQ subscale scores for Alagille syndrome and the normative population and differences between the cohorts. Difference between cohorts: † $p<0.05$, ‡ $p<0.01$, *stands for a p<0.005. (Reproduced with permission from Elisofon et al. [30])

Wilson Disease (WD)

Cognitive Outcomes

WD can present with progressively worsening symptoms that are not diagnosed for months or years or with acute decompensation of neurological, psychiatric, and/or liver functioning. Thus, outcomes may be quite variable, in part related to the progression of the disease prior to treatment initiation, the stage of treatment, and the type of symptoms at presentation. A retrospective review of WD patients ($n=129$) suggested a pattern of early improvement following treatment, in both hepatic and neuropsychiatric symptoms with a subsequent plateau in these symptoms [31]. However, two smaller studies ($n<10$) did not show significant neurological improvement or change in IQ with therapy [32, 33]. Imaging studies suggest that patients with WD have cognitive deficits in areas such as processing speed, executive functioning, attention, learning/memory, visuoconstructive ability, and verbal fluency that appear to be associated with brain abnormalities in basal ganglia, brainstem, thalamus, frontal lobes, and general cognitive atrophy [34, 35].

Psychosocial and HRQOL Outcomes

There are limited studies assessing the emotional health of children with WD. This may be due to the focus on and difficulty differentiating between neurological symptoms and emotional disorders. In fact, patients with primarily neurological or psychiatric symptoms (such as personality change) are typically diagnosed later than those with primarily hepatic symptoms and may actually be misdiagnosed initially, leading to more disease progression prior to treatment [36]. Case reports detail hyperactivity, poor sleep, and bad temper in undiagnosed children with WD that improve after therapy [37]. A small study ($n=23$) of adults with WD found they had an increased prevalence of major depressive disorders as well as bipolar disorder [38].

Svetel et al. [39] conducted a cross-sectional study to identify clinical and demographic factors influencing health-related quality of life in 60 treated, clinically stable patients with WD using the SF-36. The level of disability and grading of WD severity were assessed by the Global Assessment Scale for WD [GAS for WD]; cognitive impairment and depressive features were assessed respectively by the Mini Mental State Examination [MMSE] and the 21-item Hamilton Depression Rating Scale [HDRS]. Lower scores on the SF-36 domains were found in patients with neurological and psychiatric symptoms compared with those with a predominantly hepatic form of WD. SF-36 scores were also lower in those who were depressed, who had cognitive impairment, and had a longer latency from appearance of symptoms to treatment initiation.

Metabolic Liver Disease

Cognitive Outcomes

Outcomes vary widely depending on the disease presentation. Certain disorders such as urea cycle defects (UCD) and tyrosinemia present in early infancy with severe hyperammonemia that may result in profound brain damage. Thimm et al. assessed the cognitive and motor fdevelopment

of nine patients who ranged in age from 1 to 8.5 years with tyrosinemia type I using the Bayley Scales, Snijders-Oomen test, Kaufman Assessment Battery for Children (KABC), and Movement Assessment Battery for Children. Six of the nine patients tested below normal with one patient testing in the mild to moderate mental disability range. Four of seven patients tested for motor abilities also scored below the normal range [40]. Krivitzky et al. in an analysis of 92 patients with UCD reported 30 % of patients as having intellectual disabilities with a greater proportion of those affected having neonatal onset of disease. The number of hyperammonemic episodes was not a significant factor in IQ levels in these subjects. All patients had difficulties in social interactions, attention deficit, and executive functioning [41]. A report of 28 patients with ornithine carbamoyltransferase deficiency (OTC), the most common UCD, revealed 18 with disabling neurological conditions including seven with focal neurological deficits [42]. In a report assessing the effects of early vs. late transplantation in five patients with UCDs, all the children had below average developmental scores on Griffiths scales. Three of the five patients improved after transplant; however, they remained greater than one standard deviation below normal [43]. A review of 88 patients with urea cycle defects reveals that neonatal screening has improved survival; however, 2/3 continue to suffer severe neurological damage [44].

Psychosocial and HRQOL Outcomes

It appears that metabolic disorders in particular exact a very high emotional toll on the family. A survey of parents of children with urea cycle defects reported that almost half thought of their children dying every day and a quarter of parents did not feel they could change jobs due to insurance [45].

Mitochondrial disorders frequently present with liver failure but also can cause chronic liver disease. The majority of these patients are not transplant candidates because they suffer from progressive neurologic injury as well and succumb to these complications even following successful transplantation [46]. However, there are a small number of these disorders that are associated with more chronic neurological injury which is not life-limiting. Isolated case reports of these survivors do not detail cognitive status or functional outcomes, and the rarity of these patients limits accumulated experience [47].

Hepatitis C Virus

Cognitive Outcomes

Hepatitis C virus (HCV) is generally considered to be asymptomatic in childhood; however, in a study of treatment-naïve patients ($n = 114$) using the Behavior Rating Inventory of Executive Function (BRIEF), 18 % had clinically significant impairment in executive function, including working memory. However, overall they performed better than children with attention-deficit/hyperactivity disorder (ADHD) [11]. Adults with HCV also have decreased cognitive [48] and psychological functioning [49].

Psychosocial and HRQOL Outcomes

Once therapy is initiated, the use of interferon alfa, which remains the standard of care, increases the risk of psychiatric disorders such as depression and anxiety [49]. Although the use of newer agents such as protease inhibitors has led to promising outcomes, unless treatment strategies change, it is likely that children who are infected with HCV today will face these same side effects when they are treated in adulthood. In the absence of functional impairment, children and adolescents may not experience any behavioral or emotional sequelae that may be linked to their medical diagnosis [12]. This perception of well-being seems to negate parental concern of future morbidity and has led to a lack of services to provide support for these families.

Hepatitis C is considered to have an asymptomatic course in early childhood. HCV, especially in the early stages, may not cause any significant impairment in physical functioning, social activities, and bodily discomfort. However, Nydegger et al. using the Child Health Questionnaire (CHQ) have reported marked reductions in emotional, general health, parent

impact-emotional, and time scores. Children self-reported scores comparable to their healthy peers with the exception of lower scores in physical functioning. While 73 % of parents reported being worried "a lot" about their child's future health, only 10 % of children were concerned about being treated differently or about the risk of future medical complications such as liver cancer [50]. In a study of 114 treatment naïve children with HCV, parents reported significant emotional impact from their child's illness as well as a perception that they were less healthy on the CHQ. Family activities and cohesion were unaffected as was mental health and self-esteem. Mothers who transmitted the disease to their child reported lower emotional scores on the SF-36 compared to parents who did not transmit the virus. Good caregiver emotional and mental health was associated with patient psychosocial health with only two children scoring in the depressed range on the Childhood Depression Inventory [11].

Liver Transplantation

Liver transplant recipients now enjoy survival rates exceeding 85 % at 5 years [51]. Although "cured" of their underlying disease, these patients continue to face the long-term effects of chronic immunosuppression, fear of graft failure, and the need for lifelong medical surveillance.

Cognitive Outcomes

Studies examining post-LT cognitive functioning over the past decade have mostly reinforced earlier findings. IQ is nearly universally below published norms [13, 52–55]. Pediatric LT patients clearly have a downward shift in IQ, with mean IQ scores typically in the mid-80s to low-90s and an increased prevalence (up to 27 % vs. 2 % expected) of scores falling below 70 [13, 52, 56, 57]. One study of long-term LT survivors aged 3–9 years revealed that almost 20 % had an IQ of less than 70 [58]. Patients who experience liver disease earlier in life appear to be at higher risk for developmental delay which may be further exacerbated during the transplant process. An analysis of 40 infants with biliary atresia who were assessed before transplant and again 3 and 12 months after transplant revealed that mean Bayley scores for both mental and psychomotor development, which were in the low average range, dropped by another standard deviation at 3 months following transplant. One year after transplant 35 % were diagnosed as developmentally delayed and mean scores had improved only to the pre-transplant level. Delayed development was associated with decreased weight at transplant, low albumin, length of hospital stay, and younger age at transplant [59].

Studies of cognitive outcomes following LT have primarily focused on IQ, and therefore, less is known about other cognitive domains. Most have found similarly delayed verbal and nonverbal IQ [13, 52, 57]. However, one study [56] found significantly weaker language processing on the Clinical Evaluation of Language Fundamentals-Preschool or Revised (CELF-P/CELF-R) compared with controls with cystic fibrosis. Only two other studies of children with liver disease have suggested a relative weakness in language: in children under age 2 with a large proportion of non-English speakers [23] and in a retrospective review [25].

In contrast, a recent study ($n=18$; age 7–16) [60] reported poorer nonverbal IQ ($M=88.9$), but not verbal IQ ($M=99.6$) or full scale IQ (94.0) compared with WISC-III norms. This study also found poorer performance on visuospatial, visuoconstructive, and social perception tasks on the NEPSY-II, but not in language, attention/executive function, or memory and learning. Other studies have reported deficits in visuomotor skills ($M=82$) [57] and lower nonverbal IQ ($M=84.5$) than verbal IQ ($M=90.6$) in 30 % of patients on the Wechsler scales [52]. Such findings are in line with prior report by the Stewart group [61] and recent report of MHE outcomes [6].

The few pediatric studies of attention and executive functioning post-LT have typically found deficits. Several studies using the KABC have found deficits in both sequential (i.e., working memory) and simultaneous (i.e.,

nonverbal reasoning) performance relative to norms [53–55]. In a recent study by the same group ($n=137$; age 6–18 years), LT patients demonstrated poorer attention compared with norms in alertness, working memory, sustained attention, and divided attention [62]. A large multicenter study ($n=144$) [13] reported significant executive deficits relative to norms on the BRIEF, particularly by teacher report (Global Executive Composite = 58) (Fig. 7.2). Consistent with earlier findings by Stewart and colleagues, recent studies have also documented significant problems with learning and school functioning. Achievement was found to be below norms in several studies [13, 53, 57], but not different as compared to controls with cystic fibrosis [56]. Sorensen et al. [13] found that young LT patients demonstrated school readiness concepts consistent with peers on the Bracken Basic Concept Scale, Revised, but differed from norms in both word reading ($M=92.7$) and math ($M=93.1$) on the Wide Range Achievement Test, 4th edition.

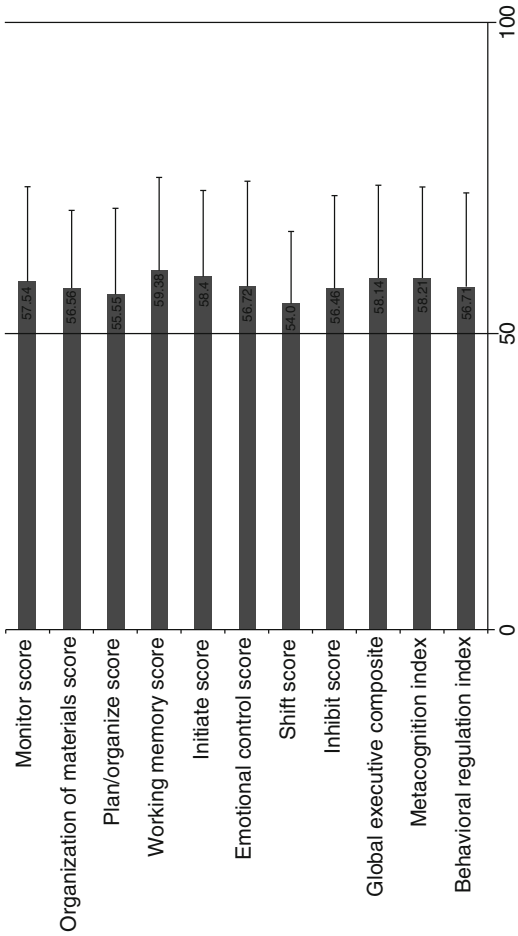

Fig. 7.2 All teacher BRIEF T scores for the pediatric liver transplant sample ($n=72$) were significantly different from the normative population ($p<0.005$). The normative population for the BRIEF has a mean T score of 50 and a standard deviation of 10, with higher scores reflecting poorer executive functioning. The Hochberg adjustment was used to control for multiple comparisons (Data from Sorensen et al. [13])

In the largest study of academic outcomes to date in pediatric LT patients ($n=823$; age 6–18) [63], 34 % of patients were receiving special education services, 11 % had received accommodations, and 20 % had repeated a grade by parent report. Diagnosis of learning disability was reported in 17.4 % and mental retardation in 5.2 %. The other large multicenter study of pediatric LT patients [13] similarly reported that 31 % had received special education in the past year and 25 % had profiles suggesting learning disability. These results are consistent with earlier findings despite substantial improvement in post-transplant survival and management over the past 15 years [58].

While recent studies have examined predictors of cognitive and academic outcomes after LT in children, the results remain mixed. Younger age at LT was found to be an important factor leading to poorer outcomes in one study [56], but not in another [53]. A retrospective review ($n=40$; age less than 6 months at LT) found "long-term" outcomes of "regular mental development" in only 28 % of participants [64]. In contrast, another study reported that younger age at transplant predicted *better* performance, but only for nonverbal IQ and achievement, not for working memory on the KABC [54].

Although one study did not find a significant effect of diagnosis or time since transplant [52], LT patients with BA performed better than those with other diagnoses in another [57]. A large study found worse attention/executive function in patients with "diagnoses affecting the brain" (Crigler-Najjar, citrullinemia, Alagille, metabolic disorders, WD, tyrosinemia) compared

with those who had diagnoses that presumably do not directly affect the brain (BA, α_1-AT, oxalosis, cholestasis, autoimmune hepatitis, liver tumor) [62]. It should be noted that this distinction is debatable.

In a moderately large sample ($n=44$), longer duration of illness and height deficient at LT predicted nonverbal IQ and achievement [54]. Another study found that 45 % of variance in nonverbal IQ was explained by growth deficits pre-LT and elevated serum ammonia, while 23 % of variance in verbal IQ was due to elevated calcineurin inhibitor levels [57]. Language deficits have been found to be associated with disease severity and peri- and post-LT complications as reflected in more days in intensive care, more days in the hospital post-LT, and elevated bilirubin pre-LT [56].

In a large multicenter school outcomes study [63], the strongest predictor of special education was pre-LT special education (odds ratio 22.5). Posttransplant factors were also predictive, including type of immunosuppression (cyclosporine or other was worse than tacrolimus) and cytomegalovirus post-LT. In a smaller study ($n=29$), age (younger better) and more normal height at LT explained 66 % of variance in achievement [53]. Slow reaction time and poor sustained attention were predicted by type of LT (deceased donor), longer duration of disease, older age at LT, and gender, although the amount of variance explained was modest (14–25 %) [62].

Consistent with previous literature, recent evidence suggests that progressive cognitive decline may be halted or even reversed after LT. A case series of patients with Crigler-Najjar syndrome type 1 suggested that earlier LT (i.e., prior to brain injury) results in better outcome [65]. A 4-year-old without brain injury remained cognitively intact post-LT, whereas children aged 7 and 12 years who had mild to moderate deficits pre-LT improved incompletely following LT. Similarly, a small series ($n=14$) of patients with maple syrup urine disease (MSUD) showed stable IQ in 57 % and improved IQ in 36 % post-LT [66]. Cognitive functioning in another metabolic disorder, propionic acidemia, also stabilized or improved post-LT according to a retrospective review ($n=12$) [67]. Stable or improved functioning up to 15 years post-LT in WD patients has also been reported ($n=32$; age 6–40) [68].

Other ESLD and BA groups were found to demonstrate stable functioning post-LT vs. pre-LT. A follow-up to an earlier report [69] on 25 infants (<1 year) undergoing LT found a slight dip in some areas of cognitive functioning on the Griffiths initially but a return to pre-LT levels by 4 years post-LT [70]. One case report suggested improved cognitive and/or academic functioning post-LT in a child with BA [71]. The child's school functioning was reduced relative to her healthy identical twin by the 2nd year of school, but following LT in middle school, her performance steadily and dramatically improved until she was performing at the level of her twin in the 3rd year of high school.

Data on cognitive outcomes and predictors in pediatric LT recipients are mixed. IQ is most clearly skewed lower than normal, although there is some evidence for deficits in other cognitive domains such as attention/executive function, learning/memory, visuospatial/nonverbal abilities, language, as well as academic functioning. Certain factors such as age at transplant have been both positively and negatively associated with improved cognitive outcomes. Other factors relating to pre-transplant disease (e.g., disease type, deficient growth, liver function, HE) as well as peri-/posttransplant issues (e.g., immunosuppressants, infection, transplant complications) may also play a role. Children with metabolic diseases may have worse outcomes when compared to those with biliary atresia; however, LT may prevent further neurological decline. The heterogeneous patient population with respect to disease category and age, as well as typically small samples, makes comparisons between studies difficult. LT recipients struggle with various neurologic deficits and further research is needed to identify factors to improve their cognitive outcomes. Additional details on cognitive outcomes in pediatric patients with liver disease and transplantation can be found in a recent review by Sorensen [72].

Psychosocial and HRQOL Outcomes

The very first studies of behavioral outcomes following LT suggested that 50 % of children show maladaptive behavior, such as temper tantrums, impulsiveness, poor concentration, defiance, and aggressive behavior [18, 73]. Furthermore, adolescents have been noted to have attention and conduct problems, particularly boys [74]. Behavioral problems rarely present in the early post-LT period, more typically manifesting in the later post-LT period, especially in those transplanted at an early age [74]. More recently, two studies demonstrated that between 1/3 and 1/2 of transplant recipients assessed with the CBCL scored in the pathological range for total problems. The most affected problem scales included withdrawal, thought problems, aggressive behavior, and attention problems [55, 75].

LT is a stressful experience for many children. However, by 5 years post-LT, children often have better emotional adjustment, compared to those with other chronic illnesses [76, 77]. In a large single-center study of 51 patients with a median time since transplant of over 10 years, parents regarded their children as having psychological health that was comparative to a normal population using the CHQ [78]. However, other studies show that transplant recipients continue to lag behind their healthy peers, with 55 % thought to have some emotional problems [52]. Parents self-report higher levels of psychological symptoms pre- and posttransplant with fathers showing greater distress than mothers [79]. Post-traumatic stress disorder (PTSD) has also been demonstrated in 16 % of adolescent patients that receive transplantation [80] and 27 % of parents of childhood LT recipients [81].

Several studies have explored the impact of liver transplantation on the parents and family. Using the Family Environment Scale, Fredericks et al. did not find a difference in cohesion among family members of transplant patients but did find higher levels of parental stress and total difficulty on the CHQ [82]. This is significant as an older study reported that more than a third of divorced or separated parents of pediatric transplant patients claimed the stress of raising a chronically ill child contributed to marital discord [18]. A recent study using the Family Assessment Device revealed similar levels of family dysfunction as a reference sample [83]. Analysis of risk factors associated with lower reported family function identified demographic factors, such as lower parental education level, and medical complications related to biliary tract obstruction as potentially important determinants of this outcome. The daily adjustments required to accommodate a child posttransplantation have also been associated with maternal depression and anxiety [84]. Given the previous research detailing altered functioning of families of children with chronic illness or disability [85], the extent to which families modify or adjust routines to accommodate children following transplantation warrants further investigation.

The largest study on school outcomes consisting of 823 children in the Studies of Pediatric Liver Transplantation (SPLIT) registry has shown that 96 % of children are able to attend school, although a significant number require special education [63]. In addition to cognitive deficits, missed school days may also contribute to compromised academic functioning. Data from the SPLIT network suggests that 30–40 % of patients in long-term follow-up miss more than 10 days of school per year, with teens having the highest rate of absences. There are conflicting reports in the literature regarding participation in extracurricular activities, with some studies reporting that the majority of children participate in organized sport and integrate well in school [86], while others suggest that social functioning and participation in activities is reduced [87]. Physical abilities measured by physical summary scores of the PedsQL™ are reduced and may contribute to less physical interaction [88].

Research focused on individual aspects of psychosocial functioning indicates some positive outcomes for children after LT such as improved attendance at school and increases in social activity and the ability to cope with everyday stress. However, there is also evidence suggesting that children continue to have psychosocial difficulties such as behavioral problems, depression, anxiety, and reduced self-esteem [55]. The risk factors for psychosocial problems post-LT remain poorly understood. Furthermore, adolescents are underrepresented in many of the studies and may present particular vulnerability or treatment challenges.

Pediatric liver transplant recipients have significantly lower HRQOL compared to healthy

controls [89–91]. The SPLIT Functional Outcomes Group (FOG) conducted a large cross-sectional analysis of generic HRQOL in 873 (363 self-report) pediatric LT recipients between the ages of 2 and 18 years using the PedsQL™ (Mapi Research Institute, Lyon, France) generic core scales. Patients in the sample had a mean age of 8.2 ± 4.4 years and 55 % were female. The median interval from transplant to survey was 3.1 years. Outcomes were compared to a sample of healthy children randomly matched by age group, gender, and race/ethnicity; see Fig. 7.3. The physical and psychosocial functioning of the LT recipients compared favorably with children with other

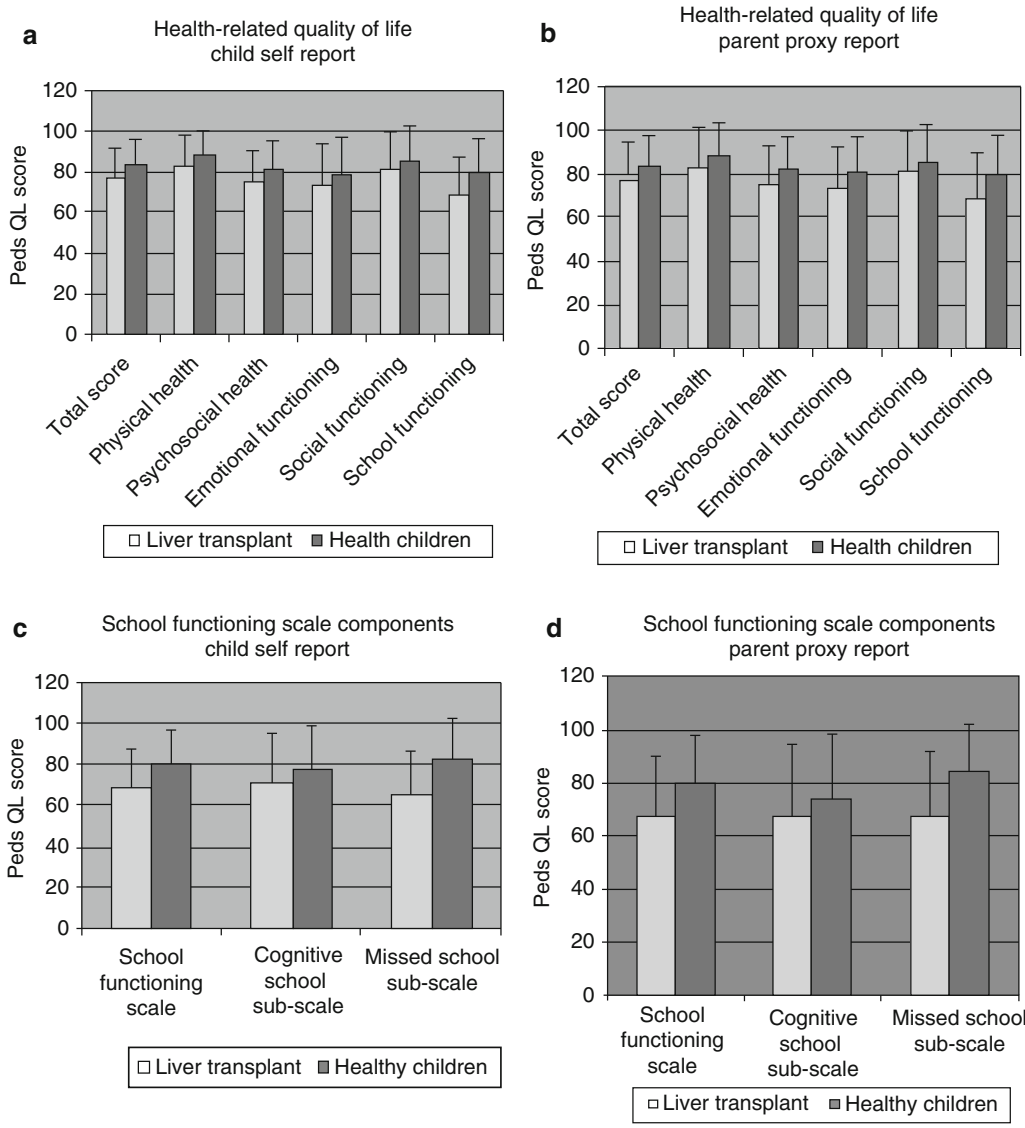

Fig. 7.3 (**a–d**) Using the PedsQL generic core scales to assess quality of life in liver transplant recipients compared to a healthy sample matched for gender, race, and age. (**a**) Patient self-report ($n = 363$) patients report significantly lower scores when compared to healthy controls. (**b**) Parent proxy report ($n = 869$) parents report lower scores compared to healthy controls. (**c**) School functioning scale by patient self-report ($n = 361$) parents of liver transplant recipients report significantly lower scores than parents of healthy children with an effect size of 0.68. (**d**) School functioning scale parent proxy report ($n = 746$) parents of liver transplant recipients report significantly lower scores than parents of healthy children (Reproduced with permission from [96])

chronic pediatric illnesses but was not equal to the healthy sample. The total scale score and subscales of the PedsQL™ 4.0 generic core scales were all significantly lower than those of healthy children ($p<0.001$) with effect sizes ranging from 0.25, for self-reported emotional functioning, to 0.68 for self-reported school functioning [89, 90]. Effect sizes greater than 0.5 are considered moderate with those approaching 0.8 considered large. The altered school functioning that is observed in this group may be secondary to an increased prevalence of cognitive deficits and learning disabilities.

Demographic as well as medical variables may predict levels of HRQOL in this population [21, 91, 92]. The impact of age on HRQOL in pediatric LT recipients has been considered in several studies. In a small, multicenter report, younger survivors (less than age 5 years) had physical and psychosocial health that was comparable to age-matched controls and higher than what was reported for older children in the same study. The SPLIT/FOG cross-sectional data set was analyzed to examine the impact of age at testing on parent report of HRQOL. Results suggest that age at testing may indeed have an important impact on HRQOL with younger children having the highest scores (Table 7.1). In fact, the impact of age at testing appears to be more significant than interval from transplant. Initial results from multivariate analysis examining the impact of various factors on parent reported HRQOL in the SPLIT/FOG study identified single-parent household, length of initial hospitalization after transplant, older age, history of seizures, lower height z score at transplant, and days hospitalized in recent follow-up as negative predictors. A large multicenter report demonstrated strong correlation between impaired cognitive functioning and lower HRQOL [93]. Additionally, the relationship between the patient's HRQOL and family dynamics bears further consideration. Studies that have included assessment of the impact of the child's health state on the parents have shown a considerable negative influence on parental emotional state and family life [52, 78, 83].

However, when formally measured, family function was found to be equal to that reported by a reference population [83]. These preliminary results suggest that services that support the parent's ability to cope with their child's health condition would likely improve the child's HRQOL. This strategy is especially important as the patients' level of HRQOL has been linked to adherence behaviors and possibly maintenance of graft function [94].

Implications for Practice and Research Opportunities

As the number of patients surviving pediatric liver disease/LT increases, many questions with regards to the long-term psychosocial, cognitive, and HRQOL outcomes remain unanswered. Appropriate interventions for the abnormalities described in this chapter have not been determined as we continue to assess the scope and determinants of the problem. Nevertheless, health-care providers need to be vigilant for signs of distress among patients with CLD and their families. Patient/parent interview should incorporate questions around cognitive and school functioning, as well as psychosocial functioning and overall HRQOL. Parental stress, adjustment,

Table 7.1 PedsQL™ 4.0 generic core scale scores by age at testing*

	Age at testing			
	<2 years ($n=259$)	2–4 years ($n=254$)	5–7 years ($n=244$)	≥8 years ($n=169$)
Scale score	**Median (interquartile range)**			
Total score ($p<0.0001$)	85.7 (73.8–94.4)	79.4 (63.0–90.2)	73.9 (59.8–84.2)	76.1 (59.8–88.0)
Physical health ($p<0.0001$)	93.8 (78.1–100.0)	87.5 (68.8–96.9)	83.3 (62.5–93.8)	81.3 (62.5–93.8)
Psychosocial health ($p<0.0001$)	82.7 (70.0–92.3)	76.7 (61.7–88.3)	69.2 (56.7–81.7)	73.3 (56.7–90.0)

*Unpublished data from the Functional Outcomes Group (FOG) research group, part of the Studies of Pediatric Liver Transplantation (SPLIT) collaborative

and family functioning should also be assessed (Table 7.2). Providers need to be particularly alert for these concerns in very young and very growth deficient patients, those who are the most ill, have a complicated course, or have an illness that causes more serious impairment (e.g., metabolic disorders). Providers need to recognize and prepare parents for the likelihood that any cognitive and academic challenges seen pre-transplant will persist following transplantation.

Table 7.2 Suggested interview/survey approach to screen for psychosocial, cognitive, and HRQOL concerns in pediatric patients with liver disease in the medical setting

Area of functioning	Parent ("Does your child have difficulty…")
Cognitive/motor	
Age 0–5	Speaking or understanding?
	Performing gross motor or fine motor activities such as walking, running, jumping, skipping, buttoning, tying laces, or drawing?
	[Use the Ages & Stages Questionnaires®]
Elementary school years	Concentrating, following directions, or remembering things?
	Understanding others or putting their thoughts into words?
	Understanding ideas that are not concrete or literal (things that are not obviously stated)?
	Performing gross motor or fine motor activities?
Middle/high school years (include patient in interview)	[Same as for elementary]
	Keeping their belongings organized?
	Using strategies to solve problems?
	Performing gross motor or fine motor activities?
Academic	
Preschool	Drawing or writing?
	Learning letters and letter sounds, numbers and counting, colors, and shapes?
	Learning phone number, address, parents' names?
	Writing their name?
Elementary school	With homework: taking longer, needing more help, working harder than same age peers?
	Remembering to bring home materials, complete assignments, and turn in completed work?
	Planning ahead on longer projects?
	Reading and understanding what they read?
	Putting ideas into writing?
	Understanding math concepts and problem-solving?
Middle/high school (include patient in interview)	[Same as for elementary]
	Learning new material: making it "stick"?
	Keeping school materials organized?
	Managing time efficiently?
	Studying effectively for tests?
	Checking work for careless errors?
	Working mostly independently?
Psychosocial	
Age 0–5	[Use the Ages & Stages Questionnaires®]
Elementary years	Making and keeping friends?
	Regulating mood (keeping mood "on an even keel" without getting overly excited, sad, or mad frequently)?
	Dealing with frustration?
	With fears and worries or self-esteem?
	Adjusting to changes?
	Understanding and accepting their medical condition/history?
	Controlling their behavior?

(continued)

Table 7.2 (continued)

Area of functioning	Parent ("Does your child have difficulty…")
Middle/high school	[Same as elementary]
	Adhering to medication/treatment regimen?
	Planning for future?
	Engaging in age-appropriate activities?
	Avoiding excessively risky behaviors?
	Self-advocating (e.g., asking questions, asking for help when needed, using available resources and supports)?
HRQOL	Use the PedsQL™

Patients with significant concerns should be referred for more comprehensive evaluation. Parents should also be screened for psychosocial concerns related to their child's illness and referred for treatment as needed

When cognitive/academic concerns are discovered, patients should be referred for neuropsychological evaluation or school-based testing so that they may receive special education services if warranted. Even if school performance is adequate in the early grades, parents should be vigilant for signs of increased difficulty keeping pace with academic demands and seek evaluation and/or services in a timely manner when such concerns arise. Since LT occurs most commonly prior to the start of formal education, teachers may not be aware of the child's history and the extent to which it may impact neuropsychological, academic, and psychosocial functioning.

Several websites provide information and resources regarding liver function and disorders in children. The Children's Liver Disease Foundation (www.childrensliverdisease.org), the American Liver Foundation (www.liverfoundation.org), and Children's Liver Association for Support Services (CLASS; www.classkids.org) provide informational handouts and resources. The Children's Liver Disease Foundation has appealing and kid-friendly animations explaining normal and abnormal liver functioning. The CLASS website provides numerous links to additional support organizations. UNOS (www.unos.org) provides statistics on LT and information for patients and families. The Childhood Liver Disease Research and Education Network (ChiLDREN) is a collaborative network of medical centers and patient support organizations designed to encourage and facilitate participation in research studies (http://childrennetwork.org).

Pediatric patients with liver disease clearly demonstrate deficits in IQ that typically persist even after LT. However, the longitudinal course of these deficits over the lifespan and in terms of time since LT is not well described. It is unclear which patients will improve after LT and which will remain stable. While IQ deficits are expected, more data is needed regarding the pattern of functioning across other cognitive domains. Attention/executive function is an area of particular interest given its importance to maintaining a job and independent living and its relationship to MHE. While lactulose and rifaximin are sometimes given in pediatric patients with suspected MHE, their effectiveness in children has not been examined using cognitive measures, as they have in adults [95]. These areas would benefit from further study.

Referral for a more thorough psychological evaluation may also be warranted if the child or parents express psychosocial concerns. Patients and families are negatively affected by the chronicity of liver disease and the fear and uncertainty therein. The complexity and burden of medical management of children with end-stage liver disease place enormous psychosocial and financial stress on patients and their families. Parents may be overwhelmed with the fear that their child may die suddenly or in the case of genetically acquired metabolic diseases that they are to blame. Disruption caused by frequent doctor's visits, the pain and prospects of unexpected complications associated with multiple medical procedures, and the difficulty in maintaining steady employment and a normal home

environment for the rest of their family members contribute to this struggle [1]. The demands of providing adequate health care to children with end-stage liver disease may quickly outstrip the family's resources. When this happens, social services must be involved to optimize provision of unmet psychosocial and financial needs to minimize further negative repercussions for the child. Transportation, housing, and financial arrangements can often be made to meet the family's immediate care-related needs. Early and intensive involvement of social and financial services is critical to maintaining good quality of life for these patients and families in order to ensure the best outcomes for this vulnerable patient population.

The transplant process is also a time of great stress for the entire family. Attention to the needs and concerns of the parents and other family members is essential. This may require consulting with social workers and psychologists. Further study is needed on how to best help families cope with these pressures and how they affect the medical outcome, particularly graft failure. HRQOL remains below that of the general population with school function of particular concern. The growing understanding of the relationship between HRQOL, medication adherence, and overall graft function underscores this as an area where successful interventions may lead to long-term medical and social benefits.

Once the scope and nature of the problems have been more comprehensively characterized, potential changes in policy (e.g., age at LT listing) and standard of care (e.g., use of post-LT medications, surgical interventions) can be proposed to promote optimal outcomes. Finally, when all modifiable medical variables contributing to cognitive and psychosocial outcomes have been addressed, research must pursue additional means for improving outcomes by assessing the efficacy of targeted interventions (e.g., psychostimulants for inattention). These goals can only be accomplished with more multicenter collaboration and efforts to carefully design prospective studies with large, representative samples. Use of different tests based on age in the same sample should be minimized or at least standardized as this introduces a confounder and makes interpretation more challenging. Psychosocial, cognitive, and HRQOL outcomes in children with liver disease and LT represent an underexplored frontline whose assessment will hopefully lead to a better understanding and more effective prevention and management of deficits in the coming years.

References

1. Simon NB, Smith D. Living with chronic pediatric liver disease: the parents' experience. Pediatr Nurs. 1992;18(5):453–8, 489.
2. Mastroyannopoulou K, et al. Psychological effects of liver disease and transplantation. Eur J Pediatr. 1998; 157(10):856–60.
3. Garrison WT, McQuiston S. Chronic illness during childhood and adolescence: psychological aspects. Newbury Park: Sage Publications, Inc.; 1989.
4. Foerster BR, et al. Minimal hepatic encephalopathy in children: evaluation with proton MR spectroscopy. Am J Neuroradiol. 2009;30:1610–3.
5. Yadav SK, et al. Brain MR imaging and ^{1}H-MR spectroscopy changes in patients with extrahepatic portal vein obstruction from early childhood to adulthood. Am J Neuroradiol. 2010;31:1337–42.
6. Yadav SK, et al. Encephalopathy assessment in children with extra-hepatic portal vein obstruction with MR, psychometry, and critical flicker frequency. J Hepatol. 2010;52:348–54.
7. Srivastava A, et al. Pro-inflammatory cytokines are raised in extrahepatic portal venous obstruction, with minimal hepatic encephalopathy. J Gastroenterol Hepatol. 2011;26:979–86.
8. Eroglu Y, et al. Improved neurocognitive function after radiologic closure of congenital portosystemic shunts. J Pediatr Gastroenterol Nutr. 2004;39:410–7.
9. Mack CL, et al. Surgically restoring portal blood flow to the liver in children with primary extrahepatic portal vein thrombosis improves fluid neurocognitive ability. Pediatrics. 2006;117(3):e405–12.
10. Lautz TB, et al. Management and classification of type II congenital portosystemic shunts. J Pediatr Surg. 2011;46:308–14.
11. Rodrigue JR, et al. Impact of Hepatitis C virus infection on children and their caregivers: quality of life, cognitive, and emotional outcomes. J Pediatr Gastroenterol Nutr. 2009;48:341–7.
12. Rodrigue JR, et al. Peginterferon with or without ribavirin has minimal effect on quality of life, behavioral/emotional, and cognitive outcomes in children. Hepatology. 2011;53(5):1468–75.
13. Sorensen LG, et al. Cognitive and academic outcomes after pediatric liver transplantation: Functional Outcomes Group (FOG) results. Am J Transplant. 2011; 11:303–11.

14. Amodio P, et al. Characteristics of minimal hepatic encephalopathy. Metab Brain Dis. 2004; 19(3–4):253–67.
15. Bao ZJ, et al. Assessment of health-related quality of life in Chinese patients with minimal hepatic encephalopathy. World J Gastroenterol. 2007;13(21):3003–8.
16. Wunsch E, et al. Minimal hepatic encephalopathy does not impair health-related quality of life in patients with cirrhosis: a prospective study. Liver Int. 2011;31(7):980–4.
17. Stewart SM, et al. Mental and motor development, social competence, and growth one year after successful pediatric liver transplantation. J Pediatr. 1989;114(4 Pt 1):574–81.
18. Zitelli BJ, et al. Changes in life-style after liver transplantation. Pediatrics. 1988;82(2):173–80.
19. Hoffmann 3rd RG, et al. Moderating effects of family functioning on the social adjustment of children with liver disease. Child Health Care. 1995;24(2):107–17.
20. Varni JW, et al. The PedsQL Infant Scales: feasibility, internal consistency reliability, and validity in healthy and ill infants. Qual Life Res. 2011;20(1):45–55.
21. Cole CR, et al. Impact of liver transplantation on HRQOL in children less than 5 years old. Pediatr Transplant. 2004;8(3):222–7.
22. Stewart SM, et al. Mental and motor development correlates in patients with end-stage biliary atresia. Pediatrics. 1987;79(6):882–8.
23. Caudle SE, et al. Language and motor skills are impaired in infants with biliary atresia before transplantation. J Pediatr. 2010;156:936–40.
24. Stewart SM, et al. Mental development and growth in children with chronic liver disease of early and late onset. Pediatrics. 1988;82(2):167–72.
25. Stewart SM, et al. Cognitive patterns in school-age children with end-stage liver disease. J Dev Behav Pediatr. 1992;13(5):331–8.
26. Howard ER, et al. Survival patterns in biliary atresia and comparison of quality of life of long-term survivors in Japan and England. J Pediatr Surg. 2001;36(6):892–7.
27. Crosnier C, et al. Alagille syndrome. The widening spectrum of arteriohepatic dysplasia. Clin Liver Dis. 2000;4(4):765–78.
28. Emerick KM, et al. Features of Alagille syndrome in 92 patients: frequency and relation to prognosis. Hepatology. 1999;29(3):822–9.
29. Papapetropoulos S, et al. Case of pediatric acquired chronic hepatocerebral degeneration. Pediatr Neurol. 2008;38(1):67–70.
30. Elisofon SA, et al. Health status of patients with Alagille syndrome. J Pediatr Gastroenterol Nutr. 2010;51(6):759–65.
31. Dening TR, Berrios GE. Wilson's disease: a longitudinal study of psychiatric symptoms. Biol Psychiatry. 1990;28(3):255–65.
32. El-Karaksy H, et al. A clinical study of Wilson's disease: the experience of a single Egyptian Paediatric Hepatology Unit. Arab J Gastroenterol. 2011;12(3): 125–30.
33. Medalia A, Scheinberg H. Intellectual functioning in treated Wilson's disease. Ann Neurol. 1991;29(5): 573–4.
34. Hegde S, et al. Cognitive profile and structural findings in Wilson's disease: a neuropsychological and MRI-based study. Neurol India. 2010;58(5):708–13.
35. Xu P, et al. Category and perceptual learning in subjects with treated Wilson's disease. PLoS One. 2010;5(3):e9635.
36. Lorincz MT. Neurologic Wilson's disease. Ann N Y Acad Sci. 2010;1184:173–87.
37. Lin JJ, et al. Psychological presentations without hepatic involvement in Wilson disease. Pediatr Neurol. 2006;35(4):284–6.
38. Carta MG, et al. Bipolar disorders and Wilson's disease. BMC Psychiatry. 2012;12:52.
39. Svetel M, et al. Quality of life in patients with treated and clinically stable Wilson's disease. Mov Disord. 2011;26(8):1503–8.
40. Thimm E, et al. Neurocognitive outcome in patients with hypertyrosinemia type I after long-term treatment with NTBC. J Inherit Metab Dis. 2012;35(2): 263–8.
41. Krivitzky L, et al. Intellectual, adaptive, and behavioral functioning in children with urea cycle disorders. Pediatr Res. 2009;66(1):96–101.
42. Nicolaides P, et al. Neurological outcome of patients with ornithine carbamoyltransferase deficiency. Arch Dis Child. 2002;86(1):54–6.
43. Campeau PM, et al. Early orthotopic liver transplantation in urea cycle defects: follow up of a developmental outcome study. Mol Genet Metab. 2010;100 Suppl 1:S84–7.
44. Bachmann C. Long-term outcome of patients with urea cycle disorders and the question of neonatal screening. Eur J Pediatr. 2003;162 Suppl 1:S29–33.
45. Cederbaum JA, et al. Psychosocial issues and coping strategies in families affected by urea cycle disorders. J Pediatr. 2001;138(1 Suppl):S72–80.
46. Lee WS, Sokol RJ. Liver disease in mitochondrial disorders. Semin Liver Dis. 2007;27(3):259–73.
47. Salviati L, et al. Mitochondrial DNA depletion and dGK gene mutations. Ann Neurol. 2002;52(3):311–7.
48. Forton DM, et al. Hepatitis C and cognitive impairment in a cohort of patients with mild liver disease. Hepatology. 2002;35(2):433–9.
49. Lim JK, et al. The impact of chronic hepatitis C and comorbid psychiatric illnesses on health-related quality of life. J Clin Gastroenterol. 2006;40(6): 528–34.
50. Nydegger A, et al. Health-related quality of life in children with hepatitis C acquired in the first year of life. J Gastroenterol Hepatol. 2008;23(2):226–30.
51. Ng VL, et al. Outcomes of 5-year survivors of pediatric liver transplantation: report on 461 children from a north american multicenter registry. Pediatrics. 2008;122(6):e1128–35.
52. Adeback P, Nemeth A, Fischler B. Cognitive and emotional outcome after pediatric liver transplantation. Pediatr Transplant. 2003;7:385–9.

53. Schulz K-H, et al. Cognitive performance of children who have undergone liver transplantation. Transplantation. 2003;75(8):1236–40.
54. Kaller T, et al. Cognitive abilities in children after liver transplantation. Transplantation. 2005;79(9):1252–6.
55. Kaller T, et al. Cognitive abilities, behaviour, and quality of life in children after liver transplantation. Pediatr Transplant. 2010;14(4):496–503.
56. Krull K, et al. Neurocognitive outcome in pediatric liver transplant recipients. Pediatr Transplant. 2003;7: 111–8.
57. Gilmour S, et al. Assessment of psychoeducational outcomes after pediatric liver transplant. Am J Transplant. 2009;9:294–300.
58. Kennard BD, et al. Academic outcome in long-term survivors of pediatric liver transplantation. J Dev Behav Pediatr. 1999;20(1):17–23.
59. Wayman KI, Cox KL, Esquivel CO. Neurodevelopmental outcome of young children with extrahepatic biliary atresia 1 year after liver transplantation. J Pediatr. 1997;131(6):894–8.
60. Haavisto A, et al. Visuospatial impairment in children and adolescents after liver transplantation. Pediatr Transplant. 2011;15:184–92.
61. Stewart SM, et al. Neuropsychological outcome of pediatric liver transplantation. Pediatrics. 1991;87(3): 367–76.
62. Kaller T, et al. Attention and executive functioning deficits in liver transplanted children. Clin Transl Res. 2010;90(12):1567–73.
63. Gilmour SM, et al. School outcomes in children registered in the studies for pediatric liver transplant (SPLIT) consortium. Liver Transpl. 2010;16(9): 1041–8.
64. Grabhorn E, et al. Liver transplantation in infants younger than 6 months old. Transplant Proc. 2002;34: 1964–5.
65. Schauer R, et al. Treatment of Crigler-Najjar type I disease: relevance of early liver transplantation. J Pediatr Surg. 2003;38(8):1227–31.
66. Shellmer DA, et al. Cognitive and adaptive functioning after liver transplantation for maple syrup urine disease: a case series. Pediatr Transplant. 2011;15: 58–64.
67. Barshes NR, et al. Evaluation and management of patients with propionic acidemia undergoing liver transplantation: a comprehensive review. Pediatr Transplant. 2006;10:773–81.
68. Yoshitoshi EY, et al. Long-term outcomes for 32 cases of Wilson's disease after living-related donor liver transplantation. Transplantation. 2009;87(2):261–7.
69. Beath SV, et al. Long term outcome of liver transplantation (LTx) in babies aged less than 12 months. J Pediatr Gastroenterol Nutr. 1997;24(4):485.
70. van Mourik IDM, et al. Long-term nutritional and neurodevelopmental outcome of liver transplantation in infants aged less than 12 months. J Pediatr Gastroenterol Nutr. 2000;30(3):269–75.
71. Ikegami T, et al. Effect of liver transplantation in a twin for biliary atresia on physical development and intellectual performance: report of a case. Surg Today. 2000;30:841–3.
72. Baron IS, Rey-Casserly C. Pediatric neuropsychology: medical advances and lifespan outcomes. Oxford, New York: Oxford University Press; 2013.
73. Chin SE, et al. Survival, growth and quality of life in children after orthotopic liver transplantation: a 5 year experience. J Paediatr Child Health. 1991;27(6):380–5.
74. Tornqvist J, et al. Long-term psychosocial adjustment following pediatric liver transplantation. Pediatr Transplant. 1999;3(2):115–25.
75. Gritti A, et al. Emotional and behavioral problems after pediatric liver transplantation: a quantitative assessment. Pediatr Transplant. 2006;10(2):205–9.
76. Wise BV. In their own words: the lived experience of pediatric liver transplantation. Qual Health Res. 2002;12(1):74–90.
77. Gritti A, et al. Psychological impact of liver transplantation on children's inner worlds. Pediatr Transplant. 2001;5(1):37–43.
78. Sundaram SS, et al. Adolescent health-related quality of life following liver and kidney transplantation. Am J Transplant. 2007;7(4):982–9.
79. Tarbell SE, Kosmach B. Parental psychosocial outcomes in pediatric liver and/or intestinal transplantation: pretransplantation and the early postoperative period. Liver Transpl Surg. 1998;4(5):378–87.
80. Mintzer LL, et al. Traumatic stress symptoms in adolescent organ transplant recipients. Pediatrics. 2005; 115(6):1640–4.
81. Young GS, et al. Symptoms of posttraumatic stress disorder in parents of transplant recipients: incidence, severity, and related factors. Pediatrics. 2003;111(6 Pt 1):e725–31.
82. Fredericks EM, et al. Psychological functioning, nonadherence and health outcomes after pediatric liver transplantation. Am J Transplant. 2007;7(8):1974–83.
83. Alonso EM, et al. Health-related quality of life and family function following pediatric liver transplantation. Liver Transpl. 2008;14(4):460–8.
84. Rodrigue JR, et al. Transplantation in children. A longitudinal assessment of mothers' stress, coping, and perceptions of family functioning. Psychosomatics. 1997;38(5):478–86.
85. Maynard A, Martini MI. Learning in cultural context: family, peers, and school, International and cultural psychology series. New York: Kluwer Academic/ Plenum Publishers; 2005. p. 280, viii.
86. Schulz K, et al. Comparison of quality of life and family stress in families of children with living-related liver transplants versus families of children who received a cadaveric liver. Transplant Proc. 2001;33(1–2):1496–7.
87. DeBolt AJ, et al. A survey of psychosocial adaptation in long-term survivors of pediatric liver transplants. Child Health Care. 1995;24(2):79–96.
88. Limbers CA, et al. Health-related quality of life in pediatric liver transplant recipients compared with other chronic disease groups. Pediatr Transplant. 2011;15(3):245–53.

89. Alonso EM, et al. Cross-sectional analysis of health-related quality of life in pediatric liver transplant recipients. J Pediatr. 2010;156:270–6.e1.
90. Alonso EM, et al. Functional outcomes of pediatric liver transplantation. J Pediatr Gastroenterol Nutr. 2003;37(2):155–60.
91. Bucuvalas JC, et al. Health-related quality of life in pediatric liver transplant recipients: a single-center study. Liver Transpl. 2003;9(1):62–71.
92. Bucuvalas JC, Britto M. Health-related quality of life after liver transplantation: it's not all about the liver. J Pediatr Gastroenterol Nutr. 2003;37(2):106–8.
93. Varni JW, et al. PedsQL Cognitive Functioning Scale in pediatric liver transplant recipients: feasibility, reliability, and validity. Qual Life Res. 2011; 20(6):913–21.
94. Fredericks EM, et al. Adherence and health-related quality of life in adolescent liver transplant recipients. Pediatr Transplant. 2008;12(3): 289–99.
95. Prasad S, et al. Lactulose improves cognitive functions and health-related quality of life in patients with cirrhosis who have minimal hepatic encephalopathy. Hepatology. 2007;45(3):549–59.
96. Mohammad S, Alonso EM, Approach to Optimizing Growth, rehabilitation and neurodevelopmental Outcomes in Children After Solid Organ Transplantation. Pediatric Clinics of North America. 2010;57(2).

Part II

Disorders

Metabolic Liver Disease: Part 1

8

James E. Squires and James E. Heubi

Introduction

The liver is known to have an expansive role in the synthesis, degradation, and regulation of pathways involved in the metabolism of carbohydrates, proteins, lipids, trace elements, and vitamins. Subsequently, abnormalities that affect these pathways as well as specific enzyme deficiencies often affect the liver either primarily or secondarily. The spectrum of liver injury results when there is absence or blockage of an enzyme in the metabolic pathway often resulting in accumulation of unmetabolized toxic substrates and/or deficiencies in essential substances normally produced distal to the dysfunctional enzyme. Clinical presentations of children with metabolic liver disease vary from acute, life-threatening illness in the neonatal period to a more indolent, chronic disease process that becomes evident in adolescence or adulthood. Disease progression often results from the failure of metabolic functions resulting in hepatocyte injury or cellular derangement and hepatomegaly with advancement to cirrhosis and/or tumor development. A detailed history can often elicit the possibility of a metabolic dysfunction and assist in the guidance of further clinical investigations while treatment is dependent of the particular metabolic defect that is identified.

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Disorders of Bilirubin Metabolism

Bilirubin is formed by the breakdown of heme-containing products – most notably red blood cells (RBCs). This two-step process begins with the degradation of heme into carbon monoxide and biliverdin. Biliverdin is then metabolized to bilirubin by the enzyme biliverdin reductase. The majority of bilirubin is bound to albumin and transported to the liver where it is taken up by hepatic cells for secretion into the biliary ductal system after undergoing conjugation via the enzyme uridine diphosphogluconurate glucuronosyltransferase (UGT). The majority of neonatal jaundice is non-pathologic and is due to normal changes in bilirubin metabolism that result in increased production, decreased clearance, and increased intrahepatic circulation.

Breast Milk Jaundice

Introduction

Breast milk jaundice is a term used to describe the jaundice that results from the normal physiologic

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mechanisms contributing to elevated circulating bilirubin levels.

Pathogenesis

Breast milk jaundice is thought to result from several physiologic mechanisms unique to the newborn infant: Increased bilirubin production results from the increased breakdown and turnover of RBCs [1]. This results from elevated hematocrit levels with accompanying decreased RBC half-life [2]. UGT, the enzyme responsible for bilirubin conjugation and subsequent secretion into the biliary system, is deficient in newborns, often operating at 0.1–1 % of the adult liver capacity and not reaching full efficiency until 14 weeks of life [3, 4]. Increased intrahepatic circulation occurs in the newborn secondary to relative sterility of the neonatal gut. Under normal conditions, gut flora is responsible for converting the conjugated bilirubin secreted in bile into stercobilin – the material that gives stool its brown color. In the absence of sufficient intestinal flora, bilirubin is de-conjugated by beta-glucuronidase and reabsorbed resulting in increased intrahepatic circulation. Breast milk composition provides additional elements that contribute to hyperbilirubinemia. Both progesterone metabolites and lipoprotein lipase have been isolated in breast milk and shown to inhibit the action of UGT thus leading to decreased bilirubin clearance [5, 6].

Clinical Manifestations and Diagnosis

Secondary to placental clearance, infants do not exhibit jaundice over the first day or two of life. However, as the production begins to exceed the infants' ability to process and clear the bilirubin, the stereotypical yellowing of the skin and sclera becomes clinically evident. Jaundice progresses in a cephalocaudal manner with symptoms appearing first over the head and progressing to the palms of the hands and soles of the feet. Bilirubin elevation can be correlated with degree of infant jaundice with whole body jaundice occurring with levels >15 mg/dL. Moderate jaundice (>12 mg/dL) occurs in at least 12 % of breast-fed infants with severe jaundice (>15 mg/dL) occurring in 2 % [7]. Kernicterus, a bilirubin-derived brain dysfunction resulting from deposition of the neurotoxic bilirubin in the grey matter of the newborn brain, is not thought to be associated with breast milk jaundice. However, kernicterus has been reported in otherwise healthy newborn infants with no other identified etiology for their jaundice [8]. Measurement of the total serum bilirubin concentration allows quantification of jaundice with a diagnosis of hyperbilirubinemia given to infants whose total bilirubin levels are elevated on the hour-specific Bhutani nomogram (see Fig. 8.1) [9].

Treatment, Management, and Outcomes

The hyperbilirubinemia associated with breast milk jaundice will improve with cessation of breast-feeding for 24–48 h. However, given the lack of specificity and the breadth of potential etiologies for elevated serum bilirubin, jaundice should always be considered a sign of potentially more serious disease. Immediate investigations should be considered as to the etiology when [1] jaundice occurs before 36 h of age [2], jaundice persists beyond 10 days of age [4], serum bilirubin >12 mg/dL, and [10] elevation of the direct reacting fraction of bilirubin [1].

Gilbert Syndrome

Introduction

Gilbert syndrome (GS) is the most common inherited disorder of bilirubin glucuronidation and is characterized by chronic and recurrent elevations in unconjugated bilirubin in the setting of normal liver function and normal aminotransferases. The typical inheritance pattern is autosomal recessive as only individuals who are homozygous for promoter gene mutations express symptoms (see Pathogenesis). However, individuals who have heterozygous mutations do average higher bilirubin levels when compared to the general population [11].

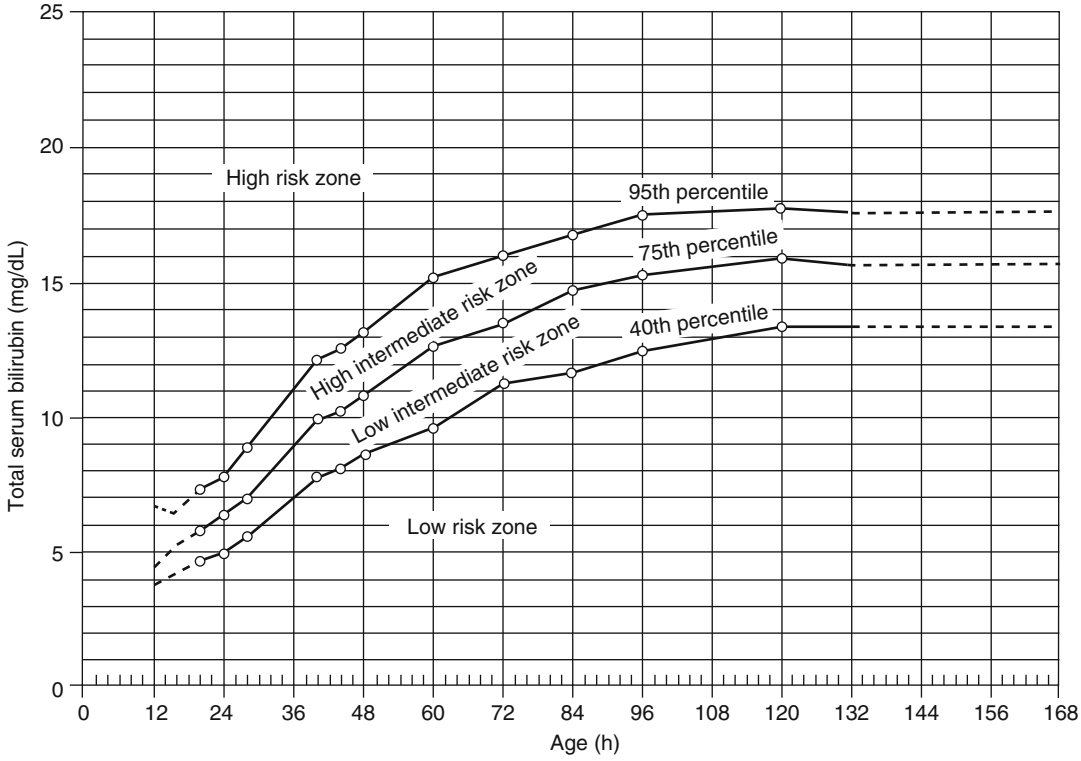

Fig. 8.1 Bhutani nomogram. Hour-specific nomogram developed to predict infants most at risk for developing severe hyperbilirubinemia (Reprinted with permission from publisher of Peds (American Academy of Pediatrics))

Pathogenesis

Uridinediphosphoglucuronate glucuronosyltransferase 1 (UGT1) is a gene whose enzyme is responsible for the glucuronidation of several compounds in the body, including bilirubin. Bilirubin-specific UGT (UGT1A1 or BUGT) activity is decreased in patients with GS often secondary to mutations appreciated on the gene's promoter region (see Fig. 8.2), leading to elevations in serum unconjugated bilirubin. The expression of the GS phenotype is likely multifactorial as not all individuals who carry homozygous mutations develop unconjugated hyperbilirubinemia [11].

Clinical Manifestations and Diagnosis

Patients diagnosed with GS present in adolescence or young adulthood with mild, intermittent elevations in unconjugated bilirubin levels without evidence additional liver disease or dysfunction. Conjugated bilirubin levels seldom rise above 4 mg/dL. Often, patients are first identified on screening blood chemistries, or mild jaundice is appreciated during periods of fasting or in accompaniment of a viral illness. The majority of patients are asymptomatic. Physical exam findings may exhibit only mild scleral icterus, and yellowing of the skin is rare in patients with GS. Critical to the diagnosis of GS is the clinician's suspicion and the avoidance of unnecessary invasive and investigatory testing. A presumptive diagnosis can be made when patients exhibit (1) unconjugated hyperbilirubinemia on repeat testing; (2) a normal complete blood count, blood smear, and reticulocyte count; and (3) no additional evidence of liver dysfunction or liver disease. While several tests can be used when GS is suspected, such as fasting or nicotinic acid administration with subsequent measurement of

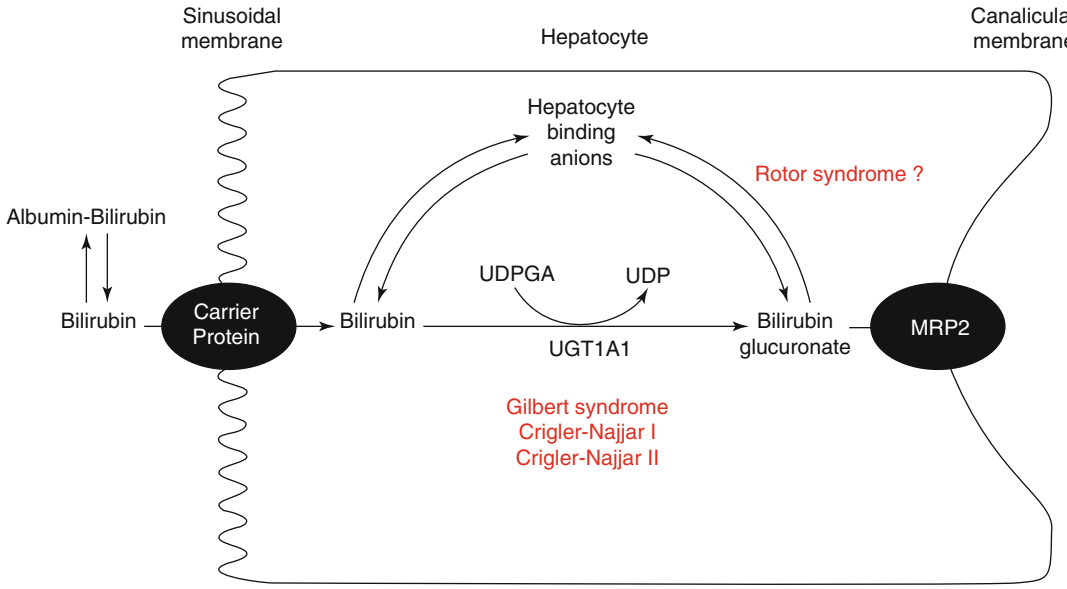

Fig. 8.2 Bilirubin metabolism. Mutations in bilirubin-uridine diphosphate glucuronosyltransferase (UGT1A1) are responsible for genetic errors in bilirubin conjugation that cause Gilbert syndrome as well as Crigler-Najjar syndromes 1 and 2. Rotor syndrome is caused by defective binding anions with deficient intracellular storage capacity of bilirubin

unconjugated bilirubin levels, clinical awareness and recognition remains the most important factor when entertaining a diagnosis of GS.

Treatment, Management, and Outcomes

GS is considered a benign condition. No specific treatment is required for patients with GS, and the most important aspect of the diagnosis is a recognition of the disorder and the understanding of its favorable outcomes.

Crigler-Najjar Syndrome

Introduction

Crigler-Najjar syndrome (CN) is a rare autosomal recessive disease characterized by elevated unconjugated bilirubin levels. Bilirubin elevations in CN result from defects in the gene that is responsible for bilirubin conjugation – bilirubin-uridine diphosphate glucuronosyltransferase (UGT1A1). Two distinct forms of the disease have been identified depending of the severity of UGT1A1 enzyme dysfunction (See Fig. 8.2).

Type 1 disease is more severe and associated with impressive, early jaundice with neurologic impairment and the development of kernicterus. Type 2 disease is often milder, with lower bilirubin levels and with improved patient survival.

Pathogenesis

UGT1A1 is the gene responsible for conjugation of bilirubin in the hepatocyte. The degree of genetic disruption of UGT1A1 results in the clinical severity that defines CNI and CNII. In the more severe CNI, there is complete absence of functional UGT1A1 activity, whereas in CNII UGT1A1 activity is markedly reduced. While mutations in the promoter region of the UGT1A1 gene result in the Gilbert's phenotype, the mutations associated with CN are located primarily in the exon-coding regions of the gene. Patients with CN thus have a profound decrease in their ability to conjugate and subsequently excrete bilirubin in the bile.

Clinical Manifestations and Diagnosis

Patients with CN often present in the first few days of life with jaundice and serum bilirubin

elevations, often greater than 20 mg/dL. Bilirubin elevations are primarily attributable to the unconjugated fraction. Pigmented stool is present as CN is not associated with biliary obstruction; however, fecal urobilinogen excretion is decreased secondary to reduced bilirubin conjugation and stool presence [12]. With exception of elevated bilirubin levels, liver function tests in children are usually normal and liver histology may only reveal deposition of bile pigments. Bilirubin-induced brain dysfunction, or kernicterus, is always a concern in children with CN. Bilirubin deposition in the grey matter of various central nervous system structures can result in a myriad of acute symptoms including lethargy, decreased feeding, hypo- or hypertonia, a high-pitched cry, spasmodic torticollis, opisthotonus, fever, seizures, and death.

Differentiating between CNI and CNII can be difficult. One major differing characteristic is the response to drugs that stimulate hyperplasia of the endoplasmic reticulum – such as phenobarbital. Serum unconjugated bilirubin can be reduced by the administration of phenobarbital in most patients with CNII but not CNI.

CN should be suspected in any infant with persistently elevated unconjugated bilirubin levels after evaluation for more common etiologies such as hypothyroidism, infection, and hemolysis. Genetic testing can be performed to assess UGT1A1 mutations, and liver biopsy can be used to obtain tissue for UGT1A1 function assays.

Treatment, Management, and Outcomes

The mainstay of treatment in patients with CN is phototherapy and plasmapheresis in order to lower serum bilirubin levels and prevent neurological sequelae. Additional therapeutic options are the oral administration of binding agents such as cholestyramine, agar, or calcium phosphate that trap unconjugated bilirubin in the gut and prevent enterohepatic recirculation [1]. Patients with CNII may respond to daily administration of phenobarbital; however, the response is often variable.

While bilirubin conjugation is abnormal, additional liver synthetic function is preserved in children with CN making them ideal candidates for orthotopic liver transplantation. Future therapies may include isolated hepatocyte cell transplant with donor hepatocytes containing functional UGT1A1; however, this therapy remains investigational.

The effects of elevated bilirubin concentrations on the central nervous system and the development of kernicterus remains the most important indicator of patient outcome. Patients with CNII whose UGT1A1 function may be preserved often are spared the neurological devastation seen in CNI. However, long-term effects of chronic bilirubin elevations have been shown to cause variable degrees of brain damage in older patients with CN [13].

Rotor Syndrome

Introduction

Rotor syndrome is a benign, autosomal recessive disorder resulting in chronic elevations of both conjugated and unconjugated bilirubin levels without evidence of hemolysis. The primary defect in Rotor syndrome is in the liver's ability to store conjugated bilirubin resulting in elevated plasma concentrations.

Pathogenesis

While the exact abnormality resulting in Rotor syndrome remains unknown, the primary deficiency is thought to be related to a defective intracellular storage capacity of the liver for binding anions involved in the excretion of conjugated bilirubin (See Fig. 8.2).

The resulting deficiency of hepatocellular biliary storage allows for seepage of bilirubin back into the plasma resulting in the elevations of conjugated bilirubin that define Rotor syndrome.

Clinical Manifestations and Diagnosis

Rotor syndrome should be suspected in children presenting with jaundice and scleral icterus in the setting of normal liver function tests and no evidence of biliary obstruction or hemolysis. On laboratory evaluation, the conjugated bilirubin

fraction is over half of the total serum bilirubin concentration. Total bilirubin levels generally reside in the 2–7 mg/dL range but occasionally may reach 20 mg/dL. A confirmatory diagnosis can be made when urinary coproporphyrin levels are measured to be 2.5–5 times higher than normal [14].

Treatment, Management, and Outcomes

Patients with Rotor syndrome require no specific therapy. Elevated serum bilirubin with jaundice may be chronic findings; however, no treatment is needed, and only reassurance and education should be offered.

Disorders of Carbohydrate Metabolism

A primary function of the liver is the management of glucose homeostasis. Glucose production can occur through a variety of metabolic pathways including gluconeogenesis as well as degradation of glycogen – the major storage form of glucose. Glucose along with galactose and fructose constitute the majority of dietary carbohydrates that are absorbed and processed to produce the energy required to sustain cellular function. Deficiencies in the metabolism of carbohydrates result in significant morbidity and mortality often affecting infants and neonates.

Galactosemia

Introduction

Galactosemia is an autosomal recessive disorder, with and incidence of 1:60,000 live births, characterized by an inability to affectively metabolize galactose. Galactosemia results from an enzymatic deficiency in one of three proteins in the metabolic pathway through which galactose is converted to glucose: galactokinase (GALK), galactose-1-phosphate uridyltransferase (GALT), and uridine diphosphate galactose-4-epimerase (GALE).

Pathogenesis

Galactose is metabolized into glucose-1-phosphate by a series of reactions involving the enzymes GALK, GALT, and GALE (See Fig. 8.3).

Although galactosemia can result from dysfunction of any enzyme involved in the pathway, "classical" galactosemia constituting the majority of cases involves a complete deficiency of GALT activity often due to the missense mutation on chromosome 9. Dysfunctional metabolism results in accumulation of the toxic by-products galactose-1-phosphate and galactitol that produce the pathological changes found in the liver, brain, kidney, and lens of the eye [1].

Clinical Manifestations and Diagnosis

Classic galactosemia presents in the first weeks of life after ingestion of breast milk or milk-based formulas containing lactose. Failure to thrive and jaundice are the most common presenting symptoms often accompanied by difficulty feeding, vomiting, and diarrhea. The range of presentation can be from an acute, life-threatening decompensation often associated with *E. coli* sepsis to a more subtle appearance with nonspecific manifestations. Physical exam reveals jaundice with hepatomegaly, ascites, edema, excessive bruising, hypotonia, and a full fontanelle. Laboratory investigations show multisystem abnormalities with direct hyperbilirubinemia, aminotransferase elevation, hypoalbuminemia, and coagulopathy.

While newborn screening (NBS) has allowed early detection of children with suspected galactosemia, the genetic complexity and nonstandardization of screening techniques has complicated matters for general pediatricians who are often left to interpret the results. Although classic galactosemia is indeed a medical emergency, most positive NBS results occur due to false positives and Duarte variants – a genotypic variant that results in varying degrees of GALT phenotypic activity [2]. The demonstration of complete absence of GALT activity via a quantitative assay remains the gold standard for diagnosis [4].

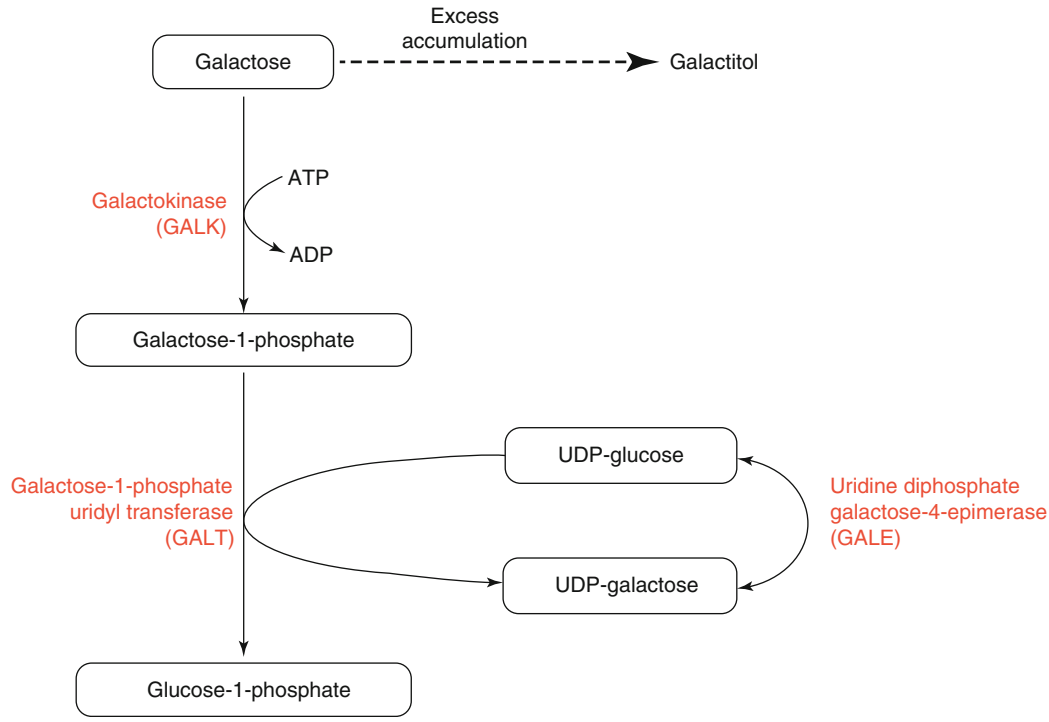

Fig. 8.3 Galactose metabolism. Galactokinase (*GALK*), galactose-1-phosphate uridyltransferase (*GALT*), and uridine diphosphate galactose 4-epimerase (*GALE*) function to metabolize glucose from galactose. "Classical" galactosemia results from GALT deficiency

Treatment, Management, and Outcomes

The immediate management of a child with galactosemia is urgent removal of galactose from the diet. Further acute management should be aimed at treating comorbidities present at diagnosis – such as jaundice, infection, coagulopathy, and acidosis. Antibiotics, intravenous fluids, blood products, and vitamin K are often needed. Acute symptoms often correct soon after galactose restriction. Infants should be placed on an appropriate nutritional alternative such as a soy-based formula with referral to a dietary specialist. Once solids are introduced, continued avoidance of lactose-containing foods is recommended.

Patients with galactosemia should be followed to ensure dietary compliance and assess end-organ damage. Most affected children will have some degree of intellectual deficit with speech and language delays. Abnormalities in coordination, gait, and balance are also seen [10].

Hereditary Fructose Intolerance

Introduction

Hereditary fructose intolerance (HFI) is an autosomal recessive disorder, with an incidence of 1:20,000, characterized by an inability to effectively metabolize fructose-1-phosphate. HFI results from a deficiency of the catalytic enzyme fructose-1-phosphate aldolase, otherwise known as aldolase B, located in the liver, kidney, and small intestine.

Pathogenesis

Fructose is a widely available monosaccharide often found naturally but also from the hydrolysis of the disaccharide sucrose into fructose and glucose. Once absorbed, fructose is taken up mostly by the liver (75 %) but also by the kidney and small intestines (remaining 25 %) where enzymes are able to further metabolize the sugar (See Fig. 8.4).

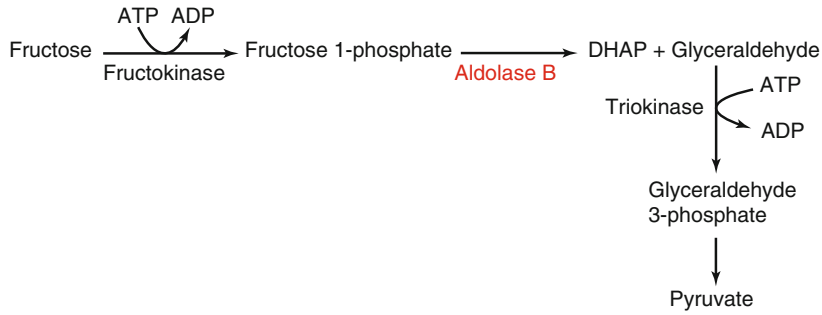

Fig. 8.4 Fructose metabolism. Metabolic pathway of fructose to pyruvate. Deficiency of aldose B results in toxic accumulation of fructose-1-phosphate and hereditary fructose intolerance

The biochemical toxicity results from a deficiency of aldose B and subsequent accumulation of fructose-1-phosphate [1]. Increased fructose-1-phosphate inhibits both gluconeogenesis and glycogenolysis evident in the manifestation of hypoglycemia, renal, and hepatic dysfunction in affected patients.

Clinical Manifestations and Diagnosis

In general, children with HFI are healthy until they ingest fructose and/or sucrose. Patients commonly present with vomiting, hepatomegaly, decreased oral intake, and failure to thrive. A detailed nutritional history is critical as symptom onset frequently correlates with intake of fructose-containing foods. Laboratory investigations may reveal evidence of acute liver failure, proximal renal tubular dysfunction, and hypoglycemia. The presence of reducing substances in the urine may result from fructosuria. More than 95 % of patients with HFI can be diagnosed with noninvasive DNA amplification with a limited number of allele-specific oligonucleotides [15].

Treatment, Management, and Outcomes

Treatment consists of fructose and sucrose removal from the diet. Particular attention should be paid to food additives, pill coatings, and medication suspensions. Complete elimination is rarely a therapeutic goal given the wide distribution and high concentration of fructose in foods. Optimal levels of restriction have not been established, and while some patients are able to demonstrate normalization of hepatic and renal function with low fructose intake, others may suffer with chronic, nonspecific symptoms despite treatment [16]. Multivitamin supplementation, particularly forms with ascorbic acid and folates, should be prescribed due to the need for fruit and vegetable restriction [17]. Once appropriate dietary restriction is implemented, clinical progression is rarely encountered and the majority of children exhibit normal growth and intellectual development.

Fructose-1,6-Bisphosphatase Deficiency

Introduction

Fructose-1,6-bisphosphatase (FDPase) deficiency (also known as fructose 1,6-diphosphatase deficiency) is a rare autosomal recessive disorder of gluconeogenesis with an estimated incidence of 1:350,000.

Pathogenesis

FDPase is important in the processes of gluconeogenesis, and its deficiency results in impaired formation of glucose from precursors – including fructose. Importantly, glycogenolysis is not affected in these individuals (See Fig. 8.5).

Clinical Manifestations and Diagnosis

Infants with FDPase deficiency often present within the first days of life with hypoglycemia, lactic acidosis, and compensatory hyperventilation, while older children can present with acute ataxia and lethargy [18]. Hepatomegaly may be present, but transaminases are normal. Fasting may trigger attacks of hypoglycemia.

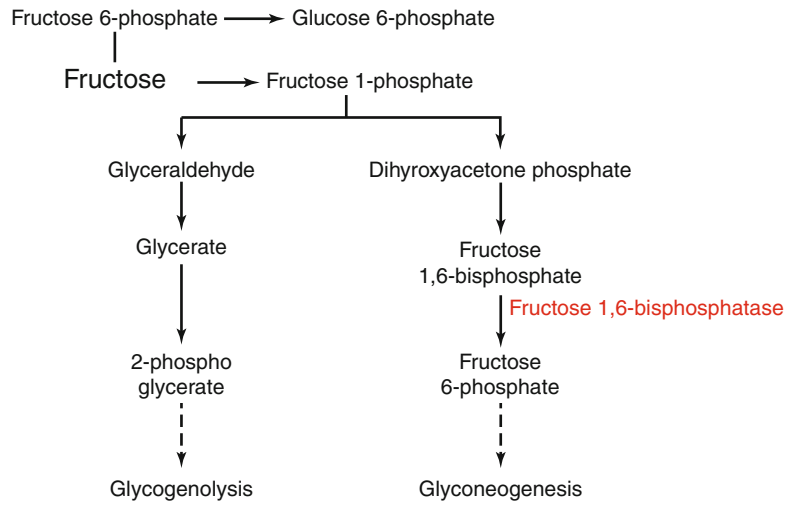

Fig. 8.5 Fructose-1, 6-bisphosphatase. Fructose metabolism resulting in gluconeogenesis and glycogenolysis. Fructose-1, 6-bisphosphatase is involved in gluconeogenesis. Deficiency results in fructose-1,6-biphosphatase (FDPase) deficiency

Lactate, pyruvate, and ketones may be elevated. Aminoaciduria with 2-oxoglutaric acid is seen on urine biochemical analysis. Diagnosis can be made with DNA analysis [17]. If results are inconclusive, a liver biopsy confirming decreased enzyme activity or mutation analysis is diagnostic.

Treatment, Management, and Outcomes

In acute crisis, patients with FDPase deficiency respond rapidly to intravenous or oral glucose with sodium bicarbonate administration to correct acidosis. Long-term therapy consists of fasting avoidance. Frequent feedings and the use of slowly absorbed carbohydrates such as cornstarch are helpful [17]. With appropriate treatment, complications are often avoided while growth and development is normal.

Glycogen Storage Diseases

Introduction

Glycogen is a polysaccharide that constitutes the primary carbohydrate storage compound and is the principal source of energy providing substrates for the generation of ATP. Although particularly prevalent in liver and muscle tissues, glycogen is present in nearly all animal cells and is processed during fasting resulting in release of glucose needed to sustain cellular processes and glucose homeostasis. The complexity of this process is underscored by the multitude of enzymes involved, deficiencies in which result in the 12 recognized forms of glycogen storage diseases (GSD). The overall incidence of GSD is estimated to be 1:20,000–40,000 cases per live birth. The discussion here is limited to types I, III, and IV because their clinical expression primarily involves the liver (See Fig. 8.6).

GSD I (Von Gierke Disease)

Pathogenesis

GSD I is an autosomal recessive disease of glycogen metabolism with an incidence of approximately 1:100,000 live births. Several enzymatic defects have been identified resulting in the subclassification of GSD I. Types Ia and Ib constitute the majority of cases, while Ic and Id are extremely rare and will not be discussed in this chapter. Patients with GSD Ia are noted to have complete absence of glucose-6-phosphatase – an enzyme involved in the final process of both gluconeogenesis and glycogenolysis. Patients with GSD Ib have a defect in the enzyme glucose-6-phosphatase translocase which transports glucose-6-phosphatase from the cytoplasm into microsomes [19].

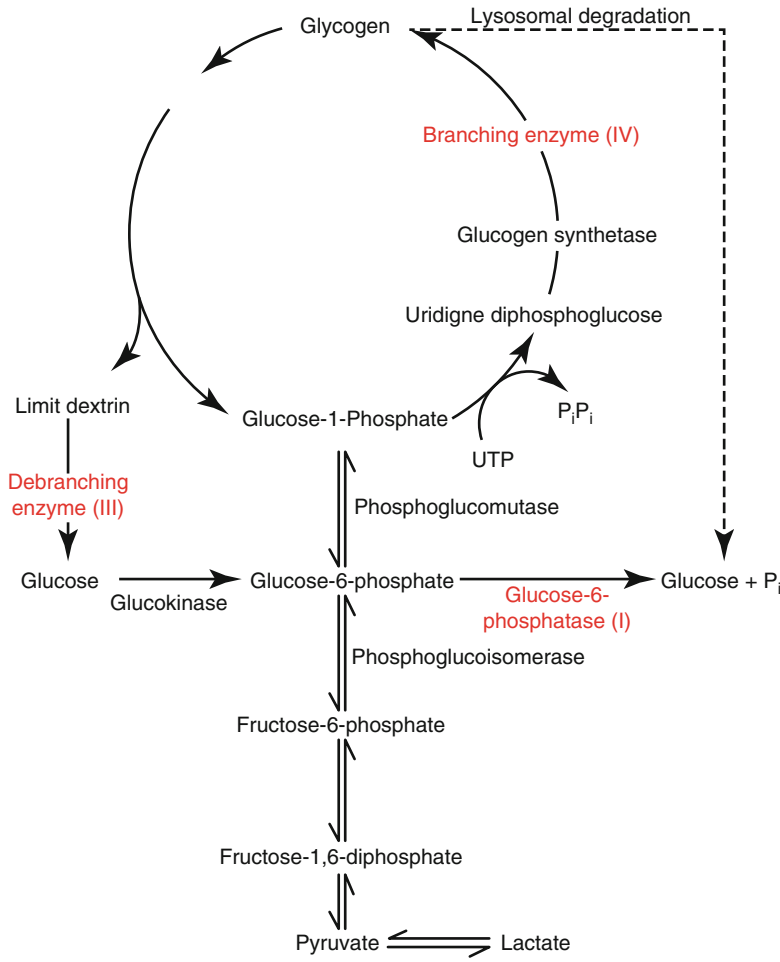

Fig. 8.6 Glycogen metabolism. Metabolic pathway of glycogen metabolism. Deficiencies in glucose-6-phosphatase, debranching enzyme, and branching enzyme result in the clinical features of glycogen storage diseases I, III, and IV whose clinical features primarily involve the liver

Clinical Manifestations and Diagnosis

Children with GSD1 typically present in early infancy with profound hepatomegaly and hypoglycemia associated with fasting. Oftentimes, symptoms may be first appreciated once a child begins to sleep through the night – a consequence of prolonged fasting. Other presenting signs and symptoms include protuberant abdomen due to hepatomegaly, metabolic derangement, seizures, growth failure, recurrent infections, muscular hypotonia, epistaxis, and delayed psychomotor development [20]. In addition to profound hypoglycemia, other laboratory findings include lactic acidosis, hyperuricemia, hypophosphatemia, hyperlipidemia, and platelet dysfunction. Neutropenia is a unique manifestation of GSD Ib. Confirming the diagnosis with DNA testing for common mutations is recommended. In the absence of identifiable genetic abnormality, liver enzyme activity can be measured.

Treatment, Management, and Outcomes

The main goal of therapy in the management of GSD Ia is to maintain normal blood glucose concentrations and correct metabolic abnormalities. A carbohydrate-balanced diet with frequent feedings over the day paired with continuous nocturnal feeds and/or the addition of uncooked cornstarch is the mainstay of treatment. Cornstarch is known to provide a "time-release" source of glucose given its slow degradation to glucose by α-amylase. However, decreased α-amylase levels in young infants may lead to suboptimal management with resulting growth

failure; therefore, high-starch meals while awake coupled with continuous overnight enteral feeds with a high-glucose, low-fat formula should be used. Published guidelines discussing appropriate biochemical targets are available to assist in the management of patients with GSD I [21]. Further treatment strategies may be required for the management of secondary complications such as hyperuricemia, lactic acidosis, hyperlipidemia, and hypertension. Management of GSD Ib is identical to Ia with the addition of granulocyte colony-stimulating factor (G-CSF) to correct neutropenia and defects in phagocytic cell function.

Current treatment strategies have allowed for patients with GSD to survive well into the third decade with normal growth and pubertal development. Hepatocellular adenomas may develop in patients with GSD I, and the malignant degeneration of the adenomas has been reported. Annual serum alpha-fetoprotein levels and liver ultrasounds are recommended. While adequate metabolic control can result in normal neurological development, repeated episodes of hypoglycemia and lactic acidosis can also result in mental handicaps. Additionally, GSD 1 patients have increased rates of cardiovascular disease, pancreatitis, and renal insufficiency.

GSD III (Forbes Disease, Cori Disease, Glycogen Debrancher Deficiency)

Pathogenesis

GSD III is an autosomal recessive disease resulting from mutations in the gene that codes for amylo-1, 6-glucosidase – a glycogen debranching enzyme. The two main subclassifications are GSD IIIa that involves both the liver and muscle and constitutes the majority of cases and GSD IIIb that affects the liver only.

Clinical Manifestations and Diagnosis

Children with GSD III often have similar clinical presentations to those with GSD I with hepatomegaly, hypoglycemia, hyperlipidemia, and acidosis [22]. Muscular involvement manifesting as weakness, muscle wasting, and cardiac dysfunction can be used to clinically distinguish GSD I and IIIa; however, the onset of muscular symptoms usually does not occur until adulthood. Furthermore, children with GSD IIIb do not develop muscular manifestations as the affected RNA isoform is only expressed in the liver.

Biochemically, children with GSD III will have similar, but less severe, abnormalities compared to children with GSD I with the exception that GSD III can develop hyperuricemia and elevated lactate levels. Elevated creatinine kinase levels indicating muscular involvement are present in GSD IIIa.

Although DNA testing of the aminoglucosidase gene is available, the diagnosis should be confirmed with direct measurement of amylo-1, 6-glucosidase activity in liver (IIIa/b) and muscle (IIIa) tissue samples. Liver biopsy may reveal the presence of fibrous septa and the paucity of fat that is unique to GSD III [1].

Treatment, Management, and Outcomes

Treatment of GSD III is directed toward avoidance of hypoglycemia. Just as the manifestations of GSD III are less severe than GSD I, so too is the demands to maintain strict dietary measures. As gluconeogenesis is not affected in GSD III, high-protein diets can be used in order to manage the disease [23].

Overall, hepatic involvement in GSD III is considered mild and self-limiting and improves with age usually with resolution by the second decade of life [17]. Hepatocellular carcinoma and cirrhosis have been reported, and practitioners should monitor with at least annual serum alpha-fetoprotein and liver ultrasound.

GSD IV (Andersen Disease, Glycogen Branching Enzyme Deficiency)

Pathogenesis

GSD IV is an autosomal recessive disorder resulting from mutations in the gene encoding the glycogen branching enzyme (GBE). GBE catalyzes the attachment of glucosyl chains to glycogen.

GSD IV results from a functional deficiency of GBE and subsequent abnormal glycogen structure. Various mutations result in a spectrum of disease ranging from intrauterine hydrops to perinatal hypotonia and cardiomyopathy. Four main phenotypic variants have been described based on age of presentation [24] (see below).

Clinical Manifestations and Diagnosis

The earliest manifestation may be in the perinatal period with fetal akinesia deformation sequence (FADS) that is characterized by contractures, hydrops fetalis, cardiac dysfunction, and death. A congenital form is defined by hypotonia, muscle atrophy, cardiomyopathy, and rapid progression to death. A juvenile variant has been described with muscular cardiomyopathy. An adult form of GSD IV is described primarily with neuromuscular manifestations such as myopathy, upper and lower neuron dysfunction, and dementia.

GSD IV is a unique disorder of glycogenolysis in that hypoglycemia is uncommon. Elevated transaminases are accompanied by elevations in alkaline phosphatase. A diagnosis of GSD IV is confirmed by the demonstration of absent branching enzyme activity in skin fibroblasts. Mutations analysis of the GBE gene can also reveal the diagnosis.

Treatment, Management, and Outcomes

Liver transplantation is the sole medical treatment for patients with diagnosed GSD IV. Without prompt diagnosis and transplant, most patients rapidly progress to end-stage liver disease, cirrhosis, and death by the third year of life.

Disorders of Amino Acid and Organic Acid Metabolism

Introduction

Although rare, these disorders may be encountered in practice, and an approach to the diagnosis, management, and outcomes is essential. The most common and important disorders that have hepatic manifestations include disorders of tyrosine, with its fulminant presentation in early infancy, and methylmalonic acid, isovaleric acid, and maple syrup urine disease which may manifest as recurrent episodes of liver dysfunction and alterations in neurocognitive function in infancy or early childhood.

Type I Tyrosinemia

Introduction

Type I tyrosinemia is a key element in the differential diagnosis of neonatal fulminant hepatic failure. Inherited as an autosomal recessive trait, it has an incidence of 1:100,000 with varying incidence by geography and an incidence of 1:1,846 in the Saguenay-Lac-Saint-Jean region of Quebec. Although historically type 1 tyrosinemia was uniformly fatal, the development of a treatment strategy with NTBC [2-(2-nitro-4-trifluromethylbenzoyl)-1,3-cyclohexanedione] has dramatically changed the long-term prognosis for these patients if identified and treated early in the course.

Pathogenesis

The clinic-pathologic manifestations of the disease relate to the metabolic defect which is deficiency of the enzyme fumarylacetoacetate hydrolase, the last enzyme in the pathway of tyrosine degradation. The consequent accumulation of fumarylacetoacetate and maleylacetoacetate and their by-products, succinylacetone and succinylacetoacetate, is responsible for the hepatocellular injury, propensity to hepatocellular carcinoma (HCC), and the development of neurotoxicity (see Fig. 8.7).

Clinical Manifestations

Most patients present with the acute form of disease characterized by findings of acute liver failure with poor growth, vomiting, ascites, coagulopathy, hypoglycemia, hypoalbminemia, and hyperbilirubinemia. This condition must be differentiated from other forms of acute liver failure since life-saving therapy is available for tyrosinemia. Patients with the chronic form have

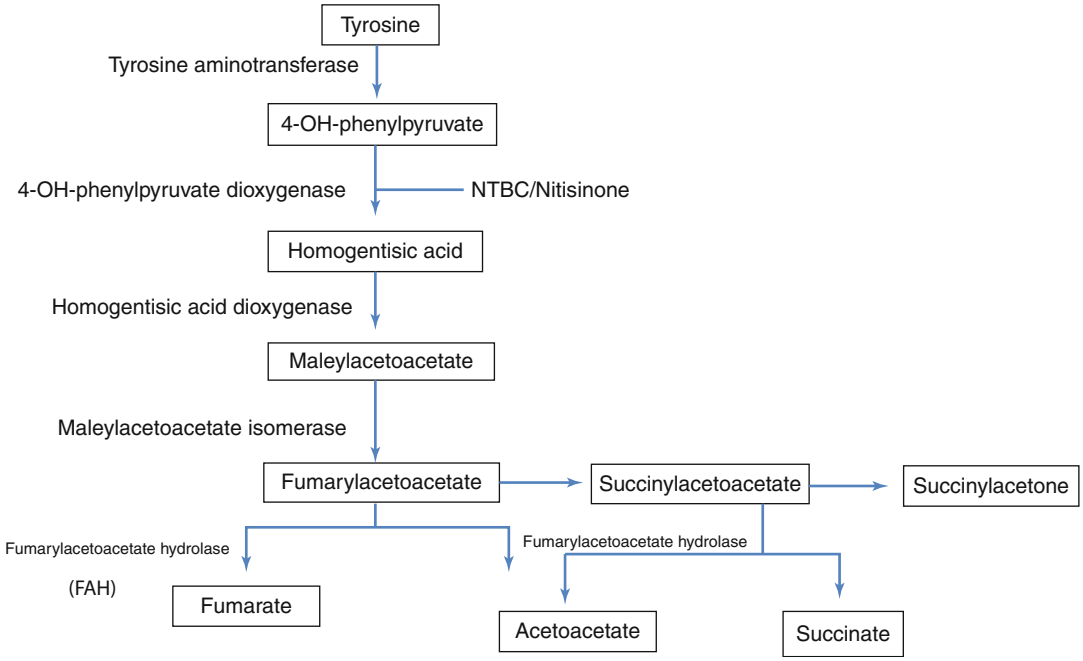

Fig. 8.7 Tyrosine degradation pathway including site of action of NTBC [2-(2-nitro-4-trifluromethylbenzoyl)-1, 3-cyclohexanedione]

growth failure; renal tubular dysfunction including Fanconi syndrome; and neurologic crises with pain and paresthesias associated with evidence of liver dysfunction including hepatomegaly, transaminasemia, hyperbilirubinemia, and coagulopathy with long-term risk of HCC. The diagnosis is made by identifying elevated urinary succinylacetone. Serum α-fetoprotein may be markedly elevated at diagnosis and hemolytic anemia may be present. Renal tubular dysfunction is common with increased excretion of phosphate, glucose, protein and amino acids. Liver histologic findings in acute disease include fatty infiltration and changes of pseudoacinar formation of lobules found in metabolic disorders, hepatocellular necrosis. Chronic forms lead to micronodular cirrhosis with regenerative nodules which may include dysplasia or HCC.

Treatment, Management, and Outcomes

Neonates presenting with acute liver failure should be treated aggressively to prevent and treat complications such as hypoglycemia, sepsis, and coagulopathy. Active bleeding should be treated with fresh frozen plasma, platelets and cryoprecipitate based upon the laboratory findings. Infusions of intravenous glucose should be given to prevent hypoglycemia. Dietary restriction of tyrosine with formulas such as Tyrex® or Tyros® should be used. NTBC ((2-(2-nitro-4-trifluromethylbenzoyl)-1,3-cyclohexanedione, nitisinone) which inhibits 4-hydroxyphenyl dioxygenase, the enzyme proximal to the deficient enzyme in type I tyrosinemia, is effective in the majority of patients with reversal of hepatic and renal dysfunction. Patients should be monitored with annual eye exams to assess eye changes associated with treatment and periodic liver chemistries and semiannual abdominal ultrasound and α-fetoprotein to assess for evidence of neoplasia. Survival with NTBC treatment is excellent with remarkable improvement over a <40 % survival at 1 year with diet alone in those presenting at less than age 2 months [25–27]. Liver transplantation should be considered for patients unresponsive to NTBC with excellent overall 1-year survival rates exceeding 85 % [28].

Methylmalonic Acid, Isovaleric Acid, HMG-CoA Lyase, and Maple Syrup Urine Disease

Introduction

These disorders are collectively quite rare but important to the practicing gastroenterologists and pediatricians because affected infants/children may present with recurrent episodes of vomiting, acidosis, modest transaminasemia, hyperammonemia, and variable neurocognitive dysfunction. Collectively, these conditions, the urea cycle defects, and defects of fatty acid oxidation were conditions that previously were part of the differential diagnosis of Reye syndrome and might present to the gastroenterologist or practicing pediatrician.

Pathophysiology

Each of the conditions has its unique metabolic profile found in serum and urine. As examples, accumulation of methylmalonic acid (MMA) occurs in methylmalonic academia; isovaleric acid, 3-hydroxyvaleric acid, *N*-isovalerylglycine, and isovalerylcarnitine in isovaleric academia; branched chain amino acids (valine, leucine, and isoleucine) in maple syrup urine disease; and 3-hydroxy-3-methylglutaric, 3- methylglutaconic, 3-methylglutaric, and 3-hydroxyisovalaeric acids in HMG-CoA lyase deficiency.

Clinical Manifestations

The diagnosis of each of these conditions is usually made in the newborn period (although some present later in life) by identifying typical urinary organic acid or serum amino acid patterns in the presence of acidosis, hypoglycemia, and alterations in consciousness. Older infants and children may present with recurrent episodes of alterations in consciousness. Liver chemistries may be abnormal with elevated serum aminotransferases and normal serum bilirubin. Acidosis, hypoglycemia, and hyperammonemia are commonly present. In patients with isovaleric acidemia, the patient has a "sick sweet" smell of sweaty feet or cheese during episodes. These conditions are commonly part of the newborn screen performed in all US states. All are inherited as autosomal recessive traits and family history may be revealing.

Treatment, Management, and Outcomes

Treatment for each of these conditions is dictated by the specific defect. Typically, for each condition intravenous glucose is administered with the intent of minimizing ketosis. Protein restriction with avoidance of catabolism is essential in the management of these conditions. If marked elevations of serum ammonia are found, use of intravenous sodium phenylacetate, sodium benzoate, and arginine should be considered or even hemodialysis for refractory episodes. In selected circumstances of MMA, patients are responsive to vitamin $B_{12,}$ and this should be considered when a patient is acutely ill with MMA. Chronic management includes diets tailored for specific defects. The prognosis of these conditions is guarded with morbidity and mortality from the acute episodes and associated morbidities accompanying the diseases. Because of recurrent metabolic crises and associated neurodevelopmental delay associated with repeated central nervous system injury, liver transplantation has been considered an option for some patients.

Fatty Acid Oxidation Defects

Mitochondrial fatty acid oxidation (FAO) defects are a group of inherited metabolic disorders that contribute greatly to pediatric morbidity and mortality. FAO provides most of the energy supplied to the heart and skeletal muscle and is crucial in the maintenance of glucose homeostasis during periods of fasting. The end product of FAO is ketones that constitute an important secondary energy source for tissues when glucose supplies are depleted.

MCAD (Medium-Chain Acyl-CoA Dehydrogenase) Deficiency

Introduction

MCAD is a nucleus-encoded mitochondrial matrix enzyme that catalyzes the initial dehydrogenation step in the β-oxidation of medium-chain fatty acids. MCAD deficiency is inherited in an

autosomal recessive pattern with a prevalence of 1 in 10,000–25,000. It is considered to be an important disorder of FAO and is the most common β-oxidation defect.

Pathogenesis

MCAD is responsible for the initial metabolism of acyl-CoAs with chain lengths of 4–12 carbon atoms. Most patients with MCAD deficiency have a missense mutation with ensuing enzyme inactivity. MCAD deficiency leads to fasting-induced hypoglycemia and accumulation of toxic acyl-CoA compounds.

Clinical Manifestations and Diagnosis

The major role of β-oxidation is to supplement energy during fasting. Historically, the inherited deficiency of MCAD with resulting dysfunction of the β-oxidation process often presented in the first months to years of life with recurrent emesis, lethargy, coma, and even death provoked by fasting or illness. MCAD deficiency is now part of newborn screening. Hepatomegaly may be present during acute decompensation, which is also characterized by hypoketosis and hypoglycemia, anion gap acidosis, hyperuricemia, elevated liver enzymes, and hyperammonemia. Increased metabolites (acylcarnitines) may be present. Characteristic urine gas chromatographic findings include elevated levels of sebacic, sebaric, and adipic acid. Liver biopsy reveals microvesicular fatty changes. Fibroblast enzymatic assays and/or genetic testing of the MCAD gene can make a confirmatory diagnosis [1].

Treatment, Management, and Outcomes

The mainstay of treatment for patients with MCAD deficiency is avoidance of fasting. Supplementation of the diet with carbohydrates or glucose can also assist in the avoidance of catabolism and symptom development during acute illness [29]. Unidentified patients with this disorder have a significant risk of sudden death in early childhood, and survivors have a significant risk of developmental disability and chronic somatic illnesses [30].

LCHAD (Long-Chain 3-Hydroxyacyl-CoA Dehydrogenase) Deficiency

Introduction

LCHAD deficiency is a rare, autosomal recessive inborn error of fatty acid metabolism. Long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) is an enzyme that constitutes part of the mitochondrial trifunctional (TFP) complex. This complex is composed of 3 separate enzymes whose main function is to metabolize long-chain fatty acids often found in milk and oils. Complete TFP deficiency is rare and more commonly patients are found to have isolated LCHAD deficiency with the remaining two enzymes of the TFP complex functioning at or above 60 % or normal [31].

Pathogenesis

The multienzyme mitochondrial TFP complex consists of 4 α- and 4 β-subunits that together work to catalyze the last three steps in β-oxidation of long-chain fatty acids. The α-subunit contains the LCHAD enzyme as well as the long-chain enoyl-CoA hydratase enzyme, while the β-subunit contains the long-chain 3-ketothiolase (LKAT) enzyme [32]. The most common genetic mutation associated with LCHAD deficiency is the mutation in the HADHA gene that results in isolated LCHAD dysfunction. The resulting pathology in patients with LCHAD deficiency results from inadequate energy supply and toxic accumulation of metabolites.

Clinical Manifestations and Diagnosis

The classic presentation of a child with LCHAD deficiency is a newborn with acute onset feeding difficulties, lethargy, hypotonia, and hypoketotic hypoglycemia. Neuropathy and retinopathy are two unique sequelae of LCHAD deficiency (these are also found in TFP complex deficiency) that often occur in older individuals, and LCHAD deficiency in a fetus is now well recognized to predispose mothers to gestational complications such as HELLP (hemolysis, liver dysfunction, and low platelets) syndrome and AFLP (acute fatty liver of pregnancy).

Newborn screening programs now test for LCHAD deficiency. Confirmation of the diagnosis is possible by measuring LCHAD activity in lymphocytes, fibroblasts, and muscle or liver biopsies and by mutational analysis [33].

Treatment, Management, and Outcomes

The primary treatment of LCHAD deficiency is dietary restriction with a low-fat, carbohydrate-rich diet. Supplementation with a medium-chain triglyceride-enriched diet can also be beneficial. L-carnitine has been given to patients with LCHAD deficiency; however, there have been several reports of adverse effects of this treatment strategy due to accumulation of long-chain acyl-CoA esters. Long-term complications include progressive peripheral neuropathy and retinopathy. Despite early recognition and dietary treatments, the morbidity in LCHAD deficiency remains high.

HMG (3-Hydroxy-3-Methylglutaryl) CoA Synthase Deficiency

Introduction

Mitochondrial HMG-CoA synthase is an enzyme that regulates the formation of ketone bodies. It is the only disorder exclusively effecting hepatic ketogenesis. Deficiency of HMG-CoA synthase is inherited in an autosomal recessive pattern.

Pathogenesis

HMG-CoA synthase catalyzes the rate-limiting step in the formation of ketones. Under normal conditions, when the body undergoes stressors such as acute illness or fasting, ketone production is increased, and the resulting product is utilized as an alternative energy source by the brain. In patients with HMG-CoA synthase deficiency, the ensuing enzyme inactivity results in failure to produce ketones during stress with resultant hypoketotic hypoglycemia often progressing to encephalopathy and coma.

Clinical Manifestations and Diagnosis

Patients often present initially with hepatomegaly, encephalopathy, and hypoketotic hypoglycemia often associated with acute illness or prolonged fasting. In the majority of cases, patients are asymptomatic prior to their acute decompensation. Clinically, these patients can present similarly to those with MCAD deficiency; however, in HMG-CoA synthase deficiency, lactate is often normal as are liver transaminases. Currently, few diagnostic tests indicate the diagnosis of HMG-CoA synthase deficiency, and high clinical suspicion is required. While the combination of normal acylcarnitine and absence of urinary ketones suggests the diagnosis, definitive testing can only be accomplished with molecular testing [34].

Treatment, Management, and Outcomes

Prompt clinical recognition along with patient and family education on the importance of fasting avoidance is critical in the management of patients with HMG-CoA synthase deficiency. No long-term organ dysfunction has been appreciated in patients with HMG-CoA synthase deficiency.

CACT (Carnitine-Acylcarnitine Translocase) Deficiency

Introduction

Carnitine-acylcarnitine translocase (CACT) deficiency is a rare β-oxidation defect often associated with cardiomyopathy and early childhood death.

Pathogenesis

CACT is one of the enzymes that constitute the carnitine shuttle needed to transport long-chain fatty acids from the cytosol into the mitochondria where β-oxidation occurs. CACT is the enzyme responsible for the transfer of fatty acids into the mitochondria in exchange for free carnitine. Subsequent oxidation of long-chain fatty acids produces ATP used for energy or is converted to ketone bodies. Enzyme dysfunction impairs mitochondrial fatty acid oxidation.

Clinical Manifestations and Diagnosis

Patients often present during the neonatal period with hypoketotic hypoglycemia and significant

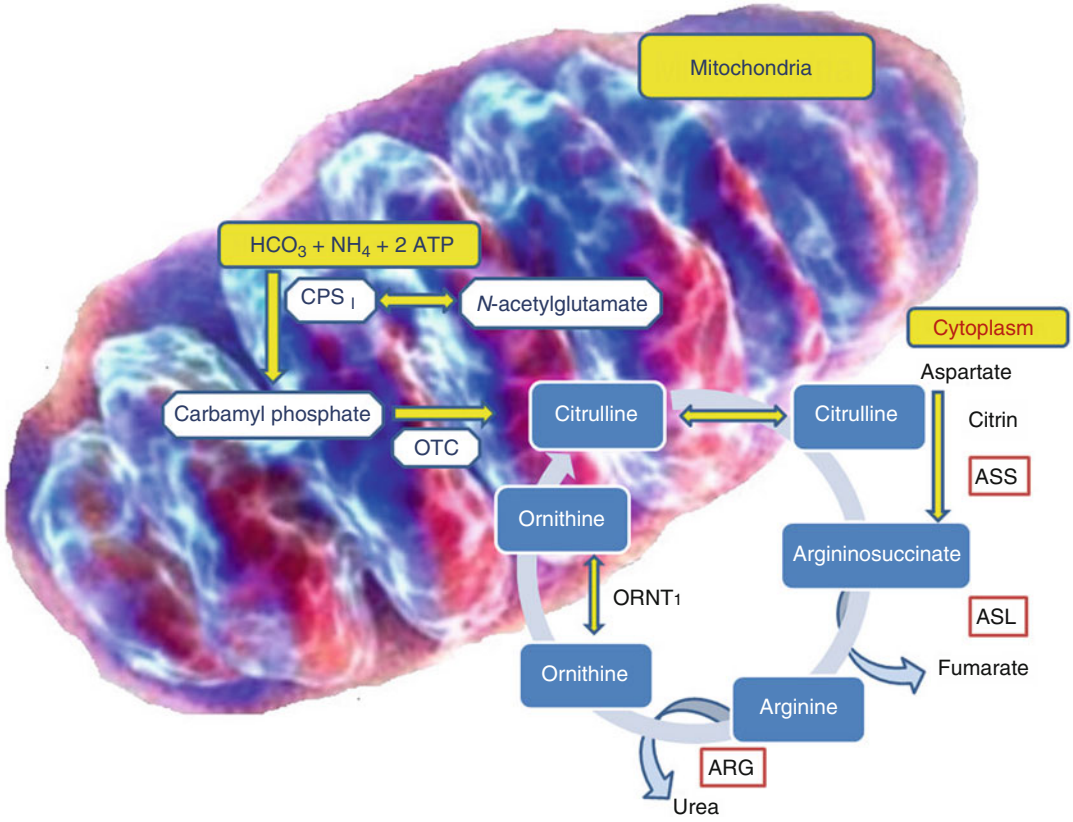

Fig. 8.8 Urea cycle with depiction of pathway within mitochondrion and cytoplasm with highlighted enzymatic steps commonly identified in inborn errors of ureagenesis

cardiomyopathy often resulting in death. Additional manifestations include hepatic dysfunction, ventricular arrhythmias, seizures, and apnea [1]. There have been few reports of a milder phenotype often associated with the absence of significant cardiac involvement. Laboratory investigations often reveal hyperammonemia, elevated creatinine kinase, elevated transaminases, dicarboxylic aciduria, decreased carnitine, and elevations of long-chain acylcarnitines. Many states now include CACT analysis on their newborn screens. A definitive diagnosis is made with mutational analysis of the CACT gene.

Treatment, Management, and Outcomes

Treatment strategies include low-fat diet, medium-chain triglyceride supplementation, intravenous glucose to assist in the prevention of lipolysis, and carnitine supplementation [1]. Medium-chain triglycerides are utilized because they do not require transport via CACT across the mitochondrial membrane. Morbidity in patients with CACT deficiency when accompanied cardiac involvement remains high, and most children will die within the first year of life. In contrast, children with CACT deficiency without cardiac involvement have been reported to survive the neonatal period and into childhood [35].

Urea Cycle Defects

Introduction

Ureagenesis is an essential hepatic function that allows degradation of protein to its end product of ammonia to be rendered nontoxic (See Fig. 8.8).

Ammonia and other precursor metabolites are known to be neurotoxic with the developing

central nervous system particularly susceptible to injury. These conditions have an incidence of 1:8,200–1:30,000. All are inherited in autosomal recessive manner except ornithine transcarbamylase (OTC) deficiency which is inherited as a sex-linked trait.

Pathophysiology

Disorders of ureagenesis lead to accumulation of ammonia and glutamine and other products proximal to the point of block of the cycle associated with each disorder. The accumulation of ammonia and its by-products leads to hepatocellular dysfunction with associated liver enzyme elevation and, in some cases, synthetic dysfunction with profound effects on the central nervous system with direct toxic effects on the brain with attendant brain swelling.

Clinical Manifestations

The most common disorders of ureagenesis including ornithine transcarbamylase deficiency (OTC), carbamoyl phosphate synthetase (CPS), and argininosuccinate synthetase (ASS) or citrullinemia commonly present in the neonatal period (see Fig. 8.8). Infants with urea cycle defects are normal at birth and rapidly develop hyperammonemia and cerebral edema and progressive coma in the first days of life. These conditions are commonly mistaken to be septic because of lethargy, hypothermia, hyper- or hypoventilation, and seizures. Repeated episodes of hyperammonemia can lead to variable long-term neurologic impairment ranging from mild cognitive dysfunction to severe cerebral palsy. There may be a family history of neonatal deaths particularly in males with OTC deficiency. In older children, particularly in females who have hemizygous ornithine transcarbamylase deficiency, recurrent episodes of vomiting with variable alterations in consciousness or aversion to protein may be presenting complaints. During episodes of hyperammonemia, there may be mild elevation in the aminotransferases with minimal evidence of other hepatic dysfunction [36, 37]. Some older children may present with marked elevation of aminotransferases without hyperbilirubinemia and coagulopathy suggestive of acute liver failure. The diagnosis is made by a high index of suspicion in the presence of elevated plasma ammonia levels and reduced blood urea nitrogen and serum amino acid patterns typical of specific defects and the presence of orotic acid with OTC deficiency (Fig. 8.9). Definitive diagnosis of a urea cycle defect can be made by molecular genetic testing of measurement of enzyme activity in the liver. Molecular genetic testing is available for all of the urea cycle enzyme defects and is the diagnostic method of choice. Many states have newborn screening for selected urea cycle defects; however, CPS1 and OTC deficiencies are not reliably detected and manifestations of disease may precede the return of the results to the physician. For pregnancies at risk for an affected infant with a family history, prenatal testing via testing of DNA from amniocentesis or chorionic villus sampling.

Treatment, Management, and Outcomes

Treatment should be tailored to the specific urea cycle disorder and should be directed by a metabolic specialist at a referral center. Initial therapy should be directed at returning elevated plasma ammonia levels to the normal range. Reversing catabolism is essential, and nutritional support with hypertonic glucose should be initiated immediately. Searches for causes of catabolic state such as sepsis should be made and presumptive treatment given. Initial pharmacologic interventions with alternative pathway excretion of excessive nitrogen should be considered using intravenously administered sodium phenylacetate and sodium benzoate with arginine. Neomycin and lactulose tend not to be very effective and should not be used. If rapid improvement in plasma ammonia is not observed or if plasma ammonia is markedly increased, immediate treatment with hemodialysis should be considered. If dialysis is initiated, it should be continued until plasma ammonia falls below

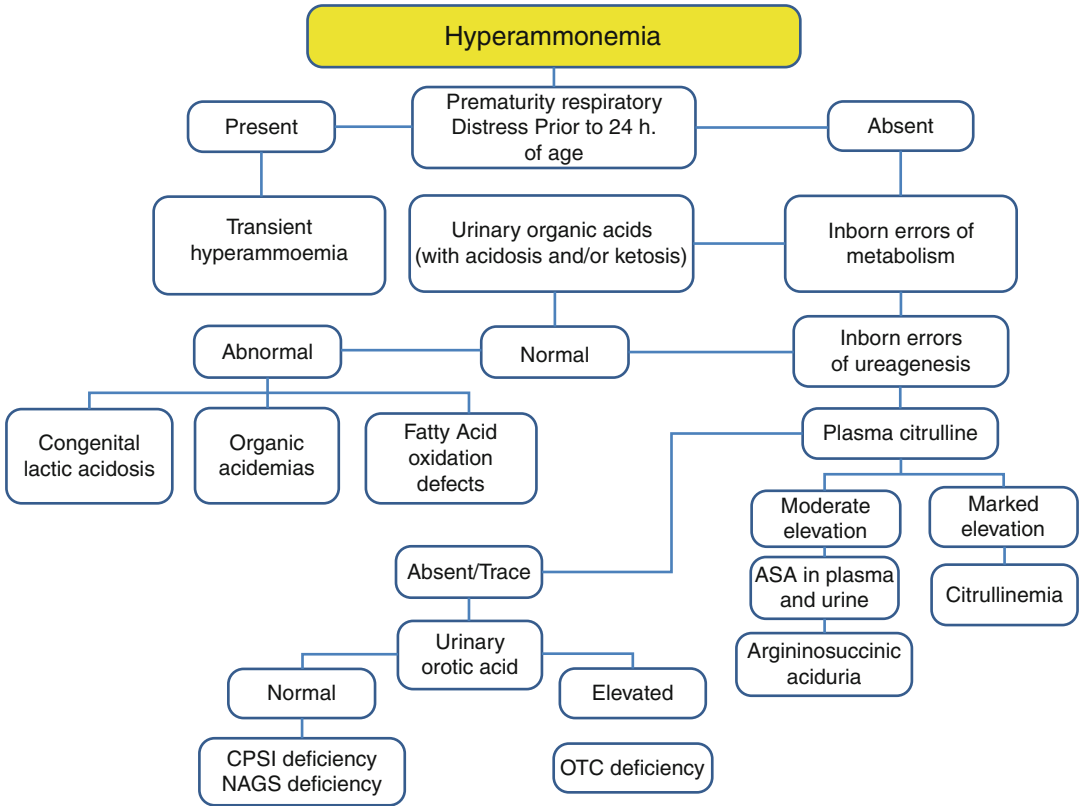

Fig. 8.9 Algorithm depicting the approach to the assessment of the infant/child with hyperammonemia

150 μmol/L. Once the acute hyperammonemic crisis is resolved, feeding should be commenced with a protein restricted formula with at least ½ of protein as essential amino acids. Sodium phenylbutyrate (Buphenyl®) should be initiated, and a newer product glycerol butyrate, which is more palatable than Buphenyl, may be available soon. Citrulline or arginine should be added based upon the location of the block in the urea cycle. Long-term support should include surveillance of illnesses that may precipitate catabolism and hyperammonemia protein restriction. In patients with severe, recurrent episodes of hyperammonemia, liver transplantation is an effective means to prevent future episodes. Currently, no effective protocols for gene therapy exist. After transplantation, there should be no further deterioration in neurologic dysfunction. With effective alternate pathway therapy, the 1-year survival for urea cycle defects is 92 %. Of these survivors, 79 % have mental retardation with a mean intelligence quotient of 43, 46 % have cerebral palsy, and 4 % are blind [38]. The IQ is correlated with the duration of elevated plasma ammonia not the peak concentration [39]. Hemizygote OTC females have a good prognosis but may have recurrent episodes of hyperammonemia which will compromise neurocognitive function.

Bile Acid and Biliary Transport Defects

Biliary Transport Defects

Introduction

Normal bile flow is dependent in part upon specific membrane transporters found in the liver and intestine. Inherited defects in the genes for some of these transporters lead to cholestasis and as a group comprise conditions termed progressive familial intrahepatic cholestasis (PFIC). PFIC

diseases are inherited as autosomal recessive traits and generally present as cholestasis in the first year of life leading to progressive liver injury associated with a family history of similarly affected infants/children/adults.

Pathophysiology

Three conditions comprise the currently known group of diseases referred to as PFIC 1, 2, and 3. Patients with PFIC 1 have mutations in the FIC1 gene (ATP8B1) which causes progressive disease. This disease was initially described as two distinct clinical entities, Byler disease and benign recurrent intrahepatic cholestasis (BRIC). Both diseases are the result of abnormalities in FIC1 but differ in severity of the underlying defect in FIC1, with milder defects being present in BRIC. Other gene modifiers may also be responsible for variations in the PFIC phenotype. FIC1 mediates the flipping of aminophospholipids from the outer to inner hemi-leaflet of the canalicular membrane. The exact nature of how FIC1 deficiency causes disease is not known. FIC1 is located on other tissues including the pancreas and intestine leading to other extrahepatic symptoms and signs.

Patients with PFIC 2 deficiency have defects in the canalicular bile salt export pump caused by mutation in *ABCB11*. BRIC-like disease (BRIC2) and intrahepatic cholestasis of pregnancy (ICP) have been observed in children and adults with genetic defects in bile salt excretory pump (BSEP). BSEP is responsible for transporting bile acids from inside the hepatocyte into the bile canaliculus.

PFIC 3 is caused by mutations in ABC B4 which encodes multidrug resistance-associated protein 3 (MDR3) and mediates the flopping of aminophospholipids from the inner to outer hemi-leaflet of the canalicular lipid bilayer. ABCB4 mutations have also been associated with low phospholipid-associated cholelithiasis syndrome and ICP.

Pathophysiology

Liver disease in PFIC results from the effects of hepatocellular accumulation of bile acids. The liver disease may be mild or severe, depending on the specific gene defect present. In FIC1 deficiency, biliary excretion of bile acids is diminished. The rate of progression to end-stage liver disease in FIC1 deficiency may be slower than in BSEP deficiency. In severe forms of BSEP deficiency, BSEP expression and function are completely absent. Hepatocellular bile acids accumulation is pronounced causing rapidly progressive liver disease. End-stage liver disease in severe BSEP deficiency can occur in the first 1–2 years of life. In MDR3 deficiency, phospholipids in canalicular bile are either deficient or absent leading to the formation of a toxic bile that likely causes the pathogenesis of a progressive intrahepatic cholangiopathy. The resulting liver disease is a consequence of the cholestasis and inflammatory response generated by this cholangiopathy. Hepatocellular injury in MDR3 deficiency also results from intracellular bile salt accumulation.

Clinical Manifestations

Patients with severe forms of these diseases present with jaundice and/or pruritus. Life-threatening hemorrhage, secondary to cholestasis-related vitamin K deficiency, can also be a dramatic early presentation of PFIC. Profound, medical therapy-resistant pruritus is one of the most common early manifestations of all three of these forms of PFIC. Typically scratching begins between ages 6 and 12 months. Patients with PFIC I and 2 have markedly elevated serum bile acid levels with mildly elevated serum bilirubin, normal serum gamma glutamyl transpeptidase (gGTP), normal serum cholesterol, and only mild elevation in serum aminotransferase values. FIC1 deficiency is a systemic condition, and affected children may have growth failure, hearing problems, recurrent respiratory problems, elevated sweat chloride, recurrent pancreatitis, and diarrhea that is independent of the cholestasis. PFIC 2 has a strong association with hepatocellular carcinoma even in the first years of life. Affected patients with BRIC related to defects in BSEP may be associated with cholelithiasis [40–42]. MDR3 deficiency should be suspected in children with progressive cholestasis who have an elevated serum gGTP and no evidence of extrahepatic biliary tract disease. In patients with severe disease, biliary phospholipid

concentrations are markedly reduced. PFIC 3 may present with intrahepatic cholestasis of pregnancy, drug-induced cholestasis, and a form of benign recurrent intrahepatic cholestasis. Low phospholipid-associated cholestasis syndrome is caused by a mutation in MDR3 and presents as cholesterol gallstones and intrahepatic cholelithiasis in adults younger than 40 years [43]. As liver disease in children with PFIC I, 2, and 3 progresses, the biochemical parameters become more typical for chronic liver disease and can include elevated bilirubin and aminotransferase values. Children with PFIC develop end-stage liver disease like other forms of progressive cholestatic liver disease. Portal hypertension develops secondary to the development of biliary cirrhosis. Definitive diagnosis of a specific form of PFIC is dependent upon identification of characteristic genetic defects. CLIA-certified molecular testing laboratories for genes of interest may be found at www.genetests.org. Histologic and ultrastructural analysis of the liver may be useful in distinguishing FIC1 from BSEP deficiency. Hepatocytes in FIC1 deficiency tend to be tidy and compact as opposed to BSEP deficiency in which there is more pronounced hepatocellular disarray, edema, giant-cell change, and hepatocellular necrosis ("neonatal hepatitis"). Portal and lobular fibrosis is more often seen at presentation in BSEP deficiency, but both FIC1 and BSEP deficiencies can progressively develop increasing amounts of fibrosis and a subset result in cirrhosis. Transmission electron microscopy in FIC1 deficiency may identify coarsely granular "Byler bile." MDR3 deficiency can display expanded portal tracts and ductular proliferation with mixed inflammatory infiltrate mimicking a biliary obstruction pattern of injury or extensive portal fibrosis and biliary cirrhosis.

Treatment, Management, and Outcomes

The treatment of PFIC includes standard nutritional approaches for fat and fat-soluble vitamin malabsorption due to cholestasis and therapies for end-stage liver disease. Certain aspects of the management are unique. Initially, the pruritus associated with PFIC may be quite debilitating. Standard treatment including ursodeoxycholic acid, cholestyramine, rifampin, and opioid antagonists may not be successful. Surgical interruption of the enterohepatic circulation of bile acids with either ileal bypass or diversion may be effective with improvement in pruritus, serum liver chemistries, and prevention of progression of liver disease. Ursodeoxycholic acid is effective therapy in milder cases of MDR3 deficiency with many patients normalizing liver function with therapy. Ursodeoxycholic acid is successful in resolution of gallstones in adult patients with low phospholipid-associated cholelithiasis syndrome.

The prognosis for children with PFIC can be quite variable and influenced by the genetic abnormality (both the specific gene mutated and the impact of the mutation on the transporter protein) and the therapeutic approaches used. Severe defects in BSEP are associated with a high risk of hepatocellular carcinoma. Severe defects in BSEP and MDR3 deficiency are typically associated with an unremitting form of cholestasis that are unresponsive to medical and surgical therapies and eventuate in liver transplantation. End-stage liver disease typically evolves in the first 5–10 years of life. Recurrent BSEP deficiency has been described after liver transplant. FIC1 is a systemic disease, and the posttransplant course can be problematic including intractable diarrhea, liver steatosis leading to cirrhosis, and recurrent pancreatitis [44]. Liver transplantation, while effective for pruritus, may not be an optimal therapy for children with FIC1 deficiency. Surgical interruption of the enterohepatic circulation appears to be preferable to liver transplantation in FIC1 deficiency. Overall, with optimal surgical intervention, the long-term prognosis for children with PFIC is excellent.

Inborn Errors of Bile Acid Metabolism

Introduction

Bile acids are the natural detergents that the liver makes that help with bile flow and efficient fat and fat-soluble vitamin absorption. The primary bile acids, cholic acid and chenodeoxycholic

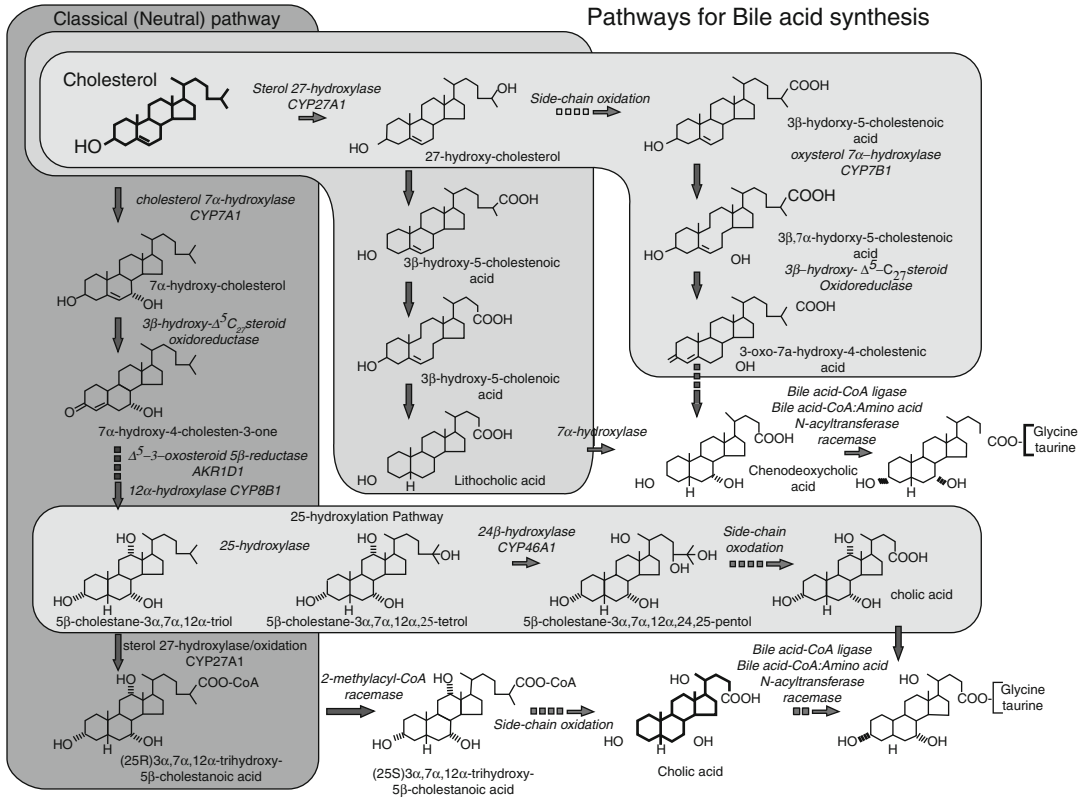

Fig. 8.10 Classical and acidic pathways of bile acid synthesis including enzymatic defects associated with inborn errors of bile acid metabolism (Reprinted with permission from publisher of Liver Disease in Children, 3e (Cambridge University Press))

acid, are synthesized by the liver from cholesterol through a series of 17 enzymatic steps, which results in the production taurine or glycine conjugates. An alteration in each of these steps can lead to the blockage of the bile acid production pathway with failure to produce "normal bile acids" and the accumulation of intermediary metabolites above the point at which the block in the pathway occurs. Because each of the enzymes in the pathway is regulated by a gene, it is believed that abnormalities in any of the steps of the pathway are inherited. Inborn errors of bile acid metabolism cause liver disease or may be a portion of more generalized diseases which include neurologic disease (See Fig. 8.10).

Pathophysiology

For all of the defects for which the genes have been identified, the inheritance of the bile acid defect is believed to be autosomal recessive. Blockage in the bile acid production pathway leads to accumulation of materials in the pathway prior to the block and few normal bile acids produced. This may result in mild to severe liver disease depending upon which of the enzymes is affected. Some conditions are associated with jaundice and significantly impaired function, while with others there may be mild changes in liver enzymes with poor bile flow and associated malabsorption of fat and fat-soluble vitamins with attendant complications (Δ^4-3-oxosteroid 5-β-reductase deficiency and 3β-OH steroid dehydrogenase/isomerase deficiency [3-HSD]). Some are associated predominantly with fat-soluble vitamin malabsorption and growth failure (bile acid-acyl transferase deficiency). In some conditions, there is liver disease associated with neurologic dysfunction (racemase deficiency, cerebrotendinous xanthomatosis {CTX}). Patients with conditions caused by peroxisomal

diseases including Zellweger and neonatal adrenoleukodystrophy have abnormalities in bile acid production because part of the production of bile acids requires steps within the peroxisome. These disorders affect many body organs since peroxisomes are present throughout the body. Liver disease may be variable in severity and is accompanied by neurologic disease which may include mental retardation, seizures, deafness, blindness, and muscular weakness.

There are two principal causes of liver injury associated with inborn errors of bile acid metabolism. The first relates to the failure to make "normal" bile acids which are a major force propelling bile out of the liver. This leads to impaired bile flow with attendant reduction of biliary excretion of individual components including cholesterol and other fats, proteins, drugs, and environmental toxins out of the liver. Secondly, the intermediary metabolites produced because of the block in the bile acid production pathway may themselves lead to liver injury. The combination of poor bile flow coupled with the production of potential toxic bile acid intermediates most likely is responsible for the injury to the liver seen in these conditions.

Clinical Manifestations

The most common defects including 3-HSD and Δ^4 -3-oxosteroid 5-β-reductase deficiencies commonly present with neonatal jaundice, poor growth, liver or spleen enlargement, bleeding, rickets (vitamin D deficiency), or liver disease of unknown cause. Liver chemistries measured in the blood are abnormal and serum vitamin levels may be reduced. There is elevation in the serum ALT and AST with normal gGTP concentrations. Pruritus is usually absent. There may be variable alterations in the prothrombin time and serum 25-OH vitamin D and retinol and tocopherol levels. Serum bile acids measured by standard clinical methods are either normal or low. Patients with CTX may present in the neonatal period with neonatal cholestasis or diarrhea in infancy prior to any of the neurologic deterioration or xanthomata accumulation seen in adulthood. Patients with conjugation defects (bile acid acyl transferase deficiency) may present with transient neonatal cholestasis or infancy and childhood with growth failure and fat-soluble vitamin deficiencies. Diagnosis of these rare conditions requires a high index of suspicion by the treating physician. In the right clinical setting in which an infant or child has jaundice, liver disease of unknown cause, or abnormalities of fat or fat-soluble vitamin absorption, the treating physician needs to consider the diagnosis. In the presence of jaundice, elevated serum bile acid levels usually exclude the diagnosis. If serum bile acids are low or normal, urine should be sent for analysis to specialized laboratories for measurement of urinary bile acids by methods such as fast atom bombardment mass spectrometry (FAB-MS). These techniques allow identification of the "profile" of the bile acids in urine that would determine the potential presence of an inborn error of bile acid metabolism. If the urine FAB-MS study is suggestive of an abnormality, additional evaluation (including measurement of bile acids in blood and bile and evaluation of the microscopic appearance of the liver) would be necessary in order to establish the diagnosis. Liver biopsy findings for the most common conditions including 3-HSD, Δ^4 -3-oxosteroid 5-β-reductase deficiencies do not show disease-specific findings but show evidence of cholestasis with canalicular bile plugs, giant-cell transformation, extramedullary hematopoiesis, and mild inflammation without bile duct proliferation [45–49].

Treatment, Management, and Outcomes

The treatment for inborn errors of bile acid metabolism not due to conjugation defects focuses on restoring normal bile flow with reduction in the injury to the liver by the toxic intermediates of the bile acid production pathway. Bile acid therapy with a specific bile acid, cholic acid, is currently available as an investigational drug approved by the US Food and Drug Administration. Another bile acid, ursodeoxycholic acid, is a drug approved by the FDA but not for this indication. UDCA is not an ideal treatment because it does not suppress synthesis of abnormal, potentially toxic metabolites, as cholic acid does. It may be prescribed by physicians as

an "off label" indication which means that a drug approved by the FDA for a specific disease may be used for other diseases at the discretion of the treating physician. When first diagnosed, patients with inborn errors of metabolism malabsorb fat and fat-soluble vitamins (vitamins A, D, E, and K) and infant formulas containing medium-chain triglycerides (Alimentum®, Pregestimil®) and supplemental vitamins may be prescribed to overcome initial deficits of these nutrients. Although most affected patients have a complete resolution of the jaundice and liver biochemistry abnormalities with bile acid treatment, the liver disease may progress to end-stage liver disease with cirrhosis and a liver transplant may be required. Patients treated with orally administered bile acids (cholic acid and ursodeoxycholic acid) usually correct all abnormalities of liver function including jaundice, growth failure, fat and vitamin malabsorption, and blood liver chemistry abnormalities. This occurs over a period of several weeks after starting therapy. Cholic acid is the preferred treatment since it suppresses the production of toxic bile acid intermediates whereas ursodeoxycholic acid does not. Occasionally, patients have worsening of liver disease despite treatment with the development of cirrhosis and liver failure. Patients who are not treated with bile acids have progressive worsening until cirrhosis develops. If cirrhosis develops, the only effective therapy is liver transplantation. Patients with conjugation defects respond to treatment with glycocholic acid, which is available under an IND from the FDA. The liver disease in these patients tends to be mild, but they commonly have fat-soluble vitamin deficiency and growth failure that is corrected with bile acid therapy. With bile acid therapy, the prognosis for children with conjugation defects is excellent [50].

Lysosomal Storage Disorders

Introduction

Lysosomal storage disorders are encountered by the pediatric gastroenterologist as hepatosplenomegaly with other systemic manifestations. The most common and important of these include Gaucher disease, Niemann-Pick type C disease, and lysosomal acid lipase deficiency (cholesterol ester storage disease and Wolman disease).

Gaucher Disease

Introduction

Gaucher disease (GD) is the most common lysosomal storage disease and is inherited as an autosomal recessive trait that affects the recycling of cellular glycolipids. It occurs in approximately 1/75,000 births worldwide but is more prevalent in individuals of Ashkenazi Jewish descent. Approximately 90 % of patients have type 1 Gaucher disease (GD1), the nonneuronopathic form, and discussion will be limited to it.

Pathophysiology

Gaucher disease results from deficiency of a lysosomal enzyme glucocerebrosidase (also known as glucosylceramide or acid beta-glucosidase, GBA) leading to accumulation glucocerebroside and other glycolipids within the lysosomes of macrophages in the spleen, liver, bone marrow, bone, and other tissues/organs.

Clinical Manifestations

GD1 is characterized by variability in signs, symptoms, severity, and progression even among siblings with the same genotype and monozygotic twins. Symptomatic patients have visceral involvement, bone disease, and bleeding and may present to the gastroenterologist with hepatosplenomegaly. Splenomegaly is the most common presenting sign. The spleen can be markedly enlarged. Hepatomegaly is universal, but the liver size increases relatively less than the spleen. Hepatic fibrosis commonly occurs, but hepatic failure, cirrhosis, or portal hypertension is uncommon. Liver biopsy findings include macrophages filled with lipid material known as Gaucher cells which have a characteristic appearance of wrinkled tissue paper. Thrombocytopenia and anemia typically are commonly present. Liver chemistries may be mildly elevated. The diagnosis of GD is confirmed by the finding of reduced glucocerebrosidase activity, usually in peripheral leukocytes, in a patient with clinical

features consistent with GD. Mutation analysis provides additional confirmation of the diagnosis and can help predict clinical manifestations and identify undiagnosed affected family members and heterozygote carriers. Prenatal diagnosis can be performed by enzyme analysis by chorionic villus sampling or amniocentesis.

Treatment, Management, and Outcome

The goals of treatment of GD disease are elimination of symptoms, prevention of irreversible damage, and improvement in the overall health and quality of life. Treatment of GD is tailored to the individual patient because of the variability in the manifestations, severity, and progression of the disease. The decision to offer enzyme replacement therapy (ERT) for GD1 is based upon disease severity and disease progression. ERT with recombinant glucocerebrosidases (imiglucerase or velaglucerase alfa) is recommended for all symptomatic children. ERT is individualized. The availability and efficacy of ERT has limited the indications for splenectomy and hematopoietic cell transplantation. Splenectomy is indicated if other measures fail to control life-threatening thrombocytopenia [51, 52].

Niemann-Pick Disease

Introduction

Niemann-Pick disease type C (NPD-C) is an autosomal recessive disease caused by mutations of the NPC1 and NPC2 genes. NPD-C can present in infants, children, or adults with an estimated prevalence of 1:150,000 in Europe.

Pathophysiology

Mutations in the NPC1 and NPC2 genes result in impaired cellular processing and transport of low-density lipoprotein (LDL) cholesterol. Abnormal lipid trafficking and storage of lipids in lysosomes of affected cells results in progressive neurocognitive and visceral disease.

Clinical Manifestations

More than 90 % of affected individuals have splenomegaly and or hepatomegaly particularly those presenting in infancy and early childhood. Over half present with infantile cholestatic liver disease similar to neonatal hepatitis of PFIC 1, 2, or 3. A small fraction present with liver failure. Older patients may have cerebellar involvement characterized by clumsiness and gait problems progressing to frank ataxia, and slow cognitive deterioration. Progressive dystonia, dysarthria, and dysphagia occur, eventually impairing oral feeding, and approximately one-third of patients develop seizures. Death typically occurs from aspiration pneumonia in the second or third decade of life. The laboratory findings may include lipid abnormalities such as decreased high-density lipoprotein (HDL) cholesterol, hypertriglyceridemia, and increased LDL cholesterol. Large lipid-laden foam cells are seen in the reticuloendothelial system of the spleen, liver, bone marrow, lymph nodes, blood vessels, Schwann cells in peripheral nerves, CNS, and retinal cells [53–55]. The diagnosis of NPD-C is suspected on the basis of the clinical features and confirmed by fibroblast cell culture. Genetic testing is commercially available. A mutation in the NPCI gene is found in approximately 90 % with an NPC2 gene mutation found in less than 5 % of cases of NPD-C.

Management, Outcome, and Prognosis

There is no specific treatment for NPD-C and treatments are symptomatic and directed to neurologic and psychiatric symptoms. Lipid lowering treatment and bone marrow transplantation have not been shown to affect outcome. Similarly, liver transplantation improves hepatic function but does not alter the natural history of the neurological disease.

Lysosomal Acid Lipase Deficiency (Wolman Disease and Cholesteryl Ester Storage Disease) (CESD)

Introduction

Lysosomal acid lipase deficiency results in two diseases with considerably different phenotypes. Both are associated with accumulation of cholesteryl esters, triglycerides, and other lipids in lysosomes, and disease manifestation is heavily dependent upon how much acid lipase enzyme

activity is present in the affected patient. The gene for lysosomal acid lipase (LIPA) codes for acid lipase activity for which mutations lead to minimal or absent enzyme activity in Wolman disease with mutations in cholesterol ester storage disease resulting in variable amounts of residual enzyme activity.

Pathophysiology

Absence of lysosomal acid lipase activity leads to accumulation of cholesterol esters, triglycerides, and other lipids in lysosomes in liver and other affected organs including the intestine in CESD and lymph nodes, bone marrow, intestine, liver, spleen, and adrenal glands in Wolman disease leading to remarkable variations in organ dysfunction.

Clinical Manifestations

Wolman disease is a severe disease with rapidly progressive course. Infants typically present in the first weeks of life with steatorrhea, failure to thrive, hepatosplenomegaly, and jaundice with death in the first year of life. Calcifications of the adrenal gland are evident on plain film of the abdomen. There is variable liver dysfunction with cholestasis, serum aminotransferase elevation, and worsening evidence of synthetic dysfunction as the disease progresses. Plasma lipids are generally normal, and liver biopsy has a characteristic orange-yellow in appearance and found to contain triglycerides and cholesterol esters on microscopic examination. Acid lipase activity in liver, leukocytes, or fibroblasts is markedly reduced. CESD is characterized by hepatomegaly without splenomegaly and hyperbetalipoproteinemia identified in older children, adolescent, or adult. There may be mild increases in aminotransferases without jaundice or evidence of synthetic dysfunction. Characteristically, the liver is orange macroscopically and there is accumulation of cholesterol ester and triglycerides on light microscopy.

Management, Outcome, and Prognosis

Limited success has been observed in patients with Wolman disease treated with hematopoietic cell transplantation [56]. Early success has been demonstrated using a plant-derived human lysosomal acid lipase for treatment in mice [57]. Currently, there is no FDA-approved therapy for CESD or Wolman disease. Phase 3 trials of recombinant lysosomal acid lipase began in 2012. Without specific treatment, Wolman disease is uniformly fatal in the first year of life, and only symptomatic therapy may be of any benefit. Patients with CESD have a normal life expectancy with some developing fibrosis but not end-stage liver disease in adulthood. Some adults may benefit from diets low in cholesterol and triglycerides and the use of HMG-CoA reductase inhibitors [58].

Peroxisomal Disorders

Introduction

Disorders of peroxisomal biogenesis affect multiple organ systems. Zellweger syndrome, neonatal adrenoleukodystrophy, and infantile Refsum disease comprise the majority of patients with disorders of biogenesis. With each of these conditions, the numbers of peroxisomes in liver are markedly reduced or absent.

Pathophysiology

Absence or marked reduction in the numbers of peroxisomes in multiple organs leads to characteristic manifestations. Peroxisomes play a role in multiple metabolic pathways including β-oxidation of fatty acids, α-oxidation of plant products, bile acid synthesis, ether lipid biosynthesis, cholesterol and isoprenoid biosynthesis, amino acid metabolism, and catalases for decomposing H_2O_2. Accumulation of by-products above the block in the pathway related to peroxisomal metabolism or inability to complete the steps in the pathway may be responsible for the clinical manifestations of disease.

Clinical Manifestations

Zellweger syndrome (ZS), neonatal adrenoleukodystrophy, and infant Refsum disease form a spectrum of features from ZS being most severe to infantile Refsum disease as the mildest. ZS includes typical craniofacial features such as

wide anterior fontanelle, prominent forehead, anteverted nostrils, epicanthal folds, and narrow upper lip. Severe neurocognitive function is present with hypotonia, mental retardation, and seizures. Visual and hearing impairment are common. Typically, no peroxisomes can be identified in the liver. Infants with ZS commonly have cholestatic liver disease with varying degrees of synthetic dysfunction with progression to end-stage liver disease at variable rates. Infants with neonatal adrenoleukodystrophy have dysmorphic features, deafness, psychomotor retardation, hypotonia, and seizures. A few residual peroxisomes may be found in the liver. The liver disease tends to be milder than ZS with better long-term prognosis. Patients with infantile Refsum disease have dysmorphic features but milder than ZS. Similarly, hypotonia is not as severe as in ZS. There is psychomotor delay and deafness. Hepatomegaly is common and liver disease may manifest as neonatal cholestasis. Liver disease may progress over time. Hepatic peroxisomes are absent or very few in number. The diagnosis of a peroxisomal disorder may be made in specialized laboratories by identifying metabolites in urine and serum that are present because of absent or diminished peroxisomal function such as very long-chain fatty acids, pipecolic acid, phytanic acid, and pristanic acid [59]. Skin fibroblast analysis may be performed to better characterize the metabolic pathways affected through examination of de novo plasmalogen synthesis, fatty acid beta-oxidation, phytanic acid alpha oxidation, and catalase immunofluorescence. Qualitative analysis of urine by fast atom bombardment mass spectrometry can identify characteristic bile acids with long acyl side chains of the sterol such as trihydroxycholestanoic acid (THCA) and dihydrocholestanoic acid (DHCA).

Treatment, Management, and Outcomes

There remain no specific treatment options for any of the defects of peroxisomal biogenesis. Limited experience with the use of cholic acid has suggested that there may be some benefit in treating cholestatic liver disease in patients with biogenesis defects. Clinical and biochemical benefits for non-hepatic manifestations of these conditions have been suggested with the use of docosahexaenoic acid (DHA) or induction of peroxisomal proliferation with agents such as 4-phenylbutyrate [60, 61]. Prognosis for these conditions is variable with death in ZS patients in the first years of life and longer survival with the other disorders.

Mitochondrial Hepatopathies

Mitochondria are intracellular organelles that contain the respiratory chain complex, the site of oxidative phosphorylation. A main function of the mitochondria is to produce adenosine triphosphate (ATP) by the respiratory chain located on the inner membrane. ATP subsequently functions to transport chemical energy within cells for metabolism and is critical for all intracellular processes. As a metabolically active organ, hepatocytes require continuous synthesis of ATP and contain elevated amounts of mitochondria to achieve this demand [62]. Alterations within the mitochondrial structure and function can result in clinical hepatopathies often presenting in children. The overall incidence of all respiratory chain defects is approximately 1 in 20,000 children with liver involvement occurring in about 10–20 %.

Neonatal Liver Failure

Introduction

Mitochondrial hepatopathies presenting as acute liver failure in the neonatal period can often be attributed to defects in the respiratory chain complex.

Pathogenesis

The respiratory chain protein complex of the mitochondria reduces equivalents derived from glycolysis, fatty acid oxidation, and the tricarboxylic acid (TCA) cycle and produces ATP via a system of electron carriers (protein complexes I through IV, coenzyme Q, and cytochrome c) in the inner mitochondrial membrane. This results in the generation of a transmembrane proton

gradient that drives the synthesis of ATP by complex V [61]. Most commonly, a deficiency in complex IV of the respiratory chain complex is causative; however, decreased activity of complexes I and III has also been associated with the development of neonatal liver failure.

Clinical Manifestations and Diagnosis

Neonatal liver failure often presents within the first weeks of life with transient hypoglycemia, neurological involvement with hypotonia, seizure activity, and psychomotor retardation in the setting of early hepatic dysfunction and failure [63]. Biochemical analysis reveals marked serum lactate elevations with a lactate-pyruvate ratio often >30. Although respiratory chain complex analysis will generally show decreased activity of complex I, III, or IV, no definitive diagnostic test is available.

Treatment, Management, and Outcomes

Mortality remains high in children presenting with neonatal liver failure, and most children progress to death within weeks to months after presentation.

Navajo Neurohepatopathy

Introduction

Navajo neurohepatopathy (NNH) is an autosomal recessive sensorimotor neuropathy associated with progressive liver disease that affects full-blooded Navajo children [64].

Pathogenesis

NNH results from a depletion of mitochondrial DNA (mtDNA) whose product, *MPV 17*, is involved in the regulation of oxidative phosphorylation and subsequent ATP production.

Clinical Manifestations and Diagnosis

There are three clinical presentations of NNH. The classical NNH presentation develops in Navajo children and presents with dominant, progressive neurologic disease accompanied by liver dysfunction. The neurological symptoms most associated with NNH are weakness, hypotonia, areflexia, and loss of extremity sensations. The infantile presentation is demonstrated in children who develop failure to thrive, jaundice, and progressive liver failure over the first 2 years of life. These children may or may not have associated neurological findings. A childhood presentation clinically appears between 1 and 5 years of age with hepatic dysfunction and rapid progression to liver failure [62].

Treatment, Management, and Outcomes

The liver disease in children with NNH is progressive, often advancing to liver failure and death within several months to years after diagnosis.

Alpers-Huttenlocher Syndrome

Introduction

Alpers-Huttenlocher syndrome is disorder of oxidative metabolism related to mitochondrial dysfunction.

Pathogenesis

Alpers-Huttenlocher syndrome is caused by a mutation in the *POLG* gene whose product, DNA polymerase-γ, is essential for mtDNA replication and repair

Clinical Manifestations and Diagnosis

Children with Alpers-Huttenlocher syndrome typically present with jaundice, hepatomegaly, hypoglycemia, and coagulopathy between 2 and 8 months of life [65]. Liver disease is often found in association with failure to thrive, emesis, ataxia, hypotonia, and seizure activity. [62] A diagnosis of Alpers-Huttenlocher syndrome can be made clinically with (1) the presence of refractory seizures that have a focal component, (2) psychomotor regression often triggered by infection, and (3) hepatopathy with or without

acute liver failure. Molecular testing for *POLG* mutations is also available.

Treatment, Management, and Outcomes

No specific treatments have shown to be effective in the management of Alpers-Huttenlocher syndrome. However, it should be emphasized that while multiple antiepileptic medications may be indicated in order to control the refractory seizures, valproic acid should be strictly avoided in children with Alpers-Huttenlocher syndrome secondary to exacerbations of the hepatic dysfunction and the risk of precipitating fulminant liver failure.

Pearson Syndrome

Introduction

Pearson syndrome is a generalized mitochondriopathy that presents in infancy and is attributed to defects in mtDNA.

Pathogenesis

Pearson syndrome is now known to result from mtDNA rearrangements that result in dysfunctional protein products. The most common deletion results in dysfunction of the respiratory chain complexes I, IV, and V.

Clinical Manifestations and Diagnosis

The clinical presentation of children with Pearson syndrome involves multiple organs including the hematopoietic system, exocrine pancreas, liver, and kidneys. Hepatic disease is manifested by liver enlargement, steatosis, hemosiderosis, and cirrhosis [62].

Treatment, Management, and Outcomes

No effective therapy exists for the treatment of Pearson syndrome, and death generally occurs in infancy secondary to metabolic disorders or infection. Symptomatic management with RBC transfusions and carbohydrate restriction has been utilized in so*me* patients.

References

1. Suchy FJ, Sokol RJ, Balistreri WF. Liver disease in children. 3rd ed. Cambridge/New York: Cambridge University Press; 2007.
2. Beutler E, Baluda MC, Sturgeon P, Day R. A new genetic abnormality resulting in galactose-1-phosphate uridyltransferase deficiency. Lancet. 1965; 1:353–4.
3. Kawade N, Onishi S. The prenatal and postnatal development of UDP-glucuronyltransferase activity towards bilirubin and the effect of premature birth on this activity in the human liver. Biochem J. 1981;196: 257–60.
4. Ridel KR, Leslie ND, Gilbert DL. An updated review of the long-term neurological effects of galactosemia. Pediatr Neurol. 2005;33:153–61.
5. Arias IM, Gartner LM, Seifter S, Furman M. Prolonged neonatal unconjugated hyperbilirubinemia associated with breast feeding and a steroid, pregnane-3(alpha), 20(beta)-diol, in maternal milk that inhibits glucuronide formation in vitro. J Clin Invest. 1964; 43:2037–47.
6. Poland RL, Schultz GE, Garg G. High milk lipase activity associated with breast milk jaundice. Pediatr Res. 1980;14:1328–31.
7. Schneider 2nd AP. Breast milk jaundice in the newborn. A real entity. JAMA. 1986;255:3270–4.
8. Maisels MJ, Newman TB. Kernicterus in otherwise healthy, breast-fed term newborns. Pediatrics. 1995; 96:730–3.
9. Bhutani VK, Johnson L, Sivieri EM. Predictive ability of a predischarge hour-specific serum bilirubin for subsequent significant hyperbilirubinemia in healthy term and near-term newborns. Pediatrics. 1999;103:6–14.
10. Waggoner DD, Buist NR, Donnell GN. Long-term prognosis in galactosaemia: results of a survey of 350 cases. J Inherit Metab Dis. 1990;13:802–18.
11. Bosma PJ, Chowdhury JR, Bakker C, Gantla S, De Boer A, Oostra BA, Lindhout D, Tytgat GN, Jansen PL, Oude Elferink RP, et al. The genetic basis of the reduced expression of bilirubin UDP-glucuronosyltransferase 1 in Gilbert's syndrome. N Engl J Med. 1995;333:1171–5.
12. Crigler Jr JF, Najjar VA. Congenital familial nonhemolytic jaundice with kernicterus. Pediatrics. 1952;10:169–80.
13. Van Der Veere CN, Sinaasappel M, Mcdonagh AF, Rosenthal P, Labrune P, Odievre M, Fevery J, Otte JB, Mcclean P, Burk G, Masakowski V, Sperl W, Mowat AP, Vergani GM, Heller K, Wilson JP, Shepherd R, Jansen PL. Current therapy for Crigler-Najjar syndrome type 1: report of a world registry. Hepatology. 1996;24:311–5.
14. Wolkoff AW, Wolpert E, Pascasio FN, Arias IM. Rotor's syndrome. A distinct inheritable pathophysiologic entity. Am J Med. 1976;60:173–9.

15. Cross NC, De Franchis R, Sebastio G, Dazzo C, Tolan DR, Gregori C, Odievre M, Vidailhet M, Romano V, Mascali G, et al. Molecular analysis of aldolase B genes in hereditary fructose intolerance. Lancet. 1990;335:306–9.
16. Stanbury JB. The metabolic basis of inherited disease. 5th ed. New York: McGraw-Hill; 1983.
17. Mayatepek E, Hoffmann B, Meissner T. Inborn errors of carbohydrate metabolism. Best Pract Res Clin Gastroenterol. 2010;24:607–18.
18. Saudubray JM, Sedel F, Walter JH. Clinical approach to treatable inborn metabolic diseases: an introduction. J Inherit Metab Dis. 2006;29:261–74.
19. Hiraiwa H, Pan CJ, Lin B, Moses SW, Chou JY. Inactivation of the glucose 6-phosphate transporter causes glycogen storage disease type 1b. J Biol Chem. 1999;274:5532–6.
20. Rake JP, Visser G, Labrune P, Leonard JV, Ullrich K, Smit GP. Glycogen storage disease type I: diagnosis, management, clinical course and outcome. Results of the European Study on Glycogen Storage Disease Type I (ESGSD I). Eur J Pediatr. 2002;161 Suppl 1: S20–34.
21. Rake JP, Visser G, Labrune P, Leonard JV, Ullrich K, Smit GP. Guidelines for management of glycogen storage disease type I – European Study on Glycogen Storage Disease Type I (ESGSD I). Eur J Pediatr. 2002;161 Suppl 1:S112–9.
22. Geberhiwot T, Alger S, Mckiernan P, Packard C, Caslake M, Elias E, Cramb R. Serum lipid and lipoprotein profile of patients with glycogen storage disease types I, III and IX. J Inherit Metab Dis. 2007;30:406.
23. Goldberg T, Slonim AE. Nutrition therapy for hepatic glycogen storage diseases. J Am Diet Assoc. 1993; 93:1423–30.
24. Bruno C, Van Diggelen OP, Cassandrini D, Gimpelev M, Giuffre B, Donati MA, Introvini P, Alegria A, Assereto S, Morandi L, Mora M, Tonoli E, Mascelli S, Traverso M, Pasquini E, Bado M, Vilarinho L, Van Noort G, Mosca F, Dimauro S, Zara F, Minetti C. Clinical and genetic heterogeneity of branching enzyme deficiency (glycogenosis type IV). Neurology. 2004;63:1053–8.
25. Mckiernan PJ. Nitisinone in the treatment of hereditary tyrosinaemia type 1. Drugs. 2006;66:743–50.
26. Holme E, Lindstedt S. Tyrosinaemia type I and NTBC (2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione). J Inherit Metab Dis. 1998;21: |507–17.
27. Masurel-Paulet A, Poggi-Bach J, Rolland MO, Bernard O, Guffon N, Dobbelaere D, Sarles J, De Baulny HO, Touati G. NTBC treatment in tyrosinaemia type I: long-term outcome in French patients. J Inherit Metab Dis. 2008;31:81–7.
28. Mohan N, Mckiernan P, Preece MA, Green A, Buckels J, Mayer AD, Kelly DA. Indications and outcome of liver transplantation in tyrosinaemia type 1. Eur J Pediatr. 1999;158 Suppl 2:S49–54.
29. Matern D, Rinaldo P. Medium-chain acyl-coenzyme A dehydrogenase deficiency. In: Pagon RA, editor. Gene reviews. Seattle: University of Washington; 1993.
30. Iafolla AK, Thompson Jr RJ, Roe CR. Medium-chain acyl-coenzyme A dehydrogenase deficiency: clinical course in 120 affected children. J Pediatr. 1994;124: 409–15.
31. Hayes B, Lynch B, O'keefe M, Monavari AA, Treacy EP. Long chain fatty acid oxidation defects in children: importance of detection and treatment options. Ir J Med Sci. 2007;176:189–92.
32. Olpin SE, Clark S, Andresen BS, Bischoff C, Olsen RK, Gregersen N, Chakrapani A, Downing M, Manning NJ, Sharrard M, Bonham JR, Muntoni F, Turnbull DN, Pourfarzam M. Biochemical, clinical and molecular findings in LCHAD and general mitochondrial trifunctional protein deficiency. J Inherit Metab Dis. 2005;28:533–44.
33. Wanders RJ, IJlst L, Van Gennip AH, Jakobs C, De Jager JP, Dorland L, Van Sprang FJ, Duran M. Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency: identification of a new inborn error of mitochondrial fatty acid beta-oxidation. J Inherit Metab Dis. 1990;13:311–4.
34. Zschocke J, Penzien JM, Bielen R, Casals N, Aledo R, Pie J, Hoffmann GF, Hegardt FG, Mayatepek E. The diagnosis of mitochondrial HMG-CoA synthase deficiency. J Pediatr. 2002;140:778–80.
35. Morris AA, Olpin SE, Brivet M, Turnbull DM, Jones RA, Leonard JV. A patient with carnitine-acylcarnitine translocase deficiency with a mild phenotype. J Pediatr. 1998;132:514–6.
36. Summar ML, Dobbelaere D, Brusilow S, Lee B. Diagnosis, symptoms, frequency and mortality of 260 patients with urea cycle disorders from a 21-year, multicentre study of acute hyperammonaemic episodes. Acta Paediatr. 2008;97:1420–5.
37. Tuchman M, Lee B, Lichter-Konecki U, Summar ML, Yudkoff M, Cederbaum SD, Kerr DS, Diaz GA, Seashore MR, Lee HS, Mccarter RJ, Krischer JP, Batshaw ML. Cross-sectional multicenter study of patients with urea cycle disorders in the United States. Mol Genet Metab. 2008;94:397–402.
38. Enns GM, Berry SA, Berry GT, Rhead WJ, Brusilow SW, Hamosh A. Survival after treatment with phenylacetate and benzoate for urea-cycle disorders. N Engl J Med. 2007;356:2282–92.
39. Krivitzky L, Babikian T, Lee HS, Thomas NH, Burk-Paull KL, Batshaw ML. Intellectual, adaptive, and behavioral functioning in children with urea cycle disorders. Pediatr Res. 2009;66:96–101.
40. Davit-Spraul A, Fabre M, Branchereau S, Baussan C, Gonzales E, Stieger B, Bernard O, Jacquemin E. ATP8B1 and ABCB11 analysis in 62 children with normal gamma-glutamyl transferase progressive familial intrahepatic cholestasis (PFIC): phenotypic differences between PFIC1 and PFIC2 and natural history. Hepatology. 2010;51:1645–55.

41. Folmer DE, Van Der Mark VA, Ho-Mok KS, Oude Elferink RP, Paulusma CC. Differential effects of progressive familial intrahepatic cholestasis type 1 and benign recurrent intrahepatic cholestasis type 1 mutations on canalicular localization of ATP8B1. Hepatology. 2009;50:1597–605.
42. Strautnieks SS, Byrne JA, Pawlikowska L, Cebecauerova D, Rayner A, Dutton L, Meier Y, Antoniou A, Stieger B, Arnell H, Ozcay F, Al-Hussaini HF, Bassas AF, Verkade HJ, Fischler B, Nemeth A, Kotalova R, Shneider BL, Cielecka-Kuszyk J, Mcclean P, Whitington PF, Sokal E, Jirsa M, Wali SH, Jankowska I, Pawlowska J, Mieli-Vergani G, Knisely AS, Bull LN, Thompson RJ. Severe bile salt export pump deficiency: 82 different ABCB11 mutations in 109 families. Gastroenterology. 2008;134:1203–14.
43. Davit-Spraul A, Gonzales E, Baussan C, Jacquemin E. The spectrum of liver diseases related to ABCB4 gene mutations: pathophysiology and clinical aspects. Semin Liver Dis. 2010;30:134–46.
44. Englert C, Grabhorn E, Richter A, Rogiers X, Burdelski M, Ganschow R. Liver transplantation in children with progressive familial intrahepatic cholestasis. Transplantation. 2007;84:1361–3.
45. Setchell KD, Heubi JE. Defects in bile acid biosynthesis – diagnosis and treatment. J Pediatr Gastroenterol Nutr. 2006;43 Suppl 1:S17–22.
46. Heubi JE, Setchell KD, Bove KE. Inborn errors of bile acid metabolism. Semin Liver Dis. 2007;27:282–94.
47. Sundaram SS, Bove KE, Lovell MA, Sokol RJ. Mechanisms of disease: inborn errors of bile acid synthesis. Nat Clin Pract Gastroenterol Hepatol. 2008; 5:456–68.
48. Gonzales E, Gerhardt MF, Fabre M, Setchell KD, Davit-Spraul A, Vincent I, Heubi JE, Bernard O, Jacquemin E. Oral cholic acid for hereditary defects of primary bile acid synthesis: a safe and effective long-term therapy. Gastroenterology. 2009; 137(1310–1320):e1311–3.
49. Clayton PT. Disorders of bile acid synthesis. J Inherit Metab Dis. 2011;34:593–604.
50. Setchell KDR, Heubi JE, Shah S, Levine JE, Suskind D, Al-Edreesi M, Potter C, Russell DW, O'Connell NC, Wolfe B, Jha P, Zhang W, Bove KE, Knisely A, Hofmann AF, Rosenthal P, Bull LN. Genetic defects in bile acid conjugation cause fat-soluble vitamin deficiency. Gastroenterology. 2013;144(5):945–55. e6; quiz e14–5.
51. Grabowski GA. Recent clinical progress in Gaucher disease. Curr Opin Pediatr. 2005;17:519–24.
52. Grabowski GA, Andria G, Baldellou A, Campbell PE, Charrow J, Cohen IJ, Harris CM, Kaplan P, Mengel E, Pocovi M, Vellodi A. Pediatric non-neuronopathic Gaucher disease: presentation, diagnosis and assessment. Consensus statements. Eur J Pediatr. 2004;163: 58–66.
53. Vanier MT, Wenger DA, Comly ME, Rousson R, Brady RO, Pentchev PG. Niemann-Pick disease group C: clinical variability and diagnosis based on defective cholesterol esterification. A collaborative study on 70 patients. Clin Genet. 1988;33:331–48.
54. Fink JK, Filling-Katz MR, Sokol J, Cogan DG, Pikus A, Sonies B, Soong B, Pentchev PG, Comly ME, Brady RO, et al. Clinical spectrum of Niemann-Pick disease type C. Neurology. 1989;39:1040–9.
55. Yerushalmi B, Sokol RJ, Narkewicz MR, Smith D, Ashmead JW, Wenger DA. Niemann-pick disease type C in neonatal cholestasis at a North American Center. J Pediatr Gastroenterol Nutr. 2002;35:44–50.
56. Tolar J, Petryk A, Khan K, Bjoraker KJ, Jessurun J, Dolan M, Kivisto T, Charnas L, Shapiro EG, Orchard PJ. Long-term metabolic, endocrine, and neuropsychological outcome of hematopoietic cell transplantation for Wolman disease. Bone Marrow Transplant. 2009;43:21–7.
57. Du H, Cameron TL, Garger SJ, Pogue GP, Hamm LA, White E, Hanley KM, Grabowski GA. Wolman disease/cholesteryl ester storage disease: efficacy of plant-produced human lysosomal acid lipase in mice. J Lipid Res. 2008;49:1646–57.
58. Leone L, Ippoliti PF, Antonicelli R. Use of simvastatin plus cholestyramine in the treatment of lysosomal acid lipase deficiency. J Pediatr. 1991;119:1008–9.
59. Moser HW, Moser AB. Peroxisomal disorders: overview. Ann N Y Acad Sci. 1996;804:427–41.
60. Paker AM, Sunness JS, Brereton NH, Speedie LJ, Albanna L, Dharmaraj S, Moser AB, Jones RO, Raymond GV. Docosahexaenoic acid therapy in peroxisomal diseases: results of a double-blind, randomized trial. Neurology. 2010;75:826–30.
61. Wei H, Kemp S, Mcguinness MC, Moser AB, Smith KD. Pharmacological induction of peroxisomes in peroxisome biogenesis disorders. Ann Neurol. 2000;47:286–96.
62. Lee WS, Sokol RJ. Mitochondrial hepatopathies: advances in genetics and pathogenesis. Hepatology. 2007;45:1555–65.
63. Cormier-Daire V, Chretien D, Rustin P, Rotig A, Dubuisson C, Jacquemin E, Hadchouel M, Bernard O, Munnich A. Neonatal and delayed-onset liver involvement in disorders of oxidative phosphorylation. J Pediatr. 1997;130:817–22.
64. Holve S, Hu D, Shub M, Tyson RW, Sokol RJ. Liver disease in Navajo neuropathy. J Pediatr. 1999;135: 482–93.
65. Narkewicz MR, Sokol RJ, Beckwith B, Sondheimer J, Silverman A. Liver involvement in Alpers disease. J Pediatr. 1991;119:260–7.

Metabolic Liver Disease: Part 2

9

Michael R. Narkewicz and Christine Waasdorp Hurtado

Alpha-One Antitrypsin Deficiency

Background

Alpha-1 antitrypsin deficiency (A1-ATD) is the most common genetic cause of liver disease in children, with a prevalence of 1:1,600–1:2,000 per live birth [1–3]. One in 5,000 North Americans has severe A1-ATD [4]. It is an autosomal codominant condition resulting in an 85–90 % reduction in serum alpha-1 antitrypsin (A1-AT). A1-AT is a member of the serine protease inhibitor (serpin) family that inhibits destructive neutrophil proteases, elastase, cathepsin G, and proteinases. The protein is produced in the liver and increases in response to injury and inflammation [5–7].

Deficiency of the A1-AT protein results in both liver and lung injury. Adults develop chronic lung disease felt to be due to unregulated elastolytic attack of the connective tissue due to decreased circulating levels of A1-AT [2]. Lung injury is accelerated by smoke and pollution exposure [8–10]. In adults the disease is also associated with a variety of liver disorders, including chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Children also demonstrate a broad spectrum of liver involvement. A1-AT deficiency is the most common metabolic disease causing neonatal hepatitis syndrome. The presentation is easily confused with biliary atresia and cystic fibrosis; however, the pathology can differ. The pathophysiology of liver disease is not clearly understood. The current belief is that liver injury results from retention of misfolded A1-AT molecules in the endoplasmic reticulum (ER) of hepatocytes [11].

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Structure of Alpha-1 Antitrypsin

The normal A1-AT molecule is designated by its electrophoretic migration pattern as M (middle). The various structural variants of A1-AT are identified by agarose electrophoresis or isoelectric focusing of plasma in polyacrylamide gel at acid pH [12]. Letters are assigned to variants based on distance of migration using alphabetical assignments from low to high isoelectric points [12]. The most common severe deficiency, PiZZ, has a A1-AT variant that moves the slowest [2]. PiZZ is responsible for 95 % of cases of severe A1-ATD [13].

Function of Alpha-1 Antitrypsin

The major physiologic function of A1-AT is inhibition of the serine proteases, released by activated

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Table 9.1 Clinical and laboratory findings in alpha-1 antitrypsin deficiency by age

Age	Newborn	Infant and child	Adult
Laboratory findings	Cholestasis	Elevated transaminases	Elevated transaminases
	Elevated transaminases	Liver synthesis dysfunction	Liver synthesis dysfunction
	Liver synthesis dysfunction		
Clinical findings	Jaundice	Jaundice	Hepatomegaly
	Hepatomegaly	Hepatomegaly	Hepatosplenomegaly
	Hepatosplenomegaly	Hepatosplenomegaly	Portal hypertension
	Ascites	Portal hypertension	Cirrhosis
		Cirrhosis	Tumor

neutrophils, to include elastase, cathepsin G, and proteinase 3 [14].

Biosynthesis and Regulation

The primary site of A1-AT production is the liver. This is demonstrated by synthesis of the donor A1-AT phenotype in patients following an orthotopic liver transplant and the basis for correction with liver transplantation [2]. A1-AT is synthesized and secreted to a lesser degree from human blood monocytes and bronchoalveolar and breast milk macrophages [2].

Plasma concentration of A1-AT increases three- to fivefold in response to inflammation and/or tissue injury mediated by IL 6 and lipopolysaccharide (LPS) stimulation [2, 3, 15]. Levels of A1-AT also rise during pregnancy and with oral contraceptive therapy [2].

Variants

There are more than 120 identified allelic variants. The majority of variants are associated with a reduction in serum A1-AT protein levels. There is a null variant, with no measurable A1-AT in the serum, associated with emphysema, but not liver disease [16]. PiMM (protease inhibitor), the normal variant, is the phenotype in 95 % of the population and is associated with normal serum levels of A1-AT. The S and Z variants are the most common. Some of the variants, such as S, are not associated with disease in homozygous state. Other familial variants result in a deficiency of serum A1-AT and demonstrate variable presentations, including emphysema and liver disease.

PiZZ has less elastase inhibition capability and is cleared sooner due to its instability [17]. The mutation is due to a single AA nucleotide change (lysine for glutamic acid) resulting in decreased secretion and accumulation of the mutated form in the endoplasmic reticulum (ER). The protein becomes trapped due to increased folding and decreased stability [18]. PiSS has no decrease in function or duration of function [19, 20].

Liver Disease

Liver involvement and disease, first reported in 1968 by Freir et al. and in 1969 by Sharp, is quite variable in affected individuals [21]. Data and animal models are most consistent with accumulation of abnormal A1-AT in ER resulting in hepatocyte damage. Interestingly not all patients with ER accumulation of the mutant protein develop injury. This suggests a role for inherited traits and environmental factors [2, 3].

Liver disease develops in PiZZ and PiSZ variants and very rarely in PiMZ. Liver disease does not occur with the other variants (e.g., PiSS). The PiMZ state, which has a 50 % reduction in serum A1-AT, may predispose to more severe liver disease in other hepatic disorders such as hepatitis B and C, alcoholic liver disease, cystic fibrosis-associated liver disease, and NAFLD [22, 23]. The clinical spectrum of liver disorders is shown in Table 9.1.

The natural history of liver disease has been described in a longitudinal follow-up study from Sweden [6, 7, 24]. Of the 176 with A1-ATD after

screening of 200,000 infants in 1972–1974, 125 were PiZZ, 48 PiSZ, 2 PiZnull, and 1 PiSnull. Forty-five percent of the PiZZ infants were small for gestational age. Only 11 % of the affected infants had obstructive jaundice in infancy. Of the 14 PiZZ infants with cholestasis, 9 demonstrated severe clinical and laboratory evidences and 5 had only laboratory evidence of liver disease. Four of these infants went on to develop cirrhosis by 2 years of age. Eighty-three percent demonstrated no clinical signs of liver disease, but 50 % still had abnormal LFTs at 6 months of age. Two thirds of those with hepatic anomalies were male. Only 25 % of the PISZ infants had abnormal LFTs. By 18 years of age, 85 % had normal aminotransferases and no signs of liver disease [6]. Three children died before age eight of liver disease. Further follow-up of the same population identified that only 5–10 % of all PiZZ children develop significant liver disease [6, 24].

Other studies have screened all infants with liver disease for A1-ATD [25, 26]. In 43 infants with A1-ATD identified over 10 years, 24 were PiZZ [25]. Liver pathology was available on 14 of the PiZZ infants in whom 57 % had periodic acid-Schiff (PAS) inclusion bodies at less than 3 months of age. Of the PiZZ infants, 10 % were born premature, 37 % were SGA, and 31 % had failure to thrive with 83 % identified and diagnosed by 3 months of age. Laboratory studies showed elevated transaminases in 59 and 91 % were cholestatic. Physical findings demonstrated hepatomegaly in 87 % and splenomegaly in 43 % with only 5 % having a normal physical exam. Heterozygotes were more likely to have normal laboratory values, with only 23 % having elevated transaminases and 39 % with cholestasis. Hepatomegaly was present in only 43 % and splenomegaly in 7 % of heterozygotes. In a large pediatric liver practice, 36 children with A1-ATD were identified from 424 with liver disease [26]. Nineteen were PiZZ, all of whom had prolonged cholestasis. All demonstrated PAS inclusions on liver biopsy. All demonstrated hepatomegaly and elevated LFTs, and all but one had resolution of cholestasis by 7 months of age. Three developed cirrhosis by 3 years of age. Cirrhosis and portal hypertension may have been present in 5 others with hepatosplenomegaly and fibrosis. The 16 affected with heterozygous disease (MS, MZ, SZ) demonstrated less severe liver involvement with cholestasis in 5. Interestingly, many were found to have other liver disease including biliary atresia. One sibling was identified as PiZZ with no evidence of liver disease, again showing the variability in disease course.

Lung Disease

Lung injury in A1-ATD is due to multiple factors. Due to low levels of A1-AT in the lungs, the elastase created by neutrophils is not neutralized, and proteolytic activity goes unchecked. Cigarette smoke and pollution exposure along with pulmonary infections significantly affect the elastase levels and therefore the severity of lung damage [27].

Lung disease is commonly associated with A1-AT deficiency with 60–70 % of PiZZ subjects developing lung injury as adults. The typical presentation is insidious development of dyspnea in the 30–40s. Lung disease peaks in the fourth and fifth decades [22, 28]. One half will have a cough and history of recurrent infections with progression to decreasing FEV (forced expiratory volume) and increasing total lung capacity [8]. Chest X-ray demonstrates hyperinflation with base atelectasis [29]. The correlation between cigarette smoking and decreasing survival is clear [13]. The National Heart Lung and Blood Institute reports a mortality of 3 % per year in A1-ATD with 72 % dying from lung disease and only 10 % from liver disease [13].

Diagnosis

A1-ATD is diagnosed by both serology and histology. Screening with serum levels is acceptable, but levels can be elevated in acute infection. PiZZ phenotypes rarely have serum levels over 50–60 mg/dL [30]. Serum A1-AT phenotyping (Pi phenotyping) should be performed in children

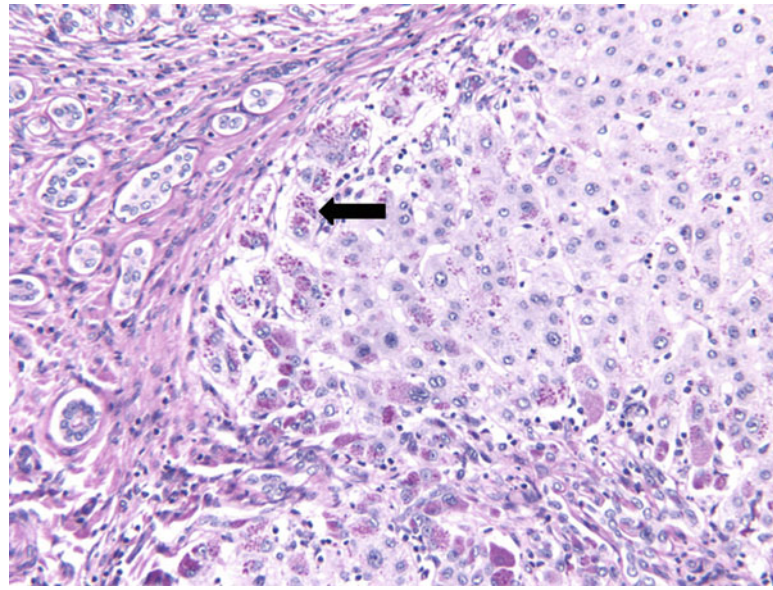

Fig. 9.1 PAS-positive diastase-resistant eosinophilic globules (*black arrow*) in a liver biopsy from a child with A1-ATD (40×)

Table 9.2 Diagnosis of alpha-1 antitrypsin

Who to screen	Liver disease of unknown etiology
	Family member with A1-ATD (PiZZ)
	Early onset emphysema
	Irreversible airflow obstruction
	Necrotizing panniculitis of unknown etiology
How to screen	Serum A1-AT level (serum level <1.1 g/L). Consider simultaneous CRP to rule out inflammation
	A1-AT phenotype
	A1-AT genotype – rarely needed

with low levels (<1.1 g/L) or those with a high suspicion despite low normal levels [30]. Genotyping is available, but rarely needed. Histology can be useful for diagnosis, but is not necessary. PAS-positive diastase-resistant eosinophilic globules can be visualized in the endoplasmic reticulum in the periportal hepatocytes (Fig. 9.1) [31, 32].

Similar findings are seen in other liver diseases and therefore are not diagnostic [32]. In addition, the histology may demonstrate necrosis, inflammation, fibrosis, cirrhosis, bile duct proliferation, bile duct destruction, and possible bile duct paucity [32]. Individuals where screening for A1-ATD is appropriate are shown in Table 9.2.

Treatment

Treatment of patients with A1-ATD is primarily supportive care and aggressive prevention and treatment of lung disease. At this time there are no specific therapies for A1-ATD liver disease. Infants with cholestasis may benefit from fat-soluble vitamin supplementation. Ursodeoxycholic acid is often used to improve bile flow, but no study has shown benefit. There has been a suggestion that breastfeeding provides some protection. It has been postulated that breast milk contains active A1-AT [33]. However, no difference was found between breast- and formula-fed babies in another report [34]. Avoidance of cigarette smoke (primary and secondary) and environmental pollution is vital as this accelerates lung disease and has a clear association with shortened life.

Liver transplantation is appropriate for individuals with end-stage liver disease. The recipient assumes the donor's Pi phenotype. A1-ATD accounts for 3.5 % of all pediatric and 1.1 % adult liver transplants [35]. More males were transplanted than females. One-year survival in children and adults was 89 and 92 % and 5-year survival 83 and 90 %. In addition to improvement in liver disease, there is a decrease in the risk of lung disease.

Gene therapy has also been evaluated and may hold hope for lung disease [36]. Pluripotent stem cell therapy is also currently being evaluated with promising early results [37].

Recombinant A1-AT administered by inhalation or infusion is used in A1-ATD-associated lung disease but not the liver disease. Many other drugs and therapies are currently being evaluated. Of these, carbamazepine is in clinical safety and efficacy trials [38].

A1-AT and Other Organs

There have been some reports and small studies that have suggested a role of A1-AT deficiency in the development of other disease to include inflammatory bowel disease, glomerulonephritis, and pancreatitis, but no definitive study has been completed. Further study is needed to confirm associations and understand mechanisms.

A1-AT Summary

A1-ATD should be considered in all adults and children with liver disease of unclear etiology. Most affected individuals develop liver disease. A1-AT deficiency is associated with both liver and lung injury and possible other organ involvement. Liver damage is due to retained A1-AT in the endoplasmic reticulum. There is currently no accepted medical therapy for liver disease, but gene and stem cell therapy continue to be investigated. Liver transplant does resolve the liver disease and improve lung function.

Wilson Disease

Background

Wilson disease (WD) is a rare autosomal recessive disorder of copper metabolism. Mutations of the copper-transporting P-type ATPase, ATP7B, impair the biliary excretion of copper and cellular utilization resulting in copper overload and copper accumulation in tissue. First described in 1912 as a central nervous system disease (CNS) with asymptomatic liver involvement [39], it is now understood that WD affects many organ systems with CNS and liver being the most significant. The prevalence is estimated at 1 in 30,000 persons worldwide with a carrier frequency of 1 in 90 [40, 41]. Symptoms often begin in childhood and adolescence. WD is universally fatal if not treated.

Genetics

The gene for WD, located on chromosome 13, encodes a transmembrane copper-transporting P-type ATPase, ATP7B [42, 43]. There are more than 500 identified polymorphisms in the ATP7B gene, with more than 250 confirmed as disease-causing mutations [44]. Newer studies show a correlation between genotype and phenotype. Patients with nonsense or frameshift mutations have earlier and more severe disease [45]. A database of the polymorphisms can be found at www.wilsondisease.med.ualberta.ca/database.asp [46]. The variability in timing of onset and severity of symptoms suggests involvement of modifier genes and environmental factors. One example is the MURR1 gene, which has been associated with earlier onset of disease [47].

Pathophysiology

Copper is an essential trace element functioning as a coenzyme for many key enzymes [47]. Following ingestion, copper diffuses across the intestinal mucosa and is carried across the enterocyte by the copper-transporting enzyme, ATP7A, into the portal circulation where it binds to albumin allowing transport into the hepatocytes by transporter HCTR1. Inside the hepatocyte it is chaperoned to the Golgi network where ATP7B, an ATPase transporter, transports copper for incorporation into ceruloplasmin. It is then exported in the bile [47]. In WD, ceruloplasmin secretion is decreased due to decreased copper delivery, but apoceruloplasmin, which has a shorter half-life, is normal.

The resultant inadequate excretion in WD results in excessive copper accumulation in the brain, liver, kidney, and other organs [48]. The exact mechanisms of copper toxicity are not fully understood. It has been suggested that copper accumulation induces cellular damage via oxidative stress, increased apoptosis, or mitochondrial damage [44, 47].

Clinical Manifestations

The initial presentation can occur between 5 and 35 years of age (See Table 9.3). The youngest reported new diagnosis was at 3 years and the oldest in their 80s [47]. Typically, manifestations are related to liver and CNS copper deposition. The frequency of the primary presentations were hepatic in 42 %, CNS in 44 % (neurologic 34 % and psychiatric 10 %), and hematologic and endocrine in 12 % [48–50]. One fourth of patients have multiorgan involvement [48]. In children it is common for hepatic involvement to precede neurologic manifestations. After the age of 20 years, the neurologic (75 %) presentation is more common than hepatic (25 %) [51]. In children evaluation is often triggered by elevated transaminases, hepatomegaly, or family history of WD [51]. Syriopoulou's study of 57 pediatric patients showed 1/3 to have hepatic symptoms triggering evaluation [51].

Table 9.3 Manifestations of Wilson disease

Organ system	Presentation
Hepatic	Mild hepatitis, chronic hepatitis, acute liver failure, chronic liver disease with cirrhosis and portal hypertension, cholelithiasis
Neurologic/ psychiatric	Tremor, dystonia, gait disturbances – ataxia
	Parkinsonian-like syndrome – rigidity, hypokinesia, tremor
	Open jaw with drooling, dysarthria
	Emotional lability
	Cognitive decline (school performance), writing difficulties, dysarthria, clumsiness, migraines, insomnia, choreiform movements, dystonia, depression, psychoses
Renal	Nephrolithiasis, aminoaciduria
Cardiac	Left ventricle hypertrophy, ST depression, arrhythmia sudden cardiac death
Ophthalmologic	KF rings, sunflower cataracts
Skeletal	Osteopenia, arthritis
Endocrine	Hypoparathyroid
Reproductive	Infertility, increased frequency of miscarriage
Others	Hemolysis, pancreatitis

Hepatic Manifestations

Liver involvement can vary from a self-limited acute hepatitis through a chronic hepatitis to acute liver failure. Younger children may present with acute liver failure with jaundice, coagulopathy, ascites, and hepatic encephalopathy commonly with massive hemolysis. Acute liver failure due to WD accounts for approximately 4 % of all acute liver failure in children over 3 years of age [52] and occurs in up to 12 % of all cases [47]. Children with liver failure demonstrate a female predominance, likely related to hormonal influences [41]. Children and adolescents may also present with evidence of chronic liver disease with cirrhosis, portal hypertension, and ascites. Hepatomegaly is found in many children with WD with one study identifying hepatomegaly in 77 % of WD children [51]. Cholelithiasis is another complication of WD and warrants a WD evaluation in the undiagnosed. The stones are mixed pigment and cholesterol [48].

Central Nervous System Manifestations

Central nervous system (CNS) involvement results in a spectrum of neurologic sequelae. Typically neurologic issues present in the second and third decade but have presented as early as 6 years of age [40]. Kaiser Fleischer rings are typically found in patients with CNS symptoms, but not always. Onset of symptoms is gradual and progressive. Typical presentation includes tremors and difficulty with fine motor tasks. As the disease progresses, patients may develop masklike facies, rigidity, and gait disturbances. Cognitive decline is also documented. Psychiatric

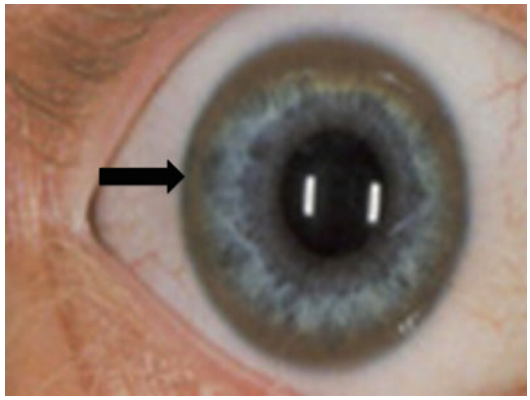

Fig. 9.2 Kayser-Fleischer ring indicated by the *solid black arrow*

disturbances occur in up to 25 % of patients. Many patients are given a psychiatric diagnosis prior to identification of WD. Manifestations can include anxiety, affective disorders, depression, compulsive behaviors, phobias, aggression, poor school performance, and even psychosis [47, 48].

Ophthalmologic Manifestations

Kayser-Fleischer (KF) rings are associated with WD, but are not limited to WD. KF rings are described as golden brown, brownish green, bronze, tannish green, and greenish yellow in the zone of Descemet's membrane in the limbic region of the cornea (Fig. 9.2). KF rings can be appreciated on gross exam, but slit-lamp examination is required for confirmation. The rings represent deposited copper granules throughout the layers of the cornea. They may be found in asymptomatic patients [48] and fade with treatment. Sunflower cataracts can be seen in WD [53]. These are a greenish gray or golden disk in the anterior capsule of the lens with spokes radiating to the lens periphery and resolve with therapy [48].

Renal Manifestations

Renal involvement is characterized by proximal tubular dysfunction and is common in WD. This results in aminoaciduria, glycosuria, and increased excretion of uric acid (with resultant low serum uric acid levels) and calcium, as well as a decrease in filtration rate. Many WD patients are unable to acidify their urine resulting in potassium loses and hypokalemia [54]. The combination of inadequate acidification and hypercalciuria results in the common occurrence of renal stones [48]. Renal function improves with therapy [48].

Cardiac Manifestations

In a study of 53 WD patients [55], 34 % percent demonstrated electrocardiographic (ECG) abnormalities to include left ventricle hypertrophy, ST depression, premature ventricular contractions, sink atrial block, T-wave inversion, and atrial fibrillation. Compared to a control population, there was an increased rate of arrhythmia, which may be associated with sudden death [50, 55].

Other Manifestations

Bone, hemolytic, endocrine, reproductive, and biliary abnormalities are also found. The skeletal changes include osteoporosis, rickets, osteomalacia, spontaneous fractures, and osteoarthritis [48]. Demineralization is the most common abnormality resulting from hypocalcemia and hyperphosphatemia [48]. Hemolysis is another common issue which can be transient or progress to chronic anemia [48]. Endocrine dysfunction presents as hypothyroidism and hypoparathyroidism [47]. Reproductive issues include infertility and increased frequency of miscarriage [47].

Diagnosis

The diagnosis can be made in the face of the classic triad of hepatic disease, neurologic symptoms, and KF rings (see Table 9.4). If this triad does not exist, there is no single test that is 100 % sensitive and specific, especially in children. A high index of suspicion, combined with a complete history and physical exam, as well as laboratory and genetics studies is used in diagnosis. Physical exam may demonstrate findings

Table 9.4 Scoring system to establish diagnosis (8th International Meeting on Wilson Disease, Leipzig, 2001) [56]

Typical clinical symptoms		Other tests	
KF rings		*Liver copper content*	
Present	2	Normal (<0.8 μmol/g)	−1
Absent	0	0.8–4 μmol/g	1
Neurologic symptoms		>5× ULN (>4 μmol/g)	2
Severe	2	Rhodanine-positive granules	1
Mild	1	*Urinary copper*	
Absent	0	Normal	0
Serum ceruloplasmin		1–2× ULN	1
Normal (>0.2 g/L)	0	>2× ULN	2
0.1–0.2 g/L	1	Normal, but >5× ULN s/p D-penicillamine	2
<0.1 g/L	2	*Mutation analysis*	
Coombs-negative hemolytic anemia		On both chromosomes	4
Present	1	One (1) chromosome	1
Absent	0	None	0
Score			
4 or more = diagnosis established			
3 = diagnosis possible. More tests			
2 or less = diagnosis very unlikely			

of chronic liver disease including fluid retention, ascites, jaundice, hepatic encephalopathy, and hepatosplenomegaly.

Ferenci incorporated comments on the Leipzig score to create a scoring system for diagnosis that includes clinical symptoms, laboratory findings, and mutational analysis [46, 57]. Points are assigned for presence of KF rings, neurologic symptoms, low serum ceruloplasmin levels, anemia, liver copper, urine copper, and presence of mutations. A score of greater than 4 establishes diagnosis and greater than 3 requires mutation analysis for confirmation (Table 9.4) [46]. Two recent studies in pediatric patients retrospectively identified 53/54 and 55/57 WD patients with this tool [51, 58]. Another retrospective study identified 28/30 asymptomatic WD patients [59].

The score is not needed in straightforward cases. This would include a patient with extrapyramidal symptoms, KF rings, and typical labs (low ceruloplasmin, high urine copper excretion) and, similarly, a patient with evidence of hepatic disease with KF rings and typical labs (low ceruloplasmin, high urine copper excretion). However, clinical symptoms and KF rings are not always present. It is in these cases the scoring system assists with diagnosis.

Laboratory Testing

Serum ceruloplasmin is decreased (<20 mg/dL) in 95 % of patients presenting with WD [47]. Decreased levels are not specific for WD and may represent other conditions associated with decreased hepatic synthetic function, protein loss, and hereditary hypoceruloplasmin [48]. Ceruloplasmin is an acute phase reactant and may be normal in patients with WD at times of hepatic inflammation [47].

Evaluation of urinary copper excretion, which is normally less than 100 μg/24 h, is also used in diagnosis. In WD urine copper excretion is typically greater than 100 μg/24 h and can increase into the thousands but may be normal in asymptomatic patients. Using a level of 40 μg/24 h increases the sensitivity in children [47]. Elevation of urinary copper excretion may also be found in other causes of cholestasis [49]. Urinary copper excretion is also used to document effectiveness of chelation treatment, with increasing levels identifying successful therapy. One retrospective study found that a ceruloplasmin less than 20 mg/dL and urinary copper greater than 40 μg/24 h in an asymptomatic child diagnosed WD with a sensitivity of 95 % and a

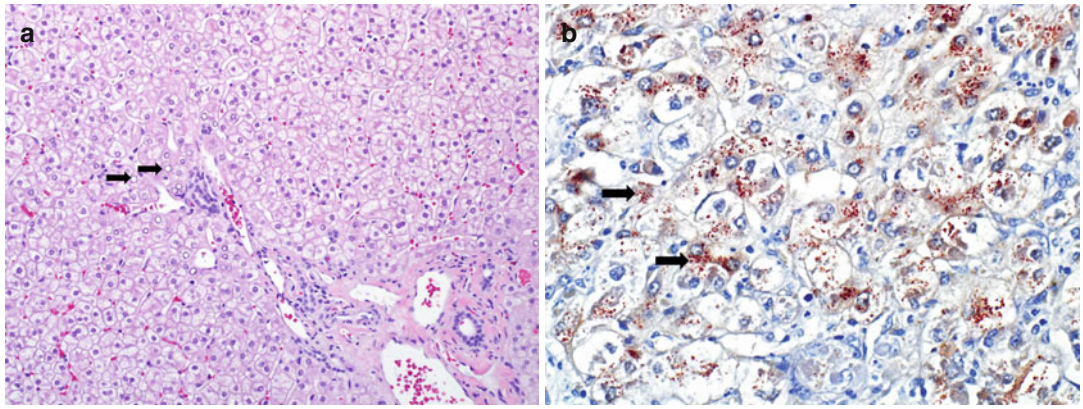

Fig. 9.3 (**a**) Periportal glycogenated nuclei (*solid black arrows*) in a liver biopsy from a patient with Wilson disease. (**b**) Rhodanine stain of a liver biopsy from a patient with Wilson disease demonstrating positive staining (*black arrows*)

specificity of 84.5 %. The positive predictive value was 93 % and the negative predictive value was 91.6 % [59]. Children with normal urinary copper excretion in whom there is still suspicion of WD may require a D-penicillamine challenge.

Serum copper concentrations are useful in the diagnosis. Serum copper levels can be used to follow therapy with a goal level of 5–15 μg/dL during chelation.

Liver functions are also helpful in identifying patients with possible hepatic WD, but are generally not specific enough for diagnosis. Typically serum transaminases remain elevated and the alkaline phosphatase is low, and some authors have suggested a high specificity for WD with an elevated AST:ALT ratio and alkaline phosphatase elevation to total bilirubin ratio in acute liver failure [47, 60].

Genetic testing can be very useful in the confirmation of diagnosis. Molecular genetic testing is utilized in asymptomatic siblings where the mutation(s) in the affected sibling is known. Testing for common mutations is recommended in cases of nonfamilial WD, as direct sequencing is costly and time consuming.

Liver Pathology

Liver biopsy for quantification of hepatic copper is included in the scoring system. Hepatic copper measurement of >250 μg/g dry weight is consistent with WD, while <50 μg/g dry weight excludes WD [48]. Some experts recommend using levels of greater than 75 μg/g of dry liver tissue for diagnosis to increase sensitivity. Elevated hepatic copper may be seen in normal healthy infants less than 6 months of age, cholestasis, cirrhosis, and cholangitis [48].

Liver histology is also helpful in diagnosis with changes seen even in the very young. Cirrhosis has been identified as early as 5 years of age [61]. Fat deposition is seen early in the disease with progression to steatosis. Evidence of chronic active hepatitis is seen with fibrosis leading to cirrhosis. Periportal glycogenated nuclei are found in liver biopsies in WD (Fig. 9.3a). Copper stores are seen in less than 10 % of patients, despite special stains (rhodanine, Fig. 9.3b) [44]. Positive staining is a factor in the scoring system.

Ophthalmologic Evaluation

Slit-lamp examination for KF rings is supportive of a diagnosis, but not pathognomonic. KF rings are seen in approximately 50 % of patients with hepatic involvement and 95 % of those with neurologic and psychiatric presentations. KF may also be seen in chronic hepatitis, primary biliary cirrhosis, and intrahepatic cholestasis [53, 56]. KF rings are rarely seen in children younger than 7 years [51].

Table 9.5 Medical treatment of Wilson disease

Medication	Treatment dose for symptomatic patients	Maintenance dose	Monitoring	Side effects
Penicillamine	*Adults*: 750–1,500 mg divided BID–QID 20 mg/kg/day to max of 2 g/day *Children*: 20 mg/kg/day divided BID–QID	Adults and children: 15 mg/kg/day	Free Cu 5–15 μg/dL Urine Cu 250–500 μg/24 h	Fever, rash Pancytopenia Bone marrow toxicity Elastosis cutis Nephrotoxicity, Goodpasture syndrome Lupus-like syndrome Optic neuritis Colitis (rare)
Trientine	*Adults*: 750–1,500 mg divided BID–QID *Children*: 20 mg/kg/day divided BID–QID	Adults and children: 15 mg/kg/day	Free Cu 5–15 μg/dL Urine Cu 100–500 μg/24 h	Sideroblastic anemia Colitis (rare)
Zinc Salts	*Adults*: 150 mg divided TID *Children* (<50 kg): 75 mg/day divided TID Dose in mg of elemental zinc	Adults: 75–150 mg divided TID Children: 50–75 mg divided TID	Free Cu 5–15 μg/dL Urine Cu 100–500 μg/24 h Urine zinc >1,000 μg/24 h	GI intolerance Elevation of amylase and lipase (non-pancreatic)

Modified from Rosencrantz and Schilsky [47]

Neurologic Examination

A thorough neurologic exam and imaging of the brain (CT or MRI) should be considered in all patients with suspected or confirmed WD. A baseline neurologic assessment is helpful while following response to therapy. MRI and CT may identify changes in the basal ganglia, pons, or thalamus suggesting the diagnosis [47].

Screening of Asymptomatic Relatives

Siblings have a 1 in 4 chance and children of a WD parent have a 1 in 180 chance of inheriting the disease [47]. It is recommended that asymptomatic relatives have a thorough screening at 3 years of age [52]. This includes a complete history and physical exam and ophthalmologic slit-lamp exam along with measurement of serum ceruloplasmin, serum copper, and 24-h urinary copper excretion and evaluation of transaminases. The likelihood of diagnosis with all the testing returning normal is exceedingly small. If any of the testing returns abnormal, further testing with a liver biopsy for quantification of copper and histology is prudent. Genetic testing is recommended to confirm diagnosis.

Treatment

Therapy for WD is lifelong (See Table 9.5). Treatment may vary between initial therapy and maintenance therapy following successful de-coppering. Foods high in copper (shellfish, grains, nuts, mushrooms, legumes, chocolate, and organ meats) should be avoided. The three therapies are D-penicillamine, trientine, and zinc salts [47, 48]. D-penicillamine and trientine chelate copper allowing urinary excretion. Dosage is slowly increased to maximize chelation and then reduced to a maintenance therapy. Significant improvement is seen within weeks of starting therapy. Neurologic symptoms may initially worsen with treatment of D-penicillamine, due to

mobilization of large amounts of copper and subsequent deposition in the brain. D-penicillamine has more side effects that lead to therapy changes (Table 9.5). Trientine is typically better tolerated and may have a lower risk of neurologic worsening. Zinc functions as an antagonist to intestinal copper absorption [48]. Zinc increases intestinal metallothionein, leading to copper binding and retention in the intestinal cell which is sloughed with the stool. It takes 2 weeks to increase intestinal metallothionein levels, and thus zinc is not a good option in symptomatic patients. To be most effective, zinc should be taken at least 1 h away from meals at least three times a day. The newest experimental therapy is ammonium tetrathiomolybdate with two anti-copper mechanisms, reduced absorption and decreasing availability due to binding. The complications and side effects are significantly less than D-penicillamine.

Long-term and comparative studies of the various available therapies are lacking, making the selection of therapy challenging despite the safety profiles. Weiss did directly compare zinc to chelators in patients with neurologic disease and found zinc to have a higher rate of worsening liver function tests and decreased urinary copper excretion [44]. Future prospective trials are needed to determine the best therapies for patients with neurologic and hepatic predominant disease.

Urine copper levels can be used to monitor success in all three therapies. With chelation therapy, urinary copper excretion should rise. Tapering the dose of the chelator to maintenance levels or converting to zinc should only be considered when the urinary copper excretion subsequently declines. With zinc therapy, copper removal is slower and will not rise but should eventually decline. Spot urine zinc can be used to assess compliance. A small study of 16 children with WD who received trientine found that 10/16 had improvement in LFTs. Thirteen of 16 continued on trientine with 1 stopping for allergic reaction, 1 with low copper excretion, and 1 going to transplant [62].

Liver transplantation is indicated in WD patients who have (1) acute liver failure and (2) severe hepatic decompensation not responding to therapy and (3) responded to D-penicillamine but have developed severe progressive hepatic insufficiency with therapy discontinuation [35, 58, 63]. Transplantation changes the hepatic genotype and improves copper excretion. The role of transplant in severe neurologic disease is unclear. Survival following transplant is good. Patients will experience normalization in serum copper and ceruloplasmin as well as 24-h urinary copper excretion 1–2 months following transplant [48].

Natural History and Clinical Outcome

The prognosis is excellent for patients with early diagnosis who are compliant with therapy. Similarly, patients with mild symptoms will likely experience improvement with therapy. Patients with neurologic symptoms may have initial worsening in the first 4 weeks of therapy due to initial increases in serum copper levels. In patients with significant neurologic and psychiatric manifestation, studies show a slow progressive benefit over 3 years or longer. Two recent studies with long follow-up found that on therapy, 61–76 % of subjects remained clinically stable and 2–18 % progressed, with few deaths associated with WD [43, 44, 64]. Patients are more likely to have progression associated with neuropsychiatric involvement than liver involvement [43, 44, 64]. King's College studied 74 children with WD including 57 with liver disease and 17 asymptomatic siblings. Of this group 32 remained on long-term chelation, 10 had liver transplants, and 15 died from acute liver failure (10 days or less from symptoms to death) [58]. There is a revised prognostic index score that is used to predict response to treatment. The score is based on serum bilirubin, INR, AST, white blood cell count, and albumin (scores 0–20). A pediatric evaluation predicted favorable response to chelation in children with a score of less than 11. Those with scores of greater than 11 died without transplant [58].

Summary

Wilson disease is an autosomal recessive disease of copper metabolism with dysfunction of the

P-type ATPase, ATP7B, copper transporter. This results in copper overload and injury to multiple organ system but most notable the CNS and liver. Presentation varies significantly in both time course and severity. Diagnosis is complex and based on several laboratory studies. Liver biopsy adds both copper content and histology to assist with the diagnosis. There is a scoring system that is showing increasing promise for the diagnosis of WD. There are currently three main medical therapies with few large studies or comparative studies to direct therapy selection. A revised scoring system can assist with selection of medical therapy versus transplantation and has been shown to be predictive in children. Liver transplant is curative.

Cystic Fibrosis Liver Disease

Cystic fibrosis (CF) is a common lethal genetic disease in the North American population, affecting 1:2,500 births. There are about 30,000 individuals in the USA with CF and approximately 1,000 new cases annually. Although pulmonary-related factors are the most common cause of death in CF [65–67], liver disease is the third leading cause of death, accounting for 2.5 % of overall mortality [68, 69].

The liver is likely involved to some degree in all individuals with CF; however, clinically significant liver disease (multilobular cirrhosis with or without portal hypertension) only occurs in 5–10 % of individuals.

The term cystic fibrosis liver disease has been commonly used. However, there have been variable definitions of this entity, and this has led to confusion about the natural history, clinical impact, and outcome of liver disease in CF. We prefer to use the following classification of liver involvement in CF put forth at a CF Foundation Williamsburg conference. In this scheme, advanced liver disease is reserved for *CF-related liver disease with cirrhosis with or without portal hypertension*, based on clinical examination, imaging, or histology. Other liver disease is classified as *liver involvement without cirrhosis/portal hypertension*. The various indications of this could include one or any combination of persistent or intermittent AST, ALT, or GGT >2 times upper limit of normal; hepatic steatosis as determined by liver biopsy or imaging; hepatic fibrosis as determined by liver biopsy; cholangiopathy demonstrated by MRCP or ERCP; and other ultrasound abnormalities not consistent with cirrhosis.

