Review

Histopathology of intestinal villi in neonatal and paediatric age: main features with clinical correlation - Part I

Chiara Rossi[1], Gloria Simoncelli[2], Giovanni Arpa[1], Alessandra Stracuzzi[3], Paola Parente[4], Matteo Fassan[5,6], Alessandro Vanoli[1], Vincenzo Villanacci[2]

[1] Unit of Anatomic Pathology, Department of Molecular Medicine, Fondazione IRCCS Policlinico San Matteo, University of Pavia, Pavia, Italy; [2] Institute of Pathology, Spedali Civili di Brescia, Brescia, Italy; [3] Pathological Anatomy Unit, Department of Diagnostic and Laboratory Medicine, IRCCS Bambino Gesù Children's Hospital, Rome, Italy; [4] Pathology Unit, Fondazione IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy; [5] Department of Medicine (DIMED), Surgical Pathology Unit, University of Padua, Padua, Italy; [6] Veneto Institute of Oncology, IOV-IRCCS, Padua, Italy

Summary

The neonatal and paediatric spectrum of small bowel disorders encompass a wide variety of conditions, ranging from food allergies to life-threatening surgical emergencies or life-long medical conditions and, as such, it comes with a whole set of diagnostic challenges for the non-paediatric pathologist. Histologic examination is a cornerstone of diagnosis in a large number of diseases and may still provide important diagnostic clues in the appropriate clinical context. In this review, divided in two sections, we aim to provide a comprehensive histopathological summary of paediatric small bowel alteration and their differential diagnoses with a reference to the main clinical aspects required for appropriate interpretation. Specifically, in this first part, we describe congenital and metabolic disorders, intestinal lymphangiectasia, immunodeficiencies, GVHD, and necrotising enterocolitis.

Key words: congenital disorders, GVHD, histopathology, immunodeficiencies, intestinal lymphangiectasia, necrotizing enterocolitis, pediatric diseases, small bowel

Introduction

Histopathologic analysis of small intestinal biopsies plays an important role in the diagnostic work-up of most enteropathies affecting children, as it does in adult intestinal disorders. Indeed, a correct diagnosis is of pivotal importance to establish tailored treatments, such as immunosuppression or, in selected cases, even small bowel transplantation. However, paediatric small bowel disorders may represent diagnostic challenges for both pathologists and clinicians. The aim of this review is to describe the histopathology of the main small intestinal disorders affecting neonates and/or children.

During embryogenesis, small bowel villi appear in the 8th gestational week, first in the duodenum and then more distally, reaching the ileum by the 12th week. Columnar absorptive cells with a brush border develop by 9th week, goblet cells appear within crypts in the 8th week, followed by Paneth cells and neuroendocrine cells in the 9th week. Stem cells within the crypts renew such epithelial cells every 3-4 days. The process of intestinal villous formation is orchestrated by a complex endodermal-mesodermal cross-talking, involving the Notch pathway. Therefore, germline mutations in several genes which play a role in epithelial differentiation

and specification of absorptive or secretory (goblet, Paneth and neuroendocrine cells) lineages may result in clinically relevant enteropathy.

The main indications for small bowel biopsy sampling in neonates and children are malabsorption syndrome with chronic diarrhoea, failure to thrive and abdominal pain. Small bowel biopsy samples for histopathologic examination in paediatric patients with gastrointestinal symptoms suspicious for enteropathies are most often obtained from the duodenum during oesophago-gastro-duodenoscopy. Beyond samples for routine histology, additional biopsy specimens may be snap-frozen or submitted for ultrastructural analysis by electron microscopy, when indicated by clinical hypothesis (e.g. microvillous inclusion disease). It should be stressed that the distribution of infancy enteropathies is often patchy; thus, multiple biopsy sampling, including both proximal and distal duodenum, is recommended. In addition, endoscopically inflamed and normal areas should be biopsied, as relevant histologic findings may be identified also in apparently normal mucosa (e.g. microgranulomas). Duodenal biopsy may appear completely normal in some disorders (mainly related to congenital transport and enzymatic deficiencies), or may show villous atrophy or specific histopathologic findings. The main paediatric disorders causing villous atrophy include coeliac disease, autoimmune enteropathy, immunodeficiency-related enteropathy, bacterial overgrowth, gastrointestinal food-allergic disease and Crohn's disease. Characteristic histopathologic lesions indicating a specific clinico-pathologic entity encompass i) fat-filled enterocytes (typical of abetalipoproteinaemia or Anderson disease), ii) ectasic lymphatics (found in primary or secondary lymphangiectasia), iii) dense inspissated mucous (suggesting cystic fibrosis), iv) epithelial abnormalities (typical of microvillous inclusion disease and tufting enteropathy), v) increased eosinophilic density (which may suggest a eosinophilic gastroenteritis) [1], and vi) absence or paucity of inflammatory cells or plasma cells (as found in some immunodeficiency syndromes).

Congenital transport and enzymatic deficiencies

Several congenital disorders of substrate digestion, absorption and transport have been described. The main subgroups included in this family are: i) disaccharidase deficiencies, ii) lipid trafficking disorders, and iii) ion and nutrient transport deficiencies. Apart from fat processing disorders, which may show rela-

tively specific histopathologic features, small bowel histology of these enteropathies is generally normal or near normal and genetic analysis is required for their definitive diagnosis.

Congenital disaccharidase deficiencies are rare, genetically determined entities, that comprise: i) congenital lactase deficiency, ii) sucrase-isomaltase deficiency, iii) maltase-glucoamylase deficiency, and iv) trehalase deficiency. Lactase-phlorizin hydrolase, sucrase-isomaltase, maltase-glucoamylase and trehalase are enzymes responsible for degradation of milk lactose, sucrose, starch, and mushroom trehalose, respectively. These enzymes are expressed on the brush border of villous absorptive cells in a time-dependent pattern (e.g. lactase is mainly expressed in neonates). Disaccharidase deficiencies cause a severe, osmotic-type diarrhoea in children. Importantly, small bowel mucosa looks histologically normal. Congenital disaccharidase disorders should be distinguished from secondary disaccharidase defects due to mucosal damage occurring in various enteropathies of different aetiology (e.g. coeliac or Crohn's disease) and from other disorder leading to carbohydrate malabsorption such as congenital fructose malabsorption, due to mutation in GLUT5 gene, which encodes for a hexose transporter at enterocyte basolateral membrane.

