Classification and Pathology of Allied Hirschsprung's Disease

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33.1 Synonym

Variants of Hirschsprung's disease [1], Pseudo-Hirschsprung's disease [2].

33.2 Classification

"Allied disorders of Hirschsprung's disease (ADHD)" are a disease group that clinically resembles Hirschsprung's disease (HD), such as delayed passage of meconium and abdominal distention in the newborn period or severe chronic constipation in a young child, despite the presence of enteric ganglion cells [3]. HD is a defined entity, and the diagnosis is confirmed by histological and histochemical evaluation of rectal mucosal biopsies, which demonstrate the absence of ganglion cells in the submucosa and increased acetylcholinesterase (AchE) activity in the muscularis mucosae and lamina propria mucosae. However, ADHD are less well defined, and disease entities of ADHD have not been unified internationally [4, 5].

ADHD has been classified into two categories based on pathology by hematoxylin and eosin (HE) and AchE staining: (1) with abnormalities of ganglion cells, including immaturity of ganglia (IG), isolated hypoganglionosis (IHG), intestinal neuronal dysplasia (IND), diffuse intestinal ganglioneuromatosis, and absence of argyrophil plexus, and (2) without abnormalities of ganglion cells, including megacystis-microcolon-intestinal hypoperistalsis syndrome (MMIHS), segmental dilatation of the intestine (SD), internal anal sphincter achalasia (IASA), and chronic idiopathic intestinal pseudo-obstruction (CIIP) [4–6] (Table 33.1). Current diagnostic algorithm for ADHD is shown in Fig. 33.1 [4, 7].

Chronic idiopathic intestinal pseudo-obstruction (CIIP) is a bowel disorder that shows recurrent or persistent functional intestinal obstruction with normal histology by conventional staining of HE and AchE. Megacystis microcolon intestinal hypoperistalsis syndrome (MMIHS) has been considered to be the severe form of CIIP. However, MMIHS can be distinguished from CIIP by clinical characteristics [5, 8]. MMIHS and segmental dilatation of the intestine are also present with normal histology of intestinal ganglia and normal AchE activity.

33.3 Pathological Examination

33.3.1 Rectal Mucosal Biopsy (RMB)

RMB provides a larger and deeper biopsy than standard endoscopic forceps biopsies which are usually inadequate to obtain sufficient submucosa for the diagnosis. It is important that the biopsy is not taken too close to the dentate line because a normal zone of submucosal hypoganglionosis

Table 33.1 Classification for allied disorders of Hirschsprung's disease (modified Taguchi et al. [5])

(1) Abnormal ganglia (abnormal histology in hematoxylin and eosin or acetylcholinesterase staining)
• Immaturity of ganglia (or immature ganglionosis)
• Isolated hypoganglionosis (congenital, acquired)
• Intestinal neuronal dysplasia
• Diffuse intestinal ganglioneuromatosis[a]
• Absence of argyrophil plexus[a]
(2) Normal ganglia (normal histology in hematoxylin and eosin or acetylcholinesterase staining)
• Megacystis-microcolon-intestinal hypoperistalsis syndrome
• Segmental dilatation of the intestine
• Internal anal sphincter achalasia
• Chronic idiopathic intestinal pseudo-obstruction

[a]Included in the review by Friedmacher and Puri [4]