The definitions, clinical presentations, and prevalence data for each of the categories are described below.

Cystic Fibrosis-Related Cirrhosis with and Without Portal Hypertension

Multilobular Cirrhosis

Multilobular cirrhosis is indicated by the presence of multiple regenerative nodules and diffuse involvement of the liver. On physical exam a hard nodular liver that may or may not be enlarged detects multilobular cirrhosis. On imaging, there is an irregular nodular liver edge and coarse heterogeneous parenchyma. Prior to the development of portal hypertension, there are often no other clinical features. Once portal hypertension is present, splenomegaly, esophageal or gastric varices, or ascites may be the first suggestion of previously unsuspected cirrhosis. Liver biopsy can show features consistent with cirrhosis but may not be sensitive due to the patchy nature of the nodular involvement and the large regenerative nodules that can be mistaken for normal hepatic parenchyma. Multilobular cirrhosis in CF is a pediatric disorder. The median age of discovery is 10 years with very few new cases identified after 20 years of age, and no increased prevalence with increased life span. The prevalence of cirrhosis and its complications have been reported in multiple studies that include 4,446 subjects from Europe, Canada, Australia, and Israel in the last 20 years. In total, there was an average prevalence of multilobular cirrhosis of 5.6 % portal hypertension 4.2 % and varices 2.4 % [68, 70–80].

Portal Hypertension Without Cirrhosis

Non-cirrhotic portal hypertension has been reported in CF [78, 81, 82].

Complications of Cirrhosis with Portal Hypertension

The primary complications of CFLD are restricted to individuals with multilobular cirrhosis with portal hypertension and include the complications expected in portal hypertension with hypersplenism and esophageal or gastric variceal hemorrhage the predominant issues. Variceal hemorrhage occurs in 40–50 % of patients and may occur even more frequently as survival with CF continues to improve. Hepatic decompensation is rare and appears much more slowly than in primarily hepatocellular diseases. Ascites is a late and ominous complication, and synthetic liver failure with coagulopathy is very rare. While liver disease is listed as the third leading cause of death among CF patients, some studies report no increase in mortality among patients with multilobular cirrhosis [70, 76, 77]. Two recent studies report a trend towards younger age of death in those with cirrhosis [80, 83]. In a large 18-year retrospective review of 1,108 patients with CF, 53 developed cirrhosis, 23 with portal hypertension, 14 with varices, 8 with coagulopathy, and 6 with overt liver failure resulting in 3 liver transplants, but only one reported liver-related death [73]. The incidence rate of major complications of cirrhosis (bleeding, ascites, encephalopathy) among a cohort of 177 CF patients (17 who developed cirrhosis) followed longitudinally was 0.4 %, with an all-cause mortality rate of 1.6 % among cirrhotic patients [70]. This is in contrast to older reports that showed 11–19 % mortality from variceal bleeding or liver failure in CF cirrhosis [84]. Once portal hypertension develops in CFLD, some studies report an increased risk of malnutrition, osteoporosis/hepatic osteodystrophy, and decline in lung function. However, a recent retrospective study of 59 CF patients with cirrhosis and portal hypertension found no decline in lung function associated with portal hypertension as compared to age- and gender-matched CF-specific reference values for lung function [85]. Malnutrition associated with portal hypertension is likely multifactorial with decreased nutrient absorption, increased resting energy expenditure, anorexia, and decreased caloric intake. There may be a link between CFLD and increased insulin resistance leading to a higher incidence of CF-related diabetes [86]. A single-center retrospective case-control study showed an odds ratio of 4.8 (95 % CI 2.49, 9.17) for CF-related diabetes in those with cirrhosis and portal hypertension, using a surrogate marker (thrombocytopenia) for cirrhosis with portal hypertension [87]. A case-control study of CF patients with and without CFLD found lower weight, height, and mid-upper arm circumference and lower FEV1 scores in CFLD patients [88].

Liver Involvement Without Cirrhosis or Portal Hypertension

There are a variety of other forms of liver involvement in CF. Some of these may occur together in individual patients.

Focal Biliary Cirrhosis

FBC is primarily a histologic diagnosis. Autopsy data has demonstrated liver involvement in 25–72 % of adults with CF, with the majority showing focal biliary cirrhosis [89, 90]. FBC has been found on postmortem exam in 11 % of infants, 27 % at 1 year, and 25–70 % of adults [89, 90]. It is the pathognomonic histopathologic liver lesion in CF and is often clinically silent without abnormalities in AST, ALT, or GGT. On ultrasound, there are thickened (>2 mm), hyperechoic periportal tissues. On MRI, there is high-intensity signal in the periportal area on T1-weighted imaging [91]. On liver biopsy, FBC is characterized by focal portal fibrosis and inflammation, cholestasis, and bile duct proliferation. It has been suggested that FBC is part of the progression to multilobular cirrhosis, but given the much lower frequency of multilobular cirrhosis compared to FBC, the progression is very rare [68, 70–72, 74–79].

Elevations in AST, ALT, and/or GGT

Forty to 50 % of CF patients have intermittent elevations in AST, ALT, or GGT that are not predictive of the development or presence of significant fibrosis. In a longitudinal study of over 250 children identified by newborn screen in

Colorado, followed for up to 20 years, 90 % had at least one abnormal ALT and 30 % had persistently (>6 months) elevated ALT. Persistent elevations of AST, ALT, or GGT more than 3 times the upper limit of normal were very rare [92]. In a study of 376 children in 3 clinical trials with frequent determination of biochemistries, 25 % had emergence of at least one abnormal AST, ALT, or GGT over an average of 8 months of follow-up [93]. Persistently elevated ALT or GGT (for more than 6 months) are common and warrant further investigation but are not diagnostic of any particular disorder [94].

Hepatic Steatosis

Steatosis is likely the most common hepatic finding in CF with a prevalence of 23–75 % of CF patients in all age categories [95]. Steatosis was present in 70 % of children undergoing liver biopsy for suspected liver disease [76, 81]. Hepatic steatosis has been associated with malnutrition and deficiencies of essential fatty acid, carnitine, and choline. However, steatosis is also found in CF patients with adequate nutritional status [69]. It presents as smooth mild hepatomegaly without signs of portal hypertension. The appearance on ultrasound is typically uniform hyperechogenicity, but it may also have a heterogeneous appearance on ultrasound or as one or several "pseudomasses," which are lobulated fatty structures 1–2 cm in size [96, 97]. In one study, 57 % of cases of steatosis detected on ultrasound were associated with elevation in aminotransferases [98].

Biliary Tract Disease

Cholangiopathy

Magnetic resonance cholangiography (MRC) demonstrates abnormalities in the intrahepatic bile ducts in a significant number of CF patients, with stricturing, beading, and areas of narrowing and dilatation similar to primary sclerosing cholangitis [96]. MRC-detected intrahepatic biliary anomalies were reported in 69 % of CF patients regardless of laboratory or clinical evidence of liver disease, with isolated or multiple dilations in the biliary tree noted [99].

Cholestasis

This is the earliest manifestation of liver involvement in CF and may mimic biliary atresia. Less than 2 % of infants with CF present with neonatal cholestasis. In a large study of infants with cholestasis, only 9 of 1,474 (0.6 %) had CF as their etiology, so it is an uncommon cause of neonatal cholestasis [100]. Liver biopsy can mimic findings seen in biliary obstruction and can be confused with biliary atresia. Meconium ileus is a known risk factor for the development of cholestasis [69, 95, 97]. While cholestasis generally resolves within 3 months with no sequelae, some studies have suggested an increased risk for cirrhosis in children with meconium ileus [68, 70, 71].

Gallbladder Involvement

Gallbladder abnormalities are found in 24–50 % of CF patients by US, MRI, or MRC. Microgallbladder is reported in 5–45 % of CF patients and 3–20 % have gallbladder distention and evidence of gallbladder dysfunction. Older studies reported that gallstones develop in 3–25 % of pediatric CF patients [95, 96, 101]. These stones are more commonly calcium bilirubinate stones [102]. Coinheritance of the Gilbert syndrome-associated UGT1A1 mutation appears to increase the risk for gallstones in CF [103]. Cholecystectomy is indicated for the management of symptomatic cholelithiasis. Calcium bilirubinate stones do not respond to ursodeoxycholic acid treatment. No treatment is required for microgallbladder.

Pathogenesis

The pathogenesis of CF liver disease is largely unknown. In the liver, CFTR is localized to the apical surface of bile duct epithelium and is not found in hepatocytes [104]. CFTR in biliary epithelium increases apical biliary chloride secretion primarily increasing bile acid-independent bile flow. In the pancreas and lung, CFTR is similarly located in the apical epithelium. These organ systems are affected to varying degrees by a dysfunctional or absent chloride channel leading to thickened mucus secretions and plugging.

A similar pathologic mechanism has been suggested in the liver, with inspissated bile leading to obstruction of small intrahepatic bile ducts, subsequent inflammation, and progression to fibrosis.

Screening for Liver Disease

The goals of screening for liver disease in CF are twofold. The first would be to identify individuals at risk for cirrhosis prior to its development in order to institute therapy to prevent or reduce progression to cirrhosis. The second would be to detect patients who have developed clinically silent cirrhosis to allow monitoring and interventions to reduce or mitigate complications.

No tests reliably identify individuals with CF who are at high risk for the development of cirrhosis. In contrast, several methods have shown promise for the detection of clinically silent cirrhosis. A combination of physical examination for hepatomegaly, annual testing of AST/ALT and GGT, and abdominal imaging (ultrasound, CT, or MRI/MRC) has been thought to hold the best promise for detecting clinically relevant liver disease in CF (cirrhosis with or without portal hypertension or advanced hepatic fibrosis). However, these tests are not sensitive for early stages of hepatic fibrosis or the identification of individuals who are at high risk for cirrhosis. In a prospective cohort study of CF patients with suspected CFLD, dual-pass core-needle liver biopsy increased the sensitivity of the detection of hepatic fibrosis by 22 % compared to a single-pass liver biopsy. In that study, the finding of more advanced hepatic fibrosis was the only factor independently associated with the future development of portal hypertension. Clinical exam, ultrasound, and ALT were not predictive of development of portal hypertension. Clinical hepatomegaly and ultrasound abnormalities were found to be sensitive, but not specific, and ALT elevation specific but not sensitive for the detection of advanced fibrosis on biopsy [81].

The CF guidelines from 1999 recommend an annual physical exam for liver span and texture, spleen size, and annual AST, ALT, and GGT determination but contained no definitive recommendations on screening imaging or liver biopsy. A recent best practice guideline paper from the European Cystic Fibrosis Society recommends hepatic ultrasound for patients with persistent elevation of AST, ALT, or GGT on three consecutive occasions over 12 months and/or clinical hepatomegaly or splenomegaly. They proposed that abnormalities in ultrasound could be followed by liver biopsy [94].

Liver biopsy remains the "gold standard" for the detection and staging of hepatic fibrosis and the diagnosis of cirrhosis. The risks, cost, and lack of ability to perform serial measurements with liver biopsy in large part explain the emphasis on developing reliable and validated noninvasive tests for liver disease in CF.

Routine liver biochemistries are unreliable as indicators of cirrhosis or the risk of development of cirrhosis. Several studies have suggested the use of serum markers of hepatic fibrogenesis for early detection of CF liver disease, such as TIMP-1, collagen type IV, prolyl hydroxylase, and glutathione s-transferase [105–108].

Radiologic Imaging

The three main imaging modalities: ultrasound, CT, and MRI can detect cirrhosis with or without findings of portal hypertension [109]. Ultrasound can demonstrate multilobular nodularity indicative of cirrhosis but is unreliable at detecting earlier stages of hepatic fibrosis. Steatosis and hepatic fibrosis may be indistinguishable. Children with normal hepatic ultrasounds can have advanced fibrosis [76]. In a single-center study, the ultrasound finding of hyperechogenicity was associated with an increased risk for the development of multilobular cirrhosis [75]. However, ultrasound heterogenicity can be intermittent [75], and consistency in interpretation of echogenicity and homogeneity may be center dependent leading to increased interobserver variability [75, 98, 110]. However some centers perform serial routine abdominal ultrasound to detect early imaging abnormalities such as parenchymal heterogeneity that may indicate an increased risk for progression to cirrhosis [75].

Acoustic Transient Elastography

Acoustic transient elastography uses a low-frequency acoustic wave transmitted through the

liver via a probe placed on the skin over the liver. The velocity of the wave propagation is directly proportional to the stiffness of the liver due to its collagen fiber content. There are limited studies of elastography in CF. Two studies of children and adults with CF found increased liver stiffness in clinical and biochemical CFLD and elastography compared favorably to ultrasound for the detection of advanced fibrosis [108, 111]. Two studies in CF found a good correlation between transient elastography values and the presence of esophageal varices [108, 112].

Acoustic Radiation Forced Impulse Imaging

Acoustic radiation forced impulse imaging (ARFI) uses ultrasound to measure liver stiffness and shear wave velocities. A potential advantage is that ARFI may not be influenced by hepatic steatosis [113, 114]. Initial studies show a good correlation with transient elastography. One report in a small cohort of CF subjects suggests that ARFI is similar to transient elastography [115].

Treatment and Outcome

To date, there is no effective therapy in CF to prevent or treat fibrosis. Thus, most efforts are directed at supportive care and management of complications.

Infants

Infants with cholestasis and prolonged jaundice are at risk for malnutrition, fat-soluble vitamin deficiency, and growth failure. This risk is higher in those infants who have undergone bowel resection. Close monitoring of growth and fat-soluble vitamin status with institution of higher-calorie feedings and fat-soluble vitamin supplementation is often required. Ursodeoxycholic acid (UCDA) increases bile flow and has been used in this setting despite the lack of published evidence in this age group.

Children and Adults

In older children and adults with CF and cirrhosis, the main issues in care are screening for and management of complications of portal hypertension (splenomegaly, ascites, varices) and optimization of their nutritional status and lung function.

Ursodeoxycholic Acid

UDCA at present is the only therapy available that may possibly prevent or delay progression of CFLD. It increases bile flow, may replace potentially toxic bile acids, acts as a cytoprotective agent, and possibly stimulates bicarbonate secretion in the biliary tract [116–118]. UDCA at 15–20 mg/kg/day has been shown to improve AST and ALT, bile drainage, liver histology, and nutritional and essential fatty acid status in CF liver disease. However, a 2000 Cochrane review on the use of UCDA in CF found few suitable randomized trials assessing UDCA efficacy, and there have not been any randomized studies conducted since that review [119]. They concluded data are insufficient to justify routine use in CF, and no data on impact of UCDA on death or liver transplant are available. In summary, there is significant disagreement about the use of UDCA in CF [94, 118, 120]. There is no well-controlled randomized study of UDCA in CF and no strong evidence for benefit or harm in CF. Further study is needed to clarify the potential role of UDCA in CF. Other potential therapies such as essential fatty acid supplementation and antioxidants have not been sufficiently investigated to provide any recommendations.

Management of CF Cirrhosis with Portal Hypertension

Screening

Standard follow-up for sequelae of cirrhosis is appropriate in CF. There is some evidence for an increased risk of GI cancers in CF [121], but not hepatocellular carcinoma (HCC), although there are isolated case reports of HCC [122].

Esophageal and Gastric Varices

In CF patients with cirrhosis, varices have been reported to be present in anywhere from 30 to 100 % [68, 70–73, 77]. In total these studies

reported on 3,057 patients with CF with 111 patients (3.6 %) with cirrhosis identified of whom 54 (48 %) had varices. The risk of variceal bleeding in CF cirrhosis has not been well studied. In two studies of a total of 67 patients with CF and cirrhosis, 28 (42 %) developed variceal hemorrhage [80, 84]. There have not been any studies of primary or secondary variceal prophylaxis in CF. Transjugular intrahepatic portosystemic shunt (TIPS) has been used in CF and reported in case reports and one small case series [123]. TIPS is effective in management of variceal bleeding in CF and can stabilize patients for over 5 years. Some authors have used partial splenic embolization or partial or total splenectomy in an attempt to reduce portal venous flow and improve thrombocytopenia [124, 125]. Indeed, partial splenectomy with splenorenal shunt had favorable outcome in 15 of 19 CF patients with portal hypertension, with improvement in liver function and portal hypertensive symptoms, significantly delaying or obviating the need for liver transplantation [124].

Ascites

The development of ascites represents a poor prognostic sign and is indicative of relative hepatic decompensation and the need for evaluation for possible liver transplant. Diuretics such as furosemide or spironolactone may be utilized as first-line therapy in concert with a salt- and fluid-restricted diet in attempts to decrease the ascitic fluid load. However, care must be taken to not overly restrict salt in CF, as hypochloridia may result especially in the summer months.

Liver Transplantation

Liver Transplantation Which patients with CF and cirrhosis require liver transplant and optimal timing of transplantation remains a controversial subject. Reserving liver transplant only for those with liver synthetic failure (a relatively rare late event in CF cirrhosis) versus early transplantation following the development of portal hypertensive complications varies by center. The established indications for liver transplant in CF include cirrhosis with evidence for hepatic decompensation or uncontrollable variceal bleeding. Some authors feel that evaluation and transplantation should occur earlier in the disease course, before lung function is severely compromised when outcomes are likely to be better [126, 127]. The relatively preserved hepatic synthetic function in the CF cirrhosis population affects prioritization for liver transplant, and clear guidelines on selection of CF patients for liver transplantation are lacking, although a scoring system has been proposed [128].

Of 203 liver transplants in CF patients performed in the USA from 1987 to 2008, 148 were performed in children. There was a significant survival advantage in both adults and children with CF cirrhosis receiving liver transplant compared to those with cirrhosis who did not receive a transplant, with further survival advantage in children compared to adults [129, 130]. Outcomes of liver transplant in CF have generally been favorable with 1-year patient and graft survival (89 %/83 %, respectively), and 5-year patient survival rates (85.8 %) are not significantly different than non-CF liver transplant patients [130, 131]. However, for CF patients without liver synthetic failure or intractable GI bleeding, their 1-year survival without transplant would likely be higher. In Europe, the median age of CF liver transplant recipients was 12 years and the 3-year survival was approximately 80 % [132]. Thus pediatric CF patients in the USA and Europe who undergo liver transplant experience survival rates similar to the 5-year unadjusted survival rates of 86 % in US non-CF pediatric liver transplant recipients [130].

Relative contraindications to an isolated liver transplant in CF include infection with multidrug-resistant organisms (Pseudomonas, Burkholderia), poor pre-transplant pulmonary function (FEV1 <50 % predicted) and/or elevated resting arterial pCO2, extensive pulmonary fibrosis on imaging, and severe pulmonary hypertension [132, 133]. Short-term improvements in lung function are reported in pediatric patients following liver transplant, and poorer lung function prior to surgery is associated with mortality risk [133]. Small single-center series have reported both favorable [127] and unfavorable pulmonary function outcomes following liver transplantation [134]. A recent analysis of the CF registry found

no difference in the rate of decline in FEV1 in the 3 years following liver transplant in the CF subjects who underwent liver transplant compared to CF controls without liver disease [135]. This suggests that liver transplantation does not lead to a significant long-term change in pulmonary outcome. The effect of liver transplant on the nutritional status of CF patients is also uncertain, with reports of improvements [136, 137] and reports of no improvement [135].

Combined lung and liver transplant may be considered in patients with cirrhosis and portal hypertension and extensive pulmonary fibrosis, lower FEV1, and resting hypercapnia. Eleven CF patients underwent combined liver-lung transplantation between 1987 and 2004 in the USA. The median age was 15 years, and 1-, 3-, and 5-year patient survival rates were 79, 63, and 63 %, respectively [138]. Eleven combined liver-pancreas transplants in CF patients with cirrhosis and CFRD were performed between 1987 and 2010, with 100 % 5-year survival reported in follow-up of 7 patients, with no further requirement for exogenous insulin or pancreatic enzyme replacement therapy [131].

Hemochromatosis and Hemosiderosis

Iron overload states can be classified as primary or secondary. There are many disorders that can lead to iron overload (Table 9.6). This discussion will focus on hereditary hemochromatosis (HHC), juvenile hemochromatosis (JHC), and secondary iron overload (primarily transfusion associated) in the pediatric patient. For a discussion of the rarer entities, the reader is referred to a recent review [140].

Table 9.6 Classification of iron overload states

Primary forms of iron overload
Hereditary hemochromatosis, *HFE* associated
C282Y homozygotes
C282Y/H63D compound heterozygotes
Hereditary hemochromatosis, non-*HFE* associated
Hereditary hemochromatosis, *TFR2* associated
Juvenile hemochromatosis (hemojuvelin associated (HJV), hepcidin associated (HAMP))
Ferroportin-associated hemochromatosis (FPN autosomal dominant)
Heavy-chain ferritin disease
Neonatal hemochromatosis
Autosomal dominant hemochromatosis (Solomon Islands)
Aceruloplasminemia
Atransferrinemia
Friedreich ataxia
DMT-1 deficiency
Secondary iron overload
Iatrogenic (transfusional iron overload)
Anemias (sideroblastic anemia, hereditary spherocytosis, β-thalassemia)
Dietary or medicinal iron overload
Long-term hemodialysis
Chronic liver disease (hepatitis B and C, alcoholic liver disease, NASH, post portocaval shunt)
Porphyria cutanea tarda
Cystic fibrosis
Tyrosinemia
Zellweger syndrome

Adapted from Pietrangelo [144]

Physiology and Pathophysiology of Iron Overload

Iron is one of the more tightly regulated nutrients in the body. Humans have no significant excretory pathway for iron. Iron stores are normally controlled at the level of absorption, matching absorption to physiologic requirements. Under normal circumstances, only about 1 mg of elemental iron is absorbed per day, in balance with gastrointestinal losses. Intestinal iron absorption is increased by low body iron stores (storage regulation), increased erythropoiesis (erythropoietic regulation), anemias associated with ineffective erythropoiesis (thalassemias, congenital dyserythropoietic anemias, and sideroblastic anemia), and acute hypoxia. Both dietary iron intake (dietary regulation) and systemic inflammation can temporarily decrease iron absorption and availability, even in the presence of iron deficiency [141].

Iron is absorbed in the duodenal and proximal jejunal enterocytes via divalent metal transporter 1 (DMT-1), where it is either stored as ferritin or

moved across the basolateral membrane to reach the plasma, where it is bound to transferrin. The regulation of iron status hinges on hepcidin and ferroportin. Hepcidin is produced in the liver and is elevated in iron-sufficient states. High levels of hepcidin decrease intestinal iron absorption [142, 143]. When hepcidin is low, iron absorption is increased. Mutations in both the hepcidin (juvenile hemochromatosis) and ferroportin (autosomal dominant hemochromatosis) genes have been described [144]. Hepcidin expression is regulated by the HFE protein (mutated in HHC type 1), transferrin receptor 2 (TfR2 mutated in some forms of iron overload), and hemojuvelin (mutated in juvenile hemochromatosis). In animal models and in humans with hemochromatosis, the normal increase in hepcidin expression with iron loading is lost, leading to lower hepcidin levels and continued iron absorption in the face of iron overload [139].

In humans, iron in the circulation is tightly bound to transferrin. Diferric transferrin binds to the transferrin receptor on the cellular plasma membrane. This complex is then endocytosed into the cell, where iron is released by the acid environment of the endocytic vesicle. The iron is then transported across the endosomal membrane to the cytoplasm. In iron overload states, more of transferrin is in the diferric state, or the more readily absorbable state. The uptake of iron is primarily regulated by the expression of the transferrin receptor on the cell surface. In iron deficiency, iron regulatory proteins are increased and upregulate expression of transferrin receptor 1 increasing iron uptake and decrease the synthesis of ferritin. In states of iron repletion or excess, there is a reduced level of iron-binding proteins, leading to less transferrin receptor production and an increase in ferritin and hepcidin synthesis.

A second transferrin receptor (TfR2) binds holotransferrin/diferric transferrin and mediates the uptake of transferrin-bound iron. TfR2 is predominately expressed in the liver, where, in contrast to TfR1, it is not downregulated by dietary iron overload. Hepatic TfR2 provides an explanation for the continued hepatic iron uptake in HHC despite the downregulation of TfR1. TfR2 also regulates hepcidin synthesis in the liver.

In HHC and JHC, net iron absorption is increased above endogenous losses. In addition, in both disorders, there is loss of the normal downregulation of iron absorption as iron accumulates in the body. This is directly related to inhibition hepatic hepcidin expression by the mutations in the hepcidin regulatory proteins responsible for HHC and JHC [139, 144]. The result is a gradual increase in total body iron. In HHC, the net increase in total body iron has been estimated at 4–7 mg/day. The increased iron absorption in HHC is the result of inappropriate transfer of iron in the intestine due to suppression of hepatic hepcidin secretion [144]. In JHC, iron accumulates more rapidly than in HHC, due in part to the more significant role of hemojuvelin in the regulation of hepcidin [145]. Excessive iron intake in the diet can accelerate the accumulation of iron in both HHC and JHC. In a similar manner, increased iron losses (most commonly in menstruating females) can slow the accumulation of iron. In secondary iron overload due to hemoglobinopathies, iron overload is related to both the anemia and increased iron absorption and excess iron provided by transfusions. In contrast, in aplastic anemias, iron overload is primarily related to transfusion.

Genetics of Hereditary Hemochromatosis

Hereditary hemochromatosis is an autosomal recessive disorder due to a mutation in *HFE* [146]. The *HFE* gene encodes a novel major histocompatibility (MHC) class 1-like molecule. Two principal missense mutations of *HFE* have been identified (C282Y and H63D). Upward of 85–90 % of patients with classic HHC are homozygous for C282Y, and another 5 % are compound heterozygotes (C282Y/H63D). The H63D mutation is quite common (15–20 % heterozygote state in the general population), but H63D homozygotes generally do not have iron loading. Ten to 15 % of patients with a clinical syndrome of typical HHC do not have either the C282Y or H63D mutation. However, 55 % of patients initially felt to have HHC who carried

neither mutation were found to have previously unrecognized causes for secondary iron overload. There remains a small subgroup of *HFE* mutation-negative iron-overloaded patients with typical HHC. Some of these patients have mutations in other hepcidin regulatory proteins. Several new mutations in *HFE* have been described in patients with iron overload, suggesting that other *HFE* mutations may be found in "wild-type" HHC.

HFE is widely expressed throughout the body; the highest levels are found in the liver and small intestine. *HFE* is involved in an as yet unknown complex regulation of hepcidin secretion, with subsequent lack of hepcidin mRNA expression and secretion in the face of excessive total body iron stores [147].

Epidemiology

Hereditary hemochromatosis is one of the most common genetic diseases in the white population, with a prevalence of the C282Y homozygous state of 1 in 200 to 1 in 400 [148]. The disease is most common in individuals of northern European descent. The frequency of the C282Y mutation is highest in subjects from northwest Europe (10–20 %), less frequent in southern and eastern European populations (2–4 %), and rare in natives of Africa, Central or South America, Eastern Asia, and the Pacific Islands [149]. The H63D mutation has a distribution similar to that of C282Y, but it is more common in European groups (15–40 %). In a large population-based study of 3,011 unrelated white adults in Busselton, Australia, 14.1 % were heterozygous for the C282Y mutation and 0.5 % were homozygous [149].

About 6.8 % of the US white population is heterozygous for the C282Y mutation and 0.5 % is homozygous. This is fairly similar to the estimates of 1 in 200 incidence of iron overload in the worldwide white population [150]. HHC and the C282Y mutation are uncommon in African Americans or Asian Americans [149], the prevalence of clinically diagnosed HHC in African Americans being about 1 in 1,000 [148].

Thus, HHC remains predominately a disease of individuals of northern European descent. Although mutation analysis has assisted in defining the prevalence of the disease in this population, it has not proved useful in other selected populations. Nevertheless, HHC remains one of the more common, if not the most common, inherited disorder in humans.

Clinical Features

In adults, HHC may present with the clinical syndrome of diabetes, cirrhosis, and increased skin pigmentation as initially described in 1865. Although the genetic defect is present at birth, years of increased iron absorption and tissue accumulation (usually >5 g of excess total body iron) are required for the development of clinical symptoms. Clinical symptoms are rare before adulthood. Before the discovery of the gene for HHC, most adults were diagnosed with clinical symptoms that included liver disease (fibrosis or cirrhosis), diabetes, skin pigmentation, heart failure, arthritis, and endocrinologic disturbances. Since the discovery of the *HFE* gene, it is recognized that many adults who are homozygous for the C282Y mutation are asymptomatic [149]. HHC can be classified into four stages: (1) a genetic predisposition with no abnormality other than possibly an elevated serum transferrin saturation, (2) iron overload (2–5 g) without symptoms, (3) iron overload with early symptoms (lethargy, arthralgia), and (4) iron overload with organ damage. Almost all children with HHC are in the first stage and may rarely be in the second stage. For further data on the clinical manifestations in adults, the reader is referred to a recent review [151]. As iron accumulates in the tissues, there is progressive fibrosis and injury. As organ failure results, the classic clinical consequences of hemochromatosis are recognized. The organs involved and pathologic findings are listed in Table 9.7.

Disease expression is dependent not only on the mutation but on other genetic and environmental factors, such as sex, age, dietary iron, and other factors affecting iron balance. Males

Table 9.7 Organ involvement in hereditary hemochromatosis

Organ	Histology
Liver	Periportal iron deposition, fibrosis, cirrhosis
Pancreas	Fibrosis with normal exocrine and β-cell function Abnormal β-cell function
Skin	Bronzing secondary to increased melanin
Heart	Dilated and restrictive cardiomyopathy
Joints	Hip, shoulder, knee, metacarpophalangeal joint involvement, and chondrocalcinosis
Pituitary	Fibrosis leading to hypogonadotropic hypogonadism

with HHC typically present earlier than females, presumably because of the ongoing iron losses in menstruating females. In addition, the association of symptoms with the mutations is quite variable and less common than previously thought.

Most children and adolescents with HHC are asymptomatic. Although most have abnormal transferrin saturation, a normal ferritin is the rule. Reports of elevated ferritin and death from congestive heart failure in pediatric patients have not been confirmed by genotype analysis and may represent JHC, a truly distinct disorder [152]. A study screening the children of 179 homozygotes found that even at a mean age of 37 years, most affected children of parents with HHC were asymptomatic [153].

Unless another illness is present, heterozygotes do not have clinically important iron overload. Heterozygotes do have slightly higher transferrin saturations than those in normal individuals (men 38 % vs. 30 %; women 32 % vs. 29 %). This suggests that the heterozygote state may be protective for iron deficiency in women.

Diagnosis and Screening

Prior to the discovery of *HFE*, the diagnosis of HHC required the documentation of iron overload or HLA linkage to an affected individual. Criteria for iron overload consistent with HHC include (a) grade 3 or 4 stainable iron on liver biopsy, (b) hepatic iron concentration greater than 4,500 μg (80 μmol)/g dry weight, (c) a hepatic iron index (iron concentration in micromoles per gram of dry weight divided by age in years) of more than 1.9, and (d) evidence of iron overload of more than 5 g. These criteria are rarely encountered in children because in the absence of confounding factors (high dietary intake, hepatitis C viral infection, etc.), children typically have not developed this degree of iron overload. These criteria are probably not acceptable for individuals identified by family screening, who may be early in the course of iron overload. Some investigators have suggested that any individual with an abnormal hepatic iron concentration (>30 μmol/g or 1,500 μg/g dry weight) who has no other reason for iron overload should be suspected of having HHC. A variety of disorders can lead to hepatic iron overload, including chronic liver diseases such as alcoholic liver disease, nonalcoholic steatohepatitis (NASH), chronic viral hepatitis, cystic fibrosis, and porphyria cutanea tarda. There is no association with an increased prevalence of the *HFE* mutations in either alcoholic liver disease or viral hepatitis [154]. However, in both NASH and porphyria cutanea tarda, a higher frequency of the C282Y mutation has been observed [155].

In adults, transferrin saturation is used to screen individuals, with the threshold for further investigation of 45 % in men and 42 % in premenopausal women. Abnormal transferrin saturation has been reported in children as young as 2 years. However, fasting transferrin saturation and ferritin level in affected children can be normal, even in known homozygous subjects [156]. Up to 30 % of women with HHC who are under 30 years of age have normal transferrin saturation [156]. Thus, transferrin saturation is helpful in phenotypic screening in children when it is abnormal but does not exclude HHC when normal. In contrast, ferritin may be elevated in many inflammatory liver diseases such as chronic viral hepatitis and NASH in the absence of HHC and is less helpful in phenotypic screening for HHC in children and adults.

Liver biopsy has been primarily studied in adults and should not be used for the diagnosis of HHC in children. Increased hepatic iron can be

demonstrated by Prussian blue staining. Hepatic iron quantitation with the determination of the hepatic iron index (micromoles of iron per gram of dry weight divided by age in years) has been considered one of the more sensitive and specific tests for HHC. A hepatic iron index of greater than 1.9 in the absence of secondary iron overload is indicative of HHC with iron overload. However, 10–15 % of HHC patients identified by genetic testing will have a hepatic iron index of less than 1.9, calling into question the use of this test for diagnosis in children [156]. In children with HHC, an abnormal hepatic iron index has been reported in those as young as 7 years. Liver biopsy has given way to magnetic resonance imaging (MRI) quantitation of hepatic iron content. Liver biopsy should reserved for assessment of hepatic fibrosis in HHC and ruling out other causes of liver disease [157].

Genetic testing is available on a commercial basis. In 150 family members of 61 white American probands, 34 family members had an HHC phenotype. Among the family members, 92 % of the C282Y homozygotes and 34 % of the C282Y/H63D compound heterozygotes had the HHC phenotype. None of the H63D homozygotes had an HHC phenotype. A few individuals were heterozygous for one mutation and had iron overload. Thus, testing for HFE mutations should include both the C282Y and the H63D mutations. Heterozygosity may contribute to iron overload with an associated condition, but it should not be considered the sole cause of iron overload. Only C282Y/C282Y and compound heterozygosity (C282Y/H63D) should be considered indicative of HHC. However, not all compound heterozygotes will develop HHC. In most cases, these individuals will not require liver biopsy for confirmation of the diagnosis. However, C282Y homozygotes with evidence of liver disease (elevated aminotransferases or hepatomegaly) or with serum ferritin levels greater than 1,000 μg/L should undergo a liver biopsy to assess the degree of liver injury and the possible contribution of other liver disorders to the clinical picture. Liver biopsy is also recommended in suspected iron overload in non-C282Y homozygotes (C282Y heterozygotes, C282Y/H63D, or no mutations).

Approach to the Child with a Parent with HHC

The preferred clinical assessment of a child whose parent has HHC is open to debate. Because symptomatic end-stage organ disease from HHC is easily preventable with early therapy, screening of potentially affected children has been advocated. However, the majority of patients present with clinical disease after the age of 20 years. Biochemical screening requires repeated determination of transferrin saturation and ferritin. With the advent of genetic mutation testing, Adams et al. [153] have shown that it is cost-effective to screen the unaffected parent with mutation analysis. A suggested strategy is shown in Fig. 9.4. If the unaffected parent is either heterozygous or homozygous for C282Y, the potentially affected children are then screened with mutation analysis following appropriate counseling and consent [153]. Subsequently, at-risk children are followed with fasting transferrin saturation, ferritin, and liver blood tests. This strategy was found to be more cost-effective when compared with the phenotypic strategy [158]. When the ferritin is greater than 200 μg/L or aminotransferases increase, phlebotomy therapy is then initiated.

Iron Overload in Children with Liver Dysfunction

In pediatric patients, iron overload should be considered in the differential diagnosis of liver disease. Testing for transferrin saturation and ferritin should be considered in the evaluation of hepatic dysfunction in children (Fig. 9.5). If liver biopsy is performed as part of the evaluation, staining for iron should be done and quantitative hepatic iron determination should be considered. There are older reports of children presenting as young as 5 years with iron overload and presumed HHC that may have been JHC, as these reports predate *HFE* testing. White children with evidence of iron overload (elevated transferrin saturation of >45 %, elevated ferritin, increased stainable iron or hepatic iron concentration) would be candidates for possible *HFE* mutation analysis. The

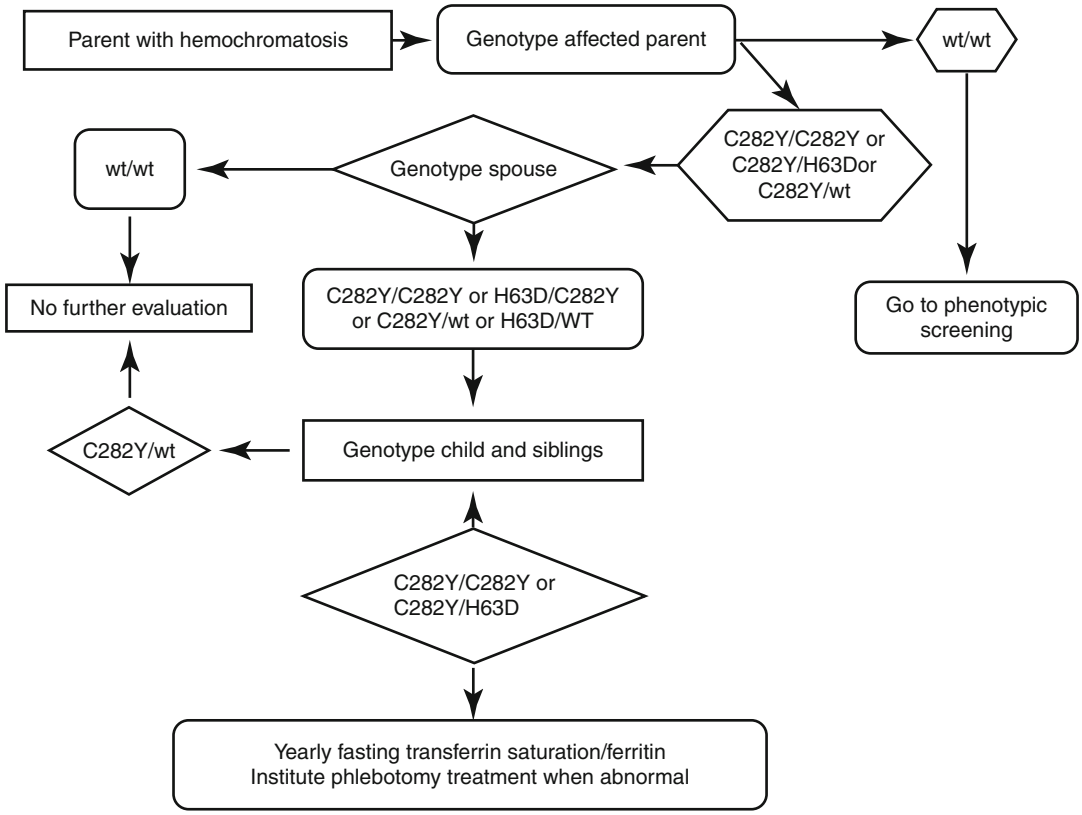

Fig. 9.4 Strategy for screening for hereditary hemochromatosis in a child with a parent with hemochromatosis

yield of *HFE* analysis in African American and Asian American children should be quite low. *HFE* mutation analysis may be helpful in patients with other diseases that can lead to iron overload (NASH, porphyria cutanea tarda).

The role of liver biopsy has not been studied in children. Data from one large adult study has shown that 50 % of HHC patients with a ferritin level greater than 1,000 μg/L, abnormal aspartate aminotransferase (AST), or hepatomegaly had significant fibrosis on liver biopsy [159]. In contrast, none of the HHC patients without those factors had significant fibrosis [159]. Even before genotyping was available, it was shown that patients diagnosed in the precirrhotic stage who were treated with venisection had a normal life expectancy. Thus, in patients homozygous for the C282Y mutation, these noninvasive measures may be used to avoid liver biopsy.

Because HHC is a common genetic disorder whose effects can be prevented by presymptomatic intervention, screening of the general population has been considered. Until the cost of the testing is reduced and the implications for insurability and management are clarified, newborn testing has not been recommended [160]. In adults, screening strategies have been suggested using a combination of transferrin saturation and confirmation with *HFE* testing.

Treatment

The goals of treatment are to reduce total body iron overload and prevent reaccumulation of iron. When instituted before end-organ injury, successful treatment would ideally prevent the development of cirrhosis and other end-organ disease and reduce the incidence of hepatocellular carcinoma. Current recommendations are that venisection is indicated if serum ferritin is greater than 300 μg/L in males or 200 μg/L in females [161]. The goal of therapy is to maintain a ferritin level less than 50 μg/L. There are little published

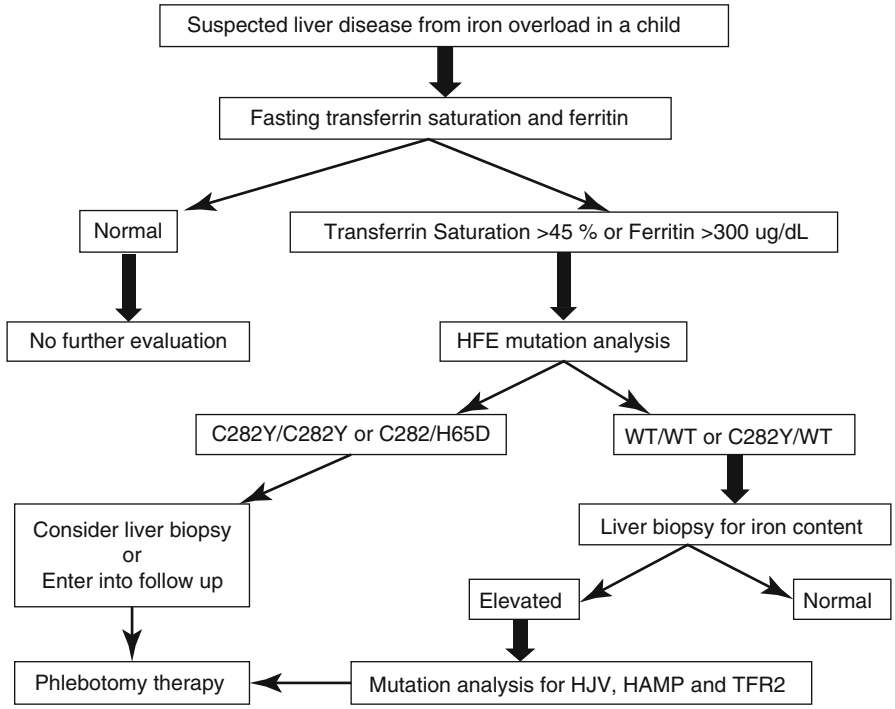

Fig. 9.5 Strategy for screening and diagnosis of iron overload in a child with liver disease and suspected iron overload

data on phlebotomy therapy in children as children with HHC rarely meet the preceding criteria. Initial therapy in symptomatic children should include weekly or every-other-week phlebotomy of 5–8 mL/kg until the ferritin has decreased to <300 μg/L. Thereafter, two to four sessions per year will probably be required. In our experience, therapeutic phlebotomy may need to be less frequent in children with HHC who have asymptomatic iron overload, probably because of their lower total body iron load. Careful attention to the development of iron deficiency is required when treating children. Once identified, children with HHC should be counseled to maintain a low-iron diet and avoid ascorbic acid and vitamin supplements containing iron.

Juvenile Hemochromatosis (OMIM 602390)

Juvenile hemochromatosis, or hemochromatosis type 2, is a rare iron-loading disorder that leads to severe iron loading and organ failure, typically before 30 years of age [152]. Even though this is rarer than HHC, JHC may be a more common cause of symptomatic iron overload in children. JHC is characterized by autosomal recessive inheritance and a pattern of iron distribution and tissue injury similar to that of HHC. However, JHC is not associated with mutations in *HFE*. In contrast to HHC, males and females are equally affected, and hypogonadism and cardiac dysfunction are the most common symptoms at presentation. The disorder has been reported in several ethnic groups. Most patients present in the second and third decades of life, likely the result of a higher rate of iron accumulation in JHC as compared with HHC. As such, the phlebotomy requirements to maintain normal iron balance are significantly higher in JHC as compared with HHC. Most cases of JHC are due to mutations in the gene *HJV* which encodes hemojuvelin, a key regulatory protein for hepcidin, and accounts for about 90 % of the cases reported to date [152]. The most frequent mutation is G320V, which was reported to account for 60 % of the mutations in the original description and all individuals identified in a French Canadian study.

However, many mutations have now been reported (reviewed in [152]). *HAMP*, which encodes hepcidin, accounts for 10 % of the reported cases [162]. Combined mutations in *HFE* and *TFR2* may also present with a phenotype of JHC without mutations in either *HJV* or *HAMP* [140, 152].

As a consequence, this disorder should be considered when children and adolescents present with clinically significant liver disease and iron overload. A family history of iron overload and hypogonadism in the absence of *HFE* mutations should raise the clinical suspicion for JHC. It is likely that some of the earlier reports of children with clinically apparent liver disease and iron overload had JHC and not HHC. With the identification of candidate genes involved in JHC, genetic diagnosis is available for *HJV*, *HAMP*, and the *HFE*/*TFR2* combination. These offer the opportunity for presymptomatic diagnosis in children from affected families. However, the overall incidence of this disorder must be small in comparison with classic HHC, as in the Australian population study, only 0.5 % of the adults with the wild-type/wild-type hemochromatosis genotype had an elevated (>45 %) fasting transferrin saturation.

A recommended approach to children with suspected iron overload and no family history of HHC is shown in Fig. 9.5.

Secondary Iron Overload

Secondary iron overload is a common problem for children with transfusion-dependent diseases such as the thalassemias, sickle cell disease, and aplastic anemias. This disorder has been referred to as hemosiderosis, primarily because the accumulation of iron begins in the reticuloendothelial cells in the liver. The pathophysiology of secondary iron overload is related to the provision of parenteral iron that bypasses the normal intestinal regulation of iron absorption. In addition, the anemias of ineffective erythropoiesis (thalassemias, congenital dyserythropoietic anemias, and sideroblastic anemias) stimulate iron absorption, resulting in an additional 2–5 g of iron absorbed from the diet per year. Tissue iron overloading of parenchymal cells is apparent after only 1 year of transfusion therapy. In patients who are receiving transfusions without chelation therapy, symptomatic heart disease has been reported within 10 years [163]. Iron-induced liver disease is a common cause of death in older patients, often aggravated by hepatitis C infection. Hepatic fibrosis is present as early as 2 years after starting transfusions, with cirrhosis reported in the first decade of life. With improved survival with iron chelation, iron loading of the anterior pituitary and associated disturbed sexual maturation are quite common.

Although the amount of excess iron that has been provided in the form of transfused iron can be calculated from the volume of red blood cells administered, the biochemical markers of iron overload may be unreliable. Some researchers advocate following ferritin levels for signs of iron overload. Under normal conditions, 1 μg/L of ferritin is equivalent to 8–10 mg of storage iron. Thus, a ferritin of 1,000 μg/L is equal to about 10,000 mg of storage iron. When ferritin is >4,000 μg/L, there is no longer a correlation between ferritin and iron stores. In addition, ferritin may be increased with hepatic inflammation that may be related to other factors, such as hepatitis C infection. This has led others to suggest that ferritin concentrations are not an accurate reflection of tissue iron accumulation [164]. More accurate estimations of tissue iron burden are obtained by liver or cardiac biopsy and iron quantitation by MRI [165] or by investigative SQUID (superconducting quantum interference device) biomagnetometry, which may be better at assessing high tissue iron concentrations.

Treatment

Treatment of transfusional iron overload centers on chelation therapy or, when possible, exchange transfusion. Deferoxamine is generally given by continuous subcutaneous administration and is capable of inducing negative iron balance. In general, ferritin should begin to decrease by the end of 1 year of treatment. The

goal is to maintain a ferritin level of less than 1,000 μg/L on treatment. Deferoxamine prevents early cardiac death, arrests the progression to cirrhosis, and stabilizes or reduces total body iron load [166]. The major risks of deferoxamine are growth failure, hearing impairment, and bone abnormalities. These are more common in patients with low iron loads. Direct dose-related toxicities involve the kidneys, lungs, and visual loss. The early use of deferoxamine in an amount proportional to the transfusional iron overload reduces iron burden and the risk of the development of diabetes mellitus, cardiac disease, and early death in patients with thalassemia major [166]. When possible, such as in some cases of sickle cell disease, exchange transfusion can reduce the iron burden from transfusions [167]. Other strategies include intravenous deferoxamine, which can rapidly reduce tissue iron burden in situations of severe iron overload. Newer oral chelation agents are available. Deferiprone, used for years in Europe and now approved in the USA, seems to be more effective at removal of cardiac iron load than deferoxamine with similar or slightly less efficacy for hepatic iron reduction. The major side effects of deferiprone are bone marrow suppression, arthropathy, gastrointestinal symptoms, and zinc deficiency. Deferasirox, another oral chelation therapy approved for use in the USA and several other countries, appears to be very efficient in liver and cardiac iron removal in adults and children. Side effect includes gastrointestinal disease. Recent reports suggest a small but increased risk of renal or hepatic failure and gastrointestinal hemorrhage. Combination treatment with deferoxamine and deferiprone or other oral chelators, such as deferasirox, suggests an additive effect in the presence of adequate renal function [168].

In the past, researchers have recommended serial liver biopsy to follow iron overload in transfusion-dependent patients. MRI is the test of choice to quantitate hepatic iron overload and longitudinal determinations [165]. Liver biopsy should be reserved for assessment of hepatic injury and fibrosis and for the assessment of the relative contribution of non-iron-related disorders to the process.

References

1. Perlmutter DH. Alpha-1-antitrypsin deficiency. Semin Liver Dis. 1998;18(3):217–25.
2. Perlmutter DH. Alpha-1 antitrypsin deficiency. In: Walker WA, editor. Pediatric gastrointestinal disease. 3rd ed. Hamilton: B C Decker; 2000.
3. Perlmutter DH. Alpha-1 antitrypsin deficiency. In: Suchy F, Sokol R, editors. Liver disease in children. 3rd ed. Cambridge: Cambridge University Press; 2007. p. 545–71.
4. Marciniuk DD, et al. Alpha-1 antitrypsin deficiency targeted testing and augmentation therapy: a Canadian Thoracic Society clinical practice guideline. Can Respir J. 2012;19(2):109–16.
5. Silverman EK, Sandhaus RA. Clinical practice. Alpha1-antitrypsin deficiency. N Engl J Med. 2009; 360(26):2749–57.
6. Sveger T. Liver disease in alpha1-antitrypsin deficiency detected by screening of 200,000 infants. N Engl J Med. 1976;294(24):1316–21.
7. Sveger T. Alpha 1-antitrypsin deficiency in early childhood. Pediatrics. 1978;62(1):22–5.
8. Eriksson S. Alpha 1-antitrypsin deficiency. J Hepatol. 1999;30 Suppl 1:34–9.
9. Gadek JE, et al. Antielastases of the human alveolar structures. Implications for the protease-antiprotease theory of emphysema. J Clin Invest. 1981;68(4): 889–98.
10. Perlmutter DH, Pierce JA. The alpha 1-antitrypsin gene and emphysema. Am J Physiol. 1989;257(4 Pt 1):L147–62.
11. Teckman JH, Perlmutter DH. Retention of mutant alpha(1)-antitrypsin Z in endoplasmic reticulum is associated with an autophagic response. Am J Physiol Gastrointest Liver Physiol. 2000;279(5): G961–74.
12. Pierce JA, Eradio BG. Improved identification of antitrypsin phenotypes through isoelectric focusing with dithioerythritol. J Lab Clin Med. 1979;94(6):826–31.
13. Ranes J, Stoller JK. A review of alpha-1 antitrypsin deficiency. Semin Respir Crit Care Med. 2005;26(2): 154–66.
14. Travis J, Salvesen GS. Human plasma proteinase inhibitors. Annu Rev Biochem. 1983;52:655–709.
15. Perlmutter DH. Alpha-1-antitrypsin deficiency: importance of proteasomal and autophagic degradative pathways in disposal of liver disease-associated protein aggregates. Annu Rev Med. 2011;62:333–45.
16. Hofker MH, et al. Cloning and characterization of an alpha 1-antitrypsin like gene 12 KB downstream of the genuine alpha 1-antitrypsin gene. Biochem Biophys Res Commun. 1988;155(2):634–42.
17. Laurell CB, Nosslin B, Jeppsson JO. Catabolic rate of alpha1-antitrypsin of Pi type M and Z in man. Clin Sci Mol Med. 1977;52(5):457–61.
18. Lomas DA, et al. The mechanism of Z alpha 1-antitrypsin accumulation in the liver. Nature. 1992;357(6379):605–7.

19. Bathurst IC, et al. Structural and functional characterization of the abnormal Z alpha 1-antitrypsin isolated from human liver. FEBS Lett. 1984;177(2):179–83.
20. Ogushi F, et al. Z-type alpha 1-antitrypsin is less competent than M1-type alpha 1-antitrypsin as an inhibitor of neutrophil elastase. J Clin Invest. 1987;80(5):1366–74.
21. Rosenthal P, Liebman WM, Thaler MM. Alpha 1-antitrypsin deficiency and severe infantile liver disease. Am J Dis Child. 1979;133(11):1195–6.
22. Waasdorp Hurtado C, Sokol R, Rosen H. Metabolic liver disease. In: Friedman's handbook of liver disease. 3rd ed. Philadelphia, PA: Elsevier/Mosby; 2011.
23. Propst T, et al. High prevalence of viral infection in adults with homozygous and heterozygous alpha 1-antitrypsin deficiency and chronic liver disease. Ann Intern Med. 1992;117(8):641–5.
24. Sveger T, Eriksson S. The liver in adolescents with alpha 1-antitrypsin deficiency. Hepatology. 1995;22(2):514–7.
25. Topic A, Prokic D, Stankovic I. Alpha-1-antitrypsin deficiency in early childhood. Fetal Pediatr Pathol. 2011;30(5):312–9.
26. Odievre M, et al. Alpha1-antitrypsin deficiency and liver disease in children: phenotypes, manifestations, and prognosis. Pediatrics. 1976;57(2):226–31.
27. Larsson C. Natural history and life expectancy in severe alpha1-antitrypsin deficiency, Pi Z. Acta Med Scand. 1978;204(5):345–51.
28. Stoller JK, Aboussouan LS. A review of alpha1-antitrypsin deficiency. Am J Respir Crit Care Med. 2012;185(3):246–59.
29. Guenter CA, et al. The pattern of lung disease associated with alpha antitrypsin deficiency. Arch Intern Med. 1968;122(3):254–7.
30. Kalsheker NA. Alpha1-antitrypsin deficiency: best clinical practice. J Clin Pathol. 2009;62(10):865–9.
31. Ghishan FK, Greene HL. Liver disease in children with PiZZ alpha 1-antitrypsin deficiency. Hepatology. 1988;8(2):307–10.
32. Qizilbash A, Young-Pong O. Alpha 1 antitrypsin liver disease differential diagnosis of PAS-positive, diastase-resistant globules in liver cells. Am J Clin Pathol. 1983;79(6):697–702.
33. Udall Jr JN, et al. Liver disease in alpha 1-antitrypsin deficiency. A retrospective analysis of the influence of early breast- vs bottle-feeding. JAMA. 1985;253(18):2679–82.
34. Labrune P, Odievre M, Alagille D. Influence of sex and breastfeeding on liver disease in alpha 1-antitrypsin deficiency. Hepatology. 1989;10(1):122.
35. Kemmer N, et al. Alpha-1-antitrypsin deficiency: outcomes after liver transplantation. Transplant Proc. 2008;40(5):1492–4.
36. Stecenko AA, Brigham KL. Gene therapy progress and prospects: alpha-1 antitrypsin. Gene Ther. 2003;10(2):95–9.
37. Yusa K, et al. Targeted gene correction of alpha1-antitrypsin deficiency in induced pluripotent stem cells. Nature. 2011;478(7369):391–4.
38. Carbamazepine in severe liver disease due to alpha-1 antitrypsin deficiency. Clinical Trials 2012. Available from: http://www.ClinicalTrials.gov/ct2/show/NCT01379469?term=alpha-1+antitrypsin. Cited 2 Nov 2012.
39. Wilson SA. Progressive lenticular degeneration: a familial nervous disease associated with cirrhosis of the liver. Brain. 1912;34:295–509.
40. Scheinberg I, Sternleib I. Wilson's disease. Philadelphia: WB Saunders; 1984.
41. Schilsky M, Scheinberg I, Sternleib I. Liver transplantation for Wilson's disease: indications and outcomes. Hepatology. 1994;19:583–7.
42. Frydman M, et al. Assignment of the gene for Wilson disease to chromosome 13: linkage to the esterase D locus. Proc Natl Acad Sci U S A. 1985;82(6):1819–21.
43. Merle U, et al. Clinical presentation, diagnosis and long-term outcome of Wilson's disease: a cohort study. Gut. 2007;56(1):115–20.
44. Weiss KH, Stremmel W. Evolving perspectives in Wilson disease: diagnosis, treatment and monitoring. Curr Gastroenterol Rep. 2012;14(1):1–7.
45. Lee BH, et al. Distinct clinical courses according to presenting phenotypes and their correlations to ATP7B mutations in a large Wilson's disease cohort. Liver Int. 2011;31(6):831–9.
46. Wilson's Disease Database. 2012. Available from: http://www.wilsondisease.med.ualberta.ca/database.asp. Cited 2 Nov 2012.
47. Rosencrantz R, Schilsky M. Wilson disease: pathogenesis and clinical considerations in diagnosis and treatment. Semin Liver Dis. 2011;31(3):245–59.
48. Chitkara D, Pleskow R, Grand R. Wilson disease. In: Walker WA, editor. Pediatric gastrointestinal disease. 3rd ed. Hamilton: BC Decker; 2000.
49. Walshe J, Briggs J. Ceruloplasmin in liver disease, a diagnostic pitfall. Lancet. 1962;ii:263–5.
50. Factor SM, et al. The cardiomyopathy of Wilson's disease. Myocardial alterations in nine cases. Virchows Arch A Pathol Anat Histol. 1982;397(3):301–11.
51. Manolaki N, et al. Wilson disease in children: analysis of 57 cases. J Pediatr Gastroenterol Nutr. 2009;48(1):72–7.
52. Yamaguchi Y, et al. Mass screening for Wilson's disease: results and recommendations. Pediatr Int. 1999;41(4):405–8.
53. Wiebers D, Hollenhurst R, Goldstein N. The ophthalmologic manifestations of Wilson's disease. Mayo Clinic Proc. 1977;52:409–16.
54. Chu CC, Huang CC, Chu NS. Recurrent hypokalemic muscle weakness as an initial manifestation of Wilson's disease. Nephron. 1996;73(3):477–9.
55. Kuan P. Cardiac Wilson's disease. Chest. 1987;91:579–83.
56. Werlin SL, et al. Diagnostic dilemmas of Wilson's disease: diagnosis and treatment. Pediatrics. 1978;62(1):47–51.
57. Ferenci P, et al. Diagnosis and phenotypic classification of Wilson disease. Liver Int. 2003;23(3):139–42.

58. Dhawan A, et al. Wilson's disease in children: 37-year experience and revised King's score for liver transplantation. Liver Transpl. 2005;11(4):441–8.
59. Nicastro E, et al. Re-evaluation of the diagnostic criteria for Wilson disease in children with mild liver disease. Hepatology. 2010;52(6):1948–56.
60. European Association for Study of Liver. EASL clinical practice guidelines: Wilson's disease. J Hepatol. 2012;56(3):671–85.
61. Dorney SF, et al. Wilson's disease in childhood. A plea for increased awareness. Med J Aust. 1986; 145(10):538–41.
62. Taylor RM, Chen Y, Dhawan A. Triethylene tetramine dihydrochloride (trientine) in children with Wilson disease: experience at King's College Hospital and review of the literature. Eur J Pediatr. 2009;168(9): 1061–8.
63. Narumi S, et al. Liver transplantation for Wilson's disease in pediatric patients: decision making and timing. Transplant Proc. 2012;44(2):478–80.
64. Bem RS, et al. Wilson's disease in southern Brazil: a 40-year follow-up study. Clinics (Sao Paulo). 2011; 66(3):411–6.
65. Corey M, et al. Longitudinal analysis of pulmonary function decline in patients with cystic fibrosis. J Pediatr. 1997;131(6):809–14.
66. Corey M, Farewell V. Determinants of mortality from cystic fibrosis in Canada, 1970–1989. Am J Epidemiol. 1996;143(10):1007–17.
67. Kerem E, et al. Prediction of mortality in patients with cystic fibrosis. N Engl J Med. 1992;326(18): 1187–91.
68. Efrati O, et al. Liver cirrhosis and portal hypertension in cystic fibrosis. Eur J Gastroenterol Hepatol. 2003; 15(10):1073–8.
69. Colombo C. Liver disease in cystic fibrosis. Curr Opin Pulm Med. 2007;13(6):529–36.
70. Colombo C, et al. Liver disease in cystic fibrosis: a prospective study on incidence, risk factors, and outcome. Hepatology. 2002;36(6):1374–82.
71. Lamireau T, et al. Epidemiology of liver disease in cystic fibrosis: a longitudinal study. J Hepatol. 2004; 41(6):920–5.
72. Scott-Jupp R, Lama M, Tanner MS. Prevalence of liver disease in cystic fibrosis. Arch Dis Child. 1991; 66(6):698–701.
73. Bhattacharjee R, et al. The natural history of liver disease in cystic fibrosis. J Cyst Fibros. 2006;5:S61.
74. Williams SM, et al. Ultrasound evaluation of liver disease in cystic fibrosis as part of an annual assessment clinic: a 9-year review. Clin Radiol. 2002;57(5): 365–70.
75. Lenaerts C, et al. Surveillance for cystic fibrosis-associated hepatobiliary disease: early ultrasound changes and predisposing factors. J Pediatr. 2003; 143(3):343–50.
76. Lindblad A, Glaumann H, Strandvik B. Natural history of liver disease in cystic fibrosis. Hepatology. 1999;30(5):1151–8.
77. Desmond CP, et al. The benign course of liver disease in adults with cystic fibrosis and the effect of ursodeoxycholic acid. Liver Int. 2007;27(10):1402–8.
78. Lewindon PJ, Ramm GA. Cystic fibrosis-cirrhosis, portal hypertension, and liver biopsy: reply. Hepatology. 2011;53(3):1065–6.
79. Feigelson J, et al. Liver cirrhosis in cystic fibrosis–therapeutic implications and long term follow up. Arch Dis Child. 1993;68(5):653–7.
80. Chryssostalis A, et al. Liver disease in adult patients with cystic fibrosis: a frequent and independent prognostic factor associated with death or lung transplantation. J Hepatol. 2011;55:1377–82.
81. Lewindon PJ, et al. Importance of hepatic fibrosis in cystic fibrosis and the predictive value of liver biopsy. Hepatology. 2011;53(1):193–201.
82. Witters P, et al. Noncirrhotic presinusoidal portal hypertension is common in cystic fibrosis-associated liver disease. Hepatology. 2011;53(3):1064–5.
83. Rowland M, et al. Outcome in cystic fibrosis liver disease. Am J Gastroenterol. 2011;106(1):104–9.
84. Debray D, et al. Outcome of cystic fibrosis-associated liver cirrhosis: management of portal hypertension. J Hepatol. 1999;31(1):77–83.
85. Polineni D, et al. Pulmonary function (FEV1) in cystic fibrosis patients with and without severe liver disease with portal hypertension (CFLD). Am J Respir Crit Care Med. 2009;A1779.
86. Minicucci L, et al. Liver disease as risk factor for cystic fibrosis-related diabetes development. Acta Paediatr. 2007;96(5):736–9.
87. Sullivan KM, Moran A, Schwarzenberg S. Cystic fibrosis related diabetes in CF patients with cirrhosis. Pediatr Pulmonol. 2009;44:414.
88. Corbett K, et al. Cystic fibrosis-associated liver disease: a population-based study. J Pediatr. 2004;145(3): 327–32.
89. Blanc WA, Di Sant'Agnese PA. A distinctive type of biliary cirrhosis of the liver associated with cystic fibrosis of the pancreas; recognition through signs of portal hypertension. Pediatrics. 1956;18(3): 387–409.
90. Vawter GF, Shwachman H. Cystic fibrosis in adults: an autopsy study. Pathol Annu. 1979;14(Pt 2): 357–82.
91. Robertson MB, Choe KA, Joseph PM. Review of the abdominal manifestations of cystic fibrosis in the adult patient. Radiographics. 2006;26(3):679–90.
92. Woodruff SA, et al. Prevalence of elevated liver function tests in children with cystic fibrosis diagnosed by newborn screen. J Pediatr Gastroenterol Nutr. 2007; 45:E27–8.
93. Mayer-Hamblett N, et al. Incidence and clinical significance of elevated liver function tests in cystic fibrosis clinical trials. Contemp Clin Trials. 2012; 34(2):232–8.
94. Debray D, et al. Best practice guidance for the diagnosis and management of cystic fibrosis-associated liver disease. J Cyst Fibros. 2011;10 Suppl 2:S29–36.

95. Herrmann U, Dockter G, Lammert F. Cystic fibrosis-associated liver disease. Best Pract Res Clin Gastroenterol. 2010;24(5):585–92.
96. Akata D, Akhan O. Liver manifestations of cystic fibrosis. Eur J Radiol. 2007;61(1):11–7.
97. Diwakar V, Pearson L, Beath S. Liver disease in children with cystic fibrosis. Paediatr Respir Rev. 2001;2(4):340–9.
98. Patriquin H, et al. Liver disease in children with cystic fibrosis: US-biochemical comparison in 195 patients. Radiology. 1999;211(1):229–32.
99. Durieu I, et al. Sclerosing cholangitis in adults with cystic fibrosis: a magnetic resonance cholangiographic prospective study. J Hepatol. 1999;30(6):1052–6.
100. Lykavieris P, Bernard O, Hadchouel M. Neonatal cholestasis as the presenting feature in cystic fibrosis. Arch Dis Child. 1996;75(1):67–70.
101. Wilschanski M, Durie PR. Patterns of GI disease in adulthood associated with mutations in the CFTR gene. Gut. 2007;56(8):1153–63.
102. Angelico M, et al. Gallstones in cystic fibrosis: a critical reappraisal. Hepatology. 1991;14(5):768–75.
103. Wasmuth HE, et al. Coinheritance of Gilbert syndrome-associated UGT1A1 mutation increases gallstone risk in cystic fibrosis. Hepatology. 2006;43(4):738–41.
104. Cohn JA, et al. Localization of the cystic fibrosis transmembrane conductance regulator in human bile duct epithelial cells. Gastroenterology. 1993;105(6):1857–64.
105. Gerling B, et al. Prediction of liver fibrosis according to serum collagen VI level in children with cystic fibrosis. N Engl J Med. 1997;336(22):1611–2.
106. Pereira TN, et al. Serum markers of hepatic fibrogenesis in cystic fibrosis liver disease. J Hepatol. 2004;41(4):576–83.
107. Sidlova K, et al. Serum alpha-glutathione S-transferase as a sensitive marker of hepatocellular damage in patients with cystic fibrosis. Physiol Res. 2003;52(3):361–5.
108. Rath T, et al. TIMP-1/-2 and transient elastography allow non invasive diagnosis of cystic fibrosis associated liver disease. Dig Liver Dis. 2012;44(9):780–7.
109. Williams SG, et al. An ultrasound scoring system for the diagnosis of liver disease in cystic fibrosis. J Hepatol. 1995;22(5):513–21.
110. Mueller-Abt PR, et al. Comparison of ultrasound and biopsy findings in children with cystic fibrosis related liver disease. J Cyst Fibros. 2008;7(3):215–21.
111. Witters P, et al. Non-invasive liver elastography (Fibroscan) for detection of cystic fibrosis-associated liver disease. J Cyst Fibros. 2009;8(6):392–9.
112. Malbrunot-Wagner AC, et al. Transient elastography and portal hypertension in pediatric patients with cystic fibrosis Transient elastography and cystic fibrosis. J Cyst Fibros. 2011;10(5):338–42.
113. Boursier J, et al. Acoustic radiation force impulse: a new ultrasonographic technology for the widespread noninvasive diagnosis of liver fibrosis. Eur J Gastroenterol Hepatol. 2010;22(9):1074–84.
114. Baranova A, et al. Non-invasive markers for hepatic fibrosis. BMC Gastroenterol. 2011;11:91.
115. Manco M, et al. Pilot study on the use of acoustic radiation force impulse imaging in the staging of cystic fibrosis associated liver disease. J Cyst Fibros. 2012;11:427–32.
116. Colombo C, et al. Liver disease in cystic fibrosis. J Pediatr Gastroenterol Nutr. 2006;43 Suppl 1:S49–55.
117. Moyer K, Balistreri W. Hepatobiliary disease in patients with cystic fibrosis. Curr Opin Gastroenterol. 2009;25(3):272–8.
118. Sokol RJ, Durie PR. Recommendations for management of liver and biliary tract disease in cystic fibrosis. Cystic Fibrosis Foundation Hepatobiliary Disease Consensus Group. J Pediatr Gastroenterol Nutr. 1999;28 Suppl 1:S1–13.
119. Cheng K, Ashby D, Smyth RL. Ursodeoxycholic acid for cystic fibrosis-related liver disease. Cochrane Database Syst Rev. 2012;10:CD000222.
120. Ooi CY, et al. Ursodeoxycholic acid in cystic fibrosis-associated liver disease. J Cyst Fibros. 2012;11(1):72–3.
121. Neglia JP, et al. The risk of cancer among patients with cystic fibrosis. Cystic Fibrosis and Cancer Study Group. N Engl J Med. 1995;332(8):494–9.
122. O'Donnell DH, et al. Hepatocellular carcinoma complicating cystic fibrosis related liver disease. J Cyst Fibros. 2009;8(4):288–90.
123. Pozler O, et al. Transjugular intrahepatic portosystemic shunt in five children with cystic fibrosis: long-term results. Hepatogastroenterology. 2003;50(52):1111–4.
124. Louis D, et al. Partial splenectomy for portal hypertension in cystic fibrosis related liver disease. Pediatr Pulmonol. 2007;42(12):1173–80.
125. Harned 2nd RK, et al. Partial splenic embolization in five children with hypersplenism: effects of reduced-volume embolization on efficacy and morbidity. Radiology. 1998;209(3):803–6.
126. Fridell JA, et al. Liver transplantation in children with cystic fibrosis: a long-term longitudinal review of a single center's experience. J Pediatr Surg. 2003;38(8):1152–6.
127. Milkiewicz P, et al. Transplantation for cystic fibrosis: outcome following early liver transplantation. J Gastroenterol Hepatol. 2002;17(2):208–13.
128. Noble-Jamieson G, et al. Liver transplantation for hepatic cirrhosis in cystic fibrosis. J R Soc Med. 1996;89 Suppl 27:31–7.
129. Gridelli B. Liver: benefit of liver transplantation in patients with cystic fibrosis. Nat Rev Gastroenterol Hepatol. 2011;8(4):187–8.
130. Mendizabal M, et al. Liver transplantation in patients with cystic fibrosis: analysis of United Network for Organ Sharing data. Liver Transpl. 2011;17(3):243–50.

131. Lu BR, Esquivel CO. A review of abdominal organ transplantation in cystic fibrosis. Pediatr Transplant. 2010;14:954–60.
132. Melzi ML, et al. Liver transplant in cystic fibrosis: a poll among European centers. A study from the European Liver Transplant Registry. Transpl Int. 2006;19(9):726–31.
133. Genyk YS, et al. Liver transplantation in cystic fibrosis. Curr Opin Pulm Med. 2001;7(6):441–7.
134. Nash KL, et al. Cystic fibrosis liver disease: to transplant or not to transplant? Am J Transplant. 2008; 8(1):162–9.
135. Miller MR, et al. Pulmonary function in individuals who underwent liver transplantation: from the US cystic fibrosis foundation registry. Liver Transpl. 2012;18(5):585–93.
136. Bartlett JR, et al. Genetic modifiers of liver disease in cystic fibrosis. JAMA. 2009;302(10):1076–83.
137. Nightingale S, et al. Isolated liver transplantation in children with cystic fibrosis–an Australian experience. Pediatr Transplant. 2010;14(6):779–85.
138. Barshes NR, et al. Combined lung and liver transplantation: the United States experience. Transplantation. 2005;80(9):1161–7.
139. De Domenico I, Ward DM, Kaplan J. Hepcidin and ferroportin: the new players in iron metabolism. Semin Liver Dis. 2011;31(3):272–9.
140. Pietrangelo A, Caleffi A, Corradini E. Non-HFE hepatic iron overload. Semin Liver Dis. 2011;31(3):302–18.
141. Evstatiev R, Gasche C. Iron sensing and signalling. Gut. 2012;61:933–52.
142. Donovan A, et al. The iron exporter ferroportin/Slc40a1 is essential for iron homeostasis. Cell Metab. 2005;1(3):191–200.
143. Vaulont S, et al. Of mice and men: the iron age. J Clin Invest. 2005;115(8):2079–82.
144. Pietrangelo A. Hepcidin in human iron disorders: therapeutic implications. J Hepatol. 2011;54(1):173–81.
145. Huang FW, et al. A mouse model of juvenile hemochromatosis. J Clin Invest. 2005;115(8):2187–91.
146. Feder JN, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet. 1996;13(4):399–408.
147. Babitt JL, Lin HY. The molecular pathogenesis of hereditary hemochromatosis. Semin Liver Dis. 2011;31(3):280–92.
148. Phatak PD, et al. Prevalence of hereditary hemochromatosis in 16031 primary care patients. Ann Intern Med. 1998;129(11):954–61.
149. Olynyk JK, et al. A population-based study of the clinical expression of the hemochromatosis gene. N Engl J Med. 1999;341(10):718–24.
150. Edwards CQ, et al. Prevalence of hemochromatosis among 11,065 presumably healthy blood donors. N Engl J Med. 1988;318(21):1355–62.
151. Pietrangelo A. Hereditary hemochromatosis: pathogenesis, diagnosis, and treatment. Gastroenterology. 2010;139(2):393–408, 408 e1–2.
152. Pietrangelo A. Juvenile hemochromatosis. J Hepatol. 2006;45(6):892–4.
153. Adams PC, Kertesz AE, Valberg LS. Screening for hemochromatosis in children of homozygotes: prevalence and cost-effectiveness. Hepatology. 1995; 22(6):1720–7.
154. Grove J, et al. Heterozygotes for HFE mutations have no increased risk of advanced alcoholic liver disease. Gut. 1998;43(2):262–6.
155. Bonkovsky HL, et al. Non-alcoholic steatohepatitis and iron: increased prevalence of mutations of the HFE gene in non-alcoholic steatohepatitis. J Hepatol. 1999;31(3):421–9.
156. Kowdley KV, et al. Utility of hepatic iron index in American patients with hereditary hemochromatosis: a multicenter study. Gastroenterology. 1997; 113(4):1270–7.
157. Martin DR, Semelka RC. Magnetic resonance imaging of the liver: review of techniques and approach to common diseases. Semin Ultrasound CT MR. 2005;26(3):116–31.
158. Adams PC. Implications of genotyping of spouses to limit investigation of children in genetic hemochromatosis. Clin Genet. 1998;53(3):176–8.
159. Guyader D, et al. Noninvasive prediction of fibrosis in C282Y homozygous hemochromatosis. Gastroenterology. 1998;115(4):929–36.
160. Whitlock EP, et al. Screening for hereditary hemochromatosis: a systematic review for the U.S. Preventive Services Task Force. Ann Intern Med. 2006;145(3):209–23.
161. Kumpe DA, et al. Partial splenic embolization in children with hypersplenism. Radiology. 1985; 155(2):357–62.
162. Gehrke SG, et al. HJV gene mutations in European patients with juvenile hemochromatosis. Clin Genet. 2005;67(5):425–8.
163. Wolfe L, et al. Prevention of cardiac disease by subcutaneous deferoxamine in patients with thalassemia major. N Engl J Med. 1985;312(25):1600–3.
164. Nielsen P, et al. Liver iron stores in patients with secondary haemosiderosis under iron chelation therapy with deferoxamine or deferiprone. Br J Haematol. 1995;91(4):827–33.
165. Carneiro AA, et al. Liver iron concentration evaluated by two magnetic methods: magnetic resonance imaging and magnetic susceptometry. Magn Reson Med. 2005;54(1):122–8.
166. Brittenham GM, et al. Efficacy of deferoxamine in preventing complications of iron overload in patients with thalassemia major. N Engl J Med. 1994; 331(9):567–73.
167. Cabibbo S, et al. Chronic red blood cell exchange to prevent clinical complications in sickle cell disease. Transfus Apher Sci. 2005;32(3):315–21.
168. Cappellini MD, Musallam KM, Taher AT. Overview of iron chelation therapy with desferrioxamine and deferiprone. Hemoglobin. 2009;33 Suppl 1:S58–69.

Neonatal Hemochromatosis and Gestational Alloimmune Liver Disease

10

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Neonatal hemochromatosis (NH) is a clinical phenotype consisting of severe liver disease in the newborn accompanied by siderosis of extrahepatic tissues in the pattern seen in hereditary hemochromatosis [1, 2]. NH was described in the mid-twentieth century as a probable inborn error in iron metabolism [3, 4], and synonyms appearing in the literature since, including neonatal iron storage disease and perinatal hemochromatosis, imply the role of iron overload in the pathogenesis of the disease. NH has been classified as being part of the family of hereditary hemochromatosis disorders (OMIM 231100). However, recent advances in understanding have made it clear that NH never results from primary iron overload due to an inherited disturbance in the regulation of iron homeostasis as do the other members of this family. Instead NH represents a form of secondary iron overload in which severe fetal liver disease disturbs maternofetal iron homeostasis and in fact is a phenotypic expression of severe fetal liver disease [5, 6]. It has now been well established that Gestational Alloimmune Liver Disease (GALD) is the cause of nearly all instances of NH [7]. In addition, GALD may cause acute liver failure in the fetus and neonatal acute liver failure in the absence of extrahepatic siderosis. Thus, GALD has emerged as the cause of most instances of NH, whereas NH is a phenotype that is symptomatic of GALD and other fetal liver diseases.

The changing landscape of the NH field has inevitably produced problems in the lexicon. It will probably take many years for clinicians to not refer to *neonatal hemochromatosis* as the disease, if that ever occurs, and to not think of it as an iron overload disorder. In this chapter, GALD is described as the fetal disease leading to most NH, NH is the phenotype in neonates that has been described in many publications and may have more than one etiology, and GALD-NH is proven GALD with the NH phenotype.

Etiology and Pathogenesis of NH

It has been suspected for several decades that NH stems from fetal liver disease [8–10]. Yet, until recently the cause of NH-related fetal liver disease remained undetermined. The unusual pattern and high rate of recurrence in affected maternal sibships led to the hypothesis that maternofetal alloimmunity might be involved [11]. After the index case in a maternal sibship, the probability that each subsequent baby born to that mother will be affected is over 90 % [12]. A woman may have unaffected offspring before having the first child with NH, but thereafter most pregnancies terminate in either a fetal loss or a child born with NH. There are several documented instances of a woman

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giving birth to affected babies with different male parentage. It has never been recorded that female siblings of women having a baby with NH themselves have had an affected baby. In addition, several women who survived NH as infants have themselves had healthy unaffected children. These findings are not consistent with any known pattern of inheritance. Thus, NH appears to be congenital and familial, but not hereditary [13].

Complement-mediated hepatocyte injury was recently identified as mechanistically involved in liver injury associated with NH. Immunohistochemical staining demonstrated deposition of C5b-9 complex (the neoantigen created during terminal complement cascade activation and culmination) in nearly all hepatocytes in a large cohort of NH cases [14]. Finding C5b-9 complex on or in a cell provides indisputable evidence that the terminal complement cascade has been activated on that cell. Maternofetal alloimmunity is mediated by IgG as that is the only component of maternal immunity that freely crosses the placenta [15]. IgG subclasses IgG1 and IgG3 are both capable of crossing the placenta and activating the terminal complement cascade via the classical pathway. Since activation of the terminal complement cascade in these circumstances must involve binding of maternal IgG to a fetal hepatocyte antigen, maternofetal alloimmunity was thus identified for certain as the cause of fetal liver disease leading to NH [7]. The specific alloantigen in fetal liver has not been identified to date, and how exposure to the maternal circulation occurs is not known. However, once sensitization has occurred, maternal antifetal liver IgG may pass to the fetus where it binds to the liver antigen and result in immune injury of fetal hepatocytes, thus producing *Gestational Alloimmune Liver Disease* (GALD).

NH may result from fetal liver diseases other than GALD. Such diseases must seriously impair fetal liver function at some interval before birth in order to impair regulation of placental iron flux and cause iron overload and tissue siderosis in the newborn [6]. Few non-GALD diseases have been firmly associated with the NH phenotype: trisomy 21 [16–18], mitochondrial DNA depletion due to deoxyguanosine kinase deficiency (DGUOK gene mutations) [19–21], and the bile acid synthetic defect delta 4-3-oxosteroid 5 beta-reductase deficiency (SRD5B1 mutations) [22–24].

Pathology of NH and GALD

The liver pathology in NH has been widely described and while not pathognomonic for the disease is highly characteristic and determines the clinical and associated pathologic findings [1, 2, 8, 25–29]. It should be remembered that nearly all NH is due to GALD, so the published descriptions of NH over the years are likely descriptions of GALD-NH. As evidence of this, the 33 cases of anatomically proven NH studied to show complement-mediated hepatocyte injury had histopathology consonant with that of NH reported in the earlier literature [14]. The liver injury associated with NH is among the most destructive described in any human liver disease. It can be classified as being subacute or chronic on pathologic grounds, and cirrhosis is evident in most cases (Fig. 10.1).

The injury in GALD is directed at hepatocytes. Clinical and other evidence suggest that GALD-associated injury begins as early as 18–20 weeks' gestation and results in progressive loss of hepatocyte mass [5, 30]. The hepatocyte volume density (measure of fraction of hepatic mass made up by hepatocytes) in NH liver is reduced to about 25 % of that in normal newborn liver [30]. Since the liver in NH cases is typically very small, the hepatocyte mass may be reduced to 10 % or less of normal. Many specimens contain no identifiable hepatocytes. Residual hepatocytes often exhibit giant-cell or pseudoacinar transformation and in GALD-NH contain large amounts of C5b-9 complex (membrane attack complex) on immunohistochemistry (Fig. 10.1) [14]. This latter finding is very specific for GALD and permits the causative diagnosis to be established using formalin-fixed paraffin-embedded liver tissue [31]. With the loss of hepatocyte mass, most liver specimens show collapse of the reticulin framework and reduced interportal spacing. Lobular fibrosis is pronounced (Fig. 10.1). Residual

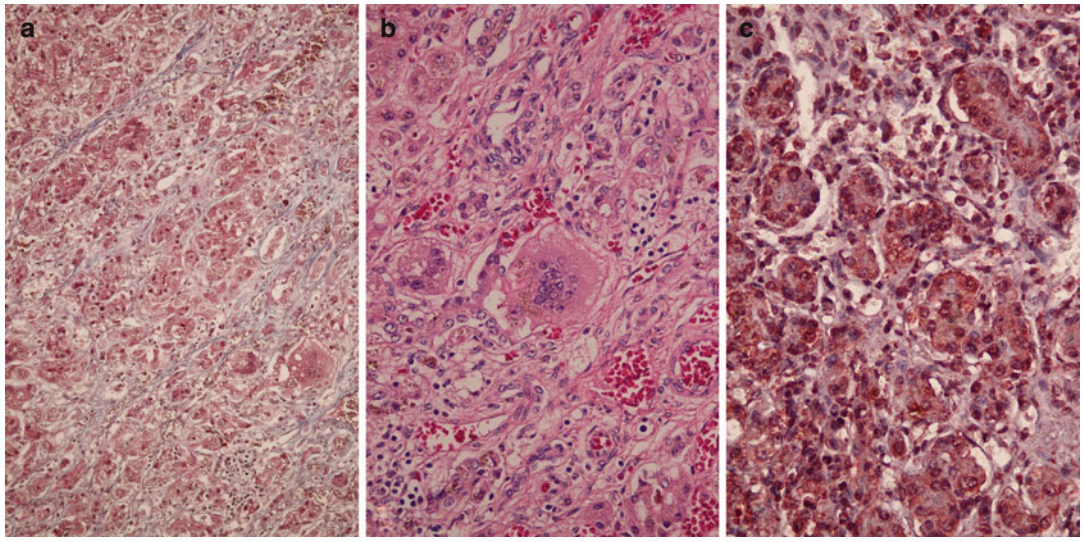

Fig. 10.1 Liver histopathology from a typical case of GALD. This infant was born at term. The mother had oligohydramnios, and the infant showed intrauterine growth restriction. Liver failure was evident immediately after birth. The infant died at 4 days of age without diagnosis. Postmortem examination was limited to the liver. When the mother was again pregnant, the liver specimen was referred for evaluation of possible GALD. Panel (**a**) shows the liver architecture to be highly deranged with extensive parenchymal fibrosis. The edge of a regenerative nodule is seen at the upper left (trichrome stain, 100× original magnification). Panel (**b**) shows the lobular architecture in greater detail. There has been extensive loss of hepatocytes. Residual hepatocytes appear as giant cells and pseudorosettes. The pigment in the central giant cell stained positively for hemosiderin (H&E, 200× original magnification). Panel (**c**) shows the pattern of staining for membrane attack complex. All residual hepatocytes in the specimen stained positively (C5b-9 immunohistochemistry, 200× original magnification). While a diagnosis of NH could not be established because of the limited autopsy, the diagnosis of GALD was made, and gestational treatment was recommended for the current pregnancy

hepatocytes often show siderosis, while Kupffer cells are spared. The siderosis is coarsely granular, which is in contrast to the hazy iron staining of normal newborn liver. Pathologic siderosis of hepatocytes is seen in many neonatal liver diseases and is not considered a diagnostic feature of NH [32]. Evidence of attempted regeneration is abundant in the liver of most cases of NH. Oval cells and neotubules appear throughout most specimens, and many cases show regenerative nodules. Paucity of inflammation is a notable feature probably related to the immature adaptive immune system in the fetus and newborn. The only inflammatory cells consistently seen in these cases are macrophages and to a lesser degree neutrophils. These cells are part of the innate immune system, and C3a and C5a, which are released during activation of the terminal complement cascade, act as potent chemokines for both.

GALD is defined by complement-mediated hepatocyte injury and is the only known liver disease to have this as the primary disease mechanism [14, 33]. The anatomic pathology of the liver in most cases of GALD-NH is the same as has been described for NH in general. GALD, however, may produce acute liver injury, which has not been included in the NH literature. GALD may produce severe acute liver injury in fetuses leading to fetal death [33]. This is in effect acute liver failure of the fetus. The pathology in such cases shows acute hepatocyte necrosis with no collapse and no fibrosis, indicating the hyperacuity of the process (Fig. 10.2). Many of these cases have no siderosis of the liver or other tissues. In addition, several term newborns with acute liver failure have been found to have GALD with pathology similar to that in cases of fetal death due to GALD (Fig. 10.3). It is not clear why some cases present with acute liver failure, while others

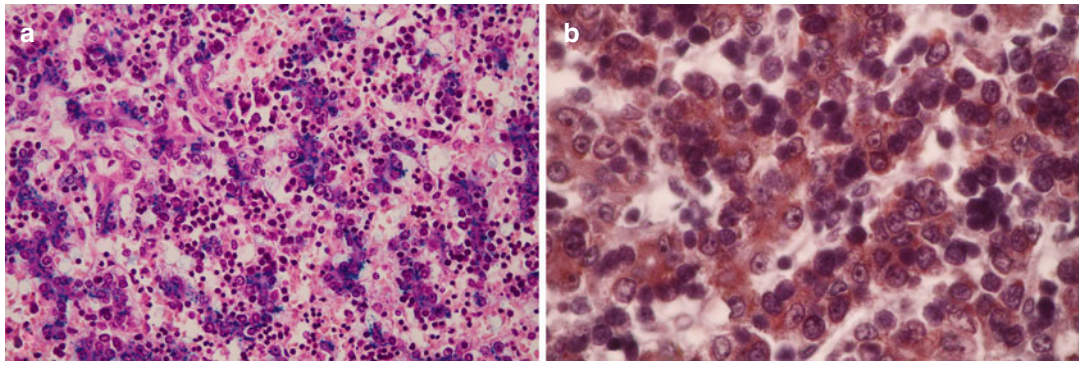

Fig. 10.2 Liver histopathology from a case of fetal death at 23 weeks' gestational age. Other findings included growth restriction and fetal hydrops. Panel (**a**) shows the overall parenchymal architecture. Extensive loss of hepatocytes is evident. Residual hepatocytes retain the cord orientation and contain pathologic hemosiderin (Perls' Prussian blue, 200× original magnification). Extensive collapse of reticulin was observed in some areas of the specimen. Panel (**b**) shows the pattern of staining for membrane attack complex. All residual hepatocytes in the specimen stained positively (C5b-9 immunohistochemistry, 400× original magnification). Siderosis was not detected in sections from thyroid, pancreas, and heart. The diagnosis of GALD with acute hepatic necrosis was established as the cause of fetal death

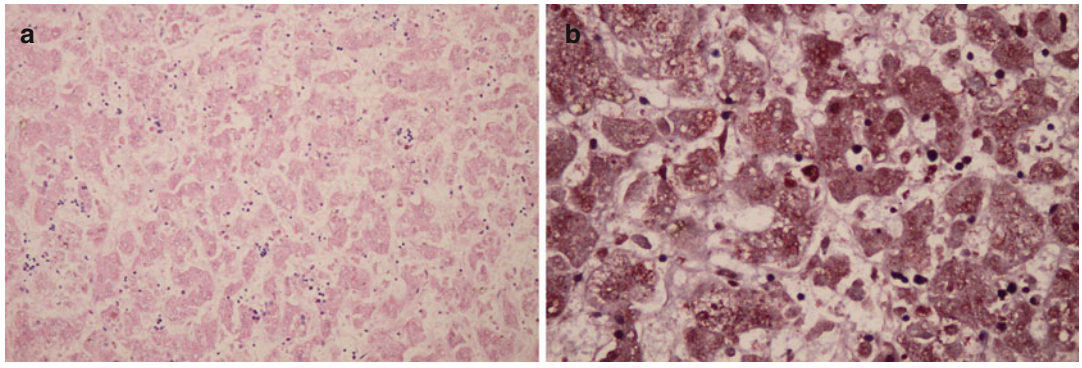

Fig. 10.3 Liver histopathology from a case of neonatal acute liver failure due to GALD. This infant was delivered by C-section at 35 weeks' gestation because of fetal distress. The infant had hydrops and died at 3 days of age. No diagnosis was established at postmortem examination. When the mother was again pregnant, the liver specimen was referred for evaluation of possible GALD. The mother had a subsequent stillbirth. Panel (**a**) shows the overall parenchymal architecture. Extensive loss of hepatocytes is evident. Residual hepatocytes retain the cord orientation (H&E, 100× original magnification). Panel (**b**) shows the pattern of staining for membrane attack complex. All residual hepatocytes in the specimen stained positively (C5b-9 immunohistochemistry, 200× original magnification). Tissues were not available for examination for extrahepatic siderosis. The diagnosis of GALD with acute hepatic necrosis was established as the cause of liver failure and death

present with congenital cirrhosis. We have found maternal siblings, one with acute liver pathology and no siderosis and another with cirrhosis and diffuse extrahepatic siderosis. So, the antigen target and the IgG response would appear to be the same, but the effect on the fetal liver is disparate.

NH is defined in part by the finding of siderosis of tissues outside the liver. The most consistently affected are the acinar epithelium of the exocrine pancreas, myocardium, the epithelia of the thyroid follicles, and the mucosal ("minor salivary") glands of the oronasopharynx and respiratory tree. Reticuloendothelial cells are spared. The mechanism by which GALD and perhaps other fetal liver diseases produce fetal iron overload involves the failure of control of placental iron flux. The fetal liver controls the flow of iron from the mother to the fetus by sensing iron sufficiency and producing hepcidin as a regulatory feedback molecule. Livers from fetus and newborn GALD

patients express hepcidin at a small fraction of that expressed by normal fetal and newborn liver [5]. The liver injury-induced failure to produce adequate hepcidin to appropriately regulate placental iron flux fully explains iron overload in GALD and probably also in other fetal liver disease leading to the NH phenotype [6]. The specific distribution of iron in extrahepatic tissues is determined by their ability to take up non-transferrin-bound iron (NTBI) via expression of ZIP14 and inability to export iron because of lack of ferroportin expression [5]. Tissues expressing ZIP14 and not ferroportin are uniquely susceptible to siderosis in conditions of excess NTBI. It should be remembered that ferroportin is fully expressed in GALD-NH despite iron overload because of the liver's incapacity to make hepcidin. This explains why reticuloendothelial cells do not show siderosis despite iron overload.

Isolated case reports have suggested an association between NH and renal hypoplasia with dysgenesis of proximal tubules and paucity of peripheral glomeruli [34–37]. Correlation with the process of normal renal development dates this arrest of renal development to about 24 weeks' gestation. This final stage of renal development is dependent upon liver function, specifically the synthesis of angiotensinogen. In a study of unselected cases of GALD-NH, liver angiotensinogen expression correlated closely with the mass of hepatocytes as determined by quantitative morphometry, and hepatocyte mass and angiotensinogen gene expression were markedly reduced relative to normal [30]. All GALD-NH cases had a degree of dysgenesis of proximal tubules, and the density of proximal tubules correlated closely with hepatic angiotensinogen gene expression. Thus, it appears that GALD alloimmune injury leads to reduced hepatocyte mass, which results in reduced angiotensinogen production, which in turn leads to defective renal development.

Clinical Presentation and Findings in NH and GALD

NH by definition is a neonatal disease, and its dominant presentation is with liver failure. The medical literature shows NH to be the prevalent if not dominant cause of neonatal liver failure [38–41]. In most series, NH has been the most common indication for liver transplantation in this age group [39, 41–45]. Despite this, it is probably underrepresented as a cause of neonatal illness for several reasons. First, liver failure is not easily diagnosed in the context of a sick neonate as many of the signs, symptoms, and laboratory abnormalities of sepsis and liver failure overlap. The diagnosis of "liver failure" in any age group depends upon demonstration of failure of one or more functions of the liver. Hepatic encephalopathy, the cardinal feature of liver failure in older children and adults, cannot be reliably identified in the neonate. By default, vitamin K-resistant coagulopathy is the defining feature of synthetic failure in neonates. Hypoglycemia is also a common feature of neonatal liver failure. However, sepsis in the neonate also manifests in both of these features. Thus, affected babies are frequently misdiagnosed as having overwhelming sepsis even with negative cultures. In addition, liver failure may produce symptom complexes that are confusing to clinicians and not traced to the liver at all. Neonatal hydrops and "bleeding diathesis" are two such symptom complexes from which neonates die without having been diagnosed with liver failure. Secondly, NH has been considered to be a rare disease and thus does not appear high in the differential diagnosis of a sick newborn. Neonatologists are generally unaware of its existence. Indeed, only in the past few years has a question about NH appeared on the neonatology board examination. Thus, even if a diagnosis of liver failure has been established, NH remains a remote consideration in most neonatology units. Thirdly, NH is difficult to diagnose with currently available tools. The diagnosis requires demonstration of siderosis in extrahepatic tissues, which proves difficult to accomplish in a sick newborn. Finally and perhaps most importantly, NH is itself a symptom complex, the phenotypic expression of fetal liver disease. NH should not be registered as a final diagnosis. Rather, the diagnosis line should read: liver failure due to or as a result of NH, due to or as result of fetal liver disease, due to or the result of "a primary disease capable of causing fetal liver injury." The "primary disease" in most cases will

Table 10.1 Contrasting clinical findings in the newborn with NH versus perinatal viral infection

	NH	Perinatal viral infection
Premature birth	Most (70–90 %)	Usual population incidence
Oligohydramnios	Most (70–90 %)	Exceedingly rare
Intrauterine growth restriction	Most (70–90 %)	Rare
Ascites	Common (40–60 %)	Exceedingly rare–never
Anasarca or hydrops	Uncommon (10–20 %)	Exceedingly rare–never
Patent ductus venosus	Most (70–90 %)	Exceedingly rare–never
Hepatomegaly	Uncommon (10–20 %)	Common
Hard liver	Always when palpable	Exceedingly rare–never
Splenomegaly	Uncommon (10–20 %)	Common though often mild
Maternal history of stillbirth or an infant with liver failure	Common (up to 30 %)	Rare

Table 10.2 Blood test results in the newborn with NH versus perinatal viral infection

	NH	Perinatal viral infection
Hypoglycemia	Usual	Usual
Coagulopathy	Nearly always (INR 2–6)	Often
ALT	Usually normal and almost always <100 IU/l	Nearly always >100 IU/l and often >1,000
Bilirubin	Elevated total and direct in nearly all cases after DOL 3	Usually normal or minimally elevated especially direct
Ferritin	Almost always >800 ng/ml, up to and exceeding 15,000	Usually <800 ng/ml
Alpha-fetoprotein	Almost always >80,000 mg/dl; typically >300–600,000	Almost always <80,000 mg/dl
Iron saturation	Almost always >90 %	Almost always <80 %
Plasma amino acids	Tyrosine and methionine often elevated	Usually normal

be GALD, but others also deserve consideration. That GALD can produce severe liver disease without the NH phenotype adds another layer of confusion.

It is important to suspect NH in order to diagnose it. All liver failure in the neonate is considered to be "acute liver failure" by the definition of time elapsed from onset of symptoms to the diagnosis of failure. However, NH presents in a manner that is distinctly different from other common causes of neonatal acute liver failure. NH results from fetal liver injury, and the image of NH presented in the bulk of published literature is one of congenital cirrhosis, where there is evidence of fetal insult *and* neonatal liver failure [46]. This association serves reasonably well to distinguish NH from liver failure due to causes acquired perinatally, such as viral infection (Table 10.1). Blood findings also help in distinguishing NH cases from acute liver failure due to perinatal viral infections (Table 10.2). The other major causes of neonatal liver failure such as mitochondrial disease (especially respiratory chain defects and mitochondrial DNA depletion due to DGUOK gene mutations), bile acid synthetic defects (especially delta 4-3-oxosteroid 5 beta-reductase deficiency), ABCB11 gene mutations (type 2 progressive familial intrahepatic cholestasis), tyrosinemia type 1, hereditary galactosemia, hereditary fructose intolerance, and hemophagocytic lymphohistiocytosis should be kept in mind and excluded by appropriate testing when indicated.

In summary, a baby with NH typically is sick from birth and shows evidence of fetal insult. A maternal history of child lost by stillbirth or neonatal illness compatible with liver failure is an important finding. Signs of liver failure such as coagulopathy and hypoglycemia should be present. While babies with NH develop cholestasis, it is usually not evident at birth and is not a primary symptom. Cholestasis without coagulopathy

does not add up to NH. ALT (and AST) values are typically much lower than in other diseases presenting with liver failure. Hyperferritinemia and elevated alpha-fetoprotein are consistent but nonspecific findings. This is the classic presentation, but there are variations. In rare cases the liver disease takes a prolonged course and is manifest days to weeks after birth. There is a spectrum of severity too as evidenced by twins with disparate clinical findings, one severely affected and the other minimally so [47].

While most NH results from GALD, GALD produces a spectrum of fetal and neonatal liver disease that is far broader than prototypic NH. One of its most common presentations is late-second and third trimester fetal loss. The gestational histories of women who have had a baby diagnosed with NH show that approximately 1 in 7 of their pregnancies end with fetal loss [12]. Study of the livers of 8 stillborn infants showed acute necrosis similar to that seen in acute liver failure and widespread C5b-9 complex inclusion in hepatocytes and hepatocyte ghosts [33]. Five of the eight infants had extrahepatic siderosis consistent with NH, but three did not. This proof of principle suggests that GALD can produce acute liver failure in the fetus and is probably the cause of the high rate of stillbirth in the maternal sibships of infants with NH. GALD can also produce acute liver failure with an acute pattern of liver injury in newborns. This is a confusing diagnostic scenario in that the typical clinical signs of NH are absent. A few such cases have presented with "nonimmune hydrops" and astutely diagnosed in postmortem examination as having acute hepatic necrosis. With the emerging knowledge that GALD can produce this pattern of injury, specimens were referred for study, which showed widespread C5b-9 complex inclusion in residual hepatocytes.

Diagnosis

The approach to diagnosing GALD-NH relies mainly on diagnosing NH, since nearly all NH is due to GALD. It should be suspected in infants who manifest liver disease antenatally or very shortly after birth. It should also be suspected in unexplained cases of stillbirth and newborn death. Demonstration of extrahepatic siderosis is necessary to make the diagnosis of NH. Finding siderosis in the liver is not diagnostic. The normal newborn liver contains sufficient stainable iron to be confused with pathologic siderosis, although they are qualitatively different to the eyes of experienced pathologists. Furthermore, pathologic hepatic siderosis has been described in several neonatal liver diseases, usually not in combination with extrahepatic siderosis. The absence of stainable iron in the liver does not exclude the diagnosis since in many cases few if any hepatocytes remain, and hepatic siderosis in NH involves hepatocytes exclusively.

NH is often diagnosed at autopsy, where siderosis of many tissues can be demonstrated if looked for. Proper stains for iron (Prussian blue, Perls' stain) should be performed on the tissues typically involved when autopsy is performed on any baby with liver failure or suspected liver disease and in unexplained stillbirths and newborn deaths. Demonstration of extrahepatic siderosis in living babies can be by tissue biopsy or by magnetic resonance imaging (MRI). Biopsy of the oral mucosa is a clinically useful approach to obtain glandular tissue in which to demonstrate siderosis [48]. Differences in magnetic susceptibility between iron-laden and normal tissues on T2-weighted MRI can document siderosis of various tissues, particularly the pancreas and liver [49, 50]. The diagnostic utility of these approaches has never been formally evaluated. Oral mucosal biopsy often fails because an inadequate specimen not containing submucosal glands is obtained. Experience suggests that each of these tests has about 60 % sensitivity for diagnosing NH relative to autopsy. A recommended diagnostic approach is where one is done (depending on local ease and availability), and only if that is negative, the other is done. There is no need to do both simultaneously.

GALD can be diagnosed in the absence of NH, which is to say in the absence of extrahepatic siderosis. Liver biopsy immunohistochemistry for C5b-9 has been used to diagnose GALD in an infant with liver failure who could not be shown to have extrahepatic siderosis by MRI and oral mucosal biopsy [31]. This infant survived with

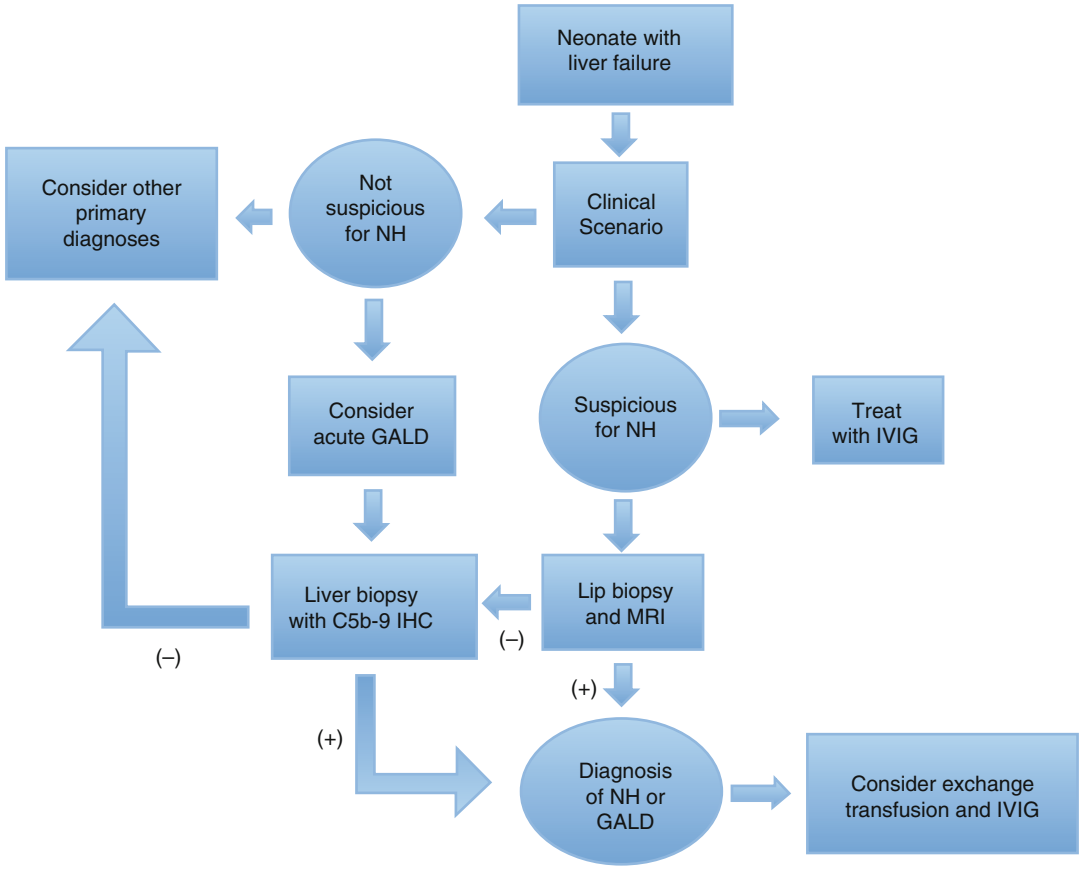

Fig. 10.4 Diagnostic paradigm for the diagnosis of GALD and NH in a newborn. The diagnosis should be considered in a neonate with acute liver failure. The clinical scenario (Table 10.1) determines suspicion for NH and triggers treatment with IVIG while diagnosis is pursued. Lip (oral mucosal) biopsy and MRI are used to demonstrate extrahepatic siderosis but have limited sensitivity. GALD should be considered along with other diagnoses in a clinical scenario not suspicious for NH. Liver biopsy with immunohistochemistry for C5b-9 complex can establish the diagnosis of GALD in cases suspicious for NH but with negative studies for extrahepatic siderosis and in cases not suspicious for NH. Once the diagnosis of GALD or NH has been established, additional treatment may be considered

treatment, so it cannot be said with certainty that there was no extrahepatic siderosis. This case points out the utility of this diagnostic approach when usual means of diagnosis are exhausted. GALD has also been diagnosed by this approach in cases of fetal death in which there was no siderosis of liver or extrahepatic tissues [33].

The foregoing leads to the following diagnostic approach, which if followed should minimize missed diagnoses (Fig. 10.4). In a newborn with liver failure or other suspicious clinical circumstances (e.g., nonimmune hydrops), an attempt should be made to demonstrate extrahepatic siderosis by oral mucosal biopsy and/or MRI. If positive, the diagnosis of NH is established. One can consider sending appropriate tests to rule out the non-GALD causes of NH at this point (i.e., bile acid synthesis defect and mitochondrial DNA depletion due to DGUOK mutations). If extrahepatic siderosis cannot be demonstrated, liver biopsy for C5b-9 staining can be considered. However, any newborn with liver failure and no identifiable other cause (e.g., perinatal herpes) should be considered to have GALD and treated accordingly (see below). Autopsy diagnosis is likewise straightforward. Stillbirths

undergoing postmortem should be examined for extrahepatic siderosis unless there is a clear cause for fetal death identified. Stillborn liver is often macerated, so a diagnosis of acute liver injury is obscured. C5b-9 staining is difficult to perform and interpret in macerated liver, so it cannot be recommended in such cases. Newborns dying of liver failure or under other suspicious circumstances and undergoing postmortem examination should be examined for extrahepatic siderosis. Liver disease can usually be diagnosed by histopathology in these circumstances; however, the changes of acute GALD are frequently ascribed to "postmortem change." Thus, in cases suspicious for GALD but with no extrahepatic siderosis, C5b-9 staining should be considered.

Treatment and Outcome

The older literature demonstrated a very poor prognosis for NH with medical therapy [44, 51, 52], and NH became a frequent indication for liver transplantation in the first 3 months of life [39, 41, 42, 44, 45]. When performed for NH, the difficulties attendant to transplanting newborns are frequently compounded by prematurity, small size for gestational age, and multiorgan failure, which have led to reduced survival in infants receiving a transplant for this indication [53].

Outcome with medical therapy using a cocktail of antioxidants and an iron chelator has been very poor, and it has become clear that this approach has no role in treating NH [54]. This therapy was based on the hypothesis that oxidative injury due to iron overload was central to disease pathogenesis, which appears not to be the case. Since GALD is the primary cause of NH, treatment applied successfully to other gestational alloimmune and passive autoimmune diseases has been used with much improved success [53]. The treatment employs the combination of double-volume exchange transfusion to remove existing reactive antibody and administration of high-dose IVIG (1 g/kg) to block antibody action (i.e., interfere with complement activation). The published experience with this approach in 16 infants with severe NH showed marked improvement in survival as compared to historical controls (published results with the antioxidant/chelation cocktail) [53]. Twelve subjects (75 %) had good outcome, defined as survival without liver transplantation, whereas good outcome was achieved in only 17 % (23/131) of historical control patients ($P < .001$). Four subjects died, 2 without and 2 after liver transplants. To date nearly 50 infants with severe NH have been treated in this way with about 80 % survival without liver transplant. The results of this tally may have been biased by non-report of poor outcomes or treatment of infants not having NH or only mild disease, since infants have been treated around the world not on protocol and the tally relies on often spontaneous reporting by email. Yet, this appears to be an important advance in treating NH, and it can be applied in less than advanced medical environments. Based on this experience, the recommended approach to treating NH is as follows. When presented with an infant in liver failure, if one even thinks it could be NH, a dose of IVIG should be given at once. This biological is very benign, and a single dose poses little or no risk to a newborn, no matter the disease. Then a formal evaluation can begin. If NH is proven and the infant has not shown significant clinical improvement, then exchange transfusion can be performed followed by a second dose of IVIG. Improvement in liver function may be long-coming because this approach might be expected to reduce ongoing immune-mediated injury, but would not necessarily be effective in reversing liver disease, which must occur before function improves. Normalization of the INR takes on average 4–6 weeks.

Limited published experience exists in regard to the outcome of babies who survive severe NH with medical therapy. Experience with the aforementioned immune therapy suggests that full recovery is likely. All 12 infants reported to survive were in clinical liver failure shortly after birth (average INR > 4), and most had clinical evidence of cirrhosis including a hard liver and ascites [53]. One had biopsy-proven cirrhosis. Recovery from liver failure to ability to discharge to home varied from 1 to 4 months. All of these infants have recovered fully with no residual

clinical liver disease after a period of 3–5 years. In a unique experience, two siblings with NH underwent liver biopsy as neonates and again after 2–4 years [55]. The initial biopsies demonstrated typical histology of severe NH with a pathologic diagnosis of cirrhosis. The repeat biopsies demonstrated normal histology with no pathologic findings. While a very limited experience, this suggests that the neonatal liver affected with NH is quite plastic and capable of recovery even from severe injury.

Prevention of GALD-NH

Accumulating experience indicates that recurrence of severe NH can be prevented by treatment during gestation [12, 56]. The current guideline for treatment consists of IVIG administered at a dose of 1 g/kg body weight at 14 weeks, 16 weeks, and weekly from the 18th week until the end of gestation. Women who have had a gestation affected with proven NH should be treated in lieu of any other marker for high risk of recurrence. A woman who has had a stillbirth and/or neonatal death in her gestational history is very suspicious for being affected by GALD-alloimmunity. If postmortem examinations have been performed, reexamination of the remains may establish a diagnosis of GALD (see Figs. 10.1, 10.2, and 10.3 as examples). The results of gestational treatment in 110 pregnancies recorded to date are as follows. One pregnancy was lost at 22 weeks due to severe acute GALD (treatment started at 18 weeks on an older protocol). Two infants were born prematurely, at 26 and 32 weeks, and both survived intact. Otherwise, no IUGR, fetal liver disease, or other evidence of fetal distress has been detected in any case. Five babies born after gestational treatment have had significant clinical liver disease. One of these died at 2 months of age from post-viral encephalomyelitis. In sum, 108 of 110 pregnancies resulted in children who are currently healthy and normal. This growing experience suggests that treatment with high-dose IVIG during gestation modifies recurrent GALD so that it is not lethal to the fetus or the newborn. It should be noted that without gestational treatment, the rate of recurrence of lethal disease is >90 % [12].

References

1. Knisely AS, Mieli-Vergani G, Whitington PF. Neonatal hemochromatosis. Gastroenterol Clin North Am. 2003;32:877–89, vi–vii.
2. Whitington PF. Fetal and infantile hemochromatosis. Hepatology. 2006;43:654–60.
3. Cottier H. A hemochromatosis similar disease in newborn. Schweiz Med Wochenschr. 1957;87:39–43.
4. Laurendeau T, Hill JE, Manning GB. Idiopathic neonatal hemochromatosis in siblings. An inborn error of metabolism. Arch Pathol. 1961;72:410–23.
5. Bonilla S, Prozialeck JD, Malladi P, Pan X, Yu S, Melin-Aldana H, Whitington PF. Neonatal iron overload and tissue siderosis due to gestational alloimmune liver disease. J Hepatol. 2012;56:1351–5.
6. Zoller H, Knisely AS. Control of iron metabolism – lessons from neonatal hemochromatosis. J Hepatol. 2012;56:1226–9.
7. Whitington PF. Gestational alloimmune liver disease and neonatal hemochromatosis. Semin Liver Dis. 2012;32:325–32.
8. Thaler MM. Fatal neonatal cirrhosis: entity or end result? A comparative study of 24 cases. Pediatrics. 1964;33:721–34.
9. Witzleben CL, Uri A. Perinatal hemochromatosis: entity or end result? Hum Pathol. 1989;20:335–40.
10. Hoogstraten J, de Sa DJ, Knisely AS. Fetal liver disease may precede extrahepatic siderosis in neonatal hemochromatosis. Gastroenterology. 1990;98:1699–701.
11. Whitington PF, Malladi P. Neonatal hemochromatosis: is it an alloimmune disease? J Pediatr Gastroenterol Nutr. 2005;40:544–9.
12. Whitington PF, Kelly S. Outcome of pregnancies at risk for neonatal hemochromatosis is improved by treatment with high-dose intravenous immunoglobulin. Pediatrics. 2008;121:e1615–21.
13. Whitington PF. Neonatal hemochromatosis: a congenital alloimmune hepatitis. Semin Liver Dis. 2007;27:243–50.
14. Pan X, Kelly S, Melin-Aldana H, Malladi P, Whitington PF. Novel mechanism of fetal hepatocyte injury in congenital alloimmune hepatitis involves the terminal complement cascade. Hepatology. 2010;51:2061–8.
15. Hoftman AC, Hernandez MI, Lee KW, Stiehm ER. Newborn illnesses caused by transplacental antibodies. Adv Pediatr. 2008;55:271–304.
16. Ruchelli ED, Uri A, Dimmick JE, Bove KE, Huff DS, Duncan LM, Jennings JB, et al. Severe perinatal liver disease and Down syndrome: an apparent relationship. Hum Pathol. 1991;22:1274–80.
17. Cheung PC, Ng WF, Chan AK. Neonatal haemochromatosis associated with Down syndrome. J Paediatr Child Health. 1995;31:249–52.

18. Neil E, Cortez J, Joshi A, Bawle EV, Poulik J, Zilberman M, El-Baba MF, et al. Hepatic failure, neonatal hemochromatosis and porto-pulmonary hypertension in a newborn with trisomy 21 – a case report. Ital J Pediatr. 2010;36:38.
19. Hanchard NA, Shchelochkov OA, Roy A, Wiszniewska J, Wang J, Popek EJ, Karpen S, et al. Deoxyguanosine kinase deficiency presenting as neonatal hemochromatosis. Mol Genet Metab. 2011;103:262–7.
20. Nobre S, Grazina M, Silva F, Pinto C, Goncalves I, Diogo L. Neonatal liver failure due to deoxyguanosine kinase deficiency. BMJ Case Rep. 2012;2012.
21. Pronicka E, Weglewska-Jurkiewicz A, Taybert J, Pronicki M, Szymanska-Debinska T, Karkucinska-Wieckowska A, Jakobkiewicz-Banecka J, et al. Post mortem identification of deoxyguanosine kinase (DGUOK) gene mutations combined with impaired glucose homeostasis and iron overload features in four infants with severe progressive liver failure. J Appl Genet. 2011;52:61–6.
22. Shneider BL, Setchell KD, Whitington PF, Neilson KA, Suchy FJ. Delta 4-3-oxosteroid 5 beta-reductase deficiency causing neonatal liver failure and hemochromatosis. J Pediatr. 1994;124:234–8.
23. Siafakas CG, Jonas MM, Perez-Atayde AR. Abnormal bile acid metabolism and neonatal hemochromatosis: a subset with poor prognosis. J Pediatr Gastroenterol Nutr. 1997;25:321–6.
24. Ueki I, Kimura A, Chen HL, Yorifuji T, Mori J, Itoh S, Maruyama K, et al. SRD5B1 gene analysis needed for the accurate diagnosis of primary 3-oxo-Delta4-steroid 5beta-reductase deficiency. J Gastroenterol Hepatol. 2009;24:776–85.
25. Silver MM, Beverley DW, Valberg LS, Cutz E, Phillips MJ, Shaheed WA. Perinatal hemochromatosis. Clinical, morphologic, and quantitative iron studies. Am J Pathol. 1987;128:538–54.
26. Knisely AS. Neonatal hemochromatosis. Am J Gastroenterol. 1988;83:1312–3.
27. Witzleben CL, Uri AK. Neonatal hemochromatosis. Am J Gastroenterol. 1988;83:1429.
28. Knisely AS. Neonatal hemochromatosis. Adv Pediatr. 1992;39:383–403.
29. Silver MM, Valberg LS, Cutz E, Lines LD, Phillips MJ. Hepatic morphology and iron quantitation in perinatal hemochromatosis. Comparison with a large perinatal control population, including cases with chronic liver disease. Am J Pathol. 1993;143:1312–25.
30. Bonilla SF, Melin-Aldana H, Whitington PF. Relationship of proximal renal tubular dysgenesis and fetal liver injury in neonatal hemochromatosis. Pediatr Res. 2010;67:188–93.
31. Debray FG, de Halleux V, Guidi O, Detrembleur N, Gaillez S, Rausin L, Goyens P, et al. Neonatal liver cirrhosis without iron overload caused by gestational alloimmune liver disease. Pediatrics. 2012;129:e1076–9.
32. Knisely AS. Iron and pediatric liver disease. Semin Liver Dis. 1994;14:229–35.
33. Whitington PF, Pan X, Kelly S, Melin-Aldana H, Malladi P. Gestational alloimmune liver disease in cases of fetal death. J Pediatr. 2011;159:612–6.
34. Bale PM, Kan AE, Dorney SF. Renal proximal tubular dysgenesis associated with severe neonatal hemosiderotic liver disease. Pediatr Pathol. 1994;14:479–89.
35. Johal JS, Thorp JW, Oyer CE. Neonatal hemochromatosis, renal tubular dysgenesis, and hypocalvaria in a neonate. Pediatr Dev Pathol. 1998;1:433–7.
36. Morris S, Akima S, Dahlstrom JE, Ellwood D, Kent A, Falk MC. Renal tubular dysgenesis and neonatal hemochromatosis without pulmonary hypoplasia. Pediatr Nephrol. 2004;19:341–4.
37. Waters PJ, Khashu M, Lillquist Y, Senger C, Mattman A, Demos M, Setchell K, et al. Neonatal hyperphenylalaninemia, perinatal hemochromatosis, and renal tubulopathy: a unique patient or a novel metabolic disorder? Mol Genet Metab. 2005;86 Suppl 1:S148–52.
38. Shneider BL. Neonatal liver failure. Curr Opin Pediatr. 1996;8:495–501.
39. Durand P, Debray D, Mandel R, Baujard C, Branchereau S, Gauthier F, Jacquemin E, et al. Acute liver failure in infancy: a 14-year experience of a pediatric liver transplantation center. J Pediatr. 2001;139:871–6.
40. Squires Jr RH, Shneider BL, Bucuvalas J, Alonso E, Sokol RJ, Narkewicz MR, Dhawan A, et al. Acute liver failure in children: the first 348 patients in the pediatric acute liver failure study group. J Pediatr. 2006;148:652–8.
41. Sundaram SS, Alonso EM, Whitington PF. Liver transplantation in neonates. Liver Transpl. 2003;9:783–8.
42. Grabhorn E, Richter A, Burdelski M, Rogiers X, Ganschow R. Neonatal hemochromatosis: long-term experience with favorable outcome. Pediatrics. 2006;118:2060–5.
43. Grabhorn E, Richter A, Fischer L, Ganschow R. Emergency liver transplantation in neonates with acute liver failure: long-term follow-up. Transplantation. 2008;86:932–6.
44. Rodrigues F, Kallas M, Nash R, Cheeseman P, D'Antiga L, Rela M, Heaton ND, et al. Neonatal hemochromatosis – medical treatment vs. transplantation: the King's experience. Liver Transpl. 2005;11:1417–24.
45. Woodle ES, Millis JM, So SK, McDiarmid SV, Busuttil RW, Esquivel CO, Whitington PF, et al. Liver transplantation in the first three months of life. Transplantation. 1998;66:606–9.
46. Whitington PF, Kelly S, Ekong UD. Neonatal hemochromatosis: fetal liver disease leading to liver failure in the fetus and newborn. Pediatr Transplant. 2005;9:640–5.
47. Ekong UD, Kelly S, Whitington PF. Disparate clinical presentation of neonatal hemochromatosis in twins. Pediatrics. 2005;116:e880–4.
48. Knisely AS, O'Shea PA, Stocks JF, Dimmick JE. Oropharyngeal and upper respiratory tract mucosal-gland siderosis in neonatal hemochromatosis: an approach to biopsy diagnosis. J Pediatr. 1988; 113:871–4.

49. Hayes AM, Jaramillo D, Levy HL, Knisely AS. Neonatal hemochromatosis: diagnosis with MR imaging. AJR Am J Roentgenol. 1992;159:623–5.
50. Udell IW, Barshes NR, Voloyiannis T, Lee TC, Karpen SJ, Carter BA, Finegold M, et al. Neonatal hemochromatosis: radiographical and histological signs. Liver Transpl. 2005;11:998–1000.
51. Flynn DM, Mohan N, McKiernan P, Beath S, Buckels J, Mayer D, Kelly DA. Progress in treatment and outcome for children with neonatal haemochromatosis. Arch Dis Child Fetal Neonatal Ed. 2003;88:F124–7.
52. Vohra P, Haller C, Emre S, Magid M, Holzman I, Ye MQ, Iofel E, et al. Neonatal hemochromatosis: the importance of early recognition of liver failure. J Pediatr. 2000;136:537–41.
53. Rand EB, Karpen SJ, Kelly S, Mack CL, Malatack JJ, Sokol RJ, Whitington PF. Treatment of neonatal hemochromatosis with exchange transfusion and intravenous immunoglobulin. J Pediatr. 2009; 155:566–71.
54. Leonis MA, Balistreri WF. Neonatal hemochromatosis: it's OK to say "NO" to antioxidant-chelator therapy. Liver Transpl. 2005;11:1323–5.
55. Ekong UD, Melin-Aldana H, Whitington PF. Regression of severe fibrotic liver disease in 2 children with neonatal hemochromatosis. J Pediatr Gastroenterol Nutr. 2008;46:329–33.
56. Whitington PF, Hibbard JU. High-dose immunoglobulin during pregnancy for recurrent neonatal haemochromatosis. Lancet. 2004;364:1690–8.

Alagille Syndrome

11

Binita M. Kamath and David A. Piccoli

Nomenclature	
JAG1	Italicized gene
Notch	Signaling pathway
Notch	Receptor
NOTCH2, NOTCH3	Italicized gene

Introduction

Nearly 50 years ago, hepatologist Daniel Alagille recognized that a significant number of his patients with bile duct paucity also had abnormalities of the face, heart, eye, and spine [1]. He realized that this constellation was inherited in an autosomal dominant fashion in many families [2]. Alagille termed the disease "syndromic paucity of the interlobular ducts," in which the systemic manifestations were variable but included a particular facies, cholestasis, a heart murmur, posterior embryotoxon, and butterfly vertebrae. Watson and Miller reported patients with the same syndrome from the perspective of familial pulmonary artery stenoses with associated liver disease and termed the syndrome "arteriohepatic dysplasia" [3]. Alagille defined the diagnostic criteria for the syndrome as the combination of paucity and at least three of those five major criteria, providing a definition that proved useful and durable [4]. Over time, other organs have been recognized to be common or sometimes rare manifestations of the disease, and as more individuals were identified, the range of manifestations within an organ system was recognized to be as diverse as the number of organs potentially affected [5–9]. Alagille realized that the manifestations in the heart, liver, and spine were highly variable and furthermore that some family members had only minor involvement of a few organs. Over time, other names (including intrahepatic atresia, biliary hypoplasia, intrahepatic biliary dysgenesis, and Watson–Alagille syndrome) were used to describe the syndrome, but eventually the term Alagille syndrome (ALGS) became synonymous with this constellation of findings. Although the hepatic manifestations predominate in most patients, the term Alagille syndrome shifts the emphasis from a liver disease to a generalized developmental disorder.

While the genetic basis of ALGS was recognized very early on, it was not until the 1980s, when a number of patients with ALGS were noted to have deletions in chromosome 20p, that the site of the gene was proposed. In 1997, Li et al. [10] and Oda et al. [11] identified that mutations in *JAG1* cause Alagille syndrome. The discovery of this causative gene led to a

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tremendous increase in the knowledge about the manifestations of ALGS. *JAG1* is a ligand in the Notch signaling pathway, which is involved in the embryogenesis of many human organs, including those affected in ALGS. Mutations in *JAG1* are now found in nearly all patients with clinical ALGS [12]. A small number of patients with ALGS have been found to have mutations in *NOTCH2* (rather than *JAG1*) [13, 14], underscoring the importance of the Notch pathway in human development. With the current availability of clinical mutation and deletion analysis, genetic testing has supplanted the need for histologic documentation of paucity in many individuals. Furthermore, it has greatly expanded recognition of the number of minimally affected mutation carriers that have an "incomplete syndrome" yet have the risk of producing severely affected progeny. The spectrum of *JAG1*mutation carriers is now recognized to include individuals who have no apparent disease and others who have a major manifestation in other organs such as heart or vasculature, without clinically apparent liver disease or cholestasis. The prevalence of ALGS has been reported to be 1 in 70,000 births, but with the introduction of molecular testing and the identification of mildly affected individuals, this is likely to be a significant underestimate [15]. All of the early descriptive clinical studies focused only on patients who met the classical clinical definition of the syndrome. As a result, the reported clinical outcomes of morbidity and mortality were adversely biased toward the severest end of the spectrum. With the advent of genetic screening of the relatives of a proband, it has become apparent that many mutation carriers are minimally affected, and it is difficult to ascribe the term "syndrome" as it has been classically defined [15]. Some individuals with *JAG1* or *NOTCH2* mutations have manifestations that do not resemble ALGS [15–17]. Furthermore, a number of other diseases of Notch signaling have predominant manifestations in one organ system (bone, vasculature) that bear resemblance to the manifestations of ALGS in that organ, but without common effects on the other sites typically affected in ALGS [16, 18, 19].

Genetics

ALGS was previously reported to affect 1 in 70,000 newborns; however, this likely represents an underestimate of its true prevalence as molecular screening has identified mildly affected individuals with subtle or atypical ALGS manifestations [15]. The true prevalence is likely closer to 1 in 30,000. The majority of patients with ALGS (>90 %) carry a mutation in *JAG1*, located in the short arm of chromosome 20 [12]. To date more than 430 different mutations have been described. Sixty percent of ALGS individuals have sporadic mutations, and the remainder have inherited disease [20]. A few patients have a total gene deletion (3–7 %), and the rest have intragenic deletions, most of which are protein truncating. Nine percent of affected individuals have splicing mutations and 9 % have missense mutations [12]. A small percentage (approximately 1 %) of patients who fulfill the clinical criteria for ALGS, but do not carry a *JAG1* mutation, have been found to have a mutation in another gene, *NOTCH2* [14].

JAG1 encodes for a ligand in the Notch signaling pathway, which is a highly evolutionarily conserved intercellular signaling mechanism. There are five ligands (Dll1, Dll3, Dll4, *JAG1*, and 2) and four Notch [1–4] receptors known to date in mammals. *JAG1* is a single-pass type I membrane protein with an extracellular domain made of a N-terminal region, a Delta/Serrate/LAG2 (DSL) domain, 16 EGF tandem repeats, and a cysteine-rich region [21]. The Notch receptor consists of an extracellular segment, formed by multiple epidermal growth factor (EGF)-like repeats; a transmembrane part; and an intracellular domain. Once the receptor–ligand interaction has occurred, the intracellular domain is cleaved from the inner surface of the membrane and translocates into the nucleus where it regulates the transcription of different downstream genes, such as Hes1/Hey2 [21].

A small fraction (3–5 %) of ALGS individuals have deletions of chromosome 20p. Genome-wide SNP analysis of 25 patients with ALGS revealed 21 deletions ranging from 95 kb to 14.62 Mb [22]. 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. Interestingly deletions up to 5.4 MB did not confer additional clinical findings although there was haploinsufficiency for several genes other than *JAG1*.

The identification of individuals in the same family sharing the same *JAG1* mutation with different disease manifestations strongly supports the existence of genetic modifiers. Current research strategies are under way to identify these modifiers in different organ systems. Given the complexity and numerous members of the Notch signaling pathway, there are many candidate genes.

Clinical Manifestations and Management

Liver Disease in ALGS

Duct Paucity and Hepatic Histopathology

The hallmark of the liver disease in Alagille syndrome is bile duct paucity, along with the usual severe cholestasis that accompanies paucity (Fig. 11.1). Many patients, however, do not have paucity due to a variety of factors. The presence of paucity is no longer considered essential for diagnosis. Furthermore, bile duct paucity is not specific for ALGS. Paucity is seen as either a common or an occasional histologic feature of a highly diverse group of infectious, metabolic, immunologic, and genetic diseases. Paucity is also recognized to be a late histologic pattern of a number of diseases that have neonatal hepatitis or even bile duct proliferation on earlier biopsies. The progression to paucity is highly variable, from months to years depending on the etiology. Nevertheless, the finding of bile duct paucity in patients of any age should raise the possibility of ALGS, as it is the single most important and most common cause of paucity. It is an important caveat, however, that the presence of apparently normal duct number, or rarely even bile duct proliferation, can be seen in patients with ALGS and thus does not fully eliminate the syndrome from consideration.

The normal bile duct to portal tract ratio undergoes a developmental maturation, which is of particular consideration in a preterm infant, where the number is normally diminished [23]. In full-term infants and older children, the normal bile duct to portal tract ratio ranges between 0.9 and 1.8. The precise ascertainment of this ratio can be difficult in needle biopsies, particularly

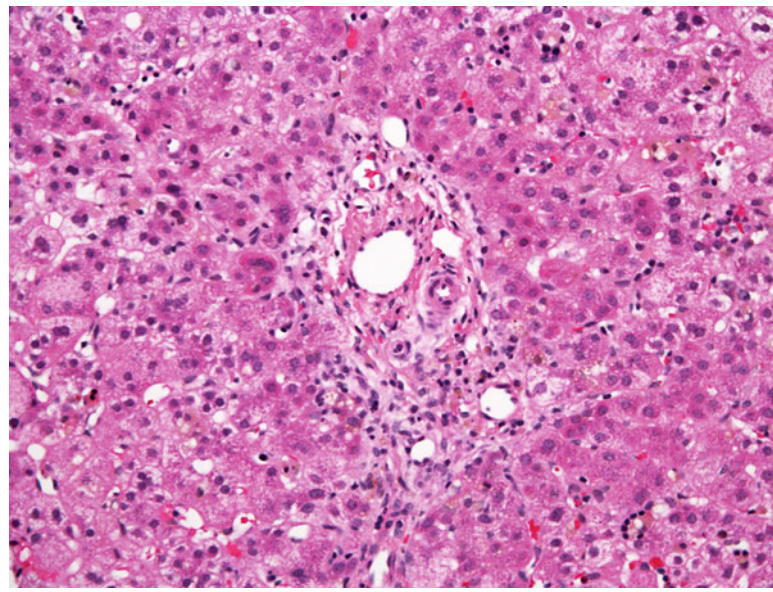

Fig. 11.1 Histology: Bile duct paucity in a large portal tract demonstrating several branches of portal vein and hepatic artery, from a 2-month-old infant with ALGS. Magnification × 200 (Courtesy of Dr. Pierre Russo, CHOP)

if the number of portal tracts is limited. It is important to only include ducts, but not ductules, in this ratio. The minimal number of portal tracts necessary for an accurate ratio can only reliably be achieved by a wedge biopsy. However, a reasonable assessment of the ratio can be obtained with most needle biopsy specimens containing at least six portal tracts [24]. A bile duct to portal tract ratio of less than 0.9 is suggestive of ALGS, but most older infants with ALGS have a ratio that is in the range of 0.5–0.75 [2]. In infants, the timing of the biopsy is commonly dictated by the need to discriminate biliary atresia from ALGS. Unfortunately, as many as 40 % of infants less than 6 months of age will not have established paucity recognized on early biopsy, although paucity is generally expected (up to 95 %) after that age in symptomatic children [7]. For diagnostic evaluations of neonatal cholestasis in the first 2 months of life, a biopsy is performed to identify bile duct proliferation, which suggests biliary atresia, other forms of obstruction, and certain metabolic liver diseases, but only very rarely ALGS. If proliferation is identified in an infant who has clinical features of Alagille syndrome, a careful and cautious evaluation should be undertaken, as a non-excreting DISIDA scan or a noncommunicating intrahepatic cholangiogram can each occur in ALGS, leading to an error in diagnosis and an unnecessary Kasai portoenterostomy. A number of ALGS infants have been misdiagnosed as biliary atresia [25, 26]. The overlap of ALGS and concomitant biliary atresia is controversial, but it appears to be, at the most, extraordinarily rare. More commonly, postoperative histologic assessment of the central and extrahepatic ducts demonstrates them to be patent yet extremely hypoplastic. The ultimate value of a needle biopsy at this age for ALGS infants, therefore, is to keep them out of the operating room, where a cholangiogram in the best of situations may be noncommunicating and misleading.

The importance of identifying paucity, and the role of the liver biopsy in establishing the diagnosis of ALGS, is diminishing as molecular diagnosis is increasingly available. While paucity was a required criterion for the diagnosis for many years, newer studies focusing on patients with *JAG1* mutations or with systemic disease identify paucity in approximately 80–85 % of patients. Many patients identified in this fashion have minimal clinical hepatic manifestations, and some have negligible biochemical disease. It is reasonable to assume that the hepatic manifestations of paucity and cholestasis as are as variable in incidence as cardiac, renal, ocular, or vascular findings. The role of biopsy in older children and adults may not only include the assessment of bile duct and portal tract number but also the identification of fibrosis and to rule out other concomitant disease.

The progression to cirrhosis in ALGS is not typical, although significant fibrosis or cirrhosis has been reported to occur in 10–50 % of patients in different series representing inclusion of the most severely affected individuals and also referral patterns to transplantation centers [6–9, 27] (see Fig. 11.2). The actual incidence of progressive liver failure or intractable portal hypertension has not been well characterized in the molecular era but is undoubtedly significantly lower.

Hepatic Clinical Disease

The vast majority of patients with symptomatic ALGS will present in the first 3 months of life, although the manifestations and severity of presentation can vary considerably. Presentation with severe cardiac disease such as tetralogy of Fallot or pulmonary atresia will be evident at birth, if not previously identified by fetal ultrasound. The presence of severe cardiac disease commonly obscures the importance of hyperbilirubinemia or aminotransferase elevations, thereby delaying diagnosis in some infants. Neonatal renal failure has occasionally been seen. Infants with ALGS may be small for gestational age. Facies, embryotoxon, and butterfly vertebrae are rarely sought until ALGS is already a major consideration.

Most symptomatic ALGS patients present with hepatic disease, frequently within the first month of life. ALGS is one of the more common etiologies of neonatal cholestasis and conjugated hyperbilirubinemia. At this age, it must be rapidly and correctly discriminated from biliary atresia

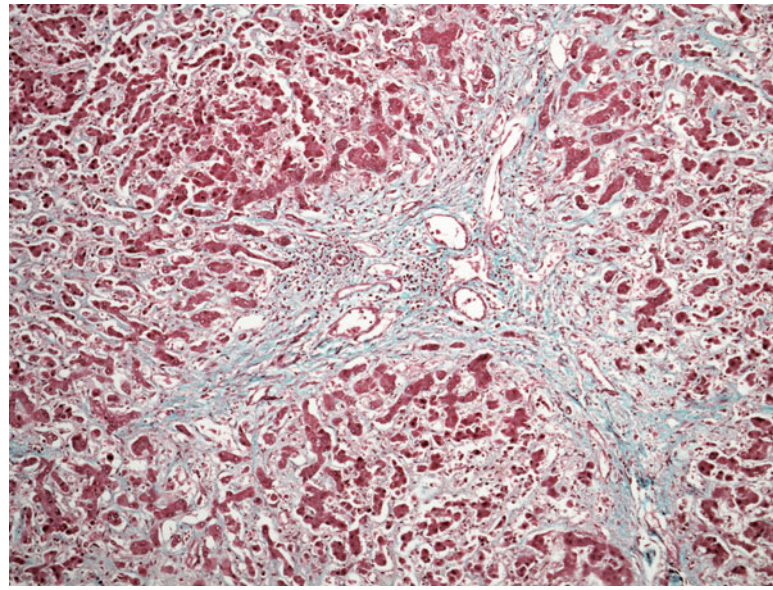

Fig. 11.2 Histology: Liver biopsy demonstrating portal fibrosis, with portal to portal bridging, focal nodule formation, and extensive sinusoidal fibrosis (Courtesy of Dr. Pierre Russo, CHOP)

and other treatable causes of neonatal cholestasis. ALGS, biliary atresia, other extrahepatic obstructions, cystic fibrosis, FIC3, and other disorders may present with conjugated hyperbilirubinemia and a rising GGTP level. A prompt assessment for other manifestations of the syndrome should be undertaken, usually culminating in a percutaneous liver biopsy to assess ductular histology. As stated above, extreme caution should be taken to differentiate ALGS from biliary atresia, as the therapy is different and surgical excision of the extrahepatic tree may worsen long-term hepatic outcome [25].

Essentially all patients with hepatic ALGS have some degree of conjugated hyperbilirubinemia. The bilirubin levels typically increase over the first months or years of life. In severely affected children, the bilirubin levels may be as high as 30-fold elevated. While modest bilirubin elevations may improve significantly in later childhood, extreme elevations generally portend the need for liver transplantation at some point, either for intractable pruritus, portal hypertension, or synthetic liver failure. The aminotransferases are commonly elevated, but to varying degrees that do not seem to correlate with excretory function or with outcome. Some patients have normal aminotransferase levels in infancy. More characteristically, the biochemical measures associated with cholestasis are significantly elevated. In addition to bilirubin, and sometimes even more striking, bile salt levels can be markedly elevated, reaching up to 100-fold elevations. Bile salt elevations can also be seen in older patients with normal total bilirubin. The gamma-glutamyl transpeptidase level is also commonly strikingly elevated, at times 50-fold or higher. Alkaline phosphatase is also quite elevated but can be also affected by vitamin D deficiency, bone disease, and the increased incidence of fractures in various stages of healing.

The apparent obstruction to bile flow and the resulting cholestasis progress over the first months to years and then in many patients improve thereafter. In addition to the jaundice, a striking and clinically disfiguring feature is the development of xanthomas (see Fig. 11.3). Hypercholesterolemia and hypertriglyceridemia are common features of ALGS. The elevations of cholesterol may exceed 1,000 mg/dl. In severely affected infants, xanthomas typically start to occur as the cholesterol level surpasses 500 mg/dl. As the level further rises, these xanthomas characteristically occur on the extensor surfaces of fingers and toes and in areas of minor trauma, including the abdomen, buttocks, inguinal area, and neck. In some patients, the face can be severely involved, particularly the ears, eyes, and nose. The xanthomas

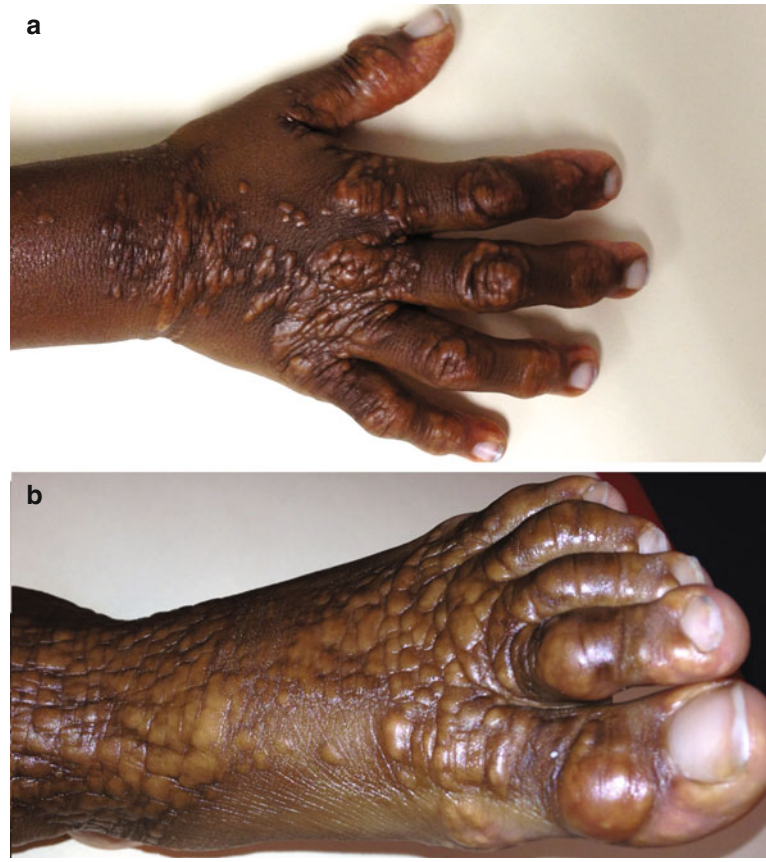

Fig. 11.3 (**a** and **b**) Profound disfiguring xanthomas of the hand and foot in a 4-year-old toddler with thousands of xanthomas and a serum cholesterol level of 825 mg/dl

can be particularly upsetting because they itch and are commonly traumatically injured, leading to bleeding, scabbing, and scarring. Patients can have hundreds, or even thousands of xanthomas, which can lead to disfigurement and emotional distress for patients and parents alike. As cholesterol levels decline, either with age progression, medical therapy, partial external biliary diversion, or liver transplantation, the xanthomas can resolve, although pigmentary abnormalities may mark the site of prior lesions (see Fig. 11.4).

Hepatic synthetic function is generally well preserved in patients with ALGS, despite profound deficits in excretion capability. Metabolic regulation is generally intact. Albumin and ammonia levels are generally normal. The prothrombin time is commonly elevated unless vitamin K supplementation is adequate, which in some very severely affected individuals requires repeated parenteral therapy. Worsening synthetic and metabolic functioning can occur, as can progression to cirrhosis and liver failure in approximately 10–20 % of patients whose onset of liver disease was in infancy.

A large number of *JAG1* mutation carriers have mild or even nonexistent liver disease. Some of these patients are identified because they have major manifestations in other organs (heart, moyamoya, kidney, etc.). Others identified by family screenings are the asymptomatic parents or siblings of a newly identified affected proband. For these individuals with minimal liver disease, progression to cirrhosis or liver failure (without another toxic or infectious event) is extraordinarily uncommon, and they should be reassured that their liver function will remain intact, even though other manifestations (kidney, vascular, etc.) should be considered.

Patients with severe cholestatic liver disease may have profound problems with growth, weight gain, and fat-soluble vitamin absorption. Diminished bile salt excretion and low

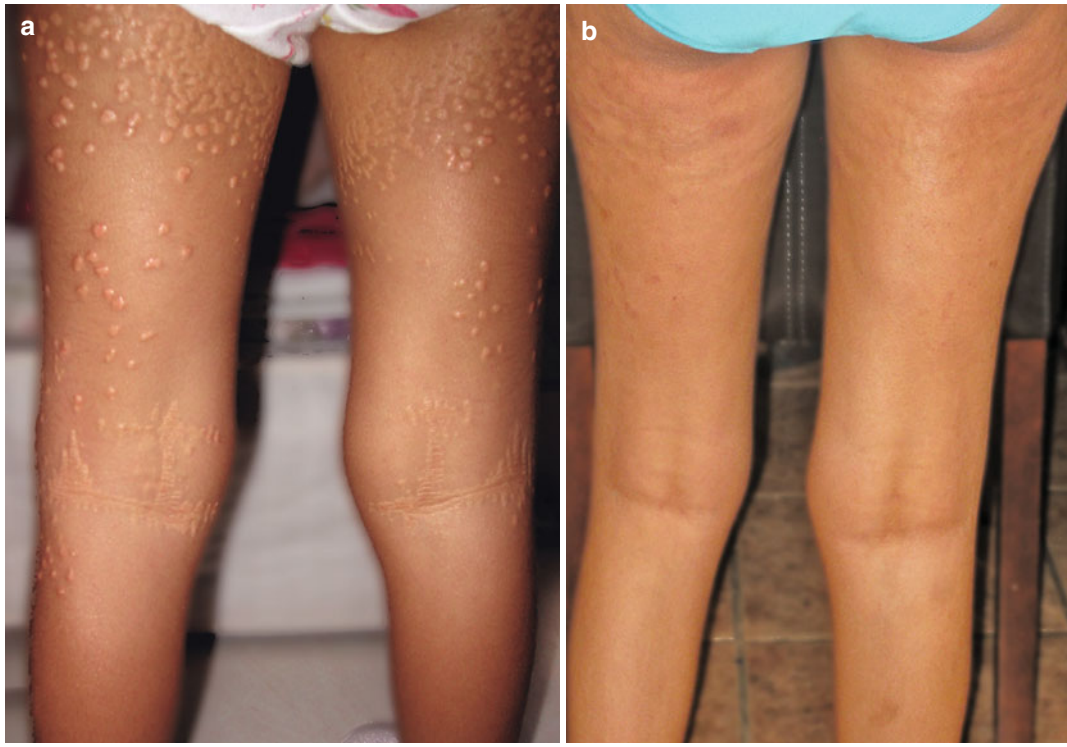

Fig. 11.4 Spontaneous resolution of severe xanthomas after a decade of medical therapy for hypercholesterolemia and pruritus. (**a**) Posterior thigh and popliteal fossa xanthomas, representative of thousands of large, elevated xanthomas at 3.5 years of age, with a serum cholesterol level of 1,260 mg/dl. (**b**) Spontaneous resolution of all xanthomas 11 years later, at age 14, with a serum cholesterol level of 200 mg/dl. Resolution occurred with medical therapy alone (Courtesy Dr. Joshua R. Friedman, CHOP)

intraluminal bile salt concentrations result in ineffective solubilization and absorption of dietary lipids, essential fatty acids, and the fat-soluble vitamins A, D, E, and K. Vitamin levels should be checked regularly, particularly in cholestatic infants. Excellent vitamin preparations are available to treat deficiencies, although sometimes the amount required and the uncovered cost of the supplements can be an impediment to successful therapy. Combination as well as individual vitamin preparations should be used in a customized fashion, as an individual patient's needs vary significantly. At times, therapy with preparations such are parenteral vitamin K or calcitriol is necessary to treat severe deficiencies. It should be recognized that other medications (e.g., cholestyramine) and special diets may contribute to refractory deficits. Essential fatty acid deficiency has occurred in ALGS. Many children do well with formula composed of or fortified with medium-chain triglycerides (MCT), which are better absorbed than long-chain fat. However, some children seem to do better with a low-fat diet, with an emphasis on supplementing essential fats. For severely affected infants, a gastrostomy tube is useful to augment intake and promote growth and development. This also has an advantage for infants and toddlers taking numerous doses of supplements, antipruritics, choleretics, sequestrants, and other medications.

Pruritus in ALGS

Pruritus is a particularly significant and commonly intractable feature of ALGS. Severe pruritus can occur in infancy and progress with age. For many patients, this is the single most

important and overarching feature of ALGS. The presence of pruritus generally trends with the levels of cholesterol and bile salts, and some antipruritic therapy is directed at those substances. Local cutaneous therapy has significant value. The avoidance of drying soaps, the copious administration of emollients and ointments, and careful attention to fingernail length are useful. Many medications have utility for the pruritus of ALGS. Antihistamines may decrease itching and if given only at night may help with sleep as well. Rifampin has been shown to decrease itching and in many cases is particularly effective [28, 29]. Ursodeoxycholic acid is a potent choleretic and can have a dramatic effect on reducing symptomatic cholestasis [30] although in some patients it may exacerbate pruritus. Bile acid-binding resins may increase the elimination of bile salts and secondarily decrease itching. Naltrexone, an opioid antagonist, can also be effective in some cases [31].

Refractory pruritus is an indication for liver transplantation consideration. Diversion surgeries have been developed to provide relief from intractable pruritus without the risks and mortality associated with liver transplantation, particularly for patients with preserved synthetic function and without cirrhosis. In partial external biliary diversion (PEBD), a biliary conduit is constructed from a segment of resected jejunum, and an anastomosis is made between the proximal portion of the conduit and the most dependent portion of the gallbladder. The distal roux limb is used to form an ostomy. For ALGS patients with severe, mutilating pruritus, the majority have a dramatic improvement in their pruritus score at 1-year post-diversion [32]. In addition, the subset of patients with extensive xanthomas had complete resolution within a year, concomitant with significant decreases in mean bile salt levels and mean cholesterol. In one patient who requested reversal, there was prompt recurrence of severe pruritus [32]. It has been estimated that PEBD diverts as much as 50 % of bile, whereas maximal therapy with resins such as cholestyramine can only divert about 5 % of the bile acid pool each day [33]. For many ALGS patients, PEBD has provided sustained and substantial improvement in quality of life and reduction of symptoms and serves an important role for patients who might otherwise require transplantation.

Some patients will decline PEBD since it requires the construction of a permanent draining ostomy. An alternative surgical approach is terminal ileal exclusion, which avoids the need for an external fistula. In this technique, approximately 15 % of the terminal ileum is bypassed via a direct anastomosis of the more proximal ileum to the ascending colon. This approach has been reported less frequently in ALGS [34, 35] but has been used successfully in familial intrahepatic cholestasis. In a small study on three ALGS patients, there was significant reduction of pruritus and improvement (but not resolution) of xanthomas, but the improvement in biochemical cholestasis was much less than PEBD, and some values worsened with follow-up [35]. Nevertheless, ileal exclusion may provide an alternative to PEBD for certain situations. The effect of diminished effective bowel surface area on nutrition and malabsorption has not been extensively studied.

Liver Transplantation in ALGS

Liver transplantation is a highly effective therapy for the hepatic disease of ALGS, and it is generally estimated to be necessary in approximately 20–30 % of patients with ALGS [7, 8], although some studies report much higher rates [9]. There are a number of reports from single centers mostly documenting 1-year survival rates in the 71–92 % range [7, 9, 27, 36–38], although some studies are limited by small patient numbers and relatively short mean follow-up.

Two recent large studies of transplant databases provide a broad overview of the outcomes and the complications of transplantation for ALGS, with a comparison to data for biliary atresia [39, 40]. Between 1987 and 2008, 461 ALGS patients (4 % of the total recipients) recorded in the UNOS database underwent hepatic transplantation. The 1-year ALGS patient survival rate was 82.9 %, and the 5-year survival rate was 78.4 %. The 1- and 5-year graft survival rates were 74.7

and 61.5 %. Each of these rates is lower than those seen for biliary atresia, where 1- and 5-year patient survival rates are 89.9 and 84 % [39]. There was a significantly increased rate of both graft loss and patient mortality in the first 30 days, compared to patients with BA. Early (<30 days) graft loss for ALGS was 11.7 % compared to 4.8 % for BA, and early mortality for ALGS was 9.6 % compared to 4.8 % for BA. In this series, graft failure and infection were the leading causes of death in ALGS transplants. Neurologic and cardiac complications also contributed to the excess mortality in ALGS [39]. Kaplan Meier analysis of 10-year patient survival was approximately 78 %, with contributions to mortality from pre-transplant creatinine elevations, extended cold ischemic time, and repeat transplantation. Although the data for survival was presented in aggregate, a sub-analysis demonstrated (for both ALGS and BA) that the percentage of deaths and the graft loss were significantly better in the newest quartile data reflecting a later era of transplantation care. This type of analysis has not been performed in most single-center studies, but it can be reasonably assumed that the data quoted above represents minimal estimates for patient and graft survival in the current era.

In a subsequent study utilizing the SPLIT (Studies on Pediatric Liver Transplantation) transplant database, 91 patients with ALGS (2.9 % of the total population of recipients) were compared to 236 age- and gender-matched BA patients transplanted between 1995 and 2009 [40]. The 1-year patient survival rate for ALGS patients was 87 % (compared to 96 % for matched biliary atresia patients). Nearly all of the excess mortality for ALGS patients occurred in the first 6 months after transplantation, and then subsequently the Kaplan–Meier curves are parallel. Early death in this cohort was associated with biliary, vascular, central nervous system (seizures, cerebral hemorrhage, edema), and renal (requiring dialysis, hemofiltration) complications. There is a high frequency of renal complications in ALGS both before and after transplantation. Posttransplant renal insufficiency worsened in many ALGS patients. At 1 year after transplant, 22 % had GFR less than 90 ml/min/1.73 m^2. In this study, ALGS patients were more growth-impaired overall than BA transplant patients, and although the height deficit persisted, they did demonstrate good catch-up growth (not seen in other smaller studies) [38, 40, 41]. Occasionally, concomitant liver–kidney transplantation or rarely liver–heart transplantation has been successfully performed.

The results of living-related transplants in ALGS are highly favorable. Studies of long-term 10-year survival after living-related transplantation for ALGS have results as high as 80 %. In one series, the survival rates were similar for living-related transplantations for ALGS and other pediatric liver diseases [41]. One caveat, however, is that ALGS is a dominantly inherited disease in which approximately 40 % of probands will have a *JAG1* mutation-carrying parent who may be minimally affected. It is imperative to fully assess living-related donor candidates for unapparent ALGS, because donors with unsuspected but severe biliary involvement have been identified only at the time of surgery [42]. In the era of molecular diagnosis, and with sufficient lead time, all potential living-related transplant recipients with ALGS should have exhaustive mutation analysis (including *NOTCH2* if *JAG1* is negative), which should identify a mutation in 95 % of individuals. All potential related donors should have targeted mutation analysis to supplement the other typical donor screening studies.

Indications for Liver Transplantation

The indications for transplantation, the timing of transplantation, and the assessment of associated risk factors are more complex compared to other pediatric liver diseases. Cholestasis and its complications are the most common indication for transplantation. In BA patients with a failed Kasai, the progression of liver disease is essentially inevitable, leading to worsening hepatic synthetic function, intractable portal hypertension, malnutrition, and worsening quality of life. For ALGS patients, the magnitude of the cholestasis is commonly much larger, but many ALGS infants have cholestasis that worsens and then improves over time (Fig. 11.5). The specific indications for transplant in ALGS include cholestasis, intractable pruritus, complications

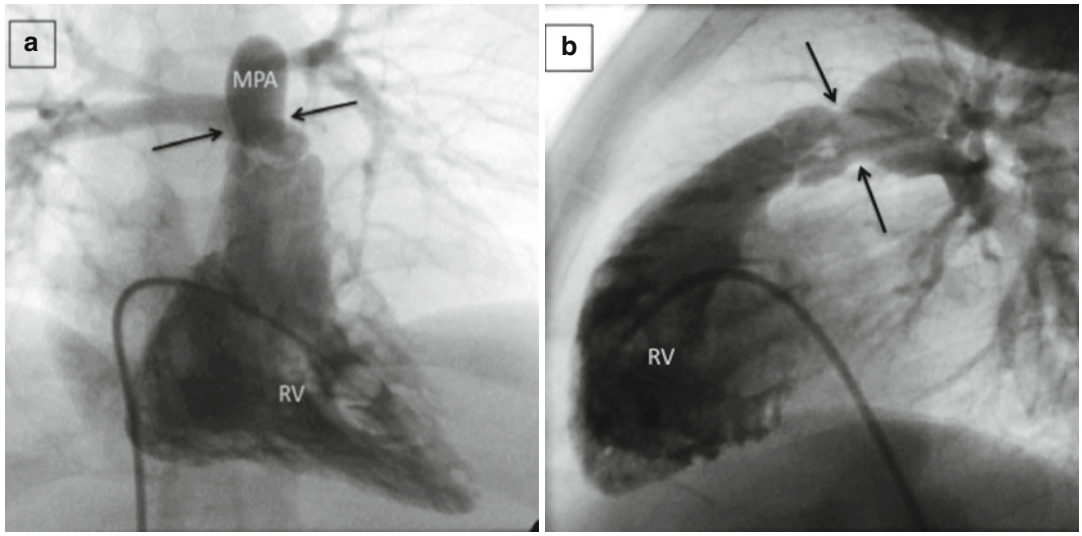

Fig. 11.5 Figure 1 is a right ventriculogram taken in anteroposterior (panel **a**) and lateral (panel **b**) views of a typical patient with Alagille syndrome. The *arrows* denote supravalvar pulmonary stenosis. There is post-stenotic dilation of the MPA, and the proximal branch pulmonary arteries are diffusely small. *RV* right ventricle, *MPA* main pulmonary artery

of portal hypertension, synthetic liver failure, growth failure from chronic malnutrition and vitamin deficiency, recurrent fractures and bone disease, and occasionally severe xanthomatosis [7, 9, 27, 39, 40]. Commonly, several of these are present concomitantly. Other therapies, however, can be implemented that can obviate or diminish the need for transplantation in some patients. For severe cholestasis with intractable pruritus and good synthetic function, ALGS patients may improve significantly with combination medical therapy, partial external biliary diversion, or ileal exclusion therapy. Surgical strategies to treat pruritus should generally be offered to patients who have a good chance of avoiding transplantation otherwise. If transplant is inevitable for other reasons, then a direct approach to transplant is generally advised.

Preoperative Assessment for Liver Transplantation

The progression to transplantation generally follows a long trajectory, with time to address and possibly treat many of the factors that could worsen outcome [43]. As a multisystem disease, ALGS presents many non-hepatic issues, which must be addressed. Malnutrition should be aggressively treated, with either nasogastric or gastrostomy supplemental nutrition if not already being administered. The most important part of the transplant assessment is the evaluation for cardiac and renal disease. A formal current cardiopulmonary assessment will help to predict challenges and identify problems that can be addressed preoperatively. For example, critical pulmonary artery stenoses can be addressed in the cardiac catheterization lab in order to decrease right heart pressures or to improve differential pulmonary perfusion. A protocol for cardiac assessment recommendations has been provided by the faculty at King's College [44]. The presence of pre-transplant renal disease should influence the posttransplant choice of immunosuppression. If significant renal impairment is present, renal-sparing immunosuppressive protocols with low target levels of calcineurin inhibitors and early introduction of other strategies should be considered. Dental disease, which is common in ALGS, should be addressed completely. Immunizations should be reviewed and updated. Because of the occasional vascular abnormalities seen in ALGS, patients should have careful imaging by CT or MR angiography to anticipate abnormalities. Although

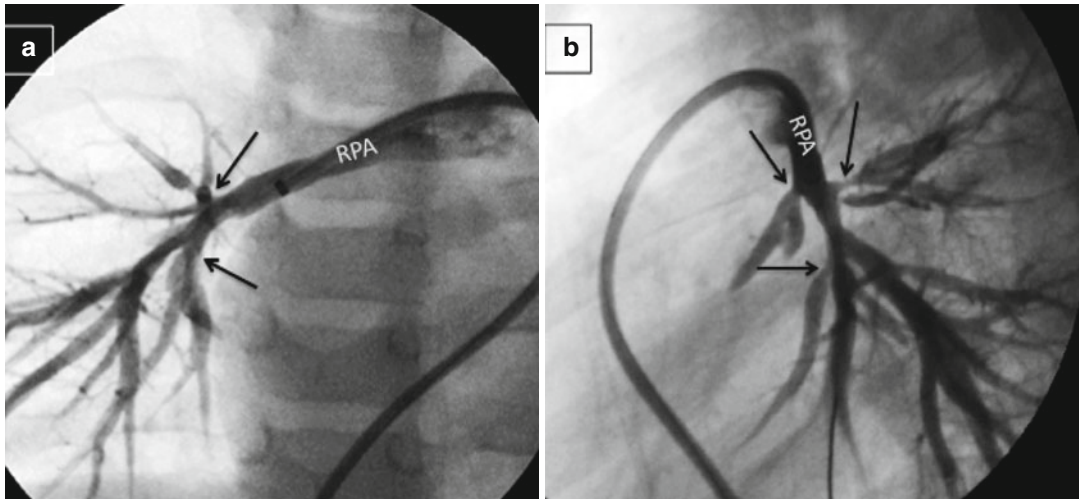

Fig. 11.6 Figure 6 is an angiogram of the right pulmonary artery (*RPA*) in anteroposterior (panel **a**) and lateral (panel **b**) views. The proximal RPA is diffusely small, and there are multiple focal areas of subsegmental arterial stenosis (*arrows*) (Courtesy, Matthew Gillespie, MD, CHOP)

there are no formal indications, brain and CNS vascular imaging seems prudent in children and adults who are being evaluated, as the presence of moyamoya, a vascular lesion, or a prior infarct may influence preoperative strategies.

Cardiac Involvement

In a comprehensive evaluation of 200 ALGS subjects, cardiovascular involvement was present in 94 % [45], with right-sided lesions being the most prevalent. Pulmonary artery anomalies are the most common abnormality identified (76 %) and may occur in isolation or in combination with structural intracardiac disease [45] (see Figs. 11.5 and 11.6). Intracardiac lesions were present in 24 % of 92 patients with ALGS [7]. The most common congenital defect is tetralogy of Fallot (TOF), which occurs in 7–12 % [7, 45]. It appears that severe forms of TOF (especially TOF with pulmonary atresia) occur with greater frequency in the ALGS population than in the general population of individuals with TOF. Approximately 40 % of patients with ALGS demonstrating TOF have pulmonary atresia. There is no correlation between the type of *JAG1* mutation and the nature of the cardiovascular involvement.

The management of cardiac anomalies in ALGS is largely driven by the same algorithms as in non-syndromic children; however, outcomes differ. In a large ALGS series, cardiac surgery was performed in infancy in 11 % [7]. 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 due to concomitant significant peripheral pulmonary artery stenosis or other systemic manifestations of the syndrome. Asymmetric peripheral pulmonic stensoses can result in markedly asymmetric perfusion of the lungs (see Fig. 11.7). Nonsurgical invasive techniques have been used successfully for patients with ALGS, including valvuloplasty, balloon dilatation, and stent implantation. Heart–lung transplantation has also been performed in combination with liver transplantation in a child with ALGS, though this is a rare occurrence.

Cardiac disease accounts for nearly all of the early deaths in ALGS. Patients with intracardiac disease have an approximately 40 % rate of survival to 6 years of life, compared with a 95 % survival rate in patients with ALGS without intracardiac lesions [7].

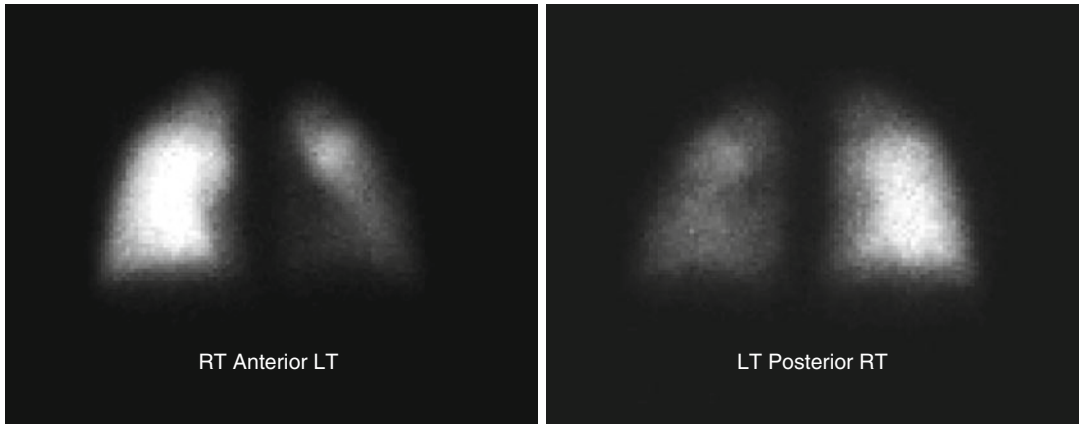

Fig. 11.7 Perfusion scan demonstrating markedly asymmetric flow to the lungs, with 75 % to the right and only 25 % to the left lung, due to asymmetric pulmonary arteriolar resistance

Renal Involvement

Renal involvement in ALGS has been widely reported, and 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. This clinical finding is supported by the known role of the Notch signaling pathway in nephron and glomerular development [46]. In a large retrospective study, there was a prevalence of 39 % of renal anomalies or disease, and the most common renal involvement was renal dysplasia (58.9 %), with renal tubular acidosis (9.5 %), vesicoureteric reflux (8.2 %), and urinary obstruction (8.2 %) following [47]. Hypertension in patients with ALGS could be of cardiac, vascular, or renal etiology, but the frequency of this has not been systematically studied.

The frequency of renal insufficiency in ALGS has also not been formally characterized though renal replacement therapy and transplantation are reported. In the study of liver transplantation outcomes in ALGS from the SPLIT cohort, a high frequency of renal complications in ALGS, both pre- and posttransplant, was noted [40]. Renal complications, GFR, and serum creatinine were worse in ALGS as compared to biliary at different time points. Most of the renal insufficiency in ALGS post-liver transplant was present at the time of transplant. ALGS children with preexisting renal insufficiency were less likely to have renal improvement, implicating intrinsic renal disease, which is not reversed by liver transplantation.

Functional and structural evaluation of the kidneys should be undertaken in all patients. The role of renal tubular acidosis in early growth failure is unclear, but administration of bicarbonate is necessary in some individuals. Renal function should clearly be reassessed during the hepatic transplant evaluation and calcineurin-sparing immunosuppressive regimes are recommended following liver transplantation.

Vascular Involvement

Unexplained intracranial bleeding is a devastating complication in ALGS. Intracranial bleeds occur in approximately 15 % of patients, and in 30–50 % of these events, the hemorrhage is fatal [7, 8]. There does not seem to be any pattern to the location or severity of intracranial events, which range from massive fatal bleeds to asymptomatic cerebral infarcts. Of note, the majority of intracerebral hemorrhagic episodes in ALGS occur in the absence of coagulopathy. Head trauma, typically of a minor degree, has been associated with the bleeding in a number of patients. The majority of cases of bleeding are spontaneous, however, with no clear risk factors.

Lykavieris studied a cohort of 174 individuals with ALGS and identified 38 patients (22 %) who had 49 bleeding episodes at multiple locations in the body [48]. All these hemorrhages occurred in the presence of normal platelet counts and prothrombin times, suggesting that ALGS patients may be at particular risk for bleeding.

Structural intracranial vascular abnormalities that could explain the occurrence of bleeding and stroke in ALGS have been described in some patients [8, 49, 50]. Aneurysms of the basilar and middle cerebral arteries and various internal carotid artery anomalies have been described. Moyamoya syndrome (progressive intracranial arterial occlusive disease) has also been reported in several children with ALGS (see Fig. 11.8). Emerick et al. prospectively studied 26 patients with ALGS using magnetic resonance angiography (MRA) of the head and identified cerebrovascular abnormalities in ten patients (38 %). This cohort consisted of asymptomatic and asymptomatic patients. One hundred percent of symptomatic patients had detected abnormalities, and, of note, 23 % of asymptomatic patients had anomalies detected [49]. These results suggest that MRA is useful in detecting these lesions and may have a valuable role in screening, although this remains somewhat controversial. The authors' current recommendation is for all asymptomatic ALGS patients to have a screening MRA as a baseline and for physicians to have a low threshold for reimaging ALGS patients in the event of any symptoms, head trauma, or suspicious neurologic signs.

Systemic vascular abnormalities have also been well documented in ALGS. Aortic aneurysms and coarctations and renal artery, celiac artery, superior mesenteric artery, and subclavian artery anomalies have all been described. In a large retrospective study, 9 % (25 of 268) of ALGS individuals had noncardiac vascular anomalies or events [50]. In addition, vascular accidents accounted for 34 % of the mortality in this cohort (surpassing mortality from hepatic or cardiac causes). These findings suggest that vascular abnormalities have been under-recognized as a potentially devastating complication of ALGS. The presence of vasculopathy in ALGS is consistent with the intricate role of the Notch signaling pathway in vascular development [21].

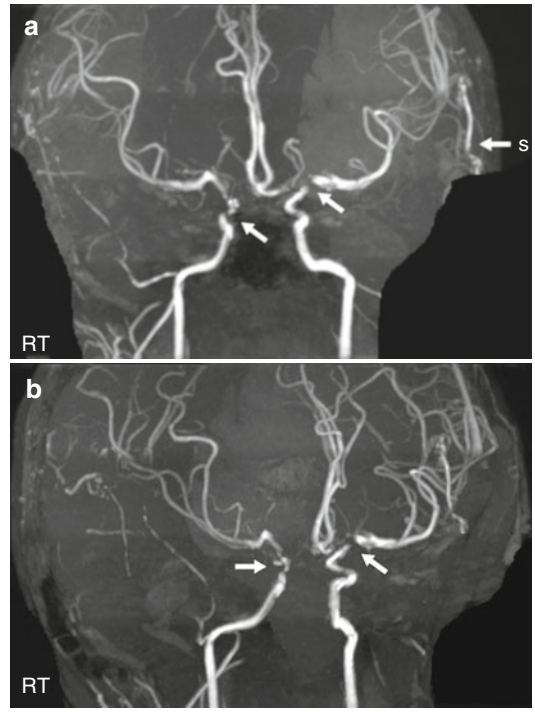

Fig. 11.8 Moyamoya **a** and **b**: MR angiogram demonstrating severe bilateral moyamoya in a 12-year-old boy with ALGS. (**a**) Severe stenoses of the left and the right internal carotid arteries (*arrows*). Anteroposterior view, also demonstrating successful reperfusion of the brain via a bitemporal synangiosis seen well on the left (*S*). (**b**) Oblique view better demonstrating multiple stenoses (*arrows*). The patient had previously suffered an ischemic infarct (Courtesy of Dr. Erin Simon, CHOP)

Facial Features and Skeletal and Ophthalmologic Involvement

These three features of ALGS are considered together since they rarely have clinical significance for the patient but are often important as diagnostic tools.

Facial Features

A characteristic facial appearance is probably one of the most penetrant features of the syndrome [51]. These features include a prominent forehead, deep-set eyes with moderate

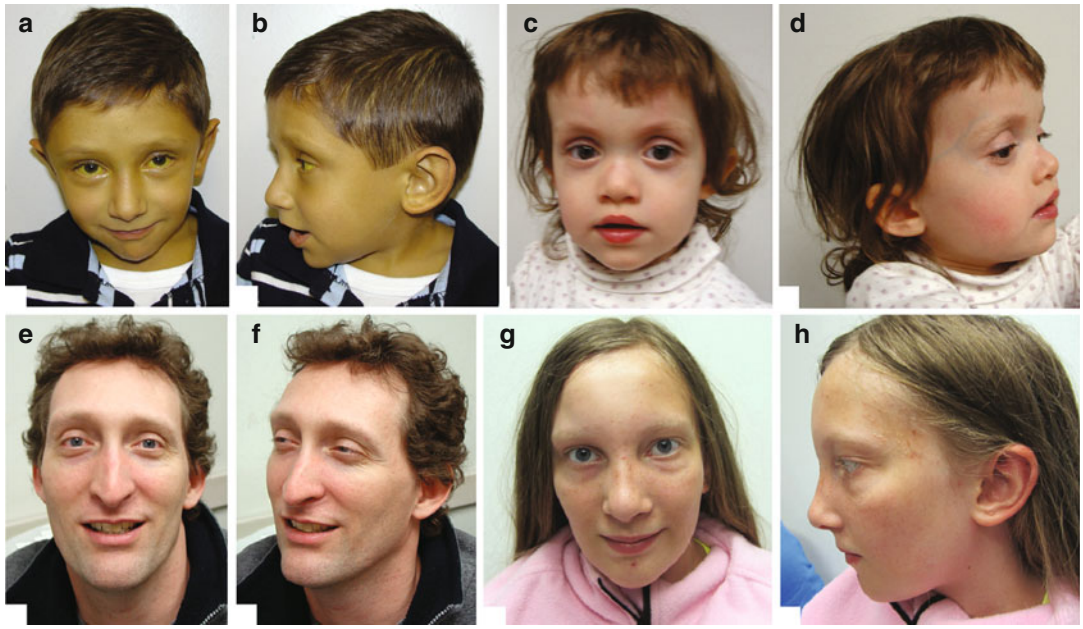

Fig. 11.9 Facies: Representative Alagille syndrome facial features. (**a**, **b**) A 5-year-old boy and (**c**, **d**) 2-year-old girl with typical features including broad and prominent forehead with pointed chin, rounded nasal tip, and seemingly wide-spaced eyes. (**e**, **f**) Father of child depicted in (c–d) with typical adult facial appearance with deep-set eyes, less prominence of forehead, and prognathism. (**g**, **h**) A 12-year-old girl showing facial features intermediate between those of childhood and adult (Courtesy of Dr. Ian Krantz, CHOP)

hypertelorism, a pointed chin, and a saddle or straight nose with a bulbous tip. The combination of these features gives the face an inverted triangular appearance. The facies are difficult to detect early in infancy but become more apparent with increasing age. In adults the facial characteristics of ALGS appear to change; the forehead is less prominent and the protruding chin is more noticeable (see Fig. 11.9). The correct identification of these adults, who commonly have minimal signs and symptoms of ALGS, would help physicians in the evaluation of adults with apparently idiopathic cardiac, hepatic, or renal disease. It should be noted that amongst the few patients reported to date, there appears to be a lower penetrance of characteristic facial features in ALGS patients with *NOTCH2* mutations [13].

Skeletal Involvement

The most characteristic skeletal finding in ALGS is the sagittal cleft or butterfly vertebrae, which is found in 33–87 % of patients [6–9] (see Fig. 11.10). This anomaly may occur in normal individuals and is also seen in other multisystem abnormalities, such as 22q deletion syndrome. The affected vertebral bodies are split sagittally

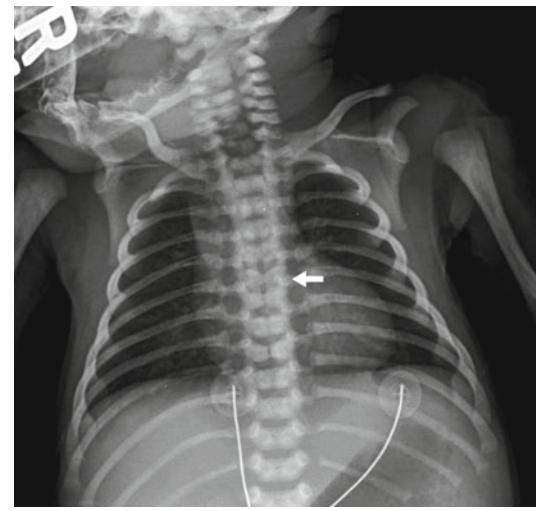

Fig. 11.10 Babygram in a neonate with congenital cardiac disease, demonstrating spinal abnormalities including a clear butterfly vertebra (*arrow*) (Courtesy of Dr. Sabah Servaes, CHOP)

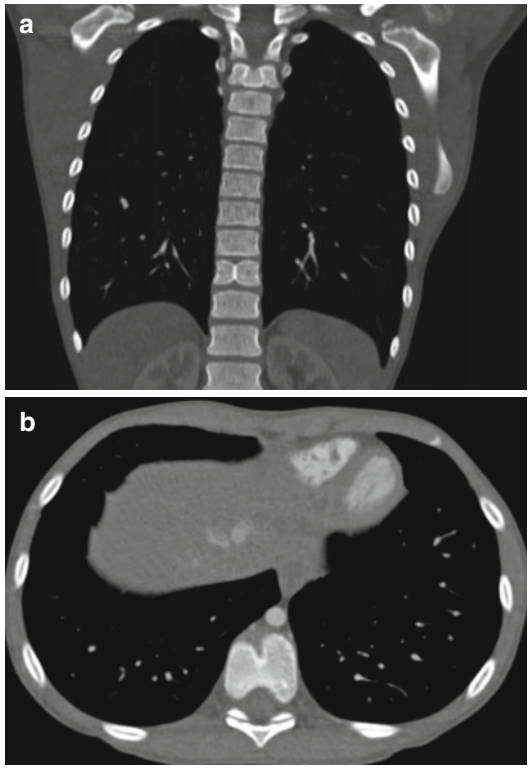

Fig. 11.11 CT scan with vertical reconstruction (**a**) and cross section (**b**) demonstrating butterfly vertebra at T10 (Courtesy of Dr. Sabah Servaes, CHOP)

into paired hemivertebrae because of a failure of the fusion of the anterior arches of the vertebrae (see Fig. 11.11). Generally, these are asymptomatic and of no structural significance. Other associated skeletal abnormalities include an abnormal narrowing of the interpedicular space in the lumbar spine, fusion of the adjacent vertebrae, hemivertebrae, the absence of the twelfth rib, and the presence of a bony connection between ribs. In addition, supernumerary digital flexion creases have been described in one-third of patients [52].

Severe metabolic bone disease with pathologic fractures is common in patients with ALGS. Recurrent fractures, particularly of the femur, have been cited as a major indication for hepatic transplantation. Preliminary survey data suggests that there is a propensity toward pathologic lower-extremity long-bone fractures in ALGS [53] (see Fig. 11.12). A number of factors may contribute to osteopenia and fractures, including severe chronic malnutrition and vitamin D and vitamin K deficiencies. There may also be an intrinsic defect in cortical or trabecular structure of the bones in patients with ALGS.

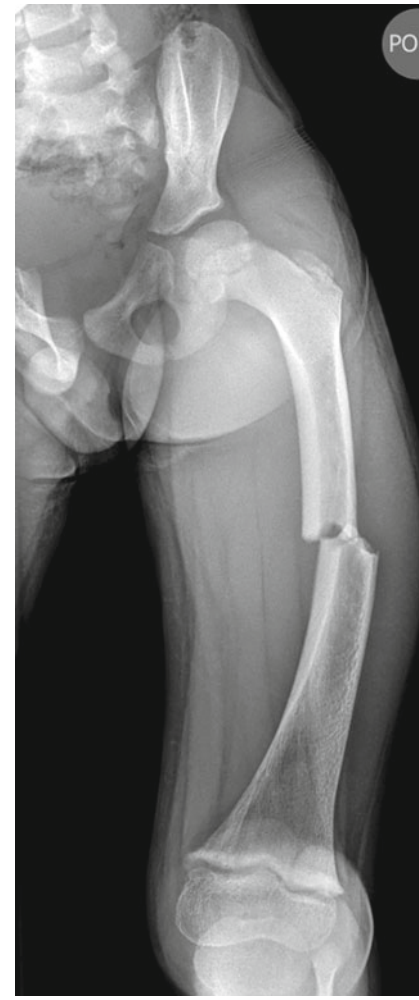

Fig. 11.12 A transverse fracture of the mid-shaft of the left femur, sustained atraumatically while running at play, in a 4-year-old with ALGS (Courtesy of Dr. Christina B. Bales, MD, CHOP)

ALGS patients frequently have short stature; and this is likely multifactorial in origin, resulting from cholestasis and malabsorption, congenital heart disease, and genetic predisposition.

Ocular Involvement

A large and varied number of ocular abnormalities have been described in ALGS, though posterior embryotoxon is the most common. Posterior embryotoxon is a prominent, centrally positioned

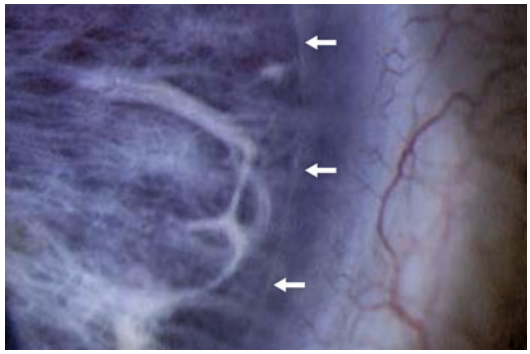

Fig. 11.13 Slit-lamp examination demonstrating posterior embryotoxon (*arrows*) in an infant with ALGS (Courtesy of Dr. William Anninger and Dr. Brian Forbes, CHOP)

Schwalbe's ring (or line) at the point at which the corneal endothelium and the uveal trabecular meshwork join and is most clearly identified during slit-lamp examination (see Fig. 11.13). Posterior embryotoxon occurs in 56–88 % of patients with ALGS but can also be detected in 22 % of children evaluated in a general ophthalmology clinic [54]. Posterior embryotoxon is also seen in other multisystem disorders such as chromosome 22q deletion. Other reported ALGS ocular anomalies include Axenfeld anomaly (seen in 13 % of ALGS patients), microcornea, keratoconus, congenital macular dystrophy, ectopic pupil, band keratopathy, cataract, iris hypoplasia, choroidal folds, and anomalous optic disks. In a large series of patients with ALGS studied systematically, Hingorani et al. identified posterior embryotoxon in 95 % of 22 patients, iris abnormalities in 45 %, diffuse fundic hypopigmentation in 57 %, speckling of the retinal pigment epithelium in 33 %, and optic disk abnormalities in 76 % [55]. Though not widely performed, ocular ultrasound can detect optic disk drusen in at least one eye in 95 % and bilateral disk drusen in 80 % of patients with ALGS [56].

Diagnostic Considerations

The diagnosis of ALGS has traditionally been based on clinical criteria defined as the presence of paucity of intrahepatic bile ducts in addition to three of the following major criteria: (1) cholestasis, (2) evidence of cardiac disease, (3) skeletal abnormalities, (4) ocular anomalies, and (5) characteristic facial features [2, 4]. The phenotype of ALGS has clearly broadened to include renal and vascular involvement, and these should now be included as disease-defining criteria.

The classical clinical diagnosis of ALGS rests on the presence of bile duct paucity and therefore requires a liver biopsy. However, a liver biopsy is no longer considered mandatory to make a diagnosis of ALGS. A liver biopsy is often necessary, however, to distinguish ALGS from biliary atresia in an infant with high-GGT cholestasis. When a liver biopsy is performed to differentiate between ALGS and BA, it should be noted that bile duct paucity is not always seen early on in life, whereas it is much more prevalent after 6 months of age [7]. Early biopsies of patients with ALGS may reveal bile duct proliferation or even giant cell hepatitis, thus further confusing the clinical picture with BA. Following a biopsy revealing bile duct proliferation and suggestive of obstruction, the interpretation of diisopropyl iminodiacetic acid (DISIDA) scans should be made with caution, as patients with ALGS can have non-draining scans. An intraoperative cholangiogram in the hands of an experienced surgeon remains the gold standard to differentiate BA from ALGS, since mutational analysis for ALGS cannot usually be performed rapidly.

It is now thought that three or four of the expanded clinical criteria are required to make the diagnosis of ALGS in children younger than 6 months of age. If there is a first-degree relative with a definitive diagnosis of ALGS, then only one or two criteria are needed. Of course the advent of molecular screening has clarified the diagnostic challenges in many subtle or atypical ALGS cases.

Molecular sequencing is now widely commercially available for *JAG1*. *NOTCH2* screening is more limited as a clinical test. A molecular diagnosis is not mandatory but 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 (>90 %). Individuals that have clinical features of ALGS but are not found to be carrying *JAG1* mutations should have sequence analysis of *NOTCH2* [14].

If a mutation is identified in a child with ALGS, then the parents should be offered screening for the same mutation. *JAG1* mutations are inherited in 40 % of cases and are de novo in the remainder. If parents are found not to carry their child's mutation, the risk of disease recurrence in new offspring is very low. The only exception is in the case of germline mosaicism, which has been reported in patients with ALGS.

Prenatal genetic testing has been used to aid in the diagnosis of ALGS of a fetus. This requires amniocentesis or chorionic villous sampling and a known *JAG1* mutation in the family. 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 of mutation in the family or on the severity of disease in other family members.

Prognosis of Alagille Syndrome

The prognosis of Alagille syndrome varies tremendously depending on the extent and severity of the manifestations of the disease. The single most important predictive factor is complex congenital cardiac disease, which has been shown to contribute significantly to patient mortality in the first years of life [7]. The major long-term population studies of patient course and outcome have been performed at large hepatology centers, focusing mainly on patients with symptomatic liver disease diagnosed by having at least three of the major clinical criteria. In addition to this significant selection bias, a number of patients seen in these centers were referred expressly for transplantation, thus further artificially increasing the frequency of hepatic morbidity and mortality. Finally, most studies of outcomes include patients born decades ago, when cardiac, hepatic, nutritional, and transplant therapies were significantly less effective or not even available. During this time, it is also certain that many patients were not accurately diagnosed with Alagille syndrome, further complicating an accurate analysis. Since the advent of molecular diagnosis of mildly affected probands and less affected relatives, the denominator of Alagille "syndrome" *JAG1* mutation carriers has at least doubled, and the average outcome and severity have apparently proportionately improved, although this has not been formally reported. It is, however, this number that a *JAG1* mutation carrier should consider with a genetic counselor in order to make decisions about family planning.

In the largest series of long-term follow-up, Lykavieris et al. [27] report on their experience with symptomatic hepatic disease. For this study, all ALGS patients without hepatic disease were excluded. From 1960 to 2000, there were 163 patients with ALGS and liver disease, divided into two groups: 132 patients who presented with neonatal cholestatic jaundice and 31 who presented with hepatic disease later in life. Of the neonatal jaundice group, 102 remained jaundiced, 112 had poorly controlled pruritus, and 40 had xanthomas. Liver transplantation was performed on 33 %. Symptoms were considerably less in the 31 who presented at a later age, and none were referred for transplantation. For the total population of hepatic ALGS, the actuarial survival rates with native liver were 51 % at 10 years. and 38 % at 20 years. Overall survival rates were 68 % at 10 years and 62 % at 20 years. Surprisingly, the 10-year. survival rates for patients born before and after 1986 were the not different, at 67 versus 70 %, respectively. Of the 44 patients who underwent liver transplantation at a median age of 7 years old, indications were refractory pruritus in 36, xanthomas in 32, bone fractures in 15, and signs of end-stage liver disease in only five. The 10-year survival after liver transplantation in this study was 77 %. These results demonstrate that the liver disease in patients with ALGS, particularly those presenting in infancy, is severe in many instances, with significant morbidity and mortality.

In a report on 92 patients diagnosed with ALGS by classical clinical criteria between 1974 and 1997, Emerick et al. [7] found that the only early feature correlated with significant mortality was the presence of structural congenital cardiac disease. Patients and relatives who did not meet full clinical criteria were not included in this study, and essentially all patients had cholestatic liver disease. Liver transplantation for hepatic decompensation was necessary in 21 %, and 1-year posttransplant survival in this group was 79 %. The 20-year predicted life expectancy in this study was 75 % but only 60 % for the subset who required liver transplantation. Other significant factors contributing to mortality were intracranial bleeding and stroke [7].

Summary

In the nearly 50 years since Alagille described the syndrome, there have been significant advances in the diagnosis and treatments for Alagille syndrome. The finding that ALGS is caused by single-gene defects in the Notch signaling led to new insights into the role the pathway plays in controlling human embryogenesis. The easy and affordable availability of accurate mutation analysis has led to a more comprehensive understanding of the spectrum of Alagille "syndrome" and an expansion of the clinical presentations of *JAG1* mutation. Gene testing has provided clarity to patients who do not meet classical clinical criteria for the syndrome, and it has facilitated family planning and prenatal diagnosis. The role of Notch signaling is being extensively studied, and its role in bile duct development and tubulogenesis is predictable, given the manifestations of the disease. Vitamin and nutritional support greatly enhances the lives of children with ALGS, improving outcomes and preventing complications. Partial external biliary diversion can greatly alleviate pruritus in some patients. Advances in liver transplantation and immunosuppressive therapy have greatly improved the hepatic outcome, so that liver disease is a much less common cause of death and disability for patients.

References

1. Alagille D, Gautier M, Habib EC, Dommergues JP. Pre- and postoperative hepatic biopsy data in prolonged cholestasis in infants. Study of 128 cases. Arch Fr Pediatr. 1969;26:283–96.
2. Alagille D, Odievre M, Gautier M, Dommergues JP. Hepatic ductular hypoplasia associated with characteristic facies, vertebral malformations, retarded physical, mental, and sexual development, and cardiac murmur. J Pediatr. 1975;86:63–71.
3. Watson GH, Miller V. Arteriohepatic dysplasia: familial pulmonary arterial stenosis with neonatal liver disease. Arch Dis Child. 1973;48:459–66.
4. Alagille D, Estrada A, Hadchouel M, Gautier M, Odievre M, Dommergues JP. Syndromic paucity of interlobular bile ducts (Alagille syndrome or arteriohepatic dysplasia): review of 80 cases. J Pediatr. 1987;110:195–200.
5. Crosnier C, Lykavieris P, Meunier-Rotival M, Hadchouel M. Alagille syndrome. The widening spectrum of arteriohepatic dysplasia. Clin Liver Dis. 2000;4:765–78.
6. Deprettere A, Portmann B, Mowat AP. Syndromic paucity of the intrahepatic bile ducts: diagnostic difficulty; severe morbidity throughout early childhood. J Pediatr Gastroenterol Nutr. 1987;6:865–71.
7. Emerick KM, Rand EB, Goldmuntz E, Krantz ID, Spinner NB, Piccoli DA. Features of Alagille syndrome in 92 patients: frequency and relation to prognosis. Hepatology. 1999;29:822–9.
8. Hoffenberg EJ, Narkewicz MR, Sondheimer JM, Smith DJ, Silverman A, Sokol RJ. Outcome of syndromic paucity of interlobular bile ducts (Alagille syndrome) with onset of cholestasis in infancy. J Pediatr. 1995;127:220–4.
9. Quiros-Tejeira RE, Ament ME, Heyman MB, Martin MG, Rosenthal P, Hall TR, McDiarmid SV, Vargas JH. Variable morbidity in Alagille syndrome: a review of 43 cases. J Pediatr Gastroenterol Nutr. 1999;29:431–7.
10. Li L, Krantz ID, Deng Y, Genin A, Banta AB, Collins CC, Qi M, Trask BJ, Kuo WL, Cochran J, et al. Alagille syndrome is caused by mutations in human Jagged1, which encodes a ligand for Notch1. Nat Genet. 1997;16:243–51.
11. Oda T, Elkahloun AG, Pike BL, Okajima K, Krantz ID, Genin A, Piccoli DA, Meltzer PS, Spinner NB, Collins FS, et al. Mutations in the human Jagged1 gene are responsible for Alagille syndrome. Nat Genet. 1997;16:235–42.
12. Warthen DM, Moore EC, Kamath BM, Morrissette JJ, Sanchez P, Piccoli DA, Krantz ID, Spinner NB. Jagged1 (JAG1) mutations in Alagille syndrome: increasing the mutation detection rate. Hum Mutat. 2006;27:436–43.
13. Kamath BM, Bauer RC, Loomes KM, Chao G, Gerfen J, Hutchinson A, Hardikar W, Hirschfield G, Jara P, Krantz ID, et al. NOTCH2 mutations in Alagille syndrome. J Med Genet. 2012;49:138–44.

14. McDaniell R, Warthen DM, Sanchez-Lara PA, Pai A, Krantz ID, Piccoli DA, Spinner NB. NOTCH2 mutations cause Alagille syndrome, a heterogeneous disorder of the notch signaling pathway. Am J Hum Genet. 2006;79:169–73.
15. Kamath BM, Bason L, Piccoli DA, Krantz ID, Spinner NB. Consequences of JAG1 mutations. J Med Genet. 2003;40:891–5.
16. Eldadah ZA, Hamosh A, Biery NJ, Montgomery RA, Duke M, Elkins R, Dietz HC. Familial tetralogy of Fallot caused by mutation in the jagged1 gene. Hum Mol Genet. 2001;10:163–9.
17. Le Caignec C, Lefevre M, Schott JJ, Chaventre A, Gayet M, Calais C, Moisan JP. Familial deafness, congenital heart defects, and posterior embryotoxon caused by cysteine substitution in the first epidermal-growth-factor-like domain of jagged 1. Am J Hum Genet. 2002;71:180–6.
18. Garg V, Muth AN, Ransom JF, Schluterman MK, Barnes R, King IN, Grossfeld PD, Srivastava D. Mutations in NOTCH1 cause aortic valve disease. Nature. 2005;437:270–4.
19. Joutel A, Corpechot C, Ducros A, Vahedi K, Chabriat H, Mouton P, Alamowitch S, Domenga V, Cecillion M, Marechal E, et al. Notch3 mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia. Nature. 1996;383:707–10.
20. Krantz ID, Colliton RP, Genin A, Rand EB, Li L, Piccoli DA, Spinner NB. Spectrum and frequency of jagged1 (JAG1) mutations in Alagille syndrome patients and their families. Am J Hum Genet. 1998;62:1361–9.
21. Gridley T. Notch signaling in vascular development and physiology. Development. 2007;134:2709–18.
22. Kamath BM, Thiel BD, Gai X, Conlin LK, Munoz PS, Glessner J, Clark D, Warthen DM, Shaikh TH, Mihci E, et al. SNP array mapping of chromosome 20p deletions: genotypes, phenotypes, and copy number variation. Hum Mutat. 2009;30:371–8.
23. Kahn E, Markowitz J, Aiges H, Daum F. Human ontogeny of the bile duct to portal space ratio. Hepatology. 1989;10:21–3.
24. Kahn E. Paucity of interlobular bile ducts. Arteriohepatic dysplasia and nonsyndromic duct paucity. Perspect Pediatr Pathol. 1991;14:168–215.
25. Kaye AJ, Rand EB, Munoz PS, Spinner NB, Flake AW, Kamath BM. Effect of Kasai procedure on hepatic outcome in Alagille syndrome. J Pediatr Gastroenterol Nutr. 2010;51:319–21.
26. Markowitz J, Daum F, Kahn EI, Schneider KM, So HB, Altman RP, Aiges HW, Alperstein G, Silverberg M. Arteriohepatic dysplasia. I. Pitfalls in diagnosis and management. Hepatology. 1983;3:74–6.
27. Lykavieris P, Hadchouel M, Chardot C, Bernard O. Outcome of liver disease in children with Alagille syndrome: a study of 163 patients. Gut. 2001;49:431–5.
28. Cynamon HA, Andres JM, Iafrate RP. Rifampin relieves pruritus in children with cholestatic liver disease. Gastroenterology. 1990;98:1013–6.
29. Yerushalmi B, Sokol RJ, Narkewicz MR, Smith D, Karrer FM. Use of rifampin for severe pruritus in children with chronic cholestasis. J Pediatr Gastroenterol Nutr. 1999;29:442–7.
30. Balistreri WF. Bile acid therapy in pediatric hepatobiliary disease: the role of ursodeoxycholic acid. J Pediatr Gastroenterol Nutr. 1997;24:573–89.
31. Zellos A, Roy A, Schwarz KB. Use of oral naltrexone for severe pruritus due to cholestatic liver disease in children. J Pediatr Gastroenterol Nutr. 2010;51:787–9.
32. Emerick KM, Whitington PF. Partial external biliary diversion for intractable pruritus and xanthomas in Alagille syndrome. Hepatology. 2002;35:1501–6.
33. Whitington PF, Whitington GL. Partial external diversion of bile for the treatment of intractable pruritus associated with intrahepatic cholestasis. Gastroenterology. 1988;95:130–6.
34. Dingemann C, Baumann U, Petersen C, Lentze MJ, Ure B. Ileal exclusion for intractable pruritus in Alagille syndrome. Eur J Pediatr Surg. 2012;22:251–3.
35. Modi BP, Suh MY, Jonas MM, Lillehei C, Kim HB. Ileal exclusion for refractory symptomatic cholestasis in Alagille syndrome. J Pediatr Surg. 2007;42:800–5.
36. Cardona J, Houssin D, Gauthier F, Devictor D, Losay J, Hadchouel M, Bernard O. Liver transplantation in children with Alagille syndrome – a study of twelve cases. Transplantation. 1995;60:339–42.
37. Englert C, Grabhorn E, Burdelski M, Ganschow R. Liver transplantation in children with Alagille syndrome: indications and outcome. Pediatr Transplant. 2006;10:154–8.
38. Kasahara M, Kiuchi T, Inomata Y, Uryuhara K, Sakamoto S, Ito T, Fujimoto Y, Ogura Y, Oike F, Tanaka K. Living-related liver transplantation for Alagille syndrome. Transplantation. 2003;75:2147–50.
39. Arnon R, Annunziato R, Miloh T, Suchy F, Sakworawich A, Hiroshi S, Kishore I, Kerkar N. Orthotopic liver transplantation for children with Alagille syndrome. Pediatr Transplant. 2010;14:622–8.
40. Kamath BM, Yin W, Miller H, Anand R, Rand EB, Alonso E, Bucuvalas J. Outcomes of liver transplantation in Alagille syndrome: the split experience. Liver Transpl. 2012;18(8):940–8.
41. Hori T, Egawa H, Takada Y, Oike F, Kasahara M, Ogura Y, Sakamoto S, Ogawa K, Yonekawa Y, Nguyen JH, et al. Long-term outcomes after living-donor liver transplantation for Alagille syndrome: a single center 20-year experience in Japan. Am J Transplant. 2010;10:1951–2.
42. Gurkan A, Emre S, Fishbein TM, Brady L, Millis M, Birnbaum A, Kim-Schluger L, Sheiner PA. Unsuspected bile duct paucity in donors for living-related liver transplantation: two case reports. Transplantation. 1999;67:416–8.
43. Kamath BM, Schwarz KB, Hadzic N. Alagille syndrome and liver transplantation. J Pediatr Gastroenterol Nutr. 2010;50:11–5.
44. Razavi RS, Baker A, Qureshi SA, Rosenthal E, Marsh MJ, Leech SC, Rela M, Mieli-Vergani G. Hemodynamic response to continuous infusion of dobutamine in Alagille's syndrome. Transplantation. 2001;72:823–8.

45. McElhinney DB, Krantz ID, Bason L, Piccoli DA, Emerick KM, Spinner NB, Goldmuntz E. Analysis of cardiovascular phenotype and genotype-phenotype correlation in individuals with a JAG1 mutation and/or Alagille syndrome. Circulation. 2002;106:2567–74.
46. Kamath BM, Spinner NB, Rosenblum ND. Renal involvement and the role of Notch signalling in Alagille syndrome. Nat Rev Nephrol. 2013;9:409–18.
47. Kamath BM, Podkameni G, Hutchinson AL, Leonard LD, Gerfen J, Krantz ID, Piccoli DA, Spinner NB, Loomes KM, Meyers K. Renal anomalies in Alagille syndrome: a disease-defining feature. Am J Med Genet A. 2011;158A(1):85–9.
48. Lykavieris P, Crosnier C, Trichet C, Meunier-Rotival M, Hadchouel M. Bleeding tendency in children with Alagille syndrome. Pediatrics. 2003;111:167–70.
49. Emerick KM, Krantz ID, Kamath BM, Darling C, Burrowes DM, Spinner NB, Whitington PF, Piccoli DA. Intracranial vascular abnormalities in patients with Alagille syndrome. J Pediatr Gastroenterol Nutr. 2005;41:99–107.
50. Kamath BM, Spinner NB, Emerick KM, Chudley AE, Booth C, Piccoli DA, Krantz ID. Vascular anomalies in Alagille syndrome: a significant cause of morbidity and mortality. Circulation. 2004;109:1354–8.
51. Kamath BM, Loomes KM, Oakey RJ, Emerick KE, Conversano T, Spinner NB, Piccoli DA, Krantz ID. Facial features in Alagille syndrome: specific or cholestasis facies? Am J Med Genet. 2002;112:163–70.
52. Kamath BM, Loomes KM, Oakey RJ, Krantz ID. Supernumerary digital flexion creases: an additional clinical manifestation of Alagille syndrome. Am J Med Genet. 2002;112:171–5.
53. Bales CB, Kamath BM, Munoz PS, Nguyen A, Piccoli DA, Spinner NB, Horn D, Shults J, Leonard MB, Grimberg A, et al. Pathologic lower extremity fractures in children with Alagille syndrome. J Pediatr Gastroenterol Nutr. 2010;51(1):66–70.
54. Rennie CA, Chowdhury S, Khan J, Rajan F, Jordan K, Lamb RJ, Vivian AJ. The prevalence and associated features of posterior embryotoxon in the general ophthalmic clinic. Eye (Lond). 2005;19:396–9.
55. Hingorani M, Nischal KK, Davies A, Bentley C, Vivian A, Baker AJ, Mieli-Vergani G, Bird AC, Aclimandos WA. Ocular abnormalities in Alagille syndrome. Ophthalmology. 1999;106:330–7.
56. Nischal KK, Hingorani M, Bentley CR, Vivian AJ, Bird AC, Baker AJ, Mowat AP, Mieli-Vergani G, Aclimandos WA. Ocular ultrasound in Alagille syndrome: a new sign. Ophthalmology. 1997;104:79–85.

Idiopathic Neonatal Hepatitis and Its Differential Diagnoses

12

Kathleen B. Schwarz

Introduction

The aim is to provide a cost-effective diagnostic approach targeted to the common causes of the three different conditions as opposed to the blanket application of the outdated "TORCH" acronym, useful in the past for diagnosing Toxoplasmosis, Other (Syphilis), Rubella, Cytomegalovirus, and Herpes. The proportion of cholestatic infants given this diagnostic label continues to decrease as both molecular genetics and infectious disease become more technologically sophisticated. Indeed the proportion of cholestatic infants given this "diagnosis" has decreased from 65 % in 1970 to 15 % in 2004 [1]. Given that cholestatic infants (including those ultimately given the label of "INH") may present with acute liver failure [2], transient cholestasis, or chronic liver disease, and that the differential diagnosis of each presentation is lengthy, this chapter is written from the "real-world" perspective – the manner in which the cholestatic infant presents to the clinician. After all that is the major determinant of both diagnostic and treatment algorithms. Detailed descriptions of many individual nosologic entities are covered elsewhere in this book as are treatment algorithms. Thus this chapter is written mainly from the perspective of the differential diagnosis of each of these three different clinical presentations. The aim is to provide a cost-effective diagnostic approach targeted to the common causes of the three different conditions as opposed to the blanket application of the outdated "TORCH" acronym, useful in the past for diagnosing toxoplasmosis, other (syphilis), rubella, cytomegalovirus, and herpes.