Congenital lipid trafficking disorders are characterized by primary defects in lipid transport within absorptive cells, resulting in fat malabsorption, steatorrhoea, failure to thrive and various neurologic symptoms in neonates. They include i) abetalipoproteinaemia, ii) hypolipoproteinaemia, and iii) Anderson disease. They are caused by defects of some apolipoproteins; thus, fatty acids accumulate in the cytoplasm of enterocytes. In particular, abetalipoproteinaemia, which is caused a MTTP gene mutation, results in the lack of apo-B-containing lipoproteins [2]. Laboratory findings show absence of very-low-density lipoproteins. Small bowel biopsies are helpful for diagnosis, as they may show fat-filled, multivacuolated enterocytes, containing cytoplasmic lipid droplets. As a rule, villous atrophy is not observed. In addition, liver steatosis and fibrosis may be present. Electron microscopy examination may confirm that enterocyte vacuolisation is due to lipid accumulation. It should be kept in mind that lipid droplets can be also seen in normal individuals after a fat-rich meal.

Hypobetalipoproteinaemia is an autosomal dominant disorder due to an APOB gene mutation, which results in a truncated apo-B protein. While homozygous patients display a phenotype resembling abetalipoproteinaemia, heterozygous individuals may have a milder presentation. Anderson disease (chylomicron

retention disease) is caused by a mutation of SARB1 gene, which encodes for a guanosine triphosphatase (GTP-ase) involved in intracellular chylomicron trafficking. Clinically and histologically, Anderson disease resemble abetalipoproteinaemia, although it usually shows less severe neurologic manifestations and less enterocyte vacuolisation. In addition, lipid droplets may also be identified in the intercellular/extracellular spaces after feeding in Anderson disease, unlike abetalipoproteinaemia. Differential diagnosis of lipid trafficking disorders includes lipid storage disorders, which may feature lipid-containing macrophages in the small bowel lamina propria. Such macrophages may be also observed in Tangier disease, a rare disorder caused by deposition of cholesteryl ester in the reticulo-endothelial system and very low plasma levels of high-density lipoproteins (HDL).

Ion and nutrient transport deficiencies are a large and genetically complex family of disorders and their detailed description is beyond the scope of this review. Small bowel biopsies may show mild villous atrophy in rare instances; however, in the majority of cases they are unremarkable [3]. An example of ion transport deficiency is the so-called "acrodermatitis enteropathica", a rare autosomal recessive syndrome, that will be discussed in another section. Another relevant example of electrolyte transport defect is cystic fibrosis, an autosomal recessive disorder affecting 1 of 2,500 live birth. It is caused by CFTR gene mutation, resulting in the production of dense secretions in both the airways and digestive system.

Congenital defects of small intestine epithelial differentiation

They include: i) microvillous inclusion disease, ii) tufting enteropathy, iii) enteroendocrine cell dysgenesis, and iv) trico-hepato-enteric syndrome [4].

MICROVILLOUS INCLUSION DISEASE

Microvillous inclusion disease is an autosomal recessive disorder, caused by mutations of the gene MYO5B, which encodes for the protein myosin 5b, involved in apical membrane recycling [5,6]. Affected neonates usually develop a severe form of refractory diarrhoea during the first weeks of life [7].

Small intestinal biopsy specimens usually show severe villous atrophy, relatively mild crypt hyperplasia and increased of lamina propria mononuclear cellularity. The periodic-acid Schiff (PAS)-positive brush border of absorptive cells is not clearly recognizable; instead, PAS-positive dense inclusions on the apex of

enterocytes are observed. Immunohistochemistry for CD10, an enterocyte brush border marker, may help identify these inclusions. Electron microscopy analysis is diagnostic, as it shows characteristic ultrastructural features, i.e. absent (or very small) surface microvilli and the presence of microvilli-containing vesicles and other inclusions with dense material within the apical cytoplasm of absorptive cells. Similar inclusions may be present in the large bowel, as well as in kidney. Neonates with microvillous inclusion disease are treated with total parental nutrition or small bowel transplantation.

CONGENITAL TUFTING ENTEROPATHY

Congenital tufting enteropathy, also known as "congenital epithelial dysplasia", is a rare defect of epithelial differentiation (ranging from 1/50,000 to 1/100,000 live births in Europe), inherited with an autosomal recessive pattern [8-10]. Frequently, a parental consanguinity is observed, especially among Arabic patients. It is a cause of neonatal severe watery diarrhoea and may associate with other malformations, such as facial dysmorphism, micrognathia, ocular abnormalities and anal imperforation, as well as autoimmune disorders, such as autoimmune haemolytic anaemia or thrombocytopenia [11]. Treatments include total parental nutrition and even small bowel transplantation. The genetic basis of this disorder has been elucidated by Sivagnanam at al. [12], who discovered that EPCAM (epithelial cell adhesion molecule) gene encoding for a cell adhesion receptor associated with tight junction proteins, is involved in tufting enteropathy pathogenesis.