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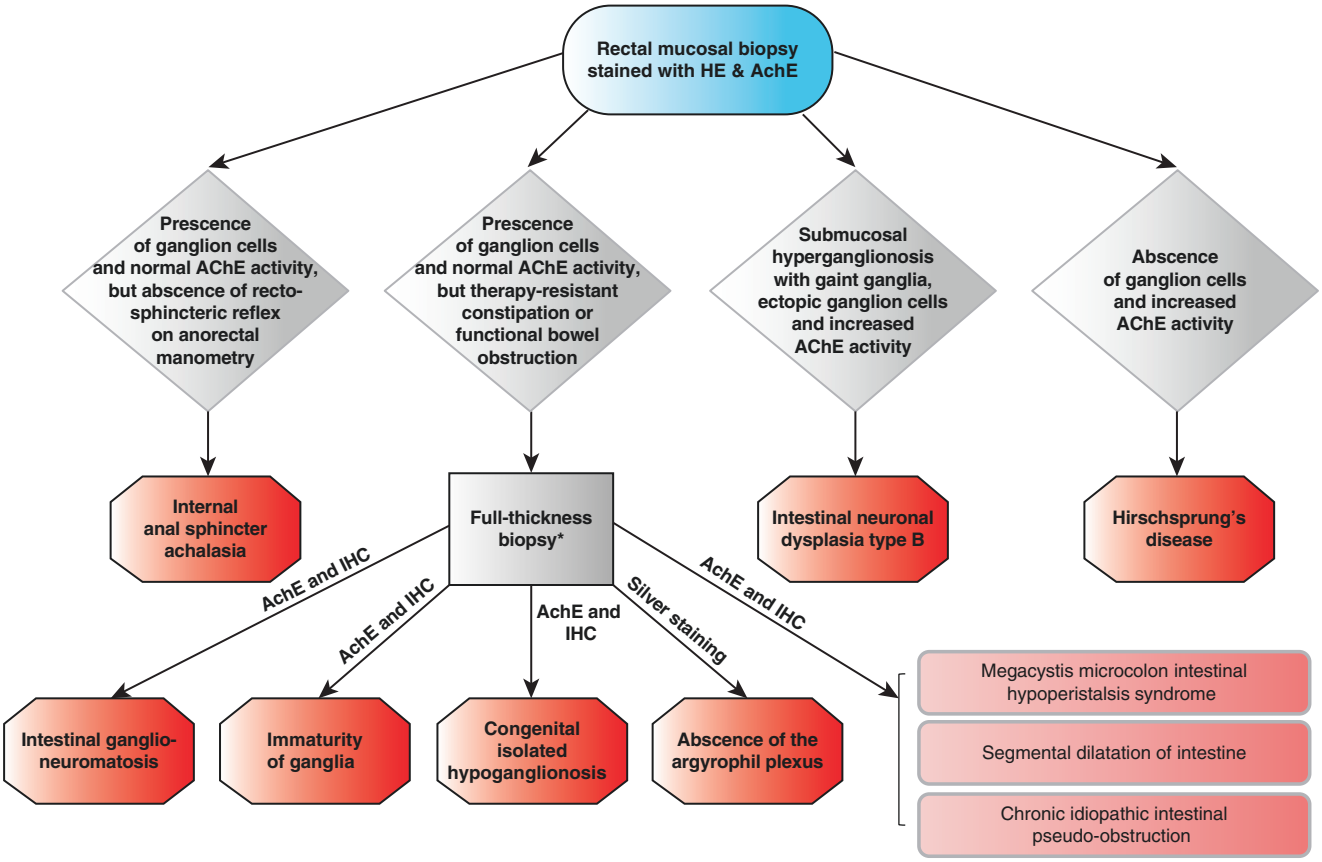

Fig. 33.1 Diagnostic algorithm for Allied disorders of Hirschspurung's disease by Friedmacher et al. with modification. *AchE* acetylcholinesterase staining, *IHC* immunohistochemistry. *Full-thickness biopsy from three locations; (1) at artificial anus that is enough oral side from narrow segment (caliber change), (2) terminal ileum, and (3) sigmoid colon

extends for approximately 10–25 mm above this point, increasing from 10 mm in neonates to 25 mm in 3-year-olds and above. Since accurate positioning of the RMB device at appropriate distance above the dentate line is sometimes difficult, some laboratories recommend that biopsies are obtained routinely from three separate levels (e.g., 1, 2, and 3 cm superior to the pectinate line).The primary goal of RMB is to exclude or confirm a diagnosis of HD. The frozen sections stained with HE plus AchE enzyme histochemistry and HE-stained paraffin-embedded section should be used as the standard techniques for the diagnosis of HD in rectal biopsies from children. The diagnostic utility of very few immunohistochemical markers has been tested in RMB. Calretinin is present normally in the cytoplasm and nerve processes of a subset of enteric ganglion cells. In the aganglionic segment of HD, calretinin immunoreactivity in muscularis mucosae and superficial submucosa is lost [9].