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Neonatal Acute Liver Failure

Cholestatic infants presenting with acute liver failure are critically ill, often with multisystem disease (Fig. 12.1). In addition to cholestasis they may have hyper- or hypothermia, hepatosplenomegaly, occasionally rash, obtundation, and/or seizures. Laboratory studies may show evidence of coagulopathy, hypoglycemia, and hyperammonemia. Table 12.1 contains a list of the many causes arranged according to the frequency they occur.

Infection is a frequent cause of this condition. The two most common viral causes include herpes simplex [3] and enterovirus [4]. Herpes simplex II is the most common treatable viral cause, although cases with HSV I have been described. The incubation is 5–21 days, so a high index of suspicion is needed given that the infected newborn may be thought to be completely normal in the first few days of life, and one-third of the infected infants never have the classical vesicular skin lesions. There are three clinical presentations: (1) skin, eyes, and mouth; (2) disseminated

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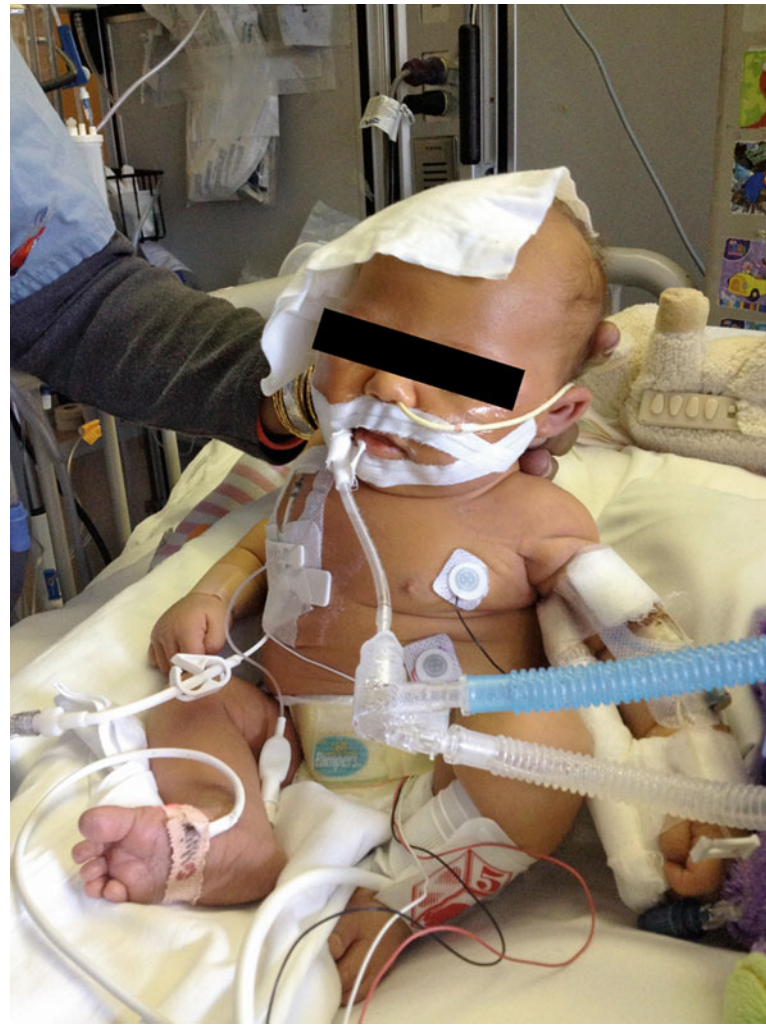

Fig. 12.1 Neonate with acute liver failure

especially to liver and lungs; and (3) central nervous system. Mortality with the cholestatic form is correlated to the height of elevation of aspartate aminotransferase, >10× the upper limit of normal carrying the worst prognosis. Diagnosis is by IgM serology, polymerase chain reaction assay, or the gold standard of viral culture, since polymerase chain reaction is not 100 % sensitive [5]. Prompt institution of acyclovir has dropped mortality from 90 to 31 %. Enterovirus infection can be devastating in preterm infants, which may present with hepatitis, coagulopathy, and thrombocytopenia. The infection carries with it a dramatic 31 % fatality. Management is supportive. Unfortunately the drug pleconaril, which inhibits viral attachment, is not available at the present time.

Much rarer viral causes are listed in Table 12.1. Cytomegalovirus (CMV) only rarely presents with this fulminant form, but liver transplantation for fulminant CMV has been reported [6]. Although rubella can present with this form, it more frequently presents as congenital rubella with malformations. The infant with congenital varicella presents with disseminated vesicular lesions, pneumonia, hepatitis, and meningoencephalitis and should be treated with intravenous acyclovir [7].

The most common causes of bacterial sepsis in the cholestatic newborn with acute liver failure are listed in Table 12.1; *group B Streptococcus pyogenes* is the most common of all [8]. Broad-spectrum antibiotic coverage for all of these

Table 12.1 Causes of acute liver failure in the neonate

Common
Infectious
Viral: enterovirus and herpes simplex I
Bacterial: *group B Streptococcus pyogenes*, *group D Enterococcus*, *Staphylococcus aureus*, *coagulase-negative Staphylococcus*, *Escherichia coli*
Rare
Infectious
Viral: adenovirus, cytomegalovirus, coxsackie virus, Epstein-Barr virus, hepatitis A, B, E, human herpes virus VI, human papilloma virus, paramyxovirus (syncytial giant cell hepatitis), parvovirus B19, rubella, varicella, yellow fever
Bacterial: *Listeria monocytogenes*, tuberculosis, and syphilis
Protozoa: malaria, *Toxoplasma gondii*, leishmaniasis
Metabolic/genetic
Neonatal hemochromatosis, galactosemia, hepatorenal tyrosinemia, fructose-1-6-diphosphatase deficiency, mitochondrial hepatopathy – esp DGUOK with nystagmus and hypotonia
Unclassified
Budd-Chiari syndrome, hemophagocytic lymphohistiocytic syndrome, hepatoblastoma, histiocytosis X, neonatal leukemia, neuroblastoma, spontaneous biliary perforation, adrenal insufficiency with hypovolemia, hyponatremia, hypoglycemia secondary to adrenal hemorrhage
INH

organisms should be initiated until results of blood and cerebrospinal fluid culture are available. Less common causes include *Listeria monocytogenes*, although hepatitis is rare with this infection. Cholestatic liver disease secondary to tuberculosis can present with fever, respiratory distress, poor feeding, and hepatomegaly. Diagnosis is by chest radiograph, gastric aspirates, gamma interferon-releasing assays, and/or liver biopsy showing caseating granulomas. Skin tests give a poor yield in this age group. Treatment is with a four-drug regimen × 2 months followed by a two-drug regimen × 4 months. The neonate with acute liver failure secondary to the spirochete bacterium *Treponema pallidum* presents with hepatosplenomegaly, hepatitis, disseminated intravascular coagulation (DIC), condyloma acuminata, rhinitis, meningitis, and lymphadenopathy. This form of syphilis is treatable with penicillin.

The protozoal causes of acute liver failure in the cholestatic neonate are all rare in North America. Malaria presents with fever, anemia, jaundice, and splenomegaly at 3–6 weeks of age. *Toxoplasma gondii* presents with severe illness if the infant is infected in the third trimester: DIC, hepatitis, and severe pneumonitis are characteristic. Infection in the first trimester leads to ophthalmologic disease. It is treatable with pyrimethamine, sulfadiazine, and folinic acid [9]. Leishmaniasis usually presents with cutaneous rather than hepatic lesions, although the latter has been reported [10].

Fulminant liver failure in the neonate can be secondary to noninfectious causes such as adrenal hemorrhage [11], neonatal hemochromatosis (congenital alloimmune hepatitis) [12], or mitochondrial hepatopathy [13].

Transient Neonatal Cholestasis

Transient causes of neonatal cholestasis commonly include extrahepatic bacterial infection or sepsis, drug-induced liver injury, perinatal asphyxia/hypoxia, total parenteral nutrition, and INH. Much rarer causes of this syndrome include fetal arrhythmia/congestive heart failure, inspissated bile syndrome following massive neonatal hemolysis, and neonatal lupus erythematosus. In this syndrome, there is asymptomatic elevation of liver enzymes in 10–25 % with cholestasis, mild hepatomegaly less commonly splenomegaly, anti-Ro and anti-La antibodies, and idiopathic neonatal giant cell hepatitis on liver biopsy [14].

The major issue with this broad classification of "transient" is that at the time of presentation, the clinician does not know how long the condition will last. It would of course be mandatory to provide appropriate antibiotic coverage if signs of infection are present. Supportive care, progression of enteral feeds to meet caloric goals as rapidly as the clinical condition permits, and only a minimal diagnostic work-up are indicated initially. The pattern of jaundice noted in Fig. 12.2 may be helpful

since the transient form would manifest itself as an exaggeration of physiologic jaundice only extended over a longer period of time.

Chronic Neonatal Cholestasis

Infants with chronic cholestasis usually present with pruritus, fat-soluble vitamin deficiency, and failure to thrive (Fig. 12.3). Occasionally they may develop acute intracerebral hemorrhage secondary to vitamin K deficiency [15]. The differential diagnosis of these conditions is much longer than for the transient and includes both intra- and extrahepatic causes [16, 17]. Intrahepatic chronic cholestasis can be secondary to infections, metabolic/genetic disorders, unclassified causes, and INH. CMV is the most common viral infection of infants (0.4–2.3 % live births) [18]. If the infant develops hepatitis, this presentation may be accompanied by hypoalbuminemia, coagulopathy, elevated alkaline phosphatase, and γ-glutamyl transpeptidase. Liver biopsy may show giant cell hepatitis and ductopenia. Rarely CMV hepatitis can progress to cirrhosis and portal hypertension. Intravenous ganciclovir improves outcome. Orally administered valganciclovir can be administered after 35 days of intravenous ganciclovir even in very low birth weight infants. Hyperimmune globulin may also useful for hepatitis [19]. Rare viral causes include human immunodeficiency virus [20], which may present with nonimmune hydrops and resolve with HAART therapy, and congenital rubella, which presents with cataracts, irritability, microphthalmia, retinopathy, patent ductus arteriosus, low birth weight, deafness, cardiomyopathy, and only rarely cholestasis. Although maternal hepatitis C virus (HCV) has been reported associated with low birth weight preterm infants with cholestasis and congenital anomalies [21], this would be the rare exception since most infants with HCV appear completely normal in the newborn period. Likewise, infants who acquire hepatitis B virus

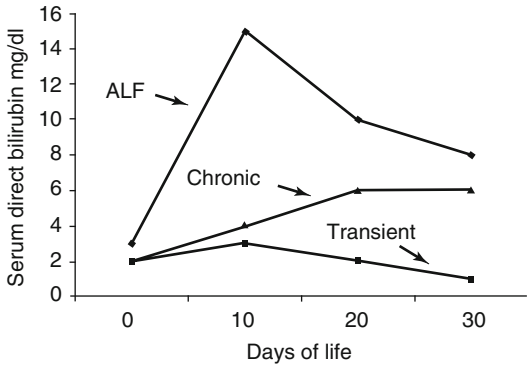

Fig. 12.2 Patterns of neonatal cholestasis

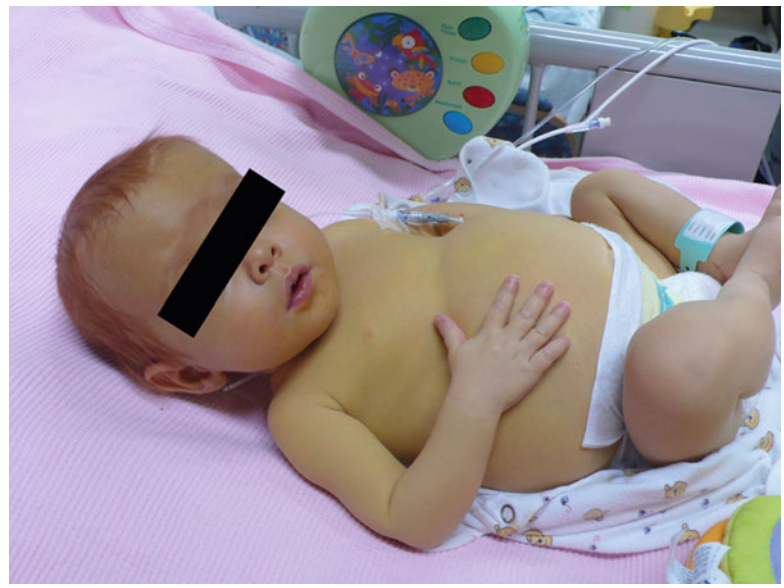

Fig. 12.3 Neonate with chronic cholestasis, prominent abdomen secondary to hepatosplenomegaly

Table 12.2 Intrahepatic causes of chronic neonatal cholestasis

Common
Infectious
CMV
Metabolic/genetic
Alagille syndrome, alpha-1-antitrypsin deficiency, citrin deficiency, cystic fibrosis, hypothyroidism, non-syndromic bile duct paucity, panhypopituitarism, progressive familial intrahepatic cholestasis 1, 2, 3
Unclassified
Total parenteral nutrition, drug-induced liver injury
INH
Rare
Infectious
Viral: HIV, rubella, HCV, HBV, varicella
Metabolic/genetic
Arthrogryposis renal dysfunction cholestasis syndrome (ARC), autosomal dominant polycystic liver disease (ADPLD), autosomal recessive polycystic kidney disease (ARPKD), bile acid synthetic defects, cholesteryl ester storage disease (Wolman disease), dehydrated hereditary stomatocytosis, Dubin -Johnson syndrome, familial hypercholanemia, Gaucher disease, glycogen storage disease IV, lymphedema cholestasis syndrome (Aagenaes syndrome), neonatal sclerosing cholangitis (NISCH); Niemann-Pick disease type A or C, North American childhood cirrhosis (cirhin deficiency), and peroxisomal disorders (Zellweger syndrome, neonatal adrenoleukodystrophy)
Unclassified
Fetal alcohol syndrome, McCune-Albright syndrome, villin functional defect, Down syndrome with myeloproliferative disorder

(HBV) from their mothers are usually asymptomatic early on, but both fulminant and chronic cholestatic neonatal HBV infections have been reported [21]. Varicella is more likely to present with hepatitis if the infection is prenatal, and it does respond to acyclovir. Infants with congenital varicella may have cicatricial skin lesions, limb hypoplasia, ocular defects, and central nervous system abnormalities including microcephaly and seizures.

A list of common and more rare infectious and other causes of chronic neonatal cholestasis is provided in Table 12.2. Alagille syndrome is one of the most common genetic causes of cholestasis and can be easily confused with INH since ~25 % of the time the liver biopsy shows nonspecific hepatitis [22]. Alpha-1-antitrypsin deficiency accounts for about 10 % of cholestatic neonates and is the most common metabolic cause of this clinical scenario. Citrin deficiency (type II citrullinemia) has been increasingly recognized as a common cause of neonatal cholestasis in Asian countries and may be more common in North America than previously appreciated [23]. The diagnosis should be suspected in the cholestatic neonate manifesting abnormalities of plasma and/or urine amino acids, galactosemia, hypoproteinemia, and hypoglycemia. Generally the disease is not severe and can be managed using nutritional support. The disease usually becomes asymptomatic within a year although some unfortunate children remain symptomatic for one or more decades. Occasionally the disease is severe enough to require liver transplantation. Panhypopituitarism (septo-optic dysplasia) presents with failure to thrive, hypoglycemia, micropenis, and cryptorchidism. The liver biopsy shows canalicular cholestasis. Treatment with thyroxine, growth hormone, and hydrocortisone usually resolves the cholestasis [24].

As noted above, INH has declined to about 15 % of causes of neonatal cholestasis and frequently the cholestasis resolves in a couple of months. A more severe form is familial and accompanied by protein-losing enteropathy, reversible by liver transplantation [25].

Of the more rare conditions to present with chronic cholestasis, dehydrated hereditary stomatocytosis is noteworthy as it presents with perinatal ascites and resolving jaundice [26]. Bile acid synthetic defects may manifest giant cell hepatitis on liver biopsy. The presentation of neonatal Gaucher disease is fairly striking and includes a history of an abnormal pregnancy, hydrops fetalis/hydramnios, splenomegaly, joint contractures, neurological abnormalities, and ichthyosis or dysmorphic facies noticeable at birth [27]. Neonatal sclerosing cholangitis should also be suspected when ichthyosis is present. Neonates with this condition exhibit leukocyte vacuoles and alopecia (NISCH). Niemann-Pick disease type A or C

presents with chronic cholestasis and progressive hepatosplenomegaly. Fetal hydrops or ascites can occur and a few infants succumb to chronic liver failure within the first 6 months of onset. Diagnosis is by electron microscopy of the liver biopsy showing characteristic deposits of sphingomyelin. Cultured skin fibroblasts can be used for the filipin test. The disease is autosomal recessive secondary to mutations in *npc1* or *npc2*. Genetic sequencing is confirmative for NPC. To make the diagnosis of NPA, one needs to demonstrate deficiency of sphingomyelinase or causative mutations in the *SMPD1* gene. Early hematopoietic stem cell transplant is under development as is treatment for *ncp2* with miglustat which has been approved in Europe.

Extrahepatic causes of chronic cholestasis include extrahepatic biliary atresia, Caroli syndrome, choledochal cyst, choledocholithiasis, hairlike bile duct syndrome, and spontaneous bile duct perforation. An important principle in guiding diagnostic evaluation of the cholestatic neonate is to attempt to distinguish between intra- and extrahepatic causes. This important area will be discussed further in the section on diagnosis.

Diagnostic Approach (Fig. 12.4)

Blood tests are generally used initially to establish that the jaundice is hepatic in origin, secondary to direct or conjugated hyperbilirubinemia. Serum aminotransferases are usually elevated. γ-glutamyl transpeptidase is elevated in most causes of neonatal cholestasis, including INH, with the exception of PFIC 1 and 2, bile acid synthetic defects, arthrogryposis renal dysfunction cholestasis syndrome (ARC), and lymphedema

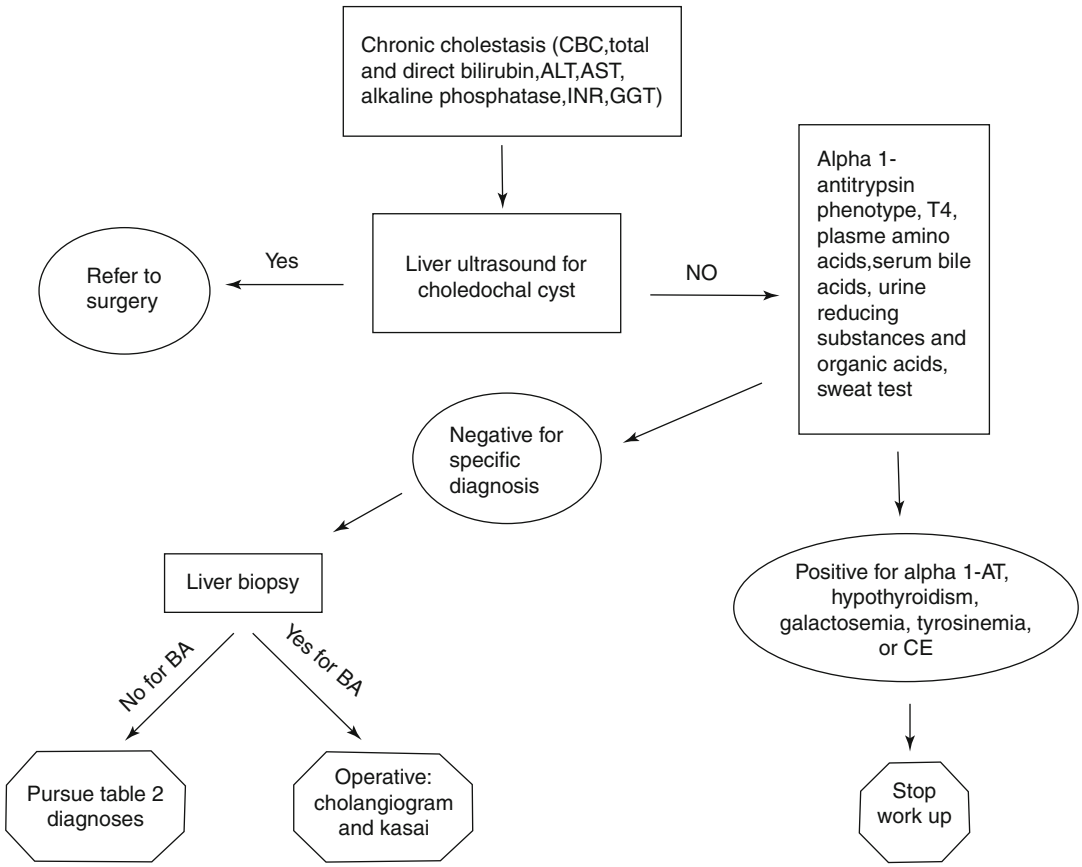

Fig. 12.4 Suggested management of an infant with chronic cholestasis

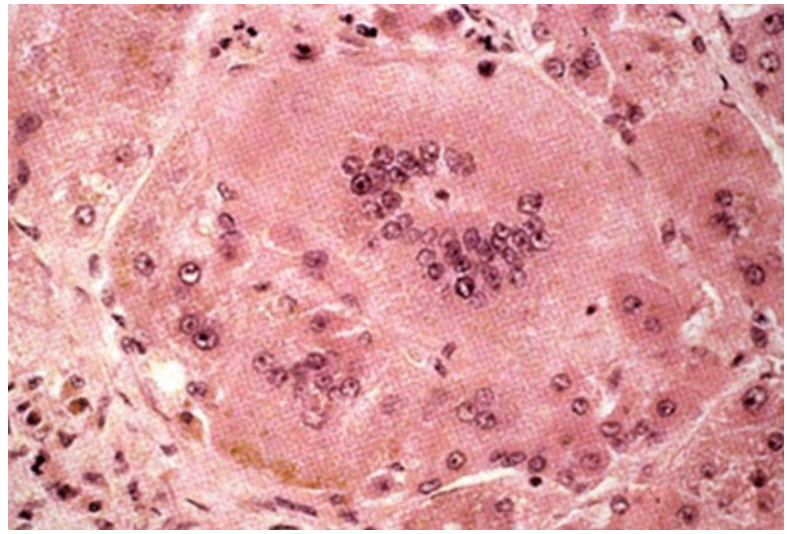

Fig. 12.5 Hepatic histology of idiopathic neonatal giant cell hepatitis (hematoxylin and eosin stain 40×)

cholestasis syndrome (LCS). Likewise serum bile acids are usually elevated unless the infant has an inborn error of bile salt synthesis. Given that α-1-antitrypsin deficiency is the most common metabolic cause of cholestasis, analysis of serum level and Pi typing should be performed. Urine-reducing substances should be checked to rule out galactosemia if the infant is consuming a lactose-containing formula or breast milk. Plasma and urine amino acids and urine organic acids (including urinary succinyl acetone to rule out tyrosinemia) should be done. A pilocarpine iontophoresis sweat chloride should be done to rule out cystic fibrosis if genetic screening was not done. The neonatal thyroid screening test results should be checked.

If the infant shows signs of infection, blood, urine, and cerebrospinal fluid should be cultured and a nasopharyngeal swab cultured for viruses. PCR and IgM should be done for putative viral infection especially the most common infections including HSV I and II, enterovirus, and CMV. In rare cases histology of the placenta may be useful for in utero infections.

Liver ultrasound should be performed as the initial imaging test. If a choledochal cyst is identified, the diagnostic work-up should be concluded and the infant referred to a pediatric surgeon for removal of the cyst, which should be considered 100 % premalignant and which can be followed by the development of biliary cirrhosis if not removed promptly.

There is controversy as to whether one should proceed directly to a liver biopsy early in the evaluation (Fig. 12.5). One of the principal concerns in determining the speed of the work-up of the cholestatic infant is to identify biliary atresia as quickly as possible. Dehghani et al. [28] compared several different diagnostic modalities as to the accuracy of these tests to diagnose biliary atresia. The diagnostic accuracy of different methods was as follows: liver biopsy, 96.9 %; clinical evaluation, 70.8 %; ultrasonography, 69.2 %; hepatobiliary scintigraphy, 58.5 %; and liver enzymes, 50.8 %. Yang et al. [29] performed a similar study to determine the optimal way to differentiate between biliary atresia and INH. The diagnostic accuracy of different methods was as follows: liver biopsy, 97.1 %; HBS single-photon emission computer tomography (HBS SPECT), 91.30 %; magnetic resonance cholangiopancreatogram (MRCP), 71.01 %; hepatobiliary scintigraphy 66.67 %; and ultrasound, 65.22 %. The authors concluded that biopsy of the liver was the most reliable method to differentiate INH from BA. The accuracy of HBS SPECT is higher than that of MRCP, hepatobiliary scintigraphy, and ultrasound.

However, even liver biopsy is not 100 % accurate, and several authors have attempted to refine the interpretation of the liver biopsy. Aiad [30]

reported that portal tract changes, such as bile duct and ductular proliferation documented by CK7 and Ki67 staining and angiogenesis documented by CD34 staining, favored the diagnosis of biliary atresia over INH. Also, copper-associated protein detected by orcein staining favored the diagnosis of biliary atresia. However, parenchymal changes such as giant cell transformation, positive iron deposition, and Kupffer cell proliferation documented by vimentin staining favored a diagnosis of INH. Torbenson et al. [31] studied a large series of liver biopsies in infants initially diagnosed with INH. Of note, the biopsy findings did not readily distinguish between the different etiologies which were ultimately diagnosed, with the exception of bile duct hypoplasia, which was more common in hypopituitarism.

If the liver biopsy is consistent with biliary atresia, then the infant should have a confirming intraoperative cholangiogram and, if consistent with atresia, a Kasai hepatic portoenterostomy. Especially when the diagnosis is uncertain, endoscopic retrograde cholangiopancreatogram (ERCP) in experienced hands can rarely lead to the diagnosis of biliary atresia or alternatively show a choledochal cyst, biliary stones, dilated bile ducts, or normal findings [32].

In summary, the most expeditious diagnostic approach to the cholestatic infant is to first establish the presence of cholestasis by verifying that direct or conjugated bilirubin is elevated and to perform a liver ultrasound (US) to rule out obvious structural abnormalities. If the US is normal, then testing for treatable causes is indicated: infections, cystic fibrosis, galactosemia, tyrosinemia, and panhypopituitarism. Alpha-1-antitrypsin levels should also be obtained given that this deficiency is the most common metabolic cause of cholestasis. Whether or not further imaging is indicated or whether one should proceed directly to a liver biopsy depends on the experience of the institution with the various radiologic techniques. If available, HBS SPECT appears to be the most accurate of the noninvasive techniques. In the event biliary atresia is suspected, then a liver biopsy should be performed promptly. If the biopsy is inconclusive and ERCP is available, then that technique could be used. However, unless the biopsy points away from biliary atresia, most would proceed to an operative cholangiogram as the "gold standard" for biliary atresia. If the biopsy is consistent with INH, the infant should still be followed closely in the event that the biopsy was done too early in the course to detect the biliary atresia which is developing.

Future Research

Given that the proportion of cholestatic infants diagnosed with "INH" has decreased dramatically over the last four decades, it seems highly likely that more genetic and metabolic causes of cholestasis will be elaborated and that "INH" will become vanishingly rare. In the meantime investigators continue to attempt to determine the molecular basis for a cholestatic infant to exhibit biliary atresia or INH. In one such study by Shih et al. [33], the authors demonstrated that infants with either condition exhibited increased frequencies of the T allele and T/T homozygosity of the CD14/_159 endotoxin receptor. Promoter polymorphism was significantly higher in patients with biliary atresia (T allele, 61.7 %; T/T genotype, 42.2 %) and in patients with INH (T allele, 67.9 %; T/T genotype, 53.6 %) compared to control populations. The authors concluded that endotoxin susceptibility may play a role in the pathogenesis of infantile cholestasis.

References

1. Balistreri WF, Bezerra JA. Whatever happened to "neonatal hepatitis"? Clin Liver Dis. 2006;10:27–53.
2. Correa KK, Nanjundiah P, Wirtschafter DD, Alshak NS. Idiopathic neonatal giant cell hepatitis presenting with acute hepatic failure on postnatal day one. J Perinatol. 2002;22:249–51.
3. Anita S. Congenital and perinatal infections: throwing new light with an old TORCH. Indian J Pediatr. 2011;78:88–95.
4. Abzug MJ. Presentation, diagnosis, and management of enterovirus infections in neonates. Pediatr Drugs. 2004;6(1):1–10.
5. Colletti JE, Homme JL, Woodridge V. Unsuspected neonatal killers in emergency medicine. Emerg Med Clin North Am. 2004;22:929–60.
6. Hasosah MY, Kutbi SY, Al-Amri AW, Alsahafi AF, Sukkar GA, Alghamdi KJ, Jacobson K. Perinatal

cytomegalovirus hepatitis in Saudi infants: a case series. Saudi J Gastroenterol. 2012;18(3):208–13.
7. Yu HR, Huang YC, Yang KD. Neonatal varicella frequently associated with visceral complications: a retrospective analysis. Acta Paediatr Taiwan. 2003;44(1):25–8.
8. Emmanuel PJ, Chamyan G, Fisher JE, Barness EG, Pomerance HH. Clinico-pathologic conference: 17 day old girl with hypothermia, decreased feedings and liver and kidney failure. Fetal Pediatr Pathol. 2004;23:39–50.
9. Armstrong L, Isaacs D, Evans N. Severe neonatal toxoplasmosis after third trimester maternal infection. Pediatr Infect Dis. 2004;23(10):968–9.
10. McGovern LM, Boyce TG, Fischer PR. Congenital infections associated with international travel during pregnancy. J Travel Med. 2007;14:117–28.
11. Koklu E, Kurtoglu S, Akcakus M, Koklu S. Adrenal haemorrhage with cholestasis and adrenal crisis in a newborn of a diabetic mother. J Pediatr Endocrinol Metab. 2007;20:441–4.
12. Pan X, Kelly S, Melin-Aldana H, Malladi P, Whitington PF. Novel mechanism of fetal Hepatocyte injury in congenital alloimmune hepatitis involves the terminal complement cascade. Hepatology. 2010;51:2061–8.
13. Ji JQ, Dimmock D, Tang L-Y, Descartes M, Gomez R, Rutledge SL, Schmitt ES, Wong L-J. A novel c.592–4_c.592–3delTT mutation in DGUOK gene causes exon skipping. Mitochondrion. 2010;10:188–91.
14. Silverman E, Jaeggi E. Non-cardiac manifestations of neonatal lupus erythematosus. Scand J Immunol. 2010;72:223–5.
15. Okada Y, Masaki H. Vitamin K deficiency bleeding with intracranial hemorrhage: focus on secondary form. Emerg Radiol. 2007;14:323–9.
16. Moyer V, Freese DK, Whitington PF, Olson AD, Brewer F, Colletti RB, Heyman MB. Guideline for the evaluation of cholestatic jaundice in infants: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. J Pediatr Gastroenterol Nutr. 2004;39:115–28.
17. Roberts EA. Neonatal hepatitis syndrome. Semin Neonatol. 2003;8:357–74.
18. Mikiko M, Shunsuke N, Hirokazu S, Yoshiyuki T, Masayuki K, Osamu M, Ikuko O, Kyoko K, Reiko O, Chihiro T, Müller A, Eis-Hübinger AM, Brandhorst G, Heep A, Bartmann P, Franz AR. Oral valganciclovir for symptomatic congenital cytomegalovirus infection in an extremely low birth weight infant. J Perinatol. 2008;28:74–6.
19. Yasuda K, Sugiura K, Ishikawa R, Kihira M, Negishi Y, Iwayama H, Ito K, Kimura H, Kosugi I, Akiyama M. Perinatal cytomegalovirus-associated bullae in an immunocompetent infant. Arch Dermatol. 2012; 148(6):770–1.
20. Kadrofske M, Parimi P, Myers M, Kumar ML, Abughali N. Nonimmune hydrops fetalis and hepatitis in a neonate with congenital human immunodeficiency virus infection. Pediatr Infect Dis J. 2006;25(10):952–3.
21. Connell LE, Salihu HM, Salemi JL, August EM, Weldeselasse H, Mbah AK. Maternal hepatitis B and hepatitis C carrier status and perinatal outcomes. Liver Int. 2011;31:1163–70.
22. Ovchinsky N, Moreira RK, Lefkowitch JH, Lavine JE. Liver biopsy in modern clinical practice: a pediatric point-of-view. Adv Anat Pathol. 2012;19:250–62.
23. Saheki T, Kobayashi K, Iijima M, Horiuchi M, Begum L, Jalil A, Li MX, Lu YB, Ushikai M, Tabata A, Moriyama M, Hsiao K-J, Yang Y. Adult-onset type II citrullinemia and idiopathic neonatal hepatitis caused by citrin deficiency: involvement of the aspartate glutamate carrier for urea synthesis and maintenance of the urea cycle. Mol Genet Metab. 2004;81:S20–6.
24. Binder G, Martin D, Kanther I, Schwarze CP, Ranke MB. The course of neonatal cholestasis in congenital combined pituitary hormone deficiency. J Pediatr Endocrinol Metab. 2007;20:695–701.
25. Alkhouri N, Carter-Kent C, Mayacy S, Hupertz V, Eghtesad B, Quintini C, Fung J, Radhakrishnan K. Reversal of protein-losing enteropathy after liver transplantation in a child with idiopathic familial neonatal hepatitis. Liver Transpl. 2009;15:1894–6.
26. Rees DC, Portmann B, Ball C, Mieli-Vergani G, Nicolaou A, Chetty MC, Stewart GW. Dehydrated hereditary stomatocytosis is associated with neonatal hepatitis. Br J Haematol. 2004;126:272–6.
27. Spear GS, Beutler E, Hungs M. Congenital Gaucher disease with nonimmune hydrops/erythroblastosis, infantile arterial calcification and neonatal hepatitis/fibrosis. Clinicopathologic report with enzymatic and genetic analysis. Fetal Pediatr Pathol. 2007;26: 153–68.
28. Dehghani SM, Haghighat M, Imanieh MH, Geramizadeh B. Comparison of different diagnostic methods in infants with cholestasis. World J Gastroenterol. 2006;12(36):5893–6.
29. Yang J-G, Mab D-Q, Peng Y, Song L, Lia C-L. Comparison of different diagnostic methods for differentiating biliary atresia from idiopathic neonatal hepatitis. Clin Imaging. 2009;33:439–46.
30. Aiad HAS, Kandil MAH, Samaka RM, Sultan MM, Badr MT, Nada GEM. The role of ck7, ki-67, cd34 and vimentin in the differentiation between biliary atresia and idiopathic neonatal hepatitis in Egyptian cholestatic neonates. APMIS. 2012;120:529–38.
31. Torbenson M, Hart J, Westerhoff M, Azzam RK, Elgendi A, Mziray-Andrew HC, Kim GE, Scheimann A. Neonatal giant cell hepatitis: histological and etiological findings. Am J Surg Pathol. 2010;34:1498–503.
32. Shteyer E, Wengrower D, Benuri-Silbiger I, Gozal D, Wilschanski M, Goldin E. Endoscopic retrograde cholangiopancreatography in neonatal cholestasis. J Pediatr Gastroenterol Nutr. 2012;55:142–5.
33. Shih H-H, Lin T-M, Chuang J-H, Eng H-L, Juo S-H, Huang F-C, Chen C-L, Chen H-L. Promoter polymorphism of the CD14 endotoxin receptor gene is associated with biliary atresia and idiopathic neonatal cholestasis. Pediatrics. 2005;116:437.

Biliary Atresia

13

Hong-Yuan Hsu and Mei-Hwei Chang

Introduction

Biliary atresia (BA) is a severe hepatobiliary disorder in infancy characterized by a progressive, inflammatory process of extrahepatic as well as intrahepatic bile ducts leading to fibrosis and obliteration of the biliary tracts [1, 2]. It is the most common cause of severe chronic liver disease in infants and the most frequent indication for pediatric liver transplantation. BA is uniformly fatal within 3 years if left untreated.

Reported incidence of BA varies in different geographic areas (Table 13.1). The annual incidences (per 10,000 live births) was 0.5–0.6 in Europe (UK, France, and the Netherlands) [3–5]; 0.65–0.74 in the southeast region of the USA [6, 7]; 1.06 in Hawaii [8]; 0.7 in Victoria, Australia [9]; 0.74–0.8 in Japan [10]; 1.5–1.7 in Taiwan [11]; and 3.2 in French Polynesia [12]. The highest incidence of BA occurs in Asians. BA is more common in females compared to males at a ratio of 1.5:1.

The abnormal anatomy in affected patients varies markedly and is classified into three types according to the level of extrahepatic obstruction of the biliary tree. Type I (about 5 %) and type II (about 2 %) refer to the segmental obliteration of common bile duct and common hepatic duct, respectively, and type III (>90 %) involves the whole extrahepatic biliary tree to the level of porta hepatis (Fig. 13.1) [13].

Two clinical phenotypes of BA have also been described. Most patients with "classical" BA (about 80–90 %) have no associated extrahepatic congenital anomalies. About 10–20 % of infants with BA have variable combinations of associated extrahepatic congenital abnormalities, including situs inversus, asplenia/polysplenia, preduodenal portal vein, absence of inferior vena cava, intestinal malrotation, and cardiac malformations. These patients are classified as having biliary

Table 13.1 Reported incidence of biliary atresia in different geographic regions over the world

Region	Incidence of BA/10,000 live births	Author
Europe		
UK		
British islets	0.6	McKiernan et al. [3]
France	0.51	Chardot et al. [4]
Netherlands	0.5	Houwen et al. [5]
USA		
Texas	0.65	Strickland et al. [6]
Atlanta	0.74	Yoon et al. [7]
Hawaii	1.06	Shim et al. [8]
Victoria, Australia	0.7	Danks et al. [9]
Asia		
Japan	0.74–0.8	Chiba et al. [10]
Taiwan	1.5	Lin et al. [11]
French Polynesia	3.2	Vic et al. [12]

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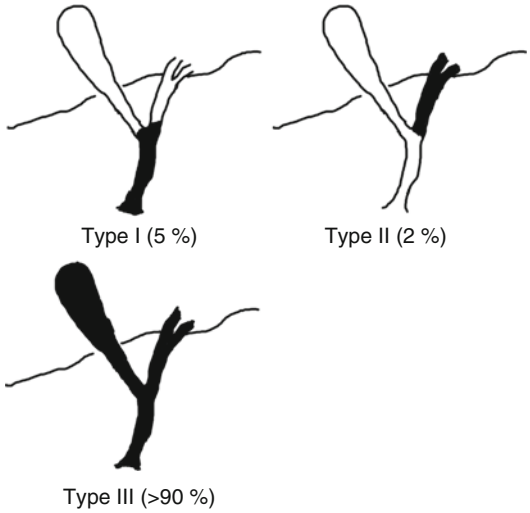

Fig. 13.1 Macroscopic types of biliary atresia. Type I and type II refer to the segmental obliteration of common bile duct and common hepatic duct, and type III involves the whole biliary tree to the level of porta hepatis

atresia splenic malformation (BASM) [14, 15]. BASM occurs more frequently in females and is less common in the Far East and Asia. The two types of BA appear to be different in pathogenesis and timing of disease onset. "Classical" BA is also named "perinatal," "sporadic," or "acquired" form. BASM syndrome is also called the "fetal," "embryonic," or "congenital" form.

Etiology and Pathogenesis of Biliary Atresia

The etiology of BA remains unclear. One hypothesis is that an acquired inflammatory disease of the bile ducts with subsequent damage to segments of the biliary tree results in obliteration of the extrahepatic bile duct and abnormal intrahepatic ducts [1, 2]. Viral infection may trigger an immune response with a continuing inflammatory process targeted at extrahepatic bile ducts. Three agents including cytomegalovirus (CMV), reovirus, and rotavirus have been extensively studied in animal models and in patients with BA [16–19]. Reovirus can induce intrahepatic cholangitis and extrahepatic duct dilatation but without the obstruction of extrahepatic bile duct when inoculated into newborn mice [20]. Inoculation of rhesus rotavirus in newborn mice triggers an inflammatory obstruction of extrahepatic bile ducts with features similar to those found in BA [21]. However, the identification of viruses in children with BA in different studies has been inconsistent [22–24].

In BA infants, the inflammatory response in the liver is periductal infiltration of mononuclear cells (T lymphocytes and macrophage) and amplification of HLA-DR expression on vascular and biliary epithelium, with increased expression of cytokines and receptors relevant to activated mononuclear cells [25]. In liver tissues from infants with BA, increased activation of interferon-γ, osteopontin, tumor necrosis factor-α, and other inflammatory mediators were found [26, 27]. A recent study found that liver T-cell response to CMV exists in the majority of BA patients at diagnosis and deficiency of T regulatory cells may decrease inhibition of inflammation and autoreactivity, potentially allowing for exaggerated bile duct injury [28]. In the rotavirus-induced newborn mouse model of biliary atresia, both immune and possible autoimmune mechanisms appear to mediate bile duct injury, and apoptosis of biliary epithelial cells is induced through the synergistic role of IFN-γ and TNF-α [29, 30].

Situs abnormalities in the congenital form of BA suggest that laterality gene may be related [31]. Mutations in the inversin gene have been found in experimental mice; however, such an association has not been found in the congenital form of human BA [32]. Recent studies have also revealed overexpression of five imprinted genes in children with the embryonic form compared with perinatal form of BA [33]—the significance has yet to be elucidated.

Clinical Features

BA typically occurs in normal birth weight infants who are discharged uneventfully from the newborn nursery. These infants may or may not have a history of physiologic jaundice, but cholestatic jaundice is usually detected by 2 weeks of age, and pale stool, icteric sclera, dark urine, and mild hepatomegaly are usually observed between 2 and 6

weeks of age. At this time, laboratory examinations show conjugated hyperbilirubinemia (direct bilirubin around 2–7 mg/dL and total bilirubin around 5–12 mg/dL), mildly elevated alanine aminotransferase (ALT) (around 80–200 IU/L), and elevated alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT) levels. Splenomegaly secondary to portal hypertension may be evident, but commonly develops at a later time. Ascites or cutaneous signs of chronic liver diseases are rare at this early stage. Thereafter, the patients become progressively ill with failure to thrive and signs of chronic liver disease and eventually with chronic liver failure and death by the age of 2–3 years unless surgical intervention (hepatic portoenterostomy) is performed and successful [34, 35].

Screening for Cholestatic Infants and Early Identification of Biliary Atresia

Newborn infants have high incidence of jaundice. If the jaundice persists beyond 2 weeks of age, it is called prolonged jaundice. Prolonged jaundice in the neonates is usually unconjugated hyperbilirubinemia (such as breast-feeding-related jaundice) and resolves. Prolonged jaundice with conjugated hyperbilirubinemia (cholestasis) occurs in a wide variety of disease entities during the neonatal period. Extrahepatic cholestasis such as biliary atresia and intrahepatic cholestasis such as neonatal hepatitis syndrome are among the most common causes of neonatal cholestasis. Early detection of BA can prevent additional liver damage due to the delay of referral and surgical treatment [35]. Thus, the primary goal in the evaluation of prolonged neonatal jaundice should be early detection of BA.

Screening tests make it possible to detect neonatal cholestasis at an early age. Our previous study revealed that 95.2 % of infants with BA had persistently clay-colored or light yellowish stools [36]. Careful observation of stool color may help to identify infants who need additional assessment to exclude the possibility of BA. Screening of newborns for BA using stool color cards was initiated by Matsui et al. in the early 1990s [37, 38]. They designed an infant stool color card to increase the efficacy of the 1-month check in identifying BA in Japan. Based on this experience, we designed our own stool color card in Taiwan, which initially imprints pictures of 6 different stool colors from infants to educate the caretakers and the medical personnel. We then conducted a nationwide screening program using this stool color card from January 2004 [39]. In this program, parents were asked to observe the stool color of their infants in accordance with this card and notified their doctors of the results at 1 month of age (30 days) during routine health check. An increased proportion of infants undergoing portoenterostomy before 60 days of age (74.3 % in 2005, compared with historical rates of 23 % from 1976 to 1989 and 47 % from 1976 to 2000) was found [39–41]. The sensitivity and specificity of stool color card screening tests in Taiwan were 72–97 % and 99.9 %, respectively [40]. The results confirmed that stool color card is a simple, efficient, and applicable mass screening method for the early diagnosis of BA and has effectively increased the rate of hepatic portoenterostomy before 60 days of age (Fig. 13.2). The screening and register process using stool color card are shown in Table 13.2.

Other screening methods, such as conjugated bilirubin measured in liquid neonatal screening bloods between 6 and 10 days of age, have been proved to be a sensitive and specific marker of neonatal liver disease including BA in the UK [42] (Table 13.2). Measurement of total and direct bilirubin in infants with jaundice at 3 weeks of age was also recommended by the American Academy of Pediatrics (AAP) [43]. If conjugated hyperbilirubinemia was found within the first weeks of life, infants with cholestasis would be identified at earlier age. Fractionation of the total bilirubin to identify the conjugated bilirubin level should therefore be done in any infantile jaundice beyond 2 weeks of age.

Diagnosis

For infants with prolonged jaundice, the initial steps should include (1) confirmation that the conjugated bilirubin is >2 mg/dL or >20 %

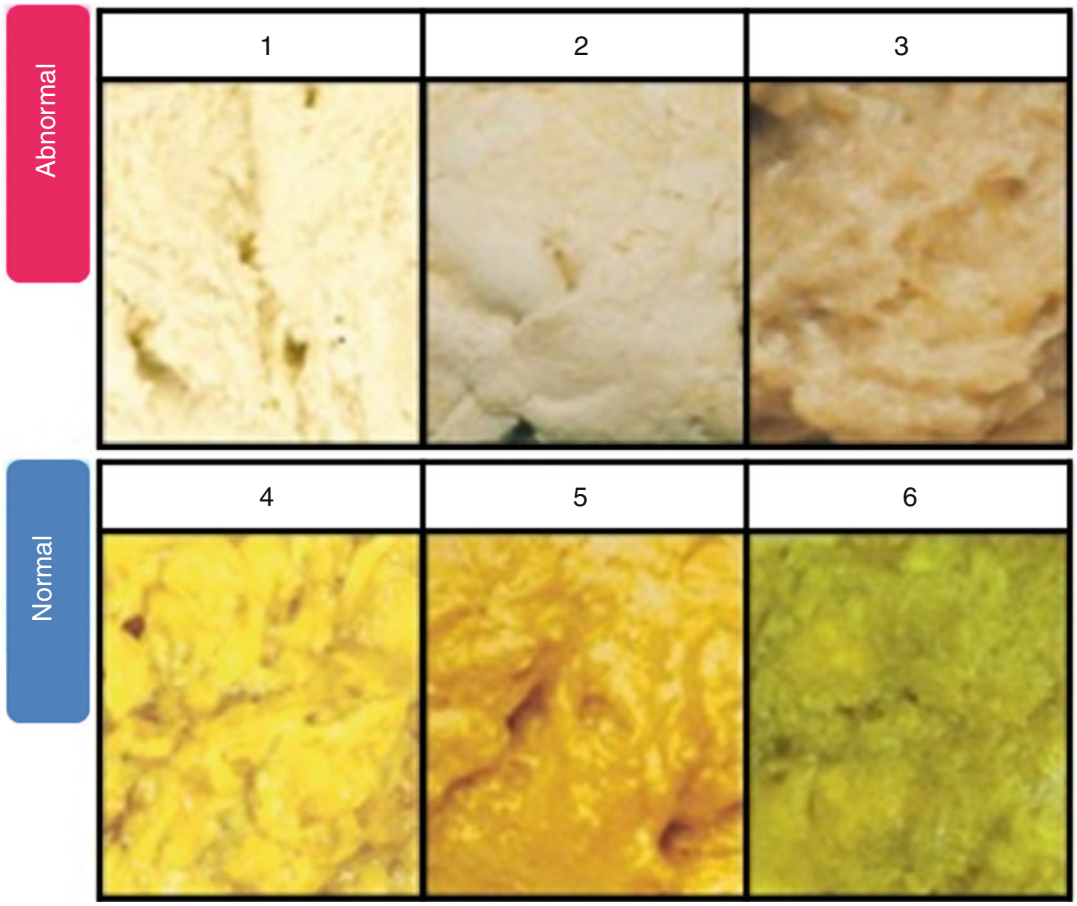

Fig. 13.2 The stool color card used in Taiwan for screening of neonatal cholestasis including biliary atresia. Images *1–3* represent acholic stool, whereas images *4–6* represent normal pigmented infant stool color

of the total bilirubin level; (2) identification of potentially treatable causes such as sepsis, hypothyroidism, or metabolic conditions; and (3) differentiating intrahepatic cholestasis from biliary atresia or obstructive condition such as choledochal cyst.

The evaluation of neonatal cholestasis usually begins with conventional liver function profile (total and direct bilirubin, alkaline phosphatase, *r*-glutamyl transpeptidase (GGT), aspartate aminotransferase, alanine aminotransferase, albumin, and prothrombin time). This evaluation alone provides very limited value in differentiating BA from other causes of neonatal cholestasis, although serum GGT is usually higher in BA, especially when correlated with age [2].

Because history taking, physical examination, and basic laboratory examinations do not reliably distinguish BA from other causes of cholestasis, the initial work-up should also include (1) serum bile acid determination; (2) cultures of blood and urine; (3) TORCH (toxoplasmosis, other agents [such as syphilis, varicella, parvovirus B19], rubella, CMV, and HSV); (4) alpha-1-antitrypsin phenotype; (5) metabolic screening including measurement of urine and serum amino acids and urinary organic acids with urinary succinyl acetone (to exclude tyrosinemia) and testing of urine-reducing substances (to exclude galactosemia); (6) thyroxine- and thyroid-stimulating hormone and cortisol level; (7) sweat chloride testing (needed only in prevalent regions of cystic fibrosis); (8) ultrasonography; (9) hepatobiliary

Table 13.2 Methods of screening and early identification of neonatal cholestasis including biliary atresia

Stool color card program
Stool color card is attached to child health booklet
Parents observe the infant stool color and report to registry center, if it is abnormal (acholic); or notify doctors the results at 1-month health check
Medical staffs check the number of the picture chosen by the parents and collect the card at 1-month health check. If the number is 4–6 (normal), the card is collected and sent to registry center. If the number is 1–3 (abnormal), the card is forwarded to register center by fax or telephone within 24 h
Registry center mail the screening results to collaborating hospitals and clinics, and contact the parents to provide related information
Newborn testing for conjugated bilirubin level
Conjugated bilirubin is measured in liquid neonatal screening bloods collected during routine newborn home visits by public health nurses between 6 and 10 days after delivery
Pediatricians and primary care providers are educated about the role of routine testing for conjugated bilirubin level measurement during the assessment of jaundiced infants more than 1-week old. A finding that the fraction of direct bilirubin more than 20 % of the total bilirubin concentrations prompts further evaluation and referral

Table 13.3 NTUH 3 days protocol for cholestasis in early infancy

Examinations on day 1
Biochemistry (3 cc): AST, ALT, ALP, *r*-GT, Alb, Ca, P
Alpha-fetoprotein
Bile acid (3–5 cc)[a]
PT/PTT (2.7 cc)
CBC/DC+ blood smear
Check results of newborn screen
Urine tests: urinalysis, bacterial culture, CMV isolation, urine GC mass
Examinations on day 2
Abdominal sonography[a]
Serum tandem mass and amino acid
Serology for CMV (IgM), CMV (IgG), rubella (IgM), herpes, and toxoplasma antibody (5 cc)
VDRL(2 cc)
Alpha-1-antitrypsin (1 cc)[b]
Urine tandem mass and bile acid[b]
Examinations on day 3
Liver biopsy[b]
Hepatobiliary scanning (MRCP), if BA or choledochocyst highly suspected

[a]Fasting at least 4 h, may consider IV fluid supplement if fasting time more than 4 h required. Ursodeoxycholic acid should be withdrawn for at least 3 days before bile acid measurement

[b]Optional, according to the decision of attending gastroenterologist, especially when patient is older than 60 days of age

scintigraphy and/or magnetic resonance cholangiopancreatography (MRCP); and (10) liver biopsy. The first seven tests largely exclude infectious and metabolic causes of cholestasis, leaving anatomic abnormalities and idiopathic neonatal hepatitis to be identified and differentiated. These evaluations should be completed within a few days. A "3-day protocol" is currently the author's work-up in differentiating BA from other causes of neonatal cholestasis to facilitate timely diagnosis and surgical intervention for BA (Table 13.3). It is also very helpful to accelerate the process of differential diagnosis for infants with cholestasis.

Real-time ultrasonography can demonstrate biliary anatomy and is useful in identifying anatomic abnormalities other than BA, such as choledochal cyst, that might be responsible for the obstructive cholestasis. Ultrasonography may detect associated anomalies such as polysplenia, vascular malformations, and situs inversus. In BA, the gall bladder is usually small or absent. Failure to find the gall bladder after an adequate fast is highly suggestive of biliary atresia. Changes in gall bladder size on sonography after a milk feeding occur in nonobstructive causes of neonatal cholestasis because of patency of the common hepatic and common bile duct. Gall bladder contractility is unlikely in patients with BA, as the biliary tree is obstructed [44]. Additionally, a fibrotic remnant of extrahepatic bile duct is echogenic on sonography and may be noted in the porta hepatis in BA. This finding has been termed "triangular cord sign" (seen as an area of increased echogenicity anterior to the bifurcation of the portal vein or a linear cord of echogenicity along the right portal vein) with a reported sensitivity up to 73 % [45, 46] (Table 13.4).

Hepatobiliary scintigraphy with technetium-labeled iminodiacetic acid derivatives is also used to differentiate BA from nonobstructive causes of cholestasis. In BA, prompt uptake of tracer by the hepatic parenchyma but no excretion into bowel is observed. Although administration of

Table 13.4 Diagnostic tests to differentiate biliary atresia from other causes of cholestasis

Procedure	Result significance	Comments
Ultrasound	The following findings are suggestive of BA: 1. After 4–6 h fasting, an absent gall bladder or one with irregular outline 2. Triangular cord sign 3. No changes of gall bladder size after feeding	Accuracy is operator-dependent; help to exclude anatomic structural abnormalities such as choledochocysts or vascular anomalies consistent with polysplenia syndrome but not diagnostic for BA
Radionuclide scanning	Excretion of tracer into bowel in 24 h in general excludes BA. Slow excretion of tracers (>24 h) may be seen in early phase of BA	High sensitivity but lower specificity because other cholestatic disease also impair excretion of the tracers. Disadvantage is time delay and cost
Percutaneous liver biopsy	Bile ductular proliferation with bile plugs is highly suggestive of BA. Absence of this finding does not exclude BA	Help to exclude other diseases like paucity of intrahepatic bile ducts, metabolic and storage disease, neonatal giant cell hepatitis, infection, and neonatal sclerosing cholangitis. Biopsies should be read by an experienced pathologist. Liver biopsy done early in the course of BA may be indistinguishable from hepatitis
MRCP	Visible extrahepatic bile ducts and gall bladder in general exclude BA	Requires deep sedation or general anesthesia and higher cost. High accuracy reported in a few studies

MRCP magnetic resonance cholangiopancreatography

phenobarbital (5 mg/kg/day) for 5 days before the scan can enhance biliary excretion of the isotope, it may delay performance of the test and hence the time of diagnosis. Hepatobiliary scintigraphy is a sensitive but not specific test for BA [47]. MRCP in the diagnosis of neonatal hepatitis is based on demonstration of well-visualized extrahepatic bile duct, thereby excluding biliary atresia as a diagnosis [48].

Liver histological study can accurately predict extrahepatic biliary obstruction in more than 90 % of cases. Liver histology in BA patients shows varying degrees of portal tract fibrosis, ductal proliferation, and cholestasis with bile plugs. However, typical histological features for BA may be absent if liver biopsy was taken early in the development of BA [49] or if performed too late, after the hepatic damage is severe.

If the abovementioned procedures fail to rule out BA, surgical exploration is indicated. When BA is suspected, intraoperative cholangiogram remains the gold standard for diagnosis. The definite diagnosis is made when the atretic biliary tree is clearly observed at laparotomy or when operative cholangiography fails to show a patent biliary tree. When BA is identified hepatic portoenterostomy (HPE) should be undertaken. Final diagnosis of BA is confirmed by histological examination of excised biliary remnants.

Management for Biliary Atresia

Surgical Management for Biliary Atresia

Kasai hepatic portoenterostomy is currently the standard surgical procedure for BA worldwide. This operation involves excision of the extrahepatic bile ducts and anastomosis of a limb of jejunum to the liver. Distal duodenum is anastomosed to the jejuna limb to create Roux-en-Y. After the Kasai operation, restoration of adequate bile flow, defined by the disappearance of jaundice with achievement of a normal bilirubin level within 3 months of the procedure, is the earliest indicators of success. If bile flow is not rapidly established in the first months of life, progressive obliteration and cirrhosis will ensue.

Although late Kasai portoenterostomy is frequently unsuccessful in reestablishing bile flow, the rate of success is greatest if done early, at younger than 2 months of age [50]. A retrospective cohort study in the USA showed that patients with a good outcome (survival with the native

liver and a bilirubin <2.0 mg/dL at 24 months of age) had Kasai operation at an earlier average age (57 days) than those with a poor outcome (64 days), but the difference was not statistically significant [51]. Other studies showed that the increasing age at Kasai operation may be accompanied by the progression of liver fibrosis and the obliteration of the biliary tree [52]. A study in Japan showed a significant survival advantage for BA infants operated on before 30 days of age and a significant disadvantage for those operated on later than 90 days of age [53]. Consistent results were also observed in studies from Canada and France [54]. Other factors affecting the outcome after HPE are size and patency of residual bile ducts at the transected porta hepatis and the experience of the center [3, 55].

The benefit of Kasai operation is the restoration of bile flow which may prevent or delay the onset of cirrhosis and sustain growth. Unsuccessful HPE usually requires liver transplantation and is the most common indication for liver transplantation in children.

Postoperative Complications and Management

Cholangitis

Cholangitis is a common complication in BA after Kasai operation and has an adverse effect on bile flow. Clinical features of cholangitis include recurrence or aggravation of jaundice, abdominal pain, fever, and elevated C-reactive proteins, serum bilirubin, and aminotransferase levels. Most episodes of cholangitis develop in the first 2 years after Kasai operation and usually respond well to intravenous antibiotics such as ceftriaxone continued for 14 days if pathogen was identified by septic work-up before antibiotic therapy [56, 57]. The most commonly identified pathogen is *E. coli*, occurring in 50 % of first and second episode. Recurrent cholangitis may be due to intrahepatic cystic dilatations (bile lakes) which can be detected by ultrasonography [58]. Multiple bile lakes with repeated cholangitis may indicate a worse prognosis. Prevention of cholangitis is an important issue because of reduced survival rate in patients with repeated episodes of cholangitis [57]. The authors use trimethoprim-sulfamethoxazole (4 mg/kg/day) or neomycin (25 mg/kg/day) as prophylactic antibiotics after the first episode of cholangitis [56]. This appears to be effective against the recurrence of cholangitis after the Kasai operation and benefit short-term survival.

Portal Hypertension

Patients with BA usually have a persistent inflammation of intrahepatic biliary tree, which continues in some infants who have initial restoration of bile flow after HPE [59]. This suggests that biliary atresia is a dynamic process involving an entire hepatobiliary system. Mild portal hypertension may have been present at the time of initial surgery. Progressive fibrosis and liver dysfunction occur in about 70 % of children whose jaundice resolves after the Kasai procedure. This may account for the ultimate development of portal hypertension with or without esophageal varices [60]. Although variceal bleeding sometimes resolves spontaneously, rebleeding is a common problem without therapy. Variceal bleeding without spontaneous resolution is frequently controlled by octreotide and/or endoscopic therapy. Esophageal variceal ligation or injection of sclerosing agents is the treatment of choice. While ligation is as effective as sclerotherapy with less complications, the size of the device limits its introduction in small children and infants. Other interventions such as transjugular intrahepatic portosystemic shunts (TIPS) can be used to bridge to transplantation, when octreotide and variceal ligation/sclerotherapy are insufficient to control variceal bleeding. Although splenic shunt or beta-blockers have been used as other options, supportive data are limited, and therefore their routine use is not widely accepted as standard of care for children with BA.

Ascites

Ascites is a poor prognostic sign in BA patients with chronic liver disease. Treatment includes low-sodium diet (1–2 mEq/kg/day) with or without fluid restriction, diuretics (spironolactone, hydrochlorothiazide, or furosemide),

and paracentesis. Refractory ascites may be effectively treated with TIPS, but is an indication for liver transplantation.

Other Complications

Hepatopulmonary syndrome may occur when incompletely metabolized vasoactive substances cause abnormal shunting in the pulmonary vascular bed and lead to hypoxia [55]. Malignancy such as hepatocellular carcinoma has been found in native liver [61].

Adjuvant Medical Therapy

Steroid has been used for improving bile flow after hepatic portoenterostomy, but its effect remains controversial [2]. Steroid has anti-inflammatory and immunomodulatory effects on continuous inflammation of intrahepatic bile ducts after operation. These are thought to increase bile salt-independent bile flow. Beneficial effect has been shown in some retrospective studies [62, 63], but was not confirmed in a recent prospective, randomized, placebo-controlled study [64].

Oral ursodeoxycholic acid (10–20 mg/kg/day) has also been used for stimulating bile flow and its possible liver protective effect after operation. Its beneficial effect was shown in a recent prospective study using withdrawal and reintroduction method [65].

Nutrition Support

Nutrition is one of the most important problems after Kasai operation, particularly in the first 2 years of life. Chronic liver inflammation and cholestasis lead to increased caloric requirement and malabsorption, resulting in growth failure in the first year of life. Energy expenditure is increased in chronic liver disease [66]. Malabsorption in BA infants is due to inadequate bile flow and passive congestion of intestine because of portal hypertension. Therefore, children with BA may need estimated caloric needs up to 150 % that of a normal healthy young child [66]. However, excess free water intake may worsen ascites. Enteral nutrition, such as nasogastric tube feeding, may be instituted to meet their caloric needs in those with poor intake, before the development of malnutrition. With diminished bile flow, BA children usually have inadequate digestion and absorption of dietary long-chain triglycerides and fat-soluble vitamins. Breast milk, infant formula, and food can be supplemented with medium-chain triglyceride (MCT) oil at least in the first year of life. MCT is directly absorbed into the portal venous system and do not require emulsification by bile acids in the duodenum. A formula such as Portagen, Pregestimil, or Alfare, in which major component of the fat is MCT, improves weight gain in cholestatic infants.

Deficiencies of vitamins A, D, E, and K should be suspected if cholestasis lasts 6 months or longer. All BA children should receive fat-soluble vitamins, and their vitamin levels should be monitored frequently to adjust supplement appropriately. Fat-soluble vitamin supplementation should include vitamin A (5,000–15,000 IU/day), vitamin D (alfacalcidol) (50 ng/kg/day), water-miscible form of vitamin E (TPGS, d-α tocopheryl polyethylene glycol-1000 succinate) (25 IU/kg/day), and vitamin K (2.5–5 mg/day) [67]. Poor nutrition and growth while awaiting liver transplantation is associated with increased risk of death and graft failure after liver transplantation [68].

Liver Transplantation

BA is the most common indication for liver transplantation (LT) in children. LT is reserved for those with failed Kasai operation. There are other indications relevant to advanced stage of liver disease, including progressive cholestasis, growth failure, refractory ascites, intractable pruritus, deteriorating coagulopathy, repeated gastrointestinal bleeding due to portal hypertension, repeated episodes of cholangitis, and multiple bile lakes or bilomas in the liver [69]. LT should be delayed when BA children are relatively healthy to allow for maximal growth. This is because of favorable techniques to perform surgery, more possibility to receive all necessary

vaccines, and fewer complications such as lower risk of posttransplant lymphoproliferative disease (PTLD) in older children [70]. However, LT is often required within 2 years of life for patients with poor bile drainage after Kasai operation and still be required beyond the age of 5 because of deteriorating portal hypertension and intractable biliary tract infection [41]. Many children in the world die from the complications of BA while waiting for liver transplantation due to the shortage of liver donation.

Biliary atresia patients can receive whole or split cadaveric livers or segments from living donors. Living-related liver transplantation has been frequently used in countries with shortage of cadaveric liver donation and was reported to have a high 5-year survival rate (98 %) in the recipients [71]. For pediatric patients with BA who underwent primary liver transplantation, the 1-year patient and liver-graft survival rates were 92.1 and 83.6 %, respectively, and 10-year patient and actuarial graft survival were 86 and 73 %, respectively [72]. The advances of surgical technique and immunosuppressive therapies have markedly improved outcomes in BA patients following liver transplantation. However, infants or children who undergo liver transplantation still face long-term immunosuppressive therapy, which can affect life quality, renal function, and life expectancy [73].

Prognosis After Kasai Operation

In Europe and North America, the native liver survival rate after HPE is ranging from 25 to 60 % during a period of 2–10 years' follow-up [3, 4, 7]. A Canadian study showed the native liver survival rates of 46 % at 2 years, 36 % at 4 years, and 26 % at 10 years and the overall survival rate of 77 % after HPE [54]. In a report from Japan, the overall rate of clearance of jaundice after Kasai procedure is 60 %, the 10-year survival rate with the native liver is 50 %, and the overall 10-year survival rate (with or without transplantation) is more than 90 % [53]. Another report from Japan found that more than 80 % of those who have had a successful Kasai procedure survive longer than 10 years with their native liver with good quality of life [74].

More recently, the stool color card screening program for BA in Taiwan enhances early Kasai operation (age at Kasai operation <60 days) (66 % vs. 49 %), increases the jaundice-free rate with native liver at 3 months postsurgery (57 % vs. 32 %), and markedly improves the 5-year jaundice-free survival with native liver (64 % vs. 27 %) and the 5-year overall survival rate (89 % vs. 56 %) of BA patients screened by stool color card, as compared to BA patients born before the stool card screening program [75].

Conclusion

While the improved medical and surgical management and liver transplantation have prolonged survival, further investigation on the etiology and pathogenesis of BA will hopefully provide effective therapy to intervene the development and progression of the disease in the future.

References

1. Balistreri WF, Grand R, Hoofnagle JH, Suchy FJ, Ryckman FC, Perlmutter DH, et al. Biliary atresia: current concepts and research directions: summary of a symposium. Hepatology. 1996;23:1682–92.
2. Hartley JL, Davenport M, Kelly DA. Biliary atresia. Lancet. 2009;374:1704–13.
3. McKiernan PJ, Bajer AJ, Kelly DA. The frequency and outcome of biliary atresia in the UK and Ireland. Lancet. 2000;355:25–9.
4. Chardot C, Carton M, Spire-Bendelac N, Le Pommelet C, Golmard JL, Auvert B. Epidemiology of biliary atresia in France: a national study. J Hepatol. 1999;31:1006–13.
5. Houwen RH, Kerremans II, van Steensel-Moll HA, van Romunde LK, Bijleveld CM, Schweizer P. Time-space distribution of extrahepatic biliary atresia in the Netherlands and West Germany. Z Kinderchir. 1988;43:68–71.
6. Strickland AD, Shannon K. Studies in the etiology of extrahepatic biliary atresia: time-space clustering. J Pediatr. 1982;100:749–53.
7. Yoon PW, Bresee JS, Olney RS, James LM, Khoury MJ. Epidemiology of biliary atresia: a population-based study. Pediatrics. 1997;99:376–82.
8. Shim WK, Kasai M, Spence MA. Racial influence on the incidence of biliary atresia. Prog Pediatr Surg. 1974;6:53–62.

9. Danks DM, Campbell PE, Jack I, Rogers J, Smith AL. Studies of the etiology of neonatal hepatitis and biliary atresia. Arch Dis Child. 1977;52:360–7.
10. Chiba T, Ohi R, Kamiyama T, Nio M, Ibrahim M. Japanese biliary atresia registry. In: Ohi R, editor. Biliary atresia. Tokyo: ICOM Associates; 1991. p. 79–86.
11. Lin YC, Chang MH, Liao SF, et al. Decreasing rates of biliary atresia in Taiwan: a survey, 2004–2009. Pediatrics. 2011;128:e530–6.
12. Vic P, Gestas P, Mallet EC, Arnaud JP. Biliary atresia in French Polynesia. Retrospective study of 10 years. Arch Pediatr. 1994;1:646–51.
13. Karrer FM, Lilly JR, Stewart BA, et al. Biliary atresia registry, 1976–1989. J Pediatr Surg. 1990;35:1076–81.
14. Carmi R, Magee CA, Neill CA, Karrer FM. Extrahepatic biliary atresia and associated anomalies: etiologic heterogeneity suggested by distinctive patterns of associations. Am J Med Genet. 1993;45:683–93.
15. Davenport M, Tizzard S, Undrehill J, et al. The biliary atresia splenic malformation syndrome: a 28-year single center retrospective study. J Pediatr. 2006;149:393–400.
16. Fjaer RB, Bruu AL, Nordbo SA. Extrahepatic bile duct atresia and viral involvement. Pediatr Transplant. 2005;9:68–73.
17. Hsu HY, Chang MH, Ni YH, et al. Cytomegalovirus infection and proinflammatory cytokine activation modulate the surface immune determinant expression and immunogenicity of cultured murine extrahepatic bile duct epithelial cells. Clin Exp Immunol. 2001;126:84–91.
18. Morecki R, Glaser JH, Cho S, Balistreri WF, Horwitz MS. Biliary atresia and reovirus type 3 infection. N Engl J Med. 1984;310:1610.
19. Riepenhoff-Talty M, Gouvea V, Evans MJ, Svensson L, Hoffenberg E, Sokol RJ, et al. Detection of group C rotavirus in infants with extrahepatic biliary atresia. J Infect Dis. 1996;174:8–15.
20. Szavay PO, Leonhardt J, Czech-Schmidt G, Petersen C. The role of reovirus type 3 infection in an established murine model for biliary atresia. Eur J Pediatr Surg. 2002;12:248–50.
21. Ripenhoff-Talty M, Schaekel K, Clark HF, Muller W, Uhnoo I, Rossi T, et al. Group A rotaviruses produce extrahepatic biliary obstruction in orally inoculated newborn mice. Pediatr Res. 1993;33:394–9.
22. Jevon GP, Dimmick JE. Biliary atresia and cytomegalovirus infection: a DNA study. Pediatr Dev Pathol. 1999;2:11–4.
23. Saito T, Shinozaki K, Matsunaga T, et al. Lack of evidence for reovirus infection in tissues from patients with biliary atresia and congenital dilatation of the bile duct. J Hepatol. 2004;40:203–11.
24. Bobo L, Ojeh C, Chiu D, Machado A, Colombani P, Schwarz K. Lack of evidence for rotavirus by polymerase chain reaction/enzyme immunoassay of hepatobiliary samples from children with biliary atresia. Pediatr Res. 1997;41:229–34.
25. Kahn E. Biliary atresia revisited. Pediatr Dev Pathol. 2004;7:109–24.
26. Bezerra JA, Tiao G, Ryckman FC, Alonso M, Sabla GE, Shneider B, et al. Genetic induction of proinflammatory immunity in children with biliary atresia. Lancet. 2002;360:1653–9.
27. Mack CL, Tucker RM, Sokol RJ, Karrer FM, Kotzin BL, Whitington PF, et al. Biliary atresia is associated with CD+ Th1 cell-mediated portal tract inflammation. Pediatr Res. 2004;56:79–87.
28. Brindley SM, Lanham AM, Karrer FM, Tucker RM, Fontenot AP, Mack CL. Cytomegalovirus-specific T-cell reactivity in biliary atresia at the time of diagnosis is associated with deficits in regulatory T cells. Hepatology. 2012;55:1130–8.
29. Shivakumar P, Campbell KM, Sabla GE, Miethke A, Tiao G, Mcneal MM, et al. Obstruction of extrahepatic ducts by lymphocytes is regulated by IFN-gamma in experimental biliary atresia. J Clin Invest. 2004;114:322–9.
30. Erickson N, Mohanty SK, Shivakumar P, Sabla G, Chakraborty R, Bezerra JA. Temporal-spatial activation of apoptosis and epithelial injury in murine experimental biliary atresia. Hepatology. 2008;47:1567–77.
31. Mazziotti MV, Willis LK, Heuckeroth RO, LaRegina MC, Swanson PE, Overbeek PA, et al. Anomalous development of the hepatobiliary system in the Inv mice. Hepatology. 1999;30:372–8.
32. Schon P, Tsuchiya K, Lenoir D, Mochizuki T, Guichard C, Takai S, et al. Identification, genomic organization, chromosomal mapping and mutation analysis of the human INV gene, the ortholog of a murine gene implicated in the left-right axis development and biliary atresia. Hum Genet. 2002;110:157–65.
33. Zhang DY, Sabla G, Shivakumar P, Tiao G, Sokol RJ, Mack C, et al. Coordinate expression of regulatory genes differentiates embryonic and perinatal forms of biliary atresia. Hepatology. 2004;39:954–62.
34. Balistreri WF. Neonatal cholestasis. J Pediatr. 1985;106:171–84.
35. Mieli-Vergani G, Howard ER, Portman B, Mowat AP. Late referral for biliary atresia-missed opportunities for effective surgery. Lancet. 1989;1:421–3.
36. Lai MW, Chang MH, Hsu SC, Hsu HC, Su CT, Kao CL, et al. Differential diagnosis of extrahepatic biliary atresia from neonatal hepatitis: a prospective study. J Pediatr Gastroenterol Nutr. 1994;18:121–7.
37. Matsui A, Ishikawa T. Identification of infants with biliary atresia in Japan. Lancet. 1994;343:925.
38. Matsui A, Dodorki M. Screening for biliary atresia. Lancet. 1995;345:1181.
39. Chen SM, Chang MH, Du JC, Lin CC, Chen AC, Lee HC, et al. Screening for biliary atresia by infant stool color card in Taiwan. Pediatrics. 2006;117:1147–54.
40. Hsiao CH, Chang MH, Chen HL, et al. Universal screening for biliary atresia using an infant stool color card in Taiwan. Hepatology. 2008;47:1233–40.
41. Hung PY, Chen CC, Chen WJ, Lai HS, Hsu WM, Lee PH, et al. Long-term prognosis of patients with biliary atresia: a 25 year summary. J Pediatr Gastroenterol Nutr. 2006;42:190–5.
42. Powell JE, Keffler S, Kelly DA, Green A. Population screening for neonatal liver disease: potential for

a community-based programme. J Med Screen. 2003;10:112–6.
43. Palmer RH, Ezhuthachan S, Newman C, Maisels MJ, Testa MA. Management of hyperbilirubinemia in newborns: measuring performance using a benchmarking model. Pediatrics. 2004;114:902.
44. Ikeda S, Sera Y, Ohshiro H, et al. Gall bladder contraction in biliary atresia: a pitfall of ultrasound diagnosis. Pediatr Radiol. 1998;28:451–3.
45. Lee HJ, Lee SM, Park WH, Choi SO. Objective criteria of triangular cord sign in biliary atresia on US scan. Radiology. 2003;229:395–400.
46. Roquete ML, Ferreira AR, Fagundes ED, Gastro LP, Penna FJ. Accuracy of echogenic periportal enlargement image in ultrasonographic exams and histopathology in differential diagnosis of biliary atresia. J Pediatr. 2008;84:331–6.
47. Gilmour SM, Hershkop M, Reifen R, Gilday D, Roberts EA. Outcome of hepatobiliary scanning in neonatal hepatitis syndrome I. Nucl Med. 1997;38:1279–82.
48. Jaw TW, Kuo YT, Liu GC, Chen SH, Wang CK. MR cholangiography in the evaluation of neonatal cholestasis. Radiology. 1999;212:249–56.
49. Azar G, Beneck D, Lane B, Markowitz J, Daum F, Kahn E. Atypical morphologic presentation of biliary atresia and value of serial biopsies. J Pediatr Gastroenterol Nutr. 2002;34:212–5.
50. Ohi R. Surgery for biliary atresia. Liver. 2001;21:175–82.
51. Shneider BL, Brown MB, Haber B, Whitington PF, Schwarz K, Squires R, et al. A multicenter study of the outcome of biliary atresia in the United States, 1997–2000. J Pediatr. 2006;148:467–74.
52. Serinet MO, Wildhaber BE, Broue P, et al. Impact of age at Kasai operation on its results in late childhood and adolescence: a rational basis for biliary atresia screening. Pediatrics. 2009;123:1280–6.
53. Nio M, Ohi R, Miyano T, et al. Five- and 10 year survival rates after surgery for biliary atresia: a report from the Japanese Biliary Atresia Registry. J Pediatr Surg. 2003;38:997–1000.
54. Schreiber RA, Barker CC, Roberts EA, Martin SR, Alvarez F, Smith L, et al. Biliary atresia: the Canadian experience. J Pediatr. 2007;151:659–65.
55. Tan CE, Davenport M, Driver M, et al. Does morphology of the extrahepatic remnants in biliary atresia influence survival? A review of 205 cases. J Pediatr Surg. 1994;29:1459–64.
56. Bu LN, Chen HL, Chang CJ, et al. Prophylactic oral antibiotics in prevention of recurrent cholangitis after the Kasai portoenterostomy. J Pediatr Surg. 2003;38:590–3.
57. Wu ET, Chen HL, Ni YH, et al. Bacterial cholangitis in patients with biliary atresia: impact on short-term outcome. Pediatr Surg Int. 2001;17:390–5.
58. Watanabe M, Kaneko TH, Komuro H, et al. Intrahepatic biliary cyst in children with biliary atresia who have had a Kasai operation. J Pediatr Surg. 2007;42:1185–9.
59. Narayanaswamy B, Gonde C, Tredger JM, Hussain M, Vergani D, Davenport M. Serial circulating markers of inflammation in biliary atresia-evolution of the post-operative inflammatory process. Hepatology. 2007;46:180–7.
60. Lykavieris P, Chardot C, Sokhn M, Gauthier F, Valayer J, Bernard O. Outcome in adulthood of biliary atresia: a study of 63 patients who survived for over 20 years with their native liver. Hepatology. 2005;41:366–71.
61. Hol L, van den Bos IC, Hussain SM, Zondervan PE, de Man RA. Hepatocellular carcinoma complicating biliary atresia after Kasai portoenterostomy. Eur J Gastroenterol Hepatol. 2008;20:227–31.
62. Muraji T, Nio M, Ohhama Y, Hashimoto T, Iwanaka T, Takamatsu H, et al. Postoperative corticosteroid therapy for bile drainage in biliary atresia – a nationwide survey. J Pediatr Surg. 2004;39:1803–5.
63. Escobar MA, Jay CL, Brooks RM, et al. Effects of corticosteroid therapy on outcomes in biliary atresia after Kasai portoenterostomy. J Pediatr Surg. 2006;41:99–103.
64. Davenport M, Stringer MD, Tizzard SA, et al. Randomized, double-blind, placebo-controlled trial of corticosteroids after Kasai portoenterostomy for biliary atresia. Hepatology. 2007;46:1821–7.
65. Willot S, Uhlen S, Michaud L, et al. Effect of ursodeoxycholic acid on liver function in children with surgery for biliary atresia. Pediatrics. 2008; 122:e1236–41.
66. Pierro A, Loletzko B, Carnielli V, et al. Resting energy expenditure is increased in infants and children with extrahepatic biliary aresia. J Pediatr Surg. 1989;24:534–8.
67. Kelly DA, Dvenport M. Current management of biliary atresia. Arch Dis Child. 2007;92:1132–5.
68. De Russo PA, et al. Growth failure and outcomes in infants with biliary atresia: a report from the Biliary Atresia Research Consortium. Hepatology. 2007;46:1632–8.
69. Utterson EC, Shepherd RW, Sokol RJ, et al. Biliary atresia: clinical profiles, risk factors, and outcomes of 755 patients listed for liver transplantation. J Pediatr. 2005;147:180–5.
70. Schneider BL, Mazariegos GV. Biliary atresia: a transplant perspective. Liver Transpl. 2007;13:1482–95.
71. Chen CL, Concejero A, Wang CC, et al. Living donor liver transplantation for biliary atresia: a single-center experience with first 100 cases. Am J Transplant. 2006;6:2672–9.
72. Barshes NR, Lee TC, Balkrishnan R, et al. Orthotopic liver transplantation for biliary atresia: the U.S. experience. Liver Transpl. 2005;11(10):1193–200.
73. Otte JB. History of pediatric liver transplantation. Where are we coming from? Where do we stand? Pediatr Transplant. 2002;6(5):378–87.
74. Kuroda T, Saeki M, Morikawa N, Fuchimoto Y. Biliary atresia and pregnancy: puberty may be an important point for predicting the outcome. J Pediatr Surg. 2005;40:1852–5.
75. Lien TH, Chang MH, Wu JF, et al. Effects of the infant stool color card screening program on 5-year outcome of biliary atresia in Taiwan. Hepatology. 2011;53:202–8, 71.

Choledochal Cysts and Fibrocystic Diseases of the Liver

14

Karen F. Murray

Introduction

Cholangiocyte differentiation and biliary development rely on complex interactions between cellular signaling, cholangiocyte biology, genetics, and extra-biliary influences. Alteration of or disruptions to any of the normal developmental processes may result in one of a number of liver and biliary diseases characterized by fibrosis. Many of the primary biliary fibrosing diseases also present with variable cystic dilation of the biliary system, commonly contributing to the symptoms that bring these conditions to clinical attention. This chapter will review the normal biliary development and most basic of cholangiocyte biology as a background to understand the pathogenesis of the most common biliary cystic conditions and fibrocystic diseases of the liver. As separate chapters are devoted to biliary atresia, conditions associated with intrahepatic paucity of the bile ducts, and chronic inflammatory hepatopathies that can lead to fibrosis, these conditions will not be reviewed here.

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Normal Biliary Development and Cholangiocyte Biology

In the first weeks of gestation, the liver develops from an outpouching of the ventral foregut and is composed of the endoderm of the ventral foregut and mesenchyme of the adjacent septum transversum (see Fig. 14.1). At approximately 3 weeks gestation, signaling from the adjacent cardiac mesoderm (fibroblast growth factor) and septum transversum mesenchyme (bone morphogenic protein) results in the underlying endoderm taking on a hepatic destiny [1, 2]. Cords of hepatoblasts, derived from the endoderm, invade the mesenchyme within days and lay the foundation for the development of the intrahepatic biliary system. Between 8 and 12 weeks gestation, the hepatoblasts close to the portal vein mesenchyme begin to express biliary-specific cytokeratins, and between 11 and 12 weeks gestation, these bile duct precursor cells divide to form a continuous layer of cells around the portal veins (the ductal plate, Fig. 14.1), which subsequently becomes a bilayer. 12 weeks later, at 25 weeks gestation, focal dilations in the bilayer form. These dilations will ultimately become the bile ducts as the ductal plate between them regresses; a process that continues beyond birth. It is now known that the arborization of the biliary tree, from the porta hepatis to the periphery of the liver, and final regression of the residual ductal plate, continues until between birth and roughly 4 weeks of age [3].

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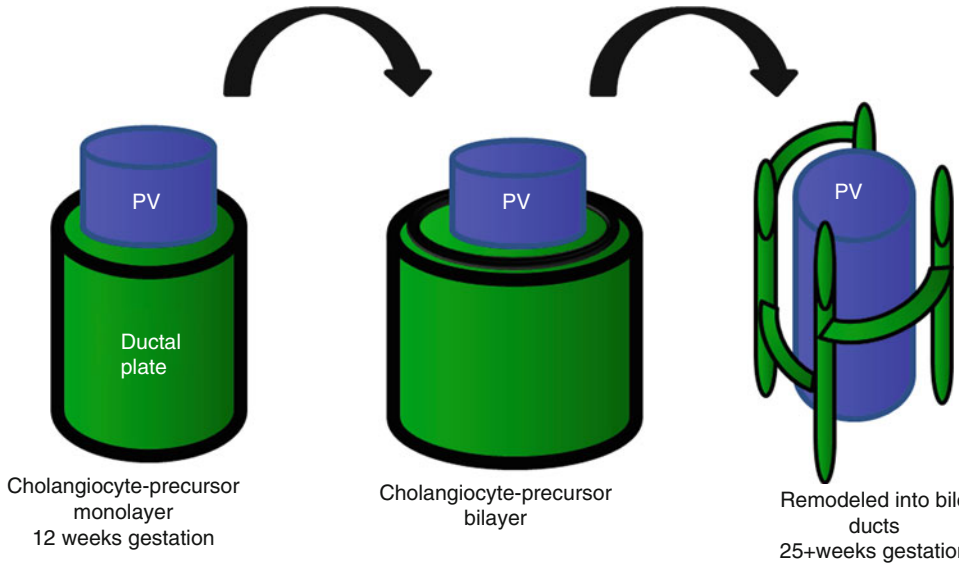

Fig. 14.1 The embryologic progression of the ductal plate monolayer through remodeling into the normal biliary ductal system. *PV* portal vein

The extrahepatic biliary tree is also thought to arise as an outpouching of the ventral foregut, but emerges just caudal to the hepatic bud, and takes its ultimate form through a remodeling process similar to that of the intrahepatic ducts. It assumes continuity with the intrahepatic system of developing ducts while still in its primitive form, converging at the hepatic ducts [4].

The cells that line the mature biliary system are cholangiocytes, cells that change their morphology and function depending on their location within the biliary tree. In the small interlobular ducts they are cuboidal and able to participate in inflammatory responses and proliferate in response to biliary damage. Cholangiocytes lining the larger interlobular or more major ducts, on the other hand, are columnar and secretory, with those lining the largest intrahepatic ducts additionally secreting mucus.

Although bile formation starts at the hepatocyte canalicular membrane, its composition is regulated by the intrahepatic biliary epithelium through absorption and secretion under paracrine and endocrine signaling control. As in most mammalian cells, the driving force for facilitated membrane transport is the Na+/K+ ATPase, creating a Na+ gradient to fuel the Na+/H+ and Cl−/HCO− exchangers that regulate acid extrusion, and the Na+/K+/2Cl− cotransporter that regulates chloride uptake and fluid secretion on the basolateral membrane. On the apical membrane, chloride efflux is mediated by chloride channels regulated by the CFTR, cystic fibrosis transmembrane conductance regulator. Efflux of chloride creates an osmotic gradient leading to the release of water into the biliary lumen. The chloride gradient itself regulates the Cl−/HCO− exchanger, in turn releasing bicarbonate into the lumen [5, 6]. Transporters for glucose, glutathione, and bile acids on the apical membrane of cholangiocytes further regulate the composition of bile.

Cholangiocyte biology is further regulated by primary cilia located on the apical domain of the cells. These nonmotile structures are moved or bent in response to luminal flow changes, and in turn cause an intracellular calcium signal. The resulting cascade of intracellular signaling ultimately influences the cells differentiation, proliferation, and secretion [7].

Disruption at any step in biliary development or defect in normal cholagiocyte biology may result in one of a number of disorders affecting the human biliary system. In the remainder of this chapter, the major conditions resulting in

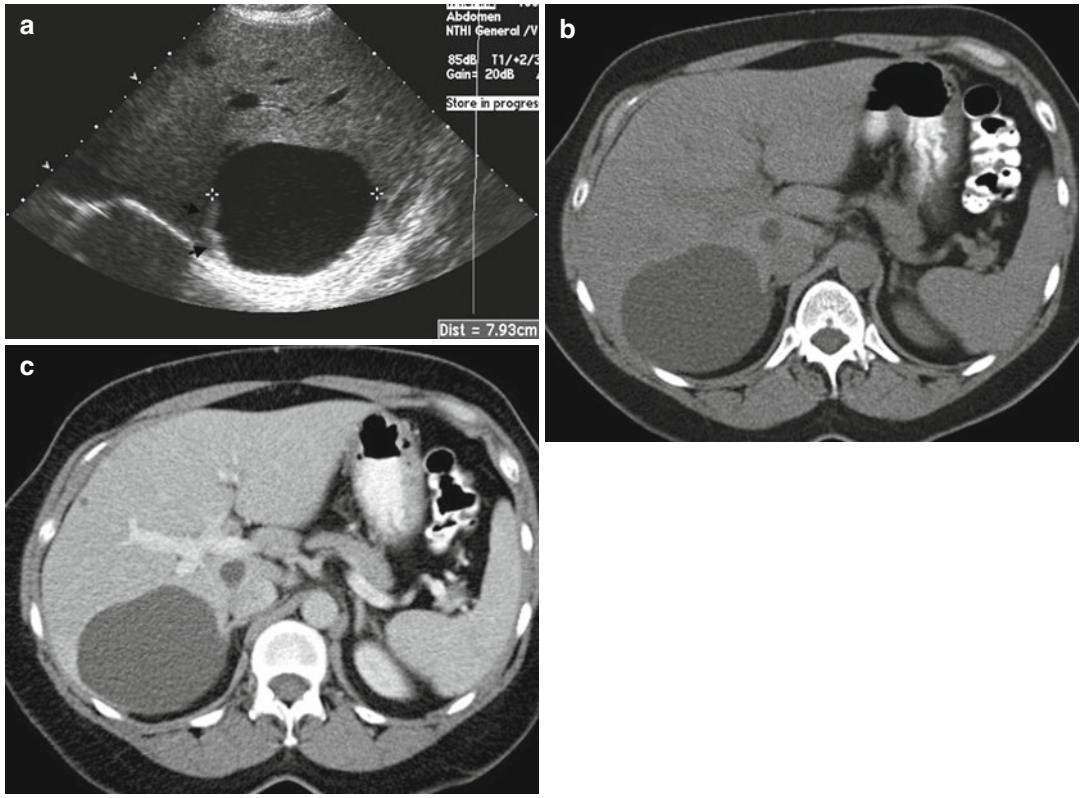

Fig. 14.2 Large subcapsular simple cyst in segment six of the liver. (**a**) Ultrasound (*black arrows* delineate a hyperechoic artifact). (**b**) Non-contrast-enhanced CT demonstrates large homogeneously hypodense cyst in the right lobe of the liver without perceptible wall. Note presence of other smaller cysts. (**c**) Contrast-enhanced CT reveals absent enhancement of cyst wall or contents characterizing this lesion as a simple cyst (Reproduced with permission from Murray and Larson [3])

cystic or fibrocystic changes in the liver and biliary tree commonly affecting children will be discussed.

Simple Hepatic Cysts

The most common congenital hepatic cysts in childhood have an epithelial lining and are characterized as simple hepatic cysts or choledochal cysts. Simple hepatic cysts are usually found incidentally at surgery or during cross-sectional imaging for other reasons. Slightly more common in females, the overall incidence in the literature is 0.1–4.75 % [8]. Although the pathogenesis is not clear, simple hepatic cysts are thought to originate from the abnormal development of an isolated intrahepatic bile duct. Patients are generally asymptomatic, but rarely develop clinical signs attributable to the cyst due to compressive obstruction of the biliary system or infection of the cyst. Picked up most commonly by cross-sectional imaging, laboratory investigations are typically normal (Fig. 14.2a–c).

Treatment is considered in relation to the symptoms caused by the cyst. As most are asymptomatic, the vast majority do not require therapy when the diagnosis is not in question. On the other hand, when symptoms are attributable to the cyst, or when the size suggests the possibility of rupture, aspiration with sclerosis, or surgical excision, fenestration, or lobectomy are rarely undertaken. When sclerotherapy is undertaken (ethanol or hypertonic saline), assurance that the cyst does not communicate with the biliary system is imperative so as to not damage the biliary system.

Choledochal Cysts

Choledochal cysts (CDCs) are congenital dilations of a part or the entire biliary system. First described by Vater in 1723[9], they were classified in 1959[10], with refinement to the current classification in 1977 [11]. Occurring more commonly in females of any race (4:1), CDCs are most common in Asians (Japanese, 1/1,000 live births) and occur at a rate of 1/13,000–15,000 live births in the West [12].

CDCs are now classified into 5 different types (Fig. 14.3a–e):

Type I, accounting for 85 %, are cystic or fusiform dilations of the common bile duct.

Type II, the most rare at 2 %, is a diverticular sacculation of the common bile duct.

Type III, a choledochocele, 2 %, is a cystic or fusiform dilation of the duodenal portion of the common bile duct only.

Type IV, 10 %, are multiple in number and involve the intra- and extrahepatic biliary ducts (subtype A, Fig. 14.4a–c) or extrahepatic ducts only (subtype B).

Type V are multiple intrahepatic cysts alone (Caroli disease) or in combination with congenital hepatic fibrosis (Caroli syndrome).

Pathogenesis

The most popular theories of the underlying mechanisms causing CDC depend on the types being described. For types I–III, the prevailing theory suggests that an abnormal pancreatobiliary junction (outside of the duodenal wall) creates a long common channel proximal to the ampulla of Vater, promoting reflux of pancreatic fluid into the more proximal portions of the biliary system, resulting in inflammation, weakening of the biliary wall, and consequent saccular dilation. On the other hand, types IV and V are better explained by disruption in the normal embryonic remodeling of the biliary ductal plate.

Clinical Presentation

Most CDCs are diagnosed in infancy or early childhood when they present with symptoms of cholestasis (jaundice), cholangitis (abdominal pain, puritis, fever, jaundice), and/or pancreatitis (abdominal pain, vomiting). Sometimes CDCs are identified during prenatal ultrasound and rarely incidentally postnatally after 3-dimensional radiological imaging for other reasons, in which cases the diagnosis is made prior to the onset of symptoms due to complications.

Diagnosis

Radiographic evaluation with ultrasonography (US) is the test of choice for visualizing the intra- and extrahepatic biliary system and identifying clinically significant biliary abnormalities. Although not always needed, 99mtechnetium-labelled hepato imino diacetic acid (HIDA) scan may additionally be used when necessary to demonstrate communication of the cystic mass with the biliary system; this study may also demonstrate delayed biliary flow from the liver to the intestine. When more complicated cysts are identified by US, computed tomography (CT) or magnetic resonance imaging (MRI)/magnetic resonance cholangiopancreatography (MRCP) can elucidate the extent of involvement in the liver and define better the relationships of the biliary abnormalities with the hepatic parenchyma.

Laboratory abnormalities are more likely when the CDC are large or are multiple. In these cases, the γ-glutamyl transpeptidase (GGT) elevation reflects biliary inflammation, with variable elevation to the serum aminotransferases. Conjugated hyperbilirubinemia reflects biliary obstruction, and elevated serum lipase would be indicative of pancreatitis (Table 14.1).

Treatment

In the short term, treatment is utilized to clear any cholangitis (see Chap. 20), allow pancreatitis to resolve, and relieve any biliary obstruction. More definitively, surgical treatment is aimed at resolving biliary stasis and obstruction and hence impact on the hepatocytes and decreasing the risk of potential complications in the future. In addition to cholangitis, choledocholithiasis, and

pancreatitis as complications of CDC, long term there is increased risk of malignant degeneration from chronic inflammation of the cyst wall (Fig. 14.5). The most common cancer of concern is cholangiocarcinoma; however, squamous cell carcinoma has also been described, although relatively rarely. The risk of developing cancer increases with age and these cancers carry poor prognoses when they develop [13–15]. As a consequence, the standard of care is now complete

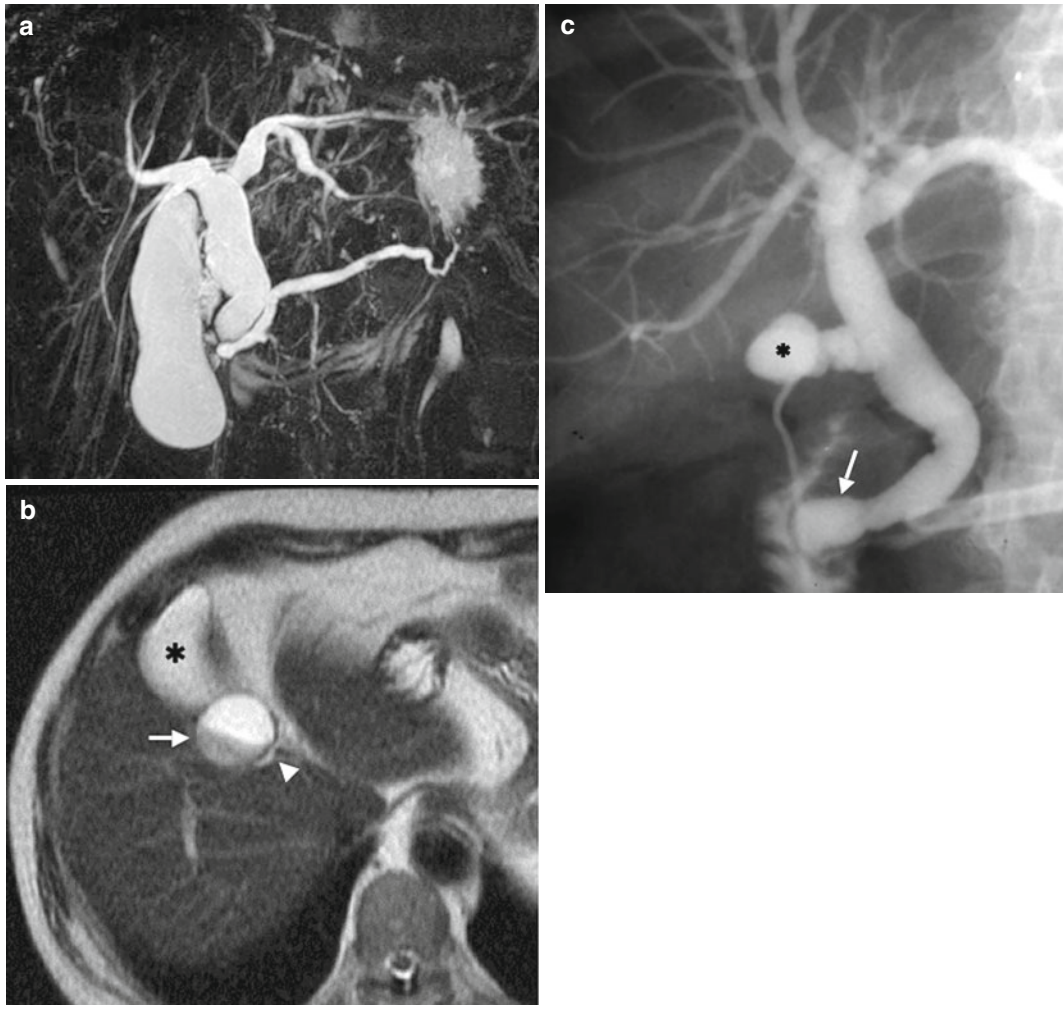

Fig. 14.3 (**a**) Choledochal cyst type I, confirmed by surgery: magnetic resonance cholangiogram (3D technique, coronal view) demonstrates diffusely and fusiformly dilated CBD with mild transition of dilatation into the right and left hepatic duct, but no separate intrahepatic dilatation. Note mass effect on main pancreatic duct, which is also mildly dilated. (**b**) Choledochal cyst type II of the common hepatic duct: T2-weighted MRI axial images of the liver at the level of the gallbladder (*) demonstrates well-defined cystic structure with debris/fluid level (*arrow*) anterior to the non-dilated common hepatic duct. (*arrowhead*). (**c**) Choledochocele: tube cholangiogram through the stump (*) of the cystic duct following cholecystectomy: incidental finding of a small choledochocele (*arrow*) with no to minimal mass effect on the duodenal wall, outlined by adjacent contrast. Subtle dilatation of the CBD might also be compensatory effect secondary to cholecystectomy. (**d**) ERCP demonstrates large cyst type IV of the CBD associated with massive bile duct dilatation of the entire intrahepatic bile duct system. Note contrast filling defects in the left ductal system (*arrows*) representing accumulation of debris. (**e**) Caroli disease: T-tube cholangiogram in a patient following cholecystectomy demonstrates non-dilated central intra- and extrahepatic bile ducts, but massively ectatic terminal bile ducts peripherally within both lobes, proving communication to "cysts" (Reproduced with permission from Murray and Larson [3])

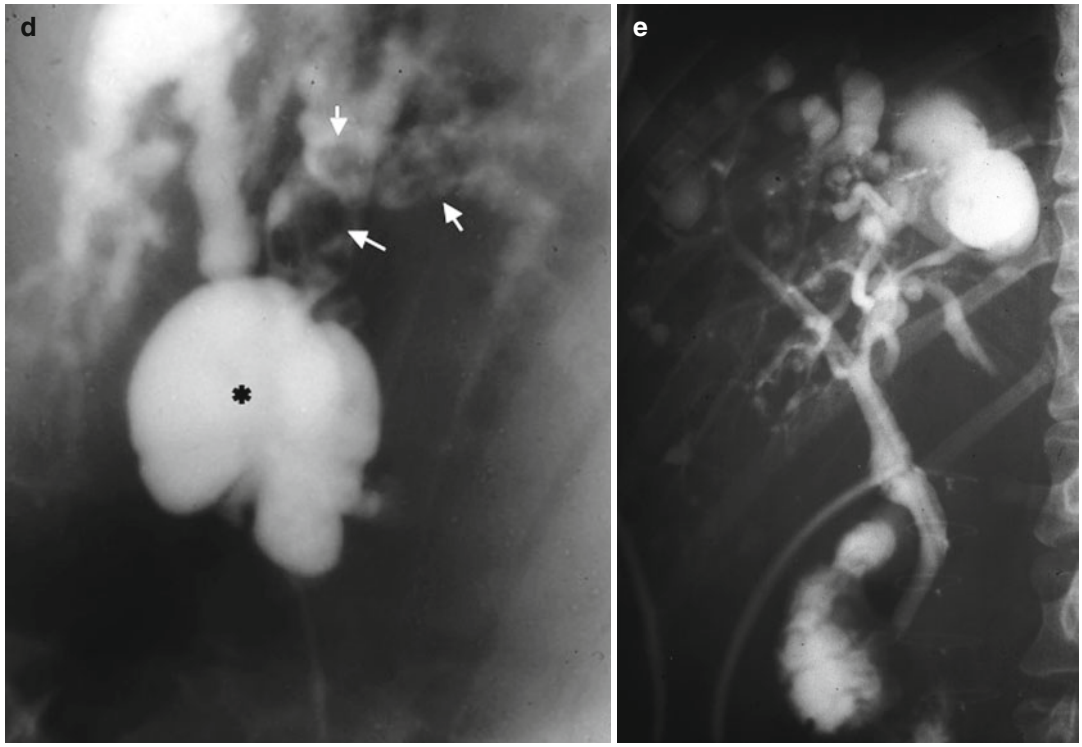

Fig. 14.3 (continued)

excision of the cyst (Fig. 14.6), and any patient who previously underwent palliative cystenterostomy, with retained cyst mucosa, should now be considered for surgical revision and complete cyst excision.

Surgery by an experienced pediatric liver surgeon is important since the procedure needs to be tailored to the specific anatomic involvement of the CDC. Type I, II, and IV cysts are amenable to complete excision and repair of biliary-intestinal continuity via a Roux-en-Y hepaticojejunostomy, when primary biliary reanastomosis is not possible. Type III cysts generally require transduodenal resection or marsupialization to provide adequate drainage of the pancreaticobiliary ducts. Focal or unilobular intrahepatic cysts may be removed via hepatic resection. Diffuse intrahepatic cystic involvement, on the other hand, precludes the ability for straightforward resection. In these patients with diffuse intrahepatic involvement, treatment of recurrent choledocholithiasis or cholangitis may be acutely aided by endoscopic retrograde cholangiopancreatography (ERCP) or transhepatic drainage, and longer-term drainage provided by bypass surgery such as hepatojejunostomy if obstructive symptoms are present; liver transplantation is less commonly required but can be effective in severe cases [16].

Congenital Hepatic Fibrosis

First described by Kerr in 1961 [17], congenital hepatic fibrosis (CHF) is a hereditary malformation of the bile ducts. Specifically caused by a premature arrest in the remodeling of the embryonic ductal plate into bile ducts, this autosomal recessive disorder can occur in isolation, as a steriotypical feature of one of a number of syndromes (Jeune, Joubert's, COACH, Berdet-Biedl, Ivemark, and congenital disorder of glycosylation-1b), associated with intrahepatic cysts (Caroli Syndrome) or, most commonly, with autosomal recessive polycystic kidney disease (ARPKD, Fig. 14.7a–b). Most cases of the latter

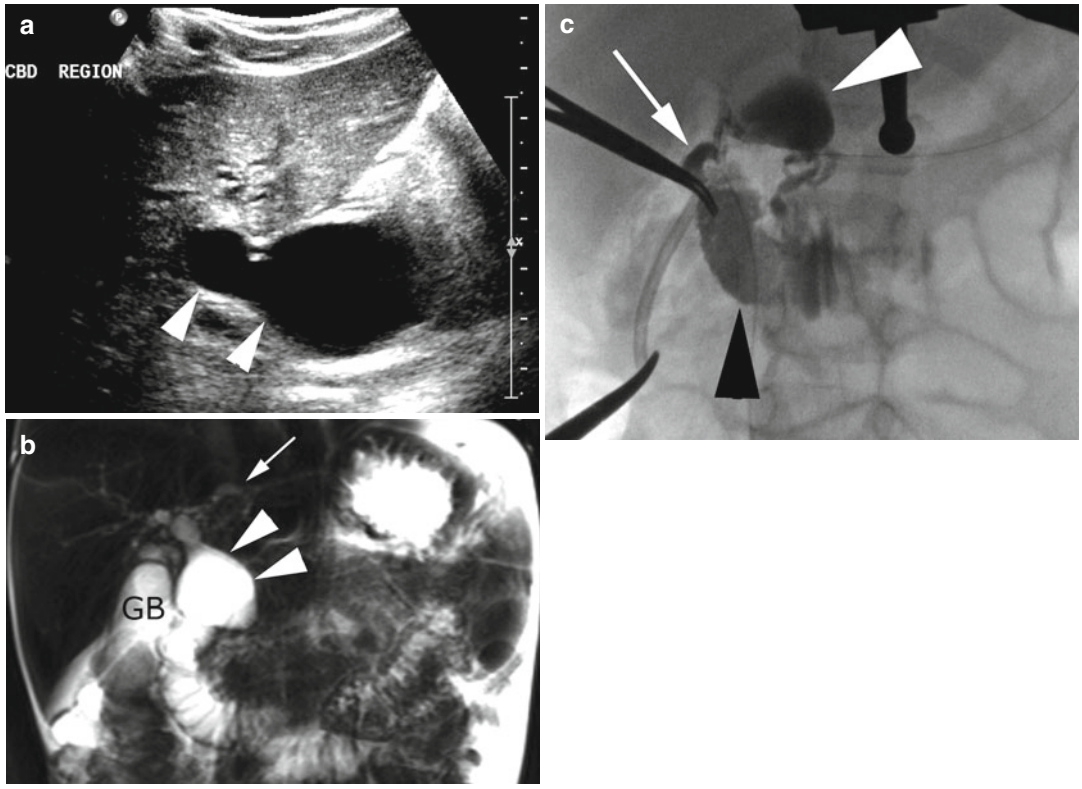

Fig. 14.4 Choledochal Cyst, type 4a. (**a**) Longitudinal sonogram of the liver shows fusiform dilatation of the common bile duct (*arrowheads*). Mild extension was noted into the right hepatic duct (not shown). (**b**) Coronal thick slab image from MRCP confirms fusiform dilatation of the common bile duct and common hepatic duct (*arrowheads*), adjacent to the gallbladder (*GB*), with slight extension into the left greater than right intrahepatic ducts (*arrow*). (**c**) Intraoperative cholangiogram at the time of resection of the choledochal cyst shows injection of contrast through the cystic duct (*white arrow*) and opacification of the cystically dilated common hepatic and proximal common bile ducts (*white arrowhead*). The more distal common bile duct has a more normal caliber. The opacified portion of the duodenum (*black arrowhead*) is unremarkable (Images provided by Grace S. Phillips MD, Associate Professor, Department of Radiology, University of Washington and Seattle Children's Hospital, Seattle, WA)

Table 14.1 Relative values of evaluations typical of congenital hepatic fibrosis (CHF) versus choledochal cysts (CDC)

Evaluations	CHF	CDC
WBC	↓	Normal
Platelets	↓	Normal
Conjugated bilirubin	Normal	↑
GGT	Normal	↑
ALT	Normal	↑
US	Diffuse echogenicity, variable ductal cystic dilation, evidence of PHTN	Cystic dilation of the biliary ducts

WBC white blood cell count, *GGT* γ-glutamyl transpeptidase, *ALT* alanine aminotransferase, *US* ultrasonography, *PHTN* portal hypertension

composite condition (CHF/ARPKD) are positive for the "polycystic kidney and hepatic disease 1" (PKHD1) gene on chromosome 6p12 that encodes for fibrocystin/polyductin localized in the primary cilia, suggesting this organelle's dysfunction in a causative role in this disease.

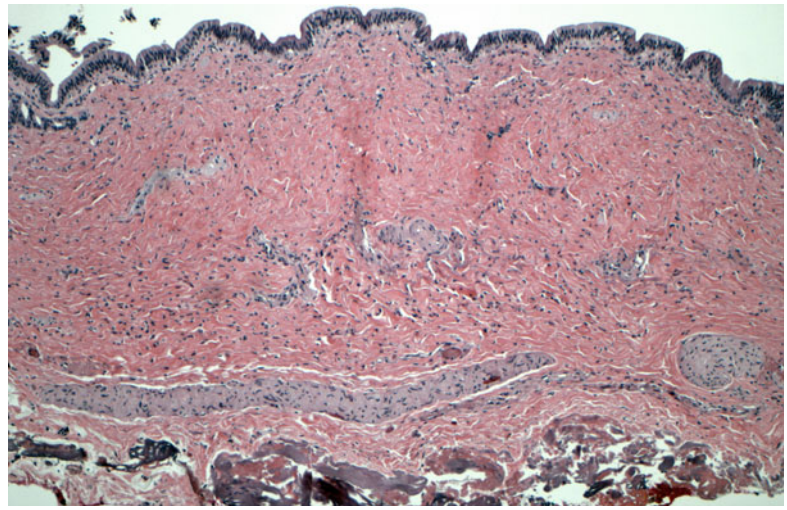

Fig. 14.5 The cyst wall of a choledochal cyst is fibrotic, thickened, and chronically inflamed. The epithelial lining of the cyst consists of columnar epithelial cells (Reproduced with permission from Murray and Larson [3])

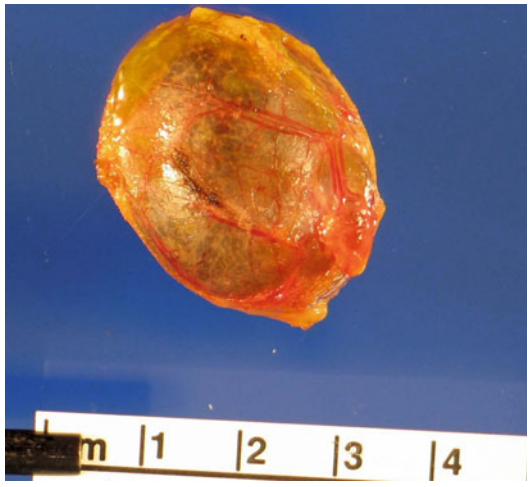

Fig. 14.6 A gross photo of a choledochal cyst. There are numerous blood vessels overlying the surface (Image provided by Matthew M. Yeh, M.D., Ph.D., Professor of Pathology, University of Washington, Seattle, WA)

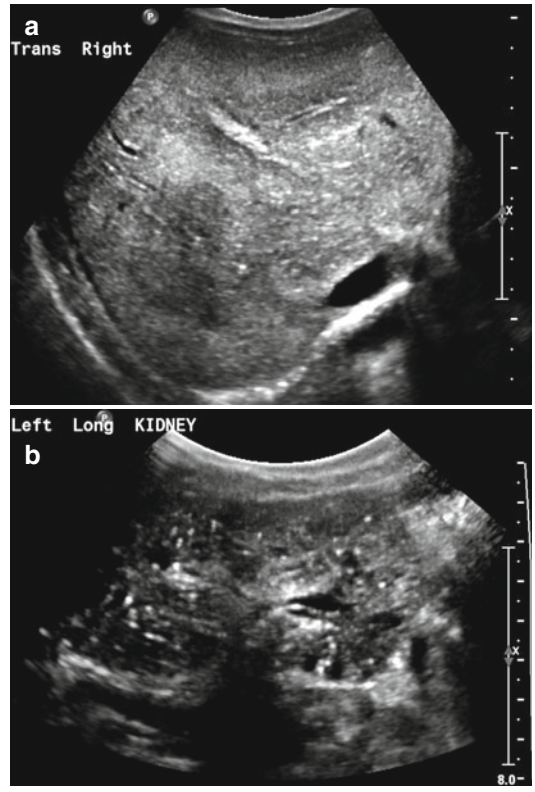

Fig. 14.7 Congenital hepatic fibrosis in association with autosomal recessive polycystic kidney disease (ARPKD). (**a**) Transverse sonogram of the liver shows heterogeneous liver parenchyma and periportal fibrosis. (**b**) Longitudinal sonogram of the native left kidney shows nephromegaly and heterogeneously increased renal echotexture characteristic of ARPKD (Images provided by Grace S. Phillips MD, Associate Professor, Department of Radiology, University of Washington and Seattle Children's Hospital, Seattle, WA)

Caroli Disease/Caroli Syndrome

Jacques Caroli first described two forms of congenital intrahepatic biliary dilation in 1958. The most commonly observed is a combination of CHF with ductal ectasia and cystic dilations, called Caroli syndrome (CS). Less commonly, the ductular ectasia occurs without CHF involvement, termed Caroli disease (CD, Fig. 14.8a–c). In both cases, the pathogenesis is thought to reflect arrest of the normal embryonic ductular remodeling, with CHF resulting when involve-

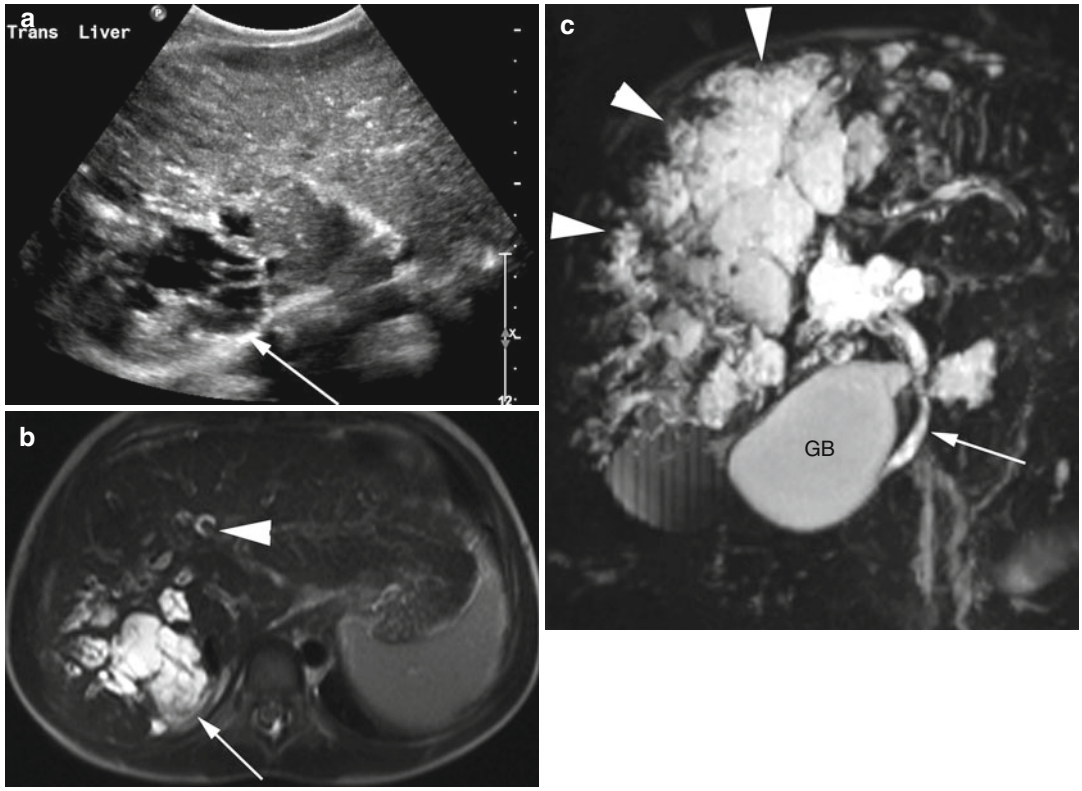

Fig. 14.8 Caroli disease. (**a**) Transverse sonogram of the liver shows heterogeneous liver parenchyma and a septated cystic structure (*arrow*) within the right lobe of the liver. (**b**) Axial MRI image further delineates the cystic structure of the right hepatic lobe as saccular dilatation of intrahepatic bile ducts (*arrow*). The left hepatic lobe is also involved to a lesser extent. Central dot sign (*arrowhead*) is noted, which is characteristic of biliary ductal dilatation. (**c**) Coronal image from MRCP depicts the saccular dilatation (*arrowheads*) of the intrahepatic ducts. The common bile duct (*arrow*) is normal in caliber GB (*gallbladder*) (Images provided by Grace S. Phillips MD, Associate Professor, Department of Radiology, University of Washington and Seattle Children's Hospital, Seattle, WA)

ment is of the interlobular ducts and CD when involvement includes the intrahepatic ducts. In addition to CHF alone, CS is commonly associated with ARPKD and less commonly autosomal dominant polycystic kidney disease (Fig. 14.9).

Although the inheritance of CS is autosomal recessive, that for isolated CD is less clear and suggested at times to have an autosomal dominant pattern of inheritance, with an incidence of approximately 1 in 1,000,000 births.

In CD, there are segmental saccular dilations of the intrahepatic bile ducts causing cystic malformations lined by hyperplastic (Fig. 14.10) or commonly ulcerated biliary epithelium. Involvement may be limited, most commonly to the left lobe of the liver, or diffuse. Due to the cystic formation, biliary flow is commonly impaired in these areas predisposing to the development of biliary sludge, stones, and cholangitis.

Clinical Presentation

Although CHF, CD, and CS can present from early childhood into the sixth decade of life, most present in childhood or early adulthood with signs of portal hypertension (PHTN), cholestasis, or both. Individuals who survive infancy with PKHD1-defined ARPKD eventually develop CHF with PHTN 44 % of the time, correlating with their age [18]. In a recent review of patients with CHF/ARPKD presenting to the National Institute of Health from 2003 to 2009,

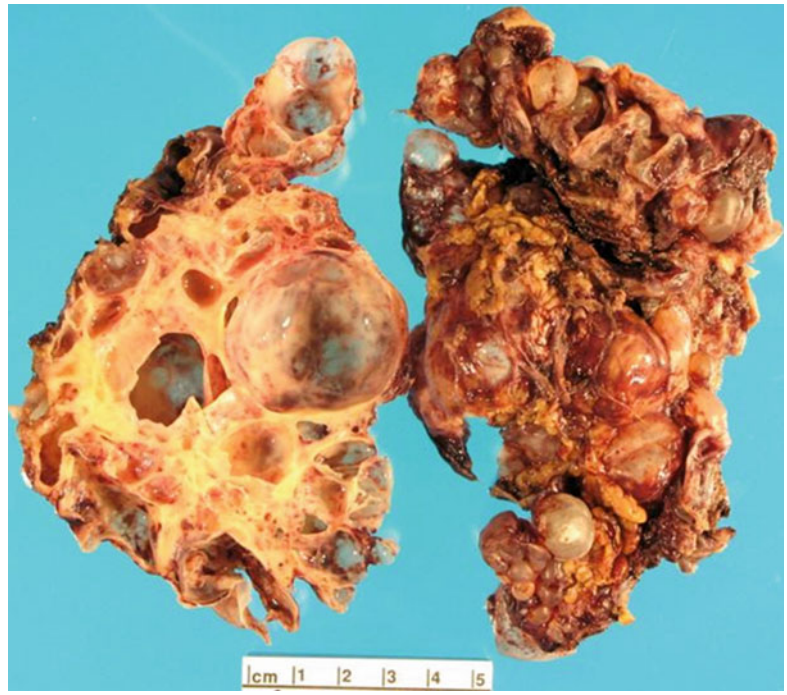

Fig. 14.9 A gross photo showing an autosomal dominant polycystic kidney disease (ADPKD)-associated polycystic liver with many variable-sized cysts that contain serous fluid (Image provided by Matthew M. Yeh, M.D., Ph.D., Professor of Pathology, University of Washington, Seattle, WA)

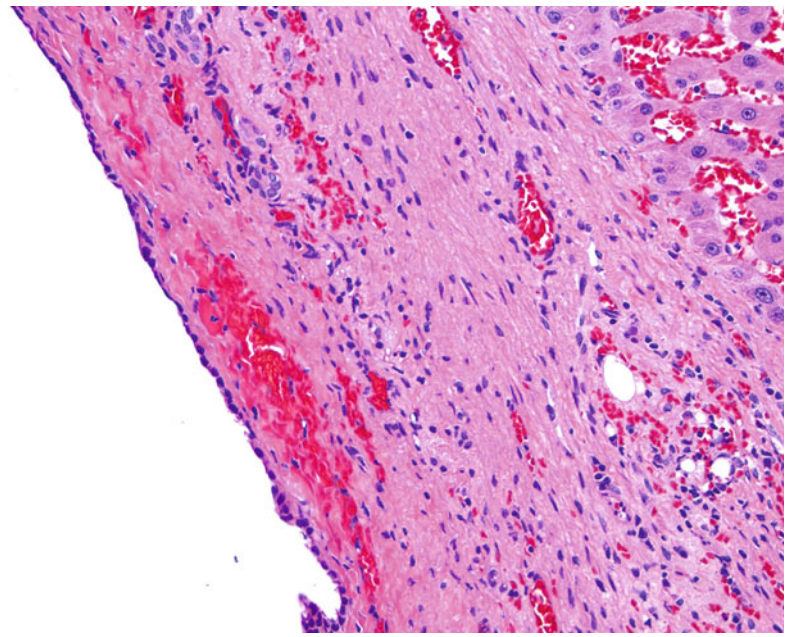

Fig. 14.10 The photomicrograph shows polycystic liver with the cystic cavity lined by cuboidal, columnar to flat epithelium (Hematoxylin and Eosin stain, 200× magnification) (Image provided by Matthew M. Yeh, M.D., Ph.D., Professor of Pathology, University of Washington, Seattle, WA)

73 subjects were evaluated, presenting between 1 year and 56 years of age. The average age at presentation was 12.7 years, with 26 % presenting due to liver-related causes. Even without liver-specific symptoms, however, 92 % had increased echogenicity on US, 69 % had enlarged left lobes by MRI, and 40 % had associated Caroli syndrome [19].

A portal hypertensive presentation may manifest as any sign or symptom of PHTN (see Chap. 25) but most commonly includes splenomegaly or esophageal variceal bleeding [19]. Of those with

CHF/ARPKD, 65 % of the 73 patients had splenomegaly at the time of presentation. Of the children presenting less than 5 years of age, 60 % had enlarged spleens, and as is commonly seen in other forms of portal hypertension, spleen volume was inversely correlated with platelet count. Of 31 patients who underwent endoscopy, 22 were found to have varices, with five suffering variceal bleeding and two undergoing portal systemic shunt surgery [19]. Similar findings were elucidated in a review of ARPKD-associated liver disease where information on 1,230 patients was extracted from the literature [20]. In this review, the mean age at diagnosis was 11.2 years (median 2 years), with 409 subjects suffering sequelae of portal hypertension, 164 with varices, 74 bleeding varices, and 81 undergoing portosystemic shunt surgery.

The pathogenesis of the PHTN is unclear, but is theorized to be from compression of the portal vein (PV) radicles by the fibrous bands and anomalous PV arborization, and associated vascular hypoplasia in CHF. Although suffering from signs and symptoms of portal hypertension, individuals who manifest their CHF in this way usually have preserved hepatic function and growth.

Individuals who have cholangitis as their predominant manifestation (see Chap. 20), indicative of extra- and intrahepatic biliary involvement, are more likely to also have synthetic dysfunction and may have suffered growth impairment or failure to thrive. Only 4–12 % of CHF present with cholangitis [21, 22], although approximately 64 % of patients with CD present with this complication [21]. Similar rates were found in the analysis by Srinath and Shneider where 152 patients developed cholangitis out of 1,230 patients with CHF/ARPKD [20]. Despite the overall relatively low rates, 3 of 23 children who developed this infectious complication after renal transplantation had a fatal outcome, suggesting that increased vigilance in monitoring for this complication is warranted, especially when the patient is most immunosuppressed.

Physical Examination (PE)

The vast majority of patients have physical examination evidence of PHTN at presentation, splenomegaly with a firm liver and prominent left hepatic lobe. If also with cholangitis they may be icteric, febrile, and/or experience pruritus. Less commonly, children will have evidence of stunted growth.

Diagnosis

With the telltale comorbidity of ARPKD, diagnosis of CHF can now be confirmed by mutational analysis of the PKHD1 gene or polymorphisms at a detection rate of 80–85 % [23]. Without ARPKD, clinical suspicion based on the presenting signs and symptoms and PE findings is of utmost importance. Characteristic of CHF, and distinguishing of CHF relative to other intrahepatic causes of PHTN, is preservation of hepatic function, most typically with normal transaminases. Presences of ductal dilation may provide a nidus for cholangitis resulting in elevation of the conjugated bilirubin and GGT values, as well as indicators of bacterial infection.

Cross-sectional imaging is critical in defining the involvement and extent of disease. US provides a readily available and relatively inexpensive assessment of hepatic echogenicity, heterogeneity, and spleen size and with Doppler interrogation can elucidate disordered hepatic-associated vascular flow suggestive of advanced PHTN. With CHF without biliary cystic dilation, the hepatic parenchyma appears echogenic, and there may be right lobe atrophy and segment IV hypertrophy. When cysts are present, the larger of these too can be identified and the extent of biliary involvement suggested. The "central dot sign" is seen when branches of the portal vein are seen as small, nondependent, echogenic dots surrounded by the dilated biliary ductules (Fig. 14.8b). CT and MRI/MRCP provide further definition and clarity on the nature and extent of the disease. These techniques can elucidate the relative components of cystic versus fibrotic involvement at a macroscopic level and identify smaller dilations of the biliary system. In all cases, cystic involvement of the kidneys, if present, can be seen.

Histological evaluation is rarely required unless the diagnosis is in question (Tables 14.2 and 14.3). When done, bands of fibrous tissue of

Table 14.2 Comparison of pathologic features of fibrocystic liver diseases

Disease	Gross appearance	Fibrosis	Cirrhosis	Cholestasis	Bile Ducts	Inflammation	Ductular Reaction	Other features
ARPKD in infancy	Enlarged liver	Yes. Features of CHF and Caroli disease	No	Usually not	Dilated		Yes	Hypoplasia of portal vein branches leading to portal hypertension
Congenital hepatic fibrosis	Enlarged, extremely firm liver; fibrotic cut surfaces	Diffuse; periportal; "jigsaw pattern"	No	Usually not	Numerous; Interrupted circular arrangement (feature of ductal plate malformation)	"Portal hypertensive form": little or no inflammation. "Cholangitic form": marked neutrophilic inflammation involving bile ducts and fibrotic tissue bands, can have microabscess	Yes	Bile ducts can also contain traces of mucin
Caroli disease and Caroli syndrome	Liver with cystic cavities arising from dilated intrahepatic bile ducts	CD – no CHF. CS – associated CHF, diffuse, "jigsaw pattern"	No	Usually not	Numerous; dilated, thickened walls; ductal fibrosis (degree varies); ductal plate malformation may be present	Severe ductal chronic inflammation; can see acute inflammation within dilated bile ducts		CD – mucous glands may be present within dilated bile ducts CS – bilirubin calculi can be present within lumen of cystic cavities

Adapted with permission from Murray and Larson [3]
ARPKD autosomal recessive polycystic kidney disease, *CHF* congenital hepatic fibrosis, *CD* Caroli disease, *CS* Caroli syndrome

Table 14.3 Comparison of pathologic features of cystic components

Disease	Gross	Cyst content	Wall	Lining	Stroma	Other features
Simple hepatic cyst	Well-demarcated cyst. Usually less than 4 cm in diameter	Clear serous fluid. Hemorrhage can be seen	Thin	Cuboidal or low columnar biliary epithelial cells		Complications: intracystic hemorrhage, infection. Squamous cell carcinoma, adenocarcinoma have been reported
Choledochol cyst	Multiple cysts. Size varies	Up to 10 l of bile	Thickened, fibrotic cyst wall. Bile may be present within wall	Columnar epithelium (usually denuded). Goblet and Paneth cells		Risk of developing cholangiocarcinoma. Squamous cell carcinomas, anaplastic carcinomas, and adenocarcinomas with sarcomatous features have been reported
ADPKD and PLD	Multiple cysts. Size varies (<1 mm to 12 cm)	Serous fluid. Exudate and abscess when infected and ruptured	Collapsed cyst has corrugated wall	Cuboidal or columnar epithelium. Flat epithelium in larger cysts	Scanty stroma. Dense and/or hyalinized in area with von Meyenburg complexes. Mild chronic inflammatory cell infiltrate may be present	Cysts have no communication with biliary tract Von Meyenburg complex is a frequent feature. Associated cholangiocarcinoma, congenital hepatic fibrosis, mitral valve prolapsed have been reported
Microhamartoma (von Meyenburg complexes)	Usually multiple, small, less than 5–10 mm in diameter	Bile	Very thin wall. Bile ducts are dilated, branching, irregular	Cuboidal epithelium	Dense fibrous stroma	

Reproduced with permission from Murray and Larson [3]
ADPKD autosomal dominant polycystic kidney disease, *PLD* polycystic liver disease

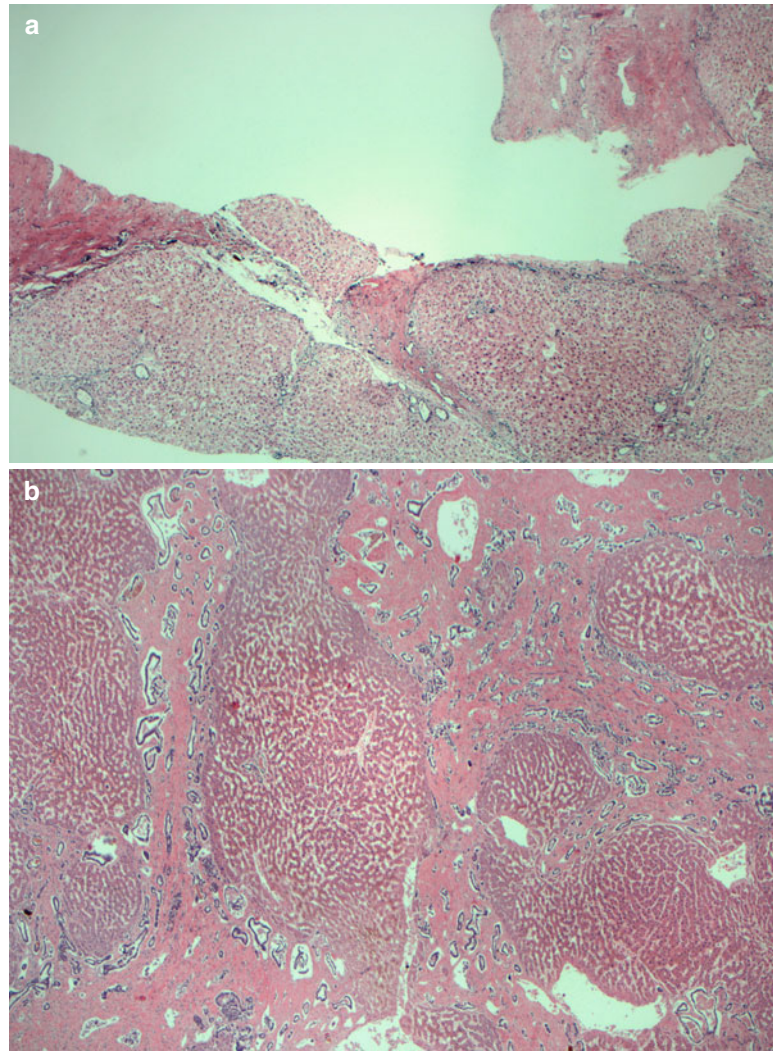

Fig. 14.11 Congenital hepatic fibrosis. (**a**) At low power magnification, islands of unremarkable liver parenchyma are separated by bands of fibrous tissue of varying thickness. (**b**) At higher magnification, the fibrous bands contain numerous small, uniform bile ducts, some of which can be dilated, irregularly shaped, and contain bile and traces of mucin. The ductal lining consists of cuboidal or low columnar epithelial cells (Reproduced with permission from Murray and Larson [3])

variable width with elongated biliary structures separating islands of hepatic parenchyma with normal vascular relationships are found in CHF (Fig. 14.11a, b). Additional intrahepatic ectasia of the bile ducts with evidence of cholangitis would suggest CS, and bile duct hamartomas (von Meyenburg complex) are commonly seen as clusters of irregularly shaped duct-like structures, embedded in fibrous stroma (Fig. 14.12a, b).

Treatment and Prognosis

Therapy is directed at support and treating the complications of the disease (see Chaps. 20 and 25 for the treatment of cholangitis and complications of PHTN). The use of prophylactic antibiotics is controversial and does not clearly lower the rates of recurrent bacterial cholangitis. The one possible exception is in the high-risk period immediately after renal transplantation [20]. Acute treatment with intravenous antibiotics, however, is indicated for acute cholangitis, to prevent or treat sepsis, and decrease biliary inflammation. Endoscopic retrograde cholangiopancreatography (ERCP) with sphincterotomy or percutaneous transhepatic cholangiogram (PTC) can be useful to extract biliary stones and hence augment bile flow, and ursodeoxycholic acid (10–20 mg/kg/day) can further facilitate bile flow when cystic dilations cause stasis.

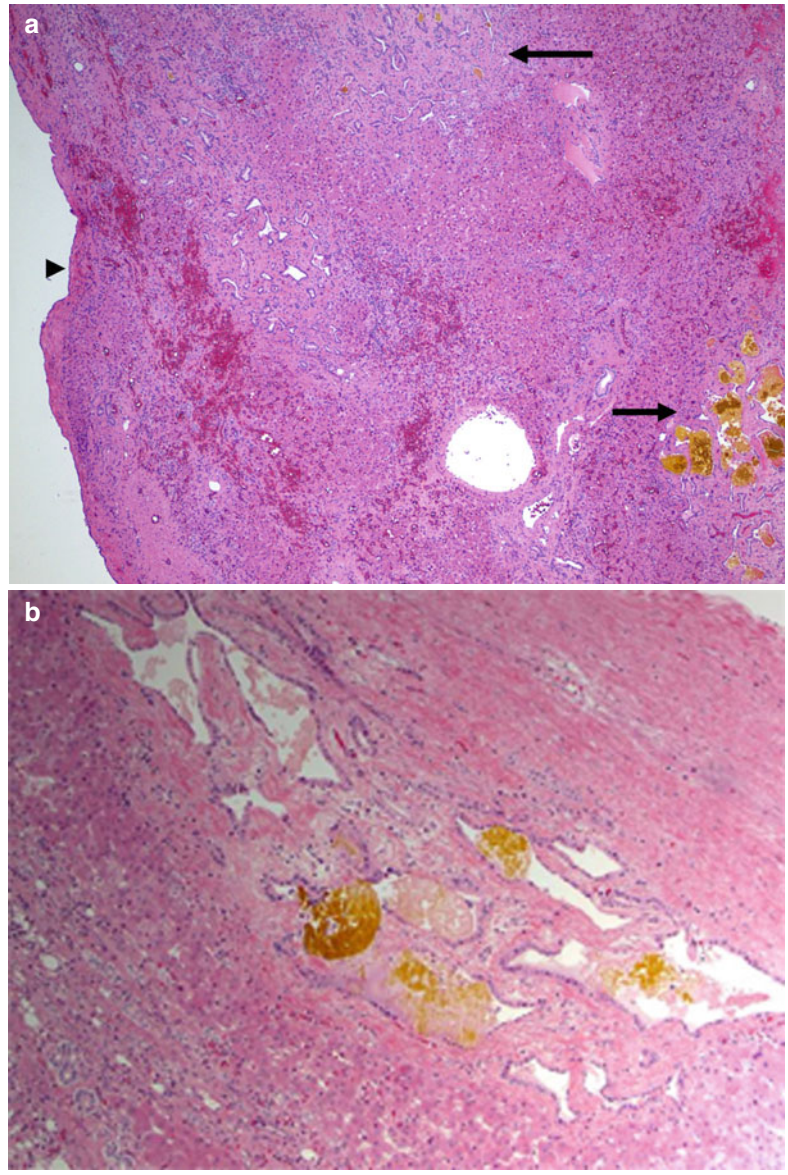

Fig. 14.12 Von Meyenburg complex. This is a frequent feature associated with polycystic liver disease. (**a**) The photomicrograph shows the presence of polycystic liver (*arrowhead*) along with a von Meyenburg complex (*arrows*, cluster of irregularly shaped, duct-like structures embedded in the fibrous stroma), (Hematoxylin and Eosin stain, 40× magnification). (**b**) Variable number of ductal structures embedded in a hyalinized stroma may have microcystic dilatation with bile in the ductal lumens. The ductal lumena are lined by a flattened or cuboidal epithelium (Hematoxylin and Eosin stain, 100× magnification) (Image provided by Matthew M. Yeh, M.D., Ph.D., Professor of Pathology, University of Washington, Seattle, WA)

Surgical options directed at the primary liver disease include lobectomy, hepatojejunostomy in select cases, and liver transplantation. In the case of CD with involvement isolated to a lobe, lobectomy may resolve symptoms and decrease the ongoing complications of the disease. Diffuse involvement with CD or CS may be improved with bypass surgery such as hepatojejunostomy if obstructive symptoms are present. Liver transplantation is less commonly required but is considered when subjects develop end-stage liver disease or otherwise uncontrollable complications. Studies show that only 4 % of patients with CHF/ARPKD/CS require liver transplantation [16, 18]. Although the rates for transplantation are relatively low, the survival outcomes are good for CD/CS (1-, 3-, and 5-year survival was 86.3, 78.4, and 77 % respectively in 104 patients) overall [24] and in pediatrics [16]. For those with ARPKD, combined liver/kidney transplants have also been successful.

References

1. Jung J, et al. Initiation of mammalian liver development from endoderm by fibroblast growth factors. Science. 1999;284(5422):1998–2003.
2. Rossi JM, et al. Distinct mesodermal signals, including BMPs from the septum transversum mesenchyme, are required in combination for hepatogenesis from the endoderm. Genes Dev. 2001;15(15):1998–2009.
3. Murray KF, Larson AM. Embryology and development of the ductal plate. In: Wu GY, editor. Fibrocystic diseases of the liver. 1st ed, vol. 1. Farmington: Humana Press; 2010. p. 3–22.
4. Roskams T, Desmet V. Embryology of extra- and intrahepatic bile ducts, the ductal plate. Anat Rec. 2008;291(6):628–35.
5. Lecchi S, et al. Cholangiocyte biology as relevant to cystic liver diseases. In: Murray KF, Larson AM, editors. Fibrocystic diseases of the liver. Farmington: Humana Press; 2010. p. 23–43.
6. Strazzabosco M, Fabris L. Functional anatomy of normal bile ducts. Anat Rec. 2008;291:653–60.
7. Masyuk AI, et al. Cholangiocyte cilia detect changes in luminal fluid flow and transmit them into intracellular Ca^{2+} and cAMP signaling. Gastroenterology. 2006;131:911–20.
8. Caremani M, et al. Ecographic epidemiology of non-parasitic hepatic cysts. J Clin Ultrasound. 1993;21:115–8.
9. Vater A, Ezler C. Dissertatio de scirhis viscerum occasione sectionis viri tympanite defunte. Wittenburgae. 1723;881:22.
10. Alonso-Lej F, Revor WB, Pessagno DJ. Congenital choledochal cyst, with a report of 2, and an analysis of 94 cases. Surg Gynecol Obstet Int Abstr Surg. 1959;108:1–30.
11. Todani T, et al. Congenital bile duct cyst: classification, operative procedures, and review of 37 cases including cancer arising from choledochal cyst. Am J Surg. 1977;137:263–9.
12. Kim SH. Choledochal cysts: survey by the surgical section of the American Academy of Pediatrics. J Pediatr Surg. 1981;16:402–7.
13. Todani T, et al. Carcinoma related to choledochal cysts with internal drainage operations. Surg Gynecol Obstet. 1987;164(1):61–4.
14. Bismut H, Krissat J. Choledochal cysts malignancies. Ann Oncol. 1999;10 Suppl 4:S94–8.
15. Voyles CR, et al. Carcinoma in choledochal cysts: age-related incidence. Arch Surg. 1983;118(8):986–8.
16. Ko JS, et al. Pediatric liver transplantation for fibropolycystic liver disease. Pediatr Transplant. 2012;16:195–200.
17. Kerr DNS, et al. Congenital hepatic fibrosis. Q J Med. 1961;30:91–117.
18. Bergmann C, et al. Clinical consequences of PKHD1 mutations in 164 patients with autosomal recessive polycystic kidney disease (ARKPD). Kidney Int. 2005;67(3):829–48.
19. Gunay-Aygun M, et al. Characteristics of congenital hepatic fibrosis and a large cohort of patients with autosomal recessive polycystic kidney disease. Gastroenterology. 2013;144(1):112–21.
20. Srinath A, Shneider BL. Congenital hepatic fibrosis and autosomal recessive polycystic kidney disease. J Pediatr Gastroenterol Nutr. 2012;54(5):580–7.
21. Giovanardi RO. Monolobar Caroli's disease in an adult. Case report. Hepatogastroenterology. 2003; 50(54):2185–7.
22. Guay-Woodford LM, Desmond RA. Autosomal recessive polycystic kidney disease: the clinical experience in North America. Pediatrics. 2003;111 (5 Pt 1):1072–80.
23. Gunay-Aygun M, et al. Autosomal recessive polycystic kidney disease and congenital hepatic fibrosis: summary statement of a first National Institutes of Health/Office of Rare Diseases conference. J Pediatr. 2006;149(2):159–64.
24. Millwala F, Segev DL, Thuluvath PJ. Caroli's disease and outcomes after liver transplantation. Liver Transpl. 2008;14(1):11–7.

Infections of the Liver

15

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Introduction

The portal vein carries blood from the gastrointestinal tract to the liver and in so doing carries microbes as well. The liver may therefore be involved in infections with a myriad number of microbial organisms. While some of these infections most commonly occur in the immunocompromised host, others affect the immune competence. Hepatic infections may be primary in nature or secondary, as part of systemic or contagious disease. The purpose of this chapter is to provide a brief overview of the various infections of the liver in the pediatric patient.

Bacterial Infection Involving the Liver

Gram-Positive and Gram-Negative Infections

There are several gram-positive and gram-negative bacterial infections that may lead to hepatic compromise. This includes infections with *Staphylococcus aureus* or the group A streptococcal species [1]. Common risk factors for *Staphylococcus aureus* infections include surgical wounds and the use of tampons in adolescents. Complications such as hypotensive shock may ensue. Hepatic manifestations of disease include elevation of serum transaminases and the appearance of jaundice. Progression of disease may lead to extensive hepatic necrosis and liver failure. On histology the liver may be punctuated with microabscesses and granuloma formation. Treatment of infection with clindamycin is recommended and antibiotics such as vancomycin or linezolid may be required for methicillin-resistant infections.

In a study in cirrhotic patients from Spain, extended-spectrum β-lactamase-producing *Enterobacteriaceae* were the main organism identified in sepsis, followed by bacteria such as *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus*, and *Enterococcus faecium*. Septic shock (26 % vs. 10 %; $P<0.0001$) and mortality rate (25 % vs. 12 %; $P=0.001$) were significantly higher in infections caused by multiresistant strains. Third-generation cephalosporins were found to be clinically ineffective for treatment in these patients [2].

Clostridium perfringens has also been associated with hepatic disease. Clostridial infections in children may occur in the newborn period in the setting of necrotizing enterocolitis as well as in children receiving chemotherapy or following infections of puncture wounds. The development of gas gangrene is associated with a high mortality rate. Jaundice may develop in about 20 % of these patients and hepatic disease includes

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abscess formation and the appearance of gas in the portal vein [3, 4]. Surgical debridement of the affected tissue and treatment with penicillin and clindamycin is warranted.

Listeria monocytogenes

Infections with *Listeria* in the neonatal period are described as severe, infrequent, and often associated with hepatic abscess formation. Predisposing factors include prematurity and in older children immunosuppression, diabetes mellitus, and cirrhosis. Treatment of infection is with ampicillin and gentamicin while a vaccine against the organism is being developed for use in immunocompromised hosts [5].

Salmonella and *Shigella*

In the developing world, *Salmonella* and *Shigella* infections are common. Typhoid fever in particular leads to systemic disease that frequently involves the liver. *Salmonella typhi* infection is acquired through contaminated food/water and presents with high fever and abdominal pain. While the serum transaminases rise, the bilirubin in contrast is minimally elevated. Cholecystitis and liver abscess formation may occur. The bacterial endotoxin-mediated hepatic compromise is treated with ciprofloxacin and ceftriaxone as first-line agents [6–8]. Blood cultures, enzyme immunoassays, and bone marrow cultures assist in diagnosis. Hepatitis may also occur with *Salmonella paratyphi A* and *B*, related paratyphoid fever. Treatment is recommended with cephalosporins or fluoroquinolones.

Shigella infections lead to dysentery or an acute diarrheal illness and are acquired from contaminated food and water. The infection is common in developing countries and hepatic manifestations include a cholestatic hepatitis [9, 10].

Yersinia

Yersinia enterocolitica infection in children presents with diarrhea, abdominal discomfort, and often a terminal ileitis that mimics Crohn disease. Hepatic involvement may occur with infections in the immunocompromised host such as those with cirrhosis or diabetes. It may manifest as multiple hepatic abscess formation. The mortality in such instances is as high as 50 % and treatment with fluoroquinolones is indicated [11].

Actinomyces

Pediatric infections with *Actinomyces israelii* occur in the immunocompromised host and include cranial, thoracic, and abdominal disease. It is a gram-positive anaerobic bacterium that leads to hepatic infection in 15 % of abdominal actinomycosis cases and often spreads from other contiguous abdominal sites. The disease process is usually indolent with nonspecific elevation of inflammatory markers and leukocytosis. The liver may be dotted with multiple abscesses and treatment includes intravenous penicillin or oral tetracycline. Surgical resection may be required for large abscesses [12, 13].

Legionella

Legionnaire disease, caused by infections with *Legionella pneumophila*, manifests as pneumonia and hepatitis. Characteristically, jaundice is minimal and hepatic steatosis and necrosis may be demonstrated on biopsy. Treatment of infection is with fluoroquinolones or azithromycin [14].

Gonococci

Gonococcal infections as seen in the Fitz-Hugh–Curtis syndrome present with perihepatitis and the associated right upper quadrant pain and fever. Patients frequently have a history of pelvic inflammatory disease with gonococci demonstrated on vaginal culture. Ceftriaxone is used in treatment of infection with resolution of the perihepatitis with treatment [15].

Brucellosis

There are multiple species of *Brucella* that may cause human disease. Acquired from contaminated sheep, pigs, cattle, and goat, clinical manifestations comprise an acute onset of fever, abdominal discomfort, and jaundice. Noncaseating hepatic granulomas are identified on biopsy. Surgical drainage of the abscesses

may be required and a combination of streptomycin and doxycycline is used for treatment [16–18].

Coxiella burnetii

Infections with the organism lead to Q fever, which is characterized by relapsing fevers, pneumonitis, endocarditis, and hepatitis. Characteristically, the serum alkaline phosphatase is elevated disproportionately to the mild rise in serum bilirubin and transaminases. Fibrin ring granulomas are seen on liver biopsy and treatment is with doxycycline [19, 20].

Bartonella

Bartonella henselae infection, as in "cat scratch disease," is associated with hepatosplenic necrotizing granulomas. Peliosis hepatis or blood-filled cysts are seen in infections in patients with concomitant AIDS. A papular dermatitis and pulmonary and neurological symptoms may also occur. The bacillary angiomatosis is treated with erythromycin, while doxycycline may be considered for treatment of visceral disease [21].

Other Gram-Negative Infections

Chlamydia trachomatis infections have been described in perihepatitis (Fitz-Hugh–Curtis syndrome) similar to that seen with gonococcal disease. The liver function tests are usually normal and azithromycin and doxycycline are used for treatment of the infection.

Rocky Mountain spotted fever is a tick-borne rickettsial infection which is characterized by the development of a maculopapular rash, fever, and hepatic disease. The latter consists of portal inflammation and vasculitis. Jaundice and increased liver enzymes are seen with disease. Hemolysis may lead to hyperbilirubinemia and biliary obstruction. In contrast, Ehrlichia (another tick-borne intracellular bacteria) infections are associated with liver injury due to proliferation within the hepatocyte and a concomitant immune response. Focal necrosis, fibrin ring granulomas, and cholestasis are seen. Treatment with doxycycline is indicated.

Spirochetes

Leptospirosis

Leptospirosis is carried by a variety of domestic and wild animals with human infection on exposure to urine or contaminated soil and water. Anicteric leptospirosis occurs in more than 90 % of cases. There is usually a biphasic illness, with the first phase characterized by fever and conjunctival injection. The second phase is associated with myalgias, nausea, vomiting, and abdominal pain. It is at this time that aseptic meningitis may occur and an increase in serum liver enzymes and jaundice is seen.

Weil disease is the icteric form of the infection and occurs in 5–10 % of patients. It too has a biphasic illness with an earlier phase that is marked by jaundice. High fever and renal manifestations with acute tubular necrosis develop in the second phase and often lead to renal failure. There is a high mortality. Hemorrhagic complications are frequent and follow immune complex deposition leading to capillary injury. Serological tests and cultures help in diagnosis. Doxycycline is usually used for treatment [22, 23].

Lyme Disease

Lyme infection occurs in various parts of the USA on exposure to ticks. It is caused by a tick-borne spirochete, *Borrelia burgdorferi*. Hepatic disease manifests with anorexia, nausea, vomiting, weight loss, and right upper quadrant pain. There is an increase in liver enzymes and the appearance of a rash called erythema migrans. The diagnosis of Lyme disease is confirmed by serology and the typical clinical history. Treatment is with oral doxycycline or azithromycin [24–27].

Tuberculosis

In developing countries where tuberculosis is endemic, hepatic disease is often seen. Granulomas with central caseation necrosis are

found on liver biopsy in 25 % of patients with pulmonary tuberculosis and in about 80 % of those with extrapulmonary disease. Similar granulomas may be found in immunocompromised individuals who have been vaccinated with the Bacillus Calmette–Guérin vaccine. Diagnosis is based on the identification of the acid-fast bacilli and treatment is with 4-drug therapy. Jaundice and an increase in serum alkaline phosphatase may also occur when there is miliary disease [28].

Syphilis

Infrequently seen in children, hepatic involvement may occur in secondary syphilis. Cholestatic hepatitis with congenital syphilis has been described. The frequency of hepatitis then ranges from 1 to 50 %. In addition to nonspecific symptoms such as anorexia and weight loss, a characteristic maculopapular rash involving the palms and soles is seen. Jaundice as well as hepatomegaly and right upper quadrant pain is described. Histological examination of the liver reveals hepatitis with spirochetes demonstrated on silver stain. Treatment of the infection is with penicillin [29].

Liver Abscess

Liver abscess formation usually follows an underlying problem such as immunocompromise, diabetes mellitus, surgery, or malignancy. Pyogenic abscesses may follow an episode of appendicitis, perforated bowel, or inflammatory bowel disease. A pyogenic liver abscess may also be the initial manifestation of hepatic malignancy.

Most abscesses are due to infections with *Escherichia coli*, *Klebsiella*, *Proteus*, *Pseudomonas*, and *Streptococcus*. Anaerobic infections with *Bacteroides* may also occur. Additionally, *Clostridium*, *Actinomyces*, *Yersinia*, *Haemophilus influenzae*, *Listeria*, and *Staphylococcus* may lead to liver abscess formation. Disseminated fungal infections may lead to micro- or macro-abscess formation in an immunocompromised host. The patient typically presents with abdominal discomfort, severe right upper abdominal pain, and high fever. Physical examination identifies the tender hepatomegaly.

Ultrasound and CT scans are the initial imaging modalities of choice and allow small abscesses up to 1 cm in diameter to be identified. A CT scan identifies localization of the abscess and defines whether there is gas in the abscess. It allows for aspiration for culture or insertion of a drain when necessary. The treatment of pyogenic liver abscess is by drainage of the pus and initiation of appropriate antibiotic treatment. An indwelling catheter may be placed in the abscess cavity when the abscess is larger than 5 cm in size. Intermittent needle aspiration may be attempted. With multiple abscesses, it is only the large ones that need to be aspirated. Small abscesses are treated by antibiotic therapy alone.

The initial antibiotic treatment before culture results are available should be broadened to cover a number of organisms. It is usually with amoxicillin and an aminoglycoside as well as a third-generation cephalosporin. Anaerobic coverage may also be required with metronidazole. Treatment is intravenously for at least 2 weeks and then orally for up to 6 weeks. For streptococcal infection high-dose oral antibiotics may be required for 6 months. The mortality rate despite treatment is high and complications of delayed diagnoses include shock, pleural effusion with sepsis, and multiorgan dysfunction [30–37].

Parasitic Infections of the Liver

Protozoa

Malaria

Malaria is an infection by the protozoan species, *Plasmodia*. It is estimated that about 300–500,000,000 people are infected globally with these parasites every year. The major species differ in their life cycle as well as presence or absence of a hepatic phase. *Plasmodium falciparum* and *Plasmodium malariae* are not associated with a hepatic cycle, while *Plasmodium vivax* and *ovale* do have a persistent exoerythrocytic phase in the liver. The extent of hepatic

injury relates to the species and the severity of infection. Active hemolysis leads to an increase in the unconjugated fraction of bilirubin. However, once hepatocellular dysfunction sets in, the conjugated fraction of bilirubin begins to rise as well. Liver failure leads to liver synthetic function impairment and a decrease in serum albumin and prolonged prothrombin time. Hyperglycemia and lactic acidosis are late complications. There is a high mortality with disease. Congenital malaria, although rare, may also lead to severe and even fulminant liver disease in the newborn infant. The diagnosis of malaria requires history, physical examination, and the identification of the parasite on blood smear. Rapid antigen detection assays are now available. Treatment depends on the species of *Plasmodium*. Chloroquine may be effective, but in many parts for the world chloroquine resistance is increasingly seen, and in these areas treatment requires mefloquine and quinine. Pyrimethamine-sulfadoxine and proguanil are also used for treatment [38, 39].

Babesiosis

Babesia is transmitted by the deer tick *Ixodes scapularis*. Infection manifests as fever, anemia, and hepatosplenomegaly. Infected individuals typically have anemia, hemoglobinuria, and hemophagocytosis on bone marrow examination. Treatment is with azithromycin and atovaquone or clindamycin and quinine. In complicated cases exchange transfusions may be required.

Leishmaniasis

Leishmaniasis, caused by *Leishmania donovani*, is transmitted by the sand fly and is endemic in the Mediterranean, Central Asian, and South Asian regions as well as Africa, South America, and New Guinea. A papular or ulcerative lesion occurs at the bite site and is followed over a period of time, ranging from months to years, with fever, weight loss, diarrhea, and massive hepatosplenomegaly. Hepatocyte necrosis is followed by cirrhosis and complications of chronic liver disease. The diagnosis is based on characteristic clinical features, history, and the identification of the parasite on Wright, Giemsa, Leishman, or Jenner staining of buffy-coat preparations of peripheral blood or aspirates from marrow, spleen, liver, lymph nodes, or skin lesions spread on a slide to make a thin smear. Pancytopenia and an increased predisposition to secondary bacterial infections such as pneumococcal infections and tuberculosis may occur. Cutaneous pigmentation may also be seen. Treatment of infection is with the pentavalent antimonials. Alternative parenteral agents include liposomal amphotericin B and paromomycin. Miltefosine, a phosphocholine analogue, has also been used for treatment [40–45].

Toxoplasmosis

Toxoplasma gondii infection may be acquired congenitally or by the ingestion of oocytes from contaminated meat, soil, and water. Hepatic involvement may occur with severe disseminated infection. Patients typically present with fever, headache, lymphadenopathy, and hepatosplenomegaly. Myocarditis and encephalitis may also occur. The diagnosis is made by the identification of specific antibody on enzyme immune assay, and treatment is with a combination of pyrimethamine, sulfadiazine, and folinic acid [46–49].

Entamoeba

Amoebic Liver Abscess

Amoebic infections of the liver occur commonly in the developing world. Patients present with abdominal pain and an enlarged liver. There may be a history of a preceding diarrhea. Pulmonary features as well as pericardial involvement and peritonitis may occur but are very rare. The diagnosis of amoebic liver abscess is based on a good history, clinical exam, imaging, and serological analysis. However, it is important to note that serological testing may need to be interpreted with caution as antibodies may remain elevated for several years after treatment. Aspiration may be attempted when the diagnosis is unclear. There is a characteristic reddish-brown anchovy paste on aspiration. Treatment includes antibiotics such as metronidazole for a month or if necessary intravenously for 7–10 days. Tinidazole or

chloroquine may be used [50–53]. An oral luminal amebacide, such as diloxanide furoate or iodoquinol, is used following the course of metronidazole for an additional 7–10 days.

Helminths

Although liver infections with helminthes occur infrequently in developed countries, in contrast, hepatic disease with nematodes is seen often in the developing world.

Nematodes

Toxocariasis

Toxocara canis and *cati* infect dogs and cats, respectively, and may ultimately lead to human disease. Visceral larva migrans is seen commonly in children with a history of pica ingestion. It presents with fever, hepatomegaly, urticaria, and an increased eosinophil count. The infection leads to cholestatic hepatitis and liver abscess formation. Pulmonary disease with asthma and pneumonia may be seen and neurological involvement with seizures and encephalopathy may also occur. Migration into the eyes is associated with visual loss. A liver biopsy may be necessary and enzyme immunoassays confirm diagnosis. Treatment of infection is with mebendazole or albendazole [54, 55].

Ascariasis

Ascaris lumbricoides affects at least one million people in the developing world. Infection is through ingestion of contaminated fruits and vegetables. There may be respiratory symptoms with cough and wheezing and occasional hepatomegaly. Infection of the biliary tree leads to calculus formation. Obstructive jaundice, cholangitis, and liver abscesses may be seen. Treatment is with mebendazole, albendazole, or pyrantel pamoate. Endoscopic or surgical interventions may be required for intestinal or biliary obstruction [56].

Strongyloidiasis

Strongyloides stercoralis is prevalent mostly in the tropics. The larvae penetrate the skin, are carried to the lungs, and then are swallowed to reach the intestine where maturation occurs. Symptoms of acute infection include pruritus, abdominal discomfort, and diarrhea. Hepatic infection leads to jaundice and cholestasis. Periportal inflammation and eosinophilic granulomas are seen on biopsy. Diagnosis is achieved by serological tests or the identification of the larvae in the stool or intestinal biopsy specimens. The treatment for acute infection is ivermectin or albendazole.

Trichinosis

Trichinella spiralis is acquired through the ingestion of contaminated raw pork. The larvae may be found in the liver and gallbladder. The clinical manifestations include diarrhea fever, marked eosinophilia, and obstructive jaundice. Diagnosis is suggested by the characteristic history and serological analysis. A muscle biopsy may help confirm the diagnosis. Treatment is with antihelminthics such as albendazole or mebendazole [57, 58].

Trematodes

Schistosomiasis

Schistosoma infection is found in various parts of the world, most commonly in areas of Africa and the Middle East. *Schistosoma intercalatum* in particular causes liver disease. The cercariae penetrate skin and proceed to the lungs and in the liver. The adult fluke may also migrate to the mesenteric vasculature. The eggs induce a granulomatous response. Clinical features of schistosomiasis include headache, fever, cough, a tender liver, and diarrhea. Chronic liver disease is described with portal hypertension, gastroesophageal varices, ascites, and splenomegaly. Chronic infection may be associated with an increased susceptibility to Salmonella infections

and frequently coexist with hepatitis B and C viral infections in endemic areas. This leads to progression of liver disease and an increased predisposition to hepatocellular carcinoma. Stool tests and serological analysis aid in diagnosis. A liver biopsy demonstrates periportal fibrosis. The infection is treated with praziquantel.

Fascioliasis

Fasciola hepatica is caused by a liver fluke and leads to acute, chronic, or obstructive hepatic disease. Clinical features are marked by fever, right upper quadrant pain, and eosinophilia. An enlarged liver is appreciated on exam. The chronic obstructive phase is characterized by intrahepatic and extrahepatic bile duct inflammation. This leads to cholangitis, stone formation, and obstruction. The diagnosis is by antibody detection or the identification of the eggs in stool, duodenal aspirate, or bile. Treatment is with triclabendazole.

Clonorchiasis

Clonorchis sinensis infection is acquired by the ingestion of contaminated seafood. The clinical features are those of fever, abdominal pain, and diarrhea. On physical exam a tender hepatomegaly is appreciated. Biliary obstruction and stone formation may also occur. There may be recurrent pyogenic cholangitis and cholangiocarcinoma may arise. The diagnosis is by the detection of characteristic eggs in the stool or the identification of the flukes in the bile ducts or gallbladder. Treatment is with praziquantel [59–64].

Cestodes

Echinococcosis

Echinococcus granulosus is also found in many areas of the world. Infection occurs through the ingestion of contaminated food. The liver is most commonly involved followed by the lungs, kidney, spleen, and brain. Cysts in the liver are called hydatid cysts. The cyst has characteristic layers on imaging with most patients being asymptomatic. However, as the cyst increases in size, it may lead to abdominal pain and a tender enlarged liver. The cyst may rupture into the lungs or may induce a life-threatening anaphylactic reaction. It may also rupture into the biliary tree and may induce cholangitis or obstruction. Rare complications include pancreatitis, portal hypertension, and the Budd–Chiari syndrome. Infection of the bile ducts may lead to liver abscess formation as well. Diagnosis requires the identification of ring-like hepatic calcifications on plain X-ray and further imaging with an ultrasound or computed tomography (CT) scan for confirmation of diagnosis. Percutaneous aspiration of the cyst may be associated with anaphylactic reactions and therefore was traditionally discouraged. However, aspiration may be attempted under carefully controlled circumstances. Treatment has included surgical cystectomy, marsupialization, and in advanced cases hepatic resection. Calcified cysts do not need to be removed. Percutaneous drainage may be attempted for complicated cysts. Administration of albendazole for 8 weeks is recommended [65].

Fungal Infections of the Liver

Candidiasis

Candida albicans is an infection that is frequently seen in severely immunocompromised individuals. It may lead to hepatic abscess formation and multiorgan disease with dissemination. Seeding of the portal vein leads to the formation of small abscesses in the liver. A CT scan of the abdomen is the most useful test to identify these tiny abscesses. Clinical features include fever, abdominal discomfort, and tender hepatomegaly. The serum aminotransferases, bilirubin, and alkaline phosphatase levels are elevated. There is a very high mortality rate. Treatment of infection is with intravenous amphotericin B [66, 67]. Alternative treatment includes fluconazole, liposomal amphotericin, caspofungin, micafungin, and anidulafungin.

Histoplasmosis

Histoplasma capsulatum infection is acquired via the respiratory tract in patients with immunodeficiency and may manifest with fever, hepatosplenomegaly, and lymphadenopathy. A liver biopsy can be done to identify yeast in areas of caseation necrosis or granulomas. Treatment is amphotericin B or fluconazole [68, 69].

Viral Infections of the Liver

Hepatitis A

Hepatitis A virus (HAV) is an enterovirus belonging to the *Picornaviridae* family. It has an icosahedral shape and lacks an envelope. The virion measures 27–28 nm in diameter and there is only one known serotype. The HAV genome consists of a positive sense RNA that is 7.48 kb long, single stranded, and linear. HAV RNA has a long open reading frame consisting of 6,681 nucleotides [70].

Numerous strains of the virus exist with considerable sequence variability. Human HAV is grouped into genotypes 1, 2, 3, and 7. Simian strains belong to genotypes 4, 5, and 6. The antigenic structure of human HAV is highly conserved [71]. Differences in the genome may play a role in the development of fulminant hepatic failure [72].

The incidence of hepatitis A virus infection has declined in the USA since 1995. Since some infections are asymptomatic, the true number of infections may be underreported. Most infections occur among children aged 5–14 years. In the USA the epidemiology of infection in 2006 was unknown in about 65 % of patients; 15 % was attributed to international travel, 12 % contact with the patient who had hepatitis, 10 % men having sex with men, 9 % from contaminated food water, 7 % children or employee in a day-care center, 4 % contact with a day-care child or employee, and 2 % with injection drug use [73]. In the developing world where there are poor hygiene conditions, children are infected early in life. It has been shown that almost 100 % of children in these countries have immunity by about 5 years of age. It has also been shown that symptomatic infection is more common in older children [74–77].

The primary transmission is fecal–oral, by person-to-person contact, or through ingestion of contaminated food/water. Parenteral transmission has been described but is rare. The risk of perinatal transmission is very small and transmission of the infection during the neonatal period is also rare. However, it has been described in neonatal intensive care units attributed to transfusion of contaminated blood or plasma and horizontal spread from infected persons [78, 79].

The virus infects the liver and the pathogenesis of injury is not yet well defined. Immunologically mediated injury to hepatocytes seems to be the likely mechanism.

HAV hepatitis is an acute infection and does not develop into chronic disease. There may be a prolonged or relapsing course with significant cholestasis. A relapsing course, occurring over a 6–10 week period, is observed in approximately 10 % of patients with acute hepatitis. Rarely acute hepatic failure may occur. The incubation period is short from 2 to 4 weeks. In previously healthy children, the morbidity and mortality are low but are higher in older children and adults. Younger children are usually asymptomatic and jaundice may develop in only 20 % of these patients. Icterus is seen more often in children older than 5 years of age. HAV infection is not associated with a higher mortality in pregnant woman.

The clinical features are those of anorexia, nausea, vomiting, and abdominal pain. Myalgias and diarrhea may also occur. Dark urine precedes other symptoms in approximately 90 % of infected people. The symptoms of abdominal discomfort last from a few days to several weeks and usually decline as jaundice becomes manifest. Complete clinical recovery is achieved in about 60 % of patients within 2 months. The overall prognosis is excellent although a few infections may be associated with fatal complications. Acute hepatic failure is seen rarely in children. The case fatality rate in individuals older than 49 years is 1.8 % compared to an overall rate

of 0.3 % in persons of all ages. The fulminant failure usually becomes obvious in the first week of illness in the majority of patients. There is an increase morbidity and high risk of liver failure in the elderly and those with chronic liver disease and HIV [80–85].

Unlike hepatitis B virus infection, arthralgias, rashes, vasculitis, and glomerular nephritis are uncommon with acute hepatitis A. Extrahepatic manifestations include myocarditis, renal failure, optic neuritis, transverse myelitis, polyneuritis, and cholecystitis. Aplastic anemia and red cell aplasia may occur. Autoimmune hepatitis has also been described following infection with this virus.

Diagnosis

The diagnosis of infection is by the detection of specific antibodies in serum. An elevation of anti-HAV IgM indicates an acute infection and is detectable in the serum with the onset of symptoms and may remain positive for approximately 4 months. Some patients may have low levels of IgM antibody for almost a year after the initial infection. IgG antibody remains present for life and indicates previous infection or vaccination. Testing for the RNA is limited to research. The RNA can be detected in body fluid such as serum, stool, and liver tissue.

Vaccination against the infection is now licensed for use after 12 months of age. Universal childhood vaccination was adopted in 2006 with the hope of eliminating transmission in the USA. This led to a decline in incidence rates among children in high-risk population for the virus. In the USA, greatest risk is to healthy individuals traveling to endemic areas, men who have sex with men, patients who are positive for HIV, and patients with underlying chronic liver disease or drug use. There are no specific medications to treat acute hepatitis A.

Administration of serum immunoglobulins have been the mainstay in preventing infection. The availability of the vaccine has rendered the use of immunoglobulin for preexposure prophylaxis relatively unnecessary. The vaccine has also been used for post-exposure prophylaxis since the early 2000s. The Advisory Committee on Immunization Practices suggests that those who have recently been exposed to the virus and have not been vaccinated previously be given a single dose of the vaccine or immunoglobulin as soon as possible ideally within 2 weeks of exposure. Since immunoglobulin preparations are derived from blood products, there is concern with their use. Post-exposure prophylaxis with immunoglobulin can be administered at the same time as the initiation of active immunization with the vaccine. Patients who have concomitant chronic liver disease particularly need to be protected against HAV and the vaccine is recommended for these individuals [86–89].

Hepatitis E

The hepatitis E (HEV) virus is a small RNA virus that is 32–34 nm in diameter. It is a non-enveloped, icosahedral particle that belongs to the family Hepeviridae. It contains three open reading frames (ORF). The open reading frame [ORF] 1 includes nonstructural proteins. ORF2 encodes the virus capsid protein and ORF3 encodes a protein of unknown function. Details of replication in the liver cells and release from infected cells remain unknown. They are four different genotypes. Genotype 1 is found in Asia and genotype 2 from Mexico and Western Africa. The USA has genotype 1, 2, and 3 infections. Genotype 3 has also been reported in several European countries and genotype 4 from China, Taiwan, Japan, and Vietnam. All genotypes belong to a single serotype [90–93].

There have been several epidemics of the virus in the Indian subcontinent as well as Central Asia, Middle East, and Africa. The overall attack rates ranges from 1 to 15 % and is higher for adults than for children. In children attack rates are from 0.2 to 10 %. There is a high mortality among pregnant women. In endemic areas hepatitis E accounts for 50–70 % of cases of sporadic acute hepatitis. In those countries that are non-endemic, the infection is related to travel to

endemic areas. A few patients are asymptomatic and are not jaundiced although most patients are icteric [94].

The virus is transmitted through the ingestion of contaminated food and water. The outbreaks frequently follow the monsoon season. Secondary attack rates among household contacts range from 0.7 to 2.2 %. It is thought that contamination of water sources, subclinical infection, animal reservoirs, and prolonged fecal shedding lead to persistence of the virus in communities.

In countries such as India, antibodies to the hepatitis A virus are almost universally detectable by early childhood or adolescence. However, antibody development to hepatitis E in contrast remains uncommon.

The infection has an incubation period of about 4–5 weeks. The virus can be detected in the stool approximately 1 week before the onset of illness and for up to 2 weeks after that. Fecal shedding continues for about 4 weeks from the onset of illness. Histopathologic features of the infection are similar to those of other forms of acute hepatitis. Massive necrosis is seen rarely. Chronic HEV viremia with the genotype 3 virus has been reported in some kidney and liver transplant patients in Europe, and it has been suggested that such infection may potentially lead to chronic liver disease [94].

The symptoms of hepatitis include fever, abdominal pain, anorexia, nausea, and vomiting. There is a period of cholestasis where the stools are clay colored and the urine is dark. Pruritus and a transient macular rash may be seen. Physical exam demonstrates jaundice and an enlarged tender liver. Serum transaminases are elevated and there is a conjugated hyperbilirubinemia. Ultrasound demonstrates a mildly enlarged liver.

Viral infection with HEV is self-limited and case fatality rates of 0.5–4 % have been described. Pregnant women particularly those in the second and third trimester are affected more frequently and have a worse outcome. The mortality in these patients is from 5 to 25 %. In an epidemic in India, clinical hepatitis developed in 17.3 % of pregnant women. Fulminant hepatic failure was seen in approximately 22 % of the infected pregnant women with an increased frequency of complications.

The diagnosis requires the detection of the virus in stool and serum. PCR is done to look for HEV RNA. Enzyme immune assays for the detection of IgM and IgG antibody have been developed. The presence in serum of IgM antibody indicates acute infection, and the presence of IgG indicates past disease.

Treatment is supportive with no specific medication. Prevention of hepatitis E in endemic areas comprises of improved hygiene. The antibody is not fully protective as seen with the occurrence of large epidemics among adults living in endemic areas. Candidate vaccines have been developed and are being currently studied in Nepal. An IgG antibody was observed after the third vaccine dose although only 56 % of the volunteers had a high antibody titer by the end of the study [95]. The vaccine may potentially be useful for travelers to endemic areas.

Hepatitis B

Hepatitis B virus (HBV) infection is a worldwide health problem, which can cause acute liver failure, acute hepatitis, chronic hepatitis, cirrhosis, and liver cancer. It is most prevalent in Asia, Africa, southern Europe, and Latin America, where the hepatitis B surface antigen (HBsAg) positive rate in the general population ranges from 2 to 20 %. Approximately two billion people in the world have been infected by HBV and more than 350 million are chronic HBsAg carriers. In endemic areas, HBV infection occurs mainly during infancy and early childhood. Mother-to-infant transmission accounts for approximately half of the chronic HBV infections. In contrast to infection in adults, HBV infection during early childhood results in a much higher rate of persistent infection and long-term serious sequelae such as liver cirrhosis and hepatocellular carcinoma (HCC). HBV carriers have a lifetime risk of developing HCC in up to 25 % and an incidence of cirrhosis of 2–3 %/year [96, 97].

Hepatitis B virus is an enveloped DNA virus that is a member of the Hepadnaviridae family. It has several important structures that include the surface antigen, the core antigen, and e antigen. Various tests have been developed to these different structures to help make a diagnosis of hepatitis B. Ten genotypes (A to J) and several subtypes have been described. Genotype A is prevalent in North America and Europe. The virus is transmitted predominantly by the parenteral route, sexual contact, and perinatal exposure [98].

Acute illness may have varying manifestations, and while the majority developed antibodies to the infection and immunity, 10–15 % go on to develop chronic disease and approximately 1 % will have fulminant failure. After exposure, the risk of developing chronic infection is indeed higher for newborns (90 %) than for infants and children <5 years of age (25–30 %) or adolescents and adults (<5 %) [99]. To reduce mother-to-child transmission, the World Health Organization recommends the administration of both the vaccine and hepatitis B immunoglobulins (HBIG) to newborns of HBsAg-positive mothers within 24 h from birth (90–98 % protection rate) [100, 101]. HBsAg and HBV DNA can be detected in breast milk of chronic carriers, but no increased risk of transmission to a breast-fed infant has been shown and breastfeeding is currently recommended after proper infant immunization [102].

Neonates with the infection rarely show any signs of disease. There may be a transient mild acute hepatitis or chronic persistent hepatitis. There is no specific therapy for acute infection for neonates.

Three phases of chronic hepatitis B have been identified: the immune-tolerant phase, the immune-active phase, and the inactive phase. Most children with chronic HBV infection are immune tolerant, with high viral replication, positive hepatitis B envelope antigen (HBeAg), high HBV deoxyribonucleic acid (DNA) levels, and normal levels of aminotransferases [103]. This pattern is mainly seen in children infected at birth. The immune-tolerant phase may last long into adulthood; however, some children go into the immune-active phase. This phase is marked by active inflammation and elevated aminotransferases and may develop into fibrosis over time.

Most individuals with sudden elevations of aminotransferases undergo spontaneous HBeAg/anti-HBe seroconversion. After HBeAg clearance, aminotransferase levels gradually return to normal limits, with anti-HBe developing spontaneously. The majority of individuals who demonstrate this clearance enter an "inactive carrier" state with normalization of aminotransferases, a reduction in HBV DNA levels, and improvement in hepatic inflammation. A fraction of patients retain hepatic inflammation with elevated aminotransferases and HBV DNA and remain in the immune-active state. There is a greater risk for the development of cirrhosis and HCC. Risk factors that have been associated with progressive hepatic inflammation and subsequent complications include HBV genotype, persistent viremia, and specific mutations in the HBV genome. The optimal goal of antiviral therapy for chronic HBV infection is to eradicate HBV and to prevent its related liver complication by shortening the duration of liver inflammation. However, due to the limited effect of available therapies in viral eradication, the goal of current antiviral therapy for hepatitis B is to reduce viral replication, minimize the liver injury, and reduce infectivity [104] (Table 15.1).

The guidelines for treatment are represented in Fig. 15.1.

Treatment Options

Interferon-Alpha

IFN-alpha is delivered by subcutaneous injection and was the first of the approved therapies for HBV. Predictors of IFN responsiveness include active hepatitis, low HBV DNA levels (<1,000 pg/mL), high serum alanine aminotransferase ALT(>2× ULN), short duration of disease, non-Asian ethnic origin, and horizontal transmission. On the basis of European experience, consensus recommendations for the use of IFN-alpha for HBV infected children were developed. The main goals of therapy, according to these recommendations, were to accelerate HBeAg clearance in children with HBeAg and HBV DNA positivity, with low-intermediate HBV DNA levels and

Table 15.1 Phases of chronic hepatitis B infection

Phase	Laboratory results and histology	Note
Immune tolerant	HBsAg and HBeAg detectable	Biopsy not indicated
	HBV DNA >20,000 IU/mL (>105 copies/mL)	Antiviral therapies generally ineffective
	ALT normal	Risk of drug resistance if treated with nucleos(t)ide analogs
	Absent or minimal liver inflammation and fibrosis	Continued monitoring
HBeAg + immune active	HBsAg and HBeAg remain detectable	Most asymptomatic
	HBV DNA >20,000 IU/mL (>105 copies/mL)	Biopsy
	ALT persistently elevated	Rule out other liver diseases
Inactive HBsAg	HBsAg present	Age at serconversion influenced by HBV genotype
	HBeAg undetectable, anti-HBe present	Risk of developing cirrhosis declines
	HBV DNA <2,000 IU/mL (>104 copies/mL) or undetectable	Risk of developing HCC
	ALT normal	Biopsy generally not indicated
	Absent or minimal liver inflammation, fibrosis regresses over time	Continued monitoring
Reactivation of HBeAg-negative, immune active	HAsAg present	Occurs in 20–30 % of patients
	HBeAg remains negative and anti-HBe positive	"e-antigen-negative" hepatitis B
	HBV DNA levels >2,000 IU/mL (>104 copies/mL)	Liver biopsy indicated, especially if ALT raised
	ALT normal or elevated	Treatment should be considered if moderate/severe inflammation of fibrosis present
	Active liver inflammation ± fibrosis	

Modified from Jonas et al. [104]

abnormal aminotransferase enzymes, ages 2 years or older. IFN therapy is less likely to be of benefit in children with perinatally acquired infection who have normal or minimally elevated aminotransferases. The recommended treatment regimen for IFN-alpha is five to ten million units per square meter thrice weekly by subcutaneous injection for 4 to 6 months. The response rates are variable, depending on route of acquisition, ethnic origin, disease activity, and treatment regime. Adult data suggest that HBeAg-negative chronic disease should be treated for 12 months, whereas others demonstrate that longer durations of treatment of 24 months increased sustained response rates [105]. Pretreatment with corticosteroids ("priming") and their withdrawal before commencing IFN-alpha may exacerbate the host immune response, facilitating seroconversion. The benefit, however, remains unproven and is associated with the risk of precipitating fulminant liver failure. IFNα is thought to simply accelerate seroconversion, as many patients who do not respond to treatment may still seroconvert to anti-HBe later in life [106]. In a series, 74 children were followed for 7 years. In treated patients with elevated baseline ALT levels HBsAg clearance was observed in 4–15 % of children treated with IFN (15–25 % of responders), compared to 0–10 % of controls [106].

IFNα is contraindicated in children with decompensated cirrhosis, cytopenias, and autoimmune disease. Side effects of IFNα treatment included fever and flu-like symptoms, behavioral disorders, gastrointestinal disorders, and

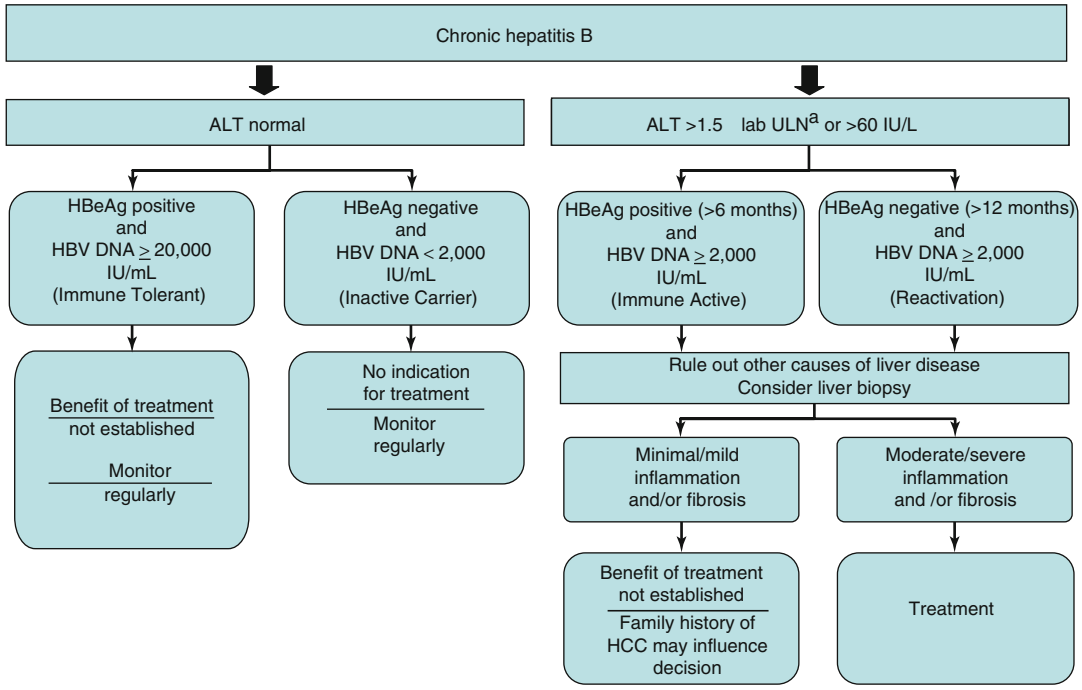


Fig. 15.1 HBV antiviral treatment in children (Modified from Jonas et al. [104])

neutropenia. Furthermore, IFNα was shown to temporarily affect growth. Pegylated interferon-alpha (PegIFN) has not yet been approved for the treatment of chronic hepatitis B in children.

Lamivudine

Lamivudine is an orally administered pyrimidine nucleoside analogue. It prevents replication of HBV in infected hepatocytes, is incorporated into viral DNA leading to chain termination, and competitively inhibits viral reverse transcriptase. Viral response was achieved by 23 % of children receiving lamivudine after 1-year treatment (compared to 13 % in the control group). The response increased to 35 % in children with ALT levels of at least twice the ULN [107]. At the end of 2–3 years of treatment, the response rate was 56 % for children receiving lamivudine in the absence of resistant mutations. Resistance rates increased over time (24 % after 1 year of treatment, 49 % at 2 years, and 64 % at 3 years) [108].

The recommended treatment dose is 3 mg/kg/day (maximum 100 mg/day), administered orally once daily. Longer treatment leads to higher resistance rates and it is therefore recommended that lamivudine be discontinued after 6 months for lack of complete viral suppression or if YMDD mutants emerge. Alternative therapy should be considered with severe and protracted transaminitis.

Combination therapy with lamivudine/IFNa (either concurrent or sequential) proved to be more effective than single drugs alone in adult patients with elevated ALT levels [109]. Three studies in children investigated therapy in treatment-naïve children with elevated ALT levels. Although no difference was found between different combination strategies, the children reached 30–60 % seroconversion to anti-HBe and 9–17 % to anti-HBs [110]. As no large clinical trials have been conducted so far, advantages of combination therapy over monotherapy in children are still unclear.

Adefovir

Adefovir dipivoxil is a purine analogue approved to treat children with chronic hepatitis B aged 12 years and older. 23 % of patients

aged 12–17 years achieved viral response after a 48-week treatment with adefovir (compared to 0 % of placebo-treated subjects). The efficacy on HBV DNA suppression and ALT normalization was less significant in younger children (15 % vs. 3 %) [111]. While mutations are rare in children, adefovir-resistant mutations are reported in more than 20 % of HBeAg-positive adults after a 5-year treatment [112]. A proximal renal tubular toxicity is a side effect of adefovir, which has been rarely reported in adults, but not in children. Patients with HBeAg-positive chronic HBV infection should continue on treatment for at least 6 months after seroconversion with discontinuation of treatment if there is incomplete viral suppression after 24 weeks.

Entecavir

Entecavir is a carbocyclic analogue of 20-deoxyguanosine that has proved to be effective in adult patients [113]. Resistance is rare, even after 5 years of treatment [114]. It has been approved by the FDA for treatment of adolescents aged 16 years or older. The recommended dose is 0.5 mg once daily for nucleoside-naïve patients and 1 mg/day for lamivudine-resistant patients. A phase III clinical trial in children as young as 2 years old is underway.

New Drugs

Telbivudine is an L-nucleoside analogue with a potent antiviral activity and a safety profile similar to lamivudine (although myopathy and peripheral neuropathy were reported in adults). Resistance rate is lower than lamivudine but higher than adefovir. Therefore, telbivudine is only used in combination with other antiviral drugs. A phase I clinical trial is ongoing on children 2–18 years of age [115].

Tenofovir disoproxil fumarate is a nucleoside analogue originally licensed for treatment for HIV infection. The dose for tenofovir in adults is higher than that of adefovir (300 mg/day) and has a greater antiviral activity than adefovir in clinical trials (undetectable HBV DNA in 76 % of patients vs. 13 % of adefovir-receiving subjects after 48 weeks of treatment) [116]. No genotypic resistance to tenofovir has yet been confirmed. A phase III trial is ongoing on 12–17-year-old patients [117] (Table 15.2).

Hepatitis D

Hepatitis delta virus (HDV) is closely associated with hepatitis B virus (HBV) infection. The simultaneous presence of HBV is required for complete virion assembly and secretion. HBV replication is suppressed in most HDV-infected individuals. The HDV genome is a small RNA molecule and expresses the HDV antigen.

Coinfection of HBV and HDV results in acute hepatitis that is usually transient and self-limited. However, a high incidence of liver failure has been reported among drug users [118].

HDV superinfection of a chronic HBsAg carrier may present as severe acute hepatitis in a previously unrecognized HBV carrier or as an exacerbation of preexisting chronic hepatitis B. Progression to chronic HDV infection occurs, while HBV replication is usually suppressed [119].

In the Western world, where the predominant HDV genotype is genotype 1 [120], acute hepatitis D has an increased risk of a fulminant disease. Chronic HDV infection exacerbates the preexisting liver disease in patients with hepatitis B with rapidly progressive cirrhosis [121]. In the Far East, where the predominant genotype is genotype 2, there is a less frequent fulminant hepatitis with acute HDV infection and rapidly progressive liver disease with chronic HDV. Severe outbreaks of acute hepatitis D with a high incidence of liver failure have been reported in South America with genotype 3.

Treatment

Interferon

The only drug approved at present for treatment of chronic hepatitis D is IFNα. In the largest multicenter trial, 61 Italian patients with chronic hepatitis D were randomly assigned to receive IFNα in doses of 5 MU/m^2 three times weekly for 4 months, followed by 3 MU/m^2 three times weekly for an additional 8 months or placebo [122]. They were followed for another 12 months.

Table 15.2 Available treatments for chronic hepatitis B in children

Treatment	Licensing	Dose	Duration	Advantages	Disadvantages
IFNa	≥12 months	5–10 M units/m^2 SC 3×/week	6 months	No resistance Usable in young children	Side effects Parenteral administration Not for use in decompensated cirrhosis or transplantation
Lamivudine	≥3 years	3 mg/kg po once daily (max 100 mg/day)	≥1 year	Few side effects Usable in young children Oral administration	High resistance rate
Adefovir	≥12 years	10 mg po once daily	≥1 year (+6 months after HBeAG seroconversion)	Partially effective in lamivudine resistant patients Oral administration	
Entecavir	≥16 years + Phase III (2–17 years)	0.5 mg/day once Daily (1 mg/day for Lamivudine-resistant pts)	≥1 year (+6 months after HBeAG seroconversion)	Partially effective in lamivudine resistant patients Oral administration	Not approved for children < 12 years resistant mutations
PeglFN	Phase III (2–18 years)	180 ug/week	6 months	No resistance Once weekly administration	Side effects Parenteral administration
Telbivudine	Phase 1 (2–18 years)	600 mg/day once daily	≥1 year	Few side effects Oral administration	High resistance
Tenofovir	Phase III (12–17 years)	300 mg/day once daily	≥1 year	High response rate No resistance identified Few side effects Oral administration	No available preparation for young children Reduced mineral density

Modified from Paganelli et al. [98]

Twenty-five percent of the 31 treated patients had normal serum transaminases versus none of the 30 controls at the end of the study. All but one of the responders had biochemical relapse after discontinuation of treatment.

Pegylated Interferon

There is little data with pegylated interferon in the treatment of chronic hepatitis D. The largest published study included 38 patients who were treated with pegylated IFN alfa-2b (1.5 MU/kg/week) alone or in combination with ribavirin for 48 weeks [123]. Patients were maintained on pegylated IFN for an additional 24 weeks and then followed for 24 weeks. At the end of follow-up, HDV RNA was not detectable in 21 %. The response rate was similar in the monotherapy and combination therapy groups suggesting that ribavirin had no effect on viral clearance. A higher virologic response rate (43 %) was found in another study with 12 months of pegylated IFN [120].

Combination therapies with nucleoside and nucleotide analogues have not been encouraging and there are currently no standard recommendations for the treatment of pediatric HDV infection. The mainstay of prevention of HDV infection is vaccination against its helper virus, HBV.

Hepatitis C

HCV is an RNA virus that affects >180 million individuals worldwide. In the USA, antibodies to HCV are present in approximately 0.2 % of children ages 6–12 and 0.4 % of those ages 12–19. Overall, approximately 28,000 new HCV infections occur in the USA each year, although the specific incidence in children is unknown [124]. Chronic HCV infection is estimated to affect 0.1–2 % of children in the USA.

Perinatal transmission is a common source of infection in children. Adolescents may be exposed through intravenous or intranasal drug use and use of shared tattoo needles. The incidence of HCV vertical transmission is approximately 2–5 % in HCV RNA positive mothers, with the highest risk in mothers with high HCV viral load. The risk also is increased fourfold for mothers with a concomitant HIV coinfection [125–130].

Six major genotypes of HCV have been defined with greater than 50 subtypes. Genotype 1 is most common (60–70 %) in the USA and Europe; genotypes 2 and 3 are less common in these areas, while genotypes 4, 5, and 6 are rare. Genotype 3 is most common in India, the Far East, and Australia. Genotype 4 is most common in Africa and the Middle East and genotype 5 is most common in South Africa. Genotype 6 is common in Hong Kong, Vietnam, and Australia. The most common subtypes are 1a, 1b, 2a, and 2b.

Viral genotypes affect the response to interferon therapy. The sustained virologic response to pegylated interferon plus ribavirin ranges from about 40 to 50 percent with genotype 1 (including 1a and 1b) to as high as 70–80 % with genotypes 2 and 3 [131–136].

Infections acquired during infancy are more likely to resolve on their own. Spontaneous clearance rates ranging from 20 to 45 % have been described [137–143]. In follow-up of perinatally acquired HCV in 266 children, approximately 20 % cleared the infection, while 80 % had chronic disease [144]. The disease progresses slowly and therefore advanced liver disease is uncommon in children. However, progression to advanced fibrosis and cirrhosis during childhood has been reported.

Screening

Patients suspected of having chronic hepatitis C virus (HCV) infection or with risk factors for HCV should be tested for HCV antibodies. HCV RNA testing should be performed in those with a positive antibody test to confirm infection and in those with unexplained liver disease whose anti-HCV test is negative and who are immunocompromised. HCV RNA quantitation (viral load) should be obtained in patients being considered for treatment. Quantitative and qualitative tests are used during treatment to assess response. HCV genotype should be determined in all infected persons prior to treatment to determine

Table 15.3 Screening for HCV infection

Group	Screening
Injected illicit drug use	Antibody
Persons with conditions associated with a high prevalence of HCV infection including:	Antibody or RNA
HIV infection	
On hemodialysis	
Unexplained abnormal amionotransferases	
Earlier recipients of transfusions or organ transplants before July 1992 including:	Antibody or RNA
Children born to HCV-infected mothers	Antibody after 18 months of age. RNA for younger ages
Health care, emergency medical, and public safety workers after a needle stick injury or mucosal exposure to HCV-positive blood	Antibody or RNA
Present sexual partners of HCV-infected persons	Antibody
Children from a region with high prevalence of HCV infection	Antibody

Modified from Mack et al. [146]

the duration of therapy, dose of ribavirin, and the likelihood of response (Table 15.3).

The NASPGHAN guidelines recommend testing for anti-HCV antibodies in the infant after 18 months of age and confirmation by PCR if positive. If the parents desire, HCV RNA may be tested in the first year of life; however, the infant must be older than 2 months of age at testing [145, 146].

Chronic infection with HCV has been associated with hepatocellular carcinoma (HCC), in those with cirrhosis [147]. As a result, HCC is rare among children infected with HCV but appears to be more common in children who developed HCV after treatment for childhood leukemia.

Treatment

Children with hepatitis C with persistently elevated serum aminotransferases or those with fibrosis on liver histology should be considered for treatment. Presently available treatments are IFNα or PEG-IFNα and ribavirin. AASLD and NASPGHAN recommend the FDA-approved combination of PEG-IFNα with ribavirin as first-line treatment for children ages 3–17 years [148–153].

Table 15.4 Investigational therapy for hepatitis C infection

Direct acting antiviral therapy
Ns3/4a protease inhibitors
Boceprevir/Telaprevir
BI201335
Danoprevir
TMC 435
Asunaprevir
NS5B polymerase inhibitors
GS7977
NS5A inhibitors
Daclatasvir
Combinations without peginterferon
Treatment targeting host encoded factors
HCV vaccine
Complementary medicine

Triple Therapy

As of May 2011, two protease inhibitors, boceprevir and telaprevir, are licensed in the USA for use in combination with pegylated interferon and ribavirin in adults with chronic HCV genotype 1. This therapy is associated with a significantly higher rate of sustained virologic response as compared with the combination of pegylated interferon and ribavirin. A pediatric trial of combination therapy with boceprevir is underway and a trial of telaprevir is being planned.

Practice recommendations for children with chronic HCV are similar to those for adults [154]. However, because of limited data in the pediatric age group, treatment decisions may vary with the child's age and individual disease characteristics. Combination treatment with pegylated interferon plus ribavirin is considered for children with chronic HCV infection who are older than 3 years and who appear to have progressive disease or advanced histological features. For these children, the course of action depends on the HCV genotype.

Newer potential treatments include direct acting antivirals and possible options that may minimize the use of injectable therapy (Table 15.4).

Hepatitis G

Hepatitis G virus (HGV) was discovered incidentally and was named after a 35-year-old surgeon who developed jaundice. His serum led to the identification of GBV-A, GBV-B, and GBV-C. Subsequent studies have not identified any association between infections with GBV or acute hepatitis. Therefore, the term hepatitis G virus has been questioned. It is a positive-strand RNA virus belonging to the Flaviviridae family and GB V-C shares 27 % homology with HCV [155].

The virus is found worldwide with five different genotypes. The development of antibodies correlates with loss of viremia and suggests past exposure and clearance of the infection [156]. About 16 % of healthy blood donors are positive for antibodies with much lower rate of active viremia. The rate of natural clearance of the virus is higher than that for hepatitis C. Although GBV-C is detected in many patients with chronic hepatitis of unclear etiology, it does not appear to cause any liver disease [157–159]. The duration of infection depends on the immune status and the age of the patient. No association has been found with malignancy or aplastic anemia [160]. Antibodies can be checked to document past infection and PCR analysis may be done.

TT Virus

This virus was first identified in 1977 and is also known as the transfusion transmitted virus. It is a non-enveloped single-stranded DNA virus that is believed to be hepatotropic. It is found worldwide and is common. It has been found in 1–40 % of healthy blood donors and is transmitted by parenteral routes [161]. Although it was associated with hepatitis in the first patient in whom it was identified, studies have not supported a relationship between liver disease and the virus [162].

Several similar viruses, Sanban, Yonban and Sen viruses, have also been identified. Their clinical significance remains controversial.

Epstein–Barr Virus

Infection with this virus has myriad clinical presentations. While most infants and young children are asymptomatic or have nonspecific complaints, adolescents may have fever, lymphadenopathy, and pharyngitis. Subclinical liver disease may occur and overt disease ranges from mild transaminitis to hepatic necrosis with fulminant failure. Serum lactic dehydrogenase may be elevated to three times ULN. Alkaline phosphatase is elevated and hyperbilirubinemia is also seen in about 45 % of patients. The laboratory abnormalities are slow to resolve. Cholestatic jaundice with pruritus may occur in women with EBV hepatitis taking the oral contraceptive pill. Fatal fulminant hepatitis has been described in both immunocompromised patients as well as the immunocompetent. Fever, hepatosplenomegaly, liver failure, and bone marrow suppression with hyperferritinemia may develop in the hemophagocytic syndrome associated with EBV infection [163].

The diagnosis of EBV hepatitis is based on clinical features as well as the tests that support the diagnosis. There are predominant lymphocytosis, monocytosis, and thrombocytopenia [164]. The monospot test may be positive and IgM antibodies are seen early in disease with IgG antibodies indicating possible past infection. It is important to note that the monospot test however may be falsely negative. An abdominal ultrasound frequently demonstrates hepatosplenomegaly, lymphadenopathy, and gallbladder thickening. A liver biopsy is rarely necessary for diagnosis. There is no specific therapy for EBV hepatitis. Acyclovir inhibits replication of EBV but has no effect on clinical symptoms or outcome [165]. Improvement in acute and chronic EBV hepatitis has been reported with ganciclovir treatment, but this is not yet well studied [166]. Liver transplantation has been performed for fulminant hepatitis caused by EBV.

Cytomegalovirus

Cytomegalovirus or CMV is a member of the herpes family and persists for life in a

non-replicative state. Clinically disease may occur as a primary infection or reactivation in immunocompromised patients. In those who are immunocompetent, primary infection is usually subclinical or may mimic infection with EBV. Hepatic disease is characterized by an increase in liver enzymes and alkaline phosphatase with or without organomegaly. The clinical course is usually mild; however, occasionally there may be hepatic necrosis. In congenital infections with CMV, jaundice, hepatosplenomegaly, thrombocytopenic purpura, and severe neurological impairment have been described [167]. Portal vein thrombosis may occur [168]. Disseminated disease can occur in the immunocompromised host and may manifest as hepatitis, pancreatitis, and rarely gangrenous cholecystitis. CMV has been described in AIDS-associated cholangiopathy and primary sclerosing cholangitis [169, 170]. CMV hepatitis may be difficult to distinguish from graft rejection in liver transplant patients, but the differentiation is crucial to appropriate management. Diagnosis requires serological analysis and liver biopsy. IgM antibodies demonstrate acute infection and PCR is used for confirmation particularly in immunocompromised patients. Multinucleated giant cells and mononuclear portal infiltrate are seen on liver biopsy. There are characteristics intranuclear inclusions that are called "owl's eye" inclusions. Ganciclovir is used for treatment, and alternative agents include foscarnet and cidofovir.

Herpes Simplex Virus

HSV hepatitis is seen in immunocompromised individuals and neonates. The infection may be rapidly progressive and life threatening. The neonate may be exposed to infected maternal secretions at the time of delivery. In pregnant women the hepatitis may be fulminant. There is therefore a high maternal and perinatal mortality of approximately 40 %. Prompt diagnosis and treatment is extremely important [171, 172].

A liver biopsy is required for diagnosis. Extensive hemorrhagic necrosis may be seen on biopsy with intranuclear inclusions (Cowdry A bodies). The hepatocytes may have a ground glass appearance [173]. Treatment is with acyclovir but liver transplantation may be indicated for severe disease.

Varicella-Zoster Virus

Children infected with the virus may demonstrate hepatitis with an increase in liver enzymes, albeit rarely. Dissemination of infection leading to visceral involvement has been described before the onset of cutaneous features in patients who have received solid organ transplantation and other forms of immunodeficiency. If visceral involvement is suspected, high-dose intravenous acyclovir is required [174].

Infectious Complications in Liver Transplantation

In transplant patients, morbidity and mortality due to infections remains a persistent problem. Infections include those with bacterial, viral, or fungal pathogens. It is important to note that in solid organ transplant recipient, the signs and symptoms of infection are often blunted in the setting of immune suppression. In addition, antibiotics or antimicrobials used may have interactions with immunosuppressive medications. An important risk factor is the presence of latent infections in either the transplant recipient or donor. All potential transplant donors are screened for infection with cytomegalovirus, herpes virus, tuberculosis, hepatitis B and C, syphilis, and the human immunodeficiency virus. Pretransplant infections with methicillin-resistant Staphylococcus aureus or MRSA or vancomycin-resistant enterococcus VRE lead to posttransplant infections [175, 176]. However, colonization with these pathogens is not a contraindication to transplantation [177].

An additional risk factor includes surgical complications at transplant. For example, the risk of bacterial infections is increased in those who undergo a Roux-en-Y biliary anastomosis and in

those with multiple abdominal surgeries [178–180]. Those patients who develop rejection or have poor graft function are also at increased risk of infection because of the aggressive immunosuppression required.

Minimizing infections includes strategies for appropriate vaccination prior to transplantation. Particularly since antirejection immunosuppressive medications prevent the development of an optimal response to vaccines, certain vaccines such as the pneumococcal and influenza vaccines may need to be repeated after transplantation. Live vaccines are to be avoided in transplant recipients due to potential risk of disseminated disease.

There is a higher chance of developing surgical site infections in liver transplant recipient compared to other solid organ transplants. Treatment with trimethoprim sulfamethoxazole is generally used after liver transplantation for 3–12 months to reduce the risk of Pneumocystis but also to prevent infections with Listeria, Nocardia, Toxoplasma, and many other common infections [181–183]. The dose is a single-strength tablet taken daily or double-strength tablets taken thrice a week. The most common adverse effect is allergy and myelosuppression. It is also important to note that higher doses may increase the nephrotoxicity associated with cyclosporin or tacrolimus.

