

Review of Current Applications of Immunohistochemistry in Pediatric Nonneoplastic Gastrointestinal, Hepatobiliary, and Pancreatic Lesions

Joseph de Nanassy^{1,2} and Dina El Demellawy^{1,2}

¹Department of Anatomical Pathology, Children's Hospital of Eastern Ontario (CHEO), Ottawa, ON, Canada. ²Department of Pathology, University of Ottawa, Ottawa, ON, Canada.

Analytical Chemistry Insights
Volume 12: 1–11
© The Author(s) 2017
Reprints and permissions:
sagepub.co.uk/journalsPermissions.nav
DOI: 10.1177/1177390117690140



ABSTRACT: Immunohistochemical (IHC) stains are widely used by pathologists for a variety of considerations in the diagnostic workup of pediatric nonneoplastic lesions in gastrointestinal (GI), hepatic, biliary, and pancreatic lesions. The pathologic changes cover a wide range and types of presentations, including inflammatory (bacterial and viral), metaplastic, posttransplant lymphoproliferative, autoimmune, metabolic, degenerative, developmental, and genetic conditions, among others. The everyday practical value of IHC stains covers primary identification, confirmation, differential, and/or exclusionary roles in the hands and eyes and minds of the practitioners. This article is intended to review and discuss the currently available IHC stains for a variety of pediatric GI, hepatobiliary, and pancreatic lesions as encountered in the day-to-day practice of pathologists and clinicians. It reflects the most recent methods and types of IHC stains with the stated aim of helping to provide a quick reference for diagnostic considerations and thereby facilitate the workup of a broad range of GI and related conditions in a pediatric population. The tables provide a handy reference on a wide range of IHC stains for commonly encountered lesions covering a variety of pediatric GI, hepatobiliary, and pancreatic conditions that are amenable to light microscopic diagnostic interpretation.

KEYWORDS: Immunohistochemistry, pediatric, gastrointestinal, hepatobiliary, nonneoplastic

RECEIVED: July 21, 2016. **ACCEPTED:** January 2, 2017.

PEER REVIEW: Six peer reviewers contributed to the peer review report. Reviewers' reports totaled 551 words, excluding any confidential comments to the academic editor.

TYPE: Review

FUNDING: The author(s) received no financial support for the research, authorship, and/or publication of this article.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

CORRESPONDING AUTHOR: Joseph de Nanassy, Department of Anatomical Pathology, Children's Hospital of Eastern Ontario (CHEO), 401 Smyth Road Ottawa, ON K1H 8L1, Canada. Email: jdenanassy@cheo.on.ca

Overview

Pediatric gastrointestinal (GI) lesions have come to represent an ever-increasing component of the practice of a number of anatomical pathology departments in children's hospitals in numerous locations on every continent. The reasons for this growth include, among others, a larger number of patients with a variety of GI symptoms coming to medical attention sooner, improved clinical diagnostic methods, more practitioners entering the field of pediatric gastroenterology/hepatology and nutrition, and also a wider array of diagnostic tools available to the pathologists involved in assessing such specimens. In pediatric GI pathology, the routine hematoxylin-eosin (H&E) stain is supplemented by additional techniques such as "special stains," immunohistochemistry (IHC), electron microscopy, polymerase chain reaction, and metabolic studies, to mention a few. The focus of this review is the current state of immunohistochemical stains used in characterizing a variety of nonneoplastic GI and hepatic lesions in pediatric patients (<18 years of age).

In applying the currently available latest IHC stains and techniques, practitioners are provided with a useful and practical tool in their diagnostic workup of cases that can be challenging at times. The concise summary of stains in table format will enable the pathologist to identify at a glance the most useful stains for differential diagnostic considerations. One relevant group of stains for each of a variety of diagnostic entities will allow for the application of IHC stains to differentiate related diagnostic options, using a list of IHC stains as summarized in the tables.

It is likely that not all pathology departments will have all listed stains available, for a variety of valid reasons. Some of the stains as listed are the latest ones currently available commercially, whereas others are more routine, well-established stains that may have been in use for longer periods of time and have proven to be useful in daily practice. Although newer stains are the result of the most advanced techniques that have led to their discovery, other, more established stains are also of diagnostic value. Only stains of diagnostic usefulness are listed in the tables. Individual practitioners are then free to choose any stain or panel of stains that is available in their department, which will lead to a diagnostic interpretation. One stain is not superior to other(s), and the provided lists are flexible enough to allow for application in any or all pathology departments.

Nonneoplastic Lesions

Conditions in this category can be further listed according to their anatomic site of occurrence into those arising in or affecting the esophagus, stomach, small intestines, colon, and rectum.

Esophagus

Esophagitis can be subdivided into gastroesophageal reflux disease-associated (reflux) esophagitis and eosinophilic esophagitis (EoE) (Figure 1). Immunohistochemistry may play a role in everyday diagnostic workup in differentiating EoE from reflux esophagitis. ALOX15 (arachidonate 15-hydroxygenase) is a sensitive marker for EoE and separates it from



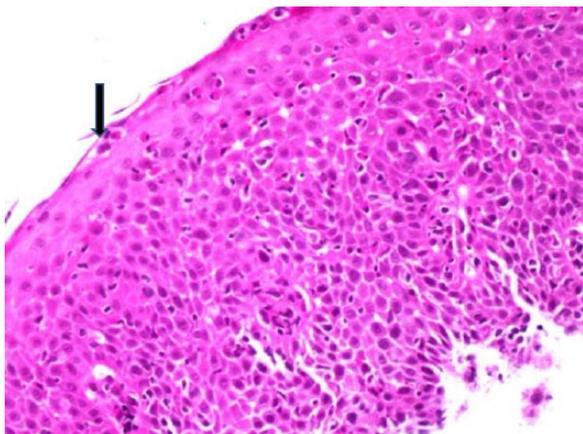


Figure 1. Eosinophilic esophagitis. Esophageal squamous epithelium with proliferative changes and marked increase in intraepithelial eosinophils and abscess formation (HE x400).

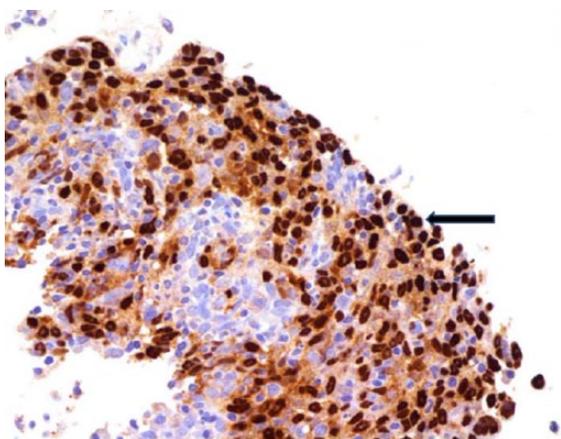


Figure 2. Post-transplant lymphoproliferative disorder, EBV infected cells (EBER x400).