33.3.2 Full-Thickness Biopsy

When therapy-resistant constipation or functional bowel obstruction is continuously present, in spite of the fact that the presence of ganglion cells and normal AchE activity is confirmed by RMB, full-thickness rectal biopsy is required for differential diagnosis (Fig. 33.1). It is recommended to take enough size of intestinal specimen with 10 mm of longitudinal length and 5 mm of short axis. Immunohistochemistry is a very helpful and good diagnostic adjunct to be replaced for enzyme histochemistry to delineating the immature neurons (BCL2), the size of the enteric ganglion cell and neuromuscular innervation (S-100 protein, synaptophysin, and CD56, PGP9.5), and the intestinal cells of Cajal (c-kit) and myopathy (SMA) [5, 10]. Immunolocalization of the RNA-binding proteins, HuC/HuD, has been advocated as an excellent method for ganglion cell quantitation because HuC/HuD appears to be expressed in the cell body of virtually every mature and immature enteric ganglion cell [11]. The criteria to identify ganglion cells by Swaminathan et al. are as follows: an area of dark brown perikaryal staining in a cell that contains a nucleus and the granular stain must cover the nucleus or encircle at least 50% of circumference of the nucleus. If there was any ambiguity about the presence of a nucleus, the cell was not included [12]. In the evaluation of the size of the myenteric plexus by CD56 immunohistochemical staining, the CD56-

positive area between the circular and longitudinal layer of the muscularis externa is defined as the myenteric plexus [7]. In clinical practice, ADHD often develop a neonatal ileus and require an urgent surgery as is the case with intestinal atresia or HD. In such cases including isolated hypoganglionosis and immaturity of ganglia, intraoperative full-thickness biopsy is indispensable for diagnosis. Full-thickness biopsy is desirable to be taken from the three locations: (1) at the artificial anus that has enough oral side from a narrow segment (caliber change), (2) terminal ileum, and (3) sigmoid colon (Fig. 33.2). Immunohistochemical stainings for HuC/HuD and Sox10 are useful to evaluate the size and density of enteric ganglion cells and to identify enteric glia cells, respectively [7, 12, 13]. Immunohistochemical staining for CD56 is useful to evaluate the size of the myenteric plexus [7].A prerequisite is an understanding of the normal neuronal density, which varies depending on the following: (1) age, with an inverse relation between ganglion cells/plexus and age; (2) region of the examined bowel, fewer myenteric ganglion cells in the small intestine than the colon; (3) degree of intestinal dilatation, especially when the specimen is taken transversely along the long axis of the bowel; (4) type of biopsy and preparation (tissue sections vs whole mount preparation); and (5) mode of staining (marker). Hence, the diagnosis of quantitative abnormalities such as hypoganglionosis or hyperganglionosis in the myenteric plexus can be difficult, and a consensus in the detailed diagnostic criteria still remains to be found. Pathology of ADHD with abnormalities of ganglion cells is briefly described in the next.

33.4 Immaturity of Ganglia (IG)

The diagnosis of IG can be made from suction rectal biopsy. The immature ganglion cells appear very small and have a high nuclear-cytoplasmic ratio. The nucleolus is inconspicuous. It is often not possible to distinguish between these small ganglion cells and the enteric glial cells by HE morphology or