Although cytomegalovirus remains an important viral infection posttransplant, other viruses such as HSV I and 2 and VZV are significant pathogens. Ganciclovir and valganciclovir are used for posttransplant viral prophylaxis [184]. CMV-negative transplant recipients who receive an organ from a CMV seropositive donor are at increased risk to develop CMV reactivation posttransplant. CMV seropositive recipients have a lower risk and CMV donor/recipient negative patients the least risk of CMV activation [185, 186]. Infection with the CMV virus increases risk for bacterial and fungal infections and leads to an almost fourfold increased risk of death by a year posttransplant [187–191].

Fungal infections are also problematic in the posttransplant setting. *Candida albicans* is the most commonly seen fungal pathogen although infections with non-albicans Candida may occur [192]. The role of antifungal treatment for prophylaxis is not well studied; however, fluconazole or liposomal amphotericin B for 14–27 days is used for postoperative antifungal prophylaxis for high-risk liver transplant recipients such as those with renal failure, fulminant hepatic failure, prolonged hospitalization, prolonged antibiotic use, or large transfusion requirements [193, 194].

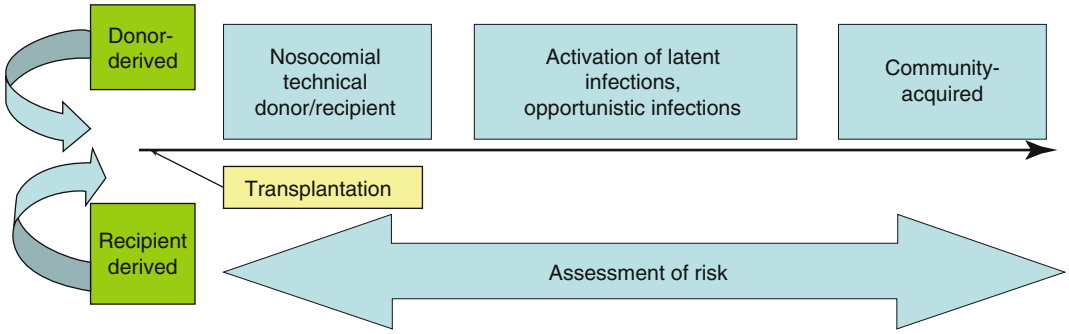
Patients with liver transplantation remain at increased risk for the development of active *Mycobacterium tuberculosis* infection. Although it is optimal to treat latent TB prior to transplantation, it can be very difficult to do because of the significant hepatotoxicity of isoniazid. Treatment can sometimes be attempted after transplantation [195].

In the first 3 months immediately following transplantation, bacterial infections predominate. They usually have a nosocomial source such as indwelling catheters and drains. Prolonged intubation increases risk of infections. Abdominal abscesses, cholangitis, wound infections, and nosocomial pneumonias are common. Clostridium difficile colitis may also occur [196, 197] (Fig. 15.2).

If bacterial infection is suspected in the transplant recipient, empiric broad-spectrum antibiotics are used, until sensitivities are identified. Aminoglycosides are usually avoided due to their nephrotoxicity particularly if calcineurin inhibitors are being used for immunosuppression. Common organisms isolated in bacteremic patients are methicillin-resistant *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.

Beyond the immediate posttransplant period, other infections with cytomegalovirus, VZV, Epstein–Barr virus, respiratory syncytial virus, human herpesvirus 6, influenza virus, and adenovirus may occur [198]. Infections with EBV are very important because of the potential to induce posttransplant lymphoproliferative disease.

Invasive aspergillosis is associated with a very high mortality [199]. Opportunistic infections become uncommon beyond the first 6 months after transplant. However, since these patients are immunocompromised, they are at increased risk for infections with pathogens such as *Cryptococcus neoformans*, Legionella, and also



Common infections in solid organ transplantation recipients

Antimicrobial-**resistant species:**	**With PCP and antiviral (CMV, HBV) Prophylaxis:**	**Community-acqurired pneumonia, urainary tract infection:**
• MRSA • VRE • Candida species (non-albicans) Aspiration Line infection Wound infection Anastamotic leaks/ischemia Clostridium difficile colitis **Donor-derived:** HSV, CMV **Recipient-derived (colonization):** Aspergilus, Pseudomonas	• Clostridium difficile colitis • Hepatitis C virus • Adenovirus, Influenza • Cryptococcus neoformans • M. tuberculosis **Without Prophylaxis:** Penumocystis Herpes viruses (HSV, VZV, CMV, EBV) Hepatitis B virus Listeria, Nocardia, Toxoplasma, Strongyloides, Leishania, Trypanosoma	Aspergillus, atypical molds, Mucor species Nocardia **Late viral:** • CMV • Hepatitis • HSV encephalitis • Community-acquired (SARS, West Nile) Skin cancer, lymphoma

Fig. 15.2 Common patterns of opportunistic infection. Abbreviations: *CMV* cytomegalovirus, *EBV* Epstein–Barr virus, *HSV* herpes simplex virus, *MRSA* methicillin-resistant Staphylococcus aureus, *SARS* severe acute respiratory syndrome, *VRE* vancomycin-resistant enterococcus, *VZV* varicella-zoster virus (Modified from Fishman and AST Infectious Diseases Community of Practice [202]. Copyright © 2009 American Society of Transplantation and the American Society of Transplant Surgeons)

West Nile virus. Respiratory infections due to pathogens such as *Streptococcus pneumonia* and *Haemophilus influenza* may be life threatening if not promptly treated. Recurrent infections with EBV, CMV, and herpesvirus 6 and 7 may occur and may be devastating. Fungal infections with *Histoplasma*, *Coccidioides*, and *Blastomyces* may be seen in the late posttransplant period. Listeria infection may manifest as hepatitis, bacteremia, and meningitis [200, 201].

References

1. Stevens DL. Group A streptococcal sepsis. Curr Infect Dis Rep. 2003;5:379–86.
2. Fernández J, Acevedo J, Castro M, et al. Prevalence and risk factors of infections by multiresistant bacteria in cirrhosis: a prospective. Hepatology. 2012;55(5):1551–61.
3. Bryant AE, Chen RY, Nagata Y, et al. Clostridial gas gangrene. I. Cellular and molecular mechanisms of microvascular dysfunction induced by exotoxins of Clostridium perfringens. J Infect Dis. 2000;182:799–807.
4. Chen YM, Lee HC, Chang CM, et al. Clostridium bacteremia: emphasis on the poor prognosis in cirrhotic patients. J Microbiol Immunol Infect. 2001;34:113–8.
5. Le DT, Brockstedt DG, Nir-Paz R, Hampl J, Mathur S, Nemunaitis J, Sterman DH, Hassan R, Lutz E, Moyer B, Giedlin M, Louis JL, Sugar EA, Pons A, Cox AL, Levine J, Murphy AL, Illei P, Dubensky Jr TW, Eiden JE, Jaffee EM, Laheru DA. A live-attenuated Listeria vaccine (ANZ-100) and a live-attenuated Listeria vaccine expressing mesothelin (CRS-207) for advanced cancers: phase I studies of safety and immune induction. Clin Cancer Res. 2012;18(3):858–68.
6. El-Newihi HM, Alamy ME, Reynolds TB. Salmonella hepatitis: analysis of 27 cases and

comparison with acute viral hepatitis. Hepatology. 1996;24:516–9.
7. Chaudhry R, Mahajan RK, Diwan A, et al. Unusual presentation of enteric fever: three cases of splenic and liver abscesses due to Salmonella typhi and Salmonella paratyphi a. Trop Gastroenterol. 2003;24:198–9.
8. Kamath PS, Jalihal A, Chakraborty A. Differentiation of typhoid fever from fulminant hepatic failure in patients presenting with jaundice and encephalopathy. Mayo Clin Proc. 2000;75:462–6.
9. Hsueh PR, Kao JH, Chen YC, et al. Paratyphoid hepatitis: an emerging clinical entity. Am J Med. 2002;113:257–8.
10. Rajagopal A, Ramasamy R, Mahendran G, Thomas M. Hepatic abscess complicating paratyphoid infection. Trop Gastroenterol. 2002;23:181–2.
11. Bergmann TK, Vinding K, Hey H. Multiple hepatic abscesses due to Yersinia enterocolitica infection secondary to primary haemochromatosis. Scand J Gastroenterol. 2001;36:891–5.
12. Wong JJ, Kinney TB, Miller FJ, Rivera-Sanfeliz G. Hepatic actinomycotic abscesses: diagnosis and management. AJR Am J Roentgenol. 2006;186:174–6.
13. Uehara Y, Takahashi T, Yagoshi M, Shimoguchi K, Yanai M, Kumasaka K, Kikuchi K. Liver abscess of Actinomyces israelii in a hemodialysis patient: case report and review of the literature. Intern Med. 2010;49(18):2017–20. Review.
14. Pedro-Botet ML, Sabria M. Legionellosis. Semin Respir Crit Care Med. 2005;26:625–34.
15. Holmes KK, Counts GW, Beaty HN. Disseminated gonococcal infection. Ann Intern Med. 1971;74:979–93.
16. Akritidis N, Tzivras M, Delladetsima I, et al. The liver in brucellosis. Clin Gastroenterol Hepatol. 2007;5:1109–12.
17. Colmenero Jde D, Queipo-Ortuno MI, Maria Reguera J, et al. Chronic hepatosplenic abscesses in brucellosis. Clinico-therapeutic features and molecular diagnostic approach. Diagn Microbiol Infect Dis. 2002;42:159–67.
18. Ariza J, Pigrau C, Canas C, et al. Current understanding and management of chronic hepatosplenic suppurative brucellosis. Clin Infect Dis. 2001;32:1024–33.
19. Zaidi SA, Singer C. Gastrointestinal and hepatic manifestations of tickborne diseases in the United States. Clin Infect Dis. 2002;34:1206–12.
20. Tissot-Dupont H, Raoult D. Q fever. Infect Dis Clin North Am. 2008;22:505–14.
21. VanderHeyden TR, Yong SL, Breitschwerdt EB, Maggi RG, Mihalik AR, Parada JP, Fimmel CJ. Granulomatous hepatitis due to Bartonella henselae infection in an immunocompetent patient. BMC Infect Dis. 2012;12:17.
22. Palaniappan RU, Ramanujam S, Chang YF. Leptospirosis: pathogenesis, immunity, and diagnosis. Curr Opin Infect Dis. 2007;20:284–92.
23. Feigin RD, Anderson DC. Human leptospirosis. CRC Crit Rev Clin Lab Sci. 1975;5:413–67.
24. Steere AC. Lyme disease. N Engl J Med. 2001;345:115–25.
25. Nadelman RB, Wormser GP. Lyme borreliosis. Lancet. 1998;352:557–65.
26. Goellner MH, Agger WA, Burgess JH, Duray PH. Hepatitis due to recurrent Lyme disease. Ann Intern Med. 1988;108:707–8.
27. Horowitz HW, Dworkin B, Forseter G, et al. Liver function in early Lyme disease. Hepatology. 1996;23:1412–7.
28. Alvarez SZ. Hepatobiliary tuberculosis. J Gastroenterol Hepatol. 1998;13:833–9.
29. Peeters L, Van Vaerenbergh W, Van der Perre C, et al. Tertiary syphilis presenting as hepatic bull's eye lesions. Acta Gastroenterol Belg. 2005;68:435–9.
30. Yu SC, Ho SS, Lau WY, et al. Treatment of pyogenic liver abscess: prospective randomized comparison of catheter drainage and needle aspiration. Hepatology. 2004;39:932–8.
31. Zerem E, Hadzic A. Sonographically guided percutaneous catheter drainage versus needle aspiration in the management of pyogenic liver abscess. AJR Am J Roentgenol. 2007;189:W138–42.
32. Lee KT, Wong SR, Sheen PC. Pyogenic liver abscess: an audit of 10 years' experience and analysis of risk factors. Dig Surg. 2001;18:459–65.
33. Alvarez JA, Gonzalez JJ, Baldonedo RF, et al. Single and multiple pyogenic liver abscesses: etiology, clinical course, and outcome. Dig Surg. 2001;18:283–8.
34. Alvarez Perez JA, Gonzalez JJ, Baldonedo RF, et al. Clinical course, treatment, and multivariate analysis of risk factors for pyogenic liver abscess. Am J Surg. 2001;181:177–86.
35. Wong WM, Wong BC, Hui CK, et al. Pyogenic liver abscess: retrospective analysis of 80 cases over a 10-year period. J Gastroenterol Hepatol. 2002;17:1001–7.
36. Chemaly RF, Hall GS, Keys TF, Procop GW. Microbiology of liver abscesses and the predictive value of abscess gram stain and associated blood cultures. Diagn Microbiol Infect Dis. 2003;46:245–8.
37. Serste T, Bourgeois N, Vanden Eynden F, et al. Endoscopic drainage of pyogenic liver abscesses with suspected biliary origin. Am J Gastroenterol. 2007;102:1209–15.
38. Kochar DK, Agarwal P, Kochar SK, et al. Hepatocyte dysfunction and hepatic encephalopathy in plasmodium falciparum malaria. QJM. 2003;96:505–12.
39. Devarbhavi H, Alvares JF, Kumar KS. Severe falciparum malaria simulating fulminant hepatic failure. Mayo Clin Proc. 2005;80:355–8.
40. Artan R, Yilmaz A, Akcam M, Aksoy NH. Liver biopsy in the diagnosis of visceral leishmaniasis. J Gastroenterol Hepatol. 2006;21:299–302.
41. Moreno A, Marazuela M, Yebra M, et al. Hepatic fibrin-ring granulomas in visceral leishmaniasis. Gastroenterology. 1988;95:1123–6.
42. Sundar S, Jha TK, Thakur CP, et al. Injectable paromomycin for visceral leishmaniasis in India. N Engl J Med. 2007;356:2571–81.
43. Sindermann H, Engel J. Development of miltefosine as an oral treatment for leishmaniasis. Trans R Soc Trop Med Hyg. 2006;100 Suppl 1:S17–20.

44. Bhattacharya SK, Sinha PK, Sundar S, et al. Phase 4 trial of miltefosine for the treatment of Indian visceral leishmaniasis. J Infect Dis. 2007;196:591–8.
45. Myles O, Wortmann GW, Cummings JF, et al. Visceral leishmaniasis: clinical observations in 4 U.S. Army soldiers deployed to Afghanistan or Iraq, 2002-2004. Arch Intern Med. 2007;167:1899–901.
46. Jones JL, Kruszon-Moran D, Sanders-Lewis K, Wilson M. Toxoplasma gondii infection in the United States, 1999 2004, decline from the prior decade. Am J Trop Med Hyg. 2007;77:405–10.
47. Montoya JG, Liesenfeld O. Toxoplasmosis. Lancet. 2004;363:1965–76.
48. Hurt C, Tammaro D. Diagnostic evaluation of mononucleosis-like illnesses. Am J Med. 2007;120: 911.e1-8.
49. Remington JS, Thulliez P, Montoya JG. Recent developments for diagnosis of toxoplasmosis. J Clin Microbiol. 2004;42:941–5.
50. Botterel F, Ichai P, Feray C, et al. Disseminated toxoplasmosis, resulting from infection of allograft, after orthotopic liver transplantation: usefulness of quantitative PCR. J Clin Microbiol. 2002;40:1648–50.
51. Stanley Jr SL. Amoebiasis. Lancet. 2003;361: 1025–34.
52. Soentjens P, Ostyn B, Clerinx J, et al. A case of multiple amoebic liver abscesses: clinical improvement after percutaneous aspiration. Acta Clin Belg. 2005;60:28–32.
53. Salles JM, Salles MJ, Moraes LA, Silva MC. Invasive amebiasis: an update on diagnosis and management. Expert Rev Anti Infect Ther. 2007;5:893–901.
54. Leone N, Baronio M, Todros L, et al. Hepatic involvement in larva migrans of Toxocara canis: report of a case with pathological and radiological findings. Dig Liver Dis. 2006;38:511–4.
55. Kaplan KJ, Goodman ZD, Ishak KG. Eosinophilic granuloma of the liver: a characteristic lesion with relationship to visceral larva migrans. Am J Surg Pathol. 2001;25:1316–21.
56. Shah OJ, Zargar SA, Robbani I. Biliary ascariasis: a review. World J Surg. 2006;30:1500–6.
57. Hurtado RM, Sahani DV, Kradin RL. Case records of the Massachusetts General Hospital. Case 9-2006. A 35-year-old woman with recurrent right-upper-quadrant pain. N Engl J Med. 2006;354:1295–303.
58. Rana SS, Bhasin DK, Nanda M, Singh K. Parasitic infestations of the biliary tract. Curr Gastroenterol Rep. 2007;9:156–64.
59. Gryseels B, Polman K, Clerinx J, Kestens L. Human schistosomiasis. Lancet. 2006;368:1106–18.
60. Manzella A, Ohtomo K, Monzawa S, Lim JH. Schistosomiasis of the liver. Abdom Imaging. 2008;33:144–50.
61. Bottieau E, Clerinx J, de Vega MR, et al. Imported Katayama fever: clinical and biological features at presentation and during treatment. J Infect. 2006;52:339–45.
62. Maclean JD, Cross J, Mahanty S. Liver, lung, and intestinal fluke infections. In: Guerrant RL, Walker DH, Weller PF, editors. Tropical infectious diseases: principles, pathogens, and practice. 2nd ed. Philadelphia: Churchill Livingstone; 2006. p. 1349.
63. Sezgin O, Altintas E, Disibeyaz S, et al. Hepatobiliary fascioliasis: clinical and radiologic features and endoscopic management. J Clin Gastroenterol. 2004;38:285–91.
64. Marcos LA, Tagle M, Terashima A, et al. Natural history, clinicoradiologic correlates, and response to triclabendazole in acute massive fascioliasis. Am J Trop Med Hyg. 2008;78:222–7.
65. Choi D, Lim JH, Lee KT, et al. Cholangiocarcinoma and Clonorchis sinensis infection: a case-control study in Korea. J Hepatol. 2006;44:1066–73.
66. Paksoy Y, Odev K, Sahin M, et al. Percutaneous treatment of liver hydatid cysts: comparison of direct injection of albendazole and hypertonic saline solution. AJR Am J Roentgenol. 2005;185:727–34.
67. Sora F, Chiusolo P, Piccirillo N, et al. Successful treatment with caspofungin of hepatosplenic candidiasis resistant to liposomal amphotericin B. Clin Infect Dis. 2002;35:1135–6.
68. Kontoyiannis DP, Luna MA, Samuels BI, Bodey GP. Hepatosplenic candidiasis. A manifestation of chronic disseminated candidiasis. Infect Dis Clin North Am. 2000;14:721–39.
69. Assi MA, Sandid MS, Baddour LM, et al. Systemic histoplasmosis: a 15-year retrospective institutional review of 111 patients. Medicine (Baltimore). 2007;86:162–9.
70. Kauffman CA. Diagnosis of histoplasmosis in immunosuppressed patients. Curr Opin Infect Dis. 2008;21:421–5.
71. Robertson BH, Jansen RW, Khanna B, et al. Genetic relatedness of hepatitis A virus strains recovered from different geographic regions. J Gen Virol. 1992;73:1365–77.
72. Mathiesen LR, Feinstone SM, Purcell RH, Wagner JA. Detection of hepatitis A antigen by immunofluorescence. Infect Immun. 1977;18:524–30.
73. Centers for Disease Control and Prevention. Surveillance for acute viral hepatitis, United States-2006. MMWR. 2008;57(SS02).
74. Emerson SU, Huang YK, Nguyen H, et al. Identification of VP1/2A and 2C as a virulence genes of hepatitis A virus and demonstration of genetic instability of 2C. J Virol. 2002;76:8551–9.
75. Gust ID. Epidemiological patterns of hepatitis A in different parts of the world. Vaccine. 1992;10:S56–8.
76. Gandolfo GM, Ferri GM, Conti L, et al. Prevalence of infections by hepatitis A, B, C and E viruses in two different socio-economic groups of children from Santa Cruz, Bolivia. Med Clin (Barc). 2003;120:725–7.
77. Prevention of hepatitis A through active or passive immunization. Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR. 1996;45(RR-15):1.
78. Coulepis AG, Locarnini SA, Lehmann NI, Gust ID. Detection of HAV in feces. J Infect Dis. 1980;141: 151–6.

79. Rosenblum LS, Villarino ME, Nainan OV, et al. Hepatitis A outbreak in a neonatal intensive care unit: risk factors for transmission and evidence of prolonged viral excretion among preterm infants. J Infect Dis. 1991;164:476–82.
80. Ostapowicz G, Fontana R, Schiødt FV, et al. Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. Ann Intern Med. 2002;137:947–54.
81. Schiødt FV, Atillasoy E, Shakill AO, et al. Etiology and outcome for 295 patients with acute liver failure in the United States. Liver Transpl Surg. 1999;5:29–34.
82. Tagle Arrospide M, Leon Barua R. Viral hepatitis A as a triggering agent of autoimmune hepatitis report of a case and review of literature. Rev Gastroenterol Peru. 2003;23:134–7.
83. Rezende G, Roque-Alsonso M, Samuel D, et al. Viral and clinical factors associated with fulminant course of hepatitis A infection. Hepatology. 2003;38:613–8.
84. Fujiwara K, Yokosuka O, Imazeki F, et al. Analysis of the genotype-determining region of hepatitis A viral RNA in relation to disease severities. Hepatol Res. 2003;25:124–34.
85. Shah U, Habib Z, Kleinman RE. Liver failure due to hepatitis A virus infection in a developing country. Pediatrics. 2000;105(2):436–8.
86. American Academy of Pediatrics. Committee on infectious disease: hepatitis A vaccine recommendations. Pediatrics. 2007;120:189–99.
87. Center of Disease Control and Prevention. Prevention of hepatitis A after exposure to hepatitis A virus and in international travelers. MMWR. 2007;56:1080–4.
88. Victor JC, Monto AS, Surdina TY, et al. Hepatitis A vaccine versus immune globulin for postexposure prophylaxis. N Engl J Med. 2007;357:1685–94.
89. Diaz-Mitoma F, Law B, Subramanya A, Hoet B. Long-term antibody persistence induced by a combined hepatitis A and B vaccine in children and adolescents. Vaccine. 2008;26:1759–63.
90. Yamashita T, Mori Y, Miyazaki N, et al. Biological and immunological characteristics of hepatitis E-like particles based on the crystal structure. Proc Natl Acad Sci U S A. 2009;106:12986–91.
91. Guu TS, Liu Z, Ye Q, et al. Structure of the hepatitis E virus-like particle suggests mechanism for virus assembly and receptor binding. Proc Natl Acad Sci U S A. 2009;106:12992–7.
92. Berke T, Matson DO. Reclassification of the Caliciviridae into distinct genera and exclusion of hepatitis E virus from the family on the basis of comparative phylogenetic analysis. Arch Virol. 2000; 145:1421–36.
93. Arankalle VA, Chobe LP, Joshi MV, et al. Human and swine hepatitis E viruses from Western India belong to different genotypes. J Hepatol. 2002;36:417–25.
94. Aggarwal R, Krawczynski K, Hepatitis E. Sleisenger and Fordtran's gastrointestinal and liver disease. 9th ed. Boston: Saunders Elsevier; 2010. p. 1337–42.
95. Shrestha MP, Scott RM, Joshi DM, et al. Safety and efficacy of a recombinant hepatitis E vaccine. N Engl J Med. 2007;356:895–903.
96. Shah U, Kelly D, Chang MH, Fujisawa T, Heller S, González-Peralta RP, Jara P, Mieli-Vergani G, Mohan N, Murray KF. Management of chronic hepatitis B in children. J Pediatr Gastroenterol Nutr. 2009;48(4):399–404.
97. McMahon BJ. The natural history of chronic hepatitis B virus infection. Hepatology. 2009;49:S45–55.
98. Paganelli M, Stephenne X, Sokal EM. Chronic hepatitis B in children and adolescents. J Hepatol. 2012;57(4):885–96.
99. McMahon BJ, Alward WL, Hall DB, Heyward WL, Bender TR, Francis DP, et al. Acute hepatitis B virus infection: relation of age to the clinical expression of disease and subsequent development of the carrier state. J Infect Dis. 1985;151:599–603.
100. World Health Organization. Hepatitis B vaccines – WHO position paper. Weekly epidemiological record. 2009;89:405–20.
101. Chen H-L, Lin L-H, Hu F-C, Lee J-T, Lin W-T, Yang Y-J, et al. Effects of maternal screening and universal immunization to prevent mother-to infant transmission of HBV. Gastroenterology. 2012;142(4):773–81.e2.
102. Shi Z, Yang Y, Wang H, Ma L, Schreiber A, Li X, et al. Breastfeeding of newborns by mothers carrying hepatitis B virus: a meta-analysis and systematic review. Arch Pediatr Adolesc Med. 2011;165:837–46.
103. Chang MH, Hsu HY, Hsu HC, et al. The significance of spontaneous HBeAg seroconversion in childhood: with special emphasis on the clearance of HBeAg before three years of age. Hepatology. 1995;22:1387–92.
104. Jonas MM, Block JM, Haber BA, Karpen SJ, London WT, Murray KF, Narkewicz MR, Rosenthal P, Schwarz KB, McMahon BJ. Hepatitis B foundation treatment of children with chronic hepatitis B virus infection in the United States: patient selection and therapeutic options. Hepatology. 2010;52(6):2192–205. doi:10.1002/hep.23934. Epub 2010 Oct 1.
105. Lampertico P, Del Ninno E, Vigano M, et al. Long term suppression of HBeAg negative chronic hepatitis B by 24 months interferon therapy. Hepatology. 2003;37:756–63.
106. Vo Thi Diem H, Bourgois A, Bontems P, Goyens P, Buts J-P, Nackers F, et al. Chronic hepatitis B infection: long-term comparison of children receiving interferon alpha and untreated controls. J Pediatr Gastroenterol Nutr. 2005;40:141–5.
107. Jonas MM, Kelly DA, Mizerski J, Badia IB, Areias JA, Schwarz KB, et al. Clinical trial of lamivudine in children with chronic hepatitis B. N Engl J Med. 2002;346:1706–13.
108. Sokal EM, Kelly DA, Mizerski J, Badia IB, Areias JA, Schwarz KB, et al. Long-term lamivudine therapy for children with HBeAg-positive chronic hepatitis B. Hepatology. 2006;43:225–32.
109. Sarin SK, Kumar M, Kumar R, Kazim SN, Guptan RC, Sakhuja P, et al. Higher efficacy of sequential

therapy with interferon-alpha and lamivudine combination compared to lamivudine monotherapy in HBeAg positive chronic hepatitis B patients. Am J Gastroenterol. 2005;100:2463–71.
110. Akman SA, Okcu SC, Halicioglu O, Sutcuoglu S, Anil M, Kizilgunesler A, et al. Therapeutic efficacy of sequential and simultaneous treatments with interferon-alpha and lamivudine in children with chronic hepatitis B. Pediatr Int. 2007;49:848–52.
111. Jonas MM, Kelly D, Pollack H, Mizerski J, Sorbel J, Frederick D, et al. Safety, efficacy, and pharmacokinetics of adefovir dipivoxil in children and adolescents (age 2 to <18 years) with chronic hepatitis B. Hepatology. 2008;47:1863–71.
112. Marcellin P, Chang T-T, Lim SGL, Sievert W, Tong M, Arterburn S, et al. Long term efficacy and safety of adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. Hepatology. 2008;48:750–8.
113. Leung N, Peng C-Y, Hann H-W, Sollano J, Lao-Tan J, Hsu C-W, et al. Early hepatitis B virus DNA reduction in hepatitis B e antigen-positive patients with chronic hepatitis B: a randomized international study of entecavir versus adefovir. Hepatology. 2009;49:72–9.
114. Sherman M, Yurdaydin C, Simsek H, Silva M, Liaw Y-F, Rustgi VK, et al. Entecavir therapy for lamivudine-refractory chronic hepatitis B: improved virologic, biochemical, and serology outcomes through 96 weeks. Hepatology. 2008;48:99–108.
115. Novartis Pharmaceuticals. Pharmacokinetics and safety of single-dose telbivudine in children and adolescents with chronic hepatitis B. In: ClinicalTrials.gov [Internet]. Bethesda: National Library of Medicine (US); 2000 – [cited 18.09.11]. Available from: http://clinicaltrials.gov/show/NCT00907894. NLM Identifier: NCT00907894.
116. Marcellin P, Heathcote EJ, Buti M, Gane E, de Man RA, Krastev Z, et al. Tenofovir disoproxil fumarate versus adefovir dipivoxil for chronic hepatitis B. N Engl J Med. 2008;359:2442–55.
117. Gilead Sciences. Evaluation of tenofovir disoproxil fumarate in adolescents with chronic hepatitis B infection. In: ClinicalTrials.gov. Bethesda: National Library of Medicine (US); 2000. [cited 18.09.11]. http://clinicaltrials.gov/show/NCT00734162. NLM Identifier: NCT00734162.
118. Lau JY, King R, Tibbs CJ, et al. Loss of HBsAg with interferon-alpha therapy in chronic hepatitis D virus infection. J Med Virol. 1993;39:292.
119. Battegay M, Simpson LH, Hoofnagle JH, et al. Elimination of hepatitis delta virus infection after loss of hepatitis B surface antigen in patients with chronic delta hepatitis. J Med Virol. 1994;44:389.
120. Castelnau C, Le Gal F, Ripault MP, et al. Efficacy of peginterferon alpha-2b in chronic hepatitis delta: relevance of quantitative RT-PCR for follow-up. Hepatology. 2006;44:728.
121. Ferenci P, Formann E, Romeo R. Successful treatment of chronic hepatitis D with a short course of peginterferon alfa-2a. Am J Gastroenterol. 2005;100:1626.
122. Rosina F, Pintus C, Meschievitz C, Rizzetto M. A randomized controlled trial of a 12-month course of recombinant human interferon-alpha in chronic delta (type D) hepatitis: a multicenter Italian study. Hepatology. 1991;13:1052.
123. Niro GA, Ciancio A, Gaeta GB, et al. Pegylated interferon alpha-2b as monotherapy or in combination with ribavirin in chronic hepatitis delta. Hepatology. 2006;44:713.
124. Alter MJ. Epidemiology of hepatitis C. Hepatology. 1997;26(3 Suppl 1):62S–5.
125. Ohto H, Terazawa S, Sasaki N, et al. Transmission of hepatitis C virus from mothers to infants. The vertical transmission of hepatitis C virus collaborative study group. N Engl J Med. 1994;330:744–50.
126. Tajiri H, Miyoshi Y, Funada S, et al. Prospective study of mother-to-infant transmission of hepatitis C virus. Pediatr Infect Dis J. 2001;20:10–4.
127. Steininger C, Kundi M, Jatzko G, et al. Increased risk of mother-to infant transmission of hepatitis C virus by intrapartum infantile exposure to maternal blood. J Infect Dis. 2003;187:345–51.
128. Minola E, Maccabruni A, Pacati I, et al. Amniocentesis as a possible risk factor for mother-to-infant transmission of hepatitis C virus. Hepatology. 2001;33:1341–2.
129. Ducarme G, Ceccaldi PF, Bernuau J, et al. Amniocentesis and viral risk (hepatitis B, C virus and HIV). J Gynecol Obstet Biol Reprod (Paris). 2009;38:469–73.
130. Ferrero S, Lungaro P, Bruzzone BM, et al. Prospective study of mother-to-infant transmission of hepatitis C virus: a 10-year survey (1990–2000). Acta Obstet Gynecol Scand. 2003;82:229–34.
131. Simmonds P, Alberti A, Alter HJ, Bonino F, Bradley DW, Brechot C, Brouwer JT, Chan SW, Chayama K, Chen DS, et al. A proposed system for the nomenclature of hepatitis C viral genotypes. Hepatology. 1994;19(5):1321–4.
132. Simmonds P, Bukh J, Combet C, et al. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. Hepatology. 2005; 42:962.
133. Lau JY, Davis GL, Prescott LE, et al. Distribution of hepatitis C virus genotypes determined by line probe assay in patients with chronic hepatitis C seen at tertiary referral centers in the United States. Hepatitis interventional therapy group. Ann Intern Med. 1996;124:868.
134. Dusheiko G, Schmilovitz-Weiss H, Brown D, et al. Hepatitis C virus genotypes: an investigation of type-specific differences in geographic origin and disease. Hepatology. 1994;19:13.
135. Davis GL, Lau JY. Factors predictive of a beneficial response to therapy of hepatitis C. Hepatology. 1997;26:122S.
136. Martinot-Peignoux M, Marcellin P, Pouteau M, et al. Pretreatment serum hepatitis C virus RNA levels and

hepatitis C virus genotype are the main and independent prognostic factors of sustained response to interferon alfa therapy in chronic hepatitis C. Hepatology. 1995;22:1050.
137. Minola E, Prati D, Suter F, et al. Age at infection affects the long-term outcome of transfusion-associated chronic hepatitis C. Blood. 2002;99:4588.
138. Casiraghi MA, De Paschale M, Romanò L, et al. Long-term outcome (35 years) of hepatitis C after acquisition of infection through mini transfusions of blood given at birth. Hepatology. 2004;39:90.
139. Matsuoka S, Tatara K, Hayabuchi Y, et al. Serologic, virologic, and histologic characteristics of chronic phase hepatitis C virus disease in children infected by transfusion. Pediatrics. 1994;94:919.
140. Vogt M, Lang T, Frösner G, et al. Prevalence and clinical outcome of hepatitis C infection in children who underwent cardiac surgery before the implementation of blood-donor screening. N Engl J Med. 1999;341:866.
141. Tovo PA, Pembrey LJ, Newell ML. Persistence rate and progression of vertically acquired hepatitis C infection. European paediatric hepatitis C virus infection. J Infect Dis. 2000;181:419.
142. Mohan P, Colvin C, Glymph C, et al. Clinical spectrum and histopathologic features of chronic hepatitis C infection in children. J Pediatr. 2007;150:168.
143. Matsuoka S, Tatara K, Hayabuchi Y, et al. Post-transfusion chronic hepatitis C in children. J Paediatr Child Health. 1994;30:544.
144. European Paediatric Hepatitis C Virus Network. Three broad modalities in the natural history of vertically acquired hepatitis C virus infection. Clin Infect Dis. 2005;41:45.
145. Indolfi G, Resti M. Perinatal transmission of hepatitis C virus infection. J Med Virol. 2009;81:836.
146. Mack C, Gonzales-Peralta RP, Gupta N, Leund D, et al. NASPGHAN practice guidelines. Diagnosis and management of hepatitis C infection in infants, children and adolescents. J Pediatr Gastroenterol Nutr. 2012;54:838–55.
147. González-Peralta RP, Langham Jr MR, Andres JM, et al. Hepatocellular carcinoma in 2 young adolescents with chronic hepatitis C. J Pediatr Gastroenterol Nutr. 2009;48:630.
148. Gonzalez-Peralta RP, Kelly DA, Haber B, et al. International Pediatric Hepatitis C Therapy Group Interferon alfa-2b in combination with ribavirin for the treatment of chronic hepatitis C in children: efficacy, safety, and pharmacokinetics. Hepatology. 2005;42:1010–8.
149. Schwarz KB, Gonzalez-Peralta RP, Murray KF, et al. The combination of ribavirin and peginterferon is superior to peginterferon and placebo for children and adolescents with chronic hepatitis C. Gastroenterology. 2011;140:450–8.
150. Wirth S, Lang T, Gehring S, et al. Recombinant alfa-interferon plus ribavirin therapy in children and adolescents with chronic hepatitis C. Hepatology. 2002; 36:1280–4.
151. Wirth S, Pieper-Boustani H, Lang T, et al. Peginterferon alfa-2b plus ribavirin treatment in children and adolescents with chronic hepatitis C. Hepatology. 2005;41:1013–8.
152. Wirth S, Ribes-Koninckx C, Calzado MA, et al. High sustained virologic response rates in children with chronic hepatitis C receiving peginterferon alfa-2b plus ribavirin. J Hepatol. 2010;52:501–7.
153. Christensson B, Wiebe T, Akesson A, et al. Interferon-alpha and ribavirin treatment of hepatitis C in children with malignancy in remission. Clin Infect Dis. 2000;30:585–6.
154. Ghany MG, Strader DB, Thomas DL, et al. Diagnosis, management, and treatment of hepatitis C: an update. Hepatology. 2009;49:1335.
155. Muerhoff AS, Leary TP, Sathar MA, et al. African origin of GB virus C determined by phylogenetic analysis of a complete genotype 5 genome from South Africa. J Gen Virol. 2005;86(Pt 6):1729–35.
156. Schaluder GG, Dawson GJ, Simons JN, et al. Molecular and serologic analysis in the transmission of the GB hepatitis agents. J Med Virol. 1995;46: 81–90.
157. Tan D, Matsumoto A, Conry-Cantilena C, et al. Analysis of hepatitis G virus (HGV) RNA, antibody to HGV envelope protein, and risk factors for blood donors coinfected with HGV and hepatitis C virus. J Infect Dis. 1999;179:1055–61.
158. Alter MJ, Gallagher M, Morris TT, et al. Acute non-A-E hepatitis in the United States and the role of hepatitis G virus infection. Sentinel counties viral hepatitis study team. N Engl J Med. 1997;336: 741–6.
159. Tanaka E, Alter HJ, Nakatsuji Y, et al. Effect of hepatitis G virus infection on chronic hepatitis C. Ann Intern Med. 1996;125:740–3.
160. Kanda T, Yokosuka O, Imazeki F, et al. GB virus-C RNA in Japanese patients with hepatocellular carcinoma and cirrhosis. J Hepatol. 1997;27:464–9.
161. Simmonds P, Davidson F, Lycett C, et al. Detection of a novel DNA virus (TTV) in blood donors and blood products. Lancet. 1998;352:191–5.
162. Gimenez-Barcons M, Forns X, Ampurdanes S, et al. Infection with a novel human DNA virus (TTV) has no pathogenic significance in patients with liver diseases. J Hepatol. 1999;30:1028–34.
163. Rouphael NG, Talati NJ, Vaughan C, et al. Infections associated with haemophagocytic syndrome. Lancet Infect Dis. 2007;7:814–22.
164. Edwards JM, Vandervelde EM, Cohen BJ, McSwiggan DA. Laboratory diagnosis of EB virus infection in some cases presenting as hepatitis. J Clin Pathol. 1978;31:179–82.
165. van der Horst C, Joncas J, Ahronheim G, et al. Lack of effect of peroral acyclovir for the treatment of acute infectious mononucleosis. J Infect Dis. 1991;164:788–92.
166. Adams LA, Deboer B, Jeffrey G, et al. Ganciclovir and the treatment of Epstein-Barr virus hepatitis. J Gastroenterol Hepatol. 2006;21:1758–60.

167. Domiati-Saad R, Dawson DB, Margraf LR, et al. Cytomegalovirus and human herpesvirus 6, but not human papillomavirus, are present in neonatal giant cell hepatitis and extrahepatic biliary atresia. Pediatr Dev Pathol. 2000;3:367–73.
168. Squizzato A, Ageno W, Cattaneo A, Brumana N. A case report and literature review of portal vein thrombosis associated with cytomegalovirus infection in immunocompetent patients. Clin Infect Dis. 2007;44:e13–6.
169. Benhamou Y, Caumes E, Gerosa Y, et al. AIDS-related cholangiopathy. Critical analysis of a prospective series of 26 patients. Dig Dis Sci. 1993;38:1113–8.
170. Mahajani RV, Uzer MF. Cholangiopathy in HIV-infected patients. Clin Liver Dis. 1999;3:669–84.
171. Chatelain S, Neumann DE, Alexander SM. Fatal herpetic hepatitis in pregnancy. Infect Dis Obstet Gynecol. 1994;1:246–8.
172. Bissig KD, Zimmermann A, Bernasch D, et al. Herpes simplex virus hepatitis 4 years after liver transplantation. J Gastroenterol. 2003;38:1005–8.
173. Fink CG, Read SJ, Hopkin J, et al. Acute herpes hepatitis in pregnancy. J Clin Pathol. 1993;46:968–71.
174. Fehr T, Bossart W, Wahl C, Binswanger U. Disseminated varicella infection in adult renal allograft recipients: four cases and a review of the literature. Transplantation. 2002;73:608–11.
175. McNeil SA, Malani PN, Chenoweth CE, et al. Vancomycin-resistant enterococcal colonization and infection in liver transplant candidates and recipients: a prospective surveillance study. Clin Infect Dis. 2006;42:195.
176. Russell DL, Flood A, Zaroda TE, et al. Outcomes of colonization with MRSA and VRE among liver transplant candidates and recipients. Am J Transplant. 2008;8:1737.
177. Avery RK. Recipient screening prior to solid-organ transplantation. Clin Infect Dis. 2002;35:1513.
178. Collins LA, Samore MH, Roberts MS, et al. Risk factors for invasive fungal infections complicating orthotopic liver transplantation. J Infect Dis. 1994;170:644.
179. Castaldo P, Stratta RJ, Wood RP, et al. Clinical spectrum of fungal infections after orthotopic liver transplantation. Arch Surg. 1991;126:149.
180. Gladdy RA, Richardson SE, Davies HD, Superina RA. Candida infection in pediatric liver transplant recipients. Liver Transpl Surg. 1999;5:16.
181. Fishman JA, Rubin RH. Infection in organ-transplant recipients. N Engl J Med. 1998;338:1741.
182. Fishman JA. Pneumocystis carinii and parasitic infections in transplantation. Infect Dis Clin North Am. 1995;9:1005.
183. Martin SI, Fishman JA, AST Infectious Diseases Community of Practice. Pneumocystis pneumonia in solid organ transplant recipients. Am J Transplant. 2009;9 Suppl 4:S227.
184. Park JM, Lake KD, Arenas JD, Fontana RJ. Efficacy and safety of low-dose valganciclovir in the prevention of cytomegalovirus disease in adult liver transplant recipients. Liver Transpl. 2006;12:112.
185. Paya C, Humar A, Dominguez E, et al. Efficacy and safety of valganciclovir vs. oral ganciclovir for prevention of cytomegalovirus disease in solid organ transplant recipients. Am J Transplant. 2004;4:611.
186. Singh N, Dummer JS, Kusne S, et al. Infections with cytomegalovirus and other herpesviruses in 121 liver transplant recipients: transmission by donated organ and the effect of OKT3 antibodies. J Infect Dis. 1988;158:124.
187. Razonable RR. Cytomegalovirus infection after liver transplantation. Liver Transpl. 2010;16:S45.
188. Munoz-Price LS, Slifkin M, Ruthazer R, et al. The clinical impact of ganciclovir prophylaxis on the occurrence of bacteremia in orthotopic liver transplant recipients. Clin Infect Dis. 2004;39:1293.
189. George MJ, Snydman DR, Werner BG, et al. The independent role of cytomegalovirus as a risk factor for invasive fungal disease in orthotopic liver transplant recipients. Boston Center for Liver Transplantation CMVIG-Study Group. Cytogam, MedImmune, Inc. Gaithersburg, Maryland. Am J Med. 1997;103:106.
190. Falagas ME, Snydman DR, Griffith J, et al. Effect of cytomegalovirus infection status on first-year mortality rates among orthotopic liver transplant recipients. The Boston Center for Liver Transplantation CMVIG Study Group. Ann Intern Med. 1997;126:275.
191. Singh N, Paterson DL, Gayowski T, et al. Cytomegalovirus antigenemia directed pre-emptive prophylaxis with oral versus I.V. ganciclovir for the prevention of cytomegalovirus disease in liver transplant recipients: a randomized, controlled trial. Transplantation. 2000;70:717.
192. Husain S, Tollemar J, Dominguez EA, et al. Changes in the spectrum and risk factors for invasive candidiasis in liver transplant recipients: prospective, multicenter, case-controlled study. Transplantation. 2003;75:2023.
193. Pappas PG, Kauffman CA, Andes D, et al. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. Clin Infect Dis. 2009;48:503.
194. Reed A, Herndon JB, Ersoz N, et al. Effect of prophylaxis on fungal infection and costs for high-risk liver transplant recipients. Liver Transpl. 2007;13:1743.
195. Singh N, Wagener MM, Gayowski T. Safety and efficacy of isoniazid chemoprophylaxis administered during liver transplant candidacy for the prevention of posttransplant tuberculosis. Transplantation. 2002;74:892.
196. Albright JB, Bonatti H, Mendez J, et al. Early and late onset Clostridium difficile-associated colitis following liver transplantation. Transpl Int. 2007;20:856.
197. Riddle DJ, Dubberke ER. Clostridium difficile infection in solid organ transplant recipients. Curr Opin Organ Transplant. 2008;13:592.

198. Arthurs SK, Eid AJ, Pedersen RA, et al. Delayed-onset primary cytomegalovirus disease after liver transplantation. Liver Transpl. 2007;13:1703.
199. Singh N, Avery RK, Munoz P, et al. Trends in risk profiles for and mortality associated with invasive aspergillosis among liver transplant recipients. Clin Infect Dis. 2003;36:46.
200. Freifeld AG, Iwen PC, Lesiak BL, et al. Histoplasmosis in solid organ transplant recipients at a large Midwestern university transplant center. Transpl Infect Dis. 2005;7:109.
201. Peetermans WE, Endtz HP, Janssens AR, van den Broek PJ. Recurrent Listeria monocytogenes bacteraemia in a liver transplant patient. Infection. 1990;18:107.
202. Fishman JA, AST Infectious Diseases Community of Practice. Introduction: infection in solid organ transplant recipients. Am J Transplant. 2009;9 Suppl 4:S3.

Key References

Aggarwal R, Krawcyznski K. Sleisenger and Fordtran's gastrointestinal and liver disease, pathophysiology/diagnosis/management. 2013;1337–42.
Feld JJ, Heathcote EJ. Hepatitis caused by other viruses. Sleisenger and Fordtran's gastrointestinal and liver disease, pathophysiology/diagnosis/management. 2013;1343–50.
Kim AY, Chung RT. Bacterial, parasitic and fungal infections of the liver, including liver abscess. Sleisenger and Fordtran's gastrointestinal and liver disease, pathophysiology/diagnosis/management. 2013;1351–69.
Sjogren MH, Cheatham JG, Hepatitis A. Sleisenger and Fordtran's gastrointestinal and liver disease, pathophysiology/diagnosis/management. 2013;1279–85.

Autoimmune Hepatitis and Sclerosing Cholangitis

16

Giorgina Mieli-Vergani and Diego Vergani

Introduction

Autoimmune hepatitis (AIH) and sclerosing cholangitis are major causes of liver disease in children and adolescents and should always be considered in the differential diagnosis of childhood hepatopathies. Both conditions have clinical and laboratory features, response to treatment and outcome different from their adult counterparts. A common form of sclerosing cholangitis in childhood has strong autoimmune features and is referred to as autoimmune sclerosing cholangitis (ASC). The presentation of AIH and sclerosing cholangitis is nonspecific and can mimic most other liver disorders. As prompt treatment, particularly in AIH, is life saving, it is imperative to suspect these conditions and perform appropriate investigations in all children who present with a cryptogenic liver disorder.

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Autoimmune Hepatitis

AIH is a progressive inflammatory liver disorder characterized serologically by high levels of transaminases and immunoglobulin G (IgG), and presence of autoantibodies, and histologically by interface hepatitis (Fig. 16.1a), in the absence of a known etiology [1]. In children and adolescents, AIH often presents acutely and has a more aggressive course than in adults. AIH usually responds satisfactorily to immunosuppressive treatment, even when it presents with features of acute liver failure [2]. If left untreated, it progresses rapidly to cirrhosis and liver failure. Seventy-five percent of patients are girls.

Two types of AIH are recognized: AIH type 1 (AIH-1), which also affects adults, is characterized by the presence of smooth muscle antibody (SMA) and/or antinuclear antibodies (ANA); AIH type 2 (AIH-2), which is mainly a pediatric condition, is positive for antibodies to liver-kidney microsomal type 1 (anti-LKM-1) [3] and/or anti-liver cytosol type 1 (anti-LC1) [4, 5].

AIH-1 accounts for two thirds of the cases and presents often around puberty, whereas AIH-2 tends to present at a younger age and also during infancy. IgG is usually raised at disease onset in both types, though 15 % of children with AIH-1 and 25 % of those with AIH-2 have normal levels. IgA deficiency is common in AIH-2 [3]. Severity of disease is similar in the two types, but anti-LKM-1-positive children have higher levels of bilirubin and transaminases at onset than those who are ANA/SMA positive and

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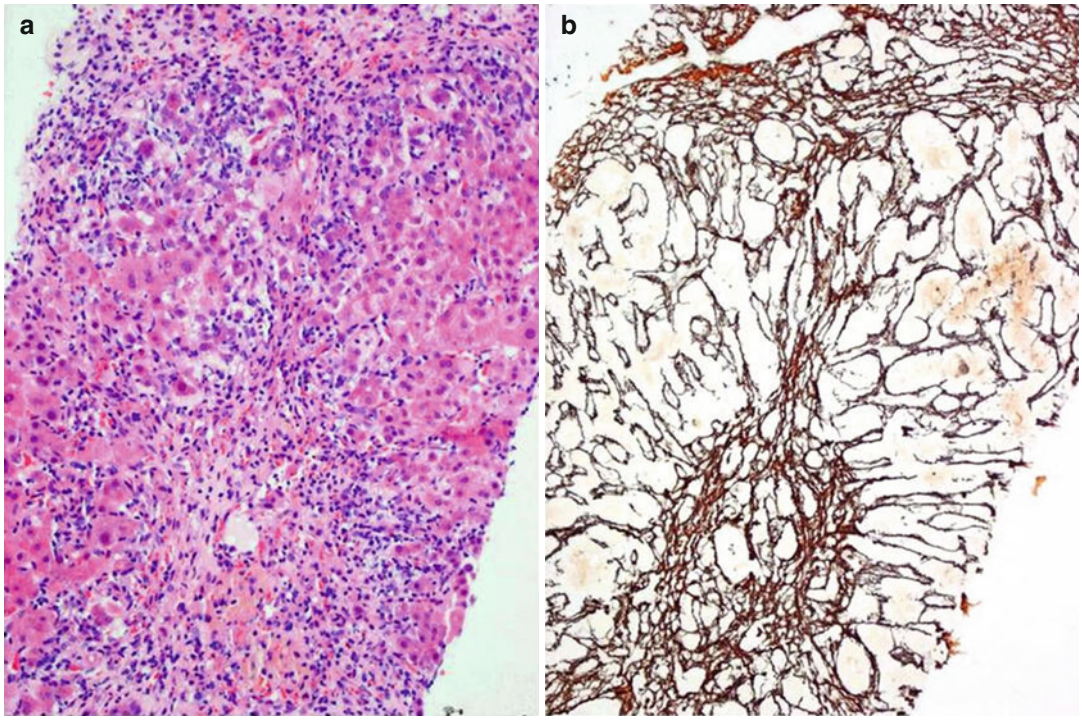

Fig. 16.1 Panel (**a**): portal and periportal lymphocyte and plasma cell infiltrate, extending to and disrupting the parenchymal limiting plate (interface hepatitis). Swollen hepatocytes, pyknotic necroses, and acinar inflammation are present. Hematoxylin and eosin staining. Panel (**b**): bridging collapse of the hepatic stroma following hepatocellular necrosis. Reticulin staining (Pictures kindly provided by Dr Alberto Quaglia)

present significantly more frequently with fulminant hepatic failure [3]. 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), which are present in approximately half of the patients with AIH-1 or AIH-2 at diagnosis (Table 16.1) [7, 9]. In both types, 20 % of patients have associated autoimmune disorders—including thyroiditis, vitiligo, type 1 diabetes, inflammatory bowel disease (IBD), and nephrotic syndrome—and 40 % have a family history of autoimmune disease (Table 16.1) [3].

There are three clinical patterns of AIH presentation [3]: (a) in at least 40 % of patients, the presentation is indistinguishable from that of an acute viral hepatitis (nonspecific symptoms of malaise, nausea/vomiting, anorexia, and abdominal pain, followed by jaundice, dark urine, and pale stools). Some children, particularly those who are anti-LKM-1 positive, develop acute hepatic failure with grade II to IV hepatic encephalopathy (fulminant hepatitis) within 2–8 weeks from onset of symptoms. (b) In 25–40 % of patients, the onset is insidious, with an illness characterized by progressive fatigue, relapsing jaundice, headache, anorexia, amenorrhea, and weight loss, lasting for several months and even years before diagnosis. (c) In about 10 % of patients, there is no history of jaundice, and the diagnosis follows presentation with complications of portal hypertension, such as

Table 16.1 Clinical, immunological, and histological features at presentation of autoimmune hepatitis type 1 (AIH-1), autoimmune hepatitis type 2 (AIH-2), and autoimmune sclerosing cholangitis (ASC) among patients referred to the King's College Hospital Tertiary Paediatric Liver Centre [3, 6]

	AIH-1	AIH-2	ASC
Median age in years	11	7	12
Females (%)	75	75	55
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 immune diseases (%)	22	20	48
IBD (%)	20	12	44
Family history of autoimmune disease (%)	43	40	37
Bile duct changes on cholangiography (%)	0	0	100
ANA/SMA (%)	100	25	96
Anti-LKM-1 (%)	0	100	4
pANNA (%)	45	11	74
Anti-SLA (%)[a]	58	58	41
Increased IgG level (%)	84	75	89
Partial IgA deficiency (%)	9	45	5
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
Histology			
Interface hepatitis (%)	92	94	60
Biliary features (%)	28	6	35

IBD inflammatory bowel disease, *ANA* antinuclear antibodies, *SMA* anti-smooth muscle antibody, *anti-LKM-1* anti-liver-kidney microsomal type 1 antibody, *pANNA* peripheral antinuclear neutrophil antibody, *anti-SLA* anti-soluble liver antigen antibody, *IgG* immunoglobulin G, *IgA* immunoglobulin A, *C4* C4 component of complement, *HLA* human leukocyte antigen

[a]Measured by radioligand assay [7]

[b]But increased in HLA *DR*0701* negative patients [8]

splenomegaly, hematemesis from esophageal varices, bleeding diathesis, chronic diarrhea, and weight loss.

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. The majority of children, however, on physical examination have clinical signs of an underlying chronic liver disease, including cutaneous stigmata (spider naevi, palmar erythema, leukonychia, striae), firm liver, and splenomegaly. At ultrasound, the liver parenchyma of these patients is often nodular and heterogeneous.

Epidemiology and Genetic Predisposition

The epidemiology of childhood AIH has not been studied. Data collected at the King's College Hospital Pediatric Hepatology tertiary referral center show an increase in the yearly incidence of juvenile autoimmune liver disease, only partially explained by a referral bias: In the 1990s, it represented 2.3 % of 400 children older than 4 months who were newly referred yearly; since 2000, the yearly incidence has increased to 12 %.

In northern Europe, pediatric AIH-1, similar to adult AIH, is associated with the possession of the human leukocyte antigen (HLA) *DRB1*03* [3, 10]. In contrast to adult patients, possession of *DRB1*04* does not predispose to AIH in childhood and can even exert a protective role [3]. AIH-2 is associated with possession of *DRB1*07* [8, 11] and, in DR7 negative patients, with possession of *DRB1*03* [8]. In Egypt, AIH-2 appears to be associated also with possession of *HLA-DRB1*15* [11]. 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 [11, 12]. 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 [13, 14]. 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 [15].

AIH-2 can be part of the autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) syndrome, an autosomal recessive monogenic disorder [16, 17] in which the liver disease is reportedly present in over 20 % of cases [18, 19].

Diagnosis

The diagnosis of AIH is based on a series of inclusion and exclusion criteria [20, 21]. Liver biopsy is necessary to establish the diagnosis, the typical histological picture including a dense mononuclear and plasma cell infiltration of the portal areas, which expands into the liver lobule; destruction of the hepatocytes at the periphery of the lobule with erosion of the limiting plate ("interface hepatitis") (Fig. 16.1a); connective tissue collapse resulting from hepatocyte death and expanding from the portal area into the lobule ("bridging collapse") (Fig. 16.1b); and hepatic regeneration with "rosette" formation. In addition to the typical histology, other positive criteria include elevated serum transaminase and IgG levels and presence of ANA, SMA, or anti-LKM-1.

The diagnosis of AIH has been advanced by the scoring systems developed by the International Autoimmune Hepatitis Group (IAIHG) for adult patients [20, 21] where negative criteria such as evidence of infection with hepatitis B or C virus, Wilson disease, or alcohol, 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 has published a simplified scoring system based on autoantibodies, IgG, histology, and exclusion of viral hepatitis that is better suited to clinical application [22]. 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 [23–25]. In addition, neither system can distinguish between AIH and ASC (see below) [6, 26], which can only be differentiated if a cholangiogram is performed at presentation.

A key diagnostic criterion for all AIH scoring systems is the detection of autoantibodies (ANA, SMA, and anti-LKM-1), which not only assists in the diagnosis, but also allows differentiation of AIH types. ANA and SMA that characterize AIH-1 and anti-LKM-1 that defines AIH-2 are practically mutually exclusive; in those rare instances when they are present simultaneously, the clinical course is similar to that of AIH-2 [27]. ANA, SMA, and anti-LKM-1 should be sought by indirect immunofluorescence using rodent stomach, kidney, and liver as substrate, as other techniques, e.g., commercially available ELISAs, remain to be fully validated [27]. 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. Positivity for autoantibodies, however, 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 [28, 29], Wilson disease [30], and nonalcoholic steatohepatitis [31].

Other autoantibodies less commonly tested but of diagnostic importance include anti-liver cytosol type 1 (LC-1), peripheral antinuclear neutrophil antibody (atypical pANCA or pANNA), and anti-SLA. Anti-LC-1, detected by indirect immunofluorescence, can be present on its own, but frequently occurs in association with anti-LKM-1, and is an additional marker for AIH-2 [5, 32]. 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 [33], is also found in some 50 % of patients with AIH-1, AIH-2, and ASC, where it defines a more severe course [7]. Anti-SLA is not detectable by immunofluorescence, but the definition of its molecular target as UGA transfer RNA (tRNA) suppressor-associated antigenic protein (SepSecS) [34, 35] has enabled the establishment of molecularly based diagnostic assays. However, it should be noted that commercial

enzyme-linked immunosorbent assays (ELISAs) are less sensitive than radioligand assays available in research laboratories [7, 9].

There is a small proportion of patients with AIH without detectable autoantibodies. This condition, which responds to immunosuppression like the seropositive form, represents seronegative AIH [36], a rare type of AIH in adults, whose prevalence and clinical characteristics remain to be defined in children.

Treatment

Definition of Remission/Relapse

Remission is defined as clinical recovery, normal transaminase and IgG levels, negative or very low-titer autoantibodies by immunofluorescence (≤1:20 for ANA and SMA; ≤1:10 for anti-LKM-1), and histological resolution of inflammation. The histological response lags behind the biochemical response [37], and clinical/biochemical remission does not necessarily reflect histological resolution. After a mean duration of 4 years of treatment, improvement of the intensity of portal inflammation is observed in up to 95 % of AIH cases and is accompanied by an improvement of fibrosis scores [37]. Relapse is characterized by an increase of serum aminotransferase levels above normal values after remission has been achieved. Relapse during treatment is common, occurring in about 40 % of patients and requiring a temporary increase in the steroid dose [3]. An important role in relapse is played by nonadherence, particularly in adolescents [38]. 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 belief that it has a less negative effect on the child's growth. Small daily doses are more effective in maintaining disease control and minimize the need for high-dose steroid pulses during relapses (with consequent more severe side effects) and do not affect final height [39].

When to Treat

AIH should be suspected and sought in all children with evidence of liver disease after exclusion of infectious and metabolic etiologies. With the exception of a fulminant presentation with encephalopathy, where liver transplant is usually required, AIH responds satisfactorily to immunosuppressive treatment whatever the degree of liver impairment, with a reported remission rate exceeding 80 % [3, 6, 40, 41]. Treatment should be initiated promptly to avoid progression of disease.

The goals of treatment are to reduce or eliminate liver inflammation, to induce remission, to improve symptoms, and to prolong survival [42]. The rapidity and degree of the response depend on the disease severity at presentation. Though cirrhosis is found in between 44 and 80 % of children at the time at diagnosis, [3, 43] development of end-stage liver disease requiring liver transplantation is rare, most children remaining clinically stable, with a good quality of life on long-term treatment.

How to Treat

Standard Treatment

Conventional treatment of AIH consists of an initial dose of prednisolone (or prednisone) of 2 mg/kg/day (maximum 60 mg/day), which is gradually decreased over a period of 4–8 weeks, in parallel to the decline of transaminase levels, to a maintenance dose of 2.5–5 mg/day, depending on the child's age and weight [42, 44]. In most patients an 80 % decrease of the aminotransferase levels is achieved in the first 2 months, but their complete normalization may take several months [3]. During the first 6–8 weeks of treatment, liver function tests should be checked often to allow weekly dose adjustments, avoiding severe steroid side effects. In our center, azathioprine is added as a steroid-sparing agent if the transaminase levels stop decreasing on steroid treatment alone or in the presence of early serious steroid side effects (e.g., psychosis), at a starting dose of 0.5 mg/kg/day, which in the absence of signs of toxicity is increased up to a maximum of 2.0–2.5 mg/kg/day until biochemical control is achieved. The timing for the addition of azathioprine varies in different centers. In some centers, azathioprine is added in all cases at a dose of 0.5–2 mg/kg/day after a few weeks of steroid treatment. Other centers use a combination of steroids and azathioprine from the

beginning, but caution is recommended because azathioprine can be hepatotoxic, particularly in severely jaundiced patients. Whatever the protocol, 85 % of the patients eventually require the addition of azathioprine.

Measurement of thiopurine methyltransferase activity level before initiating azathioprine therapy has been advocated to predict azathioprine toxicity. However, only patients with near-zero erythrocyte concentrations of thiopurine methyltransferase activity are at risk for myelosuppression during azathioprine treatment [45], and determination of the enzyme activity is warranted only when there is pre- or intra-treatment cytopenia, or the need of higher than conventional doses [46]. Measurement of the azathioprine metabolites 6-thioguanine 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 [47], though an ideal therapeutic level for AIH has not been determined.

Alternative Treatments

Induction of remission has been obtained in treatment-naïve children using cyclosporine A alone for 6 months, followed by the addition of prednisone and azathioprine. One month later the cyclosporine was discontinued [40, 41]. Cyclosporine was used at the dose of 4 mg/kg/day in three divided doses, increased if necessary every 2–3 days to achieve a whole blood concentration of 250 ± 50 ng/ml for 3 months. If there was clinical and biochemical response in the first months, cyclosporine was reduced to achieve a concentration of 200 ± 50 ng/ml for the following 3 months, before discontinuing it. Whether this mode of induction has any advantage over the standard treatment has yet to be evaluated in controlled studies.

Tacrolimus is a more potent immunosuppressive agent than cyclosporine, but it also has significant toxicity. There is limited evidence supporting its role as initial treatment of AIH apart from anecdotal reports in adults.

Budesonide has a hepatic first-pass clearance of >90 % of oral dose and fewer side effects than predniso(lo)ne, but cannot be used in cirrhotic patients, who represent a large proportion of AIH patients. In a large European study, a combination of budesonide and azathioprine had fewer adverse effects compared to medium-dose standard prednisone and azathioprine [48]. In this 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]. However, the results within the paediatric cohort of this study are disappointing, with similarly low remission rates in the budesonide/azthioprine and prednisone/azathioprine arms (16 % and 15 % after 6 months of treatment and 50 % and 42 % after 12 months of treatment respectively) [49]. The poor response rate to prednisolone/azathioprine in this study compared to that observed with standard treatment (80–90 %) is likely to depend on the low fixed initial dose of prednisone, decreased by protocol and not according to response, used in the trial [50]. Nevertheless, budesonide could be a valid alternative in selected non-cirrhotic patients who are at risk of adverse effects from steroids.

Maintenance with azathioprine monotherapy has been advocated once remission is achieved [51], but whether this is effective long term and whether it offers any benefit on possible side effects compared to low-dose prednisolone/azathioprine maintenance are unclear.

Treatment of Refractory Cases

Mycophenolate mofetil (MMF) is the prodrug of mycophenolic acid. Its effect on purine synthesis leads to decreased T- and B-lymphocyte proliferation. In patients (up to 10 %) in whom standard immunosuppression is unable to induce stable remission, or who are intolerant to azathioprine, MMF at a dose of 20 mg/kg twice daily (total daily dose 40 mg/kg), together with predniso(lo)ne, is successfully used [52]. If there is a persistent lack of response or if there is intolerance for MMF (headache, diarrhea, nausea, dizziness, hair loss, and neutropenia), the use of calcineurin inhibitors should be considered. In our center, tacrolimus, in combination with prednisolone,

has been successful in inducing remission in difficult-to-treat patients.

Duration of Treatment and Prognosis

The optimal duration of immunosuppressive treatment for AIH is unknown. Treatment withdrawal is successful only if there is histological resolution of inflammation. Hence, cessation of treatment should be considered if a liver biopsy shows minimal or no inflammatory changes after 1–2 years of normal liver function tests, normal IgG levels, and negative or low-titer autoantibodies. However, it is advisable not to attempt to withdraw treatment within 3 years of diagnosis or during or immediately before puberty, when relapses are more common. It has been reported that 20 % of patients with AIH-1 can successfully and permanently stop treatment, while this is rarely achieved in AIH-2 [3]. Long-term treatment is required for the majority of patients, and parents and patients should be counselled accordingly. In the pediatric setting, an important role in monitoring the response to treatment is the measurement of IgG levels and autoantibody titers, the fluctuation of which correlates with disease activity [53]. In particular, for patients with high IgG levels, their decrease is a reliable, objective, and inexpensive measure of disease control.

The prognosis of those children with AIH who respond to immunosuppressive treatment is generally good, with most patients surviving long term with excellent quality of life on low-dose medication. Development of end-stage liver disease requiring liver transplantation despite treatment, however, has been reported 8–14 years after diagnosis in 8.5 % of children with AIH [3].

Sclerosing Cholangitis

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 [6, 54–57] there are well-defined forms of sclerosing cholangitis. In the neonatal period, pathological features of severe sclerosing cholangitis characterize biliary atresia as well as neonatal sclerosing cholangitis (NSC), a condition inherited in an autosomal recessive manner [58]. 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 [59, 60]; moreover 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 anaemia. Moreover, an overlap syndrome between AIH and sclerosing cholangitis, ASC, is significantly more common in children than in adults. In only a relatively small number of pediatric patients, sclerosing cholangitis occurs without any of the above defining features. The term of PSC should be confined to the latter.

With the increased usage of biliary imaging in the form of endoscopic retrograde cholangiopancreatography (ERCP), percutaneous cholangiography, and, more recently, noninvasive magnetic resonance cholangiography (MRCP), sclerosing cholangitis is diagnosed with increasing frequency in pediatric age and is an important cause of morbidity and mortality, accounting for some 2 % of the pediatric liver transplants in the USA between 1988 and 2008 [United Network for Organ Sharing (UNOS) Data Report—October 2009. http://www.unos.org/data/].

There are five relatively large studies of sclerosing cholangitis in childhood [6, 54–57] describing a total of 236 cases. In these reports the incidence of the various clinical forms of sclerosing cholangitis differs depending upon the year when and the center where the study was conducted, reflecting different study design, patterns of referral, and diagnostic protocols (Table 16.2). In four of these series, cholangiographic studies, performed by ERCP, percutaneous cholangiography, or, more recently, MRCP, were prompted by biochemical and/or histological features of cholestatic disease [53–56]. Interestingly, in the most recent series [56], where cholangiographic studies were mainly performed by MRCP, no radiological biliary involvement was detected, despite histological evidence of sclerosing cholangitis, in a high proportion

Table 16.2 Comparison of the different forms of sclerosing cholangitis in five published pediatric series

	Debray et al. [54]	Wilschanski et al. [55]	Gregorio et al. [6]	Feldstein et al. [56]	Miloh et al. [57]
Total number of patients	56	32	49	52	47
Immunodeficiency	8 (14 %)	2 (6 %)	6 (12 %)	0	0
Langerhans cell histiocytosis	14 (25 %)	0	2 (4 %)	0	0
Neonatal SC	15 (27 %)	0	5 (10 %)	0	0
Psoriasis	1 (2 %)	0	0	0	0
PSC	10 (18 %)	10 (31 %)	9 (18 %)	38 (73 %)	35 (75 %)
AIH/SC overlap	2 (4 %)	9 (28 %)	27 (55 %)	14 (27 %)	12 (25 %)
IBD	7 (13 %)	17 (53 %)	15 (31 %)	42 (81 %)	28 (59 %)
Ulcerative colitis	4	14	8	30	20
Crohn disease	3	3	3	8	8
Indeterminate colitis			4	4	

SC sclerosing cholangitis, *AIH* autoimmune hepatitis, *IBD* inflammatory bowel disease

(36 %) of patients ("small-duct PSC"). Whether this finding is due to a lower sensitivity of the MRCP compared to the ERCP in detecting biliary changes remains to be verified.

Our own study, published in 2001, differs from all the other series, as it was prospective and aimed at establishing the relative incidence of AIH and AIH/sclerosing cholangitis overlap syndrome (autoimmune sclerosing cholangitis, ASC) among children presenting with liver disease and positive autoimmune serology (autoantibodies; increased levels of IgG) [6], by performing cholangiograms at disease onset, irrespective of biochemical or histological evidence of cholestatic disease. Other forms of sclerosing cholangitis seen over the same period of observation were excluded from the prospective study.

In all published series, boys are more affected than girls, 20–40 % of patients have intrahepatic cholangiopathy with normal extrahepatic bile ducts, and IBD is strongly associated with the diagnosis of sclerosing cholangitis, being found in some 63 % overall [6, 54–57]. More than two thirds of the cases had ulcerative colitis. The prevalence of IBD was higher in those centers where surveillance enteroscopy was performed and 23 % of the cases presented after the diagnosis of sclerosing cholangitis and even in the absence of clinical symptoms of IBD. It is, therefore, advisable to consider diagnostic colonoscopy in children who are newly diagnosed with sclerosing cholangitis and to have a low threshold for performing this procedure in those who have symptoms consistent with IBD (e.g., diarrhea, growth failure, anemia).

Autoimmune Sclerosing Cholangitis

In all the pediatric series described above, sclerosing cholangitis is often associated with florid autoimmune features, including elevated titers of autoantibodies, in particular ANA and SMA; elevated IgG levels; and interface hepatitis (Table 16.1 and Fig. 16.2a) [6, 54–57]. Whether these children respond to immunosuppressive treatment and whether their prognosis is different from that of children with AIH is controversial. In an attempt to clarify this, the King's prospective study was initiated in 1984 and conducted over a period of 16 years [6]. Interim results were published in 2001, but the patient cohort is being followed up to date. In this study, all children with serological (i.e., positive autoantibodies, high IgG levels) and histological (i.e., interface hepatitis) features of autoimmune liver disease underwent a cholangiogram at the time of presentation, independently from the presence of biochemical or histological evidence of cholestasis. Surveillance enteroscopy to investigate for possible IBD was

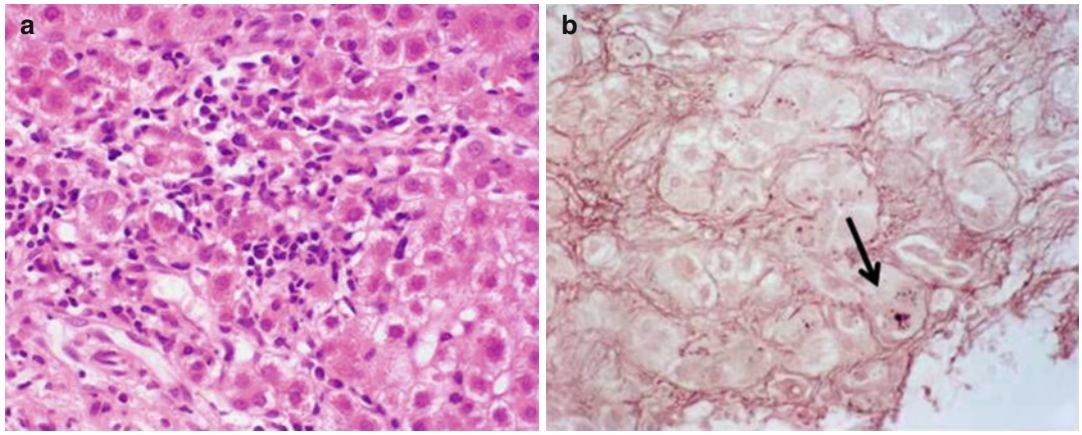

Fig. 16.2 Panel (**a**): portal plasma cell infiltrate in a child with autoimmune sclerosing cholangitis. Hematoxylin and eosin staining. Panel (**b**): orcein staining of the same biopsy shows copper-associated protein deposition (*arrow*) suggesting chronic cholestasis (Pictures kindly provided by Dr Yoh Zen)

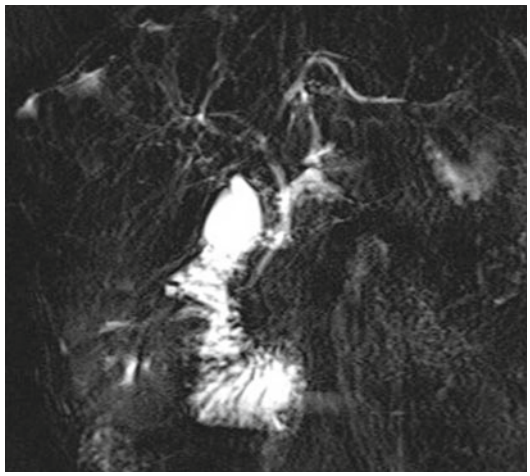

Fig. 16.3 Magnetic resonance cholangiography of a child with autoimmune sclerosing cholangitis showing a diffuse cholangiopathy with ductal changes in both lobes. The extrahepatic bile ducts have normal appearance

performed in all cases, independently from symptoms. Approximately 50 % of the patients enrolled in this prospective study had alterations of the bile ducts characteristic of sclerosing cholangitis, although they were generally less advanced than those observed in adult PSC (Fig. 16.3) and were diagnosed as having ASC. A quarter of the children with ASC, despite abnormal cholangiograms, had no histological features that suggested bile duct involvement, and the diagnosis of sclerosing cholangitis was only possible because of the cholangiographic studies. Virtually all ASC patients were seropositive for ANA and/or SMA. In contrast to AIH, which had a clear female preponderance, ASC was diagnosed in a similar proportion of boys and girls. The mode of presentation of ASC was similar to that of AIH-1. Inflammatory bowel disease was present in 45 % of children with ASC compared to 20 % of those with typical AIH, and 90 % of children with ASC had greatly increased serum IgG levels. At the time of presentation, standard liver function tests did not help in discriminating between AIH and ASC, although the alkaline phosphatase/aspartate aminotransferase ratio was significantly higher in ASC (Table 16.3). pANNA was present in 74 % of patients with ASC compared with 45 % of patients with AIH-1 and 11 % of those with AIH-2. Anti-SLA was found in some 50 % of patients with ASC, and also in this condition it defines a more severe disease course [7]. Evolution from AIH to ASC was documented in one patient during the published prospective series [6] and has been observed in two further patients during follow-up [62], suggesting that AIH and ASC are part of the same pathogenic process.

Clinical, laboratory, and histological features of types 1 and 2 AIH and ASC are compared in Tables 16.1 and 16.3.

Currently, in our center imaging of the biliary system by MRCP, followed by ERCP if MRCP is

Table 16.3 Biochemical indices at presentation in children with autoimmune hepatitis (AIH) and autoimmune sclerosing cholangitis (ASC) referred to the King's College Hospital Tertiary Paediatric Liver Centre [3, 6]

	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 (24–4,830)	102 (18–1,215)
INR (<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 (104–1,710)
AP/AST ratio	1.14 (0.05–14.75)	3.96 (0.20–14.20)

Modified from [61]
AST aspartate aminotransferase, *INR* international normalized prothrombin ratio, *GGT* gamma glutamyl transpeptidase, *AP* alkaline phosphatase, *nv* normal values

Table 16.4 Response to treatment and outcome in patients with autoimmune hepatitis type 1 (AIH-1), autoimmune hepatitis type 2 (AIH-2), and autoimmune sclerosing cholangitis (ASC) treated at the King's College Hospital Tertiary Paediatric Liver Centre [3, 6, 62]

	AIH-1	AIH-2	ASC
Remission rate (%)	97	87	89
Median time to remission (months)	6	9	2
Relapse rate (%)	42	46	45
Cessation of treatment (%)	19	0	5
Liver transplant rate (%)	6	13	23
Disease recurrence posttransplant (%)	0	0	67

Modified from [61]

not informative, as well as colonoscopy is part of the evaluation of all children with liver disease associated with autoimmune features.

The IAIHG scoring systems for the diagnosis of AIH, as currently formulated, do not distinguish AIH from ASC [6, 26], as they do not include cholangiographic investigations at presentation.

HLA studies have shown that in the UK susceptibility to ASC is conferred by the possession of HLA *DRB1*1301* [13].

Treatment and Prognosis

Treatment and prognosis of sclerosing cholangitis depends on the underlying pathology. Management of sclerosing cholangitis associated to immunodeficiency syndromes, LCH, or metabolic/genetic disorders is closely related to the ability of controlling the primary disease. For sclerosing cholangitis without associated pathologies, no standard mode of treatment is presently advocated [63]. Based on a reported beneficial effect in adult PSC, ursodeoxycholic acid (UDCA) is used also for the treatment of childhood sclerosing cholangitis, but whether it is helpful in arresting the progression of the bile duct disease remains to be established. In adults with PSC, high-dose UDCA was reported as more beneficial than standard doses [64], but a randomized double-blind controlled study from the Mayo Clinic shows that high-dose UDCA has a negative effect [65]. It is prudent, therefore, to use doses not higher than 15–20 mg/kg/day.

A beneficial effect of oral vancomycin (500 mg tds) has been reported in 14 patients with sclerosing cholangitis and IBD [66]. All patients showed improvement of liver function tests and erythrocyte sedimentation rate, which was more marked in those without cirrhosis. These results await confirmation in a larger number of patients. Whether vancomycin acts through its antibiotic or immunomodulatory [67] properties remains to be elucidated.

The King's prospective study shows that ASC responds well to the same immunosuppressive treatment described above for AIH if started early, with resolution of liver test abnormalities within a few months in most patients (Table 16.4), but 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 20 % of them eventually requiring liver transplantation (Table 16.4) [6, 62]. Similarly, in the series by Miloh et al [57], though all patients with overlap AIH/sclerosing cholangitis syndrome were reported to have a favorable biochemical response to immunosuppression and UDCA treatment, 25 % required liver transplantation during the 12-year observation period. Response to immunosuppressive drugs

was less satisfactory in sclerosing cholangitis patients with autoimmune features described by Wilschanski et al. [55] and Feldstein et al. [56], possibly because of long-standing liver disease before starting treatment.

Reactivation of the liver disease often follows flares of the intestinal disease in sclerosing cholangitis patients with IBD. It is therefore essential to control efficiently the bowel disease to avoid progression of liver disease.

Liver Transplantation

Liver transplantation is indicated in patients with AIH who present with fulminant hepatic failure (with encephalopathy) 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 (Table 16.4). After transplantation, recurrent AIH has been described in about 20 % of cases [68] and recurrent sclerosing cholangitis in 27 % of transplanted patients in Feldstein's series [56], but in as many as 67 % of the patients with ASC followed up prospectively at King's [62]. 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 generally recommended. While recurrence of AIH does not usually affect posttransplant outcome, recurrence of ASC leads to retransplantation in a high proportion of patients [62]. Recurrence of sclerosing cholangitis after transplantation appears to be associated to uncontrolled IBD [69]. In this context it is of interest that PSC recurrence in adults with IBD can be prevented by pre-liver transplant colectomy [70–72].

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 had not been transplanted for autoimmune liver disease. The 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 [73], de novo AIH after liver transplant has been confirmed by several studies both in adult and pediatric patients [74, 75] [76]. 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 graft and patient survival. It is of interest that these patients 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 [77].

Conclusion

Over the past two decades, there has been a sharp increase in the diagnosis of both AIH and sclerosing cholangitis in children. Whether this is due to a real increase in prevalence or to an increased awareness of these conditions remains to be clarified. If diagnosed and treated early, AIH has an excellent prognosis, with only a minority of the children who achieve remission with immunosuppression requiring liver transplantation 10–20 years after presentation. The prognosis is worse in patients with sclerosing cholangitis, in whom a higher proportion requires transplantation medium term and in whom the risk of disease recurrence after transplant is very high, particularly for those who have strong autoimmune features and associated inflammatory bowel disease. A better understanding of the pathogenic mechanisms leading to AIH and sclerosing cholangitis will hopefully lead to a targeted, more efficient, and less toxic therapeutic approach.

References

1. Mieli-Vergani G, Vergani D. Autoimmune hepatitis. Nat Rev Gastroenterol Hepatol. 2011;8:320–9.
2. Cuarterolo ML, Ciocca ME, Lopez SI, de Davila MT, Alvarez F. Immunosuppressive therapy allows recovery from liver failure in children with autoimmune hepatitis. Clin Gastroenterol Hepatol. 2011;9:145–9.
3. Gregorio GV, et al. Autoimmune hepatitis in childhood: a 20-year experience. Hepatology. 1997;25:541–7.
4. Martini E, et al. Antibody to liver cytosol (anti-LC1) in patients with autoimmune chronic active hepatitis type 2. Hepatology. 1988;8:1662–6.
5. Bridoux-Henno L, et al. Features and outcome of autoimmune hepatitis type 2 presenting with isolated positivity for anti-liver cytosol antibody. Clin Gastroenterol Hepatol. 2004;2:825–30.
6. Gregorio GV, et al. Autoimmune hepatitis/sclerosing cholangitis overlap syndrome in childhood: a 16-year prospective study. Hepatology. 2001;33:544–53.
7. Ma Y, et al. Antibodies to conformational epitopes of soluble liver antigen define a severe form of autoimmune liver disease. Hepatology. 2002;35:658–64.
8. Ma Y, et al. Polyclonal T-cell responses to cytochrome P450IID6 are associated with disease activity in autoimmune hepatitis type 2. Gastroenterology. 2006;130:868–82.
9. Vitozzi S, Djilali-Saiah I, Lapierre P, Alvarez F. Anti-soluble liver antigen/liver-pancreas (SLA/LP) antibodies in pediatric patients with autoimmune hepatitis. Autoimmunity. 2002;35:485–92.
10. Donaldson P. Genetics in autoimmune hepatitis. Semin Liver Dis. 2002;22:353–64.
11. Elfaramawy AA, Elhossiny RM, Abbas AA, Aziz HM. HLA-DRB1 as a risk factor in children with autoimmune hepatitis and its relation to hepatitis A infection. Ital J Pediatr. 2010;36:73.
12. Oliveira LC, et al. Autoimmune hepatitis. HLA and extended haplotypes. Autoimmun Rev. 2011;10:189–93.
13. Fainboim L, et al. Protracted, but not acute, hepatitis A virus infection is strongly associated with HLA-DRB*1301, a marker for pediatric autoimmune hepatitis. Hepatology. 2001;33:1512–7.
14. Pando M, et al. Pediatric and adult forms of type I autoimmune hepatitis in Argentina: evidence for differential genetic predisposition. Hepatology. 1999;30:1374–80.
15. Vergani D, et al. Genetically determined low C4: a predisposing factor to autoimmune chronic active hepatitis. Lancet. 1985;2:294–8.
16. Liston A, Lesage S, Gray DH, Boyd RL, Goodnow CC. Genetic lesions in T-cell tolerance and thresholds for autoimmunity. Immunol Rev. 2005;204:87–101.
17. Simmonds MJ, Gough SC. Genetic insights into disease mechanisms of autoimmunity. Br Med Bull. 2004;71:93–113.
18. Ahonen P, Myllarniemi S, Sipila I, Perheentupa J. Clinical variation of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) in a series of 68 patients. N Engl J Med. 1990;322:1829–36.
19. Meloni A, et al. Autoimmune polyendocrine syndrome type 1: an extensive longitudinal study in Sardinian patients. J Clin Endocrinol Metab. 2012;97:1114–24.
20. Johnson PJ, McFarlane IG. Meeting report: International Autoimmune Hepatitis Group. Hepatology. 1993;18:998–1005.
21. Alvarez F, et al. International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. J Hepatol. 1999;31:929–38.
22. Hennes EM, et al. Simplified criteria for the diagnosis of autoimmune hepatitis. Hepatology. 2008;48:169–76.
23. Ebbeson RL, Schreiber RA. Diagnosing autoimmune hepatitis in children: is the International Autoimmune Hepatitis Group scoring system useful? Clin Gastroenterol Hepatol. 2004;2:935–40.
24. Ferri PM, Ferreira AR, Miranda DM, Simoes E Silva AC. Diagnostic criteria for autoimmune hepatitis in children: a challenge for pediatric hepatologists. World J Gastroenterol. 2012;18:4470–3.
25. Mileti E, Rosenthal P, Peters MG. Validation and modification of simplified diagnostic criteria for autoimmune hepatitis in children. Clin Gastroenterol Hepatol. 2012;10(417–21):e1–2.
26. Hiejima E, Komatsu H, Sogo T, Inui A, Fujisawa T. Utility of simplified criteria for the diagnosis of autoimmune hepatitis in children. J Pediatr Gastroenterol Nutr. 2011;52:470–3.
27. Vergani D, et al. Liver autoimmune serology: a consensus statement from the committee for autoimmune serology of the International Autoimmune Hepatitis Group. J Hepatol. 2004;41:677–83.
28. Gregorio GV, et al. Autoantibody prevalence in chronic hepatitis B virus infection: effect in interferon alfa. Hepatology. 1996;24:520–3.
29. Gregorio GV, et al. Autoantibody prevalence in children with liver disease due to chronic hepatitis C virus (HCV) infection. Clin Exp Immunol. 1998;112:471–6.
30. Dhawan A, et al. Wilson's disease in children: 37-year experience and revised King's score for liver transplantation. Liver Transpl. 2005;11:441–8.
31. Cotler SJ, Kanji K, Keshavarzian A, Jensen DM, Jakate S. Prevalence and significance of autoantibodies in patients with non-alcoholic steatohepatitis. J Clin Gastroenterol. 2004;38:801–4.
32. Lapierre P, Hajoui O, Homberg JC, Alvarez F. Formiminotransferase cyclodeaminase is an organ-specific autoantigen recognized by sera of patients with autoimmune hepatitis. Gastroenterology. 1999;116:643–9.
33. Manns M, Gerken G, Kyriatsoulis A, Staritz M, Meyer zum Buschenfelde KH. Characterisation of a new subgroup of autoimmune chronic active hepatitis by autoantibodies against a soluble liver antigen. Lancet. 1987;1:292–4.
34. Costa M, Rodriguez-Sanchez JL, Czaja AJ, Gelpi C. Isolation and characterization of cDNA encoding the antigenic protein of the human tRNP(Ser)Sec complex recognized by autoantibodies from patients

withtype-1 autoimmune hepatitis. Clin Exp Immunol. 2000;121:364–74.
35. Wies I, et al. Identification of target antigen for SLA/LP autoantibodies in autoimmune hepatitis. Lancet. 2000;355:1510–5.
36. Gassert DJ, Garcia H, Tanaka K, Reinus JF. Corticosteroid-responsive cryptogenic chronic hepatitis: evidence for seronegative autoimmune hepatitis. Dig Dis Sci. 2007;52:2433–7.
37. Ferreira AR, et al. Effect of treatment of hepatic histopathology in children and adolescents with autoimmune hepatitis. J Pediatr Gastroenterol Nutr. 2008;46:65–70.
38. Kerkar N, et al. Prospective analysis of nonadherence in autoimmune hepatitis: a common problem. J Pediatr Gastroenterol Nutr. 2006;43:629–34.
39. Samaroo B, Samyn M, Buchanan C, Mieli-Vergani G. Long-term daily oral treatment with prednisolone in children with autoimmune liver disease does not affect final adult height. Hepatology. 2006;44:438A.
40. Alvarez F, et al. Short-term cyclosporine induces a remission of autoimmune hepatitis in children. J Hepatol. 1999;30:222–7.
41. Cuarterolo M, et al. Follow-up of children with autoimmune hepatitis treated with cyclosporine. J Pediatr Gastroenterol Nutr. 2006;43:635–9.
42. Manns MP, et al. Diagnosis and management of autoimmune hepatitis. Hepatology. 2010;51:2193–213.
43. Ferreira AR, Roquete ML, Penna FJ, Toppa NH, Castro LP. Type 1 autoimmune hepatitis in children and adolescents: assessment of immunosuppressive treatment withdrawal. J Pediatr (Rio J). 2005;81:343–8.
44. Mieli-Vergani G, Vergani D. Autoimmune hepatitis in children. Clin Liver Dis. 2002;6:335–46.
45. Lennard L, Van Loon JA, Weinshilboum RM. Pharmacogenetics of acute azathioprine toxicity: relationship to thiopurine methyltransferase genetic polymorphism. Clin Pharmacol Ther. 1989;46:149–54.
46. Czaja AJ. Safety issues in the management of autoimmune hepatitis. Expert Opin Drug Saf. 2008;7:319–33.
47. Rumbo C, Emerick KM, Emre S, Shneider BL. Azathioprine metabolite measurements in the treatment of autoimmune hepatitis in pediatric patients: a preliminary report. J Pediatr Gastroenterol Nutr. 2002;35:391–8.
48. Manns MP, et al. Budesonide induces remission more effectively than prednisone in a controlled trial of patients with autoimmune hepatitis. Gastroenterology. 2010;139:1198–206.
49. Woynarowski M, et al. Budesonide vs prednisone with azathioprine for the treatment of autoimmune hepatitis in children and adolescents. J Pediatr. 2013. pii: S0022-3476(13)00658-6. doi: 10.1016/j.jpeds.2013.05.042. [Epub ahead of print].
50. Mieli-Vergani G, Vergani D. Budesonide for juvenile autoimmunehepatitis?Notyet.JPediatr.2013.pii:S0022-3476(13)00823-8. doi: 10.1016/j.jpeds.2013.06.064. [Epub ahead of print].
51. Banerjee S, Rahhal R, Bishop WP. Azathioprine monotherapy for maintenance of remission in pediatric patients with autoimmune hepatitis. J Pediatr Gastroenterol Nutr. 2006;43:353–6.
52. Aw MM, Dhawan A, Samyn M, Bargiota A, Mieli-Vergani G. Mycophenolate mofetil as rescue treatment for autoimmune liver disease in children: a 5-year follow-up. J Hepatol. 2009;51:156–60.
53. Gregorio GV, McFarlane B, Bracken P, Vergani D, Mieli-Vergani G. Organ and non-organ specific autoantibody titres and IgG levels as markers of disease activity: a longitudinal study in childhood autoimmune liver disease. Autoimmunity. 2002;35:515–9.
54. Debray D, Pariente D, Urvoas E, Hadchouel M, Bernard O. Sclerosing cholangitis in children. J Pediatr. 1994;124:49–56.
55. Wilschanski M, et al. Primary sclerosing cholangitis in 32 children: clinical, laboratory, and radiographic features, with survival analysis. Hepatology. 1995;22:1415–22.
56. Feldstein AE, et al. Primary sclerosing cholangitis in children: a long-term follow-up study. Hepatology. 2003;38:210–7.
57. Miloh T, Arnon R, Shneider B, Suchy F, Kerkar N. A retrospective single-center review of primary sclerosing cholangitis in children. Clin Gastroenterol Hepatol. 2009;7:239–45.
58. Baker AJ, et al. Neonatal sclerosing cholangitis in two siblings: a category of progressive intrahepatic cholestasis. J Pediatr Gastroenterol Nutr. 1993;17:317–22.
59. Jacquemin E, et al. The wide spectrum of multidrug resistance 3 deficiency: from neonatal cholestasis to cirrhosis of adulthood. Gastroenterology. 2001;120:1448–58.
60. Ziol M, et al. ABCB4 heterozygous gene mutations associated with fibrosing cholestatic liver disease in adults. Gastroenterology. 2008;135:131–41.
61. Best Pract Res Clin Gastroenterol. 2011;25:783–95.
62. Scalori A, Heneghan M, Hadzic N, Vergani D, Mieli-Vergani G. Outcome and survival in childhood onset autoimmune sclerosing cholangitis and autoimmune hepatitis: a 13-year follow up study. Hepatology. 2007;46(Suppl):555A.
63. Ibrahim SH, Lindor KD. Current management of primary sclerosing cholangitis in pediatric patients. Paediatr Drugs. 2011;13:87–95.
64. Mitchell SA, et al. A preliminary trial of high-dose ursodeoxycholic acid in primary sclerosing cholangitis. Gastroenterology. 2001;121:900–7.
65. Lindor KD, et al. High-dose ursodeoxycholic acid for the treatment of primary sclerosing cholangitis. Hepatology. 2009;50:808–14.
66. Davies YK, et al. Long-term treatment of primary sclerosing cholangitis in children with oral vancomycin: an immunomodulating antibiotic. J Pediatr Gastroenterol Nutr. 2008;47:61–7.
67. Abarbanel DN, et al. Immunomodulatory effect of vancomycin on Treg in pediatric inflammatory bowel disease and primary sclerosing cholangitis. J Clin Immunol. 2013;33:397–406.

68. Duclos-Vallee JC, et al. A 10 year follow up study of patients transplanted for autoimmune hepatitis: histological recurrence precedes clinical and biochemical recurrence. Gut. 2003;52:893–7.
69. Miloh T, et al. Pediatric liver transplantation for primary sclerosing cholangitis. Liver Transpl. 2011;17: 925–33.
70. Alabraba E, et al. A re-evaluation of the risk factors for the recurrence of primary sclerosing cholangitis in liver allografts. Liver Transpl. 2009;15:330–40.
71. Vera A, et al. Risk factors for recurrence of primary sclerosing cholangitis of liver allograft. Lancet. 2002; 360:1943–4.
72. Cholongitas E, et al. Risk factors for recurrence of primary sclerosing cholangitis after liver transplantation. Liver Transpl. 2008;14:138–43.
73. Kerkar N, Hadzić N, Davies ET, Portmann B, Donaldson PT, Rela M, et al. De-novo autoimmune hepatitis after liver transplantation. Lancet. 1998;351: 409–13.
74. Vergani D, Mieli-Vergani G. Autoimmunity after liver transplantation. Hepatology. 2002;36:271–6.
75. Mieli-Vergani G, Vergani D. De novo autoimmune hepatitis after liver transplantation. J Hepatol. 2004; 40:3–7.
76. Cho JM, et al. De novo autoimmune hepatitis in Korean children after liver transplantation: a single institution's experience. Transplant Proc. 2011;43: 2394–6.
77. Kerkar N, et al. Rapamycin successfully treats post-transplant autoimmune hepatitis. Am J Transplant. 2005;5:1085–9.

17 Parenteral Nutrition-Associated Liver Disease in Pediatric Patients: Strategies for Treatment and Prevention

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Introduction

Parenteral nutrition (PN) has revolutionized the nutritional care of patients who cannot fully use the gastrointestinal tract [1]. Today more than 30,000 patients, young and old, depend on long-term PN for survival, and more than 350,000 patients in the USA receive PN on a yearly basis [2]. Despite its common usage, PN is associated with a number of significant morbidities. Parenteral nutrition-associated liver disease (PNALD) has become one of the most challenging complications associated with prolonged administration of PN. As PNALD progresses to a more permanent injury to the liver, patients are at high risk of additional morbidity and even mortality [3, 4]. A number of recognized risk factors have been attributed to PNALD, but it appears that no single factor is fully responsible as a causative agent or factor. Thus, the multifactorial nature of PNALD has been an incredible challenge to both clinicians and researchers, and the process unfortunately remains incompletely understood. This chapter discusses the current knowledge of PNALD, the potential causative factors, as well as treatment and preventive strategies.

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Background

Parenteral nutrition (PN) describes the intravenous administration of complete and balanced nutrition in order to support anabolism and maintain or promote weight gain whenever the gastrointestinal tract cannot and should not be used for adequate nutrition in patients with or at risk for malnutrition. PN is composed of the energy-yielding macronutrients [amino acids, dextrose, intravenous fat emulsions (IVFEs)], micronutrients (vitamins and trace elements), electrolytes, and fluids. PN components are tailored to individual patient needs based on nutritional status and nutritional requirements, underlying clinical conditions, concomitant medication therapy, and laboratory and diagnostic parameters [5]. Although PN is a life-saving therapy in patients with intestinal failure, it can be associated with infectious and metabolic complications. PNALD is one of the most

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challenging and morbid complications associated with PN. PNALD is typically associated with long-term PN use and includes cholestasis, steatosis, and cholelithiasis. This chapter reviews the epidemiology, risk factors, and etiologies of PNALD and discusses the different approaches to its prevention and treatment.

Epidemiology

The reported overall frequency of PNALD in clinical studies varies from 7.4 to 84 %. This wide variation is due to the heterogeneity of study subjects, differences in the definition of PNALD, and the variation in the composition and duration of PN therapy. For instance, overall liver complications were reported in 40–60 % of home-dependent PN children [6], whereas 33 % of premature infants who received PN for more than 7 days reportedly developed PNALD [7]. In another study of premature neonates (median gestational age of 26 weeks), the frequency of PNALD was considerably higher and ranged from 56 to 85 % [8]. In a retrospective review of 176 premature infants who received PN, cholestasis occurred in 24 % of infants especially in those with lower gestational age (34 vs. 36 weeks; $p < 0.01$) and those who received longer duration of PN (76 vs. 21 days; $p < 0.001$) [9].

Clinical Features

A transient elevation of liver transaminases and alkaline phosphatase may occur within 1–2 weeks of PN initiation, but levels return to normal upon termination of PN without necessarily denoting permanent liver damage. However, prolonged PN use increases the risk for severe liver disease that may progress to liver failure if not appropriately addressed [10, 11].

Histopathology

The liver histologic changes related to PN vary in relation to the extent and type of PNALD. Pathologic features may include portal inflammation, canalicular and intralobular cholestasis, periportal inflammation, pseudoacinar formation, portal-portal bridging, steatosis, portal and pericellular fibrosis, and cirrhosis [12, 13]. Some of the earlier processes of PNALD are discussed below.

Steatosis

Hepatic steatosis, or fat accumulation in the hepatocytes, is mostly the result of excessive carbohydrate administration. Excess dextrose increases insulin levels, and when the amount of carbohydrates exceeds the metabolic rate of the substrate, this results in carbohydrate conversion to fat (lipogenesis) in the hepatocytes. Overfeeding from intravenous fat emulsion may also cause hepatic steatosis by causing excessive fat accumulation in the liver, but is a far less common process. Other possible causes may include choline and carnitine deficiencies. Hepatic steatosis is mostly asymptomatic and liver transaminases are poor clinical markers of the degree of fatty infiltration [14]. Therefore, the diagnosis of hepatic steatosis is mostly incidental but should be ruled out in PN-dependent patients who present with malaise, abdominal pain, and hepatomegaly. Hepatic steatosis can be reversed with rebalancing carbohydrate and IVFE intake and through avoidance of overfeeding before advanced liver disease occurs. A balanced PN typically provides 50–60 % of total daily calories from dextrose; 20–30 % of calories from IVFEs, with the lipid dose not exceeding 3 g/kg/day in infants and 2 g/kg/day in other children; and the remaining 10–20 % of calories from amino acids [15].

Cholelithiasis

Cholelithiasis or gallstone formation in PN-dependent patients is the result of bile accumulation secondary to decreased gallbladder contractility when fasting. The lack of oral or enteral feeding results in decreased secretion of cholecystokinin (CCK), a peptide hormone that is secreted in the duodenum in response to food

and stimulates gallbladder emptying. In the absence of gallbladder contractility during fasting, bile accumulates in the gallbladder, which facilitates the formation of calcium bilirubinate sludge and cholesterol gallstones [16, 17]. Approximately 10 % of infants receiving chronic PN develop gallstone disease [18]. While the etiology of cholelithiasis in PN patients was initially presumed to be due to the lack of CCK secretion, a prospective randomized controlled trial showed that treatment with cholecystokinin-octapeptide (CCK-OP), a synthetic peptide derivative of CCK, failed to reduce the incidence of gallstone formation [19]. This suggests that the etiology of these stones may actually be due to an abnormality of bile production itself. Patients with short bowel syndrome (SBS) are at increased risk for cholelithiasis due to impaired enterohepatic cycling with ileal resection, decreased bile flow during fasting, and the canalicular accumulation of toxic bile acids [20, 21]. Cholelithiasis may be prevented or delayed by early initiation of oral or enteral feeding. Cholecystectomy may be considered in symptomatic cases.

Cholestasis

PN-associated cholestasis (PNAC) is the most clinically challenging form of PNALD. Its time to onset is difficult to accurately predict due to its association with different risk factors. In a retrospective study of premature infants who received PN, the median time to development of cholestasis was 23 days, with 77 % of infants developing cholestasis within 5 weeks of PN initiation [9]. Biochemical markers of PNAC typically include elevations of serum bilirubin and γ-glutamyl transpeptidase (GGT) concentrations. More specifically, serum conjugated bilirubin concentrations of 2 mg/dL or higher are commonly used in clinical practice as a biochemical marker of cholestasis [22, 23]. Patients with progressive liver disease develop jaundice, hepatosplenomegaly, and ascites. The presence of jaundice along with steady elevation of serum bilirubin concentrations is a predictor of higher mortality risk [4].

Outcomes

While most patients with PNALD undergo resolution of the condition once off PN, the disorder is associated with a significant morbidity and mortality rate in those who remain on PN. Despite biochemical resolution of PNALD signs in most patients, normalized biochemical markers of PNALD are not always indicative of a normalization of liver histology. Liver fibrosis may continue to be apparent despite normal biomarkers [24]. For those infants who have progressive PNALD, the disease will eventually result in overt liver failure and death. In a study of surgical neonates, almost one-third of patients died with long-standing PNALD [25]. In a more recent study of children with SBS, PNALD was the greatest risk factor for patient mortality [26]. Patients with PNALD should be treated aggressively with a multimodal approach in an attempt to ameliorate the progression of the disease and to avoid invasive surgical procedures, intestinal transplantation, and death. For children with intestinal failure, a multidisciplinary approach to care has been associated with better outcomes.

Risk Factors

Risk factors associated with PNALD are predominately related to the underlying clinical condition(s), but PN components (e.g., soybean-based intravenous fat emulsions) may also contribute. Several risk factors have been identified that contribute to PNALD including prematurity and low birth weight, prolonged PN duration, sepsis, SBS, bacterial overgrowth and translocation, bowel rest, and lack of enteral feeding [23]. Necrotizing enterocolitis (NEC) is a significant risk factor for PNALD [27]. In neonates with NEC who underwent surgical treatment, independent risk factors for PNALD [defined as serum direct bilirubin concentration of 2 mg/dL or higher, or serum *alanine aminotransferase* (*ALT*) of at least twice the upper limit of normal] also included small bowel resection or presence of a proximal jejunostomy, PN duration, and preoperative exposure to PN especially for 4 weeks or longer [8]. Gestational age and cholestasis in

neonates receiving PN are also independent risk factors to poor postnatal growth [28]. A systematic review of the risk factors associated with PNALD that was conducted by the American Pediatric Surgical Association Outcomes and Clinical Trials Committee provides a critical appraisal of the evidence relating the nutrient and non-nutrient risk factors and liver disease [27].

Prematurity and low birth weight are risk factors for PNALD considering the physiologic immaturity of the liver excretory systems. PNALD was reported in 50 % of premature infants with a birth weight below 2,000 g [29]. The frequency of PNALD gradually increased from 1.4 to 13.7 % in infants with increasing prematurity from over 36 weeks' to before 32 weeks' gestation, respectively [30]. In a study of 24 premature infants (mean gestational age of 32.5 weeks and birth weight of 1,840 g), PN duration, fasting, gastrointestinal surgery, and maximum caloric and carbohydrate intake in PN were significant risks of PNALD [7]. Considering the available data, it remains debatable whether prematurity alone is an independent risk factor for PNALD considering the presence of confounding variables in premature infants that may also influence the development of PNALD and the lower quality of available studies [27].

The duration of PN administration is a strong predictor of developing PNALD [27]. In a study of surgical neonates, the frequency of PNALD was 35 % in those who received PN for at least 2 weeks, but increased to 58 and 75 % with PN administration for at least 30 and 90 days, respectively. All neonates developed PNALD whenever PN was given for more than 180 days [25].

The number of septic episodes in PN patients also increases the risk of cholestasis [27]. In one study, surgical neonates had a 30 % increase in plasma bilirubin concentrations during recurrent episodes of sepsis [31]. Sepsis may induce cholestasis possibly via the toxic effects of endotoxins or lipopolysaccharides on the hepatobiliary system. Endotoxin may cause direct hepatocellular injury or possibly mediate the formation of cytotoxic bile acids and stimulate the release of hepatotoxic inflammatory mediators such as tumor necrosis factor (TNF) and interleukins 1 and 6 [23]. Endotoxins also downregulate critical canalicular transporter proteins, including bile salt export protein, which may change the composition of bile or result in the accumulation of potentially cytotoxic substances within the liver [32–34].

SBS [35] resulting from extensive resection of the small intestine is associated with malabsorption and metabolic abnormalities that require long duration of PN. PNALD including cholestasis, hepatic fibrosis, and liver failure are the leading causes of death in patients with SBS [36]. Predisposing factors to PNALD in SBS patients include prolonged duration of PN, reduced intestinal length and loss of epithelial barrier integrity, intestinal bacterial overgrowth, and abnormal bile acid excretion in patients with ileal resection [23]. The shorter the remaining small bowel length, the higher is the risk for developing PNALD [4]. Conversely, for any length of small bowel, the presence of cholestasis in a child with SBS markedly increases the risk of death attributable to PNALD [26].

Bacterial translocation from the intestinal tract especially gram-negative bacteria producing endotoxins has been also postulated to be a risk factor for PNALD. Animal studies have shown that the lack of enteral feeding during PN infusion along with intestinal dysmotility leads to bacterial overgrowth that causes intestinal inflammation and disruption of the gut barrier. It has been hypothesized that intestinal-derived lipopolysaccharide (LPS) permeates through this disrupted gut barrier, enters the portal circulation, and binds to Toll-like receptor 4 (TLR4). This leads to Kupffer activation, resulting in the release of a cascade of proinflammatory cytokine signaling in early stages of PNALD. Figure 17.1 shows a schematic concept of this theoretical mechanism driving the development of PNALD. Potentially, the use of broad-spectrum antibiotics to suppress the intestinal microflora or the ablation of TLR4 signaling may attenuate this liver injury [37]. Combined small bowel atrophy, decreased intestinal immunoglobulin A (IgA) levels, production of hepatotoxic cytokines, and disruption of the intestinal microflora and bacterial overgrowth all may occur as a result of bowel rest. Therefore, bacterial overgrowth combined with intestinal permeability could result in bacterial translocation

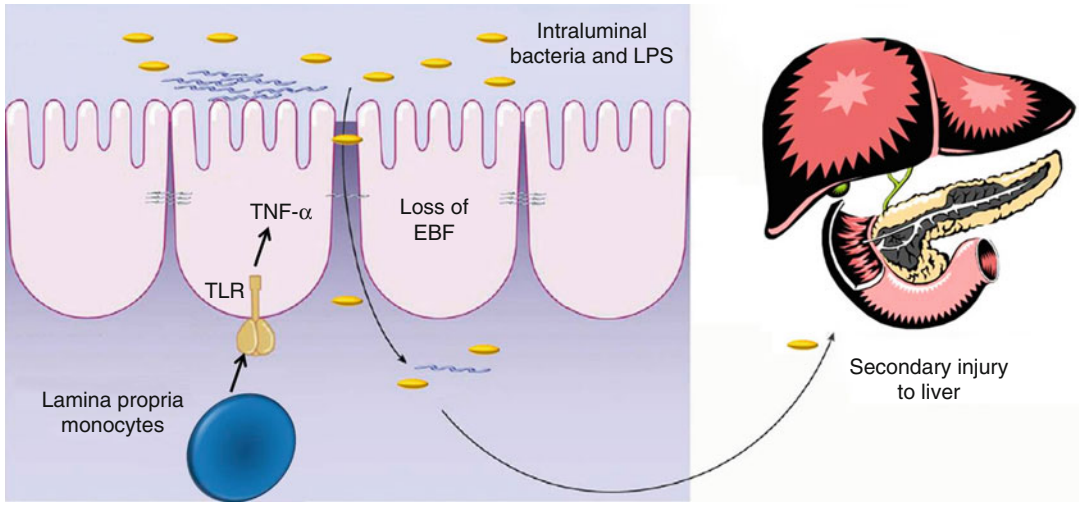

Fig. 17.1 Schematic of the potential mechanism which may drive the development of PNALD. Loss of epithelial barrier function (*EBF*) occurs from a loss of enteral feeding or injury to the bowel from inflammatory processes leading to an upregulation of tumor necrosis factor-alpha (*TNF-α*) from lamina propria monocytes. This results in translocation of bacterial and lipopolysaccharide (*LPS*) which pass into the portal venous system and secondarily incurs injury to the liver

(passage of intestinal microflora from the intestines into the mesenteric lymph nodes, blood, or organs) leading to direct toxic effects of bacteria on the liver or cytokines causing hepatocyte injury. Significant correlation has been reported between bacterial overgrowth and cholestasis and PN dependence [38, 39]. In 1985, Freund and colleagues examined the effect of oral metronidazole on hepatic dysfunction during PN administration in rats [40]. The administration of metronidazole at a dose of 15 mg/kg/day significantly decreased the hepatic lipid content, suggesting the possible involvement of anaerobic bacteria as part of the multifactorial pathogenesis of PNALD. Although the toxic effects of bacterial infections on the liver are established, the evidence remains lacking for the routine use of oral antibiotic therapy to prevent bacterial translocation and decrease hepatocyte damage in PN patients [27].

Trace Elements and PNALD

Manganese, Copper, and Aluminum

Manganese and copper are trace minerals that are routinely supplemented in PN. They are mainly eliminated via the bile and therefore may accumulate during cholestasis. Manganese accumulation especially in patients with hyperbilirubinemia may have direct toxicity on the canalicular membrane [41]. In a group of 57 children who received PN for more than 14 days, there was a significant correlation between blood manganese and plasma bilirubin and AST concentrations [42]. Although an association has been reported between elevated blood manganese and increased plasma GGT and alkaline phosphatase concentrations [43, 44], no firm conclusion could be drawn that high manganese levels cause cholestasis in PN patients. In clinical practice, manganese should be monitored on a monthly basis in patients receiving long-term PN and restricted if levels are elevated.

Although liver toxicity is a feature of copper accumulation, copper toxicity has not been reported in PN patients. However, copper accumulation may still occur. Autopsies from patients with SBS who received copper supplementation in their home PN revealed copper accumulation in the liver and kidneys, especially in those who died of liver failure [45]. Potentially subclinical copper accumulation may occur in home PN patients with cholestasis especially when higher copper doses are given [46]. However, copper deficiencies may

occur and have been reported in PN-dependent patients when copper intake is restricted, some of which can be lethal [47]. Copper levels, along with clinical markers of copper deficiency and/or overload, should be monitored every 1–3 months for patients on long-term PN therapy with the provided dose adjusted accordingly. While historical recommendations indicate empiric reduction in manganese and copper provision in PN in the setting of PNALD, current evidence does not support this practice. Rather, individualized dosing of manganese and copper should be guided by clinical status and measured serum concentrations in order to detect any accumulation and prevent any toxicity or deficiency [27].

Aluminum is a known contaminant of PN solutions [48]. Aluminum accumulation may cause bone, liver, blood, and central nervous system toxcixicities. Although aluminum-induced liver toxicity has been not reported in PN patients, animal studies have reported that aluminum in PN may induce portal inflammation and liver injury by possibly blunting the bile canaliculi microvilli in a way similar to findings in PNALD patients [49, 50]. Although the Food and Drug Administration has mandated PN component product labeling for aluminum and recommended maximum daily exposure limits [48], aluminum contamination in PN remains problematic [51]. Therefore, periodic monitoring of serum aluminum is recommended especially in patients at risk for aluminum accumulation such as premature infants, PN-dependent patients, and those with decreased renal function.

Non-pharmacologic Management Strategies

Early Initiation of Enteral Feeding

The most effective therapy for PNALD is achievement of enteral nutrition either orally or via a feeding tube [52]. Cholestasis develops more commonly in pediatric patients who are unable to tolerate any enteral feeding [27]. Enteral feeding exposes the intestinal tract to nutrients causing the release of endogenous hormones that promote intestinal epithelial cell growth, intestinal adaptation, and reversal of mucosal hypoplasia induced by starvation [23, 53]. Even small, trophic volumes of feedings via continuous administration or small bolus amounts have shown clinical benefit by reducing intestinal stasis and translocation of bacteria, improving bile flow, and avoiding oral aversion [23]. Trophic feeding exposes the intestinal tract to nutrient and hormonal stimulation which results in intestinal epithelial cell growth, enzyme activity, and motility [53]. Once tolerance to trophic feedings is established, advancing slowly toward caloric goal over a period of weeks to months may be achieved with a proportional decrease in PN administration, as clinically tolerated. When intolerances (e.g., abdominal distension or high stool output of 30–40 mL/kg/day) to enteral feedings occur, the rate of enteral feeding may be decreased, the enteral formula may be changed when indicated, and pharmacologic management of diarrhea may be initiated [54]. If dependence on PN is a result of intestinal resection or SBS, specialized enteral formulas may be chosen to optimize nutrient absorption depending on the length and segment of the remaining bowel.

Cycled Parenteral Nutrition

A proposed method for reducing PNALD is to "cycle" daily PN infusion, that is, to have a PN-free period during the day. Cycling PN promotes the cyclic release of gastrointestinal hormones and better approximates feeding rhythms or normal eating patterns that avoids the continuous compulsive effects of nutrients on the liver [5, 52]. Although originally cyclic PN was used in adults to free patients from the infusion apparatus especially those on long-term PN, a clinical benefit of this practice has been also demonstrated in its effects on improving serum bilirubin levels in PN-dependent patients [55]. However, maintaining euglycemia during PN-free periods is a concern in young children, especially in neonates and infants due to the immaturity of their protective metabolic functions such as decreased gluconeogenesis, glycogenolysis, and ketogenesis

and limited glycogen stores. Nevertheless, it remains an approach for stable older infants, children, and adolescents for the management of PNALD. Cycled PN is achieved by ramping up and ramping down the infusion over 1–2 h, with the remainder of the volume infused at a continuous rate over the remaining time of the cycle [5]. For patients who cannot tolerate PN cycling, slow advancement of PN cycle (e.g., starting with 22-h infusion and then advancing the cycle slowly by 2 h every few days) with close monitoring is recommended. If cyclic PN is employed, it is important to monitor blood glucose control throughout the cycle, often by capillary blood glucose measurements. One approach is to monitor blood glucose levels three times daily while on cycled PN: immediately before the start of PN (lowest value), 4 h into the cycle (highest value), and 30 min post-PN infusion in order to check hypoglycemia at the end of the cycle. Closer monitoring of fluid and electrolyte balance is also indicated as the PN cycle is advanced. In general, one should avoid cycling PN until at least 25 % of nutrients are concomitantly given via the enteral route in order to avoid hypoglycemia during PN off times.

The benefit of cyclic PN in preventing PNALD in young infants receiving PN has not been consistently demonstrated. In a study that evaluated the effects of cyclic PN on infants with gastroschisis, 107 patients were analyzed, 36 of which received a cyclic PN and 71 of which did not [56]. The duration of the PN cycle was initially 20 h with a dextrose-containing solution infused during the off period to avoid hypoglycemia. The cycle was extended up to 6–12-h off of PN daily as patients grew and became older; however, a standard cycle for all included patients was not defined. The time to onset to hyperbilirubinemia was found to be significantly longer in those patients receiving cyclic PN as compared to those on continuous PN: cycled 5.7 % (95 % CI 0–13.1 %) versus continuous 22.3 % (95 % CI 9.9–33 %), $p = 0.005$ at 25 days of therapy. At any time during the course of PN, after adjusting for other confounding factors, patients who received continuous PN were 2.86 times more likely to develop hyperbilirubinemia as compared to those patients on cycled PN; however, this result was not statistically significant. Conversely, in another study of cyclic PN in very-low-birth-weight infants (weight ≤1,250 g), early cycling of PN did not significantly reduce the development of cholestasis [57]. Rather, other factors such as duration of PN as well as the time to full enteral feedings were identified as independent risk factors for the development of PNALD. For very young patients, if cycling is considered, it cannot be implemented unless the patient is already tolerating some amount of enteral feedings or a dextrose-containing solution is infused during off PN times so as to avoid hypoglycemia [27].

Further, the risks involved with cycling PN in critically ill patients including metabolic abnormalities such as electrolyte, acid–base, and fluid balance abnormalities make continuous infusion preferred in this group of patients [58]. For older, clinically stable patients, or those tolerating concomitant enteral administration of nutrients, cyclic PN may be a consideration as a preventative strategy for PNALD and as an improvement in quality of life for long-term PN patients.

Alternative Lipid Strategies

IVFEs are a source of calories and essential fatty acids (linoleic and alpha-linolenic acid). Currently, the available IVFE products in the USA are derived from soybean oil. These formulations have been considered as an independent risk factor in the development of PNALD [59]. Interest in intravenous fat emulsion and its association with PNALD has mounted within the last decade, and much work has recently been done investigating novel approaches to lipid therapy. Although the exact mechanism whereby IVFEs cause PNALD is not yet fully elucidated, several mechanisms have been proposed including the effects of proinflammatory omega-6 fatty acids possibly causing hepatocyte damage or apoptosis or the cell membrane peroxidation induced by long-chain polyunsaturated fatty acids [60, 61]. Soybean-based fat emulsions contain high levels of phytosterols, which have been shown to impair

bile drainage and contribute to hepatobiliary dysfunction [27]. Phytosterols are contaminants of IVFEs and are inefficiently metabolized by the liver. The accumulation of one plant phytosterol, in particular stigmasterol, may be a major contributor to the development of liver toxicity and PNALD by binding to membrane proteins and reducing bile synthesis and flow [62]. While liver enzyme levels may remain close to normal, liver biopsies of patients with high serum plant sterol levels have shown liver fibrosis indicating that elevated serum plant sterol levels may mirror the formation of liver fibrosis, although a definite cause-effect relationship between phytosterols and PNALD has not been proven [24]. An additional mechanism by which IVFE may contribute to PNALD is that soybean-based fat emulsions are rich in omega-6 fatty acids, which may cause hepatocyte damage or apoptosis due to a proinflammatory mechanism [27]. Due to these factors and the available data, several strategies can be undertaken to minimize the effects of IVFEs on the liver and include (1) soybean-based FE minimization or (2) the use of alternatives IVFE such as fish oil-based emulsions and combination fat emulsions. However, data supporting either approach are mostly reliant upon retrospective or uncontrolled studies, therefore limiting the strength of supporting evidence. Results of studies for each approach are promising, but long-term outcomes and safety data are needed from well-designed trials.

IVFE Minimization

The definition of IVFE minimization has not been consistently described; however, typically it is defined as receipt of less than 1 g/kg/day of IVFE [63]. The benefit of IVFE minimization was first described in the adult literature in 1982, by Allardyce [64]. For pediatric patients, this technique was first described by Colomb et al., in a group of ten infants with severe cholestasis [65]. The authors acutely terminated IVFE administration and noted the marked decline in bilirubin levels in these patients. A follow-up, prospective study by Cober and Teitelbaum supported this IVFE minimization strategy for the prevention of PNALD [66, 67]. In this single-center prospective study, patients who developed PNALD (direct bilirubin >2.5 mg/dL) on standard soybean-based IVFE at a dose of 3 g/kg/day received an IVFE regimen of 1 g/kg/day twice weekly. These patients were compared to a well-matched historical control group that received standard IVFE dosing. Thirty-one patients were evaluated, and in the serum direct bilirubin levels in those treated with IVFE minimization were found to significantly decline over time compared to the control group. Specifically, the group receiving IVFE minimization was found to have a downward trend in bilirubin (estimated slope −0.73 mg/dL/week) as compared to the standard cohort, which showed a rise in bilirubin levels across study weeks (estimated slope 0.29 mg/dL/week). A significant difference in the slopes was identified between the groups, $p = 0.0017$. Additionally, the number of patients achieving resolution of PNALD was significantly greater in the IVFE minimization group ($n = 13$) as compared to the control group ($n = 3$), $p = 0.013$. It is important to note that in this study, eight patients developed reversible, mild essential fatty acid deficiency (EFAD) while receiving twice weekly IVFE. There were no physical manifestations of EFAD observed other than biochemical markers (triene-to-tetraene ratio ≥0.05). For those patients who developed EFAD, however, the lipid regimen was altered to 1 g/kg/day three days per week and then again to 2 g/kg/day three days per week, if needed. These changes resulted in resolution of EFAD in all patients. Finally, there were no significant differences in overall growth between the two groups.

Conflicting results were demonstrated in a retrospective study conducted by Nehra and colleagues [61]. Neonates requiring long-term parenteral nutrition support, defined as ≥21 days of therapy, were divided into two groups: (1) [$n = 29$] those receiving IVFE at a dose of 1 g/kg/day and (2) [$n = 32$] those receiving IVFE at a dose between 2 and 3 g/kg/day and who were evaluated for the primary outcome of development of cholestasis, defined as direct bilirubin of >2 mg/dL for ≥2 consecutive weeks. The incidence of cholestasis was not statistically significant between the two groups, with 15/29 patients

in the group receiving 1 g/kg/day and 14/32 patients in the group receiving 2–3 g/kg/day developing cholestasis, $p = 0.61$. Likewise, the time to develop cholestasis was found to be similar between both groups. In this study, once cholestasis developed, patients were expeditiously attempted to transition to full enteral feeds or transitioned to a fish oil-based fat emulsion. A limitation to this study is its retrospective nature as well as slight differences in study populations between the two groups.

These two studies regarding IVFE minimization reveal important, but conflicting, findings relevant to reduced doses of fat emulsion and the effect on prevention of PNALD. Each study has its own limitations, and several questions still remain regarding this approach to treatment such as optimal dose of IVFE, timing of initiation of IVFE minimization, as well as short- and long-term outcomes as well as the safety associated with this treatment approach. These questions may be answered in a well-designed prospective study that randomizes patients in a matched fashion to IVFE minimization and standard therapies in order to evaluate effectiveness of this strategy in reducing the development of PNALD. These studies must also be designed to evaluate important findings such as EFAD, growth restriction, and both short- and long-term neurodevelopmental effects.

Use of Fish Oil-Based IVFE

In contrast to soybean-based fat emulsions which contain omega-6 fatty acids, fish oil-based emulsions are almost completely omega-3 fatty acids, which are believed to have several beneficial effects and to play a role in the resolution of PNALD [63]. The benefits of fish oil-based emulsions in preventing PNALD as compared to soybean-based IVFEs may be due to several factors which include a reduction in detrimental phytosterols and proinflammatory mediators. Fish oil FE may have an additive benefit of being a substrate for the formation of a favorable prostaglandin composition and also contain an antioxidant alpha-tocopherol, which has immunomodulatory effects. The only available fat emulsion composed of fish oil is Omegaven® (Fresenius Kabi Deutschland GmbH, Bad Homburg, Germany). A recent study that compared Omegaven to four other fat emulsions in a murine model demonstrated that inclusion of fish oil had positive effects on hepatic outcomes [68]. The first clinical report of fish oil-based emulsion, published in 2006, demonstrated complete reversal of PNALD in two infants with intestinal failure-associated liver disease [69]. The clinical utility has subsequently been demonstrated in several case reports [70–76]. Further, the use of fish oil-based emulsion was studied in 42 infants who developed PNALD while receiving soybean-based FE who were predicted to require PN for a minimum of 30 days [77]. These infants were compared to a cohort of 59 infants that received standard soybean-based fat emulsion. Infants who received fish oil-based fat emulsion received it at a dose of 1 g/kg/day, whereas the historical cohort received soybean-based fat emulsion at a dose ranging from 1 to 4 g/kg/day. Direct bilirubin levels decreased over time in the fish oil cohort as compared to an increase in levels seen in the standard soybean cohort ($p < 0.0001$). Forty-five percent ($n = 19$) of the patients in the fish oil group versus 4.1 % ($n = 2$) in the soybean oil group demonstrated reversal of cholestasis during the study period. Further, the risk of death or the need for transplantation was lower in the group receiving fish oil-based fat emulsion. From a safety standpoint, fish oil-based emulsion was, overall, well tolerated with fewer patients developing hypertriglyceridemia; however, two patients did develop EFAD in the fish oil group. Continued safety has been substantiated with continued use under a compassionate-use open study [78]. This study is limited by its short follow-up period as well as the fact that a quarter of the patients received PN for less than 3 weeks [77]. Additionally, it is not known whether the beneficial effects were due to the fish oil emulsion itself or due to the reduced dose (1 g/kg/day) of fat emulsion. However, a prospective randomized trial is underway that will examine conventional soybean oil-based fat emulsion versus fish oil-based fat emulsion at a goal dose of 1 g/kg/day, which may help to answer this question.

Another study evaluated the effects of fish oil IVFE on 12 children with SBS and severe PNALD (direct bilirubin >2.9 mg/dL) [79]. Study results showed that nine patients had complete resolution of hyperbilirubinemia within 24 weeks of treatment. Four of these patients were receiving a combination of fish oil and soybean oil, whereas five patients were receiving fish oil alone. Despite the small sample size, results indicate a possible clinical use of combined fish- and soybean-based IVFE to prevent PNALD while preserving adequate caloric and fatty acid intake. Combination fat emulsion may more closely approximate the optimal fatty acid intake and may lead to more physiologic outcomes as well as benefits in growth and development [63].

While not intravenous, the use of enteral fish oil has also recently been examined and deserves a brief mention [80, 81]. While benefits were seen in a small subset of patients, the use of enteral fish oil relies on at least a partially functional intestine. Therefore, the beneficial effects may be confounded by the fact that a functional intestine may also tolerate enteral feeds and demonstrate intestinal adaptation [63]. Therefore, resolution of PNALD may be due to improved intestinal adaptation and tolerance of enteral feeds rather than the presence of enteral fish oil.

Composite Intravenous Fat Emulsion Formulation

In addition to single-component fat emulsions, a few novel fat emulsions have been developed. SMOFlipid (Fresenius Kabi) is a combination fat emulsion that contains soybean oil, medium-chain triglycerides, olive oil, as well as fish oil resulting in an omega-6 to omega-3 fatty acid ratio of 2.5:1 [63]. While SMOFlipid is not currently available in the USA, several studies have shown safety and efficacy of this emulsion [82–85]. A study of 28 children between the ages of 5 months and 11 years requiring long-term home PN compared the liver effects of SMOFlipid and standard soybean-based fat emulsion at a target dose of 2 g/kg/day [82]. At 4 weeks, patients who received SMOFlipid had lower mean serum concentrations of ALT, AST, and GGT as compared with the soybean-based IVFE group. Further, the mean change in serum total bilirubin was significantly greater in the SMOFlipid group ($p<0.01$) with a decrease seen in the SMOFlipid group as compared with an increase in the standard fat emulsion group. In addition to these benefits, several case series have been published that demonstrate a reversal of elevated direct bilirubin with the use of SMOFlipids [86–88].

IVFEs available outside of theUSA, such as Lipofundin MCT (B. Braun), which is a combination of soy and coconut oil, and Clinoleic (Baxter), which is a combination of olive and soy oils, may also play a role in the management of PNALD [63]. There have been a few clinical studies evaluating the efficacy of these agents; however, more data is warranted before use of these agents can be universally recommended. The American Society of Parenteral and Enteral Nutrition recently published a position paper on alternative fat emulsions, which calls for expanded availability of and further research on the alternative fat emulsions so that the ideal formula may be identified for special patient populations [89].

Pharmacologic Management

Phenobarbital

Phenobarbital is a barbiturate, a sedative hypnotic, and an anticonvulsant medication that has been used to stimulate choleresis in patients with various forms of cholestatic liver disease [90]. For the treatment of PNALD, several theoretical mechanisms exist. Its effect is thought to be associated with the activation of enzymes, which stimulate the flow of bile [91, 92]. Additionally, phenobarbital may increase bile salt production, decrease serum bile acids and bilirubin, increase the conjugation of bilirubin and accelerate hepatic clearance of bile acids, and possibly affect the hepatic activity of the Na, K-ATPase pump [92, 93]. In an early case report, phenobarbital efficacy was demonstrated 36 h after phenobarbital initiation at a dose of 5 mg/kg/day in a patient with progressive liver disease and

cirrhosis secondary to PNALD [93]. However, despite its theoretical benefit and effectiveness seen in individual cases, other case reports have not shown similar benefit. A retrospective study performed in 1986 evaluated the effects of phenobarbital in PN-dependent neonates weighing less than 1,500 g [92]. Two groups of patients were evaluated: (1) those who received parenteral nutrition ($n=21$) and (2) those who received parenteral nutrition plus phenobarbital at an initial intravenous dose of 5 mg/kg/day with therapeutic drug monitoring to maintain serum phenobarbital concentrations between 15 and 25 mcg/dL ($n=10$). For infants who received phenobarbital therapy, indications included seizure treatment or prophylaxis. Interestingly, more patients in the phenobarbital group developed PNALD as compared to those who did not receive phenobarbital (60 % vs. 33 %). Thus, phenobarbital did not offer protection against the development of PNALD and, in fact, may have contributed to its development [91, 92].

The use of phenobarbital for the treatment of PNALD has fallen out of favor due to conflicting and minimal evidence supporting its use, in addition to advancements made in other more efficacious therapies. However, in severe cases, phenobarbital may be considered on an individual basis when other alternatives are not possible or have been exhausted. Patients should be monitored for possible phenobarbital-associated adverse effects such as hypotension, bradycardia, respiratory depression, sedation, and thrombophlebitis with intravenous use and vitamin D deficiency especially with extended use, to name a few [90].

Ursodiol

Ursodiol, or ursodeoxycholic acid, is a hydrophilic dihydroxylated bile acid, which typically comprises a small fraction (1–3 %) of the secondary bile acid pool in humans, is released by the gallbladder, and is then solubilized and absorbed in the jejunum and terminal ileum, respectively [94, 95]. After oral administration of exogenous ursodiol, passive absorption in the small and large intestines (30–60 %) and the colon (20 %) results, followed by conjugation in the liver, secretion into the biliary tree and the intestines, and then extensive enterohepatic recirculation and reabsorption in the terminal ileum [94–96]. While the exact mechanism of action of ursodiol in the treatment of PNALD is unknown, proposed mechanisms include correction of bile acid deficiency, improvement in and stimulation of bile flow, and displacement of toxic hydrophobic bile acids such as chenodeoxycholic acid, in addition to the provision of immunomodulatory and cytoprotective effects [94, 95, 97–99]. During chronic administration, ursodiol becomes the primary biliary and plasma bile acid, ultimately displacing cytotoxic bile acids and increasing bile secretion and flow [95, 96, 100].

After administration of ursodiol (10–30 mg/kg/day in 2–3 divided doses) to pediatric patients with PNALD, a marked and sustained improvement in liver biochemistry levels has been demonstrated, including direct bilirubin, serum ALT, aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transpeptidase (GGT), with the earliest improvements seen in direct bilirubin and GGT [100–102]. Ursodeoxycholic acid has shown to decrease the extent and duration of PNALD in both medical and surgical populations and in very-low-birth-weight infants, neonates, infants, and older children and in those children with short bowel syndrome (SBS). By and large the results have been positive, with results showing a decreased extent and the duration of PNALD [101–105]. Early response may be seen within the first 2 weeks of therapy; however, complete resolution may take up to 4 months [102]. Interestingly, in a recent prospective study, those children with SBS had an earlier and more pronounced response to ursodiol as compared to those children without SBS, indicating that even those children with significant intestinal loss respond well to treatment with ursodiol at a higher dose of 30 mg/kg/day in divided doses [105].

Tauroursodeoxycholic acid (TUDCA), an agent similar to ursodeoxycholic acid, was evaluated by Heubi et al. in a prospective, randomized clinical trial designed to evaluate TUDCA's

prophylactic effects on lowering peak conjugated bilirubin levels in infants on long-term PN [106]. The dose evaluated was 30 mg/kg/day in two divided doses. Study results showed that TUDCA was ineffective in preventing or reducing the severity of PNALD in neonates. Further studies may be needed to further delineate the role of TUDCA in the treatment and prevention of PNALD.

The timing of initiation of ursodiol therapy has yet to be fully defined, and a major limitation is that the drug must be given enterally. While some centers may initiate at the earliest signs of cholestasis, others wait until full enteral feeding has been achieved. However, evidence suggests benefit with initiation at the earliest onset of PNALD, if enteral tolerance is possible [104, 105]. While there is no consensus on the minimal amount of enteral feeds required prior to initiating oral ursodiol, it may be recommended to achieve tolerance of at least 5 mL/h prior to initiating oral medications. Likewise, the optimal duration of therapy is still unknown. A rebound rise in markers of cholestasis may be seen after discontinuation of ursodiol; however, normalization occurs after re-initiation of therapy [105]. In general, ursodiol is very well tolerated; however, mild diarrhea has been described in some cases. The long-term effect of ursodiol on progression and prognosis on PNALD is unknown. Further, it is uncertain how the well-controlled study on TUDCA failed to show efficacy, where the less controlled studies were beneficial. Thus, evidence from large, prospective, randomized, and placebo-controlled studies is still needed.

Other Therapies

Cholecystokinin-octapeptide (CCK-OP) is the active portion of cholecystokinin gastrointestinal peptide that can improve intrahepatic bile flow [107]. Although early investigations showed promise for the role of CCK-OP in the management of PNALD [107–109], later larger well-designed studies showed no clinical role of CCK-OP in the prevention or treatment of PNALD. In a double-blind, multicenter, randomized control trial conducted at eight centers, CCK was studied in a total of 243 infants at a dose of 0.04 mcg/kg intravenously twice daily and failed to show a significant effect in conjugated bilirubin levels, incidence of sepsis, time to enteral feedings, length of intensive care unit stay, or hospital length of stay [110]. Since that time, no additional trials have been published with alternative dosing strategies or differing results [27]. Thus, routine use of CCK is not recommended for treatment of PNALD in pediatric patients.

Glutamine, one of the most abundant amino acids in the plasma and human milk, has been suggested as an exogenous supplement to reduce sepsis, enhance gastrointestinal integrity, and improve immune function [111]. Glutamine supplementation may also have protective effects on the liver, especially in those patients receiving long-term PN through the increase of glutathione stores in the liver. In 2010, the effects of intravenous glutamine supplementation on hepatic function and mortality and the time to full enteral nutrition were evaluated in 28 neonates. Significant decreases in total bilirubin and AST concentrations were observed in patients supplemented with glutamine, although no other study endpoints were found to be significant. The effects of enteral glutamine are currently being investigated. At this time, however, the routine use of parenteral or enteral glutamine, for the prevention or treatment of PNALD, is not recommended until substantive data are available.

Amino acids such as cysteine and taurine have been presumed to be nonessential in older children and adults; however, they are conditionally essential in neonates and infants [112]. However, taurine deficiencies have been noted in individuals with short bowel syndrome receiving long-term PN, and taurine is needed for the successful conjugation of bilirubin to bilirubin diglucuronide. Thus, efforts to increase plasma taurine levels have been investigated. One investigation by Helms et al. revealed that plasma taurine concentrations may be selectively increased with the addition of cysteine (40 mg/g of amino acids) to infant parenteral nutrition [112]. Supplemental taurine has also been investigated in the setting of PNALD [113]. In a multicenter, prospective

study conducted between 1996 and 2001, the effect of supplemental taurine on the development of PNALD was evaluated. When evaluated overall, taurine did not demonstrate benefit; however, when stratified by gestational age and indication, taurine showed benefit in those patients who were premature and those with a history of necrotizing enterocolitis (NEC). Specifically, the conjugated bilirubin was reduced in premature infants receiving taurine 0.5 mg/dL [0.17–1.18] versus 3.45 mg/dL [1.79–5.11] in those not receiving taurine, $p=0.07$. For those patients with NEC, conjugated bilirubin was 4.04 mg/dL [2.85–5.23] in those receiving taurine versus 8.29 mg/dL [5.61–10.96] in those who did not receive taurine, $p=<0.01$. Therefore, the investigators concluded that taurine supplementation may be beneficial in a subset of higher risk patients but may also be considered as a standard in all neonatal amino acid solutions.

Prevention and Treatment of Sepsis

Recurrent sepsis has been closely associated with the development or worsening of PNALD [31]. Recurrent sepsis was associated with a 30 % increase in bilirubin levels. Thus, several strategies can be undertaken to decrease the risk or frequency of sepsis in patients on long-term PN. The prevention of sepsis should be a priority in the treatment of patients dependent on long-term PN.

Enteral Antibiotics

For patients on long-term PN, translocation of bacteria from a disused intestine increases the risk of systemic and catheter-related bloodstream infections (CRBSIs) due to decreased gut barrier integrity [114, 115]. The theory that lack of intestinal stimulation during periods of prolonged fasting and PN administration may lead to a change in the gut microbiology (i.e., microbiota) and increased risk of bacterial translocation possibly leading to sepsis has led to the use of enteral antibiotics to treat intestinal microbial overgrowth and ultimately prevent bacterial translocation. However, this practice is not universally accepted due to a relative lack of evidence-based data. While intravenous medications such as metronidazole have not been shown to be effective [116], cycled enteral antibiotics (CEA) as a preventative strategy have demonstrated efficacy. Eradication of abnormal flora, selective decontamination via CEA, may be considered in high risk patients, such as patients with missing ileocecal valve and those with frequent episodes of bacteremia or CRBSIs [115, 117]. While it is recognized that total eradication of intestinal bacteria is not possible, therapy should be aimed at reducing symptoms as well as decreasing abnormal flora as well as decreasing the number of potential pathogens. There are several approaches to CEA. Typically CEA is a combination or successive administration of enteral antibiotics, followed by an antibiotic-free period, which allows the microbiota of the gut to recover [115]. In a recent study by Dobson and colleagues, the use of enteral metronidazole 7.5 mg/kg three times daily for 2 weeks, followed by a combination of enteral colistin 25 mg four times daily with enteral tobramycin 20 mg four times daily for 2 weeks, followed by a 2-week antibiotic-free period before restarting the cycle, was retrospectively evaluated in pediatric patients receiving PN for at least 28 days [115]. A significant reduction in sepsis rates for those patients treated with CEA was observed.

The ideal choice of enteral antibiotics has not yet been fully elucidated; however, the combination should target organisms typically found in the gut, such as anaerobes (i.e., metronidazole, amoxicillin-clavulanate) and gram-negative organisms (i.e., aminoglycosides, ciprofloxacin, or colistin). In some cases, antifungal coverage (i.e., fluconazole, nystatin) may be considered. Preferably, the combination of antibiotics should be nonabsorbable from the gut so as to limit systemic exposure and minimize adverse effects. One approach may be to continuously administer a combination of antibiotics, while a second approach consists of sequential, cyclic administration of antibiotics with an antibiotic-free period in between cycles.

Antibiotic-Lock Therapy

Catheter-related bloodstream infections (CRBSIs) account for a major source of morbidity and mortality among patients receiving long-term parenteral nutrition. Indeed, the National Nosocomial Infections Surveillance System notes that CRBSIs occur 2.7–9.1 per 1,000 catheter days, on average [118]. Systemic treatment with antibiotics may last from 7 to 21 days, depending on the type of infection, and treatment success rates are less than 100 %, due to difficulty in eradication of organisms from biofilms that form in the catheter lumen [119]. The biofilm is a protein-fibrin matrix that, in essence, traps bacteria and then allows for bacterial duplication and continued shedding into the bloodstream [120]. One technique that may be employed to prevent difficult infections is antibiotic-lock technique, a technique that involves filling the central venous access device with a specific antibiotic solution. The antibiotic is in direct contact with the catheter lumen and thus the biofilm, allowing for greater eradication of infectious pathogens, typically in a shorter amount of time (i.e., 1–2 weeks) [121]. The Infectious Diseases Society of America recognizes this technique as an approach to treating and salvaging central venous access devices [122]. Implementation of this technique requires knowledge of the infectious organism for treatment, and for prophylaxis, the decision to use a specific antibiotic based on previous infection history dictates the antibiotic of choice. There are many types of antibiotic-lock therapy, typically consisting of vancomycin, gentamicin or tobramycin, or fluconazole, to name a few. Solutions usually contain an anticoagulant such as heparin, as well, in order to maintain catheter patency as well as aid in degradation of the fibrin sheath in the biofilm layer [121]. Antibiotic-lock therapies have been studied in several patient populations with long-term indwelling central venous access devices with overall favorable results.

Ethanol-Lock Therapy

In addition to targeted antibiotic-lock therapy, the use of ethanol-lock therapy has been shown to be an effective therapy to prevent infections [114]. Ethanol-lock therapy was first introduced in 2003 for use in oncology patients; however, use has expanded to include several other patient populations, including those patients with intestinal failure-associated liver disease (IFALD) on long-term PN [123, 124]. Ethanol is antimicrobial and fungicidal (protein denaturation) as well as fibrinolytic (prevention of fibrin sheath formation) at concentrations ranging from 40 to 100 %, and it offers the advantage of covering multiple organisms rather than targeting specific organisms with specific antibiotic-lock therapy [125, 126]. The risk of development of antimicrobial resistance is also limited with the use of ethanol-lock rather than antibiotic-lock therapy. A meta-analysis published in 2012 evaluated the use of ethanol-lock therapy as compared to heparin-lock therapy in a total of 53 patients from four observational studies [114]. Overall, while some variability exists in study methods, a 70 % ethanol solution dwelled in the catheter for at least 2 h ranging from daily to 3 days per week at a volume of 0.2–3 mL is reported. The results of the meta-analysis revealed a mean rate difference of CRBSIs of 7.67 (95 % CI 5.87, 9.47; $p<0.0001$) and a reduction in risk of CRBSIs by 81 % (RR 0.19, 95 % CI 0.12, 0.32; $p<0.0001$). Despite these more robust findings, the combined results revealed only weak evidence that ethanol-lock therapy decreases the need for catheter replacement. The number needed to treat (NNT) in included studies in the meta-analysis ranged from 108 to 150 ethanol-lock days to prevent one CRBSI, a clinically relevant effect. Interestingly, the microbiological cause of infection, when infections do occur while receiving ethanol-lock therapy, may be altered, as demonstrated by Cober et al. [127]. Specifically, infections were caused by *Staphylococcus aureus* and *S. epidermidis* only, rather than by some of the more common pathogens such as *Enterococcus* spp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Candida* spp.

Safety concerns with ethanol-lock therapy include the loss of catheter integrity as well as systemic exposure to ethanol. Ethanol's effect on both polyurethane and silicone catheters has been reported in the literature; reports of a lower

breakage force as well as decreased elasticity have been reported [114]. The clinical significance of these observations is not yet known; however, the greater observed effects on polyurethane have led to avoidance of concomitant use with polyurethane catheters and to limit use of ethanol-lock therapy to silicone-based catheters only [125–128]. Additionally, ethanol-lock therapy has not been well studied in peripherally inserted central venous catheters or implantable ports [126]. It is of the utmost importance to determine the type of catheter a patient has prior to initiating ethanol-lock therapy. Similarly, the clinical significance of systemic exposure to small volumes of ethanol is unknown. One study allowed systemic flushing of ethanol without reported adverse effects [126]; however, it is recommended to withdraw the ethanol solution whenever able [125]. Of note, upon administration of the first dose of ethanol-lock therapy, the solution must be withdrawn to prevent introduction of bacteria into the patient after initial disruption of the biofilm layer [127]. Furthermore, Cober et al. provide estimated blood ethanol levels if the entire ethanol lock is infused into patients, which allows for a targeted safety margin where the ethanol-lock volume will not exceed half of the anticipated intoxication blood level [127]. Finally, by limiting use to patients who weigh at least 5 kg, systemic effects of ethanol, should exposure occur, will be limited [125, 127, 128]. Additionally, studies have not reported any observable signs or symptoms of intoxication [126, 127]. Other adverse effects of ethanol-lock therapy are rare and may include thrombotic events [114, 126–129].

Ethanol-lock therapy is typically compounded using a 98 % dehydrated ethanol, USP, and sterile water for injection to make a final concentration of 70 % ethanol [125, 127]. This solution has been found to be stable at room temperature for up to 14 days [130]. It is incompatible with citrate and heparin, so catheters must be flushed with normal saline before and after administration in order to prevent incompatibilities [130]. The volume of the ethanol lock should be individualized based on the volume of the patient's central venous access device. This may be done by aspirating from the catheter until blood return is noted and then using this volume plus 0.1–0.2 mL as the ethanol-lock volume [125, 127].

Supportive data for ethanol-lock therapy comes mainly from retrospective studies as well as case reports and case series. More robust data from randomized, controlled trials is warranted. It has been suggested that these studies should focus on the lowest effective concentration of ethanol, optimal dwell time, optimal administration regimen (i.e., daily vs. 3 days/week), as well as clinically relevant side effects [129]. At this time, ethanol-lock therapy is not endorsed by the Infectious Diseases Society of America, despite published convincing data; however, it is noted that supporting evidence is growing and the role in prevention in CRBSI is becoming clearer [122]. Interestingly, in a recent investigation that evaluated the effects of a national shortage of the ethanol product used to compound medicinal ethanol-lock therapy (98 % dehydrated ethanol), failure of CRBSI prophylaxis was demonstrated when use was rationed, further supporting the use of this therapeutic modality [131].

Surgical Options

Surgical therapy in intestinal failure consists of maintaining bowel length at initial presentation and subsequent autologous intestinal reconstructive surgery. In those patients in whom bowel adaptation does not occur and whose liver disease progresses while on parenteral nutrition, small bowel or multivisceral transplantation remains the therapeutic option.

Bowel Conservation

Patient outcomes in intestinal failure (IF) patients are dependent on the remaining length of the small bowel [26, 132]. But the combination of PNALD and loss of small bowel represents an additive risk factor for mortality in infants with SBS (Fig. 17.2) [26]. It is, therefore, crucial at initial operation that every effort be made to maintain as much bowel length as possible. This often requires multiple explorations with the resection of only frankly necrotic bowel at each procedure.

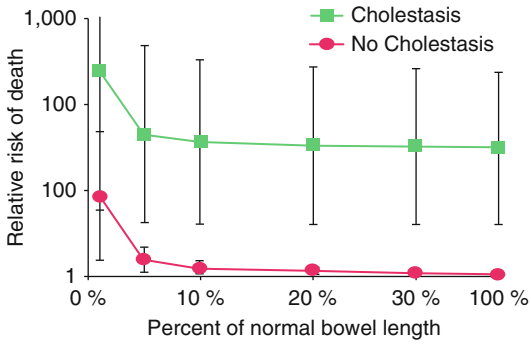

Fig. 17.2 Relative risk of death in a cohort of 102 infants and children with SBS stratified by their percent of normal small bowel length. Note an almost 100-fold great risk of death for any length of small bowel in infants with associated PN-associated cholestasis (Modified from previously reported data [27])

Enteral feeds allow intestinal adaptation and bowel growth that can allow for expedited weaning from PN. The process of adaptation, however, often results in significant small bowel dilation with those segments characterized by poor transit and significant stasis [133]. While ostomies are often crucial in the management of neonates and children with SBS, early ostomy closure can improve electrolyte and fluid balance and has been shown to be associated with a faster wean from PN [134–136]. Other derangements that occur in IF include decreased intestinal transit time that can lead to stasis, bacterial overgrowth, and malabsorption as well as diarrhea and electrolyte derangements. These problems cannot always be treated adequately with supplementation or changes in enteral formulas. Surgical interventions in IF are aimed at eliminating or minimizing these complications with an ultimate goal of restoring enteral autonomy [133].

Autologous Intestinal Reconstruction Surgery

While the goals of early surgical and continued medical management in IF involve maintaining bowel length, initiating early enteral feeds, and avoiding the potentially fatal complications of long-term PN, a significant number of children will progress to surgical therapy in an attempt to increase their bowel length [137]. Surgical bowel lengthening is primarily indicated when children have reached a plateau in the ability to advance enteral feeding and a continued dependence on PN [136].

Over the years numerous surgical procedures have been used to increase bowel length and/or slow intestinal transit time. Earliest among these procedures were reversed intestinal segments, recirculating bowel loops, colonic interposition, as well as tapering or plicating dilated bowel segments [138–141]. Intestinal tapering as a primary surgical modality in pediatric IF is often not an option given the significant number of patients with limited short bowel length. Most of the promising data regarding reversed intestinal segments was obtained in dogs, and although some successful data exist for adults, these results were not replicated in children perhaps because the preferred length of bowel to be used was variable across procedures [142, 143]. Recirculating bowel loops involved creation of a circular loop of bowel to effectively increase mucosal surface area and allow for increased nutrient absorption. These procedures, however, were often complicated by obstruction, volvulus, and stasis that limited their widespread use [139, 144]. Colonic interposition procedures – which involved inserting an isoperistaltic segment of colon along the small bowel length – were first described in dogs before being applied to children [140, 145]. Success in these first case series to be reported were mixed and related to the remnant small bowel length as well as the length of colon which was interposed [146, 147]. Long-term success in the pediatric population using this procedure has not been defined although some isolated case reports exist that show that, at least in isolated instances, success is possible [148, 149]. Due to the complex nature of some of these procedures as well as only modest clinical success, these have essentially been replaced by a variety of autologous intestinal reconstruction procedures with small bowel transplantation remaining a viable option in children with progressive liver disease and PN dependence [150, 151].

Autologous intestinal reconstruction surgery (AIRS) consists of a number of different surgical

procedures, all of which use the patient's own bowel to improve length and absorptive capacity. The three main AIRS procedures in use today include the Bianchi procedure and its modifications, the Iowa procedure, and the serial transverse enteroplasty (STEP).

The longitudinal intestinal lengthening and tailoring operation (LILT) was first described by Bianchi in 1980 [152]. This procedure takes advantage of the fact that the small bowel is supplied by a mesentery which can be divided in half without compromising intestinal blood supply. In order to perform a Bianchi procedure, the mesentery is carefully divided into its two halves along the length of dilated small bowel. The bowel is then stapled to create two new lumens along the mesenteric sheets. The bowel is then anastomosed in an isoperistaltic manner. The result is a doubled length of bowel of half its original diameter. The obvious benefits of this procedure include its ability to easily double the length of small bowel and to halve its diameter – in this manner dramatically increasing the mucosal absorptive surface available to nutrients and decreasing the stasis and potential bacterial overgrowth of intestinal contents. The biggest drawback to this procedure is the technical skill required and the increased morbidity inherent in the number of bowel anastomoses required as well as the suture or staple line along the small bowel [153]. In addition, the Bianchi procedure can only be performed once on a given segment of bowel given its reliance on longitudinal division of the mesentery [154]. Numerous authors have reported long-term success using this procedure although complications consisting of adhesive bowel obstruction, anastomotic strictures, and redilation of the involved bowel are not uncommon [133, 155–157]. A modification of the original Bianchi procedure has been reported which decreases the number of anastomoses required, but details of the long-term success of this procedure have not yet been reported in the literature [153].

The most recently developed and widely used intestinal lengthening procedure is the serial transverse enteroplasty (STEP) [158]. In this technique a stapling device is used to create alternating mesenteric and antimesenteric divisions in a length of dilated small bowel (Fig. 17.3). The advantages of this procedure are that it both lengthens and tapers dilated small bowel, it is relatively easy to perform, and it can be used in a bowel that does not exhibit uniform dilation [159, 160]. Other advantages include the ability to repeat the procedure when the small bowel, invariably, dilates again [154, 161, 162]. Using a registry of STEP procedures performed worldwide, long-term results were published in 2007. In this cohort of 38 patients from 19 centers, small bowel length was increased from 68 ± 44 cm pre-STEP to 115 ± 87 cm post-STEP, a relative increase of 67 %. All but three patients had improved enteral nutrition tolerance following the procedure. Operative complications were considered relatively minor and consisted of leakage from a staple line in two patients and two bowel obstructions that were managed conservatively. Five patients developed progressive liver dysfunction and were referred for transplantation. Three patients died of progressive liver failure and/or sepsis [160]. A second report on the long-term outcomes of the STEP procedure demonstrated that these children exhibit improved

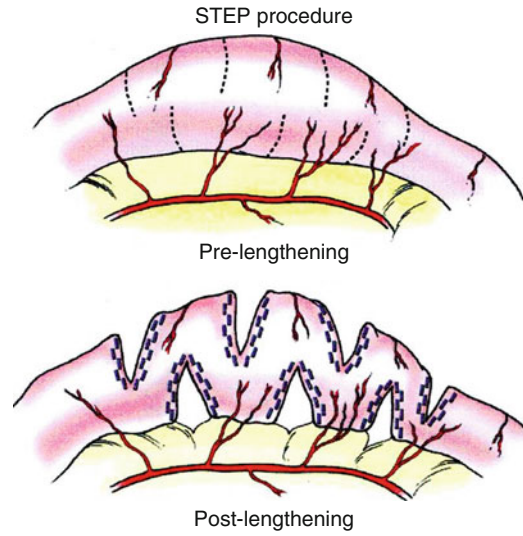

Fig. 17.3 Diagram showing the pre- and postoperative appearance of small bowel before and after serial transverse enteroplasty (STEP) procedure (Reproduced with permission from Keith Georgeson)

growth parameters including increased weight-for-age, height-for-age, and weight-for-height Z scores [159, 163]. Two reports have been published that describe the ability to repeat the STEP procedure following a previous lengthening procedure [154, 162].

Conclusion/Summary

In summary, PNALD remains a serious and potentially life-threatening disease process. While it appears that the mechanisms of action may be multifactorial, many approaches can impact the severity of this disease. Concerted efforts to modify intravenous fat emulsion administration, reduce the incidence of bacterial translocation, and prevent the occurrence of sepsis, use of bile acid therapies and conserve intestinal length all can effectively reduce or even prevent the development of this very difficult hepatic process.

References

1. Suita S, Yamanouchi T, Masumoto K, Ogita K, Nakamura M, Taguchi S. Changing profile of parenteral nutrition in pediatric surgery: a 30-year experience at one institute. Surgery. 2002;131(1 Suppl): S275–82. Epub 2002/02/01.
2. HCUPnet. National Center for Health Statistics Data on PN and EN Use. From the Agency for Healthcare Research and Quality (AHRQ). 2010. http://hcupnet.ahrq.gov/.
3. Willis TC, Carter BA, Rogers SP, Hawthorne KM, Hicks PD, Abrams SA. High rates of mortality and morbidity occur in infants with parenteral nutrition-associated cholestasis. JPEN J Parenter Enteral Nutr. 2010;34(1):32–7. Epub 2009/07/10.
4. Teitelbaum DH, Drongowski R, Spivak D. Rapid development of hyperbilirubinemia in infants with the short bowel syndrome as a correlate to mortality: possible indication for early small bowel transplantation. Transplant Proc. 1996;28(5):2699–700.
5. Btaiche IF, Khalidi N, Kovacevich DS, editors. The University of Michigan Hospital and Health Centers – parenteral and enteral nutrition manual. Ann Arbor: The University of Michigan; 2010.
6. Kelly DA. Liver complications of pediatric parenteral nutrition-epidemiology. Nutrition. 1998;14:153–7.
7. Koseesirikul P, Chotinaruemol S, Ukarapol N. Incidence and risk factors of PN-associated liver disease in newborn infants. Pediatr Int. 2012;54:434–6.
8. Duro D, Mitchell PD, Kalish LA, Martin C, McCarthy M, Jaksic T, et al. Risk factors for PN-associated liver disease following surgical therapy for necrotizing enterocolitis: a Glaser Pediatric Research Network Study. J Pediatr Gastroenterol Nutr. 2011;52: 595–600.
9. Javid P, Malone FR, Dick AA, Hsu E, Sunseri M, Healey P, Horslen SP. A contemporary analysis of parenteral nutrition-associated liver disease in surgical infants. J Pediatr Surg. 2011;46(10):1913–7.
10. Nanji AA, Anderson FH. Sensitivity and specificity of liver function tests in the detection of parenteral nutrition associated cholestasis. J Parenter Enteral Nutr. 1985;9(3):307–8.
11. Beath SV, Booth IW, Murphy MS, Buckels JA, Mayer AD, McKiernan PJ, Kelly DA. Nutritional care and candidates for small bowel transplantation. Arch Dis Child. 1995;73(4):348–50.
12. Naini BV, Lassman CR. Total parenteral nutrition therapy and liver injury: a histopathologic study with clinical correlation. Hum Pathol. 2012;43(6): 826–33.
13. Beath SV, Needham SJ, Kelly DA, Booth IW, Raafat F, Buick RG, Buckels JA, Mayer AD. Clinical features and prognosis of children assessed for isolated small bowel or combined small bowel and liver transplantation. J Pediatr Surg. 1997;32(3): 459–61.
14. Sax HC, Talamini MA, Brackett K, Fischer JE. Hepatic steatosis in total parenteral nutrition: failure of fatty infiltration to correlate with abnormal serum hepatic enzyme levels. Surgery. 1986;100(4):697–704.
15. Btaiche IF, Khalidi N. Metabolic complications of parenteral nutrition in adults, part 2. Am J Health Syst Pharm. 2004;61(19):2050–9.
16. Gurll NJ, Meyer PD, DenBesten L. The effect of cholesterol crystals on gallbladder function in cholelithiasis. Surg Forum. 1977;28:412–3.
17. Allen B, Bernhoft R, Blanckaert N, Svanvik J, Filly R, Gooding G, Way L. Sludge is calcium bilirubinate associated with bile stasis. Am J Surg. 1981;141(1): 51–6.
18. King DR, Ginn-Pease ME, Lloyd TV, Hoffman J, Hohenbrink K. Parenteral nutrition with associated cholelithiasis: another iatrogenic disease of infants and children. J Pediatr Surg. 1987;22(7):593–6.
19. Tsai S, Strouse PJ, Drongowski RA, Islam S, Teitelbaum DH. Failure of cholecystokinin-octapeptide to prevent TPN-associated gallstone disease. J Pediatr Surg. 2005;40(1):263–7.
20. Hofmann AF. Defective biliary secretion during parenteral nutrition: probable mechanisms and possible solutions. J Pediatr Gastroenterol Nutr. 1995;20(4): 376–90.
21. Palmer RH, Ruban Z. Production of bile duct hyperplasia and gallstones by lithocholic acid. J Clin Invest. 1966;45(8):1255–67.
22. Vileisis RA, Inwood RJ, Hunt CE. Laboratory monitoring of parenteral nutrition-associated hepatic dysfunction in infants. J Parenter Enteral Nutr. 1981;5(1):67–9.
23. Btaiche IF, Khalidi N. Parenteral nutrition-associated liver complications in children. Pharmacotherapy. 2002;22(2):188–211.

24. Kurvinen A, Nissinen MJ, Gylling H, Miettinen TA, Lampela H, Koivusalo A, et al. Effects of long-term parenteral nutrition on serum lipids, plant sterols, cholesterol metabolism, and liver histology in pediatric intestinal failure. J Pediatr Gastroenterol Nutr. 2011;53(4):440–6.
25. Ginn-Pease ME, Pantalos D, King DR. TPN-associated hyperbilirubinemia: a common problem in newborn surgical patients. J Pediatr Surg. 1985;20(4):436–9.
26. Spencer AU, Neaga A, West B, Safran J, Brown P, Btaiche I, et al. Pediatric short bowel syndrome: redefining predictors of success. Ann Surg. 2005;242(3):403–9; discussion 9–12. Epub 2005/09/02.
27. Rangel SJ, Calkins CM, Cowles RA, Barnhart DC, Huang EY, Abdullah F, et al. Parenteral nutrition–associated cholestasis: an American Pediatric Surgical Association Outcomes and Clinical Trials Committee systematic review. J Pediatr Surg. 2012;47(1):225–40.
28. Fallon EM, Mitchell PD, Potemkin AK, Nehra D, Arsenault DA, Robinson EM, et al. Cholestasis and growth in neonates with gastroschisis. J Pediatr Surg. 2012;47(8):1529–36.
29. Beale EF, Nelson RM, Bucciarelli RL, Donnelly WH, Eitzman DV. Intrahepatic cholestasis associated with parenteral nutrition in premature infants. Pediatrics. 1979;64(3):342–7.
30. Pereira GR, Sherman MS, DiGiacomo J, Ziegler M, Roth K, Jacobowski D. Hyperalimentation-induced cholestasis: increase frequency and severity in premature infants. Am J Dis Child. 1981;135(9):842–5.
31. Beath SV, Davies P, Papadopoulou A, Khan AR, Buick RG, Corkery JJ, et al. Parenteral nutrition related cholestasis in postsurgical neonates: multivariate analysis of risk factors. J Pediatr Surg. 1996;31(4):604–6.
32. Moseley RH, Wang W, Takeda H, Lown K, Shick L, Ananthanarayanan M, et al. Effect of endotoxin on bile acid transport in rat liver: a potential model for sepsis-associated cholestasis. Am J Physiol. 1996;271(1 Pt 1):G137–46.
33. Trauner M, Arrese M, Lee H, Boyer JL, Karpen SJ. Endotoxin downregulates rat hepatic ntcp gene expression via decreased activity of critical transcription factors. J Clin Invest. 1998;101(10):2092–100.
34. Trauner M, Arrese M, Soroka CJ, Ananthanarayanan M, Koeppel TA, Schlosser SF, Suchy FJ, Keppler D, Boyer JL. The rat canalicular conjugate export pump (Mrp2) is down-regulated in intrahepatic and obstructive cholestasis. Gastroenterology. 1997;113(1):255–64.
35. Simmons MG, Georgeson KE, Figueroa R, Mock 2nd DL. Liver failure in parenteral nutrition dependent children with short bowel syndrome. Transplant Proc. 1996;28(5):2701.
36. Stanko RT, Nathan G, Mendelow H, Adibi SA. Development of hepatic cholestasis and fibrosis with massive loss of intestine supported by prolonged parenteral nutrition. Gastroenterology. 1987;92(1):197–202.
37. El Kasmi KC, Anderson AL, Devereaux MW, Fillon SA, Harris JK, Lovell MA, et al. Toll-like receptor 4-dependent Kupffer cell activation and liver injury in a novel mouse model of PN and intestinal injury. Hepatology. 2012;55(5):1518–28.
38. Colomb V, Goulet O, Rambaud C, De Potter S, Saddoun E, Ben-Hariz M, et al. Long-term parenteral nutrition in children: liver and gallbladder disease. Transplant Proc. 1992;24(3):1054–5.
39. Kaufman SS, Loseke CA, Lupo JV, Young RJ, Murray ND, Pinch LW, Vanderhoof JA. Influence of bacterial overgrowth and intestinal inflammation on duration of parenteral nutrition in children with short bowel syndrome. J Pediatr. 1997;131(3):356–61.
40. Freund HR, Muggia-Sullam M, La France R, Enrione EB, Popp MB, Bjornson HS. A possible beneficial effect of metronidazole in reducing TPN-associated liver function derangements. J Surg Res. 1985;38(4):356–63.
41. Plaa GL, de Lamirande E, Lewittes M, Yousef IM. Liver cell plasma membrane lipids in manganese-bilirubin-induced intrahepatic cholestasis. Biochem Pharmacol. 1982;31(22):3698–701.
42. Fell JM, Reynolds AP, Meadows N, Khan K, Long SG, Quaghebeur G, Taylor WJ, Milla PJ. Manganese toxicity in children receiving long-term parenteral nutrition. Lancet. 1996;347(9010):1218–21.
43. Forbes A, Jawhari A. Manganese toxicity and parenteral nutrition [letter]. Lancet. 1996;347(9017):1774.
44. Jawhari A, Ong C, Wood S, Forbes A. Hypermanganesemia and TPN: a cause for cholestasis? Gastroenterology. 1995;108:A732.
45. Howard L, Ashley C, Lyon D, Shenkin A. Autopsy tissue trace elements in 8 long-term parenteral nutrition patients who received the current U.S. Food and Drug Administration formulation. J Parenter Enteral Nutr. 2007;31(5):388–96.
46. Blaszyk H, Wild PJ, Oliveira A, Kelly DG, Burgart LJ. Hepatic copper in patients receiving long-term parenteral nutrition. J Clin Gastroenterol. 2005;39(4):318–20.
47. Blackmer AB, Bailey E. Management of copper deficiency in cholestatic infants: review of the literature and a case series. Nutr Clin Pract. 2012;28(1):75–86. Epub ahead of print, 2012 Oct 15.
48. Department of Health and Human Services, Food and Drug Administration. Aluminum in large and small volume parenterals used in total parenteral nutrition. Fed Regist. 2000;(6):4103–11.
49. Alemmari A, Miller GG, Bertolo RF, Dinesh C, Brunton JA, Arnold CJ, Zello GA. Reduced aluminum contamination decreases parenteral nutrition associated liver injury. J Pediatr Surg. 2012;47(5):889–94.
50. Demircan M, Ergun O, Coker C, Yilmaz F, Avanoglu S, Ozok G. Aluminum in total parenteral nutrition solutions produces portal inflammation in rats. J Pediatr Gastroenterol Nutr. 1998;26(3):274–8.
51. Poole RL, Schiff L, Hintz SR, Wong A, Mackenzie N, Kerner Jr JA. Aluminum content of parenteral nutrition in neonates: measured versus calculated levels. J Pediatr Gastroenterol Nutr. 2010;50(2):208–11.
52. Ching YA, Gura K, Modi B, Jaksic T. Pediatric intestinal failure: nutrition, pharmacologic, and surgical approaches. Nutr Clin Pract. 2007;22(6):653–63.

53. Le HD, Fallon EM, de Meijer VE, Malkan AD, Puder M, Gura KM. Innovative parenteral and enteral nutrition therapy for intestinal failure. Semin Pediatr Surg. 2010;19(1):27–34.
54. Wessel JJ, Kocoshis SA. Nutritional management of infants with short bowel syndrome. Semin Perinatol. 2007;31(2):104–11.
55. Stout SM, Cober MP. Metabolic effects of cyclic parenteral nutrition infusion in adults and children. Nutr Clin Pract. 2010;25(3):277–81.
56. Jensen AR, Goldin AB, Koopmeiners JS, Stevens J, Waldhausen JH, Kima SS. The association of cyclic parenteral nutrition and decreased incidence of cholestatic liver disease in patients with gastroschisis. J Pediatr Surg. 2009;44(1):183–9.
57. Salvador A, Janeczko M, Porat R, Sekhon R, Moewes A, Schutzman D. Randomized controlled trial of early parenteral nutrition cycling to prevent cholestasis in very low birth weight infants. J Pediatr. 2012;161(2):229–33.
58. Friel C, Bistrian B. Cycled total parenteral nutrition: is it more effective? Am J Clin Nutr. 1997;65(4): 1078–9.
59. Kumpf VJ. Parenteral nutrition-associated liver disease in adult and pediatric patients. Nutr Clin Pract. 2006;21(3):279–90.
60. Tillman EM. Review and clinical update on parenteral nutrition-associated liver disease. Nutr Clin Pract. 2013;28(1):30–9. Epub 2012 Oct 19.
61. Nehra D, Fallon EM, Carlson SJ, Potemkin AK, Hevelone ND, Mitchell PD, et al. Provision of soybased intravenous lipid emulsions at 1 g/kg/day does not prevent cholestasis in neonates. J Parenter Enteral Nutr. 2013;37:498–505. [Epub ahead of print], PMID: 22767698.
62. Kurvinen A, Nissinen MJ, Andersson S, Korhonen P, Ruuska T, Taimisto M, et al. Parenteral plant sterols and intestinal failure-associated liver disease in neonates. J Pediatr Gastroenterol Nutr. 2012;54(6):803–11.
63. Diamond IR, Pencharz PB, Feldman BM, Ling SC, Moore AM, Wales PW. Novel lipid based approaches to pediatric intestinal failure-associated liver disease. Arch Pediatr Adolesc Med. 2012;166(5):473–8.
64. Allardyce DB. Cholestasis caused by lipid emulsions. Surg Gynecol Obstet. 1982;154(5):641–7.
65. Colomb V, Jobert-Giraud A, Lacaille F, Goulet O, Fournet J, Ricour C. Role of lipid emulsions in cholestasis associated with long-term parenteral nutrition in children. J Parenter Enteral Nutr. 2000;24(6):345–50.
66. Cober MP, Killu G, Brattain A, Welch KB, Kunisaki SM, Teitelbaum DH. Intravenous fat emulsions reduction for patients with parenteral nutrition-associated liver disease. J Pediatr. 2012;160(3):421–7.
67. Cober MP, Teitelbaum DH. Prevention of parenteral nutrition-associated liver disease: lipid minimization. Curr Opin Organ Transplant. 2010;15(3):330–3.
68. Meisel JA, Le HD, de Meijer VE, Nose V, Gura KM, Mulkern RV, et al. Comparison of 5 intravenous lipid emulsions and their effects on hepatic steatosis in a murine model. J Pediatr Surg. 2011;46(4):666–73.
69. Gura KM, Duggan CP, Collier SB, Jennings RW, Folkman J, Bistrian BR, Puder M. Reversal of parenteral nutrition associated liver disease in two infants with short bowel syndrome using parenteral fish oil: implications for future management. Pediatrics. 2006;118(1):e197–201.
70. Jain SK, Pahwa M. Management of short gut syndrome by omega 3 fatty acid infusion. Clin Nutr. 2007;26(6):810.
71. Calhoun AW, Sullivan JE. Omegaven for the treatment of parenteral nutrition associated liver disease: a case study. J Ky Med Assoc. 2009;107(2):55–7.
72. Cheung HM, Lam HS, Tam YH, Lee KH, Ng PC. Rescue treatment of infants with intestinal failure and parenteral nutrition-associated cholestasis (PNAC) using a parenteral fish-oil based lipid. Clin Nutr. 2009;28(2):209–12.
73. Chung PH, Wong KK, Wong RM, Tsoi NS, Chan KL, Tam PK. Clinical experience in managing pediatric patients with ultra-short bowel syndrome using omega-3 fatty acid. Eur J Pediatr Surg. 2010;20(2):139–42.
74. Pawlik D, Lauterback R, Turyk E. Fish-oil fat emulsion supplementation may reduce the risk of severe retinopathy in VLBW infants. Pediatrics. 2011;127(2):223–8.
75. Sigalet D, Boctor D, Brindle M, Lam V, Robertson M. Elements of successful intestinal rehabilitation. J Pediatr Surg. 2011;46(1):150–6.
76. Ekema G, Falchetti D, Boroni G, Tanca AR, Altana C, Righetti L, et al. Reversal of severe parenteral nutrition associated liver disease in an infant with short bowel syndrome using parenteral fish oil (omega-3 fatty acids). J Pediatr Surg. 2008;43(6):1191–5.
77. Puder M, Valim C, Meisel JA, Le HD, de Meijer VE, Robinson EM, et al. Parenteral fish oil improves outcomes in patients with parenteral nutrition-associated liver injury. Ann Surg. 2009;250(3):395–402.
78. Fallon EM, Le HD, Puder M. Prevention of parenteral nutrition-associated liver disease: role of omega-3 fish oil. Curr Opin Organ Transplant. 2010;15(3):334–40.
79. Diamond IR, Sterescu A, Pencharz PB, Kim JH, Wales PW. Changing the paradigm; Omegaven for the treatment of liver failure in pediatric short bowel syndrome. J Pediatr Gastroenterol Nutr. 2009;48(2):209–15.
80. Tillman EM, Crill CM, Black DD, Hak EB, Lazar LF, Christensen ML, et al. Enteral fish oil for treatment of parenteral nutrition-associated liver disease in six infants with short bowel syndrome. Pharmacotherapy. 2011;31(5):503–9.
81. Rollins MD, Scaife ER, Jackson WD, Meyers RL, Mulroy CW, Book LS. Elimination of soybean lipid emulsion in parenteral nutrition and supplementation with enteral fish oil improve cholestasis in infants with short bowel syndrome. Nutr Clin Pract. 2010;25(2):199–204.
82. Goulet O, Antebi H, Wolf C, Talbotec C, Alcindor LG, Corriol O, et al. A new intravenous fat emulsion containing soybean oil, medium-chain triglycerides,

olive oil, and fish oil: a single-center, double blind randomized study on efficacy and safety in pediatric patients receiving home parenteral nutrition. J Parenter Enteral Nutr. 2010;34(5):485–95.
83. Tomsits E, Pataki M, Tolgyesi A, Fekete G, Rischak K, Szollar L. Safety and efficacy of a lipid emulsion containing a mixture of soybean oil, olive oil, medium-chain triglycerides and fish oil: a randomized double-blind clinical trial in preterm infants requiring parenteral nutrition. J Pediatr Gastroenterol Nutr. 2010;51(4):514–21.
84. Rayyan M, Devlieger H, Jochum F, Allegaert K. Short-term use of parenteral nutrition with a lipid emulsion containing a mixture of soybean oil, olive oil, medium chain triglycerides, and fish oil: a randomized double blind study in preterm infants. J Parenter Enteral Nutr. 2012;36(1 Suppl):81S–94.
85. Skouroliakou M, Konstantinou D, Koutri K, Kakavelaki C, Stathopoulou M, Antonidai M, et al. A double-blind, randomized clinical trial of the effect of omega-3 fatty acids on the oxidative stress of preterm neonates fed through parenteral nutrition. Eur J Clin Nutr. 2010;64(9):940–7.
86. Muhammed R, Bremner R, Protheroe S, Johnson T, Holden C, Murphy MS. Resolution of parenteral nutrition associated jaundice on changing from a soy-oil emulsion to a complex mixed-lipid emulsion. J Pediatr Gastroenterol Nutr. 2012;54(6):797–802.
87. Ferreira N, Falconer J, Harley C, Rawat D, Fell J, Koglmeier J. Improved serum bilirubin with SMOF lipid in children with intestinal failure on long term parenteral nutrition. Paper presented at: British Society of Paediatric Gastroenterology, Hepatology and Nutrition Winter Meeting, Sheffield; 28–30 Jan 2009.
88. Anish A, Lawson A, Cram H, et al. SMOF lipid in intestinal failure associated liver disease: experience in a regional centre. Paper presented at: British Society of Paediatric Gastroenterology, Hepatology and Nutrition Winter Meeting, Liverpool; 27–29 Jan 2010.
89. Vanek VW, Seidner DL, Allen P, Bistrian B, Collier S, Gura K, et al. A.S.P.E.N. position paper: clinical role for alternative intravenous fat emulsions. Nutr Clin Pract. 2012;27(2):150–92. Epub 2012 Feb 29.
90. Phenobarbital. Lexi-Comp Online™, Pediatric & Neonatal Lexi-Drugs Online™. Hudson: Lexi-Comp, Inc.; 2013.
91. Berger HM. Phenobarbital therapy and parenteral nutrition. J Parenter Enteral Nutr. 1987;11(3):331.
92. Gleghorn EE, Merritt RJ, Subramanian N, Ramos A. Phenobarbital does not prevent total parenteral nutrition-associated cholestasis in noninfected neonates. J Parenter Enteral Nutr. 1986;10(3):282–3.
93. South M, King A. Parenteral nutrition-associated cholestasis: recovery following phenobarbitone. J Parenter Enteral Nutr. 1987;11(2):208–9.
94. San Luis VA, Btaiche IF. Ursodiol in patients with parenteral nutrition-associated cholestasis. Ann Pharmacother. 2007;41(11):1867–72.
95. Trauner M, Graziadei IW. Review article: mechanisms of action and therapeutic applications of ursodeoxycholic acid in chronic liver diseases. Aliment Pharmacol Ther. 1999;13(8):979–96.
96. Ursodiol. Lexi-Comp Online™, Pediatric & Neonatal Lexi-Drugs Online™. Hudson: Lexi-Comp, Inc.; 2013.
97. Kumar D, Tandon RK. Use of ursodeoxycholic acid in liver disease. J Gastroenterol Hepatol. 2001;16(1):3–14.
98. Angulo P. Use of ursodeoxycholic acid in patients with liver disease. Curr Gastroenterol Rep. 2002;4(1):37–44.
99. Lebensztejn DM. Application of ursodeoxycholic acid (UDCA) in the therapy of liver and biliary duct disease in children. Med Sci Monit. 2000;6(3):632–6.
100. Kowdley KV. Ursodeoxycholic acid therapy in hepatobiliary disease. Am J Med. 2000;108(6):481–6.
101. Chen CY, Tsao PN, Chen HL, Chou HC, Hsieh WS, Chang MH. Ursodeoxycholic acid (UDCA) therapy in very-low-birth-weight infants with parenteral nutrition-associated cholestasis. J Pediatr. 2004;145(3): 317–21.
102. Al-Hathlol K, Al-Madani A, Al-Saif S, Abulaimoun B, Al-Tawil K, El-Demerdash A. Ursodeoxycholic acid therapy for intractable total parenteral nutrition-associated cholestasis in surgical very low birth weight infants. Singapore Med J. 2006;47(2):147–51.
103. Levine A, Maayan A, Shamir R, Dinari G, Sulkes J, Sirotta L. Parenteral nutrition-associated cholestasis in preterm neonates: evaluation of ursodeoxycholic acid treatment. J Pediatr Endocrinol Metab. 1999;12(4):549–53.
104. Spagnuolo MI, Iorio R, Vegnente A, Guarino A. Ursodeoxycholic acid for treatment of cholestasis in children on long-term total parenteral nutrition: a pilot study. Gastroenterology. 1996;111(3):716–9.
105. De Marco G, Sordion D, Bruzzese E, Di Caro S, Mambretti D, Tramontano A, et al. Early treatment with ursodeoxycholic acid for cholestasis in children on parenteral nutrition because of primary intestinal failure. Aliment Pharmacol Ther. 2006;24(2):387–94.
106. Heubi JE, Wiechmann DA, Creutzinger V, Setchell KD, Squires Jr R, Couser R, et al. Tauroursodeoxycholic acid (TUDCA) in the prevention of total parenteral nutrition-associated liver disease. J Pediatr. 2002;141(2):237–42.
107. Teitelbaum DH, Han-Markey T, Schumacher RE. Treatment of parenteral nutrition-associated cholestasis with cholecystokinin-octapeptide. J Pediatr Surg. 1995;30(7):1082–5.
108. Rintala RJ, Lindahl H, Pohjavuori M. Total parenteral nutrition-associated cholestasis in surgical neonates may be reversed by intravenous cholecystokinin: a preliminary report. J Pediatr Surg. 1995;30(6):827–30.
109. Teitelbaum DH, Han-Markey T, Drongowski R, Coran AG, Bayar B, Geiger JD, et al. Use of cholecystokinin to prevent the development of parenteral nutrition-associated cholestasis. J Parenter Enteral Nutr. 1997;21(2):100–3.
110. Teitelbaum DH, Tracey Jr TF, Aouthmany MM, Llanos A, Brown MB, Yu S, et al. Use of cholecystokinin-octapeptide for the prevention

of parenteral nutrition-associated cholestasis. Pediatrics. 2005;115(5):1332–40.
111. Wang Y, Tao YX, Cai W, Tang QY, Feng Y, Wu J. Protective effect of parenteral glutamine supplementation on hepatic function in very low birth weight infants. Clin Nutr. 2010;29(3):307–11.
112. Helms RA, Storm MC, Christensen ML, Hak EB, Chesney RW. Cysteine supplementation results in normalization of plasma taurine concentrations in children receiving home parenteral nutrition. J Pediatr. 1999;134(3):358–61.
113. Spencer AU, Yu S, Tracey TF, Aouthmany MM, Llanos A, Brown MB, et al. Parenteral nutrition–associated cholestasis in neonates: multivariate analysis of the potential protective effect of taurine. J Parenter Enteral Nutr. 2005;29(5):337–44.
114. Oliveira C, Nasr A, Brindle M, Wales PW. Ethanol locks to prevent catheter-related bloodstream infections in parenteral nutrition: a meta-analysis. Pediatrics. 2012;129(2):318–29.
115. Dobson R, McGuckin C, Walker G, Lucas C, McGrogan P, Russell RK, et al. Cycled enteral antibiotics reduce sepsis rates in paediatric patients on long-term parenteral nutrition for intestinal failure. Aliment Pharmacol Ther. 2011;34(8):1005–11.
116. Kubota A, Okada A, Imura K, Kawahara H, Nezu R, Kamata S, et al. The effect of metronidazole on TPN-associated liver dysfunction in neonates. J Pediatr Surg. 1990;25(6):618–21.
117. Pierro A, van Saene HK, Jones MO, Brown D, Nunn AJ, Lloyd DA. Clinical impact of abnormal gut flora in infants receiving parenteral nutrition. Ann Surg. 1998;227(4):547–52.
118. National Nosocomial Infections Surveillance System. National nosocomial infections surveillance (NNIS) system report, data summary from January 1992 through June 2004, issued October 2004. Am J Infect Control. 2004;32(8):470–85.
119. Bagnall-Reeb H. Evidence for the use of the antibiotic lock technique. J Infus Nurs. 2004;27(2):118–22.
120. Onder AM, Kato T, Simon N, Rivera-Hernandez M, Chandar J, Montane B, et al. Prevention of catheter-related bacteremia in pediatric intestinal transplantation/short gut syndrome children with long-term central venous catheters. Pediatr Transplant. 2007;11(1):87–93.
121. Bestul MB, Vandenbussche HL. Antibiotic lock technique: review of the literature. Pharmacotherapy. 2005;25(2):211–27.
122. Mermel LA, Allon M, Bouza E, Craven DE, Flynn P, O'Grady NP, et al. Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 update by the infectious diseases society of America. Clin Infect Dis. 2009;49(1):1–45.
123. Ball PA, Brokenshire E, Parry B, Merrie A, Gillanders L, McIllroy K, Plank L. Ethanol locking as a possible treatment for microbial contamination of long-term central venous catheters. Nutrition. 2003;19(6):570.
124. Dannenberg C, Bierbach U, Rothe A, Beer J, Korholz D. Ethanol-lock technique in the treatment of bloodstream infections in pediatric oncology patients with broviac catheter. J Pediatr Hematol Oncol. 2003;25(8):616–21.
125. Jones BA, Hull MA, Richardson DS, Zurakowski D, Gura K, Fitzgibbons SC, et al. Efficacy of ethanol locks in reducing central venous catheter infections in pediatric patients with intestinal failure. J Pediatr Surg. 2010;45(6):1287–93.
126. Mouw E, Chessman K, Lesher A, Tagge E. Use of an ethanol lock to prevent catheter-related infections in children with short bowel syndrome. J Pediatr Surg. 2008;43(6):1025–9.
127. Cober MP, Kovacevich DS, Teitelbaum DH. Ethanol-lock therapy for the prevention of central venous access device infections in pediatric patients with intestinal failure. J Parenter Enteral Nutr. 2011;35(1):67–73.
128. Wales PW, Kosar C, Carricato M, de Silva N, Lang K, Avitzur Y. Ethanol lock therapy to reduce the incidence of catheter-related bloodstream infections in home parenteral nutrition patients with intestinal failure: preliminary experience. J Pediatr Surg. 2011;46(5):951–6.
129. Wong T, Clifford V, McCallum Z, Shalley H, Peterkin M, Paxton G, et al. Central venous catheter thrombosis associated with 70% ethanol locks in pediatric intestinal failure patients on home parenteral nutrition: a case series. J Parenter Enteral Nutr. 2012;36(3):358–60.
130. Cober MP, Johnson CE. Stability of 70% alcohol solutions in polypropylene syringes for use in ethanol-lock therapy. Am J Health Syst Pharm. 2007;64(23):2480–2.
131. Ralls MW, Blackwood RA, Arnold MA, Partipilo ML, Dimond J, Teitelbaum DH. Drug shortage-associated increase in catheter related blood stream infection in children. Pediatrics. 2012;130(5):e1369–73.
132. Goulet OJ, Revillon Y, Jan D, De Potter S, Maurage C, Lortat-Jacob S, et al. Neonatal short bowel syndrome. J Pediatr. 1991;119(1 Pt 1):18–23. Epub 1991/07/01.
133. Barksdale EM, Stanford A. The surgical management of short bowel syndrome. Curr Gastroenterol Rep. 2002;4(3):229–37. Epub 2002/05/16.
134. Andorsky DJ, Lund DP, Lillehei CW, Jaksic T, Dicanzio J, Richardson DS, et al. Nutritional and other postoperative management of neonates with short bowel syndrome correlates with clinical outcomes. J Pediatr. 2001;139(1):27–33. Epub 2001/07/11.
135. Gertler JP, Seashore JH, Touloukian RJ. Early ileostomy closure in necrotizing enterocolitis. J Pediatr Surg. 1987;22(2):140–3. Epub 1987/02/01.
136. Chaet MS, Farrell MK, Ziegler MM, Warner BW. Intensive nutritional support and remedial surgical intervention for extreme short bowel syndrome. J Pediatr Gastroenterol Nutr. 1994;19(3):295–8. Epub 1994/10/01.

137. Diamanti A, Basso MS, Castro M, Calce A, Pietrobattista A, Gambarara M. Prevalence of life-threatening complications in pediatric patients affected by intestinal failure. Transplant Proc. 2007;39(5):1632–3. Epub 2007/06/21.
138. Venables CW, Ellis H, Smith AD. Antiperistaltic segments after massive intestinal resections. Lancet. 1966;2(7478):1390–4. Epub 1966/12/24.
139. Altman DP, Ellison EH. Massive intestinal resection: inadequacies of the recirculating loop. Surg Forum. 1965;16:365–7. Epub 1965/01/01.
140. Hutcher NE, Salzberg AM. Pre-ileal transposition of colon to prevent the development of short bowel syndrome in puppies with 90 percent small intestinal resection. Surgery. 1971;70(2):189–97. Epub 1971/08/01.
141. de Lorimier AA, Harrison MR. Intestinal plication in the treatment of atresia. J Pediatr Surg. 1983;18(6):734–7. Epub 1983/12/01.
142. Priebe Jr CJ. Reversed intestinal segments in young puppies with massive intestinal resections. J Pediatr Surg. 1970;5(2):215–23. Epub 1970/04/01.
143. Warner B, Chaet M. Nontransplant surgical options for management of the short bowel syndrome. J Pediatr Gastroenterol Nutr. 1993;17(1):1–12.
144. Muraki T, Singleton AO Jr. Effects of recirculating jejunal loops on absorption and transit after massive bowel resection in dogs. Surg Forum. 1964;15:291–2. PMID:14189373.
145. Hutcher NE, Mendez-Picon G, Salzberg AM. Prejejunal transposition of colon to prevent the development of short bowel syndrome in puppies with 90 per cent small intestine resection. J Pediatr Surg. 1973;8(5):771–7. Epub 1973/10/01.
146. Garcia VF, Templeton JM, Eichelberger MR, Koop CE, Vinograd I. Colon interposition for the short bowel syndrome. J Pediatr Surg. 1981;16(6):994–5. Epub 1981/12/01.
147. Glick PL, de Lorimier AA, Adzick NS, Harrison MR. Colon interposition: an adjuvant operation for short-gut syndrome. J Pediatr Surg. 1984;19(6):719–25. Epub 1984/12/01.
148. Devesa JM, Botella-Carretero JI, Lopez Hervas P, Rey A, Die J, Calero A. Ultrashort bowel syndrome: surgical management and long-term results of an exceptional case. J Pediatr Surg. 2008;43(3):E5–9. Epub 2008/03/25.
149. Sudan D, DiBaise J, Torres C, Thompson J, Raynor S, Gilroy R, et al. A multidisciplinary approach to the treatment of intestinal failure. J Gastrointest Surg. 2005;9(2):165–76; discussion 76–7. Epub 2005/02/08.
150. Jones BA, Hull MA, McGuire MM, Kim HB. Autologous intestinal reconstruction surgery. Semin Pediatr Surg. 2010;19(1):59–67. Epub 2010/02/04.
151. Nayyar N, Mazariegos G, Ranganathan S, Soltys K, Bond G, Jaffe R, et al. Pediatric small bowel transplantation. Semin Pediatr Surg. 2010;19(1):68–77. Epub 2010/02/04.
152. Bianchi A. Intestinal loop lengthening – a technique for increasing small intestinal length. J Pediatr Surg. 1980;15(2):145–51. Epub 1980/04/01.
153. Chahine AA, Ricketts RR. A modification of the Bianchi intestinal lengthening procedure with a single anastomosis. J Pediatr Surg. 1998;33(8):1292–3. Epub 1998/08/29.
154. Ehrlich PF, Mychaliska GB, Teitelbaum DH. The 2 STEP: an approach to repeating a serial transverse enteroplasty. J Pediatr Surg. 2007;42(5):819–22. Epub 2007/05/16.
155. Thompson JS, Pinch LW, Young R, Vanderhoof JA. Long-term outcome of intestinal lengthening. Transplant Proc. 2000;32(6):1242–3. Epub 2000/09/21.
156. Waag KL, Hosie S, Wessel L. What do children look like after longitudinal intestinal lengthening. Eur J Pediatr Surg. 1999;9(4):260–2. Epub 1999/10/26.
157. Bianchi A. From the cradle to enteral autonomy: the role of autologous gastrointestinal reconstruction. Gastroenterology. 2006;130(2 Suppl 1):S138–46. Epub 2006/02/14.
158. Kim HB, Fauza D, Garza J, Oh JT, Nurko S, Jaksic T. Serial transverse enteroplasty (STEP): a novel bowel lengthening procedure. J Pediatr Surg. 2003;38(3):425–9.
159. Ching YA, Fitzgibbons S, Valim C, Zhou J, Duggan C, Jaksic T, et al. Long-term nutritional and clinical outcomes after serial transverse enteroplasty at a single institution. J Pediatr Surg. 2009;44(5):939–43. Epub 2009/05/13.
160. Modi BP, Javid PJ, Jaksic T, Piper H, Langer M, Duggan C, et al. First report of the international serial transverse enteroplasty data registry: indications, efficacy, and complications. J Am Coll Surg. 2007;204(3):365–71. Epub 2007/02/28.
161. Piper H, Modi BP, Kim HB, Fauza D, Glickman J, Jaksic T. The second STEP: the feasibility of repeat serial transverse enteroplasty. J Pediatr Surg. 2006;41(12):1951–6. Epub 2006/12/13.
162. Andres AM, Thompson J, Grant W, Botha J, Sunderman B, Antonson D, et al. Repeat surgical bowel lengthening with the STEP procedure. Transplantation. 2008;85(9):1294–9. Epub 2008/05/14.
163. Duggan C, Piper H, Javid PJ, Valim C, Collier S, Kim HB, et al. Growth and nutritional status in infants with short-bowel syndrome after the serial transverse enteroplasty procedure. Clin Gastroenterol Hepatol. 2006;4(10):1237–41. Epub 2006/08/15.

Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis

18

Nidhi P. Goyal and Jeffrey B. Schwimmer

Nonalcoholic fatty liver disease, or NAFLD, is the leading cause of chronic liver disease in children [1]. NAFLD was first described in children in 1983 [2] and includes a histologic spectrum of disease. NAFLD ranges from macrosteatosis, accumulation of triglycerides in the hepatocyte, to steatosis with inflammation, to fibrosis. Nonalcoholic steatohepatitis, or NASH, is the term used for steatosis accompanied by inflammation and cellular injury and lies within the spectrum of NAFLD. Liver fibrosis, including cirrhosis, lies at the extreme end of NAFLD. NAFLD can lead to hepatocellular carcinoma as well as end-stage liver disease ultimately requiring liver transplantation.

The diagnosis of NAFLD is one made by the combination of clinical and histologic findings. NAFLD is frequently suspected based on elevated serum alanine aminotransferase (ALT) in obese children; however, the differential diagnosis of elevated ALT should be appropriately evaluated and other etiologies of liver disease must be ruled out. Histologic evaluation is required to confirm NAFLD and to identify the severity of disease.

This chapter will highlight current research in pediatric NAFLD and focus on the epidemiology, pathogenesis, genetics, clinical presentation, diagnosis, treatment, and associated comorbidities that are relevant to a pediatric clinician.

Epidemiology

Prevalence

Estimating the prevalence of NAFLD is difficult, as diagnosis requires a liver biopsy. The Study of Child and Adolescent Liver Epidemiology (SCALE) was a population-based autopsy study designed to estimate the prevalence of NAFLD. Autopsy data from 742 children aged 2–19 between 1993 and 2003 in San Diego County was evaluated, and the prevalence of fatty liver, after adjusting for age, race, gender, and ethnicity, was 9.6 % [1]. The prevalence of NAFLD was higher (38 %) for obese children. Additionally, the prevalence was higher for older children, with a range from 0.7 % for 2–4-year-olds to 17.3 % for 15–19-year-olds.

Some studies have used alanine aminotransferase (ALT) as a putative surrogate marker for NAFLD when biopsy data was not available. According to National Health and Nutrition Examination Survey (NHANES) data on over 5,500 adolescents from 1999 to 2004, ALT >30 U/L was present in 8 % of adolescents [3]. In a

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large cohort of over 16,000 European children and adolescents from Germany, Austria, and Switzerland, the prevalence of ALT >50 U/L was 12.4 % [4] and varied by BMI, with 6.7 % of normal weight children having an ALT >50 U/L and 17 % of children with BMI >99.5 % for age having these higher ALT levels.

Race and Ethnicity

The prevalence of NAFLD also varies with respect to race and ethnicity with the highest prevalence in Hispanic children and lowest in black children. In the SCALE study, NAFLD was present in 1.5 % of black children, 8.6 % of white children, 10.2 % of Asian children, and 11.8 % of Hispanic children [1]. There were similar racial trends with prevalence of elevated ALT according to NHANES data: 6 % in blacks, 7.4 % Caucasians, and 11.5 % in Hispanics [3].

Sex

There are also notable differences in the prevalence of NAFLD in boys compared to girls, with boys having a higher prevalence. In the SCALE study, the prevalence was 11.1 % in males in San Diego and 9.7 % in females [1]. The ALT trends are similar in NHANES data with 12.4 % of male adolescents with elevated ALT compared to 3.5 % of female adolescents [3].

Prevalence of NASH

Children with NASH are at greatest risk for severe complications and morbidity related to their disease. The prevalence of NASH, however, varies based on the clinical setting and has been a moving target as definitions of NASH have evolved over time. In fatty liver clinics in San Diego and Rome in the years 2003 to 2006, the prevalence of NASH of all patients that were biopsied was 84 % [5] and 86 % [6], respectively, with 5–8 % of these children having advanced fibrosis. This initial high prevalence of NASH may have been due in part to referral bias. In later studies, when NASH was better defined and screening uncovered patients with wider disease severity, the prevalence was lower ranging from about 25–40 %. In the SCALE study, 23 % of children with NAFLD had NASH, with 2 % having advanced fibrosis [1]. In a series of patients with bariatric surgery, NASH was demonstrated in 24 % of intraoperative biopsies [7]. In the NASH CRN, 36 % of children with NAFLD had definite NASH [8].

Pathogenesis

Understanding the pathogenesis of NAFLD is useful for informed treatment of fatty liver disease in children. Therefore, pathogenesis relevant to treatment will be discussed here.

Insulin Resistance

Insulin resistance is one of the primary mechanisms proposed in the pathogenesis of NAFLD, thus many of the proposed treatments of NAFLD target insulin resistance. Insulin resistance can be defined as the impaired response of glucose to a given concentration of insulin. In a study of pediatric biopsy-proven NAFLD, 95 % met criteria for insulin resistance using HOMA-IR (Homeostatic Model of Assessment – Insulin Resistance) and 75 % had fasting hyperinsulinemia [5]. Thus, systemic insulin resistance may be important to the pathogenesis of pediatric NAFLD. Insulin decreases the output of glucose from the liver directly by decreasing glycogenolysis and indirectly by decreasing gluconeogenesis. Insulin also acts by decreasing the flux of free fatty acids (FFA) to the liver by decreasing adipocyte lipolytic activity. Impaired tissue response to insulin thus causes an increased adipose lipolytic response than would be expected, leading to increased peripheral FFA, which are the predominant source of intrahepatic triglycerides [9]. It has been shown that in patients with NAFLD and insulin resistance, insulin does not suppress adipose tissue lipolysis as it does in healthy patients without insulin resistance [10]. In a small study of obese adolescents with steatosis on magnetic resonance spectroscopy (MRS), it was demonstrated that children with increased hepatic

triglyceride content had increased adipose lipolytic activity and increased serum FFA levels [11]. Additionally, children with insulin resistance have more de novo lipogenesis (DNL) [5]. Due to peripheral insulin resistance, the combination of increased FFA circulation and DNL is implicated in the pathogenesis of NAFLD.

Adipokines and Inflammation

TNFα and Resistin

TNFα and resistin induce insulin resistance and inflammation via stress-related protein kinases (JNK-1) and the NFκB pathway. NFκB translocation into the nucleus induces chronic hepatic inflammation via enhancing synthesis of IL-6, TNFα, and IL1β leading to chronic inflammation and insulin resistance [12, 13]. To translate to potential therapeutic options, antagonism of these pathways may help reduce insulin resistance.

IL-6 and Leptin

Leptin, TNFα, and IL-6 also promote hepatic fibrogenesis through hepatic stellate cells (HSC) activation, both directly by binding to HSC receptors and indirectly through transforming growth factor-β secretion by Kupffer cells [14–16]. IL-6 levels have been demonstrated to be elevated in those with NASH compared to those with isolated steatosis or healthy controls [17].

Free Fatty Acids

Plasma free fatty acids (FFA) have also been implicated in the pathogenesis of NAFLD as pediatric patients with NAFLD have demonstrated elevated levels of circulating FFA. It is also known that increased circulating FFA impairs insulin signaling and increases skeletal muscle and hepatic insulin resistance [18]. Current "lipotoxicity" theories indicate that hepatic neutral triglyceride (TG) accumulation may not be toxic, but rather it protects the liver by buffering the toxic FFA, and the hepatic TG accumulation is a by-product. The hepatotoxicity of FFA is theorized to be generated by increased hepatocyte apoptosis [19], NFκB pathway activation [20], and triggering endoplasmic reticulum stress and the unfolded protein response [21, 22]. Translating this to potential therapies for NAFLD, in adults the use of acipimox, which inhibits hepatic FFA uptake, improved hepatic injury and insulin sensitivity without affecting liver triglyceride stores [23].

PPAR-γ Signaling

Peroxisome proliferator-activated receptor-γ (PPAR-γ) signaling is essential for adipocyte maturation. It is a nuclear receptor that is involved in mediating adipocyte plasticity and the adipocyte's ability to adapt to overfeeding via hypertrophy. This mechanism potentially protects other tissues from exposure to FFA [24]. PPAR-γ agonists may play a role in multiorgan anti-inflammatory effect as PPAR-γ, in addition to distribution in adipose, are also present in macrophages, stromal vascular cells, and the liver. Thiazolidinediones (TZDs), such as rosiglitazone and pioglitazone, have been used for treatment of diabetes and primarily act via activating PPAR-γ. TZDs were used for therapeutic NAFLD trials because of their ability to target Kupffer cells and exert an anti-inflammatory response in NASH [25, 26].

Adiponectin

Adiponectin may be involved in the pathogenesis of NAFLD; however, unlike other adipokines mentioned previously, adiponectin may be protective. Adiponectin is a protein that is secreted by adipocytes and is involved in glucose homeostasis and fatty acid catabolism [27]. Obese children with elevated ALT have lower levels of adiponectin compared to obese and normal weight controls with normal ALT [28]. Similarly, obese adolescents with high hepatic fat (as measured by MRI) have lower levels of adiponectin [29]. Interestingly, treatment with pioglitazone for 6 months increased levels of plasma adiponectin and improved insulin resistance in adults with NASH [30]. Additionally, in the same study increased adiponectin was also significantly associated with histologic improvement with improved steatosis, necroinflammation, and most importantly fibrosis.

The pathogenesis of NAFLD and NASH is complex and involves an intricate interplay of insulin resistance and adipocytokines/inflammatory cytokines as well as FFA. These intermeshed pathways are the subjects of numerous treatment trials that are ongoing. However, the precise mechanism of progressive NASH and why some patients with steatosis progress to NASH and others do not is yet to be understood.

Genetics

While current treatments focus on modifying environmental factors, it is likely that NAFLD has a strong genetic component as evidenced by two observations. The first observation is the racial and ethnic differences in the prevalence of NAFLD discussed in the Epidemiology section, and the second was that NAFLD tends to cluster in families. In a heritability study by Schwimmer and colleagues, 33 obese children with biopsy-proven NAFLD, 11 obese children without NAFLD, and 152 of their family members (parents, siblings, 2nd- or 3rd-degree relatives) were studied [31]. Hepatic fat fraction of family members was evaluated by MRI. In children without NAFLD, 17 % of siblings and 37 % of parents had NAFLD compared to 59 % of siblings and 78 % of parents of children with biopsy-proven NAFLD. The heritability estimates (with 0 being no heritability and 1 representing a trait that is completely heritable) were 0.85 for the unadjusted dichotomous variable for NAFLD and 1.0 after adjusting for age, gender, race, and BMI. For the continuous measurement of NAFLD, the heritability estimates were 0.58 and 0.39 for the unadjusted and adjusted estimates, respectively. As of yet, the mechanism for the progression from steatosis to steatohepatitis remains unclear. Additionally, it is not known why NAFLD occurs in some obese individuals and not others, and this suggests that genetics may be a modifying factor. Thus, there is likely an interplay of environment and genetics that is involved in the pathogenesis of NAFLD.

PNPLA3

The hope in understanding the genetics behind NAFLD is the possibility that this knowledge can help with diagnosis and guide treatment. Recently, a genome-wide association study (GWAS) resulted in the discovery of a single-nucleotide polymorphism (SNP) in the gene *PNPLA3* that confers susceptibility to NAFLD [32].

The Dallas Heart Study was a multiethnic, population-based study in adults ($n = 1{,}032$ African American, 696 European American, and 383 Hispanic) that evaluated hepatic fat content via proton magnetic resonance spectroscopy and performed a GWAS to search for sequence variations [32]. A single variant in *PNPLA3* (rs739409), a cytosine to guanine substitution of codon 148 resulting in a non-synonymous change to methionine from isoleucine, was highly associated with hepatic fat content independent of BMI, diabetes, or alcohol use. Notably, the highest frequency of this allele was present in Hispanics (0.49) followed by European Americans (0.23) and African Americans (0.17), reflecting the pattern seen in NAFLD. Additionally, another study has shown an association with *PNPLA3* and histologic severity in adults, with the minor allele associated with increased steatosis, NASH, and fibrosis [33]. These results indicate that the *PNPLA3* gene locus is not only associated with steatosis but likely also with steatohepatitis, or NASH.