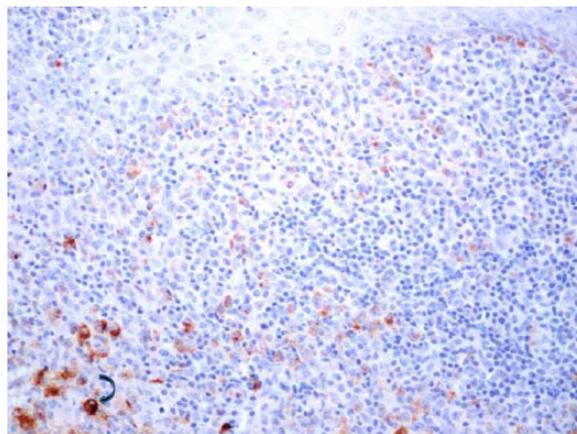


Figure 3. Post-transplant lymphoproliferative disorder, EBV infected cells (LMP x400).

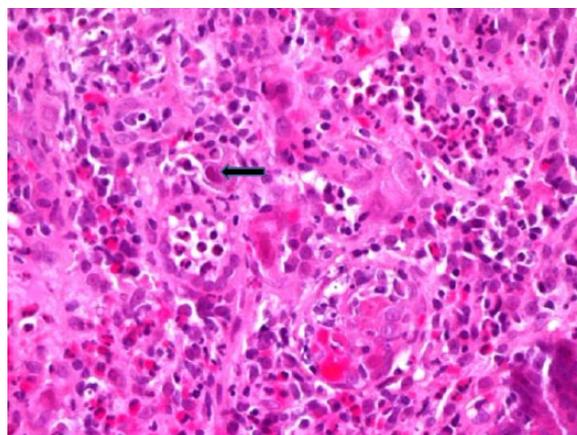


Figure 4. CMV infection. Actively inflamed colonic mucosa with ulceration. Note CMV viral inclusions (HE x400)

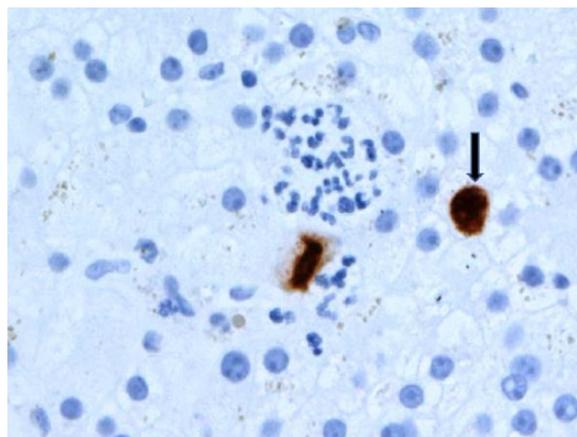


Figure 5. CMV hepatitis (CMV x600).

reflux-associated esophagitis in more than 90% of cases.¹ Major basic protein can also be helpful in highlighting the eosinophilic granules both within the cytoplasm and in extracellular locations if degranulated.² The Barrett esophagus (BE) and the cervical inlet patch (CIP) can both be defined with the CK7/CK20 and acid mucin (MUC6, MUC5AC, and MUC5B) immunohistochemical stains to rule in or rule out intestinal (columnar) metaplasia or gastric ectopia, respectively. However, MUC2 is usually positive in BE, but rarely so in CIP.³

One disease entity where IHC does play a major role is in identifying Epstein-Barr virus (EBV) or cytomegalovirus (CMV) infection, the most obvious examples being in immunocompromised patients, whether post transplant or in the context of HIV/AIDS-associated opportunistic infection. EBV-EBER (EBV-encoded RNA probe) detects EBV using in situ hybridization and will stain the nucleus (Figure 2), whereas EBV-LMP1 (latent membrane protein 1) detects EBV using an immunohistochemical stain and will stain the cytoplasm (Figure 3).⁴ The CMV nuclear inclusions (Figure 4) and cytoplasmic granules will stain intensely on IHC for CMV (Figure 5). Candida esophagitis is readily identified on

silver impregnation stains (eg, Gomori methenamine silver [GMS]) and does not require IHC.

Stomach

Helicobacter pylori infection may elicit a nonspecific gastritis. The IHC to highlight the organism is available (anti-*H. pylori*

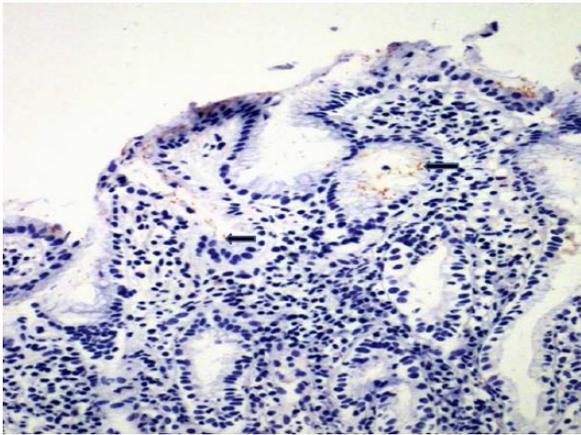


Figure 6. *Helicobacter pylori* bacteria in gastric antrum mucosa (*H. pylori* x200).

antibody) (Figure 6). The elongated, spiral-like bacteria are readily visible in the mucus on the surface of the gastric mucosa. The IHC staining is highly sensitive and specific, but the cost (compared with other histochemical stains, eg, H&E, Giemsa, or Warthin-Starry) may act as a limiting factor in the adoption of *H. pylori* IHC as a routine stain.⁵ Focally-enhanced gastritis as seen in association with inflammatory bowel disease (IBD) does not have a specific IHC stain, and staining for *H. pylori* by IHC will be negative⁶.