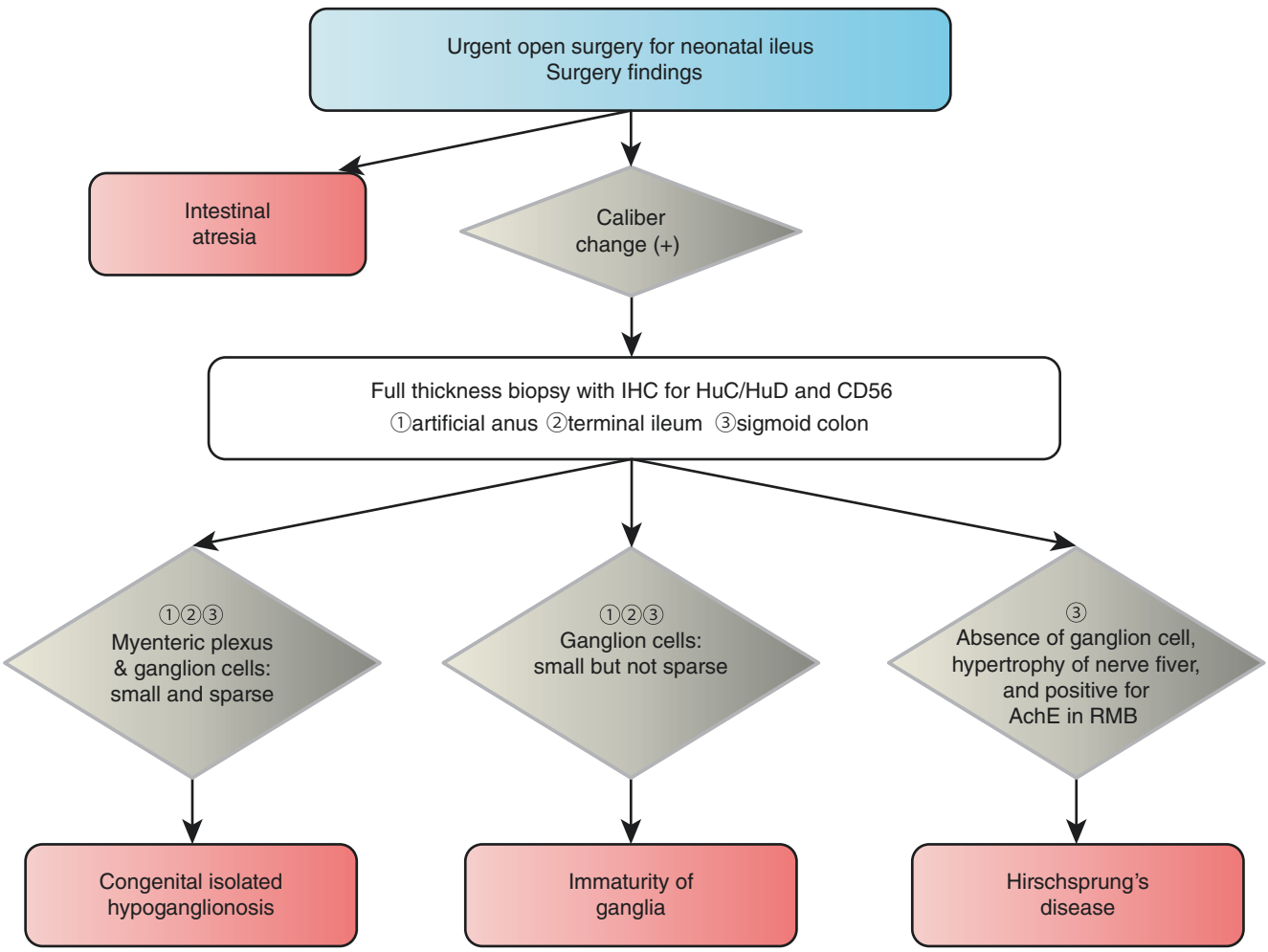

Fig. 33.2 Diagnostic algorithm for the differential diagnosis in urgent surgery for neonatal ileus. *IHC* immunohistochemistry, *AchE* acetylcholinesterase staining, *RMB* rectal mucosal biopsy

AchE histochemistry. HuC/HuD immunohistochemistry is helpful to identify immature small ganglion cells [12]. The density of ganglion cells is within normal range or may be highly cellular (Fig. 33.3). It has been demonstrated that this immaturity is a physiological, age-dependent phenomenon and maturation of ganglia strongly correlates with the age of the patient [3]. At the time of stoma closure, maturation of ganglion cells should be confirmed histologically.

33.5 Congenital Isolated Hypoganglionosis (IHG)

A full-thickness biopsy is required for the definitive diagnosis of isolated hypoganglionosis. To differentiate congenital IHG from HD accurately, full-thickness biopsy should be taken from the three locations (Fig.33.2). The myenteric plexus and ganglion cells are small and sparse (Fig. 33.4). AchE activity in lamina propria is absent or low and hypertrophy of the muscularis mucosae and circular muscle is also seen [4]. However, no consensus has been given on the detailed diagnostic criteria.

Meier-Ruge et al. found significant histological differences between resected bowel specimens from patients with congenital IHG and normal bowel tissue using AchE staining. They showed a 42% reduction in the number of ganglion cells in the plexus area and a 55% decrease of the ganglion cell number per mm length of the colon. The number and area of the myenteric plexus showed a decrease of 59% and a doubling of the plexus distances [14]. Although these observations form the basis for the histopathological diagnosis of congenital IHG, the evaluation of hypoganglionosis using coiled colon frozen section is technically difficult in practical diagnostic pathology. The consensus of diagnostic criteria using formalin-fixed paraffin-embedded specimen with immunohistochemistry is expected.