The histologic characteristic features of this entity are villous atrophy, associated with "tufts" of teardrop-shaped surface enterocytes towards the small bowel lumen, with a preserved brush border. The degree of villous atrophy is usually severe; however, it can change over time. Although there may be mild inflammation of lamina propria, crypt hyperplasia and even crypt cystic dilatation, there is no intraepithelial lymphocytosis, differently from coeliac disease. It should be kept in mind that histology is not entirely specific for this defect, as other congenital disorders, such as congenital sodium diarrhoea due to SPINT2 mutation, can occasionally mimic the histologic picture of tufting enteropathy, as well as the more common autoimmune entheropaty [13] (Fig. 1). Therefore, immunohistochemistry showing reduced expression of EPCAM may support the morphologic diagnosis [14]. Ultrastructure analysis, which usually shows non-specific desmosome irregularities, is not recommended for diagnosis.
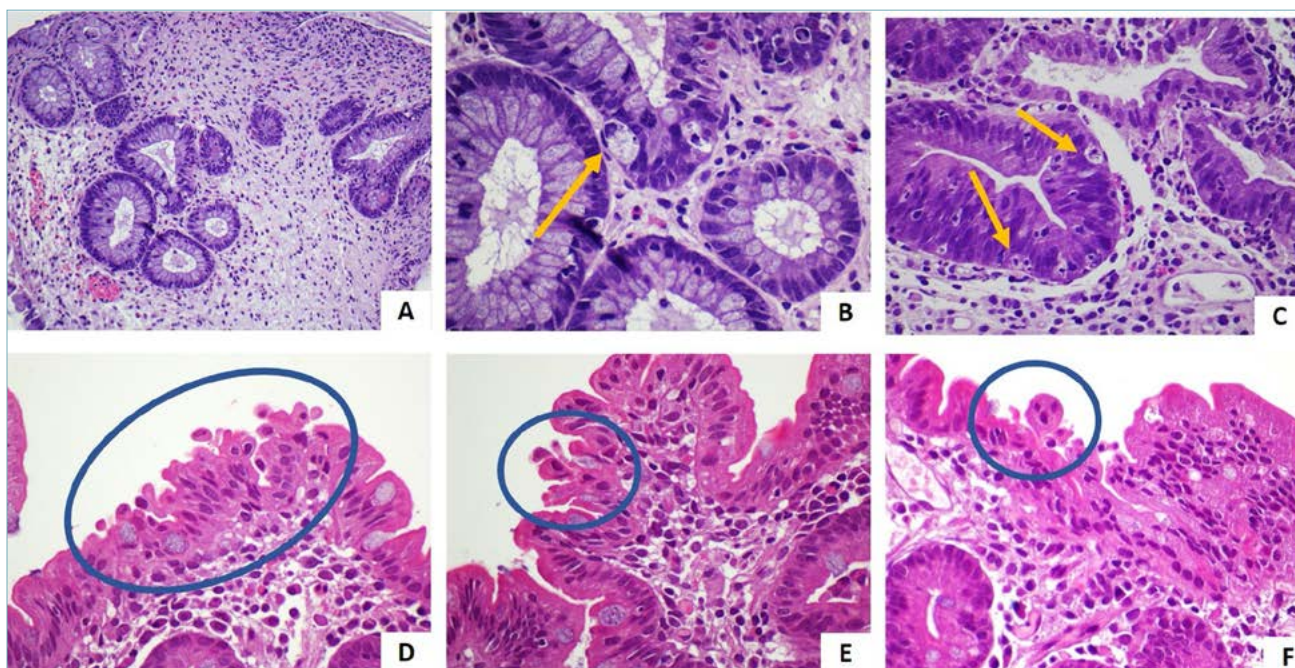
Figure 1. (A, B, C): autoimmune enteropathy with crypt atrophy and hyperplasia and apoptotic bodies (arrows) A H&E 10x, B H&E 40x, C H&E 20x; (D, E, F): tufting enteropathy with the typical "tufts" on the superficial epithelium (blue circle) H&E 40x;

ENTEROENDOCRINE CELL DYSGENESIS

Enteroendocrine cell dysgenesis is characterised by the complete lack of neuroendocrine cells in the intestine and is caused by mutations in NEUROG3 gene, which encodes for neurogenin-3, a protein essential for differentiation of gut and pancreatic neuroendocrine cells [15,16]. This disease, which is inherited in an autosomal recessive manner, results in a congenital malabsorptive diarrhoea and in insulin-dependent diabetes.

Small bowel histology may be unremarkable in some cases, while it may show villous atrophy and crypt hyperplasia in other neonates. As a rule, no significant increase in lamina propria cellularity is noted. Immunohistochemistry for pan-neuroendocrine markers, like synaptophysin or chromogranin-A, is helpful to confirm the lack of enteroendocrine cells. Differential diagnosis includes microvillous inclusion disease; however, in enteroendocrine cell dysgenesis, the brush border is regular, and no inclusions are seen in either light or electron microscopy. It should also be remembered that in some cases of autoimmune enteropathy small bowel endocrine cells may be destroyed by immune cells. Patients are usually managed with predominantly parental nutrition.

TRICO-HEPATO-ENTERIC SYNDROME

This extremely rare syndrome is characterised by trichomalacia (thin and sparse hair), hypertelorism, and chronic diarrhoea [17,18]. Infants may have a history of intrauterine growth retardation, neonatal hemochromatosis, and platelet dysfunctions. The genetic defect underlying this syndrome are mutations in gene TTC37, which encodes for a tetratricopeptide repeat protein. A genetically and phenotypically similar syndrome, characterised by dysmorphic features, trichorrhexis nodosa, intractable diarrhoea and immunodeficiency, has been more recently described by Girault et al. as "syndromatic intractable diarrhoea" [19,20]. Most children are of Middle Eastern origin with consanguineous parents. Small bowel histology usually shows villous atrophy, without increase in lamina propria inflammatory cellularity.

Metabolic disorders/thesaurismoses

Several metabolic disorders display, among many systemic symptoms, also GI tract involvement; however, comprehensive data on the histopathology of villi in these conditions is lacking, owing to the rarity of this group of diseases and to the systemic involvement which, most of the times, overshadows GI symptoms. Among the congenital disorders of glycosylation (CGD), deficit of phosphomannose isomerase (*MPI-CGD, fomer CGD type Ib*) and mutations in the gene

encoding alpha-1,3-glucosyltransferase (*ALG6-CDG, formerly CDG type Ic*) are the biochemical defects usually associated with prominent GI tract symptoms, usually manifesting as a protein-losing enteropathy and failure to thrive in infancy [21,22].

Histological findings are usually non-specific: shortened, blunted villi with increased number of inflammatory cells in the lamina propria, non-responsive to food protein withdrawal. Electron microscopy performed on the epithelial cells, lymphocytes and fibroblasts shows a dilation of smooth endoplasmic reticulum with presence of abnormal lipid inclusions [21,23].

Mucopolysaccharidoses represent another class of disorders of lysosomal storage characterised by prominent musculoskeletal and neurological manifestations, and burdened by a dire prognosis. Splenomegaly and diarrhoea may occur during infancy, and they are mostly ascribed to impairment of the autonomic nervous system rather than alterations of the intestinal mucosa. Histologic descriptions are scarce; Sibilio et al. [24] describes the presence of dilated lymphatic vessels in the lamina propria of the ileum in an 11-year-old patient with a concomitant diagnosis of *mucopolysaccharidosis type IIIB* (Sanfilippo syndrome).

Wolman disease is the most severe form of lysosomal acid lipase deficiency (LAL-D) and it is characterised by an onset in the first weeks of life of intractable diarrhoea and malabsorption, hepatosplenomegaly, jaundice, and adrenal calcifications. On histology, villi appearance is reported to vary from normal to blunted and club-shaped, with infiltration of the lamina propria by foamy PAS-positive macrophages [25,26]. The same histological picture is reported in *cholesteryl ester storage disorder (CESD)*, the less severe form of LAL-D with paediatric-age or even adulthood onset [27,28]. An analogue presentation with PAS-negative foamy macrophages in the lamina propria is reported in *Tangier disease* [29], a high-density lipoprotein deficiency syndrome. In all these conditions, the cellular deposits stain positive for Sudan Black and Oil Red O on frozen section.