Small Intestine

Duodenum

Celiac disease (CD) is a classic condition affecting the duodenum in pediatric patients. The term *CD* per se is a clinical diagnosis rather than a purely pathologic one (because CD can be diagnosed clinically, without the need for confirmation by histopathology if the serum tissue transglutaminase is >100 IU/mL⁷); however, for the purpose of this review, the term CD will be applied to the features diagnosed by light microscopy. The typical findings of increased numbers of intraepithelial lymphocytes of greater than 25-30/100 epitheliocytes in the tips of villi, villous shortening and widening (so-called blunting) due to inflammatory edema and cellular infiltration in the lamina propria, decreased villus-to-crypt ratio, crypt hyperplasia, and increased numbers of mononuclear inflammatory cells (mainly plasma cells and lymphocytes, but also eosinophils, histiocytes, and mast cells) do not in and of themselves require IHC. However, the histologic features observed in CD have a range of differential diagnostic considerations (drugs, food hypersensitivity, infections, immune dysregulation, etc).⁸

Therefore, to better characterize the T lymphocytes in the surface epithelium, IHC for CD3 and CD8 will identify them as belonging to the mucosa-associated lymphoid tissue. About 90% of these will express the $\alpha\beta$ T-cell surface receptor (TCR) and 10% will have the $\gamma\delta$ TCR. The lamina propria contains CD4⁺ T lymphocytes. Anti-endomysial antibodies can be detected by immunofluorescence, with a positive predictive

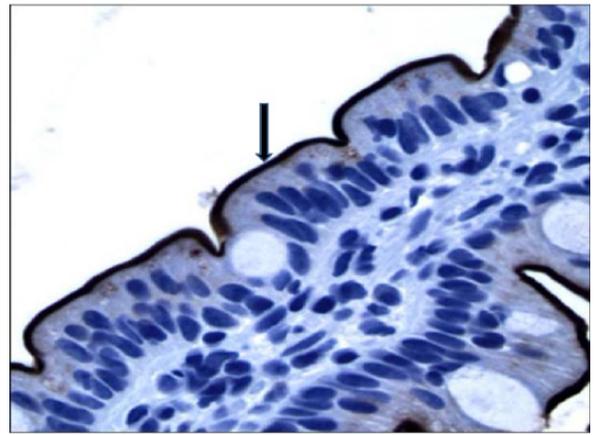


Figure 7. Normal small bowel brush border (CD10 x600)

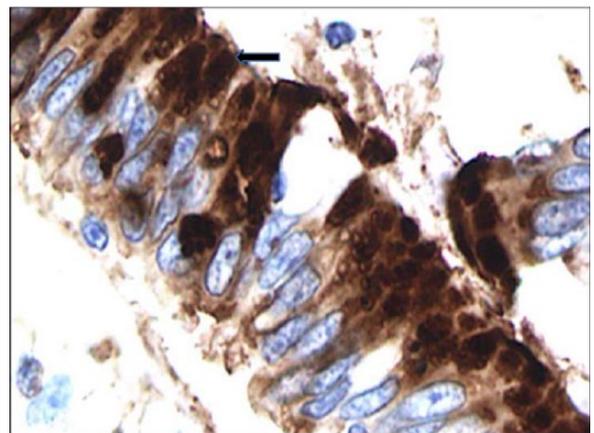


Figure 8. Microvillus inclusion disease (CD10 x600).

value of 85%.⁹ The presence of microorganisms (eg, *Giardia*, *Cryptosporidium*, *Helicobacter*, viruses) does not require the use of IHC for identification.

Dilated lacteals in the tips of villi and the submucosa, as may be seen in protein-losing enteropathy, can be differentiated from air insufflation artifact during endoscopy by way of the endothelial cells of the lacteals being immunoreactive for D2-40¹⁰ or CD38.¹¹

Microvillus inclusion disease (MID) results from an autosomal recessive defect in apical plasma membrane recycling. The light microscopic appearance is usually that of villous atrophy, variable degrees of crypt hyperplasia, and a mild-to-moderate increase in inflammatory cells in the lamina propria. A distinct feature in contrast to normal (Figure 7), in MID there is the absence of an obvious brush border on luminal enterocytes (Figure 8). Although the periodic acid-Schiff (PAS) stain will suggest an absent positive staining at the apex of epithelial cells, along with the presence of cystic structures of variable size in the apical cytoplasm, the CD10 (Figure 8), carcinoembryonic antigen, and alkaline phosphatase IHC stains are all more sensitive.¹² Antibodies against a protein on the inner lining of endosomes, Rab11a, a guanosine triphosphatase protein, also give similar positive results.¹³ Electron microscopy is

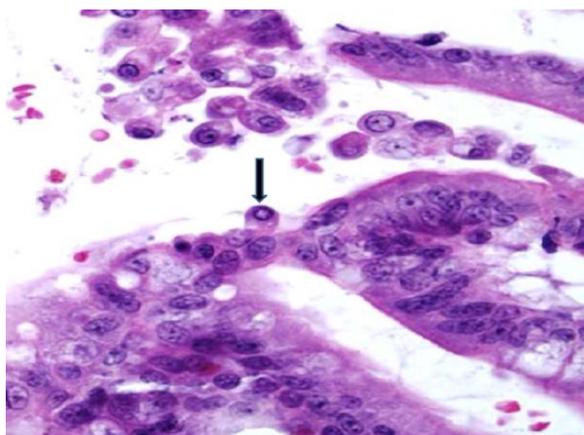


Figure 9. Tufting enteropathy with tufted enterocytes. Note preserved brush border in contrast to microvillus inclusion disease (HE x600).

usually confirmatory, but not necessary, if the above IHC stains are diagnostic. Electron microscopy will demonstrate both the denuded brush border and the endocytic vesicles with microvilli protruding inward.

Tufting enteropathy is an autosomal recessive condition, presenting shortly after birth, observed most frequently in newborns of Arabic descent. Other malformations and autoimmune manifestations may also be present in these patients. The abnormality in tufting enteropathy is a defect in the epithelial cell adhesion molecule (EpCAM), normally present in tight junction proteins between epithelial cells. The absence of a functional, morphologically intact tight junction results in rounded, “tufted” enterocytes (Figure 9). On light microscopy, the changes in epithelial cells evolve over time. The IHC shows abnormal staining for basement membrane laminin and heparan sulfate glycoprotein, along with decreased EpCAM gene expression and absent MOC31 (anti-EpCAM protein) staining.¹⁴

Enteric neuroendocrine cells are usually present deep in the crypts, admixed with enterocytes (Figure 10). They decrease in number or become undetectable in enteroendocrine cell dysplasia (ECD). ECD is an autosomal recessive condition affecting both the small and large bowels due to a mutation in the *NEUROG3* gene, which is a prerequisite for the phenotypic differentiation from enterocytes to endocrine cells. The endocrine cells will stain in a nonspecific fashion for chromogranin A (Figure 11). Loss of staining can be encountered in autoimmune enteropathy or autoimmune polyglandular syndrome I. Absent staining on IHC is also seen to specific endocrine products of the enteroendocrine cells, eg, lack of demonstrable cholecystikinin in autoimmune polyendocrinopathy candidiasis ectodermal dystrophy.