Yoshimaru et al. reported that neither HuC/HuD-positive cells nor CD56-positive myenteric plexus was

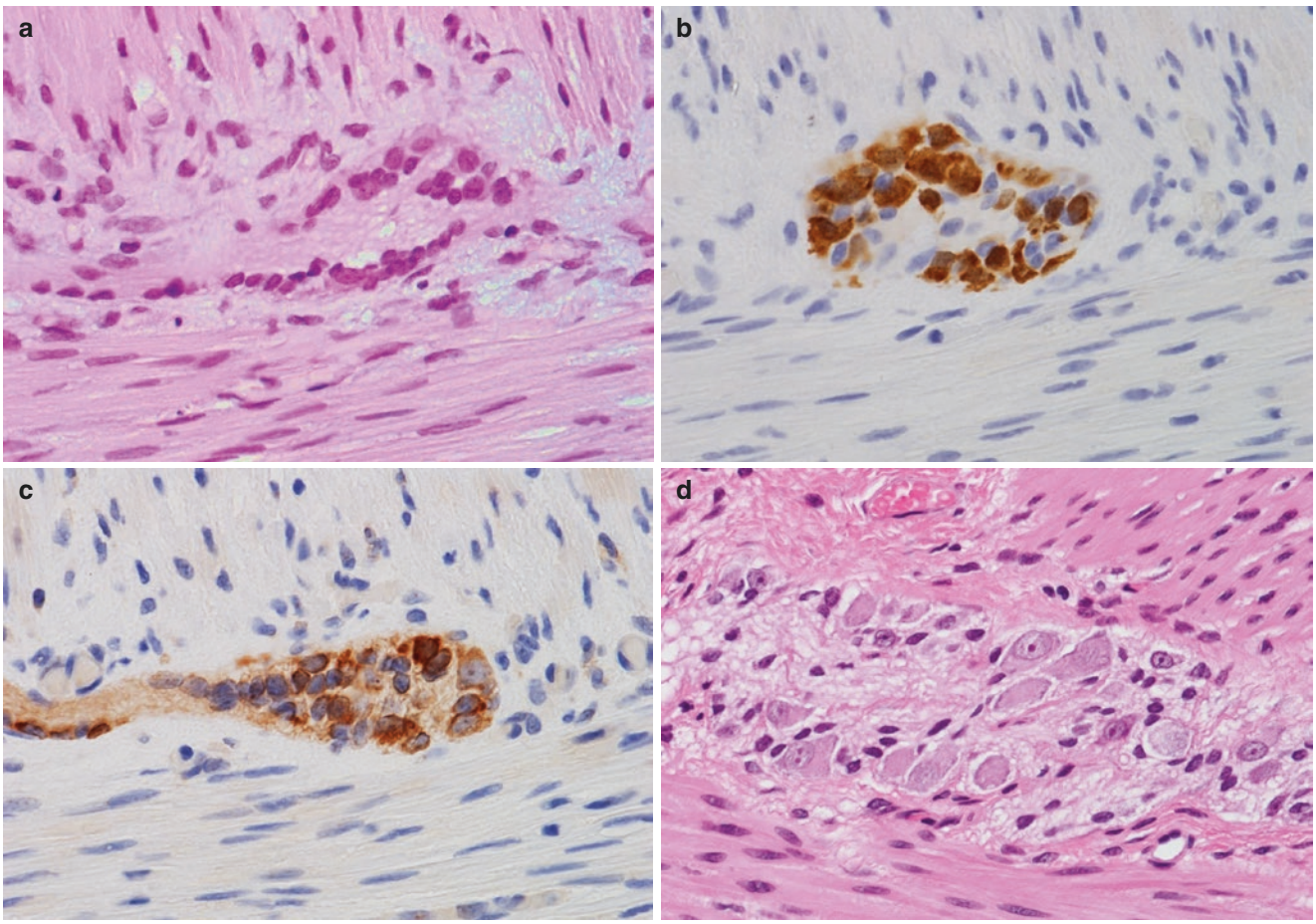

Fig. 33.3 Immaturity of ganglia. Myenteric plexus of the newborn include many immature ganglion cells with a high nuclear-cytoplasmic ratio and inconspicuous nucleoli. These cells are positive for HuC/HuD and BCL2 (**a**: HE staining, **b**: immunostaining for HuC/HuD, **c**: immunostaining for BCL2). Re-biopsy from the same patient at the age of 18 months shows the myenteric plexus including mature ganglion cells with abundant cytoplasm and prominent nucleoli (**d**: HE staining)