PNPLA3 belongs to the patatin-like phospholipase domain-containing family of proteins, and it encodes a 481 amino acid protein called adiponutrin [32]. While it is unclear what the exact role of *PNPLA3* is in the liver, it is likely involved in lipid metabolism and abnormal accumulation of triglycerides in the human liver. A recent study demonstrated that human carriers of the minor *PNPLA3* allele have decreased hepatic secretion of VLDL [34]. Further studies are needed to determine the activity of PNPLA3 and its function in humans as this may help further elucidate the pathogenesis of NAFLD.

To date, there have been several pediatric studies published regarding *PNPLA3*. These are summarized in Table 18.1. In a study of 475

Table 18.1 Studies evaluating association of *PNPLA3* genotype and pediatric NAFLD

Study	Year	Location	*N*	Age	Population	Outcome measure
PNPLA3 and ALT						
Lin et al. [35]	2011	Taiwan	520	6–18	Obese school-aged children enrolled	AST and ALT
Romeo et al. [36]	2010	Rome, Italy	475	10 (mean)	Obesity Clinic	AST and ALT
PNPLA3 and imaging						
Goran et al. [37]	2010	Los Angeles, CA	188	8–18	Hispanic children in General Clinical Research Center	MRI
Santoro et al. [38]	2010	New Haven, CT	85	8–18	Obesity Clinic	MRI
PNPLA3 and histology						
Rotman et al. [39]	2010	US multicenter	223	8–17	NASH CRN	Liver biopsy
Valenti et al. [40]	2010	Rome, Italy	149	6–13	NAFLD Clinic	Liver biopsy

Abbreviations: *PNPLA3* patatin-like phospholipase domain-containing protein 3, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *MRI* magnetic resonance imaging, *NASH CRN* Nonalcoholic Steatohepatitis Clinical Research Network, *NAFLD* nonalcoholic fatty liver disease

obese or overweight children, children who were homozygous for the minor allele (148 M) had higher levels of circulating ALT compared to those with homozygous wild-type allele (148I). The ALT value was higher for each variant allele of the *PNPLA3* locus present, with the highest ALT in children homozygous for the minor allele [36]. When stratified by genotype, of those subjects with homozygous minor alleles for *PNPLA3*, 32 % had ALT values >30 U/L versus 10 % in those with homozygous wild-type alleles. In a study of 520 obese Taiwanese children, ALT level was also associated with *PNPLA3* genotype in an additive effect, with the highest ALT in children who were homozygous for the variant allele. The variant allele was also associated with ultrasound evidence of hepatic steatosis [35].

Santoro and colleagues evaluated 85 obese children and studied the association of the *PNPLA3* allele with hepatic steatosis, as determined via MRI. They found a positive association of hepatic fat fraction (HFF) with presence of at least one 148 M allele in Caucasian and African American children [38]. In Hispanic children, similar association of *PNPLA3* genotype with hepatic fat fraction by MRI was demonstrated by Goran and colleagues [37].

In terms of histologic association and *PNPLA3*, a study by Rotman and colleagues in 2010 included 223 pediatric patients from the NASH CRN. Unexpectedly, there was no association of the *PNPLA3* locus with the histologic severity of NAFLD [39]. Valenti and colleagues, in a study of 149 Italian pediatric patients with biopsy-proven NAFLD, also evaluated histologic severity [40]. In contrast, this study demonstrated that the *PNPLA3* variant allele was associated with steatosis severity, lobular inflammation, hepatocellular ballooning, and fibrosis. The prevalence of grade 2 and 3 steatosis was greater in children with homozygous variant alleles compared to heterozygotes. Lobular necroinflammation was observed in 3 % of the children with wild-type homozygous alleles, 30 % in carriers of the allele, and 70 % in children with homozygous variant alleles. The variant genotype was associated with higher grade and perivenular fibrosis.

Clinical Presentation

Symptoms

Symptoms of NAFLD can be difficult to assess, as they can be vague and may not be necessarily specific to fatty liver alone. As most studies evaluating symptoms of NAFLD are cross-sectional, causality of these symptoms cannot always be attributable to NAFLD. Longitudinal studies would be needed to assess the timing of development of NAFLD and report of symptoms. Abdominal pain has been a commonly

reported symptom in NAFLD and was an individual-reported symptom in 42 % of children with biopsy-proven NAFLD [5]. In a multicenter study from five centers in North America and Canada, abdominal pain was reported by 18 % of children with biopsy-proven NAFLD [41]. Abdominal pain can be nonspecific and relating it directly to NAFLD may be difficult without prospective studies. In a cross-sectional study from the NASH Clinical Research Network, the most predominantly reported symptoms reported in children with biopsy-proven NAFLD were fatigue and irritability, which were reported in 68 and 73 % of children with NAFLD, respectively [42].

Physical Exam

As children with NAFLD can be asymptomatic or have vague symptomatology, they can go unrecognized. When suspecting NAFLD, the physical exam should be systematic and focus on key findings.

Careful measurement and interpretation of height, weight, and blood pressure are particularly important. The prevalence of NAFLD increases with body mass index (BMI): 38 % for obese children, 16 % in overweight children, and 5 % in normal weight children [1]. BMI should be calculated and plotted at each health visit beginning at the age of two. In contrast to the absolute values for BMI in adults, obesity in children is defined as a BMI percentile ≥95th percentile for age and sex. For the older adolescent female, a BMI of 30 or greater should be considered as obese regardless of percentile, as around age 17 there is a crossover with the adult criteria. Blood pressure measurement with the proper cuff size is important for the obese child, as a small cuff size may falsely overestimate blood pressure. About 13 % of overweight children have systolic hypertension, and nearly 10 % have diastolic hypertension based on age and height percentiles [43]. Three readings of systolic or diastolic measurements >95 % for height percentile indicate stage 1 hypertension and require further evaluation.

A complete head to toe physical exam should follow the vital signs measurements. This section will highlight those parts of the exam focused on NAFLD and obesity. The head and neck exam should assess for tonsillar hypertrophy as an evaluation of possible obstructive sleep apnea. Thyromegaly or the presence of a goiter should prompt diagnostic testing of thyroid disease. The abdominal exam should focus on liver size as nearly half of patients with biopsy-proven NAFLD have hepatomegaly. Palpating for hepatomegaly is difficult in obese children but can be achieved with a methodical approach. The child should be supine, with knees flexed to relax abdominal musculature. Starting with applying gentle pressure to palpate in the right lower quadrant near the anterior superior iliac spine, the exam should then proceed up towards the rib cage. Any extension of the liver edge below the costal margin is suggestive of hepatomegaly and merits closer evaluation. Similarly, palpation and percussion for splenomegaly should be performed, as splenomegaly can be a consequence of liver disease.

A thorough musculoskeletal exam, including assessment of gait, passive range of motion, and examination for bowing of the lower extremities, can uncover several comorbidities of obesity that may otherwise be asymptomatic including Blount disease and slipped capital femoral epiphysis (SCFE).

Finally, a complete skin exam should include assessment of acanthosis nigricans (AN), which is a marker of hyperinsulinemia and has been observed in about 50 % of children with biopsy-proven NAFLD. It appears as a velvety thickening and hyperpigmentation of the skin that is usually assessed at the neck but can also be seen in other skin folds such as the axilla. AN can be graded from 0 to 4, based on severity, with zero being no AN visible on the neck [44]. Grade 1 is slight hyperpigmentation visible with close inspection, grade 2 is mild hyperpigmentation on the posterior neck, grade 3 extends to the sternocleidomastoid, and grade 4 extends circumferentially around the neck. With weight loss, AN can recede and become more spotty in appearance, at which time the grading system may be more difficult.

Labs

The laboratory work-up for NAFLD includes screening for NAFLD, diagnostic evaluation of NAFLD, and screening for NAFLD comorbidities. These are summarized in Tables 18.2 and 18.3.

Table 18.2 Labs for screening and diagnosis of NAFLD and its comorbidities

Lab	Abnormal value
AST	>25 U/L
ALT	>25 U/L
GGT	>30 U/L
Fasting lipids	
Total cholesterol	≥170 mg/dL
LDL	≥110 mg/dL
Triglycerides	
0–9 years	≥75 mg/dL
10–19 years	≥90 mg/dL
HDL	≤45 mg/dL
Endocrine	
Fasting glucose	≥100 mg/dL
Fasting insulin	>17 mU/mL
Uric acid	>5 mg/dL
Vitamin D 25-OH	<30 ng/mL

Abbreviations: *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *GGT* gamma-glutamyl transpeptidase, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *vitamin D 25-OH* 25-hydroxyvitamin D

Table 18.3 Laboratory evaluation in the differential diagnosis of chronically elevated transaminases

Diagnosis	Labs
Autoimmune hepatitis	Smooth muscle antibody, anti-LKM, ANA, and quantitative immunoglobulins
Viral hepatitis	IgM for hepatitis C, HBsAg, Anti-HBcAg, Anti-HBsAg, HBeAg
Wilson disease	Serum ceruloplasmin
Alpha-1 antitrypsin deficiency	AAT phenotype or level
Metabolic disease, cystic fibrosis, hemochromatosis, celiac disease	Evaluation as clinically indicated

Abbreviations: *Anti-LKM* anti-liver kidney microsomal antibody, *ANA* antinuclear antibody, *HBsAg* hepatitis B surface antigen, *anti-HBcAg* anti-hepatitis B core antigen, *HBeAg* hepatitis B e Antigen, *AAT* alpha-1 antitrypsin

Screening

When screening for NAFLD serum ALT, AST (aspartate aminotransferase), and GGT (gamma-glutamyl transpeptidase) should be measured. National guidelines recommend screening with ALT and AST in children ≥10 years with BMI ≥95 % for age [45]. Interpretation of AST and ALT should be done cautiously as many labs continue to use inappropriate ranges for "normal." The liver SAFETY study demonstrated the 95 % levels for ALT in healthy weight, and liver disease-free children are 25 U/L (boys) and 22 U/L (girls) [46], yet the median upper limit of normal for ALT in use at children's hospitals is 53 U/L. In this study, it was noted that populations tested for establishing the range for "normal" ALT values likely include children with undiagnosed NAFLD or other liver disease, and the cutoff values in most laboratories are therefore set too high to reliably detect chronic liver disease. National guidelines suggest that values greater than two times the upper limit of normal warrants consultation with a pediatric gastroenterologist. According to the biology-based thresholds, this would be a value of >50 U/L (Table 18.2).

Diagnostic Evaluation

Once it is determined that a patient has chronic elevation of serum aminotransferases, a diagnostic evaluation to uncover the etiology of liver disease should be performed. Elevated serum ALT and AST may unmask other causes of occult liver disease, such as autoimmune hepatitis, viral hepatitis, Wilson disease, and alpha-1 antitrypsin deficiency (Table 18.3). Evaluation for autoimmune hepatitis includes smooth muscle antibody, liver kidney microsomal antibody (anti-LKM), antinuclear antibody (ANA), and quantitative immune globulins. Testing for viral hepatitis should include antibodies for hepatitis B and C. Wilson disease testing with serum ceruloplasmin should also be performed. Alpha-1 antitrypsin (AAT) deficiency can also be associated with liver disease and elevated transaminases, warranting AAT phenotype or level. There are, however, additional etiologies of occult liver disease such as celiac disease,

hereditary hemochromatosis, metabolic diseases, and cystic fibrosis that should be screened for as clinically indicated.

Screening Comorbidities of NAFLD

Obese children with NAFLD can have a pattern of dyslipidemia with normal to mildly elevated total cholesterol, elevated serum triglycerides, and low serum HDL cholesterol. Lipid abnormalities increase the risk for atherosclerotic heart disease, and early diagnosis and control of dyslipidemia has been shown to reduce the risk of cardiovascular disease in adulthood. Mild elevations may be amenable to physical exercise and nutrition with repeat evaluation in 3–6 months; however, more aberrant values, persistent abnormalities despite lifestyle modifications, and mild persistent elevations in patients with a positive family history of cardiovascular disease may require pharmacotherapy.

Type 2 diabetes is another comorbidity of NAFLD. A serum fasting glucose ≥126 mg/dL meets diagnostic criteria for diabetes, and these children should be referred to an endocrinologist. Impaired fasting glucose (≥100 mg/dL) indicates an increased risk for developing diabetes as well as associated morbidities such as hypertension. Thus, more intensive medical management is indicated. Fasting insulin is still controversial in use and interpretation; however, it is helpful as it is a potentially relevant biomarker in NAFLD.

In addition to lipid abnormalities and type 2 diabetes, children with NAFLD may also have elevated uric acid levels and vitamin D deficiency. Hyperuricemia has been associated with metabolic syndrome, cardiovascular disease, and insulin resistance. Recent studies have shown that it may be associated with NAFLD independent of metabolic syndrome [47]. Uric acid should be evaluated in children with NAFLD given its association with these comorbidities. Children with NAFLD also have been demonstrated to have low vitamin D 25-OH levels [48] as well as low bone mineral density [49]. Checking vitamin D 25-OH levels is important and appropriate treatment of vitamin D deficiency or insufficiency is warranted.

Diagnosis

NAFLD is a clinicopathologic diagnosis and it encompasses a spectrum of liver injury that spans isolated steatosis to steatohepatitis with progressive liver injury including fibrosis, cirrhosis, and hepatocellular carcinoma. Exclusion of other etiologies of primary liver disease in the pediatric population such as viral hepatitis, alpha-1 antitrypsin (AAT) deficiency, Wilson disease, and autoimmune hepatitis is also important to the diagnosis of NAFLD. Although the initial workup of NAFLD may include biochemical and radiographic tests, biopsy is the clinical standard for diagnosis. The minimum criterion for diagnosis of NAFLD is the finding of 5 % or more hepatocytes with macrovesicular steatosis.

Histology

Histologic lesions of NAFLD include steatosis, inflammation, hepatocellular ballooning, and fibrosis [50]. The steatosis is predominantly macrovesicular. Although smaller clusters of microvesicular fat may be visible, they should not be the predominant pattern. Hepatocellular ballooning is indicative of hepatocyte injury and refers to enlarged hepatocytes with cytoplasmic rarefaction [50].

There are numerous histologic differences between children and adults with NAFLD including more severe steatosis, lower degree of ballooning and Mallory's hyaline (or Mallory-Denk bodies), increased portal inflammation, decreased amount of perisinusoidal (zone 3) fibrosis, and an increased amount of portal fibrosis [51]. Given that the histologic characteristics of pediatric NAFLD were not well defined, Schwimmer and colleagues studied 100 consecutive pediatric NAFLD patients (ages 2–18) with two pathologists evaluating biopsies [52]. This study defined two types of pediatric histologic disease: type 1 was a more classic adult type pattern with steatosis, ballooning, and perisinusoidal fibrosis, and type 2 was characterized by steatosis, portal inflammation, and portal fibrosis. Type 1 NASH was present in 17 %, while type 2 was the more

predominant pattern in the pediatric age group as it was present in 51 % of subjects. There were also gender and racial differences where males and Caucasians were more likely to have type 1 pattern. Type 2 NASH was more common in Hispanics, Native Americans, and Asians. Type 2 was also more commonly associated with advanced fibrosis. In a study of pediatric NAFLD in 80 Korean children, type 2 NASH was present in 44 % of subjects and type 1 in 34 % [53]. Subsequent studies have shown a more overlapping pattern of disease with a mix of type 1 and type 2 as well as a higher percentage of ballooning. A study from Italy in 84 patients, with a single pathologist evaluating the biopsies, demonstrated an overlap of type 1 and type 2 pattern in 52 % of patients with ballooning present in nearly half of patients [6]. Type 2 pattern was present in 27 % of patients. Most of the children had portal injury (69 %). In a multicenter study from the USA and Canada in 2009, 130 pediatric NAFLD biopsies were evaluated, and 82 % had an overlapping pattern, 85 % of patients demonstrated portal injury most commonly in zone 3, and 73 % had ballooning [41]. The differences in the percentages of ballooning and overlap of type 1 and type 2 pattern may be attributable to racial and ethnic differences or to the differences in histopathologic interpretation. What is similar across studies, however, is that type 1, or the adult pattern of injury in NAFLD, is not predominant in the pediatric population. Additionally, portal-based injury is also a common theme. Kleiner and colleagues in 2005, when designing a histologic scoring criteria for NAFLD, found that portal fibrosis was much more common in the pediatric age group and that pediatric NAFLD has a distinct histologic pattern [54].

The most widely used histologic criteria, the NAFLD activity index (NAS), for defining histologic disease severity for NAFLD was developed in 2005 by Kleiner and colleagues. The NAS was validated by the NASH Clinical Research Network (CRN) Pathology Committee. The NAS is a sum of scores for steatosis (0–3), lobular inflammation (0–3), and ballooning (0–2), where a higher score reflects worse disease activity [54]. Subsequent to the development of the NAS, it was inadvertently used in publications as a possible replacement for the diagnosis of steatohepatitis if the NAS score was ≥5; however, this use of NAS has not been validated. NAS scoring was developed to assess change in clinical trials. Thus, in 2010, Brunt and colleagues assessed the validity of using NAS scores to grade histologic severity of NASH where biopsy data from 976 adults from the NASH CRN was evaluated [55]. In this study, only 75 % of patients with definite histologic steatohepatitis had a NAS of ≥5. Furthermore, NAS ≤4 was not representative of benign histology, and of those patients, 29 % had steatohepatitis and only 42 % had no steatohepatitis [55]. Thus, the NAS score does not correlate with histologic diagnosis and should not be used for diagnosis. Importantly, NAS does not include portal inflammation, a common feature in children, in the scoring system.

Biomarkers

At present diagnosis of NAFLD requires liver biopsy; however, a biopsy is not always possible. Additionally, many children with NAFLD go undiagnosed due to the requirement for biopsy. A noninvasive, sensitive, and specific biomarker would be helpful. A good biomarker should also be able to accurately classify the stage of fibrosis, because this makes an important difference clinically. Thus, an ideal biomarker would reflect both presence of the disease and severity of disease to be clinically useful.

NASH and Fibrosis

In a review of children with biopsy-proven NAFLD in San Diego, CA, from 1999 to 2002 clinical characteristics of NAFLD were correlated to liver pathology. In this study, the strongest predictive factor of portal inflammation was the combination of fasting insulin and ALT [5]. Portal fibrosis was predicted by abdominal pain and HOMA-IR.

Stemming from adult studies, several biomarkers have emerged as potential candidates for predicting fibrosis in pediatric NAFLD. Hyaluronic acid (HA) is a component of extra-

cellular matrix and thus biologically relevant to hepatic fibrosis. Cytokeratin 18 (CK18) is a marker of apoptosis, which may have a potential role in the pathogenesis of NAFLD [56]. Fifty-two children with biopsy-proven NAFLD were evaluated to study the correlation of histologic fibrosis with serologic hyaluronic acid and cytokeratin-18 levels [57]. When used independently to predict fibrosis, the AUC was 0.67. When both markers were used in combination, the PPV was 56 % with NPV 63 %, which is too low for clinical use. Additionally, CK18 level was not significantly different from a healthy control population of children, which limits its use as a solitary biomarker for screening individuals for fibrosis. In a separate study by Fitzpatrick and colleagues from 2010, serum levels of CK18 and HA were evaluated in 45 children with biopsy-proven NAFLD [58]. In this study, there was no association of HA with pediatric NAFLD. CK18 as a predictor of significant or severe fibrosis (F>2) had an AUC of 0.66 and a sensitivity of 83 % and specificity of 40 %, which are too low to be clinically useful.

Hyaluronic acid as a predictor of severity of fibrosis was studied in 100 children from Italy with 35 children without fibrosis, 50 with stage 1 fibrosis, 11 with stage 2 fibrosis, 2 with stage 3 fibrosis, and 2 with stage 4 fibrosis based on histologic criteria [59]. The AUC for use of HA to predict the presence of liver fibrosis was 0.88. Data are still needed on diagnostic accuracy in unselected populations.

Several scoring systems have also been developed for predicting the likelihood of fibrosis in patients with NAFLD. Nobili and colleagues evaluated the PNFI (Pediatric NAFLD Fibrosis Index) and the ELF (enhanced liver fibrosis) test as indices of predicting liver fibrosis in the pediatric age group. The PNFI is obtained from three measures: age, waist circumference, and triglycerides. According to their numerical scale from these three values, values less than three could "rule out" fibrosis and values of nine or higher could be used to "rule in" fibrosis. It was not reported, however, how many children fell into the 4–8 range, which would be helpful in determining the practicality of using this as a biomarker. The ELF test included a combination of three extracellular matrix components: hyaluronic acid, amino terminal propeptide of type III collagen (PIIINP), and inhibitor of metalloproteinase 1 (TIMP-1). In a study of 111 children with biopsy-proven NAFLD, Nobili and colleagues evaluated the ability of these two scores at differentiating any fibrosis from no fibrosis in these patients [60]. In this study, ELF was better at predicting any level of fibrosis versus no fibrosis compared to PNFI with an AUC of 0.92 versus 0.76, respectively. The combination of the two scores predicted the presence or absence of fibrosis (without grading fibrosis) in 86.4 % of children with NAFLD. Although some of these scales may be helpful in combination with biopsies, at this point these measures cannot be used as a substitute for biopsy as they cannot be used to stage fibrosis. Additionally, data are needed for the general population to better determine the potential utility of these for screening or diagnosis.

Radiology

There are several radiologic modalities that may be considered in the evaluation of children with NAFLD including ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI). This section will review the different radiologic techniques and their accuracy in evaluating NAFLD.

Ultrasonography

Ultrasonography is a widely used tool in evaluating NAFLD, due to its ease of availability, low cost, and lack of radiation, which is important in the pediatric age group. Ultrasound (US) of steatosis relies on ultrasound wave propagation through liver tissue to differentiate normal hepatic tissue from abnormal. Distortion of the hepatocyte by steatosis or the parenchyma by inflammation or fibrosis will scatter the ultrasound beam resulting in a brighter and more echogenic liver. The echotexture of the liver has been compared to the echotexture of the kidney, which should be similar to that of a normal liver

[61]. Abnormalities of the hepatocyte or parenchyma may also attenuate the ultrasound beam, blurring the margin between the diaphragm and the liver as well as vascular blurring. The differential echogenicity of the liver and kidney along with deep attenuation and vascular blurring have been proposed as useful in the assessment of hepatic steatosis [61].

There have been a few studies evaluating steatosis as determined by US and comparing this to MRI. The first study in 2006 by Pacifico and colleagues evaluated 50 obese children and determined that US evidence of mild steatosis did correlate to hepatic fat fraction (HFF) as determined by MRI [62]. Moreover, MRI HFF had a wide range (from <5 to 40 %) in subjects with moderate or severe steatosis on ultrasound. Additionally, US was subject to more interobserver variation than was MRI. A pediatric study in 2011 by Bohte and colleagues compared US to proton MR spectroscopy in 104 severely obese adolescents [63]. In this study, the PPV of US to detect steatosis in severely obese adolescents was 62 %. In a study of 78 obese or overweight children from Egypt, the PPV to detect histologic NAFLD was 47 % [64].

There are several limitations in using US for detection of steatosis, which makes this tool imperfect in diagnostic capacity for NAFLD. Firstly, US may only be able to detect moderate to severe hepatic steatosis, when biopsies demonstrate >33 % HFF, and is not reliable for detection of lower levels of steatosis [65], which means it cannot be used to exclude steatosis. Also, obesity affects the accuracy of US diagnosis as extrahepatic fat attenuates the wave before it reaches the liver [65]. Interobserver variability is also an issue leading to difficulty with interpreting the results [62, 63, 65].

Although US has been recommended as a diagnostic tool, the data do not support its use as the PPV is quite low, it cannot be used to grade steatosis, the NPV is unknown, and hence it is not a good longitudinal tool. Given these data, at present, ultrasound diagnosis of steatosis should be interpreted cautiously. US, however, may be a potential screening tool.

US elastography, also known as transient elastography (TE), has been proposed to estimate hepatic fibrosis in NAFLD. TE uses a small probe placed in the intercostal space that propagates waves through the liver parenchyma. The velocity of the emitted wave may be related to liver stiffness, which has been found to have a nonlinear relationship to the degree of fibrosis [66]. Although there are multiple studies evaluating this modality with chronic liver disease in the adult population, there is only one study in the pediatric age group using histology as a basis for diagnostic accuracy of TE for liver fibrosis. This pediatric study demonstrated that TE may have a role in diagnosis of fibrosis at the extremes of disease for predicting absence of fibrosis or severe fibrosis; however, TE was limited in predicting the degrees of fibrosis [67]. Studies have shown that BMI is an important factor for failure of TE measurements as thick subcutaneous and perihepatic fat can make the TE measurement difficult [68, 69].

Computed Tomography

Computed tomography (CT) has very limited use in children due to ionizing radiation. CT should not be used as a diagnostic modality for pediatric NAFLD. Hepatic steatosis in children can be found incidentally on CT scans performed for other reasons. Hounsfield units decrease with hepatic fat accumulation, and this can be used to estimate presence of hepatic steatosis [70]. CT is not reliable at detecting mild steatosis and is not adequately quantitative. Furthermore, contrast CT can be less accurate in determining hepatic steatosis than a non-contrast scan [71].

MRI

MR imaging may be an important tool for evaluation of NAFLD as it is noninvasive, free of ionizing radiation, and has a high potential for accuracy. The triglyceride molecule has multiple proton-containing moieties that resonate at a different frequency than that from the protons of the water molecule, thus the triglyceride molecule generates frequency peaks that are different from the water frequency peak. This theory is utilized

with proton MR spectroscopy (MRS) to determine the different proton densities and calculate a hepatic fat fraction (HFF). Determination of HFF via proton MRS has been shown in adults to have a diagnostic accuracy of 80–85 % [72, 73]. What makes MRS unique is the ability to detect HFF below 10 % [74–76]. With MRS, however, the HFF is calculated in a single voxel covering about 8 cm^3, thus it may not be representative of the full organ, much like a liver biopsy [77]. MRS was used to document liver fat content in pediatric NASH in a pilot study of metformin treatment by Schwimmer and colleagues in San Diego, CA [78].

MR imaging (MRI) offers the potential advantage of assessment of the whole liver because in contrast to MRS, MRI also provides a structural image. The Dixon method, also known as chemical shift imaging, utilizes the signals from fat protons and water protons that are "in-phase" or "out-of-phase" and comparing these signal intensities to estimate hepatic fat content [79]. There have been studies that demonstrate correlation between MRI fat fraction, MRS fat fraction, and histology. The first pediatric study to correlate MRI fat fraction using chemical shift imaging with histology was done by Pacifico and colleagues in 2011. They demonstrated that HFF was highly correlated with histologic steatosis (correlation coefficient $(r) = 0.88$); however, none of the controls have biopsies, so it is difficult to assess this reported diagnostic accuracy [80]. MRI, however, is limited by potential confounding factors (TI and T2* relaxation) [81].

MR elastography (MRE) is an emerging technique to evaluate hepatic fibrosis. MRE can be performed with standard MR machines with a driver placed on the abdominal wall that is used to transmit a vibrational energy that is passed through the tissue of interest. The acquired wave images are then used to assess tissue stiffness via an elastogram that is displayed as a color-coded scale throughout the entire liver. Although there has not yet been published data for MRE in pediatric NAFLD, MRE may have advantages over transient elastography for delineating various stages of fibrosis [82].

Treatment

Treatment trials thus far for pediatric NAFLD have focused on lifestyle modifications and medications targeted at insulin resistance and oxidative damage. These treatments have had varying degrees of effect. There remains to be found, however, a definitive treatment for pediatric NAFLD. Given the burgeoning epidemic of pediatric NAFLD and subsequent economic and social ramifications, it is important to have a therapeutic option for children with NAFLD.

Lifestyle

Given that pediatric NAFLD is often associated with obesity, dietary and exercise treatments have been universally recommended. Many pediatric intervention trials have focused on obesity itself and lifestyle modifications for weight loss; however, there are very few pediatric trials focusing on lifestyle modification in the context of a diagnosis of NAFLD [6, 83–88]. These trials are summarized in Table 18.4.

One of the first lifestyle interventions trials was conducted by Nobili and colleagues [6]. This trial consisted of 84 children ranging in age from 3 to 18 years with a mean BMI z score of 1.85. All subjects had biopsy-proven NAFLD prior to entering the study. The uncontrolled intervention consisted of a 1-h nutritional counseling session by a dietician where they were prescribed a low-calorie diet and a moderate exercise regimen that was individually tailored. Medical examinations and laboratory measurements were performed in 3-month intervals with a liver ultrasound to assess hepatic steatosis at the 12-month endpoint. Compliance was evaluated with monthly phone interviews and personal interview at the 3-month medical examination. Of the 84 subjects, 52 obese or overweight children completed the trial and 17 children lost greater than 10 % of their body weight. Of these 17 children, only 5 had normal abdominal ultrasounds at the completion of the study. ALT was significantly decreased in most patients, with the greatest decrease in those subjects who lost at least 5 % of their body weight.

Table 18.4 Lifestyle intervention studies for pediatric NAFLD

Study	Year	Location	*N*	Biopsy-proven NAFLD	Length	Study design	Steatosis assessment post treatment
Gronback et al. [88]	2012	Denmark	117/117	No	10 weeks	Uncontrolled	Ultrasound
Pozatto et al. [87]	2010	Italy	26/26	No	1 year	Uncontrolled	MRI
de Piano et al. [86]	2010	Brazil	34/43	No	12 weeks	Uncontrolled	Ultrasound
Vos et al. [85]	2009	USA	10/10	Yes in 7/10	6 months	Randomized, controlled, open-label	None
Reinehr et al. [84]	2009	Germany	152/160	No	2 years	Controlled	None
Wang et al. [83]	2008	China	76/76	No	1 month	Randomized controlled	None
Nobili et al. [6]	2006	Italy	52/84	Yes	1 year	Uncontrolled	Ultrasound

Abbreviations: *N* children completing study/children enrolled, *US* ultrasound, *MRI* magnetic resonance imaging

Fructose consumption has been implicated as a risk factor for NAFLD [90]. The association of fructose consumption to pathogenesis and progression of pediatric NAFLD, however, is yet to be determined. A pilot study evaluated the efficacy of a low-fructose diet over a 6-month period on aminotransferase levels [85]. Ten subjects with suspected NAFLD based on ALT and ultrasound (biopsy-proven NAFLD in 7) were placed on either a low-fructose diet ($n=6$) or low-fat diet ($n=4$). No significant change was noted in the ALT in those on the low-fructose diet.

The outcome measures used in lifestyle treatment trials vary. Histology is a cornerstone of diagnosis and should be used as the outcome measure to assess efficacy of interventions. Given the current level of evidence, it is difficult to assess how much weight loss is required for an improvement in NAFLD. In the study from Rome, weight loss was potentially inadequate [6]. In 12 of the 17 children that lost 10 % of total body weight, their US did not normalize at the end of the year. Weight loss with diet and exercise should continue to be recommendations for overweight and obese children; however, weight loss may not be sufficient to improve NAFLD in all children.

Pharmacologic

Drugs Targeting Insulin Resistance

Insulin resistance has been implicated in the pathogenesis of NAFLD, and as such, there have been several trials evaluating the efficacy of metformin treatment in pediatric NAFLD. There was an open-label pilot study of metformin treatment in ten children with biopsy-proven NASH with MRS imaging as an outcome measure of hepatic steatosis [78]. In this study, there was normalization of ALT in 40 % of subjects and a significant reduction in hepatic fat fraction from a mean of 30–23 % in 9 out of 10 subjects after 24 weeks of treatment.

Another open-label pilot study from Rome by Nobili and colleagues evaluated the effect of 24 months of metformin treatment on 30 children with biopsy-proven NAFLD [89]. Only 40 % had a follow-up biopsy; however, of those subjects with biopsies, several histologic features including steatosis, ballooning, and lobular inflammation improved after metformin treatment.

Antioxidant and Cytoprotective Agents

Reactive oxygen species and mitochondrial dysfunction have been implicated in NAFLD pathogenesis, thus several trials have focused on antioxidants. The most data to date are for vitamin E. An initial open-label pilot study with vitamin E in 11 children with suspected NAFLD based on ultrasound demonstrated a decrease in ALT with treatment [91]. Subsequently, Nobili and colleagues evaluated the effect of vitamin E, vitamin C, or placebo in a 1-year treatment in 88 children with biopsy-proven NAFLD with endpoints of decrease in aminotransferase values or prevalence of NAFLD by ultrasonography [92]. The study

subjects also participated in monthly sessions with a dietician. There was no statistically significant difference in the decrease in ALT between groups. In a follow-up study by the same group, about 60 % of these patients underwent liver biopsy, and there was significant improvement in steatosis, hepatocellular ballooning, and lobular inflammation in both groups without a difference between placebo or vitamin groups [93].

Cysteamine is an aminothiol agent that may act as an antioxidant by scavenging reactive oxygen intermediates such as free radicals and by increasing glutathione, the most abundant intracellular antioxidant agent [94]. In an open-label pilot study of 11 children with biopsy-proven NAFLD and serum ALT ≥60 U/L, the effect of twice-daily enteric-coated cysteamine for 24 weeks on serum ALT was evaluated [94]. At the 24-week time point, 7 out of the 11 subjects had a decrease in serum ALT by at least 50 % of baseline, and this effect was maintained at 48 weeks.

Cytoprotective agents, such as ursodeoxycholic acid (UDCA), have been investigated as potential treatment for NAFLD. UDCA is a secondary bile acid formed by intestinal bacteria. Treatment trials began with adult studies and there has been one pediatric study to date. In a small study from Italy of 31 obese children with suspected NAFLD based on elevated ALT and ultrasound, the effect of UDCA on ALT values was determined in four groups: diet alone ($n=11$), UDCA treatment ($n=7$), UCDA and diet ($n=7$), and untreated controls ($n=6$) [95]. ALT and hepatic steatosis, as determined by abdominal ultrasound, improved for those who lost weight, irrespective of the treatment group. UDCA alone was not effective in lowering ALT. There have been no trials in children evaluating histologic disease improvement after treatment in UDCA.

TONIC Trial

The Treatment of NAFLD in Children (TONIC) trial was a large National Institutes of Health NASH CRN multicenter, randomized, placebo-controlled, double-blind trial that was completed in 2010 [96]. In this trial, 173 children ages 7–17 with biopsy-proven NAFLD were randomized to receive metformin, or vitamin E, or placebo for 96 weeks. The primary outcome measure was decrease in ALT by 50 % compared to baseline or less than 40 U/L. Compared to placebo, neither metformin treatment nor vitamin E resulted in a significant decrease in ALT. In a secondary analysis, 85 % of children had a liver biopsy after 2 years of treatment. For this subgroup, 44 % had improvement in hepatocellular ballooning in both the metformin and vitamin E group compared with 21 % in the placebo group. There was no change in any other histologic feature for either treatment. Although neither vitamin E nor metformin was effective for the treatment groups, as a whole there may be subpopulations with potential for benefit.

Dietary Supplements

Although there is no supporting data, probiotics and omega-3 polyunsaturated fatty acids are being used in treatment of adult NAFLD. Pediatric data at this point are also lacking. These dietary supplements generally consist of compounds such as yogurt and fish oil. The theory underlying dietary supplement treatment is that certain nutrients may increase intestinal permeability to gut flora endotoxins leading to an immune-mediated proinflammatory response, which may lead to liver damage [97]. There has been one double-blind, placebo-controlled pilot study of probiotics in children with suspected NAFLD. Ten obese Italian children with suspicion of NAFLD based on ultrasound and elevated transaminases were treated with *Lactobacillus rhamnosus* strain GG (12 billion CFU/day) for 8 weeks. This resulted in a significant decrease in mean ALT from a baseline of 70 U/L to posttreatment value of 40 U/L [98]. No improvement was noted by ultrasound. Other clinical trials of probiotics in children with NAFLD are currently underway and pending results.

Thus far there has been one trial of long-chain omega-3 polyunsaturated fatty acids in pediatric NAFLD. In this randomized controlled trial of docosahexaenoic acid (DHA) treatment for 6 months in 60 children with biopsy-proven NAFLD, the subjects were randomly assigned to one of three groups: DHA 250 mg/day, DHA 500 mg/day, or placebo [99]. No effect was noted on serum ALT.

Surgery

As NAFLD is associated with obesity, bariatric surgery has a potential for treatment of NAFLD. There are several studies in adults that demonstrated some histologic improvement in NASH after bariatric surgery [100–102]. To date there is a lack of pediatric histologic data regarding bariatric surgery and NAFLD. What further complicates studying the issue of bariatric surgery in children with NAFLD is that children with the diagnosis of NAFLD tend to be younger and less obese than adolescents undergoing bariatric surgery. Although bariatric surgery has been shown to produce substantial weight loss in adolescents, there are no studies that evaluate the resolution of NAFLD. There is one study that demonstrated decreased AST and ALT 1 and 2 years after surgery; however, there are no histologic or imaging outcomes for NAFLD [103].

Although many studies exist for treatment of pediatric NAFLD, there are very few that use histologic criteria as an endpoint. Thus, there is insufficient evidence to determine the effect of these treatments on pediatric NAFLD. Most studies have been pilot studies with an open-label design and are not powered to assess the treatment effect. Future drug therapeutic studies with a placebo-controlled, randomized, and double-blinded design in children with a histologic primary outcome are needed. Furthermore, clinical trials should focus on inclusion of patients with NASH, which confers the most morbidity and mortality.

Associated Morbidity

Pediatric NAFLD has a number of associated morbidities encompassing multiple organ systems including hepatic, cardiovascular, metabolic, and psychosocial. These comorbidities have the potential to increase the risk of premature death as an adult.

Hepatic

It is difficult to assess the hepatic outcomes in children with pediatric NAFLD as there is a paucity of longitudinal histologic data looking at these clinical outcomes in the pediatric population. Much more data are available, however, looking at the long-term hepatic outcomes in adults with NAFLD, in which about one-third of adults with NAFLD demonstrate disease progression to fibrosis over 5 years [104]. Less is known about the progression of disease in children. In a series of 106 children undergoing biopsies for suspected NAFLD, the prevalence of fibrosis and cirrhosis was 60 and 3 %, respectively [52]. In a NASH CRN study of 176 children with suspected NAFLD, 74 % had fibrosis at initial biopsy, with 13.6 % having bridging fibrosis [8]. In longitudinal studies with adults, about 1 in 4 patients with NASH goes on to have cirrhosis, in contrast to only 4 % of patients with isolated steatosis progressing to cirrhosis [105].

NAFLD also carries a risk of development of hepatocellular carcinoma (HCC). According to one study, about 1,000 cases of HCC per year in the USA can be attributed to NAFLD [106]. The risk of HCC and childhood NAFLD is not well studied as longitudinal data are lacking. It is not known if duration of disease and onset in childhood is associated with risk of HCC or worse outcomes. It is presumed that most cases of HCC are associated with cirrhotic liver disease, but the field is seeing HCC even without cirrhosis. The incidence of HCC with NASH alone is not known in the pediatric age group. There are only limited outcomes data for pediatric NAFLD with respect to mortality. One retrospective study in Minnesota found children with NAFLD to be at higher risk for mortality compared to the general population [107]. Obtaining these outcomes data are critical for long-term patient counseling and clinical management.

Cardiovascular

As more children with NAFLD are identified, more data are emerging about the associated cardiac and metabolic risks. NAFLD has been demonstrated to be more frequent in children with metabolic syndrome than in those without [108]. In this series, 300 children were evaluated and

children with biopsy-proven NAFLD had a much higher cardiovascular risk profile including higher fasting glucose, insulin, triglycerides, and LDL, as well as higher systolic blood pressure. In a study of 72 obese adolescent children, children with MRI evidence of fatty liver were three times as likely to have metabolic syndrome compared to those without evidence of NAFLD on MRI [29]. Carotid intima-media thickness (CIMT) has been used as a quantifiable intermediate cardiovascular phenotype and has been studied in a few studies of pediatric NAFLD. A Turkish group evaluated CIMT in children with suspected NAFLD based on ultrasound and found a significantly higher CIMT in children with suspected NAFLD compared to healthy controls and obese controls without NAFLD [109]. This was similar to findings by Pacifico and colleagues who reported highest CIMT in obese children with suspected NAFLD based on ultrasound [110]. A subsequent study out of Italy, however, found no association with NAFLD and CIMT in children with biopsy-proven NAFLD compared to obese controls [111]. Children with NAFLD should be counseled on lifestyle management for improvement of cardiovascular health.

Metabolic

In addition to the association of NAFLD with metabolic syndrome, there may be strong associations with diabetes as well. Given that insulin resistance is important in the pathogenesis of NAFLD, the development of type 2 diabetes mellitus in children with NAFLD seems to be logical progression. About 50 % of children with type 2 diabetes have suspected NAFLD based on elevated ALT [112]. In cohorts of children with biopsy-proven NAFLD, about 8–10 % have type 2 diabetes at the time of their NAFLD diagnosis [5, 113].

Bone mineral density is another metabolic outcome associated with pediatric NAFLD. In a study of 38 children with biopsy-proven NAFLD, obese children with NAFLD had significantly lower bone mineral density as measured by DEXA compared to obese controls without NAFLD [49]. Additionally, the bone mineral density for children with NASH was significantly lower compared to children with NAFLD without NASH. Awareness of this risk for fractures is important to recognize in the clinical management of children with NAFLD.

Psychosocial

In addition to the physical outcomes associated with NAFLD, psychosocial outcomes can lead to tremendous morbidity and affect the quality of life in these children. In a study of 239 children with biopsy-proven NAFLD enrolled in the NASH CRN, impaired quality of life was present in 39 % [42]. Fatigue, trouble sleeping, and sadness were the symptoms that accounted for nearly half of the variance in quality of life scores compared to controls. In a recent study of psychosocial outcomes in children with NAFLD, children with NAFLD had higher levels of depression compared to obese controls [114]. Screening for symptoms of depression is important in the management of children with NAFLD.

Pediatric NAFLD is a complex disease with potential for multiorgan complications and involvement. Along with developing curative treatments, more studies are needed to assess the long-term outcomes in children with NAFLD in order to reduce the disease burden and associated morbidities that have the potential to significantly reduce life expectancy in these children.

References

1. Schwimmer JB, et al. Prevalence of fatty liver in children and adolescents. Pediatrics. 2006;118(4):1388–93.
2. Moran JR, et al. Steatohepatitis in obese children: a cause of chronic liver dysfunction. Am J Gastroenterol. 1983;78(6):374–7.
3. Fraser A, Longnecker MP, Lawlor DA. Prevalence of elevated alanine aminotransferase among US adolescents and associated factors: NHANES 1999–2004. Gastroenterology. 2007;133(6):1814–20.
4. Wiegand S, et al. Obese boys at increased risk for nonalcoholic liver disease: evaluation of 16,390 overweight or obese children and adolescents. Int J Obes (Lond). 2010;34(10):1468–74.

5. Schwimmer JB, et al. Obesity, insulin resistance, and other clinicopathological correlates of pediatric nonalcoholic fatty liver disease. J Pediatr. 2003;143(4):500–5.
6. Nobili V, et al. NAFLD in children: a prospective clinical-pathological study and effect of lifestyle advice. Hepatology. 2006;44(2):458–65.
7. Xanthakos S, et al. Histologic spectrum of nonalcoholic fatty liver disease in morbidly obese adolescents. Clin Gastroenterol Hepatol. 2006;4(2):226–32.
8. Patton HM, et al. Clinical correlates of histopathology in pediatric nonalcoholic steatohepatitis. Gastroenterology. 2008;135(6):1961–71.e2.
9. Donnelly KL, et al. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. J Clin Invest. 2005;115(5):1343–51.
10. Sanyal AJ, et al. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. Gastroenterology. 2001;120(5):1183–92.
11. Fabbrini E, et al. Alterations in fatty acid kinetics in obese adolescents with increased intrahepatic triglyceride content. Obesity (Silver Spring). 2009;17(1):25–9.
12. Cai D, et al. Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. Nat Med. 2005;11(2):183–90.
13. Musso G, Gambino R, Cassader M. Non-alcoholic fatty liver disease from pathogenesis to management: an update. Obes Rev. 2010;11(6):430–45.
14. Connolly MK, et al. In liver fibrosis, dendritic cells govern hepatic inflammation in mice via TNF-alpha. J Clin Invest. 2009;119(11):3213–25.
15. Nieto N. Oxidative-stress and IL-6 mediate the fibrogenic effects of [corrected] Kupffer cells on stellate cells. Hepatology. 2006;44(6):1487–501.
16. Wang J, et al. Kupffer cells mediate leptin-induced liver fibrosis. Gastroenterology. 2009;137(2):713–23.
17. Wieckowska A, et al. Increased hepatic and circulating interleukin-6 levels in human nonalcoholic steatohepatitis. Am J Gastroenterol. 2008;103(6):1372–9.
18. Lomonaco R, et al. Effect of adipose tissue insulin resistance on metabolic parameters and liver histology in obese patients with nonalcoholic fatty liver disease. Hepatology. 2012;55(5):1389–97.
19. Malhi H, et al. Free fatty acids induce JNK-dependent hepatocyte lipoapoptosis. J Biol Chem. 2006;281(17):12093–101.
20. Boden G, et al. Free fatty acids produce insulin resistance and activate the proinflammatory nuclear factor-kappaB pathway in rat liver. Diabetes. 2005;54(12):3458–65.
21. Wang D, Wei Y, Pagliassotti MJ. Saturated fatty acids promote endoplasmic reticulum stress and liver injury in rats with hepatic steatosis. Endocrinology. 2006;147(2):943–51.
22. Puri P, et al. Activation and dysregulation of the unfolded protein response in nonalcoholic fatty liver disease. Gastroenterology. 2008;134(2):568–76.
23. Rigazio S, et al. The lowering of hepatic fatty acid uptake improves liver function and insulin sensitivity without affecting hepatic fat content in humans. Am J Physiol Endocrinol Metab. 2008;295(2):E413–9.
24. Cusi K. Role of obesity and lipotoxicity in the development of nonalcoholic steatohepatitis: pathophysiology and clinical implications. Gastroenterology. 2012;142(4):711–25.e6.
25. Uchimura K, et al. Activation of retinoic X receptor and peroxisome proliferator-activated receptor-gamma inhibits nitric oxide and tumor necrosis factor-alpha production in rat Kupffer cells. Hepatology. 2001;33(1):91–9.
26. Enomoto N, et al. Protective effect of pioglitazone against endotoxin-induced liver injury through prevention of Kupffer cell sensitization. Alcohol Clin Exp Res. 2005;29(12 Suppl):216S–9.
27. Diez JJ, Iglesias P. The role of the novel adipocyte-derived hormone adiponectin in human disease. Eur J Endocrinol. 2003;148(3):293–300.
28. Louthan MV, et al. Decreased serum adiponectin: an early event in pediatric nonalcoholic fatty liver disease. J Pediatr. 2005;147(6):835–8.
29. Burgert TS, et al. Alanine aminotransferase levels and fatty liver in childhood obesity: associations with insulin resistance, adiponectin, and visceral fat. J Clin Endocrinol Metab. 2006;91(11):4287–94.
30. Gastaldelli A, et al. Pioglitazone in the treatment of NASH: the role of adiponectin. Aliment Pharmacol Ther. 2010;32(6):769–75.
31. Schwimmer JB, et al. Heritability of nonalcoholic fatty liver disease. Gastroenterology. 2009;136(5):1585–92.
32. Romeo S, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet. 2008;40(12):1461–5.
33. Valenti L, et al. Homozygosity for the patatin-like phospholipase-3/adiponutrin I148M polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease. Hepatology. 2010;51(4):1209–17.
34. Pirazzi C, et al. Patatin-like phospholipase domain-containing 3 (PNPLA3) I148M (rs738409) affects hepatic VLDL secretion in humans and in vitro. J Hepatol. 2012;57:1276–82.
35. Lin YC, et al. A common variant in the PNPLA3 gene is a risk factor for non-alcoholic fatty liver disease in obese Taiwanese children. J Pediatr. 2011;158(5):740–4.
36. Romeo S, et al. The 148M allele of the PNPLA3 gene is associated with indices of liver damage early in life. J Hepatol. 2010;53(2):335–8.
37. Goran MI, et al. Effects of PNPLA3 on liver fat and metabolic profile in Hispanic children and adolescents. Diabetes. 2010;59(12):3127–30.
38. Santoro N, et al. A common variant in the patatin-like phospholipase 3 gene (PNPLA3) is associated with fatty liver disease in obese children and adolescents. Hepatology. 2010;52(4):1281–90.
39. Rotman Y, et al. The association of genetic variability in patatin-like phospholipase domain-containing protein 3 (PNPLA3) with histological severity of nonalcoholic fatty liver disease. Hepatology. 2010;52(3):894–903.

40. Valenti L, et al. I148M patatin-like phospholipase domain-containing 3 gene variant and severity of pediatric nonalcoholic fatty liver disease. Hepatology. 2010;52(4):1274–80.
41. Carter-Kent C, et al. Nonalcoholic steatohepatitis in children: a multicenter clinicopathological study. Hepatology. 2009;50(4):1113–20.
42. Kistler KD, et al. Symptoms and quality of life in obese children and adolescents with non-alcoholic fatty liver disease. Aliment Pharmacol Ther. 2010;31(3):396–406.
43. Freedman DS, et al. Relationship of childhood obesity to coronary heart disease risk factors in adulthood: the Bogalusa Heart Study. Pediatrics. 2001;108(3):712–8.
44. Burke JP, et al. A quantitative scale of acanthosis nigricans. Diabetes Care. 1999;22(10):1655–9.
45. Barlow SE, Expert C. Expert committee recommendations regarding the prevention, assessment, and treatment of child and adolescent overweight and obesity: summary report. Pediatrics. 2007;120 Suppl 4:S164–92.
46. Schwimmer JB, et al. SAFETY study: alanine aminotransferase cutoff values are set too high for reliable detection of pediatric chronic liver disease. Gastroenterology. 2010;138(4):1357–64, 1364.e1–2.
47. Sirota JC, et al. Elevated serum uric acid levels are associated with non-alcoholic fatty liver disease independently of metabolic syndrome features in the United States: Liver ultrasound data from the National Health and Nutrition Examination Survey. Metabolism. 2013;62:392–9.
48. Manco M, Ciampalini P, Nobili V. Low levels of 25-hydroxyvitamin D(3) in children with biopsy-proven nonalcoholic fatty liver disease. Hepatology. 2010;51(6):2229; author reply 2230.
49. Pardee PE, Dunn W, Schwimmer JB. Non-alcoholic fatty liver disease is associated with low bone mineral density in obese children. Aliment Pharmacol Ther. 2012;35(2):248–54.
50. Brunt EM, Tiniakos DG. Pathological features of NASH. Front Biosci. 2005;10:1475–84.
51. Brunt EM. Nonalcoholic steatohepatitis. Semin Liver Dis. 2004;24(1):3–20.
52. Schwimmer JB, et al. Histopathology of pediatric nonalcoholic fatty liver disease. Hepatology. 2005;42(3):641–9.
53. Ko JS, et al. Clinical and histological features of nonalcoholic fatty liver disease in children. Dig Dis Sci. 2009;54(10):2225–30.
54. Kleiner DE, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005;41(6):1313–21.
55. Brunt EM, et al. Nonalcoholic fatty liver disease (NAFLD) activity score and the histopathologic diagnosis in NAFLD: distinct clinicopathologic meanings. Hepatology. 2011;53(3):810–20.
56. Valva P, et al. Apoptosis markers in liver biopsy of nonalcoholic steatohepatitis in pediatric patients. Hum Pathol. 2008;39(12):1816–22.
57. Lebensztejn DM, et al. Cytokeratin-18 and hyaluronic acid levels predict liver fibrosis in children with non-alcoholic fatty liver disease. Acta Biochim Pol. 2011;58(4):563–6.
58. Fitzpatrick E, et al. Serum levels of CK18 M30 and leptin are useful predictors of steatohepatitis and fibrosis in paediatric NAFLD. J Pediatr Gastroenterol Nutr. 2010;51(4):500–6.
59. Nobili V, et al. Hyaluronic acid predicts hepatic fibrosis in children with nonalcoholic fatty liver disease. Transl Res. 2010;156(4):229–34.
60. Alkhouri N, et al. A combination of the pediatric NAFLD fibrosis index and enhanced liver fibrosis test identifies children with fibrosis. Clin Gastroenterol Hepatol. 2011;9(2):150–5.
61. Yajima Y, et al. Ultrasonographical diagnosis of fatty liver: significance of the liver-kidney contrast. Tohoku J Exp Med. 1983;139(1):43–50.
62. Pacifico L, et al. MRI and ultrasound for hepatic fat quantification:relationships to clinical and metabolic characteristics of pediatric nonalcoholic fatty liver disease. Acta Paediatr. 2007;96(4):542–7.
63. Bohte AE, et al. US cannot be used to predict the presence or severity of hepatic steatosis in severely obese adolescents. Radiology. 2012;262(1):327–34.
64. El-Koofy N, et al. Ultrasonography as a non-invasive tool for detection of nonalcoholic fatty liver disease in overweight/obese Egyptian children. Eur J Radiol. 2012;81(11):3120–3.
65. Saadeh S, et al. The utility of radiological imaging in nonalcoholic fatty liver disease. Gastroenterology. 2002;123(3):745–50.
66. Sandrin L, et al. Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. Ultrasound Med Biol. 2003;29(12):1705–13.
67. Nobili V, et al. Accuracy and reproducibility of transient elastography for the diagnosis of fibrosis in pediatric nonalcoholic steatohepatitis. Hepatology. 2008;48(2):442–8.
68. Foucher J, et al. Prevalence and factors associated with failure of liver stiffness measurement using FibroScan in a prospective study of 2114 examinations. Eur J Gastroenterol Hepatol. 2006;18(4):411–2.
69. Petta S, et al. Reliability of liver stiffness measurement in non-alcoholic fatty liver disease: the effects of body mass index. Aliment Pharmacol Ther. 2011;33(12):1350–60.
70. Bydder GM, et al. Computed tomography attenuation values in fatty liver. J Comput Tomogr. 1981;5(1):33–5.
71. Jacobs JE, et al. Diagnostic criteria for fatty infiltration of the liver on contrast-enhanced helical CT. AJR Am J Roentgenol. 1998;171(3):659–64.
72. Kim H, et al. Comparative MR study of hepatic fat quantification using single-voxel proton spectroscopy, two-point dixon and three-point IDEAL. Magn Reson Med. 2008;59(3):521–7.
73. Thomas EL, et al. Hepatic triglyceride content and its relation to body adiposity: a magnetic resonance imaging and proton magnetic resonance spectroscopy study. Gut. 2005;54(1):122–7.

74. Longo R, et al. Proton MR spectroscopy in quantitative in vivo determination of fat content in human liver steatosis. J Magn Reson Imaging. 1995;5(3):281–5.
75. Browning JD, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology. 2004;40(6):1387–95.
76. Szczepaniak LS, et al. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. Am J Physiol Endocrinol Metab. 2005;288(2):E462–8.
77. Taouli B, Ehman RL, Reeder SB. Advanced MRI methods for assessment of chronic liver disease. AJR Am J Roentgenol. 2009;193(1):14–27.
78. Schwimmer JB, et al. A phase 2 clinical trial of metformin as a treatment for non-diabetic paediatric non-alcoholic steatohepatitis. Aliment Pharmacol Ther. 2005;21(7):871–9.
79. Mazhar SM, Shiehmorteza M, Sirlin CB. Noninvasive assessment of hepatic steatosis. Clin Gastroenterol Hepatol. 2009;7(2):135–40.
80. Pacifico L, et al. T1-weighted dual-echo MRI for fat quantification in pediatric nonalcoholic fatty liver disease. World J Gastroenterol. 2011;17(25):3012–9.
81. Yokoo T, et al. Estimation of hepatic proton-density fat fraction by using MR imaging at 3.0 T. Radiology. 2011;258(3):749–59.
82. Huwart L, et al. Magnetic resonance elastography for the noninvasive staging of liver fibrosis. Gastroenterology. 2008;135(1):32–40.
83. Wang CL, et al. Effect of lifestyle intervention on nonalcoholic fatty liver disease in Chinese obese children. World J Gastroenterol. 2008;14(10):1598–602.
84. Reinehr T, et al. Lifestyle intervention in obese children with non-alcoholic fatty liver disease: 2-year follow-up study. Arch Dis Child. 2009;94(6):437–42.
85. Vos MB, et al. Fructose and oxidized low-density lipoprotein in pediatric nonalcoholic fatty liver disease: a pilot study. Arch Pediatr Adolesc Med. 2009;163(7):674–5.
86. de Piano A, et al. Metabolic and nutritional profile of obese adolescents with nonalcoholic fatty liver disease. J Pediatr Gastroenterol Nutr. 2007;44(4):446–52.
87. Pozzato C, et al. Liver fat change in obese children after a 1-year nutrition-behavior intervention. J Pediatr Gastroenterol Nutr. 2010;51(3):331–5.
88. Gronbaek H, et al. Effect of a 10-week weight loss camp on fatty liver disease and insulin sensitivity in obese Danish children. J Pediatr Gastroenterol Nutr. 2012;54(2):223–8.
89. Nobili V, et al. Metformin use in children with nonalcoholic fatty liver disease: an open-label, 24-month, observational pilot study. Clin Ther. 2008;30(6):1168–76.
90. Ouyang X, et al. Fructose consumption as a risk factor for non-alcoholic fatty liver disease. J Hepatol. 2008;48(6):993–9.
91. Lavine JE. Vitamin E treatment of nonalcoholic steatohepatitis in children: a pilot study. J Pediatr. 2000;136(6):734–8.
92. Nobili V, et al. Effect of vitamin E on aminotransferase levels and insulin resistance in children with non-alcoholic fatty liver disease. Aliment Pharmacol Ther. 2006;24(11–12):1553–61.
93. Nobili V, et al. Lifestyle intervention and antioxidant therapy in children with nonalcoholic fatty liver disease: a randomized, controlled trial. Hepatology. 2008;48(1):119–28.
94. Dohil R, et al. Enteric-coated cysteamine for the treatment of paediatric non-alcoholic fatty liver disease. Aliment Pharmacol Ther. 2011;33(9):1036–44.
95. Vajro P, et al. Lack of efficacy of ursodeoxycholic acid for the treatment of liver abnormalities in obese children. J Pediatr. 2000;136(6):739–43.
96. Lavine JE, et al. Effect of vitamin E or metformin for treatment of nonalcoholic fatty liver disease in children and adolescents: the TONIC randomized controlled trial. JAMA. 2011;305(16):1659–68.
97. Alisi A, Carsetti R, Nobili V. Pathogen- or damage-associated molecular patterns during nonalcoholic fatty liver disease development. Hepatology. 2011;54(5):1500–2.
98. Vajro P, et al. Effects of Lactobacillus rhamnosus strain GG in pediatric obesity-related liver disease. J Pediatr Gastroenterol Nutr. 2011;52(6):740–3.
99. Nobili V, et al. Docosahexaenoic acid supplementation decreases liver fat content in children with non-alcoholic fatty liver disease: double-blind randomised controlled clinical trial. Arch Dis Child. 2011;96(4):350–3.
100. Barker KB, et al. Non-alcoholic steatohepatitis: effect of Roux-en-Y gastric bypass surgery. Am J Gastroenterol. 2006;101(2):368–73.
101. Dixon JB, Bhathal PS, O'Brien PE. Weight loss and non-alcoholic fatty liver disease: falls in gamma-glutamyl transferase concentrations are associated with histologic improvement. Obes Surg. 2006;16(10):1278–86.
102. Mathurin P, et al. Prospective study of the long-term effects of bariatric surgery on liver injury in patients without advanced disease. Gastroenterology. 2009;137(2):532–40.
103. Nadler EP, et al. Laparoscopic adjustable gastric banding for morbidly obese adolescents affects android fat loss, resolution of comorbidities, and improved metabolic status. J Am Coll Surg. 2009;209(5):638–44.
104. Harrison SA, Torgerson S, Hayashi PH. The natural history of nonalcoholic fatty liver disease: a clinical histopathological study. Am J Gastroenterol. 2003;98(9):2042–7.
105. Matteoni CA, et al. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. Gastroenterology. 1999;116(6):1413–9.
106. Rubinstein E, Lavine JE, Schwimmer JB. Hepatic, cardiovascular, and endocrine outcomes of the histological subphenotypes of nonalcoholic fatty liver disease. Semin Liver Dis. 2008;28(4):380–5.
107. Feldstein AE, et al. The natural history of non-alcoholic fatty liver disease in children: a follow-up study for up to 20 years. Gut. 2009;58(11):1538–44.
108. Schwimmer JB, et al. Cardiovascular risk factors and the metabolic syndrome in pediatric nonalcoholic

fatty liver disease. Circulation. 2008;118(3): 277–83.
109. Demircioglu F, et al. Intima-media thickness of carotid artery and susceptibility to atherosclerosis in obese children with nonalcoholic fatty liver disease. J Pediatr Gastroenterol Nutr. 2008;47(1):68–75.
110. Pacifico L, et al. Nonalcoholic fatty liver disease and carotid atherosclerosis in children. Pediatr Res. 2008;63(4):423–7.
111. Manco M, et al. Intima-media thickness and liver histology in obese children and adolescents with non-alcoholic fatty liver disease. Atherosclerosis. 2010;209(2):463–8.
112. Nadeau KJ, Klingensmith G, Zeitler P. Type 2 diabetes in children is frequently associated with elevated alanine aminotransferase. J Pediatr Gastroenterol Nutr. 2005;41(1):94–8.
113. Aygun C, et al. Evaluation of metabolic syndrome frequency and carotid artery intima-media thickness as risk factors for atherosclerosis in patients with nonalcoholic fatty liver disease. Dig Dis Sci. 2008;53(5):1352–7.
114. Kerkar N, et al. Psychosocial outcomes for children with nonalcoholic fatty liver disease over time and compared to obese controls. J Pediatr Gastroenterol Nutr. 2013;56:77–82.

19 Drug-Induced Liver Injury in Children: A Structured Approach to Diagnosis and Management

M. James Lopez and Jacob L. Bilhartz

Introduction

Drug-induced liver injury (DILI) can be separated into idiosyncratic (unpredictable and without dose dependence) or intrinsic (predictable and dose dependent). This chapter is focused on the evaluation and management of idiosyncratic DILI. To help practitioners develop a standard approach for identifying DILI, we include a proposed algorithm for evaluation based on available information in adults and children with DILI (Fig. 19.1). Additional information is included to amplify on the algorithm and add information relevant to the evaluation and management of patients with DILI.

DILI is rare, even in patients exposed to medications with a higher risk for liver injury. In retrospective studies, the risk ranges from 1:5,000 to 1:100,000 [1, 2]. In one prospective study from France (which allowed a population-based estimate), however, the risk of DILI was much higher with an estimated incidence of 13.9 cases per 100,000 (approximately 16 times higher than estimates based on spontaneous reporting) [3]. The authors extrapolated (not observed), based on their population-based incidence, that there would be approximately 8,000 cases of DILI per year in France and that such an incidence would be associated with approximately 500 deaths per year.

Rigorous estimates from pediatric populations are lacking and conclusions about pediatric DILI are based on small series or case reports. Pediatric patients do not appear to be at greater risk than adults for DILI. Most pediatric exposure to hepatotoxic drugs is from limited but frequent exposure to antimicrobials (e.g., beta-lactams or amoxicillin-clavulanate); however, patients followed for chronic diseases by pediatric subspecialists are exposed to a broader range of medications including many with limited pediatric data on which to base risk assessment (e.g., multiple anticonvulsants, antidepressants). However, no matter what the incidence or risk in children, it remains an important clinical issue as illustrated by the fact that at least 5 % of cases of pediatric acute liver failure (non-acetaminophen) result from idiosyncratic drug-induced liver injury [4]. Given its relative rarity and overlap with other clinical diseases, evaluation must begin with a high index of suspicion for DILI in the appropriate clinical context.

Suspicion of Drug-Induced Liver Injury

Patients with DILI initially present with laboratory abnormalities or signs and symptoms that overlap with other hepatobiliary diseases. There is no specific single clinical presentation

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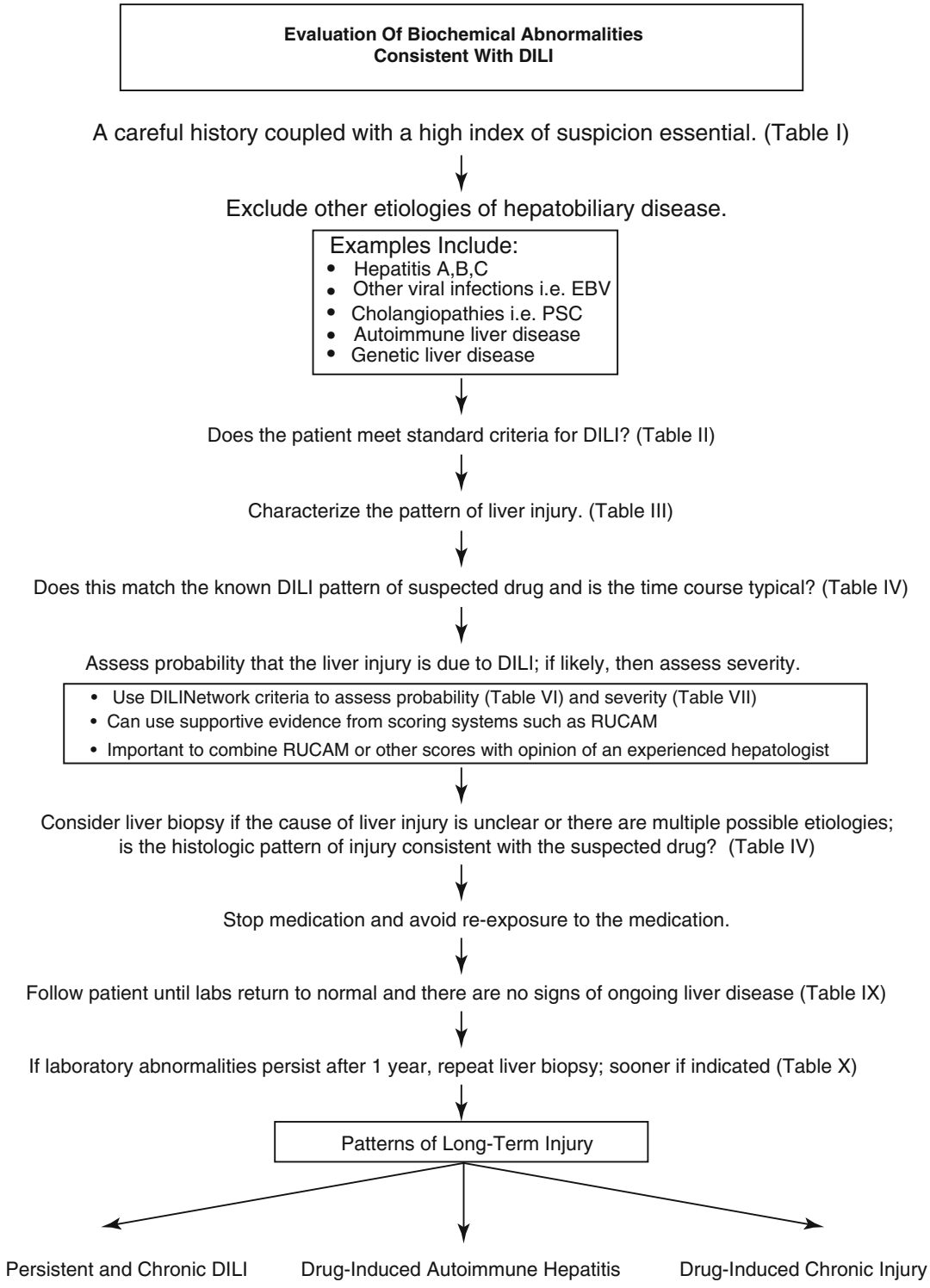

Fig. 19.1 Algorithm for standardized assessment and management of pediatric drug-induced liver injury

associated with DILI. In fact, DILI can simulate almost all forms of acute and chronic liver injury, even after assessment of complete clinical, biochemical, and histological information. Usually, the evidence of possible DILI arises between 1 and 12 weeks following initiation of a drug [2]. Approximately, a quarter of adults present with "allergic" symptoms including fever, rash, and eosinophilia. Although a small series of 39 children with DILI from Asia, also, noted that hypersensitivity features were common (41 %; anticonvulsants $n=10$, dapsone $n=4$, nimesulide $n=1$, clotrimazole $n=1$) [5], a recent multicenter report of pediatric DILI in the USA did not report any patients who presented with "allergic" symptoms, and none of those biopsied had evidence of eosinophilia [6]. Thus, the prevalence of hypersensitivity in pediatric DILI is unknown, and differences in studies may reflect bias related to small sample size, geographic variation, or referral patterns at tertiary care centers.

In general, each individual drug presents with characteristic patterns and pathology, but there is not complete fidelity to the typical pattern in all patients. The range of patterns and severity can be illustrated with the following examples: acute hepatic necrosis, isoniazid; acute liver failure, nitrofurantoin; viral hepatitis-like, halothane; autoimmune hepatitis-like, minocycline; bland cholestasis, estrogens; cholestatic hepatitis, amoxicillin/clavulanate; immunoallergic hepatitis, phenytoin; nodular regeneration, azathioprine; and vanishing bile duct syndrome, β-lactam antibiotics.

Thus, a careful history with exclusion of other hepatobiliary diseases is necessary in the clinical setting of DILI. The history needs to include specific historical elements which are relevant to DILI (Table 19.1). An exhaustive age-appropriate differential for hepatobiliary disease is beyond the scope of this chapter but can be reviewed in other chapters of this textbook (Fig. 19.1).

For those children under 2 years of age, attribution is particularly difficult as most of the potentially hepatotoxic drugs are used in settings where acute viral illnesses or other infections could be responsible for the laboratory and clinical abnormalities and the duration of drug treatment is brief with resolution of biochemical abnormalities occurring in time frames that would be consistent with either acute illness or DILI. In the current DILI Network prospective trial in the USA, children in this age range are excluded [6].

In addition, drugs associated with DILI are often used in clinical contexts where the history suggests multiple potential contributors to the liver test abnormalities. As an example, antifungals are used primarily in very ill populations in which elevations in transaminases and bilirubin could be related to issues other than a medication [7].

Table 19.1 Clinical history for suspected DILI

What is/was the daily dose of the drug in question? (Calculate amount per kilogram or BSA.)
What was the start date of drug treatment?
If drug already stopped at the time of evaluation, when was it stopped?
When, in relation to treatment course, did symptoms/abnormal labs become apparent?
Has the patient ever previously been on the drug in question?
What other drugs, vitamins, herbal supplements, complementary, and/or alternative treatments is/has the patient been on while taking suspected drug?
Are there other patient-specific risk factors – excessive alcohol use, obesity/metabolic syndrome, heart or kidney disease, and age?
Does the patient have any history of serious illness or hospitalization?
Is there symptomatic hepatitis – jaundice, right upper quadrant pain, fatigue, nausea/vomiting, and encephalopathy?
Are there other symptoms – e.g., rash with features of an immune reaction?
Have other potential causes of liver injury been excluded?

Clinical History (Table 19.1)

The clinical history of those with possible DILI should take note of the following issues. Most idiosyncratic drug reactions are seen between 1 and 12 weeks after initiation of the medication [2]. Although DILI can be seen presenting beyond 3 months, it becomes increasingly unlikely. If individuals were previously exposed to a drug, a shorter latency from drug exposure to

presentation of 1–2 days can be seen (sensitized) – an immunoallergic reaction [8]. It is important to specifically ask about exposure to medications consistent with the clinical presentation, especially those commonly prescribed. For example, amoxicillin-clavulanate [9], erythromycins [10], and chlorpromazine [11] commonly present with cholestasis, whereas allopurinol, minocycline, nitrofurantoin, and phenytoin [8] predominantly present with a hepatocellular pattern.

As DILI can result in severe acute hepatitis, acute liver failure, and chronic liver disease, it is important to consider DILI at the time of presentation. Examples of both chronic and acute liver injury from drug exposure: (1) atomoxetine, acute hepatitis and autoimmune-like hepatitis [12]; (2) minocycline, autoimmune-like hepatitis [13]; (3) and lamotrigine, acute hepatitis and acute liver failure [14–16]. Although DILI is rare in the general population, the likelihood of DILI may be higher in subsets of patients with specific clinical presentations. As examples, in patients presenting with serious acute hepatitis, the incidence of DILI was 7.4 per 100,000 [17]. In 800 jaundiced patients presenting to a single center (3.5 % attributed to DILI), the estimated incidence of DILI was 1.27 per 100,000 [18]. Thus, it is important to consider DILI early in those who are ill or have jaundice.

DILI: Do the Biochemical Data Fit the Pattern?

If the clinical history is consistent with DILI, application of more standard criteria is recommended for assessment of biochemical data. We suggest that use of the inclusion criteria proposed by the DILI Network (Table 19.2) based on biochemical criteria at presentation represents a reasonable starting point [6, 19]. These criteria are strict; thus, there may be cases that do not meet criteria for DILI but in the judgment of a practitioner are secondary to DILI. However, until a diagnostic test is available, application of these strict biochemical criteria should reduce the inappropriate attribution of liver injury to a drug where no association exists.

Table 19.2 Biochemical criteria consistent with DILI [19]

AST or ALT >5 times the upper limit of normal on two discrete occasions (or >5 times pretreatment baseline if previously elevated)
Alkaline phosphatase >2× ULN on two discrete occasions (or >2× pretreatment baseline if previously elevated)
Total bilirubin >2.5 mg/dL with any elevation in AST, ALT, or alkaline phosphatase
INR >1.5 with any elevation in AST, ALT, or alkaline phosphatase

Modified from Fontana et al. [19] with permission from Springer International Publishing Switzerland (© 2009. All rights reserved)
AST aspartate aminotransferase, *ALT* alanine aminotransferase, *INR* international normalized ratio, *ULN* upper limits of normal

Table 19.3 Characterizing pattern of liver injury [19]

Hepatocellular injury
R ratio >5[a]
Examples: minocycline, isoniazid, atomoxetine
Mixed pattern of injury
R ratio >2 but <5[a]
Examples: amoxicillin, amoxicillin-clavulanate, NSAID's, lamotrigine
Cholestatic injury
R ratio <2[a]
Examples: macrolides, sulfamethoxazole, amoxicillin-clavulanate

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ALT alanine aminotransferase, *ULN* upper limit of normal
[a]R *ratio* = (ALT/ULN)/(alkaline phosphatase/ULN) [19]

Once DILI is established as the most likely diagnosis, the pattern of injury should be determined. This should be done using biochemical data from as early in the clinical course as possible to avoid missing an initial stereotypic pattern of injury consistent with DILI; such a pattern could be masked/missed by relying on data from either an evolving or resolving injury. There are three described patterns seen in DILI: hepatocellular, cholestatic, and a mixed pattern [19, 20]; these are based on laboratory values for ALT (AST if not available) and alkaline phosphatase at presentation (Table 19.3). Not all forms of liver injury would meet the biochemical threshold for DILI, e.g., mitochondrial toxicity (valproate), but these criteria work well for the majority of cases.

Medications and Patterns of Injury in DILI (Table 19.4)

The most commonly implicated medications in DILI include antimicrobials, anticonvulsants, NSAIDs, and herbal medications [21, 22]. Table 19.5 lists the most common categories of medications implicated in DILI in several series and includes the prospective pediatric data from the DILI network; the categories in this table are reported in pediatric patients from the DILI Network [6] and represent classes of medications commonly used in pediatrics.

Prospective pediatric data (DILI Network) identified the following classes as most common in children: antimicrobials (~40 %), anticonvulsants (~22 %), drugs for ADHD (11 %), and psychoactive drugs (8 %); importantly, close to half of all children had been exposed to multiple drugs during the 2 months prior to presentation, and six children were on two or more drugs when DILI was diagnosed [6]. Amoxicillin-clavulanate is the most commonly implicated single agent in adult or pediatric patients [21, 22]. Single agents that together accounted for 27 % of all implicated drugs were minocycline, isoniazid, azithromycin, atomoxetine, and lamotrigine. Notable presenting features included: 60 % had jaundice, 30–40 % had abdominal pain or itch as primary complaints, 43 % had histories of drug allergies, and there was a frequent presence of comorbidities (e.g., neurological, cardiac, and pulmonary disease).

Use of herbal medications, as part of a growing movement to integrate complementary and alternative medicine into the medical regimen, undoubtedly, is underreported to the medical team. In one study, 69 % of those using non-vitamin dietary supplements did not discuss their use of supplements with a medical practitioner [87]. Usage may be common in pediatric and adult patients: 30–80 % of pediatric patients with asthma reported use [88]; 38 % of adults in the USA are estimated to use complementary or alternative medication [89]; and 26 % of university students reported use of herbal medications [90]. In general, the highest use of non-vitamin, non-mineral supplement use is associated with the following demographic factors: education beyond high school, higher income, older age, white race, and female gender [90–94]. Children whose parents use herbal/dietary drugs are much more likely to receive supplements (23.9 %) than those whose parents do not use supplements (5.1 %) [89]. Herbal/dietary remedies are used more commonly in some ethnic communities. Hispanics (Mexican-American background) are an example of such a group where traditional reliance on these remedies and low cost predispose to high usage [95]. In El Paso, Texas, up to 80 % of patients were found to use herbal/dietary supplements as part of their medical regimen [96]. Finally, up to two-thirds of patients report a belief that these are safe drugs/medications which is a likely contributor to increased use [97, 98].

Thus, the typical pediatric academic hospitals would reside in areas with a population likely to use herbal/dietary supplements as do providers who practice in areas with ethnic groups culturally predisposed to use herbal/traditional remedies.

Unfortunately, there has not been any systematic examination of herbal/dietary drug use in children. Despite this, there are clearly known toxicities for a subset of these medications and their increased use makes consideration of their potential impact important in a patient with liver injury.

Although the risk for DILI is relatively low for all who use herbal and dietary supplements (<1 %) [99], other data from registries and prospective trials suggest that their importance in DILI may be greater than previously thought. In patients, consecutively enrolled in the DILI network, approximately 10 % had herbal/dietary supplements implicated in their liver injury [21]. In a very small series of patients with acute liver failure, 35 % had herbal/dietary supplements as the only identifiable cause of liver failure [100]. In a Spanish registry of DILI, approximately 2 % of all patients had hepatic injury attributable to herbal or dietary supplements [101]. Thus, these studies suggest that this class needs to be considered as a potential cause of DILI at any age, and given the under reporting, their usage should be purposefully solicited.

As a starting point for assessing toxicity of herbal/dietary supplements, websites with information are readily accessible and have reliable

Table 19.4 DILI presentation of drugs commonly prescribed in pediatrics

Drug category and specific agent	Time frame of liver injury	Biochemical pattern of injury	Histological pattern of injury	Hypersensitivity reaction	VBDS	Ref.
Anti-infectives						
Amoxicillin-clavulanate[a]	Weeks	Cholestatic; mixed	Cholestatic hepatitis	Yes (rare)	Yes	[23–28]
Minocycline	Delayed	Hepatocellular	Chronic autoimmune hepatitis[b]	Yes	No	[13, 28–31]
Isoniazid	Variable – days to months	Hepatocellular	Lobular hepatitis; hepatocyte necrosis	No	No	[28, 32–36]
Azithromycin	Days to weeks	Cholestatic	Cholestasis with proportionately less inflammation	Yes	No	[28, 37–39]
Erythromycin	Days to weeks	Cholestatic	Cholestatic hepatitis	Yes	Yes	[10, 28, 40–42]
Amoxicillin	***	Hepatocellular; mixed	Cholestatic hepatitis	No	Yes	[28, 43, 44]
Sulfamethoxazole	Days to weeks	Cholestatic	Cholestasis +/– hepatitis	Yes	Yes	[28, 41, 44–46]
Cephalosporins	Days	Cholestatic	Chronic cholestasis; biliary sludging with ceftriaxone	No	No	[28, 44, 47, 48]
Azole antifungals	Variable	Hepatocellular; cholestatic	Hepatocellular injury; cholestatic hepatitis	No	No	[7, 28, 44]
Rifampin[c]	Weeks to months	Cholestatic	Cholestatic hepatitis	No	No	[28, 36, 49]
CNS-acting agents						
Atomoxetine	Variable	Hepatocellular	Lymphocytic portal inflammation	No	No	[12, 50, 51]
Lamotrigine	Weeks	Mixed	Hepatocyte injury and necrosis; bile duct injury	Yes	No	[14–16, 28, 52]
Valproic acid	Months	Hepatocellular	Steatosis; steatotic hepatitis	No	No	[28, 53–57]
Carbamazepine	Weeks	Cholestatic; hepatocellular	Cholestatic and granulomatous hepatitis	Yes	Yes	[42, 58–62]
Phenytoin	Variable – days to months	Mixed; hepatocellular	Hepatocyte necrosis; granulomatous inflammation	Yes	No	[6, 28, 58, 63, 64]
Nonsteroidal anti-inflammatory drugs						
Indomethacin	***	Hepatocellular; cholestatic	Hepatocyte necrosis; steatosis	No	No	[65–68]
Ibuprofen	***	Hepatocellular; mixed	Steatosis	Yes	Yes	[65, 69, 70]

Naproxen	***	Hepatocellular; cholestatic; mixed	Hepatocyte necrosis; cholestatic hepatitis	Yes	No	[65, 71, 72]
Herbal supplements						
Hydroxycut[d]	Weeks to months	Hepatocellular; mixed	Hepatocyte necrosis; cholestatic hepatitis	?[e]	No	[5, 73–75]
Pyrrolizidine alkaloids	Weeks	***	Veno-occlusive disease[f]	No	No	[76–82]

VBDS vanishing bile duct syndrome
[a]Hepatotoxicity of this combination drug is heavily due to the clavulanate component
[b]Minocycline and other tetracyclines can also be associated with hepatic steatosis
[c]DILI due to rifampin is rare on its own, but rifampin increases the hepatotoxic potential of other drugs
[d]A weight loss supplement that has now been withdrawn from the market; this was, however, the only herbal supplement identified in the pediatric DILIN paper from Molleston et al.
[e]Associated with autoantibodies (ANA, a-SM) thus unclear if may be an immune-mediated reaction
[f]DILI from pyrrolizidine alkaloids typically manifests with the complications of VOD and portal hypertension rather than a specific laboratory pattern

Table 19.5 Most common drug classes associated with DILI

Series	Era	*N*	Antimicrobials (%)	CNS agents (%)	Medicinal herbs	Analgesics	Immunomodulatory agents (%)	Antihypertensives
Devarbhavi et al. [5]	2004–2009	30[a]	50	50	10 %	N/A	6.7	N/A
Molleston et al. [6]	1997–2010	39[b]	74.4	41	2.6 %	5.1 %	2.6	N/A
Chalasani et al. [21]	2003–2008	300	45.5	15	9 %	5 %[c]	5	5 %
Bjornsson and Olsson [83]	1968–2003	4,690[d]	4.6	4	N/A	8.9 %	3.5	N/A
Bjornsson and Olsson [84]	1970–2004	784	29	5.2	N/A	5.7 %[c]	1.1	1 %
Andrade et al. [22]	1994–2004	446	32	17	2 %	16.1 %[c]	1.3	N/A
Russo et al. [85]	1990–2002	137[e]	29	21.9	5.1 %	2.9 %	1.5	2.2 %
De Abajo et al. [86]	1994–1999	128	30.5	24.2	N/A	7.8 %[c]	1.6	N/A
Sgro et al. [3]	1997–2000	34	29.4	17.6	N/A	11.8 %[c]	2.9	N/A
Ibáñez et al. [17]	1993–1998	107[b]	33.6	18.7	10.3 %	63.6 %	7.5	26.2 %

Classes primarily those observed in Molleston, et al. with addition of analgesics and antihypertensives to cover most reported classes relevant to pediatrics

N/A not available or no data

[a]This is the only solely pediatric case series presented here. Some patients were on more than one drug at the time of liver injury and thus percentages add up to >100 %

[b]Percentage of patients with suspected DILI who were exposed to a particular drug class. Many patients were on more than one drug at the time of liver injury and thus percentages add up to >100 %

[c]Non-acetaminophen analgesics

[d]This series was compiled from data submitted to the WHO on suspected drug-related hepatotoxicity. No specific causality assessment was performed. Of these 4,690 patients with suspected DILI, only 1,808 had a suspect drug which had been reported >50 times

[e]Non-acetaminophen related acute liver failure

information for this class of drugs/supplements (the Natural Standard, http://www.naturalstandard.com/; Facts & Comparisons – Review of Natural Products http://online.factsandcomparisons.com/index.aspx; NIH – NCCAM Herbal Medicine http://health.nih.gov/topic/HerbalMedicine).

Causality Assessment

Attribution of causality is difficult. Thus, attempts have been made to construct systematic scoring methods to assess the probability of DILI in an individual patient. The most widely used assessment tool is the Roussel Uclaf Causality Assessment Method (RUCAM; now also called CIOMS scale) [102]. This score provides a semiquantitative assessment of causality. Six domains are assigned a score with the final cumulative score used to categorize the likelihood of causality, e.g., highly probable >8 and excluded <0 [103]. It is somewhat cumbersome to use, so is rarely used in the clinical setting [104]. Nonetheless, the domains of the RUCAM included as part of clinical evaluation serve as a useful guide for important information that must be considered at the time of evaluation: (1) time to onset of reaction, (2) course of the reaction, (3) risk factors for drug reaction, (4) concomitant drug use, (5) nondrug-related causes, (6) previous information on the drug, and (7) response to readministration (readministration rarely occurs in clinical practice, so for all practical purposes is irrelevant).

When RUCAM was initially tested, it was reported to have a sensitivity of 86 % and specificity of 89 % [105]. Subsequent evaluation suggests its reliability to be much lower even when rescoring the same case over several months [106]. There is subjectivity in scoring, as well, noted by poor inter-rater reliability (between 0.34 and 0.45; 1 = perfect inter-rater reliability) [106]. Thus, no one should rely on this tool as an absolute measure of causality in either research or the clinical setting.

Given the lack of reliability of existing tools, the DILI Network devised a scoring system (Table 19.6) and, also, utilized a system of expert opinion (panel) to improve reliability [19]. An example of such use is found in the publication from this group on pediatric DILI [6]. The rating system remains semiquantitative and requires careful consideration of all the available data to arrive at a conclusion. If there is more than one possible medication, then a global score for causality is assigned. Scores were, also, included for up to three medications for those on multiple drugs. Thus, there is no simple way to assign specific causality. However, the structured thinking that goes into these scoring systems can be instructive and help ensure a careful clinical evaluation that facilitates thoughtful assessment of DILI causality.

Table 19.6 Assessing causality in suspected DILI [19]

Probability (%)	Expert clinical assessment
>95	Patient with a classic pattern, time course, and overall clinical presentation for the drug in question
75–95	"Clear and convincing" evidence for causality of drug in question
50–74	"Preponderance of evidence" supports causality for drug in question
25–49	"Preponderance of evidence" does not support causality for drug in question
<25	"Highly unlikely" that drug in question is cause of symptoms / lab abnormalities
Insufficient data	Critical points missing from the history (Table 19.1) or alternate diagnosis present

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Once causality has been assessed, an estimate of severity should be made [19, 107] (Table 19.7). The assumption is that more severe cases require more intensive follow-up and monitoring. However, severity scores in this setting do not have the same predictive power other scores do, such as Ranson's criteria in acute pancreatitis.

Risk Factors

There have been multiple studies directed toward identifying nongenetic and genetic risk factors for DILI. The data do not identify specific risks

for pediatric patients, but the risks identified for adults should be considered in the postpubertal patient. It is not clear, however, if these help in delineating likely prognosis, severity, or even actual risk in an individual case.

Nongenetic Risks

Age

Age is only a risk for certain medications. Thus, younger age appears to present the greatest risk for valproate toxicity (under 3 years) and aspirin-related Reye syndrome [1, 2]. For other drugs, e.g., erythromycin, nitrofurantoin, and isoniazid, the risk increases with age [1, 2]. However, some data suggest no predisposition for DILI in older adults for any medications [108].

Table 19.7 Severity of DILI [19]

Severity	Clinical features
Mild	Elevated transaminases and/or alkaline phosphatase, with total bilirubin <2.5 mg/dL *and* INR <1.5
Moderate	Elevated transaminases and/or alkaline phosphatase, with total bilirubin ≥2.5 mg/dL *or* INR ≥1.5
Moderate–Severe	Elevated transaminases, alkaline phosphatase, bilirubin, and/or INR *and* patient is hospitalized due to DILI
Severe	Elevated transaminases and/or alkaline phosphatase, total bilirubin ≥2.5 mg/dL, and liver failure *or* other organ failure related to DILI
Fatal	Final outcome of death or liver transplantation

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INR international normalized ratio

Gender (Sex)

Although women do not appear to be at greater risk of DILI than men [108, 109], women who develop DILI may be at risk for more severe outcomes. Lucerna et al. noted that of those who developed fulminant liver failure, 90 % were women [108]. In the USA, 67 % of those transplanted for fulminant hepatic failure from drug toxicity were women [85]. Additionally, more women develop hepatocellular injury [21, 110]. There is no clear mechanism to explain this difference. There are no published data suggesting a gender bias during childhood for DILI or severe outcomes from DILI.

Daily Dose

Dose may be important even in idiosyncratic DILI. There is evidence that higher doses of amoxicillin-clavulanate, flucloxacillin, and diclofenac are associated with DILI [86]. In fact, recent analysis suggests that the highest daily doses for medications commonly prescribed in the USA have a higher risk for DILI [111]. Data from a Spanish registry, also, supported a relationship between higher daily doses and DILI [108]. Given that amoxicillin-clavulanate is commonly used in pediatrics, those on higher doses may be at slightly increased risk (Table 19.8) [111].

Table 19.8 Relationship between drug dosage, hepatic metabolism, and DILI outcomes

	Drug dose*			Degree of hepatic metabolism†	
Outcome	≤10 mg/day (%)	11–49 mg/day (%)	≥50 mg/day (%)	>50 % (significant) (%)	<50 % (insignificant) (%)
ALT >3× ULN	19	27	31[a]	35	11[e]
Liver failure	17	12	32[b]	28	9[f]
Death	11	11	28[c]	23	4[g]
Transplant	0	2	13[d]	9	2[h]

p values compared to lowest dose for drug dose; [a]$p=0.1$, [b]$p=0.042$, [c]$p=.021$, [d]$p=.003$ (comparison of ≥50 mg with <50 mg); *p* values for hepatic metabolism; [e]$p=0.001$, [f]$p=0.004$, [g]$p=0.001$, [h]$p=0.11$
*Modified from Lammert et al. [111] with permission from John Wiley and Sons (© 2008. All rights reserved)
†Modified from Lammert et al. [112] with permission from John Wiley and Sons (© 2009. All rights reserved)

Metabolism Characteristics

Studies note that medications prescribed in the USA with 50 % or greater hepatic metabolism were much more likely to be associated with DILI [112]; those drugs with high liver metabolism were much more likely to be associated with markedly elevated ALT (38 % vs. 10 %) and liver failure (28 % vs. 9 %) (Table 19.8). This may be most problematic for those children who require multiple drugs for treatment of their chronic disease all of which have high hepatic metabolism.

Drug Interactions

Idiosyncratic drug injury is attributed to toxic reactive metabolites [113, 114]. Thus, drugs that alter the metabolism of drugs such as inducers or inhibitors of P450 enzymes can influence the risk for DILI. Potential interactions between medications should be considered for those requiring multiple drugs.

Alcohol Consumption

Alcohol should be considered as a potential risk for liver injury, although DILI in either its acute or chronic manifestations has not been specifically associated to any level of alcohol consumption. Alcohol appears to have a limited effect on idiosyncratic DILI resulting from select drugs such as methotrexate [115, 116] and isoniazid [117, 118], but not for other drugs. Even for these drugs, especially the antituberculosis drugs, the link is confounded by poor nutrition in many subjects in the studies [119, 120]. Thus, the influence of alcohol consumption on DILI remains poorly defined, and it is not a clear risk factor for most drugs.

Chronic Liver Disease

Although chronic liver disease is often cited as a risk for DILI, there are no clear data to support an increased risk [121, 122]. What does appear true is that there is a higher risk for a more complicated or severe course, if DILI occurs in the setting of chronic liver disease. As an example, limited data suggest that nonalcoholic fatty liver disease may increase the risk for DILI when compared to other chronic liver diseases such as hepatitis C; in at least one study of middle-aged men, the risk of DILI was increased fourfold relative to those with hepatitis C [123, 124], but rigorous studies need to be done. Such an increased risk would be consistent with observations of alterations in enzymatic activity in human and animal studies; both phase I and phase II drug-metabolizing enzymes are affected [125]. Thus, careful monitoring of those with underlying liver disease placed on potentially hepatotoxic drugs should take place, but the actual risk is unknown.

Genetic Risks

There are likely to be genetic susceptibilities associated with most drug-induced liver injury. To date, only a few associations have been elucidated via either genome-wide association studies or candidate gene studies. At present, there is no direct clinical utility of genetic testing. In addition, there have not been studies done to replicate initial results implicating potential gene associations. Thus, brief mention will suffice to acquaint the practitioner with a developing area in the field that may become important as we move toward individualized medicine.

HLA haplotypes have been associated with toxicity from two drugs; flucoxacillin liver injury has been associated with HLA-B*5701 in GWAS studies [126], and HLA-DRB1*1501 was found via a candidate gene search to be associated with amoxicillin/clavulanate liver injury [127–129]. Additionally, isoniazid liver injury is clearly related to polymorphisms of N-acetyltransferase 2 (NAT2), and early studies using phenotype analysis suggested that slow acetylators were at increased risk. More recently, discovered genetic variants associated with slow acetylation show increased risk for liver injury [130–132]. Other genes may play a role in both isoniazid and/or amoxicillin/clavulanate hepatotoxicity, such as isoforms of glutathione S-transferases [133, 134]. There is, also, growing evidence that UDP glucuronyltransferases, drug transporters such as ABCB11 and ABCC2, superoxide dismutase, and cytokine genes may be involved (e.g., IL-10 and IL-4) in susceptibility to DILI [135–138]. Undoubtedly, as we move toward personalized medicine, this area of genetic risks will become more prominent in the assessment of DILI, but for now it remains a laboratory curiosity.

Liver Biopsy

The primary utility of liver biopsy is to help differentiate between DILI and other potential liver diseases. The patterns observed on liver biopsy in DILI mimic other hepatobiliary diseases but can help limit the differential diagnosis. In general, the most common patterns observed on liver biopsy are necroinflammatory and cholestatic injury [28]. Necroinflammatory injury can be zonal, non-zonal, or massive. In general, idiosyncratic DILI is non-zonal. Examples of how pathology can create a more focused differential follow: (1) Acute hepatitis pattern 1 – sinusoidal beading of lymphocytes – EBV hepatitis vs. phenytoin. (2) Acute hepatitis pattern 2 – multiple small areas of degeneration "spotty" necrosis with predominantly lobular, lymphocytic infiltrate with scattered isolated apoptotic hepatocytes – acute viral hepatitis vs. isoniazid/sulfonamides. (3) Acute cholestatic pattern (bland cholestasis) – hepatocellular cholestasis, canalicular cholestasis, hepatocyte swelling, occasionally mild parenchymal injury, and mild portal inflammation – sepsis or acute large duct obstruction vs. contraceptive steroids (usually no injury) or erythromycin. (4) Chronic cholestatic pattern – duct sclerosis and loss, periportal cholestasis, and portal-based fibrosis – primary biliary cirrhosis, autoimmune cholangitis, or primary sclerosing cholangitis vs. amoxicillin/clavulanate, imipramine, or trimethoprim-sulfamethoxazole. Table 19.9 lists common patterns on liver biopsy for drugs commonly reported to cause DILI.

There are no clear data suggesting a specific relationship between pathological features and prognosis. Thus, the main utility remains identification of the histological pattern and severity of injury, exclusion of other hepatobiliary or systemic diseases, comparison of pattern to candidate drugs (time after exposure is important), identification of chronic liver disease, and considering the likelihood of DILI based on clinical data and pathological findings [139].

Prognosis

The majority of patients with DILI recover following drug removal. However, patients presenting with jaundice fare worse than those without jaundice [21, 22, 84]. Recent studies have confirmed what is called "Hy's Rule"; original studies by Hyman Zimmerman suggested that a total bilirubin ≥2.5 mg/dL and a hepatocellular pattern of injury were associated with a mortality of about 10 % (now includes liver transplantation) [140]. More recent case series from Spain, Sweden, and the USA observed mortalities in those with jaundice to be similar (12–15 %) [21, 22, 84] (Table 19.9).

Table 19.9 Mortality rates and biochemical pattern

Series	Hepatocellular (%)	Cholestatic (%)	Mixed (%)
Chalsani et al. [21][a]	7.5	14.3	2.1
Andrade et al. [22]	7	5	2
Bjornsson and Olsson [84]	12.7	7.8	2.4

Modified from Chalasani et al. [21] with permission from Elsevier (© 2008. All rights reserved)
Series include transplantation as an outcome; deaths are in those not transplanted
[a]11/18 deaths unrelated to liver failure

In contrast, two important additional observations were made in the recent US series. First, there was no difference in outcome between those with a hepatocellular pattern and those with a cholestatic pattern. Second, only 38 % of the reported mortality was related to liver dysfunction or disease (18 deaths). Other reported causes of death included malignancy, pulmonary/respiratory complications, renal failure, and congestive heart failure.

The specific drug causing the hepatic injury is also important to the outcome. Patients presenting with erythromycin-associated hepatic injury do not have increased mortality [84], but this contrasts with most medications causing cholestasis (e.g., amoxicillin/clavulanate) in which cholestasis is associated with increased mortality and progressive liver disease including vanishing bile duct syndrome [84]. Drugs used for central nervous system disorders or cardiovascular disease were the most likely to cause chronic liver damage [22]. Finally, those drugs presenting with clinical hypersensitivity features and eosinophilia on biopsy were unlikely to have a severe presentation, an increased mortality, or develop chronic liver disease [5, 141].

Table 19.10 Drugs associated with chronic liver disease

Drug	Class	Reference
Flucloxacillin	Antibiotic	[141]
Sulfonamides	Antibiotic	[46]
Nitrofurantoin	Antibiotic	[142–144]
Amiodarone	Antiarrhythmic	[145]
Alpha-methyldopa	Antihypertensive	[146]
Aspirin (acetylsalicylic acid)	Analgesic	[147]
Diclofenac	Analgesic (NSAID)	[148]
Methotrexate	Anti-inflammatory	[115]
Chlorpromazine	Antipsychotic	[149]
Fluoxetine	Antidepressant	[150]

There are limited prognostic data in children, but a recent prospective series documented two deaths and 7 % of patients had continued biochemical abnormalities at 6-month follow-up. For those children presenting with acute liver failure, 30 % died and 30 % were transplanted at 2-week follow-up [4]. Additionally, pediatric data suggest that presentation with encephalopathy, ascites, or hyperbilirubinemia is associated with an increased mortality, whereas presentation with hypersensitivity is not associated with increased mortality [5]. Thus, in all ages, DILI can have serious and long-term consequences and requires aggressive early evaluation and close monitoring (Table 19.10).

Management

Once a drug is identified as the likely cause of DILI, it must be stopped immediately. The only exception might be for drugs with a hepatocellular pattern – without evidence of cholestasis – where the drug is necessary to treat a primary chronic disease or problem for which there are no other or limited options – e.g., pulmonary hypertension. In such cases, the patient must be followed closely with a minimum of monthly laboratory assessments to monitor for worsening liver injury.

After a suspected drug is stopped, the patient must be followed closely, particularly, if jaundiced at presentation. Usually, for the first several months, a monthly interval is indicated for monitoring – if relatively well (lab abnormality only) this could consist primarily of laboratory testing. However, monitoring plans should be individualized given the variability in presentation and severity; if the patient has systemic signs and symptoms, then monitoring should be at a shorter interval for both clinical and laboratory assessment. In general, most hepatocellular injury will show a continued although slow pattern of improvement over the first 3 months, but cholestatic injury often will persist up to 6 months following presentation.

If there is persistence of laboratory abnormalities, then chronic liver injury needs to be considered (Fig. 19.1); usually this is defined as evidence of persistent injury for greater than 3 months for hepatocellular injury and greater than 6 months for cholestatic injury [152, 153]. However, data from a multicenter prospective trial suggest that persistent biochemical abnormalities are common with 42 % of subjects having continued elevation of liver enzymes at 3 months and 17 % at 12 months [154]. In general, three predominant types of chronic injury are recognized: (1) persistent (slow resolution) or chronic DILI (this type may ultimately resolve but needs close follow-up and possible liver biopsy for assessment of fibrosis or other chronic injury), (2) drug-induced autoimmune hepatitis (may require treatment for autoimmune liver disease; in clinical practice may be more responsive to treatment and not require long-term medication use) [30], and (3) drug-associated chronic liver disease (these usually are those who have persistent elevation of liver enzymes beyond 1 year) [154]. However, some patients develop fibrosis as early as 3 months after the injury [155]. Thus, the most important aspects of management include: (1) drug removal; (2) careful monitoring to document return to normal on biochemical testing and clinical evaluation with judicious early biopsy for those with confusing patterns of injury or early concerns for chronic disease; (3) if there is persistent evidence of injury, consider liver biopsy to document status

and exclude other diseases, especially fibrosis or vanishing bile duct syndrome (if jaundiced); and (4) if patients develop chronic liver disease, they require continued long-term follow-up.

Summary (Fig. 19.1)

The key initial step is to consider drug-induced liver injury as part of the routine differential in those with abnormal liver tests or acute liver failure. The first step is to obtain an appropriate history that includes all the essential information for assessing possible DILI. The practitioner must exclude other hepatobiliary diseases and use an age-appropriate differential for the pediatric population. Diagnosis of DILI in children under 2 years is exceedingly difficult but should still be considered in the appropriate clinical context. Use of a standard semiquantitative scoring system can help focus the decision making about the probability of a drug-related injury. Once the practitioner is convinced that DILI is likely, an assessment of the pattern of injury should be done – hepatocellular, cholestatic, or mixed pattern. Next, assess severity with thought being given to need for rapidity of evaluation and frequency of monitoring based on severity. Finally, as part of the early evaluation, decide if a liver biopsy might help determine a more precise pattern of injury (e.g., on multiple drugs) and help focus the differential. After drug removal, most patients should normalize lab studies between 3 and 6 months following cessation of drug use. If there are persistent abnormalities, careful monitoring for continued improvement out to at least a year should be done. If still abnormal at that time, reconsideration of a liver biopsy to document evidence of chronic/permanent injury should be done.

References

1. Bell LN, Chalasani N. Epidemiology of idiosyncratic drug-induced liver injury. Semin Liver Dis. 2009;29(4):337–47.
2. Larrey D. Epidemiology and individual susceptibility to adverse drug reactions affecting the liver. Semin Liver Dis. 2002;22(2):145–55.
3. Sgro C, et al. Incidence of drug-induced hepatic injuries: a French population-based study. Hepatology. 2002;36(2):451–5.
4. Squires Jr RH, et al. Acute liver failure in children: the first 348 patients in the pediatric acute liver failure study group. J Pediatr. 2006;148(5):652–8.
5. Devarbhavi H, et al. Drug-induced liver injury with hypersensitivity features has a better outcome: a single-center experience of 39 children and adolescents. Hepatology. 2011;54(4):1344–50.
6. Molleston JP, et al. Characteristics of idiosyncratic drug-induced liver injury in children: results from the DILIN prospective study. J Pediatr Gastroenterol Nutr. 2011;53(2):182–9.
7. Song JC, Deresinski S. Hepatotoxicity of antifungal agents. Curr Opin Investig Drugs. 2005;6(2):170–7.
8. Kaplowitz N. Idiosyncratic drug hepatotoxicity. Nat Rev Drug Discov. 2005;4(6):489–99.
9. Garcia Rodriguez LA, Stricker BH, Zimmerman HJ. Risk of acute liver injury associated with the combination of amoxicillin and clavulanic acid. Arch Intern Med. 1996;156(12):1327–32.
10. Derby LE, et al. Erythromycin-associated cholestatic hepatitis. Med J Aust. 1993;158(9):600–2.
11. Selim K, Kaplowitz N. Hepatotoxicity of psychotropic drugs. Hepatology. 1999;29(5):1347–51.
12. Lim JR, et al. Severe liver injury after initiating therapy with atomoxetine in two children. J Pediatr. 2006; 148(6):831–4.
13. Goldstein NS, et al. Minocycline as a cause of drug-induced autoimmune hepatitis. Report of four cases and comparison with autoimmune hepatitis. Am J Clin Pathol. 2000;114(4):591–8.
14. Mecarelli O, et al. Acute hepatitis associated with lamotrigine and managed with the molecular adsorbents recirculating system (Mars). Epilepsia. 2005;46(10):1687–9.
15. Arnon R, et al. Acute hepatic failure in a child treated with lamotrigine. Pediatr Neurol. 1998;18(3):251–2.
16. Fayad M, Choueiri R, Mikati M. Potential hepatotoxicity of lamotrigine. Pediatr Neurol. 2000;22(1): 49–52.
17. Ibanez L, et al. Prospective surveillance of acute serious liver disease unrelated to infectious, obstructive, or metabolic diseases: epidemiological and clinical features, and exposure to drugs. J Hepatol. 2002;37(5):592–600.
18. Vuppalanchi R, Liangpunsakul S, Chalasani N. Etiology of new-onset jaundice: how often is it caused by idiosyncratic drug-induced liver injury in the United States ? Am J Gastroenterol. 2007;102(3):558–62.
19. Fontana RJ, et al. Drug-induced liver injury network (DILIN) prospective study rationale, design and conduct. Drug Saf. 2009;32(1):55–68.
20. Chang CY, Schiano TD. Review article: drug hepatotoxicity. Aliment Pharmacol Ther. 2007;25(10):1135–51.
21. Chalasani N, et al. Causes, clinical features, and outcomes from a prospective study of drug-induced liver injury in the United States. Gastroenterology. 2008;135(6):1924–34.

22. Andrade RJ, et al. Drug-induced liver injury: an analysis of 461 incidences submitted to the Spanish registry over a 10-year period. Gastroenterology. 2005;129(2):512–21.
23. Cundiff J, Joe S. Amoxicillin-clavulanic acid-induced hepatitis. Am J Otolaryngol. 2007;28(1):28–30.
24. Gresser U. Amoxicillin-clavulanic acid therapy may be associated with severe side effects – review of the literature. Eur J Med Res. 2001;6(4):139–49.
25. Hautekeete ML, et al. Liver injury related to amoxycillin-clavulanic acid: interlobular bile-duct lesions and extrahepatic manifestations. J Hepatol. 1995;22(1):71–7.
26. Isabel Lucena M, et al. Determinants of the clinical expression of amoxicillin-clavulanate hepatotoxicity: a prospective series from Spain. Hepatology. 2006; 44(4):850–6.
27. Larrey D, et al. Hepatitis associated with amoxycillin-clavulanic acid combination report of 15 cases. Gut. 1992;33(3):368–71.
28. Lewis JH, Kleiner DE. Hepatic injury due to drugs, herbal compounds, chemicals and toxins. In: Burt A, Portmann B, Ferrell LD, editors. MacSween's pathology of the liver. New York: Churchill Livingstone Elsevier; 2012. p. 645–760.
29. Chamberlain MC, et al. Minocycline-induced autoimmune hepatitis with subsequent cirrhosis. J Pediatr Gastroenterol Nutr. 2006;42(2):232–5.
30. Czaja AJ. Drug-induced autoimmune-like hepatitis. Dig Dis Sci. 2011;56(4):958–76.
31. Teitelbaum JE, et al. Minocycline-related autoimmune hepatitis: case series and literature review. Arch Pediatr Adolesc Med. 1998;152(11):1132–6.
32. Severe isoniazid-associated liver injuries among persons being treated for latent tuberculosis infection – United States, 2004–2008. MMWR Morb Mortal Wkly Rep. 2010;59(8):224–9.
33. Black M, et al. Isoniazid-associated hepatitis in 114 patients. Gastroenterology. 1975;69(2):289–302.
34. Devrim I, et al. Risk factors for isoniazid hepatotoxicity in children with latent TB and TB: difference from adults. Chest. 2010;137(3):737–8.
35. Nolan CM, Goldberg SV, Buskin SE. Hepatotoxicity associated with isoniazid preventive therapy: a 7-year survey from a public health tuberculosis clinic. JAMA. 1999;281(11):1014–8.
36. Steele MA, Burk RF, DesPrez RM. Toxic hepatitis with isoniazid and rifampin. A meta-analysis. Chest. 1991;99(2):465–71.
37. Chandrupatla S, Demetris AJ, Rabinovitz M. Azithromycin-induced intrahepatic cholestasis. Dig Dis Sci. 2002;47(10):2186–8.
38. Longo G, et al. Azithromycin-induced intrahepatic cholestasis. Am J Med. 1997;102(2):217–8.
39. Suriawinata A, Min AD. A 33-year-old woman with jaundice after azithromycin use. Semin Liver Dis. 2002;22(2):207–10.
40. Andrade RJ, Tulkens PM. Hepatic safety of antibiotics used in primary care. J Antimicrob Chemother. 2011;66(7):1431–46.
41. Carson JL, et al. Acute liver disease associated with erythromycins, sulfonamides, and tetracyclines. Ann Intern Med. 1993;119(7 Pt 1):576–83.
42. Ferrajolo C, et al. Drug-induced hepatic injury in children: a case/non-case study of suspected adverse drug reactions in VigiBase. Br J Clin Pharmacol. 2010;70(5):721–8.
43. Fontana RJ, et al. Acute liver failure due to amoxicillin and amoxicillin/clavulanate. Dig Dis Sci. 2005;50(10):1785–90.
44. Thiim M, Friedman LS. Hepatotoxicity of antibiotics and antifungals. Clin Liver Dis. 2003;7(2): 381–99, vi–vii.
45. Bell TL, Foster JN, Townsend ML. Trimethoprim-sulfamethoxazole-induced hepatotoxicity in a pediatric patient. Pharmacotherapy. 2010;30(5):539.
46. Tonder M, Nordoy A, Elgjo K. Sulfonamide-induced chronic liver disease. Scand J Gastroenterol. 1974; 9(1):93–6.
47. Chen J, Ahmad J. Cefdinir-induced hepatotoxicity: potential hazards of inappropriate antibiotic use. J Gen Intern Med. 2008;23(11):1914–6.
48. Yilmaz B, et al. Cefixime-induced hepatotoxicity. Turk J Gastroenterol. 2011;22(4):445.
49. van Hest R, et al. Hepatotoxicity of rifampin-pyrazinamide and isoniazid preventive therapy and tuberculosis treatment. Clin Infect Dis. 2004;39(4):488–96.
50. Bangs ME, et al. Hepatic events associated with atomoxetine treatment for attention-deficit hyperactivity disorder. Drug Saf. 2008;31(4):345–54.
51. Erdogan A, et al. Idiosyncratic liver failure probably associated with atomoxetine: a case report. J Child Adolesc Psychopharmacol. 2011;21(3):295–7.
52. Makin AJ, et al. Fulminant hepatic failure induced by lamotrigine. BMJ. 1995;311(7000):292.
53. Delima SI, Walsh LE, Golomb MR. Simultaneous toxicities in a child on multiple anticonvulsants. J Child Neurol. 2008;23(9):1054–7.
54. Goto S, et al. Potential relationships between transaminase abnormality and valproic acid clearance or serum carnitine concentrations in Japanese epileptic patients. J Pharm Pharmacol. 2008;60(2):267–72.
55. McFarland R, et al. Reversible valproate hepatotoxicity due to mutations in mitochondrial DNA polymerase gamma (POLG1). Arch Dis Child. 2008;93(2): 151–3.
56. Price KE, et al. Effects of valproic acid on organic acid metabolism in children: a metabolic profiling study. Clin Pharmacol Ther. 2011;89(6):867–74.
57. Verrotti A, et al. Nonalcoholic fatty liver disease in adolescents receiving valproic acid. Epilepsy Behav. 2011;20(2):382–5.
58. Bjornsson E. Hepatotoxicity associated with antiepileptic drugs. Acta Neurol Scand. 2008;118(5):281–90.
59. Chen P, et al. Carbamazepine-induced toxic effects and HLA-B*1502 screening in Taiwan. N Engl J Med. 2011;364(12):1126–33.
60. Dertinger S, et al. Immunosuppressive therapy for carbamazepine-induced hypersensitivity syndrome and hepatitis. J Hepatol. 1998;28(2):356–7.

61. Hsu HF, Huang SY. Severe hepatitis associated with administration of oxcarbazepine. Pediatr Int. 2010; 52(4):677–8.
62. Ozeki T, et al. Genome-wide association study identifies HLA-A*3101 allele as a genetic risk factor for carbamazepine-induced cutaneous adverse drug reactions in Japanese population. Hum Mol Genet. 2011;20(5):1034–41.
63. Howard PA, Engen PL, Dunn MI. Phenytoin hypersensitivity syndrome: a case report. DICP. 1991; 25(9):929–32.
64. Roy AK, Mahoney HC, Levine RA. Phenytoin-induced chronic hepatitis. Dig Dis Sci. 1993;38(4): 740–3.
65. Agundez JA, et al. Assessment of nonsteroidal anti-inflammatory drug-induced hepatotoxicity. Expert Opin Drug Metab Toxicol. 2011;7(7):817–28.
66. Boardman PL, Hart FD. Side-effects of indomethacin. Ann Rheum Dis. 1967;26(2):127–32.
67. Jacobs JS. Sudden death in arthritic children receiving large doses of indomethacin. JAMA. 1967; 199(12):932–4.
68. Kelsey WM, Scharyj M. Fatal hepatitis probably due to indomethacin. JAMA. 1967;199(8):586–7.
69. Argentieri J, Morrone K, Pollack Y. Acetaminophen and Ibuprofen overdosage. Pediatr Rev. 2012;33(4): 188–9.
70. Nanau RM, Neuman MG. Ibuprofen-induced hypersensitivity syndrome. Transl Res. 2010;155(6): 275–93.
71. Ali S, Pimentel JD, Ma C. Naproxen-induced liver injury. Hepatobiliary Pancreat Dis Int. 2011;10(5): 552–6.
72. Victorino RM, et al. Jaundice associated with naproxen. Postgrad Med J. 1980;56(655):368–70.
73. Fong TL, et al. Hepatotoxicity due to hydroxycut: a case series. Am J Gastroenterol. 2010;105(7): 1561–6.
74. Sharma T, et al. Hydroxycut((R)) (herbal weight loss supplement) induced hepatotoxicity: a case report and review of literature. Hawaii Med J. 2010;69(8): 188–90.
75. Stickel F, et al. Review of liver injury associated with dietary supplements. Liver Int. 2011;31(5): 595–605.
76. Bah M, Bye R, Pereda-Miranda R. Hepatotoxic pyrrolizidine alkaloids in the Mexican medicinal plant Packera candidissima (Asteraceae: Senecioneae). J Ethnopharmacol. 1994;43(1):19–30.
77. Dai N, et al. Gynura root induces hepatic veno-occlusive disease: a case report and review of the literature. World J Gastroenterol. 2007;13(10): 1628–31.
78. Li N, et al. Hepatotoxicity and tumorigenicity induced by metabolic activation of pyrrolizidine alkaloids in herbs. Curr Drug Metab. 2011;12(9): 823–34.
79. Lin G, et al. Hepatic sinusoidal obstruction syndrome associated with consumption of Gynura segetum. J Hepatol. 2011;54(4):666–73.
80. Prakash AS, et al. Pyrrolizidine alkaloids in human diet. Mutat Res. 1999;443(1–2):53–67.
81. Ridker PM, McDermott WV. Comfrey herb tea and hepatic veno-occlusive disease. Lancet. 1989;1(8639): 657–8.
82. Stedman C. Herbal hepatotoxicity. Semin Liver Dis. 2002;22(2):195–206.
83. Bjornsson E, Olsson R. Suspected drug-induced liver fatalities reported to the WHO database. Dig Liver Dis. 2006;38(1):33–8.
84. Bjornsson E, Olsson R. Outcome and prognostic markers in severe drug-induced liver disease. Hepatology. 2005;42(2):481–9.
85. Russo MW, et al. Liver transplantation for acute liver failure from drug induced liver injury in the United States. Liver Transpl. 2004;10(8):1018–23.
86. de Abajo FJ, et al. Acute and clinically relevant drug-induced liver injury: a population based case–control study. Br J Clin Pharmacol. 2004;58(1): 71–80.
87. Gardiner P, et al. Factors associated with dietary supplement use among prescription medication users. Arch Intern Med. 2006;166(18):1968–74.
88. Slader CA, et al. Complementary and alternative medicine use in asthma: who is using what? Respirology. 2006;11(4):373–87.
89. Barnes P.M, Bloom B, Nahin R.L. Complementary and alternative medicine use among adults and children: United States, 2007. Natl Health Stat Report. 2008;(12):1–23.
90. Perkin JE, et al. Prevalence of nonvitamin, nonmineral supplement usage among university students. J Am Diet Assoc. 2002;102(3):412–4.
91. Schaffer DM, et al. Nonvitamin, nonmineral supplement use over a 12-month period by adult members of a large health maintenance organization. J Am Diet Assoc. 2003;103(11):1500–5.
92. Bailey RL, et al. Dietary supplement use in the United States, 2003–2006. J Nutr. 2011;141(2): 261–6.
93. Timbo BB, et al. Dietary supplements in a national survey: prevalence of use and reports of adverse events. J Am Diet Assoc. 2006;106(12):1966–74.
94. Nayga Jr RM, Reed DB. Factors associated with the intake of dietary supplements. Fam Econ Nutr Rev. 1999;12(1):43.
95. Loya AM, Sias JJ, Rivera JO. Medication use practices and perceptions among patients in a U.S.-Mexico border community. J Am Pharm Assoc (2003). 2012;52(6):e194–8.
96. Rivera JO, et al. Herbal product use in non-HIV and HIV-positive Hispanic patients. J Natl Med Assoc. 2005;97(12):1686–91.
97. Marinac JS, et al. Herbal products and dietary supplements: a survey of use, attitudes, and knowledge among older adults. J Am Osteopath Assoc. 2007; 107(1):13–20; quiz 21–3.
98. Gray DC, Rutledge CM. Herbal supplements in primary care: patient perceptions, motivations, and effects on use. Holist Nurs Pract. 2013;27(1):6–12.

99. Melchart D, et al. Liver enzyme elevations in patients treated with traditional Chinese medicine. JAMA. 1999;282(1):28–9.
100. Estes JD, et al. High prevalence of potentially hepatotoxic herbal supplement use in patients with fulminant hepatic failure. Arch Surg. 2003;138(8):852–8.
101. Garcia-Cortes M, et al. Liver injury induced by "natural remedies": an analysis of cases submitted to the Spanish Liver Toxicity Registry. Rev Esp Enferm Dig. 2008;100(11):688–95.
102. Garcia-Cortes M, et al. Causality assessment methods in drug induced liver injury: strengths and weaknesses. J Hepatol. 2011;55(3):683–91.
103. Danan G, Benichou C. Causality assessment of adverse reactions to drugs–I. A novel method based on the conclusions of international consensus meetings: application to drug-induced liver injuries. J Clin Epidemiol. 1993;46(11):1323–30.
104. Hayashi PH. Causality assessment in drug-induced liver injury. Semin Liver Dis. 2009;29(4):348–56.
105. Benichou C, Danan G, Flahault A. Causality assessment of adverse reactions to drugs–II. An original model for validation of drug causality assessment methods: case reports with positive rechallenge. J Clin Epidemiol. 1993;46(11):1331–6.
106. Rochon J, et al. Reliability of the Roussel Uclaf Causality Assessment Method for assessing causality in drug-induced liver injury. Hepatology. 2008;48(4):1175–83.
107. Agarwal VK, McHutchison JG, Hoofnagle JH. Important elements for the diagnosis of drug-induced liver injury. Clin Gastroenterol Hepatol. 2010;8(5):463–70.
108. Lucena MI, et al. Phenotypic characterization of idiosyncratic drug-induced liver injury: the influence of age and sex. Hepatology. 2009;49(6):2001–9.
109. Shapiro MA, Lewis JH. Causality assessment of drug-induced hepatotoxicity: promises and pitfalls. Clin Liver Dis. 2007;11(3):477–505, v.
110. De Valle MB, et al. Drug-induced liver injury in a Swedish University hospital out-patient hepatology clinic. Aliment Pharmacol Ther. 2006;24(8):1187–95.
111. Lammert C, et al. Relationship between daily dose of oral medications and idiosyncratic drug-induced liver injury: search for signals. Hepatology. 2008;47(6):2003–9.
112. Lammert C, et al. Oral medications with significant hepatic metabolism at higher risk for hepatic adverse events. Hepatology. 2010;51(2):615–20.
113. Knowles SR, Uetrecht J, Shear NH. Idiosyncratic drug reactions: the reactive metabolite syndromes. Lancet. 2000;356(9241):1587–91.
114. Pessayre D. Role of reactive metabolites in drug-induced hepatitis. J Hepatol. 1995;23 Suppl 1:16–24.
115. Whiting-O'Keefe QE, Fye KH, Sack KD. Methotrexate and histologic hepatic abnormalities – a meta-analysis. Am J Med. 1991;90(6):711–6.
116. Malatjalian DA, et al. Methotrexate hepatotoxicity in psoriatics: report of 104 patients from Nova Scotia, with analysis of risks from obesity, diabetes and alcohol consumption during long term follow-up. Can J Gastroenterol. 1996;10(6):369–75.
117. Cross FS, et al. Rifampin-isoniazid therapy of alcoholic and nonalcoholic tuberculous patients in a U.S. public health service cooperative therapy trial. Am Rev Respir Dis. 1980;122(2):349–53.
118. Pande JN, et al. Risk factors for hepatotoxicity from antituberculosis drugs: a case–control study. Thorax. 1996;51(2):132–6.
119. Dossing M, et al. Liver injury during antituberculosis treatment: an 11-year study. Tuber Lung Dis. 1996;77(4):335–40.
120. Schaberg T, Rebhan K, Lode H. Risk factors for side-effects of isoniazid, rifampin and pyrazinamide in patients hospitalized for pulmonary tuberculosis. Eur Respir J. 1996;9(10):2026–30.
121. Lewis JH. The rational use of potentially hepatotoxic medications in patients with underlying liver disease. Expert Opin Drug Saf. 2002;1(2):159–72.
122. Russo MW, Watkins PB. Are patients with elevated liver tests at increased risk of drug-induced liver injury? Gastroenterology. 2004;126(5):1477–80.
123. Khorashadi S, Hasson NK, Cheung RC. Incidence of statin hepatotoxicity in patients with hepatitis C. Clin Gastroenterol Hepatol. 2006;4(7):902–7; quiz 806.
124. Tarantino G, et al. A prospective study of acute drug-induced liver injury in patients suffering from non-alcoholic fatty liver disease. Hepatol Res. 2007;37(6):410–5.
125. Merrell MD, Cherrington NJ. Drug metabolism alterations in nonalcoholic fatty liver disease. Drug Metab Rev. 2011;43(3):317–34.
126. Daly AK, et al. HLA-B*5701 genotype is a major determinant of drug-induced liver injury due to flucloxacillin. Nat Genet. 2009;41(7):816–9.
127. Hautekeete ML, et al. HLA association of amoxicillin-clavulanate-induced hepatitis. Gastroenterology. 1999;117(5):1181–6.
128. O'Donohue J, et al. Co-amoxiclav jaundice: clinical and histological features and HLA class II association. Gut. 2000;47(5):717–20.
129. Lucena MI, et al. Susceptibility to amoxicillin-clavulanate-induced liver injury is influenced by multiple HLA class I and II alleles. Gastroenterology. 2011;141(1):338–47.
130. Ohno M, et al. Slow N-acetyltransferase 2 genotype affects the incidence of isoniazid and rifampicin-induced hepatotoxicity. Int J Tuberc Lung Dis. 2000;4(3):256–61.
131. Possuelo LG, et al. Association of slow N-acetyltransferase 2 profile and anti-TB drug-induced hepatotoxicity in patients from Southern Brazil. Eur J Clin Pharmacol. 2008;64(7):673–81.
132. Huang YS, et al. Polymorphism of the N-acetyltransferase 2 gene as a susceptibility risk factor for antituberculosis drug-induced hepatitis. Hepatology. 2002;35(4):883–9.
133. Roy B, et al. Increased risk of antituberculosis drug-induced hepatotoxicity in individuals with glutathione S-transferase M1 'null' mutation. J Gastroenterol Hepatol. 2001;16(9):1033–7.

134. Lucena MI, et al. Glutathione S-transferase m1 and t1 null genotypes increase susceptibility to idiosyncratic drug-induced liver injury. Hepatology. 2008;48(2):588–96.
135. Lang C, et al. Mutations and polymorphisms in the bile salt export pump and the multidrug resistance protein 3 associated with drug-induced liver injury. Pharmacogenet Genomics. 2007;17(1):47–60.
136. Daly AK, et al. Genetic susceptibility to diclofenac-induced hepatotoxicity: contribution of UGT2B7, CYP2C8, and ABCC2 genotypes. Gastroenterology. 2007;132(1):272–81.
137. Aithal GP, et al. Hepatic adducts, circulating antibodies, and cytokine polymorphisms in patients with diclofenac hepatotoxicity. Hepatology. 2004;39(5):1430–40.
138. Huang Y-S, et al. Genetic polymorphisms of manganese superoxide dismutase, NAD(P)H : quinone oxidoreductase, glutathione S-transferase M1 and T1, and the susceptibility to drug-induced liver injury. J Hepatol. 2007;47(1):128–34.
139. Kleiner DE. The pathology of drug-induced liver injury. Semin Liver Dis. 2009;29(4):364–72.
140. Bjornsson E. Drug-induced liver injury: Hy's rule revisited. Clin Pharmacol Ther. 2006;79(6):521–8.
141. Bjornsson E, Kalaitzakis E, Olsson R. The impact of eosinophilia and hepatic necrosis on prognosis in patients with drug-induced liver injury. Aliment Pharmacol Ther. 2007;25(12):1411–21.
142. Olsson R, et al. Liver-damage from flucloxacillin, cloxacillin, and dicloxacillin. J Hepatol. 1992;15(1–2):154–61.
143. Iwarson S, Lindberg J, Lundin P. Nitrofurantoin-induced chronic liver disease. Clinical course and outcome of five cases. Scand J Gastroenterol. 1979;14(4):497–502.
144. Stricker BHC, et al. Hepatic-injury associated with the use of nitrofurans – a clinicopathological study of 52 reported cases. Hepatology. 1988;8(3):599–606.
145. Volbeda F, et al. Liver cirrhosis due to chronic use of nitrofurantoin. Ned Tijdschr Geneeskd. 2004;148(5):235–8.
146. Oikawa H, et al. Liver cirrhosis induced by long-term administration of a daily low dose of amiodarone: a case report. World J Gastroenterol. 2005;11(34):5394–7.
147. Arranto AJ, Sotaniemi EA. Histologic follow-up of alpha-methyldopa-induced liver-injury. Scand J Gastroenterol. 1981;16(7):865–72.
148. Seaman WE, Ishak KG, Plotz PH. Aspirin-induced hepatotoxicity in patients with systemic lupus erythematosus. Ann Intern Med. 1974;80(1):1–7.
149. Mazeika PK, Ford MJ. Chronic active hepatitis associated with diclofenac sodium therapy. Br J Clin Pract. 1989;43(3):125–6.
150. Russell RI, Allan JG, Patrick R. Active chronic hepatitis after chlorpromazine ingestion. Br Med J. 1973;1(5854):655–6.
151. Johnston DE, Wheeler DE. Chronic hepatitis related to use of fluoxetine. Am J Gastroenterol. 1997;92(7):1225–6.
152. Seeff LB. Drug-induced chronic liver disease, with emphasis on chronic active hepatitis. Semin Liver Dis. 1981;1(2):104–15.
153. Benichou C. Criteria of drug-induced liver disorders. Report of an international consensus meeting. J Hepatol. 1990;11(2):272–6.
154. Borraz Y, et al. Would it be desirable to modify the cut-off point for definition of chronicity in drug-induced liver injury (Dili)? Hepatology. 2010;52(4):457a–8.
155. Aithal PG, Day CP. The natural history of histologically proved drug induced liver disease. Gut. 1999;44(5):731–5.

Gallstone Disease in Children

20

Matthew J. Giefer and Richard A. Kozarek

Introduction

Gallstone disease or cholelithiasis refers to the presence of pathologic concretions in the gallbladder or bile ducts and is one of the most common digestive diseases among adults. The degree of disease burden is reflected by the fact that over 700,000 cholecystectomies are performed annually in the USA [1]. Historically, gallstone disease in children was thought to be rare and typically associated with hemolytic conditions [2]. Over the last few decades, however, an increasing number of children have been found to have gallstones and related complications. Gallstones can be classified by their composition (cholesterol, pigment, calcium carbonate) and by their location (gallbladder, intrahepatic ducts, common biliary duct). The increasing prevalence of pediatric gallstone disease appears to be largely due to obesity and an increasing prevalence of cholesterol stones [3]. The approach to diagnosis and management of pediatric gallstone disease varies in a number of important ways from adults due to unique considerations relating to the etiology and natural history in this population.

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Pathophysiology

Cholesterol Stones

Normal human bile contains water, cholesterol, phospholipids, bile salts, bile pigments, protein, and a number of soluble elements. Cholesterol is nearly insoluble in water, and because bile is an aqueous solution, its hydrophobic properties would limit its biliary excretion. Bile salts, however, are highly soluble due their amphiphilic nature, meaning that they have both hydrophilic and hydrophobic regions. Bile salts are the majority constituent of the dry mass of bile and account for about two thirds of solute mass. Bile salt monomers spontaneously aggregate into disklike formations called simple micelles. The hydrophobic interior of micelles allows for cholesterol solubility, while the hydrophilic exterior allows for the micelle itself to be soluble in aqueous bile. Mixed micelles are larger micelles that have incorporated both phospholipids and cholesterol into the bile salt membrane and are capable of solubilizing three times the amount of cholesterol in their hydrophobic core compared to simple micelles [4].

The maximal solubility of cholesterol in bile relates to the relative proportions of cholesterol, phospholipids, bile salts, and water. In unsaturated

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bile, all cholesterol can be solubilized within simple and mixed micelles. In supersaturated bile, the cholesterol concentration exceeds that which can be isolated within hydrophobic micelle cores and the unbound cholesterol may form liquid or solid monohydrate crystals. Cholesterol supersaturation may result from excessive hepatic secretion of cholesterol or decreased rates of bile acid or phospholipid secretion. In this supersaturated state, solid cholesterol monohydrate crystals can precipitate in the bile and slowly form into visible cholesterol stones.

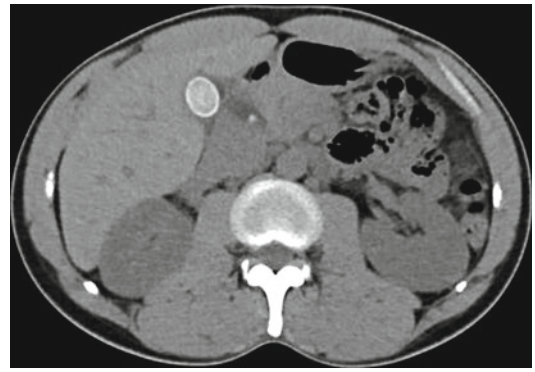

Fig. 20.1 Intrahepatic gallstone: CT image of a large intrahepatic, pigmented, and partially calcified stone located in the left hepatic duct

Black Pigment Stones

Black pigment stones may form in patients with sickle-cell disease, hereditary spherocytosis, thalassemia, or any other condition that leads to hemolysis. The processing of heme-containing compounds by the liver results in secretion of conjugated bilirubin into the biliary tree. Conjugated bilirubin is freely soluble in bile and not prone to stone formation. However, the activity of endogenous beta-glucuronidases converts a small fraction of conjugated bilirubin into unconjugated monohydrogenated bilirubin, which is poorly water soluble [5]. High concentrations of unconjugated bilirubin in the bile lead to precipitation of bilirubin and formation of black stones consisting of either pure calcium bilirubinate or polymer-like complexes of unconjugated bilirubin, calcium bilirubinate, calcium, and copper [6]. Unlike cholesterol stones, black pigment stones do not contain a regular crystalline structure.

Brown Pigment Stones

Brown pigment stones are associated with infection of the biliary tree and bile stasis. The stones are often reddish brown or dark brown in color and are softer and more easily fragmented than either cholesterol or black pigment stones. Brown stones are mostly composed of calcium salts of unconjugated bilirubin along with a variable composition of other bile components such as cholesterol, fatty acids, and mucin. Similar to black pigment stones, the conversion of conjugated bilirubin to unconjugated bilirubin by beta-glucuronidase is critical to stone formation. But rather than endogenous beta-glucuronidase acting on conjugated bilirubin, the beta-glucuronidase in brown pigment stone formation comes predominantly from bacteria infecting the biliary tree. *E. coli* is the bacteria most commonly associated with brown stone formation, and this species also produces other enzymes such as phospholipase and bile acid hydrolase, thus creating other poorly soluble components that are incorporated into the stone [7–9]. Brown pigment stones can form anywhere in the biliary tree, but the intrahepatic ducts are a particularly common location (Fig. 20.1).

Calcium Carbonate Stones

Calcium carbonate stones are extremely rare in adults, but recent infrared microspectroscopy analysis of pediatric stones suggests that they may represent up to a quarter of biliary stones in children [10]. The etiology and natural history of calcium carbonate stones remain unclear, but it has been suggested that they may form in response to transient cystic duct obstruction by viscous bile [10].

Epidemiology/Risk Factors

Age and Gender

Gallstones are uncommon in infancy but have been observed in cases of prematurity, sepsis, and total parenteral nutrition (TPN) use [11]. Most gallstones diagnosed in prepubescent children are associated with hemolytic disease, congenital abnormalities of the biliary tree (such as biliary cysts), or in the inspissated bile syndrome seen in acutely ill patients and those with severe congenital heart disease. Young children are at a low risk for cholesterol stones.

After the onset of puberty and throughout adulthood, the risk of stone formation increases linearly with age. By age 70, almost 50 % of women are found to have cholesterol gallstones [12].

In prepubescent children, gallstones occur equally in males and females. However, after the onset of puberty, women are about twice as likely to form cholesterol stones [13]. The unequal gender distribution has been at least partially explained by the effects of estrogen, which appears to make bile more lithogenic by increasing hepatic secretion of cholesterol [14, 15].

Obesity and Diet

Obesity is a significant risk factor for cholesterol cholelithiasis, but is not thought to directly relate to black or brown gallstone formation. Due to the historical observation that most pediatric stone disease was of hemolytic origin, obesity was thought to play a limited role in pediatric gallstone disease [2, 16, 17]. However, the increasing prevalence of pediatric obesity appears to be translating to an increased incidence of cholesterol stone disease among children [3]. A review by Mehta et al. of over 400 cholecystectomies in the southern USA showed that half were performed for cholesterol stone disease and, of those, almost 70 % of the children were overweight or obese [18]. A second study by Koebnick et al. showed a strong correlation between obesity and nonhemolytic stone disease with odds ratios for stone development over three for extremely obese teenage males and almost eight for extremely obese females [19]. Non-western countries also seem to be reporting more cases of cholesterol stone disease in children. A multicenter review of cholecystectomies performed on children in India showed that over 90 % had no evidence of hemolytic disease [20].

A western diet with high amounts of total calories, saturated fats, and cholesterol is thought to predispose to cholesterol supersaturation in bile and subsequent stone formation. Some non-western countries, such as China and Japan, have slowly adopted a more western-like diet and have had corresponding increases in cholesterol cholelithiasis, although separating this trend from the obesity associated with western diets is difficult [21, 22].

Hemolysis

All of the major hemolytic anemias including sickle-cell disease, spherocytosis, and thalassemia are predisposing factors for black pigment stone formation, but subclinical hemolysis has also been reported to convey increased risk. Ultrasound examination in 146 children with sickle-cell disease showed gallbladder sludge or stones in 83 (57 %) [23]. In another study, 44 asymptomatic children with hereditary spherocytosis were screened via ultrasonography, and 18 (41 %) were found to have gallstones. All but two of these children were 13 years old or younger [24]. An Italian study of 858 consecutive patients with thalassemia major demonstrated gallstones in 30 % [25]. Erythrocyte injury from prosthetic heart valve replacements, malaria infection, hypersplenism, and even repetitive foot trauma in long-distance runners have been linked to black pigment gallstone formation [26–28].

Total Parenteral Nutrition

TPN predisposes to cholestasis, biliary sludge, and stone formation. Over 40 % of children on

TPN for 3 or more months have been found to have gallstones [29]. Even shorter courses of TPN (1–2 weeks) may predispose to gallbladder sludge formation [16, 30]. Infusions of cholecystokinin (CCK) octapeptide or injections of CCK analogs have been shown to be a safe and effective way to decrease complications related to biliary stasis in adults on chronic TPN, but safety and efficacy in children have not been examined [31, 32].

Biliary Sludge

Biliary sludge is a common finding on imaging studies in both children and adults. Sludge is an intermediate step in gallstone formation, and the presence of biliary sludge increases the chance of subsequent stone formation. Despite this, the vast majority of adults with biliary sludge experience resolution and do not go on to develop biliary stones [33].

Drugs

Few drugs have been directly linked to gallstone formation. Prolonged use of ceftriaxone, particularly at high doses, has been associated with biliary sludge and microlithiasis. Unmetabolized ceftriaxone is concentrated in the bile and can precipitate after complexing with calcium to form an insoluble salt [34]. The antiretroviral, atazanavir, inhibits the bilirubin-conjugating enzyme UGT1A1. It has been linked to hyperbilirubinemia and has occasionally been reported to lead to cholelithiasis [35]. Furosemide has also been linked to gallstone formation in neonates, but distinguishing furosemide from other risk factors for stone formation in this age group has been difficult [36, 37].

Ileal Disease

The terminal ileal mucosa contains bile salt transporters responsible for maintaining the bile salt pool. Loss of these transporters leads to depletion of the bile salt pool and subsequent relative cholesterol supersaturation in bile. Disruptions of bile salt homeostasis also lead to increased intraluminal solubility of bilirubin in the colon and subsequent absorption and re-excretion into bile. This process increased the bilirubin concentration in bile and favors precipitation of pigmented stones [4]. Ileal disease or surgical resection has therefore been linked to an increased risk for both cholesterol and pigmented stones [38, 39].

Genetics

Despite controlling for multiple independent risk factors such as age, gender, and obesity, certain populations are at a higher risk for stone formation, indicative of a genetic component to gallstone disease. The Pima tribe of Native Americans are at a particularly high risk for stone disease and upwards of 50 % have evidence of gallstones [40]. Hispanic ethnicity has been shown to be an independent risk factor for stone disease in children [18]. Over a dozen genes related to hepatobiliary lipid transport and gallbladder motility have been implicated in gallstone disease [41]. ATP-binding cassette transporter mutations, such as those seen with progressive familial intrahepatic cholestasis, seem to confer an especially high risk for intrahepatic sludge, chronic cholestasis, and gallbladder cholesterol stones [42, 43]. Patients with cystic fibrosis are three to six times more likely than age-matched controls to develop black pigmented stones [44]. Gilbert syndrome has also been associated with an increased risk for gallstone formation, and coinheritance of this genotype with other risk factors for stone formation confers an even higher risk [45].

Biliary Dyskinesia

Poor gallbladder contractility may be secondary to impaired function or release of CCK or increased fibroblast growth factor [46]. Biliary dyskinesia may lead to stasis and stone formation but has also been implicated in right upper

quadrant pain, nausea, and vomiting without evidence of gallstones. The natural history of biliary dyskinesia remains poorly understood and successful treatment in children has been described with both conservative strategies and cholecystectomy [47, 48].

Asymptomatic Cholelithiasis

Consideration of the natural history of gallstones is important, particularly when asymptomatic gallbladder stones are incidentally discovered. Healthy population screening studies have shown that most gallstones are asymptomatic or cause only mild symptoms that do not lead to medical investigation [49–51]. Up to 2 % of children have gallstones, and the majority of them have no symptoms [11]. A North American study of patients with incidentally discovered gallstones revealed that after 15 years of follow-up, only 18 % had developed biliary pain. None had serious complications such as acute cholecystitis, gallstone pancreatitis, or cholangitis. The authors determined that there is only a 2 % annual risk of biliary pain developing in patients who have been incidentally discovered to have gallstones and that this risk decreases after 5 years [49]. Other investigations have suggested that the risk of asymptomatic biliary stones causing a major complication such as pancreatitis or cholangitis before the onset of biliary pain is very low (0–3 %) [51].

Treatment of asymptomatic gallstones is generally not necessary, but in situations where treatment is felt to be indicated, care needs to be taken to not expose patients to treatment-related risks that are more significant than the minor risks of untreated, asymptomatic stones. This consideration typically excludes endoscopic or surgical treatment of asymptomatic cholelithiasis.

Ursodeoxycholic acid (ursodiol) is the drug of choice for oral dissolution therapy. It is well tolerated and has no important side effects. Its major limitation in pediatric gallstone disease is that it is only effective for cholesterol stones and has essentially no effect on pigmented stones. Ursodiol works as a choleretic and by decreasing biliary cholesterol secretion thereby desaturating bile. The surface to volume ratio of the stones is an important factor affecting the rate of dissolution and is the reason why small stones respond more quickly and reliably to treatment. Ursodiol is typically dosed at 10–15 mg/kg/day at night. Therapy should be continued for at least 4–6 months but is often continued indefinitely. Oral dissolution therapy is fairly reliable for stones under 5 mm in diameter (70 % resolution rate), but drops to below 30 % for stones over 1 cm [52].

Symptomatic Cholelithiasis

Biliary pain, sometimes called biliary colic, is often the initial sign of a symptomatic gallstone. The term colic is somewhat of a misnomer as typical biliary pain starts rapidly, increases in intensity, and lasts for hours. The pain is typically localized to the right upper quadrant, but the right flank or epigastric region are other commonly reported locations. It may radiate to the back or shoulders and is commonly accompanied by nausea and vomiting. Patients often report that fatty foods can induce repeated episodes, but this association is relatively nonspecific. Recurrent biliary pain is quite common and is estimated to affect up to 70 % of patients with a history of known or suspected gallstone-related pain [53]. The complication risk associated with stones that cause biliary pain differs from those that are asymptomatic. The annual major complication rate is estimated at around 2 % per year and seems to remain constant over time [54].

Symptomatic cholelithiasis requires treatment, but the specific approach can be tailored to frequency and severity of symptoms. Patients with mild and infrequent biliary pain and suspected cholesterol stones are often good candidates for oral dissolution therapy (discussed above), which historically was combined with extracorporeal shock wave lithotripsy if there is a large (1–2 cm) solitary stone. Patients with more severe or frequent symptoms should be referred to surgery for cholecystectomy.

Gallstone-Related Complications

Acute Cholecystitis

Acute cholecystitis is characterized by gallbladder wall inflammation typically in the presence of cholelithiasis. It is the most common complication of symptomatic gallstone disease. A 2012 review by Peery et al. of over 39 million pediatric and adult inpatient admissions in the United States Nationwide Inpatient Sample database found cholecystitis to be the second leading gastrointestinal discharge diagnosis, trailing only acute pancreatitis [55].

Diagnosis

Patients with acute cholecystitis often present with fevers, leukocytosis, and right upper quadrant pain. Murphy's sign is a relatively specific finding and consists of inspiratory arrest with right subcostal palpation by the examiner while the patient attempts to take a deep breath. Serum aminotransferases may be mildly elevated and the patient may have mild jaundice with a total bilirubin up to 6 mg/dL. Bilirubin levels above 6 mg/dL are highly suggestive of common bile duct obstruction [56].

Ultrasound examination is often the most useful additional study when acute cholecystitis is suspected after initial examination and laboratory testing. Although the exam can be quite operator dependent in children, a sonographic Murphy's sign (focal gallbladder tenderness under the transducer) carries a 90 % positive predictive value in adults [57]. Cholescintigraphy is less commonly available and more time-consuming to obtain yet may be helpful in situations where acute cholecystitis seems unlikely but cannot be excluded due to the presence of stones in the gallbladder. A normal scintigraphy exam which shows isotope uptake in the intra- and extrahepatic ducts, gallbladder, and excretion into the intestine excludes the possibility of acute cholecystitis [58]. Computed tomography (CT) is typically not necessary to diagnose acute cholecystitis, but may be helpful in cases where local complications such as perforation are suspected. Magnetic resonance imaging (MRI) has not been formally studied for this indication in children, but adult studies show it to have equivalent sensitivity and specificity to US.

Treatment

Left untreated, the majority of patients with acute cholecystitis will experience uncomplicated resolution, yet an important minority, approximating 17 %, go on to develop serious complications including gangrenous cholecystitis, perforation, gallbladder empyema, or emphysematous cholecystitis [59].

The initial treatment approach to patients suspected of having acute cholecystitis should be focused on supportive care with intravenous hydration and electrolyte management and pain control as needed. Oral intake should be withheld and a nasogastric tube should be placed if there is persistent emesis or abdominal distension. For most cases, empiric antibiotic coverage is not necessary, but antibiotics are warranted if there is concern for perforation, gangrenous cholecystitis, and emphysematous cholecystitis or if the patient appears toxic. In these situations, broad-spectrum gram-negative antibacterial coverage is indicated with agents such as ampicillin/sulbactam, piperacillin/tazobactam, or a third-generation cephalosporin with metronidazole. After stabilization of the patient, definitive surgical therapy with cholecystectomy should be performed. A laparoscopic approach has been shown to be safe in children when done by experienced pediatric surgeons [60].

Choledocholithiasis

Choledocholithiasis is defined as the presence of stones in the bile ducts. A small percentage (15 %) of adult patients with cholelithiasis are found to have choledocholithiasis, but almost all patients with choledocholithiasis have concurrent cholelithiasis [61]. Cholelithiasis in the presence of choledocholithiasis suggests that most stones

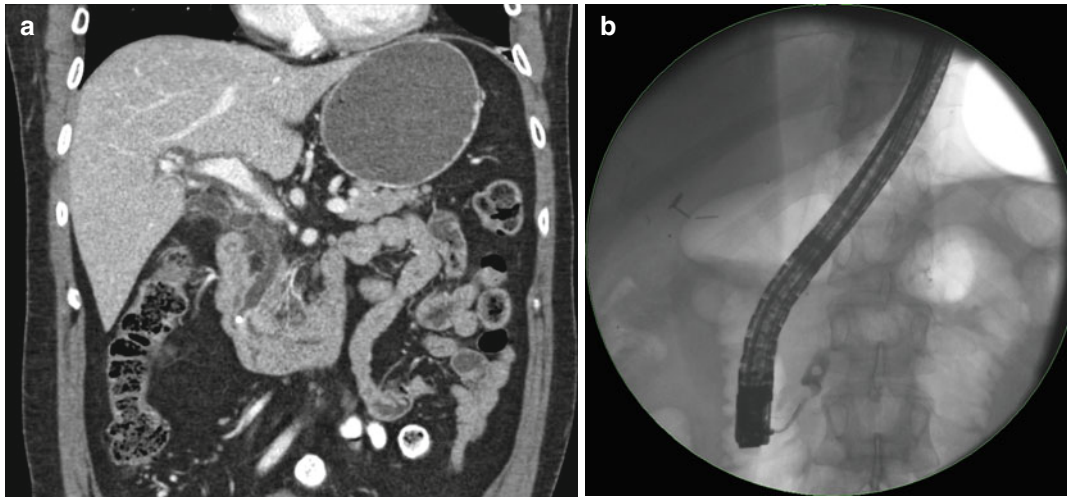

Fig. 20.2 (**a**) Choledocholithiasis: distal common bile duct stone noted on CT after cholecystectomy. (**b**) ERCP cholangiogram showing the same stone prior to endoscopic removal

found in the bile ducts are of gallbladder origin and it is believed that all cholesterol stones are formed in gallbladder (Fig. 20.2). On the other hand, most brown pigmented stones develop within the bile ducts. This is reflected clinically in patients with symptomatic cholesterol stone disease who have undergone endoscopic retrograde cholangiopancreatography (ERCP) with sphincterotomy and cholecystectomy, only to return months or years later with recurrence of choledocholithiasis. These newly found stones are likely brown pigment stones that can form within the bile ducts due to retrograde movement of intestinal bacteria into the biliary tree and the actions of bacterial beta-glucuronidase and deconjugation of bilirubin.

Diagnosis

Choledocholithiasis becomes symptomatic when stones partially or completely obstruct the common bile duct, often in its distal segment at the level of the ampulla. This obstruction can be persistent or transient with spontaneous expulsion of the stone into the duodenum or with ball-valve-like movement of the stone which remains in the duct but only intermittently occludes bile drainage.

Patients with acute stone impaction often present with right upper quadrant pain and jaundice (Fig. 20.3). Laboratory evidence of cholestatic hepatitis is remarkably sensitive (>90 %) for choledocholithiasis and may consist of elevations of serum aminotransferases, alkaline phosphatase, and gamma-glutamyl transferase (GGT). GGT is the most sensitive parameter of these laboratory markers [62]. The level of bilirubin elevation typically reflects the degree of biliary obstruction. Impacted stones in the distal common bile duct may also prevent drainage from the pancreatic duct resulting in laboratory and clinical evidence of gallstone pancreatitis.

Abdominal ultrasound is much less sensitive for choledocholithiasis compared to cholecystitis. Although common bile duct dilation with the presence of cholelithiasis is suggestive of choledocholithiasis, ultrasound alone led to a definitive diagnosis of only 22 % of the time in one study [63]. Because of the poor sensitivity of ultrasound, other diagnostic studies may be necessary depending upon the clinical suspicion for a bile duct stone. In cases where choledocholithiasis is highly likely proceeding to ERCP, laparoscopic ultrasound or intraoperative cholangiography can definitively establish the diagnosis while offering treatment options. In less clear cases, additional imaging may be necessary. Magnetic

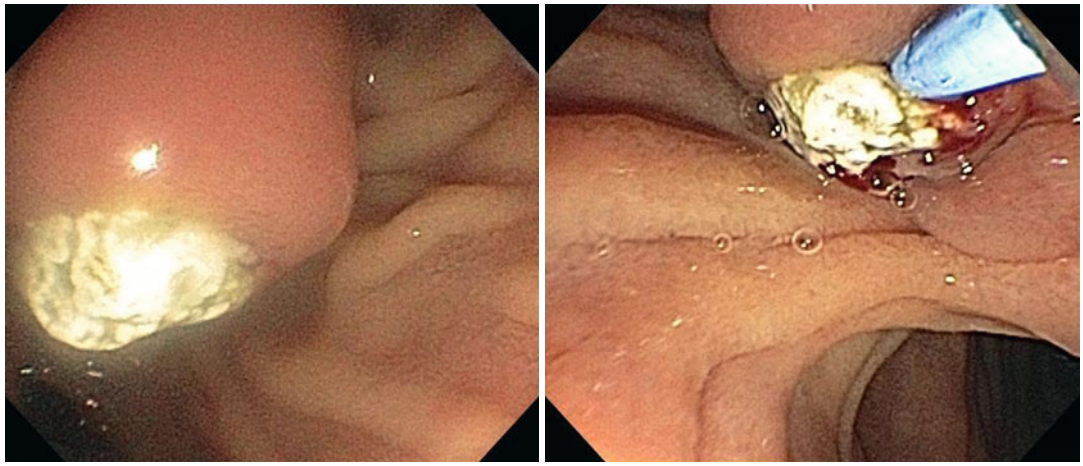

Fig. 20.3 Impacted stone: endoscopic images of an impacted cholesterol stone at the duodenal ampulla in a patient who presented with jaundice, biliary-type pain, and gallstone pancreatitis. Signs of pancreaticobiliary obstruction resolved after removal and the patient underwent cholecystectomy prior to discharge

resonance cholangiopancreatography (MRCP) is often the modality of choice, but more invasive options such as endoscopic ultrasound (EUS) and percutaneous transhepatic cholangiography (PTC) offer sensitivity similar to that of ERCP [64, 65].

Treatment

Although choledocholithiasis may be asymptomatic, and small stones may pass spontaneously into the duodenum, when stones in the common bile duct are found, intervention is necessary due to the risk of serious complications such as cholangitis and gallstone pancreatitis. The approach to stone removal may depend on when choledocholithiasis is discovered during the clinical course, the presence or absence of a gallbladder, and local endoscopic/surgical expertise. Options for stone removal include ERCP and surgical common bile duct exploration via either an open or laparoscopic trans-cystic approach. Because most common bile duct stones originate in the gallbladder, both cholecystectomy and removal of ductal stones are necessary. Resolution of choledocholithiasis is of higher priority than cholecystectomy, but the exact sequence and timing of the two procedures remains debatable.

Many institutions with the appropriate endoscopic expertise have adopted a strategy of initial ERCP for endoscopic sphincterotomy and stone removal followed by interval cholecystectomy within 6 weeks [66]. This approach allows for early identification of patients that require surgical bile duct exploration for treatment of stones that cannot be endoscopically removed. Early cholecystectomy with intraoperative cholangiography is appropriate when cholelithiasis has been proven, but the presence of choledocholithiasis remains uncertain. This approach, however, raises the possibility that a third procedure (surgical bile duct exploration) will be required to address the stone if it cannot be flushed into the duodenum on the intraoperative cholangiogram and if ERCP removal attempts are unsuccessful. Intraoperative ERCP at the time of cholecystectomy may present coordination and logistical issues but allows for definitive treatment during one anesthetic session [67].

Cholangitis

The term cholangitis typically refers to acute bacterial cholangitis. This bacterial infection of the biliary tree is the most serious and worrisome complication of gallstone disease. A high index

of suspicion is necessary in all patients with biliary obstruction because signs and symptoms of cholangitis may be initially subtle.

In adults, about 85 % of cholangitis cases are due to choledocholithiasis [68], but data regarding the proportional etiologies in pediatrics have not been well delineated. Any condition that causes biliary stasis or obstruction can lead to cholangitis including choledochal cysts, biliary strictures, parasitic infections, neoplasm, or a history of hepatobiliary surgery. The enteric flora most often responsible for cholangitis, include *E. coli*, *Pseudomonas*, *Proteus*, *Klebsiella*, *Bacteroides*, and *Clostridium* [69, 70].

Diagnosis

The gold standard for cholangitis diagnosis requires direct confirmation of biliary infection. This level of diagnostic certainty is rare, however, as it requires culture of liver tissue or directly aspirated bile. Cholangitis is more typically diagnosed clinically on the presence of Charcot's triad of right upper quadrant pain, fever, and jaundice or Reynolds' pentad which also includes septic shock and confusion. There are few reports comparing the sensitivity of these clinical markers to the gold standard, but available data suggests that less than one quarter of patients with cholangitis present with all three elements of Charcot's triad [71]. Because of the need for a standardized diagnostic definition of acute cholangitis, the Tokyo guidelines were adopted in 2006. They state that a definitive diagnosis of cholangitis is based on the presence of either all three elements of Charcot's triad or two elements of the triad plus laboratory evidence of an inflammatory response, abnormal liver function tests, or imaging evidence of biliary abnormalities. A suspected diagnosis was defined as two or more of the following: upper abdominal pain, jaundice, fever/chills, or a history of biliary disease [72].

Multiple imaging options are available to examine for duct dilation or etiologies predisposing to cholangitis including abdominal ultrasound, CT, MRI/MRCP, EUS, and ERCP. Only ERCP offers the possibility of therapeutic intervention and it is also the modality of choice for identifying biliary stones and strictures. The most significant downfall of ERCP relates to its invasive nature and potential complications. CT and MRI/MRCP are superior to abdominal ultrasound and are the preferred noninvasive studies. EUS is superior to CT and MRI/MRCP at identifying stones and obstructive lesions but is rarely obtained prior to cross-sectional imaging [73].

Management

Inpatient hospital management is indicated for almost all patients with cholangitis. The two mainstays of treatment are antibiotics and biliary decompression. After initial fluid resuscitation, blood cultures should be obtained and empiric antibiotics started promptly. There are multiple appropriate initial antibiotic options, but all include broad-spectrum gram-negative and anaerobic bacterial coverage targeting the most common pathogens noted above. Institutional and regional antibiotic choices may differ based on the local formulary and resistance patterns. Common initial regimens may be a penicillin with β-lactamase inhibitor such as ampicillin/sulbactam or piperacillin/tazobactam.

Gallstone Pancreatitis

As alluded to earlier, gallstones present in the common bile duct may become lodged at the level of the major duodenal ampulla. In addition to causing biliary obstruction, stones in this region may also compress and obstruct pancreatic drainage resulting in gallstone pancreatitis. Gallstone pancreatitis should be suspected in any patient with pancreatitis who is found to have cholelithiasis on initial ultrasound examination. The initial treatment of gallstone pancreatitis focuses on appropriate supportive care. Cholecystectomy should not be delayed and should be performed during the same hospital admission after improvement of pancreatitis symptoms [74]. Occasionally, gallstones can become tightly impacted at the

major ampulla, and ERCP is required to allow for biliary and pancreatic ductal decompression prior to cholecystectomy.

Mirizzi Syndrome

Mirizzi syndrome refers to obstruction of the common hepatic or common bile duct as a consequence of direct compression by a gallstone impacted in the cystic duct. Secondary inflammatory changes may exacerbate the biliary obstruction and lead to biliary strictures or even erosion of the stone into the common duct with resultant fistulous connection. Treatment includes cholecystectomy with or without common bile duct exploration or ERCP. Repair of cholecystocholedochal fistulas may require a hepaticojejunostomy. Mirizzi syndrome is a rare complication of gallstone disease and typically seen in elderly patients but has been reported in children [75, 76].

Sphincter of Oddi Dysfunction

Although not related to gallstones, sphincter of Oddi dysfunction (SOD) is another obstructive disorder of the bile duct that may result in biliary pain similar to that of gallstone disease. In SOD, there is resistance to bile excretion in the distal aspect of the common bile duct where it is enveloped by the sphincter of Oddi, just proximal to its entry to the duodenum. Other terms used in the medical literature and clinical practice which are often used interchangeably with SOD include ampullary stenosis, postcholecystectomy syndrome, biliary spasm, biliary dyssynergia, papillary stenosis, and sclerosing papillitis.

Diagnosis of SOD is based on the Modified Milwaukee Criteria [77] (Table 20.1). This classification system is extremely important in determining the need for ERCP, sphincter of Oddi manometry, and possible biliary sphincterotomy. Over 90 % of patients with SOD type I will have improvement or resolution of their biliary pain after sphincterotomy regardless of endoscopic manometric sphincter pressures. Hypertension of the sphincter, however, can predict clinical response to sphincterotomy for SOD types II and III. In SOD type II, 85 % of those with elevated sphincter pressures will respond to sphincterotomy, while only 35 % of those with normal pressures will note improvement. In type III, about half of patients with elevated sphincter pressures will respond to sphincterotomy, while few patients with normal pressures will have any change in their symptoms, suggesting a functional etiology [78].

Table 20.1 Modified Milwaukee Criteria for diagnosis of sphincter of Oddi dysfunction

Sphincter of Oddi dysfunction	Criteria
Type I	1. Biliary-type pain 2. Serum ALT, AST, or alkaline phosphatase ≥2 times the upper limit of normal on two or more occasions 3. Common bile duct diameter ≥10 mm
Type II	1. Biliary-type pain 2. One of the other two criteria for Type I
Type III	1. Biliary-type pain alone

Acute Hydrops of the Gallbladder

Acute hydrops of the gallbladder is defined by distension of the organ without associated cholelithiasis, infection, or related anatomic abnormality. Gallbladder hydrops is distinguished from acalculous cholecystitis by the absence of a coexistent local inflammatory process. The condition may be seen in acute systemic infections and with prolonged use of TPN, but in many cases, a specific cause is not identified.

Affected patients may present with right upper quadrant pain, and US examination typically shows a markedly distended, echo-free gallbladder without wall thickening and a normal biliary tree. Gallbladder hydrops is a benign condition and typically resolves with conservative management and appropriate supportive care of any intercurrent illness. Cholecystectomy or percutaneous drainage is rarely necessary.

References

1. Russo MW, Wei JT, Thiny MT, et al. Digestive and liver diseases statistics, 2004. Gastroenterology. 2004; 126(5):1448–53.
2. Calabrese C, Pearlman DM. Gallbladder disease below the age of 21 years. Surgery. 1971;70(3):413–5.
3. Kaechele V, Wabitsch M, Thiere D, et al. Prevalence of gallbladder stone disease in obese children and adolescents: influence of the degree of obesity, sex, and pubertal development. J Pediatr Gastroenterol Nutr. 2006;42(1):66–70.
4. Wang DQ, Afdhal NH. Gallstone disease. In: Feldman M, Friedman LS, Brandt LJ, editors. Sleisenger and Fordtran's gastrointestinal and liver disease, vol. 1. 9th ed. Philadelphia: Elsevier; 2010.
5. Lambou-Gianoukos S, Heller SJ. Lithogenesis and bile metabolism. Surg Clin North Am. 2008;88(6):1175–94, vii.
6. Vitek L, Carey MC. New pathophysiological concepts underlying pathogenesis of pigment gallstones. Clin Res Hepatol Gastroenterol. 2012;36(2):122–9.
7. Vitetta L, Sali A. Primary bile duct stones and bacterial activity. HPB Surg. 1992;6(1):23–32; discussion 33.
8. Cetta FM. Bile infection documented as initial event in the pathogenesis of brown pigment biliary stones. Hepatology. 1986;6(3):482–9.
9. Nakano T, Tabata M, Nakayma F. Unconjugated bilirubin in hepatic bile with brown pigment gallstones and cholangitis. Dig Dis Sci. 1988;33(9):1116–20.
10. Stringer MD, Soloway RD, Taylor DR, Riyad K, Toogood G. Calcium carbonate gallstones in children. J Pediatr Surg. 2007;42(10):1677–82.
11. Debray D, Franchi-Abella S, Irtan S, Girard M. Cholelithiasis in infants, children and adolescents. Presse Med. 2012;41(5):466–73.
12. Sama C, Labate AM, Taroni F, Barbara L. Epidemiology and natural history of gallstone disease. Semin Liver Dis. 1990;10(3):149–58.
13. Diehl AK. Epidemiology and natural history of gallstone disease. Gastroenterol Clin North Am. 1991; 20(1):1–19.
14. Wang HH, Afdhal NH, Wang DQ. Estrogen receptor alpha, but not beta, plays a major role in 17beta-estradiol-induced murine cholesterol gallstones. Gastroenterology. 2004;127(1):239–49.
15. Angelin B, Olivecrona H, Reihner E, et al. Hepatic cholesterol metabolism in estrogen-treated men. Gastroenterology. 1992;103(5):1657–63.
16. Wesdorp I, Bosman D, de Graaff A, Aronson D, van der Blij F, Taminiau J. Clinical presentations and predisposing factors of cholelithiasis and sludge in children. J Pediatr Gastroenterol Nutr. 2000;31(4):411–7.
17. Lobe TE. Cholelithiasis and cholecystitis in children. Semin Pediatr Surg. 2000;9(4):170–6.
18. Mehta S, Lopez ME, Chumpitazi BP, Mazziotti MV, Brandt ML, Fishman DS. Clinical characteristics and risk factors for symptomatic pediatric gallbladder disease. Pediatrics. 2012;129(1):e82–8.
19. Koebnick C, Smith N, Black MH, et al. Pediatric obesity and gallstone disease. J Pediatr Gastroenterol Nutr. 2012;55(3):328–33.
20. Punia RP, Garg S, Bisht B, Dalal U, Mohan H. Clinico-pathological spectrum of gallbladder disease in children. Acta Paediatr. 2010;99(10):1561–4.
21. Huang YC, Zhang XW, Yang RX. Changes in cholelithiasis in Tianjin in the past 30 years. Chin Med J (Engl). 1984;97(2):133–5.
22. Nagase M, Tanimura H, Setoyama M, Hikasa Y. Present features of gallstones in Japan. A collective review of 2,144 cases. Am J Surg. 1978;135(6):788–90.
23. Suell MN, Horton TM, Dishop MK, Mahoney DH, Olutoye OO, Mueller BU. Outcomes for children with gallbladder abnormalities and sickle cell disease. J Pediatr. 2004;145(5):617–21.
24. Tamary H, Aviner S, Freud E, et al. High incidence of early cholelithiasis detected by ultrasonography in children and young adults with hereditary spherocytosis. J Pediatr Hematol Oncol. 2003;25(12):952–4.
25. Origa R, Galanello R, Perseu L, et al. Cholelithiasis in thalassemia major. Eur J Haematol. 2009;82(1):22–5.
26. Azemoto R, Tsuchiya Y, Ai T, et al. Does gallstone formation after open cardiac surgery result only from latent hemolysis by replaced valves? Am J Gastroenterol. 1996;91(10):2185–9.
27. Chawla LS, Sidhu G, Sabharwal BD, Bhatia KL, Sood A. Jaundice in plasmodium falciparum. J Assoc Physicians India. 1989;37(6):390–1.
28. Diehl AK, Schwesinger WH, Holleman Jr DR, Chapman JB, Kurtin WE. Clinical correlates of gallstone composition: distinguishing pigment from cholesterol stones. Am J Gastroenterol. 1995;90(6):967–72.
29. Roslyn JJ, Berquist WE, Pitt HA, et al. Increased risk of gallstones in children receiving total parenteral nutrition. Pediatrics. 1983;71(5):784–9.
30. Messing B, Bories C, Kunstlinger F, Bernier JJ. Does total parenteral nutrition induce gallbladder sludge formation and lithiasis? Gastroenterology. 1983;84(5 Pt 1):1012–9.
31. Sitzmann JV, Pitt HA, Steinborn PA, Pasha ZR, Sanders RC. Cholecystokinin prevents parenteral nutrition induced biliary sludge in humans. Surg Gynecol Obstet. 1990;170(1):25–31.
32. Prescott Jr WA, Btaiche IF. Sincalide in patients with parenteral nutrition-associated gallbladder disease. Ann Pharmacother. 2004;38(11):1942–5.
33. Lee SP, Maher K, Nicholls JF. Origin and fate of biliary sludge. Gastroenterology. 1988;94(1):170–6.
34. Schaad UB, Wedgwood-Krucko J, Tschaeppeler H. Reversible ceftriaxone-associated biliary pseudolithiasis in children. Lancet. 1988;2(8625):1411–3.
35. Jacques AC, Giguere P, Zhang G, Touchie C, la Porte CJ. Atazanavir-associated choledocholithiasis leading to acute hepatitis in an HIV-infected adult. Ann Pharmacother. 2010;44(1):202–6.

36. Randall LH, Shaddy RE, Sturtevant JE, Reid BS, Molteni RA. Cholelithiasis in infants receiving furosemide: a prospective study of the incidence and one-year follow-up. J Perinatol. 1992;12(2):107–11.
37. Brill PW, Winchester P, Rosen MS. Neonatal cholelithiasis. Pediatr Radiol. 1982;12(6):285–8.
38. Lapidus A, Bangstad M, Astrom M, Muhrbeck O. The prevalence of gallstone disease in a defined cohort of patients with Crohn's disease. Am J Gastroenterol. 1999;94(5):1261–6.
39. Brink MA, Slors JF, Keulemans YC, et al. Enterohepatic cycling of Bilirubin: a putative mechanism for pigment gallstone formation in ileal Crohn's disease. Gastroenterology. 1999;116(6):1420–7.
40. Grimaldi CH, Nelson RG, Pettitt DJ, Sampliner RE, Bennett PH, Knowler WC. Increased mortality with gallstone disease: results of a 20-year population-based survey in pima Indians. Ann Intern Med. 1993; 118(3):185–90.
41. Marschall HU, Katsika D, Rudling M, Einarsson C. The genetic background of gallstone formation: an update. Biochem Biophys Res Commun. 2010;396(1): 58–62.
42. Rosmorduc O, Hermelin B, Boelle PY, Parc R, Taboury J, Poupon R. ABCB4 gene mutation-associated cholelithiasis in adults. Gastroenterology. 2003;125(2): 452–9.
43. Rosmorduc O, Hermelin B, Poupon R. MDR3 gene defect in adults with symptomatic intrahepatic and gallbladder cholesterol cholelithiasis. Gastroenterology. 2001;120(6):1459–67.
44. Wasmuth HE, Keppeler H, Herrmann U, Schirin-Sokhan R, Barker M, Lammert F. Coinheritance of Gilbert syndrome-associated UGT1A1 mutation increases gallstone risk in cystic fibrosis. Hepatology. 2006;43(4):738–41.
45. Fretzayas A, Moustaki M, Liapi O, Karpathios T. Gilbert syndrome. Eur J Pediatr. 2012;171(1):11–5.
46. Portincasa P, Di Ciaula A, Wang HH, et al. Coordinate regulation of gallbladder motor function in the gut-liver axis. Hepatology. 2008;47(6):2112–26.
47. Scott Nelson R, Kolts R, Park R, Heikenen J. A comparison of cholecystectomy and observation in children with biliary dyskinesia. J Pediatr Surg. 2006; 41(11):1894–8.
48. Constantinou C, Sucandy I, Ramenofsky M. Laparoscopic cholecystectomy for biliary dyskinesia in children: report of 100 cases from a single institution. Am Surg. 2008;74(7):587–92; discussion 593.
49. Gracie WA, Ransohoff DF. The natural history of silent gallstones: the innocent gallstone is not a myth. N Engl J Med. 1982;307(13):798–800.
50. Attili AF, Pazzi P, Galeazzi R. Prevalence of previously undiagnosed gallstones in a population with multiple risk factors. Dig Dis Sci. 1995;40(8):1770–4.
51. Attili AF, De Santis A, Capri R, Repice AM, Maselli S. The natural history of gallstones: the GREPCO experience. The GREPCO group. Hepatology. 1995;21(3): 655–60.
52. May GR, Sutherland LR, Shaffer EA. Efficacy of bile acid therapy for gallstone dissolution: a meta-analysis of randomized trials. Aliment Pharmacol Ther. 1993; 7(2):139–48.
53. Thistle JL, Cleary PA, Lachin JM, Tyor MP, Hersh T. The natural history of cholelithiasis: the national cooperative gallstone study. Ann Intern Med. 1984; 101(2):171–5.
54. Ransohoff DF, Gracie WA. Treatment of gallstones. Ann Intern Med. 1993;119(7 Pt 1):606–19.
55. Peery AF, Dellon ES, Lund J, et al. Burden of gastrointestinal disease in the United States: 2012 update. Gastroenterology. 2012;143(5):1179–87.e3.
56. Dumont AE. Significance of hyperbilirubinemia in acute cholecystitis. Surg Gynecol Obstet. 1976;142(6): 855–7.
57. Nino-Murcia M, Jeffrey Jr RB. Imaging the patient with right upper quadrant pain. Semin Roentgenol. 2001;36(2):81–91.
58. Kiewiet JJ, Leeuwenburgh MM, Bipat S, Bossuyt PM, Stoker J, Boermeester MA. A systematic review and meta-analysis of diagnostic performance of imaging in acute cholecystitis. Radiology. 2012;264(3):708–20.
59. Bedirli A, Sakrak O, Sozuer EM, Kerek M, Guler I. Factors effecting the complications in the natural history of acute cholecystitis. Hepatogastroenterology. 2001;48(41):1275–8.
60. Chen K, Cheung K, Sosa JA. Surgeon volume trumps specialty: outcomes from 3596 pediatric cholecystectomies. J Pediatr Surg. 2012;47(4):673–80.
61. Soloway RD, Trotman BW, Ostrow JD. Pigment gallstones. Gastroenterology. 1977;72(1):167–82.
62. Pereira-Lima JC, Jakobs R, Busnello JV, Benz C, Blaya C, Riemann JF. The role of serum liver enzymes in the diagnosis of choledocholithiasis. Hepatogastroenterology. 2000;47(36):1522–5.
63. Einstein DM, Lapin SA, Ralls PW, Halls JM. The insensitivity of sonography in the detection of choledocholithiasis. AJR Am J Roentgenol. 1984;142(4):725–8.
64. Ralls PW, Colletti PM, Lapin SA, et al. Real-time sonography in suspected acute cholecystitis. prospective evaluation of primary and secondary signs. Radiology. 1985;155(3):767–71.
65. NIH state-of-the-science statement on endoscopic retrograde cholangiopancreatography (ERCP) for diagnosis and therapy. NIH Consens State Sci Statements. 2002;19(1):1–26.
66. Li VK, Yum JL, Yeung YP. Optimal timing of elective laparoscopic cholecystectomy after acute cholangitis and subsequent clearance of choledocholithiasis. Am J Surg. 2010;200(4):483–8.
67. ElGeidie AA, ElEbidy GK, Naeem YM. Preoperative versus intraoperative endoscopic sphincterotomy for management of common bile duct stones. Surg Endosc. 2011;25(4):1230–7.
68. Lillemoe KD. Surgical treatment of biliary tract infections. Am Surg. 2000;66(2):138–44.
69. Leung JW, Ling TK, Chan RC, et al. Antibiotics, biliary sepsis, and bile duct stones. Gastrointest Endosc. 1994;40(6):716–21.

70. Leung JW, Liu YL, Lau GC, et al. Bacteriologic analyses of bile and brown pigment stones in patients with acute cholangitis. Gastrointest Endosc. 2001;54(3): 340–5.
71. Csendes A, Diaz JC, Burdiles P, Maluenda F, Morales E. Risk factors and classification of acute suppurative cholangitis. Br J Surg. 1992;79(7):655–8.
72. Lee JG. Diagnosis and management of acute cholangitis. Nat Rev Gastroenterol Hepatol. 2009;6(9):533–41.
73. Kondo S, Isayama H, Akahane M, et al. Detection of common bile duct stones: comparison between endoscopic ultrasonography, magnetic resonance cholangiography, and helical-computed-tomographic cholangiography. Eur J Radiol. 2005;54(2):271–5.
74. Tang E, Stain SC, Tang G, Froes E, Berne TV. Timing of laparoscopic surgery in gallstone pancreatitis. Arch Surg. 1995;130(5):496–9; discussion 499–500.
75. Obata K, Ohta M, Yamagiwa I, Naito S, Washio M. Cholecystolithiasis causing Mirizzi's syndrome with a rare anomaly of the extrahepatic bile duct in a child. Report of a case. Nihon Geka Gakkai Zasshi. 1988; 89(5):781–5.
76. Kim J, Jarboe MD, Arnold MA, DiPietro MA, Bloom DA, Teitelbaum DH. Biliary duplication cyst with heterotopic gastric mucosa resulting in obstruction of the biliary system: a case report. J Pediatr Surg. 2012; 47(6):e5–8.
77. Hogan WJ, Geenen JE. Biliary dyskinesia. Endoscopy. 1988;20 Suppl 1:179–83.
78. Eversman D, Fogel EL, Rusche M, Sherman S, Lehman GA. Frequency of abnormal pancreatic and biliary sphincter manometry compared with clinical suspicion of sphincter of Oddi dysfunction. Gastrointest Endosc. 1999;50(5):637–41.

Vascular Liver Disease

21

Ines Loverdos and Simon C. Ling

Anatomy and Embryology

The liver has a dual blood supply: the portal vein and the hepatic artery. The portal vein originates and ends in a capillary system and carries venous blood from the mesenteric and splenic veins to the liver. The branches of the portal vein define the eight segments of the liver and divide sequentially into terminal branches ending in the hepatic sinusoids.

The hepatic artery supplies oxygenated blood from the celiac trunk and divides sequentially along the distribution of the portal vessels. Oxygenated blood thus circulates to the hepatic sinusoids as well as to the periportal capillary plexus, the peribiliary capillary plexus, the vasa vasorum, and the capillaries of Glisson's capsule.

Blood from the hepatic sinusoids flows to the central veins which merge into the three hepatic veins and thence to the inferior vena cava.

The intrauterine pattern of the hepatic venous circulation is established by the end of the 6th week of gestation. During the fetal period, the liver has two afferent veins: the umbilical vein (from the umbilical vascular system) and the portal vein (from the vitelline veins). The umbilical vein is the predominant vessel accounting for approximately 80 % of the fetal liver blood supply. Involution of the umbilical vein begins at birth and is completed by 15–20 days of age.

The development of the arterial hepatic circulation begins slightly later, at the eighth gestational week. It is dependent on the development of the intrahepatic portal system and coordinated with that of the biliary system [1–3].

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Extrahepatic Portal Vein Obstruction

The portal vein is responsible for approximately two thirds of the hepatic blood supply. When thrombosis in the portal system occurs, two mechanisms develop to maintain the hepatic blood flow: hepatic artery vasodilatation and creation of a portal cavernoma. Portal cavernoma is a network of hepatopetal collaterals around the thrombosed portal vein, which attempt to bypass the block and allow blood flow to the liver. Cavernous transformation of the portal vein begins to develop within days of the thrombosis occurring. Nevertheless, these new collaterals are not sufficient to carry all the portal blood flow and thus presinusoidal, prehepatic portal hypertension occurs [1, 4, 5].

Definition

Extrahepatic portal vein obstruction (EHPVO) is a vascular disorder of the liver defined by obstruction of the extrahepatic portal vein with

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or without involvement of the intrahepatic portal veins or mesenteric veins. Isolated occlusion of the splenic vein or superior mesenteric vein does not constitute EHPVO, but it can extend to the portal branches or to the splenic, superior mesenteric, or coronary veins. The term EHPVO usually refers to portal vein thrombosis with portal cavernoma in the absence of liver cirrhosis or abdominal neoplasm. EHPVO implies chronicity and therefore acute thrombosis of the portal vein should be considered as a different entity [6–9].

Epidemiology

EHPVO is the second most common cause of portal hypertension in the world, after cirrhosis. It is responsible for up to 30 % of all variceal bleeding in developing countries and is the most common cause of variceal bleeding in children [6, 8, 10].

Etiology

The cause of EHPVO in children remains unclear in approximately 50 % of patients. Several variables are relevant to understanding the cause in individual children.

Local Factors

The presence of local factors such as omphalitis, umbilical vein catheterization (UVC), dehydration, and neonatal or abdominal sepsis has been documented in a variable percentage of cases. The role of UVC as a risk factor for problematic EHPVO is controversial because the associated thrombi will often resolve spontaneously during infancy and may be limited to the left portal vein without important clinical sequelae. The percentage of portal vein thrombosis following neonatal UVC ranges from 0 to 43 % in the literature [11–14].

Prothrombotic State

It is unclear whether or not a hypercoagulable condition plays a role in pediatric EHPVO [6, 8, 15–17]. Case–control studies report odds ratios for children with EHPVO having a thrombophilic state compared to controls ranging from 1 to 11.9. The most common reported abnormalities are factor V Leiden mutation and protein C and/or S deficiency [17–19]. The concurrence of more than one thrombophilic disorder is reported in up to 12.5 % of children with EHPVO [20]. However, not all reports have found thrombophilic abnormalities [15]. Apparent deficiency of coagulation-inhibitor proteins should be interpreted with caution, because restoration of portal flow by performing mesenteric-to-left portal vein bypass surgery has been shown to improve previously depressed circulating levels of protein C, protein S, and prothrombin time in children with EHPVO [21]. Therefore genetic testing is preferred over functional testing to assess for thrombophilic disorders in this setting.

Congenital Anomaly

The presence of abnormalities in other systems, such as cardiovascular or urinary tract, suggests potential for congenital anomaly of the portal vein to be associated with EHPVO in some children [17, 22].

Clinical Presentation and Natural History

Acute portal vein thrombosis should be suspected in patients presenting with abdominal pain, ascites, fever, or symptoms consistent with intestinal ischemia in the absence of portal cavernoma. Identifying portal vein thrombosis in the acute stage is rare in pediatrics, and its management will not be further considered here.

EHPVO in children is usually a chronic condition that is not associated with intrinsic liver disease. The symptoms of EHPVO can be classified into those derived from portal hypertension and those secondary to spontaneous portosystemic shunting.

EHPVO may present coincidentally when splenomegaly is felt on physical examination or noted on an abdominal ultrasound ordered for other reasons. In other children, acute variceal bleeding may be the first presenting symptom. In a cohort of 108 children with EHPVO, 79 %

of the patients had an episode of gastrointestinal bleeding, and in 42 % the bleeding occurred before 4 years of age [5, 6, 8, 17].

Ascites is usually absent. Abnormal cell counts resulting from hypersplenism may be noted. In the majority of children, liver enzyme levels in blood are normal.

Children with EHPVO may have growth retardation and decreased growth velocity. Reduced blood flow to the liver might reduce the hepatic supply of hepatotrophic hormones generated in the splanchnic circulation. Whether resistance to growth hormone plays a role in the setting of EHPVO is unclear [23, 24].

Neurocognitive function may be impaired by covert hepatic encephalopathy. Mack et al. studied eight children with EHPVO who underwent mesenteric-to-left portal vein bypass (Rex bypass), which restores portal blood flow to the liver. Surgery resulted in improvement of fluid cognitive ability indices, implying that portosystemic shunting is responsible for cognitive deficits in some children with EHPVO [25].

Rarely, portal biliopathy may cause clinical, biochemical, and/or imaging features of biliary obstruction or stricturing. In these children, imaging studies reveal the presence of intra- and/or extrahepatic bile duct dilatation and strictures, which may arise due to extrinsic compression of the bile ducts by the portal cavernoma or due to ischemic damage secondary to portal vein thrombosis. Symptomatic portal biliopathy is a serious complication that can lead to biliary cirrhosis [5, 17, 26–28].

Other associated morbidities include hepatopulmonary syndrome and portopulmonary hypertension that may be identified by reduced exercise tolerance and oxygen saturation levels.

Diagnosis

EHPVO is best diagnosed by Doppler ultrasound, which reveals the presence of a portal vein obstruction, intraluminal material, or portal cavernoma (Table 21.1). Computerized tomography (CT) and magnetic resonance imaging (MRI) may help to determine the extent of thrombosis and plans for future intervention. The patency of intrahepatic portal veins can be demonstrated by transjugular retrograde or transhepatic portal venography. Assessment of underlying thrombophilic disorders should be undertaken, relying where possible on genetic rather than functional testing. Liver biopsy is only indicated if there is suspicion of intrinsic liver disease. In children with other clinical findings or hypoxemia, echocardiography is useful to rule out the presence of associated congenital heart defects and the presence of concomitant hepatopulmonary syndrome or portopulmonary hypertension [5, 6, 8, 29].

Table 21.1 EHPVO workup

Physical exam	Splenomegaly, jaundice, vascular spiders, palmar erythema, abdominal wall veins
Medical history	Umbilical vein catheterization, omphalitis, pancreatitis, appendicitis, abdominal surgery, inflammatory bowel disease, liver abscess
	Family history of thrombosis
Imaging studies	Doppler ultrasound
	Second-line studies: CT scan, MRI
Initial laboratory investigations	CBC, coagulation, liver function tests
Thrombophilia panel	Protein C, protein S, antithrombin, factor VIII level, factor V Leiden mutation, prothrombin gene mutation, anticardiolipin antibody, lupus anticoagulant antibody
Enhanced contrast echocardiography	Hepatopulmonary syndrome
	Portopulmonary syndrome
Neuropsychologist assessment	Neurocognitive testing

Treatment

Acute Portal Vein Thrombosis

There is a lack of evidence to guide therapeutic choices for children with acute portal vein thrombosis. Due to the low probability of spontaneous recanalization, anticoagulation is usually recommended. Treatment is usually continued for 3–6 months as complete recanalization may be delayed. Limited experience with other therapeutic

approaches such as thrombectomy, thrombolysis, or transjugular intrahepatic portosystemic shunt (TIPS) has been reported, but the appropriate clinical indications and contraindications have not yet been defined [9, 30].

Chronic Portal Vein Thrombosis

There is no role for anticoagulation therapy in the management of chronic portal vein thrombosis, unless a prothrombotic disorder has been identified [8]. The approach to management is focused on the alleviation of portal hypertension and/or the reduction of portosystemic shunting. There is widespread agreement that therapeutic interventions are indicated when significant morbidity occurs, for example, due to variceal hemorrhage. The role of therapy to prevent complications is more controversial [21, 25, 31, 32].

The meso-Rex bypass (mesenteric-to-left portal vein bypass) is the surgical procedure of choice in children with EHPVO who fulfill criteria for treatment. This procedure results in physiological restoration of portal blood flow to the liver, without portosystemic shunting (Fig. 21.1). An autologous internal jugular vein graft is used as the conduit from the superior mesenteric vein to the intrahepatic left portal vein. Patency of the left portal vein can sometimes be established preoperatively by Doppler ultrasound, CT, or MR venography but may require invasive hepatic venography. When successful, the meso-Rex bypass provides a corrective treatment for EHPVO that resolves symptoms related to portal hypertension and portosystemic shunting, including variceal bleeding, hypersplenism, hepatopulmonary syndrome, and encephalopathy. The adaptation of a hypotrophic intrahepatic portal venous system to the restored blood flow seems to be better in younger children; therefore, some authors suggest that meso-Rex bypass surgery should be performed early if possible.

If a meso-Rex bypass cannot be performed, then a distal splenorenal shunt should be considered as an alternative. Children with complications due to spontaneous portosystemic shunting are not candidates for surgical portosystemic shunt procedures, which may worsen symptoms of encephalopathy, portopulmonary hypertension, or hepatopulmonary syndrome.

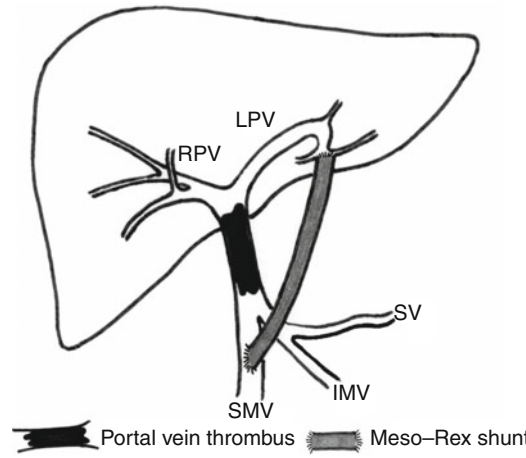

Fig. 21.1 Meso-Rex bypass procedure. An autologous venous graft is used to bypass the portal vein thrombosis by connecting the superior mesenteric vein with the left portal vein. Portal blood flow is thereby restored to both right and left portal veins. *LPV* left portal vein, *RPV* right portal vein, *SV* splenic vein, *IMV* inferior mesenteric vein, *SMV* superior mesenteric vein

Liver transplantation is an alternative therapeutic option for these children, although it requires a careful case-by-case determination of risks and benefits. Reports suggest that insertion of TIPS may successfully reduce portal pressure in EHPVO and thereby reduce the risk of further variceal bleeding. However, the place of this therapy for children with EHPVO remains unclear as it can reduce the success of a future meso-Rex bypass [8, 32].

Acute variceal bleeding in the setting of EHPVO is rarely fatal in the absence of intrinsic liver disease but is often associated with significant morbidity. There is little high-quality evidence to guide clinical decision making for the prevention and management of variceal bleeding in children with EHPVO. The use of endoscopic treatment as primary prophylaxis for children needs to be evaluated as well as the appropriate timing of endoscopic surveillance [5, 8, 33].

For secondary prophylaxis after the first episode of bleeding, surgical therapy is recommended as described above. If surgical intervention is not possible, then repeated endoscopic surveillance and variceal ligation may be an effective long-term option.

Sinusoidal Obstruction Syndrome (Hepatic Veno-occlusive Disease)

Sinusoidal obstruction syndrome (SOS) is a non-thrombotic obstruction of the hepatic sinusoids that can extend to the adjacent central veins but in the absence of an underlying disorder of the hepatic veins. The initial injury is to the hepatic sinusoids and involvement of the central vein can be absent. In fact, occlusion of the central vein was only seen in 55 % of patients with mild to moderate SOS and in 75 % of patients with severe SOS. Therefore, the previous term of "hepatic veno-occlusive disease" is considered inaccurate [9, 30].

SOS occurs most commonly after hematopoietic stem-cell transplantation (HSCT), when it is caused by toxicity from the conditioning therapy. The most hepatotoxic regimens are those that contain cyclophosphamide in combination with busulfan or total body irradiation. The overall incidence of SOS after myeloablative therapy is around 14 % [34]. SOS also occurs in patients receiving radiotherapy and other drugs, including gemtuzumab ozogamicin, 6-thioguanine, cytosine arabinoside, urethane, 6-mercaptopurine, azathioprine, actinomycin D, dacarbazine, oxaliplatin, and tacrolimus.

SOS can be caused by the ingestion of plants (*Senecio*, *Crotalaria*, *Heliotropium*, *Symphytium*) and herbal teas containing pyrrolizidine alkaloids, that can be ingested from contaminated food or as herbal remedies. The severity of the liver damage depends on the ingested dose and individual susceptibility [35].

Discussion here refers only to SOS secondary to myeloablative therapies.

Clinical Presentation

SOS typically presents with a clinical syndrome that includes tender hepatomegaly, abdominal pain localized to the right upper quadrant, weight gain secondary to fluid retention, ascites, and jaundice. Depending on the severity, McDonald et al. classified SOS as mild (no requirement for treatment and self resolving), moderate (need for treatment to manage fluid balance or pain but resolves completely), or severe (leading to death or failure to resolve after day 100 post-initiation of therapy) [36]. Severe SOS is associated with a mortality rate of more than 84 % [36, 37].

Risk factors for developing SOS are younger age, the presence of preexisting liver disease (e.g., chronic hepatitis B and C, nonalcoholic steatohepatitis, cirrhosis, cholestatic disease), HLA mismatched or unrelated donor transplant, the use of certain concomitant drugs (itraconazole, sirolimus, norethisterone), previous abdominal irradiation, high-dose conditioning regimens, and previous HSCT [35, 38]. Primary diagnoses associated with a greater risk of SOS include osteopetrosis, neuroblastoma, adrenoleukodystrophy, and inherited hemophagocytic lymphohistiocytosis [37, 39, 40]. The increased risk in these conditions is most likely related to the younger patient age and the need for more aggressive ablation therapy.

Pathophysiology

SOS is primarily a vascular disorder that causes liver dysfunction as a secondary phenomenon. The first morphological change of SOS to appear is ballooning of the endothelial cells of hepatic sinusoids, detected by electron microscopy. This change causes detachment of the endothelial cells, allowing penetration of red blood cells to the space of Disse which encourages dissection of endothelial cells into the sinusoidal lumen. Thus, resistance to sinusoidal blood flow increases and portal hypertension ensues. These initial changes are followed by deposition of collagen in the sinusoids and central and sublobular veins.

Diagnosis

The diagnosis of SOS is reached on clinical criteria because there is no specific laboratory marker that has adequate diagnostic or prognostic value. SOS should be suspected in any patient who has received a hepatotoxic myeloablative regimen and who has the clinical signs and symptoms

Table 21.2 Diagnostic criteria for SOS

Baltimore criteria [41]	Modified Seattle Criteria [36]
Hyperbilirubinemia >2 mg/dl (34.2 μmol/l) and ≥2of the following:	*Two of three findings within 20 days of HSCT:*
Hepatomegaly (usually painful)	Hepatomegaly or R upper quadrant pain
≥5 % weight gain	Sudden >2 % weight gain (due to fluid retention)
Ascites	Bilirubin >2 mg/dl (34.2 μmol/l)

described above. There are two widely accepted diagnostic criteria for SOS, the Seattle criteria and the Baltimore criteria, each based on the clinical features of a large cohort of patients with SOS (Table 21.2). The Seattle criteria have chronologic consideration that can only be applied to cyclophosphamide-containing regimens because other drugs can induce SOS beyond 20 days post-HSCT. Therefore, the concept of late SOS, presenting beyond 20 days after HSCT, and the coexistence of multiorgan failure (especially renal and pulmonary failure) have been introduced as features of the clinical spectrum of SOS [30, 36, 41].

The diagnosis can be difficult because of the presence of confounding factors in children who often have multiple comorbidities. The differential diagnosis should include sepsis, graft-versus-host disease, hemolysis, total parenteral nutrition-induced cholestasis, congestive heart failure, and toxicity due to the concomitant use of other hepatotoxic drugs.

The poor sensitivity of the Baltimore and Seattle criteria must be remembered when trying to reach a diagnosis.

Imaging studies are mainly useful to rule out other conditions such as biliary obstruction, portal vein thrombosis, or liver abscess. In SOS, imaging typically shows hepatomegaly, splenomegaly, ascites, and gallbladder wall edema. Doppler ultrasound may show reversal of portal venous flow, attenuation of hepatic venous flow, and increase hepatic artery resistive index [30, 35].

When clinical and radiological features do not provide enough information to secure the diagnosis of SOS, liver biopsy may be warranted. However, the high risk of this procedure makes most practitioners reluctant to undertake liver biopsy early after HSCT when pancytopenia is present. The histological features of SOS include dilatation of sinusoids, extravasation of red cells through the space of Disse and widening of the subendothelial zone in the central veins. As the disease progresses, hemorrhage in zones 2 and 3 of the liver acinus, dislodgement of hepatocytes into portal and hepatic venules, collagenization of sinusoids and vein walls, and cirrhosis may appear. The liver involvement may be patchy and the histological features evolve with time [9, 35].

Measurement of the hepatic venous pressure gradient may improve diagnostic accuracy. A pressure gradient above 10 mmHg was highly specific for SOS in one study [42]. The transjugular, intrahepatic approach to this measurement allows performance of a liver biopsy at the same time in those children in whom the diagnosis is unclear.

Prognosis

In the Reiss pediatric cohort, the survival rate in children with severe SOS was 38 % after 1 year, and the survival rate in children with SOS at day 100 post-HSCT was 77 %. A poor prognosis is predicted by multiorgan failure, high serum alanine aminotransferase levels, and high hepatic venous pressure gradient. Death is usually related to complications secondary to the multiorgan failure rather than hepatic insufficiency [30, 38].

Prophylaxis

Prevention of SOS involves the identification of children at high risk and the use of less toxic myeloablative regimens that do not decrease the chances of engraftment or increase the relapse rate of the primary disease. Several groups have obtained good results in the prevention of SOS by using reduced intensity myeloablative regimens that do not contain cyclophosphamide [43, 44].

Several treatments have been used in the hope of preventing SOS but without clear evidence for

their efficacy. Prospective studies have not shown any benefit from the use of unfractionated or low molecular weight heparin, antithrombin III, pentoxifylline, or prostaglandin E1 [35]. A meta-analysis of three randomized trials of ursodeoxycholic acid showed that this drug is effective for the prevention of SOS [45].

Defibrotide is a single-stranded polydeoxyribonucleotide with antithrombotic and profibrinolytic effects that has been studied as an anti-ischemic, endothelium-protective agent. Preliminary studies showed that it might offer benefit for SOS prophylaxis. Corbacioglu et al. presented the first pediatric prospective, open-label, randomized trial to evaluate the prophylactic use of defibrotide in children at high risk for SOS. They showed a reduction of the incidence of SOS and lower incidence of graft-versus-host disease and renal failure in the defibrotide treatment group, without significant increase in adverse effects [30, 37].

Prophylactic regimens therefore include the use of reduced intensity myeloablative regimens, ursodeoxycholic acid, and defibrotide.

Treatment

The majority of patients with SOS will resolve spontaneously. The treatment of SOS is focused on the management of sodium and fluid balance, ascites and preservation of renal blood flow. Studies have explored approaches that modulate endothelial cell injury while hopefully not increasing the risk of bleeding. Unfortunately, either no benefit or too high risks have been shown for treatment with tissue plasminogen activator, N-acetylcysteine, antithrombin III, prostaglandin E1, prednisone, or vitamin E with glutamine.

The use of defibrotide for severe SOS is promising; in a cohort of 88 patients including children, 36 % showed complete resolution of SOS with 35 % survival after day 100 post-HSCT [46]. A randomized phase II dose-ranging trial showed a complete response rate (total bilirubin <2 mg/dL with resolution of multiorgan failure) of 41 % in patients with severe SOS treated with defibrotide. Pediatric rates of survival were higher compared to adults [47].

TIPS have been used to reduce portal hypertension with no improvement in outcome. Liver transplantation has been undertaken for selected children with HSCT for benign conditions [35, 48].

Budd-Chiari Syndrome (Hepatic Venous Outflow Obstruction)

George Budd, an English internist from King's College Hospital, first described the triad of hepatomegaly, abdominal pain, and ascites in 1845 and then in 1899 the Austrian pathologist Hans Chiari reported the histological features of what we know as Budd-Chiari syndrome (BCS) [49].

Definition

BCS is an obstruction of the hepatic venous outflow, which can be located at any level from the small hepatic veins to the junction of the inferior vena cava (IVC) with the right atrium. BCS does not include hepatic congestion secondary to heart failure, pericardial disease or obstruction confined to the sinusoids secondary to toxic drugs or irradiation [5, 30].

BCS is considered secondary when the obstruction occurs as a result of an invasion or extrinsic compression of the hepatic veins by a malignant or benign tumor, amoebic or pyogenic abscess, parasitic cyst, and polycystic liver disease or when it is due to blunt abdominal trauma or following liver transplantation [30]. Otherwise BCS is caused by thrombosis and is considered primary, which forms the focus of this section [5].

BCS may present acutely or as an acute decompensation after chronic venous obstruction has caused cirrhosis [30, 50].

Epidemiology

Primary BCS is a rare disease whose incidence in children is unknown. The estimated overall incidence in adults ranges from 0.2 to 0.8 per million

per year, with anatomical variation depending on geographical location. In Asian countries BCS is commonly associated with obstruction of the inferior vena cava, whereas in Western countries involvement of the hepatic veins is more common [51, 52].

Etiology

Primary BCS is often associated with one or several risk factors for thrombosis. In adults 84 % of the patients have at least one risk factor [53]. Among the causes of acquired thrombophilia, myeloproliferative disorders (MPD) are found most frequently and account for 50 % of BCS cases in adults. MPD should always be sought even when peripheral blood counts are unexceptional, because hypersplenism, hemodilution, and iron deficiency in BCS may mask the peripheral blood count abnormalities. Adult guidelines therefore recommend that all BCS patients should undergo genetic testing for a particular mutation in the janus tyrosine kinase-2 (JAK-2) gene in granulocytes (V617FJAK2 mutation testing) which is positive in about 80 % of adults with MPD and BCS. If the genetic testing is negative, a bone marrow biopsy should be considered to assess for MPD [5, 51, 54]. There is inadequate data to determine if this approach is also appropriate in children.

Other acquired thrombophilic conditions associated with BCS include antiphospholipid antibodies and hyperhomocysteinemia, but it remains unclear whether they are a cause or consequence. Paroxysomal nocturnal hemoglobinuria and Behçet disease have also been described in association with BCS [54].

The major causes of inherited thrombophilia can be classified as gain of function mutations (factor V Leiden G1691A and prothrombin G20210A polymorphism) or loss of anticoagulant function (deficiencies of protein C, protein S, or antithrombin). Factor V Leiden mutation is the most common thrombophilia marker in the general population; the relative risk for venous thrombosis is 5–10-fold for heterozygotes and 50–100-fold for homozygotes. Factor V Leiden mutation has been found in 16–26 % of adults with BCS and seems to be more common in patients presenting with inferior vena cava obstruction. However, this abnormality is commonly associated with other prothrombotic risk factors, and its individual role in promoting BCS is therefore unclear [54, 55]. Inherited deficiencies of protein C, protein S, and antithrombin have been implicated in BCS, but low levels of these proteins in serum should be carefully interpreted because impaired liver function or thrombosis may also reduce the circulating levels of these anticoagulant proteins.

In a series of 22 children with BCS, JAK2 mutations were negative in the 5 children who were tested, 2 patients had protein C deficiency, 1 had antiphospholipid antibodies, and 1 had antithrombin III deficiency [56].

In some patients, venous obstruction is localized to a small area of the IVC or hepatic veins, appearing as a membrane or web. Although these webs have sometimes been considered as congenital malformations, they are now more commonly explained as sequelae of prior thrombus, resulting in fibrous thickened regions which may form a valve-like membrane several centimeters in length [51, 57, 58].

Clinical Presentation

The presentation of BCS varies along a spectrum from asymptomatic to acute or acute-on-chronic liver failure, depending on the extent of thrombosis, the rate of onset of the obstruction, and the development of collateral vessels. The typical clinical signs are abdominal pain, hepatosplenomegaly, and ascites. Prominent cutaneous abdominal veins may be seen. Liver enzymes, albumin, and INR may be normal or abnormal and jaundice is uncommon. BCS is a cause of portal hypertension and patients can therefore present with variceal bleeding or hypersplenism. The type of clinical presentation correlates poorly with the duration of the disease [30].

In a historical French pediatric series of 22 cases of BCS, symptoms were detected at an average of 3 years of age (range 9 months to

11 years). The initial symptoms or signs were hepato- or hepatosplenomegaly, abdominal distention, abdominal pain, and acute ascites. Physical examination revealed hepatomegaly, splenomegaly, ascites, and prominent superficial abdominal veins. Most of the patients had normal or slightly abnormal liver enzymes [59].

Diagnosis

Doppler ultrasound is the most reliable method for identification of venous obstruction in the hepatic veins or suprahepatic IVC. The typical ultrasonographic features for BCS are the presence of a hepatic vein with absent flow signal, reversed or turbulent flow, collateral veins connecting the hepatic veins or the diaphragmatic or intercostal veins, a weblike appearance near the hepatic vein ostia, or the replacement of a normal vein by a hyperechoic cord. MRI is not as effective as Doppler ultrasound to assess the presence of intrahepatic collaterals and does not provide information on the blood flow direction; nevertheless, it may provide useful complimentary information about obstructed hepatic veins and IVC and the presence of webs. On CT scan the absence of visualization of the hepatic veins suggests obstruction, but indeterminate results are frequent. The accuracy of multiphasic helical CT seems to be better but needs further evaluation in the setting of BCS. Venography has little role in the diagnostic phase but may have a role in evaluating the response to therapy [5, 9].

Two further features are highly suggestive of BCS on imaging studies: the presence of regenerative hypervascular nodules, explained by impaired hepatic perfusion, and hypertrophy of the caudate lobe, due to this lobe having separate venous drainage directly to the IVC [30].