The autoimmune endocrinopathies are a mixed group of heterogeneous conditions, characterized by a variety of circulating anti-enterocyte autoantibodies, often associated with immunodeficiency disorders. The best known entity in this group is the IPEX syndrome (immune dysregulation, polyendocrinopathy, enteropathy, X-linked), with variants known as

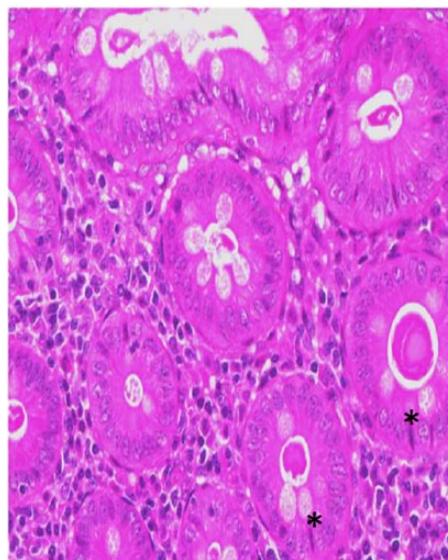


Figure 10. Normal colonic mucosa with neuroendocrine cells highlighted by * (HE x400).

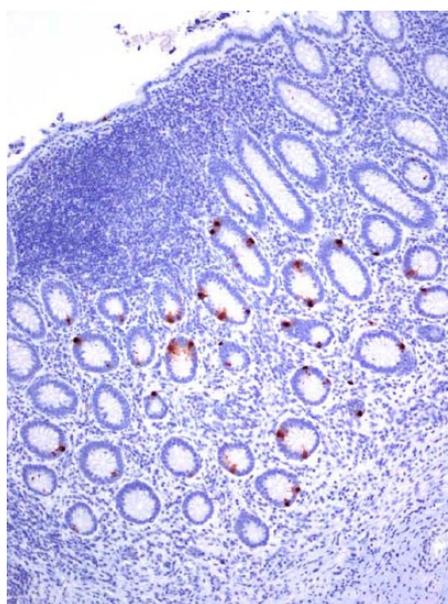


Figure 11. Normal colonic mucosa with neuroendocrine cells staining positive (Chromogranin x200).

IPEX-like conditions. The light microscopic appearance resembles that of graft-versus-host disease with marked increase in epithelial apoptosis (Figure 12) or CD-like changes of the intestinal epithelium, displaying villous atrophy, crypt hyperplasia, and a lymphocyte-predominant inflammatory infiltrate in the lamina propria. The simultaneous presence of colitis and gastritis makes IBD a differential diagnostic consideration. Paneth cells and goblet cells can be decreased in number or are absent altogether. In autoimmune enteropathy, (AIE) there is a mutation of the *FOXP3* gene; hence, immunostaining with antibodies against the protein product of the *FOXP3* gene might be used in patients suspected of having the IPEX syndrome. An IHC panel of CD10, EpCAM, and chromogranin is

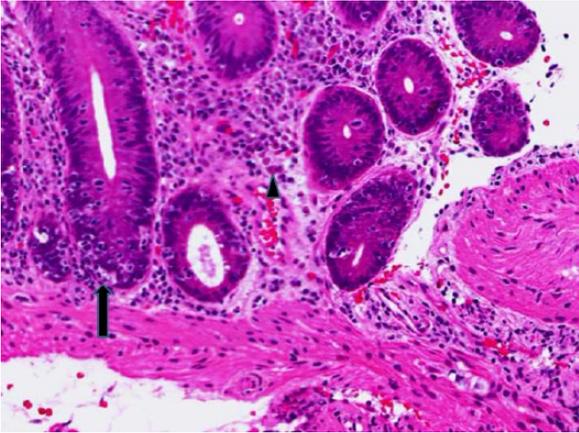


Figure 12. Autoimmune enteropathy. Small intestinal mucosa showing marked increase in apoptosis, loss of goblet cells and Paneth cells. Note normal lamina propria rich in plasma cells (HE x200).

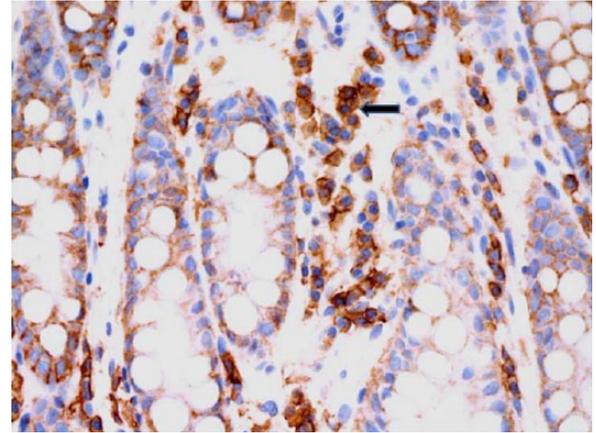


Figure 14. Normal colonic mucosa. Note ample plasma cells in lamina propria (CD138 x200).

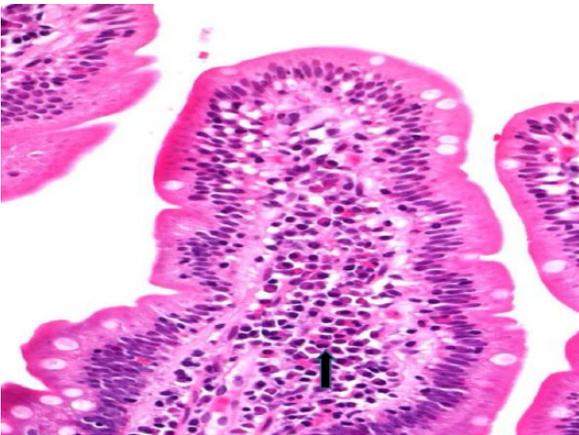


Figure 13. Normal duodenal mucosa. Note ample plasma cells in lamina propria (HE x200).

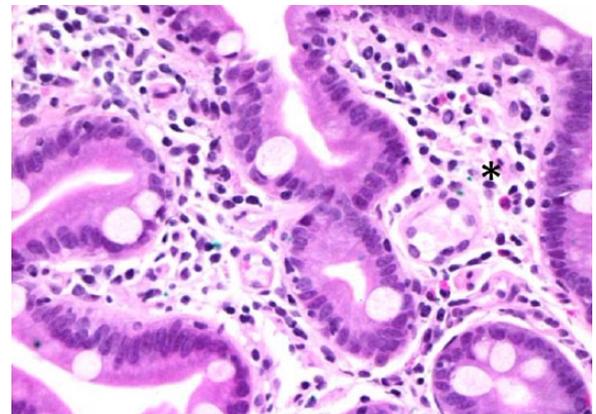


Figure 15. Common variable immunodeficiency with absence of plasma cells in the lamina propria (HE x400).

a useful screening tool for pediatric enteropathies.¹⁵ In addition, the IHC stains, harmonin and villin, have been described in IPEX syndrome.^{16,17}

Gastrointestinal mucosal pathology can be seen in association with primary immunodeficiency states as in patients with common variable immunodeficiency (CVID). In contrast to normal (Figures 13 and 14), the hallmark in these children is the absence of plasma cells in the lamina propria in the intestinal mucosa (Figure 15) and absence of villous architecture in the small intestine. In addition, opportunistic infectious diarrhea, most commonly related to *Giardia lamblia*, can be seen in CVID, X-linked hyper-IgM syndrome, X-linked agammaglobulinemia, and selective IgA deficiency. In the latter, an overlapping morphological finding identical to CD can be noted as well.