Glycogen storage disease type Ib is recognised to be associated with a Crohn-like enteritis [30,31], putatively associated with transient neutropenia; histological finding in the small intestine are terminal ileitis and mild blunting of duodenal villi [32].

Fabry disease is a glycolipid storage disorder caused by a mutation in the GLA gene, leading to a lacking or reduced alpha-galactosidase A activity and lysosomal accumulation of glycosphingolipids. Data from the Fabry Outcome Survey revealed GI symptoms to be predominant among all Fabry patients, chiefly among children, with the most frequent complaints being abdominal pain and diarrhoea [33]. Glycolipid accumulation

in the ganglion cells of the Auerbach and Meissner plexuses with consequent GI tract dysmotility has been proposed as the causative lesion [34], with enlarged ganglion cells displaying a foamy appearance due to the presence of intracytoplasmic lipid inclusions [35].

Intestinal lymphangiectasias

Intestinal lymphangiectasia is distinguished into primary or secondary lymphangiectasia. Primary lymphangiectasias are further classified into localized to intestinal lymphatics (Waldmann syndrome) or associated with other syndromes, involving multiple organs and systems. Secondary lymphangiectasias may be either local, due to an obstruction of local lymphatic capillaries form neoplastic processes or inflammatory diseases, or central, correlated with a cardiac aetiology or a reduced venous return.

Primary lymphangiectasia is a rare hereditary condition, usually affecting children and young adults, characterised by developmental anomalies of lymphatics resulting in the presence of tortuous, dilated lymphatic channels causing stasis of lymph in the intestinal tract and consequent loss of proteins and immunoglobulins into the lumen.

It clinically presents as a protein-losing enteropathy with varying degrees of hypoalbuminemia, peripheral oedema and secondary immunodeficiency with lymphopenia and hypogammaglobulinaemia. CT scan and endoscopic findings are usually sufficient to establish suspicion in a patient with an appropriate clinical picture [36].

Histologically, both primary and secondary lymphangiectasias are characterised by the presence of blunted villi with dilated lymphatic channels lined by endothelium in the superficial and/or deep mucosa. These channels may either be empty or filled with proteinaceous, weakly eosinophilic material. Histology can also be unremarkable if the abnormalities are very focal or present in deeper layers of the bowel wall [37].

Care must be taken not to overdiagnose this condition on the basis of isolated dilated lymphatics, often present in the normal, healthy population [38].

Immunodeficiencies

Many disorders of the immune system have GI tract manifestations, broadly grouped under manifestations linked to increased infection susceptibility, chronic inflammation, and increased risk of neoplasia, with the first two categories playing a major role during infancy. *Common variable immune deficiency* (CVID) is per-

haps the immunodeficiency that most commonly manifests with GI involvement. The classic histopathological picture in the small bowel is villous blunting, with increase of intraepithelial CD3+ lymphocytes and a lamina propria inflammatory infiltrate with conspicuous absence of plasma cells, closely resembling the histological appearance of coeliac disease [39,40]. The fact that the two disorders arise in the same age group, and the difficulty in confirming the coeliac disease diagnosis through anti-gliadin and endomysial IgA measurements in CVID patients, further complicate the differential diagnosis, and despite absence of plasma cells and response to a gluten-free diet orient the diagnosis towards CVID, sometimes histology cannot help differentiating between a refractory coeliac disease and CVID [41]. Coeliac disease will be discussed more in detail in part II of this review [42]. Nodular lymphoid hyperplasia (NLH) is another histological finding of CVID in small bowel biopsies, with hyperplastic lymphoid nodules found in the lamina propria and submucosa probably as a result of chronic antigen stimulation; also in this case, plasma cells are absent from the extramantle zone of the nodule, in contrast with NLH in immunocompetent patients. An infectious aetiology, particularly *Giardia lamblia*, must also be taken into differential when examining small bowel samples from a patient with suspected coeliac disease or CVID in the presence of villous blunting and an increased lymphocytic intraepithelial infiltrate. *Selective IgA deficiency*, a milder immunodeficiency disease on the same spectrum of CVID, shows an analogous histological picture [43].

Chronic granulomatous disease is characterized by a defect in the phagocytic mechanisms in macrophages leading to an accumulation of pigment-laden histiocytes in the lamina propria throughout the digestive tract; in small bowel samples the granulomata are usually located deep in the crypts, but may extend to the villus when particularly florid. These granulomata are usually less florid than those seen in Crohn's disease, and they don't usually cause the same degree of architectural chronic distortion that is seen in Crohn's or ulcerative colitis [43-45].

Other immunodeficiencies are characterized by a more varied and less specific constellation of gastrointestinal symptoms and histological findings, and careful correlation with clinical data is always mandatory to reach a conclusive diagnosis.

Graft-versus-host disease

Graft-versus-host disease (GHVD) is one of the most common complications of hematopoietic stem cells transplantation and it frequently affects the GI tract. The pathologist remains a key figure in the diagnosis of this important complication, for a bioptic confirmation of the disease is always encouraged.

Current advances in transplant and conditioning regimens has rendered the classification of acute versus chronic GVHD based on the time elapsed since transplant obsolete; a new definition had been put forward in 2005 by the NIH and then revised in 2014 and is reported in Table I [46,47].

Table I. Criteria for the diagnosis of acute vs chronic GVHD.

Classification	Time of onset	Features
Classic acute GVHD	< 100 days	Cutaneous rash, nausea, vomiting, anorexia, severe diarrhoea, ileus, cholestatic hepatitis
Persistent, recurrent, or late acute GVHD	> 100 days	Classic acute GVHD features without key symptoms of chronic GVHD
Classic chronic GVHD	No time limit	At least one key symptom of chronic GVHD without symptoms characteristics of acute GVHD
Overlap chronic and acute GVHD	No time limit	Presence of one or more acute GVHD symptoms in a patient with a diagnosis of chronic GVHD

In the GI tract, chronic GVHD manifestations include oesophageal web and stricture and pancreatic atrophy and exocrine insufficiency; symptoms like nausea, vomiting, diarrhoea, failure to thrive and wasting syndrome are shared with the acute counterpart of the disease.