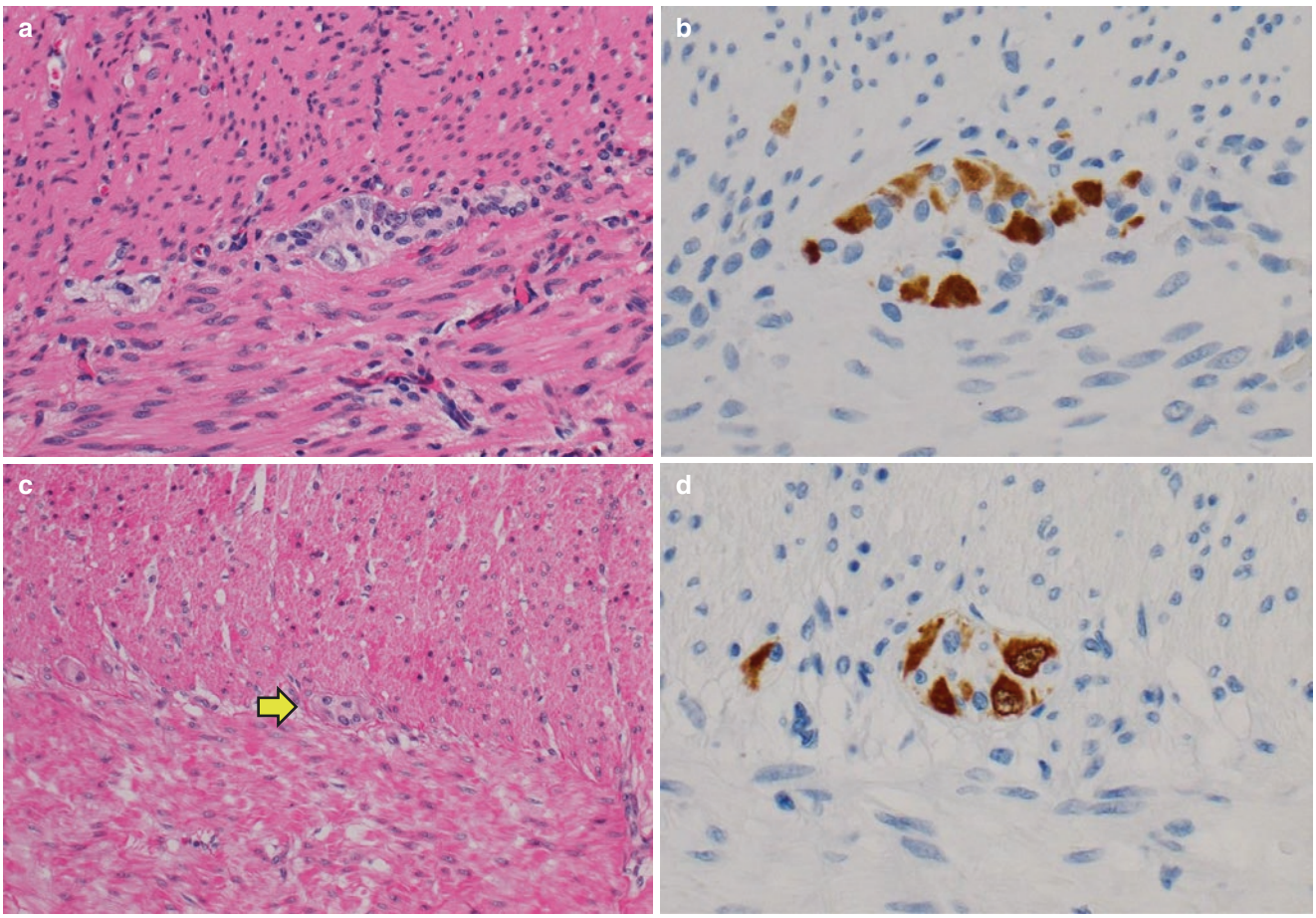

Fig. 33.4 Myenteric plexus of normal colon including mature ganglion cells (**a**: HE staining, **b**: immunostaining for HuC/HuD). In congenital isolated hypoganglionosis, myenteric plexus (arrow) are small and sparse with a few ganglion cells (**c**: HE staining, **d**: immunostaining for HuC/HuD)