Appendix, Colon, Rectum

Nonspecific acute and chronic inflammation of the lower GI tract may be due to drugs (eg, antibiotics); microorganisms, including viruses such as CMV, parasites such as *Giardia*, and worms such as *Enterobius vermicularis* (Figure 16); or

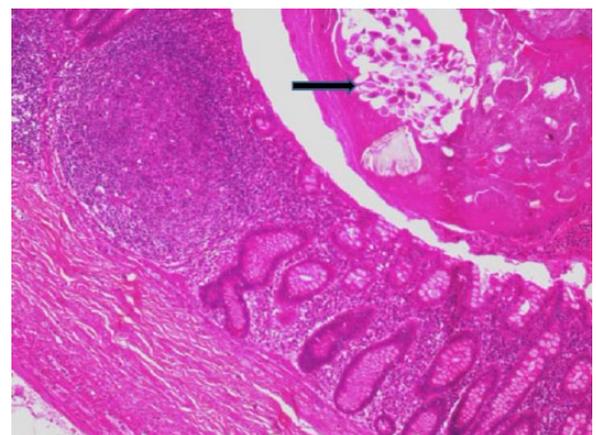


Figure 16. *Enterobius vermicularis* (pinworm) with several ova, present in lumen of appendix. (HE x200).

idiopathic. IHC is usually not required in the diagnosis of these conditions.

Inflammatory bowel disease (Crohn disease or ulcerative colitis) can involve the entire length of the large bowel, from appendix to anorectal area. The diagnosis rests on features identified on H&E sections, and IHC is usually not necessary.

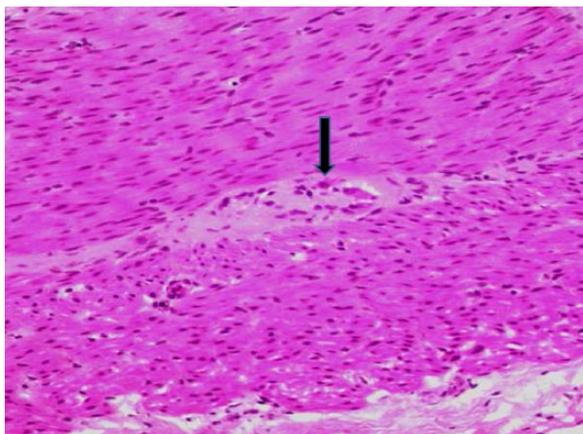


Figure 17. Normal muscularis propria with myenteric plexus formed of neuropil and ganglion cells (HE x200).

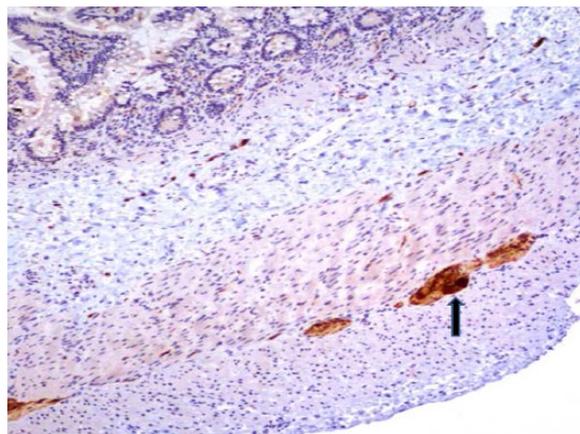


Figure 19. Normal muscularis propria with myenteric plexus including ganglion cells (Calretinin x200).

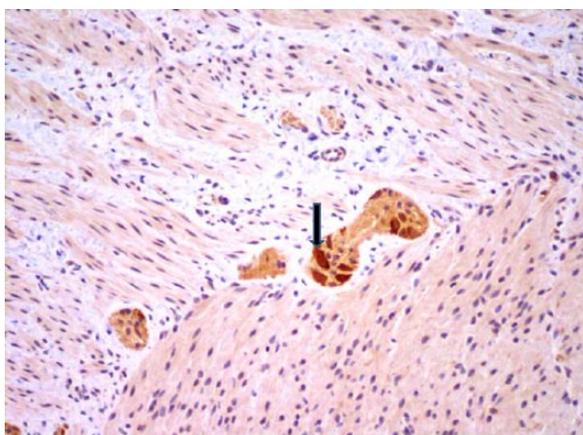


Figure 18. Normal muscularis propria with myenteric plexus including ganglion cells (MAP-2 x200).

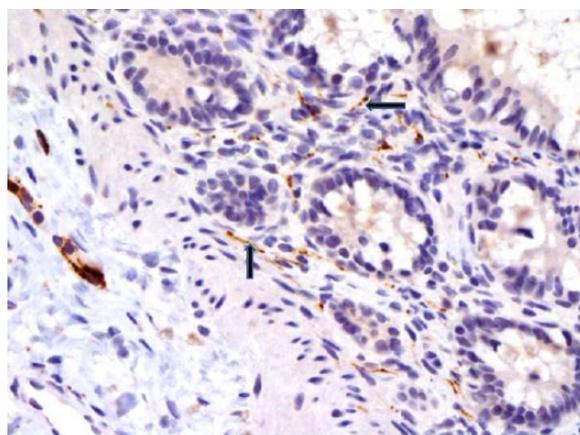


Figure 20. Normal colonic wall with mucosal Calretinin-positive thin nerve twigs (Calretinin x400).

The macrophages in pediatric Crohn disease can be highlighted by CD68 and CD40.¹⁸ Specific infections, such as granulomatous colitis or opportunistic infections, in immunocompromised patients can also be diagnosed without resorting to IHC stains. Histiocytes can be highlighted using the CD68 stain. Microorganisms may stain for special stains specific to them (eg, Gram, PAS, GMS, and Ziehl-Neelsen).