Involvement of the GI tract is not site-specific, patchy, and may involve the whole length of the digestive system; epithelial apoptosis is the hallmark of the acute form of the disease, and it localizes in the proliferating compartments of the epithelium. In the duodenum and other small bowel tracts, the manifestations of the disease can be subtle, with apoptotic changes localizing mainly in the neck and crypts of the villi [48]; a sparse mononucleated infiltrate usually accompanies the apoptotic changes. Mild villous blunting is commonly observed. Loss of Paneth cells seems to correlate with increased severity of the disease. The NIH 2014 Pathology Working Group Report document suggests the presence of ≥ 1 apoptotic body/piece for crypt as

the minimal criterion for acute/active GVHD diagnosis [49]. In chronic GVHD, fibrosis of the lamina propria with minimal mucosal changes have been reported [50]. The same 2014 Pathology Working Group Report issues recommendations for the final diagnosis of GVHD into three categories: i) *not GVHD*, ii) *possible GVHD*, if there is histological evidence for GVHD but other possible causes, and iii) *likely GVHD*, if there is clear evidence of GVHD with no other cause, there are mitigating factors, or the history of the patient is lacking.

Other competing causes that should be excluded are infections associated with increased enterocytes apoptosis, such as CMV or cryptosporidiosis. Another important mimic to be excluded is toxicity from either the conditioning regimen (very early manifestation, up to day 20 after transplant) [51] or mycophenolate mofetil, an immunosuppressive drug used in the post-transplant period. The changes in mycophenolate mofetil mucosal injury can closely resemble GHVD [52], with a more prominent eosinophilic infiltrate and loss of neuroendocrine cells [53], but a definite diagnosis is usually reached integrating the clinical and histological suspicion with regression of the symptoms upon discontinuation of the drug.

Necrotising enterocolitis

Necrotising enterocolitis (NEC) is a surgical emergency in the new-born and the major cause of morbidity and mortality in neonatal intensive care units [54]. Most cases (> 90%) occurs in preterm infants, especially in those with very low birth weight (< 1,500 grams); the incidence is inversely proportional to gestational age at birth and the time to onset is longer the more premature the infant is [55]. The mortality rate for preterm infants who have extremely low birth weight (< 1,000 grams) is 30-50% while for infants with a very low birth weight is 10-30% [56].

Early signs and symptoms are subtle and vague, including feeding intolerance, abdominal distention and, after 8 to 10 days of age, bloody stools. Characteristic radiological findings are pneumatosis intestinalis, portal venous gas, or both. The universally recognised risk factors for NEC are formula feeding, intestinal dysbiosis, low birth weight, and prematurity [57]. Its aetiopathogenesis can be considered multifactorial and the major factors involved are nitric oxide (NO) imbalance, intestinal ischaemia, dysbiosis and reduced activity of intestinal stem cells. A key role is played by Toll-like-receptor 4 (TLR4), a receptor that takes part in innate immunity; its increased expression in premature infants is triggered by intestinal Gram-negative bacteria, disrupting and invading the intestinal epithelium at the tips of intestinal villi. Endotoxins bind to TLR4 on the intestinal epithelial cells, activating pathogen-associated molecular pattern receptors, which facilitate the breakdown of the gut barrier and allow bacteria to translocate. This process leads to an intense inflammatory response in the lamina propria mediated by inflammatory cytokines and vasoactive substances. Intestinal inflammation also activates complement and coagulation systems, that adhere to the endothelium, preventing blood flow in the microvascular structure of the small intestine, thus leading to tissue injury. Additional damage to the endothelium from adherent neutrophils and platelets also impairs NO generation needed for vasorelaxation. TLR4 are inhibited by probiotic bacteria that activate Toll-like-receptor 9 (TLR9) and can prevent goblet cell differentiation, which is needed to maintain the physical mucous intestinal barrier to pathogenic bacteria [56].

In neonatal pathology, three form of intestinal injury can be observed: i) *conditions primarily seen in term infants,* ii) *spontaneous intestinal perforations,* and iii) *classic NEC.*

Necrotising enterocolitis-like symptoms are also known to happen during the first week after birth, both in term and late preterm infants; in the latter, the condition is more often associated with other comorbidities, such as maternal drug abuse, intestinal anomalies (e.g., aganglionosis or atresias), congenital heart disease, and perinatal stress that may affect mesenteric blood flow [58].

The lack of reliable diagnostic criteria often makes it difficult to establish the diagnosis. Bell's classification is the currently and most widely used tool in early assessment of the disease. This staging system was first described by Bell et al. in 1978 [59] and subsequently refined by Walsh in 1986 [60]; it is divided into III stages (suspected, definite and advanced NEC) based on systemic, abdominal and radiographic signs [61].

Usually the affected segments are the distal ileum and right colon (80% of cases) which represent vascular watersheds of perfusion. NEC can be considered as ischaemic colitis, as its histopathological hallmarks are similar to those observed in others type of ischaemic colitis both in adults and children (Tab. II).

Table II. Ischaemic colitis in children: differential diagnosis.

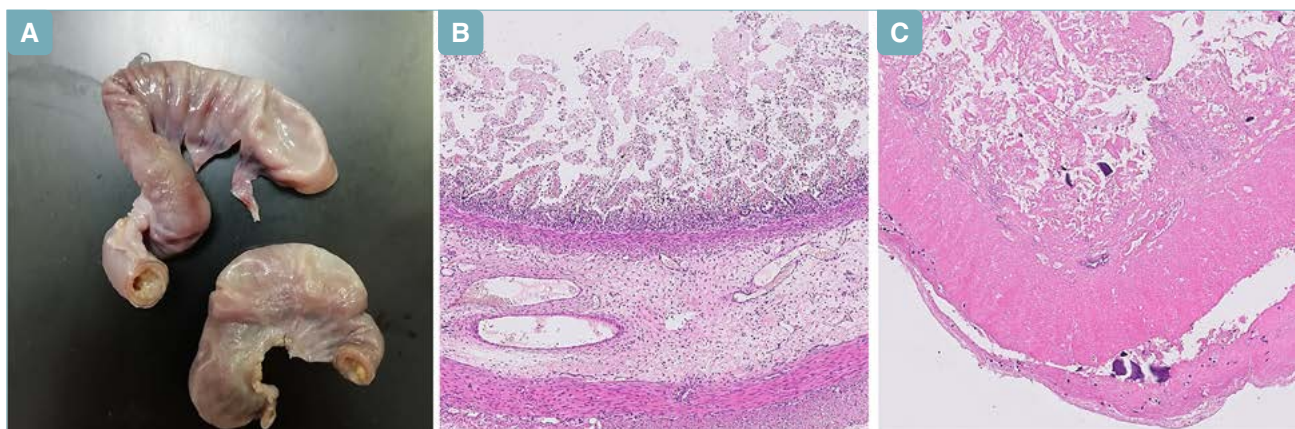| Ischaemic colitis in children |
|---|
| Necrotising enterocolitis (NEC) |
| Henoch-Schonlein purpura |
| Haemolytic-uraemic syndrome |
| Hirschprung-associated enterocolitis (late) |
| Neutropenic colitis |

Figure 2. Total colectomy and ileal resection for NEC and intestinal perforation in a 23 days preterm infant born at 30 weeks of gestation. (A): surgical resection of necrotic distal ileum; (B): H&E necrotic ileal mucosa with oedema and severe inflammatory infiltrate in the submucosa 2x; (C): H&E transmural necrosis of the colon 2x.