present, but CD56-positive nerve bundles, which indicated an extrinsic innervation, were present in the aganglionic segment in HD. In the congenital IHG, a quantitative analysis revealed that the number of ganglion cells and the size of myenteric plexus were significantly smaller than that in the normoganglionic segment (NGS) of HD, while the number of ganglion cells was significantly smaller, but the size of the myenteric plexus was equivalent to NGS of HD in acquired IHG. They clearly showed that immunostaining using HuC/HuD and CD56 was useful to distinguish acquired IHG from congenital IHG; however, they could not achieve a quantitative cutoff value in comparison to the normal findings [7].

33.6 Intestinal Neuronal Dysplasia (IND)

IND has been classified into two clinical and histological distinct subtypes: IND type A and type B. IND type A is characterized by congenital aplasia, hypoplasia, or immaturity of the sympathetic innervation affecting the myenteric plexus as well as the submucosal arterial vessels and the mucosa. The biopsy specimen shows an ulcerative colitis or inflammatory changes and a total destruction of the muscularis mucosae. Aplasia of adrenergic innervation allows uninhibited overstimulation of cholinergic structures in the muscular layers, and dilatation of blood vessels leads to an increased mucosal permeability [3].

RMB with AchE enzyme histochemistry is the method of choice for the diagnosis of IND type B. RMB from IND type B shows hyperganglionosis, giant neural plexus (ganglia), and increased AchE activity in the lamina propria mucosae. However, the AchE activity in the lamina propria mucosae has been shown to be an age-dependent phenomenon that disappears on maturation of the submucosal plexus. Thus, the most commonly used diagnostic criteria is that more than 20% of 25 submucosal ganglia must be giant ganglia containing nine or more ganglion cells in patients older than 1 year, as before that age, giant ganglia may be misinterpreted due to the fact that immature ganglia often have an incomplete differentiation in ganglion cells. These criteria were developed with

15 μm-thick frozen section and AchE enzyme histochemistry. The correlation with counts on paraffin-embedded sections stained by HE or immunohistochemistry is unclear [4].

33.7 Diffuse Intestinal Ganglioneuromatosis

Intestinal ganglioneuromas are divided into three subgroups: (1) solitary polypoid ganglioneuromas that are typically asymptomatic tumors of the mucosa and submucosa with features similar to adenomas or juvenile polyps; (2) ganglioneuromatous polyposis characterized by multiple small mucosal polyps comprised of loose collections of mature ganglia, often resembling familial adenomatous polyposis and typically found in the colon and terminal ileum; and (3) diffuse ganglioneuromatosis (GNM). Diffuse intestinal GNM is characterized by diffuse proliferation of nerve fibers with significant hyperplasia of submucosal and myenteric ganglion cells causing thickening of the bowel wall. The patients with diffuse intestinal GNM present with severe chronic constipation and abdominal distention due to intestinal obstruction. This extremely rare condition is frequently associated with multiple endocrine neoplasia type 2B (MEN 2B), neurofibromatosis 1, or Cowden syndrome [15]. Mutation analysis in patients with MEN 2B further confirmed a de novo germline mutation of the *RET* proto-oncogene [16].

RMB or full-thickness biopsies of diffuse intestinal GNM show massive proliferation of submucosal and myenteric plexuses comprising thick nerve trunks with scattered mature ganglion cells, giant ganglia with often 15–40 ganglion cells, and a high AchE activity. Diffuse intestinal GNM appears to be largely confined to the colon and rectum, unlike neurofibromatosis, which occurs more commonly in the small intestine and stomach [17].