Hirschsprung disease (HD) (aganglionosis) or hypoganglionosis is a classic neonatal/pediatric condition that requires quantitative assessment of the intestinal ganglion cells in the myenteric (Figure 17) and/or submucosal plexus. An IHC panel that enhances the accuracy of diagnostic interpretation includes Map2 (Figure 18), calretinin (Figures 19 and 20), Glut-1, and S-100,¹⁹ and perhaps c-kit (Figure 21). This panel is sufficiently specific and sensitive in most cases to obviate the need for enzyme histochemical staining for acetylcholinesterase that requires frozen biopsy material. Faced with indeterminate or nonspecific findings, it is advisable to request additional biopsies for correct diagnostic interpretation. In HD, there will be reduced or absent staining of ganglion cells for antibodies against Map2 and calretinin, whereas the concomitant presence of hypertrophied nerve

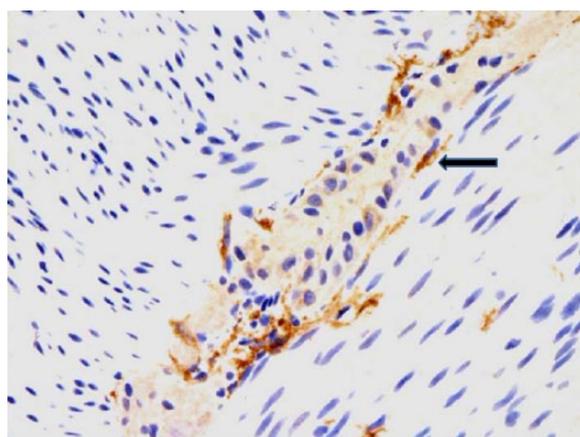


Figure 21. Normal muscularis propria with myenteric plexus bordered by cells of Cajal (CD117 x400).

fibers will be seen as a gain in staining of the perineurium by antibodies for Glut-1 and S-100. In addition, c-kit may reveal an altered distribution of the interstitial cells of Cajal.²⁰

Primary, isolated, intestinal neuronal dysplasia presents as abnormal nerve differentiation affecting the submucosal plexus,

characterized by hyperganglionosis, giant ganglia, and ectopic ganglion cells. IHC as used for HD (Map2, calretinin, Glut-1, S-100, c-kit) will be helpful in providing confirmatory details on light microscopy.

Hollow visceral myopathy is an uncommon entity in the everyday practice of pediatric pathology. It is characterized by attenuation or absence of degenerating smooth muscle fibers affecting mostly the external longitudinal layer of the muscularis propria. Anti-smooth muscle antibodies, such as alpha-smooth muscle actin and desmin, will define the partial or complete absence of smooth muscle fibers. Vimentin may be helpful in demonstrating any fibrosis replacing the preexisting smooth muscle fibers.

Posttransplant lymphoproliferative disease (PTLD) is a diagnostic consideration in pediatric patients who underwent stem cell or solid organ transplantation. One of the common sites of involvement by PTLD is the GI tract. Biopsy material obtained at endoscopy should be evaluated by the EBV latent membrane protein staining in the monotonous proliferation of lymphocytes. A panel of markers for the lymphocytic population is helpful in indicating the possible presence of nascent lymphoma, Burkitt lymphoma being most common in pediatric patients (Table 1). Flow cytometric analysis has limited value in double-hit lymphomas.

Histiocytic disorders of the GI tract in pediatric patients include a range of benign and neoplastic conditions (eg, xanthomatosis, juvenile xanthogranuloma, Whipple disease, malakoplakia, Rosai-Dorfman disease, Erdheim-Chester disease, and Langerhans cell histiocytosis²¹). A panel of immunohistochemical markers is found to be useful in the differential diagnosis of such a wide range of histiocytic disorders²² (see Table 1).

Metabolic disorders affecting the GI tract (eg, glycogen storage disorders, mucopolysaccharidoses, congenital disorders of glycosylation, Wolman disease, Tangier disease, Fabry disease, infantile Refsum disease, abetalipoproteinemia, and infantile systemic hyalinosis) can be characterized with special stains specific to the underlying condition(s) and do not require the use of IHC stains.

Liver

The most common pediatric nonneoplastic hepatic lesions, for which needle core or wedge biopsies may be submitted for microscopic evaluation, include primary sclerosing cholangitis (PSC), autoimmune hepatitis (AIH), various overlap syndromes, and duct paucity conditions, both sporadic (biliary atresia) and syndromic (eg, Alagille syndrome). Alpha-1 antitrypsin deficiency enters the differential, particularly for biliary atresia, ductopenia, or PSC as it may produce an obstructive pattern. In those instances, correlation with serum alpha-1 antitrypsin levels is crucial. Nevertheless, salient morphological findings of alpha-1 antitrypsin deficiency can be seen in the biopsy and IHC can be somewhat helpful (Figure 22). A caveat

is that morphological findings of alpha-1 antitrypsin deficiency in neonates are not well developed because the periodic acid-Schiff with diastase-positive periportal globules noted in hepatocytes (Figure 23) and not in macrophages (Figure 24) take about a period of 3 months to develop.

Rarely, if ever, is a biopsy obtained for infectious hepatitis in pediatric patients. Should such a specimen come to the pathologist, IHC for antibodies against hepatitis A (Vp3 antibody), hepatitis B (HBcAg, HBsAg), and hepatitis C (core antigen, envelope antigen, NS3 antigen) is available, if needed.

Primary sclerosing cholangitis in pediatric patients is a chronic cholestatic liver disease of unknown cause (likely autoimmune) with progressive inflammation and worsening fibrosis and cirrhosis of the intrahepatic and extrahepatic bile ducts. Recently, an increase in IgG4-positive plasma cells has been observed in hilar areas of explanted livers.²³ The status of the interlobular bile ducts on liver biopsies can be assessed with the CK7 IHC stain (Figure 25). A ratio of IgM to IgG >1 in inflammatory cells (lymphocytes and plasma cells) in periportal areas is reported as being able to reliably distinguish PSC from primary biliary cirrhosis (PBC) and the AIH-PBC overlap syndrome in more than 90% of cases.²⁴

Autoimmune hepatitis is a chronic inflammatory condition of the liver of unknown cause, more common in females, and is a diagnosis of exclusion (once PBC, PSC, and overlap syndromes have been excluded).²⁵ There is a mononuclear portal inflammation (lymphocytes, sometimes plasma cells predominating) and fibrosis with interface hepatitis and hepatocellular necrosis and bridging fibrosis or cirrhosis. The bile ducts are spared by the necroinflammatory process. The lymphocytes will be positive for CD3, and the plasma cells are predominantly IgG positive with a lesser number positive for IgM.²⁶

Originally, the overlap syndrome was considered an entity with some but not all features of PBC, on one hand, and of AIH, on the other hand, representing the 2 extremes. Over time, the concept of overlap syndrome has evolved and now includes conditions “overlapping” not only PBC and AIH but also PSC and AIH and even PSC and PBC. Presently, there are no clearly defined specific clinicopathologic features for either of these overlap conditions. The PBC and PSC tend to affect the bile ducts preferentially, whereas the AIH causes damage to the hepatocellular parenchyma that is the obvious feature on microscopy. Treatment has to be balanced and usually goes along the lines of the dominant entity. The IHC includes all stains as applied to both of the overlapping entities (CK7, CD3, IgG, IgM, etc).²⁷

Conditions displaying various degrees of bile duct proliferation (eg, neonatal hepatitis) or duct paucity or those conditions that enter in the differential diagnosis as TPN (total parenteral nutrition) may benefit from highlighting the interlobular bile ducts with the CK7 IHC stain (Figures 26-28). This characterization is particularly useful in conditions

Table 1. Immunohistochemical stains in nonneoplastic pediatric GI conditions.