The histopathology of early-stage NEC is difficult to examine, as obtaining small biopsies during the initial phase of the disease is challenging [2]. Resected specimens may show patchy or diffuse haemorrhagic necrosis of the mucosa and coagulative necrosis of the muscular layers; a transmural damage leads to intestinal perforation which is often multifocal and represents a major cause of peritonitis in these patients (Fig. 2 A-C). Pneumomatosis intestinalis is frequently found, while vascular thrombosis can be occasionally observed and is secondary to necrosis [62]. Considering the age of onset, the major differential diagnosis is Hirchsprung-Associated Enterocolitis (HAEC), that can occur before surgical treatment and from 3 weeks to 20 months after the pull-through operation. Neonates may also present with pneumatosis intestinalis and a clinical pictures suggesting NEC [62].

When the diagnosis is confirmed, it requires medical or surgical procedures based on the clinical presentation; the former may include bowel rest, broad spectrum intravenous antibiotics, and intravenous hyperalimentation while the latter are required for intestinal perforation or if sepsis is suspected [58].

The prognosis of infants who recover from NEC depends on the severity of the disease and is worse for infants who underwent surgical procedures; mortality rate in these patients is 35%, while it decreases to 20% for those who are treated with medical therapies. Moreover, survival correlates with degree of prematurity and weight. Approximately 50% of NEC survivors develop early postoperative complications that include wound infection or dehiscence of the anastomosis, compartment syndrome and complications secondary to stomas. Intermediate and long-term complications,

such as intestinal strictures, frequently occur in the colon. Short-gut syndrome can develop in the first month after recovery [61] and requires long-term nutritional management for malabsorption and growth deficiencies [55,63]. Moreover, these infants are at increased risk of poor neurodevelopmental outcomes, including cerebral palsy and cognitive impairment. The poor neurodevelopmental outcomes in infants with NEC may be secondary to a systemic inflammatory response and/or alterations in the gut-brain axis [64].

NEC is a serious and complex inflammatory bowel disease, and therefore clinical strategies are mainly aimed at its prevention by promoting breast feeding and skin-to-skin care [65]. It is known that breast milk reduces the incidence of NEC because it is demonstrated to have anti-inflammatory properties, to contain various factors promoting intestinal epithelial growth and to influence the developing intestinal microbiome [55].

The future therapeutic perspectives include breast milk component, prophylactic probiotics and stem cell administration [56].

References

1   Campora M, Mastracci L, Carlin L, et al. Pathologist's approach to paediatric and neonatal eosinophilic gastrointestinal disorders. Pathologica 2021 In Press.

2   Wetterau JR, Aggerbeck LP, Bouma ME, et al. Absence of microsomal triglyceride transfer protein in individuals with abetalipoproteinemia. Science 1992;258(5084):999-1001. https://doi.org/10.1126/science.1439810

3   Keller KM, Wirth S, Baumann W, et al. Defective jejunal brush border membrane sodium/proton exchange in association with lethal familial protracted diarrhoea. Gut 1990;31:1156-1158. https://doi.org/10.1136/gut.31.10.1156

4   Ensari A, Kelsen J, Russo P. Newcomers in paediatric GI pathology: childhood enteropathies including very early onset monogenic IBD. Virchows Arch 2018;472:111-123. https://doi.org/10.1007/s00428-017-2197-9

5   Davidson GP, Cutz E, Hamilton JR, et al. Familial enteropathy: a syndrome of protracted diarrhea from birth, failure to thrive, and hypoplastic villus atrophy. Gastroenterology 1978;75(5):783-790.

6   Erickson RP, Larson-Thomé K, Valenzuela RK, et al. Navajo microvillous inclusion disease is due to a mutation in MYO5B. Am J Med Genet A 2008;146A:3117-3119. https://doi.org/10.1002/ajmg.a.32605

7   Ruemmele FM, Schmitz J, Goulet O. Microvillous inclusion disease (microvillous atrophy). Orphanet J Rare Dis 2006;1:22. https://doi.org/10.1186/1750-1172-1-22

8   Reifen RM, Cutz E, Griffiths AM, et al. Tufting enteropathy: a newly recognized clinicopathological entity associated with refractory diarrhea in infants. J Pediatr Gastroenterol Nutr. 1994;18:379-385.

9   Goulet O, Salomon J, Ruemmele F, et al. Intestinal epithelial dysplasia (tufting enteropathy). Orphanet J Rare Dis 2007;2:20. https://doi.org/10.1186/1750-1172-2-20

10   Abely M, Hankard GF, Hugot JP, et al. Intractable Infant Diarrhea with Epithelial Dysplasia Associated with Polymalformation: J Pediatr Gastroenterol Nutr 1998;27:348-352. https://doi.org/10.1097/00005176-199809000-00016

11   El-Matary W, Dalzell AM, Kokai G, et al. Tufting enteropathy and skeletal dysplasia: is there a link? Eur J Pediatr 2007;166:265-268. https://doi.org/10.1007/s00431-006-0231-z

12   Sivagnanam M, Mueller JL, Lee H, et al. Identification of EpCAM as the Gene for Congenital Tufting Enteropathy. Gastroenterology 2008;135:429-437. https://doi.org/10.1053/j.gastro.2008.05.036

13   Francalanci P, Cafferata B, Alaggio R, et al. Pediatric autoimmune disorders with gastrointestinal expression: from bench to bedside. Pathologica 2021 Epub December 2. https://doi.org/10.32074/1591-951X-339