Liver biopsy is usually not necessary when a venous obstruction has been clearly detected by imaging studies, but it may be necessary when the small intrahepatic veins are primarily involved. The histological features of BCS show marked regional variation in severity, depending on the affected vessel(s), and, therefore, two biopsies from different lobes are preferred to improve sensitivity. Liver biopsy can be normal in the early stages of acute BCS. The initial features are dilatation of veins and sinusoids with a variable degree of necrosis. Following the acute phase, the sinusoids become collagenized and dilated, and the hepatic veins become incorporated into the septa and disappear. These septa lead to venocentric cirrhosis with relative sparing of the portal triads [60].

Nagral et al. described 16 cases of BCS in children. Abdominal Doppler ultrasound led to the diagnosis in ten children. Liver biopsy was performed in the six children in whom ultrasound was not diagnostic; histology confirmed BCS in four. A diagnosis of BCS was reached in the other two children following a hepatic venogram. Collaterals between the hepatic veins and caudate lobe hypertrophy were found in half of the patients. Once the diagnosis was established, invasive venography was undertaken in 14 cases to enable image-guided therapy [56].

Treatment

Clinical trials for therapy of BCS are lacking, and the optimal therapeutic approach is therefore based on expert opinion arising from the experience of adult hepatologists. The main goal of treatment is decompression of the liver and restoration of the hepatic blood flow. Anticoagulation should be commenced in all patients unless there is a major contraindication. A previous gastrointestinal bleeding episode related to portal hypertension is not considered a contraindication for anticoagulation. There is no consensus on the optimal duration of treatment except for patients with an underlying thrombophilia, for whom lifelong anticoagulation is usually recommended [5, 9]. A short-length stenosis of the IVC or hepatic veins should be recanalized by percutaneous angioplasty or stenting techniques. If this approach to therapy is unsuccessful, TIPS is the next treatment of choice in adults. Liver transplantation is considered in patients unresponsive to the above procedures and in those who present with acute liver failure [5, 9].

Literature regarding treatment in children with BCS is scarce and composed only of small case series and case reports. In a series of 16 children reported by Nagral et al. from India, 11 of 16 children underwent radiological interventions that included angioplasty in 4 children, stenting in 2 children, and TIPS in 6 children (one child had both angioplasty and TIPS). The outcome seemed better with stenting and TIPS than with angioplasty [56]. Thrombolytic therapy, pericardial patch atriocavoplasty, surgical repair, surgical portosystemic shunting, and liver transplant have also been described in case series of pediatric BCS [59, 61–64].

Idiopathic Non-cirrhotic Portal Hypertension

Idiopathic non-cirrhotic portal hypertension (INCPH) was first recognized in 1889 by the Italian pathologist Guido Banti who presented a disease characterized by splenomegaly and anemia in the absence of hematological disorder. The so-called Banti syndrome was one of the diseases included under the term INCPH [65].

Definition of Idiopathic Non-cirrhotic Portal Hypertension

INCPH comprises a group of conditions characterized by portal hypertension in the absence of cirrhosis that share many common histological and clinical features [66–68]. These conditions are likely to be caused by intrahepatic small blood vessel abnormalities. Nomenclature about this condition is confusing, and different terms have been reported in the literature regarding INCPH: non-cirrhotic intrahepatic portal hypertension, non-cirrhotic portal hypertension, idiopathic portal hypertension, non-cirrhotic portal fibrosis, nodular regenerative hyperplasia, obliterative portal venopathy, hepatoportal sclerosis, and incomplete septal cirrhosis (Table 21.3).

Epidemiology

The prevalence of INCPH varies by region, accounting for 3–5 % of cases of portal hypertension in Europe and North America and

Table 21.3 Entities corresponding to INCPH [9]

Non-cirrhotic intrahepatic portal hypertension	*Definition*: Increased portal pressure due to liver disease other than cirrhosis, with patent portal and hepatic veins. *Diagnosis*: Evidence of portal hypertension + Doppler US showing patent portal and hepatic vessels + liver biopsy with absence of cirrhosis + exclusion of conditions causing cirrhosis.
Idiopathic portal hypertension	*Definition*: Disorder of unknown etiology presenting with splenomegaly. anemia and portal hypertension in the absence of cirrhosis, hematological disease, parasites, granulomas, congenital hepatic fibroesis, occlusion of portal or hepatic veins and other diseases. *Diagnosis*: clinical or hemodynamic evidence for portal hypertension + liver biopsy and other tests showing no underlying cause.
Nodular regenerative hyperplasia	*Definition*: *M*icronodular transformation of the hepatic parenchyma without fibrous septa between the nodules. *Diagnosis*: *H*epatocellular nodules measuring less than 3 mm not surrounded by fibrous tissue with different contrast between interlobular tissue.
Obliterative portal venopathy	*Definition*: severe portal hypertension with intimal thickening and luminal narrowing of the intrahepatic branches of the portal vein, in the absence of cirrhosis or obstruction of extrahepatic portal vein. *Diagnosis*: *Liver* biopsy with >2/3 portal tracts showing portal venules with reduced caliber and intimal thickening (biopsy should be longer than 1 cm with more than 5 portal tracts).
Hepatoportal sclerosis	*Definition*: Fibrous intimal thickening of the portal vein or its branches in patients with non-cirrhotic portal hypertension. *Diagnosis*: Thrombosis or sclerosis of small portal vein branches and/or intrahepatic aberrant vessels.

Table 21.3 (continued)

Incomplete septal cirrhosis	*Definition*: Macronodular hepatic parenchyma with incomplete fibrous septa delimiting the nodules. *Diagnosis*: Liver biopsy showing nodular parenchyma + thin incomplete septa + hypoplastic portal tracts + increase in venous channels + abnormal spacing between portal tracts and veins + crowding of reticulin fibers + hyperplastic hepatocytes + dilated sinusoids.

15–30 % in India [65, 66]. The explanation for these geographical variations has not been determined. The prevalence and incidence in children is unknown.

Etiology

INCPH is the result of a primary vascular lesion of the small portal veins of unknown etiology. Several etiological factors are involved [66, 69–72]:

Immunological Disorders

INCPH is associated with immunological disorders such as systemic lupus erythematosus, systemic sclerosis, celiac disease, and primary hypogammaglobulinemia.

Infections

It has been hypothesized that intestinal bacterial infections may give rise to septic embolization to the portal circulation causing small portal vein obliteration.

Medications and Toxins

INCPH occurs in a subgroup of patients treated with azathioprine or 6-thioguanine and following prolonged ingestion of arsenic.

Genetic Disorders

A genetic background to some cases of INCPH is suggested by its familial recurrence and its association with congenital disorders such as Turner, Felty, and Adams-Oliver syndromes. There is a high frequency of HLA-DR3 in patients with INCPH.

Thrombophilia

The frequent finding of thrombophilic risk factors points towards a contribution to the development of INCPH.

Diagnosis

The diagnosis of INCPH is based on the presence of clinical manifestations of portal hypertension, exclusion of known causes of portal hypertension, and the absence of cirrhosis on the liver biopsy.

Imaging studies help to demonstrate portal hypertension, to rule out other causes of portal hypertension, and to confirm portal vein patency. Abdominal ultrasound findings of thickened portal vein walls and nodularity of the liver surface are suggestive of INCPH. Recently, transient elastography has shown that the liver stiffness of adult patients with INCPH is elevated in the intermediate range between normal and values found in cirrhosis [73].

Liver biopsy is required to confirm the diagnosis of INCPH. However, interpretation of the biopsy may be difficult because this is a rare disease in pediatrics with changes that are often subtle and patchy. The histological lesion includes obliteration of small intrahepatic portal veins [68]. Many portal tracts lack a normal-sized portal vein, and portal veins that are present have thickening of the media of the wall. Inflammation is absent or mild. Reduction of portal blood flow leads to atrophy of liver cell plates in the perivenular areas, resulting in portal tracts being abnormally close to each other. Compensatory hyperplasia of hepatocytes in the areas that remain well perfused results in regenerative small nodules from 1 to 3 mm (nodular regenerative hyperplasia). Fine fibrous septa may appear in the areas of parenchymal atrophy but do not link to each other (incomplete septal cirrhosis). Biliary features may be present in the form of periductular fibrosis without loss of bile ducts [67, 71].

Clinical Presentation

INCPH presents with splenomegaly, which is often massive and may cause abdominal pain and distension. Complications from portal hypertension such as gastrointestinal bleeding and ascites may occur and for some patients may be the initial reason for seeking medical attention. Liver enzymes are frequently normal or slightly altered, and synthetic liver function is preserved. Cytopenias may occur in the peripheral blood count, secondary to hypersplenism. Hyperammonemia and hepatopulmonary syndrome have been reported in children with INCPH.

Although most published data relates to INCPH in adults, a comparative series found no significant differences between clinical presentation or venographic findings in 11 children compared to 140 adults [65, 66, 71, 74, 75].

Outcome

Although INCPH is rarely fatal, significant morbidity may occur related to the complications of portal hypertension. The long-term natural history may depend on the cause and the ongoing presence of the initial trigger for INCPH. However, among children with nodular regenerative hyperplasia following 6-thioguanine treatment for acute lymphoblastic leukemia, gradual progression in the severity of portal hypertension may be observed for many years following cessation of cancer therapy [76].

Treatment

Treatment of INCPH consists of the management of the complications of portal hypertension. It is currently unclear whether there is a role for anticoagulation therapy to address the apparent primary pathology of small-vessel thrombosis [66, 77, 78].

Congenital Vascular Malformations

Liver vascular malformations cause abnormal shunting of blood in the liver. Shunting can occur from the hepatic artery to the hepatic vein (arteriovenous or arteriohepatic), from the hepatic artery to the portal vein (arterioportal), or from the portal vein to the systemic circulation (portosystemic). In hereditary hemorrhagic telangiectasia, these three types of shunts may coexist.

All these conditions are most often congenital but may be acquired after abdominal blunt trauma, liver biopsy, cholangiography, or surgery.

Arteriovenous Malformation (AVM)

Isolated AVM is a rare malformation presenting in the perinatal period with the clinical triad of hepatomegaly, anemia, and cardiac congestive failure. Persistent pulmonary hypertension has also been reported. The literature is scarce and in many publications the term AVM is used to refer to infantile hepatic hemangioma. The mortality rate among reported cases is high, and it is therefore important to strive for early diagnosis before cardiopulmonary vascular complications become irreversible. The treatment options include embolization, surgical ligation, or liver transplantation [79–81].

Liver Involvement in Hereditary Hemorrhagic Telangiectasia

Hereditary hemorrhagic telangiectasia (HHT), or Rendu-Osler-Weber disease is an autosomal dominant disease characterized by cutaneous and mucosal telangiectasias and visceral vascular malformations, especially involving the lungs, brain, and liver.

Diagnosis of HHT

HHT is diagnosed by clinical criteria and genetic testing. The Curaçao criteria are based on four diagnostic clinical features: spontaneous and recurrent epistaxis; multiple telangiectasias located in the oral cavity and lips, fingers, or nose; the presence of visceral lesions such as gastrointestinal telangiectasia and pulmonary, hepatic, cerebral, or spinal AVMs; and the presence of a first-degree relative with HHT. Patients who fulfill three or more criteria are considered to be definite HHT, two criteria indicate possible HHT, and the diagnosis is unlikely if only one or none of the criteria are present [82].

Genetic testing is used to identify a specific mutation in a family, allowing for diagnostic screening among relatives. Mutations in the endoglin gene (ENG) and the activin receptor type II-like 1 gene (ALK-1 or ACVRL1) account for the majority of cases. These genes encode for transmembrane proteins involved in the TGF-β signaling pathway and are expressed in the vascular endothelium. Mutations in the SMAD4 gene are responsible for a syndrome in which HHT is associated with juvenile polyposis [83, 84].

Epidemiology

The estimated prevalence is 1–2 cases per 10,000 population. Liver vascular malformations are present in 32–78 % of the patients, but only about 8 % are symptomatic [83–85].

Liver Involvement

Hepatic vascular lesions range from small telangiectasias to the coexistence of the three types of intrahepatic shunting (arteriovenous, arterioportal, and portosystemic). Usually one type of shunt is predominant, although the type of predominant shunt may change with time. The diagnosis of liver involvement is often made while screening for the presence of visceral lesions.

Diagnosis

Doppler ultrasound shows the presence of a dilated hepatic artery with elevated hepatic artery flow and intrahepatic vascularity that is suggestive of vascular malformations. With CT scan, the hepatic artery appears dilated, and the presence of multiple telangiectasias leads to a heterogeneous enhancement of the hepatic parenchyma. Irregular blood flow to different areas of the liver may cause nodular regenerative hyperplasia and a nodular appearance on imaging. Liver biopsy is not necessary and carries a high risk of bleeding [86, 87].

Clinical Presentation

Symptoms of liver involvement usually manifest around the age of 30 years, but cases of symptomatic liver lesions in the neonatal and pediatric age range have been reported [88]. Suspicion may arise in the presence of a thrill in the right upper quadrant. The clinical features are high-output heart failure, portal hypertension, and jaundice. High-output heart failure is the most common clinical presentation and is the main indication for liver transplant. Portal hypertension occurs in the presence of arterioportal shunting or from the presence of nodular regenerative hyperplasia and may present with gastrointestinal bleeding or ascites. Biliary disease occurs secondary to a "steal syndrome" in which arterial blood flows preferentially to the intrahepatic arteriovenous shunts instead of the peribiliary plexus. The resultant ischemic cholangiopathy presents with biliary strictures, dilatations, and cysts mimicking primary sclerosing cholangitis or Caroli disease. Most commonly liver synthetic function is normal, and gamma-glutamyl transpeptidase and alkaline phosphatase levels are elevated [30, 83–85, 89, 90].

Treatment of Liver Manifestations

The balance between risks and benefits of hepatic arterial embolization may not be favorable, because the beneficial effects on cardiac symptoms may only be temporary and there are considerable risks for ischemic hepatobiliary complications. Liver transplantation should be considered in the setting of ischemic biliary necrosis, intractable heart failure, or intractable portal hypertension [83].

Bevacizumab, an anti-vascular endothelial growth factor antibody, may be a therapeutic option in the treatment of associated complications of liver vascular malformations in HHT. Initial studies suggest that it reduces cardiac output in patients with hepatic vascular malformations and potentially avoids the need for liver transplantation. Further studies are needed to more clearly determine its role [91, 92].

Congenital Portosystemic Shunts

Congenital portosystemic shunts (CPSS) are rare vascular malformations that allow intestinal blood to bypass the hepatic sinusoids. Intrahepatic shunts can occur in one or in both lobes and consist of one or multiple connections between branches of the portal and hepatic veins [93]. Extrahepatic portosystemic shunts are also known as the "Abernethy malformation" and may be associated with apparent absence (Abernethy

type 1) or preservation (Abernethy type 2) of portal venous flow to the liver [94]. A patent ductus venosus is sometimes included within the classification of intrahepatic CPSS, despite its course in the ligamentum venosum from the left portal vein to a hepatic vein.

Clinical Presentation

CPSS may present in children or adults and are often found following abdominal imaging that was ordered for other reasons. Associated laboratory abnormalities include elevated blood ammonia, galactose, conjugated bilirubin, bile acids, and aminotransferases. However, the clinical presentation of CPSS may include neurodevelopmental, hepatic, and cardiopulmonary manifestations. Portal hypertension is not usually a feature of CPSS, in which shunting of blood is not associated with increased resistance to flow.

CPSS may cause hepatic encephalopathy that can present with learning difficulties, behavioral issues, or developmental delay. Approximately a quarter of children with CPSS develop benign liver masses, most commonly associated with extrahepatic shunts [95]. Rare occurrence of malignant tumors has also been reported, necessitating careful imaging and possible biopsy when hepatic masses are identified [96, 97]. Both hepatopulmonary syndrome and portopulmonary hypertension have been reported in association with CPSS [98].

Treatment

Intrahepatic CPSS may close spontaneously within the first year of life, and it is unclear whether all persistent CPSS will eventually cause problems. Therefore, the decision to provide an intervention to close a shunt should be based on the presence of deleterious associated clinical manifestations in a child in whom spontaneous closure is not expected. Current therapeutic options include interventional radiology embolization or closure devices, surgical ligation, or liver transplantation [99, 100]. Recent experience suggests that closure may be successful even when the intrahepatic venous circulation appears to be absent [101].

Liver Vascular Tumors

In 1982, Mulliken and Glowacki presented a classification of pediatric vascular malformations, in which they defined hemangiomas as lesions with cellular proliferation and hyperplasia and vascular malformations as entities with normal endothelial turnover. The differentiation of these two entities is important because the approach to their treatment differs. Liver hemangiomas may regress, but arteriovenous malformations do not regress and are associated with higher morbidity and mortality [79, 102].

Infantile Hepatic Hemangiomas (IHH)

Infantile hemangiomas are the most common benign tumor in infancy, affecting 1–2 % of newborns and around 10 % of infants by 1 year of age [79]. The majority of the lesions involve the skin and subcutaneous tissue, but a subset of infants may also present with lesions elsewhere, including the liver. The presence of more than 5 cutaneous hemangiomas should prompt screening for visceral involvement by imaging liver, intestine, brain, and lungs. Diffuse neonatal hemangiomatosis is diagnosed when three or more organs are involved [79, 80, 103].

Infantile hepatic hemangioma (IHH) is the most common pediatric liver tumor. This tumor may also be called a hemangioendothelioma and has been divided into three categories: focal, multifocal, and diffuse [104]. IHH presents before 6 months of age in almost all cases and follows a natural history of initial proliferation (from birth to 9–12 months), a variable period of stability, and then slow involution (2–10 years). This pattern is similarly seen in soft tissue hemangiomas [105].

Focal Lesions

Single focal IHH are usually asymptomatic and diagnosed as an incidental finding when a liver ultrasound scan is performed for other reasons, including routine prenatal ultrasound scans. Usually there are no associated skin

hemangiomas. In larger lesions containing high-flow arteriovenous shunts, high-output cardiac failure may occur [103, 106].

Liver biopsy of vascular lesions is rarely performed due to the risks of bleeding. Imaging studies therefore play an essential role in diagnosis [80]. IHH appears as a well-defined hypo- or hyperechoic lesion with heterogeneous echotexture on ultrasound scan. Doppler findings vary depending on the presence and type of vascular shunt. MRI scan reveals a well-defined, hypointense lesion on T1-weighted images and hyperintense on T2-weighted scans, with centripetal enhancement after gadolinium contrast. Areas of necrosis, thrombosis, or hemorrhage lead to a heterogeneous appearance on imaging studies. Calcifications are present in about 16 % of cases.

Focal IHH is a congenital lesion that does not proliferate in the postnatal period and which can be diagnosed prenatally. Males and females are equally affected, the tumor does not express Glut-1 when examined histologically, and there is only an infrequent association with skin hemangiomas [105–107].

Multifocal Lesions

Multifocal and diffuse IHH affect girls more frequently than boys, stain positively for Glut1, and are often associated with multiple cutaneous hemangiomas. These two forms are not present at birth and develop within the first weeks of life [108].

Multifocal IHH may be asymptomatic and diagnosed only when visceral screening is undertaken in a child with multiple skin lesions. Among those children with symptoms, approximately 80 % present with a triad of hepatomegaly, congestive heart failure, and anemia. Anemia and thrombocytopenia occur secondary to consumption, hemorrhage, or thrombosis within the hemangiomas. Hemolysis may give rise to jaundice. High-output cardiac failure may occur in the presence of arteriovenous shunting. In rare cases, severe hepatomegaly may lead to respiratory compromise or abdominal compartment syndrome [80, 103].

In some infants, overproduction of type III iodothyronine deiodinase by the hemangiomas promotes inactivation of thyroxine and results in hypothyroidism. Other rare clinical presentations include fulminant hepatic failure and intraperitoneal hemorrhage following rupture of hemangiomas located at the liver surface [80, 107, 109].

On ultrasound imaging, the hemangiomas appear as small multifocal lesions, homogeneous, and most commonly hypoechoic. The hepatic vessels appear enlarged, and large feeding arteries and draining veins may be present. On MR imaging, multiple nodular tumors are noted to be hypointense on T1 and hyperintense on T2 signal, with flow voids sometimes present [79].

Angiography and portography are reserved for patients in whom embolization therapy is considered. The angiographic features are variable, because lesions have different vascular supply from portal vessels, hepatic artery, and systemic arterial collaterals such as intercostal, phrenic, renal, and mesenteric arteries. On portal venography, multiple small portal vein branches supplying the hemangiomas or total diversion of the portal flow may be seen [80].

Cases of hepatic angiosarcoma have been reported in association with multiple cutaneous infantile hemangiomas. Therefore, there may be a need to consider liver histological examination in hepatic vascular lesions that present after 1 year of age and in which the radiological diagnosis is unclear [110].

Diffuse Lesions

Some infants present with innumerable small lesions with extensive hepatic involvement leading to almost total replacement of hepatic parenchyma by hemangioma. The radiological features are similar to multifocal IHH. The clinical presentation is usually with massive hepatomegaly. Respiratory compromise and multiorgan failure can occur as a result of compression of the inferior vena cava and renal veins. Cardiac failure is present in more than half of these patients, and hypothyroidism is common [107].

Treatment

Asymptomatic focal lesions can be observed with serial imaging studies. Although the efficacy of medical treatment for larger single symptomatic

lesions is unknown, a trial of medical therapy (see below) is recommended before considering embolization or surgical resection [107].

Asymptomatic infants with multinodular IHH without shunts or hypothyroidism can be observed. In diffuse forms and symptomatic multinodular IHH (those with high-output cardiac failure or hypothyroidism), medical treatment is the first step of therapy [103, 107].

Propranolol has become the first-line therapy for IHH after the fortuitous discovery that it was effective in an infant with skin hemangioma treated with propanolol for obstructive hypertrophic cardiomyopathy. At a dose of 1–3 mg/kg/day, this nonselective β-blocker has been successful for treating IHH and causes few side effects. The potential mechanisms of action are vasoconstriction, decreased expression of angiogenic factors, apoptosis, and effects on the differentiation of mesenchymal stem cells. Treatment should be prolonged until the proliferation phase of IHH is completed, usually until 8–12 months of age. Second-line medical options include steroids, vincristine, and alfa 2a interferon [111–114].

Surgical resection or embolization therapy is the treatment of choice if medical therapy fails or in the presence of life-threatening complications. Embolization is performed in patients with cardiac failure related to the presence of arteriovenous or portosystemic shunts and results in improvement of the cardiac function but does not treat the IHH. Early involvement of the liver transplant team is recommended in the presence of progressive cardiac failure or severe shunting. Liver transplantation has been successfully used in these critical circumstances [103, 105].

Hepatic Angiosarcoma

Hepatic angiosarcoma is a malignant neoplasm that is very rare in childhood. The clinical presentation is often as abdominal distension or hepatomegaly secondary to the presence of a hepatic mass. In some patients, associated multiple cutaneous hemangiomas lead to the misdiagnosis of multifocal IHH. The age of presentation is variable with a range from newborns to adolescence. Pulmonary metastases are common and the overall prognosis is very poor with death often occurring within 6 months of diagnosis. Imaging characteristics are variable, often multifocal and usually showing a heterogeneous enhancement pattern. Treatment options reported in the pediatric literature are vascular ablation, chemotherapy, and resection. Liver transplantation is controversial due to reported cases of posttransplant recurrence [110, 115, 116].

Hepatic Epithelioid Hemangioendothelioma

Hepatic epithelioid hemangioendothelioma is a low-grade malignant tumor mostly seen in adults but with occasional pediatric cases also reported. The appearance on ultrasound imaging is variable, including an individual nodule, multinodular changes, or a diffusely heterogeneous echotexture of the liver [80, 118]. It is commonly associated with bone and brain involvement. Surgery is the treatment of choice, because this tumor is not responsive to chemotherapy. Liver transplantation should be considered.

References

1. Suchy FJ, Sokol RJ, Balistreri WF. Liver disease in children. 3rd ed. Cambridge: Cambridge University Press; 2007, xvii, 1030 p, 22 p, of plates p.
2. Collardeau-Frachon S, Scoazec JY. Vascular development and differentiation during human liver organogenesis. Anat Rec (Hoboken). 2008;291(6):614–27.
3. Sherlock S, Dooley J. Diseases of the liver and biliary system. 11th ed. Malden: Blackwell Science; 2002, xvi, 706 p.
4. Valla DC, Condat B. Portal vein thrombosis in adults: pathophysiology, pathogenesis and management. J Hepatol. 2000;32(5):865–71.
5. Shneider B, Emre S, Groszmann R, Karani J, McKiernan P, Sarin S, et al. Expert pediatric opinion on the report of the Baveno IV consensus workshop on methodology of diagnosis and therapy in portal hypertension. Pediatr Transplant. 2006;10(8):893–907.
6. Sarin SK, Sollano JD, Chawla YK, Amarapurkar D, Hamid S, Hashizume M, et al. Consensus on

extra-hepatic portal vein obstruction. Liver Int. 2006;26(5):512–9.
7. Senzolo M, Riggio O, Primignani M. Vascular disorders of the liver: recommendations from the Italian Association for the Study of the Liver (AISF) ad hoc committee. Dig Liver Dis. 2011;43:503–14.
8. Shneider BL, Bosch J, de Franchis R, Emre SH, Groszmann RJ, Ling SC, et al. Portal hypertension in children: expert pediatric opinion on the report of the Baveno V Consensus Workshop on Methodology of Diagnosis and Therapy in Portal Hypertension. Pediatr Transplant. 2012;16(5):426–37
9. Plessier A, Rautou PE, Valla DC. Management of hepatic vascular diseases. J Hepatol. 2012;56 Suppl 1:S25–38.
10. Garcia-Pagan JC, Hernandez-Guerra M, Bosch J. Extrahepatic portal vein thrombosis. Semin Liver Dis. 2008;28(3):282–92.
11. Williams S, Chan AK. Neonatal portal vein thrombosis: diagnosis and management. Semin Fetal Neonatal Med. 2011;16:329–39.
12. Sakha SH, Rafeey M, Tarzamani MK. Portal venous thrombosis after umbilical vein catheterization. Indian J Gastroenterol. 2007;26(6):283–4.
13. Morag I, Epelman M, Daneman A, Moineddin R, Parvez B, Shechter T, et al. Portal vein thrombosis in the neonate: risk factors, course, and outcome. J Pediatr. 2006;148(6):735–9.
14. Morag I, Shah PS, Epelman M, Daneman A, Strauss T, Moore AM. Childhood outcomes of neonates diagnosed with portal vein thrombosis. J Paediatr Child Health. 2011;47(6):356–60.
15. Seixas CA, Hessel G, Siqueira LH, Machado TF, Gallizoni AM, Annichino-Bizzacchi JM. Study of hemostasis in pediatric patients with portal vein thrombosis. Haematologica. 1998;83(10):955–6.
16. Pinto RB, Silveira TR, Bandinelli E, Rohsig L. Portal vein thrombosis in children and adolescents: the low prevalence of hereditary thrombophilic disorders. J Pediatr Surg. 2004;39(9):1356–61.
17. Abd El-Hamid N, Taylor RM, Marinello D, Mufti GJ, Patel R, Mieli-Vergani G, et al. Aetiology and management of extrahepatic portal vein obstruction in children: King's College Hospital experience. J Pediatr Gastroenterol Nutr. 2008;47(5):630–4.
18. Pietrobattista A, Luciani M, Abraldes JG, Candusso M, Pancotti S, Soldati M, et al. Extrahepatic portal vein thrombosis in children and adolescents: influence of genetic thrombophilic disorders. World J Gastroenterol. 2010;16(48):6123–7.
19. Heller C, Schobess R, Kurnik K, Junker R, Gunther G, Kreuz W, et al. Abdominal venous thrombosis in neonates and infants: role of prothrombotic risk factors – a multicentre case–control study. For the Childhood Thrombophilia Study Group. Br J Haematol. 2000;111(2):534–9.
20. El-Karaksy H, El-Koofy N, El-Hawary M, Mostafa A, Aziz M, El-Shabrawi M, et al. Prevalence of factor V Leiden mutation and other hereditary thrombophilic factors in Egyptian children with portal vein thrombosis: results of a single-center case–control study. Ann Hematol. 2004;83(11):712–5.
21. Mack CL, Superina RA, Whitington PF. Surgical restoration of portal flow corrects procoagulant and anticoagulant deficiencies associated with extrahepatic portal vein thrombosis. J Pediatr. 2003;142(2):197–9.
22. Odievre M, Pige G, Alagille D. Congenital abnormalities associated with extrahepatic portal hypertension. Arch Dis Child. 1977;52(5):383–5.
23. Sarin SK, Bansal A, Sasan S, Nigam A. Portal-vein obstruction in children leads to growth retardation. Hepatology. 1992;15(2):229–33.
24. Mehrotra RN, Bhatia V, Dabadghao P, Yachha SK. Extrahepatic portal vein obstruction in children: anthropometry, growth hormone, and insulin-like growth factor I. J Pediatr Gastroenterol Nutr. 1997;25(5):520–3.
25. Mack CL, Zelko FA, Lokar J, Superina R, Alonso EM, Blei AT, et al. Surgically restoring portal blood flow to the liver in children with primary extrahepatic portal vein thrombosis improves fluid neurocognitive ability. Pediatrics. 2006;117(3):e405–12.
26. Alvarez F, Bernard O, Brunelle F, Hadchouel P, Odievre M, Alagille D. Portal obstruction in children. I. Clinical investigation and hemorrhage risk. J Pediatr. 1983;103(5):696–702.
27. Khuroo MS, Yattoo GN, Zargar SA, Javid G, Dar MY, Khan BA, et al. Biliary abnormalities associated with extrahepatic portal venous obstruction. Hepatology. 1993;17(5):807–13.
28. Gauthier-Villars M, Franchi S, Gauthier F, Fabre M, Pariente D, Bernard O. Cholestasis in children with portal vein obstruction. J Pediatr. 2005;146(4):568–73.
29. de Franchis R, Baveno VF. Revising consensus in portal hypertension: report of the Baveno V consensus workshop on methodology of diagnosis and therapy in portal hypertension. J Hepatol. 2010;53(4):762–8.
30. DeLeve LD, Valla DC, Garcia-Tsao G. Vascular disorders of the liver. Hepatology. 2009;49(5):1729–64.
31. Superina R, Bambini DA, Lokar J, Rigsby C, Whitington PF. Correction of extrahepatic portal vein thrombosis by the mesenteric to left portal vein bypass. Ann Surg. 2006;243(4):515–21.
32. Superina R, Shneider B, Emre S, Sarin S, de Ville de Goyet J. Surgical guidelines for the management of extra-hepatic portal vein obstruction. Pediatr Transplant. 2006;10(8):908–13.
33. Ling SC, Walters T, McKiernan PJ, Schwarz KB, Garcia-Tsao G, Shneider BL. Primary prophylaxis of variceal hemorrhage in children with portal hypertension: a framework for future research. J Pediatr Gastroenterol Nutr. 2011;52(3):254–61.
34. Coppell JA, Richardson PG, Soiffer R, Martin PL, Kernan NA, Chen A, et al. Hepatic veno-occlusive disease following stem cell transplantation: incidence, clinical course, and outcome. Biology of Blood and Marrow. 2010;16(2):157–68.

35. McDonald GB. Hepatobiliary complications of hematopoietic cell transplantation, 40 years on. Hepatology. 2010;51(4):1450–60.
36. McDonald GB, Hinds MS, Fisher LD, Schoch HG, Wolford JL, Banaji M, et al. Veno-occlusive disease of the liver and multiorgan failure after bone marrow transplantation: a cohort study of 355 patients. Ann Intern Med. 1993;118(4):255–67.
37. Corbacioglu S, Cesaro S, Faraci M, Valteau-Couanet D, Gruhn B, Rovelli A, et al. Defibrotide for prophylaxis of hepatic veno-occlusive disease in paediatric haemopoietic stem-cell transplantation: an open-label, phase 3, randomised controlled trial. Lancet. 2012;379(9823):1301–9.
38. Reiss U, Cowan M, McMillan A, Horn B. Hepatic venoocclusive disease in blood and bone marrow transplantation in children and young adults: incidence, risk factors, and outcome in a cohort of 241 patients. J Pediatr Hematol Oncol. 2002;24(9):746–50.
39. Corbacioglu S, Honig M, Lahr G, Stohr S, Berry G, Friedrich W, et al. Stem cell transplantation in children with infantile osteopetrosis is associated with a high incidence of VOD, which could be prevented with defibrotide. Bone Marrow Transplant. 2006;38(8):547–53.
40. Ouachee-Chardin M, Elie C, de Saint Basile G, Le Deist F, Mahlaoui N, Picard C, et al. Hematopoietic stem cell transplantation in hemophagocytic lymphohistiocytosis: a single-center report of 48 patients. Pediatrics. 2006;117(4):e743–50.
41. Jones RJ, Lee KS, Beschorner WE, Vogel VG, Grochow LB, Braine HG, et al. Venoocclusive disease of the liver following bone marrow transplantation. Transplantation. 1987;44(6):778–83.
42. Carreras E, Granena A, Navasa M, Bruguera M, Marco V, Sierra J, et al. On the reliability of clinical criteria for the diagnosis of hepatic veno-occlusive disease. Ann Hematol. 1993;66(2):77–80.
43. Puig N, de la Rubia J, Remigia MJ, Jarque I, Martin G, Cupelli L, et al. Morbidity and transplant-related mortality of CBV and BEAM preparative regimens for patients with lymphoid malignancies undergoing autologous stem-cell transplantation. Leuk Lymphoma. 2006;47(8):1488–94.
44. de Lima M, Couriel D, Thall PF, Wang X, Madden T, Jones R, et al. Once-daily intravenous busulfan and fludarabine: clinical and pharmacokinetic results of a myeloablative, reduced-toxicity conditioning regimen for allogeneic stem cell transplantation in AML and MDS. Blood. 2004;104(3):857–64.
45. Tay J, Tinmouth A, Fergusson D, Huebsch L, Allan DS. Systematic review of controlled clinical trials on the use of ursodeoxycholic acid for the prevention of hepatic veno-occlusive disease in hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2007;13(2):206–17.
46. Richardson PG, Murakami C, Jin Z, Warren D, Momtaz P, Hoppensteadt D, et al. Multi-institutional use of defibrotide in 88 patients after stem cell transplantation with severe veno-occlusive disease and multisystem organ failure: response without significant toxicity in a high-risk population and factors predictive of outcome. Blood. 2002;100(13):4337–43.
47. Richardson PG, Soiffer RJ, Antin JH, Uno H, Jin Z, Kurtzberg J, et al. Defibrotide for the treatment of severe hepatic veno-occlusive disease and multiorgan failure after stem cell transplantation: a multicenter, randomized, dose-finding trial. Biol Blood Marrow Transplant. 2010;16(7):1005–17.
48. DeLeve LD. Vascular liver diseases. Curr Gastroenterol Rep. 2003;5(1):63–70.
49. Horton JD, San Miguel FL, Membreno F, Wright F, Paima J, Foster P, et al. Budd-Chiari syndrome: illustrated review of current management. Liver Int. 2008;28:455–66.
50. Hadengue A, Poliquin M, Vilgrain V, Belghiti J, Degott C, Erlinger S, et al. The changing scene of hepatic vein thrombosis: recognition of asymptomatic cases. Gastroenterology. 1994;106(4):1042–7.
51. Valla DC. Primary Budd-Chiari syndrome. J Hepatol. 2009;50:195–203.
52. Valla D-C. Hepatic venous outflow tract obstruction etiopathogenesis: Asia versus the West. J Gastroenterol Hepatol. 2004;19:S204–11.
53. Darwish Murad S, Plessier A, Hernandez-Guerra M, Fabris F, Eapen CE, Bahr MJ, et al. Etiology, management, and outcome of the Budd-Chiari syndrome. Ann Intern Med. 2009;151:167–75.
54. Shetty S, Ghosh K. Thrombophilic dimension of Budd Chiari syndrome and portal venous thrombosis – a concise review. Thromb Res. 2011;127:505–12.
55. Deltenre P, Denninger MH, Hillaire S, Guillin MC, Casadevall N, Briere J, et al. Factor V Leiden related Budd-Chiari syndrome. Gut. 2001;48(2):264–8.
56. Nagral A, Hasija RP, Marar S, Nabi F. Budd-Chiari syndrome in children: experience with therapeutic radiological intervention. J Pediatr Gastroenterol Nutr. 2010;50(1):74–8.
57. Vickers CR, West RJ, Hubscher SG, Elias E. Hepatic vein webs and resistant ascites. Diagnosis, management and implications. J Hepatol. 1989;8(3):287–93.
58. Kage M, Arakawa M, Kojiro M, Okuda K. Histopathology of membranous obstruction of the inferior vena cava in the Budd-Chiari syndrome. Gastroenterology. 1992;102(6):2081–90.
59. Gentil-Kocher S, Bernard O, Brunelle F, Hadchouel M, Maillard JN, Valayer J, et al. Budd-Chiari syndrome in children: report of 22 cases. J Pediatr. 1988;113(1 Pt 1):30–8.
60. MacSween RNM, Anthony PP, Scheuer PJ. Pathology of the liver. Edinburgh/New York: Churchill Livingstone; 1979. p. 458.
61. Cauchi JA, Oliff S, Baumann U, Mirza D, Kelly DA, Hewitson J, et al. The Budd-Chiari syndrome in children: the spectrum of management. J Pediatr Surg. 2006;41(11):1919–23.
62. Gomes AC, Rubino G, Pinto C, Cipriano A, Furtado E, Goncalves I. Budd-Chiari syndrome in children and outcome after liver transplant. Pediatr Transplant. 2012;16:E338–41.

63. Nezakatgoo N, Shokouh-Amiri MH, Gaber AO, Grewal HP, Vera SR, Chamsuddin AA, et al. Liver transplantation for acute Budd-Chiari syndrome in identical twin sisters with factor V leiden mutation. Transplantation. 2003;76(1):195–8.
64. Odell JA, Rode H, Millar AJ, Hoffman HD. Surgical repair in children with the Budd-Chiari syndrome. J Thorac Cardiovasc Surg. 1995;110(4 Pt 1):916–23.
65. Dhiman RK, Chawla Y, Vasishta RK, Kakkar N, Dilawari JB, Trehan MS, et al. Non-cirrhotic portal fibrosis (idiopathic portal hypertension): experience with 151 patients and a review of the literature. J Gastroenterol Hepatol. 2002;17:6–16.
66. Schouten JN, Garcia-Pagan JC, Valla DC, Janssen HL. Idiopathic noncirrhotic portal hypertension. Hepatology. 2011;54(3):1071–81.
67. Hübscher SG. Pathology of non-cirrhotic portal hypertension and incomplete septal cirrhosis. Diagn Histopathol. 2011;17(12):530–8.
68. Nakanuma Y, Hoso M, Sasaki M, Terada T, Katayanagi K, Nonomura A, et al. Histopathology of the liver in non-cirrhotic portal hypertension of unknown aetiology. Histopathology. 1996;28(3): 195–204.
69. Hillaire S, Bonte E, Denninger MH, Casadevall N, Cadranel JF, Lebrec D, et al. Idiopathic non-cirrhotic intrahepatic portal hypertension in the West: a re-evaluation in 28 patients. Gut. 2002;51(2):275–80.
70. Sarin SK, Agarwal SR. Extrahepatic portal vein obstruction. Semin Liver Dis. 2002;22(1):43–58.
71. Yilmaz G, Sari S, Egritas O, Dalgic B, Akyol G. Hepatoportal sclerosis in childhood: some presenting with cholestatic features (a re-evaluation of 12 children). Pediatr Dev Pathol. 2012;15(2):107–13.
72. Eapen CE, Nightingale P, Hubscher SG, Lane PJ, Plant T, Velissaris D, et al. Non-cirrhotic intrahepatic portal hypertension: associated gut diseases and prognostic factors. Dig Dis Sci. 2011;56(1):227–35.
73. Seijo S, Reverter E, Miquel R, Berzigotti A, Abraldes JG, Bosch J, et al. Role of hepatic vein catheterisation and transient elastography in the diagnosis of idiopathic portal hypertension. Dig Liver Dis. 2012;44(10): 855–60.
74. Moran CA, Mullick FG, Ishak KG. Nodular regenerative hyperplasia of the liver in children. Am J Surg Pathol. 1991;15(5):449–54.
75. Abramowsky C, Romero R, Heffron T. Pathology of noncirrhotic portal hypertension: clinicopathologic study in pediatric patients. Pediatr Dev Pathol. 2003;6(5):421–6.
76. De Bruyne R, Portmann B, Samyn M, Bansal S, Knisely A, Mieli-Vergani G, et al. Chronic liver disease related to 6-thioguanine in children with acute lymphoblastic leukaemia. J Hepatol. 2006;44(2): 407–10.
77. Dabritz J, Worch J, Materna U, Koch B, Koehler G, Duck C, et al. Life-threatening hypersplenism due to idiopathic portal hypertension in early childhood: case report and review of the literature. BMC Gastroenterol. 2010;10:122.
78. Al-Mukhaizeem KA, Rosenberg A, Sherker AH. Nodular regenerative hyperplasia of the liver: an under-recognized cause of portal hypertension in hematological disorders. Am J Hematol. 2004;75(4):225–30.
79. Boon LM, Burrows PE, Paltiel HJ, Lund DP, Ezekowitz RA, Folkman J, et al. Hepatic vascular anomalies in infancy: a twenty-seven-year experience. J Pediatr. 1996;129(3):346–54.
80. Burrows PE, Dubois J, Kassarjian A. Pediatric hepatic vascular anomalies. Pediatr Radiol. 2001;31(8): 533–45.
81. Alexander CP, Sood BG, Zilberman MV, Becker C, Bedard MP. Congenital hepatic arteriovenous malformation: an unusual cause of neonatal persistent pulmonary hypertension. J Perinatol. 2006;26(5): 316–8.
82. Shovlin CL, Guttmacher AE, Buscarini E, Faughnan ME, Hyland RH, Westermann CJ, et al. Diagnostic criteria for hereditary hemorrhagic telangiectasia (Rendu-Osler-Weber syndrome). Am J Med Genet. 2000;91(1):66–7.
83. Faughnan ME, Palda VA, Garcia-Tsao G, Geisthoff UW, McDonald J, Proctor DD, et al. International guidelines for the diagnosis and management of hereditary haemorrhagic telangiectasia. J Med Genet. 2011;48(2):73–87.
84. Garcia-Tsao G. Liver involvement in hereditary hemorrhagic telangiectasia (HHT). J Hepatol. 2007;46(3): 499–507.
85. Buscarini E, Leandro G, Conte D, Danesino C, Daina E, Manfredi G, et al. Natural history and outcome of hepatic vascular malformations in a large cohort of patients with hereditary hemorrhagic teleangiectasia. Dig Dis Sci. 2011;56(7):2166–78.
86. Memeo M, Stabile Ianora AA, Scardapane A, Suppressa P, Cirulli A, Sabba C, et al. Hereditary haemorrhagic telangiectasia: study of hepatic vascular alterations with multi-detector row helical CT and reconstruction programs. Radiol Med. 2005;109(1–2):125–38.
87. Buscarini E, Plauchu H, Garcia Tsao G, White Jr RI, Sabba C, Miller F, et al. Liver involvement in hereditary hemorrhagic telangiectasia: consensus recommendations. Liver Int. 2006;26(9):1040–6.
88. Al-Saleh S, John PR, Letarte M, Faughnan ME, Belik J, Ratjen F. Symptomatic liver involvement in neonatal hereditary hemorrhagic telangiectasia. Pediatrics. 2011;127(6):e1615–20.
89. Brenard R, Chapaux X, Deltenre P, Henrion J, De Maeght S, Horsmans Y, et al. Large spectrum of liver vascular lesions including high prevalence of focal nodular hyperplasia in patients with hereditary haemorrhagic telangiectasia: the Belgian Registry based on 30 patients. Eur J Gastroenterol Hepatol. 2010;22(10): 1253–9.
90. Garcia-Tsao G, Korzenik JR, Young L, Henderson KJ, Jain D, Byrd B, et al. Liver disease in patients with hereditary hemorrhagic telangiectasia. N Engl J Med. 2000;343(13):931–6.

91. Dupuis-Girod S, Ginon I, Saurin JC, Marion D, Guillot E, Decullier E, et al. Bevacizumab in patients with hereditary hemorrhagic telangiectasia and severe hepatic vascular malformations and high cardiac output. JAMA. 2012;307(9):948–55.
92. Mitchell A, Adams LA, MacQuillan G, Tibballs J, vanden Driesen R, Delriviere L. Bevacizumab reverses need for liver transplantation in hereditary hemorrhagic telangiectasia. Liver Transpl. 2008;14(2):210–3.
93. Park JH, Cha SH, Han JK, Han MC. Intrahepatic portosystemic venous shunt. AJR Am J Roentgenol. 1990;155(3):527–8.
94. Morgan G, Superina R. Congenital absence of the portal vein: two cases and a proposed classification system for portasystemic vascular anomalies. J Pediatr Surg. 1994;29(9):1239–41.
95. Kondo F. Benign nodular hepatocellular lesions caused by abnormal hepatic circulation: etiological analysis and introduction of a new concept. J Gastroenterol Hepatol. 2001;16(12):1319–28.
96. Barton 3rd JW, Keller MS. Liver transplantation for hepatoblastoma in a child with congenital absence of the portal vein. Pediatr Radiol. 1989;20(1–2): 113–4.
97. Pichon N, Maisonnette F, Pichon-Lefievre F, Valleix D, Pillegand B. Hepatocarcinoma with congenital agenesis of the portal vein. Jpn J Clin Oncol. 2003;33(6):314–6.
98. Yagi H, Takada Y, Fujimoto Y, Ogura Y, Kozaki K, Ueda M, et al. Successful surgical ligation under intraoperative portal vein pressure monitoring of a large portosystemic shunt presenting as an intrapulmonary shunt: report of a case. Surg Today. 2004;34(12):1049–52.
99. Gillespie MJ, Golden A, Sivarajan VB, Rome JJ. Transcatheter closure of patent ductus venosus with the Amplatzer vascular plug in twin brothers. Pediatr Cardiol. 2006;27(1):142–5.
100. Ohnishi Y, Ueda M, Doi H, Kasahara M, Haga H, Kamei H, et al. Successful liver transplantation for congenital absence of the portal vein complicated by intrapulmonary shunt and brain abscess. J Pediatr Surg. 2005;40(5):e1–3.
101. Franchi-Abella S, Branchereau S, Lambert V, Fabre M, Steimberg C, Losay J, et al. Complications of congenital portosystemic shunts in children: therapeutic options and outcomes. J Pediatr Gastroenterol Nutr. 2010;51(3):322–30.
102. Mulliken JB, Glowacki J. Classification of pediatric vascular lesions. Plast Reconstr Surg. 1982;70(1):120–1.
103. Dickie B, Dasgupta R, Nair R, Alonso MH, Ryckman FC, Tiao GM, et al. Spectrum of hepatic hemangiomas: management and outcome. J Pediatr Surg. 2009;44(1):125–33.
104. Frieden IJ, Haggstrom AN, Drolet BA, Mancini AJ, Friedlander SF, Boon L, et al. Infantile hemangiomas: current knowledge, future directions. Proceedings of a research workshop on infantile hemangiomas, April 7–9, 2005, Bethesda, Maryland, USA. Pediatr Dermatol. 2005;22(5):383–406.
105. Christison-Lagay ER, Burrows PE, Alomari A, Dubois J, Kozakewich HP, Lane TS, et al. Hepatic hemangiomas: subtype classification and development of a clinical practice algorithm and registry. J Pediatr Surg. 2007;42(1):62–7; discussion 7–8.
106. Hernandez F, Navarro M, Encinas JL, Lopez Gutierrez JC, Lopez Santamaria M, Leal N, et al. The role of GLUT1 immunostaining in the diagnosis and classification of liver vascular tumors in children. J Pediatr Surg. 2005;40(5):801–4.
107. Kulungowski AM, Alomari AI, Chawla A, Christison-Lagay ER, Fishman SJ. Lessons from a liver hemangioma registry: subtype classification. J Pediatr Surg. 2012;47(1):165–70.
108. Behr GG, Fishman SJ, Caty MG, Kulungowski AM, Paltiel HJ, Alomari AI. Hepatic mesenchymal hamartoma and infantile hemangioma: a rare association. J Pediatr Surg. 2012;47(3):448–52.
109. Huang SA, Tu HM, Harney JW, Venihaki M, Butte AJ, Kozakewich HP, et al. Severe hypothyroidism caused by type 3 iodothyronine deiodinase in infantile hemangiomas. N Engl J Med. 2000;343(3):185–9.
110. Nord KM, Kandel J, Lefkowitch JH, Lobritto SJ, Morel KD, North PE, et al. Multiple cutaneous infantile hemangiomas associated with hepatic angiosarcoma: case report and review of the literature. Pediatrics. 2006;118(3):e907–13.
111. Leaute-Labreze C, Dumas de la Roque E, Hubiche T, Boralevi F, Thambo JB, Taieb A. Propranolol for severe hemangiomas of infancy. N Engl J Med. 2008;24:2649–51.
112. Mhanna A, Franklin WH, Mancini AJ. Hepatic infantile hemangiomas treated with oral propranolol – a case series. Pediatr Dermatol. 2011;28(1):39–45.
113. Sans V, de la Roque ED, Berge J, Grenier N, Boralevi F, Mazereeuw-Hautier J, et al. Propranolol for severe infantile hemangiomas: follow-up report. Pediatrics. 2009;124(3):e423–31.
114. Mazereeuw-Hautier J, Hoeger PH, Benlahrech S, Ammour A, Broue P, Vial J, et al. Efficacy of propranolol in hepatic infantile hemangiomas with diffuse neonatal hemangiomatosis. J Pediatr. 2010; 157(2):340–2.
115. Geramizadeh B, Safari A, Bahador A, Nikeghbalian S, Salahi H, Kazemi K, et al. Hepatic angiosarcoma of childhood: a case report and review of literature. J Pediatr Surg. 2011;46(1):e9–11.
116. Awan S, Davenport M, Portmann B, Howard ER. Angiosarcoma of the liver in children. J Pediatr Surg. 1996;31(12):1729–32.
117. Chung EM, Lattin Jr GE, Cube R, Lewis RB, Marichal-Hernandez C, Shawhan R, et al. From the archives of the AFIP: pediatric liver masses: radiologic-pathologic correlation. Part 2. Malignant tumors. Radiographics. 2011;31(2): 483–507.

Hepatic Tumors

22

Rohit Gupta, André A. Dick, and Karen F. Murray

Abbreviations

AFP	Serum alpha-fetoprotein
CT	Computed tomography
EHE	Epithelioid hemangioendothelioma
ESL	Embryonal (undifferentiated) sarcoma of the liver
FLC	Fibrolamellar carcinoma
FNH	Focal nodular hyperplasia
HA	Hepatocellular adenoma
HB	Hepatoblastoma
HCC	Hepatocellular carcinoma
HR	Hepatobiliary rhabdomyosarcoma
IHH	Infantile hepatic hemangioma
MH	Mesenchymal hamartoma
MRI	Magnetic resonance imaging
NRH	Nodular regenerative hyperplasia
RICH	Rapidly involuting congenital hemangioma
US	Ultrasound

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Benign Tumors

Infantile Hepatic Hemangioma (IHH)

Infantile hepatic hemangioma (IHH), formerly infantile hemangioendothelioma, is the most common benign vascular hepatic tumor in children and the most common liver tumor in the first year of life [1, 2]. IHH can be focal, multifocal, or diffuse, the latter two distribution types having a slight female predominance without any racial predilection. Hemangiomas are commonly clinically silent but can be associated with serious clinical complications. There are two types of IHH [3]: type I IHHs, which are more common, generally displace the liver parenchyma but ultimately follow an indolent course with spontaneous involution within a year. Histologically, type I IHHs have prominent endothelium in close proximity to the portal tracts. In contrast, type II lesions have tortuous vascular channels with endothelial cells infiltrating the hepatic parenchyma that are aggressive and can metastasize; type II IHH are sometimes difficult to distinguish from malignant angiosarcomas. The term hemangioendothelioma, in reference to infantile hemangiomas, has fallen out of favor to prevent confusion with epithelioid hemangioendothelioma, an adult liver tumor with malignant potential, and the pediatric soft tissue kaposiform hemangioendothelioma.

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Clinical Features

Most IHHs are diagnosed within the first 6 months of life, manifesting as an asymptomatic abdominal mass. When symptomatic, the infant may have nonspecific symptoms such as nausea, vomiting, and failure to thrive. Significant complications such as high-output congestive heart failure (CHF) and thrombocytopenia may also be present. CHF occurs in approximately 60 % of patients resulting from arteriovenous shunting of blood within the IHH lesion, and Kasabach-Meritt syndrome (KMS), a consumptive coagulopathy, is due to platelet sequestration. Hypothyroidism, present in some patients with diffuse hemangiomas and some with multifocal lesions, is a result of increased activity of type 3 iodothyronine deiodinase produced directly from the tumor. When present, hypothyroidism can exacerbate any cardiac dysfunction resulting from arteriovenous shunting.

The majority of patients with multifocal liver tumors also have hemangiomas at other sites including the skin, trachea, chest, adrenal glands, and dura mater [4] which contribute to unique signs and symptoms.

Pathologic Features

Biopsies are rarely indicated to differentiate IHH from other hepatic lesions, and due to the increased risk of bleeding, biopsy of these masses is usually avoided. Histologically, IHHs are composed of closely packed small capillary-sized vessels lined by variably plump endothelial cells and adjacent pericytes. Endothelium in multifocal and diffuse lesions is typically GLUT-1 (glucose transporter 1) positive, while focal lesions are negative, consistent with a so-called rapidly involuting congenital hemangioma (RICH). Serum alpha-fetoprotein (AFP) is not elevated in IHH.

Radiologic Findings

Plain abdominal radiograph might show evidence of hepatomegaly or hepatic-associated mass and occasionally fine calcifications when present. Features suggestive of CHF on chest radiograph include cardiomegaly and pulmonary edema [5]. Ultrasound is often the first diagnostic test and plays a critical role in initial detection, localization, and follow-up of IHH. Lesions appear hypoechoic or of variable echogenicity relative to normal hepatic parenchyma by ultrasound. Doppler flow analysis can provide information regarding arteriovenous shunting, and hepatic artery and vein enlargement along with tapering of the abdominal aorta below the origin of celiac axis are features suggestive of high flow across the hemangioma. Multiphase computed tomography (CT) with arterial, portal, or venous and delayed phases classically demonstrates diffuse or ring enhancement followed by delayed filling of the center of the lesion. Magnetic resonance imaging (MRI) is the preferred modality of imaging, however, as it allows for definitive diagnosis [6]. Multifocal lesions (Fig. 22.1a) that are small show homogenous signal intensity, while the presence of central hemorrhage, necrosis, and fibrosis results in heterogeneous signal intensity. Angiography is usually reserved for patients in whom embolization therapy is anticipated.

Classification of Hepatic Hemangiomas [7]

(a) *Focal*: GLUT-1 negative; typically asymptomatic, often seen on prenatal imaging. High-flow shunting can cause CHF along with anemia and thrombocytopenia. T2-weighted MRI: hyperintense, heterogeneous enhancement due to areas of central necrosis, thrombosis, or hemorrhage. Undergoes regression by 12–14 months of age. Considered to be a RICH.

(b) *Multifocal*: GLUT-1 positive; typically asymptomatic but often has associated extrahepatic lesions and patients can develop CHF. On T2-weighted MRI: hyperintense, homogenous enhancement, aortic tapering below the origin of celiac axis suggesting arteriovenous shunting. Usually has a proliferative phase that is followed by involution.

(c) *Diffuse*: GLUT-1 positive; clinically presents as massive hepatomegaly, abdominal compartment syndrome, and hypothyroidism can be present, along with CHF and KMS. On imaging: near total hepatic parenchymal replacement (Fig. 22.1b). Clinically diffuse IHH follows a complicated course.

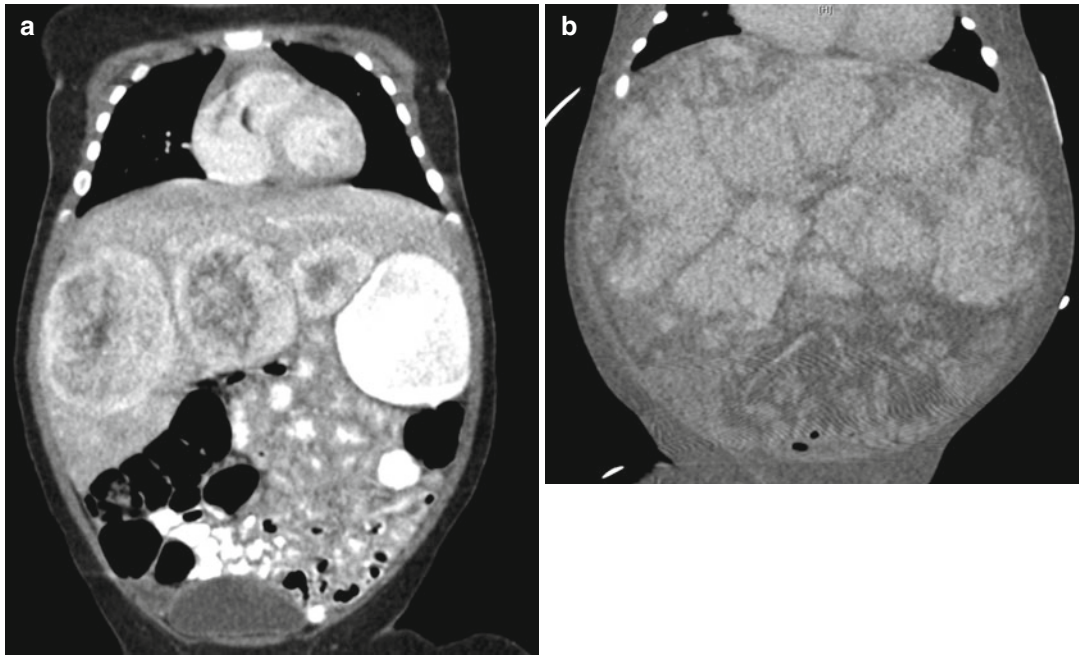


Fig. 22.1 (**a**) Multiple heterogeneous hepatic lesions with thick peripheral rim of enhancement consistent with multifocal hemangioma on CT abdomen. (**b**) Contrast-enhanced CT scan showing markedly enlarged liver with innumerable arterially enhancing areas that are present diffusely throughout the liver

Differentiation of IHH from other potential hepatic lesions is primarily based on clinical and radiologic features. Hepatoblastoma (HB) can be distinguished by imaging features of intense centripetal enhancement and elevated AFP levels [8]. A mesenchymal hamartoma (MH) is a hypovascular, multicystic, and multiloculated mass with characteristic enhancement of the septa and solid portions of the tumor. Metastatic neuroblastomas (NB) and angiosarcomas have distinguishing imaging features as well as elevated urinary catecholamines in a neuroblastoma.

Management: Treatment depends on tumor size and severity of symptoms. Asymptomatic patients are followed conservatively because the natural history of hemangiomas is to involute over time. Radiologic evaluations to assure there are no associated intracranial or pulmonary hemangiomas should be considered. Management of CHF results in improved survival as most deaths in patients with IHH are related to CHF [9]; thyroid hormone supplementation can improve cardiac dysfunction when hypothyroidism is also present. Symptomatic patients or those with multifocal and diffuse hemangiomas that have potential for a complicated clinical course have been treated with corticosteroids, propranolol, interferon-α-2a, or vincristine, but success rates are variable and potential complications of therapy must be weight against the risk of not treating. Corticosteroids, although most commonly used historically, only result in involution of hemangiomas in roughly 25 % of patients [10]. The use of vincristine is supported only by a few case reports and hence its true efficacy unknown, although its toxicities are well recognized. The use of propranolol is rapidly increasing, however, and changing the traditional treatment algorithm for patients with hepatic hemangiomas [11]. The striking effect of propranolol on growing hemangiomas is due to its role in causing vasoconstriction, inhibition of angiogenesis, and induction of apoptosis [12]. Close monitoring during propranolol administration is required because of potential side effects of bradycardia and hypoglycemia [13], however, and the imposed limitation to cardiovascular compensation must be considered when infants are treated with this β-blocker. Dual

therapy with corticosteroids and propranolol or vincristine, taking advantage of possible synergies between these medications, has been used in life-threatening presentations of IHH to achieve a rapid response [14]. Interferon-α has good efficacy for hemangiomas, but because of the potential for irreversible spastic diplegia when used in children less than 1 year of age, it is no longer considered a first-line agent [15].

Embolization can be effective when the complications of the IHH warrant invasive manipulation, when medical management is either ineffective or contraindicated, and when the lesion is focal or limited and its feeder vessels accessible. Surgical resection is reserved for those who have failed medical management and for whom the lesion is resectable. In medically resistant or severe cases where the hemangioma is unresectable, liver transplantation can be lifesaving.

Mesenchymal Hamartoma (MH)

MH is the third most common benign liver tumor in children after IHH and focal nodular hyperplasia. Most lesions are identified by the age of 5 years as a painless, progressively enlarging abdominal mass; there is a slight male predominance.

Clinical Features

These cystic lesions can present with rapid abdominal distension secondary to fluid accumulation. Other associated symptoms may include nausea, vomiting, early satiety, and anorexia, likely secondary to compression of the stomach or intestines as the lesion grows. Serum AFP levels are typically not elevated, but occasionally a mild elevation above the age-adjusted reference range can be seen.

Pathologic Features

MH is typically a large solitary mass with clear margins that contains cysts of varying sizes. The histopathologic appearance is diverse, however, with various epithelial or mesenchymal elements predominating (Fig. 22.2a). As a result multiple hypotheses have been proposed for the pathogenesis of MH including as a developmental malformation (ductal plate malformation), a vascular or toxic insult, or a neoplastic proliferation that is disorganized and limited [16]. The epithelium typically includes irregular bile ducts and ductules with peripherally placed cords of bland hepatocytes (Fig. 22.2b). Loose fibromyxomatous stroma dissects the lesion and often contains thin-walled vascular channels. A link between MH and embryonal (undifferentiated) sarcoma of the liver (ESL), an aggressive malignant liver neoplasm, has been suggested based on rare reports of a shared chromosomal translocation (19q13.4) and ESL arising within a MH.

Radiologic Findings

Imaging features are dependent upon the cellular components of the lesion. Most tumors are predominantly cystic with either thin or thick septa or predominantly solid with a few small cysts. US findings of thin mobile septa and small round hyperechoic nodules within a cyst lesion are highly suggestive of MH [17]. CT and MRI studies can provide better characterization of both solid and cystic structures within the tumor (Fig. 22.2c).

Differential Diagnosis

For predominantly solid lesions, hepatoblastoma should be considered as they also present in young children. Significant elevation of AFP levels along with a solid appearance and calcifications would favor hepatoblastoma over MH; however, overlapping features may warrant histopathologic review for diagnostic confirmation. Solitary IHH with degenerative changes and ESL are distinguished by characteristic imaging features for hemangioma and the older age of presentation for ESL. Predominantly cystic lesions must be differentiated from simple cysts, hydatid disease, hepatic abscesses, choledochal cysts, enteric duplication cysts, and pedunculated mesenteric lymphangioma.

Management

MH have the potential for rapid growth and questionably for malignant transformation, and hence, complete surgical excision is the preferred as definitive treatment. Surgical complications are rare but fatal hemorrhage, small bowel obstruction, and biliary complications have been

described [18–20]. Enucleation has been used for unresectable large tumors [21]. Patients undergoing marsupialization should be followed serially because of the risk of recurrence [22].

Focal Nodular Hyperplasia (FNH)

FNH is a benign tumor resulting from the proliferation of hepatocytes, Kupffer cells, and vascular and biliary elements. FNH has an indolent course and has no known malignant potential. It is usually seen in adult women but infrequently can occur in children and adolescents. A "central stellate scar" (coalescence of fibrous septa) is a characteristic feature of FNH, although is not present in all cases. The diagnosis of FNH is typically made incidentally during radiologic imaging for other reasons in asymptomatic children [23], seen in all ages with the youngest reported patient

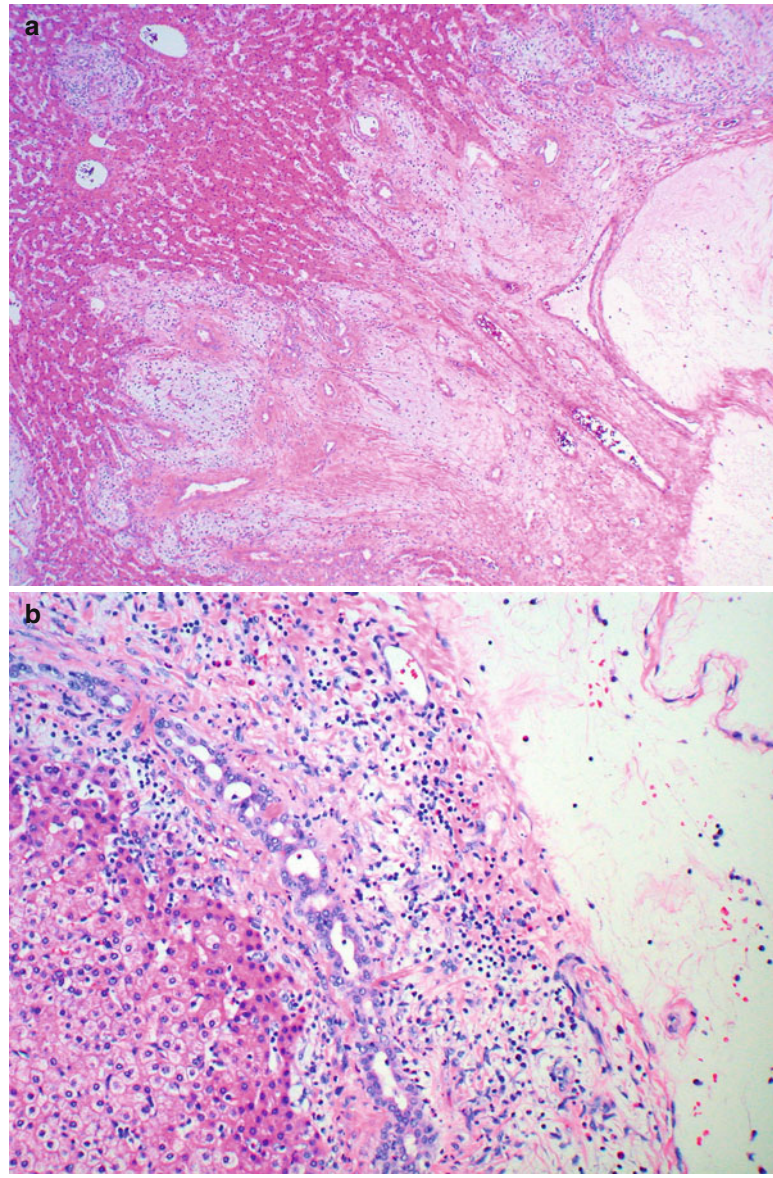


Fig. 22.2 Mesenchymal hamartoma. (**a**) A low-power view demonstrates typical heterogeneity of MH with epithelial (hepatocyte-rich) region, *left*, adjacent to myxoid region that becomes cystic, *lower right*. (**b**) At higher power, atypical ductular structures lie within fibromyxoid stroma; cyst degeneration occupies the space at the *right*. (**c**) CT abdomen showing numerous hypodense cysts with heterogeneously enhancing solid components

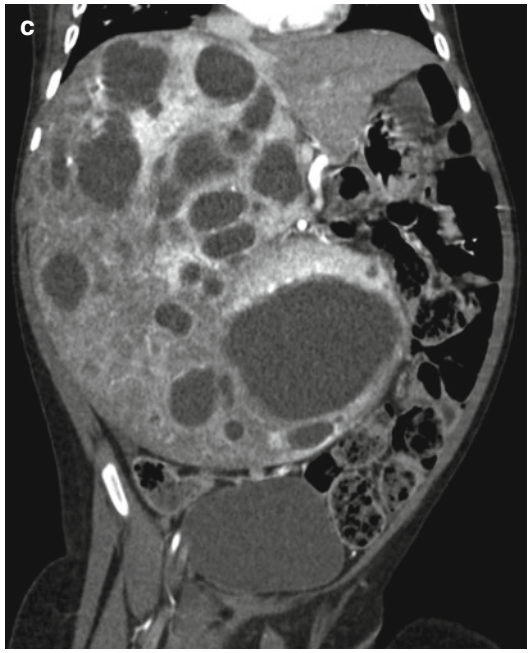


Fig. 22.2 (continued)

being only 7 months of age at diagnosis, with a slight female preponderance. Controversy exists regarding the association between FNH and oral contraceptive use [24–26].

Clinical Features

Large mass lesions can be associated with abdominal pain secondary to stretching of Glisson's capsule or compression of adjacent organs. AFP levels are not elevated in FNH, and liver blood tests are usually normal unless there is extrinsic intrahepatic bile duct compression. Presentation with hemoperitoneum secondary to intratumoral hemorrhage is rare.

Pathogenesis

The exact etiology of FNH is unknown; however, it most likely represents a hyperplastic response to a vascular abnormality [27]. An increased prevalence has been seen in children who have received chemotherapy or radiation therapy for solid malignancies, probably due to resulting vascular injury.

Pathologic Features

The tumors are typically single, well-circumscribed, unencapsulated, lobular masses that are paler than the surrounding normal liver. A central fibrous scar is invariably present from which fibrous septa radiate to separate the lesion into smaller nodules (Fig. 22.3a). Microscopically hyperplastic hepatocytes lack an acinar arrangement and are interrupted by variably inflamed fibrous septa; numerous ductules are present at the interface between hepatocytes and septa but do not communicate with the biliary tree (Fig. 22.3b, c). Numerous vessels, particularly arteries with subintimal fibrosis, course through the septa and central scar.

Radiologic Findings

On radiologic imaging, FNH may mimic other hepatic lesions such as hepatic adenoma or hepatocellular carcinoma (HCC) and as a result may require the use of multiple different imaging modalities. US demonstrates a central arterial structure with a "spoke-wheel" pattern [28, 29]; however, as these patterns can also be seen in malignant lesions, imaging with other modalities is important to confirm the diagnosis. Multiphase CT including the arterial, portal venous, and delayed phases is the test of choice for making the diagnosis of FNH (Fig. 22.3d). During the early arterial phase, FNH appears as a contrast-enhanced homogenous lesion that becomes isodense to hepatic parenchyma on delayed phase [30]. Recent use of gadoxetate disodium, a liver-specific hepatobiliary contrast agent for MRI, has shown promise in distinguishing FNH from a hepatocellular adenoma [31]. ^{99m}Tc sulfur colloid scan can help differentiate FNH from hepatic adenoma.

Management

FNH is a slow-growing tumor with no known malignant potential and rare chance of acute complications such as hemorrhage or rupture; patients who are asymptomatic should be managed conservatively [32]. Surgical resection or possibly ablative therapy or embolization can be performed in symptomatic cases only after other causes of symptoms have been ruled out.

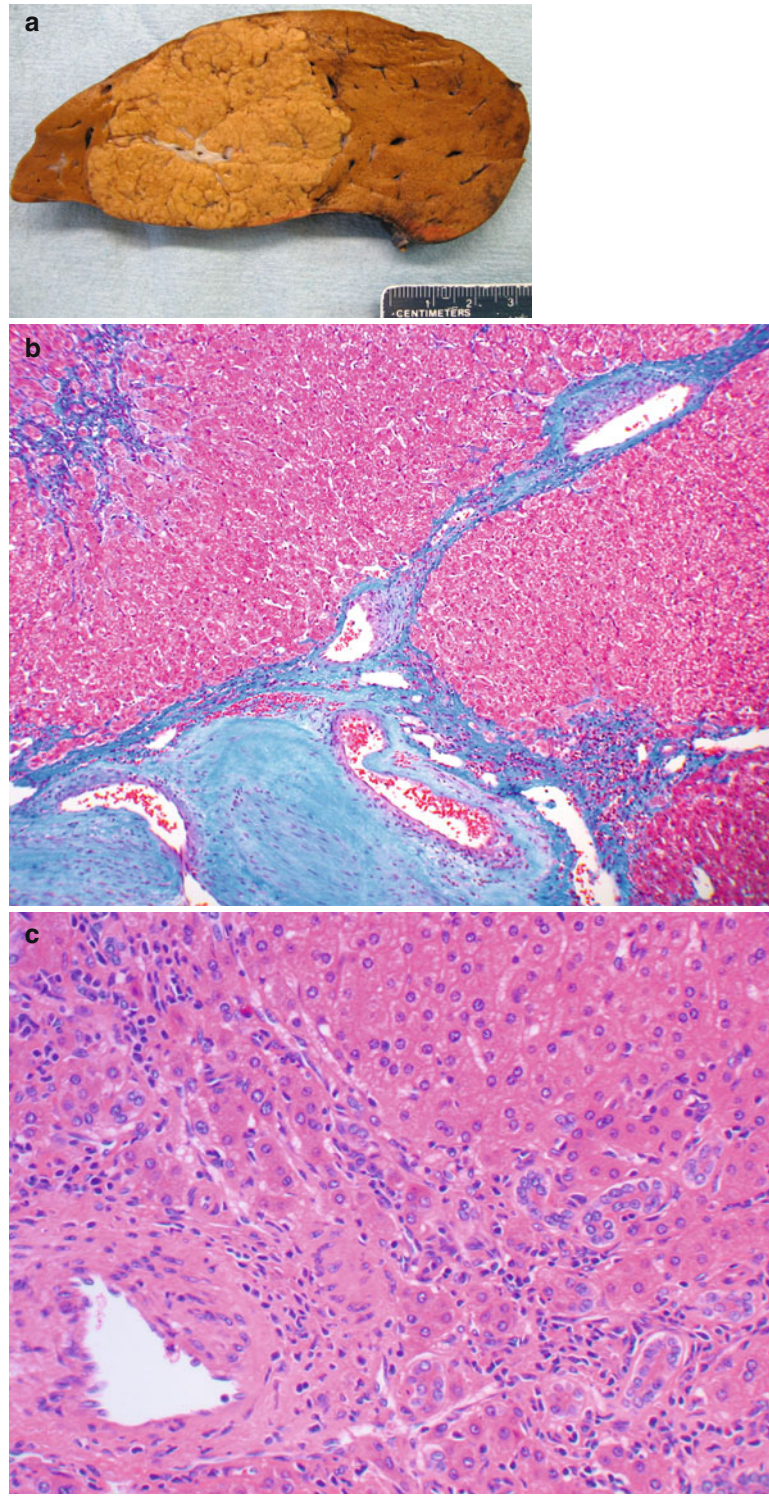


Fig. 22.3 Focal nodular hyperplasia. (**a**) The pale well-circumscribed cerebriform cut surface bulges from the adjacent normal liver parenchyma; note the slightly eccentric scared region in which vascular profiles are apparent. (**b**) A low-power view shows a "central scar" with aberrant ectatic vascular spaces and fibrous septa that radiate into the adjacent hepatocytes. (**c**) Numerous ductular profiles haphazardly interdigitate with hepatocytes, adjacent to an irregular thick-walled vessel. (**d**) MR abdomen showing a mildly hyperintense liver mass in the caudate lobe with central T2-hyperintense scar (*arrow*)

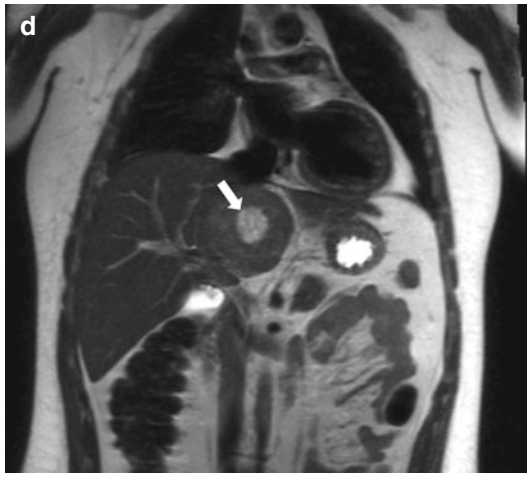


Fig. 22.3 (continued)

Hepatocellular Adenoma (HA)

HA are benign liver tumors most often related to the use of hormonal birth control in women of childbearing age. This tumor accounts for approximately 4 % of all solid liver tumors in children, and most patients are females over the age of 10 years who have a history of oral contraceptive use. HA is also associated with glycogen storage disease (I and III), galactosemia, and familial diabetes mellitus, polycythemia, anabolic steroid use, and polycystic ovary syndrome [33–36]. HCC has also been associated with HA but usually in patients with an underlying diagnosis of glycogen storage disease type 1a. When ten or more simultaneous adenomas are present, this disorder is called adenomatosis [37].

Clinical Features

Patients usually present with abdominal pain or mass and the primary clinical concern is the possibility of intratumoral hemorrhage with rupture, resulting in intraperitoneal hemorrhage and hypovolemic shock [38]; AFP levels are normal.

Pathologic Features

Most tumors (80 %) are solitary with multiple lesions associated with anabolic androgen therapy or glycogen storage disease. HA are tan or variegated (due to hemorrhage or necrosis), smooth, well circumscribed, and fleshy, varying anywhere from 1 to 30 cm, but typically measure 5–15 cm. HA are composed of sheets of hepatocytes that lack an acinar arrangement and often have cytoplasmic glycogen or lipid; reduced numbers of functional Kupffer cells, scattered thin-walled vessels, and no biliary structures (Fig. 22.4) distinguish hepatic adenomas from focal nodular hyperplasia. Discrimination from well-differentiated HCC can sometimes be difficult, however.

Radiologic Findings

Pathologic composition determines radiologic appearance. Hepatocellular adenomas are usually hyperechoic lesions on ultrasound secondary to increased lipid content or hemorrhage; however, in the setting of glycogen storage diseases or fatty infiltration of the liver, adenomas can appear hypoechoic. Hepatic arterial phase enhancement with contrast can be seen on CT, in addition to features consistent with lipid or hemorrhage [39].

Differential Diagnosis

Like HA, arterial phase hyperattenuation is also seen with FNH, hepatocellular carcinoma, and fibrolamellar carcinoma. A central stellate scar helps differentiate FNH from HA, and findings of cirrhosis and portal hypertension usually accompany hepatocellular carcinoma. Fibrolamellar carcinoma commonly has calcifications and an eccentric scar.

Management

Spontaneous regression has been seen after discontinuing oral contraceptives, after steroid use, or after instituting dietary therapy for glycogen storage disease. If these lesions remain small and the patient stays asymptomatic, they can be safely observed. Increasing size and prolonged duration of contraceptive therapy can increase the risk of hemorrhage and are valid indications for surgical resection. Sporadic cases of hepatocellular carcinoma arising from HA have been reported [40, 41]. Liver transplantation is indicated in patients with type 1 glycogen storage disease and multiple adenomas due to their risk of HA rupture and HCC.

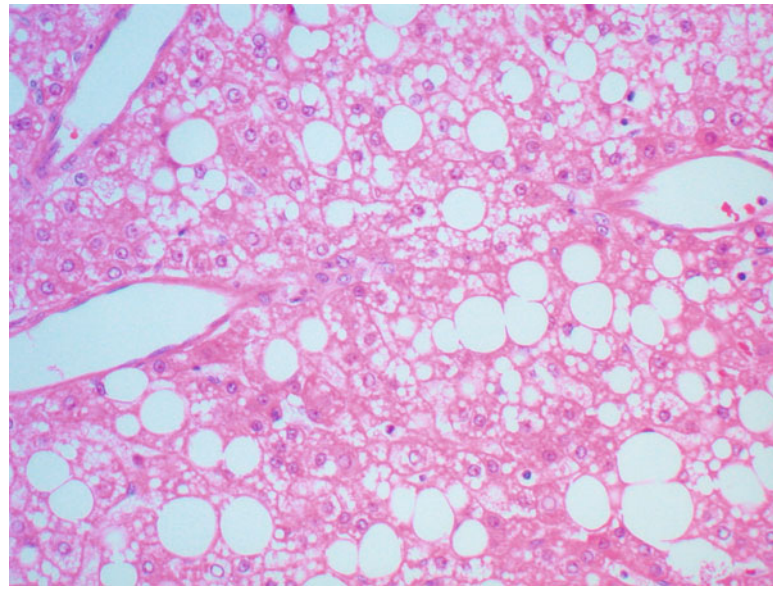


Fig. 22.4 Hepatocellular adenoma. Many hepatocytes contain variably sized cytoplasmic lipid droplets that enlarge the cells leading to compression of sinusoids. Multiple thin-walled vessels are distributed randomly within the lesion

Nodular Regenerative Hyperplasia (NRH)

NRH is a rare benign transformation of hepatic parenchyma into small regenerative nodules without fibrosis. NRH is a cause of non-cirrhotic intrahepatic portal hypertension (as are sinusoidal obstruction syndrome, perisinusoidal fibrosis, hepatoportal sclerosis, and incomplete septal cirrhosis). The etiology of the resultant portal hypertension is likely due to an intrahepatic hypercoagulable state, secondary to sinusoidal endothelial injury. Unlike causes of cirrhosis, NRH only rarely results in compromised hepatic synthetic function. NRH is an incidental finding in most cases, and most patients with NRH are asymptomatic with laboratory evaluation of liver function yielding normal results.

Pathogenesis

Etiopathogenesis of NRH is not entirely understood, although animal models suggest that endothelial injury results in a decrease in blood flow causing acinar atrophy and compensatory hyperplasia in the adjacent acini where blood supply is preserved. The inciting endothelial injury can be due to underlying autoimmune, inflammatory, or neoplastic disease or secondary toxic effects of immunosuppressive therapy. The endothelial injury subsequently results in obliteration of small venules and ultimately the decreased blood flow. In support of this proposed pathogenesis is the association of NRH with Abernathy Syndrome, a condition characterized by congenital absence of the portal vein. In this condition the splanchnic and splenic venous blood drains directly into the inferior vena cava, thus bypassing the liver entirely, and the liver relies upon high-pressure arterial perfusion. NRH is also associated with portal vein thrombosis, pulmonary hypertension, rheumatoid arthritis/Felty syndrome, HIV infection, lymphomas, and a number of extrahepatic tumors [42].

Pathologic Features

The atrophic liver is diffusely studded with small tan nodules that are typically less than 1 cm in diameter, although rarely larger lesion can be appreciated on palpation. Small nodules may be challenging to appreciate on a needle biopsy but are recognized by their usual periportal location and compression of intervening parenchyma, which is highlighted with reticulin staining. The nodules are composed of hyperplastic otherwise normal appearing hepatocytes that often acquire cytoplasmic glycogen or lipid as the nodule enlarges; they have less lipofuscin or other pigments (i.e., hemosiderin) than the internodular hepatocytes. The surrounding parenchyma often shows acinar atrophy without any fibrosis (Fig. 22.5a–c).

Radiologic Findings

The imaging appearance varies depending on the size of the nodules, although smaller nodules might remain undetected, and since the nodules are composed of normal hepatocytes, even larger nodules might not be appreciated. Findings associated with portal hypertension including splenomegaly, gastroesophageal varices, and ascites can be seen when portal hypertension is present. With intravascular contrast imaging, the absence of arterial phase enhancement with NRH can usually distinguish its nodules from those of other hepatic tumors [5].

Management

There is no specific treatment for NRH, although management of the resulting portal hypertension is commonly needed. Malignant transformation to hepatocellular carcinoma can occur rarely.

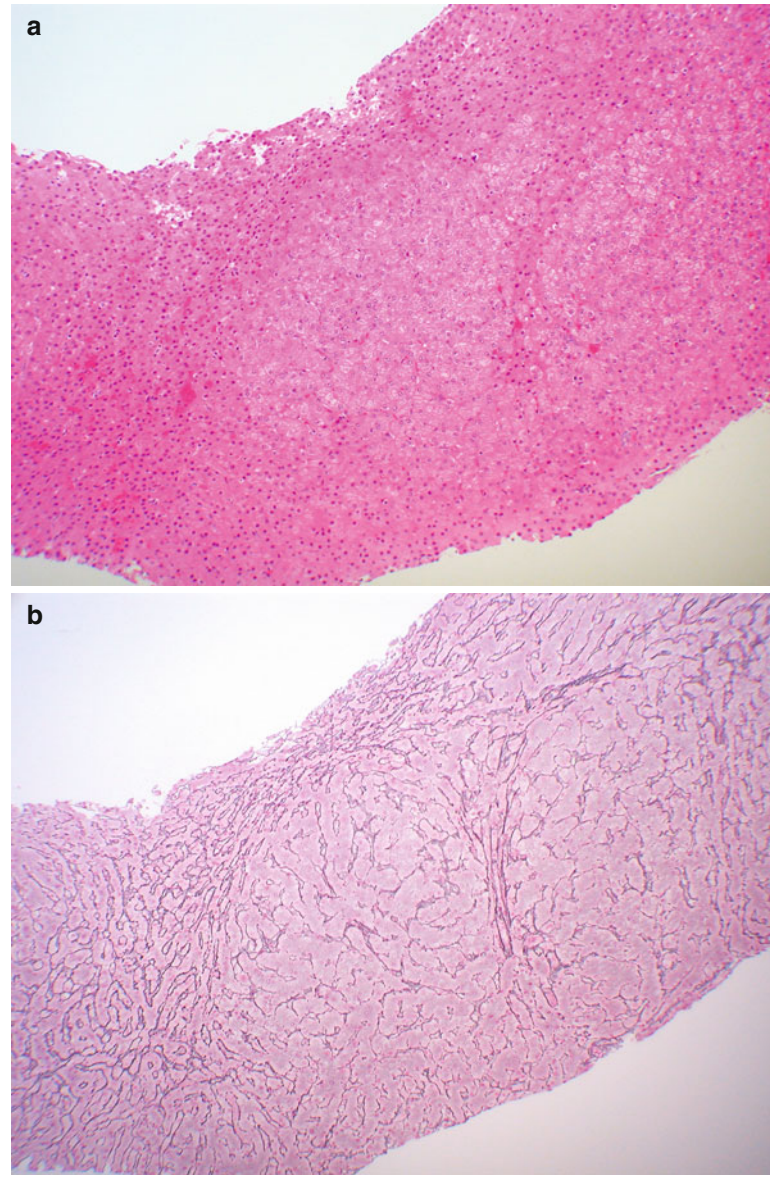
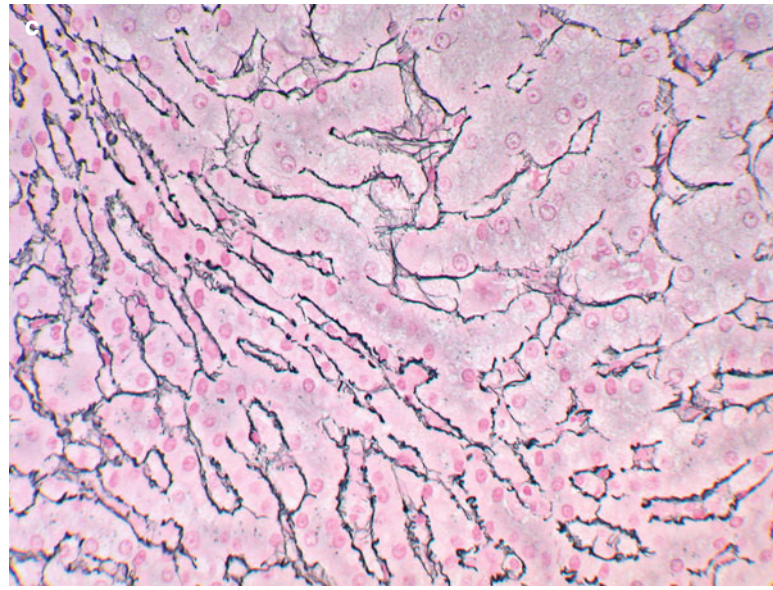


Fig. 22.5 Nodular regenerative hyperplasia. (**a**) Only slight tinctorial differences on an H&E stain hints at the distorted lobular architecture. Fibrosis is not evident. (**b**) Reticulin stain of the same field dramatically demonstrates the hyperplastic hepatocellular nodules that compress intervening parenchyma. (**c**) Higher magnification illustrates the thick hepatocellular plates of a nodule (*right*) and the nearly perpendicular orientation of its sinusoids compared to the adjacent atrophic parenchyma

Fig. 22.5 (continued)



Malignant Tumors

Hepatoblastoma (HB)

Hepatoblastoma is the most common primary hepatic tumor in preadolescent children. The majority of the cases are identified by the age of 5 years, and there is a slight male predominance. The increasing incidence of HB is most likely related to increased survival of premature patients; low birth weighted infants are at increased risk of developing HB. Multiple syndromes associated with HB include familial adenomatous polyposis [43], Beckwith-Wiedemann syndrome [44], Li-Fraumeni syndrome, trisomy 18, and glycogen storage disease type 1a.

Clinical Features

HB presents as an abdominal mass with associated weight loss, anorexia, and abdominal pain. About 90 % of patients have elevated serum AFP levels, and these levels generally correlate with disease extent [45]. Metastatic disease may involve the lungs most commonly but also the brain, bones, lymph nodes, and eyes. A high index of suspicion in the appropriate clinical setting is crucial to make the diagnosis.

Pathologic Features

HB is typically a single mass in the right lobe of the liver that is variegated tan to light brown to green on cut surface with frequent areas of hemorrhage and necrosis. Microscopically, hepatoblastomas are subdivided into either the epithelial (56 %) or the epithelial/mesenchymal (44 %) types. The epithelial type is further subdivided into fetal (31 %), embryonal (19 %), macrotrabecular (3 %), and small-cell undifferentiated (3 %) subtypes. Fetal HB is comprised of cords of hepatoid cells, while embryonal tumors have smaller cells with higher nuclear-cytoplasmic ratios, and small-cell tumors resemble other "small round blue cell" tumors of childhood; most tumors contain a mixture of fetal and embryonal cells. The most common mesenchymal elements are cartilage and osteoid in mixed hepatoblastomas (Fig. 22.6).

Radiologic Findings

Ultrasound, typically demonstrates a hyperechoic, solid, intrahepatic mass. CT scan typically shows a well-circumscribed slightly hypoattenuating mass compared to adjacent normal liver parenchyma. A more homogenous appearance on CT is associated with epithelial

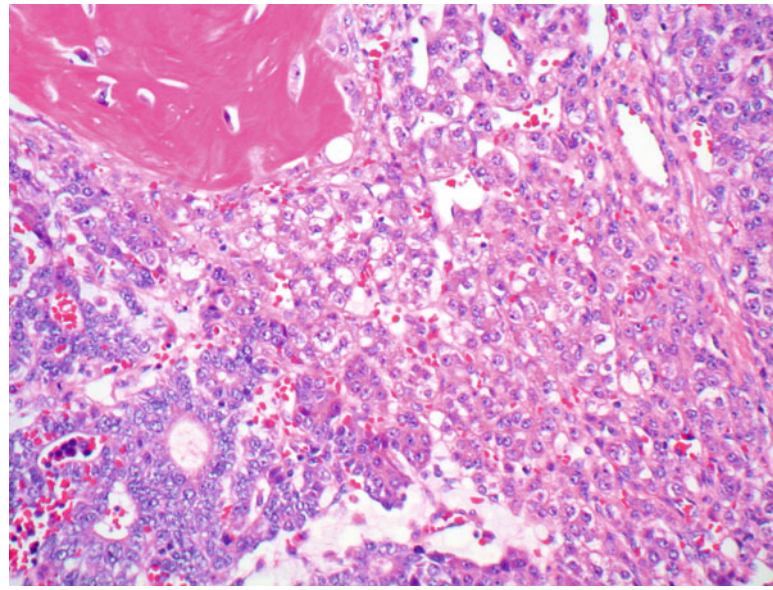


Fig. 22.6 Hepatoblastoma. The mixed (epithelial/mesenchymal) hepatoblastoma contains both fetal (*right*) and embryonal (*left*) epithelial components, the former bearing a close resemblance to normal hepatocytes. A segment of bone projects into the *upper left* portion of the field

hepatoblastoma, whereas mixed tumors are predominantly heterogeneous [46]. CT and MRI are also helpful in delineating the segmental involvement and the proximity of the tumor to the portal vein which helps determine resectability and aids with staging. Use of contrast, especially with MR-angiography, helps to define its vascular supply, again to assist in surgical planning.

Management

Treatment of hepatoblastomas requires a multidisciplinary approach that includes oncologists, pediatric surgeons, and transplant surgeons. Surgical resection is the mainstay of treatment and resectability of the tumor determines the prognosis. Pretreatment extent of disease (PRETEXT) is based on the segmental anatomy of the liver and used to determine the extent of required liver resection and hence resectability at the time of diagnosis. Posttreatment extent of disease (POST-TEXT) is used to monitor response to neoadjuvant chemotherapy and to determine resectability after chemotherapy [47, 48]. The use of primary neoadjuvant chemotherapy can frequently allow resection of pretreatment unresectable tumors, leading to improved prognosis and survival. Definitive resection or liver transplantation should occur prior to or just following the fourth cycle of chemotherapy, as there is no increased benefit to prolonged presurgical exposure to chemotherapy [49]. There are various chemotherapeutic regimens and schedules used in neoadjuvant and adjuvant treatment of patients with hepatoblastoma. The most common agents used are cisplatin, 5-fluorouracil (5-FU), carboplatin, doxorubicin, vincristine, irinotecan, etoposide, and ifosfamide. Cisplatin and doxorubicin are the two most common chemotherapeutic agents used in treating hepatoblastoma and are generally given for four to eight cycles with surgical resection mid-treatment. Pulmonary metastasis is not an absolute contraindication to liver transplantation as these lesions are sensitive to chemotherapy and amenable to surgical resection. Unilateral solitary lung lesions should undergo wedge resection, and if multiple but isolated to the same area of the lungs, patient may undergo lobectomy. Contraindications to resection include extensive bilateral liver involvement, vascular invasion of major hepatic veins and inferior vena cava, multifocal disease, and distant metastasis other than pulmonary [50]. Patients with unresectable, large, biopsy-proven hepatoblastoma with no evidence of metastatic or vascularly invasive disease should be evaluated for liver transplantation on the deceased donor wait list with a 1B priority.

Prognosis

Outcome largely depends on staging at presentation, histological type, and response to chemotherapy. Failure to decrease serum AFP levels by two logs with initial therapy suggests a poor prognosis. Pure fetal histology and the presence of mesenchymal elements are associated with a better prognosis while undifferentiated histology carries a poorer prognosis. Preoperative PRETEXT staging system utilizes the number of liver segments involved to help stage the disease [51]. Five-year survival based on the stage is as follows: stage I 100, II 91, III 68, and IV 57 %.

PRE-/POST-TEXT group
I: One segment involved, three adjoining sections are tumor-free
II: One or two sections involved with two adjoining sections tumor-free
III: Two or three sections involved with one adjoining section tumor-free
IV: Four sections involved

Hepatocellular Carcinoma (HCC)

HCC is the second most common hepatic malignancy in children although it is rare in young children [52] and peaks between the ages of 10 and 15 (mean age 12 years) with a male predominance. Unlike in adults where cirrhosis is almost always a predisposing factor for the development of HCC, non-cirrhotic liver disease is commonly present in the children who develop HCC. As in adults the incidence of HCC parallels the prevalence of hepatitis B and C viral infection rates, and children with cirrhotic chronic liver disease from any cause are at risk. Particular inborn errors of metabolism (tyrosinemia, urea cycle defects, glycogen storage disease, progressive familial intrahepatic cholestasis, Niemann-Pick type C) carry increased risk for children affected and may do so in the absence of cirrhosis.

Clinical Features

Presentation typically includes an incidental finding of a mass that accompanies other symptoms such as abdominal pain, anorexia, and weight loss. Preemptive monitoring of patients with risk factors aids in timely diagnosis and intervention. Screening with serum AFP and abdominal US can help detect tumors prior to their clinical manifestation [53].

Pathologic Features

HCC can be solitary, multinodular, or rarely a diffuse infiltrative tumor. Histologically, tumors are comprised of intermediate to large, polygonal cells with central nuclei and a moderate amount of eosinophilic to clear cytoplasm that are separated by biliary canaliculi; they may be so well differentiated as to resemble a normal liver. They typically grow as thick trabeculae that are separated by sinusoid-like spaces (Fig. 22.7) but can occasionally have a pseudoglandular architecture. Necrosis and hemorrhage, as well as vascular invasion, are commonly seen.

Radiologic Findings

Smaller lesions can be hypo-, iso-, or hyperechoic to the liver parenchyma on US, while bigger lesions tend to be heterogeneous. Vascular invasion can be analyzed with Doppler evaluation. Multiphase CT scan shows an early arterial phase enhancement as the main blood supply is via the hepatic artery. On MRI HCC is hyperintense on T2-weighted images. Larger tumors will have a mosaic pattern secondary to the presence of intratumoral hemorrhage, necrosis, fat, or calcifications.

Management

Complete surgical resection of the tumor confers the best chance for long-term survival; however, HCC is often unresectable at presentation in the pediatric population, because of metastatic disease [52, 54]. When not metastatic the ability to completely resect a HCC is related to the extent of cirrhosis, functional reserve of the liver, and the size and number of lesions. Systemic chemotherapy is not effective as HCC is chemoresistant [52] and ablative therapy has limited role in HCC therapy. Liver transplantation for HCC, however, does afford a <10 % 1-year mortality when restricted to patients with HCC <5 cm for a single lesion or multiple lesions involving no more than

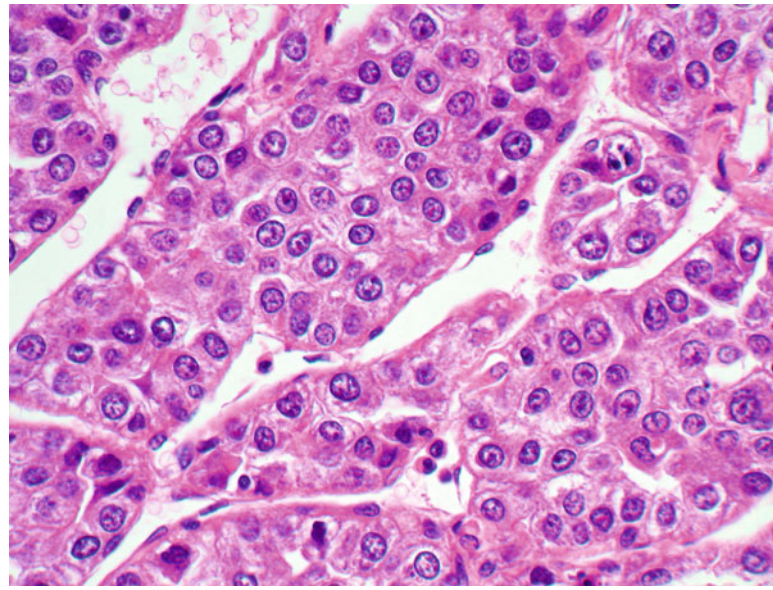


Fig. 22.7 Hepatocellular carcinoma. The tumor cells resemble small hepatocytes and grow as thick cords, mimicking cell plates of a normal liver. The trabeculae are surrounded by endothelial cells and separated by vascular spaces

three lesions with the largest measuring ≤3 cm (Milan Criteria) [55–57].

Fibrolamellar Carcinoma (FLC)

FLC is a variant of HCC seen in adolescent and young adults who lack any evidence of underlying liver disease.

Clinical Features

As with standard HCC, the typical presentation includes an abdominal mass or pain, along with constitutional symptoms of weight loss, anorexia, and malaise. Less commonly, jaundice, gynecomastia, or venous thrombosis has been reported as presenting symptoms. Unlike other HCC variants, serum AFP levels may not be elevated.

Pathologic Features

On gross examination, FLC is a well-circumscribed firm mass with characteristic radiating fibrous septa that resemble FNH. Microscopically neoplastic cells are large and polygonal with abundant granular eosinophilic cytoplasm that often contains hyaline or "ground-glass"-like inclusions. Nuclei are large hyperchromatic and vesicular with prominent nucleoli. The name is derived from the distinct feature of fibrous stroma and thick hyalinized collagen that surrounds individual or groups of cells (Fig. 22.8).

Radiologic Findings

Although a solitary circumscribed mass with heterogeneous appearance is seen on US, CT is the preferred modality of imaging and provides better definition and visualization of the central scar that commonly is calcified. Nonenhancement on arterial phase of the central scar helps differentiate FLC from FNH [58].

Management

Surgical resection is the mainstay of treatment along with adjunctive chemotherapy (e.g., sorafenib). Chemoembolization and liver transplantation can also be utilized based on tumor characteristics. The best prognosis is achieved by complete tumor resection with tumor-free surgical margins. Other indicators of better prognosis include younger age at presentation and absence of lymph node involvement or vascular invasion. *Clinical data regarding treatment outcome and prognosis of patients with FLC are scarce, probably due to the rarity of this tumor. Systematic review of pooled information of all previous published case series and reports reveal a 5-year survival rate of 44% that increases to 70% with surgical resection* [59].

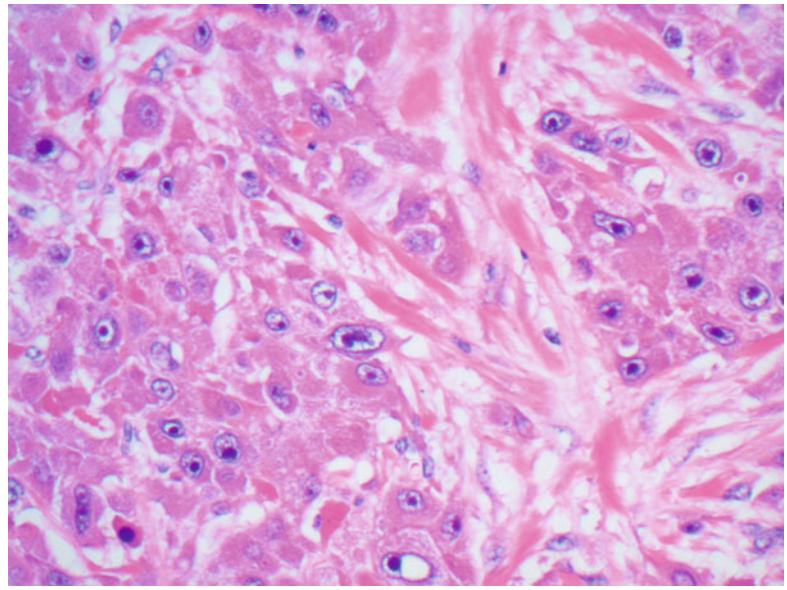


Fig. 22.8 Fibrolamellar carcinoma. Large tumor cells have abundant granular cytoplasm and vesicular nuclei with prominent nucleoli. Strands of dense hyalinized collagen interdigitate between the cells

Embryonal (Undifferentiated) Sarcoma of the Liver (ESL)

ESL is a rare but highly malignant hepatic neoplasm of mesenchymal origin seen in young children with a poor outcome. Most cases are identified in children less than 10 years of age; there is no sex predilection.

Clinical Features

There are no specific clinical features. Abdominal mass with accompanying abdominal pain is seen in majority of patients, but, rarely, it can present acutely with complications of tumor rupture. Laboratory values including serum AFP levels are usually normal.

Pathologic Features

The tumors are usually >10 cm and up to 30 cm in size, predominantly solid on the periphery, whereas the center has cystic gelatinous spaces, hemorrhage, and necrosis. It is well demarcated and often demonstrates a fibrous pseudocapsule. Microscopically, the tumor cells are spindled, oval, or stellate with hyperchromatic and often very pleomorphic nuclei and frequent mitoses (Fig. 22.9). They are arranged within fibrous or typically mucopolysaccharide-rich stroma. Multiple intra- or extracellular PAS-positive eosinophilic globules of varying size are characteristically present, and entrapped dilated bile ducts can be seen at the periphery.

Radiologic Findings

The solid tumor is either iso- or hyperechoic relative to normal liver parenchyma on US. A cyst-like appearance can be seen on CT and MR imaging which is in contrast to the US and gross pathologic appearance of ESL. MRI is the preferred modality for imaging and helps determine resectability with regard to vascular or biliary tree involvement [60].

Management

Previously considered to carry a poor prognosis, the mean 12-month survival has significantly improved to as high as 62 % [61]. This improvement has resulted from a multidisciplinary approach including adjuvant chemotherapy, radiotherapy, and surgical resection. Complete surgical resection is the mainstay of treatment. Neoadjuvant chemotherapy that utilizes chemotherapy in patients with localized cancer in order to decrease the tumor burden prior to surgical resection renders unresectable tumors amenable to surgical excision. Liver transplantation for unresectable tumors can also be considered.

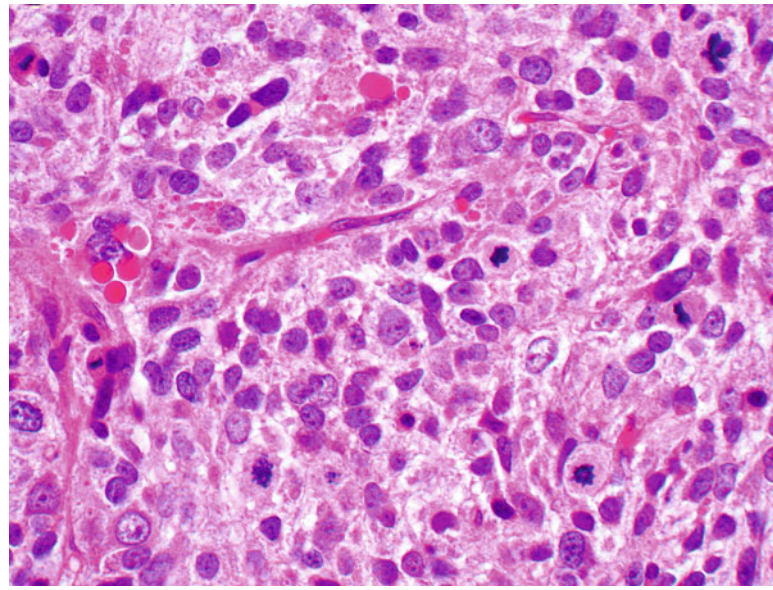


Fig. 22.9 Embryonal sarcoma. The moderately cellular tumor is comprised of hyperchromatic ovoid undifferentiated cells and has numerous mitotic figures. Eosinophilic globules (*left*) lie within the cytoplasm of a pleomorphic tumor cell

Epithelioid Hemangioendothelioma (EHE)

EHE is a rare slow-growing vascular tumor of the liver that primarily affects adult patients with only rare cases reported in late childhood (12–14 years of age). EHE is considered a low-grade malignant neoplasm in contrast to the more aggressive angiosarcoma.

Clinical Features

Nonspecific symptoms include right upper quadrant pain and weight loss. There is a slight female predominance [62]. Serum AFP level is generally not elevated.

Pathologic Features

These tumors are often multifocal involving both lobes of the liver. Peripheral masses close to the capsule cause subcapsular retraction secondary to a fibrotic reaction. Lesions are firm and white to tan and range from a few millimeters to 14 cm [62]. Histologically, EHE is comprised of dendritic and epithelioid cells that show vascular differentiation. Epithelioid cells have nuclear atypia and contain abundant eosinophilic cytoplasm. Some have a signet-ring appearance, where a cytoplasmic vacuole represents an intracellular vascular lumen. Immunohistochemistry staining for VIII, CD34, and/or CD31 is usually necessary to demonstrate the endothelial differentiation in EHE. The tumor often grows along preexisting sinusoids and hepatic or portal vein branches, creating persistence of acinar landmarks despite extensive invasion (Fig. 22.10a). As the tumor evolves, its fibromyxoid stroma becomes progressively sclerotic (Fig. 22.10b).

Radiologic Findings

Single to multiple nodules are seen peripherally in the liver with adjacent capsular retraction. EHE nodules are predominantly hypoattenuating compared to normal liver parenchyma on non-contrast CT. A target-like appearance of EHE on MRI is not infrequent and is due to reduced signal secondary to central hemorrhage, coagulative necrosis, and calcification which contrasts to the higher signal intensity at the periphery due to edematous connective tissue and viable tumor tissue [63].

Management

EHE is not readily amenable to chemotherapy, and thus, surgical resection and liver transplantation are the mainstays of treatment. Presence of multiple lesions diffusely throughout the liver parenchyma makes surgical excision challenging. Even in the face of metastatic disease, the prognosis is considered better than other hepatic

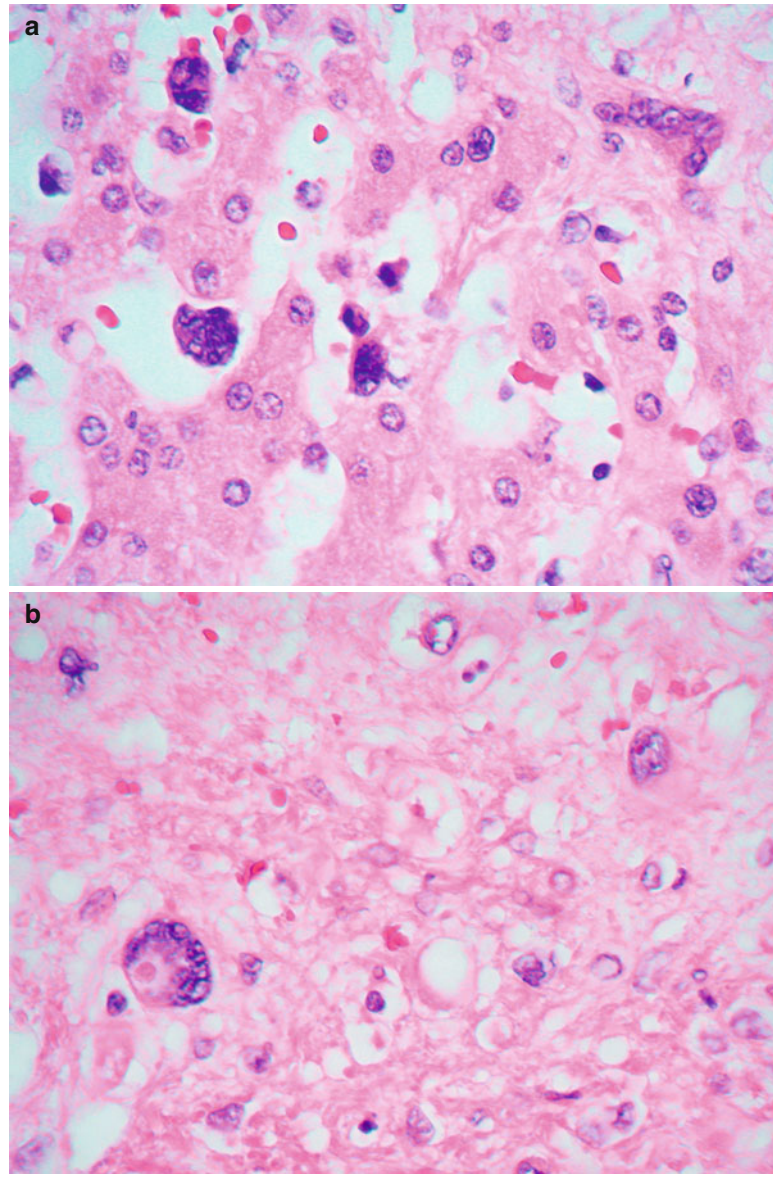


Fig. 22.10 Epithelioid hemangioendothelioma. (**a**) Hyperchromatic atypical epithelioid cells infiltrate hepatic sinusoids and destroy hepatic plates. (**b**) A sclerotic area of tumor contains only a few atypical and multinucleated tumor cells and lacks obvious vascular features

malignancies. Infiltration of the Glisson's capsule, mitoses, or nuclear atypia bears no prognostic importance; however, high cellularity correlates with poor clinical outcome.

Angiosarcoma

Angiosarcoma is a rare rapidly progressive vascular tumor of the liver that carries poor prognosis. It is the most uncommon hepatic vascular tumor in the pediatric population. It most commonly affects elderly men. Abdominal pain, anorexia, and weight loss in combination with hepatomegaly and jaundice are the most common clinical presentation. Features seen with IHH such as anemia, thrombocytopenia, and consumptive coagulopathy are commonly seen in angiosarcoma; however, the congestive heart failure component is usually absent. Hepatic involvement usually involves both lobes at presentation, thereby eliminating resection as a possibility. Biopsies are

rarely done so as to avoid the possible complication of significant bleeding. As in EHE, endothelial markers confirm vascular differentiation. Pediatric angiosarcomas often have a "kaposiform" component comprised of bundles of spindled cells with slit-like vascular spaces.

Imaging with multiphase CT or MRI is key to making the diagnosis. A hypoattenuating lesion is seen on CT in both arterial and venous phases. Delayed persistent enhancement with incomplete centripetal filling due to central fibrosis or necrosis can also be seen [64].

Arterial embolization can be utilized in appropriate clinical setting and based on tumor features. Treatment strategies including chemotherapy, radiation, surgical resection, and transplantation have had dismal outcomes.

Hepatobiliary Rhabdomyosarcoma

Rhabdomyosarcoma (RMS) is the most common sarcoma in children; however, in the liver, it is extremely rare and presents in early childhood (median age at presentation of 3.4 years). Hepatic RMS most commonly originates from the intrahepatic biliary tree and less commonly from the gall bladder, cystic duct, and the ampulla of Vater. It commonly presents with obstructive jaundice, abdominal pain, fever, vomiting, and weight loss. Laboratory evaluation discloses mild transaminitis in association with moderate elevation of serum bilirubin. US or CT scan can help delineate the hepatobiliary anatomic features and demonstrate dilatation of the bile ducts suggesting obstruction. Biliary tree dedicated MRI is the imaging test of choice. The tumor, often a "grape-like mass" (sarcoma botryoides) that projects into the duct lumen, is comprised of round to spindled cells that may have eosinophilic cytoplasm and cross-striations. Immunohistochemically, the cells are positive for muscle lineage markers such as desmin, myogenin, and MyoD. Although the tumors typically remain localized, they are not usually resectable, and thus, instead of surgery, chemotherapy or radiation therapy is initiated first [65]. Biliary obstruction might necessitate stent placement or even an external biliary drain. Neoadjuvant chemotherapeutic and multidisciplinary strategies have significantly improved the prognosis of hepatobiliary RMS. Preoperative chemotherapy, with the goal of reducing the tumor's size, may aid in improved ability to discern between normal and cancerous tissue during surgery and reduce the extent of the required surgery.

References

1. Boon LM, Burrows PE, Paltiel HJ, Lund DP, Ezekowitz RA, Folkman J, et al. Hepatic vascular anomalies in infancy: a twenty-seven-year experience. J Pediatr. 1996;129(3):346–54. S0022-3476(96)70065-3 [pii].
2. Selby DM, Stocker JT, Waclawiw MA, Hitchcock CL, Ishak KG. Infantile hemangioendothelioma of the liver. Hepatology. 1994;20(1 Pt 1):39–45. S0270913994002120 [pii].
3. Dehner LP, Ishak KG. Vascular tumors of the liver in infants and children. A study of 30 cases and review of the literature. Arch Pathol. 1971;92(2):101–11.
4. Burrows PE, Dubois J, Kassarjian A. Pediatric hepatic vascular anomalies. Pediatr Radiol. 2001;31(8):533–45.
5. Chung EM, Cube R, Lewis RB, Conran RM. From the archives of the AFIP: pediatric liver masses: radiologic-pathologic correlation part 1. Benign tumors. Radiographics. 2010;30(3):801–26. doi:10.1148/rg.303095173. 30/3/801.
6. Kassarjian A, Zurakowski D, Dubois J, Paltiel HJ, Fishman SJ, Burrows PE. Infantile hepatic hemangiomas: clinical and imaging findings and their correlation with therapy. AJR Am J Roentgenol. 2004;182(3):785–95.
7. Christison-Lagay ER, Burrows PE, Alomari A, Dubois J, Kozakewich HP, Lane TS, et al. Hepatic hemangiomas: subtype classification and development of a clinical practice algorithm and registry. J Pediatr Surg. 2007;42(1):62–7. doi:10.1016/j.jpedsurg.2006.09.041; S0022-3468(06)00654-3 [pii].
8. Ohama K, Nagase H, Ogino K, Tsuchida K, Tanaka M, Kubo M, et al. Alpha-fetoprotein (AFP) levels in normal children. Eur J Pediatr Surg. 1997;7(5):267–9. doi:10.1055/s-2008-1071168.
9. Keslar PJ, Buck JL, Selby DM. From the archives of the AFIP. Infantile hemangioendothelioma of the liver revisited. Radiographics. 1993;13(3):657–70.
10. Kuroda T, Kumagai M, Nosaka S, Nakazawa A, Takimoto T, Hoshino K. Critical infantile hepatic hemangioma: results of a nationwide survey by the Japanese infantile hepatic hemangioma study group. J Pediatr Surg. 2011;46(12):2239–43. doi:10.1016/j.jpedsurg.2011.09.007; S0022-3468(11)00746-9 [pii].
11. Mhanna A, Franklin WH, Mancini AJ. Hepatic infantile hemangiomas treated with oral propranolol – a case series. Pediatr Dermatol. 2011;28(1):39–45. doi:10.1111/j.1525-1470.2010.01355.x.

12. Storch CH, Hoeger PH. Propranolol for infantile haemangiomas: insights into the molecular mechanisms of action. Br J Dermatol. 2010;163(2):269–74.
13. Bosemani T, Puttgen KB, Huisman TA, Tekes A. Multifocal infantile hepatic hemangiomas – imaging strategy and response to treatment after propranolol and steroids including review of the literature. Eur J Pediatr. 2012;171(7):1023–8.
14. Yeh I, Bruckner AL, Sanchez R, Jeng MR, Newell BD, Frieden IJ. Diffuse infantile hepatic hemangiomas: a report of four cases successfully managed with medical therapy. Pediatr Dermatol. 2011;28(3):267–75.
15. Barlow CF, Priebe CJ, Mulliken JB, Barnes PD, Mac DD, Folkman J, et al. Spastic diplegia as a complication of interferon alfa-2a treatment of hemangiomas of infancy. J Pediatr. 1998;132(3 Pt 1):527–30.
16. Stringer MD, Alizai NK. Mesenchymal hamartoma of the liver: a systematic review. J Pediatr Surg. 2005;40(11):1681–90. doi:10.1016/j.jpedsurg.2005.07.052; S0022-3468(05)00622-6 [pii].
17. Koumanidou C, Vakaki M, Papadaki M, Pitsoulakis G, Savvidou D, Kakavakis K. New sonographic appearance of hepatic mesenchymal hamartoma in childhood. J Clin Ultrasound. 1999;27(3):164–7. 10.1002/(SICI)1097-0096(199903/04)27:3<164::AID-JCU12>3.0.CO;2-J [pii].
18. Kitano Y, Ruchelli E, Weiner S, Adzick NS. Hepatic mesenchymal hamartoma associated with mesenchymal stem villous hyperplasia of the placenta. Fetal Diagn Ther. 2000;15(3):134–8. [pii] 20992.
19. Yen JB, Kong MS, Lin JN. Hepatic mesenchymal hamartoma. J Paediatr Child Health. 2003;39(8):632–4. 250 [pii].
20. Srouji MN, Chatten J, Schulman WM, Ziegler MM, Koop CE. Mesenchymal hamartoma of the liver in infants. Cancer. 1978;42(5):2483–9.
21. Kamata S, Nose K, Sawai T, Hasegawa T, Kuroda S, Sasaki T, et al. Fetal mesenchymal hamartoma of the liver: report of a case. J Pediatr Surg. 2003;38(4):639–41. doi:10.1053/jpsu.2003.50142; S0022346802631513 [pii].
22. Meinders AJ, Simons MP, Heij HA, Aronson DC. Mesenchymal hamartoma of the liver: failed management by marsupialization. J Pediatr Gastroenterol Nutr. 1998;26(3):353–5.
23. Luciani A, Kobeiter H, Maison P, Cherqui D, Zafrani ES, Dhumeaux D, et al. Focal nodular hyperplasia of the liver in men: is presentation the same in men and women? Gut. 2002;50(6):877–80. Pubmed PMCID:PM:PMC1773241.
24. Scalori A, Tavani A, Gallus S, La VC, Colombo M. Oral contraceptives and the risk of focal nodular hyperplasia of the liver: a case–control study. Am J Obstet Gynecol. 2002;186(2):195–7. S0002937802382498 [pii].
25. Mathieu D, Kobeiter H, Maison P, Rahmouni A, Cherqui D, Zafrani ES, et al. Oral contraceptive use and focal nodular hyperplasia of the liver. Gastroenterology. 2000;118(3):560–4. S0016508500442605 [pii].
26. Kubota T, Shimizu K, Sonoyama T, Ikeda E, Kurioka H, Ouchi T, et al. Enlarged focal nodular hyperplasia of the liver under the influence of oral contraceptives. Hepatogastroenterology. 2001;48(42):1736–9.
27. Wanless IR, Mawdsley C, Adams R. On the pathogenesis of focal nodular hyperplasia of the liver. Hepatology. 1985;5(6):1194–200. S0270913985001525 [pii].
28. Golli M, Mathieu D, Anglade MC, Cherqui D, Vasile N, Rahmouni A. Focal nodular hyperplasia of the liver: value of color Doppler US in association with MR imaging. Radiology. 1993;187(1):113–7.
29. Wang LY, Wang JH, Lin ZY, Yu ML, Lu SN, Chuang WL, et al. Hepatic focal nodular hyperplasia: findings on color Doppler ultrasound. Abdom Imaging. 1997;22(2):178–81.
30. Carlson SK, Johnson CD, Bender CE, Welch TJ. CT of focal nodular hyperplasia of the liver. AJR Am J Roentgenol. 2000;174(3):705–12.
31. Bieze M, van den Esschert JW, Nio CY, Verheij J, Reitsma JB, Terpstra V, et al. Diagnostic accuracy of MRI in differentiating hepatocellular adenoma from focal nodular hyperplasia: prospective study of the additional value of gadoxetate disodium. AJR Am J Roentgenol. 2012;199(1):26–34.
32. Meyers RL. Tumors of the liver in children. Surg Oncol. 2007;16(3):195–203. doi:10.1016/j.suronc.2007.07.002; S0960-7404(07)00068-0 [pii].
33. Resnick MB, Kozakewich HP, Perez-Atayde AR. Hepatic adenoma in the pediatric age group. Clinicopathological observations and assessment of cell proliferative activity. Am J Surg Pathol. 1995;19(10):1181–90.
34. Bagia S, Hewitt PM, Morris DL. Anabolic steroid-induced hepatic adenomas with spontaneous haemorrhage in a bodybuilder. Aust N Z J Surg. 2000;70(9):686–7.
35. Toso C, Rubbia-Brandt L, Negro F, Morel P, Mentha G. Hepatocellular adenoma and polycystic ovary syndrome. Liver Int. 2003;23(1):35–7.
36. Marie-Cardine A, Schneider P, Greene V, Metayer J, Bachy B, Vannier JP. Erythrocytosis in a child with a hepatic adenoma. Med Pediatr Oncol. 2001;36(6):659–61. doi:10.1002/mpo.1148.
37. Musthafa CP, Chettupuzha AP, Syed AA, Ayshabi KA, Aravindan KP, Shobhana DR. Liver adenomatosis. Indian J Gastroenterol. 2003;22(1):30–1.
38. Rubin RA, Mitchell DG. Evaluation of the solid hepatic mass. Med Clin North Am. 1996;80(5):907–28.
39. Grazioli L, Federle MP, Brancatelli G, Ichikawa T, Olivetti L, Blachar A. Hepatic adenomas: imaging and pathologic findings. Radiographics. 2001;21(4):877–92.
40. Micchelli ST, Vivekanandan P, Boitnott JK, Pawlik TM, Choti MA, Torbenson M. Malignant transformation of hepatic adenomas. Mod Pathol. 2008;21(4):491–7. doi:10.1038/modpathol.2008.8; modpathol20088 [pii].
41. Farges O, Dokmak S. Malignant transformation of liver adenoma: an analysis of the literature. Dig Surg. 2010;27(1):32–8. doi:10.1159/000268405; 000268405 [pii].
42. Hartleb M, Gutkowski K, Milkiewicz P. Nodular regenerative hyperplasia: evolving concepts on underdiagnosed cause of portal hypertension. World J Gastroenterol. 2011;17(11):1400–9.

43. Giardiello FM, Petersen GM, Brensinger JD, Luce MC, Cayouette MC, Bacon J, et al. Hepatoblastoma and APC gene mutation in familial adenomatous polyposis. Gut. 1996;39(6):867–9. Pubmed PMCID:PM:PMC1383462.
44. Steenman M, Westerveld A, Mannens M. Genetics of Beckwith-Wiedemann syndrome-associated tumors: common genetic pathways. Genes Chromosomes Cancer. 2000;28(1):1–13. 10.1002/(SICI)1098-2264(200005)28:1<1::AID-GCC1>3.0.CO;2-# [pii].
45. Van Tornout JM, Buckley JD, Quinn JJ, Feusner JH, Krailo MD, King DR, et al. Timing and magnitude of decline in alpha-fetoprotein levels in treated children with unresectable or metastatic hepatoblastoma are predictors of outcome: a report from the Children's Cancer Group. J Clin Oncol. 1997;15(3):1190–7.
46. Chung EM, Lattin Jr GE, Cube R, Lewis RB, Marichal-Hernandez C, Shawhan R, et al. From the archives of the AFIP: pediatric liver masses: radiologic-pathologic correlation. Part 2. Malignant tumors. Radiographics. 2011;31(2):483–507. doi:10.1148/rg.312105201. 31/2/483 [pii].
47. Otte JB. Progress in the surgical treatment of malignant liver tumors in children. Cancer Treat Rev. 2010;36(4):360–71. doi:10.1016/j.ctrv.2010.02.013; S0305-7372(10)00033-2 [pii].
48. Meyers RL, Czauderna P, Otte JB. Surgical treatment of hepatoblastoma. Pediatr Blood Cancer. 2012;59(5):800–8. doi:10.1002/pbc.24220.
49. Trobaugh-Lotrario AD, Katzenstein HM. Chemotherapeutic approaches for newly diagnosed hepatoblastoma: past, present, and future strategies. Pediatr Blood Cancer. 2012;59(5):809–12. doi:10.1002/pbc.24219.
50. Herzog CE, Andrassy RJ, Eftekhari F. Childhood cancers: hepatoblastoma. Oncologist. 2000;5(6):445–53.
51. Brown J, Perilongo G, Shafford E, Keeling J, Pritchard J, Brock P, et al. Pretreatment prognostic factors for children with hepatoblastoma – results from the International Society of Paediatric Oncology (SIOP) study SIOPEL 1. Eur J Cancer. 2000;36(11):1418–25. S0959-8049(00)00074-5 [pii].
52. Czauderna P. Adult type vs. childhood hepatocellular carcinoma – are they the same or different lesions? Biology, natural history, prognosis, and treatment. Med Pediatr Oncol. 2002;39(5):519–23. doi:10.1002/mpo.10178.
53. Ferenci P, Fried M, Labrecque D, Bruix J, Sherman M, Omata M, et al. Hepatocellular carcinoma (HCC): a global perspective. J Clin Gastroenterol. 2010;44(4):239–45. doi:10.1097/MCG.0b013e3181d46ef2.
54. Yu SB, Kim HY, Eo H, Won JK, Jung SE, Park KW, et al. Clinical characteristics and prognosis of pediatric hepatocellular carcinoma. World J Surg. 2006;30(1):43–50. 10.1007/s00268-005-7965-z.
55. European Association for the Study of the Liver, European Organisation for Research and Treatment of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. J Hepatol. 2012;56(4):908–43. doi:10.1016/j.jhep.2011.12.001; S0168-8278(11)00873-7 [pii].
56. Hanish SI, Knechtle SJ. Liver transplantation for the treatment of hepatocellular carcinoma. Oncology (Williston Park). 2011;25(8):752–7.
57. Mazzaferro V, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. N Engl J Med. 1996;334(11):693–9. doi:10.1056/NEJM199603143341104.
58. McLarney JK, Rucker PT, Bender GN, Goodman ZD, Kashitani N, Ros PR. Fibrolamellar carcinoma of the liver: radiologic-pathologic correlation. Radiographics. 1999;19(2):453–71.
59. Mavros MN, Mayo SC, Hyder O, Pawlik TM. A systematic review: treatment and prognosis of patients with fibrolamellar hepatocellular carcinoma. J Am Coll Surg. 2012;215(6):820–30.
60. Crider MH, Hoggard E, Manivel JC. Undifferentiated (embryonal) sarcoma of the liver. Radiographics. 2009;29(6):1665–8. doi:10.1148/rg.296085237. 29/6/1665 [pii].
61. Noguchi K, Yokoo H, Nakanishi K, Kakisaka T, Tsuruga Y, Kamachi H, et al. A long-term survival case of adult undifferentiated embryonal sarcoma of liver. World J Surg Oncol. 2012;10:65. doi:10.1186/1477-7819-10-65. Pubmed PMCID:PM:PMC3407002 DOI: 1477-7819-10-65 [pii].
62. Makhlouf HR, Ishak KG, Goodman ZD. Epithelioid hemangioendothelioma of the liver: a clinicopathologic study of 137 cases. Cancer. 1999;85(3):562–82. 10.1002/(SICI)1097-0142(19990201)85:3<562::AID-CNCR7>3.0.CO;2-T [pii].
63. Lyburn ID, Torreggiani WC, Harris AC, Zwirewich CV, Buckley AR, Davis JE, et al. Hepatic epithelioid hemangioendothelioma: sonographic, CT, and MR imaging appearances. AJR Am J Roentgenol. 2003;180(5):1359–64.
64. Buetow PC, Buck JL, Ros PR, Goodman ZD. Malignant vascular tumors of the liver: radiologic-pathologic correlation. Radiographics. 1994;14(1):153–66.
65. Spunt SL, Lobe TE, Pappo AS, Parham DM, Wharam Jr MD, Arndt C, et al. Aggressive surgery is unwarranted for biliary tract rhabdomyosarcoma. J Pediatr Surg. 2000;35(2):309–16. S0022-3468(00)90030-7 [pii].

Part III

Management

Acute Liver Failure

23

Robert H. Squires

Scope of the Problem

Pediatric acute liver failure (PALF) is a rare and potentially life-threatening event that occurs in all age groups [1]. PALF remains one of the few conditions not related to trauma that can suddenly take the life of a previously healthy infant, child, or adolescent. The prevalence of PALF in North America is unknown, but the busiest pediatric liver transplant centers in the United States see between 5 and 12 PALF cases per year, and PALF accounts for approximately 10–12 % of all pediatric liver transplants in the United States. Despite its rarity, early recognition of PALF and communication with or referral to a pediatric liver transplant center are critical for patient survival. Diagnostic studies should be prioritized by age, potential response to directed therapy, and impact upon liver transplant (LTx) eligibility. As clinical deterioration can occur rapidly, coordinated management with a pediatric hepatologist, intensive care specialist, and liver transplant surgeon along with other supportive personnel will optimize patient outcome. Outcomes vary among and between etiologies, patient age groups, and disease severity.

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Criteria for Acute Liver Failure

A precise "definition" for PALF remains elusive. Development of hepatic encephalopathy (HE) within 8 weeks of the "first signs" of hepatic dysfunction defines acute liver failure (ALF) for adults. The onset of jaundice is often one of the first signs of liver dysfunction. However, its precise onset is difficult to determine as it is dependent on clinical observation by individuals with disparate expertise in assessing jaundice, patients' skin color and hemoglobin, as well as the ambient lighting. Therefore, jaundice may be present but unrecognized for a period of time. Although acute HE remains a hallmark of PALF as well, it is difficult to assess in children and may not be clinically apparent until the terminal stages of the disease process [2]. In addition, mental status changes occur in seriously ill children independent of hepatic dysfunction as a consequence of infection, metabolic derangements, or anxiety associated with acute illness.

Recent clinical studies of PALF have included children without clinical encephalopathy [3]. A consensus of the principal investigators for the PALF study group proposed the following entry criteria for the longitudinal cohort study: (1) children with no known evidence of chronic liver disease, (2) biochemical evidence of acute liver injury, and (3) coagulopathy not corrected by vitamin K. The presence of HE is required if the prothrombin time (PT) is between 15 and 19.9 s or the INR between 1.5 and 1.9, but if the PT is at least 20 s or INR $\geq$2.0, patients were enrolled with or without HE [4].

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Clinical Features

General

PALF presents in a previously healthy infant, child, or adolescent with a nonspecific prodrome. The duration of the prodrome is variable with symptoms similar to a "viral" syndrome such as abdominal pain, vomiting, decreased energy, and fever. With the exception of acute ingestions (e.g., mushrooms, acetaminophen), the precise onset of disease is rarely identified. Symptoms may persist or fluctuate for days or weeks before the child is brought to medical attention. In the absence of clinical evidence of liver disease such as jaundice or altered mental status, the child may receive empiric treatment to relieve symptoms. Once clinical signs of liver injury become evident, the clinical syndrome of PALF can be recognized.

Physical assessment should evaluate growth, development and nutrition status, presence of jaundice, bruises, bleeding following venipuncture, and petechiae. Hepatomegaly alone or with splenomegaly and peripheral edema can be present. Kayser-Fleischer rings are present in only 50 % of patients with Wilson disease who present with ALF. Fetor hepaticus is a sweet distinctive aroma to the breath associated with hepatic encephalopathy but is rarely present. While ascites may develop over the course of the illness, ascites detected on physical examination at presentation occurs rarely and should raise the possibility of an underlying chronic liver disease. Additional findings suggestive of chronic liver disease include digital clubbing, palmar erythema, cutaneous xanthoma, and prominent abdominal vessels suggesting long-standing portal hypertension. Altered mental status should be carefully evaluated but may be difficult to assess in infants and young children.

Biochemical features include evidence of liver injury and severe hepatic dysfunction. Serum aminotransferases, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) are markers for hepatocellular injury in the absence of muscle injury or myopathy. Elevations in both total and direct (or conjugated) bilirubin are present not only with hepatocellular injury but also intra- and extrahepatic biliary obstruction and hemolysis. Patterns of AST, ALT, and bilirubin elevation can be associated with some conditions, but one must use caution using an individual patients' value for diagnostic purposes. That said, there are some patterns of elevation worth noting [5].

Infants with gestational alloimmune liver disease will have normal or near normal ALT and AST levels despite significant hyperbilirubinemia and coagulopathy. Those with galactosemia and tyrosinemia can similarly have a "disproportionately" severe coagulopathy and hyperbilirubinemia associated with very modest ALT and AST elevations. As a group, children with metabolic disease typically have modest elevations of aminotransferase levels, usually less than 400 IU/L. Wilson disease should be considered in patients presenting with marked hyperbilirubinemia and low hemoglobin, both likely due to hemolysis, and a normal or low serum alkaline phosphatase [6, 7]. PALF patients with a known infectious cause will often have marked elevation of aminotransferase levels, often well over 750–1,000 IU/L. Other conditions associated with marked elevation of aminotransferase levels include shock, drug toxicity, and immune marker-positive and immune-mediated PALF. Children with acute acetaminophen toxicity will often present with marked elevation of aminotransferase level and a low serum bilirubin. These patterns are not diagnostic but only suggestive in an individual patient, and the absence of these patterns should not exclude a diagnosis. While these patterns may assist in diagnostic prioritization, they should never be used to limit the differential diagnosis as overlap among these patterns is significant.

Hepatic Encephalopathy

Hepatic encephalopathy (HE) is a neuropsychiatric syndrome associated with acute or chronic hepatic dysfunction. The pathogenesis of HE has long been associated with hyperammonemia, but it is likely a complex interaction between cerebral blood flow, systemic and cerebral inflammation, and metabolic disturbances including hyperammonemia [8, 9]. HE is not always clinically apparent, particularly in

Table 23.1 Stages of hepatic encephalopathy in infants and children

Stage	Clinical	Reflexes	Neurological signs	EEG changes
0	None	Normal	None	Normal
I	*Infant/child*: inconsolable crying, inattention to task Child is not acting like self to parents	Normal or hyperreflexic	Difficult or impossible to test adequately	
	Adult: confused, mood changes, altered sleep habits, forgetful	Normal	Tremor, apraxia, impaired handwriting	Normal or diffuse slowing to theta rhythm, triphasic waves
II	*Infant/child*: inconsolable crying, inattention to task Child is not acting like self to parents	Normal or hyperreflexic	Difficult or impossible to test adequately	
	Adult: drowsy, inappropriate behavior, decreased inhibitions	Hyperreflexic	Dysarthria, ataxia	Abnormal, generalized slowing, triphasic waves
III	*Infant/child*: somnolence, stupor, combativeness	Hyperreflexic	Difficult or impossible to test adequately	
	Adult: stuporous, obeys simple commands	Hyperreflexic, (+) Babinski	Rigidity	Abnormal, generalized slowing, triphasic waves
IV	*Infant/child*: comatose, arouses with painful stimuli (IVa) or no response (IVb)	Absent	Decerebrate or decorticate	
	Adult: comatose, arouses with painful stimuli (IVa) or no response	Absent	Decerebrate or decorticate	Abnormal, very slow, delta activity

infants and young children. In participants enrolled into the prospective PALF study, HE was present at study entry in 50 % of participants and increased to 65 % over the next 7 days [4].

Changes in behavior, cognition, neurological examination, and the electroencephalogram (EEG) pattern are used to characterize the patient as having one of five clinical stages of encephalopathy ranging from stage 0, with minimal or no evidence of neurological dysfunction, to stage IV coma (see Table 23.1) [10]. Clinical staging of HE was developed for adult patients with end-stage liver disease and not ALF. However, in the absence of a better clinical tool, the current scoring system has been adopted for use in PALF and has been found to have important clinical and prognostic implications. The spectrum of HE evolves from confusion, to combativeness, to somnolence and coma and can be traversed within hours or days in some cases. Efforts are ongoing to improve upon the current clinical grading of neurological dysfunction. Transcranial Doppler [11], cerebral near-infrared spectrophotometry [12], continuous EEG monitoring [13], and serum biomarkers [14] are being investigated to determine their usefulness in the setting of ALF. While neurologic morbidity remains a major determinant of outcome following PALF, further studies are needed to improve early detection of neurologic injury, standardize management of seizures and HE, and to determine whether such interventions improve outcomes.

Both generalized and focal seizures may occur in PALF [15]. The frequency of nonconvulsive seizures, or those identified only by electroencephalogram, has not been studied in detail. While seizures can occur as a consequence of cerebral inflammation or edema, other causes for seizures should be excluded including an underlying mitochondrial disease (e.g., Alpers syndrome), congenital disorder of glycosylation, and Ecstasy intoxication.

Cerebral Edema

Cerebral edema is a life-threatening complication of acute liver failure [16]. It occurs most commonly in those with advanced encephalopathy (grade III or IV) and can be rapidly progressive. Detection of cerebral edema in the early stages is difficult as noninvasive measures such as clinical assessment or radiographic studies are not sensitive. Surgical placement of an intracranial pressure (ICP) monitor is currently the most accurate measure of cerebral edema. Intracranial bleeding, while not always clinically significant, can occur in up to 20 % of patients following ICP monitor placement [17]. Use of activated factor VII in recent years is believed to have made placement of ICP monitors somewhat safer. Once in place and properly functioning, intracranial pressure monitoring is useful to assess response to treatment of increased cerebral pressure and during surgical procedures, including liver transplantation, to gage fluid and medical management of the unconscious patient.

Renal Injury

Renal insufficiency or failure occurs in patients with ALF. A classification system for pediatric renal injury has been proposed to more uniformly assess renal injury in critically ill children based upon the estimated creatinine clearance and urine output [18]. Evidence of acute kidney injury (AKI) at the time of presentation of ALF is uncommon. However, concomitant AKI and ALF [19] should raise concerns for acetaminophen toxicity, Wilson disease, hypovolemia, shock, sepsis, or exposure to potential toxins such as senna glycosides [20], lipid-lowering agents [21], or snake gallbladder ingestion [22]. Excessive fluid restriction can lead to prerenal azotemia. Acute deterioration of renal function following presentation with ALF may result from systemic hypotension due to sepsis or hemorrhage. Hepatorenal syndrome (HRS) typically occurs in patients with cirrhosis or acute hepatic decompensation of a previously stable chronic liver disease. HRS rarely occurs in the setting of ALF in previously healthy individuals. HRS is suspected when there is evidence of deteriorating renal function in the absence of bleeding, hypotension, sepsis, or nephrotoxic medications and in association with failure to improve with volume expansion. The urine sodium is typically low.

Ascites

Clinically apparent ascites is rare in PALF and, if present on physical examination at the time of presentation, should prompt consideration of an unsuspected chronic liver disease. Development of ascites during the course of the disease is more common. Precipitating factors include hypoalbuminemia, excessive fluid administration, and infection. For ascites in the setting of ALF, the primary treatment is fluid restriction. Diuretics should be reserved for patients with respiratory compromise or generalized fluid overload. Overly aggressive diuresis may precipitate HRS.

Coagulopathy

The prothrombin time (PT) and international normalization ratio (INR) are used in many prognostic schemes to assess the severity of liver injury in the setting of ALF. The coagulation profile is complex and heterogeneous in PALF [23]. Both procoagulant proteins (e.g., factors V, VII, and X and fibrinogen) and anticoagulant proteins (e.g., antithrombin, protein C, and protein S) are reduced [1]. Despite an elevated INR, normal hemostasis, as assessed by thromboelastography, is present in most adult patients with ALF [24]. This balanced reduction in the pro- and anticoagulant proteins may account for the relative infrequency of clinically important bleeding in the absence of a provocative event such as infection or increased portal hypertension. Therefore, the PT/INR may reasonably reflect the reduction of some of the liver-based coagulation proteins, but not the relative risk of bleeding.

Pancreatitis

Biochemical and clinical pancreatitis is increasingly recognized as a condition associated with

multisystem failure in critically ill children. Development of hyperglycemia should raise the possibility of concurrent pancreatitis [25]. In patients who develop pancreatitis in the setting of acute liver failure, glucose and fluid management may become even more challenging. Evidence of pancreatitis at presentation may suggest a toxic or metabolic injury such as mitochondrial disease [26], valproic acid toxicity [27], drug-related liver injury [28], or erythropoietic protoporphyria [29].

Cardiopulmonary

Excessive fluid administration may precipitate pulmonary edema. Careful fluid restriction and discrete use of diuretics may be needed in some instances but should be used with caution. Central venous pressure monitoring may assist in assessing volume needs for the child. Subclinical myocardial injury manifested by an elevated troponin I level (>.01 ng/mL) was identified in adults with ALF [30]. Inotropic support may be needed to maintain perfusion of vital organs.

Metabolic

Hypoglycemia develops as a consequence of impaired gluconeogenesis and depleted glycogen stores. A central venous catheter is required if hypertonic glucose (e.g., greater than 12.5 % dextrose) is required. Glucose infusion rates at or above 10–15 mg/kg/min may be necessary to achieve a stable serum glucose. Hypokalemia may occur secondary to dilution from volume overload, ascites, or renal wasting. Serum phosphorus should be monitored frequently as hypophosphatemia can be profound and may require periodic intravenous supplementation. Hyperphosphatemia may also develop as a consequence of renal insufficiency. Acid–base disturbances can be complicated with respiratory alkalosis from hyperventilation, respiratory acidosis from respiratory failure, metabolic alkalosis from hypokalemia, and metabolic acidosis from hepatic necrosis, shock, and increased anaerobic metabolism.

Immune/Inflammatory Dysregulation

The liver is a complex immune organ [31]. An estimated 20–40 % of the liver cell mass consists of immunogenic endothelial cells, Kupffer cells or hepatic macrophages, lymphocytes, biliary cells, and stellate cells. The immunological constituents within the liver differ from the peripheral blood compartment [32]. While both innate and adaptive immune responses are generated within the liver, the innate immune response predominates [33]. Given the complexity and relative independence of the hepatic immunological milieu, it is not surprising that ALF may occur as a consequence of missteps in the livers' general tolerance of real or perceived pathogens.

Severe liver injury, to the degree that it meets criteria for PALF, can trigger or result from an aberrant systemic immune or inflammatory response. Multisystem involvement, particularly development of pancytopenia and metabolic abnormalities that include hyperferritinemia, hypertriglyceridemia, hyponatremia, and fever, should raise suspicion of a more generalized immune dysregulation such as macrophage activation syndrome or hemophagocytic lymphohistiocytosis [34, 35].

Systemic Inflammatory Response Syndrome (SIRS)

The systemic inflammatory response syndrome (SIRS) has been recognized since 1992 as a generalized inflammatory response to what often began as a local trigger such as trauma, pancreatitis, infection (e.g., bacterial, viral, fungal), or liver injury [36]. Clinical manifestations include temperature dysregulation (e.g., hyperthermia or hypothermia), tachycardia, tachypnea, or leukocytosis/leukopenia. While most of the data are derived from adults, it is becoming evident that inflammatory responses in children differ from those in adults [37]. In addition, a recent consensus conference proposed criteria for SIRS and organ dysfunction in children [38].

SIRS can occur in the setting of ALF and likely contributes to many extrahepatic manifestations of ALF, particularly the progression of HE [39]. The extent to which hepatic immune

dysregulation may trigger SIRS is not known [40–42]. And yet, it would be expected that immune and inflammatory mechanisms are involved in both the initiation and perpetuation of PALF as well as extrahepatic manifestations of PALF such as renal dysfunction, pancreatitis, altered vascular integrity, encephalopathy, and cerebral edema.

Macrophage Activation Syndrome (MAS)

Severe liver injury, to the degree that it meets criteria for PALF, can trigger or result from an aberrant systemic immune or inflammatory response. Multisystem involvement, particularly the bone marrow, coagulation profile, cerebral function, and metabolic abnormalities that include hyperferritinemia, hypertriglyceridemia, hyponatremia, and fever, is present at or shortly after clinical presentation in MAS [34]. MAS is associated with lobular hepatitis and cholestasis histologically similar to vanishing bile duct syndrome [43]. The initial trigger is often not identified but could be viral, an underlying immune disorder such as juvenile rheumatoid arthritis or systemic lupus, or medication induced. The importance of recognizing PALF in the context of a more generalized immune or inflammatory process is that treatment strategies need to be implemented to suppress the exuberant immune response.

Etiology

The etiologies of PALF are varied and fall into a few broad categories: metabolic, immune dysregulation, drug or toxin induced, indeterminate, and a number of other rare conditions [4]. The distribution of specific etiologies within these categories differs from adults with ALF. Not surprisingly, etiologies differ among children of various age groups. Details of the specific conditions can be found in other sections of this textbook.

An indeterminate diagnosis occurs in nearly 50 % of all PALF patients but can reach as high as 60 % in children between 1 and 10 years of age. Historically, indeterminate PALF (I-PALF) cases were reported as either non-A, non-B hepatitis, neonatal hepatitis, or non-A–E hepatitis. Efforts to identify novel or unsuspected hepatotropic viruses have not yet been rigorously undertaken in children, but such searches in adults were unrevealing [44]. Studies from the PALF study group found that 12 % of children with I-PALF were found to harbor markers of acetaminophen (APAP)-induced liver injury despite lacking evidence of a clear toxic exposure to APAP [45]. This raises the possibility that unsuspected APAP toxicity may be present in some I-PALF patients; alternatively, this finding may just represent APAP exposure without any relation to the pathogenesis of I-PALF.

I-PALF likely consists of multiple patient subgroups. One subgroup reflects an incomplete diagnostic evaluation. Reasons for an incomplete evaluation include: the diagnosis was not considered, the test was ordered but the blood was not drawn, or the planned diagnostic tests were interrupted by death, liver transplantation, or clinical improvement in which further diagnostic studies are abandoned. I-PALF patients were incompletely evaluated for AIH with only 79 % of PALF participants undergoing any testing for AIH, and only 55 % had all three autoantibodies determined [5]. Another subgroup may include patients with a yet undefined pathophysiological injury that is not currently included in diagnostic strategies. We found 5 of 62 patients with a final diagnosis of AIH had no positive AIH markers reported and were deemed to have “marker-negative” AIH. Such cases may reflect an ill-defined immune dysregulation that is pathophysiologically distinct from autoimmune disease but clinically responsive to similar treatment strategies.

Infants Less Than 3 Months

A diagnosis can be established in the majority of patients with diagnoses distributed between metabolic, viral, immune, and other rare conditions [46]. Among the metabolic conditions, galactosemia is the most common followed by mitochondrial disease [47], tyrosinemia [48], and urea cycle defects [49]. Herpes simplex is by far the most common viral etiology, followed by enterovirus and cytomegalovirus. Gestational alloimmune

liver disease [50], formerly neonatal iron storage disease, accounts for almost 14 % of PALF cases. Examples of other rare causes include shock, hemophagocytic syndrome, sepsis, acetaminophen toxicity due to a therapeutic misadventure, and leukemia. An indeterminate diagnosis accounts for just under 40 % of cases.

3 to 12 Months

Metabolic conditions, particularly mitochondrial disease, are relatively common in this age group. Hemophagocytic syndrome and immune marker-positive PALF occur with some regularity. Interestingly, viral diagnoses appear to be uncommon. Indeterminate cases can account for over 40 % of cases.

1 to 5 Years

The majority of children appear to have an indeterminate diagnosis. Those with an established diagnosis include autoimmune marker-positive ALF (particularly LKM positive), hemophagocytic syndrome/macrophage activation syndrome [35], acetaminophen therapeutic misadventure [51], fatty acid oxidation defects [52], viral infections due to Epstein-Barr virus (EBV), adenovirus, hepatitis A, herpes simplex, or influenza/parainfluenza and drug-related liver injury (particularly antiepileptic medications).

5 to 10 Years

The distribution of diagnoses is similar to the 1–5-year age group, with the exception of Wilson disease [53] emerging as the most common metabolic condition.

10 to 18 Years

Acute acetaminophen toxicity, due to a single ingestion associated with an attempted suicide, is the most common diagnosed condition [4]. Wilson disease and fatty acid oxidation defects are metabolic conditions to be considered [52]. Infectious etiologies include EBV; adenovirus; hepatitis A, B, and E; as well as herpes simplex. Autoimmune marker-positive hepatitis occurs in this age group. However, autoimmune markers are found in conditions other than autoimmune hepatitis thus necessitating subjective clinical judgment to influence the final diagnosis and treatment strategy [5].

Other Diagnoses

In the course of the diagnostic evaluation, it is important to recognize rare conditions that result in PALF, particularly when a diagnosis is not forthcoming. These conditions include celiac disease [54], herbal remedies [55], drug toxicity [56], or ischemic liver injury due to primary cardiomyopathy [57], Budd-Chiari syndrome [58], or torsion of an accessory liver lobe [59].

Diagnostic Evaluation

The diagnostic evaluation should proceed quickly, and the time interval between the presentation of PALF and an outcome of death or liver transplantation can be measured in hours or days. Death, liver transplantation, or rapid clinical improvement will interrupt the evaluation [5]. In addition, daily blood volume restrictions or an incomplete or poorly prioritized differential diagnosis can delay important diagnostic tests. An age-based diagnostic approach coupled with specific tests that will likely establish the diagnosis or remove it from consideration will improve the diagnostic yield (Table 23.2). If a specific diagnosis can be secured, an effective treatment could alter the natural history of the disease.

A detailed history and physical examination are essential [1]. The history should include exposure to contacts with infectious hepatitis or blood products, and use of illicit drugs and travel within and outside the country. An inventory of prescription and over-the-counter medications in the child's residence or other homes frequently

Table 23.2 Age-based Diagnostic Considerations for PALF

Age	Diagnosis
Birth – 3 months	Herpes
	Enterovirus
	Gestational alloimmune liver disease
	Mitochondrial disease
	Galactosemia
	Urea cycle defect
	Fatty acid oxidation
	Tyrosinemia
	Cardiovascular failure
	Bacterial sepsis
Birth – 5 years	Tyrosinemia
3 months–18 years	Enterovirus
	Hepatitis A
	Hepatitis B
	Hepatitis E
	Epstein-Barr virus
	Adenovirus
	Acetaminophen (misadventure)
	Acetaminophen (chronic exposure)
	Influenza/parainfluenza
	Mitochondrial disease
	Fatty acid oxidation
	Autoimmune marker (+)
	Cardiovascular failure
	Macrophage activation syndrome
	Hemophagocytic lymphohistiocytosis
	Drug/toxin exposure
5–18 years	Wilson disease
	Acetaminophen (suicide)

visited should be compiled. A family history of Wilson disease, infectious hepatitis, unexpected or unexplained infant deaths, or autoimmune conditions might lead to a specific diagnosis. Evidence of developmental delay and/or a history of seizures should prompt an early assessment for metabolic disease. Pruritus, ascites, portal hypertension, digital clubbing, or growth failure might suggest a chronic liver condition with an acute presentation.

As over 30 % of children with PALF are less than 3 years of age, limitations on the volume of blood that can be drawn are encountered frequently. Blood for diagnostic tests will compete with other studies required to assess the health of the patient and the severity of liver injury. Required blood work in preparation for a liver transplant also competes for this limited resource. Therefore, diagnostic tests should be prioritized based upon conditions likely to occur in a particular age group, the potential for directed therapy (e.g., herpes, Wilson disease, autoimmune marker-positive hepatitis, acetaminophen toxicity, hemophagocytic lymphohistiocytosis), and conditions considered to be contraindications to LTx (e.g., systemic mitochondrial disease). Proactive coordination of laboratory and diagnostic tests among those responsible for ordering and obtaining those tests is helpful to ensure high-priority tests are performed expeditiously.

Management

Management Principles

Collaboration between gastroenterology/hepatology, intensive care, neurology, neurosurgery, nephrology, metabolic disease specialists, and transplant surgeons will afford the child the best opportunity to survive (Fig. 23.1). Patient management is best conducted along multiple parallel paths: (1) monitor and support the patient and organ systems; (2) anticipate, identify, and treat complications; and (3) develop an age-appropriate diagnostic prioritization strategy [1].

Monitor and Support

Initial admission to a pediatric intensive care unit provides a highly skilled nursing and support environment. A cardiorespiratory and oxygen saturation monitor should not substitute for careful and frequent bedside assessment by an experienced nurse or clinician. Input and output should be strictly monitored. Frequent examinations are necessary to assess changes in mental status, increased respiratory effort, changing heart rate or blood pressure which might be signs of infection, increasing cerebral edema, or electrolyte imbalance.

Laboratory monitoring should include a complete blood count with platelets, electrolytes, renal function tests, glucose, calcium, phosphorous, total

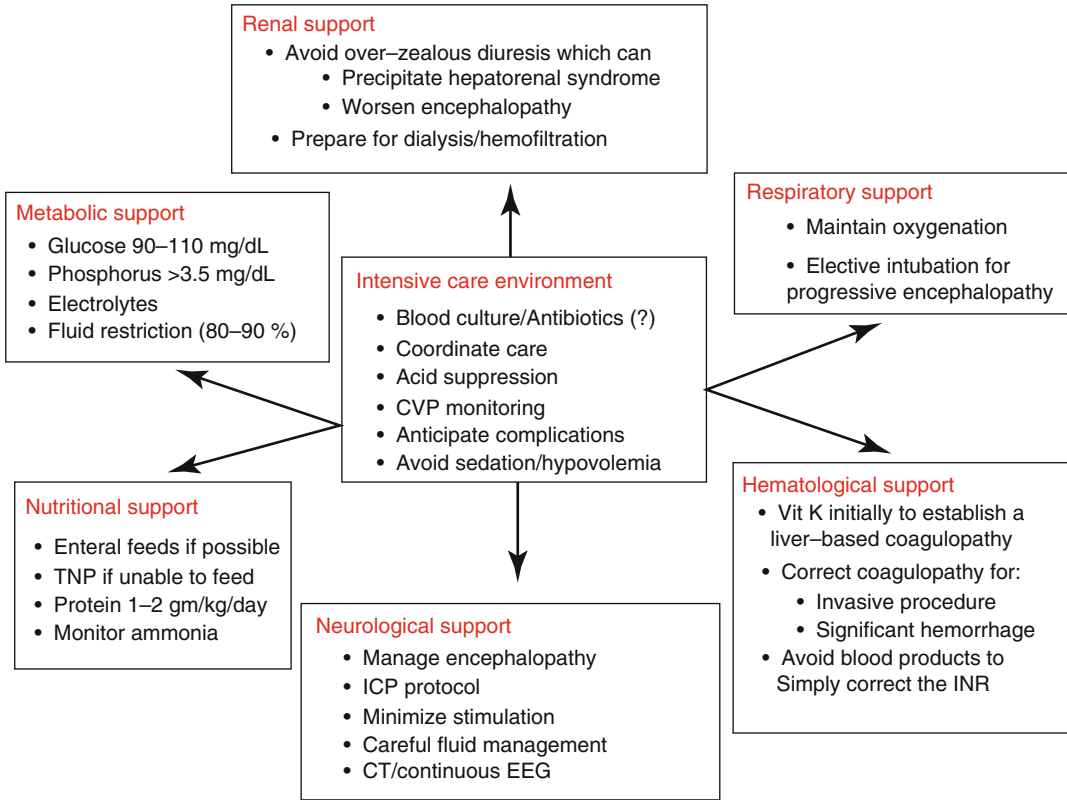

Fig. 23.1 A multisystem approach is needed to optimize care for children with acute liver failure. General measures to minimize unnecessary stimulation, avoid overhydration, maintain serum glucose and phosphorous levels, maintain oxygenation, careful use of blood products, medical management of encephalopathy, and nutritional support should be carefully coordinated

protein, albumin, ammonia, coagulation profile, and total and direct bilirubin at least once daily. While arterial ammonia measurement is ideal, it is not practical in children who do not require an arterial catheter. Therefore, venous ammonia obtained from a free-flowing catheter and promptly placed on ice and transported to the laboratory is a suitable substitute. Placement of arterial and central catheters should be reserved for patients who show signs of clinical deterioration to late stage II or stage III HE. An exception would be if hypoglycemia cannot be managed without a central catheter in the absence of progressive HE. Blood cultures should be obtained if fever develops but also for sudden changes in clinical status regardless of fever or elevated white blood cell count. Bacteremia can occur in the absence of typical systemic manifestations of sepsis.

Radiographic studies are tailored for the clinical circumstance. A chest radiograph and a Doppler sonogram of the abdomen should be performed on most patients. The chest x-ray would serve to assess heart size and the pulmonary vascular and parenchymal pattern. The sonogram would assess patency and direction of flow in the portal and hepatic vein vessels and persistence of the ductus venosus in infants. A non-enhanced computed tomography (CT) scan of the head should be considered for sudden, unexplained neurological deterioration, seizures, or localizing neurological findings.

In the absence of the need for volume resuscitation, total daily intravenous fluids should initially be restricted to between 85 and 95 % of maintenance fluids to avoid overhydration yet still provide sufficient glucose and phosphorus to achieve normal

serum values. Adjustment in fluid rates is based upon the clinical conditions, but relative fluid restriction should be an underlying principal.

Placement of a central venous catheter should be considered for multiorgan failure, inability to maintain serum glucose with peripheral fluids, and advanced encephalopathy or in conjunction with endotracheal intubation. The catheter can be used to provide high concentrations of glucose if needed as well as monitor central venous pressure to aid in fluid management.

Renal

Renal replacement therapy with continuous veno-venous hemofiltration can be useful in the setting of renal insufficiency or failure when removal of excess fluid is desired [60]. Hemodialysis may be necessary if other renal support measures have failed to adequately regulate volume overload, electrolyte abnormalities, or hyperosmolality. Liver transplantation may be necessary to reverse hepatorenal syndrome.

Nutrition

Nutritional support is needed to minimize muscle catabolism. Oral feeding is best if the patient can eat safely. Nasogastric tube feeding is possible, but the tube may not be tolerated if the patient is uncooperative or confused. If it is not safe for the child to receive oral or enteral feeding, intravenous alimentation should be initiated to provide at least 1 g/kg/day of protein. Adjustment in the protein allotment may be needed based on the serum ammonia. Micronutrients such as copper and manganese should be reduced or eliminated in the patients with liver disease, while chromium, molybdenum, and selenium should be reduced or eliminated if renal disease is also present.

Plasmapheresis/Plasma Exchange

Plasmapheresis is thought to facilitate removal of suspected toxins, but evidence of its usefulness in children with ALF is sparse [61, 62]. It has been used to control hemorrhage or improve the coagulation profile prior to surgery. It has also been used in the setting of fulminant Wilson disease [63], drug-induced liver failure [64], and immune-mediated PALF [34, 65]. While coagulation profiles may improve, the procedure has not been shown to improve neurological outcome or ability of the liver to recover spontaneously. It can be performed every 12–24 h. Potential complications include hypotension, especially in small children; reduction in cerebral perfusion pressure; and removal of some medications from the plasma. Another potential disadvantage is the nonselective removal of potentially helpful substances such as hepatocyte growth factor. The use of selective filters to facilitate retention of this potentially beneficial substance would make this therapy more attractive [66].

Membrane Adsorbent Recirculating System (MARS)

In this detoxification system, a membrane coated with albumin-binding sites separates the patients' blood from an albumin dialysate. Albumin-bound substances, such as bilirubin, aromatic amino acids, and endogenous benzodiazepine-like substances, can be transferred to the membrane-binding sites and then to the albumin within the dialysate for removal. Unbound, free low molecular weight molecules, such as ammonia, can pass freely down a concentration gradient into the dialysate and be removed. The MARS system has been used to treat children with mushroom poisoning and as a bridge for re-transplantation [67]. However, in the absence randomized trials, its relevance in the overall treatment of PALF is uncertain [68].

Complications

Encephalopathy

The patient should be assessed frequently as neurological deterioration can be devastatingly rapid. If the patient is old enough and cooperative, having him or her write his signature can be informative. Changes in handwriting style may be an early sign of

neurological impairment. Distinguishing hepatic-based encephalopathy from other causes of an altered mental status such as sepsis, hypotension, electrolyte disturbances, anxiety, or "ICU psychosis" is difficult for all age groups [69]. Hyperammonemia plays a central role in the development of HE in most cases. However, a specific level of ammonia does not reliably predict the degree of HE.

Treatment of HE begins with minimizing excess stimulation. The patient's room should be free of unnecessary stimulation as voices and lights can contribute to patient agitation and confusion. Precipitating causes for HE such as sepsis, excess protein intake, electrolyte imbalance, and sedative medications should be sought and treated. Medical therapy with lactulose is used empirically but lacks evidence of efficacy [70]. A goal of 1–2 loose bowel movements per 8 h is a reasonable goal to achieve with lactulose as excessive diarrhea can result in dehydration and electrolyte disturbances. Bowel "decontamination" with rifaximin or neomycin can be used as a second-tier treatment, but ototoxicity and nephrotoxicity are potential risks when neomycin is used and neither have been rigorously studied in ALF [71]. L-Ornithine L-aspartate, a compound known to reduce blood ammonia in cirrhotic adults, failed to improve HE or reduce blood ammonia levels in adults with ALF [72].

Seizures

In most cases, seizure treatment begins with phenytoin, but practices are variable and there is no definitive standard of care. Seizures which are refractory to phenytoin may respond to midazolam infusion, phenobarbital, levetiracetam, or topiramate. The selection of drug will depend on the patients' mental status, physiologic stability, availability of continuous EEG monitoring to titrate drug infusions, and institutional experience.

Cerebral Edema

Clinically important cerebral edema does not occur in all patients with HE but when present can have devastating consequences [16]. Direct intracranial pressure monitoring is the most sensitive and specific test when compared to less invasive neuroradiographic procedure, such as cranial CT [73]. Monitoring of intracranial pressure remains controversial due to associated complications of the procedure and no evidence of improved survival for those who were monitored.

Management of cerebral edema begins with general management principles to maintain oxygen saturation above 95 %, fluid restriction, diastolic pressure >40 mmHg, adequate sedation, head elevation of 20–30° and neutral head position, and consideration of empiric broad-spectrum antibiotics to minimize the development of bacterial infection [15, 73]. Therapies targeted specifically to improve cerebral edema have not met scientific rigor, but they include hypertonic saline to maintain serum sodium between 145 and 155 meq/L and mannitol keeping serum osmolarity <320 mOsm/L to create a more favorable osmotic gradient to extract water from the brain [74]. Hypothermia has been used in some adults with acute liver failure with some success but has not been studied rigorously [75].

Coagulopathy

Parenteral vitamin K should be administered once at the time of presentation to ensure the child has a liver-based coagulopathy and not simply vitamin K deficiency. Efforts to "correct" the PT/INR with fresh frozen plasma or other procoagulation products such as recombinant factor VII should be restricted to patients with active bleeding or in anticipation of an invasive surgical procedure. Excessive use of blood products can contribute to fluid overload. Administration of platelets to correct thrombocytopenia should be restricted for use when bleeding is present or a procedure is anticipated.

For active or hemodynamically significant bleeding, packed red blood cells (to maintain the hematocrit between 25 and 30), platelets (to maintain the platelet count over 50–100,000), and fresh frozen plasma 10 cc/kg would serve as the initial resuscitative measures. Plasma exchange

would be considered for refractory coagulopathy in the presence of active bleeding. Recombinant factor VIIa (rFactor VII) has been used in anticipation of invasive procedures (e.g., percutaneous liver biopsy, placement of intracranial pressure monitoring catheter) resulting in improvement of the INR within 1 h and lasting up to 8 h [76]. However, given the "balanced" decrease in coagulation factors, administration of rFactor VIIa may increase the risk of thrombosis [77].

Aplastic Anemia

Bone marrow failure, characterized by a spectrum of features ranging from mild pancytopenia to aplastic anemia, occurs in a minority of children with either indeterminate or viral ALF [78, 79]. It will typically become manifest 3–6 weeks after recovery from PALF or following emergent liver transplantation with a diminishing white blood cell and platelet count. Treatment includes immunomodulatory medications such as corticosteroids, cyclosporine A, antilymphocyte or antithymocyte globulin, as well as hematopoietic stem cell transplant.

Fluid

Deficits in intravascular volume should be corrected and maintained to achieve satisfactory perfusion. Once the patient is euvolemic, total fluids should be initially restricted to around 90 % of maintenance fluids and then adjusted based upon ongoing needs and losses. Overhydration may precipitate cerebral edema, ascites, pulmonary edema, and anasarca. Fluid administration can be guided by central venous pressure, left ventricular filling pressure, and cardiac output.

Glucose

Treat hypoglycemia aggressively to maintain the serum glucose between 100 and 150 mg/dL. To achieve this, in the setting of volume restriction, it may be necessary to provide a high dextrose fluid at a low infusion rate. A central catheter is required to administer fluids with a dextrose concentration greater that 12.5 %.

Sodium

Maintain sodium requirements of 2–3 meq/kg/day to minimize edema and ascites. Treat hyponatremia if it is severe (<125 meq/L), the patient is symptomatic, or when further fluid restriction is impractical. Hypernatremia has been used to treat cerebral edema (see above).

Phosphorous

Hypophosphatemia is common and is likely due to hepatic consumption as the liver tries to regenerate. Serum phosphorous should be maintained above 3.0 mg/dL (1 mmol/L) with supplemental phosphorous infusions. Hyperphosphatemia was found to be a poor prognostic sign in adult and is likely related to renal insufficiency [80].

Calcium

Citrate in blood products and plasmapheresis may cause hypocalcemia. Only ionized calcium levels should be used to guide therapy. In the absence of hyperphosphatemia and renal failure, hypocalcemia is treated with a slight increase in total 24 h calcium intake. Bolus infusion of calcium can be considered if hypocalcemia is felt to be due to citrate binding after plasmapheresis.

Ascites

Ascites develops in some but not all patients. Precipitating factors include hypoalbuminemia, excessive fluid administration, and infection. The primary treatment is fluid restriction. Diuretics should be reserved for patients with respiratory compromise or generalized fluid overload. Overly aggressive diuresis may precipitate HRS.

Bleeding

Gastrointestinal bleeding is rare despite the degree of coagulopathy. Prophylactic use of acid-reducing agents is often initiated, but their usefulness is difficult to assess. Causes for bleeding include gastric erosions or ulcers due to nonsteroidal anti-inflammatory medications or idiopathic gastroduodenal ulceration. Infection can precipitate bleeding in this vulnerable population, so blood cultures and initiation of antibiotics should also be considered if bleeding occurs. Administration of platelets, blood, and plasma is necessary if bleeding is hemodynamically significant.

Infection

Patients with ALF have an enhanced susceptibility to bacterial infection and sepsis from immune system dysfunction [1]. Bacterial infections were identified early in the course of an adult with ALF prompting an unproven recommendation to initiate a course of prophylactic antibiotics at presentation [81, 82]. In pediatric patients who received prophylactic antibiotics, infectious complications are more common beyond 2 weeks after presentation [83]. Evidence of infection may be subtle, such as tachycardia, intestinal bleeding, reduced renal output, or changes in mental status. Fever may not be present. Blood cultures should be obtained with any evidence of clinical deterioration and antibiotics initiated with a clinical concern for sepsis due to gram-positive or gram-negative organisms. Infants less than 3 months of age presenting with ALF should be assessed for herpes infection and started on appropriate antiviral medication immediately and continued until test results show no evidence of infection.

Disease Severity Assessment

Currently, there are no reliable tools to predict survival or death in children with PALF. Biochemical tests (lactate, total bilirubin, phosphorous, INR, prothrombin time, ammonia, Gc-globulin), clinical features (encephalopathy, cerebral edema), diagnosis (acetaminophen), or combinations of the three have been tried without consistent success.

Existing liver failure scoring systems including the King's College Hospital Criteria (KCHC), the Clichy score, Model for End-Stage Liver Disease (MELD) score, and Pediatric End-Stage Liver Disease (PELD) score fall well short of the ideal prognostic tool [1]. Recently, the Liver Injury Unit (LIU) score was developed from a single site experience and performed well in predicting a poor outcome defined as death or liver transplantation [84]. However, utilizing the PALF registry to test the validity of the LIU score in a large multicenter cohort where death and liver transplantation were separate outcomes, the LIU score was better at predicting transplant than death [85]. Similarly, the KCHC were not found to reliably predict death in a selected cohort of participants enrolled in the PALFG [86]. The ideal scoring system should reflect the daily clinical changes typical of PALF patients and incorporate them into a model to assess the likelihood of death or survival; such a dynamic scoring system is not yet available.

Liver Transplant

Liver transplantation has improved overall survival for children with ALF. A deceased-donor whole, split, or cutdown liver transplant was used in nearly 86 % of all transplants for ALF in children in a recent report from the Studies of Pediatric Liver Transplant (SPLIT) consortium [87]. Living-donor liver transplant (LDLT) for PALF and concurrent multiorgan failure is associated with improved 30-day and 6-month survival compared to recipients of a deceased-donor liver allograft [88]. Improved outcome for patients receiving a LDLT is likely related to a reduced cold ischemia time and wait time, resulting in a more expeditious time to transplant for those who require LTx. Auxiliary liver transplantation has been used as a "bridge" to provide need time for the native liver to regenerate, but challenges remain as to the timing for withdrawal of immunosuppression and involution of the transplanted graft [89].

Liver transplantation is contraindicated in patients with a systemic mitochondrial disorder as transplantation will not alter their clinical course. Likewise, LTx is not indicated for patients with MAS or hemophagocytic lymphohistiocytosis (HLH) as immunosuppression and, in the case of HLH, bone marrow transplantation would be the recommended therapy.

The role of hepatocyte transplantation in PALF is yet to be determined and remains an opportunity for future investigation [90]. Hepatocyte transplantation may serve as a bridge to transplant or, perhaps, a "cure" for some children with metabolic diseases. It has been used in a small number of children with ALF. However, technical challenges as well as lack of a readily available source for hepatocytes have limited the opportunity for this procedure at most centers.

Outcomes

In the pre-transplant era and an adult definition of ALF, spontaneous survival occurred in 28 % of patients overall and only 4 % of those with stage IV coma [91]. More recently, with improvements in the management of critically ill children coupled with a more lenient definition of PALF, outcomes have improved [1, 4, 46].

Findings from the PALF study revealed that 21-day outcome varied by diagnosis, age, and degree of encephalopathy [4, 46]. Spontaneous survival or survival with their native liver was highest among those with liver failure due to acetaminophen (94 %). Spontaneous survival occurred less frequently for those with liver failure due do metabolic disease (44 %), non-acetaminophen drug-induced (41 %), and those with an indeterminate diagnosis (45 %). As might be expected, those with higher coma scores had lower spontaneous survival. Unexpectedly, 21 % of patients with a peak coma score of 0 either died or received a liver transplant. Thus, death can occur in the absence of clinical HE. For children with an established diagnosis, the percent of those receiving a liver transplant ranged between 20 and 33 %. However, liver transplantation occurred in 46 % of those patients with an indeterminate diagnosis. Therefore, children who do not have a specific diagnosis are more likely to receive a liver transplant. Only 2 % of children with acetaminophen-induced PALF received a liver transplant in this cohort. The major causes of death for all who do not receive a liver transplant include multiorgan system failure, cerebral edema and herniation, and sepsis.

Both early and late graft loss and death are higher among children who undergo liver transplantation for acute liver failure than for those with chronic liver disease [92]. Reasons for these findings are uncertain, but one possibility includes immune dysregulation that may be associated with PALF which could lead to increased susceptibility to infection or graft rejection.

References

1. Squires Jr RH. Acute liver failure in children. Semin Liver Dis. 2008;28(2):153–66.
2. Rivera-Penera T, Moreno J, Skaff C, McDiarmid S, Vargas J, Ament ME. Delayed encephalopathy in fulminant hepatic failure in the pediatric population and the role of liver transplantation. J Pediatr Gastroenterol Nutr. 1997;24(2):128–34.
3. Lee WS, McKiernan P, Kelly DA. Etiology, outcome and prognostic indicators of childhood fulminant hepatic failure in the United Kingdom. J Pediatr Gastroenterol Nutr. 2005;40(5):575–81.
4. Squires Jr RH, Shneider BL, Bucuvalas J, Alonso E, Sokol RJ, Narkewicz MR, et al. Acute liver failure in children: the first 348 patients in the pediatric acute liver failure study group. J Pediatr. 2006;148(5):652–8.
5. Narkewicz MR, Dell Olio D, Karpen SJ, Murray KF, Schwarz K, Yazigi N, et al. Pattern of diagnostic evaluation for the causes of pediatric acute liver failure: an opportunity for quality improvement. J Pediatr. 2009;155(6):801–6.e1.
6. Shaver WA, Bhatt H, Combes B. Low serum alkaline phosphatase activity in Wilson's disease. Hepatology. 1986;6(5):859–63.
7. Tissieres P, Chevret L, Debray D, Devictor D. Fulminant Wilson's disease in children: appraisal of a critical diagnosis. Pediatr Crit Care Med. 2003;4(3):338–43.
8. Butterworth RF. Neuroinflammation in acute liver failure: mechanisms and novel therapeutic targets. Neurochem Int. 2011;59(6):830–6.
9. Jalan R, Olde Damink SW, Hayes PC, Deutz NE, Lee A. Pathogenesis of intracranial hypertension in acute liver failure: inflammation, ammonia and cerebral blood flow. J Hepatol. 2004;41(4):613–20.

10. Whitington PF, Alonso AE. Fulminant hepatitis and acute liver failure. In: DA Kelly, editor. Paediatric liver disease. Oxford: Blackwell; 2003. p. 107–26.
11. Aggarwal S, Brooks DM, Kang Y, Linden PK, Patzer 2nd JF. Noninvasive monitoring of cerebral perfusion pressure in patients with acute liver failure using transcranial Doppler ultrasonography. Liver Transpl. 2008;14(7):1048–57.
12. Nielsen HB, Tofteng F, Wang LP, Larsen FS. Cerebral oxygenation determined by near-infrared spectrophotometry in patients with fulminant hepatic failure. J Hepatol. 2003;38(2):188–92.
13. Hwang S, Lee SG, Park JI, Song GW, Ryu JH, Jung DH, et al. Continuous peritransplant assessment of consciousness using bispectral index monitoring for patients with fulminant hepatic failure undergoing urgent liver transplantation. Clin Transplant. 2010; 24(1):91–7.
14. Strauss GI, Christiansen M, Moller K, Clemmesen JO, Larsen FS, Knudsen GM. S-100b and neuron-specific enolase in patients with fulminant hepatic failure. Liver Transpl. 2001;7(11):964–70.
15. Shawcross DL, Wendon JA. The neurological manifestations of acute liver failure. Neurochem Int. 2012; 60(7):662–71.
16. Wendon J, Lee W. Encephalopathy and cerebral edema in the setting of acute liver failure: pathogenesis and management. Neurocrit Care. 2008;9(1): 97–102.
17. Stravitz RT, Kramer AH, Davern T, Shaikh AO, Caldwell SH, Mehta RL, et al. Intensive care of patients with acute liver failure: recommendations of the U.S. Acute Liver Failure Study Group. Crit Care Med. 2007;35(11):2498–508.
18. Akcan-Arikan A, Zappitelli M, Loftis LL, Washburn KK, Jefferson LS, Goldstein SL. Modified RIFLE criteria in critically ill children with acute kidney injury. Kidney Int. 2007;71(10):1028–35.
19. Moore K. Renal failure in acute liver failure. Eur J Gastroenterol Hepatol. 1999;11(9):967–75.
20. Vanderperren B, Rizzo M, Angenot L, Haufroid V, Jadoul M, Hantson P. Acute liver failure with renal impairment related to the abuse of senna anthraquinone glycosides. Ann Pharmacother. 2005;39(7–8): 1353–7.
21. Akoglu H, Yilmaz R, Kirkpantur A, Arici M, Altun B, Turgan C. Combined organ failure with combination antihyperlipidemic treatment: a case of hepatic injury and acute renal failure. Ann Pharmacother. 2007;41(1): 143–7.
22. Chao TC, Wu ML, Tsai WJ, Ger J, Deng JF. Acute hepatic injury and renal failure after ingestion of snake gallbladder. Clin Toxicol (Phila). 2006;44(4):387–90.
23. Agarwal B, Wright G, Gatt A, Riddell A, Vemala V, Mallett S, et al. Evaluation of coagulation abnormalities in acute liver failure. J Hepatol. 2012;57(4):780–6.
24. Stravitz RT, Lisman T, Luketic VA, Sterling RK, Puri P, Fuchs M, et al. Minimal effects of acute liver injury/acute liver failure on hemostasis as assessed by thromboelastography. J Hepatol. 2012;56(1):129–36.
25. Srinivasan A, Venkataraman S, Hansdak SG, Abraham OC, Mathai D. Hyperglycaemia as an indicator of concurrent acute pancreatitis in fulminant hepatic failure associated with hepatitis B infection. Singapore Med J. 2005;46(5):236–7.
26. Finsterer J. Mitochondriopathies. Eur J Neurol. 2004;11(3):163–86.
27. Pinkston R, Walker LA. Multiorgan system failure caused by valproic acid toxicity. Am J Emerg Med. 1997;15(5):504–6.
28. Boudreaux JP, Hayes DH, Mizrahi S, Hussey J, Regenstein F, Balart L. Fulminant hepatic failure, hepatorenal syndrome, and necrotizing pancreatitis after minocycline hepatotoxicity. Transplant Proc. 1993;25(2):1873.
29. Komatsu K, Shimosegawa T, Uchi M, Maruhama Y, Toyota T. Erythropoietic protoporphyria with severe liver dysfunction and acute pancreatitis. J Gastroenterol. 2000;35(5):391–5.
30. Parekh NK, Hynan LS, De Lemos J, Lee WM. Elevated troponin I levels in acute liver failure: is myocardial injury an integral part of acute liver failure? Hepatology. 2007;45(6):1489–95.
31. Racanelli V, Rehermann B. The liver as an immunological organ. Hepatology. 2006;43(2 Suppl 1):S54–62.
32. Crispe IN. The liver as a lymphoid organ. Annu Rev Immunol. 2009;27:147–63.
33. Gao B, Jeong WI, Tian Z. Liver: an organ with predominant innate immunity. Hepatology. 2008;47(2): 729–36.
34. Ravelli A. Macrophage activation syndrome. Curr Opin Rheumatol. 2002;14(5):548–52.
35. Castillo L, Carcillo J. Secondary hemophagocytic lymphohistiocytosis and severe sepsis/systemic inflammatory response syndrome/multiorgan dysfunction syndrome/macrophage activation syndrome share common intermediate phenotypes on a spectrum of inflammation. Pediatr Crit Care Med. 2009;10(3): 387–92.
36. Robertson CM, Coopersmith CM. The systemic inflammatory response syndrome. Microbes Infect. 2006;8(5):1382–9.
37. Wood JH, Partrick DA, Johnston Jr RB. The inflammatory response to injury in children. Curr Opin Pediatr. 2010;22(3):315–20.
38. Goldstein B, Giroir B, Randolph A. International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics. Pediatr Crit Care Med. 2005;6(1):2–8.
39. Rolando N, Wade J, Davalos M, Wendon J, Philpott-Howard J, Williams R. The systemic inflammatory response syndrome in acute liver failure. Hepatology. 2000;32(4 Pt 1):734–9.
40. Antoniades CG, Berry PA, Wendon JA, Vergani D. The importance of immune dysfunction in determining outcome in acute liver failure. J Hepatol. 2008;49(5):845–61.
41. Eksteen B, Afford SC, Wigmore SJ, Holt AP, Adams DH. Immune-mediated liver injury. Semin Liver Dis. 2007;27(4):351–66.

42. Adams DH, Ju C, Ramaiah SK, Uetrecht J, Jaeschke H. Mechanisms of immune-mediated liver injury. Toxicol Sci. 2010;115(2):307–21. PMCID: 2871750.
43. Bihl F, Emmenegger U, Reichen J, Neftel KA, Zimmermann A, Cerny A. Macrophage activating syndrome is associated with lobular hepatitis and severe bile duct injury with cholestasis. J Hepatol. 2006;44(6):1208–12.
44. Umemura T, Tanaka E, Ostapowicz G, Brown KE, Heringlake S, Tassopoulos NC, et al. Investigation of SEN virus infection in patients with cryptogenic acute liver failure, hepatitis-associated aplastic anemia, or acute and chronic non-A-E hepatitis. J Infect Dis. 2003;188(10):1545–52.
45. James LP, Alonso EM, Hynan LS, Hinson JA, Davern TJ, Lee WM, et al. Detection of acetaminophen protein adducts in children with acute liver failure of indeterminate cause. Pediatrics. 2006;118(3): e676–81.
46. Sundaram SS, Alonso EM, Narkewicz MR, Zhang S, Squires RH. Characterization and outcomes of young infants with acute liver failure. J Pediatr. 2011;159(5):813–8.e1. PMCID: 3177978.
47. Lee WS, Sokol RJ. Mitochondrial hepatopathies: advances in genetics and pathogenesis. Hepatology. 2007;45(6):1555–65.
48. Nobili V, Vento S, Dionisi C, Sartorelli MR, Russo C, Marcellini M. Acute liver failure as presenting feature of tyrosinemia type 1 in a child with primary HHV-6 infection. J Gastroenterol Hepatol. 2006;21(1 Pt 2): 339.
49. Teufel U, Weitz J, Flechtenmacher C, Prietsch V, Schmidt J, Hoffmann GF, et al. High urgency liver transplantation in ornithine transcarbamylase deficiency presenting with acute liver failure. Pediatr Transplant. 2011;15(6):E110–5.
50. Bonilla S, Prozialeck JD, Malladi P, Pan X, Yu S, Melin-Aldana H, et al. Neonatal iron overload and tissue siderosis due to gestational alloimmune liver disease. J Hepatol. 2012;56(6):1351–5.
51. Heubi JE, Barbacci MB, Zimmerman HJ. Therapeutic misadventures with acetaminophen: hepatotoxicity after multiple doses in children. J Pediatr. 1998;132(1): 22–7.
52. Shneider BL, Rinaldo P, Emre S, Bucuvalas J, Squires R, Narkewicz M, et al. Abnormal concentrations of esterified carnitine in bile: a feature of pediatric acute liver failure with poor prognosis. Hepatology. 2005;41(4):717–21.
53. Eisenbach C, Sieg O, Stremmel W, Encke J, Merle U. Diagnostic criteria for acute liver failure due to Wilson disease. World J Gastroenterol. 2007;13(11): 1711–4.
54. Kaukinen K, Halme L, Collin P, Farkkila M, Maki M, Vehmanen P, et al. Celiac disease in patients with severe liver disease: gluten-free diet may reverse hepatic failure. Gastroenterology. 2002;122(4): 881–8.
55. Stedman C. Herbal hepatotoxicity. Semin Liver Dis. 2002;22(2):195–206.
56. Chang CY, Schiano TD. Review article: drug hepatotoxicity. Aliment Pharmacol Ther. 2007;25(10):1135–51.
57. Hoffman BJ, Pate MB, Marsh WH, Lee WM. Cardiomyopathy unrecognized as a cause of hepatic failure. J Clin Gastroenterol. 1990;12(3):306–9.
58. Dilawari JB, Bambery P, Chawla Y, Kaur U, Bhusnurmath SR, Malhotra HS, et al. Hepatic outflow obstruction (Budd-Chiari syndrome). Experience with 177 patients and a review of the literature. Medicine (Baltimore). 1994;73(1):21–36.
59. Ladurner R, Brandacher G, Mark W, Iannetti C, Lottersberger C, Steurer W, et al. Complete hepatic ischemia due to torsion of a large accessory liver lobe: first case to require transplantation. Transpl Int. 2005;18(4):467–9.
60. Agarwal R, Farber MO. Is continuous veno-venous hemofiltration for acetaminophen-induced acute liver and renal failure worthwhile? Clin Nephrol. 2002;57(2): 167–70.
61. Abe T, Kobata H, Hanba Y, Kitabata Y, Narukawa N, Hasegawa H, et al. Study of plasma exchange for liver failure: beneficial and harmful effects. Ther Apher Dial. 2004;8(3):180–4.
62. Singer AL, Olthoff KM, Kim H, Rand E, Zamir G, Shaked A. Role of plasmapheresis in the management of acute hepatic failure in children. Ann Surg. 2001;234(3):418–24.
63. Jhang JS, Schilsky ML, Lefkowitch JH, Schwartz J. Therapeutic plasmapheresis as a bridge to liver transplantation in fulminant Wilson disease. J Clin Apher. 2007;22(1):10–4.
64. Aydemir S, Ustundag Y, Bayraktaroglu T, Tekin IO, Peksoy I, Unal AU. Fulminant hepatic failure associated with propylthiouracil: a case report with treatment emphasis on the use of plasmapheresis. J Clin Apher. 2005;20(4):235–8.
65. Demirkol D, Yildizdas D, Bayrakci B, Karapinar B, Kendirli T, Koroglu TF, et al. Hyperferritinemia in the critically ill child with secondary hemophagocytic lymphohistiocytosis/sepsis/multiple organ dysfunction syndrome/macrophage activation syndrome: what is the treatment? Crit Care. 2012;16(2):R52.
66. Ho DW, Fan ST, To J, Woo YH, Zhang Z, Lau C, et al. Selective plasma filtration for treatment of fulminant hepatic failure induced by D-galactosamine in a pig model. Gut. 2002;50(6):869–76.
67. Covic A, Goldsmith DJ, Gusbeth-Tatomir P, Volovat C, Dimitriu AG, Cristogel F, et al. Successful use of Molecular Absorbent Regenerating System (MARS) dialysis for the treatment of fulminant hepatic failure in children accidentally poisoned by toxic mushroom ingestion. Liver Int. 2003;23 Suppl 3:21–7.
68. Tissieres P, Sasbon JS, Devictor D. Liver support for fulminant hepatic failure: is it time to use the molecular adsorbents recycling system in children? Pediatr Crit Care Med. 2005;6(5):585–91.
69. Ely EW, Inouye SK, Bernard GR, Gordon S, Francis J, May L, et al. Delirium in mechanically ventilated patients: validity and reliability of the confusion assessment method for the intensive care unit (CAM-ICU). JAMA. 2001;286(21):2703–10.

70. Han MK, Hyzy R. Advances in critical care management of hepatic failure and insufficiency. Crit Care Med. 2006;34(9 Suppl):S225–31.
71. Rivkin A, Gim S. Rifaximin: new therapeutic indication and future directions. Clin Ther. 2011;33(7): 812–27.
72. Acharya SK, Bhatia V, Sreenivas V, Khanal S, Panda SK. Efficacy of L-ornithine L-aspartate in acute liver failure: a double-blind, randomized, placebo-controlled study. Gastroenterology. 2009;136(7): 2159–68.
73. Larsen FS, Wendon J. Prevention and management of brain edema in patients with acute liver failure. Liver Transpl. 2008;14 Suppl 2:S90–6.
74. Murphy N, Auzinger G, Bernel W, Wendon J. The effect of hypertonic sodium chloride on intracranial pressure in patients with acute liver failure. Hepatology. 2004;39(2):464–70.
75. Stravitz RT, Lee WM, Kramer AH, Kramer DJ, Hynan L, Blei AT. Therapeutic hypothermia for acute liver failure: toward a randomized, controlled trial in patients with advanced hepatic encephalopathy. Neurocrit Care. 2008;9(1):90–6.
76. Brown JB, Emerick KM, Brown DL, Whitington PF, Alonso EM. Recombinant factor VIIa improves coagulopathy caused by liver failure. J Pediatr Gastroenterol Nutr. 2003;37(3):268–72.
77. Pavese P, Bonadona A, Beaubien J, Labrecque P, Pernod G, Letoublon C, et al. FVIIa corrects the coagulopathy of fulminant hepatic failure but may be associated with thrombosis: a report of four cases. Can J Anaesth. 2005;52(1):26–9.
78. Hadzic N, Height S, Ball S, Rela M, Heaton ND, Veys P, et al. Evolution in the management of acute liver failure-associated aplastic anaemia in children: a single centre experience. J Hepatol. 2008;48(1):68–73.
79. Rauff B, Idrees M, Shah SA, Butt S, Butt AM, Ali L, et al. Hepatitis associated aplastic anemia: a review. Virol J. 2011;8:87. PMCID: 3052191.
80. Baquerizo A, Anselmo D, Shackleton C, Chen TW, Cao C, Weaver M, et al. Phosphorus as an early predictive factor in patients with acute liver failure. Transplantation. 2003;75(12):2007–14.
81. Rolando N, Harvey F, Brahm J, Philpott-Howard J, Alexander G, Casewell M, et al. Fungal infection: a common, unrecognised complication of acute liver failure. J Hepatol. 1991;12(1):1–9.
82. Rolando N, Harvey F, Brahm J, Philpott-Howard J, Alexander G, Gimson A, et al. Prospective study of bacterial infection in acute liver failure: an analysis of fifty patients. Hepatology. 1990;11(1):49–53.
83. Godbole G, Shanmugam N, Dhawan A, Verma A. Infectious complications in pediatric acute liver failure. J Pediatr Gastroenterol Nutr. 2011;53(3):320–5.
84. Liu E, MacKenzie T, Dobyns EL, Parikh CR, Karrer FM, Narkewicz MR, et al. Characterization of acute liver failure and development of a continuous risk of death staging system in children. J Hepatol. 2006; 44(1):134–41.
85. Lu BR, Zhang S, Narkewicz MR, Belle SH, Squires RH, Sokol RJ. Evaluation of the liver injury unit scoring system to predict survival in a multinational study of pediatric acute liver failure. J Pediatr. 2013;162(5): 1010–6.
86. Sundaram V, Shneider BL, Dhawan A, Ng VL, Im K, Belle S, et al. King's College Hospital criteria for non-acetaminophen induced acute liver failure in an international cohort of children. J Pediatr. 2013;162(2): 319–23.e1. PMCID: 3504621.
87. Baliga P, Alvarez S, Lindblad A, Zeng L. Posttransplant survival in pediatric fulminant hepatic failure: the SPLIT experience. Liver Transpl. 2004;10(11):1364–71.
88. Mack CL, Ferrario M, Abecassis M, Whitington PF, Superina RA, Alonso EM. Living donor liver transplantation for children with liver failure and concurrent multiple organ system failure. Liver Transpl. 2001;7(10):890–5.
89. Girlanda R, Vilca-Melendez H, Srinivasan P, Muiesan P, O'Grady JG, Rela M, et al. Immunosuppression withdrawal after auxiliary liver transplantation for acute liver failure. Transplant Proc. 2005;37(4):1720–1.
90. Soltys KA, Soto-Gutierrez A, Nagaya M, Baskin KM, Deutsch M, Ito R, et al. Barriers to the successful treatment of liver disease by hepatocyte transplantation. J Hepatol. 2010;53(4):769–74. PMCID: 2930077.
91. Psacharopoulos HT, Mowat AP, Davies M, Portmann B, Silk DB, Williams R. Fulminant hepatic failure in childhood: an analysis of 31 cases. Arch Dis Child. 1980;55(4):252–8.
92. Soltys KA, Mazariegos GV, Squires RH, Sindhi RK, Anand R. Late graft loss or death in pediatric liver transplantation: an analysis of the SPLIT database. Am J Transplant. 2007;7(9):2165–71.

Intensive Care Management of Children with Liver Failure

24

Thomas V. Brogan and Francois Aspesberro

Introduction

Advanced liver disease requiring admission to the pediatric intensive care unit (PICU) is uncommon in children. However, the importance of the liver in human homeostasis cannot be overstated. Due to its broad range of activity including maintaining metabolism of glucose and fats, synthesis of proteins including albumin and coagulation factors, and detoxification of exogenous and endogenous substances, the extent of extrahepatic organ involvement with hepatic decompensation and the rapidity of liver failure progression can be sobering. As such, children with severe liver dysfunction present a unique and challenging population.

The presentation of children with liver failure to the PICU may be dramatic or relatively subtle. Such patients are complex often with multiorgan involvement and require a team approach that extends beyond the ICU, gastroenterology, and surgical teams. In the case of acute liver failure, the severity of illness may proceed with astonishing speed. Consequently, such patients require rapid mobilization of the multidiscipline team to provide care and determine eligibility for liver transplantation.

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Acute Versus Chronic Liver Failure

Children may present with acute liver failure (ALF)/fulminant hepatic failure (FHF), acute complications of chronic liver failure, or worsening of ongoing end-stage liver failure (ESLD). Fulminant hepatic failure is defined as hepatic encephalopathy developing within 8 weeks of the onset of acute liver failure. The etiology of ALF/FHF falls into several broad categories including infectious, metabolic, toxic, autoimmune, malignancy, vascular, and undetermined. The term end-stage liver failure (ESLD) refers to severe hepatic disease with little hope of recovery. ESLD can occur due to ALF or from chronic liver failure

Complications of End-Stage Liver Disease

Hepatic Encephalopathy

Hepatic encephalopathy (HE) is a neuropsychiatric syndrome that results from severe liver dysfunction (Table 24.1). HE has been the defining criterion for fulminant hepatic failure but may also complicate chronic liver disease [1, 2]. In chronic liver failure, HE usually arises after an acute precipitating event such as GI bleeding, infection, or other complication. However, two-thirds of infants who present with ALF develop HE during the first week following initial presentation [3]. Elevated ICP is common in ALF, affecting up to 80 % of those dying from the illness. Evaluation of children, especially

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Table 24.1 Drug interaction with calcineurin inhibitors

Increased blood level		Decreased blood level	
Increased absorption	Decreased metabolism	Decreased absorption	Increased metabolism
Grapefruit juice	Ketoconazole	Phenytoin	Corticosteroids
Octreotide	Fluconazole	Carbamazepine	
Metoclopramide	Itraconazole	Barbiturates	
	Quinolones	Imipenem	
	Ganciclovir		
	Acyclovir		
	Trimethoprim-sulfamethoxazole		

young children and infants, with early stages of hepatic encephalopathy can be difficult as many of the findings of early HE such as irritability, inconsolability, and poor concentration are nonspecific. Consequently, children who present with liver failure must be followed carefully to assess the level of neurologic involvement as well as the rate of neurologic decline.

Manifestations of encephalopathy due to liver disease vary and can include minimal neurologic dysfunction to seizures, cerebral edema, or dense coma [3]. The mechanisms of cerebral dysfunction, edema, and encephalopathy are multifactorial. The pathogenic factors that appear to contribute to neurologic dysfunction include (1) accumulation of ammonia, (2) abnormal ligand(s) acting on the γ-amino butyric acid (GABA) benzodiazepine receptors, and (3) deposition of manganese in the basal ganglia [4].

A failing liver cannot adequately metabolize ammonia, much of which is produced by bacteria in the gut. The changes caused by hyperammonemia occur primarily in the astrocytes which are the only cells in the CNS that can metabolize ammonia. Ammonia is detoxified by the conversion of glutamate to glutamine by the enzyme glutamine synthetase in the astrocytes, resulting in increased levels of glutamine [5]. Glutamine acts as an osmotic agent within the brain producing cellular edema and consequent mitochondrial dysfunction. However, ammonia levels do not necessarily correlate with the presence and degree of HE. This may stem, in part, from differences in the brain uptake of ammonia which is usually high in patients with HE, independent of blood ammonia levels [5]. Glutamine concentrations in CSF, which reflect the degree of CNS ammonia metabolism, correlate well with HE in cirrhotic adults [6].

Another apparent pathogenic mechanism in patients with HE relates to an increase in endogenous benzodiazepines found in the brains of patients with HE [7]. Benzodiazepines activate the GABA receptor complex which results in inhibition of neuronal activity. Thus, increases in GABAergic compounds will reduce the sensorium. This may be exacerbated by exogenous administration of benzodiazepines. The GABA receptor complex itself may be altered as well [7]. Benzodiazepine antagonists appear only to have intermittent and short-lived benefits in HE.

In some patients with cirrhosis, abnormalities in the basal ganglia on magnetic resonance imaging suggest accumulation of manganese, and these signals resolve following liver transplantation [2]. Other possible pathologic mechanisms contributing to HE relate to changes in cerebral vasculature whereby cerebral arterioles become dilated in ALF increasing cerebral blood flow above levels found in patients who do not have cerebral edema [8]. Blood pressure autoregulation appears to be altered in ALF. Hyperemia has been associated with brain swelling and death in ALF. Increases in blood flow especially in a relatively non-compliant brain can increase ICP and lead to inadequate perfusion. The underlying cause of these abnormalities remains uncertain. Other potential contributing pathologic mechanisms include increased inflammatory cytokines and oxidative stress in the brain [8].

Treatment of Hepatic Encephalopathy

HE is the leading cause of death in children with acute liver failure. The treatment of HE must proceed deliberately but expeditiously to diminish

worsening of the neurologic dysfunction and increase chances of successful transplantation. Initial goals of therapy include decreasing the production of toxins (ammonia) that lead to neurologic dysfunction, discontinuing sedation medications that may confound the clinical picture, and keeping patients safe. When HE progresses despite these measures, cerebral edema may result with dire consequences such as uncal herniation. Cerebral edema in the context of liver failure represents a medical emergency of the greatest order.

Most therapies for HE are directed at lowering elevated levels of ammonia either by decreasing its production or limiting absorption from the gut. These methods include "bowel cleansing," limiting dietary protein intake, and hemodialysis/hemofiltration. Bowel cleansing may help decrease the load of colonic bacterial that produce ammonia. Most studies have examined the use of agents such as lactulose and neomycin in the setting of chronic liver failure, but they are also employed in ALF [8]. Lactulose is a disaccharide that is broken into its constituent sugars galactose and fructose which are then metabolized in the colon to the organic acids lactic and acetic acids. These acids decrease the colonic lumen pH to approximately 5.5 resulting in the preferential formation of the ammonium ion which is less absorbable than ammonia itself. Lactulose also promotes the growth of lactose forming bacteria in the gut, suppressing ammoniagenic organisms such as *Bacteroides*, because the acidic environment does not favor survival of these urease-producing bacteria. Lactulose was shown in a single-center study to decrease HE in adult patients with variceal bleeding [9]. Antimicrobial agents including ampicillin, metronidazole, vancomycin, or rifaximin (a synthetic analog of rifamycin) have been employed to decrease ammoniagenic colonic bacterial loads [10].

The restriction of dietary protein and the use of urea cycle activation agents have been shown to decrease the production of ammonia [10]. Administration of limited protein (0.5 g/kg/day) with a gradual increase to 1.5 g/kg/day slowly over several weeks if the liver recovers appears prudent. Vegetable proteins are tolerated better than animal proteins as they result in lower amounts of ammonia produced due to relatively low methionine and aromatic acid contents. Ornithine aspartate provides substrate for ureagenesis and glutamine, both of which can help remove ammonia from portal blood [2]. L-ORNITHINE is a substrate for ureagenesis, activating the urea cycle enzymes ornithine transcarbamylase and carbamoyl phosphate synthesis. In the hepatic perivenous scavenger cells which do not possess urea cycle enzymes, aspartate stimulates glutamine synthesis, providing another pathway for ammonia metabolism [10].

When serum ammonia levels become elevated, more aggressive efforts to lower the blood concentration center on removal from the blood by hemodialysis (HD) or continuous renal replacement therapy (CRRT) [11]. Both HD and CRRT have been shown to be effective in lowering serum ammonia levels. The choice of modality will depend upon patient clinical status including presence or absence of hemodynamic instability or renal dysfunction. Children with hemodynamic instability (e.g., hypotension, low cerebral perfusion pressure) tolerate CRRT better than HD for several reasons. First, fluid shifts occur over 24 h rather than 2–3 h. Second, CRRT permits optimization of nutrition as whatever fluid is required to deliver nutrition can be removed essentially simultaneously. However, HD can remove ammonia more rapidly than CRRT, so under circumstances of acute worsening of encephalopathy, HD is the preferred mode.

When patients experience worsening hepatic coma (stages III and IV), therapies aimed at treating intracranial hypertension should be instituted emergently. Markedly reduced sensorium renders clinical evaluation of the patient nearly impossible; thus intracranial monitoring has been described as a useful adjunct to guide therapy to treat intracranial hypertension [12]. Coagulation abnormalities should be corrected prior to monitor placement and ongoing stringent management of coagulation abnormalities, and thrombocytopenia appears prudent during intracranial monitoring. Correction of coagulopathy with ESLD often requires use of activated factor VII in addition to plasma and platelet transfusion. The ICP monitor can be used to manage the use of

accepted therapies for intracranial hypertension: assuring optimal mean arterial blood pressure, normal arterial oxygenation, avoidance of hypercarbia ($PaCO_2$ levels from 35 to 40 Torr), osmolar therapies (hypertonic saline, mannitol), sedation and analgesia, appropriate positioning of the head, and strict avoidance of hyperthermia. These measures may prove inadequate when hepatic failure progresses. Some clinicians have used artificial liver support (ALS) devices (please see below) under these circumstances with transient improvement in symptoms of hepatic encephalopathy, but symptoms appear to return with cessation of this support mode. Definitive therapy remains liver transplantation.