DISEASE/LESION	IHC STAINS	RESULTS
Eosinophilic esophagitis	ALOX15, MBP	Cytoplasmic and extracellular granules of eosinophils
Barrett esophagus	CK7/CK20, MUC6, MUC5AC, MUC5B, MUC2	Metaplastic intestinal (columnar) mucosa positive
Cervical inlet patch	CK7/CK20, MUC6, MUC5AC, MUC5B, MUC2	Ectopic gastric mucosa; MUC2 negative (no intestinal metaplasia in children)
CMV esophagitis	Anti-CMV antibody	CMV nuclear inclusion (“owl-eye”) and cytoplasmic granules
EBV esophagitis	EBV-EBER EBV-LMP1	Positive in nucleus Positive in cytoplasm
<i>Helicobacter</i> gastritis	Anti- <i>H pylori</i> antibody	<i>Helicobacter pylori</i> in surface mucus (sensitive and specific)
Celiac disease	CD3, CD8 CD4	IELs in surface epithelium Lymphocytes in lamina propria
Dilated lacteals	D2-40, CD38	Lining endothelial cells
Microvillus inclusion disease	CD10, CEA, alkaline phosphatase Rab11a	Brush border Apical cytoplasm of enterocytes Inner lining of endosomes
Tufting enteropathy	MOC31 (EpCAM) Basement membrane laminin Heparan sulfate glycoprotein	Absent stain at tight junction Absent stain at tight junction Absent stain at tight junction
Autoimmune endocrinopathies	Chromogranin A, EpCAM, CD10 Specific endocrine markers (eg, cholecystokinin)	Loss of neuroendocrine cells in crypts and infundibular epithelium
IPEX syndrome	CD10, EpCAM, chromogranin, harmonin, villin	Absent Paneth cells and goblet cells, loss of brush border, intraepithelial lymphocytosis
Crohn disease	CD68, CD40	Macrophages in lamina propria
Granulomatous colitis	CD68	Histiocytes
Hirschsprung disease	Map2, calretinin Glut-1, S-100 c-kit (CD117)	Loss of staining for ganglion cells in myenteric and submucosal plexus Intense perineural staining Altered staining of interstitial cells of Cajal
Intestinal neuronal dysplasia	Map2, calretinin, Glut-1, S-100, c-kit	Hyperganglionosis, giant ganglia, and ectopic ganglion cells in the submucosal plexus
Hollow visceral myopathy	α -SMA, desmin, vimentin	Absent staining in muscularis propria, replaced by fibrosis
PTLD	EBV latent membrane protein IHC panel for Burkitt lymphoma (CD45, CD20, CD10, CD79, TdT, BCL2, BCL6, CD3, MUM-1, Cyclin-D1, Ki-67)	EBV in sheets of lymphocytes
Histiocytic disorders	CD68, CD163, CD21, CD23, CD35, factor XIIIa, A-1-AT, A-1-ACT, fascin, HAM-56	Histiocytes
Langerhans cell histiocytosis	S-100, CD1a, langerin	Langerhans cells

Abbreviations: α -SMA, alpha-smooth muscle actin; ALOX15, arachidonate 15-hydroxygenase; CEA, carcinoembryonic antigen; CMV, cytomegalovirus; EBER, EBV-encoded RNA; EBV, Epstein-Barr virus; EpCAM, epithelial cell adhesion molecule; GI, gastrointestinal; IELs, intraepithelial lymphocytes; IPEX, immune dysregulation, polyendocrinopathy, enteropathy, X-linked; LMP1, latent membrane protein 1; MBP, major basic protein; PTLD, posttransplant lymphoproliferative disease.

in which the number of bile ducts is variable or evolves over time (eg, biliary atresia, ductopenia whether syndromic or nonsyndromic [Alagille syndrome] bile duct formation at the interface in premature newborns).

In biliary atresia, the presence of natural killer (NK) cells in the portal infiltrate around interlobular bile ducts has been found to have an immunohistochemical profile of CD56(-)CD16(+)/CD68(-).²⁸ In addition, the damaged bile ducts

strongly express CX3CL1, attracting CD16(+) NK cells with CX3CR1 expression.

The extrahepatic choledochal cystic lesions, which may be located within or outside of the pancreas, will be either denuded or have a lining of low columnar biliary epithelial cells. The IHC is usually not required because the H&E slide is diagnostic, but if pursued, the lining epithelium will be reactive to antibodies against CK7.

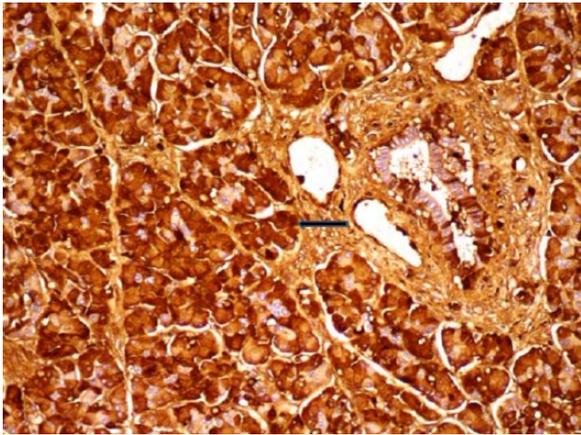


Figure 22. Liver parenchyma with Alpha-1-antitrypsin deficiency (Alpha-1-antitrypsin x400).