14   Salomon J, Espinosa-Parrilla Y, Goulet O, et al. A founder effect at the EPCAM locus in congenital tufting enteropathy in the Arabic Gulf. Eur J Med Genet 2011;54:319-322. https://doi.org/10.1016/j.ejmg.2011.01.009

15   Wang J, Cortina G, Wu SV, et al. Mutant neurogenin-3 in congenital malabsorptive diarrhea. N Engl J Med 2006;355:270-280. https://doi.org/10.1056/NEJMoa054288

16   Pinney SE, Oliver-Krasinski J, Ernst L, et al. Neonatal diabetes and congenital malabsorptive diarrhea attributable to a novel mutation in the human neurogenin-3 gene coding sequence. J Clin Endocrinol Metab 2011;96:1960-1965. https://doi.org/10.1210/jc.2011-0029

17   Stankler L, Lloyd D, Pollitt RJ, et al. Unexplained diarrhoea and failure to thrive in 2 siblings with unusual facies and abnormal scalp hair shafts: a new syndrome. Arch Dis Child 1982;57:212-216. https://doi.org/10.1136/adc.57.3.212

18   Verloes A, Lombet J, Lambert Y, et al. Tricho-hepato-enteric syndrome: further delineation of a distinct syndrome with neonatal hemochromatosis phenotype, intractable diarrhea, and hair anomalies. Am J Med Genet 1997;68:391-395. https://doi.org/10.1002/(sici)1096-8628(19970211)68:4<391::aid-ajmg3>3.0.co;2-p

19   Girault D, Goulet O, Le Deist F, et al. Intractable infant diarrhea associated with phenotypic abnormalities and immunodeficiency. J Pediatr 1994;125:36-42. https://doi.org/10.1016/S0022-3476(94)70118-0

20   Fabre A, Martinez-Vinson C, Roquelaure B, et al. Novel mutations in TTC37 associated with tricho-hepato-enteric syndrome. Hum Mutat 2011;32:277-281. https://doi.org/10.1002/humu.21420

21   Russo P. Entheropathies Associated with chronic diarrhea and malabsorption in childhood. In: Odze R, Goldblum J, eds. Odze and Goldblum Surgical Pathology of the GI Tract, Liver, Biliary Tract and Pancreas. Saunders 2014, pp. 212-213.

22   Jaeken J. Congenital disorders of glycosylation: Congenital disorders of glycosylation. Ann N Y Acad Sci 2010;1214:190-198. https://doi.org/10.1111/j.1749-6632.2010.05840.x

23   Kristianson B, Borulf S, Conradi N, et al. Intestinal, pancreatic and hepatic involvement in carbohydrate-deficient glycoprotein syndrome type I. J Pediatr Gastroenterol Nutr 1998;27:23-29. https://doi.org/10.1097/00005176-199807000-00005

24   Sibilio M, Miele E, Ungaro C, et al. Chronic diarrhea in mucopolysaccharidosis IIIB. J Pediatr Gastroenterol Nutr 2009;49:477-480. https://doi.org/10.1097/MPG.0b013e31818a082a

25   Kikuchi M, Igarashi K, Noro T, et al. Evaluation of jejunal function in wolman's disease. J Pediatr Gastroenterol Nutr 1991;12:65-69. https://doi.org/10.1097/00005176-199101000-00014

26   Boldrini R, Devito R, Biselli R, et al. Wolman disease and cholesteryl ester storage disease diagnosed by histological and ultrastructural examination of intestinal and liver biopsy. Pathol Res Pract 2004;200:231-240. https://doi.org/10.1016/j.prp.2003.11.001

27   Kyosen SO, Geocze S, Yamamoto MH, et al. Endoscopic findings in lysosomal acid lipase deficiency. J Pediatr Gastroenterol Nutr 2019;68:e105. https://doi.org/10.1097/MPG.0000000000002030

28   Bernstein DL, Hülkova H, Bialer MG, et al. Cholesteryl ester storage disease: Review of the findings in 135 reported patients with an underdiagnosed disease. J Hepatol 2013;58:1230-1243. https://doi.org/10.1016/j.jhep.2013.02.014

29   Ferrans VJ, Fredrickson DS. The pathology of Tangier disease. A light and electron microscopic study. Am J Pathol 1975;78:101-158.

30   Dieckgraefe B, Korzenik J, Husain A, et al. Association of glycogen storage disease 1b and Crohn disease: results of a North American survey. Eur J Pediatr 2002;161:S88-S92. https://doi.org/10.1007/s00431-002-1011-z

31   Visser G, Rake J-P, Fernandes J, et al. Neutropenia, neutrophil dysfunction, and inflammatory bowel disease in glycogen storage disease type Ib: results of the European Study on Glycogen Storage Disease Type I. J Pediatr 2000;137:187-191. https://doi.org/10.1067/mpd.2000.105232

32   Barker CC, Butzner JD, Woodman RC, et al. Crohn-like enteritis presenting as hypoglycemia in a patient with glycogen storage disease type 1b, Treated with granulocyte colony-stimulating factor and splenectomy. J Pediatr Gastroenterol Nutr 2001;32:197-200. https://doi.org/10.1097/00005176-200102000-00019

33   Hoffmann B, Schwarz M, Mehta A, et al. Gastrointestinal symptoms in 342 patients with fabry disease: prevalence and response to enzyme replacement therapy. Clin Gastroenterol Hepatol 2007;5:1447-1453. https://doi.org/10.1016/j.cgh.2007.08.012

34   Hilz MJ, Arbustini E, Dagna L, et al. Non-specific gastrointestinal features: could it be Fabry disease? Dig Liver Dis 2018;50:429-437. https://doi.org/10.1016/j.dld.2018.02.011

35   Zar-Kessler C, Karaa A, Sims KB, et al. Understanding the gastrointestinal manifestations of Fabry disease: promoting prompt diagnosis. Ther Adv Gastroenterol 2016;9:626-634. https://doi.org/10.1177/1756283X16642936

36   Radhakrishnan K, Rockson SG. The clinical spectrum of lymphatic disease. Ann N Y Acad Sci 2008;1131:155-184. https://doi.org/10.1196/annals.1413.015

37   Vignes S, Bellanger J. Primary intestinal lymphangiectasia (Waldmann's disease). Orphanet J Rare Dis 2008;3:5. https://doi.org/10.1186/1750-1172-3-5

38   Pai RK, Pai RK. Polyps of the small intestine. In: Odze R, Goldblum J, eds. Odze and Goldblum Surgical Pathology of the GI Tract, Liver, Biliary Tract and Pancreas. 3rd ed. Saunders 2014, p. 601.