Acute Kidney Injury/Hepatorenal Syndrome

Patients with liver failure are at risk for acute kidney injury (AKI) from a variety of sources including their increased risk for infection, cardiovascular dysfunction, hemorrhage, and receipt of nephrotoxic medications. Hepatorenal syndrome is defined as renal dysfunction or AKI in the presence of liver failure and occurs most commonly in the setting of advanced liver disease and portal hypertension, affecting nearly three-quarters of patients with ALF [4]. HRS occurs despite the absence of a clear cause of AKI such as hypotension, shock, sepsis, or administration of nephrotoxic drugs. HRS is characterized by significant arterial underfilling secondary to arterial vasodilatation in the splanchnic circulation with compensatory constriction of renal vascular bed [13, 14]. In response, the renin-angiotensin-aldosterone axis is stimulated, ultimately decreasing renal perfusion and GFR. Patients with liver failure are also at risk for prerenal AKI due to hypovolemia from bleeding or infection or other cardiovascular compromise. HRS has been divided into two categories. Type 1 HRS is an acute, rapidly progressive form that often develops after a precipitating event such as infection or GI bleed, while type 2 usually begins spontaneously and progresses slowly. HRS is marked by sodium retention and diminished urine output but normal urine sediment.

Treatment for AKI

In all liver failure patients, basic supportive measures aimed at restoring circulating volume and cardiac output should be employed. Efforts should be made to avoid or limit exposure to nephrotoxic medications. Therapies aimed directly at HRS include vasoconstrictors which are used to limit flow through the dilated splanchnic arterial bed and to suppress the endogenous vasoconstrictor system, ultimately improving renal perfusion [13]. The vasoconstrictor agents used in adults have included octreotide, which is ineffective in HRS, and the vasopressin analog terlipressin, which acts at the V1 receptor [13, 14]. Also norepinephrine has been described as effective when used in combination with albumin. Data on these therapies come from adult studies, but the effect of these agents among children remains to be analyzed further.

Transjugular intrahepatic portosystemic shunt (TIPS) lowers portal pressure and reduces vasoconstrictor activity thereby increasing renal perfusion [13]. The absence of appropriate studies in children and the potential complications of hepatic encephalopathy and shunt obstruction may limit the effectiveness of this treatment. Thus, TIPS should be considered sparingly. CRRT has been shown to be an effective approach to infants and children with HRS and fluid overload and represents a useful adjunct in fluid management.

Infection

Patients with ESLD are at risk for infection due to altered immune responses and because of the presence of medical devices (e.g., indwelling catheters) [4, 15]. Impaired PMN function, decreased cell-mediated and humoral immunity, defects in the reticuloendothelial system, and diminished opsonic and complement activity have all been documented in patients with liver failure. Risk factors for infection include the presence of indwelling catheters, H2 blockers, steroid therapy, and broad-spectrum antibiotics.

Bacteremia appears to be the most common infection in one series followed by urinary tract

infections (UTI) and lower respiratory tract infections (LRTI) [16]. The most widespread organisms were non-lactose fermenting organisms (e.g., pseudomonads), *Staphylococcus aureus* and Enterococci. The LRTI commonly result from ventilator-associated pneumonia. Surveillance cultures have not been well studied in this population and have been uncommonly used in other children with respiratory failure. False-positive rates are high and may lead to unnecessary antimicrobial therapy. Infectious complications were shown in that study to increase hospital length of stay. It appears that the use of prophylactic antifungal therapy with fluconazole led to a decrease in fungal infections among patients with liver failure.

Spontaneous Bacterial Peritonitis (SBP)

This potentially life-threatening infection occurs commonly in children with cirrhosis and ascites. Symptoms usually include abdominal distention, fever, abdominal pain, emesis, decreased feeding, and diarrhea and may encompass lethargy or encephalopathy [15]. Predisposing factors to the development of SBP in children have not yet been established. One potential contributor may be complement deficiency which was found in nearly 90 % of children with ESLD who developed SBP compared to 14 % of children with cirrhosis who did not [17]. The most common etiologic agents for SBP in children appear to be *Streptococcus pneumoniae* and other GPCs followed by gram-negative enteric bacteria [17, 18]. In other studies, however, the gram-negative organisms outranked the gram-positive organisms [19, 20].

Treatment of SBP requires rapid initiation of broad-spectrum antibiotics until cultured peritoneal fluid reveals an offending organism and its sensitivities are established. Supportive therapy which may include positive pressure ventilation to counter the effects of fluid overload and abdominal distention is required to stabilize patients. Furthermore, pneumococcal antigen vaccine, if the child is not vaccinated, and prophylactic antibiotics should be considered following resolution of the acute process as recurrent SBP has a high rate.

Hematologic Complications/ Coagulopathy

Coagulation abnormalities represent an important risk factor for bleeding in ESLD and result primarily from deficiencies in protein synthetic function in hepatocytes. Coagulopathy is evidenced primarily by prolonged prothrombin time/INR results due to low levels of vitamin K-dependent factors (II, VII, IX, and X) as well as factors V and fibrinogen [21]. Additionally, antithrombin III and levels of vitamin K-dependent anticoagulation factors, protein C and S, are low. Because of the importance of liver synthetic function, PT and INR are used in almost all prognostic schemes to assess severity of liver injury in the setting of ALF [21]. Dysfunctional coagulation factors and increased factor consumption may also contribute to coagulation abnormalities. Finally there appears to be diminished catabolism of anticoagulation factors, increased fibrinolysis, and thrombocytopenia in liver failure.

Bone marrow failure is seen uncommonly in children with ALF. It may result in findings from mild pancytopenia all the way to aplastic anemia but may not become clinically apparent until after liver transplantation. Therapy includes immune-modulating agents such as corticosteroids, cyclosporine, antithymocyte globulin (ATG), or even hematopoietic stem cell transplant (HSCT).

Despite marked coagulation abnormalities, clinical bleeding is relatively uncommon in liver failure without some precipitating event (e.g., portal hypertension or infection). Thus, correction of abnormal clotting tests should occur primarily in the presence of bleeding or prior to a procedure (e.g., placement of an intracranial monitor). However, it is imperative that clinicians be prepared to respond rapidly to the onset of acute bleeding and keep several units of crossmatched packed red blood cells readily available.

Hemorrhage

Esophageal varices are seen primarily in patients with chronic liver dysfunction, arise in response to portal venous hypertension, and can be a

source of life-threatening hemorrhage. Ongoing variceal bleeding requires a rapid but systematic approach that begins from the general and proceeds to more invasive. The first step is immediate attention to hemodynamic status and resuscitation which will usually include transfusion of packed red blood cells and replacement of factor deficiencies with plasma and cryoprecipitate as well as administration of parenteral vitamin K. Placement of a nasogastric tube helps to drain the stomach and assess ongoing blood loss. Care should be practiced while placing the tube in order not to exacerbate bleeding. In addition to resuscitation, pharmacologic therapy with continuous infusions of octreotide (1–2 mcg/g/min) should be instituted [4]. Octreotide, a vasoconstrictor, decreases splanchnic tone and thus portal venous pressure. Octreotide is usually well tolerated by children.

Approximately 30 % of pediatric patients have ongoing hemorrhage despite conservative management including use of octreotide. Evidence of bleeding that exceeds 10 mL/kg to maintain an adequate hemoglobin level (~8 g/dL) should prompt more aggressive therapy. The preferred approach to arresting bleeding includes variceal banding. Sclerotherapy may also be used, but a high rate of complications including bleeding prior to obliteration, esophageal ulceration, perforation, and stricture formation has been reported in children [13, 22]. Banding has led to significantly less recurrent bleeding and fewer complications and may be easier to perform in a potentially obscured field. Use of the technique is limited in infants due to their small esophageal size and ability to pass the banding apparatus into the esophagus. When uncontrolled variceal bleeding occurs a Sengstaken-Blakemore tube which is designed to balloon tamponade gastroesophageal variceal bleeding should be placed. The use of these tubes is associated with rebleeding upon removal and discomfort, and so sedation of patients may be needed. Finally, due to the risk of aspiration, it is recommended to secure a patient's airway when using a Sengstaken-Blakemore tube. The Sengstaken-Blakemore tube is usually a bridge to a more definitive procedure such as an emergent portosystemic shunt or TIPS.

Pulmonary Abnormalities

Patients with advanced liver failure often have multifactorial pulmonary dysfunction related to extrinsic factors (e.g., fluid overload) and intrinsic disease precipitated by liver dysfunction (e.g., hepatopulmonary syndrome) [23]. Children with ascites and abdominal distention have a component of restrictive lung disease which may inhibit lung volumes and tidal excursion. Similarly patients with fluid overload may have chest wall edema which contributes further to restrictive lung disease. Patients may develop hypoxemia and respiratory distress and require positive pressure therapy. However, when ascites progresses it can cause severe lung restriction and may require intermittent drainage procedures. Intrinsic pulmonary disease in the setting of liver failure includes hepatopulmonary syndrome and portopulmonary hypertension.

Hepatopulmonary Syndrome (HPS)

HPS is usually defined as the triad of liver disorder (chronic liver disease and/or portal hypertension), intrapulmonary vascular dilatation, and altered gas exchange producing arterial hypoxemia [23]. Gas exchange abnormalities result from intrapulmonary shunting which may be due to microvascular dilatations, direct arteriovenous connections, or angiogenesis in more severe cases. The gas exchange abnormalities found in HPS can be mild, moderate, or severe defined by arterial $PO_2 \geq 80$ mmHg, a PO_2 of 60–80 mmHg, or a $PO_2 < 60$ mmHg, respectively [24]. Bubble contrast echocardiography may aid diagnosis [23]. There are no proven therapies for HPS except for liver transplantation. The rate of HPS in children appears to be somewhere below 10 % (usually between 2 and 8 %) [24–26]. Liver transplantation results in correction of hypoxemia but may take as much as 6 months to a year [24–26].

No differences in the severity of liver disease could be detected in patients with and without HPS [24, 26]. Intrapulmonary shunts have also been reported in patients with a Glenn shunt (cavopulmonary shunt) that results in decreased portal flow to the liver. This finding has led to the concept that factors normally produced or metabolized

in the liver may influence the lung microvasculature when hepatic function or blood flow is altered. Studies suggest that alterations in nitric oxide production through the endothelin-1/ETB receptor-stimulated eNOS activation as well as iNOS induction in intravascular monocytes may contribute to the pathophysiology of HPS [23]. The presence of HPS appears to increase mortality in adults. But recent data suggest equivalent outcome following liver transplantation in children with HPS to those without HPS [26].

Portopulmonary Hypertension

Portopulmonary hypertension (PPHTN) is pulmonary artery hypertension that is associated with liver disease or portal hypertension [27, 28]. PPHTN is defined as a mean pulmonary artery pressure (PAP) >25 mmHg or elevated pulmonary vascular resistance (PVR) (>3 Woods units) at rest with normal left atrial pressure. Patients may present with subtle findings or more obvious signs of pulmonary hypertension such as dyspnea and syncope. Without therapy PPHTN is fatal. The primary goal of therapy is to optimize the potential for liver transplantation and survival. Treatment usually includes supplemental oxygen to keep arterial saturations above 92 % and diuretics to decrease fluid overload. Pretransplantation therapies to decrease pulmonary artery pressure include calcium-channel blockers in children who demonstrate good pulmonary vasoreactivity at cardiac catheterization, milrinone, or continuous prostacyclin, a prostanoid, infusion. Prostacyclin use may be complicated by systemic hypotension especially when intracardiac shunts exist. Right heart catheterization prior to its use has been recommended. Prostacyclin may also be delivered by continuous inhalation. Other prostanoids include treprostinil which can be delivered via the IV, nebulization, or subcutaneous routes and iloprost which is inhaled but requires frequent dosing due to its short half-life. Other therapies that may be useful on a long-term basis include the phosphodiesterase type 5 inhibitor, sildenafil, and the endothelin receptor antagonists bosentan (nonspecific) and ambrisentan (endothelin A-specific). Few data exist for these agents in children, but they have been successfully employed in adults [28]. Liver transplantation in children without irreversible pulmonary vascular changes is appropriate but still is marked by poor outcomes. In one adult series, the postoperative mortality was 36 % prior to hospital discharge [29].

Cardiac

Patients with severe liver disease have ongoing peripheral vasodilatation despite appropriate fluid resuscitation reminiscent of systemic inflammatory response syndrome (SIRS). They demonstrate a high-output cardiac state with low systemic vascular resistance (SVR) which may progress to cardiac failure characterized by decreased contractility and, possibly, diastolic dysfunction. Patients with hypotension may respond to α-adrenergic agents (norepinephrine) [4]. Other therapies for cardiac failure will depend on the relative degree of myocardial dysfunction in relation to vascular dysfunction.

Patients with long-standing liver failure may develop cirrhotic cardiomyopathy which is characterized by QT prolongation and both systolic and diastolic dysfunction [30]. It may contribute to hepatorenal syndrome. The reduced SVR mitigates some of the cardiac dysfunction so that it may not reveal itself until times of stress on the heart. Children with preoperative cardiac dysfunction as assessed by echocardiography have longer ICU and hospital LOS.

Hepatoadrenal Syndrome

Adrenal insufficiency (AI) during hepatic failure may worsen the proclivity for hypotension and low systemic vascular resistance. There is overlap of the symptoms of decompensated chronic liver disease and sepsis. In one series, more than 80 % of patients with ESLD had low baseline cortisol levels, but of these patients 55 % had a normal cosyntropin stimulation test [31]. Most patients with abnormal adrenal function had short gut syndrome in addition to liver failure. However, in that series, non-survivors had a lower rise of serum

cortisol in response to the CST than did survivors. Ongoing vasopressor-dependent hypotension was the best clinical indicator of AI in those patients. Corticosteroid therapy has been shown to decrease the duration of vasopressor dependence but has not been clearly shown to impact survival. However, if the steroid therapy prolongs survival, this may increase the likelihood of undergoing liver transplantation.

Extracorporeal Liver Support Systems

Nonbiologic artificial liver support devices (ALS) and biologic ALS aim to detoxify the blood in patients with liver failure [32]. Biologic ALS use hepatocytes or ex vivo whole-organ perfusion with no improvement in survival yet demonstrated. Nonbiologic ALS use dialysis-derived techniques employing either albumin or plasma separation [32]. Substances which bind albumin including bilirubins, aromatic amino acids, endogenous benzodiazepine-like substances, and medications travel across the membrane to bind to the albumin within the dialysate. The albumin either is then recycled by passing through a charcoal adsorbent filter and anion exchange resins or is simply dumped and replaced by fresh albumin [32]. Other, unbound, free low molecular weight molecules such as ammonia can travel down its concentration gradient into the dialysate. A number of such systems exist. One such system, the Molecular Adsorbent and Recycling System (MARS, Teraklin AG), has been used to treat mushroom poisoning and as a bridge to retransplantation. The MARS has been shown to attenuate the hyperdynamic circulation, hepatic encephalopathy, and intracranial hypertension in liver failure. A randomized controlled trial of MARS in adults with acute-on-chronic liver failure was stopped early because of the decrease in mortality in the study arm (30-day mortality of 8.3 % versus 50 %, $p=0.0027$) [32]. A meta-analysis of one study combination of plasma exchange and hemodialysis resulted in greater falls in serum ammonia levels [33], serum INR, and bilirubin levels compared to the MARS. Also, hepatic encephalopathy can be reversed, but when the albumin dialysis is stopped, the abnormalities return.

In plasma separation of which the Prometheus system (Fresenius Medical Care AG) is probably the most well studied to date, the patient's plasma albumin and other low molecular weight proteins (<100 kDa) are filtered by a specific albumin permeable polysulfone filter into a secondary circuit where they are purified by adsorption on a neutral resin and an anion exchanged and then returned to the circulation [32]. Also the system employs high-flux hemodialysis to remove water-soluble toxins. These systems may provide support to the patient with advanced hepatic encephalopathy, providing the team with greater time to procure a donor organ.

ICU Care Following Liver Transplantation

PICU admission is routine following liver transplantation. Patients undergoing liver transplantation represent a unique population because of the variety of potential preoperative pathology and the wide possibility of postoperative complications. Preoperative complications have been addressed above but may strongly impact postoperative course as many pretransplant issues do not resolve immediately after graft function normalizes.

Further potential complicating issues in the PICU management of liver transplant patients include the type of liver graft itself and the need for immune suppressive medications. Small children often receive reduced liver grafts which increase transplant complication and postoperative management [34]. Unlike other solid organ transplantation, liver matching is based on blood group typing not human leukocyte antigen (HLA) matching as the liver is less susceptible to HLA-mediated rejection [34]. Transplantation has been extended to include ABO-blood group mismatched. In such cases, removal of anti-A/B antibodies is required and can be achieved effectively by plasmapheresis [35].

Following transplantation, adequate fluid resuscitation is essential for organ perfusion and

to limit potential complications such as vascular thrombosis. In the multicenter Studies of Pediatric Liver Transplant (SPLIT) registry, recommendations for best practices suggested aiming for normotension and a CVP of approximately 10 mmHg [36]. Some centers use colloid such as dextran for resuscitation, but a Cochrane review has shown no superiority of any one colloid [37]. Blood products may be transfused when patients have bleeding, but hyperviscosity and aggressive correction of coagulopathy and thrombocytopenia should be avoided to limit the potential contribution to thrombosis at the vascular anastomosis. Low-dose heparin infusions have also been used but have not been clearly established to decrease vascular thrombosis in the immediate postoperative period.

These considerations combine to make intensive postoperative monitoring *de rigueur*. Such monitoring includes continuous arterial and venous blood pressure and arterial hemoglobin saturations. Patients also require frequent assessment of liver function (coagulation studies and bilirubin) and hepatocellular injury (transaminases, alkaline phosphatase, and GGT). Similarly close following of metabolic status (serum glucose, albumin, and pH) provides insights into graft recovery. Also, close monitoring of other organs (renal function, electrolyte levels, neurologic examination, hemodynamic changes) may provide additional clues of graft dysfunction.

Any evidence of worsening liver function in the early postoperative period or lack of improvement must prompt rapid and thorough diagnostic evaluation for vascular complications, bleeding, infection, primary nonfunction, rejection, or biliary complications. The study of choice is abdominal ultrasound because of ease of use and good sensitivity. However, operative exploration remains the definitive "study" and often is the only avenue for treatment.

The use of immune suppression following transplantation can place transplant patients at risk for graft or secondary organ dysfunction and for infection. Additionally, immunosuppressants may interact with other agents (e.g., antifungal medications) or exacerbate side effects of other agents (e.g., nephrotoxicity). Careful monitoring of drug levels and daily review of the medication list are needed to avoid interactions that may cause postoperative complications.

Liver Transplantation Complications

The development of new surgical techniques including reduced size, living donor, and split livers that permit the use of two functional allografts for transplantation has improved organ availability for pediatric recipients. Although segmental grafts may reduce waiting list time and mortality in the setting of ALF, they are associated with a higher rate of postoperative complications [38]. Despite this higher rate of complications, survival for recipients of technical variant grafts appears to be similar to that seen with whole liver transplants.

A significant proportion of patients who undergo liver transplantation require prolonged postoperative care because of preexisting conditions, adverse intraoperative events, or posttransplant complications. Early postoperative complications include primary nonfunction, vascular complications such as hepatic artery or portal vein thrombosis, hemorrhage, abdominal compartment syndrome, and bile leak. Late complications include infection, rejection, hypertension, renal dysfunction, and posttransplant lymphoproliferative disease (PTLD).

Primary Nonfunction

Primary nonfunction of the liver graft accounts for 25 % of graft failure within the first 30 days posttransplant and requires emergency retransplantation [39]. Primary nonfunction usually occurs within the first 48 h posttransplantation [40]. The signs and symptoms of poorly functioning allografts may be insidious. Signs of early graft dysfunction result from hepatocellular injury, extrahepatic organ dysfunction, or portal hypertension [41]. The symptoms of primary nonfunction include hepatic encephalopathy, vasoplegic shock, worsening coagulopathy, acidemia, rising liver enzymes, and cholestasis. The supportive measures employed in fulminant

hepatic failure should be initiated without delay, and the surgery team should be advised immediately upon this concern.

Vascular Thromboses

Postoperative vascular complications, either hepatic arterial or portal vein thrombosis, are responsible for 43 % of graft loss [39]. Within the initial 30 days after transplantation, hepatic artery thrombosis (HAT) occurs in 10 % of children and is the most common vascular anomaly leading to retransplantation [42, 43]. HAT may lead to acute allograft failure, biliary obstruction or leaks, strictures, intra-abdominal infection, and sepsis [44]. The most dramatic manifestation of HAT is fulminant hepatic ischemic necrosis with rapid onset of hepatic dysfunction, fever, encephalopathy, hypotension, and coagulopathy [41]. The absence of laboratory signs such as transaminitis or leukocytosis does not exclude HAT. Doppler ultrasound for diagnosis can be helpful in establishing the diagnosis, but surgical exploration may be required. Computed tomography and magnetic resonance angiography have a reported concordance of 95 % with operative findings but may delay operative management and expose patients to a contrast load [45]. Although strategies such as catheter-directed thrombolysis have been used, revision of the arterial anastomosis is usually indicated. If significant parenchymal necrosis has occurred, retransplantation is required [41].

Early portal vein thrombosis (PVT) occurs usually within the first week after transplantation in 7–15 % of patients [46, 47]. A meta-analysis evaluating the data of 1,257 pediatric liver transplants showed a 2.2 % risk of PVT in groups using postoperative aspirin versus 8 % in the nontreatment group [46]. Findings of PVT include transaminitis, ascites, intestinal congestion, systemic inflammatory responses due to bacterial translocation, and gastrointestinal bleeding [41]. Refractory ascites may indicate portal stenosis or thrombosis or stenosis of suprahepatic veins. Doppler ultrasonography remains the diagnostic test of choice. When left untreated, the mortality of PVT approaches 100 % [47]. Emergency thrombectomy may lead to successful revascularization and graft salvage, but when PVT progresses to acute liver failure, retransplantation becomes necessary [41].

Hepatic vein thrombosis (HVT) occurs infrequently, usually resulting from hepatic venous outflow compromise [48]. Recipients with underlying hypercoagulability are predisposed to HVT [41]. Signs of HVT include: acute transaminitis, severe abdominal pain, worsening jaundice, hepatomegaly, and ascites. The diagnosis is made with Doppler ultrasonography and/or surgical exploration. In cases of massive necrosis, retransplantation is indicated.

Hemorrhage

Preexisting coagulopathy and thrombocytopenia increase the risk for intra- and postoperative hemorrhage for transplant patients. The preoperative coagulopathy may be complicated by dilutional coagulopathy during the transplant [34]. In the immediate postoperative period, pediatric liver transplant patients may be started on continuous low-dose heparin infusion in an attempt to maintain patency of the vascular anastomoses. As the function of the graft recovers, bleeding becomes less of a danger. When coagulopathy does not improve or worsens, hepatic dysfunction or infection should be suspected. Aggressive diagnostic evaluation and treatment of the underlying cause is warranted.

The evaluation of the patient with suspected postoperative hemorrhage includes repeated monitoring of blood counts and coagulation studies as well as quantity and characteristics of the intra-abdominal drainage. Careful transfusion management should be employed at all times with the goal of treating anemia and coagulopathy while avoiding hyperviscosity and the risk of potential vascular thrombosis.

Intra-abdominal Compartment Syndrome

Large grafts may result in difficulties during abdominal closure and subsequent risk of abdominal compartment syndrome (elevated intra-abdominal

pressure) that may precipitate vascular thrombosis, organ hypoperfusion, or graft dysfunction. Recommendations from the SPLIT registry encourage leaving abdomen open and deferral of primary closure when the fascia appears tight [36]. Intra-abdominal pressure can be monitored through a bladder catheter. High intra-abdominal pressure with signs of organ dysfunction such as oliguria, metabolic acidosis, or respiratory compromise should raise the concern for intra-abdominal compartment syndrome and possible surgical exploration.

Infection

Infection is the leading cause of morbidity and mortality in the posttransplant population within the first year [49, 50]. More than half of patients develop infection during the first year after liver transplantation, and many of these infections require care in the ICU. In addition to the pathogen-specific antimicrobial therapy, a reduction in the degree of pharmacological immunosuppression should be regarded as a major component of the treatment of opportunistic infections.

The risk of nosocomial and opportunistic infections generally depends on the degree of immunosuppression and is greatest in the first 6 months after transplantation. The combination of calcineurin inhibitors (cyclosporine, tacrolimus) and corticosteroids places a child at risk for a broad range of infections, including sepsis syndrome [49–53]. Preoperative complications represent additional risk factors for infections including immune dysfunction described above [4]. Children with chronic cholestasis have increased risk for bacterial peritonitis and cholangitis [41]. The presence of arterial thrombosis or biliary leak significantly increases the risk of infection and abscess formation.

Transplant recipients are also at risk for viral infection because they are often immune naïve to viruses such as cytomegalovirus (CMV), Epstein-Barr virus (EBV), herpes simplex virus (HSV), and adenovirus [52]. Additionally, donor lymphocytes in the graft may act as a source of primary infection. The monitoring of active viral replication by polymerase chain reaction may identify infections before their clinical manifestation leading to the adjustment of immunosuppressive medications in patients with an acute infection [50]. Prophylactic regimens in the postoperative period should include ganciclovir, fluconazole, a β-lactam antibiotic, and trimethoprim-sulfamethoxazole [53].

Infections in the Immediate Posttransplant Period

During the postoperative period, infections are related to surgical issues, prolonged hospitalization, and immune suppression [51]. The surgical procedure itself may result in injury to the gastrointestinal tract, an area of high microbial load [51]. Extensive blood loss and increased blood transfusions have both been correlated with the risk of infection and mortality. Abdominal re-exploration surgery for retransplantation, biliary leaks, and vascular thrombosis also increase the risk of bacterial and fungal infections [51, 54]. Prolonged hospitalization, younger age, lower body weight, longer cold ischemia time, use of reduced graft, presence of indwelling urinary and vascular catheters, wound infections, and prolonged ventilation all predispose liver transplant patients to nosocomial bacterial and fungal infections [54]. These early-onset infections lead to prolonged hospitalization, which further increases the risk of infectious complications [49–51].

The most common sites of infection in children undergoing liver transplantation were abdominal (48 %) and bloodstream (26 %) [54]. The mean infection rate was 1.35 infectious episodes per patient, with 73 % bacterial, 17 % fungal, and 8 % mixed infections [54]. The clinical findings include fever, leukocytosis, erythema, purulence, drainage, and dehiscence of surgical wounds [41]. Infections have been associated with decreased allograft and patient survival [55]. The risk of infections is reduced by peri-transplant antibacterial prophylaxis with third-generation cephalosporins. Despite prevention efforts, infected bilomas, intra-abdominal abscesses, and surgical site infections caused by a variety of bacteria (*S. aureus*, coagulase-negative Staphylococci, Enterococci, gram-negative bacilli, and anaerobic organisms) and fungi (*Candida albicans*) may

occur. Latent active infections involving the donor or recipient liver with opportunistic organisms can manifest with severe atypical disease during the early postoperative period [49–51, 56, 57].

The early administration of effective empirical and broad-spectrum antimicrobial therapy is crucial in the treatment of infections and sepsis after liver transplantation [53]. Once susceptibilities are available, antimicrobial therapy should be tailored to the offending microorganism [49, 52]. Controlling the source of the infection is an essential component of therapy, and it includes the drainage of infected fluid collections (e.g., infected hematomas and abscesses), the debridement of surgical infections, and the removal of infected vascular and urinary catheters [49, 53]. Resuscitation and hemodynamic management should aim at rapidly reversing evidence of septic shock by following the guidelines for early goal-directed therapy and thus preventing the onset of severe cardiovascular failure [58, 59].

Opportunistic Infections

Opportunistic infections occur when the net state of immunosuppression is greatest from 2 to 6 months posttransplantation [49–51]. One of the most common opportunistic infection is cytomegalovirus (CMV) [60, 61]. CMV-seronegative recipients of liver allografts from CMV-seropositive donors are at greatest risk [60, 61]. The use of lymphocyte-depleting agents (e.g., antithymocyte globulin), mycophenolate mofetil, and alemtuzumab further increases the risk of CMV as well as other opportunistic infections such as human herpesvirus 6, *C. neoformans*, Aspergillus, and *Pneumocystis jirovecii* [62–65]. CMV prophylaxis should be instituted in the immediate postoperative period. CMV clinical presentation usually includes fever and myelosuppression (CMV syndrome), abdominal pain, nausea, and diarrhea [62]. Elevated transaminases may be confused with rejection or drug toxicity. CMV can also produce a pneumonia that often is severe and carries a high mortality. Herpes simplex virus (HSV) reactivation disease may occur although antiviral prophylaxis (ganciclovir) has markedly reduced its incidence [50]. Untreated HSV reactivation can lead to disseminated multiorgan dysfunction (e.g., fulminant hepatitis) with high morbidity and mortality rates.

Candida is the most common fungal disease followed by invasive aspergillosis during this opportunistic period [57, 64]. Candida can cause urinary tract infections, abscesses, and bloodstream infections. Risk factors for opportunistic fungal infections include reoperation, retransplantation, and renal replacement therapy. Prevention of fungal infections begins with fluconazole prophylaxis which was shown in a meta-analysis to reduce invasive fungal infections by 75 % [66]. However, fluconazole does not cover aspergillosis. Thus, in high-risk patients, consideration should be given to broader antifungal therapy (e.g., voriconazole). Cases of fungal infections (*Histoplasma capsulatum*, *Coccidioides immitis*, *C. neoformans*) transmitted via the graft have been reported [63]. Cryptococcosis often presents as meningoencephalitis with symptoms that include fever, neck stiffness, headache, seizures, and mental status changes, although it may occur as isolated pulmonary cryptococcoma or nonhealing cellulitis. *Pneumocystis jirovecii* infections and nocardiosis have been eliminated since the widespread use of trimethoprim-sulfamethoxazole prophylaxis during the first 6 months after transplant [49].

Infections in the Late Posttransplant Period

At 6 months after liver transplantation, the intensity of immunosuppression is reduced and the risk of opportunistic infections decreases. Community-acquired infections such as viral respiratory diseases (influenza, parainfluenza, and respiratory syncytial virus) and bacterial pathogens (e.g., pneumococcal pneumonia, staphylococcal and streptococcal cellulitis, gram-negative bacterial urinary infections, and sepsis) may occur, although the need for critical care grows much less common [53, 54]. Patients with biliary complications may present with hepatic abscesses or sepsis.

For patients with poor allograft function, recurrent chronic hepatitis, or recurrent acute or chronic rejection and those who remain on

intense immunosuppression, opportunistic infections may still occur [51]. These opportunistic infections include herpes zoster (vesicular eruption, disseminated form leads to multiorgan failure), Epstein-Barr virus-related posttransplant lymphoproliferative disorder (PTLD), and late CMV disease (gastroenteritis, intestinal bleeding, and pneumonia) [50]. *Pneumocystis* infections now present as late-onset pneumonia with severe hypoxemia and diffuse interstitial pulmonary infiltrates in patients who are no longer on trimethoprim-sulfamethoxazole prophylaxis.

Pulmonary Disease

Patients who develop pretransplant pulmonary complications may enter the transplant with HPS, PPHTN, atelectasis, pleural effusions, or diminished respiratory compliance or restrictive lung disease [23–27, 67]. Liver transplantation involves transection of the abdominal oblique and rectus muscles that aid respiratory movements. Posttransplant patients may experience diaphragmatic dysfunction, which can produce significant reduction in vital capacity (50–60 %) and functional residual capacity (30 %) [68]. In addition, the usage of anesthetics and diminished coughing, respiratory excursion, and mucous clearance due to wound pain may contribute to the development of postoperative atelectasis and other pulmonary complications. Patients may experience fluid overload with resultant decreases in chest and abdominal wall compliances. Elevation of intra-abdominal pressures may also worsen respiratory function and gas exchange. The resultant hypoxemia in collaboration with elevated intra-abdominal pressure and altered hemodynamics can severely alter graft perfusion and function. Ideally, rapid weaning from mechanical ventilation in the immediate postoperative period should be the goal for all patients.

Complications of prolonged mechanical ventilation include ventilator-associated pneumonia and neuromuscular deconditioning. Liver transplant patients often are suitable for early postoperative extubation, and they should be assessed for their readiness to be extubated as early as their postoperative admission to the ICU. Daily evaluation for extubation readiness may shorten duration of mechanical ventilation following transplant. Increasing evidence suggests that sedation results in an increased duration of mechanical ventilation [69]. Protocol-driven ventilator and sedation weaning such as daily sedation interruption result in significant reduction in the duration of mechanical ventilation, length of ICU and hospital stays, and 1-year mortality.

The use of noninvasive mechanical ventilation (NIPPV) in post-liver transplant patients has not been specifically studied in pediatric liver transplant patients. NIPPV offers a number of advantages over traditional mechanical ventilation with intratracheal intubation. Patients receiving NIPPV usually require lower doses of sedatives and analgesics than intubated patients and have greater mobility. A recent meta-analysis of 12 adult clinical trials found reduced mortality and ventilator-associated pneumonia among patients receiving NIPPV [70]. However, children may be at risk for insufflation of air into the gut resulting in abdominal distension that may compromise diaphragmatic excursion and may place tension on the surgical incision.

Acute Kidney Injury

In the postoperative period, preexisting or new AKI may necessitate renal replacement therapy (RRT) to control water balance and maintain electrolyte homeostasis. AKI reduces graft and patient survival, and about 10 % of the patients do progress to end-stage renal failure [71]. Surgical factors such as hemodynamic instability or hypovolemia from intraoperative blood loss may lead to prerenal or ischemic kidney injury. Total hepatectomy with resection of the retrohepatic IVC results in interruption of the venous return, reduces renal blood flow, and causes renal venous hypertension [72, 73]. Newer cava-preserving techniques have been reported to reduce the incidence of early renal failure after liver transplantation [74, 75]. Additionally, surgical leak, retransplantation, poor graft function,

and multiple transfusions of blood products have been associated with the development of AKI.

AKI may also arise due to postoperative factors. The most important postoperative factor in AKI remains the exposure to renal toxic medications including cyclosporine, tacrolimus, and nephrotoxic antibiotics (vancomycin and aminoglycosides) [76]. Calcineurin inhibitors have been reported to increase almost 3-fold the odds ratio for developing chronic kidney disease within 10 years [76]. Other important risk factors for AKI in the postoperative period include infection, abdominal distention, and hypovolemia [71]. In the past, renal insufficiency was identified in 33 and 77 % of 3-year and 10-year survivors of pediatric liver transplant patients, respectively [77–79].

Fluid overload and electrolyte disturbances are the most common reasons for initiation of renal replacement therapy (RRT). Despite the current consensus of early initiation of RRT for AKI, the most recent literature lacks adequately powered prospective randomized trials to show improved survival. The choice of the renal replacement modality should be guided by each patient's clinical status. Continuous renal replacement therapy (CRRT) offers superior hemodynamic stability in comparison with conventional intermittent hemodialysis, although studies have failed to confirm a survival advantage of any specific modality [80].

Graft Rejection

Despite current immunosuppressive regimens, acute rejection remains an important concern following liver transplantation. The median time to rejection after transplant was 16 days, and 40–70 % of children who experienced rejection had the first episode 7–10 days after transplantation [53]. Acute cellular rejection is diagnosed in 25–50 % of recipients within the first 6 months after transplantation [81]. Laboratory findings of acute rejection include rising serum bilirubin and alkaline phosphatase, although biliary expression of rejection is usually a late event [53]. The diagnosis of acute rejection also requires liver biopsy which may help distinguish between acute rejection, biliary obstruction, graft ischemia, and viral infection (CMV, EBV, adenovirus). Intensified immunosuppression to reverse the process and minimize graft loss includes a short course of high-dose methylprednisolone, which is effective in treating rejection in 80 % of cases [53]. T cell depletion therapies like thymoglobulin (a polyclonal lymphocyte-depleting agent) are reserved for severe or refractory episodes nonresponsive to conventional pulse methylprednisolone therapy [77].

Hyperacute rejection (antibody-mediated rejection) is a rare event resulting from the deposition of circulating, preformed antibodies present at the time of transplantation into the allograft sinusoids and vascular endothelium, with the activation of complement and the coagulation cascade culminating in thrombosis and hemorrhagic graft necrosis [82]. The use of ABO-incompatible grafts accounts for 60 % of these cases [82]. Preventive measures in the perioperative period include plasma exchange, intravenous gamma globulin (IVIG), B cell-depleting therapy, and splenectomy [82]. When transplant recipients develop acute liver failure, emergent retransplantation is required.

Complications of Immunosuppression

Immunosuppressive agents have long-term consequences that include mortality [83]. Immunosuppressive therapy includes calcineurin inhibitors, cyclosporine, tacrolimus, mTOR inhibitors such as sirolimus, and chimeric humanized monoclonal antibodies (basiliximab and daclizumab) directed at the IL-2 receptor. The most common undesirable side effects of immune suppressant agents are listed in Table 24.1. Prolonged exposure to these therapies may result in renal toxicity and risk of malignancy. The global effect of long-term immune suppression on the child's growth, development, and intellectual potential remains unclear. Of particular concern is the potential for neurotoxicity from the calcineurin inhibitors. The risks of rejection as well as hematologic, renal, and neurologic com-

plications of overdosage are determined by the drug levels. Calcineurin blood levels need to be determined on a daily basis initially in order to fine-tune the degree of immunosuppression.

Calcineurin inhibitors and corticosteroids frequently result in arterial hypertension and may cause renal failure. Conventional pharmacologic agents are used to treat arterial hypertension. Steroids in the immediate postoperative phase cause hyperglycemia that may be treated with insulin [84]. Tacrolimus is associated with an increased prevalence of new-onset diabetes. Cyclosporine and tacrolimus, especially when delivered intravenously, respectively, cause encephalopathy and seizures in 8 and 11 % of the transplanted patients [85]. Seizures are treated with antiepileptic medications, but care must be taken as many influence the metabolism of the immunosuppressive agents. Ultimately, there is a need for new immunosuppressive strategies, and the goal of achieving donor-specific tolerance will require the development of new immunosuppressive protocols and methods that measure recipient immunoreactivity [39, 84].

Biliary Complications

Biliary complications occur frequently in pediatric liver transplant recipients (anastomosis stricture, biliary sludge, and recurrent cholangitis). Approximately 15 % of transplant patients experience biliary complications within the first 30 days postoperatively, and 25 % experience this complication eventually [39]. Biliary leaks are caused by biliary anastomosis dehiscence or from surgical cuts at the surface of the liver. Cut surfaces that leak from minor biliary radicals usually resolve spontaneously, but more serious leaks from the biliary anastomosis or from larger cut surface ducts require operative management. Hepatic artery thrombosis causes bile duct ischemia that frequently results in stricture or leak that may require retransplantation. The diagnosis of bile leak is made by the appearance of bile in intra-abdominal drains or by radionucleotide scan or transhepatic contrast studies. Bile leaks increase the risk of postoperative infections that require appropriate antibiotic therapy. Rising serum bilirubin and alkaline phosphatase may signify acute rejection, although biliary expression of rejection is usually a late event.

Retransplantation

Of patients who develop graft failures, vascular thrombosis causes approximately 40 % of cases, primary graft nonfunction occurs in 17–28 %, and graft rejection produces 11–27 % [86]. Within the first 30 days following initial transplantation, 11 % of children require retransplantation [77]. Patients undergoing second or third retransplant operations have progressively higher rates of early graft dysfunction and loss [39]. In a recent review of the SPLIT registry of 461 5-year survivors, 12 % of patients required retransplantation [77]. Retransplantation occurred more frequently in children receiving primary LT before January 1, 2000 (13.9 %) than in patients transplanted after January 1, 2000 (7.9 %).

Outcomes

In a recent study, initial graft survival for 461 5-year survivors was 88 %, with 12 and 2 % of children undergoing a second and third liver transplantation [77]. Graft type did not influence outcomes. Current national average 1-year patient survival rates are 90 %, and 5-year rates are 85 % at experienced centers (The SPLIT Research Group, Annual Report, written communication, 2007) [82, 87–89]. Children who are 5-year survivors of liver transplantation have good graft function, but chronic medical conditions and posttransplantation complications affect extrahepatic organs. Probability of an episode of acute cellular rejection within 5 years after liver transplantation was 60 %. Chronic rejection occurred in 5 % patients. PTLD was diagnosed in 6 % children. Calculated glomerular filtration rate was 90 mL/min per 1.73 m^2 in 13 % of 5-year survivors.

A recent study focused specifically on the outcome of young infants [84]. Approximately 90 % of infants received deceased liver grafts, of which

47 % were reduced organs. Infants experienced prolonged hospitalizations with a mean length of stay (LOS) of 51 ± 7.6 days and mean ICU LOS of 22 ± 1.5 days and duration of mechanical ventilation (16 ± 2.7 days). The reoperation rate (61 %) was significantly greater than in older children although survival did not differ. Indication for reoperation included bleeding, wound complications, biliary complications, and sepsis. This infant cohort had an overall graft survival of 76 % and 1-year survival of 87.8 %. Graft and patient survival in infants younger than 90 days was similar to older children.

A comprehensive approach to ICU management of transplant patients requires the expertise of health-care providers within transplant centers to further optimize long-term outcomes for pediatric liver transplant recipients [83]. However, success of liver transplantation in children requires meticulous preoperative care, excellent surgical technique, careful management of the graft, and comprehensive postoperative care. Ongoing research will help clarify ongoing concerns.

References

1. Ferenci P, Lockwood A, Mullen K, Tarter R, Weissenborn K, Blei AT. Hepatic encephalopathy—definition, nomenclature, diagnosis, and quantification: final report of the working party at the 11th World Congresses of Gastroenterology, Vienna, 1998. Hepatology. 2002;35:716–21.
2. Arya R, Gulati S, Deopujari S. Management of hepatic encephalopathy in children. Postgrad Med J. 2010;86:34–41.
3. Squires Jr RH, Shneider BL, Bucuvalas J, Alonso E, Sokol RJ, Narkewicz MR, Dhawan A, Rosenthal P, Rodriguez-Baez N, Murray KF, Horslen S, Martin MG, Lopez MJ, Soriano H, McGuire BM, Jonas MM, Yazigi N, Sheperd RW, Schwarz K, Lobritto S, Thomas DW, Lavine JE, Karpen S, Ng V, Kelly D, Simonds N, Hynan LS. Acute liver failure in children: the first 348 patients in the pediatric acute liver failure study group. J Pediatr. 2006;148:652–8.
4. El-Shabrawi MH, Kamal NM. Medical management of chronic liver diseases (CLD) in children (part II): focus on the complications of CLD, and CLD that require special considerations. Paediatr Drugs. 2011;13:371–83.
5. Lockwood AH, McDonald JM, Rieman RE, Gelbard AS, Laughlin JS, Duffy TE, Plum F. The dynamics of ammonia metabolism in man. Effects of liver disease and hyperammonemia. J Clin Invest. 1979;63:449–60.
6. Watanabe A, Takei N, Higashi T, Shiota T, Nakatsukasa H, Fujiwara M, Sakata T, Nagashima H. Glutamic acid and glutamine levels in serum and cerebrospinal fluid in hepatic encephalopathy. Biochem Med. 1984;32:225–31.
7. Mas A. Hepatic encephalopathy: from pathophysiology to treatment. Digestion. 2006;73 Suppl 1:86–93.
8. D'Agostino D, Diaz S, Sanchez MC, Boldrini G. Management and prognosis of acute liver failure in children. Curr Gastroenterol Rep. 2012;14:262–9.
9. Sharma A, Agrawal A, Sharma BC, Sarin SK. Prophylaxis of hepatic encephalopathy in acute variceal bleed: a randomized controlled trial of lactulose vs no lactulose. J Gastroenterol Hepatol. 2011;26:996–1003.
10. McBryde KD, Kershaw DB, Bunchman TE, Maxvold NJ, Mottes TA, Kudelka TL, Brophy PD. Renal replacement therapy in the treatment of confirmed or suspected inborn errors of metabolism. J Pediatr. 2006;148:770–8.
11. Bunchman TE, Barletta GM, Winters JW, Gardner JJ, Crumb TL, McBryde KD. Phenylacetate and benzoate clearance in a hyperammonemic infant on sequential hemodialysis and hemofiltration. Pediatr Nephrol. 2007;22:1062–5.
12. Kamat P, Kunde S, Vos M, Vats A, Gupta N, Heffron T, et al. Invasive intracranial pressure monitoring is a useful adjunct in the management of severe hepatic encephalopathy associated with pediatric acute liver failure. Pediatr Crit Care Med. 2012;13:e33–8.
13. Debray D, Yousef N, Durand P. New management options for end-stage chronic liver disease and acute liver failure. Potential for pediatric patients. Paediatr Drugs. 2006;8:1–13.
14. Yousef N, Habes D, Ackermann O, Durand P, Bernard O, Jacquemin E. Hepatorenal syndrome: diagnosis and effect of terlipressin therapy in 4 pediatric patients. J Pediatr Gastroenterol Nutr. 2010;51:100–2.
15. Leonis MF, Balistreri WF. Evaluation and management of end-stage liver disease in children. Gastroenterology. 2008;134:1741–51.
16. Godbole G, Shanmugam N, Dhawan A, Verma A. Infectious complications in acute liver failure. J Pediatr Gastroenterol Nutr. 2011;53:320–5.
17. Larcher VF, Manolaki N, Vegnente A, Vergani D, Mowat AP. Spontaneous bacterial peritonitis in children with chronic liver disease: clinical features and etiologic factors. J Pediatr. 1985;106:907–12.
18. Rabinovitz M, Gavaler JS, Kumar S, Kalani M, Van Thiel DH. Role of serum complement, immunoglobulins, and cell-mediated immune system in the pathogenesis of spontaneous bacterial peritonitis (SBP). Dig Dis Sci. 1989;34:1547–52.
19. Vieira SM, Matte U, Kieling CO, Barth AL, Ferreira CT, Souza AF, Taniguchi A, da Silveira TR. Infected and noninfected ascites in pediatric patients. J Pediatr Gastroenterol Nutr. 2005;40:289–94.

20. Wiest R, Garcia-Tsao G. Bacterial translocation (BT) in cirrhosis. Hepatology. 2005;41:422–33.
21. Squires Jr RH. Acute liver failure in children. Semin Liver Dis. 2008;28:153–66.
22. Zargar SA, Javid G, Khan BA, Yattoo GN, Shah AH, Gulzar GM, Singh J, Rehman BU, Din Z. Endoscopic ligation compared with sclerotherapy for bleeding esophageal varices in children with extrahepatic portal venous obstruction. Hepatology. 2002;36:666–72.
23. Rodriguez-Roisin R, Krowka MJ. Hepatopulmonary syndrome – a liver induced vascular disorder. N Engl J Med. 2008;358:2378–87.
24. Noli K, Solomon M, Golding F, Charon M, Ling SC. Prevalence of hepatopulmonary syndrome in children. Pediatrics. 2008;121:e522–7.
25. Barbe T, Losay J, Grimon G, Devictor D, Sardet A, Gauthier F, Houssin D, Bernanrd O. Pulmonary arteriovenous shunting in children with liver disease. J Pediatr. 1995;126:571–9.
26. Al-Hussaini A, Taylor RM, Samyn M, Bansal S, Heaton N, Rela M, Mieli-Vergani G, Dhawan A. Long-term outcome and management of hepatopulmonary syndrome in children. Pediatr Transplant. 2010;14:276–82.
27. Condino AA, Ivy DD, O'Connor JA, Narkewicz MR, Mengshol S, Whitworth JR, Claussen L, Doran A, Sokol RJ. Portopulmonary hypertension in pediatric patients. J Pediatr. 2005;147:20–6.
28. Safdar Z, Bartolome S, Sussman N. Portopulmonary hypertension: an update. Liver Transpl. 2012;18:881–91.
29. Krowka MJ, Mandell MS, Ramsay MA, Kawut SM, Fallon MB, Manzarbeitia C, Pardo Jr M, Marotta P, Uemoto S, Stoffel MP, Benson JT. Hepatopulmonary syndrome and portopulmonary hypertension: a report of the multicenter liver transplant database. Liver Transpl. 2004;10:174–82.
30. Soler X, Myo Bui CC, Aronson L, Saied AS. Current issues in pediatric liver transplantation. Int Anesthesiol Clin. 2012;50:54–65.
31. Hauser GJ, Brotman HM, Kaufman SS. Hepatoadrenal syndrome in pediatric patients with end-stage liver disease. Pediatr Crit Care Med. 2012;13:e145–9.
32. Barshes NR, Gay AN, Williams B, Patel AJ, Awad SS. Support for the acutely failing liver: a comprehensive review of historic and contemporary strategies. J Am Coll Surg. 2005;201:458–76.
33. Nevens F, Laleman W. Artificial liver support devices as treatment option for liver failure. Best Pract Res Clin Gastroenterol. 2012;26:17–26.
34. Taylor RM, Franck LS, Gibson F, Dhawan A. Liver transplantation in children: part 1 – peri-operative issues. J Child Health Care. 2005;9:256–73.
35. Farges O, Kalil AN, Samuel D, Saliba F, Arulnaden JL, Debat P, Bismuth A, Castaing D, Bismuth H. The use of ABO-incompatible grafts in liver transplantation: a life-saving procedure in highly selected patients. Transplantation. 1995;9:1124–33.
36. Englesbe MJ, Kelly B, Goss J, Fecteau A, Mitchell J, Andrews W, Krapohl G, Magee JC, Mazariegos G, Horslen S, Bucuvalas J. Reducing pediatric liver transplant complications: a potential roadmap for transplant quality improvement initiatives within North America. Am J Transplant. 2012;12:2301–6.
37. Bunn F, Trivedi D. Colloid solutions for fluid resuscitation. Cochrane Database Syst Rev. 2012;7:CD001319.
38. Bourdeaux C, Darwish A, Jamart J, Tri TT, Janssen M, Lerut J, Otte JB, Sokal E, de Ville de Govet J, Reding R. Living-related versus deceased donor pediatric liver transplantation: a multivariate analysis of technical and immunological complications in 235 recipients. Am J Transplant. 2007;7:440–7.
39. McDiarmid SV. Current status of liver transplantation in children. Pediatr Clin North Am. 2003;50:1335–74.
40. Rand E, Olthoff K. Overview of pediatric liver transplantation. Gastroenterol Clin. 2003;32:913–29.
41. Razonable RR, Findlay JY, O'Riordan A, Burroughs SG, Ghobrial RM, Agarwal B, Davenport A, Gropper M. Critical care issues in patients after liver transplantation. Liver Transpl. 2011;17:511–27.
42. Gordon SA, Carmody I. Arterial complications after liver transplantation. In: Busuttil RW, Klintmalm GB, editors. Transplantation of the liver. 2nd ed. Philadelphia: Elsevier; 2005. p. 953–61.
43. Martin SR, Atkison P, Anand R, Linblad AS, SPLIT Research Group. Studies of pediatric liver transplantation 2002: patient and graft survival and rejection in pediatric recipients of a first liver transplant in the United States and Canada. Pediatr Transplant. 2004;8:273–83.
44. SPLIT Research Group. Studies of pediatric liver transplantation (SPLIT) year 2000 outcomes. Transplantation. 2001;72:463–76.
45. Stewart ZA, Locke JE, Segev DL, Dagher NN, Singer AL, Montgomery RA, Cameron AM. Increased risk of graft loss from hepatic artery thrombosis after liver transplantation with older donors. Liver Transpl. 2009;15:1688–95.
46. Chardot C, Herrera JM, Debray D, Branchereau S, De Dreuzy O, Devictor D, Dartayet B, Norwood P, Lambert T, Preintee D, Gauthier F, Valayer J. Portal vein complications after liver transplantation for biliary atresia. Liver Transpl Surg. 1997;3:351–8.
47. Cheng YF, Chen CL, Huang TL, Chen TY, Chen YS, Takatsuki M, Wang CC, Chiu KW, Tsang LL, Sun PL, Jawan B. Risk factors for intraoperative portal vein thrombosis in pediatric living donor liver transplantation. Clin Transplant. 2004;18:390–4.
48. Borsa JJ, Daly CP, Fontaine AB, Patel NH, Althaus SJ, Hoffer EK, Winter TC, Nghiem HV, McVicar JP. Treatment of inferior vena cava anastomotic stenoses with the Wallstent endoprosthesis after orthotopic liver transplantation. J Vasc Interv Radiol. 1999;10:17–22.
49. Blair JE, Kusne S. Bacterial, mycobacterial, and protozoal infections after liver transplantation—part I. Liver Transpl. 2005;11:1452–9.
50. Kusne S, Blair JE. Viral and fungal infections after liver transplantation—part II. Liver Transpl. 2006;12:2–11.

51. Razonable RR, Paya CV. Liver transplant patients. In: Cohen J, Powderly WG, Opal SM, editors. Infectious diseases, vol. 1. 3rd ed. London: Mosby Elsevier Science; 2010. p. 848–52.
52. Shepherd R, Turnelle Y, Nadler M, Lowell JA, Narkewicz MR, McDiarmid SC, Anand R, Song C, SPLIT Research Group. Risk factors for rejection and infection in pediatric liver transplantation. Am J Transplant. 2008;8:396–403.
53. Garzoni C, AST Infectious Diseases Community of Practice. Multiply resistant gram-positive bacteria methicillin-resistant, vancomycin-intermediate and vancomycin-resistant Staphylococcus aureus (MRSA, VISA, VRSA) in solid organ transplant recipients. Am J Transplant. 2009;9 suppl 4:S41–9.
54. Ison MG, Michaels MG. RNA respiratory viral infections in solid organ transplant recipients. Am J Transplant. 2009;9 suppl 4:S166–72.
55. Garcia S, Roque J, Ruza F, Gonzalez M, Madero R, Alvarado F, Herruzo R. Infection and associated risk factors in the immediate postoperative period of pediatric liver transplantation: a study of 176 transplants. Clin Transplant. 1998;12:190–7.
56. Kotton CN, Lattes R. Parasitic infections in solid organ transplant recipients. Am J Transplant. 2009;9 suppl 4:S234–51.
57. Pappas PG, Silveira FP. Candida in solid organ transplant recipients. Am J Transplant. 2009;9 suppl 4: S173–9.
58. Rivers E, Nguyen B, Havstad S, Ressler J, Muzin A, Knoblich B, Peterson E, Tomlanovich M, Early Goal-Directed Therapy Collaboration Group. Early goal-directed therapy in the treatment of severe sepsis and septic shock. N Engl J Med. 2001;345:1368–77.
59. Kumar A, Roberts D, Wood KE, Light B, Parillo JE, Sharma S, Suppes R, Feinstein D, Zarotti S, Taiber L, Gurka D, Kumar A, Cheang M. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Crit Care Med. 2006;34:1589–96.
60. Humar A, Snydman D. Cytomegalovirus in solid organ transplant recipients. Am J Transplant. 2009;9 suppl 4:S78–86.
61. Razonable RR. Cytomegalovirus infection after liver transplantation: current concepts and challenges. World J Gastroenterol. 2008;14:4849–60.
62. Falagas ME, Snydman DR, Griffith J, Ruthazer R, Werner BG. Effect of cytomegalovirus infection status on first-year mortality rates among orthotopic liver transplant recipients. Ann Intern Med. 1997;126: 275–9.
63. Singh N, Forrest G. Cryptococcosis in solid organ transplant recipients. Am J Transplant. 2009;9 suppl 4:S192–8.
64. Singh N, Husain S. Invasive aspergillosis in solid organ transplant recipients. Am J Transplant. 2009;9 suppl 4:S180–91.
65. Martin SI, Fishman JA. Pneumocystis pneumonia in solid organ transplant recipients. Am J Transplant. 2009;9 suppl 4:S227–33.
66. Cruciani M, Mengoli C, Malena M, Bosco O, Serpelloni G, Grossi P. Antifungal prophylaxis in liver transplant patients: a systematic review and meta-analysis. Liver Transpl. 2006;12:850–8.
67. Huang CT, Lin HC, Chang SC, Lee WC. Pre-operative risk factors predict post-operative respiratory failure after liver transplantation. PLoS One. 2011;6(8): e22689.
68. Smetana GW, Lawrence VA, Comell JE. Preoperative pulmonary risk stratification for noncardiothoracic surgery: systematic review for the American College of Physicians. Ann Intern Med. 2006;144:581–95.
69. Deeter K, King MA, Ridling D, Irby G, Lynn AM, Zimmerman JJ. Successful implementation of a pediatric sedation protocol for mechanically ventilated patients. Crit Care Med. 2011;39:683–8.
70. Burns KE, Adhikari NK, Keenan SP, Meade M. Use of non-invasive ventilation to wean critically ill adults off invasive ventilation: meta-analysis and systematic review. BMJ. 2009;338:b1574.
71. Barri YM, Sanchez EQ, Jennings LW, Melton LB, Hays S, Levy MF, Klintmalm GB. Acute kidney injury following liver transplantation: definition and outcome. Liver Transpl. 2009;15:475–83.
72. Estrin JA, Belani KG, Ascher NL, Lura D, Payne W, Najarian JS. Hemodynamic changes on clamping and unclamping of major vessels during liver transplantation. Transplant Proc. 1989;21:3500–5.
73. Davenport A, Anker SD, Mebazaa A, Palazzuoli A, Vescovo G, Bellomo R, Ponikowski P, Anand I, Aspromonte N, Bagshaw S, Berl T, Bobek I, Cruz DN, Daliento L, Haapio M, Hillege H, House A, Katz N, Maisel A, Mankad S, McCullough P, Ronco F, Shaw A, Sheinfeld G, Soni S, Zamperetti N, Zanco P, Ronco C, Acute Dialysis Quality Initiative (ADQI) Consensus Group. ADQI 7: the clinical management of the cardio-renal syndromes: work group statements from the 7th ADQI consensus conference. Nephrol Dial Transplant. 2010;25:2077–89.
74. Cabezuelo JB, Ramirez P, Acosta F, Torres D, Sansano T, Pons JA, Bru M, Montoya M, Rios A, Sánchez Bueno F, Robles R, Parrilla P. Does the standard vs piggyback surgical technique affect the development of early acute renal failure after orthotopic liver transplantation? Transplant Proc. 2003;35:1913–4.
75. Mehrabi A, Mood ZA, Fonouni H, Kashfi A, Hillebrand N, Muller SA, Encke J, Buchler MW, Schmidt J. A single center experience of 500 liver transplants using the modified piggyback technique by Belghiti. Liver Transpl. 2009;15:466–74.
76. Lee JP, Heo NJ, Joo KW, Yi NJ, Suh KS, Moon KC, Kim SG, Kim YS. Risk factors for consequent kidney impairment and differential impact of liver transplantation on renal function. Nephrol Dial Transplant. 2010;25:2772–85.
77. Ng VL, Fecteau A, Shepherd R, Magee J, Bucuvalas J, Alonso E, McDiarmid S, Cohen G, Anand R, Studies of Pediatric Liver Transplantation Research Group. Outcomes of 5-year survivors of pediatric liver transplantation: report on 461 children from a

North American multicenter registry. Pediatrics. 2008;122:e1128–35.
78. Avitzur Y, De Luca E, Cantos M, Jimenez-Rivera C, Jones N, Fecteau A, Grant D, Ng VL. Health status ten years after pediatric liver transplantation–looking beyond the graft. Transplantation. 2004;78(4): 566–73.
79. Campbell KM, Yazigi N, Ryckman FC, Alonso M, Tiao G, Balisteri WF, Atherton H, Bucuvalas JC. High prevalence of renal dysfunction in long-term survivors after pediatric liver transplantation. J Pediatr. 2006;148(4):475–80.
80. Davenport A. Continuous renal replacement therapies in patients with liver disease. Semin Dial. 2009;22: 169–72.
81. Fisher LR, Henley KS, Lucey MR. Acute cellular rejection after liver transplantation: variability, morbidity, and mortality. Liver Transpl Surg. 1995;1: 10–5.
82. Monteiro I, McLoughlin LM, Fisher A, de la Torre AN, Koneru B. Rituximab with plasmapheresis and splenectomy in ABO-incompatible liver transplantation. Transplantation. 2003;76:1648–9.
83. McDiarmid SV. Liver transplantation. The pediatric challenge. Clin Liver Dis. 2000;4:879–927.
84. Jain A, Reyes J, Kashyap R, Rohal S, Cacclarelli T, McMichael J, Rakela J, Starzl TE, Fung JJ. Liver transplantation under tacrolimus in infants, children, adults, and seniors: long-term results, survival and adverse events in 1000 consecutive patients. Transplant Proc. 1998;30:1403–4.
85. Bronster D, Emre S, Boccagni P, Sheiner PA, Schwartz ME, Miller CM. Central nervous system complications in liver transplant recipients-incidence, timing, and long-term follow-up. Clin Transplant. 2000; 14:1–7.
86. Sundaram SS, Alonso EM, Anand R, Study of Pediatric Liver Transplantation Research Group. Outcomes after liver transplantation in young infants. J Pediatr Gastroenterol Nutr. 2008;47:486–92.
87. Muiesan P, Vergani D, Mieli-Vergani G. Liver transplantation in children. J Hepatol. 2007;46(2):340–8.
88. Magee JC, Krishnan SM, Benfield MR, Hsu DT, Shneider BL. Pediatric transplantation in the United States, 1997–2006. Am J Transplant. 2008;8(4 pt 2):935–45.
89. Farmer DG, Venick RS, McDiarmid SV, et al. Predictors of outcomes after pediatric liver transplantation: an analysis of more than 800 cases performed at a single institution. J Am Coll Surg. 2007;204(5): 904–14.

25 Chronic Liver Disease, Cirrhosis, and Complications: Part 1 (Portal Hypertension, Ascites, Spontaneous Bacterial Peritonitis (SBP), and Hepatorenal Syndrome (HRS))

Ross W. Shepherd

Portal Hypertension

Introduction

Portal hypertension due to cirrhosis or pre- or posthepatic vascular events is a major cause of morbidity and mortality at all ages. Signs and symptoms of portal hypertension are primarily the result of decompression of elevated portal blood pressure through portosystemic collaterals. The major problems in children are bleeding varices, ascites and its complications (spontaneous bacterial peritonitis and hepatorenal syndrome), and malnutrition. Encephalopathy and portopulmonary hypertension, while important when they do occur, are seen less frequently in children. Splenomegaly with or without hypersplenism are common presenting features but rarely require specific intervention. Children with these conditions provide special challenges in understanding and management because of a predominance of congenital etiologies, combined with growth and developmental considerations.

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Pathophysiology

In general, portal hypertension occurs when *portal pressure rises above 10 mmHg*, most often resulting from increased portal resistance, combined in some circumstances with increased portal blood flow. The physiological basis for maintenance of portal pressure is in accordance with Poiseuille's law (or its analogue, Ohm's law), where changes in portal pressure are proportional to alteration in blood flow and resistance. For example, in cirrhosis, there is initially an increase in intrahepatic resistance and then an increase in splanchnic blood flow which maintains or further increases portal pressure, giving rise to a hyperdynamic circulatory state, with increased cardiac and decreased splanchnic arteriolar tone, both of which further increase portal inflow. These dynamic vascular alterations are effected by *humoral mediators*, such as glucagon, prostaglandins, nitric oxide, and endothelium-derived relaxing factor, *changes in intravascular volume*, and *alterations in adrenergic tone in the splanchnic system*.

A full understanding of the effects of portal hypertension requires knowledge of the anatomy and physiology of the portal system in infants and children (Fig. 25.1). In fetal life the ductus venosus connects the umbilical vein and the inferior vena cava, and the umbilical vein joins the left branch of the portal vein, providing nutrient-/hormone-rich blood to the developing liver. Importantly, these may remain patent in some situations postnatally. In post-fetal life, portal capillaries

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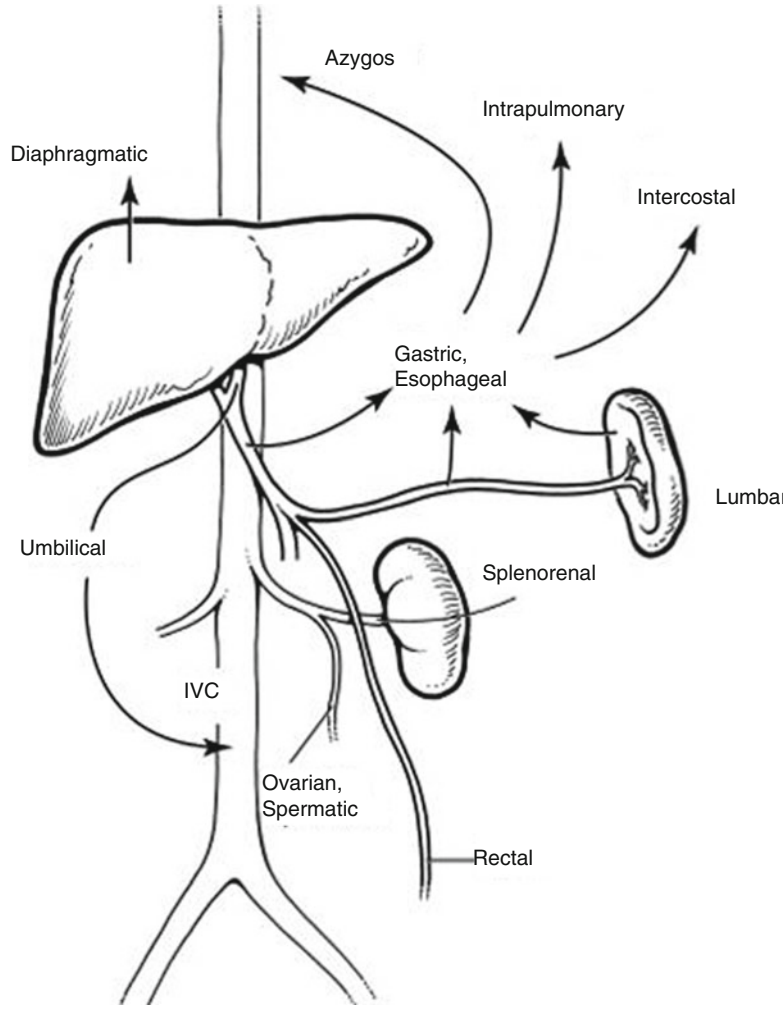

Fig. 25.1 The portal system and sites of portosystemic shunts in portal hypertension. A major consequence of portal hypertension is the development of collaterals between the portal venous system to the systemic circulation resulting in gastric and esophageal varices and other naturally occurring shunts from the splenic system, around the rectum, from the left renal vein, through the falciform ligament, and via the umbilical vein remnant into the inferior vena cava

originating in the mesentery of the intestine and spleen supply the portal vein with its nutrient-rich and hormone-rich blood supply. At the liver hilum, the portal vein divides to supply the right and left lobes of the liver, which then undergo a series of divisions supplying segments of the liver, terminating in small branches which pierce the limiting plate of the portal tract and enter the sinusoids through short channels. The partly oxygenated portal venous blood supplements the oxygenated hepatic arterial blood flow to give the liver unique protection against hypoxia.

A major pathological effect of portal hypertension is the development of collaterals carrying blood from the portal venous system to the systemic circulation in the upper part of the stomach and esophagus, the rectum, and the falciform ligament and may drain into the inferior vena cava via the umbilical vein remnant or the left renal vein (Fig. 25.1). Absence or disconnection of the inferior vena cava and/or interruption to the azygos system, such as occurs in some cases of biliary atresia, may cause special concern. Similarly, in extrahepatic portal venous malformations, the splenic vein can be small or thrombosed. Only the submucosal collaterals, such as in the esophagus and stomach, and sometimes in other parts of the intestine, particularly from stoma and anastomotic sites, are associated with gastrointestinal bleeding. Portal hypertensive gastropathy, which is suggested by dilated mucosal veins and capillaries and mucosal congestion in the stomach,

develops particularly in patients with cavernous transformation of the portal vein and may occur after esophageal variceal obliteration.

Causes of Portal Hypertension in Children (Table 25.1)

Portal hypertension may be derived from increased portal vascular resistance due to either *extrahepatic* (portal vein), *posthepatic* (hepatic vein), or *intrahepatic* block, where the block may be presinusoidal, sinusoidal, or postsinusoidal.

Extrahepatic portal venous obstruction, caused by a congenital thrombotic or atretic process or an acquired stenosis or thrombosis (most often in the context of portal vein anastomoses in liver transplantation), is an important cause of portal hypertension in children. Septic or traumatic umbilical vein injury from omphalitis and/or catheterization accounts for some cases, but most are idiopathic or perhaps a congenital malformation, where the portal vein is transformed into a cavernoma. The liver, while often small and underperfused, is histologically normal. Splenic vein or more diffuse portal system obstruction results in somewhat different hemodynamics, with an extensive collateral circulation involving a preponderance of gastric varices and sometimes ectopic varices involving paracholecystic, paracholedochal, and pancreaticoduodenal veins. Esophageal and/or gastric variceal bleeding is the most important clinical consequence, although, importantly, the occurrence of naturally occurring shunts may over time reduce the risk, usually by the second decade of life. Other features include hypersplenism with mild hemolytic anemia; easy bruising from thrombocytopenia; growth retardation, due to malabsorption, in turn due to failure of the enteropancreatic and enterohepatic circulation; and encephalopathy, due to shunting. Overt encephalopathy appears uncommon, except in the acquired forms, but subclinical signs including learning failure may occur. Although the liver may appear normal, reversible decompensation may be seen after an acute variceal hemorrhage, and functional compromise may develop in the long term.

Intrahepatic portal hypertension results from a range of presinusoidal, sinusoidal, and postsinusoidal causes of increased portal bed resistance within the liver:

Presinusoidal conditions, such as *congenital hepatic fibrosis* and *nodular regenerative hyperplasia,* do not result in impaired liver function. Congenital hepatic fibrosis is a developmental disorder that belongs to the family of hepatic ductal plate malformations and is characterized histologically by a variable degree of periportal fibrosis and irregularly shaped proliferating bile ducts. Virtually all manifestations of the disease are related to portal hypertension—especially splenomegaly and varices—often with spontaneous gastrointestinal bleeding, presenting from early childhood into adult life. Liver biopsy is highly specific for the diagnosis.

Increased sinusoidal resistance and portal hypertension occur almost invariably in cases of *cirrhosis in children*. Cirrhosis is a chronic diffuse disease characterized by irreversible widespread hepatic fibrosis with regenerative nodule formation. The prominent fibrous tissue contains vascular anastomoses, which cause hemodynamic alterations and portosystemic shunting. This diffuse pathology superimposes on the primary liver disease often obscuring the nature of the original insult. The major clinical consequences are the result of both impaired hepatic function and portal hypertension. Progression to cirrhosis and portal hypertension in pediatric liver diseases is highly variable and an important consideration in management. In some conditions, such as neonatal extrahepatic biliary atresia, the development of portal hypertension can be extraordinarily rapid, occurring by 12–16 weeks of age. Other conditions, such as cystic fibrosis-associated focal biliary cirrhosis, can be compatible with normal liver function for many years, presenting with signs of portal hypertension in the second decade of life.

Postsinusoidal intrahepatic conditions, such as sinusoidal-obstruction syndrome (*veno-occlusive disease*), are rare, most often occurring in the context of chemotherapy for

Table 25.1 Causes of portal hypertension in children

Extrahepatic
Extrahepatic portal vein obstruction/thrombosis
Congenital
Acquired – dehydration anastomotic (post-liver transplant)
Portal vein stenosis
Intrahepatic
Presinusoidal – congenital hepatic fibrosis, nodular regenerative hyperplasia
Sinusoidal – cirrhosis (biliary atresia, metabolic hepatopathies)
Postsinusoidal – veno-occlusive diseases
Posthepatic
Budd–Chiari syndrome (webs, thrombophilia, idiopathic)
Anastomotic (post-liver transplant)
Constrictive pericarditis
Post-Fontan procedure

childhood cancers or occasionally related to toxin ingestion, e.g., bush teas.

Posthepatic portal hypertension (Table 25.1) due to obstruction to hepatic venous outflow can occur after liver transplantation (hepatic vein stenosis), as an acute hepatic vein thrombosis (Budd–Chiari syndrome), or due to cardiac lesions resulting in increased right atrial pressure and/or chronic systemic venous hypertension. Budd–Chiari syndrome is rare and may occur with some thrombophilic disorders but is usually idiopathic. Of note are the effects of a *Fontan procedure*, a cavo- or atrio-pulmonary shunt which allows lifesaving systemic to pulmonary blood flow for single-ventricle syndromes in neonates but results in chronic systemic venous hypertension (pressures may be >20 mmHg) and eventually portal hypertension, commonly with protein-losing enteropathy.

Clinical Features (Table 25.2)

The main clinical features of portal hypertension are splenomegaly; the occurrence of esophageal, gastric, and rectal varices; ascites and hepatorenal syndrome; nutritional growth failure; and encephalopathy. Note that splenomegaly is not present in splenic malformation syndromes and temporarily absent during acute variceal bleeding.

Table 25.2 Clinical features of portal hypertension in children

Splenomegaly
Esophageal varices with bleeding
Portal gastropathy with bleeding
Recurrent epistaxis, easy bruising
Ascites
Malabsorption
Protein-losing enteropathy
Growth failure
Anemia
Thrombocytopenia
Cyanosis and clubbing

In extrahepatic portal hypertension, or when there is compensated liver disease, there may be no symptoms. The first indication of portal hypertension may be a gastrointestinal bleed, an incidental finding of splenomegaly, or anemia and thrombocytopenia due to hypersplenism. Commonly the liver is small and impalpable, but if due to an intrahepatic cause, it can be enlarged, hard, or nodular with palpable splenomegaly. Cutaneous features such as spider angiomata, prominent periumbilical veins (caput medusae), and palmar erythema may provide a clue. Spider angiomata may occur in healthy children under the age of 5 years and are thus not pathognomonic, but the appearance of new spiders or more than five or six may be indicative of portal hypertension. They are frequently observed in the distribution of vascular drainage of the superior vena cava and feature a central arteriole from which radiates numerous fine vessels, ranging from 2 to 5 mm in diameter. Other cutaneous features include easy bruising; fine telangiectasia on the face and upper back, white spots, most often on buttocks and arms, which when examined with a lens show the beginnings of spider angiomata; and clubbing of the fingers. On intranasal examination, prominent telangiectasia of Little's area (in the anteroinferior part of the nasal septum where four arteries come together to form a vascular plexus) is common, associated with recurrent epistaxis. Decompensated liver disease is characterized by clinical and laboratory findings

of liver synthetic failure including coagulopathy, commonly with other features including ascites and peripheral edema. Signs of hepatic encephalopathy may be subtle in children with portal hypertension. Malnutrition with reduced lean tissue and fat stores as well as poor linear growth is a well-recognized and important feature due to malabsorption and impaired protein synthesis. Portal hypertension may also be associated with changes in the systemic and pulmonary circulations, with arteriolar vasodilatation, increased blood volume, a hyperdynamic circulatory state, clubbing, and cyanosis due to intrapulmonary shunting. Renal failure is a late but serious event.

Diagnosis

While measurement of the hepatic venous pressure gradient (HVPG) is currently the best available method to evaluate the presence and severity of portal hypertension, defined as an HVPG to ≥10 mmHg, the clinical utility of HVPG measurements in managing children with portal hypertension is yet to be determined. In adults, HVPG measurements are increasingly being used for diagnosis, risk stratification, and monitoring of the efficacy of medical treatment. In the meantime, in children, confirmation of the diagnosis of portal hypertension is based on the suggestive clinical findings detailed above with or without signs of chronic liver disease and on four investigations: ultrasonography, endoscopy, liver biopsy, and angiography.

Ultrasonography with Doppler allows visualization and measurement of the size, patency, and flow of the portal vein, the occurrence of splenomegaly or a cavernoma, and information about liver size, hepatic homogeneity, and blood flow. Echocardiography is useful to exclude primary cardiac causes of hepatic venous outflow obstruction, when suspected.

Endoscopy can be performed to evaluate the presence and size of varices and the occurrence of cherry red spots (which correlate with risk of rupture) and visualize and perhaps treat the source of bleeding varices when occurring. Other features such as portal gastropathy and exclusion of other sources of GI bleeding can be visualized.

Liver biopsy either excludes liver disease, in the case of extrahepatic obstruction, or aids in the diagnosis of intrahepatic or prehepatic causes of portal hypertension. Notably, a full range of laboratory and imaging investigations should be performed prior to performing a liver biopsy to inform handling of the liver biopsy specimen with respect to specific histological and biochemical analyses. Differentiation between cirrhosis, presinusoidal, and extrahepatic causes of portal hypertension can sometimes be difficult. In presinusoidal and extrahepatic conditions, there are no signs of chronic liver disease, and transaminases and synthetic function are typically normal. In congenital hepatic fibrosis, for instance, the liver is enlarged and hard, but histologically hepatocytes are normal with prominent abnormal bile ducts in wide bands of fibrous tissue, but no nodules. In extrahepatic portal hypertension, the liver may be small but is histologically normal, although some steatosis may be evident. In contrast, obstruction to hepatic venous outflow causes centrilobular hemorrhagic necrosis with fibrosis extending from central veins to portal tracts.

Angiography, either with direct venography, MR angiography, or CT angiography, can provide important information about the site of block, the size of the liver and spleen, direction of blood flow and patency of major veins in the portal system, and relationship to the coronary, esophageal, or other varices. Suspected obstruction to hepatic venous outflow requires venography and/or cardiac catheterization which are the diagnostic procedures of choice in such cases. Pressure gradient measurements may be useful across venous blocks and to determine the magnitude of the portal pressure.

Management of Portal Hypertension

There are pharmacological, endoscopic, and/or surgical means of controlling portal pressure, which might be indicated in certain circumstances, as a bridge to transplant or as an adjunct

Table 25.3 Pharmacotherapy in portal hypertension

Short-acting splanchnic vasoactive agents
Growth hormone-inhibiting factors
Somatostatin
Octreotide (25 mg/h i.v. infusion or 50 mg/8 h s.c.)
Vasoconstrictors
Vasopressin (0.3 units/kg/h infusion for 24 h)
Terlipressin (0.3–1.0 unit/kg/h infusion)
Long-acting splanchnic vasoactive agents
Beta-adrenergic receptor blockers
Propranolol (1–5 mg/kg/day in three divided doses)
Atenolol (1 mg/kg/day in two doses)
Alpha-adrenergic receptor blockers
Clonidine[a] (10–20 mg/kg/day in three doses)
Combined alpha- and beta-blockade
Carvedilol
5HT, 5-hydroxy tryptamine tryptophan receptor antagonists
Ritanserin
Nitrovasodilators
Nitroglycerin[a] (5–10 mg patches)
Isosorbide 5 nitrate
Diuretics
Spironolactone (1.5–3.0 mg/kg/day in three doses)

[a]Reported adjunctive effect with propranolol

to therapies directed at the major complications, but predominantly, therapy of portal hypertension is directed at the major complications, i.e., the prevention and/or management of variceal hemorrhage, ascites, hepatorenal syndrome, and encephalopathy (see individual sections). In cases of end-stage liver disease, liver transplantation is the primary therapy. In cases of hepatic venous outflow block, directly treatable causes, such as vena caval webs, tumors causing obstruction, or constrictive pericarditis, need to be considered. Hypersplenism, while common, rarely results in morbidity or mortality.

Pharmacological therapy is directed towards reduction of splanchnic blood flow, with the added benefit of increasing renal blood flow (Table 25.3). In acute situations, e.g., GI bleeding, somatostatin and its longer-acting analogue octreotide (maximum dose 1 mg/kg/h i.v. or 2–4 mg/kg/dose subcutaneously 8-hourly) have fewer side effects and are the drugs of choice over other splanchnic vasoconstrictors, e.g., vasopressin, (0.3 U/kg bolus over 20 min, then continuous infusion of 0.3 U/kg/h, usually for 24 h or until the bleeding has ceased, or its inactive precursor terlipressin, 0.01 mg/kg bolus 4- to 6-hourly or 0.05 mg/kg infusion over 6 h for 24–48 h). Side effects include skin pallor, abdominal colic, and chest pain. Adjunctive vasodilators, such as nitroglycerine in the form of a 10-mg patch, may reduce these effects, although are rarely required in children. Long-acting vasoactive drugs, e.g., beta-blockers (propranolol and the more selective atenolol), at high enough doses may reduce hepatic arterial and portal vein blood flow and portal pressures to <12 mmHg, suggesting a benefit for both primary and secondary prophylaxis against GI bleeding. In adults, a combination of a nonselective beta-blocker and certain nitrates (e.g., isosorbide 5-mononitrate) or combined alpha- and beta-blockade is the drug of choice, aiming at a 25 % reduction in resting heart rate. Their use in children with portal hypertension remains unstudied, however, and concern exists with their use for young children whose main mechanism of compensation to acute hemorrhage is to increase cardiac output by increasing the heart rate. When used, major adverse effects include reactive airway disease and heart block, where they are contraindicated.

Endoscopic management of portal hypertension is directed at *the treatment of variceal hemorrhage* which clinically presents either as a need for emergency therapy or as a need for prophylaxis of initial or subsequent rebleeding. Episodes of minor variceal hemorrhage may spontaneously terminate, but endoscopic treatment of acute variceal bleeding by sclerotherapy or band ligation is indicated for continued bleeding after initial resuscitation and coagulation is achieved. In recognized cases of portal hypertension with varices which have not yet bled, controversy exists as to whether any therapy reduces the risk or prevents the occurrence of gastrointestinal bleeding. However, in all cases, it is reasonable to be prepared for the possibility by ensuring that the child's caregivers understand the importance of seeking early medical advice by attending the nearest hospital for blood crossmatching and appropriate referral to a tertiary unit. There may be a case under certain circumstances (e.g., distance from tertiary center) for

prophylactic endoscopic therapy, but the potential for bleeding of known varices which have never bled in children is conjectural. One controlled clinical trial in children found a reduced risk of bleeding, but an increase in portal gastropathy. Where bleeding has occurred, rebleeding risk is reduced by direct obliteration of the varices usually over 2–3 sessions, although consideration of the underlying liver disease is the major determinant of long-term management. Randomized controlled trials in adults have shown a reduction in the frequency of bleeding and improved survival. Although no randomized controlled trials have been performed in children, several large studies of sclerotherapy or banding in children with portal hypertension indicate that these procedures are safe and they reduce the chance of rebleeding.

Banding involves isolation and entrapment of a varix by suction and then applying a band ligature, using a special device attached to the endoscope. Sclerotherapy involves injection of sclerosant (ethanolamine, or tetradecyl sulfate) either para- or intravariceally, in volumes of 0.5–1.0 ml. Injecting or banding too high above the cardia can increase bleeding from a distal varix. Neither technique immediately reduces portal pressure, but they do reduce risk. With obliteration of varices, hypersplenism and portal gastropathy temporarily get worse, but ultimately with time, spontaneous portosystemic shunts can arise reducing portal pressures. Adult comparative trials of sclerotherapy vs ligation indicate equal efficacy in controlling bleeding, reducing rebleeding, and ablating varices but fewer adverse effects with banding. Both techniques are well described in children, and in general, they are complimentary; thus access to both is warranted. Band ligation may be technically difficult for small bleeding varices, particularly in infants, where band entrapment of part of the esophageal wall with perforation or bleeding can occur. In these circumstances, sclerotherapy is more appropriate. Broad-spectrum antibiotics should be prescribed (amoxicillin, cefuroxime, and metronidazole). Complications of both banding and sclerotherapy include bleeding, esophageal ulceration, perforation (mainly with banding in small children), stricture, and pain.

Table 25.4 Surgical options for portal hypertension

Orthotopic liver transplantation
Bridge to transplantation
TIPS (transjugular intrahepatic portosystemic shunt)
Nonselective shunts
Mesocaval (H-graft or direct)
Portacaval
Central splenorenal
Selective shunts
Distal splenorenal
Splenoadrenal
Restorative shunt
Rex shunt (mesenteric left portal bypass)
Anatomic repair
Hepatic or portal webs, stenosis
Devascularization
Sugiura procedure

Surgical management of portal hypertension is a major consideration for those in whom medical therapies have failed or as acute life-saving maneuvers. As there are various options (Table 25.4), those with primary liver disease should be evaluated and treated in a transplant center, where the range of surgical options, including liver transplantation, can be assessed. In critically ill patients with intrahepatic causes of portal hypertension, the use of a *TIPS (transjugular intrahepatic portosystemic shunt)* as a bridging procedure to liver transplant is the procedure of choice. A TIPS decompresses the high pressures in the portal circulation by placing a small stent between a portal and hepatic vein. As for all portosystemic shunts, one of the main complications of TIPS is hyperammonemia, with the potential for encephalopathy. *Surgical portosystemic shunts* may reduce the risk of gastrointestinal bleeding but decrease portal blood flow, decrease hepatic perfusion, and carry the risk of hepatic decompensation and hepatic encephalopathy, precluding or enhancing difficulty with liver transplantation. For those with preserved hepatic function who do not need transplant, e.g., patients with congenital hepatic fibrosis or extrahepatic portal hypertension, and some with CF liver disease, the choice of

the type of shunt is determined by the vascular anatomy, the size of the veins, the risk of thrombosis and failure, and the risk of encephalopathy. *Mesocaval shunts* and the more selective *distal splenorenal shunt* have been the procedures of choice in the past, though a *central splenorenal shunt with splenectomy* is sometimes advocated if there is massive hypersplenism and pain from splenic infarcts. These shunts may not be technically possible; however, because of thrombotic involvement of splenic or mesenteric veins and particularly where varices are derived from a cavernoma, these shunts may not alter variceal pressures in the coronary esophageal veins. The *Rex shunt*, usually an internal jugular vein graft mesenterico-left portal vein bypass, has the advantage of restoring portal flow to the liver, reducing risk of encephalopathy, and it obviates technical problems associated with splenic vein thrombosis. First introduced for portal vein thrombosis after liver transplant, this procedure may become the surgical procedure of choice for all causes of extrahepatic portal vein obstruction. In the rare cases of an obstructed hepatic venous outflow, vena caval webs, etc. can be anatomically repaired. In unusual circumstances, a *Sugiura procedure* (esophageal disconnection/devascularization procedure) may be lifesaving, with the added advantage of a low risk of encephalopathy. Finally, although splenectomy is sometimes considered for treatment of hypersplenism, it is usually not indicated, as it rarely results in any morbidity or mortality.

Ascites

Extravascular fluid accumulation, manifest predominantly in the peritoneal cavity as ascites and sometimes accompanied by peripheral edema or pleural effusions, is a common complication of portal hypertension and a sign of advanced liver disease. Ascites poses an increased risk for infections, particularly spontaneous bacterial peritonitis (SBP), as well as hepatorenal syndrome, and is typically a predictor of the need for liver transplantation in those with chronic liver disease.

Clinical Features

Clinical signs include pitting-dependent and facial edema, abdominal distension, and/or the development of hernias. Patients with gross or refractory ascites may have breathing difficulties, abdominal pain, or limitation of movement. There is shifting dullness on abdominal percussion. Abdominal ultrasonography may confirm clinical suspicion. Renal and circulatory dysfunction manifest as dilutional hyponatremia, low arterial blood pressure, low serum albumin, serum creatinine >1.2 mg/dl, and sodium retention (urine sodium less than 10 mEq/day or a urine sodium > urine potassium) may occur.

Pathophysiology

The two important factors in extravascular fluid accumulation are high portal venous pressure and low plasma oncotic pressure, both of which interact to result in fluid redistribution between intra- and extravascular spaces. Ascites represents a breakdown of intravascular volume homeostasis, which is controlled by capillary hydrostatic pressure and plasma colloid osmotic pressure, with *sodium retention and decreased effective plasma volume*. It may develop insidiously, e.g., in cirrhosis, particularly if malnourished, occur acutely after vascular events such as Budd–Chiari syndrome or portal vein thrombosis, or be precipitated by events such as gastrointestinal bleeding or infection.

Three related models explain the formation of ascites:

The *underfilling model* suggests that there is increased sinusoidal pressure leading to a cascade of events resulting in fluid retention from elevated portal venous pressure, increased splanchnic volume, decreased systemic vascular resistance, and decreased effective plasma volume. Resultant activation of the plasma renin–angiotensin–aldosterone system causes avid renal retention of sodium and water, leading to the accumulation of fluid. Low intravascular oncotic pressure and increased resistance to splanchnic venous outflow result in the

accumulation of ascites This is supported by the fact that expansion of the plasma volume by albumin infusion commonly reverses ascites, decreases levels of renin and aldosterone, and results in a diuresis.

The *peripheral arterial vasodilation model* suggests that, in cirrhosis, peripheral arterial vasodilation is the initiating event in ascites formation. Sodium retention is the consequence of a homeostatic response similar to the underfilling model, with underfilling of the arterial circulation secondary to arterial vasodilatation in the splanchnic vascular bed. This underfilling is sensed by arterial and cardiopulmonary receptors and activates antinatriuretic factors, resulting in hypervolemia. The retained fluid initially compensates for the disturbance in the arterial circulation and suppresses the activation of sodium-retaining mechanisms. However, as the vasodilatation in the splanchnic circulation causes more marked arterial underfilling, the retained fluid does not fill the intravascular compartment adequately. Because fluid is leaking continuously into the peritoneal cavity, the sodium-retaining mechanisms become permanently activated. This theory is supported by the fact that patients with chronic liver disease are prone to the development of arteriovenous connections, implying the presence of vasoactive hormones known to be associated with peripheral vasodilatation and renal sodium retention.

The *overflow model* suggests that inappropriate renal sodium and water retention is the primary abnormality triggered by a possible hepatorenal reflex. This is supported by animal models but is mitigated by the observation that the renin–angiotensin–aldosterone system is activated in decompensated cirrhosis. These systems should be suppressed and not activated with sodium retention and volume expansion.

These models are not necessarily mutually exclusive. Early overflow secondary to renal sodium retention may be the initiating factor, but later, diminished effective plasma volume with its accompanying hormonal changes may predominate, leading to peripheral arterial vasodilatation and further increase in sodium and water retention.

Management of Ascites (Box 25.1)

In the majority of patients, limitation of sodium ingestion, judicious use of diuretics (to increase sodium and water excretion by reducing the tubular reabsorption of sodium), and maintenance of plasma oncotic pressure (by albumin infusions) will control ascites. Reduction of fluid intake and salt restriction to reduce ascites is commonly prescribed in adults, but in children, this may have deleterious effects on growth and should be balanced by an increase in calorie content of feeds. Preferred diuretics include an aldosterone antagonist, and, if necessary, an adjunctive thiazide. The response to diuretic therapy should be evaluated by measuring body weight, urine volume, serum electrolytes, and blood urea nitrogen and sodium excretion. The initial goal of treatment is a negative fluid balance of ~10 ml/kg/day. Higher negative balances risk plasma volume depletion and decline in renal function, which may be preempted by albumin infusion. The response to spironolactone is so reliable that the plasma volume status of the patient should be investigated if no significant diuresis is achieved within 2–4 days. Loop diuretics, e.g., furosemide 1 mg/kg, are effective in conjunction with albumin infusions, but are not recommended for chronic use alone, as electrolyte and plasma volume depletion can result in prerenal failure, encephalopathy, and arrhythmias. Rarely, patients either do not respond to diuretic therapy or have diuretic-induced complications that prevent the use of high doses of these drugs.

Those with symptomatic gross ascites may require paracentesis, but this is rarely required in the treatment of children, except where there is respiratory compromise and/or abdominal compartment syndrome. Diagnostic paracentesis is indicated for unexplained fevers and suspected SBP (protein concentration <20 g/l, leukocytosis) and in the diagnosis of Budd–Chiari syndrome where an acute onset of ascites is associated with protein concentrations >20 g/l.

Box 25.1. Management of Ascites

- Mild ascites with no discomfort or difficulties with mobility or breathing requires no specific investigation or treatment in most cases
- Lab tests include liver panel, albumin electrolytes, creatinine, BUN, and urine Na/K
- Monitor weight, fluid balance, and BP if low albumin
- Nutritional support, with maintenance of adequate protein homeostasis, is important. Note: fluid/salt restriction not usually necessary in children (–>deleterious effects on nutrition). Avoid Xs dietary sodium >2–3 mmol/kg
- Diuretics: start an aldosterone antagonist +/– a thiazide diuretic, e.g., *spironolactone* 3 mg/kg bid up to 6 mg/kg <10 years and 100–200 mg bid up to 600 mg >10 years, *aldactizide* 12.5 mg qid <3 years, 50 mgqid >3 years, or furosemide (dose ratio spironolactone to furosemide 10:2, i.e., 0.6–1.2 mg/kg/day). Side effects: hyponatremia, hyperkalemia. *Note*: furosemide alone is *not* recommended for chronic use, as electrolyte and plasma volume depletion can –>prerenal failure, encephalopathy, and arrhythmias
- The goal of diuretic treatment is a negative fluid balance of ~10 ml/kg/day initially. Spironolactone takes 2–4 days to take effect. A urine Na>K or urine Na >15 mmol/day indicates a response. If diuresis occurs at a faster rate, there is risk of plasma volume depletion –>prerenal failure
- Maintenance of effective plasma volume by albumin infusions 2 g/kg + furosemide 2 mg/kg repeated as necessary for albumin <2.0
- Paracentesis: indicated for gross tense ascites –>breathing/renal difficulties (see below) or for diagnosis of unexplained fevers, e.g., spontaneous bacterial peritonitis (protein >20 g/l, leukocytosis) and in Budd–Chiari syndrome (protein <20 g/l)
- Large volume paracentesis, combined with albumin infusions (1 g/100 ml tapped), is the preferred intervention for refractory symptomatic ascites, based on controlled trials demonstrating relative safety and efficacy. Continuous drainage is not encouraged due to risk of bacterial peritonitis
- In severe refractory or recurrent cases, where portal hypertension is also an issue, a transjugular intrahepatic portosystemic shunt (TIPS) temporarily decreases portal pressure, decompresses the liver and reduces sinusoidal and splanchnic pressure, and can act as a bridge to transplant
- In chronic liver disease, the only treatment of proven value for improved long-term survival is liver transplantation
- Refractory ascites in adults has been treated with peritoneal venous shunts (Leveen or Denver), which permit flow of ascitic fluid into the venous system causing resolution of ascites and improved urinary output, but coagulopathy, infection, and cardiac failure may develop due to the shunt. Their use has not been reported in children

Spontaneous Bacterial Peritonitis

SBP is a relatively common and a potentially fatal complication of ascites in children. The condition should always be suspected in a patient with ascites and concurrent fever, abdominal pain, or neutrophilia.

Pathophysiology and Microbiology

Bacterial infections are common in chronic liver disease and may precipitate other complications,

such as encephalopathy, ascites, and hepatorenal syndrome. Immune deficits associated with chronic liver disease include abnormalities of complement fixation and opsonization, impaired function of Kupffer cells, neutropenia, and alterations in mucosal barriers, particularly the gastrointestinal tract. Portal hypertension makes patients susceptible to bacteremia and SBP, perhaps by inducing bacterial translocation of the gut in the setting of impaired immunity.

Specific risk factors for SBP are ascites, low serum albumin, gastrointestinal bleeding, intensive care unit admission for any cause, and recent therapeutic endoscopy.

Characteristically, spontaneous bacterial peritonitis in children is caused by a single species, often enteric bacteria such as *Klebsiella* spp., *E. coli,* and *Enterococcus*, although *Streptococcus pneumoniae* predominate. The presence of multiple species suggests the possibility of bowel perforation and secondary peritonitis. Culture-negative neutrocytic ascites (probable SBP) is not uncommon (the ascitic fluid culture results are negative, but the PMN count is 250 cells/μl or higher).

Prevention

In those patients with ascites, efforts to prevent and/or make a timely diagnosis of SBP are an important consideration, given the gravity of this complication. Appropriate management of ascites may minimize the risk. There is no evidence to suggest benefit from prophylactic antimicrobials, but preventative measures such as pneumococcal and *Haemophilus influenzae* vaccination, prophylactic antibiotics for invasive procedures, and nutritional support may reduce the risk of specific infection.

Clinical Features and Diagnosis

A high index of suspicion must be maintained when caring for patients with ascites. Common signs include rapid abdominal distension, fever, vomiting, and diarrhea. Examination may reveal abdominal tenderness with rebound and decreased or absent bowel sounds. Occasionally, SBP may be relatively asymptomatic except for fever.

The diagnosis is established by abdominal paracentesis, which reveals cloudy fluid with a neutrophil leukocyte count of >250/mm. A lactate level of >25 mg/dl in combination with an ascites fluid pH <7.35 is adjunctive evidence. There may be a low protein concentration <20 g/l (as distinct from secondary peritonitis due to intestinal perforation, where protein concentrations are higher). The use of reagent strips that detect leukocyte esterase, which correlates well with laboratory polymorphonuclear leukocyte counts, leads to more rapid diagnosis.

Management: While the final choice of antibiotics is dictated best by the bacteriology, early institution of therapy with a third-generation cephalosporin, such as intravenous cefotaxime, is recommended for 14 days. A randomized controlled trial found that supplemental intravenous albumin infusions, to support intravascular volume, can reduce renal impairment. Antimicrobial prophylaxis against this disorder has not been subjected to definitive trials, but clinical experience suggests that prevention may be achieved by the use of antibiotics during invasive procedures (as above) and, in cases of recurrent SBP, a prophylactic oral antibiotic such as cotrimoxazole, ciprofloxacin, or norfloxacin.

Hepatorenal Syndrome

This syndrome is a functional progressive renal failure of unknown cause occurring in patients with severe liver disease. It is a serious complication of cirrhosis and carries a poor prognosis, unless reversed or transplantation intervenes. It may be either rapidly or slowly progressive (type I and II, respectively). Clinically, there is often a progression from ascites, through diuretic-resistant ascites, to hepatorenal syndrome.

Pathophysiology

While the pathogenesis is not fully understood, reduced renal cortical blood flow is central to the

pathogenesis. There is also increased splanchnic blood pooling from portal hypertension, further decreasing renal blood flow, possibly related to upregulated endothelial nitric oxide (NO) synthase. Renal vasoconstriction may also contribute due to increased production of thromboxane, a potent vasoconstrictor, and a decrease in prostaglandin E2, a dilatory metabolite. A high incidence of glomerulosclerosis and membranoproliferative glomerulonephritis has been documented in children with end-stage liver disease at the time of liver transplant probably secondary to the chronic reduction in renal cortical blood flow. In addition to this, it has been observed that the administration of medications to counteract splanchnic vasodilation (e.g., octreotide) leads to improvement in glomerular filtration rate in patients with hepatorenal syndrome, providing further evidence that splanchnic vasodilation is important in pathogenesis. Reduced renal cortical blood flow also involves activation of the renin–angiotensin–aldosterone system, which leads to an increase in absorption of sodium from the renal tubule and is relevant to the pathogenesis of ascites. This suggests that there is a spectrum of progression from ascites to hepatorenal syndrome where splanchnic vasodilation defines both resistance to diuretic medications and the ascites (which is commonly seen in type 2 HRS) and the onset of reduced cortical blood flow leading to hepatorenal syndrome. Thus, efforts to increase glomerular filtration and renal blood flow and decrease splanchnic pooling form the basis of current approaches to supportive medical therapy.

Clinical Features and Diagnosis

Acute renal failure in children with liver disease may be due to primary renal disease, prerenal failure, acute tubular necrosis, or hepatorenal syndrome. The combination of liver failure and its associated circulatory abnormalities, refractory ascites, oliguria, and rising serum creatinine are suggestive of the diagnosis, although some patients with hepatorenal syndrome have a normal urine output. In some, there may be an added component of compartment syndrome related to tense refractory ascites.

Table 25.5 Features of hepatorenal syndrome

Low GFR, indicated by a serum creatinine level higher than 1.5 mg/dl or 24-h creatinine clearance lower than 40 ml/min and oliguria
Absence of shock, ongoing bacterial infection and fluid losses, and current treatment with nephrotoxic medications
No sustained improvement in renal function (decrease in serum creatinine to <1.5 mg/dl or increase in creatinine clearance to >40 ml/min) after diuretic withdrawal and expansion of plasma volume with plasma expander
Proteinuria less than 500 mg/day and no ultrasonographic evidence of obstructive uropathy or intrinsic parenchymal disease
Urinary/plasma indices are not absolute, but urine sodium is usually <10 mmol/l, serum sodium >130 mmol/l, urine osmolality > serum osmolality, and urine/plasma creatinine ratio rises >40 over 3–7 days

Diagnosis of hepatorenal syndrome is typically based on altered laboratory tests and exclusion of primary renal disease and prerenal failure (Table 25.5). In addition, the rate of onset, clinical picture, and urinary indices, such as urinary sodium and creatinine concentrations, and serum creatinine level may help to distinguish prerenal and types I and II hepatorenal syndrome. In type I, the onset is acute, precipitated by gastrointestinal hemorrhage, aggressive diuresis, or an associated deterioration of liver function. It is associated with oliguria, uremia, hyperkalemia, hyponatremia, and a low urinary sodium concentration (<10 mmol/l). Initial serum creatinine levels double in less than a week. These features are similar to prerenal failure, which responds to an acute volume expansion or acute tubular necrosis, where tubular casts and high urinary sodium (>30 mmol/l) are found. Plasma volume expansion alone does not improve renal function in hepatorenal syndrome. Type II is characterized by a slower development of oliguric renal failure with a marked reduction in glomerular filtration rate and hyponatremia, again with a low urinary sodium concentration. Specifically, the production of ascites that is resistant to the use of diuretic medications is characteristic of type II HRS. Serial measurement of urinary

sodium concentration and urinary osmolarity helps distinguish the condition of acute tubular necrosis, where the urinary sodium concentration may rise and the urine osmolarity is usually equal to plasma osmolarity. These measurements are unreliable if the patient is on diuretics, particularly furosemide.

Prevention

Specific precautions should be undertaken to prevent or identify and treat those at risk for HRS. Some of the triggers for HRS are induced by inappropriate treatment of ascites. The aggressive use of diuretic medications without colloid volume expansion should be avoided. Reduced renal blood flow associated with hypoalbuminemia should be minimized with colloid volume expansion. In those with refractory tense ascites, HRS can be induced by a compartment syndrome due to the pressure on the renal veins, and paracentesis (with appropriate volume expansion) may improve renal function. However, paracentesis without volume expansion can cause circulatory changes and precipitate HRS. In addition, nephrotoxic medications that are used either to treat complications (such as some antibiotics and antifungals) or other conditions may cause sufficient impairment in renal function in the cirrhotic patient to lead to HRS. Surveillance for and aggressive management of other triggers including hypovolemia, SBP, and gastrointestinal hemorrhage are advised.

Management

Renal failure is a serious problem in the setting of severe liver disease, but HRS is effectively reversed by liver transplantation. However, a liver is often not available in a timely manner in those who are candidates for transplantation. Acute oliguric renal failure should initially be managed with volume expansion as a diagnostic therapy as mentioned above. Once the diagnosis of HRS is realized, temporary improvement in renal function is possible with the use volume expansion in conjunction with splanchnic vasocontrictors. Thus, colloid volume expansion with regular albumin infusions (1–2 g/kg/day) in combinations with a splanchnic vasocontrictor by continuous infusion (octreotide, 3–5 mg/kg/day) or vasopressin analogues (terlipressin (0.04 mg/kg/day)) is recommended. There is evidence to suggest that a combination of midodrine and octreotide, respectively, a systemic vasoconstrictor and an inhibitor of splanchnic vasodilation, has advantages over the use of octreotide alone. Tense ascites should be relieved by paracentesis with colloid volume expansion. In refractory cases, particularly those with extreme fluid overload, renal replacement therapy may bridge individuals with hepatorenal syndrome to liver transplantation, although the condition of the patient may dictate the modality used. In selected refractory cases of HPS, in particular those with gastrointestinal bleeding, a TIPS may help reverse the hemodynamic component of the hepatorenal syndrome and improve renal function. Liver transplantation is tolerated reasonably well in patients with hepatorenal syndrome, and the HRS improves posttransplantation. The preexisting glomerular abnormalities and the posttransplant nephrotoxic effects of calcineurin inhibitors, however, may partly explain the high rates of renal dysfunction posttransplant (Table 25.6).

Table 25.6 Summary of management of hepatorenal syndrome

Prevention
Identify and treat risk factors
Maintain plasma oncotic pressure with colloid expanders
Avoid aggressive use of diuretics
Treat tense ascites (compartment syndrome)
High index for SBP
Avoid nephrotoxic medications
Treatment
Colloid volume expansion (albumin infusions 1–2 g/kg/day)
+ splanchnic vasoconstrictor by continuous infusion (octreotide, 3–5 mg/kg/day)
+/− systemic vasoconstrictor (midodrine)
Renal replacement therapy – bridge to transplant
TIPS – consider if also GI bleeding
Liver transplantation

26

Chronic Liver Disease, Cirrhosis and Complications: Part 2: Hepatic Encephalopathy and Other Systemic Effects

Complications of Chronic Liver Disease in Children

Naresh P. Shanmugam, Palaniswamy Karthikeyan, and Anil Dhawan

Introduction

The liver is a metabolically active organ where there is continuous synthesis and detoxification of several biologically active substances such as albumin, clotting factors, neurotransmitters and vasoactive amines. In end-stage chronic liver disease (CLD), there is an imbalance between the production and degradation of these biologically active substances. This leads to a disturbance of end-organ homeostasis particularly in the brain, kidney and lungs. The severity of end-organ dysfunction might not be proportional to the degree of liver synthetic functional impairment. It is important that these complications are anticipated and treatment is initiated in a timely manner. This chapter focuses on cerebral, renal, pulmonary, skeletal and cardiac complications associated with chronic liver disease. Complications related to portal hypertension, ascites, SBP and hepatorenal syndrome are discussed in the preceding chapter.

Neurological Complications in CLD

Neurological complications can occur in patients with CLD as a result of decreased hepatic detoxification and excretion, altered cerebral blood flow, coagulopathy and thrombocytopenia and impaired immune function. The resulting manifestations include encephalopathy with or without oedema, intracranial haemorrhage and infection [1]. The most common neurological complication in CLD is hepatic encephalopathy (HE).

Hepatic Encephalopathy

Hepatic encephalopathy (HE) is defined as a metabolically induced, potentially reversible, functional disturbance of the brain that may complicate acute and chronic liver disease [2]. Although HE is generally considered to be a reversible condition, some patients might not recover to their previous level of cognitive function after a severe episode of HE. The triggering insult may be an infection, electrolyte imbalance or hypoglycaemia on the background of CLD.

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The prerequisite for hepatic encephalopathy is inadequate hepatocyte detoxification due to either severe hepatocyte dysfunction or the presence of portosystemic shunts which allow splanchnic venous drainage to bypass hepatic first-pass detoxification mechanisms. Most of the literature on this subject is from adult studies. A working party at the 11th World Congress of Gastroenterology proposed standardised nomenclature to distinguish between various types of HE. Encephalopathy that occurs in acute liver failure (ALF) is categorised as type A, HE that occurs with a portosystemic shunt without any intrinsic hepatocellular disease as type B and in CLD, type C, recognising that end-stage CLD typically involves cirrhosis and some degree of portal hypertension and portosystemic shunting. Type C HE can be further subclassified into minimal, episodic or persistent. Minimal hepatic encephalopathy (MHE) is diagnosed only on psychometric analysis, there being no overt clinical signs and symptoms of encephalopathy. Episodic encephalopathy occurs in the presence of precipitating factors such as high protein intake, gastrointestinal bleeding or infection, and the term persistent HE is used when symptoms fail to resolve despite exclusion of exacerbating factors.

Clinical Features of Hepatic Encephalopathy

HE can be clinically graded using the West Haven criteria on a scale from 1 to 4 (Table 26.1). The Glasgow coma scale has less interobserver variability, however, and can be used to further assess conscious level [3] (Table 26.2). While severe grades can be easily diagnosed, mild to moderate encephalopathy manifests as subtle behavioural alterations and is more difficult to diagnose. Initially there is only mild intellectual impairment, with preserved verbal ability [4], and potentially altered sleep patterns. Worsening of HE leads on to asterixis (also called flapping tremor, best elicited in outstretched arms with wrists hyper extended and the fingers separated), hypertonia, hyperreflexia and lethargy. With progression of coma, hypotonia and areflexia occur. Occasionally, patients with HE have features reminiscent of Parkinson disease such as muscular rigidity, bradykinesia, hypokinesia, monotony of speech and tremors.

Table 26.1 West Haven criteria for grading of mental state [3]

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 behaviour
	Brisk reflexes and Babinski's sign
	Muscle rigidity
Grade 4	Deep coma (unresponsive to verbal or noxious stimuli)

Pathogenesis of Hepatic Encephalopathy

The pathogenesis of HE due to hepatic failure is thought to be multifactorial, and complex interactions between endogenous neurotoxins and altered neurotransmitters have been implicated. Ammonia is considered to be a marker of and a factor involved in the pathogenesis of HE, but other neurotoxins implicated include mercaptans, short- and medium-chained fatty acids, phenols and methionine derivatives. There are also altered ratios of excitatory and inhibitory neurotransmitters resulting in abnormal cerebral function; excitatory amino acids such as dopamine and norepinephrine are found in decreased quantities while inhibitory neurotransmitters such as GABA and serotonin are found in increased quantities. Additionally, there is increased formation of false neurotransmitters such as octo-

Table 26.2 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	Localises 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

pamine (β,4-dihydroxyphenethylamine) and phenylmethionine as a result of the accumulation of phenylalanine and tyrosine. Altered ratios of branched-chain amino acids to aromatic amino acids and changes in postsynaptic receptor activity are other proposed contributory mechanisms.. Increased blood–brain permeability has been documented in both acute and chronic liver failure and undoubtedly facilitates the mechanisms of HE pathogenesis discussed above.

Role of Ammonia in Hepatic Encephalopathy

Circulating ammonia is derived from several sources including small intestine enterocytes, muscle, kidney and, importantly, intestinal flora. In health, 80–90 % of the ammonia in the portal vein is cleared by the liver during first-pass metabolism and either converted to urea by periportal hepatocytes or glutamine by perivenous hepatocytes. When hepatocyte metabolism is impaired (liver failure) or bypassed (portosystemic shunt), blood ammonia levels rise.

The exact role of ammonia in the pathogenesis of HE remains elusive. It is known that glial cells metabolise ammonia to glutamine (Fig. 26.1), which then competitively binds to and inhibits glutamate receptors. Moreover, increased intracellular glutamine in astrocytes raises the intracellular osmotic pressure, leading to astrocyte swelling if occurring acutely, resulting in cerebral oedema. Furthermore, the oxidative stress triggered by ammonia toxicity in astrocytes results in increased intracellular calcium, leading to mitochondrial dysfunction, enhanced cytokine activity and impaired intracellular signalling [5].

Of the multitude of enteric bacteria, urease-producing gram-negative bacilli, such as *Enterobacter* and *Proteus*, contribute most significantly to the bacteria-induced ammonia load [6]. In the past, it was thought that ammonia in the portal circulation was exclusively produced by the gut flora, but current hypothesis suggests that small gut enterocytes produce ammonia in excess of intestinal bacteria, and hence gut decontamination alone has minimal effect on ammonia levels [7].

Although the gastrointestinal tract is the main source of ammonia and the liver is central to its elimination, the kidney is also involved in both production and elimination. Acid–base status and serum potassium levels have a direct effect on ammonia handling by the kidneys. Renal

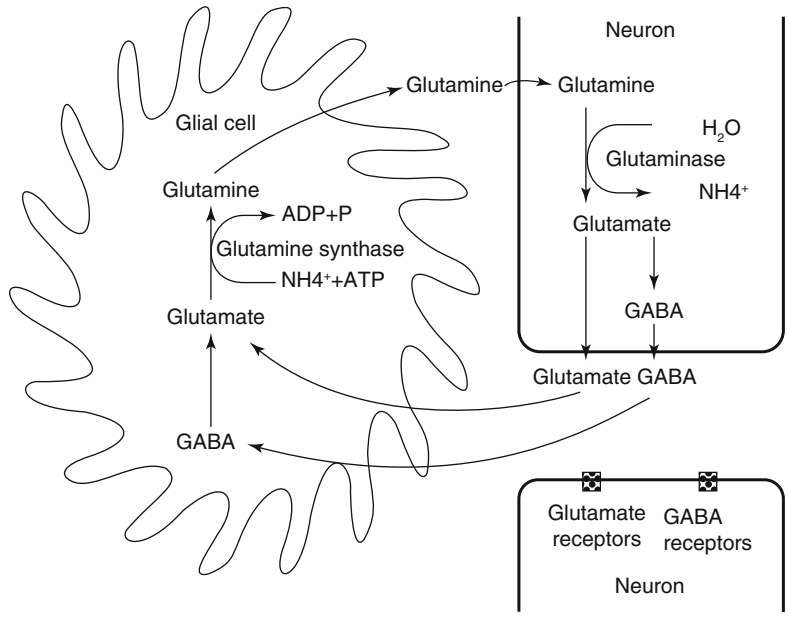


Fig. 26.1 Schematic representation of ammonia detoxification by glial (astrocytes) cells. Excess ammonia is incorporated into glutamate by glutamine synthase to form glutamine. Excess intracellular glutamine increases the osmotic load resulting in swelling of these cells causing cerebral oedema

metabolism of glutamine to glutamate produces ammonia and bicarbonate. Ammonium (NH_4^+) and urea are excreted in the urine [5]. The equilibrium between production and elimination of ammonia by the kidney mainly depends on body acid–base status. During acidosis ammonia production is increased in the kidney to facilitate excretion of hydrogen ions as NH_4^+, with bicarbonate produced during the process used as buffer. Around half the ammonia produced by the kidneys is excreted in urine and the rest returns to the circulation via the renal vein. It is believed that alkalosis decreases 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. In hypokalemia, low distal tubular potassium concentrations result in hydrogen ions being moved into cells resulting in intracellular acidosis which in turn leads to ammonia and bicarbonate formation from glutamine to buffer the acidosis; the ammonia then freely enters the circulation. Hence acidosis has a protective effect while alkalosis and hypokalemia can precipitate HE in patients with liver failure.

Myocytes can convert ammonia to nontoxic glutamine via glutamine synthetase, thereby decreasing the total ammonia load. This effect may only be temporary as glutamine can be converted back to glutamate and free ammonia, but it may explain the finding of a relative resistance to the development of HE in patients with good total muscle mass and that muscle mass depletion is an important risk factor for HE [8].

Role of Neurotoxins and Altered Neurotransmitters in Hepatic Encephalopathy

Elevated aromatic amino acids (AAA, phenylalanine, tyrosine and tryptophan) and decreased branched-chain amino acids (BCAA) are factors implicated in hepatic encephalopathy [9, 10]. The ratio of BCAA/AAA is called the Fischer ratio. There is a decrease in the Fischer ratio in liver failure due to preferential usage of BCAA by muscles and decreased clearance of AAA by the liver. The resultant elevated serum AAA levels, facilitated by increased permeability of the blood–brain barrier, causes an influx of AAA into the central nervous system leading to imbalances in neurotransmitter synthesis along with the production of false neurotransmitters such as octopamine and synephrine [11].

Thiols, commonly referred to as mercaptans, are a group of sulphur-containing organic com-

pounds derived in humans primarily by bacterial breakdown of proteins in the intestine. Usually they are found in trace amounts in blood, but in patients with liver failure, higher blood concentrations are seen. The role of mercaptans in liver cirrhosis has probably been overstated. Al Mardini et al. found that oral methionine supplemented to stable cirrhotics increased the serum methanethiol and dimethyl sulphide (mercaptans) levels but that no neurological abnormality was evident [12]. Furthermore serum levels of mercaptans were not significantly affected by neomycin and metronidazole therapy, which implies endogenous origin of mercaptans rather than as a by-product of bacterial metabolism.

Short-chain fatty acids (SCFA) such as propionate, butyrate and valerate are produced in small intestine by breakdown of carbohydrates and proteins by intestinal flora [13, 14]. These SCFAs may competitively inhibit several enzymes including urea cycle enzymes and also competitively binding to albumin thus displacing albumin-bound toxins. Table 26.3 highlights other bioactive substances imputed to play a role in HE.

Table 26.3 Other substances implicated to have a role in HE

Bioactive substances	Mechanism of action
Indole and oxindole [15]	By-products of bacterial tryptophan metabolism have sedating properties
Endogenous benzodiazepines "endozepines" [16]	Arise from gut bacterial production and act on GABAA receptors
Neurosteroids, such as allopregnanolone	Allosterically modulate the gamma-aminobutyric acid (GABA)-A receptors that suppress sensorium via increased "GABAergic tone"
Neuronal acetylcholinesterase (AChE) [17]	Causes reduction of acetylcholine by up to 50–60 %, thereby suppress sensorium
Chronic hyponatremia [18]	Depletion of intracellular organic osmolytes, like myoinositol, leading on to astrocyte swelling

Inflammatory Mediators in Hepatic Encephalopathy

Odeh et al. proposed the pro-inflammatory cytokine, tumour necrosis factor-α (TNFα), as an important contributor to hepatic encephalopathy [19]. TNFα is produced by immunologically active cells such as macrophages, monocytes, microglial cells, astrocytes and Kupffer cells. In liver failure there are several factors such as increased bacterial translocation from the gut, sites of infection and systemic inflammation that can lead to increased production of TNFα, coincident with decreased hepatic and renal clearance. The same group also showed that there is a positive correlation between TNFα levels and severity of liver failure represented by the Child-Pugh score [20]. TNFα levels were independent of aetiology of cirrhosis, infectious and non-infectious precipitating factors of HE. Additionally, a positive correlation between severity of HE and circulating levels of TNF in these patients was demonstrated [21]. TNFα increases microvascular permeability and arteriolar dilatation within the brain which can lead to capillary leakage and cerebral oedema. Goral et al. showed that other interleukins (IL-1β, IL-2R, IL-6 and IL-8), along with TNFα, were also elevated in liver failure [22].

Diagnosis

The diagnosis of encephalopathy is mainly based on clinical evaluation underscoring the importance of a good clinical history and thorough clinical examination [23]. Clinical grading using the West Haven criteria can be used in older children and the Glasgow coma scale can be used to grade the conscious levels in patients with higher encephalopathy grades [3]. Biochemical tests are of little value in making the diagnosis of HE as there is no specific biomarker. Ammonia is implicated in the pathogenesis of HE, but even the more accurate arterial levels (compared with venous levels) correlate poorly with the grade of encephalopathy and cannot be used to grade encephalopathy [3]. Neuroimaging and electrophysiological studies of the brain help in ruling

out other causes of encephalopathy, and the finding of cerebral oedema or characteristic electroencephalogram (EEG) changes can support, but not prove, the diagnosis of HE.

The clinical diagnosis of mild to moderate HE still remains a practical difficulty, particularly in children. This is due to the fact that it is practically impossible to perform detailed psychometric tests on young children.

Neuropsychological Assessment

To identify minimal hepatic encephalopathy, psychometric hepatic encephalopathy score (PHES) has been used [24]. PHES consists of a battery of tests that include the number connect test (NCT)-A and B, the line tracing test for time (*t*) and error (*e*), the serial dotting test and the digit symbol test used in adults. Portosystemic encephalopathy [25] index is a scoring system that combines mental state with arterial ammonia levels, degree of asterixis, the result of the NCT and EEG finding. In children, both of these tests are not validated and neuropsychological assessment should be made using tests such as Wechsler intelligence tests or the Dutch child intelligence test (Revisie Amsterdamse Kinder Intelligentie Test) [26]. While selecting neuropsychological tests for children, it is essential to check that validated norms exist for the selected age, sex, racial and ethnical subgroup. Neuropsychological assessment is cumbersome and time consuming and usually carried out as a part of research project or for patient-specific indications, such as poor scholastic performance in a child with liver disease, rather than as a routine investigation.

Critical Flicker Frequency

Critical flicker frequency threshold is a simple and reliable test for quantification of low-grade hepatic encephalopathy [27]. The principle of the test is to identify the point of switch over of a steady red light to a flickering light. A red light that flickers at a frequency of 60 Hz is used. At this frequency, a normal human eye perceives it as a steady light. The frequency of the light is gradually decreased to a point where the patient perceives that the light starts to flicker. The testing devise consists of a binocular apparatus and a press button, which the patient presses when the lights starts to flicker. Actual test is repeated nine times with the mean and standard deviation calculated. Using this technique it was found that patients with HE can pick up flickering only when the flicker rate falls to below 39 Hz, while cirrhotic counterparts without encephalopathy and normal individual can identify flickering at higher rates. The limitation of this test is that it can be used only on children above 8 years of age [26].

Electroencephalogram

The electrical activity of the brain, which can be recorded via an EEG, is helpful in distinguishing between various grades of HE. Using EEG, HE could be graded from 0 to 4. Grade 0 is normal with a regular alpha rhythm, whereas irregular background alpha rhythm and the appearance of a theta rhythm depict grade 1 encephalopathy. In grade 2, theta activity becomes continuous with an occasional delta wave. In grade 3 theta activity becomes prevalent with transient polyphasic complexes of spikes and slow waves. Grade 4, which is deep coma, will have continuous delta waves with abundant complexes of spikes and slow waves [23]. With availability of newer complex EEG analytical softwares, EEG can be analysed in specific regions of the brain, as a shift in beta waves from parieto-occipital areas to central areas of the cortex is seen in MHE in comparison to patients without encephalopathy [28].

Neuroimaging in HE

Apart from excluding organic causes of coma, such as intracranial bleeding, tumour and gross oedema, cerebral computer tomography is not a useful tool in diagnosis of HE. Magnetic resonance imaging (MRI) is a better modality for

the diagnosis of cerebral oedema. High signal intensity in the globus pallidum on T1-weighted images is a classic abnormality in cirrhosis and portosystemic shunt. This is thought to be due to high levels of manganese deposits in these areas. Basal ganglia structures are highly vulnerable to heavy metal deposits due to its high metabolic activity and increased blood flow. This probably could explain bradykinesia and rigidity seen in some long-term cirrhotic patients. Though this is a well-known finding, it is neither diagnostic nor prognostic of HE, because there is no apparent relationship between signal intensities and severity of HE. Conventional MRI lacks sensitivity for diagnosing milder forms of oedema and newer techniques such as such as magnetization transfer imaging (MTI), fast fluid-attenuated inversion recovery (FLAIR) imaging and diffusion-weighted imaging (DWI) are more sensitive in picking up brain tissue water content.

MTI can indirectly measure the macromolecular content in tissue, by using the differential property of free and bound water in a tissue. Decrease in MTI signal intensity directly correlates with the mild cerebral oedema, which otherwise could not be detected by standard MRI, severity of demyelination and axonal loss. High signal intensity in fast FLAIR images indicates mild brain oedema, which cannot be visualised using conventional MRI. In cirrhosis high signal intensity changes in white matter of the brain and also in the corticospinal tract can be visualised using fast FLAIR. Abnormalities in central motor pathway functions correlate with corticospinal tract changes when tested using transcranial magnetic stimulation (TMS) [29, 30]. These radiological changes revert after liver transplantation with normalisation of TMS. DWI helps in differentiating whether the excess water is in the intracellular or extracellular compartments. Proton MR spectroscopy can measure different levels of metabolites within tissues. Using this technique metabolic changes in the brain have been studied and show that glutamine/glutamate signal intensity increases in the presence of high ammonia levels and is accompanied by myoinositol depletion and decreases in the choline signal intensity.

Though routine MRI is helpful in making diagnosis of cerebral oedema and ruling out other cerebral causes of encephalopathy, specialised neuroimaging is of little benefit in routine clinical practice.

Management of Hepatic Encephalopathy

Management of HE varies with type of liver failure, i.e. acute or chronic. In acute liver failure, hepatic encephalopathy is of rapid onset and quiet resistant to medical management, often requiring urgent liver transplantation. In CLD, HE could be present as MHE for a long period without being noticed until sudden decompensation with precipitating events such as bleeding or intercurrent infection.

Assessment and Stabilisation

With the diagnosis of encephalopathy, the overall condition of the patient should be assessed and treatment should be directed according to severity of HE and any precipitating factors. As bleeding, electrolyte imbalance due to diuretics and infection are common precipitating factors, these causes should be specifically ruled out. If there is GI bleeding, blood transfusion along with emergency endoscopy should be carried out. Electrolyte disturbance has to be corrected gradually as rapid correction could lead to further neurological or cardiovascular complications. Rapid correction of hyponatremia is associated with central pontine myelinolysis, and hypokalemia can precipitate cardiac arrhythmias. Excessive use of diuretics may cause dehydration and hypovolemia, which has to be treated with fluid resuscitation. If the encephalopathy is grade 3 or 4, elective intubation must be considered to protect the patient's airway from aspiration. Once the patient has stabilised, the aim is to support all body systems until the liver recovers or transplantation can take place.

Fluids and Electrolytes

It is difficult to assess the hydration status in CLD due to the presence of ascites and oedema.

Despite increased total body water, these patients can be intravascularly fluid depleted. Hydration is best monitored by central venous pressure (CVP) measurement which ideally should be 6–8 cmH_2O. For fluid resuscitation, isotonic crystalloids or colloids can be used. For maintenance, it is better to consider fluids with low sodium such as 10 % dextrose with 1 mmol/kg of sodium and routine maintenance potassium. Although serum sodium is commonly low in patients with CLD, they are likely to have high total body sodium, and any attempt to normalise the sodium by giving additional sodium will lead to worsening oedema. Despite this supplementation with extra sodium may be required if the sodium level falls below 120 mEq/l [31]. It is advisable to start intravenous fluids at ~70 % of the total maintenance and then constantly assess fluid balance and titrate the intravenous fluids accordingly. Enteral feeds should be encouraged with no added salt.

Reducing the Ammonia Load

Lactulose

Lactulose is a non-digestible synthetic isomer of lactose, which is made of fructose and galactose monomers. Several mechanisms by which lactulose reduces ammonia level in liver failure patients have been suggested. Lactulose reaches the colon undigested where it is cleaved by colonic bacteria to fructose and galactose that are further fermented to acids such as acetic and lactic acid, which in turn decrease the luminal pH to be around 5. In an acidic environment ammonia is converted to NH_4^+ that less readily diffuses into circulation and is thereby removed along with stools. The acidic milieu is also detrimental to the majority of urease-producing organisms such as bacteroides and thus inhibits further bacterial fermentation limiting the production of short-chain fatty acids [32, 33].

Intestinal motility is enhanced by of the osmotic effect of lactulose metabolites as well as the gas formed by fermentation.

The amount of lactulose dose is generally titrated to produce three soft stools per day. Excessive dosage that produces diarrhoea should be avoided as it could lead on to electrolyte disturbances. Agrawal et al. showed that prophylactic lactulose along with probiotics in those who recovered from an episode of overt HE was significantly associated with fewer further episodes of HE [34].

Bajaj et al. showed that withdrawal of lactulose after prolonged use in CLD was associated with increased glutamine and glutamate, decreased myoinositol in brain and a reduction in stool faecal bacterium species [35]. These studies favour the use of long-term lactulose in CLD to prevent HE, but this will not change the ultimate requirement for need of liver transplantation. Lactitol (β-galactosidosorbitol) is another non-absorbable sugar alcohol which has similar action to lactulose but is less sweet and hence potentially more palatable to adults [36].

Antibiotics

Various antibiotics have been used to reduce the load of ammoniagenic bacteria in the gastrointestinal tract; neomycin, vancomycin and rifaximin are some examples. Of the antibiotics studied, rifaximin has been shown to be effective in decreasing ammonia production and has the best risk-benefit ratio [37]. When rifaximin is used as secondary prophylaxis after an overt episode of HE, it is associated with significantly fewer breakthrough HE episodes compared with placebo [38]. Furthermore, it has been suggested that the concomitant use of an appropriate antibiotic with lactulose may be more beneficial than either alone [39].

Probiotics

The concept behind the use of probiotics in hepatic encephalopathy is to replace ammoniagenic intestinal microflora with nonpathogenic bacteria that do not produce ammonia. Several bacterial stains such as *Lactobacillus acidophilus*, *Enterococcus faecium*, *Escherichia coli Nissle 1917* and *Bifidobacterium longum* have been used in HE with inconsistent results. Many studies that showed reversal of HE with probiotics also used lactulose in the treatment regimen and did so mostly in encephalopathy of minimal or mild grade [40, 41]. Systematic review and meta-analysis of randomised trials of probiotics when used as monotherapy for HE failed to

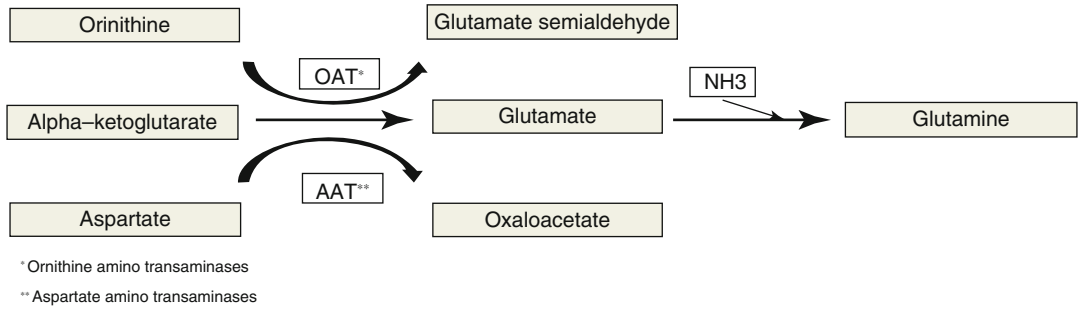


Fig. 26.2 LOLA enhances the action of ornithine and aspartate amino transaminases in the brain and peripheral tissues to produce glutamate, which helps in detoxification of ammonia

show its usefulness [42]. McGee et al. showed that though there is some reduction of plasma ammonia with probiotics, there was no change in overall outcome [43].

L-Ornithine L-Aspartate (LOLA)

LOLA is a salt of the amino acids ornithine and aspartic acid, both of which act as substrates in the urea cycle, detoxifying ammonia. Ornithine and aspartate stimulate the activity of carbamoyl phosphate synthetase I and arginase enzyme, respectively. In humans, the liver is the major site of urea cycle activity although the kidney and the intestine express some of the urea cycle enzymes. In liver failure there is a quantitative decrease in ammonia detoxification by the liver with only some of the excess ammonia converted to non-toxic glutamine by muscle [44]. LOLA enhances the action of ornithine and aspartate transaminases in brain and peripheral tissues to produce nontoxic glutamate (Fig. 26.2).

Small animal models have shown that action of LOLA is mainly peripheral, resulting in increases in plasma glutamine concentrations with no change in cerebrospinal fluid levels [45]. It was suggested that the protective effect of LOLA on brain is probably due to an overall decrease in ammonia level rather than direct effects on the brain.

Enhancing Alternative Pathways of Nitrogen Excretion

Although standard for hyperammonemia due to urea cycle defects, the use of medications that alternatively enhance nitrogen excretion, off-label, has been described for control of hyperammonemia secondary to liver failure [5]. Sodium benzoate (250 mg/kg/day) along with intravenous sodium phenylacetate (250 mg/kg/day) or oral sodium phenylbutyrate (250 mg/kg/day) has been used to eliminate ammonia by alternate pathways. Water-soluble hippuric acid and phenylacetylglutamine are formed by conjugation of benzoate with glycine and phenyl acetate with glutamine, respectively. Hippuric acid and phenylacetylglutamine are then excreted by the kidneys, thereby reducing the ammonia load [46]. These drugs, however, have high sodium content and can hence worsen the oedema in cirrhotic patients. Arginine hydrochloride (360 mg/kg/day) and L-carnitine (100 mg/kg/day) presumed to stimulate urea cycle and decreases ammonia levels [47]. In a randomised controlled trial, a significant improvement was seen on psychometric testing in adult patients treated with L-CARNITINE in comparison to patients on placebo [48]. Treatment of patients with HE with L-CARNITINE has also resulted in significant decreases in ammonia levels along with normalisation of EEG; L-carnitine also diminished muscle fatigue [48, 49].

Zinc

Zinc is an integral part of a metalloenzyme that stimulates the production of ornithine transcarbamylase, a key enzyme in the urea cycle and its deficiency might lead to decreased conversion of ammonia to urea. Serum zinc levels have been shown to be low in some patients with HE [50]. In a randomised controlled trial in adults, zinc supplementation in patients with HE was associated

with a decrease in serum ammonia and improvement in encephalopathy [51, 52]. However, there are no recommendations on dose and duration of zinc supplementation in children with HE.

Drugs of Doubtful Benefit

Several drugs have been tried to treat hepatic encephalopathy based on theories but without randomised control trials to prove benefit. In CLD there is a decrease in neurotransmitter synthesis with impaired central dopaminergic neurotransmission, resulting in parkinsonism-like features such as symmetric akinetic rigidity, postural but not resting tremor, gait impairment and neuropsychiatric symptoms along with hyperprolactinemia in HE [11, 53, 54]. Because of these findings, the use of the dopaminergic agonists, L-Dopa and bromocriptine has been tried; systematic review does not support the usefulness of these drugs in HE [55]. Similarly the benzodiazepine receptor antagonist flumazenil was not shown to be beneficial on systematic review [56]. Increased deposition of manganese in the brain in CLD and its clearance after liver transplantation led to the postulation that the use of chelators could be beneficial in HE [57] – clinical trials evaluating the effectiveness of chelation in HE are in development.

Extracorporeal Liver Support

There has long been interest in the possibility of providing extracorporeal liver support for periods of time while the liver regenerates following an acute insult, or as a bridge to liver transplantation. Liver support devices can be either "cleansing" devices or bioartificial liver support systems. Cleansing devices perform only the detoxifying function of the liver, whereas bioartificial liver support systems have the theoretical advantage of providing both the synthetic and detoxifying properties.

Cleansing devices such as the molecular adsorbent recirculating system (MARS) and fractionated plasma separation, adsorption and dialysis (Prometheus™) attempt to remove protein-bound toxins by perfusion of blood, plasma filtrate or albumin dialysate over anion exchange resins and charcoal.

The bioartificial liver support systems use bioreactors containing hepatocytes in columns. Anticoagulated whole blood or plasma is passed through a device, allowing metabolic transfer between perfusate and hepatocytes. These devices have been shown to decrease the toxins, provide hemodynamic stability and improve hepatic encephalopathy compared with standard medical therapies and could act as a support bridge while awaiting transplant in adults; there is not enough evidence of its usefulness in children [58]. High cost, nonavailability of small filters and lack of safety data in children limit the usage of these devices to clinical trial setting.

Liver Transplantation

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 in more than 85 % of children. Synthetic liver failure with encephalopathy is one of the indications for liver transplantation in chronic liver disease. Transplantation has to be done before permanent neurological damage occurs.

Pulmonary Complications

Lung disease is seen in association with chronic liver disease either as a secondary complication of chronic liver disease or because the primary disease process directly affects both liver and lung, such as in cystic fibrosis. Pulmonary complications are not only seen in cirrhotic liver disease but also in non-cirrhotic liver disease when associated with portal hypertension, probably due to hyperdynamic circulatory state or imbalance of vasoactive amines [59]. The common pulmonary complications of CLD are hepatopulmonary syndrome, portopulmonary hypertension and hydrothorax.

Hepatopulmonary Syndrome

The earliest report on association between liver disease and lung was reported by Fluckiger in eighteenth century [60]. In 1977, Kennedy and Knudson coined the term "hepatopulmonary syn-

drome" to describe the association of cirrhosis with cyanosis and clubbing. Hepatopulmonary syndrome (HPS) is characterised by the triad of liver disease, arterial hypoxemia and intrapulmonary vascular dilatation. The diagnostic criteria for HPS is the presence of CLD along with PaO_2 <70 mmHg or an alveolar-arterial oxygen gradient >15 mmHg and intrapulmonary vascular dilatation [59]. Depending on degree of intrapulmonary vascular dilatation, anatomical right to left shunting of blood can occur [61]. Capillary dilatation associated with hyperdynamic circulation in CLD leads to decreased blood transit time and acts as shunt.

The overall prevalence of HPS ranges from 4 to 30 % in cirrhotics [62]. Data in the paediatric population is scarce but data from small cohorts suggests prevalence of 8–20 % in cirrhosis [63, 64].

Pathogenesis

The development of pulmonary changes in common bile duct ligation (CBDL) animal models and absence of such changes in partial portal vein ligation models led to the hypothesis that portal hypertension (PHT) along with intrinsic liver disease is essential for development of HPS, as PHT was similar in both of the models [65]. This explains higher prevalence of HPS in disorders where there is intrinsic liver disease along with PHT such as biliary atresia splenic malformation syndrome (BASM) rather than in condition with predominant PHT such as portal vein thrombosis [66, 67]. The exact mechanism is still elusive. HPS is probably due to the effect several vasoactive substances, such as nitric oxide (NO), carbon monoxide (CO), prostaglandins, vasoactive intestinal peptide, calcitonin and glucagon, have on pulmonary vasculature. These substances are thought to be involved in angiogenesis and vasodilatation of pulmonary vasculature leading onto portopulmonary and hepatopulmonary shunts. NO is a potent vasodilator and its nitrates and nitrite metabolites are found to be high in exhaled air of patients with HPS, which normalises after liver transplantation [62, 68]. Nitric oxide synthase is an enzyme that helps in production of nitric oxide from L-arginine. It has several isoforms of which endothelial NO synthase (eNOS) was found to be increased in pulmonary small alveolar vessels in small animal models of HPS [69]. Elevated levels of endothelin 1 (ET-1) in blood, and increased expression of its receptor endothelin B (ETB) expression in the pulmonary vasculature, result in increased eNOS synthesis and thus vasodilatation [60]. NOS inhibitor N(G)-nitro-L-arginine methyl ester (L-NAME), when administered in nebulised form, decreased the exhaled NO, but failed to have any significant effect on oxygenation, implying factors other than NO is involved in HPS [70].

Zhang et al. showed in rat models that overproduction of TNFα due to endotoxin stimulation of macrophages via mitogen-activated protein kinase (MAPK) signal transduction pathway could play a role in pathologic alterations of HPS [71]. Intestinal mucosal oedema secondary to portal hypertension in cirrhosis results in increased bacterial translocation and endotoxemia, resulting in macrophage activation and TNF-alpha production. This hypothesis was further supported by the fact that gut decontamination with antibiotics or inhibition of TNFα production with pentoxifylline (a nonspecific phosphodiesterase inhibitor) decreased both the incidence and severity of HPS [72, 73]. The hypoxia in HPS is due to ventilation perfusion mismatch. In lung areas where there are capillary dilations, there is more perfusion compared to ventilation, while in areas with steal effect, the blood is diverted away from the alveoli resulting in less perfusion. Due to this variable shunting, 100 % oxygen inhalation does not improve PaO_2 in all cases of HPS; those with predominant intrapulmonary vascular dilatation would improve their PaO_2 while those with prominent shunting won't improve.

Clinical Manifestation

Clinical manifestations of HPS are those of hypoxia such as dyspnea, clubbing and cyanosis. Spider nevi have been considered to be a sensitive marker of HPS. In HPS, dyspnea

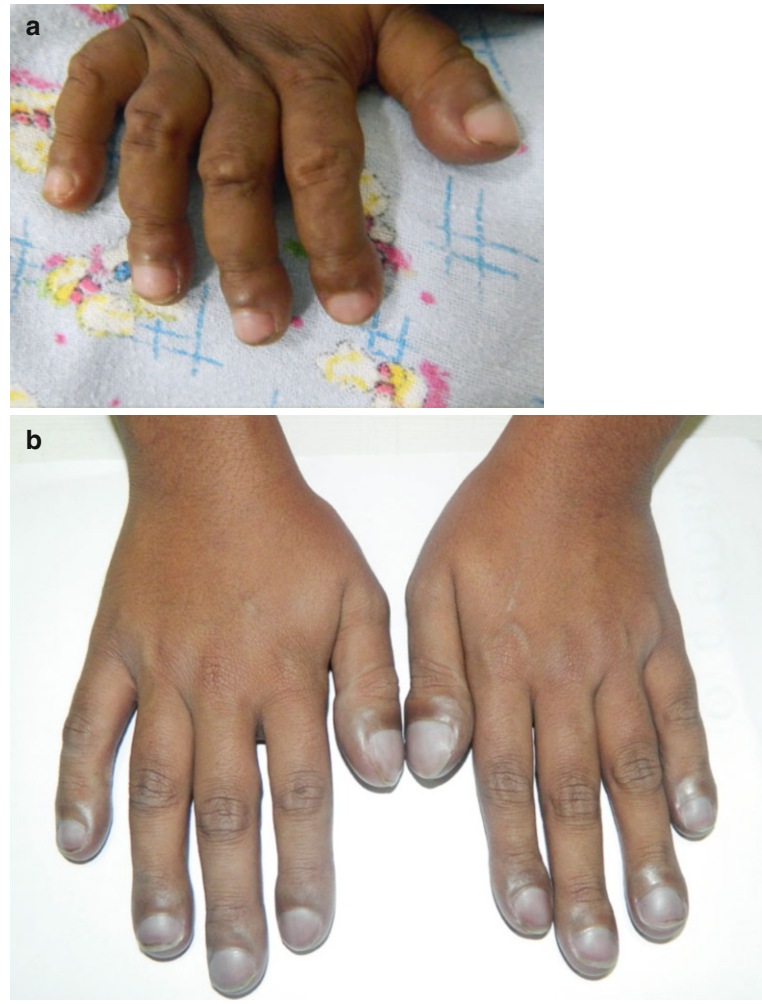


Fig. 26.3 (**a**) Shows clubbing of fingers in an 8-month-old baby with CLD due to missed biliary atresia. (**b**) Shows clubbing of fingers in a 7-year-old girl who had successful Kasai but developed severe portal hypertension

is more pronounced in the upright position because gravity exaggerates the ventilation – perfusion mismatch. Hypoxia accentuated in the upright position versus the supine position is called orthodeoxia. PaO_2 decrease of 5 % or 4 mmHg or more from the supine to upright position is defined as orthodeoxia, which is the hall mark of HPS [74]. Clubbing (Fig. 26.3a, b) seen in HPS is not due to hypoxia. It is postulated that megakaryocytes and platelet clumps traverse though the pulmonary capillary shunts and get impacted in the nail beds [75]. This causes the release of platelet-derived growth factor (PDGF) PDGF in nail beds that acts as growth factor and causes bulbous swelling of nail beds.

Diagnosis

In cirrhotic patients with hypoxia, chest roentogram should be obtained initially to rule out other causes of hypoxia such as pulmonary atelectasis, pneumonia, pulmonary oedema or hepatic hydrothorax. Roentograms will be normal in the majority of patients with HPS, but few might show interstitial infiltrates in the lung bases [76]. Contrast-enhanced echocardiography is the preferred screening test for HPS [60]. Saline is agitated to produce microbubbles and then injected intravenously. Microbubbles of at least 15 μm in diameter act as contrast when viewed with ultrasound and can be visualised in echocardiography on the right side of the

heart. When these microbubbles traverse the lung, they are trapped in the alveolar microvasculature and gradually absorbed. In individuals with either intracardiac or intrapulmonary shunts, these microbubbles avoid pulmonary entrapment and are subsequently 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 four to six heart beats for them to appear in the left side of the heart.

Although contrast echocardiography is sensitive, it lacks specificity; some cirrhotic patients with positive results on contrast echocardiography may not fulfil the diagnostic criteria for HPS [60]. Additionally, bubble echocardiography cannot quantify the shunting or differentiate between intrapulmonary vascular dilatation and direct arteriovenous communication at a pulmonary level.

An alternative method to confirm the presence of HPS is technetium-99m macroaggregated albumin (Tc-99m MAA) lung perfusion scanning, which is more sensitive and specific [59, 77]. Macroaggregated albumin with a particle size around 20 μm is tagged with technetium radioisotope. In normal individuals, macroaggregated albumin gets trapped in the lungs 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 the intrapulmonary shunt can be quantified; the degree of shunting is inversely proportional to arterial oxygen saturation [59]. Even with this technique the correlation between the shunt fraction and the response of PaO_2 after 100 % oxygen supplementation remains unpredictable. High-resolution CT may show increased ratio of segmental arterial diameter to adjacent bronchial diameter, but published data is quite scarce [78].

Angiography reveals 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 is considered to be of better prognosis with liver transplantation as there is high possibility of resolution of HPS [79]. In type 2 HPS vascular changes resembling AV shunts or vascular malformations could be seen. If amicable, embolization of feeding vessel can be done prior to liver transplantation. Algorithm for investigation of HPS is outlined in Fig. 26.4 [80, 81].

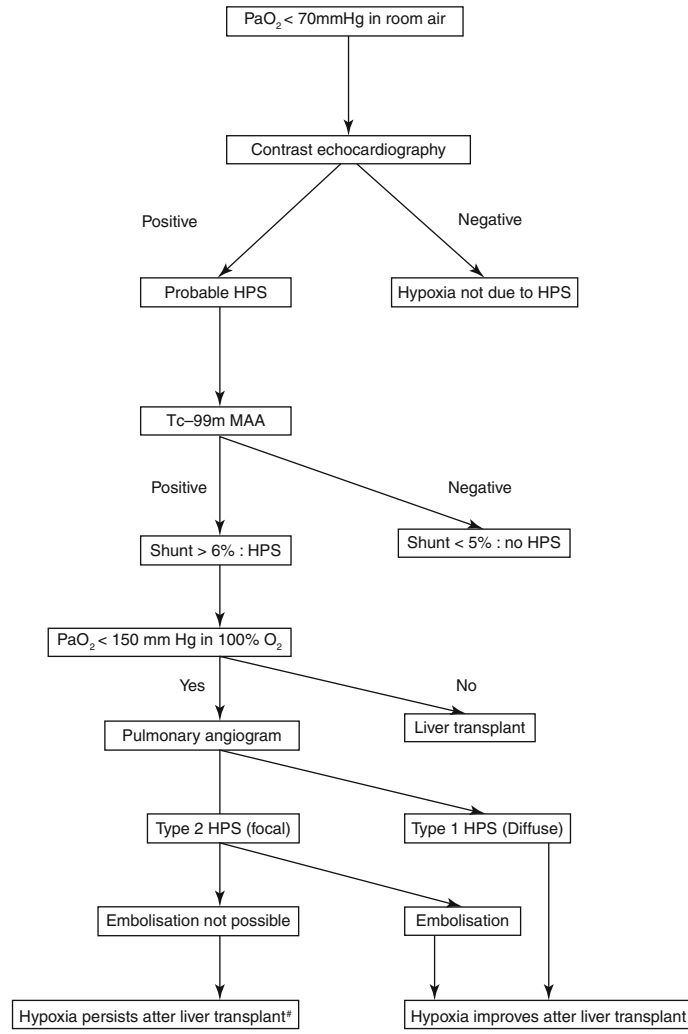
Management

Oxygen supplementation remains the main stay in HPS patients when $PaO_2 < 60$, and it generally improves the quality of life and exercise tolerance [62]. Several medications such as indomethacin, tamoxifen, somatostatin analogues, sympathomimetics, beta blockers, methylene blue and plasma exchange have been tried in HPS with abysmal results. Additionally, Martinez-Palli et al. showed that decreasing portal hypertension using transjugular intrahepatic portosystemic shunt (TIPS) had no effect on pulmonary gas exchange and thus is not recommended [82].

Liver transplantation is considered to be the definitive treatment in selective group of HPS patients as it reverses the pulmonary capillary abnormality and normalises oxygenation [83]. Overall postoperative complications in patients with HPS are higher than those without HPS. Embolic cerebral haemorrhage, worsening of hypoxia and failure of AV shunts to resolve (particularly type 2) are a few of the unique complications when HPS is present [80, 84]. Careful patient selection is essential as severe preoperative hypoxemia (PaO_2 <50 mmHg in room air) and significant intrapulmonary shunting (Tc-99m MAA shunt fraction >20 %) is associated with high mortality after liver transplantation [85].

Portopulmonary Hypertension

Portopulmonary hypertension (PoPH) is classified as pulmonary arterial hypertension associated with portal hypertension (Group 1) according to the updated clinical classification by then World Health Organization (Dana point, 2008) [86]. Pulmonary hypertension is defined



Tc–99m MAA : technetlum99m mactoaggregated albumin lung perfusion scan, HPS: Hepatopulmonary syndrome, # In majority of patients

Fig. 26.4 Algorithm for investigation of HPS. *Tc-99m MAA* technetium99m macroaggregated albumin lung perfusion scan, *HPS* Hepatopulmonary syndrome, # In majority of patients

as mean pulmonary artery pressure >25 mmHg via right heart catheterization. PoPH defined as the presence of portal hypertension along with mean pulmonary artery pressure (MPAP) >25 mmHg along with pulmonary vascular resistance (PVR) >240 dyn · s · cm^{-5} with normal pulmonary capillary wedge pressure (PCWP) <15 mmHg, in the presence of portal hypertension [87]. As flow and resistance are two factors that determine pressure, high-output states in CLD could falsely show elevated MPAP and so PVR is included in the diagnostic criteria to discern it. Castro et al. showed that in a cohort of adults awaiting liver transplantation, 20 % of them had MPAP >25 mmHg of which only 4 % had high pulmonary vascular resistance and would fit criteria for "true" PoPH [88].

Like HPS, the pathogenesis of PoPH is still elusive. It is postulated that the effluent toxins from diseased liver and bioactive substances that bypasses liver metabolism via varices in portal hypertension may lead on to pulmonary vasoconstriction, structural remodelling of the pulmonary arteries and formation of microthrombi, along with the high flow state of CLD [89]. Portal hypertension seems to be a prerequisite as PoPH is seen in disorders with no intrinsic liver disease such as extrahepatic portal vein thrombosis [90]. The histopathological changes in PoPH consist of plexogenic arteriopathy

Table 26.4 Classification of portopulmonary hypertension

Portopulmonary hypertension	Mean pulmonary arterial pressure (mmHg)	Cardiac index ($L/min/m^{-2}$)	Pulmonary vascular resistance ($dynes \cdot s \cdot cm^{-5}$)	Right arterial pressure (mmHg)
Mild	25–34	>2.5	240*–500	0–5
Moderate	35–44	>2.5	500–800	5–8
Severe	>45	<2.0	>800	>8

*Some consider pulmonary vascular resistance of 120 dynes · s · cm^{-5} as upper limit of normal in patients with liver disease

(plexiform lesions in association with medial hypertrophy and nonspecific intimal fibrous thickening) and thromboembolic (fibrous mural pads, occlusive nonvascular or vascular fibrous tissue and fibrous septa crossing the vascular lumens) disease, which is similar to that of primary pulmonary hypertension [91]. In a series of 17,901 autopsies, overall prevalence of primary pulmonary hypertension was 0.13 %, while in cirrhotics, it was 0.73 %, which was clinically significant. The data was further validated on 2,459 biopsy proven cirrhotics.

Clinical Features

The most common presenting symptom is dyspnea on excretion followed by syncope, chest pain and fatigue [92]. Sometimes it is associated with hemoptysis. In mild to moderate PoPH, the symptoms are mild or absent. The time interval between diagnosis of portal hypertension and symptoms due to PoPH is around 5 years [93]. Clinical examination would reveal systolic murmur with loud pulmonary component of second heart sound indicating tricuspid regurgitation. Oedema and ascites (features of right heart failure) are other clinical features.

Diagnosis

Diagnosis of PoPH is based on presence of pulmonary hypertension on the background of CLD, provided other causes of secondary pulmonary hypertension such as left heart diseases and interstitial, and obstructive lung disease have been ruled out. Chest roentogram might show prominent central pulmonary arteries and cardiomegaly, and the electrocardiogram may suggest right ventricular strain. Brain natriuretic peptide (BNP) is a surrogate marker of increased mortality in pulmonary hypertension. In children a level of more than 130 pg/ml was associated with increased risk of death [94]. However, this has not been validated in the specific setting of PoPH. Doppler echocardiography is an excellent screening tool for identifying PoPH and most patients are picked up during routine pretransplant cardiac assessment [87]. Using the Bernoulli equation, right ventricular systolic pressure is calculated, and this closely approximates the pulmonary artery systolic pressure in most cases. Doppler echocardiography provides only an estimated measure and right ventricular systolic pressure >50 mmHg (normal <30 mmHg) strongly suggests portopulmonary hypertension. Using this method 15 % of patients may be wrongly labelled as having PoPH due to their background high cardiac output state. Right heart catheterization remains the gold standard for accurately diagnosing pulmonary hypertension.

PoPH can be classified into mild, moderate and severe based on MPAP, PVR and cardiac index (Table 26.4) [87]. This helps in treatment initiation and prognostication for liver transplantation.

Management

Unlike HPS, PoPH may show good response to medical management, but with liver transplantation, HPS has the better prognosis when compared to PoPH. Three groups of drugs; prostacyclin analogues (e.g. epoprostenol, iloprost), endothelin receptor antagonists (e.g. bosentan) and phosphodiesterase inhibitors (e.g. sildenafil) have been used in children with primary pulmo-

nary hypertension with good response [95]. Data on usage of these medications in children specifically with PoPH is sparse, however. Continuous intravenous infusion of epoprostenol improves circulatory hemodynamics and exercise capacity in patients with PoPH. In young children the dosage ranges from 30 to 90 ng/kg/min, which is titrated according to the response [95]. Diarrhoea, jaw pain, bone pain and headaches are common side effects. There is also danger of pulmonary hypertensive crisis when the infusion gets interrupted as the half-life of the drug is only 2–5 min. Treprostinil is another prostacyclin analogue, which has been used either subcutaneously or intravenously [96].

Endothelins (ET) are potent vasoconstrictors that are found to be increased in PoPH. Bosentan is an ET receptor antagonist which when used at a dose of 31.25 mg bid (10–20 kg), 62.5 mg bid (20–40 kg) and 125 mg bid (>40 kg) in children has shown to decrease mean pulmonary artery pressure and pulmonary vascular resistance index and improve cardiac index [97]. Hepatotoxicity is an important side effect and it was found that children >12 years had higher incidence of adverse reaction when compared to younger children (2–11 years).

Sildenafil is a phosphodiesterase-5 inhibitors and causes vasodilatation of pulmonary arteries and thereby decreases pulmonary hypertension. It is usually given to children at a dose of 0.5–1 mg/kg/dose given three to four times a day [95]. Sometimes drug combinations (prostacyclin, sildenafil and bosentan) were used to produce synergistic effect and have found to be useful in selected cases [98].

Mild to moderate PoPH (high cardiac output) frequently resolves after liver transplantation while severe PoPH (low output) is associated with persistence of pulmonary hypertension and increased mortality [87]. Right heart dysfunction could further pose a risk for graft congestion and loss. Ideally patients with PoPH should be treated medically, if needed by more than one drug and, once the pulmonary hypertension is optimised, should undergo liver transplantation. Ashfaq et al. showed that patients with PoPH who responded to medical management had better out come after liver transplantation [99].

Other Causes

Low albumin levels in CLD could lead on to hydrothorax and can cause lung collapse [89]. Alpha-1 antitrypsin (AIAT) deficiency and cystic fibrosis are two important disorders in children, where both lung and liver could be affected. The above-mentioned two conditions can cause hypoxia either by destruction of lung parenchyma (intrinsic lung disease) or by causing secondary pulmonary hypertension or in combination. As the treatment options are limited, proper assessment and early intervention in the form of medical management or liver transplantation improve the outcome.

Hepatic Osteodystrophy

Hepatic osteodystrophy (HO) is a generalised term used to denote metabolic bone disease in CLD that occurs due to a combination of osteoporosis and osteomalacia [100]. In children, HO is comprised of vitamin D deficiency rickets, low bone mass, spine abnormalities and growth failure caused by malnutrition and malabsorption [101]. The pathogenesis of HO is considered to be multifactorial due to complex interaction of hormonal factors such as insulin-like growth factor-1 deficiency, vitamin D and K deficiency and hypogonadism on bone growth and differentiation in children with CLD [102]. Drugs such as cholestyramine and frusemide have the potential to further worsen this problem. The risk of bone fractures extends into the post-liver transplant period and the reported prevalence of bone fractures in children before and after OLT is 10–13 and 12–38 %, respectively [102]. Monitoring serum 25-hydroxy vitamin D (25OHD) levels and bone mineral density (BMD) by dual-energy x-ray absorptiometry (DEXA) scan may help in early detection of deficiency states and allows treatment to be initiated before pathological fractures occur.

The suggested 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 20 ng/mL. Active vitamin D metabolites α-calcidol or calcitriol increase calcium absorption in the gut but do not get stored. Furthermore these active forms can result in hypercalciuria and nephrocalcinosis in higher doses. 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 BMD [103]. It is important to optimise calcium, phosphate and vitamin D intake and treat rickets before initiating bisphosphonate therapy.

Cirrhotic Cardiomyopathy

Cardiac dysfunction in chronic liver disease as a discrete phenomenon came to light a couple of decades ago. Cirrhotic cardiomyopathy is defined as (1) baseline increased cardiac output but blunted ventricular response to stimuli, (2) systolic and/or diastolic dysfunction, (3) absence of overt left ventricular failure at rest and (4) electrophysiological abnormalities including prolonged QT interval on electrocardiography and chronotropic incompetence [104]. Not all the features are essential to make the diagnosis. As with any other end-organ dysfunction associated with liver disease, the pathogenesis of cirrhotic cardiomyopathy is not well understood and thought to be multifactorial. Altered cardiac muscle membrane properties along with impairment of stimulatory β-adrenergic receptor signalling pathways and overactive negative inotropic factors are implicated in pathogenesis of cirrhotic cardiomyopathy [105]. It affects even small children and Desai et al. showed that in a cohort of 40 children with median age of 8 months awaiting liver transplantation, 27 (74 %) had cirrhotic cardiomyopathy [106]. Severity of cardiac involvement correlates well with the severity of liver disease. There is paucity of data regarding diagnostic test and management. In overt heart failure standard treatment of congestive heart failure (bed rest, oxygen, diuretics and careful preload reduction by drugs) is initiated. Liver transplantation, though associated with increased morbidity in patients with cirrhotic cardiomyopathy when compared to those without cardiac involvement, remains the gold standard of curative therapy in most of the patients [106].

Conclusion

Liver disease affects nearly every system in the body and some are unique to children. Insight and anticipation of these problems help in planning the management. Though commonly the functionality of these organs (brain, kidney, lungs, etc.) returns back to normal after liver transplantation, advanced end-organ damage might render a patient unfit to undergo liver transplantation.

References

1. Iwasa M, et al. Decrease of regional cerebral blood flow in liver cirrhosis. Eur J Gastroenterol Hepatol. 2000;12(9):1001–6.
2. Cash WJ, et al. Current concepts in the assessment and treatment of hepatic encephalopathy. QJM. 2010;103(1):9–16.
3. Ferenci P, et al. Hepatic encephalopathy – definition, nomenclature, diagnosis, and quantification: final report of the working party at the 11th World Congresses of Gastroenterology, Vienna, 1998. Hepatology. 2002;35(3):716–21.
4. Jones EA, Weissenborn K. Neurology and the liver. J Neurol Neurosurg Psychiatry. 1997;63(3):279–93.
5. Frederick RT. Current concepts in the pathophysiology and management of hepatic encephalopathy. Gastroenterol Hepatol (N Y). 2011;7(4):222–33.
6. Vince AJ, Burridge SM. Ammonia production by intestinal bacteria: the effects of lactose, lactulose and glucose. J Med Microbiol. 1980;13(2):177–91.
7. Romero-Gomez M, et al. Gut ammonia production and its modulation. Metab Brain Dis. 2009;24(1):147–57.
8. Merli M, et al. Muscle depletion increases the risk of overt and minimal hepatic encephalopathy: results of a prospective study. Metab Brain Dis. 2013;28(2):281–4.
9. Dejong CH, et al. Aromatic amino acid metabolism during liver failure. J Nutr. 2007;137(6 Suppl 1):1579S–85; discussion 1597S–8S.
10. Fischer JE, et al. The effect of normalization of plasma amino acids on hepatic encephalopathy in man. Surgery. 1976;80(1):77–91.
11. Skowronska M, Albrecht J. Alterations of blood brain barrier function in hyperammonemia: an overview. Neurotox Res. 2012;21(2):236–44.

12. Al Mardini H, et al. Effect of methionine loading and endogenous hypermethioninaemia on blood mercaptans in man. Clin Chim Acta. 1988; 176(1):83–9.
13. Mortensen PB, et al. The degradation of amino acids, proteins, and blood to short-chain fatty acids in colon is prevented by lactulose. Gastroenterology. 1990;98(2):353–60.
14. Macfarlane S, Macfarlane GT. Regulation of short-chain fatty acid production. Proc Nutr Soc. 2003;62(1):67–72.
15. Riggio O, et al. Peripheral and splanchnic indole and oxindole levels in cirrhotic patients: a study on the pathophysiology of hepatic encephalopathy. Am J Gastroenterol. 2010;105(6):1374–81.
16. Baraldi M, et al. Natural endogenous ligands for benzodiazepine receptors in hepatic encephalopathy. Metab Brain Dis. 2009;24(1):81–93.
17. Ahboucha S, et al. Increased brain concentrations of a neuroinhibitory steroid in human hepatic encephalopathy. Ann Neurol. 2005;58(1):169–70.
18. Guevara M, et al. Hyponatremia is a risk factor of hepatic encephalopathy in patients with cirrhosis: a prospective study with time-dependent analysis. Am J Gastroenterol. 2009;104(6):1382–9.
19. Odeh M. Pathogenesis of hepatic encephalopathy: the tumour necrosis factor-alpha theory. Eur J Clin Invest. 2007;37(4):291–304.
20. Odeh M, et al. Relationship between tumor necrosis factor-alpha and ammonia in patients with hepatic encephalopathy due to chronic liver failure. Ann Med. 2005;37(8):603–12.
21. Odeh M, et al. Serum levels of tumor necrosis factor-alpha correlate with severity of hepatic encephalopathy due to chronic liver failure. Liver Int. 2004; 24(2):110–6.
22. Goral V, Atayan Y, Kaplan A. The relation between pathogenesis of liver cirrhosis, hepatic encephalopathy and serum cytokine levels: what is the role of tumor necrosis factor alpha? Hepatogastroenterology. 2011;58(107–108):943–8.
23. Quero Guillen JC, Herrerias Gutierrez JM. Diagnostic methods in hepatic encephalopathy. Clin Chim Acta. 2006;365(1–2):1–8.
24. Dhiman RK, et al. Minimal hepatic encephalopathy: consensus statement of a working party of the Indian National Association for Study of the Liver. J Gastroenterol Hepatol. 2010;25(6):1029–41.
25. Govindarajan S, et al. Immunohistochemical distribution of renal prostaglandin endoperoxide synthase and prostacyclin synthase: diminished endoperoxide synthase in the hepatorenal syndrome. Hepatology. 1987;7(4):654–9.
26. Yadav SK, et al. Encephalopathy assessment in children with extra-hepatic portal vein obstruction with MR, psychometry and critical flicker frequency. J Hepatol. 2010;52(3):348–54.
27. Kircheis G, et al. Critical flicker frequency for quantification of low-grade hepatic encephalopathy. Hepatology. 2002;35(2):357–66.
28. Kullmann F, et al. Brain electrical activity mapping of EEG for the diagnosis of (sub)clinical hepatic encephalopathy in chronic liver disease. Eur J Gastroenterol Hepatol. 2001;13(5):513–22.
29. Terao Y, Ugawa Y. Basic mechanisms of TMS. J Clin Neurophysiol. 2002;19(4):322–43.
30. Weber M, Eisen AA. Magnetic stimulation of the central and peripheral nervous systems. Muscle Nerve. 2002;25(2):160–75.
31. Arya R, Gulati S, Deopujari S. Management of hepatic encephalopathy in children. Postgrad Med J. 2010;86(1011):34–41; quiz 40.
32. Ito Y, et al. Effect of lactulose on short-chain fatty acids and lactate production and on the growth of faecal flora, with special reference to *Clostridium difficile*. J Med Microbiol. 1997;46(1):80–4.
33. Holecek M. Three targets of branched-chain amino acid supplementation in the treatment of liver disease. Nutrition. 2010;26(5):482–90.
34. Agrawal A, et al. Secondary prophylaxis of hepatic encephalopathy in cirrhosis: an open-label, randomized controlled trial of lactulose, probiotics, and no therapy. Am J Gastroenterol. 2012;107(7):1043–50.
35. Bajaj JS, et al. A longitudinal systems biology analysis of lactulose withdrawal in hepatic encephalopathy. Metab Brain Dis. 2012;27(2):205–15.
36. Patil DH, et al. Comparative modes of action of lactitol and lactulose in the treatment of hepatic encephalopathy. Gut. 1987;28(3):255–9.
37. Festi D, et al. Management of hepatic encephalopathy: focus on antibiotic therapy. Digestion. 2006;73 Suppl 1:94–101.
38. Rivkin A, Gim S. Rifaximin: new therapeutic indication and future directions. Clin Ther. 2011; 33(7):812–27.
39. Gluud LL, et al. Lactulose, rifaximin or branched chain amino acids for hepatic encephalopathy: what is the evidence? Metab Brain Dis. 2013 (in press).
40. Malaguarnera M, et al. *Bifidobacterium longum* with fructo-oligosaccharide (FOS) treatment in minimal hepatic encephalopathy: a randomized, double-blind, placebo-controlled study. Dig Dis Sci. 2007;52(11):3259–65.
41. Sharma P, et al. An open-label randomized controlled trial of lactulose and probiotics in the treatment of minimal hepatic encephalopathy. Eur J Gastroenterol Hepatol. 2008;20(6):506–11.
42. Holte K, Krag A, Gluud LL. Systematic review and meta-analysis of randomized trials on probiotics for hepatic encephalopathy. Hepatol Res. 2012;42(10):1008–15.
43. McGee RG, et al. Probiotics for patients with hepatic encephalopathy. Cochrane Database Syst Rev. 2011;(11):CD008716.
44. Olde Damink SW, et al. Interorgan ammonia and amino acid metabolism in metabolically stable patients with cirrhosis and a TIPSS. Hepatology. 2002;36(5):1163–71.
45. Rose C, et al. L-ornithine-L-aspartate lowers plasma and cerebrospinal fluid ammonia and prevents brain

edema in rats with acute liver failure. Hepatology. 1999;30(3):636–40.
46. Al Sibae MR, McGuire BM. Current trends in the treatment of hepatic encephalopathy. Ther Clin Risk Manag. 2009;5(3):617–26.
47. Shores NJ, Keeffe EB. Is oral L-acyl-carnitine an effective therapy for hepatic encephalopathy? Review of the literature. Dig Dis Sci. 2008;53(9):2330–3.
48. Malaguarnera M, et al. Acetyl-L-carnitine improves cognitive functions in severe hepatic encephalopathy: a randomized and controlled clinical trial. Metab Brain Dis. 2011;26(4):281–9.
49. Malaguarnera M, et al. Oral acetyl-L-carnitine therapy reduces fatigue in overt hepatic encephalopathy: a randomized, double-blind, placebo-controlled study. Am J Clin Nutr. 2011;93(4):799–808.
50. Loomba V, et al. Serum zinc levels in hepatic encephalopathy. Indian J Gastroenterol. 1995;14(2):51–3.
51. Takuma Y, et al. Clinical trial: oral zinc in hepatic encephalopathy. Aliment Pharmacol Ther. 2010;32(9): 1080–90.
52. Reding P, Duchateau J, Bataille C. Oral zinc supplementation improves hepatic encephalopathy. Results of a randomised controlled trial. Lancet. 1984;2(8401):493–5.
53. Burkhard PR, et al. Chronic Parkinsonism associated with cirrhosis: a distinct subset of acquired hepatocerebral degeneration. Arch Neurol. 2003;60(4):521–8.
54. Corenblum B, Shaffer EA. Hyperprolactinemia in hepatic encephalopathy may result from impaired central dopaminergic neurotransmission. Horm Metab Res. 1989;21(12):675–7.
55. Als-Nielsen B, Gluud LL, Gluud C. Dopaminergic agonists for hepatic encephalopathy. Cochrane Database Syst Rev. 2004;(4):CD003047.
56. Als-Nielsen B, Gluud LL, Gluud C. Benzodiazepine receptor antagonists for hepatic encephalopathy. Cochrane Database Syst Rev. 2004;(2):CD002798.
57. Spahr L, et al. Increased blood manganese in cirrhotic patients: relationship to pallidal magnetic resonance signal hyperintensity and neurological symptoms. Hepatology. 1996;24(5):1116–20.
58. Schaefer B, Schmitt CP. The role of molecular adsorbent recirculating system dialysis for extracorporeal liver support in children. Pediatr Nephrol. 2013;28(9):1763–9.
59. Abrams GA, et al. Use of macroaggregated albumin lung perfusion scan to diagnose hepatopulmonary syndrome: a new approach. Gastroenterology. 1998;114(2):305–10.
60. Wang YW, Lin HC. Recent advances in hepatopulmonary syndrome. J Chin Med Assoc. 2005;68(11): 500–5.
61. Whyte MK, et al. Analysis of intrapulmonary right to left shunt in the hepatopulmonary syndrome. J Hepatol. 1998;29(1):85–93.
62. Varghese J, et al. Hepatopulmonary syndrome – past to present. Ann Hepatol. 2007;6(3):135–42.
63. Sasaki T, et al. Development of intrapulmonary arteriovenous shunting in postoperative biliary atresia: evaluation by contrast-enhanced echocardiography. J Pediatr Surg. 2000;35(11):1647–50.
64. Noli K, et al. Prevalence of hepatopulmonary syndrome in children. Pediatrics. 2008;121(3):e522–7.
65. Fallon MB, et al. Common bile duct ligation in the rat: a model of intrapulmonary vasodilatation and hepatopulmonary syndrome. Am J Physiol. 1997;272(4 Pt 1):G779–84.
66. Barbe T, et al. Pulmonary arteriovenous shunting in children with liver disease. J Pediatr. 1995;126(4): 571–9.
67. Gupta D, et al. Prevalence of hepatopulmonary syndrome in cirrhosis and extrahepatic portal venous obstruction. Am J Gastroenterol. 2001;96(12):3395–9.
68. Rolla G, et al. Exhaled nitric oxide and impaired oxygenation in cirrhotic patients before and after liver transplantation. Ann Intern Med. 1998;129(5):375–8.
69. Fallon MB, et al. The role of endothelial nitric oxide synthase in the pathogenesis of a rat model of hepatopulmonary syndrome. Gastroenterology. 1997;113(2):606–14.
70. Gomez FP, et al. Effects of nebulized N(G)-nitro-L-arginine methyl ester in patients with hepatopulmonary syndrome. Hepatology. 2006;43(5):1084–91.
71. Zhang HY, et al. Experimental study on the role of endotoxin in the development of hepatopulmonary syndrome. World J Gastroenterol. 2005;11(4):567–72.
72. Sztrymf B, et al. Prevention of hepatopulmonary syndrome and hyperdynamic state by pentoxifylline in cirrhotic rats. Eur Respir J. 2004;23(5):752–8.
73. Sztrymf B, et al. Cirrhotic rats with bacterial translocation have higher incidence and severity of hepatopulmonary syndrome. J Gastroenterol Hepatol. 2005;20(10):1538–44.
74. Gomez FP, et al. Gas exchange mechanism of orthodeoxia in hepatopulmonary syndrome. Hepatology. 2004;40(3):660–6.
75. Dickinson CJ. The aetiology of clubbing and hypertrophic osteoarthropathy. Eur J Clin Invest. 1993;23(6):330–8.
76. Alves L, et al. Preoperative pulmonary assessment of children for liver transplantation. Pediatr Transplant. 2008;12(5):536–40.
77. El-Shabrawi MH, et al. (99m)Technetium-macroaggregated albumin perfusion lung scan versus contrast enhanced echocardiography in the diagnosis of the hepatopulmonary syndrome in children with chronic liver disease. Eur J Gastroenterol Hepatol. 2010;22(8):1006–12.
78. Lee KN, et al. Hypoxemia and liver cirrhosis (hepatopulmonary syndrome) in eight patients: comparison of the central and peripheral pulmonary vasculature. Radiology. 1999;211(2):549–53.
79. Kuntz E, Kuntz HD. Hepatopulmonary syndrome, in hepatology. In: Kuntz E, Kuntz HD, editors. Textbook and atlas. Heidelberg: Springer; 2008. p. 340–5.
80. Schenk P, et al. Hepatopulmonary syndrome: prevalence and predictive value of various cut offs for arterial oxygenation and their clinical consequences. Gut. 2002;51(6):853–9.

81. Herve P, et al. Pulmonary vascular disorders in portal hypertension. Eur Respir J. 1998;11(5):1153–66.
82. Martinez-Palli G, et al. Effect of transjugular intrahepatic portosystemic shunt on pulmonary gas exchange in patients with portal hypertension and hepatopulmonary syndrome. World J Gastroenterol. 2005;11(43):6858–62.
83. Battaglia SE, et al. Resolution of gas exchange abnormalities and intrapulmonary shunting following liver transplantation. Hepatology. 1997;25(5):1228–32.
84. Abrams GA, et al. Hepatopulmonary syndrome and venous emboli causing intracerebral hemorrhages after liver transplantation: a case report. Transplantation. 1999;68(11):1809–11.
85. Arguedas MR, et al. Prospective evaluation of outcomes and predictors of mortality in patients with hepatopulmonary syndrome undergoing liver transplantation. Hepatology. 2003;37(1):192–7.
86. Simonneau G, et al. Updated clinical classification of pulmonary hypertension. J Am Coll Cardiol. 2009;54(1 Suppl):S43–54.
87. Hoeper MM, Krowka MJ, Strassburg CP. Portopulmonary hypertension and hepatopulmonary syndrome. Lancet. 2004;363(9419):1461–8.
88. Castro M, et al. Frequency and clinical implications of increased pulmonary artery pressures in liver transplant patients. Mayo Clin Proc. 1996;71(6):543–51.
89. Krowka MJ. Hepatopulmonary syndromes. Gut. 2000; 46(1):1–4.
90. Budhiraja R, Hassoun PM. Portopulmonary hypertension: a tale of two circulations. Chest. 2003; 123(2):562–76.
91. Edwards BS, et al. Coexistent pulmonary and portal hypertension: morphologic and clinical features. J Am Coll Cardiol. 1987;10(6):1233–8.
92. Robalino BD, Moodie DS. Association between primary pulmonary hypertension and portal hypertension: analysis of its pathophysiology and clinical, laboratory and hemodynamic manifestations. J Am Coll Cardiol. 1991;17(2):492–8.
93. McDonnell PJ, Toye PA, Hutchins GM. Primary pulmonary hypertension and cirrhosis: are they related? Am Rev Respir Dis. 1983;127(4):437–41.
94. Lammers AE, Hislop AA, Haworth SG. Prognostic value of B-type natriuretic peptide in children with pulmonary hypertension. Int J Cardiol. 2009; 135(1):21–6.
95. Ivy DD, et al. Non-congenital heart disease associated pediatric pulmonary arterial hypertension. Prog Pediatr Cardiol. 2009;27(1–2):13–23.
96. Talwalkar JA, et al. Prevalence of spontaneous portosystemic shunts in patients with portopulmonary hypertension and effect on treatment. Gastroenterology. 2011;141(5):1673–9.
97. Barst RJ, et al. Pharmacokinetics, safety, and efficacy of bosentan in pediatric patients with pulmonary arterial hypertension. Clin Pharmacol Ther. 2003;73(4):372–82.
98. Austin MJ, et al. Safety and efficacy of combined use of sildenafil, bosentan, and iloprost before and after liver transplantation in severe portopulmonary hypertension. Liver Transpl. 2008;14(3):287–91.
99. Ashfaq M, et al. The impact of treatment of portopulmonary hypertension on survival following liver transplantation. Am J Transplant. 2007;7(5):1258–64.
100. Goel V, Kar P. Hepatic osteodystrophy. Trop Gastroenterol. 2010;31(2):82–6.
101. Klein GL, et al. Hepatic osteodystrophy in chronic cholestasis: evidence for a multifactorial etiology. Pediatr Transplant. 2002;6(2):136–40.
102. Hogler W, Baumann U, Kelly D. Growth and bone health in chronic liver disease and following liver transplantation in children. Pediatr Endocrinol Rev. 2010;7(3):266–74.
103. Hogler W, Baumann U, Kelly D. Endocrine and bone metabolic complications in chronic liver disease and after liver transplantation in children. J Pediatr Gastroenterol Nutr. 2012;54(3):313–21.
104. Baik SK, Fouad TR, Lee SS. Cirrhotic cardiomyopathy. Orphanet J Rare Dis. 2007;2:15.
105. Liu H, Song D, Lee SS. Cirrhotic cardiomyopathy. Gastroenterol Clin Biol. 2002;26(10):842–7.
106. Desai MS, et al. Cardiac structural and functional alterations in infants and children with biliary atresia, listed for liver transplantation. Gastroenterology. 2011;141(4):1264–72, 1272.e1–4.

Nutrition in Children with Liver Disease: Evaluation and Management

27

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The Role of the Healthy Liver in Nutrition Homeostasis

The liver stands both physically and functionally between the intestine and circulatory system. It plays an essential role in the maintenance of a stable nutritional state, supportive of overall biochemical homeostasis. Products of intraluminal digestion (amino acids, simple carbohydrates, short- and medium-chain triglycerides) are brought directly to the liver via the portal venous system while longer chain fats are brought to the liver via lymphatic drainage into the central circulatory system. The liver is thus provided with an abundance of nutrients which it must sort, store, transform, and transport for multiple biological functions.

Protein

Amino acids, derived from dietary proteins and transported through the portal circulation, can be used by the liver in a number of ways. They may be immediately recycled into new proteins, further metabolized into other nitrogenous compounds, or used as an energy source. Amino acids may also reach the liver via the systemic circulation, through transport of the products of proteolysis in muscle or other tissues. Thus, the liver maintains, through synthesis and degradation, serum concentration of most amino acids within a relatively narrow range. Metabolism of amino acids also takes place in other organs, such as in muscle for the branched-chain essential and nonessential amino acids.

The liver is constantly producing proteins, most of which are not stored but are continuously released into the systemic circulation. During times of health and stability, the liver produces maintenance proteins such as albumin, transferrin, retinol-binding protein, and others. These provide vascular oncotic pressure, support the transfer of substances to and from the liver, support normal coagulation, and multiple other functions. In response to stress, including inflammation or tissue injury, the liver produces acute-phase proteins which may either enhance the inflammatory response (C-reactive protein, ferritin, ceruloplasmin, haptoglobin) or limit it (alpha-2-macroglobulin, alpha-1-antitrypsin.) In this fashion, the liver exerts homeostatic influence on the physiologic stress response. New proteins are also made during physiologic stresses which presumably contribute to defense and adaptation [1]. The acute-phase response, initiated and maintained through cytokine signaling, results in a number of responses to tissue and cellular injury including fever, neuroendocrine release of hormones [2, 3], anorexia [4],

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lethargy, muscle wasting, impaired growth (in children), and anemia. Changes in micronutrient utilization such as iron, zinc, and copper have been observed as well. The liver also is a major site of nitrogen recycling into urea, eliminating potentially neurotoxic nitrogenous wastes including ammonia.

Carbohydrate

After a meal the portal venous system is rich in ingested glucose which can be used to meet acute energy demands or be stored in the liver or muscle as glycogen and ultimately be metabolized by glycolysis into lactate, pyruvate, and the amino acid alanine. When not used for further energy by oxidative phosphorylation, these compounds are returned to the liver where glucose is again formed through gluconeogenesis. The liver thus plays a central role in the tight maintenance of the competing processes of breakdown, formation, and metabolism of simple sugars and their metabolites.

Fat

There is no "first pass" effect for digested fats as there is for carbohydrates and protein. However, the liver plays an essential role in the absorption, distribution, synthesis, and storage of fats. For absorption fats must first be emulsified in the aqueous environment of the intestinal lumen. This is accomplished through the physical disruption of fats through chewing and by the coating of fats with phospholipids from both dietary sources and from bile. Triglycerides are enzymatically degraded by lingual lipase in the stomach and by pancreatic lipase in the duodenum into 2-monoglycerides and fatty acids. These are mixed with liver-derived bile salts and fat-soluble vitamins into micelles which are transported passively and by the aid of transport molecules across the apical membrane into the enterocyte. In the enterocyte fatty acids are transported to the smooth endoplasmic reticulum where triglycerides are resynthesized. The triglycerides, along with other lipid molecules such as cholesterol esters and apoproteins, are formed into chylomicrons which enter the intestinal lymphatics and eventually reach the systemic circulation. Medium and short chain fatty acids diffuse passively from the intestinal lumen to the portal system.

Free fatty acids from adipose tissue can be re-esterified into triglycerides or used as an energy substrate or expanded and desaturated to form longer chain metabolites that participate in multiple physiologic functions, including cell signaling, the inflammatory response, and mediators of mood and behavior. HDL and VLDL which are essential for fat and cholesterol transport throughout the body are also liver-derived. Finally, the liver synthesizes cholesterol which functions as a "structural" part of all cell membranes and as a precursor for bile salts and for hormones. The liver is also essential for the uptake, storage, and distribution of fat-soluble vitamins.

Chronic Liver Disease and Nutrition

Any or all of the digestive and metabolic processes of the liver may be impaired when liver function is disrupted. The clinical presentations of perturbations in nutritional homeostasis can be subtle, complex, and variable from one patient to another, for example, as an isolated correctable coagulopathy or as protein-energy malnutrition. However, when one measurable abnormality is found, there is a high likelihood that there are also other, subclinical, states of compromised nutritional integrity.

Carbohydrate

Most patients with cirrhosis have abnormal glucose tolerance related to both hyperinsulinemia and insulin resistance. Either hypoglycemia or hyperglycemia may occur. In some patients diabetes may arise [5]. All aspects of carbohydrate metabolism can be altered by liver disease. Hepatic glycogen stores may be depleted while gluconeogenesis may be enhanced [6].

High serum levels of insulin often accompany severe chronic liver disease and may be caused by portosystemic shunting of insulin away from the liver and impaired degradation of insulin by nonfunctioning hepatocytes. Uptake of glucose in the peripheral tissues may be impaired, despite the higher levels of circulating insulin, because of end-organ resistance to insulin [7, 8]. Finally, and significantly, anorexia often accompanies advancing liver disease, blunting the most basic response to depletion of energy stores. Thus, the liver may not be able to meet basic energy requirements at rest, while deficiencies may be even more profound during periods of stress and inflammation.

Protein

While hyperinsulinemia and insulin resistance have an important impact on glucose homeostasis, their role in protein metabolism is not as impaired [9]. The most common "synthetic" defects found in patients with chronic liver injury, low serum album, and uncorrectable coagulopathies are both directly related to the limited synthetic capacity of the diseased liver. The Child Pugh grading system and the Model for End-Stage Liver Disease (MELD) use albumin as part of a model to measure a patient's prognosis [10]. Serum levels of albumin are related not only to its production in the liver but also to changes in its degradation (as serum albumin levels decrease, its rate of degradation also decreases) and distribution (intra- or extravascular) throughout the body. The synthesis, degradation, or distribution may be disordered in the setting of chronic liver injury. Specific amino acid metabolism may also be abnormal, thus impacting the potential for the synthesis of new proteins. The altered synthetic capacity of the liver may negatively affect the "acute-phase" response, leading to various interruptions in the normal immune response. The patient with chronic liver disease may respond poorly, slowly, or inappropriately to infection or inflammation because of the lack of synthetic dexterity of the diseased liver (see Table, Acute Phase Reactants).

Fat

All aspects of fat metabolism may be affected by chronic liver disease. Diminished bile acid production (from diseased or reduced numbers of hepatocytes), lack of bile salt transport to the gut (in biliary obstruction such as extrahepatic biliary atresia), limited reuptake of bile salts (by compromised hepatocytes), and slowed or interrupted lymph flow into the systemic circulation (in portal hypertension) can all lead to steatorrhea.

When the synthetic function of the liver is impaired, cholesterol synthesis and lipoprotein turnover may also be impaired or altered. Loss of liver-derived lecithin-cholesterol acyltransferase results in impairment of lipid transport. Fasting plasma free fatty acids, glycerol, and ketone bodies are increased in the setting of cirrhosis. And at the same time lipids begin to serve as a preferred energy substrate [11, 12]. Despite the observed changes in lipid metabolism, plasma clearance of postprandial lipids and lipid oxidation is not impaired and storage of excessive calorie intake (above what is expended) into fat is preserved [8, 9]. Both hypercholesterolemia and hypocholesterolemia can be associated with cirrhosis. Eventually the compromised liver reaches its final end stage and serum cholesterol uniformly falls.

Thus, a patient with chronic liver disease may suffer from a variety of nutritional challenges including steatorrhea, fat-soluble vitamin deficiencies, disordered carbohydrate metabolism, or deficiencies in important serum proteins, either because of malabsorption of basic nutrients or because of disordered production or storage of liver-derived products or both.

Goals of Nutritional Therapy in Children with Liver Disease

The overall goals of nutritional therapy in children with liver disease are to (1) achieve a normal lifestyle, (2) maintain normal weight gain and linear growth, (3) prevent or manage nutritional inadequacy or imbalances, and (4) prepare for liver transplantation, if necessary.

Nutritional support in children with liver disease depends on the chronicity of liver disease. Acute insults to the liver, such as drug-induced hepatotoxicity or viral hepatitis, may not require any specific nutritional therapy unless complications from liver dysfunction arise. Supportive and symptomatic treatments for inadequate enteral intake from anorexia or nausea, a hypercatabolic state such as fever or seizures as well as fluid and nutritional replacement for gastrointestinal losses from vomiting and diarrhea, are essential to prevent significant weight loss. Enteral nutrition via nasogastric, nasojejunal, or gastric tubes or parenteral nutrition might be required if adequate oral intake cannot be achieved. While malnutrition is uncommon among children with acute liver disease who achieve complete recovery, individuals with chronic liver disease are at risk for several nutritional deficiencies from various causes. In addition, the inborn errors of metabolism that can cause chronic liver disease (e.g., galactosemia, tyrosinemia, hereditary fructose intolerance) require specific dietary interventions to prevent cirrhosis and subsequent hepatic failure.

Nutritional support of the child with chronic liver disease is also strongly related to the absence or presence of cholestasis. Absorption of fat-soluble vitamins (A, D, E, and K) and fat, including the essential fatty acids, requires adequate flow and concentration of intraluminal bile acids in the proximal small intestine for micellar solubilization. Therefore, supplementation of fat-soluble vitamins, essential fatty acids, and provision of medium-chain triglycerides (as they do not require bile acids for absorption) is usually required in children with significant chronic cholestatic liver disease [13, 14]. In cirrhosis, portal hypertension with an increased mesenteric venous pressure may cause significant enteropathy and consequently malabsorption of both macronutrients and micronutrients. Some conditions that are also associated with chronic liver disease such as cystic fibrosis (intrahepatic cholestasis), inflammatory bowel disease, or celiac disease may also worsen malabsorption (e.g., pancreatic insufficiency in cystic fibrosis, severe enteropathy/villous atrophy in inflammatory bowel disease with concomitant liver disease, and celiac disease).

Nutritional Assessment of the Children with Chronic Liver Disease

Every child with chronic liver disease requires careful and regular nutritional assessments to determine the degree of malnutrition and to establish the need for nutritional intervention. A thorough diet history, physical examination, and measurement of trend in changes in anthropometric parameters are sufficient to assess the nutritional status for most children. However, no single approach is flawlessly sensitive or specific in detecting nutritional inadequacy. Therefore, it is most common to apply various clinical, anthropometric, and laboratory measurements to help assess growth and nutritional status in children with chronic liver disease. Of note, the degree of liver synthetic dysfunction does not always correlate with the severity of nutritional imbalances. Moreover, liver disease itself can sometimes make the assessment of nutritional status inaccurate and unreliable. For example, ascites or organomegaly can be misperceived as lean body mass weight gain and therefore height, or length, and mid-arm muscle circumference may be better anthropometric parameters to define the nutritional status of these children.

Assessment by History

Details regarding both the quality and quantity of dietary macronutrients and micronutrients should be carefully obtained by experienced health care providers from the patient or primary caregiver(s). The current generally accepted method of dietary intake assessment is a 3- to 5-day diet diary to account for the daily variation in diet. Medical history such as encephalopathy, bleeding, or symptoms that can affect the oral intake such as fever, mucositis, nausea, vomiting, diarrhea, or abdominal distention due to ascites or organomegaly should be obtained. Information regarding symptoms indicating macronutrient,

vitamin, or mineral deficiency as well as current medication use and other comorbid conditions is also crucial.

Clinical Assessment

In conjunction with a detailed dietary history, a thorough physical examination for the signs associated with nutritional deficiencies should be performed. Clinical signs associated with common nutritional deficiencies in chronic liver disease are shown in Table 27.1.

Table 27.1 Acute-phase reactants

Protein	Function
Negative (lower production during inflammation)	
Albumin	Colloidal osmotic pressure, binding protein, carrier protein
Transferrin	Iron delivery from intestine to all tissues
Transthyretin	Thyroid hormone transport, retinol transport
Retinol-binding protein	Carrier protein for retinol
Antithrombin	Limits coagulation
Transcortin	Transport of glucocorticoids and progestins
Positive (higher production during inflammation)	
Plasminogen	Protease; dissolves fibrin clots
Alpha 2-macroglobulin	Antiprotease, inhibits coagulation and fibrinolysis
C-reactive protein	Bind phosphocholine, active complement cascade, opsonin
Complement factors	Chemotaxis, opsonization
Fibrinogen	Coagulation protein
Prothrombin	Part of the coagulation cascade
Hepcidin	Regulates iron homeostasis
Ceruloplasmin	Copper transport, iron homeostasis
Haptoglobin	Binds hemoglobin for degradation
Orosomucoid	Carrier of neutral-charged lipids
Alpha 1-antitrypsin	Protease inhibitor, downgrades inflammation

Anthropometric Evaluation

The measurement of growth in children can be performed both in a cross-sectional and a longitudinal fashion. The use of age-appropriate techniques and equipment for measuring these parameters are crucial for obtaining reliable values, and serial measurements should be done by one observer, if possible. Single measurements can be useful in screening children who may be at risk for nutritional deficiency and determining the need for a further comprehensive medical evaluation. The use of longitudinal measures is more valuable in determining nutritional status, and more helpful in making a decision to implement nutritional interventions/rehabilitation, than is the use of single time point measurements. The measurements should always be compared to the reference standards for age, gender, race/ethnicity, and specific health conditions/diseases (if applicable). Different comparative scales such as percentiles, *Z*-score, or percent of the median are commonly used for standardization to determine a degree of deviation from the norm, to follow the trend over time, and to implement or assess ongoing nutritional interventions.

Weight should be recorded as nude weight. Height (standing) or length (supine) is by far the most useful indicator of growth and nutritional status, although validity and reproducibility may be difficult to establish, especially in younger children. Children older than 2 years of age should be measured while standing erect, and younger children should be measured while in a supine position. Body mass index (weight/height^2 in kg/m^2) is best used to evaluate the child's adiposity, although this is more helpful in determining overweight than assisting in an evaluation of malnourished children. Weight for height (or length) is used to differentiate wasting from stunting and is independent of age and gender. Wasting is a consequence of short-term inadequate nutrition which results in a decreased weight-for-height ratio, while stunting is a longer-term consequence of malnutrition leading to growth failure, but often with an appropriate weight-for-height ratio. Triceps skin fold thickness is most commonly used to assess subcutaneous fat as an index of total body fat, and the measurement of mid-upper arm circumference

examines not only fat but also muscle. Both of these measurements provide sensitive indicators of nutritional status in children with chronic liver disease as a decline in these measurements can be seen prior to the changes in weight and height/length.

Laboratory Investigation

Laboratory studies can be helpful in evaluating and monitoring children with chronic liver disease who are at risk for or suffer from malnutrition. Children with chronic liver disease or cirrhosis are at higher risk for malabsorption of various macronutrients and micronutrients (e.g., proteins, fat, and fat-soluble vitamins). Serum protein levels depend on the rates of synthesis, degradation, and sequestration from the circulatory system and can be significantly affected by infectious and catabolic processes. The serum protein concentrations are more closely correlated with liver injury (especially acute or recent episodes) rather than the degree of malnutrition [15]. The body also tends to maintain visceral protein pools (i.e., serum, blood cells, and organs) by redistribution from skeletal muscle protein, resulting in muscle wasting with normal serum protein levels. Plasma proteins that are synthesized in the liver and often measured in clinical settings include albumin, transthyretin (prealbumin), retinol-binding protein, and transferrin. Decreased albumin levels in chronic liver disease patients often results from third spacing loss into the extravascular compartment. Malabsorption and poor oral intake may further contribute to the hypoalbuminemia. Transthyretin has a short half-life (2 days) and therefore is a good marker of the more recent status of total body protein. Generally, reduced hepatic protein synthesis is an important risk factor for mortality.

Even though nitrogen balance is difficult to accurately assess in patients with chronic liver disease, a negative balance may imply a hypercatabolic state, lean body mass breakdown, and dietary deficiencies. However, decreased urinary nitrogen can be due to impaired hepatic urea synthesis. Fortunately, intestinal peptide absorption and nitrogen status usually improve quickly with clinical recovery from stressed and/or malnourished states [16]. The creatinine-height index is another indicator of lean body mass if renal function is normal, but this index is also affected by protein intake and stress such as trauma or infection. Evaluations of fat absorption, essential fatty acid sufficiency, as well as vitamin and mineral status are discussed later in this chapter.

An additional problem with using biochemical investigations for evaluation of nutritional status in children with liver disease is that some medications may alter measured vitamin levels. For example, bile acid binding resins may deplete intestinal bile acids and impede fat-soluble vitamin absorption. Anticonvulsants such as phenytoin and phenobarbital can increase hepatic metabolism of vitamin D or shunt it into nonfunctional metabolites, thus lowering plasma 25-hydroxyvitamin D levels.

Immune status can be applied as an indirect measure of nutritional status. In children with longstanding portal hypertension, hypersplenism can result in lymphopenia and pancytopenia, delayed hypersensitivity reactions (or anergy), or hypocomplementemia irrespective of nutritional status. Therefore, these parameters are neither reliable nor accurate measures of nutritional status in children with chronic liver disease [17].

Nutritional Deficiencies in Children with Chronic Liver Disease

Fat and Essential Fatty Acids

Fat maldigestion and malabsorption are frequently observed in patients with chronic liver disease especially when associated with cholestasis. Steatorrhea is a primary symptom of fat malabsorption. Even in the absence of biliary obstruction, intraluminal bile salt concentrations are often lower than the critical micellar concentration, so micellar solutions cannot be formed. Moreover, the degree of cholestasis does not correlate well with the degree of steatorrhea [18].

In contrast to other fatty acids, short- and medium-chain triglycerides (SCTs, MCTs up

to C12 fatty acids) do not require intraluminal bile salts for micellar solubilization in order to be absorbed. Furthermore, because of increased water solubility, these are directly absorbed into the portal circulation. Supplementation with MCT containing formulas (Pregestimil [Mead Johnson, 60 % MCT], Alimentum [Ross, 50 % MCT], Portagen [Mead Johnson, 87 % MCT]), MCT oil, or MCT-enhanced diets (in older children) may be beneficial because of reduced steatorrhea and improved nutrition status and growth in children.

Essential Fatty Acids

The essential fatty acids (EFAs), linoleic (C 18:2) and linolenic (C 18:3) are fatty acids that cannot be synthesized from the chemical transformation of other fatty acids by human metabolism. They are required for synthesis of prostaglandins, eicosanoids, thromboxanes, and leukotrienes that are important for cell membrane function [19]. The most common essential fatty acid deficiency in humans with liver disease is linoleic acid deficiency, first described in 1958 in infants taking a formula deficient in linoleic acid [20]. Reports describing essential fatty acid deficiencies in patients receiving total parenteral nutrition without essential fatty acids occasionally arise as well [21]. Fat maldigestion and malabsorption in conjunction with inadequate enteral intake of the EFAs can lead to EFA deficiency which may result in growth impairment, dry scaly rash, thrombocytopenia, and impaired immune function [22].

Long-chain fatty acids are inadequately absorbed if cholestasis is present, especially when more than 30 % of dietary fat is not absorbed [23]. The risk of EFA deficiency is even higher in infants because they have lower linoleic stores compared to the older children [24]. Skin, hair, and nail changes are the most common findings in individuals with essential fatty acid deficiency, ranging from dry and scaly skin to desquamation. Dull hair and brittle nails are also described. Typically findings are most prominent in body fold regions. More rarely neurological symptoms are reported including weakness, ataxia, leg pain, numbness, and blurred vision. Docosahexaenoic acid (DHA), derived from alpha linolenic acid has been shown to be important in the development of normal vision in humans. An exogenous source of DHA may be particularly important for preterm infants [25–27].

Essential fatty acid deficiency has been reported in patients with cystic fibrosis, presumably related to pancreatic insufficiency rather than to disruption of liver function, although both can occur in cystic fibrosis. Poor wound healing, excessive desquamation, and recurrent cellulitis were reported in this population [28]. Serum fatty acid levels can be used to measure for essential fatty acid deficiency. To monitor essential fatty acid (EFA) status, the ratio 20:3ω9/20:4ω6 (triene/tetraene) is measured, called the "T/T ratio." A T/T ratio of ≥0.2–0.4 is associated with clinical essential fatty acid deficiency [29].

Essential fatty acid deficiency in chronic liver disease is not common, but has been occasionally reported. One study compared the fatty acid profiles of adults with end-stage liver disease compared with adults with upper gastrointestinal cancers. Patients with liver disease had subclinical lower serum levels of both essential fatty acids. The significance of these findings is uncertain [30]. It has also been reported that patients with end-stage liver disease have low serum levels of long-chain polyunsaturated fatty acids with carbon lengths of 20 or greater, presumably due to impaired hepatic synthesis. These "conditionally" essential and essential fatty acids can be replaced with oral supplements [31]. Foods rich in essential fatty acids include cold water fish such as salmon and tuna, flaxseeds, canola oil, soybeans, pumpkin seeds, walnuts, and walnut oil. Fish oil capsule supplements generally contain both omega-3 and omega-6 fatty acids.

To prevent EFA deficiency, at least 3–4 % of calories should be linoleic acid. Pregestimil (Mead Johnson Nutritionals) and Alimentum (Ross Laboratories) provide 7–14 % of calories as linoleic acid. On the other hand, Portagen (Mead Johnson Nutritionals) contains <3 % of calories as EFA (with 87 % as MCT) and therefore is not recommended for long-term use in children with

chronic cholestatic liver disease because EFA deficiency may arise [32]. Additives and supplementations can be given to ensure adequate EFA intake, for example, corn or safflower oil can be added to foods or a lipid emulsion (Microlipid, Novartis) can be added to formulas to provide more linoleic acid.

Fat-Soluble Vitamins

The fat-soluble vitamins A, D, E, and K also depend on the critical intraluminal bile salt concentration for intestinal absorption via enterocytes. With chronic cholestasis and steatorrhea, depletion of body stores of fat-soluble vitamins leads to biochemical and clinical features of nutritional deficiency (as shown in Table 27.2) unless proper dietary supplementation is implemented. Regular follow-up for signs and symptoms of vitamin deficiency is crucial in children with chronic cholestatic liver disease. Moreover, appropriate laboratory investigations to determine vitamin status can be useful in optimizing nutritional supplementation and follow-up.

Vitamin A

The major vitamin A (VA) compounds consist of retinol, retinal, retinoic acid, and retinyl esters. Dietary VA is mainly derived from animal sources (liver, fish liver oil, dairy products, eggs) and carotenoids (mainly beta-carotene) from brightly colored fruits and vegetables (e.g., carrots, tomatoes) and red palm oil. Retinyl esters, the source of VA when eating animal food sources, require intestinal esterase for hydrolysis that is also bile acid dependent prior to the absorption in the small intestine. VA plays important roles in maintaining normal vision, growth, cellular differentiation, and immune function.

VA status can be determined from samples of serum and liver tissue, conjunctival impression cytology, as well as dark-field adaptation tests [33]. VA deficiency has frequently been found in children with cholestatic liver disease [34]. Determinations of serum retinol and retinol-binding protein are routinely used for screening of VA status. Daily Reference Intakes (DRIs) of retinol can be given as International Units (IUs), micrograms of retinol, or "retinol equivalents" (REs). IUs refer to biologic activities of the retinol and may vary from one compound to another. One IU of retinol is roughly equal to 0.3 μg of retinol. One RE is equal to 1 μg of retinol, which is equal to 3.3 IUs of retinol. Normal fasting serum retinol concentration is >20 μg/dL (>0.7 μmol/L) with a normal fasting serum retinol-binding protein of <1 mg/dL. However, retinol-binding protein is an acute-phase reactant and is mainly produced and stored in the liver; therefore, any recent stress, inflammation, or injury can falsely elevate the values. The best noninvasive test of VA status is the relative dose–response test. If VA

Table 27.2 Clinical signs associated with nutritional deficiencies in chronic liver disease

Organ	Clinical sign (s)	Nutrient deficiency
Hair	Thin, sparse, easily fragile	Protein, energy, zinc
Face	Diffuse pigmentation	Protein, energy
Eyes	Pale conjunctivae (anemia)	Iron
	Bitot's spots, conjunctival xerosis	Vitamin A
Teeth	Dental caries	Vitamin D
Glands	Parotid enlargement	Protein, energy
Skin	Xerosis, follicular keratosis	Vitamin A, essential fatty acids
	Dry, easily pluckable hair	Protein
Nails	Koilonychia	Iron
Subcutaneous tissues	Peripheral edema	Protein
	Decreased subcutaneous fat, wasting	Protein, energy
Bone	Epiphyseal enlargement	Vitamin D
	Craniotabes, frontal bossing, rachitic rosary, bowed legs, rickets	Vitamin D
Liver	Hepatomegaly	Protein, energy
Nervous system	Psychomotor changes, confusion, irritability	Protein
	Loss of vibratory sense, decreased deep tendon reflexes	Vitamin E

status is adequate, the serum retinol concentration should not dramatically change following a relatively small oral loading dose of VA (1,500 IU). However, if hepatic VA stores are low, the retinol concentration markedly increases in response to the loading dose. To bypass the possible reduced intestinal absorption of VA, parenteral forms of VA and a modified oral form of VA (i.e., oral VA + D-ALPHA-TOCOPHERYL polyethylene glycol-1000 succinate [enhanced absorption of VA by combining it with a more water-soluble form of vitamin E]) have also been used. At 10 h, the modified relative dose response of <10 % rise is normal and >20 % rise indicates low hepatic VA stores. Conjunctival impression cytology is performed by applying cellulose acetate filter paper directly to the eye and then grading the goblet cells and epithelial cells under the light microscope.