33.8 Absence of Argyrophil Plexus

In the normal myenteric plexus, there are two distinct subtypes of ganglion cells, i.e., argyrophil cells and argentaffin cells. Argyrophil cells coordinate the activation of argentaffin cells, which secrete specific neurotransmitters and ultimately cause contraction and relaxation of muscle fibers within the bowel wall. The lack of argyrophil cells in myenteric plexus, which is also known as absence of the argyrophil plexus, is a rare cause of severe constipation and functional bowel obstruction in infants and children. The absence of argyrophil cells and their neuronal processes can only be demonstrated using silver impregnation of full-thickness biopsies, while conventional HE staining, AchE histochemistry, and immunohistochemistry with several neuronal markers fail to show this abnormality [4].

References

1. Puri P. Variant Hirschsprung's disease. J Pediatr Surg. 1997;32(2):149–57.
2. Ravitch MM. Pseudo-Hirschsprung's disease. Ann Surg. 1958;147:781–95.
3. Holschneider AM, Meier-Ruge W, Ure BM. Hirschsprung's disease and allied disorders: a review. Eur J Pediatr Surg. 1994;4(5):260–6.
4. Friedmacher F, Puri P. Classification and diagnostic criteria of variants of Hirschsprung's disease. Pediatric Sur Int. 2013;29:855–72.
5. Taguchi T, Ieiri S, Miyoshi K, Kohashi K, Oda Y, Kubota A, et al. The incidence and outcome of allied disorders of Hirschsprung's disease in Japan: results from a national survey. Asian J Surg. 2017;40:29–34.
6. Bentley JFR, Nixon HH, Ehrenpreis TH, Spencer B. Seminar on pseudo-Hirschsprung's disease and related disorders. Arch Dis Child. 1966;41:143–54.
7. Yoshimaru K, Taguchi T, Obata S, Takemoto J, Takahashi Y, Iwanaka T, et al. Immunostaining for Hu C/D and CD56 is useful for a definitive histopathological diagnosis of congenital and acquired isolated hypoganglionosis. Virchows Arch. 2017;470(6):679–85.
8. Gosemann JH, Puri P. Megacystis microcolon intestinal hypoperistalsis syndrome: systemic review of outcome. Pediatr Surg Int. 2011;27:1041–6.
9. Knowles CH, De Giorgio R, Kapur RP, Bruder E, Farrugia G, Geboes K, et al. Gastrointestinal neuromuscular pathology: guidelines for histological techniques and reporting on behalf of the Gastro 2009 International Working Group. Acta Neuropathol. 2009;118:271–301.
10. Park SH, Min H, Chi JG, Park KW, HR Y, Seo JK. Immunohistochemical studies of pediatric intestinal pseudo-obstruction: bcl2, a valuable biomarker to detect immature enteric ganglion cells. Am J Surg Pathol. 2005;29(8):1017–24.
11. Phillips RJ, Hargrave SL, Rhodes BS, Zopf DA, Powley TL. Quantification of neurons in the myenteric plexus: an evaluation of putative pan-neuronal markers. J Neurosci Methods. 2004;133:99–107.
12. Swaminathan M, Kapur RP. Counting myenteric ganglion cells in histologic sections: an empirical approach. Hum Pathol. 2010;41:1097–108.
13. Hoff S, Zeller F, von Weyhern CW, Wegner M, Schemann M, Michel K, Rühl A. Quantitative assessment of glial cells in the human and guinea pig enteric nervous system with an anti-Sox8/9/10 antibody. J Comp Neurol. 2008;509(4):356–71.
14. Meier-Ruge WA, Brunner LA, Engert J, Heminghaus M, Holschneider AM, Jordan P, et al. A correlative morphometric and clinical investigation of hypoganglionosis of the colon in children. Eur J Pediatr Surg. 1999;9(2):67–74.
15. Matthews MA, Adler BH, Arnold MA, Kumar S, Carvalho R, Besne GE. Diffuse intestinal ganglioneuromatosis in a child. J Pediatr Surg. 2013;48(5):1129–33.
16. Ichihara M, Murakumo Y, Takahashi M. RET and neuroendocrine tumors. Cancer Lett. 2004;204(2):197–211.
17. Shekitka KM, Sobin LH. Ganglioneuromas of the gastrointestinal tract: relation to Von Recklinghausen disease and other multiple tumor syndromes. Am J Surg Pathol. 1994;18(3):250–7.