39 Daniels JA, Lederman HM, Maitra A, et al. Gastrointestinal tract pathology in patients with common variable immunodeficiency (CVID): a clinicopathologic study and review. Am J Surg Pathol 2007;31:1800-1812. https://doi.org/10.1097/PAS.0b013e3180cab60c

40 Biagi F, Bianchi PI, Zilli A, et al. The Significance of duodenal mucosal atrophy in patients with common variable immunodeficiency. Am J Clin Pathol 2012;138:185-189. https://doi.org/10.1309/AJCPEIILH2C0WFYE

41 Malamut G, Verkarre V, Suarez F, et al. The enteropathy associated with common variable immunodeficiency: the delineated frontiers with celiac disease. Am J Gastroenterol 2010;105:2262-2275. https://doi.org/10.1038/ajg.2010.214

42 Rossi C, Simoncelli G, Arpa G, et al. Histopathology of intestinal villi in neonatal and paediatric age: main features with clinical correlation - Part II. Pathologica 2021 Epub December 2. https://doi.org/10.32074/1591-951X-338

43 Agarwal S, Mayer L. Diagnosis and treatment of gastrointestinal disorders in patients with primary immunodeficiency. Clin Gastroenterol Hepatol 2013;11:1050-1063. https://doi.org/10.1016/j.cgh.2013.02.024

44 Marks DJB, Miyagi K, Rahman FZ, et al. Inflammatory Bowel disease in CGD reproduces the clinicopathological features of Crohn's disease. Am J Gastroenterol 2009;104:117-124. https://doi.org/10.1038/ajg.2008.72

45 Parente P, Pastore M, Grillo F, et al. Very Early Onset-IBD: evidence for the need of a multidisciplinary approach. Pathologica 2021 Epub December 2. https://doi.org/10.32074/1591-951X-336

46 Jagasia MH, Greinix HT, Arora M, et al. National Institutes of Health Consensus Development Project on criteria for clinical trials in chronic graft-versus-host disease: I. The 2014 diagnosis and staging working group report. Biol Blood Marrow Transplant 2015;21:389-401.e1. https://doi.org/10.1016/j.bbmt.2014.12.001

47 Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health Consensus Development Project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. Biol Blood Marrow Transplant 2005;11:945-956. https://doi.org/10.1016/j.bbmt.2005.09.004

48 Washington K, Jagasia M. Pathology of graft-versus-host disease in the gastrointestinal tract. Hum Pathol 2009;40:909-917. https://doi.org/10.1016/j.humpath.2009.04.001

49 Shulman HM, Cardona DM, Greenson JK, et al. NIH Consensus Development Project on criteria for clinical trials in chronic graft-versus-host disease: II. The 2014 Pathology Working Group Report. Biol Blood Marrow Transplant 2015;21:589-603. https://doi.org/10.1016/j.bbmt.2014.12.031

50 Shulman HM, Sullivan KM, Weiden PL, et al. Chronic graft-versus-host syndrome in man. Am J Med 1980;69:204-217. https://doi.org/10.1016/0002-9343(80)90380-0

51 Epstein RJ, McDonald GB, Sale GE, et al. The diagnostic accuracy of the rectal biopsy in acute graft-versus-host disease: a prospective study of thirteen patients. Gastroenterology 1980;78:764-771.

52 Parfitt JR, Jayakumar S, Driman DK. Mycophenolate mofetil-related gastrointestinal mucosal injury: variable injury patterns, including graft-versus-host disease-like changes. Am J Surg Pathol 2008;32:1367-1372. https://doi.org/10.1097/PAS.0b013e31816bf3fe

53 Star KV, Ho VT, Wang HH, et al. Histologic features in colon biopsies can discriminate mycophenolate from GVHD-induced colitis. Am J Surg Pathol 2013;37:1319-1328. https://doi.org/10.1097/PAS.0b013e31829ab1ef

54 Horbar JD, Carpenter JH, Badger GJ, et al. Mortality and neonatal morbidity among infants 501 to 1500 grams from 2000 to 2009. Pediatrics 2012;129:1019-1026. https://doi.org/10.1542/peds.2011-3028

55 Bellodas Sanchez J, Kadrofske M. Necrotizing enterocolitis. Neurogastroenterol Motil Off J Eur Gastrointest Motil Soc 2019;31:e13569. https://doi.org/10.1111/nmo.13569

56 Alganabi M, Lee C, Bindi E, et al. Recent advances in understanding necrotizing enterocolitis. F1000Research 2019;8. https://doi.org/10.12688/f1000research.17228.1

57 Rose AT, Patel RM. A critical analysis of risk factors for necrotizing enterocolitis. Semin Fetal Neonatal Med 2018;23:374-379. https://doi.org/10.1016/j.siny.2018.07.005

58 Neu J, Walker WA. Necrotizing enterocolitis. N Engl J Med 2011;364:255-264. https://doi.org/10.1056/NEJMra1005408

59 Bell MJ, Ternberg JL, Feigin RD, et al. Neonatal necrotizing enterocolitis. Therapeutic decisions based upon clinical staging. Ann Surg 1978;187:1-7. https://doi.org/10.1097/00000658-197801000-00001

60 Walsh MC, Kliegman RM. Necrotizing enterocolitis: treatment based on staging criteria. Pediatr Clin North Am 1986;33:179-201. https://doi.org/10.1016/s0031-3955(16)34975-6

61 Rich BS, Dolgin SE. Necrotizing Enterocolitis. Pediatr Rev 2017;38:552-559. https://doi.org/10.1542/pir.2017-0002

62 Russo P, Ruchelli ED. Pathology of pediatric gastrointestinal and liver disease. New York: Springer-Verlag, Inc. 2004 https://doi.org/10.1007/978-1-4419-9066-2

63 Lin PW, Nasr TR, Stoll BJ. Necrotizing enterocolitis: recent scientific advances in pathophysiology and prevention. Semin Perinatol 2008;32:70-82. https://doi.org/10.1053/j.semperi.2008.01.004

64 Adams-Chapman I. Necrotizing enterocolitis and neurodevelopmental outcome. Clin Perinatol 2018;45:453-466. https://doi.org/10.1016/j.clp.2018.05.014

65 Meister AL, Doheny KK, Travagli RA. Necrotizing enterocolitis: It's not all in the gut. Exp Biol Med Maywood NJ 2020;245:85-95. https://doi.org/10.1177/1535370219891971