Clinical VA deficiency leads to xerophthalmia, keratomalacia, irreversible damage to the cornea, night blindness, and pigmentary retinopathy. While these findings are rare, permanent ocular damage and visual loss have occurred in children [35]. The recommended daily allowance (RDA) of VA for children 1–3 years is 300 μg/day, 4–8 years 400, and 600–900 μg/day for older children and adults. RDAs for VA are given as μg of retinol activity equivalents to account for the different bioactivities of retinol and provitamin A carotenoids. Oral supplementation of VA ranges from 5,000 to 25,000 IU/day of water-miscible VA in children >1 year. VA capsules (8,000 U/capsule, 10,000 U/capsule, 15,000 U/capsule, or 25,000 U/capsule, generic) are available. AquADEKs Pediatric Liquid (Axcan Pharma) contains 5,751 IU/mL of VA. VA is also available in parenteral form.

VA toxicity can cause fatigue, malaise, anorexia, vomiting, increased intracranial pressure, painful bone lesions, higher risk for bone fractures, hypercalcemia, dermatitis, and hepatotoxicity [36]. In order to help prevent the risk of vitamin A toxicity, the US Institute of Medicine has established Daily Tolerable Upper Levels of intake for healthy individuals [37]. According to these guidelines, the risk for VA toxicity increases when the daily dose exceeds 2,000 IUs in infants, 3,000 IUs in young children, 5,665 IUs in older children (9–13 years of age), and 10,000 IUs in teenagers. In children with liver disease, these limits may need to be exceeded. However, careful monitoring for VA toxicity is recommended when these guidelines are exceeded [38].

Vitamin D (Calciferol)

Vitamin D (VD) has two major forms: vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). Ergocalciferol is found in plants and fungi and is the form that has been used for cow milk and juice fortification. However, diet accounts for less than 10 % of VD stores in the body. VD mainly derives (>90 %) from cutaneous photosynthesis via ultraviolet-B radiation. The formation of active VD requires a first hydroxylation of cholecalciferol in the liver to 25-hydroxyvitamin D (25-OHD) and a second hydroxylation in the kidney to form the biologically active hormone −1,25-dihydroxyvitamin D. VD has a well-known biologic function in the maintenance of serum calcium and phosphorus concentrations by enhancing absorption of these two minerals from the small intestine; therefore, VD is a crucial component in maintaining normal bone homeostasis.

The most sensitive indicator of VD status is the plasma concentration of 25-OHD. A level below 12 ng/mL (<30 nmol/L) is suggestive of VD deficiency, and a level of 20 ng/mL or higher (50 nmol/L) is considered as VD sufficiency [39]. Recently, several noncalcemic effects of VD have been found in patients with chronic liver disease, including insulin resistance or diminished viral clearance of chronic hepatitis C infection [40]. Therefore, the definition of "normal" cutoff values for 25-OHD remains controversial. Periodic assessments of 25-OHD levels, sunlight exposure, intake of VD, calcium, and phosphorous from diet and supplementations may all be necessary in children with chronic cholestatic liver disease.

Severe VD deficiency manifests with hypocalcemia, hypophosphatemia, tetany, osteopenia, rickets, and pathologic fractures [41]. Furthermore, hypocalcemia results in secondary hyperparathyroidism and a subsequent

increase in bone resorption and demineralization. Although 25-OHD levels return to normal with good nutritional support, some cholestatic children continue to have suboptimal bone mass later in life [42]. Magnesium may also play a role in maintaining bone mineralization in children with cholestasis [43]. Orthotopic liver transplantation has been shown to improve bone mineral density measured by dual-energy X-ray absorptiometry and VD status of these children [44]. Serum concentrations of calcium, phosphorous, magnesium, alkaline phosphatase, and parathyroid hormone should be checked on an annual or biannual basis in long-term transplanted patients.

AI and RDA intakes, respectively, for VD are currently 10 μg/day (400 IU/day) for infants and 15 μg/day (600 IU/day) for children and adults [39]. Vitamin D deficiency can be treated with oral vitamin D supplementation (ergocalciferol 50,000 IU/capsule, 8,000 U/mL) usually at a dose range of 600–2,000 IU/day. Children with cholestasis usually require higher doses of VD to normalize the 25-OHD levels [14]; as a result larger doses of vitamin D supplementation (5,000–20,000 IU/day) may be required to correct this condition. Serum 25-OHD levels must be closely monitored along with calcium and phosphorus levels and urine calcium to creatinine ratio during supplementation to assure normalization of 25-OHD levels without causing VD toxicity. The use of parenteral VD should be done only when children fail to respond to oral supplementation in spite of excellent compliance because of the higher cost and risks for toxicity. Calcitriol injection 1 (1,25-hydroxyvitamin D) 1 μg/mL at a dose of 0.02 μg/kg can be used in clinical settings with careful monitoring of calcium, phosphorus, and PTH status.

Vitamin D toxicity includes hypercalcemia leading to central nervous system depression, ectopic calcifications, hypercalciuria, nephrocalcinosis, and nephrolithiasis. The Daily Tolerable Upper Levels of intake of vitamin D for healthy individuals varies with age. For infants it is 1,000 IUs, for young children (under 3 years) it is 2,500 IUs, for children 4–8 years of age it is 3,000 IUs, and after 8 years of age it is 4,000 IUs [45].

Vitamin E (Tocopherol)

The four major forms of vitamin E (VE) (α, β, δ, γ) differ by the position and the number of methyl group substitutions and their bioactivities. α-Tocopherol is the principal form found in food (grains, plants, and vegetable oils) and has the highest biologic activity. VE is vital for structural and functional maintenance of the neuromuscular system. VE, transported by LDL and HDL, is the most hydrophobic of the fat-soluble vitamins and requires the highest intraluminal bile acid concentrations for absorption. Furthermore, tocopheryl esters require pancreatic or intestinal esterase for hydrolysis that is also bile acid dependent [46].

A standard test of VE status in children is the ratio of serum VE to total lipids (i.e., the sum of cholesterol, triglycerides, and phospholipids) because VE also binds to lipoproteins which may be increased in cholestasis [47]. Normal vitamin E to lipids ratio is 0.8–1.0 mg of total tocopherols/g of total lipids. VE deficiency in patients older and younger than 1 year of age is defined as a ratio of <0.8 mg/g and <0.6 mg/g, respectively. Measurement of VE in adipose tissue is impractical and not readily available. Functional assays of VE can be performed by RBC hydrogen peroxide hemolysis and the RBC malondialdehyde release test [48, 49]. The ethane breath test has also been used to evaluate antioxidant status by assessing the peroxidation of fatty acids, a surrogate for VE function [50]. However, these functional tests are impractical in clinical settings and are not specific as the tests can be affected by selenium. VE absorption can be determined by oral VE tolerance test that is diminished in children with significant cholestatic liver disease and improved by coadministration of bile acids.

Peripheral neuropathy, ataxia, ophthalmoplegia, muscle weakness, and hemolytic anemia characterize VE deficiency. The neurological damage can be permanent if the supplementation is not given in a timely manner. Reversal of the findings may be accomplished before 3 years of age [51]. The RDA intake for VE is 4 mg/day in infants 0–6 months, 5 mg/day in 7–12 month infants, 6 mg/day in children 1–3 years, 7 mg/day in children 4–8 years, 11 mg/day in children

9–13 years, and 15 mg/day in adults. To prevent VE deficiency in patients with cholestasis, supplementation should always be implemented. In children with VE deficiency, 10–16 mg/kg/day (with 15–25 IU/kg/day) of VE therapy (α-tocopherol, Aqua-E [Yasoo Health 20 IU/mL], Liqui-E [TPGS-D-alpha tocopheryl polyethylene glycol 1000 succinate, 400 IU/15 mL, Twinlabs]) should be initiated as soon as VE deficiency is identified. Patients may benefit from morning dosing when bile flow reaches its peak after an overnight fast. Monitoring vitamin E to total lipids ratio and thorough neurologic examination will help determine the need for dose adjustment. If normalization of VE status is achieved, neurologic functions generally recover within 3–4 weeks of therapy [51]. VE toxicity is rare but may present as bleeding in children who take anticoagulants or be an underlying contributor to the development of sepsis in neonates.

Vitamin K

Vitamin K (VK) has major three forms. Phylloquinone (vitamin K1) is found in leafy vegetables, soybean oil, fruits, seeds, and cow's milk. Menaquinone (vitamin K2) is mainly produced by bacteria in the large intestine. Menadione (vitamin K3) is a synthetic form of VK. Absorption of vitamin K1 is an active process and requires bile salts as well as pancreatic secretion, while vitamin K2 is absorbed via passive diffusion. VK is then integrated with chylomicrons and transported via lymph into the circulation with very little storage in the liver. VK functions as a coenzyme for the synthesis of several proteins involved in the coagulation pathways (factors II, VII, IX, X, protein C, and protein S) and bone metabolism (osteocalcin) [52].

In a state of normal hepatic function, VK status can be screened for by measuring prothrombin time using the international normalized ratio (PT/INR) which depends on functions of VK-dependent clotting factors (i.e., factors II, VII, IX, and X). If PT/INR is prolonged in comparison with the activated partial thromboplastin time, VK deficiency is likely. Measurement of each individual VK-dependent clotting factors is costly and in the setting of chronic liver disease offers no added values over regular monitoring of the PT/INR for assessing VK status in children. Direct measurement of VK levels by high performance liquid chromatography is also available in many laboratories now. Of note, liver disease itself may prolong the PT/INR because of an impaired synthesis of clotting factors involved in the intrinsic pathway of the coagulation cascade, but the activated partial thromboplastin time also is usually prolonged. VK status can also be measured by the plasma proteins induced in vitamin K absence (PIVKA-II) enzyme-linked immunosorbent assay.

A PIVKA-II level greater than 3 ng/mL indicates VK deficiency. The levels have demonstrated positive correlations with plasma conjugated bilirubin, total bile acids, and severity of the liver disease. However, the test is not specific as elevated levels can be seen in normal children.

VK deficiency in infancy can cause intracranial bleeding and classic hemolytic disease of the newborn [53]. Besides malabsorption of VK in cholestatic children, VK deficiency can be precipitated or worsened by antibiotic administration due to the suppression of large intestinal flora which produces vitamin K [54]. The recommended adequate intake for VK is 20 μg/day for infants 0–6 months, 25 μg/day for infants 7–12 months, 30 μg/day for children 1–3 years, 55 μg/day for children 4–8 years, 60 μg/day for children 9–13 years, and 75 μg/day for children 14–18 years. The adequate intake for men and women is 120 and 90 μg/day, respectively. No adverse effects have been reported for individuals consuming higher amounts of VK. Supplementation with oral VK in cholestatic children should be provided (Mephyton, 5 mg vitamin K1 tablets Aton Pharma Inc.) in a daily or twice weekly dose of 2.5–10 mg, and the dose can be adjusted depending on the therapeutic response. Failure to respond to oral supplementation may indicate the need for subcutaneous or intravenous VK administration for 3 consecutive days to correct coagulopathy [Aquamephyton, Merck and Co., (vitamin K1) 2 mg/mL or 10 mg/mL]. Intravenous administration should be given slowly to avoid anaphylaxis. Failure to respond to parenteral VK administration, as indicated by inability to

correct the prolonged PT/INR (in conjunction with other symptoms such as anorexia, failure to thrive, ascites, and steatorrhea), suggests significant hepatic synthetic dysfunction.

Water-Soluble Vitamins

Water-soluble vitamin deficiencies in children with significant cholestatic liver disease are uncommon. A likely explanation is that the formulas and foods ingested by these children usually contain adequate water-soluble vitamins that are absorbed and utilized in spite of significant liver dysfunction.

Trace Minerals

Zinc

Over 100 zinc metalloenzymes have been identified since carbonic anhydrase was first described in 1940 [55]. Zinc plays an essential role in nucleic acid metabolism, mitosis, and protein synthesis. Zinc deficiency is implicated in numerous deficiency symptoms including slowed growth velocity, diarrhea, decreased appetite, abnormalities of both the humoral and cell-mediated immune response (and thus increased rates of infections), skin lesions, hair loss, nail dystrophy, slow wound healing, delayed sexual maturation, impotence, lack of dark adaptation, hypergeusia, dysgeusia, and behavior abnormalities. Although zinc is an important component of a wide range of enzyme systems, many of the features of zinc deficiency are attributed to disturbances in nucleic acid metabolism and protein synthesis.

Zinc deficiency has been described in chronic liver disease of multiple causes. Some of the mechanisms for zinc deficiency/altered metabolism include decreased dietary intake, increased urinary excretion, and alterations of certain zinc transporters such as albumin. Clinical findings associated with zinc deficiency and liver disease are similar to findings in patients without chronic liver disease including skin lesions, poor wound healing, altered mental status (which may be difficult to differentiate from hepatic encephalopathy), and altered immune function. Clinical trials in human liver disease are limited in size and quality, but it is clear that zinc supplementation can reverse clinical signs of zinc deficiency in patients with liver disease [56].

Although there is no highly reliable and clinically useful biomarker of zinc deficiency, serum and plasma zinc levels are most commonly used and are the only tests available in routine clinical practice. Plasma levels of less than 60 μg/dL are consistent with moderate zinc deficiency and levels less than 40 μg/dL suggest severe deficiency. Low levels of albumin (common in chronic liver disease) result in low levels of measured zinc because zinc is carried in the systemic circulation bound to albumin. Corrections for low albumin are not usually done because the correction is not usually large and because borderline cases of zinc deficiency should be treated. Interlaboratory differences in zinc measurements make it difficult to directly compare zinc levels measured in different laboratories. Therefore, reference levels at the local laboratory should be considered, and comparisons should be applied carefully. Because of the perceived low sensitivity of these cutoff values, and because zinc deficiency is not rare in chronic liver disease, oral supplementation should be considered if symptoms are typical, even if test results are normal.

Oral supplementation of 40–50 mg/day of elemental zinc is usually sufficient to treat deficiency in adults. Studies, from developing countries, of children with zinc deficiency and chronic diarrhea showed good tolerance and response to replacement doses of 20 mg/day [57, 58]. Many nonprescription multivitamins provide 10–20 mg/day of zinc.

Copper

Copper and iron both play essential roles in cellular respiration, particularly in oxidase enzymes. Numerous copper-dependent enzymes have been described. The frequency and severity of copper deficiency conditions in humans has been a matter of debate. In contrast, in patients with chronic cholestasis and chronic liver disease (e.g., biliary atresia, sclerosing cholangitis, pri-

mary biliary cirrhosis, Indian childhood cirrhosis, chronic active hepatitis, and with some liver tumors [59]), excessive accumulation of copper is more common than copper deficiency. Copper excretion in the urine is increased, and excessive copper accumulation in the liver may occur. The accumulation of copper in liver and other tissues is closely associated with the pathophysiology of Wilson disease.

Copper deficiency, from dietary inadequacy, results in anemia (unresponsive to iron replacement), neutropenia, and osteoporosis. Radiographic findings associated with copper deficiency included osteoporosis of the metaphyses and epiphyses and delayed bone age [60]. Copper deficiency can cause a wide variety of neurological problems including myelopathy, peripheral neuropathy, and optic neuropathy [61]. However, copper deficiency in chronic liver disease is very rare. In general it would be ill-advised to provide added copper to the dietary regimen of a patient with end-stage liver disease.

Selenium

Selenium deficiency was first clinically recognized in the 1970s when the low-selenium content of food was found to be associated with Keshan disease (a form of congestive cardiomyopathy) in China [62]. In 1973, it was shown that selenium is an integral component of glutathione peroxidase, an enzyme which reduces lipid hydroperoxides to their corresponding alcohols and free hydrogen peroxide to water [63]. Experimentally, selenium deficiency results in a cardiomyopathy characterized by multiple areas of myocardial necrosis [64]. More rarely it results in skeletal muscle injury [65].

It has been reported that patients with chronic liver disease, regardless of etiology, have low serum and hepatic levels of selenium. These same patients were not found to lack dietary selenium. Rather, it was concluded that the reduced selenium state was related to overall poor nutrition and not a specific lack of selenium intake [66]. A study of 27 children waiting for liver transplantation found low levels of serum selenium in 13 % [67].

Selenium deficiency is associated with low levels of selenium in whole blood and in plasma and also with low erythrocyte glutathione peroxidase activity [68]. The likely best treatment for selenium deficiency is appropriate nutrition. In profound deficiency such as that seen in Keshan disease, a weekly supplement of 500–1,000 μg has been given. When a child is TPN dependent, care should be taken to ensure that selenium is included in the intravenous solutions.

Chromium

It has been recognized since 1969 that chromium is a cofactor for insulin [69]. Thus, chromium deficiency is associated with impaired glucose tolerance, especially when insulin levels are normal. Weight loss, poor growth, insulin resistance, and neuropathies (similar to diabetic neuropathies) have been described as well [70, 71]. Isolated chromium deficiency in patients with chronic liver disease is exceedingly rare. More often it occurs in the setting of chronic TPN supplementation or as part of general malnutrition in the setting of chronic liver disease [72].

Chromium deficiency can be difficult to diagnosis. The lower levels of normal are so low (approximately 1 picogram/mL) that accurate measurements of below normal values exceeds the sensitivity of most laboratory methods. Thus, chromium deficiency must be considered, usually based on abnormal glucose tolerance, and then improvement demonstrated after chromium supplementation either with TPN or in oral forms.

The Role of Diet in the Treatment of Serious Complications of Liver Disease

Whenever possible, chronic cholestasis is treated according to its underlying cause. Surgical treatments, antimicrobial medications, or anti-inflammatory drugs may be used for treatments in specific instances. However, much of the treatment for chronic liver disease is supportive. Efforts are made to either slow the progression of disease or to treat specific complications as they arise. Nutrition can play an important role in several of the more common complications of chronic liver disease.

Ascites

The development of ascites is one of the most common complications of cirrhosis. Although at first it may be subtle or undetectable, it eventually becomes incapacitating, limiting a patient's respiratory effort or mobility by sheer volume and mass. This accumulation of fluid represents a breakdown of intravascular volume homeostasis. Portal hypertension is essential to the development of transudate ascites; patients without portal hypertension do not develop transudate ascites.

Competing theories about the pathogenesis of ascites in patients with cirrhosis have attempted to incorporate normal and abnormal aspects of renal blood flow and sodium balance, as well as observed responses to stimuli such as fluid challenges.

Treating children and adolescents with ascites has challenges not encountered in the treatment of adults. The goal of treatment is not only to reduce or eliminate ascites but also to encourage growth. Growth is in fact the more important consideration, especially when liver transplantation eventually will be necessary. Salt and fluid restriction results in a decrease in the rates of formation and absorption of ascites and is a foundation for the treatment of ascites in adults. However in children, such restriction may not be optimal if the result is inadequate calorie intake and growth failure. In general, diuretics may be used earlier and more aggressively in children than in adults when the goal is to achieve good growth velocity.

Adult or adolescent patients usually can tolerate a diet with daily sodium intake limited to 2,000–3,000 mg/day, and this often results in a negative sodium (and therefore water) balance. Younger children usually can be restricted successfully to 1,000 mg/day, but only if their total calorie intakes remain adequate to sustain growth and if the concomitant use of sodium-wasting diuretics does not lead to hyponatremia. Fluid intake in children and adolescents can be limited to 1,000–1,500 mL/day, but this usually is unnecessary unless the serum sodium concentration is less than 120 mEq/L. A more complete description of the treatment of ascites can be found in chapter "Neonatal Hemochromatosis and Gestational Alloimmune Liver Disease".

Hepatic Encephalopathy

Hepatic encephalopathy refers to a variety of reversible neurological abnormalities seen in patients with cirrhosis. Many neurologic functions can be affected in this syndrome, including disturbed consciousness (including coma), personality changes, intellectual deterioration, and speech and motor dysfunction. The sudden onset and rapid reversibility of encephalopathy in patients with liver disease suggest that it is of metabolic origin. The appearance of hepatic encephalopathy depends on three factors: portal-systemic shunting, alterations in the blood–brain barrier, and the interactions of toxic metabolites with the CNS.

Altered ammonium metabolism has been implicated in the pathogenesis of hepatic encephalopathy for many years. Serum ammonia concentrations are increased in subjects with liver failure and chronic liver disease. Hyperammonemia also is seen in other encephalopathies such as Reye syndrome and organic acidemias. Serum ammonia is generated from both exogenous and endogenous nitrogen sources.

In patients with liver disease, the capacity to metabolize ammonia is diminished, either because of shunting of portal blood away from the liver or because of hepatocyte dysfunction. In either case, blood ammonia levels increase. Patients with advanced liver disease often have muscle wasting and are compromised further in their ability to metabolize ammonia that escapes the liver.

Several competing theories have been proposed as the mechanism for hepatic encephalopathy including the synergistic neurotoxin hypothesis, the false neurotransmitter hypothesis, and the γ-aminobutyric acid-ergic inhibitory neurotransmitter hypothesis. Regardless of the cause for hepatic encephalopathy, the treatment is aimed at limiting the production of nitrogenous by-products and removing the by-products as promptly as possible. The first step in the

treatment of hepatic encephalopathy is to identify and treat directly any precipitating factors. All dietary and intravenous protein intake should be limited during the acute onset and treatment of encephalopathy. Protein may be liberalized as the encephalopathy subsides. Standard therapy for chronic hepatic encephalopathy in adults includes restriction of protein to 40 g/day. In children, protein restriction may result in growth failure and should be undertaken only with specific attention to the overall nutritional state and needs of the patient and attention to maintaining positive nitrogen balance whenever possible.

Lactulose (β-galactosidofructose), a semisynthetic disaccharide, is a mainstay of treatment for hepatic encephalopathy. When taken orally this disaccharide reaches the colon intact, where resident bacteria metabolize it to its component sugars, galactose and fructose, and further metabolize the sugars to lactic acid, acetic acid, and various organic acids. The acidified fecal contents trap ammonia, making it unavailable for absorption. Therapy with lactulose or with neomycin alone is successful in reducing encephalopathy in a majority of patients. In children, where growth is paramount, treating aggressively with lactulose or with neomycin, or both, may allow more intake of dietary protein, thus supporting growth.

Because the ratio of the serum concentration of aromatic amino acids (phenylalanine, tyrosine, and tryptophan) to the serum concentration of branched-chain amino acids (BCAAs)—leucine, isoleucine, and valine—is increased in subjects with cirrhosis and hepatic encephalopathy, there have been attempts to reverse encephalopathy by giving BCAAs, either intravenously [73, 74] or orally [75]. In progressive cirrhosis, amino acids are released with hepatocyte necrosis and from skeletal muscle breakdown. The excess aromatic amino acids are not cleared by the liver because of either impaired hepatocyte function or increased collateral circulation. BCAAs, on the other hand, can be oxidized by a variety of tissues and therefore are not increased in the serum [76, 77].

Brain and plasma accumulation of aromatic amino acids may result in an alteration of the synthesis of brain neurotransmitters such as norepinephrine, dopamine, and serotonin [78]. In dogs, large intravenous doses of the aromatic amino acids tryptophan and phenylalanine have caused coma; the combined infusion of tryptophan, phenylalanine, and BCAAs failed to cause coma [77]. BCAAs might treat hepatic encephalopathy successfully by reducing muscle protein breakdown or by normalizing the serum amino acid profile. A placebo-controlled study of adult patients with chronic portal-systemic encephalopathy demonstrated a decreased recurrence of acute hepatic encephalopathy in those subjects receiving diets with a BCAA supplement [75, 79]; other reports have not shown any effect, however [73, 80]. One meta-analysis of the efficacy of BCAAs in the treatment of hepatic encephalopathy concluded that further large long-term multicenter studies were needed to provide more reliable evidence [81]. BCAAs have been used as an enteral protein supplement in children with advanced cirrhosis and malnutrition. All children showed improvement in anthropometric indices and none experienced encephalopathy as a result of the diet. Although this study did not explore the use of BCAAs in the treatment of hepatic encephalopathy in children, it demonstrated the safety of using BCAA in the diets of children with liver disease [82]. Treatments for hepatic encephalopathy are discussed in detail in Chap. 26.

The Bridge to Transplantation

Nutritional assessment and counseling are important parts of the screening process for "listing" patients as potential transplant recipients. Once listed, patients are seen regularly to assess the progress of their liver disease and to update, start, or fine-tune the treatments of the primary liver disease (when possible) and the complications of liver disease, as already discussed.

Nutrition should be considered at each pretransplant visit. While liver transplantation can be carried out in a malnourished patient, the outcome of the transplant improves with better nutritional status. The preoperative nutritional status has been found to influence postoperative

morbidity for transplant surgery [83] as well as other surgeries such as tumor resection and shunting procedures [84, 85]. For example, protein-energy malnutrition is associated with variceal bleeding, refractory ascites, encephalopathy, and infection; all of these can be ameliorated to some degree by increased nutrient intake [86–88]. Poor pre-transplant nutrition has also been shown to be associated with prolonged intensive care unit stays, chronic respiratory therapy, and mortality [89, 90].

Nutrient intake should be calculated by a dietitian by means of food diaries and dietary recall. Body composition can be measured by indirect techniques such as anthropometry or bioelectric impedance. In cirrhotic patients these may be inaccurate because of excess extracellular fluid retention [91]. Urinary creatinine excretion, as an estimate of muscle or body cell mass can also be used. The validity of this measure in cirrhotics has been questioned because creatinine is itself liver-derived. However, it has been found to be adequate and is more practical and less expensive than more precise methods such as dual-energy X-ray absorptiometry or deuterium oxide dilution for determination of total body nitrogen, fat, or water [92].

Nutritional status can be improved by changes in macronutrients such as food and special formulas and micronutrients in the form of dietary supplements. A creative and invested dietitian may be able to boost intake by working within the sometimes frustrating boundaries imposed by the fussy or picky eater. Poor appetite may be related to portal hypertension and its consequences. These may respond to proton pump inhibitors or more aggressive diuretic therapy, respectively. In some cases anorexia may be related to chronic depression, and again this should be directly addressed. Direct treatment of anorexia with appetite stimulants has not been studied in great detail. One study found good responses in adults with alcoholic cirrhosis [93]. In children oxandrolone has been used to enhance the appetite of children after life-threatening and extensive burns with some success [94]. However, whether these agents result in improved lean body mass remains controversial.

All parents and caretakers understand that there are significant advantages to transplantation in larger children as compared to smaller children. These advantages include a wider pool of potential donors, increased ease of operation, and the general advantages associated with an improved nutritional state. Some patients have found success through eating more frequent small meals, including a late night meal [95].

Dietary supplements may help when a particular substrate is lacking or is desired. For example, supplementation of branched-chain amino acids has promoted positive nitrogen balance in patients at risk for development hepatic encephalopathy [75]. Liquid "meal replacements" such as Pedisure (Abbott), Ensure (Abbott) and Carnation Instant Breakfast (Nestle) may result in improved nutritional states, especially when they are used to supplement (rather than replace) a robust attempt to provide sufficient dietary calories. One study of children showed that supplementation with branched-chain amino acids resulted in improvements in both height and body weight when compared to children who did not receive the supplement [96].

Tube feeding can provide a critical bridge to transplantation when patients cannot support their caloric needs. Feeds can be given by nasogastric tube (NGT), usually safely at night, at low rates. Such a regimen preserves the hunger drive during the day and does not destroy the satisfying experience of eating. Older patients may be able to pass their own NGT at nights (sometimes daily or sometimes only on certain days), while younger children may prove to be resilient and adapt to the presence of a small feeding tube without much fuss. Some patients and their families may derive significant solace knowing that an overnight supplement of calories will "smooth over" the natural day-to-day variations in eating; it helps the family get off the "roller coaster" of dismay and delight related to the size of their child's most recent meal.

Providing NGT feeding has not been without controversy. Parents and medical providers alike may have concerns about promoting variceal bleeding because of the presence of a foreign body in the esophagus. Studies in adults have

shown that NGT feeds in patients with varices are safe and effective [88, 97]. As with all treatments, the risks and benefits of such a treatment should be carefully considered. Many factors may influence the choice to provide nutrition through an NGT including the size and appearance of the varices despite knowledge that NGT feeds are safe in the presence of varices, the prior history of bleeding, the response to therapy such as banding, the tolerance of the NGT by the patient, the degree of malnutrition, and finally the perceived proximity of the transplant itself.

Total parenteral nutrition carries the promise of nutrition support, but carries significant risks of infectious, metabolic, and mechanical complications. Progress is being made to minimize the liver injury (steatosis, chronic hepatitis, and eventually cirrhosis) which accompanies chronic parenteral nutrition. The etiology of parenteral nutrition-associated liver disease (PNALD) is not completely understood, but may be related to a number of factors including injury by the lipid component of TPN. The transition from a soybean- to a fish oil-based lipid formulation, such as the omega-3 parenteral lipid formulation Omegaven (Fresenius), has been associated with a dramatic reversal of PNALD within the pediatric population [98–100] and more recently in adults [101]. Branched-chain amino acid-enriched solutions have been used in patients with a risk for, or history of, encephalopathy. The results have been conflicting [79, 102, 103]. Micronutrient deficiency states should be addressed as discussed earlier in this chapter. Subtle deficiency states may be difficult to detect; thus, the threshold for starting treatment should be low in most cases. In general, copper and manganese should be omitted from trace element preparations used in parenteral nutrition solutions in patients with chronic obstructive biliary tract disease.

Parenteral nutrition should be reserved only for those who are not capable of tolerating oral or tube feeding. In some cases prolonged use of total parenteral nutrition itself may be the cause of the chronic liver disease such as in the case of infants with short bowel syndrome. In either case, the parenteral nutrition can be seen as a bridge to transplant of the liver or bowel or both.

Conclusion

The liver is the major organ for maintaining normal nutritional homeostasis. Children with significant liver disease, especially those with chronic cholestasis, cirrhosis, or end-stage liver disease, frequently demonstrate a wide array of impaired nutrient metabolism. The manifestations of these impairments range from subclinical isolated micronutrient deficiencies found only as a result of screening tests to classical and even life-threatening malnutrition syndromes. Comprehensive serial assessments of nutritional status, appropriate recommendations for supplementations, and aggressive nutritional intervention or rehabilitation (if needed) are crucial in providing children with cholestasis with optimal clinical outcomes. Thus, careful attention to nutrition support is required when treating children who suffer from end-stage liver disease and its complications such as hepatic encephalopathy and ascites. Nutritional support also plays an essential role in managing children with liver failure both before and after orthotopic liver transplantation.

References

1. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med. 1999;340:448–54.
2. Chrousos GP. The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. N Engl J Med. 1995;332:1351–62.
3. Karalis K, Sano H, Redwine J, Listwak S, Wilder RL, Chrousos GP. Autocrine or paracrine inflammatory actions of corticotropin-releasing hormone in vivo. Science. 1991;254:421–3.
4. Sarraf P, Frederich RC, Turner EM, et al. Multiple cytokines and acute inflammation raise mouse leptin levels: potential role in inflammatory anorexia. J Exp Med. 1997;185:171–5.
5. Muller MJ, Pirlich M, Balks HJ, Selberg O. Glucose intolerance in liver cirrhosis: role of hepatic and non-hepatic influences. Eur J Clin Chem Clin Biochem. 1994;32:749–58.
6. Petrides AS, DeFronzo RA. Glucose and insulin metabolism in cirrhosis. J Hepatol. 1989;8:107–14.
7. Muller MJ, Willman O, Rieger A, et al. Mechanism of insulin resistance associated with liver cirrhosis. Gastroenterology. 1992;102:2033–41.

8. Selberg O, Burchert W, van der Hoff J, et al. Insulin resistance in cirrhosis. Positron emission tomography scan analysis of skeletal muscle glucose metabolism. J Clin Invest. 1993;91:1897–902.
9. Petrides AS, Luzi L, Reuben A, Riely C, De Fronzo RA. Effect of insulin and plasma amino acid concentration on leucine metabolism in cirrhosis. Hepatology. 1991;14:432–41.
10. Freeman Jr RB, Wisener RH, Harper A, et al. The new liver allocation system: moving toward evidence-based transplantation policy. Liver Transpl. 2002;8:851–8.
11. Owen OE, Trapp VE, Reichard JA, et al. Nature and quantity of fuels consumed in patients with alcoholic cirrhosis. J Clin Invest. 1983;72:1821–32.
12. Merli M, Erickson SL, Hagenfeldt H, Wahren J. Splanchnic and peripheral exchange of FFA in patients with liver cirrhosis. J Hepatol. 1986;3:348–55.
13. Shneider BL, Magee JC, Bezerra JA, Haber B, Karpen SJ, Raghunathan T, et al. Efficacy of fat-soluble vitamin supplementation in infants with biliary atresia. Pediatrics. 2012;130:e607–14.
14. Ryckman F, Fisher R, Pedersen S, Dittrich V, et al. Improved survival in biliary atresia patients in the present era of liver transplantation. J Pediatr Surg. 1993;28:382–5.
15. Florez DA, Aranda-Michel J. Nutritional management of acute and chronic liver disease. Semin Gastrointest Dis. 2002;13:169–78.
16. Dowling RH. Compensatory changes in intestinal absorption. Br Med Bull. 1967;23:275–8.
17. Bahr MJ, Manns MP. Function of the immune system in liver cirrhosis. Gastroenterology. 1993;39:601–7.
18. Badley BWD, Murphy GM, Bouchier IAD, et al. Diminished micellar phase lipid in patients with chronic nonalcoholic liver disease and steatorrhea. Gastroenterology. 1970;58:781–9.
19. Sanders T. Essential fatty acids. In: Macrae R, Robinson RK, Sadler MJ, editors. Encyclopaedia of food science, food technology, and nutrition. London: Academic; 1993. p. 1651–4.
20. Hansen AE, Haggard ME, Boelsche AN, et al. Essential fatty acids in infant nutrition III. Clinical manifestations of linoleic acid deficiency. J Nutr. 1958;60:565–76.
21. Holman RT, Johnson SB, Hatch F. A case of human linolenic acid deficiency involving neurological abnormalities. Am J Clin Nutr. 1982;35:617–23.
22. Wene JD, Connor WE, Den Besten L. The development of EFA deficiency in healthy men fed fat free diets intravenously and orally. J Clin Invest. 1975;56:127–34.
23. Clandinin MT, Chappell JE, Heim T, et al. Fatty acid utilization in perinatal de novo synthesis of tissues. Early Hum Dev. 1981;5:355–66.
24. Pettei MJ, Daftary S, Levine JJ. EFA deficiency associated with the use of a MCT infant formula in pediatric hepatobiliary disease. Am J Clin Nutr. 1991;53:1217–21.
25. Neuringer M, Connor WE, Lin DS, Barstad L, Luck SJ. Biochemical and functional effects of prenatal and postnatal omega-3 fatty acid deficiency on retina and brain in rhesus monkeys. Proc Natl Acad Sci U S A. 1986;83:185–294.
26. Birch DG, Birch EE, Hoffman DR, Uauy RD. Retinal development in very low birth weight infants fed diets differing in omega-3 fatty acids. Invest Ophthalmol Vis Sci. 1992;33:2365–76.
27. Carlson SE, Werkman SH, Tolley EA. Effect of long-chain N-3 fatty acid supplementation on visual acuity and growth of preterm infants with and without bronchopulmonary dysplasia. Am J Clin Nutr. 1996;63(5):687–97.
28. Chase HP, Long MA, Lavin MH. Cystic fibrosis and malnutrition. J Pediatr. 1979;95:337–47.
29. Bender DA, Bender AE. Nutrition, a reference handbook. Oxford: Oxford University Press; 1997. p. 131–3.
30. Duerksen DR, Nehra V, Palombo JD, Ahmad A, Bristrian BR. Essential fatty acid deficiencies in patients with chronic liver disease are not reversed by short-term intravenous lipid supplementation. Dig Dis Sci. 1999;44:1342–8.
31. Burke PA, Ling PR, Forse RA, Bristrian BR. Conditionally essential fatty acid deficiencies in end-stage liver disease. Nutrition. 1999;15:302–4.
32. Kaufman SS, Scrivner DJ, Murray ND, et al. Influence of portagen and pregestimil on essential fatty acid status in infantile liver disease. Pediatrics. 1992;89:151–4.
33. Feranchak AP, Gralla J, King R, et al. Comparison of indices of vitamin A status in children with chronic liver disease. Hepatology. 2005;42:782–92.
34. Phillips JR, Angulo P, Petterson T, et al. Fat-soluble vitamin levels in patients with primary biliary cirrhosis. Am J Gastroenterol. 2001;96:2745–50.
35. Sherwin JC, Reacher MH, Dean WH, et al. Epidemiology of vitamin A deficiency and xerophthalmia in at-risk populations. Trans R Soc Trop Med Hyg. 2012;106:205–14.
36. Lippe B, Hensen L, Mendoza G, et al. Chronic vitamin A intoxication. Am J Dis Child. 1981;135:634–6.
37. Institute of Medicine, Food and Nutrition Board. Dietary reference intakes: vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington, DC: National Academy Press; 2001.
38. Penniston KL, Tanumihardjo SA. The acute and chronic toxic effects of vitamin A. Am J Clin Nutr. 2006;83:191–201.
39. Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium, Ross AC, Taylor CL, Yaktine AL, Del Valle HB, editors. Dietary reference intakes for calcium and vitamin D. Washington, DC: National Academies Press; 2011.
40. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011;96:1911–30.

41. Heubi JE, Hollis BW, Specker B, et al. Bone disease in chronic childhood cholestasis. Vitamin D adsorption and metabolism. Hepatology. 1989;9:258–64.
42. Bucuvalas JC, Heubi JE, Specker BL, et al. Calcium absorption in bone disease associated with liver cholestasis during childhood. Hepatology. 1990;12:1200–5.
43. Heubi JE, Higgins JV, Argao EA, et al. The role of magnesium in the pathogenesis of bone disease in childhood cholestatic liver disease: a preliminary report. J Pediatr Gastroenterol Nutr. 1997;25:301–6.
44. Argao EA, Balistreri WF, Hollis BW, et al. Effect of orthotopic liver transplantation on bone mineral content and serum vitamin D metabolites in infants and children with chronic cholestasis. Hepatology. 1994;20:598–603.
45. Institute of Medicine, Food and Nutrition Board. Dietary reference intakes for calcium and vitamin D. Washington, DC: National Academy Press; 2010.
46. Sokol RJ, Heubi JE, Iannaccone S, et al. Mechanisms causing vitamin E deficiency during chronic childhood cholestasis. Gastroenterology. 1983;85:1172–82.
47. Sokol RJ, Heubi JE, Iannaccone ST, et al. Vitamin E deficiency with normal serum vitamin E concentration in children with chronic cholestasis. N Engl J Med. 1984;310:1209–12.
48. Gordon HH, Nitowsky HM, Cornblath M. Studies of tocopherol deficiency in infants and children. 1. Hemolysis of erythrocytes in hydrogen peroxide. Am J Dis Child. 1955;90:669–81.
49. Cynamon HA, Isenberg JN, Nguyen CH. Erythrocyte malondialdehyde release in vitro: a functional measure of vitamin E status. Clin Chim Acta. 1985;151:169–76.
50. Refat M, Moore TJ, Kazui M, et al. Utility of breathe ethane as a noninvasive biomarker of vitamin E status in children. Pediatr Res. 1991;30:396–403.
51. Sokol RJ, Guggenheim MA, Iannaccone ST, et al. Improved neurologic function flowing correction of vitamin E deficiency in children with chronic cholestasis. N Engl J Med. 1985;313:1580–6.
52. Olson RE. The function and metabolism of vitamin K. Annu Rev Nutr. 1984;4:281–337.
53. Bancroft J, Cohen MB. Intracranial hemorrhage due to vitamin K deficiency in breast fed infants with cholestasis. J Pediatr Gastroenterol Nutr. 1993;16:78–80.
54. Yanofsky RA, Jackson VG, Lilly JR, et al. The multiple coagulopathies of biliary atresia. Am J Hematol. 1984;16:171–80.
55. Hambridge KM, Casey CE, Krebs NF. Zinc. In: Mertz W, editor. Trace elements in human and animal nutrition, vol. 2. 5th ed. Florida: Academic; 1986. p. 12–4.
56. Mohammad MK, Zhou Z, Cave M, Barve A, McClain CJ. Zinc and liver disease. Nutr Clin Pract. 2012;27:8–20.
57. Sazawal S, Black RE, Bhan MK, et al. Zinc supplementation in young children with acute diarrhea in India. N Engl J Med. 1995;333:839–44.
58. Roy SK, Tomkins AM, Akramuzzaman SM, et al. Randomised controlled trial of zinc supplementation in malnourished Bangladeshi children with acute diarrhoea. Arch Dis Child. 1997;77:196–200.
59. Ferenci P, Steindl-Munda P, Vogel W, et al. Diagnostic value of quantitative hepatic copper determination in patients with Wilson's Disease. Clin Gastroenterol Hepatol. 2005;3:811–8.
60. Grunebaum M, Horodniceanu C, Steinherz R. The radiographic manifestations of bone changes in copper deficiency. Pediatr Radiol. 1980;9:101–4.
61. Jiser SR, Winston GP. Copper deficiency myelopathy. J Neurol. 2010;2100:869–81.
62. Keshan Disease Research Group. Epidemiologic studies on the etiologic relationship of selenium and Keshan disease. Chin Med J (Engl). 1979;92:477–82.
63. Rotruck JT, et al. Selenium: biochemical role as a component of glutathione peroxidase. Science. 1973;179:588–90.
64. Fleming CR, Lie JT, McCall JT, et al. Selenium deficiency and fatal cardiomyopathy in a patient on home parenteral nutrition. Gastroenterology. 1982;83:689–93.
65. van Rij A, Thomson CD, McKenzie JM, Robinson MF. Selenium deficiency in total parenteral nutrition. Am J Clin Nutr. 1979;32:2076–85.
66. Thuluvath PJ, Triger DR. Selenium in chronic liver disease. J Hepatol. 1992;14:176–82.
67. Chin SE, Shepherd RW, Thomas BJ, et al. The nature of malnutrition in children with end-stage liver disease awaiting orthotopic liver transplantation. Am J Clin Nutr. 1992;56:164–8.
68. Levander OA. Considerations on the assessment of selenium status. Fed Proc. 1985;44:2579–83.
69. Mertz W. Chromium occurrence and function in biological systems. Physiol Rev. 1969;49:163–239.
70. Freund H, Atamiam S, Fischer JE. Chromium deficiency during total parenteral nutrition. JAMA. 1979;241:496–8.
71. Jeejeebhoy KN, Chu RC, Marliss EB, Greenberg GR, Bruce-Robertson A. Chromium deficiency, glucose intolerance, and neuropathy reversed by chromium supplementation in a patient receiving long-term total parenteral nutrition. Am J Clin Nutr. 1977;30:531–8.
72. Bavdekar A, Bhave S, Pandit A. Symposium on hospital-based nutrition: nutrition management in chronic liver disease. J Trauma Inj Infect Crit Care. 2004;56:37–44.
73. Eriksson LS, Persson A, Wahren J. Branched-chain amino acids in the treatment of chronic hepatic encephalopathy. Gut. 1982;23:801–6.
74. Wahren J, Denis J, Desurmont P, et al. Is intravenous administration of branched chain amino acids effective in the treatment of hepatic encephalopathy? A multicenter study. Hepatology. 1983;3:475–80.
75. Horst D, Grace ND, Conn HO, et al. Comparison of dietary protein with an oral, branched chain-enriched amino acid supplement in chronic portal-systemic encephalopathy: a randomized controlled trial. Hepatology. 1984;4:279–87.
76. Cascino A, Cangioano C, Calcaterra V, et al. Plasma amino acids imbalance in patients with liver disease. Am J Dig Dis. 1978;23:591–8.

77. Rossi-Francelli F, Freund H, Krause R, et al. Induction of coma in normal dogs by the infusion of aromatic amino acids and its prevention by the addition of branched-chain amino acids. Gastroenterology. 1982;83:664–71.
78. Fisher JE, Baldessarini RJ. False neurotransmitters and hepatic failure. Lancet. 1971;ii:75–80.
79. Rossi-Francelli F, Riggio O, Cangiano C, et al. Branched-chain amino acids vs lactulose in the treatment of hepatic coma: a controlled study. Dig Dis Sci. 1982;27:929–35.
80. McGhee A, Henderson JM, Millikan Jr WJ, et al. Comparison of the effects of hepatic-aid and casein modular diet on encephalopathy, plasma amino acids, and nitrogen balance in cirrhotic patients. Ann Surg. 1983;197:288–93.
81. Fabbri A, Magrini N, Bianchi G, et al. Overview of randomized clinical trials of oral branched-chain fatty acid treatment in chronic hepatic encephalopathy. JPEN J Parenter Enteral Nutr. 1996;20:159–64.
82. Charlton CP, Buchanan E, Holden CE, et al. Intensive enteral feeding in advanced cirrhosis: reversal of malnutrition without precipitation of hepatic encephalopathy. Arch Dis Child. 1992;67:603–7.
83. Helton WS. Nutritional issues in hepatobiliary surgery. Semin Liver Dis. 1994;14:140–57.
84. Fan ST, Lo CM, Lai ECS, Chu KM, Liu CL, Wong J. Perioperative nutritional support in patients undergoing hepatectomy for hepatocellular carcinoma. N Engl J Med. 1994;331:1547–52.
85. Garrison RN, Cryer HM, Howard DA, Polk HC. Clarification of risk factors for abdominal operations in patients with hepatic cirrhosis. Ann Surg. 1984;199:648–55.
86. Bonkovsky HL, Fiellin DA, Smith GS, et al. A randomized controlled trial of treatment of alcoholic hepatitis with parenteral nutrition and oxandrolone. 1. Short-term effects on liver function. Am J Gastroenterol. 1991;86:1200–8.
87. Kearns PJ, Young H, Garcia G, et al. Accelerated improvement of alcoholic liver disease with enteral nutrition. Gastroenterology. 1992;102:200–5.
88. Cabre E, Gonzalez-Huix F, Abad-Lacruz A, et al. Effect of total enteral nutrition on the short-term outcome of severely malnourished cirrhotics. A randomized controlled trial. Gastroenterology. 1990;98:715–20.
89. Moukarzel AA, Najm I, Vargas J, et al. Effect of nutritional status on outcome of orthotopic liver transplantation in pediatric patients. Transplant Proc. 1900;22:1560–3.
90. Pikul J, Sharpe MD, Lowndes R, Ghent CN. Degree of preoperative malnutrition is predictive of postoperative morbidity and mortality in liver transplant recipients. Transplantation. 1994;57:469–72.
91. Prijatmoko D, Strauss BJG, Lambert JR, et al. Early detection of protein depletion in alcoholic cirrhosis: role of body composition analysis. Gastroenterology. 1993;105:1839–45.
92. Heymsfield SB, Artega C, McManus C, Smith J, Moffitt S. Measurement of muscle mass in humans: validity of the 24-hour urinary creatinine method. Am J Clin Nutr. 1983;37:478–94.
93. Mendenhall CL, Moritz TE, Roselle GA, et al. A study of oral nutritional support with oxandrolone in malnourished patients with alcoholic hepatitis: results of a department of veterans affairs cooperative study. Hepatology. 1993;17:564–76.
94. Thomas S, Wolfe SE, Murphy KD, Chinkes DL, Herndon DN. The long-term effect of oxandrolone on hepatic acute phase proteins in severely burned children. J Trauma Inj Infect Crit Care. 2004;56:32–44.
95. Verboeket-van de Venne WPGH, Westerp KR, Van Hoek B, Swart GR. Energy expenditure and substrate metabolism in patients with cirrhosis of the liver: effects of the pattern of food intake. Gut. 1995;36:110–6.
96. Chin SE, Shepherd RW, Thomas BJ, et al. Nutritional support in children with end-stage liver disease: a randomized crossover trial of a branched-chain amino acid supplement. Am J Clin Nutr. 1992;56:158–63.
97. deLedinghen V, Beau P, Mannant PR, et al. Early feeding or enteral nutrition in patients with cirrhosis after bleeding from esophageal varices? A randomized controlled study. Dig Dis Sci. 1997; 42:536–41.
98. Gura KM, Duggan CP, Collier SB, et al. Reversal of parenteral nutrition-associated liver disease in two infants with short bowel syndrome using parenteral fish oil: implications for future management. Pediatrics. 2006;118:e197–201.
99. Le HD, deMeijer VE, Robinson EM, et al. Parenteral fish-oil-based lipid emulsion improves fatty acid profiles and lipids in parenteral nutrition-0 dependent children. Am J Clin Nutr. 2011; 94:749–58.
100. Diamond IR, Sterescu A, Pencharz PB, et al. Changing the paradigm: omegaven for the treatment of liver failure in pediatric short bowel syndrome. J Pediatr Gastroenterol Nutr. 2009;48:209–15.
101. Xu Z, Li Y, Wang J, et al. Effect of omega-3 polyunsaturated fatty acids to reverse biopsy-proven parenteral nutrition-associated liver disease in adults. Clin Nutr. 2012;31:217–23.
102. Michel H, Bories P, Aubin JP, et al. Treatment of acute hepatic encephalopathy in cirrhotics with a branched-chain amino acids enriched versus a conventional amino acid mixture: a controlled study in 70 patients. Liver. 1985;5:282–9.
103. Vilstrup H, Gluud C, Hardt F, et al. Branched-chain enriched amino acid versus glucose treatment of hepatic encephalopathy. A double-blind study of 65 patients with cirrhosis. J Hepatol. 1990;10:291–6.

Liver Transplantation

28

Evelyn Hsu and Jorge Reyes

Introduction

In the past half century, liver transplantation has become the standard of care not only for children with end-stage liver disease but also for children with metabolic diseases manifested largely outside of the liver. Significant improvements have been made in patient and graft survival, such that 1-year survival for children following liver transplantation is greater than 90 % [1].

History

The first pediatric liver transplant was attempted in 1963 by Thomas Starzl in a child with biliary atresia; the patient died from uncontrollable bleeding [2]. In the following decade, Starzl performed another eight pediatric liver transplants under prednisone and azathioprine immunosuppression, all of whom survived. His efforts in the late 1960s were joined by Moore in Boston and Calne in England [3]. Though initial 1-year survival rates were poor (<40 %) until the late 1970s, when the calcineurin inhibitor cyclosporine A was introduced, 1-year survival rates improved to 70–80 % [1, 4]. Improvements in surgical technique, organ allocation, patient selection, ICU postoperative care, as well as refinements made in new agents of immunosuppression and greater attention to viral surveillance and treatment, have further improved patient and graft survival.

Liver transplantation continues to be limited by organ scarcity. Although the number of pediatric patients listed for liver transplant has remained stable over the last decade, the total number of patients, both pediatric and adult, awaiting deceased donor liver transplant has increased in a linear fashion in the same time interval [1]. Adoption of the Pediatric End-Stage Liver Disease (PELD) and Model for End-Stage Liver Disease (MELD) (for adolescents) scoring system, which uses an objective methodology to evaluate mortality risk, hence allocating organs, has decreased mortality of pediatric patients on the wait list. The PELD scoring system, which is utilized for pediatric patients under 12 years of age, is based upon the first 884 children enrolled in the Studies for Pediatric Liver Transplantation (SPLIT) database [5] and predicts 3-month mortality. The designation of this system prioritized organ allocation based on patient's degree of illness rather than wait time and has resulted in significantly decreased wait list mortality in all age groups except for children less than 2 years of age [6]. In addition, in the USA, there have been United Network of Organ Sharing (UNOS) policy alterations to direct pediatric organs to

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pediatric recipients and to allow exception scores for specific complicating risk factors not accounted for by the PELD score, such as hepatopulmonary syndrome, hepatic malignancies, and extrahepatic metabolic disorders. Although the PELD system has worked to decrease pediatric wait list mortality, there is a perceived failure of the system in its current form because of long wait times for critically ill infants under 1 year of age [7]. Children less than 1 year of age, for example, continue to have a fivefold higher death rate on the wait list than other age groups [1]. Further organ allocation modeling and analysis will allow UNOS to apply policy modifications to continually improve access and equity in all recipients.

Indications

Indications for pediatric liver transplantation are distinct from those for adults and can be primarily grouped into four categories: (1) chronic liver diseases, (2) inborn errors of metabolism, with and without chronic liver disease, (3) fulminant hepatic failure, and (4) malignancy. Emerging transplantation indications for diagnoses such as hemophilia, graft-versus-host disease (GVHD), and portosystemic shunts occur at a low rate. More than two thirds of pediatric liver transplants occur in children under 5 years of age [1]. Chronic liver disease, primarily extrahepatic biliary atresia (EHBA), comprises the majority of pediatric patients receiving liver transplantation. EHBA alone accounts for about one third of pediatric liver transplants in the USA.

Biliary Atresia (See Also Chapter 13)

EHBA occurs rarely, with an incidence ranging from 1 to 6 per 20,000 live births, with significant ethnic and seasonal variation [8–11]. This ethnic variation persists worldwide, with an increased occurrence in Polynesian and Asian populations and decreased incidence in Caucasian populations [12]. Its etiology is unknown, however, with a nonrandom time-space clustering that is suggestive of a viral, toxin, or environmental etiology. There are two clinical patterns of EHBA, with 80 % considered "acquired EHBA" and the remaining 20 % with a syndromic (embryonic) form of EHBA that is associated with congenital anomalies, such as preduodenal portal vein, intestinal malrotation, situs anomalies, polysplenia or asplenia, and absence of the retrohepatic vena cava. Patients benefit from early hepatoportoenterostomy (HPE), with survival higher in those for whom the HPE is performed at less than 30 days of age when compared with those with HPE performed at greater than 90 days of age [13]. When the HPE does not result in adequate biliary drainage, or for whom the progression of portal hypertension becomes life threatening, liver transplantation is lifesaving.

There are significant surgical considerations in the approach to children with biliary atresia undergoing liver transplant. The presence of end-stage liver disease, coagulopathy, previous surgeries, and anatomic variations combine to make the surgery more challenging. Intestinal perforation occurs in 4–20 % [14, 15], as does reoperation for bleeding of unspecified etiology in 5–10 % [16]. Patients with biliary atresia are more likely to have hepatic arterial complications (5–15 %) [17, 18] and portal venous complications (6–14 %) [15, 17, 19], due to the anatomic abnormalities that exist in syndromic biliary atresia as well as the more common presence of a hypoplastic portal vein in the recipient. Biliary complications are also reported to occur frequently (18–20 %) [17, 18], although not more frequently than in general pediatric liver transplant population.

Despite the presence of these complications, there is excellent patient and graft survival following transplantation for biliary atresia, with 10-year patient survival ranging 81–90 % and 10-year graft survival 71–82 % [15, 17, 18, 20, 21]. Risk factors for patient death and graft loss include younger age, and the presence of growth failure and technical variant grafts contribute to an increased graft loss without increased patient mortality [20].

Other Cholestatic Liver Disease

Other cases of intrahepatic cholestasis such as sclerosing cholangitis and Alagille syndrome account for about 10 % (see Fig. 28.1) of children who receive liver transplants. These children can suffer from the effects of end-stage liver disease (susceptibility to infection, poor growth and nutrition, recurrent ascites, fat-soluble vitamin deficiency, severely debilitating pruritus), and liver transplantation can ameliorate these symptoms and allow for an improved quality of life. Additionally, children with chronic cirrhosis are at high risk for the development of hepatocellular carcinoma [22, 23], which itself may be an indication for transplantation.

Inborn Errors of Metabolism

Metabolic disease is defined as those diagnoses that are inborn errors of metabolism, i.e., they result from a single enzyme defect that alters the synthesis, transport, function, or breakdown of carbohydrate, fat, or protein. There are two groups of disease: those that result in *hepatic injury due to hepatocellular damage and have the potential to progress to cirrhosis*, *end-stage liver disease*, *and hepatocellular carcinoma* (i.e., alpha-1-antitrypsin deficiency, tyrosinemia, Wilson disease, cystic fibrosis, galactosemia, bile acid synthetic disorders, and progressive familial intrahepatic cholestasis [PFIC]) and those where the pathway and defect exists in the liver, but *there is no hepatocellular injury*; instead the damage occurs in "satellite organs" such as the brain in hyperammonemic conditions, the kidney in hyperoxaluria type 1, or the heart in familial hypercholesterolemia. These disorders, from both of the above groups, are categorized by the major intracellular pathway affected (carbohydrate, amino acid, organic acid, and fatty acid metabolism disorders) or by the primary intracellular compartment that is disrupted (peroxisomal, lysosomal, or mitochondrial). The diversity of pathways and compartments affected leads to a wide array of clinical manifestations. Liver transplantation can restore normal cellular function by replacing the genetic abnormality within the recipient liver, even in the absence of structural liver disease. Although individual metabolic disorders are rare, when aggregated, they have become the second largest indication for liver transplantation in children after EHBA [24, 25]. Diseases such as urea cycle defects, organic acidemias, and Crigler-Najjar type 1 are examples

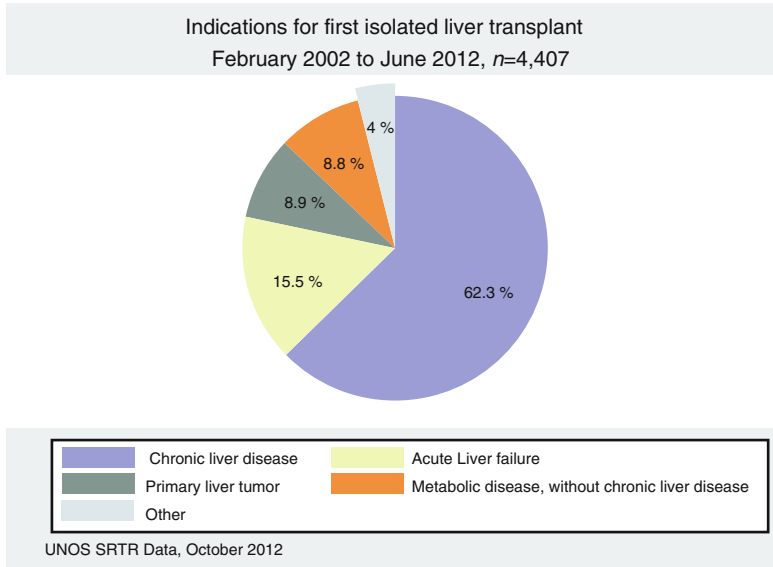

Fig. 28.1 Indications for first isolated liver transplant, pediatric patients <18 years of age, UNOS SRTR analysis of data from February 2002 to June 2012. *Note*: chronic liver disease includes diagnoses of biliary atresia, TPN-related liver disease, autoimmune hepatitis, Alagille syndrome, alpha-1-antitrypsin deficiency, cystic fibrosis, PFIC, Wilson disease, PSC, ductal plate abnormalities

of metabolic conditions that are effectively transplanted. Symptoms of these diseases are manifested by repeated metabolic crises and end-organ damage from the toxic by-products of intracellular processing. In some cases, strict dietary restriction is required to avoid these crises, particularly in the case of urea cycle defects and organic acidemias. Notably, despite adherence to these dietary regimens, patients continue to be at risk for episodes of metabolic decompensation and irreversible neurologic and other end-organ injury, particularly during times of intercurrent illness [26].

The therapeutic effect of liver transplantation in patients with inborn errors of metabolism is dependent upon the degree to which the enzyme deficiency is expressed in the liver. In urea cycle defects such as citrullinemia and ornithine transcarbamylase deficiency, liver transplantation alone is sufficient to correct the metabolic defect because the liver is the major site for the metabolism of ammonia. Primary hyperoxaluria is also corrected by liver transplantation, preventing renal failure and cardiac arrhythmia.

In metabolic defects where the enzyme deficiency also exists in extrahepatic organs, such as branched-chain amino acid defects, liver transplant does not completely correct the deficiency [27, 28]. Quality of life is generally improved after transplant in these disorders [29]. Patient and graft survival in pediatric patients transplanted for metabolic disease are superior to those who are transplanted for non-metabolic diseases, 95 and 91–92 %, respectively, compared to 89–90 and 86 % [30, 31]. Children transplanted for metabolic disease were older and less likely to be hospitalized at time of transplant or to suffer from growth failure. Patients transplanted for inborn errors of metabolism are also less likely to undergo early reoperation and have a lower rate of complications from portal vein thrombosis and intestinal perforation [30].

Fulminant Hepatic Failure

Fulminant hepatic failure accounts for an estimated 12.9 % of pediatric liver transplants in North American registry patients [32]. It is a rare occurrence in children, and even in those patients with hepatic encephalopathy, 15–20 % will recover without transplantation. A prospective study from the Pediatric Acute Liver Failure (PALF) Study Group found that in 49 % of all patients, and 54 % of those patients under 1 year of age, the etiology was unable to be determined [33].

Posttransplantation, single-center data have shown 1- and 5-year patient/graft survivals of 87/83 and 80/79 %, respectively [34]. Retrospective study of the UNOS database found 5-year patient and graft survival to be 73 and 59 %, respectively [35]. SPLIT database analysis revealed similar posttransplant patient survival [32].

Pre- and post-liver transplantation mortality remains high for patients with acute liver failure. Their poor outcome is likely to be multifactorial and related to sepsis, multiorgan failure, and delay to listing and referral. Ethnicity and age have been inconsistently implicated in contributing to poor patient and graft survival in this population [36].

Liver Tumors

Primary liver tumors are rare and account for <2 % of all pediatric malignancies [37]. Overall, transplantation for liver tumors approaches 5–9 % of all pediatric transplants in the USA [38]. Hepatoblastoma (HB) accounts for the majority of these malignancies, followed in frequency by hepatocellular carcinoma (HCC). Hepatic epithelioid hemangioendothelioma (HEH) is the third most common reason for liver transplantation for unresectable hepatic malignancies; however, infantile hemangioendothelioma and adolescent epithelioid hemangioendothelioma are grouped as one entity, even though the disease pathogenesis and prognoses are divergent [39]. Patients transplanted for HEH are the youngest (mean age 1.3 years), followed by HB (mean age 2.9 years) and then HCC (mean age 10.5 years) [38].

Overall patient and graft survival differs based upon the type of primary liver tumor. Five year patient and graft survival for HB is superior to that of HCC (72.7/63.6 % compared to

53.5/42.8 %, respectively). Recurrence-free survival is the lowest with HCC at 5 years (60.2 %) compared with HEH (80.4 %) and HB (77.3 %) [38]. Cause of death posttransplantation for both HB and HCC groups is usually due to metastatic or recurrent disease (54 and 86 %, respectively) [37].

Hepatoblastoma accounts for 75 % of primary liver tumors in childhood. Chemotherapy and surgical resection have improved survival in the last 20 years; however, complete resection of the tumor is required for cure. A large international prospective study run by the International Society of Pediatric Oncology (SIOPEL) established the Pretreatment Extent of Disease (PRETEXT) system for staging of disease. 6-year survival after "primary liver transplant," i.e., definitive surgical resection following four to six courses of PLADO chemotherapy (cisplatin and doxorubicin), approached 85 %, whereas survival after "rescue liver transplantation," where patients were transplanted after incomplete resection or recurrent disease, was 40 % [40]. A study of liver transplantation for HB at two American centers produced similar results [41].

There is limited clinical data describing the treatment of children with unresectable liver malignancies, particularly in patients with tumors other than HB. A current international registry, the Pediatric Liver Unresectable Tumor Observatory (PLUTO), has been prospectively collecting data from children (<18 years of age) receiving liver transplant for every type of primary liver malignancy and has started to fulfill the current gap in information between adults and children [42].

Contraindications to Liver Transplantation

As medical management and surgical techniques have improved over the last several decades, there remain very few absolute contraindications to pediatric liver transplantation. Absolute contraindications comprise conditions where liver transplant would be considered futile therapy and include (1) primary extrahepatic malignancy, (2) uncontrolled infection and sepsis, (3) irreversible massive brain injury, and (4) progressive terminal extrahepatic disease.

Medical Evaluation

The goal of the liver transplant evaluation process is to identify appropriate candidates for transplantation and to identify a pretransplant treatment plan that will optimize survival before and after liver transplantation. Identifying the appropriate pediatric liver transplant candidate encompasses (1) identifying and confirming the indication for transplant, (2) assessing the severity of primary disease, (3) confirming that alternative treatments have been exhausted, (4) excluding absolute contraindications to transplantation, and (5) identifying and addressing cardiopulmonary disease and vascular abnormalities that affect peri- and posttransplant survival. The pretransplantation treatment plan includes (1) assessing immunological status of the child and providing immunizations and (2) nutritional support to optimize growth, which may include the use of nasogastric tube feedings and total parenteral nutrition (TPN). Full medical evaluation requires informing the parents and the patient (if possible) of the transplant itself and issues and possible complications of and following transplant. A full social work evaluation is required to identify social concerns, potential financial issues, and to assess risk for medical nonadherence.

Immunizations Prior to Transplantation

Infectious complications contribute to a significant degree of morbidity and mortality in pediatric liver transplant recipients. These patients are often young when transplanted and rarely have completed a primary vaccination series, sometimes due to age, and at other times, they have been delayed or overlooked because of attention to their medical conditions. Vaccine responses are likely to be superior in the

Table 28.1 Suggested accelerated schedule for vaccination of SOT candidates

Vaccine	Minimum age of vaccination	Minimum interval between doses	Determination of serologic status	Review and update vaccine status of household contacts
DTaP	6 weeks	1st and 2nd, 4 weeks 2nd and 3rd, 4 weeks 3rd and 4th, 6 months 4th and 5th, 6 months	Not routinely recommended	Yes
Hepatitis A	6 months	1st and 2nd, 4 weeks	Pre- and posttransplant	Consider
Hepatitis B	Birth	1st and 2nd, 4 weeks 2nd and 3rd, 8 weeks	Pre- and posttransplant	Yes
HiB	6 weeks	1st and 2nd, 4 weeks 2nd and 3rd, 4 weeks 3rd and 4th, 8 weeks	Not routinely recommended	No
Influenza	6 months	1st and 2nd, 4 weeks	Not routinely recommended	Yes
MPV	2 years		Not routinely recommended	No
MMR	6 months	1st and 2nd, 4 weeks	Pre- and posttransplant	Yes
PCV7	6 weeks	1st and 2nd, 4 weeks 2nd and 3rd, 4 weeks 3rd, and 4th, 8 weeks	Not routinely recommended	No
PPV23	2 years		Not routinely recommended	No
Varicella	6 months	1st and 2nd, 4 weeks	Pre- and posttransplant	Yes

Adapted with permission from: Campbell and Herold [44]
Abbreviations: *DTaP* diphtheria, tetanus, and acellular pertussis vaccine, *HiB Haemophilus influenzae* type B vaccine, *MMR* measles, mumps, and rubella vaccine, *MPV* meningococcal polysaccharide vaccine, *PCV7* 7-valent pneumococcal conjugate vaccine, *PPV23* pneumococcal polysaccharide vaccine

transplant candidate prior to transplantation than after transplantation, which emphasizes the importance of verifying vaccination during the initial evaluation [43]. The young age of the candidates requires early initiation of vaccine series and shortened intervals between doses of vaccine [44]. The measles, mumps, and rubella vaccine (MMR) and the varicella zoster virus (VZV) vaccine can be given starting at 6 months of age and can help to decrease potential life-threatening infection when given before transplant [45]. A summary of the accelerated vaccination schedule is summarized in Table 28.1.

Assessment and Treatment of Malnutrition

In biliary atresia, the leading indication for pediatric liver transplant, malnutrition is a significant problem, caused by increased energy expenditure, dietary lipid malabsorption, and decreased intake secondary to early satiety from organomegaly and ascites and anorexia due to the end-stage liver disease [46, 47]. Clinical outcomes in biliary atresia and chronic liver disease patients suffering from malnutrition are worse, both before and after liver transplantation [20, 48, 49]. Nutritional assessment in chronic liver disease is difficult, as weight increase may reflect worsening ascites rather than true growth; therefore anthropometric measurements may be more accurate in tracking body composition and nutritional status [47]. High-calorie density formula and increased content of medium-chain triglycerides (MCT) are beneficial in infants with chronic cholestasis. Initiation of nasogastric tube feeding in malnourished transplant candidates should be done when required. In select patients with severe end-stage liver disease and severe malnutrition refractory to nasogastric tube feeds, parenteral nutrition (PN) has been shown to improve or

stabilize nutritional status [50, 51]. Liver disease in these patients appears to progress more rapidly in patients receiving PN [50], however, which may reflect more advanced liver disease at baseline or PN-associated liver injury, an entity well established in patients with intestinal failure, but not well described in those with preexisting chronic liver disease.

Transplant Surgery

It is beyond the scope of this chapter to review all of the details of liver transplant surgery here; however we endeavor to describe the basic idea of the processes involved to optimize the care provided by any health-care professional for these children. In-detail approach to the operations are described elsewhere [52–54].

Donor Considerations

Selection of an appropriate liver donor is the responsibility of the transplant surgeon and is of vital importance to both the short- and long-term success of the transplant procedure. The introduction of successful segmental graft transplantation has allowed for broadened donor criteria in pediatric patients. Deceased donors suitable for pediatric recipients should ideally be young (< than 45 years of age), healthy, nonobese, nondrug users, without evidence of liver disease, and free of high-risk behavior; however, variations to this algorithm inherently are guided by the severity of illness of the recipient. Hemodynamic stability is important, as are liver enzymes which should be near normal; critical events in a deceased donor may result in liver enzyme abnormalities which should be improving by the time of organ procurement.

There are three major phases of transplant surgery. The first is *recipient hepatectomy*, often the most challenging part of the transplant because of adhesions and bleeding from portal hypertension and coagulopathy. During the *implantation phase*, the three vascular anastomoses (vena cava, portal vein, and hepatic artery) are performed, beginning with vascular outflow anastomoses, followed by the vascular portal vein inflow and hepatic artery inflow. Variations at this point involve retention of the retrohepatic vena cava, the performance of a temporary portocaval shunt to decompress the splanchnic venous system, and the use of vascular homografts of donor iliac vein or artery to replace the native inflow. The use of these techniques depends upon the presence of recipient anomalies or thrombosis of native vessels. Liver reperfusion takes place during the final phase of surgery, the *neohepatic phase*, where the initial blood return to the heart from the newly perfused liver graft is cold, hyperkalemic, and acidotic as a result of necessary cold preservation technique. Cardiovascular instability, coagulopathy, and fibrinolysis can affect initial graft function. A specialized anesthesia team that possesses understanding of and the ability to manage these metabolic complexities inherent to the procedure is crucial to the success of the transplant. The biliary anastomosis is completed during the neohepatic phase. The type of biliary anastomosis depends upon the underlying diagnosis as well as the relative sizes of recipient and donor graft. In patients who lack a native biliary tree (biliary atresia), have abnormal native biliary systems (sclerosing cholangitis), or receive a segmental graft, the donor duct is implanted into a Roux-en-Y limb of small intestine. If there is relative concordance in the size of recipient and donor liver, a duct-to-duct anastomosis is possible.

Potential options for liver transplantation include deceased donor transplantation with an appropriate-sized donor, reduced or split liver transplant from a young adult donor, and living donor transplantation. The use of reduced size or segmental grafts (a right or left lobe of liver, or the left lateral segment of the left lobe) was developed in the mid-1980s to enhance the availability of liver grafts to children and to optimize the timing of transplantation; this advancement allows for the selection of a liver from a larger donor for implantation into a child and corrects the size mismatch. Reduction of a liver graft is performed ex vivo; split-liver grafts can be accomplished either ex vivo or in situ (in the hemodynamically stable brain-dead donor).

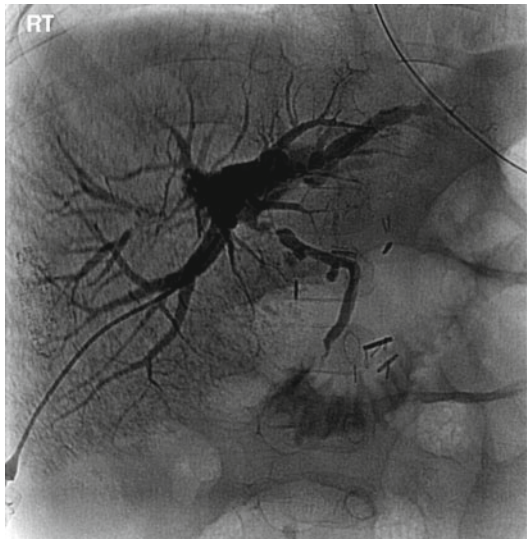

Fig. 28.2 Percutaneous cholangiogram showing ischemic cholangiopathy resulting from hepatic artery thrombosis in a 1-year-old transplant recipient

Living donor liver transplantation has greatly benefited the pediatric transplant population, particularly in those countries where the cultural or legal systems preclude deceased donor organ donation [55]. In this procedure, a left lateral segmentectomy (segments two and three, see Fig. 28.2) is performed in the donor (often a family member) and transplanted to the recipient.

Early UNOS database analysis comparing graft types and outcomes have shown improved graft survival in children under 3 years of age for live donor grafts when compared to deceased donor, whole, split, and reduced grafts [56]. Whole deceased donor grafts had relative benefit in older children. Recent UNOS data analysis, the largest to date, in recipients less than 12 years, suggested an improved immediate postoperative survival in whole-graft recipients. By 1 year, however, patient and allograft survivals were similar regardless of whether the graft was a whole liver, deceased donor segment, or living donor [57].

Operative Complications

Immediate postoperative complications can occur after liver transplantation for a variety of reasons. A complete discussion on the intensive care management of post-liver transplantation patients can be found elsewhere in this textbook. Poor clinical condition prior to surgery is associated with an increased risk of perioperative complications. *Primary nonfunction* (PNF) is a complication that occurs in all ages, but rarer in children (about 5 %) because of the selection of high-quality grafts. Despite adequate perfusion, there is an absence of function of the new liver and in the patient is clinically manifested within 72–96 h after surgery by very elevated liver enzymes, hepatic encephalopathy, uncorrectable coagulopathy, and lack of biliary drainage from the liver. This is generally a nonrecoverable complication for which the only effective treatment is early retransplantation, although some cases may initially have severe dysfunction which recovers with appropriate perioperative support.

Hepatic Artery Thrombosis

Hepatic artery thrombosis (HAT) is the most frequently encountered vascular complication, affecting 5–8 % of pediatric recipients. When HAT occurs, potential consequential problems include submassive to massive hepatic necrosis with intrahepatic abscess, extrahepatic biliary necrosis and leak, or necrosis of a portion of the intrahepatic biliary system with the formation of biliary strictures and frequently infected intrahepatic bilomas . Suspected HAT requires immediate evaluation with Doppler ultrasound, CT or MR angiography, or angiogram. If imaging studies are suspicious for thrombosis, exploration and thrombectomy are crucial. In the case of massive hepatic necrosis, retransplantation is indicated. Some cases of HAT may present with only ultrasound findings and little or no clinical impact; such cases may still require retransplantation due to chronic biliary strictures. Rarely patients with HAT remain asymptomatic and eventually develop arterial collaterals from the superior mesenteric artery.

Biliary Complications

Biliary complications occur with significant frequency (10–30 %) in pediatric liver transplant recipients, particularly in small infants [58, 59]. Biliary leaks can occur with either the

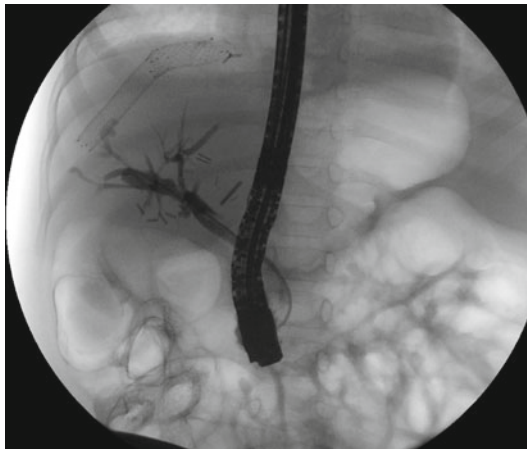

Fig. 28.3 ERCP image of anastomotic biliary stricture in a 2-year-old liver transplant recipient

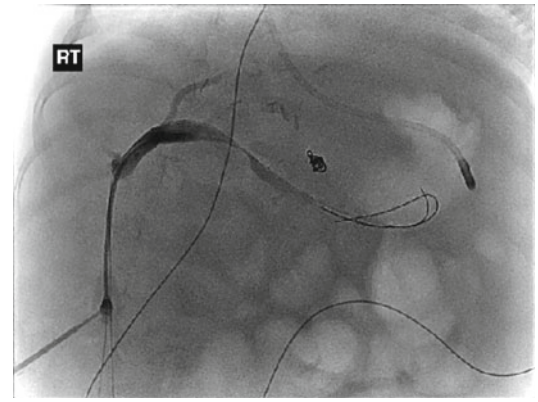

Fig. 28.4 Interventional radiology venogram showing portal vein stenosis, 6 months following transplant, in a recipient transplanted at 11 months of age for biliary atresia

Roux-en-Y or the duct-to-duct anastomoses and early on are manifested by bile-tinged fluid in the abdominal drains. Biliary leaks often spontaneously resolve, particularly with cut surface leaks, but can require stenting via endoscopic retrograde cholangiopancreatography (ERCP) or percutaneous cholangiography (PTC) (Fig. 28.3). Surgical exploration and revision of the anastomosis may be ultimately necessary.

Vascular Thromboses or Stenoses

Vascular thromboses or stenoses can occur as late surgical complications, usually the result of size issues at the time of transplantation, or ischemia. Portal vein stenosis can be seen months or years after transplantation, especially in small infants (Fig. 28.4).

Immunosuppression

The initial response of an intact immune system to liver transplantation is to recognize the graft as foreign and initiate a destructive T-lymphocyte-mediated immune response. The goal of effective clinical immunosuppression after solid organ transplantation is to inhibit the antigen-induced T-lymphocyte activation and cytokine production to interrupt allo-major histocompatibility complex recognition or to block effector responses. These effects should be accomplished while preserving immunocompetence to prevent overly weakening the host response to infection. The major emphasis is the prevention of acute and chronic rejection, as well as preserving the ability to reverse acute rejection if it occurs.

Induction Immunosuppression

Induction immunosuppression is given at the time of transplant to rapidly create a state of immunologic unresponsiveness of the recipient to donor antigens. Induction-depleting antibodies may be monoclonal or polyclonal. The most commonly used polyclonal antibody is thymoglobulin (ATG), which causes lymphocyte depletion. ATG is typically given for a short course early after transplant. ATG has also been employed in cholestatic or steroid-resistant rejection in children with good results [60]. Basiliximab, a chimeric mouse-human antibody that blocks the IL-2 receptor on the T cell, acts to prevent replication and activation of antigen-selected T cells. Its use at the time of transplant or within hours of transplant can be effective in reducing the incidence of acute rejection.

Corticosteroids

Corticosteroids were the first antirejection drugs and still comprise an important role in the immunosuppressive regimen, as they are effective in both preventing and treating rejection. They act through the suppression of antibody production

and cytokine synthesis (interleukin-2 and interferon-γ). Their use results in decreased proliferation of T cells (helper, suppressor, and cytotoxic) and B cells and decreased activity of neutrophils. Long-term steroid therapy is now avoided when possible to limit adverse effects that include increased infection and osteoporosis [61]. Metabolic and growth derangement related to corticosteroid exposure are of special concern for the pediatric transplant recipient [62, 63]. The use of tacrolimus as primary immunosuppression has been shown to allow steroid withdrawal within 2 years of transplantation without adverse effects [64].

Maintenance Immunosuppression

Calcineurin Inhibitors

Calcineurin inhibitors have been the mainstay of immunosuppression since the early 1980s and dramatically improved post-liver transplant survival rates. Cyclosporine A (CSA, Neoral, Sandimmune) became the mainstay of immunosuppression in 1983, and tacrolimus (FK-506, Prograf) was introduced in the following decade and was found to be more effective in preventing acute and steroid-resistant rejection [65].

By inhibiting calcineurin phosphatase activity, both CSA and tacrolimus interfere with the production and release of interleukin-2 (IL-2), a cytokine that plays a critical role in the cytotoxic T-cell immune response. It acts to inhibit T-cell-mediated acute cellular rejection. Tacrolimus is 100 times more potent than CSA in the inhibition of cytotoxic T-cell generation and the production of IL-2 and interferon gamma [66]. Initially, tacrolimus served as a "rescue" therapy for those who had failed, what was then a conventional therapy with CSA, high-dose steroids, antilymphocyte globulin, or azathioprine. Results were favorable [67, 68], and subsequently, tacrolimus was approved by the US Food and Drug Administration (FDA) in 1994 and now serves as the primary immunosuppressive medication in pediatric solid-organ transplantation [69].

Patients receiving tacrolimus have significantly fewer episodes of acute, steroid-resistant, or refractory rejection [65]. As opposed to CSA, tacrolimus absorption is independent of bile acid composition in the small intestine and results in more stable levels in cholestatic patients. Side effects are similar between tacrolimus and CSA and include nephrotoxicity, neurotoxicity, hypertension, infection, and gastrointestinal disturbance. Patients treated with tacrolimus have a higher incidence of hyperglycemia, hyperkalemia, and GI disturbance, but do not suffer from hirsutism or gingival hyperplasia, the more common side effects of CSA. Of greatest significance, particularly in the pediatric population, is the ability to wean and withdraw corticosteroids, which can be achieved within the first year of liver transplant, avoiding the long-term side effects of growth failure, osteoporosis, and increased risk of infection. Long-term studies of pediatric liver transplant recipients showed that patients with primary tacrolimus immunosuppressive therapy had improved patient and graft survival when compared to those on primary CSA therapy; hypertension and hyperkalemia were less marked [70]. Monitoring of blood levels is essential to assure adequate efficacy and to avoid toxicity. Levels can be altered by ingestion by certain foods and medications that induce the CYP450 systems. Additionally, both tacrolimus and CSA can bind to enteric feeding tubes so dosing in this fashion is unreliable.

CSA is still used occasionally. The most significant side effect is nephrotoxicity, mediated through acute microvascular disease, chronic progressive interstitial fibrosis, and decrease in glomerular filtration rate. Renal function following liver transplantation is a significant concern and has led to several large multicenter database studies in an attempt to find associations and subsequent modifications in posttransplant immunosuppressive protocols that may be beneficial. Cyclosporine use is associated with worse renal function when compared to tacrolimus [62, 71].

Mycophenolate Mofetil

Mycophenolate mofetil (MMF, Cellcept) is converted in the liver by ester hydrolysis to mycophenolic acid, which acts to selectively inhibit inosine monophosphate dehydrogenase, which

thereby inhibits the synthesis of guanosine, a purine nucleoside. T and B lymphocytes are uniquely dependent upon de novo purine synthesis for proliferation, whereas other cell types can utilize alternate salvage pathways. In this way, MMF selectively inhibits T- and B-cell proliferation and thus antibody formation. Drug metabolism is likely to differ between renal and liver transplant patients, and few studies have been done to examine the pharmacokinetics of MMF in pediatric liver transplant, suggesting that higher doses (approaching 750 mg/m^2/day) are needed to achieve adequate AUC levels [72–74]. Common side effects include gastrointestinal symptoms of diarrhea, vomiting, and cramping. Experience dictates starting patients at a low dose and increasing if these side effects do not arise.

Sirolimus

Sirolimus (rapamycin, Rapamune) is a macrolide that binds to the molecular target of rapamycin (mTOR) receptor, which is an intracellular regulator of protein kinases and acts to decrease interleukin-2 production and B-cell and T-cell activation and proliferation. It was initially used as rescue immunosuppressive therapy in pediatric liver and multivisceral transplant patients experiencing rejection while on calcineurin inhibitor therapy and has been subsequently used as primary immunosuppression, described in small single-center studies [75–77]. Sirolimus is not approved for use in liver transplantation because of a concern for increased risk of hepatic artery thrombosis and decreased wound healing. In subsequent adult studies, however, the concern of increased risk of HAT has not been substantiated [78, 79]. Sirolimus monotherapy in pediatric liver transplantation is associated with improved renal function [80] and decreased rates of rejection [75] when compared to tacrolimus monotherapy. Sirolimus also has shown antitumor proliferative activity, which may provide an indication for use in patients transplanted for primary liver malignancy [81]. Well-known side effects in children include worsened hyperlipidemia, sometimes requiring statin therapy, mouth ulcers, and bone marrow suppression. Emerging reports describe potentially reversible interstitial pneumonitis with sirolimus therapy [82–84]. *Everolimus* is a sirolimus derivative that acts to inhibit cell proliferation through inhibition of mTOR. It has been used in limited single centers as rescue therapy for rejection, renal insufficiency, and shares many features of the side effect profile of sirolimus [85].

Complications

Rejection (acute and chronic) is the primary medical complication of liver transplantation, but a variety of infections and other complications develop as a result of the efforts to prevent and treat rejection. Additional complications primarily related to immunosuppression include de novo autoimmune hepatitis, autoimmune-mediated hematologic derangements, allergic disease, and renal disease. There are certain conditions where the disease can recur in the transplant graft (primary sclerosing cholangitis and progressive familial intrahepatic cholestasis).

Rejection

Despite immunosuppressive medications, rejection occurs in 40–66 % of children following successful liver transplantation [24, 62, 86]. Rejection is typically suspected based upon laboratory findings before symptoms present, with rising liver-associated enzymes, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transpeptidase (GGT). Severe rejection can present clinically with fever and cholestasis. Liver biopsy is indicated in order to confirm diagnosis as well as exclude other cases of liver enzyme perturbation. Acute cellular rejection (ACR) is characterized on histology by endothelialitis, portal triad lymphocyte infiltration with bile duct injury, and hepatic parenchymal cell damage [87]. More severe rejection can be characterized by additional centrilobular necro-inflammatory changes, termed central perivenulitis with inflammation,

and perivenular hepatocyte loss [88]. Lobular inflammation is may be a feature of rejection; however, it should primarily raise the question of viral infection. Rarely, early ACR will present with centrilobular inflammatory changes in the absence of the typical appearance of the portal triad [89]. ACR is easily controlled in a majority of patients with bolus steroid therapy, optimization of calcineurin inhibitor drug levels, and possible addition of adjuvant immunosuppression such as MMF. Severe refractory cases may require treatment with a mono- or polyclonal anti-T-cell antibody infusion (ATG or OKT-3) which induces response in 90 % of patients [90].

Chronic Rejection

Chronic rejection occurs in 5–10 % of transplanted patients [24, 62] and is clinically manifested by progressive increase in alkaline phosphatase, GGT, and cholestasis. On histopathology, injury is seen to the biliary epithelium and "vanishing bile duct syndrome" where severe ductopenia is seen in at least 50 % of portal tracts [89, 91]. Reversal is unusual, although in rare cases, enhanced immunosuppressive therapy can halt progression [91]. When ductopenia is associated with ischemic necrosis and fibrosis, the clinical course is progressive and usually requires retransplantation, and recurrence of chronic rejection in the retransplanted graft can occur [91].

Unlike in kidney and heart allograft rejection, humoral mechanisms in rejection have been viewed as insignificant, as the liver is considered "immunologically privileged" and resistant to antibody-mediated rejection (AMR). Recent clinical evidence has prompted reconsideration of these views, correlating the immunohistochemical detection of the complement split product C4d in rejection with the presence of antibodies against donor HLA molecules [92, 93]. Significant C4d deposition was found in patients with bile duct injury and loss, implicating AMR in this process.

Infection

Infections remain the most significant cause of morbidity and mortality after solid organ transplantation [86]. Bacterial infections usually occur within the first 2 months after transplant, with the majority of these infections involving the abdominal cavity and surgical wound. The type of immunosuppressive regimen has an impact on the type and severity of the infections seen in the recipient. Pulmonary infections and catheter-related sepsis are also seen in the post-liver transplant population [94]. Children who have complex postoperative hospitalizations, exposure to broad-spectrum antibiotics, or have required reoperation are at high risk for fungal infection, with Candida being the most common organism. Opportunistic infections such as *Pneumocystis*, *Aspergillus*, *Toxoplasma*, *Cytomegalovirus* (CMV), and Epstein-Barr virus (EBV) are more likely to present between 1 and 6 months posttransplantation [95].

The most common cause of viral infection in the liver transplant recipient is CMV. Infection with CMV may occur in the absence of symptoms (*CMV infection*), or as mild- to life-threatening symptomatic infection (*CMV disease*). CMV disease presents as a nonspecific febrile illness with leukopenia and rash and can affect the GI tract, urinary tract, liver graft, and lungs. Diagnosis can be aided by a biopsy specimen of the involved organ showing intranuclear inclusions characteristic of CMV. Risk of infection can be stratified by serologic status of donor and recipient. Highest risk of CMV disease occurs in the CMV-negative recipient of a CMV-positive donor. Treatment of steroid-resistant rejection with mono- or polyclonal antibody to T cells is associated with CMV disease and reactivation. Ganciclovir has clinically significant antiviral activity and is available IV as well as oral as valganciclovir. Prevention of disease occurs through universal prophylaxis of all transplant recipients with IV ganciclovir or oral valganciclovir.

Epstein-Barr virus (EBV) can produce a primary or reactivation infection. Presentation is similar to CMV infection, with fever, leukopenia, and thrombocytopenia, and like CMV, patients who are seronegative at transplant and receive an organ from a positive donor are most at risk. Children are more likely than adults to be seronegative and most likely to have a primary EBV

infection. Infections range from a self-limited mononucleosis-type syndrome to severe post-transplant lymphoproliferative disease (PTLD), i.e., Burkitt's lymphoma.

PTLD can occur in up to 10 % of pediatric transplant recipients. Although most cases of PTLD are associated with EBV, some cases are associated with other viruses. PTLD arises out of a deranged immunologic response to EBV that allows unchecked proliferation of B cells. Incidence of PTLD has been documented as threefold higher in pediatric transplant recipients likely due to their EBV-naïve state at transplantation. Risk factors for poor survival are monomorphic monoclonal PTLD, young age, and tacrolimus-based immunosuppression [96]. The first approach to PTLD should be discontinuation of immunosuppression. Antiviral therapy is seldom beneficial. Rituximab, an anti-B-cell monoclonal antibody (CD20 receptor-positive cells) paired with cyclophosphamide has been used with moderate success in patients who continue to have proliferation despite immunosuppression cessation [97]. Full chemotherapy is needed for lymphomas. Donor-derived EBV-specific cytotoxic T-lymphocyte immunotherapy also holds promise for treatment of PTLD [98].

Prevention of EBV and CMV disease involves close monitoring for EBV and CMV virus levels, with close modulation of immunosuppressive therapy. Other early and severe viral infections are also caused by herpes simplex virus (HSV), varicella zoster virus (VZV), adenovirus, and human herpesvirus (HHV) types 6, 7, and 8 [99].

Autoimmune Disease

In general, in pediatrics, recurrence of primary liver disease in the graft is not common, because most liver diseases requiring transplantation in children are congenital and transplantation is curative. Primary sclerosing cholangitis can recur in 10 % of transplanted patients [100, 101] with a presentation that is identical to the original disease. De novo *autoimmune hepatitis* can occur in any patient following transplant, regardless of the original disease. It presents with autoantibodies, elevated total protein, and elevated liver-associated enzymes. Liver biopsy shows dense lymphocytic portal-tract infiltrate with plasma cells, periportal hepatitis, and bridging collapse, without the typical features of acute or chronic rejection [102]. Its pathogenesis remains unclear, with mechanisms thought to be related to multiple antigenic stimuli from viruses and toxins in addition to genetic predisposition [103]. Immunosuppressive therapies, particularly calcineurin inhibitors, may have a paradoxical effect by inducing defective de novo T-cell development that favors the emergence of autoaggressive T-cell clones. Treatment is similar to classical AIH, with intermediate dose steroids in addition to azathioprine or MMF [104].

Autoimmune cytopenias have low overall incidence but a reported incidence of 3 % in pediatric liver transplant recipients, representing a significant complication [105]. Autoimmune hemolytic anemia (AIHA) results from IgG or IgM antibodies directed towards red blood cells. In pediatric liver transplant recipients, IgM-mediated disease is more commonly reported. IgM binds complement and induces intravascular hemolysis. Idiopathic thrombocytopenic purpura (ITP) is a diagnosis of exclusion, but is characterized by increased megakaryocytes on bone marrow biopsy and presence of antiplatelet antibodies. *Evans syndrome* is the combination of at least two autoimmune cytopenias, most commonly AIHA and ITP, but may also include autoimmune neutropenia. The majority of patients presenting with autoimmune cytopenia were on tacrolimus immunosuppression and had been exposed to a viral infection prior to the onset of symptoms [105]. Treatment modalities included steroids, IVIG, rituximab, chemotherapy, or splenectomy. The mechanism behind this phenomenon is not well understood, but is thought to be similar to that of de novo autoimmune hepatitis.

Outcomes

Long-term survival in liver transplantation is favorable, and >80 % of children survive to adulthood. Providers are now facing the challenge of ensuring good long-term quality of life by

addressing and reducing the damaging effects of lifelong immunosuppression, by ensuring continued adherence to medical care as children transition from adolescents to adults, and by enhancing intellectual and emotional development in this vulnerable population.

Survival

Overall results of liver transplantation are rewarding, with SPLIT registry data (1,092 patients in North America transplanted since 1995) showing 1-year patient and graft survival of 86.3 and 80.2 %, respectively [86]. UNOS/OPTN data showed 1-year patient and graft survival for patients with a diagnosis of biliary atresia, the most common indication for pediatric liver transplantation, of 95/87 % [106]. Another study from Belgium, this one a large single-center study, found 1-year patient and graft survival of 94.6 and 95.7 %, respectively [107]. The highest risk of mortality occurs during the first year across all age groups, with the majority risk within the first 3 months following transplantation. Long-term patient and graft survival beyond 1 year is good, 84/76 % [86], with dramatically improved survival over the last decade in the infants <1 year of age or <10 kg in weight (now 65–88 % [108], previously 50–60 % [109]). Improved survival in this group likely results from technical innovations in graft preservation and transport, as well as the avoidance of the catastrophic complications of HAT and PNF. Examination of 461 5-year survivors of pediatric liver transplantation in the SPLIT registry found a 1st graft survival of 88 %, with 12 % requiring second graft transplant and 2 % requiring a third liver transplant [24]. The same group published a study of 167 10-year survivors, which again showed a 1st graft survival of 88 %, suggesting that graft survival is sustained once recipients reach the 5-year survival mark [62]. Children with malignancy have significantly higher risks of early and late graft loss, reflecting recurrence of disease [110]. Other factors that predicted late graft loss included older age at transplant, race, and insurance type, suggesting that socioeconomic factors play a role in long-term survival of these patients.

Growth

Most pediatric patients awaiting liver transplant have some form of growth failure mediated, at least in part in the children with cirrhosis, by growth hormone resistance [111]. In addition, malnutrition caused by fat malabsorption and increased energy expenditure is a well-described feature of end-stage liver disease and contributes to growth failure. Following successful transplant, growth hormone and insulin-like growth factor-1 levels normalize [112] and they will display catch-up growth within 6–12 months [113–115]. Weight is the first to normalize, but linear growth is delayed, with the posttransplant pediatric population having a height distribution below the normative values for children of comparable age [62]. Factors that predicted growth impairment were nutritional status prior to transplant and steroid exposure that extended beyond 18 months after transplant.

Cognitive Outcomes, Family Function, and Quality of Life

Improved survival and decreased surgical complications following transplantation have allowed researchers and practitioners to adjust the focus and scope of study on long-term outcomes to that of cognitive development and quality of life. Recent registry analyses of cognitive and neurodevelopment outcomes in a multicenter North American cohort of pediatric transplant recipients transplanted before 5 years of age have shown that 26 % have mild-moderate cognitive delay, with intellectual deficits that are expected to hinder academic performance as well as the ability to function independently long term [116]. Hepatic encephalopathy, malnutrition, and end-stage liver disease are believed to have a greater detrimental impact upon the rapidly developing brain of an infant in comparison to that of an older child. Younger children who are

transplanted under 6 months of age, who have therefore had a shorter duration of illness, less malnutrition, and shorter hospital stays, have a better cognitive prognosis [117, 118]. Hearing loss affects a significant number of those children transplanted between 12 and 18 months of age [119], with increased risk for those patients transplanted for hepatoblastoma and those patients with additional hospitalizations. Considering the increased prevalence of adverse cognitive outcomes, intervention services at a younger age may benefit this population of patients, as well as closer attention to earlier transplant to decrease the amount of time young infants sustain the long-term effects of end-stage liver disease and malnutrition.

Family functioning has been measured by the Family Assessment Device (FAD), which assesses problem solving, communication, roles, behavior control, affective responsiveness and involvement, and general function. The FAD was used to assess the families of 102 pediatric liver transplant recipients and found a generally healthy level of functioning and no increase in family dysfunction [120]. The only variable that significantly negatively impacted family functioning was the presence of biliary complications.

Although liver transplant is lifesaving, it is not curative, in that it produces a chronic condition with its own morbidity and lifelong need for medical care that imposes a potential burden upon survivors and their families. Health-related quality of life (HRQOL) is a multidimensional approach to assessing a patient and family's "state of well-being." The PedsQL™ is a well-validated instrument that measures HRQOL [121] that was administered to 873 pediatric liver transplant patients as well as a normative and a pediatric cancer sample [122]. Pediatric liver transplant recipients reported significantly lower HRQOL relative to healthy children, with areas of absence from school and school functioning being the areas of greatest divergence, comparable to those pediatric cancer patients in social and school function, although approaching normal values for physical function. Demographic variables may have a strong impact upon HRQOL [120]. Decreased HRQOL in adolescents is associated with older age at transplant, history of symptom distress, headaches, and presence of secondary chronic illness [123]. A recent interview-based attempt to *qualitatively describe* the experience of 42 liver transplant recipients and their families found that families perceived that the transplant played a large role in improving general health, physical activity, relationships, and overall HRQOL, with a majority of parents reporting no or few problems with health [124]. Particular barriers and areas of concern involved adherence and self-care, restriction upon physical activities, and difficulties with medical procedures and hospitalizations. A national cross-sectional study of pediatric liver transplant recipients in Finland found that posttransplant, HRQOL, and sexual health were equivalent to normal [125].

Obesity, Dyslipidemia, and Metabolic Syndrome

There is growing concern about the increasing prevalence of posttransplant metabolic syndrome within the adult liver transplant recipient population, which mirrors the increase in obesity in the general population and is exacerbated by corticosteroid treatment and the immunosuppressive regimen of calcineurin inhibitors [126]. Likewise, childhood obesity has also been climbing, increasing risk for adult obesity and morbidity. Recent UNOS data analysis showed obesity at transplant increased long-term mortality risk for pediatric liver transplant recipients [127]. The prevalence of overweight and obesity in the pediatric population assessed through the UNOS database was 20–50 %[128], comparable and possibly increased over that of healthy US children (20–30 %) [129]. The strongest predictor of overweight and obesity in this population has been showed to be weight status at transplant; however, the development of posttransplant diabetes was not related to weight status during the follow-up period [128]. SPLIT registry analysis found that 7 % of 5-year survivors had hypercholesterolemia, 10 % with hypertriglyceridemia,

and 13 % with diabetes mellitus [24]. Screening and attention towards obesity prevention are essential in the long-term care of any child following liver transplantation.

Nonadherence

Adherence

Adherence is defined by the World Health Organization as "the extent to which a person's behavior—taking medication, following a diet, and/or executing life-style changes, corresponds with agreed recommendations from a health care provider" [130]. Failure to adhere to the prescribed immunosuppressive regimen is a major cause of graft loss in pediatric liver transplant recipients, particularly to those transplanted in adolescence [131]. There have been attempts to develop an objective method of assessing adherence to reduce posttransplantation morbidity, with standard deviation (SD) of medication levels being the best predictor of nonadherence and biopsy-proven rejection episodes [132]. History of childhood abuse is linked to increased levels of nonadherence and biopsy-proven rejection [133]; however, the presence of abuse may be associated with family dysfunction, which may independently increase nonadherence. The most common reason for noncompliance was forgetfulness [132, 134]. Adolescent nonadherence has been better described, although parental noncompliance has been described in children under 10 years of age [134]. Improving access to care by opening weekend and evening clinics may be able to decrease school and work absences and improve the ability of patients and their families to access care. Developing a validated adherence assessment protocol in the clinic could assess adherence behavior and improve overall medical care [132].

Fertility and Pregnancy Posttransplantation

Female pediatric liver transplant recipients have an excellent chance of surviving into adulthood and will want to consider the option of pregnancy. The US National Transplantation Pregnancy Registry (NTPR) has reported on 238 pregnancies in 151 female liver transplant recipients and found that 70 % of these pregnancies have resulted in a successful live birth [135]. There were higher rates of hypertension, preeclampsia, gestational diabetes mellitus, and Cesarean section when compared to the general population, and rejection episodes were reported in between 6 and 10 % during pregnancy. MMF has been associated with an increased incidence of structural malformations [136]. In individuals who have undergone liver transplant before 21 years of age, there was a 75 % live birth rate. There were higher rates of prematurity and low birth weight when compared to the general population, as well as birth defects that were associated with maternal MMF use [137]. There may be a higher rate of graft loss in the first 2 years following pregnancy. The NTPR also reported upon 49 male transplant recipients, 3 of whom had undergone liver transplantation, fathering 56 pregnancies without an increase incidence of structural malformations [138].

Current recommendations for pregnant liver transplant recipients include multidisciplinary care provided by a combined transplant and obstetric management team. Before considering pregnancy, liver transplant recipients should have normal graft function and should not have experienced any episodes of rejection in the year preceding pregnancy. With careful management the majority of these pregnancies can have a successful outcome.

References

1. Kim WR, Stock PG, Smith JM, Heimbach JK, Skeans MA, Edwards EB, et al. OPTN/SRTR 2011 Annual Data Report: Liver. Am J Transplant. 2013; 13(1):73–102.
2. Starzl TE, Marchioro TL, Vonkaulla KN, Hermann G, Brittain RS, Waddell WR. Homotransplantation of the liver in humans. Surg Gynecol Obstet. 1963;117: 659–76.
3. Calne RY. Early days of liver transplantation. Am J Transplant. 2008;8(9):1775–8.
4. Starzl TE, Koep LJ, Schröter GP, Halgrimson CG, Porter KA, Weil R. Liver replacement for pediatric patients. Pediatrics. 1979;63(6):825–9.

5. Mcdiarmid SV, Anand R, Lindblad AS, Principal Investigators and Institutions of the Studies of Pediatric Liver Transplantation SPLIT Research Group. Development of a pediatric end-stage liver disease score to predict poor outcome in children awaiting liver transplantation. Transplantation. 2002;74(2):173–81.
6. Freeman RB, Wiesner RH, Roberts JP, McDiarmid S, Dykstra DM, Merion RM. Improving liver allocation: MELD and PELD. Am J Transplant. 2004;4 Suppl 9:114–31.
7. Shneider BL, Neimark E, Frankenberg T, Arnott L, Suchy FJ, Emre S. Critical analysis of the pediatric end-stage liver disease scoring system: a single center experience. Liver Transpl. 2005;11(7):788–95.
8. Caton AR. Exploring the seasonality of birth defects in the New York State Congenital Malformations Registry. Birth Defects Res A Clin Mol Teratol. 2012;94(6):424–37.
9. Chardot C, Carton M, Spire-Bendelac N, Le Pommelet C, Golmard JL, Auvert B. Epidemiology of biliary atresia in France: a national study 1986–96. J Hepatol. 1999;31(6):1006–13.
10. Livesey E, Borja MC, Sharif K, Alizai N, Mcclean P, Kelly D, et al. Epidemiology of biliary atresia in England and Wales (1999–2006). Arch Dis Child Fetal Neonatal Ed. 2009;94(6):F451–5.
11. Yoon PW, Bresee JS, Olney RS, James LM, Khoury MJ. Epidemiology of biliary atresia: a population-based study. Pediatrics. 1997;99(3):376–82.
12. Girard M, Jannot A-S, Besnard M, Jacquemin E, Henrion-Caude A. Biliary atresia: does ethnicity matter? J Hepatol. 2012;57(3):700–1.
13. Chardot C, Serinet M-O. Prognosis of biliary atresia: what can be further improved? J Pediatr. 2006;148(4): 432–5.
14. Vilca Melendez H, Vougas V, Muiesan P, Andreani P, Mieli-Vergani G, Rela M, et al. Bowel perforation after paediatric orthotopic liver transplantation. Transpl Int. 1998;11(4):301–4.
15. Chen CL, Concejero A, Wang CC, Wang SH, Lin C-C, Liu YW, et al. Living donor liver transplantation for biliary atresia: a single-center experience with first 100 cases. Am J Transplant. 2006;6(11):2672–9.
16. Broering DC, Kim J-S, Mueller T, Fischer L, Ganschow R, Bicak T, et al. One hundred thirty-two consecutive pediatric liver transplants without hospital mortality. Ann Surg. 2004;240(6):1002–12.
17. Diem HVT, Evrard V, Vinh HT, Sokal EM, Janssen M, Otte JB, et al. Pediatric liver transplantation for biliary atresia: results of primary grafts in 328 recipients. Transplantation. 2003;75(10):1692–7.
18. Fouquet V, Alves A, Branchereau S, Grabar S, Debray D, Jacquemin E, et al. Long-term outcome of pediatric liver transplantation for biliary atresia: a 10-year follow-up in a single center. Liver Transpl. 2005;11(2): 152–60.
19. Darwish AA, Bourdeaux C, Kader HA, Janssen M, Sokal E, Lerut J, et al. Pediatric liver transplantation using left hepatic segments from living related donors: surgical experience in 100 recipients at Saint-Luc University Clinics. Pediatr Transplant. 2006;10(3): 345–53.
20. Utterson EC, Shepherd RW, Sokol RJ, Bucuvalas J, Magee JC, McDiarmid SV, et al. Biliary atresia: clinical profiles, risk factors, and outcomes of 755 patients listed for liver transplantation. J Pediatr. 2005;147(2): 180–5.
21. Barshes NR, Lee TC, Balkrishnan R, Karpen SJ, Carter BA, Goss JA. Orthotopic liver transplantation for biliary atresia: the U.S. experience. Liver Transpl. 2005;11(10):1193–200.
22. Bhadri VA, Stormon MO, Arbuckle S, Lam AH, Gaskin KJ, Shun A. Hepatocellular carcinoma in children with Alagille syndrome. J Pediatr Gastroenterol Nutr. 2005;41(5):676–8.
23. Hadžić N, Quaglia A, Portmann B, Paramalingam S, Heaton ND, Rela M, et al. Hepatocellular carcinoma in biliary atresia: King's College Hospital experience. J Pediatr. 2011;159(4):617–22.e1.
24. Ng VL, Fecteau A, Shepherd R, Magee J, Bucuvalas J, Alonso E, et al. Outcomes of 5-year survivors of pediatric liver transplantation: report on 461 children from a North American Multicenter Registry. Pediatrics. 2008;122(6):e1128–35.
25. Peeters PMJG, Sieders E, De Jong KP, Bijleveld CMA, Hendriks HGD, Ten Vergert EM, et al. Comparison of outcome after pediatric liver transplantation for metabolic diseases and biliary atresia. Eur J Pediatr Surg. 2001;11(1):28–35. Georg Thieme Verlag/Masson Editeur: Stuttart, New York/Paris.
26. Leonard JV, Walter JH, McKiernan PJ. The management of organic acidaemias: the role of transplantation. J Inherit Metab Dis. 2001;24:309–11.
27. Shneider BL, Vockley J, Mazariegos GV. Trading places: liver transplantation as a treatment, not a cure, for metabolic liver disease. Liver Transpl. 2011;17(6): 628–30.
28. Vara R, Turner C, Mundy H, Heaton ND, Rela M, Mieli-Vergani G, et al. Liver transplantation for propionic acidemia in children. Liver Transpl. 2011;17(6): 661–7.
29. Whitington PF, Alonso EM, Boyle JT, Molleston JP, Rosenthal P, Emond JC, et al. Liver transplantation for the treatment of urea cycle disorders. J Inherit Metab Dis. 1998;21 Suppl 1:112–8.
30. Arnon R, Kerkar N, Davis MK, Anand R, Yin W, González-Peralta RP. Liver transplantation in children with metabolic diseases: the studies of pediatric liver transplantation experience. Pediatr Transplant. 2010; 14(6):796–805.
31. Kayler LK, Rasmussen CS, Dykstra DM, Punch JD, Rudich SM, Magee JC, et al. Liver transplantation in children with metabolic disorders in the United States. Am J Transplant. 2003;3(3):334–9.
32. Baliga P, Alvarez S, Lindblad A, Zeng L, Studies of Pediatric Liver Transplantation Research Group. Posttransplant survival in pediatric fulminant hepatic failure: the SPLIT experience. Liver Transpl. 2004; 10(11):1364–71.

33. Squires Jr RH, Shneider BL, Bucuvalas J, Alonso E, Sokol RJ, Narkewicz MR, et al. Acute liver failure in children: the first 348 patients in the pediatric acute liver failure study group. J Pediatr. 2006;148(5): 652–8.
34. Miloh T, Kerkar N, Parkar S, Emre S, Annunziato R, Mendez C, et al. Improved outcomes in pediatric liver transplantation for acute liver failure. Pediatr Transplant. 2010;14(7):863–9.
35. Futagawa Y, Terasaki PI. An analysis of the OPTN/ UNOS Liver Transplant Registry. Clin Transpl. 2004:315–29.
36. Farmer DG, Venick RS, Mcdiarmid SV, Duffy JP, Kattan O, Hong JC, et al. Fulminant hepatic failure in children: superior and durable outcomes with liver transplantation over 25 years at a single center. Ann Surg. 2009;250(3):484–93.
37. Austin MT, Leys CM, Feurer ID, Lovvorn HN, O'Neill JA, Pinson CW, et al. Liver transplantation for childhood hepatic malignancy: a review of the United Network for Organ Sharing (UNOS) database. J Pediatr Surg. 2006;41(1):182–6.
38. Guiteau JJ, Cotton RT, Karpen SJ, O'mahony CA, Goss JA. Pediatric liver transplantation for primary malignant liver tumors with a focus on hepatic epithelioid hemangioendothelioma: The UNOS experience. Pediatr Transplant. 2010;14(3):326–31.
39. Avila L, Luis A, Hernandez F, Garcia-Miguel P, Jara P, Andres A, et al. Liver transplantation for malignant tumours in children. Eur J Pediatr Surg. 2006;16(6): 411–4. Georg Thieme Verlag KG Stuttgart/Masson Editeur: New York/Paris.
40. Otte JB, Pritchard J, Aronson DC, Brown J, Czauderna P, Maibach R, et al. Liver transplantation for hepatoblastoma: results from the International Society of Pediatric Oncology (SIOP) study SIOPEL-1 and review of the world experience. Pediatr Blood Cancer. 2003;42(1):74–83.
41. Browne M, Sher D, Grant D, Deluca E, Alonso E, Whitington PF, et al. Survival after liver transplantation for hepatoblastoma: a 2-center experience. J Pediatr Surg. 2008;43(11):1973–81.
42. Otte JB, Meyers R. PLUTO First report. Pediatr Transplant. 2010;14(7):830–5.
43. Burroughs M, Moscona A. Immunization of pediatric solid organ transplant candidates and recipients. Clin Infect Dis. 2000;30(6):857–69.
44. Campbell AL, Herold BC. Immunization of pediatric solid-organ transplantation candidates: immunizations in transplant candidates. Pediatr Transplant. 2005;9(5):652–61.
45. Abuali MM, Arnon R, Posada R. An update on immunizations before and after transplantation in the pediatric solid organ transplant recipient. Pediatr Transplant. 2011;15(8):770–7.
46. Greer R, Lehnert M, Lewindon P, Cleghorn GJ, Shepherd RW. Body composition and components of energy expenditure in children with end-stage liver disease. J Pediatr Gastroenterol Nutr. 2003;36(3): 358–63.
47. Sokol RJ, Stall C. Anthropometric evaluation of children with chronic liver disease. Am J Clin Nutr. 1990;52(2):203–8.
48. DeRusso PA, Ye W, Shepherd R, Haber BA, Shneider BL, Whitington PF, et al. Growth failure and outcomes in infants with biliary atresia: a report from the Biliary Atresia Research Consortium. Hepatology. 2007;46(5):1632–8.
49. Shepherd RW, Chin SE, Cleghorn GJ, Patrick M, Ong TH, Lynch SV, et al. Malnutrition in children with chronic liver disease accepted for liver transplantation: clinical profile and effect on outcome. J Paediatr Child Health. 1991;27(5):295–9.
50. Sullivan JS, Sundaram SS, Pan Z, Sokol RJ. Parenteral nutrition supplementation in biliary atresia patients listed for liver transplantation. Liver Transpl. 2012; 18(1):120–8.
51. Guimber D, Michaud L, Ategbo S, Turck D, Gottrand F. Experience of parenteral nutrition for nutritional rescue in children with severe liver disease following failure of enteral nutrition. Pediatr Transplant. 1999;3(2):139–45.
52. Broelsch CE, Emond JC, Thistlethwaite JR, Whitington PF, Zucker AR, Baker AL, et al. Liver transplantation, including the concept of reduced-size liver transplants in children. Ann Surg. 1988;208(4):410–20.
53. Emond JC, Whitington PF, Thistlethwaite JR, Cherqui D, Alonso EA, Woodle IS, et al. Transplantation of two patients with one liver. Analysis of a preliminary experience with "split-liver" grafting. Ann Surg. 1990;212(1):14–22.
54. Broelsch CE. Application of reduced-size liver transplants as split grafts, auxiliary orthotopic grafts, and living related segmental transplants. Ann Surg. 1990;212(3):368. Lippincott, Williams, and Wilkins.
55. Strong RW, Lynch SV, Ong TH, Matsunami H, Koido Y, Balderson GA. Successful liver transplantation from a living donor to her son. N Engl J Med. 1990;322(21):1505–7.
56. Abt PL, Rapaport-Kelz R, Desai NM, Frank A, Sonnad S, Rand E, et al. Survival among pediatric liver transplant recipients: impact of segmental grafts. Liver Transpl. 2004;10(10):1287–93.
57. Becker NS, Barshes NR, Aloia TA, Nguyen T, Rojo J, Rodriguez JA, et al. Analysis of recent pediatric orthotopic liver transplantation outcomes indicates that allograft type is no longer a predictor of survivals. Liver Transpl. 2008;14(8):1125–32.
58. Sunku B, Salvalaggio PRO, Donaldson JS, Rigsby CK, Neighbors K, Superina RA, et al. Outcomes and risk factors for failure of radiologic treatment of biliary strictures in pediatric liver transplantation recipients. Liver Transpl. 2006;12(5):821–6.
59. Peclet MH, Ryckman FC, Pedersen SH, Dittrich VS, Heubi JE, Farrell M, et al. The spectrum of bile duct complications in pediatric liver transplantation. J Pediatr Surg. 1994;29(2):214–20.
60. Kerkar N, Morotti RA, Iyer K, Arnon R, Miloh T, Sturdevant M, et al. Anti-lymphocyte therapy successfully controls late "cholestatic" rejection in

pediatric liver transplant recipients. Clin Transplant. 2011;25(6):E584–91.
61. Cohran VC, Griffiths M, Heubi JE. Bone mineral density in children exposed to chronic glucocorticoid therapy. Clin Pediatr. 2008;47(5):469–75.
62. Ng VL, Alonso EM, Bucuvalas JC, Cohen G, Limbers CA, Varni JW, et al. Health status of children alive 10 years after pediatric liver transplantation performed in the US and Canada: report of the studies of pediatric liver transplantation experience. J Pediatr. 2011;160:820–6.
63. Alonso EM, Shepherd R, Martz KL, Yin W, Anand R. Linear growth patterns in prepubertal children following liver transplantation. Am J Transplant. 2009;9(6): 1389–97.
64. Diem HVT, Sokal EM, Janssen M, Otte JB, Reding R. Steroid withdrawal after pediatric liver transplantation: a long-term follow-up study in 109 recipients. Transplantation. 2003;75(10):1664–70.
65. The US Multicenter FK 506 Liver Study Group. A comparison of tacrolimus (FK 506) and cyclosporine for immunosuppression in liver transplantation. N Engl J Med. 1994;331(17):1110–5.
66. Goto T, Kino T, Hatanaka H, Nishiyama M, Okuhara M, Kohsaka M, et al. Discovery of FK-506, a novel immunosuppressant isolated from Streptomyces tsukubaensis. Transplant Soc. 1987;19(5 Suppl 6):4–8.
67. Armitage JM, et al. The clinical trial of FK 506 as primary and rescue immunosuppression in pediatric Cardiac transplantation. Transplant Proc. 1991;23(6):3058.
68. Tzakis AG, et al. Two-year experience with FK 506 in pediatric patients. Transplant Proc. 1993;25(1 Pt 1):619.
69. Horslen S, Barr ML, Christensen LL, Ettenger R, Magee JC. Pediatric transplantation in the United States, 1996–2005. Am J Transplant. 2007;7(s1):1339–58.
70. Jain A, Mazariegos G, Kashyap R, Green M, Gronsky C, Starzl TE, et al. Comparative long-term evaluation of tacrolimus and cyclosporine in pediatric liver transplantation. Transplantation. 2000;70(4):617–25.
71. Campbell K, Ng V, Martin S, Magee J, Goebel J, Anand R, et al. Glomerular filtration rate following pediatric liver transplantation – the SPLIT experience. Am J Transplant. 2010;10(12):2673–82.
72. Lobritto SJ, Rosenthal P, Bouw R, Leung M, Snell P, Mamelok RD. Pharmacokinetics of mycophenolate mofetil in stable pediatric liver transplant recipients receiving mycophenolate mofetil and cyclosporine. Liver Transpl. 2007;13(11):1570–5.
73. Barau C, Barrail-Tran A, Hemerziu B, Habes D, Taburet A-M, Debray D, et al. Optimization of the dosing regimen of mycophenolate mofetil in pediatric liver transplant recipients. Liver Transpl. 2011;17(10): 1152–8.
74. Barau C, Furlan V, Debray D, Taburet A-M, Barrail-Tran A. Population pharmacokinetics of mycophenolic acid and dose optimisation with limited sampling strategy in liver transplant children. Br J Clin Pharmacol. 2012;74(3):515–24.
75. Gibelli NEM, Tannuri U, Pinho-Apezzato ML, Tannuri ACA, Maksoud-Filho JG, Andrade WC, et al. Sirolimus in pediatric liver transplantation: a single-center experience. Transplant Soc. 2010;41(3):901–3.
76. Sindhi R, Seward J, Mazariegos G, Soltys K, Seward L, Smith A, et al. Replacing calcineurin inhibitors with mTOR inhibitors in children. Pediatr Transplant. 2005;9(3):391–7.
77. Czubkowski P, Pawłowska J, Jankowska I, Teisseyre M, Kamińska D, Markiewicz M, et al. Successful sirolimus rescue in tacrolimus-induced thrombotic microangiopathy after living-related liver transplantation. Pediatr Transplant. 2012;16(6):E261–4.
78. Dunkelberg J. Sirolimus as primary immunosuppression in liver transplantation is not associated with hepatic artery or wound complications. Liver Transpl. 2003;9(5):463–8.
79. Campsen J, Zimmerman MA, Mandell S, Kaplan M, Kam I. A decade of experience using mTor inhibitors in liver transplantation. J Transplant. 2011;2011:1–7.
80. Sanchez EQ, Martin AP, Ikegami T, Uemura T, Narasimhan G, Goldstein RM, et al. Sirolimus conversion after liver transplantation: improvement in measured glomerular filtration rate after 2 years. Transplant Proc. 2005;37(10):4416–23.
81. Jiménez-Rivera C, Avitzur Y, Fecteau AH, Jones N, Grant D, Ng VL. Sirolimus for pediatric liver transplant recipients with post-transplant lymphoproliferative disease and hepatoblastoma. Pediatr Transplant. 2004;8(3):243–8.
82. Filippone EJ, Carson JM, Beckford RA, Jaffe BC, Newman E, Awsare BK, et al. Sirolimus-induced pneumonitis complicated by pentamidine-induced phospholipidosis in a renal transplant recipient: a case report. Transplant Soc. 2011;43(7):2792–7.
83. Roberts RJ, Wells AC, Unitt E, Griffiths M, Tasker AD, Allison ME, et al. Sirolimus-induced pneumonitis following liver transplantation. Liver Transpl. 2007; 13(6):853–6.
84. Garrean S, Massad MG, Tshibaka M, Hanhan Z, Caines AE, Benedetti E. Sirolimus-associated interstitial pneumonitis in solid organ transplant recipients. Clin Transplant. 2005;19(5):698–703.
85. Nielsen D, Briem-Richter A, Sornsakrin M, Fischer L, Nashan B, Ganschow R. The use of everolimus in pediatric liver transplant recipients: First experience in a single center. Pediatr Transplant. 2011;15(5):510–4.
86. Martin SR, Atkison P, Anand R, Lindblad AS, Split Research Group. Studies of pediatric liver transplantation 2002: patient and graft survival and rejection in pediatric recipients of a first liver transplant in the United States and Canada. Pediatr Transplant. 2004; 8(3):273–83.
87. Demetris AJ, Batts KP, Dhillon AP, Ferrell L, Fung J, Geller SA, Hart J, Hayry P, Hofmann WJ, Hubscher S. Banff schema for grading liver allograft rejection: an international consensus document. Hepatology. 1997;25:658–63.
88. Ormonde DG, de Boer WB, Kierath A, Bell R, Shilkin KB, House AK, et al. Banff schema for grading liver allograft rejection: utility in clinical practice. Liver Transpl Surg. 1999;5(4):261–8.

89. Banff Working Group, Demetris AJ. Liver biopsy interpretation for causes of late liver allograft dysfunction. Hepatology. 2006;44(2):489–501.
90. Schmitt TM, Phillips M, Sawyer RG, Northup P, Hagspiel KD, Pruett TL, et al. Anti-thymocyte globulin for the treatment of acute cellular rejection following liver transplantation. Dig Dis Sci. 2010; 55(11):3224–34.
91. Ludwig J, Wiesner RH, Batts KP, Perkins JD, Krom RA. The acute vanishing bile duct syndrome (acute irreversible rejection) after orthotopic liver transplantation. Hepatology. 1987;7(3):476–83.
92. Musat AI, Agni RM, Wai PY, Pirsch JD, Lorentzen DF, Powell A, et al. The significance of donor-specific HLA antibodies in rejection and ductopenia development in ABO compatible liver transplantation. Am J Transplant. 2011;11(3):500–10.
93. Ali S, Ormsby A, Shah V, Segovia MC, Kantz KL, Skorupski S, et al. Significance of complement split product C4d in ABO-compatible liver allograft: diagnosing utility in acute antibody mediated rejection. Transpl Immunol. 2012;26(1):62–9.
94. Lumbreras C, Lizasoain M, Moreno E, Aguado JM, Gomez R, Garcia I, et al. Major bacterial infections following liver transplantation: a prospective study. Hepatogastroenterology. 1992;39(4):362–5.
95. Blair JE, Kusne S. Bacterial, mycobacterial, and protozoal infections after liver transplantation –part I. Liver Transpl. 2005;11(12):1452–9.
96. Jagadeesh D, Woda BA, Draper J, Evens AM. Post transplant lymphoproliferative disorders: risk, classification, and therapeutic recommendations. Curr Treat Options Oncol. 2012;13(1):122–36.
97. Kamdar KY, Rooney CM, Heslop HE. Posttransplant lymphoproliferative disease following liver transplantation. Curr Opin Organ Transplant. 2011;16(3): 274–80.
98. Savoldo B, Goss JA, Hammer MM, Zhang L, Lopez T, Gee AP, et al. Treatment of solid organ transplant recipients with autologous Epstein Barr virus – specific cytotoxic T lymphocytes (CTLs). Blood. 2006;108:2942–9.
99. Kusne S, Blair JE. Viral and fungal infections after liver transplantation – part II. Liver Transpl. 2005; 12(1):2–11.
100. Miloh T, Arnon R, Shneider B, Suchy F, Kerkar N. A retrospective single-center review of primary sclerosing cholangitis in children. Clin Gastroenterol Hepatol. 2009;7(2):239–45.
101. Miloh T, Anand R, Yin W, Vos M, Kerkar N, Alonso E, et al. Pediatric liver transplantation for primary sclerosing cholangitis (PSC). Liver Transpl. 2011; 17(8):925–33.
102. Hadžić N, Quaglia A, Cotoi C, Hussain MJ, Brown N, Vergani D, et al. Immunohistochemical phenotyping of the inflammatory infiltrate in de novo autoimmune hepatitis after liver transplantation in children. Pediatr Transplant. 2012;16(5):501–10.
103. Guido M, Burra P. De novo autoimmune hepatitis after liver transplantation. Semin Liver Dis. 2011; 31(1):71–81.
104. Kerkar N, Hadzic N, Davies ET, Portmann B, Donaldson PT, Rela M, et al. De-novo autoimmune hepatitis after liver transplantation. Lancet. 1998; 351(9100):409–13.
105. Miloh T, Arnon R, Roman E, Hurlet A, Kerkar N, Wistinghausen B. Autoimmune hemolytic anemia and idiopathic thrombocytopenic purpura in pediatric solid organ transplant recipients, report of five cases and review of the literature. Pediatr Transplant. 2011;15(8):870–8.
106. Shneider BL, Mazariegos GV. Biliary atresia: a transplant perspective. Liver Transpl. 2007;13(11): 1482–95.
107. Wallot M. Long-term survival and late graft loss in pediatric liver transplant recipients –a 15-year single-center experience. Liver Transpl. 2002;8(7): 615–22.
108. Magee JC, Bucuvalas JC, Farmer DG, Harmon WE, Hulbert-Shearon TE, Mendeloff EN. Pediatric transplantation. Am J Transplant. 2004;4 Suppl 9:54–71.
109. Sokal EM, Veyckemans F, De Ville de Goyet J, Moulin D, Van Hoorebeeck N, Alberti D, et al. Liver transplantation in children less than 1 year of age. J Pediatr. 1990;117(2 Pt 1):205–10.
110. Soltys KA, Mazariegos GV, Squires RH, Sindhi RK, Anand R, The SPLIT Research Group. Late graft loss or death in pediatric liver transplantation: an analysis of the SPLIT database. Am J Transplant. 2007;7(9):2165–71.
111. Maes M, Sokal E, Otte JB. Growth factors in children with end-stage liver disease before and after liver transplantation: a review. Pediatr Transplant. 1997;1(2):171–5.
112. Sarna S, Laine J, Sipila I, Kolstinen R, Holmberg C. Differences in linear growth and cortisol production between liver and renal transplant recipients on similar immunosuppression. Transplantation. 1995; 60:656–61; (Cited 29 Dec 2012).
113. Bartosh SM, Thomas SE, Sutton MM, Brady LM, Whitington PF. Linear growth after pediatric liver transplantation. J Pediatr. 1999;135(5):624–31.
114. Codoner-Franch P, Bernard O, Alvarez F. Long-term follow-up of growth in height after successful liver transplantation. J Pediatr. 1994;124(3):368–73.
115. Mcdiarmid SV, Gornbein JA, DeSilva PJ, Goss JA, Vargas JH, Martín MG, et al. Factors affecting growth after pediatric liver transplantation. Transplantation. 1999;67(3):404–11.
116. Sorensen L, Neighbors K, Martz K, Zelko F, Bucuvalas JC, Alonso EM. Cognitive and academic outcomes after pediatric liver transplantation: Functional Outcomes Group (FOG) results. Am J Transplant. 2011;11(2):303–11.
117. Kaller T, Boeck A, Sander K, Richterich A, Burdelski M, Ganschow R, et al. Cognitive abilities,

behaviour and quality of life in children after liver transplantation. Pediatr Transplant. 2009;14(4): 496–503.
118. Grabhorn E, Schulz A, Helmke K, Hinrichs B, Rogiers X, Broering DC, et al. Short- and long-term results of liver transplantation in infants aged less than 6 months. Transplantation. 2004;78(2):235–41.
119. Bucuvalas JC, O'Connor A, Buschle K, Krug S, Ryckman FC, Atherton H, et al. Risk of hearing impairment in pediatric liver transplant recipients: a single center study. Pediatr Transplant. 2003;7(4): 265–9.
120. Alonso EM, Neighbors K, Barton FB, Mcdiarmid SV, Dunn SP, Mazariegos GV, et al. Health-related quality of life and family function following pediatric liver transplantation. Liver Transpl. 2008;14(4): 460–8.
121. Varni JW, Limbers CA, Sorensen LG, Neighbors K, Martz K, Bucuvalas JC, et al. PedsQL™ cognitive functioning scale in pediatric liver transplant recipients: feasibility, reliability, and validity. Qual Life Res. 2011;20(6):913–21.
122. Alonso EM, Limbers CA, Neighbors K, Martz K, Bucuvalas JC, Webb T, et al. Cross-sectional analysis of health-related quality of life in pediatric liver transplant recipients. J Pediatr. 2010;156(2):270–1.
123. Taylor RM, Franck LS, Gibson F, Donaldson N, Dhawan A. Study of the factors affecting healthrelated quality of life in adolescents after liver transplantation. Am J Transplant. 2009;9(5):1179–88.
124. Nicholas DB, Otley AR, Taylor R, Dhawan A, Gilmour S, Ng VL. Experiences and barriers to health-related quality of life following liver transplantation: a qualitative analysis of the perspectives of pediatric patients and their parents. Health Qual Life Outcomes. 2010;8(1):150.
125. Kosola S, Lampela H, Lauronen J, Mäkisalo H, Jalanko H, Qvist E, et al. General health, health-related quality of life and sexual health after pediatric liver transplantation: a nationwide study. Am J Transplant. 2011;12(2):420–7.
126. Pagadala M, Dasarathy S, Eghtesad B, Mccullough AJ. Posttransplant metabolic syndrome: an epidemic waiting to happen. Liver Transpl. 2009;15(12): 1662–70.
127. Dick AAS, Perkins JD, Spitzer AL, Lao OB, Healey PJ, Reyes JD. Impact of obesity on children undergoing liver transplantation. Liver Transpl. 2010;16(11):1296–302.
128. Perito ER, Glidden D, Roberts JP, Rosenthal P. Overweight and obesity in pediatric liver transplant recipients: prevalence and predictors before and after transplant, United Network for Organ Sharing Data, 1987–2010. Pediatr Transplant. 2011;16(1): 41–9.
129. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of obesity and trends in body mass index among US children and adolescents, 1999–2010. JAMA. 2012;307(5):483–90.
130. Sabate E. Adherence to long-term therapies: evidence for action. Geneva, Switzerland: World Health Organization, editor. 2003:1–211.
131. Sudan DL, Shaw Jr BW, Langnas AN. Causes of late mortality in pediatric liver transplant recipients. Ann Surg. 1998;227(2):289. Lippincott, Williams, and Wilkins.
132. Shemesh E, Shneider BL, Savitzky JK, Arnott L, Gondolesi GE, Krieger NR, et al. Medication adherence in pediatric and adolescent liver transplant recipients. Pediatrics. 2004;113:825–32.
133. Shemesh E, Annunziato RA, Yehuda R, Shneider BL, Newcorn JH, Hutson C, et al. Childhood abuse, nonadherence, and medical outcome in pediatric liver transplant recipients. J Am Acad Child Adolesc Psychiatry. 2007;46(10):1280–9.
134. Falkenstein K, Flynn L, Kirkpatrick B, Casa-Melley A, Dunn S. Non-compliance in children post-liver transplant. Who are the culprits? Pediatr Transplant. 2004;8(3):233–6.
135. Coscia LA, Constantinescu S, Moritz MJ, Radomski JS, Gaughan WJ, McGrory CH, et al. Report from the National Transplantation Pregnancy Registry (NTPR): outcomes of pregnancy after transplantation. Clin Transpl. 2007. pp 29–42.
136. Hoeltzenbein M, Elefant E, Vial T, Finkel-Pekarsky V, Stephens S, Clementi M, et al. Teratogenicity of mycophenolate confirmed in a prospective study of the European Network of Teratology Information Services. Am J Med Genet A. 2012;158A(3): 588–96.
137. Coscia LA, Constantinescu S, Moritz MJ, Frank A, Ramirez CB, Maley WL, et al. Report from the National Transplantation Pregnancy Registry (NTPR): outcomes of pregnancy after transplantation. Clin Transpl. 2009:103–22.
138. Armenti VT, Radomski JS, Moritz MJ, Gaughan WJ, Philips LZ, McGrory CH, et al. Report from the National Transplantation Pregnancy Registry (NTPR): outcomes of pregnancy after transplantation. Clin Transpl. 2002:121–30.

Liver in Systemic Disease

29

Emily Rothbaum Perito and Philip Rosenthal

Abbreviations

6-MP	6-mercaptopurine
AIH	Autoimmune hepatitis
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
CGD	Chronic granulomatous disease
DIC	Disseminated intravascular coagulation
GGT	Gamma-glutamyl transferase
GVHD	Graft-versus-host disease
HLH	Hemophagocytic lymphohistiocytosis
IVC	Inferior vena cava
IVIG	Intravenous immunoglobulin
JIA	Juvenile idiopathic arthritis
LDH	Lactate dehydrogenase
MAS	Macrophage activation syndrome
NAFLD	Nonalcoholic fatty liver disease
NSAIDs	Nonsteroidal anti-inflammatory drugs
PAN	Polyarteritis nodosa
PBC	Primary biliary cirrhosis
PCR	Polymerase chain reaction
PSC	Primary sclerosing cholangitis
SCT	Stem cell transplantation
SLE	Systemic lupus erythematosus

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Introduction

As a major component of the body's filtration system and metabolic machinery, the liver is affected in many systemic diseases. Systemic diseases that manifest primarily in the liver, or with accompanying liver disease as one of the most common manifestations, have been discussed elsewhere in the text. However, hepatitis, cholestasis, or other liver disease may be secondary manifestations of many other systemic diseases. The pediatric hepatologist is often consulted to interpret liver abnormalities in children with other systemic diseases. This chapter provides an overview of the liver involvement characteristic of major systemic diseases, by organ system.

In interpreting diagnostic testing in children with systemic diseases, it is important to remember that abnormalities in the "liver function test" panel may represent problems with other organ systems, not just the liver. Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) elevation can represent muscle inflammation such as that seen acutely in rhabdomyolysis or chronic processes like dermatomyositis or muscular dystrophies. Mild hyperbilirubinemia can be seen with hemolytic disease, even with normal liver function. Alkaline phosphatase (ALP) elevation can represent arthritis or bone involvement. In addition, systemic disease can include laboratory abnormalities that suggest hepatic dysfunction, but may be partially or wholly caused by other processes. Sepsis with shock and disseminated intravascular coagulation (DIC) can lead to

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coagulopathy with elevated INR and vasodilation with hypoalbuminemia.

Hepatic Dysfunction with Systemic Infection

Pediatric patients, particularly neonates, are vulnerable to cholestasis in the setting of systemic infection and sepsis. Children may also develop elevated serum transaminases, ALP, or gamma-glutamyl transferase (GGT) in a wide variety of systemic infections including common viral infections. Isolated unconjugated hyperbilirubinemia in the setting of illness, with no evidence of hemolysis, should raise suspicion for Gilbert syndrome.

Cardiovascular Disease

Hypoxic Hepatitis

Hypoxic Hepatitis, also referred to as "ischemic hepatitis" or "shock liver," can develop following any episode of severe systemic hypotension or hypoperfusion. The term "shock liver" is not preferred because this type of liver injury can develop secondary to hypoperfusion even if the patient does not progress to full-blown shock. In the largest series of adult patients with hypoxic hepatitis, only 55 % were clinically in a shock state but 86 % had signs of systemic hypoperfusion [1]. Chronic cardiac or respiratory dysfunction increases the risk of developing hypoxic hepatitis without severe shock [1].

Hypoxic hepatitis should be suspected in children with an acute, dramatic rise in their serum AST and ALT within 24–48 h after an episode of severe, systemic hypotension, or hypoperfusion [1–3]. The triggering episode is usually easily identified given the rapid time course, but as noted may be more subtle in children with chronic heart or lung disease or following cardiopulmonary surgery. Serum LDH often rises dramatically on this same time course, although this is not a specific finding [4]. If hemodynamic stability is restored, the serum AST and ALT should drop precipitously within the following 6–10 days [1–3]. Hyperbilirubinemia and synthetic dysfunction with coagulopathy often develop but should resolve spontaneously within 7–10 days if hemodynamics remains stable [1–3]. Persistent abnormalities should raise suspicion for underlying chronic liver disease, which may be undiagnosed particularly in children with chronic heart or lung disease.

The pathophysiology of hypoxic hepatitis differs by triggering episode and underlying chronic disease in adults, although this has not been well studied in children. Henrion et al. identified four major causes in adults: decompensated congestive heart failure, acute heart failure, exacerbated chronic respiratory failure, and toxic/septic shock [1]. In both heart failure groups, liver hypoxia was due to a combination of ischemia due to left-sided heart failure and passive congestion due to right-sided heart failure. In respiratory failure, liver injury was due to arterial hypoxemia [5]. In toxic and septic shock patients, hypoxia was a result of the liver's increased oxygen demand but reduced efficiency at oxygen extraction [1]. Liver dysfunction has been recognized in children with heart failure and cyanotic heart disease, due to a combination of systemic venous congestion and low cardiac output [6].

Liver biopsy is usually not required to diagnose hypoxic hepatitis, given the characteristic clinical course. The histologic appearance varies with the pathophysiology, as described above. Low cardiac output or arterial hypoxemia may lead to centrilobular necrosis with relative preservation of the periportal zone, reflecting increased vulnerability of the centrilobular hepatocytes to ischemic injury because of the direction of oxygenated blood flow—from the portal tracts through the sinusoids to the central vein [7]. Right-sided heart failure with elevated systemic venous pressures leads to venous congestion and sinusoidal dilatation in the liver [7]. Inflammatory cells are usually not prominent. Fibrosis suggests preexisting chronic liver damage.

Although a triggering hypotensive episode and subsequent rise in serum AST and ALT suggest hypoxic hepatitis, acute hepatitis due to acetaminophen or other drug-related toxicity

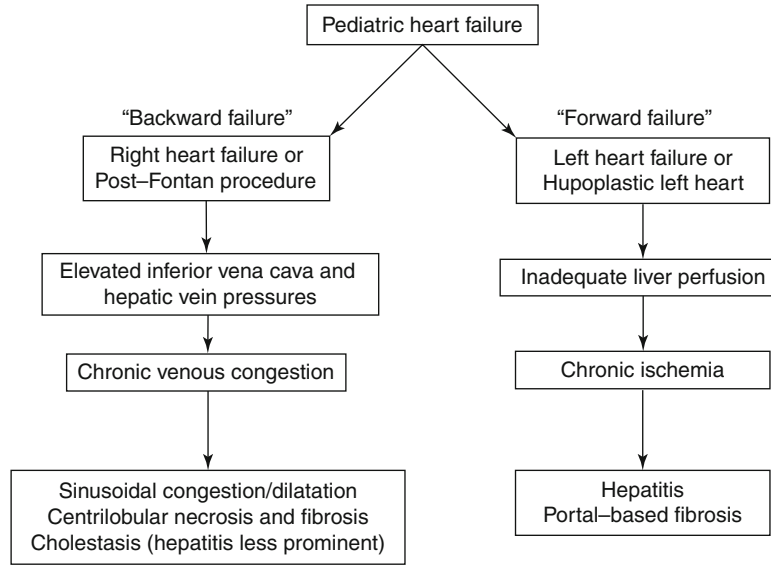

Fig. 29.1 Hepatic consequences of heart failure in children

or viral infection may present similarly. These should be considered in the child who presents with shock or hypoperfusion accompanied by fever, other signs of infection, or a suspicious or unknown recent medication history. Hypoxic injury to other organs, notably altered mental status and acute renal tubular necrosis, is frequently seen in conjunction with hypoxic hepatitis [7].

Therapy of hypoxic hepatitis should focus on reestablishing hemodynamic stability and adequate circulation. With appropriate supportive care and treatment of the triggering condition, hepatitis and liver function usually recover within 7–10 days. Worsening hyperbilirubinemia or coagulopathy can represent persistent hypoperfusion, infection with DIC or hemolysis, or liver failure and should trigger further diagnostic workup.

Pediatric Heart Disease and Chronic Liver Damage

In parallel to the acute liver damage caused by acute hypoperfusion as described above, chronic cardiac dysfunction or abnormal circulation can lead to chronic liver damage. This chronic liver damage is best characterized in adults, but is being increasingly recognized in children as therapy for congenital and acquired heart disease improves survival [8]. Chronic right-sided heart failure is associated with hepatic venous congestion, sinusoidal dilatation, centrilobular necrosis, and fibrosis [7]. Mild hyperbilirubinemia is often associated, although the cause of this is not well understood. Serum aminotransferase elevations are usually mild or absent. Chronic left-sided heart failure, or congenital abnormalities leading to low cardiac output, can lead to chronic ischemic changes. Chronic damage leading to fibrosis, cirrhosis, and portal hypertension can develop as a result of either process, or a combination of the two [6] (Fig. 29.1).

Single-Ventricle Cardiac Physiology and the Liver

Chronic liver disease in children with congenital cardiac anomalies has been most thoroughly studied in children with hypoplastic left heart syndrome or other single-ventricle physiologies that undergo a Fontan procedure. The Fontan procedure, the third and final stage of surgical repair for single-left-ventricle physiology, directs inferior vena cava (ICV) flow directly into the pulmonary artery. This relieves the child's cyanosis but elevates IVC pressures; this pressure is back-transmitted to the hepatic veins and liver,

leading to chronic hepatic congestion. Even after the Fontan procedure, cardiac output is usually below normal, which may impair forward flow to the liver [8].

Children with hypoplastic left heart syndrome or other cyanotic heart disease likely start to accumulate liver damage before corrective surgeries. As discussed above, cyanosis and systemic ischemia starting after birth predispose them to chronic liver hypoxia and damage. In one pediatric series, portal-based fibrosis was seen in over 80 % of children who died within 1 month of Fontan surgery; in these children, fibrosis severity was associated with length of hospitalizations for pre-Fontan surgeries [9]. This supports the theory that chronic liver damage predates the Fontan procedure.

Monitoring children with these cardiac problems for signs and symptoms of liver disease is crucial. New physical exam findings of hepatomegaly, splenomegaly, peripheral edema, ascites, or distended veins over the abdomen should prompt further workup for liver disease and portal hypertension. Incidental findings of thrombocytopenia or elevated INR on laboratory monitoring should prompt consideration of hepatic congestion, cirrhosis, or portal hypertension. Frequent bruising or petechiae may be caused by the coagulopathy of liver synthetic dysfunction and/or thrombocytopenia associated with congestive splenomegaly.

Rheumatologic Disease

Liver disease in patients with active or chronic rheumatologic disease is increasingly recognized. The etiology of abnormal liver tests and liver disease is incredibly varied in this population (Tables 29.1 and 29.2).

Generally, coexistent primary liver disease must be considered in patients with known rheumatologic disease. Autoimmune liver disease—including autoimmune hepatitis (AIH), primary sclerosing cholangitis (PSC), and primary biliary cirrhosis (PBC)—may develop. In adults, both AIH and PBC have been associated with Sjögren syndrome, rheumatoid arthritis, systemic sclerosis, and Hashimoto's thyroiditis [10]. In nonsystematic reviews, AIH and PBC are most commonly associated with other rheumatologic diseases, with PSC reported rarely [10, 11]. As discussed in detail in chapter "Autoimmune Hepatitis and Sclerosing

Table 29.1 Differential diagnosis of hepatitis in rheumatologic disease

Viral hepatitis
Hepatitis B, C
Chronic CMV hepatitis in immunosuppressed patients
Nonalcoholic fatty liver disease/steatohepatitis
Autoimmune disease
Autoimmune hepatitis
Primary sclerosing cholangitis
Primary biliary cirrhosis (rare in children/adolescents)
Drug-induced liver injury
Methotrexate
NSAIDs
Anakinra
Macrophage activation syndrome/hemophagocytic lymphohistiocytosis
Congestive hepatopathy (if concomitant cardiomyopathy/heart failure)
Nonspecific chronic hepatitis
See Table 29.2 for specific associations between rheumatologic diseases and other liver diseases

Table 29.2 Liver diseases associated with specific rheumatologic diseases

Disease	Characteristic liver findings
Juvenile idiopathic arthritis (systemic)	Hepatosplenomegaly Nonspecific hepatitis
Systemic lupus erythematosus	Hepatomegaly Small-vessel vasculitis Granulomatous hepatitis Nodular regenerative hyperplasia Chronic nonspecific/autoimmune hepatitis
Rheumatoid arthritis	Felty syndrome (splenomegaly, neutropenia, nodular regenerative hyperplasia)
Polyarteritis nodosa	Necrotizing hepatic arteritis
Wegener's granulomatosis	Granulomatous hepatitis
Behcet disease	Budd-Chiari syndrome
Amyloidosis	Hepatosplenomegaly with amyloid deposits

Cholangitis," elevated serum transaminases, positive serologies, and histologic hepatitis with lymphocyte and plasma cell infiltrates are often seen in AIH. Imaging revealing beaded bile ducts or liver biopsy showing periductal fibrosis is classic for PSC. Small bile duct obliteration with granulomas should raise concern for PBC, although this is very rare in the pediatric population [11].

Viral hepatitis should be ruled out in rheumatologic patients with newly elevated serum AST and ALT, particularly those with a history of blood transfusions and those on immunosuppressive medications. Fatty liver disease can be seen, particularly in children requiring chronic corticosteroid therapy for their rheumatologic disease. Hepatic congestion can lead to chronic liver disease in patients with cardiomyopathy [10].

Juvenile Idiopathic Arthritis

In juvenile idiopathic arthritis (JIA) patients, minor abnormalities in serum AST and ALT levels appear to be relatively common, although prevalence studies have been small [12].

Systemic JIA, which includes hepatosplenomegaly as a diagnostic feature, has been recognized as an entity distinct from other forms of JIA. Systemic JIA accounts for 10–20 % of childhood JIA. These children have arthritis in one or more joints and prolonged or recurrent fevers, with or without rash, lymphadenopathy, and serositis (pericarditis, peritonitis, pleuritis, meningitis) [13, 14]. Splenomegaly is seen at diagnosis in up to 15–50 % of cases, with hepatomegaly rarer [13]. Elevation of the serum transaminase levels is seen in up to 25 % at diagnosis [15] and may improve with treatment. Other laboratory findings include leukocytosis or thrombocytosis and elevated erythrocyte sedimentation rate, C-reactive protein, ferritin, aldolase, and D-dimers. Worsening hepatosplenomegaly in systemic JIA, often accompanied by proteinuria and renal dysfunction, should raise concern for secondary amyloidosis caused by ongoing inflammation [13, 16].

Of note, macrophage activation syndrome (MAS) is a life-threatening complication of systemic JIA that can present with a similar clinical picture and hepatitis. In MAS, as discussed further later in this chapter, hepatitis should be accompanied by relative leukopenia, thrombocytopenia, hypofibrinogenemia, and hypertriglyceridemia.

Rheumatoid Arthritis

Felty syndrome is a rare complication of rheumatoid arthritis with splenomegaly and neutropenia that has been case-reported in children [17]. In adults, Felty syndrome has been associated with portal hypertension and esophageal varices, with nodular regenerative hyperplasia reported in some patients, but minimal histologic changes in others [18, 19]. The etiology of the nodular regenerative hyperplasia in the liver may be related to abnormal platelet aggregation and small-vessel venous thrombosis or to venous injury from immune complex deposition.

Systemic Lupus Erythematosus

Hepatic involvement in systemic lupus erythematosus (SLE) is increasingly recognized, although it is rarely the most clinically significant locus of disease. Most studies are small and retrospective, so reported prevalence of hepatomegaly ranges from 12 to 55 % and abnormal serum transaminases from 2.4 to 55 % [18].

When hepatitis or cholestasis is detected in an SLE patient, a broad differential diagnosis must be considered. For example, abnormalities may be caused by medications or sequelae of lupus-induced damage to other organs—e.g., cardiomyopathy leading to hepatic congestion or even cardiac cirrhosis, drug-induced liver injury, or viral hepatitis acquired by blood transfusions. Many patterns of liver damage have been reported in liver biopsies from SLE patients. Hepatic small-vessel vasculitis, nonalcoholic fatty liver disease, chronic hepatitis, granulomatous hepatitis, nodular regenerative hyperplasia, and cirrhosis have all been

detected in liver biopsy or autopsy studies [10, 11, 18]. Chronic hepatitis from SLE may be histologically indistinguishable from autoimmune hepatitis [10].

Neonatal lupus erythematosus is a special case of lupus that involves hepatobiliary disease in approximately 9 % of cases [20]. The classic manifestations of neonatal lupus are congenital heart block or cutaneous lesions, with anti-Ro/SSA or anti-La/SSB in either the infant or mother. Hepatobiliary manifestations of neonatal lupus included three phenotypes in a retrospective review of the Neonatal Lupus National Research Registry: (1) severe neonatal liver failure resembling neonatal hemochromatosis, (2) conjugated hyperbilirubinemia with mild or no AST/ALT elevation in the first few weeks of life, and (3) mild AST/ALT elevation in the first 2–3 months of life. Of note, 50 % of the children with severe liver failure had no other manifestations of neonatal lupus but were suspected as cases based on family history. Whether there may be overlap with neonatal hemochromatosis-gestational alloimmune liver disease, or similar prenatal factors at play, is not well understood [21]. Early unconjugated hyperbilirubinemia can also occur in these infants and appears to self-resolve as would be expected in physiologic jaundice [20].

Autoimmune Vasculitis

Liver involvement in other autoimmune vasculitides has been reported but is thought to be rare [11, 18]. Polyarteritis nodosa (PAN) is a small and medium-vessel vasculitis that can involve necrotizing hepatic arteritis [11]. PAN is more common in patients infected with hepatitis B or C. Granulomatous hepatitis has been reported in Wegener's granulomatosis in adults. Liver involvement in Behcet disease should prompt consideration of Budd-Chiari syndrome [11, 18]. However, the literature on these associations is limited to case reports and series. There has been no systematic, published study of liver involvement in pediatric populations with these vasculitic diseases.

Autoimmune Myositis

In patients with dermatomyositis or polymyositis, serum AST, ALT, and lactate dehydrogenase (LDH) elevations may be due to the myositis and not liver disease. If serum aminotransferase or LDH elevations are detected, serum creatine kinase can be helpful to screen for muscle involvement [18].

Kawasaki Disease

Kawasaki disease is one of the most common childhood vasculitides. Suspicion for Kawasaki disease arises in children with unexplained fevers for 5 days or longer, particularly in conjunction with bilateral conjunctivitis, rash, red lips and strawberry tongue, and erythematous palms and soles. Typical laboratory findings include elevated inflammatory markers, anemia, leukocytosis, thrombocytosis, and sterile pyuria. Mild to moderately elevated transaminases and GGT are also fairly common, occurring in 30–45 % of children. Of note, in two large cohorts, elevated bilirubin was associated with IVIG-resistant Kawasaki disease, but not with other outcomes including coronary artery aneurysms [22, 23]. The classic liver manifestation of Kawasaki disease is gallbladder hydrops, thought to be caused by small-vessel vasculitis affecting the gallbladder wall. This may be accompanied by cholestasis with or without abdominal pain and vomiting, although this appears to be a relatively rare manifestation [24, 25]. There have also been case reports of cholangitis accompanying Kawasaki disease; whether this is primary bile duct inflammation or secondary as a consequence of vasculitis is not known [26].

Drug-Induced Liver Injury in Rheumatologic Disease

The medications prescribed to treat rheumatologic disease can also induce hepatotoxicity. Corticosteroids, used for disease flares or chronically in refractory cases, can contribute to weight

gain and hepatic steatosis with steatohepatitis. Aspirin has largely been replaced by other nonsteroidal anti-inflammatory drugs (NSAIDs), but if used can precipitate a mild hepatitis or Reye syndrome.

NSAIDs and methotrexate, both first-line treatments for mild rheumatologic disease in children, can be hepatotoxic. Both require regular monitoring of liver enzymes during chronic use [14]. In randomized controlled trials, 0.5–3 % of subjects on NSAIDS develop serum aminotransferase elevations above three times the upper limit of normal, with the prevalence highest in diclofenac and rofecoxib trials [27]. These elevations most commonly occur in the first 4–6 months of therapy. However, serious adverse events requiring drug discontinuation appears to be rare, with no increase over rates in placebo except with diclofenac [27].

Methotrexate, commonly used in the treatment of JIA and other rheumatologic or autoimmune conditions, can cause significant liver injury. The mechanism of hepatotoxicity is not completely understood, but may be related to folate depletion as well as presence of methotrexate metabolites in the liver. Histologic findings in methotrexate-induced liver injury include steatosis, hepatocellular necrosis, portal-based inflammation, and fibrosis. Damage can progress to cirrhosis. Serum transaminase elevation in children with JIA on methotrexate appears to be relatively rare, occurring in 2–3 % of children [28, 29], which may be significantly less than is seen in adults [29, 30]. Series of children on long-term, low-dose methotrexate have revealed little significant fibrosis, but higher-dose regimens may increase risk [31–33]. Concurrent exposure to multiple hepatotoxic drugs may increase the risk of chronic liver damage [29, 32]. Persistent elevation of serum transaminases on routine monitoring is significantly associated with fibrosis grade [34]. Some evidence suggests that obesity may exacerbate liver damage from methotrexate also, possibly secondary to coexistent NAFLD [34].

Recommended monitoring for methotrexate includes liver enzymes at baseline, within 1 month after initiating treatment or escalating the dose, and then every 3–4 months for those on a stable maintenance dose [14]. Folic acid supplementation in children on methotrexate may help minimize hepatotoxicity, but routine monitoring remains crucial. Previous guidelines recommended liver biopsy after each 1–2 g cumulative methotrexate dose. This is no longer recommended, but liver biopsy for staging of disease and consideration of dose adjustment can be helpful in patients with persistently elevated serum transaminases [14, 32].

Anakinra, an interleukin-1 antagonist more recently added to the pharmacologic armamentarium for severe juvenile rheumatologic disease [14], has also been associated with rare hepatotoxicity. Elevation of serum transaminases that resolves with cessation of the drug may be related to anakinra-mediated liver injury [35]. However, autoimmune hepatitis, JIA flare, and MAS should also be on the differential for pediatric patients on anakinra with hepatitis. Serial monitoring of clinical symptoms and laboratory tests is essential for differentiating the diagnosis in these cases. Increasing serum AST and ALT with climbing ferritin and triglycerides, and dropping white blood cell and platelet counts, should raise concern for MAS.

Amyloidosis

Systemic amyloidosis is extremely rare in children and usually presents with renal involvement as the most prominent feature. But hepatomegaly, and less commonly splenomegaly, can also develop with amyloidosis [16]. The hallmark of amyloidosis is the extracellular deposition of insoluble plasma protein precursors within organs, eventually leading to organ damage and dysfunction. Amyloidosis can be a secondary (reactive) process to chronic inflammation or a primary (familial) process. The type of protein deposited varies based on underlying cause [16].

In children, systemic amyloidosis has been reported in a variety of chronic inflammatory diseases including familial Mediterranean fever, JIA, inflammatory bowel disease, tuberculosis, and cystic fibrosis [28, 36–39]. Diagnosis of amyloidosis is made by biopsy, with amyloid

deposits visualized using Congo red staining. Treatment of secondary amyloidosis is directed at the underlying chronic disease [16]. In familial Mediterranean fever, colchicine is also used.

Familial amyloidosis, although recognized as a dominant genetic disorder, is almost exclusively a disease of middle-age onset. Up to 10 % of carriers never develop symptoms. How early affected patients begin to deposit amyloid is not known. Familial amyloidosis is most commonly caused by transthyretin mutations. The most prominent symptoms are usually neuropathy, either sensorimotor or autonomic, and cardiomyopathy. Hepatosplenomegaly can also occur. Renal involvement is less common [16].

Of interest to the hepatologist, familial amyloidosis can be an indication for liver transplantation, as the majority of transthyretin is synthesized in the liver. Liver transplant is intended to halt progression of neuropathy, but does not seem to reverse disease and may not be helpful for all mutations. Livers explanted from familial amyloidosis patients have been used for domino transplants, but de novo systemic amyloid deposition has been reported in some patients [40, 41].

Hematologic Disease

Hemoglobinopathies

Liver involvement in hemoglobinopathies, including sickle cell disease, hemoglobin C disease, and beta-thalassemia, can be due to multiple etiologies. These are genetic disorders that lead to abnormally structured or folded hemoglobin, which predisposes to microvascular occlusion and hemolysis.

Hemolytic disease crises can lead to hyperbilirubinemia and serum transaminase elevations, even without direct liver involvement. Jaundice due solely to hemolysis should be an unconjugated hyperbilirubinemia. Recurrent hemolytic crises predispose to cholelithiasis, with accumulation of pigmented gallstones. Pigmented gallstones can develop in children as young as 2–4 years of age, and 50–70 % of sickle cell patients have gallstones by adulthood [42–44]. Obstruction leading to biliary colic, cholecystitis, or cholangitis can develop in these patients and often mimics the presentation of abdominal or hepatic crisis. Cholecystectomy to prevent further complications has been the most common indication for surgery in sickle cell patients. However, surgery can precipitate sickle cell crises, including acute chest syndrome, in up to 20 % of patients; preoperative transfusion can help prevent complications [45].

Sickle cell crisis can also occur in the liver. Acute sickle hepatic crisis is also referred to in the literature as sickle cell hepatopathy or intrahepatic cholestasis [46]. Symptoms include right upper quadrant pain, low-grade fever, and hepatomegaly with increasing splenomegaly. Laboratory testing reveals conjugated hyperbilirubinemia and mild to moderate hepatitis—and the falling hematocrit commonly seen with disease crises [42]. Significant liver dysfunction with coagulopathy and encephalopathy can develop. Serum bilirubin elevations can be extreme, rising above 15 mg/dL or even 30 mg/dL [46]. Liver dysfunction is thought to be secondary to localized liver ischemia caused by sickling of erythrocytes within the liver sinusoids. Liver biopsy, if performed, reveals sinusoidal dilatation, congestion with sickled erythrocytes, and Kupffer cell hyperplasia [46]. Ischemic necrosis of hepatocytes and perisinusoidal fibrosis can also be seen [47]. Imaging to rule out bile duct obstruction and serum testing to rule out other causes of acute hepatitis are essential if this diagnosis is suspected.

Similar symptoms with active hemolytic anemia but otherwise minimal changes in liver tests can be seen in hepatic sequestration crises, in which sickled red blood cells are sequestered in the liver sinusoids in a process that echoes sequestration crises in the spleen or lungs [48].

The primary treatment for acute sickle hepatic crisis is supportive care and, if required, exchange transfusion. Acute sickle hepatic crisis can progress to fulminant liver failure [46]. Cases of liver transplantation for liver failure associated with sickle cell disease have been reported. Data on long-term outcomes is very limited, but recurrent

intrahepatic sickling or vessel thrombosis may occur [49, 50].

In children with sickle cell disease or thalassemia, hepatic complications of disease treatment must also be considered. Those that have had blood transfusions are at risk for viral hepatitis, including hepatitis B and C [47], although risk of this has dropped with improved screening of the blood supply.

Secondary Iron Overload

Chronic hemolysis and frequent blood transfusions can also lead to secondary iron overload, in hemoglobinopathies or other diseases like Diamond-Blackfan anemia. Elevated ferritin can be used as a screen for iron overload, with levels >2,500 ng/mL suggestive of high liver iron content, but sensitivity and specificity are limited [47, 51, 52]. Ferritin is also an acute-phase reactant that increases during hemolytic crises or acute illness. In the hemolytic diseases, if iron overload is suspected, chronic ferritin elevation in between crises should be confirmed before more invasive testing is pursued.

The lifetime volume of transfusions is also associated with hepatic iron content and risk of liver fibrosis in children and adolescents [53, 54]. Patients with thalassemia major seem to have higher risk of liver fibrosis than those with sickle cell disease, as well as extrahepatic iron deposition (heart, endocrine organs) with related complications [51, 55]. This is likely related to the larger transfusion requirements in thalassemia major, although higher levels of non-transferrin-bound iron and lower hepcidin levels may also play a role [56].

Magnetic resonance imaging with T2* is increasingly used to evaluate iron overload, but liver biopsy remains the definitive diagnostic technique. Total liver iron content, pattern of iron deposition, and active hepatitis or fibrosis can be assessed with a liver biopsy. Both sickle cell and thalassemia patients show typical patterns of secondary iron overload—with iron accumulating in the Kupffer cells of the liver sinusoids, but hepatocyte iron accumulation around the portal tracts can also be seen in patients with high liver iron burden [55]. Chelation therapy, with oral deferasirox or intravenous or subcutaneous deferoxamine, can decrease ferritin, ALT, and liver iron content, but long-term effects on hepatic fibrosis and the progression of chronic liver disease in children are not known [57].

Primary iron overload, or hereditary hemochromatosis, is discussed in detail in chapter "Metabolic Liver Disease: Part 2."

Oncologic Disease

Primary liver tumors are discussed in chapter "Hepatic Tumors," but systemic oncologic processes commonly have hepatic involvement. It is crucial to monitor for liver involvement and ongoing damage—either caused by infiltration of the cancer itself or secondary due to toxicity of the treatments.

Leukemia, Lymphoma, and Hepatosplenic T-cell Lymphoma

Acute lymphocytic leukemia is the most common type of childhood cancer, accounting for approximately 30 % of all childhood malignancy. Hodgkin's lymphoma is the most common malignancy in US adolescents [58]. Hepatosplenomegaly can be seen in pediatric leukemia and lymphoma at presentation, secondary to infiltration of these organs. Rarely, non-Hodgkin's lymphoma can present with liver as the primarily involved organ, though this has more often been reported in adults [59, 60]. Elevated serum transaminases and abnormal coagulation studies are common at diagnosis but are often due to hemolysis and DIC, not primary liver disease.

Hepatosplenic T-cell lymphoma has more recently been recognized as an entity distinct from primary hepatic lymphoma [59]. Hepatosplenic lymphoma has been most often reported in adolescent or young adult males, particularly those being treated with immunosuppression for inflammatory bowel disease.

Antitumor necrosis factor agents like infliximab were initially implicated as risk factors, but more recent analysis suggests that the thiopurines or combination anti-TNF/thiopurine treatment may be the major risk factor [61]. Presentation most commonly includes fever, cytopenias, and hepatosplenomegaly. Diagnosis can be difficult unless clinical suspicion is high. Prognosis is poor, even with aggressive chemotherapy [61].

Chronic liver damage in pediatric leukemia and lymphoma patients is more often due to secondary infections and chemotherapy toxicity than to the oncologic process itself. Assessment of ongoing liver damage can be challenging, as serum transaminases and bilirubin often fluctuate during chemotherapy secondary to systemic illness and medications. After initial chemotherapy, current treatment protocols include up to 3 years of methotrexate and/or 6-mercaptopurine (6-MP), both of which are associated with chronic liver damage and fibrosis. Splenomegaly and thrombocytopenia from portal hypertension are often presenting signs, noted during oncologic follow-up. The risk of methotrexate-related hepatic fibrosis appears to be highest when the cumulative dose exceeds 1.5–2 g, but many patients tolerate this without significant fibrosis [30]. 6-thioguanine (6-TG) was used in the past as long-term maintenance therapy, but associated hepatotoxicity is one factor leading to its replacement with 6-MP [62]. However, 6-MP is metabolized to 6-TG and can also be hepatotoxic [63, 64].

Graft-Versus-Host Disease

Graft-versus-host disease (GVHD) remains a significant problem after hematopoietic stem cell transplant in children, and rarely after other solid-organ transplants as well. The introduction of non-myeloablative stem cell transplantation (SCT), preventive strategies, and more judicious use of immunosuppression in solid-organ transplant has reduced GVHD prevalence and severity, but it remains a significant problem. GVHD occurs after SCT conditioning regimens cause damage to host tissue with local upregulation of antigen-presenting cells. T cells from the transplanted graft enter those damaged tissues, proliferate in response to the inflammatory milieu, and cause further damage [65]. Commonly involved systems include the skin, gastrointestinal tract, and liver.

Acute GVHD, by definition, occurs within 100 days of transplant [65]. Acute GVHD of the liver is characterized most commonly by hyperbilirubinemia, often with elevated alkaline phosphatase and GGT. Serum aminotransferases can also be elevated, but an isolated hepatitis should prompt further workup for non-GVHD etiologies, including viral infection. Cholestasis after SCT can also be caused by sepsis or infection, drug-induced injury, and veno-occlusive disease; these etiologies are discussed in chapters "Infections of the Liver," "Drug-Induced Liver Injury in Children: A Structured Approach to Diagnosis and Management," and "Vascular Liver Disease," respectively [66].

Liver biopsy can be helpful in acute GVHD diagnosis. Damage to the bile duct epithelium is a hallmark of diagnosis. Portal inflammation with endothelitis and apoptosis can also be seen [67]. But liver biopsy may be associated with increased bleeding and infection risk in these patients. Sensitivity of liver biopsy is lowest within the 4 weeks after transplant [68]. Often the characteristic skin rash and/or diarrhea, or less invasive biopsies of the skin or rectum, are sufficient and preferable, for diagnosis given the risk profile.

Chronic GVHD occurs more than 100 days after transplant, with a median onset of 6 months posttransplant [69]. B lymphocytes contribute more significantly to chronic GVHD, which can involve any organ system [69]. Children who had acute GVHD are at highest risk [69]. As in acute GVHD, hyperbilirubinemia with elevations in serum ALP is the most common laboratory manifestation of liver involvement [69]. Liver biopsy may be more helpful in the diagnosis of chronic GVHD than in acute. It typically reveals ongoing bile duct damage or ductopenia and can include lobular hepatitis and substantial fibrosis [67]. A "hepatitic" form of chronic GVHD has been reported in children and adults, presenting with

more significant serum transaminase elevation. Liver biopsy shows mild to no bile duct damage with prominent lobular inflammation and hepatocyte necrosis [70]. Liver transplantation has been performed in children for end-stage liver disease due to chronic GVHD, although posttransplant survival is decreased compared to other pediatric liver transplant recipients [71].

Treatment of both acute and chronic GVHD is systemic, with prednisone and a calcineurin inhibitor usually the first-line treatment. Other immunosuppression or T-cell-depleting therapies may be required. Ursodiol is often used as an adjunct [69, 72].

Hemophagocytic Lymphohistiocytosis

Hemophagocytic lymphohistiocytosis (HLH) is a syndrome of immune dysregulation thought to be caused by excessive T-cell activation, which triggers activation of macrophages and other innate immune cells. Diagnostic criteria include fever, splenomegaly, and cytopenias (anemia, thrombocytopenia, neutropenia). Hepatic dysfunction is a common feature at diagnosis, and HLH may progress to fulminant liver failure. Elevated serum aminotransferases and coagulopathy are often present, although concurrent DIC usually contributes to these abnormalities. Elevated fasting serum triglycerides (>265 mg/dL), elevated ferritin (>500 mg/dL), and low fibrinogen (<150 mg/dL) can also occur in acute liver failure, but should trigger suspicion for HLH. Hemophagocytosis seen on biopsy (bone marrow, lymph nodes, liver), elevated soluble IL-2 receptor (soluble CD25) levels, and low or absent NK-cell activity are more specific diagnostic criteria [73, 74].

HLH can be either primary (familial) or secondary, most often triggered by a viral infection or an underlying autoimmune disease. Primary HLH usually presents in neonates with severe systemic illness that resembles sepsis and/or fulminant liver failure, but it can have later onset [75]. Testing for genetic mutations that cause defects in cytotoxic T- or NK-cell function is available [74]. Detailed family history for HLH, or unexplained infant or child deaths or liver failure, can be helpful. Viruses known to cause acute liver failure, like Epstein-Barr virus and herpes simplex virus, can also trigger HLH so diagnosis of an active viral infection does not rule out HLH [76].

Though hepatic dysfunction is common in HLH, the pathophysiology of liver involvement is not well understood. Laboratory testing usually indicates hepatitis, cholestasis, and coagulopathy suggestive of liver synthetic dysfunction, although, as noted, severe systemic illness and DIC also contribute. The largest series reporting on liver histology includes 19 children with familial HLH [77]. They found lymphocyte-mediated bile duct injury, endothelialitis of both portal and central veins, and lymphohistiocytic portal infiltrates. The extent of endothelialitis and portal inflammation appeared to correlate with clinical severity. Hemophagocytosis was seen in all specimens, most commonly in the hepatic sinusoids or vessel lumens [77]. Though these findings are suggestive for the etiology of liver injury in HLH, liver biopsy is not generally recommended for diagnosis given the availability of serum diagnostic testing and the risk of biopsy with HLH-induced thrombocytopenia and coagulopathy.

Treatment protocols for HLH continue to evolve as experience with this syndrome increases, but induction therapy for both familial and secondary HLH includes corticosteroids and anti-thymocyte globulin or etoposide plus supportive care for the multisystem dysfunction [74]. In cases with triggering infections, treatment of the infection is essential. SCT following this induction is recommended for familial HLH or recurrent disease [74]. Liver transplant in cases with fulminant liver failure has been reported [78], but reported experience is minimal and outcomes poor. Protocols will likely require induction chemotherapy, followed promptly by liver transplant and then SCT.

Macrophage Activation Syndrome

MAS is thought to be a subtype of HLH that occurs in children with rheumatologic diseases,

most commonly JIA but also SLE and Kawasaki disease [74, 79]. Presenting symptoms are identical to HLH: fevers, cytopenias, hepatitis, hepatosplenomegaly, and DIC. Since children with autoimmune diseases may have low cell counts and elevated ferritin due to their underlying inflammatory disorder, relative changes in these values rather than the more definitive cutoffs used for other HLH diagnosis have been recommended. As in HLH generally, triggers can include infections or medications and genetic predisposition has been identified [79]. Initial MAS treatment is less aggressive, starting with increased immunosuppression with steroids or cyclosporine or high-dose intravenous immunoglobulin. Specific IL-1 and IL-6 inhibitors have also been used.

Endocrine Disorders

In neonates, endocrine hormone deficiencies—including hypothyroidism, adrenal insufficiency, and panhypopituitarism—have been associated with cholestatic jaundice and mild serum transaminase elevation (Table 29.3).

Hypothyroidism

Congenital hypothyroidism is the most common of this group. Cholestasis may be a prominent initial symptom. Large fontanelle, macroglossia, umbilical hernia, and hypotonia are other classic physical characteristics [80, 81]. Congenital hypothyroidism is usually tested for during routine newborn screening in the USA. However, reassessment of thyroid function should be part of the routine evaluation of conjugated hyperbilirubinemia in infancy. The cholestasis resolves with thyroid hormone replacement.

Table 29.3 Liver findings in endocrine disorders

Disorder	Characteristic liver findings
Hypothyroidism	Cholestasis
Hyperthyroidism	Cholestasis Mild hepatitis
Panhypopituitarism, adrenal insufficiency	Cholestasis Mild hepatitis
Type I diabetes mellitus	Glycogen hepatopathy Nonalcoholic fatty liver disease

In hyperthyroidism, both cholestatic jaundice and mild serum transaminase elevation have been reported, but neither is a common or prominent manifestation [82–84].

Hypopituitarism and Adrenal Insufficiency

Cholestatic jaundice with hypoglycemia in a neonate should prompt consideration of congenital adrenal insufficiency or hypopituitarism [85–88]. Microphallus is commonly seen in males with congenital hypopituitarism [85, 86]. Serum transaminase and GGT elevations can accompany the cholestasis, and liver biopsy has shown canalicular cholestasis with mild portal inflammation [86]. Treatment of panhypopituitarism with thyroid hormone, growth hormone, and hydrocortisone leads to resolution of the cholestasis. Some have theorized that the thyroid or growth hormone deficiency is primarily responsible for the cholestasis, although the mechanisms are not well understood. Interestingly, though, there are also reports of cholestasis with isolated ACTH deficiency [87].

Evidence is emerging on a high prevalence of relative adrenal insufficiency in patients with end-stage liver disease and cirrhosis, a so-called hepato-adrenal syndrome. Adrenal insufficiency may increase morbidity and mortality in patients with severe decompensated liver disease—in children [89, 90] and adults [91, 92]. Studies in adults suggest that adrenal stress response in compensated cirrhotics is also commonly impaired, secondary to impaired hypothalamic-pituitary response but not primary adrenal gland dysfunction [92]. The mechanism for this interaction is not known, although bile acid inhibition of hepatic glucocorticoid metabolism, leading to suppression of the hypothalamic-pituitary axis, and direct cytokine inhibition of the axis have been postulated [93, 94].

Diabetes Mellitus

This discussion will be limited to the liver disease associated with type I diabetes mellitus. Type II diabetes mellitus is strongly associated with nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis, which is discussed in detail in chapter "Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis."

Type I diabetics can develop NAFLD, but those on insulin with poor glycemic control can also develop glycogen hepatopathy—an extensive but reversible accumulation of glycogen in the hepatocytes associated with mild hepatitis [95, 96]. Patients may present with right upper quadrant pain or hepatomegaly but often are asymptomatic, with elevated transaminases as the trigger for evaluation of liver disease. A current or recent episode of diabetic ketoacidosis and an elevated hemoglobin A1C are common, indicating acute and/or chronic poor glycemic control [95, 97, 98].

On ultrasound, glycogen hepatopathy and NAFLD can both cause a nonspecific diffuse echogenicity of the liver [98]. Magnetic resonance imaging (MRI) or computed tomography (CT) may help differentiate intrahepatic glycogen from lipid [98]. However glycogen hepatopathy can only be definitively differentiated from NAFLD with liver biopsy. Liver biopsy shows hepatocytes swollen with glycogenic inclusions and glycogenated nuclei. Steatosis is absent or mild, inflammation minimal, and fibrosis rare [95]. Even on liver biopsy, this entity can be mistaken for glycogen storage disease [95]; expert pathologist consultation is important for verifying the correct diagnosis. Glycogen hepatopathy resolves with improved glycemic control; transaminases and liver histology should return to normal if glycemic control is achieved [95, 97, 99].

Primary Immunodeficiency

Children with immunodeficiency are at increased risk for a multitude of liver infections. Specific infections are discussed further in chapter "Infections of the Liver." However, children with some primary immunodeficiencies are prone to liver manifestations of disease with specific, often chronic infections or to noninfectious immune dysregulation (Table 29.4).

Table 29.4 Liver involvement in primary immunodeficiency syndromes

Disorder	Characteristic liver findings
Chronic granulomatous disease	*Most common*: liver abscesses, granulomas *Other*: nodular regenerative hyperplasia, sclerosing cholangitis, portal venopathy
Common variable immunodeficiency	*Most common*: nodular regenerative hyperplasia *Other*: autoimmune hepatitis, sclerosing cholangitis, PBC, liver granulomas
Hyper-IgM	*Most common*: chronic infectious hepatitis (cryptosporidium, CMV), sclerosing cholangitis

Chronic Granulomatous Disease

Chronic granulomatous disease (CGD) is caused by defects in phagocyte NADPH oxidase. In CGD, phagocytes are unable to destroy certain bacteria and fungi, so afflicted children are prone to infections as well as noninfectious granulomas. Liver abscesses develop in 27–35 % of CGD patients [100, 101]. They may occur early in childhood, or even as the presenting symptom leading to CGD diagnosis [102]. Presenting symptoms can be vague but often include fever and right upper quadrant abdominal pain. Staphylococcus species are found in approximately half of CGD liver abscesses, but infections are often polymicrobial and can involve fungal as well as anaerobic bacteria [92, 93].

Intravenous antibiotics are first-line treatment for liver abscesses in CGD, often in conjunction with percutaneous drainage. However, persistent and recurrent abscesses are very common. Granulomatous lesions may be difficult to resolve even if the provoking infection is treated. Surgical resection is often required for complete resolution [100, 103].

Liver enzyme elevation is common in CGD even without liver abscess. Hepatotoxicity from medications used to treat the other manifestations of CGD is an important cause. Nodular regenerative hyperplasia, sclerosing cholangitis, and chronic portal venopathy have also been reported [100]. The cause of these changes is not well understood, but may be driven by chronic inflammation and architectural distortion caused by infectious or noninfectious granulomas. Non-cirrhotic portal hypertension may be an underappreciated long-term consequence [100, 104]. Liver abscesses and chronic liver disease have been associated with increased mortality risk in CGD [105]. As prophylactic antibiotics and interferon gamma have improved survival in CGD, chronic liver disease may become an increasingly important consideration in the management of this disease.

Common Variable Immunodeficiency

Common variable immunodeficiency (CVID) is associated with low levels of immunoglobulins A, G, and M—most commonly diagnosed in young adults but also seen in children. Abnormal liver tests, most commonly elevated ALP and transaminases, occur in up to 44 % of patients by adulthood [106]. Children with X-linked agammaglobulinemia (Bruton disease) appear to have similar liver manifestations, although this is less well studied than CVID [107]. Infectious hepatitis or hepatic abscesses can develop, but rates of infection have decreased as intravenous immunoglobulin (IVIG) has become routinely used as prophylaxis [108]. CVID patients with elevated transaminases must be screened for hepatitis B and C by polymerase chain reaction (PCR) testing, as viral hepatitis can rarely be transmitted through IVIG.

In CVID, non-necrotizing granulomas can develop in the liver and other organs [108]. Chronic autoimmune disorders are emerging as another clinically significant complication of CVID. Reported liver manifestations include autoimmune hepatitis, primary biliary cirrhosis, and sclerosing cholangitis [108, 109]. However, the most common histologic finding in the liver biopsies of CVID patients is nodular regenerative hyperplasia (NRH) [106, 107]. Characteristic findings include portal and sinusoidal infiltration with T lymphocytes, alternating plates of hypertrophied and atrophic hepatocytes that give the liver a nodular appearance, and lack of fibrosis bridging between the nodules [107, 110]. Portal hypertension of intrahepatic origin may develop as a result of NRH in CVID. Small cohort studies suggest that other autoimmune diseases and abnormal peripheral T cells may be risk factors [107]. Splenomegaly can arise secondary to portal hypertension, but lymphoma should also be considered in the evaluation of splenomegaly in CVID patients as they are vulnerable to cancers [108].

Hyper-IgM Syndrome

Hyper-IgM syndrome, most commonly an X-linked disorder, is caused by mutations in the CD40-CD40 ligand pathway. B lymphocytes are unable to mature completely, leading to low serum IgG and IgA levels but normal to elevated IgM [111]. Patients are vulnerable to infections, particularly with intracellular pathogens, as well as increased incidence of autoimmune disease and cancers [112, 113]. Cryptosporidiosis is an especially common infection and may affect the liver as well as the intestinal tract; liver manifestations of cryptosporidium include cholangitis, cholecystitis, and hepatitis [111, 112]. CMV may also chronically infect the biliary tree or liver in these patients [112]. Little is known about the course of these chronic infections in hyper-IgM patients, but they may lead to a chronic cholangiopathy that resembles sclerosing cholangitis, as well as biliary cirrhosis and bile duct or liver cancers [112]. Children with hyper-IgM syndrome and elevated serum GGT, transaminases, or bilirubin should be evaluated for infection and cholangiopathy. If these are diagnosed, long-term screening for cancers with imaging, alpha-fetoprotein, and carcinoembryonic antigen should be considered.

References

1. Henrion J, Schapira M, Luwaert R, Colin L, Delannoy A, Heller FR. Hypoxic hepatitis: clinical and hemodynamic study in 142 consecutive cases. Medicine (Baltimore). 2003;82(6):392–406.
2. Jacquemin E, Saliba E, Blond MH, Chantepie A, Laugier J. Liver dysfunction and acute cardiocirculatory failure in children. Eur J Pediatr. 1992;151(10):731–4.
3. Garland JS, Werlin SL, Rice TB. Ischemic hepatitis in children: diagnosis and clinical course. Crit Care Med. 1988;16(12):1209–12.
4. Cassidy WM, Reynolds TB. Serum lactic dehydrogenase in the differential diagnosis of acute hepatocellular injury. J Clin Gastroenterol. 1994;19(2):118–21.
5. Henrion J, Minette P, Colin L, Schapira M, Delannoy A, Heller FR. Hypoxic hepatitis caused by acute exacerbation of chronic respiratory failure: a case-controlled, hemodynamic study of 17 consecutive cases. Hepatology. 1999;29(2):427–33.
6. Mace S, Borkat G, Liebman J. Hepatic dysfunction and cardiovascular abnormalities. occurrence in infants, children, and young adults. Am J Dis Child. 1985;139(1):60–5.
7. Arcidi Jr JM, Moore GW, Hutchins GM. Hepatic morphology in cardiac dysfunction: a clinicopathologic study of 1000 subjects at autopsy. Am J Pathol. 1981;104(2):159–66.
8. Rychik J, Veldtman G, Rand E, et al. The precarious state of the liver after a Fontan operation: summary of a multidisciplinary symposium. Pediatr Cardiol. 2012;33(7):1001–12.
9. Schwartz MC, Sullivan L, Cohen MS, et al. Hepatic pathology may develop before the Fontan operation in children with functional single ventricle: an autopsy study. J Thorac Cardiovasc Surg. 2012;143(4):904–9.
10. Matsumoto T, Kobayashi S, Shimizu H, et al. The liver in collagen diseases: pathologic study of 160 cases with particular reference to hepatic arteritis, primary biliary cirrhosis, autoimmune hepatitis and nodular regenerative hyperplasia of the liver. Liver. 2000;20(5):366–73.
11. Selmi C, De Santis M, Gershwin ME. Liver involvement in subjects with rheumatic disease. Arthritis Res Ther. 2011;13(3):226.
12. Rachelefsky GS, Kar NC, Coulson A, Sarkissian E, Stiehm ER, Paulus HE. Serum enzyme abnormalities in juvenile rheumatoid arthritis. Pediatrics. 1976;58(5):730–6.
13. Gurion R, Lehman TJ, Moorthy LN. Systemic arthritis in children: a review of clinical presentation and treatment. Int J Inflamm. 2012;2012:271569.
14. Beukelman T, Patkar NM, Saag KG, et al. 2011 American College of Rheumatology recommendations for the treatment of juvenile idiopathic arthritis: initiation and safety monitoring of therapeutic agents for the treatment of arthritis and systemic features. Arthritis Care Res (Hoboken). 2011;63(4):465–82.
15. Behrens EM, Beukelman T, Gallo L, et al. Evaluation of the presentation of systemic onset juvenile rheumatoid arthritis: data from the Pennsylvania systemic onset juvenile arthritis registry (PASOJAR). J Rheumatol. 2008;35(2):343–8.
16. Falk RH, Comenzo RL, Skinner M. The systemic amyloidoses. N Engl J Med. 1997;337(13):898–909.
17. Bloom BJ, Smith P, Alario AJ. Felty syndrome complicating juvenile rheumatoid arthritis. J Pediatr Hematol Oncol. 1998;20(5):511–3.
18. Abraham S, Begum S, Isenberg D. Hepatic manifestations of autoimmune rheumatic diseases. Ann Rheum Dis. 2004;63(2):123–9.
19. Stock H, Kadry Z, Smith JP. Surgical management of portal hypertension in Felty's syndrome: a case report and literature review. J Hepatol. 2009; 50(4):831–5.
20. Lee LA, Sokol RJ, Buyon JP. Hepatobiliary disease in neonatal lupus: prevalence and clinical characteristics in cases enrolled in a national registry. Pediatrics. 2002;109(1):E11.
21. Schoenlebe J, Buyon JP, Zitelli BJ, Friedman D, Greco MA, Knisely AS. Neonatal hemochromatosis associated with maternal autoantibodies against ro/SS-A and la/SS-B ribonucleoproteins. Am J Dis Child. 1993;147(10):1072–5.
22. Eladawy M, Dominguez SR, Anderson MS, Glode MP. Abnormal liver panel in acute Kawasaki disease. Pediatr Infect Dis J. 2011;30(2):141–4.
23. Tremoulet AH, Jain S, Chandrasekar D, Sun X, Sato Y, Burns JC. Evolution of laboratory values in patients with Kawasaki disease. Pediatr Infect Dis J. 2011;30(12):1022–6.
24. Ibanez-Alcalde M, Sanchez-Forte M, Gimenez-Sanchez F, Ortega-Montes A, Martinez-Espinosa G. Cholestasis as the initial feature of Kawasaki disease. Pediatr Infect Dis J. 2012;31(7):766–7.
25. Zulian F, Falcini F, Zancan L, et al. Acute surgical abdomen as presenting manifestation of Kawasaki disease. J Pediatr. 2003;142(6):731–5.
26. Bader-Meunier B, Hadchouel M, Fabre M, Arnoud MD, Dommergues JP. Intrahepatic bile duct damage in children with Kawasaki disease. J Pediatr. 1992;120(5):750–2.
27. Rostom A, Goldkind L, Laine L. Nonsteroidal anti-inflammatory drugs and hepatic toxicity: a systematic review of randomized controlled trials in arthritis patients. Clin Gastroenterol Hepatol. 2005;3(5):489–98.
28. Kahn E, Markowitz J, Simpser E, Aiges H, Daum F. Amyloidosis in children with inflammatory bowel disease. J Pediatr Gastroenterol Nutr. 1989;8(4):447–53.
29. Kocharla L, Taylor J, Weiler T, Ting TV, Luggen M, Brunner HI. Monitoring methotrexate toxicity in juvenile idiopathic arthritis. J Rheumatol. 2009;36(12):2813–8.
30. Salliot C, van der Heijde D. Long-term safety of methotrexate monotherapy in patients with rheumatoid arthritis: a systematic literature research. Ann Rheum Dis. 2009;68(7):1100–4.

31. Hashkes PJ, Balistreri WF, Bove KE, Ballard ET, Passo MH. The long-term effect of methotrexate therapy on the liver in patients with juvenile rheumatoid arthritis. Arthritis Rheum. 1997;40(12):2226–34.
32. Lahdenne P, Rapola J, Ylijoki H, Haapasaari J. Hepatotoxicity in patients with juvenile idiopathic arthritis receiving longterm methotrexate therapy. J Rheumatol. 2002;29(11):2442–5.
33. Becker J, Huber WD, Aufricht C. Short- and long-time effects of pediatric liver transplantation on serum cholesterol and triglyceride levels – the Vienna cohort. Pediatr Transplant. 2008;12(8):883–8.
34. Hashkes PJ, Balistreri WF, Bove KE, Ballard ET, Passo MH. The relationship of hepatotoxic risk factors and liver histology in methotrexate therapy for juvenile rheumatoid arthritis. J Pediatr. 1999; 134(1):47–52.
35. Mahamid M, Paz K, Reuven M, Safadi R. Hepatotoxicity due to tocilizumab and anakinra in rheumatoid arthritis: two case reports. Int J Gen Med. 2011;4:657–60.
36. Mimouni A, Magal N, Stoffman N, et al. Familial mediterranean fever: effects of genotype and ethnicity on inflammatory attacks and amyloidosis. Pediatrics. 2000;105(5):E70.
37. David J, Vouyiouka O, Ansell BM, Hall A, Woo P. Amyloidosis in juvenile chronic arthritis: a morbidity and mortality study. Clin Exp Rheumatol. 1993;11(1):85–90.
38. Castile R, Shwachman H, Travis W, Hadley CA, Warwick W, Missmahl HP. Amyloidosis as a complication of cystic fibrosis. Am J Dis Child. 1985;139(7):728–32.
39. Michels H, Linke RP. Clinical benefits of diagnosing incipient AA amyloidosis in pediatric rheumatic diseases as estimated from a retrospective study. Amyloid. 1998;5(3):200–7.
40. Stangou AJ, Hawkins PN. Liver transplantation in transthyretin-related familial amyloid polyneuropathy. Curr Opin Neurol. 2004;17(5):615–20.
41. Llado L, Baliellas C, Casasnovas C, et al. Risk of transmission of systemic transthyretin amyloidosis after domino liver transplantation. Liver Transpl. 2010;16(12):1386–92.
42. Sarnaik S, Slovis TL, Corbett DP, Emami A, Whitten CF. Incidence of cholelithiasis in sickle cell anemia using the ultrasonic gray-scale technique. J Pediatr. 1980;96(6):1005–8.
43. Schubert TT. Hepatobiliary system in sickle cell disease. Gastroenterology. 1986;90(6):2013–21.
44. Tripathi A, Jerrell JM, Stallworth JR. Clinical complications in severe pediatric sickle cell disease and the impact of hydroxyurea. Pediatr Blood Cancer. 2011;56(1):90–4.
45. Haberkern CM, Neumayr LD, Orringer EP, et al. Cholecystectomy in sickle cell anemia patients: perioperative outcome of 364 cases from the National Preoperative Transfusion study. Preoperative Transfusion in Sickle Cell Disease Study Group. Blood. 1997;89(5):1533–42.
46. Ahn H, Li CS, Wang W. Sickle cell hepatopathy: clinical presentation, treatment, and outcome in pediatric and adult patients. Pediatr Blood Cancer. 2005;45(2):184–90.
47. Traina F, Jorge SG, Yamanaka A, de Meirelles LR, Costa FF, Saad ST. Chronic liver abnormalities in sickle cell disease: a clinicopathological study in 70 living patients. Acta Haematol. 2007;118(3):129–35.
48. Hatton CS, Bunch C, Weatherall DJ. Hepatic sequestration in sickle cell anaemia. Br Med J (Clin Res Ed). 1985;290(6470):744–5.
49. Baichi MM, Arifuddin RM, Mantry PS, Bozorgzadeh A, Ryan C. Liver transplantation in sickle cell anemia: a case of acute sickle cell intrahepatic cholestasis and a case of sclerosing cholangitis. Transplantation. 2005;80(11):1630–2.
50. Gilli SC, Boin IF, Sergio Leonardi L, Luzo AC, Costa FF, Saad ST. Liver transplantation in a patient with S(beta)o-thalassemia. Transplantation. 2002;74(6):896–8.
51. Hankins JS, Smeltzer MP, McCarville MB, et al. Patterns of liver iron accumulation in patients with sickle cell disease and thalassemia with iron overload. Eur J Haematol. 2010;85(1):51–7.
52. Karam LB, Disco D, Jackson SM, et al. Liver biopsy results in patients with sickle cell disease on chronic transfusions: poor correlation with ferritin levels. Pediatr Blood Cancer. 2008;50(1):62–5.
53. Brown K, Subramony C, May W, et al. Hepatic iron overload in children with sickle cell anemia on chronic transfusion therapy. J Pediatr Hematol Oncol. 2009;31(5):309–12.
54. Porter JB. Pathophysiology of transfusional iron overload: contrasting patterns in thalassemia major and sickle cell disease. Hemoglobin. 2009;33 Suppl 1:S37–45.
55. Ghugre NR, Gonzalez-Gomez I, Butensky E, et al. Patterns of hepatic iron distribution in patients with chronically transfused thalassemia and sickle cell disease. Am J Hematol. 2009;84(8):480–3.
56. Liver transplants in the U.S. by recipient age. 1 Jan 1988–30 Apr 2012. http://optn.transplant.hrsa.gov/latestData/viewDataReports.asp. Updated 2012.
57. Meerpohl JJ, Antes G, Rucker G, et al. Deferasirox for managing iron overload in people with thalassaemia. Cochrane Database Syst Rev. 2012;(2):CD007476.
58. Linabery AM, Ross JA. Trends in childhood cancer incidence in the U.S. (1992–2004). Cancer. 2008;112(2):416–32.
59. Salmon JS, Thompson MA, Arildsen RC, Greer JP. Non-Hodgkin's lymphoma involving the liver: clinical and therapeutic considerations. Clin Lymphoma Myeloma. 2006;6(4):273–80.
60. Miller ST, Wollner N, Meyers PA, Exelby P, Jereb B, Miller DR. Primary hepatic or hepatosplenic non-Hodgkin's lymphoma in children. Cancer. 1983;52(12):2285–8.
61. Kotlyar DS, Osterman MT, Diamond RH, et al. A systematic review of factors that contribute to hepatosplenic T-cell lymphoma in patients with

inflammatory bowel disease. Clin Gastroenterol Hepatol. 2011;9(1):36–41.e1.
62. Rawat D, Gillett PM, Devadason D, Wilson DC, McKiernan PJ. Long-term follow-up of children with 6-thioguanine-related chronic hepatotoxicity following treatment for acute lymphoblastic leukaemia. J Pediatr Gastroenterol Nutr. 2011;53(5):478–9.
63. Escherich G, Richards S, Stork LC, Vora AJ, Childhood Acute Lymphoblastic Leukaemia Collaborative Group (CALLCG). Meta-analysis of randomised trials comparing thiopurines in childhood acute lymphoblastic leukaemia. Leukemia. 2011;25(6):953–9.
64. Vora A, Mitchell CD, Lennard L, et al. Toxicity and efficacy of 6-thioguanine versus 6-mercaptopurine in childhood lymphoblastic leukaemia: a randomised trial. Lancet. 2006;368(9544):1339–48.
65. Ball LM, Egeler RM, EBMT Paediatric Working Party. Acute GvHD: pathogenesis and classification. Bone Marrow Transplant. 2008;41 Suppl 2:S58–64.
66. Bertheau P, Hadengue A, Cazals-Hatem D, et al. Chronic cholestasis in patients after allogeneic bone marrow transplantation: several diseases are often associated. Bone Marrow Transplant. 1995;16(2):261–5.
67. Ferrell LD, Kakar S. Liver pathology. In: Elder DE, editor. Consultant pathology series. 4th ed. New York: Demos Medical Publishing; 2011. p. 340–2.
68. Shulman HM, Sharma P, Amos D, Fenster LF, McDonald GB. A coded histologic study of hepatic graft-versus-host disease after human bone marrow transplantation. Hepatology. 1988;8(3):463–70.
69. Baird K, Cooke K, Schultz KR. Chronic graft-versus-host disease (GVHD) in children. Pediatr Clin North Am. 2010;57(1):297–322.
70. Melin-Aldana H, Thormann K, Duerst R, Kletzel M, Jacobsohn DA. Hepatitic pattern of graft versus host disease in children. Pediatr Blood Cancer. 2007;49(5):727–30.
71. Barshes NR, Myers GD, Lee D, et al. Liver transplantation for severe hepatic graft-versus-host disease: an analysis of aggregate survival data. Liver Transpl. 2005;11(5):525–31.
72. Messina C, Faraci M, de Fazio V, et al. Prevention and treatment of acute GvHD. Bone Marrow Transplant. 2008;41 Suppl 2:S65–70.
73. Risma K, Jordan MB. Hemophagocytic lymphohistiocytosis: updates and evolving concepts. Curr Opin Pediatr. 2012;24(1):9–15.
74. Jordan MB, Allen CE, Weitzman S, Filipovich AH, McClain KL. How I treat hemophagocytic lymphohistiocytosis. Blood. 2011;118(15):4041–52.
75. Parizhskaya M, Reyes J, Jaffe R. Hemophagocytic syndrome presenting as acute hepatic failure in two infants: clinical overlap with neonatal hemochromatosis. Pediatr Dev Pathol. 1999;2(4):360–6.
76. Suzuki N, Morimoto A, Ohga S, et al. Characteristics of hemophagocytic lymphohistiocytosis in neonates: a nationwide survey in Japan. J Pediatr. 2009;155(2):235–8.e1.
77. Chen JH, Fleming MD, Pinkus GS, et al. Pathology of the liver in familial hemophagocytic lymphohistiocytosis. Am J Surg Pathol. 2010;34(6):852–67.
78. Wright G, Wilmore S, Makanyanga J, et al. Liver transplant for adult hemophagocytic lymphohistiocytosis: case report and literature review. Exp Clin Transplant. 2012;10(5):508–12.
79. Ravelli A, Grom AA, Behrens EM, Cron RQ. Macrophage activation syndrome as part of systemic juvenile idiopathic arthritis: diagnosis, genetics, pathophysiology and treatment. Genes Immun. 2012;13(4):289–98.
80. LaFranchi SH, Murphey WH, Foley Jr TP, Larsen PR, Buist NR. Neonatal hypothyroidism detected by the northwest regional screening program. Pediatrics. 1979;63(2):180–91.
81. Rastogi MV, LaFranchi SH. Congenital hypothyroidism. Orphanet J Rare Dis. 2010;5:17. doi:10.1186/1750-1172-5-17.
82. Arab DM, Malatjalian DA, Rittmaster RS. Severe cholestatic jaundice in uncomplicated hyperthyroidism treated with methimazole. J Clin Endocrinol Metab. 1995;80(4):1083–5.
83. Elias RM, Dean DS, Barsness GW. Hepatic dysfunction in hospitalized patients with acute thyrotoxicosis: a decade of experience. ISRN Endocrinol. 2012;2012:325092.
84. Yao JD, Gross Jr JB, Ludwig J, Purnell DC. Cholestatic jaundice in hyperthyroidism. Am J Med. 1989;86(5):619–20.
85. Karnsakul W, Sawathiparnich P, Nimkarn S, Likitmaskul S, Santiprabhob J, Aanpreung P. Anterior pituitary hormone effects on hepatic functions in infants with congenital hypopituitarism. Ann Hepatol. 2007;6(2):97–103.
86. Binder G, Martin DD, Kanther I, Schwarze CP, Ranke MB. The course of neonatal cholestasis in congenital combined pituitary hormone deficiency. J Pediatr Endocrinol Metab. 2007;20(6):695–702.
87. Al-Hussaini A, Almutairi A, Mursi A, Alghofely M, Asery A. Isolated cortisol deficiency: a rare cause of neonatal cholestasis. Saudi J Gastroenterol. 2012;18(5):339–41.
88. Sheehan AG, Martin SR, Stephure D, Scott RB. Neonatal cholestasis, hypoglycemia, and congenital hypopituitarism. J Pediatr Gastroenterol Nutr. 1992;14(4):426–30.
89. Hauser GJ, Brotzman HM, Kaufman SS. Hepatoadrenal syndrome in pediatric patients with end-stage liver disease. Pediatr Crit Care Med. 2012;13(3):e145–9.
90. Hogler W, Baumann U, Kelly D. Endocrine and bone metabolic complications in chronic liver disease and after liver transplantation in children. J Pediatr Gastroenterol Nutr. 2012;54(3):313–21.
91. O'Beirne J, Holmes M, Agarwal B, et al. Adrenal insufficiency in liver disease – what is the evidence? J Hepatol. 2007;47(3):418–23.
92. Fede G, Spadaro L, Tomaselli T, et al. Adrenocortical dysfunction in liver disease: a systematic review. Hepatology. 2012;55(4):1282–91.

93. McNeilly AD, Macfarlane DP, O'Flaherty E, et al. Bile acids modulate glucocorticoid metabolism and the hypothalamic-pituitary-adrenal axis in obstructive jaundice. J Hepatol. 2010;52(5):705–11.
94. Swain MG, Patchev V, Vergalla J, Chrousos G, Jones EA. Suppression of hypothalamic-pituitary-adrenal axis responsiveness to stress in a rat model of acute cholestasis. J Clin Invest. 1993;91(5):1903–8.
95. Torbenson M, Chen YY, Brunt E, et al. Glycogenic hepatopathy: an underrecognized hepatic complication of diabetes mellitus. Am J Surg Pathol. 2006;30(4):508–13.
96. Lorenz G, Barenwald G. Histologic and electronmicroscopic liver changes in diabetic children. Acta Hepatogastroenterol (Stuttg). 1979;26(6):435–8.
97. Hudacko RM, Manoukian AV, Schneider SH, Fyfe B. Clinical resolution of glycogenic hepatopathy following improved glycemic control. J Diabetes Complications. 2008;22(5):329–30.
98. Murata F, Horie I, Ando T, et al. A case of glycogenic hepatopathy developed in a patient with new-onset fulminant type 1 diabetes: the role of image modalities in diagnosing hepatic glycogen deposition including gradient-dual-echo MRI. Endocr J. 2012;59(8):669–76.
99. Fridell JA, Saxena R, Chalasani NP, Goggins WC, Powelson JA, Cummings OW. Complete reversal of glycogen hepatopathy with pancreas transplantation: two cases. Transplantation. 2007;83(1):84–6.
100. Hussain N, Feld JJ, Kleiner DE, et al. Hepatic abnormalities in patients with chronic granulomatous disease. Hepatology. 2007;45(3):675–83.
101. Winkelstein JA, Marino MC, Johnston Jr RB, et al. Chronic granulomatous disease. Report on a national registry of 368 patients. Medicine (Baltimore). 2000;79(3):155–69.
102. Marchetti F, Taddio A, L'Erario I, Ventura A. A 7-month-old boy with liver abscesses. J Pediatr Gastroenterol Nutr. 2010;50(2):117.
103. Chen LE, Minkes RK, Shackelford PG, Strasberg SM, Kuo EY, Langer JC. Cut it out: managing hepatic abscesses in patients with chronic granulomatous disease. J Pediatr Surg. 2003;38(5):709–13.
104. Feld JJ, Hussain N, Wright EC, et al. Hepatic involvement and portal hypertension predict mortality in chronic granulomatous disease. Gastroenterology. 2008;134(7):1917–26.
105. Bianchi G, Marchesini G, Marzocchi R, Pinna AD, Zoli M. Metabolic syndrome in liver transplantation: relation to etiology and immunosuppression. Liver Transpl. 2008;14(11):1648–54.
106. Ward C, Lucas M, Piris J, Collier J, Chapel H. Abnormal liver function in common variable immunodeficiency disorders due to nodular regenerative hyperplasia. Clin Exp Immunol. 2008;153(3):331–7.
107. Malamut G, Ziol M, Suarez F, et al. Nodular regenerative hyperplasia: the main liver disease in patients with primary hypogammaglobulinemia and hepatic abnormalities. J Hepatol. 2008;48(1):74–82.
108. Cunningham-Rundles C. How I, treat common variable immune deficiency. Blood. 2010;116(1):7–15.
109. Debray D, Pariente D, Urvoas E, Hadchouel M, Bernard O. Sclerosing cholangitis in children. J Pediatr. 1994;124(1):49–56.
110. Daniels JA, Torbenson M, Vivekanandan P, Anders RA, Boitnott JK. Hepatitis in common variable immunodeficiency. Hum Pathol. 2009;40(4):484–8.
111. Levy J, Espanol-Boren T, Thomas C, et al. Clinical spectrum of X-linked hyper-IgM syndrome. J Pediatr. 1997;131(1 Pt 1):47–54.
112. Hayward AR, Levy J, Facchetti F, et al. Cholangiopathy and tumors of the pancreas, liver, and biliary tree in boys with X-linked immunodeficiency with hyper-IgM. J Immunol. 1997;158(2):977–83.
113. Jesus AA, Duarte AJ, Oliveira JB. Autoimmunity in hyper-IgM syndrome. J Clin Immunol. 2008;28 Suppl 1:S62–6.

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