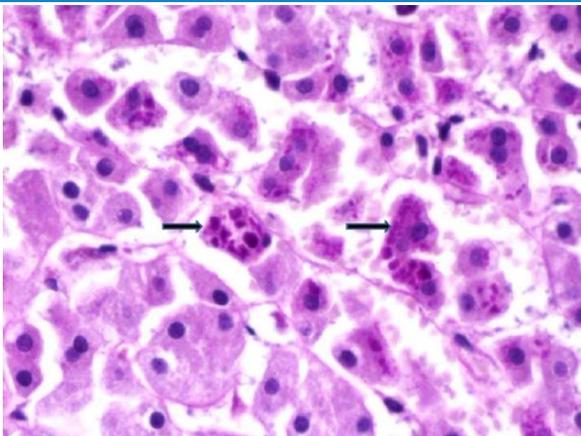


Figure 23. Liver parenchyma with Alpha-1-antitrypsin deficiency. Note periodic acid-Schiff with diastase-positive globules in hepatocytes (PASD x600).

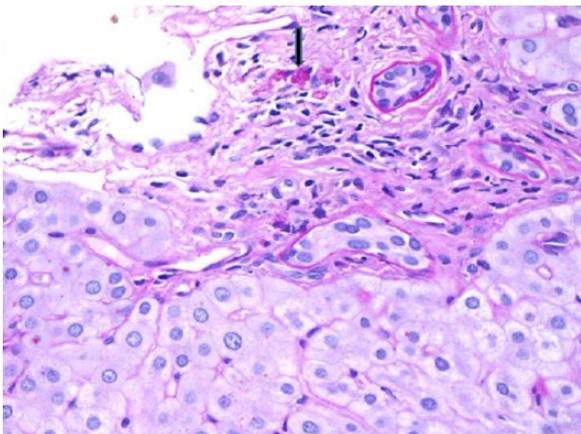


Figure 24. Ceroid macrophages in portal tract in a patient on total parenteral nutrition (PAS x400).

Pancreas

Nonneoplastic lesions of the pancreas are extremely rare and include cystic lesions, changes of cystic fibrosis, and pancreatitis. These entities usually only require routine H&E sections for diagnosis. Pancreatic cysts may need to be differentiated from mesenteric cysts. Cystic lesions in the pancreas are usually

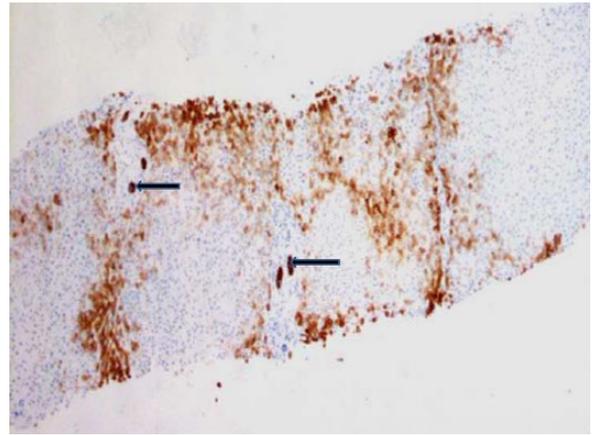


Figure 25. Primary sclerosing cholangitis. Early morphological change of obstructive pattern, manifesting as aberrant CK7 expression in hepatocytes. Note diminutive CK7-positive interlobular bile ducts (CK7 x100).

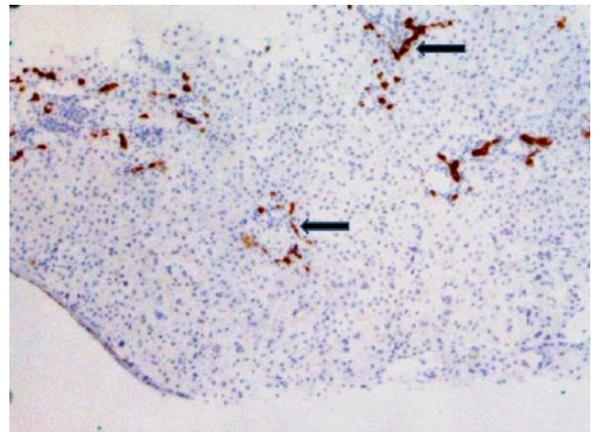


Figure 26. Primary extrahepatic biliary atresia, early stages. Note CK7 highlighting interlobular bile ducts in portal tracts, along with bile duct proliferation at the interface (CK7 x100).

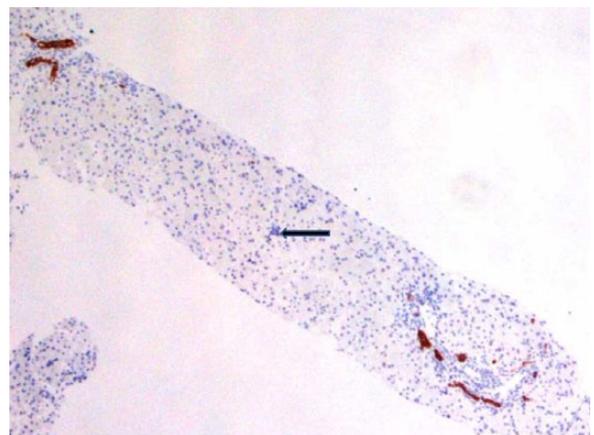


Figure 27. Alagille syndrome (syndromic ductopenia) in non-premature infant. There is absent bile duct proliferation at the interface as well as absent interlobular bile ducts in portal tracts (CK7 x100).

pseudocysts and lack an epithelial lining. The mesenteric cyst lining will be positive for CK7 and vimentin.

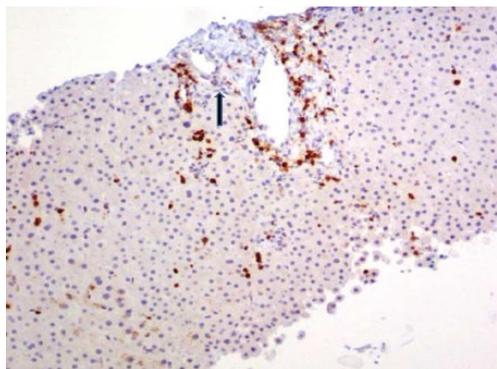
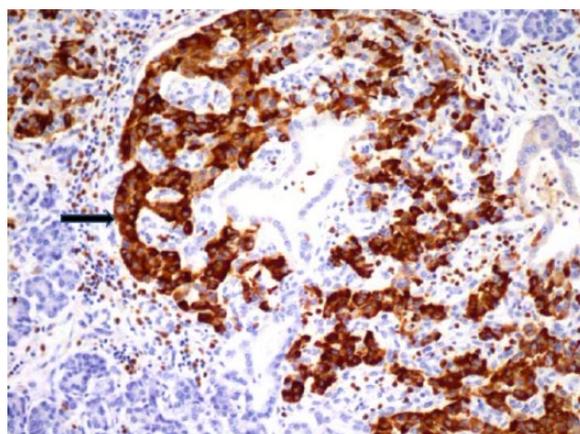
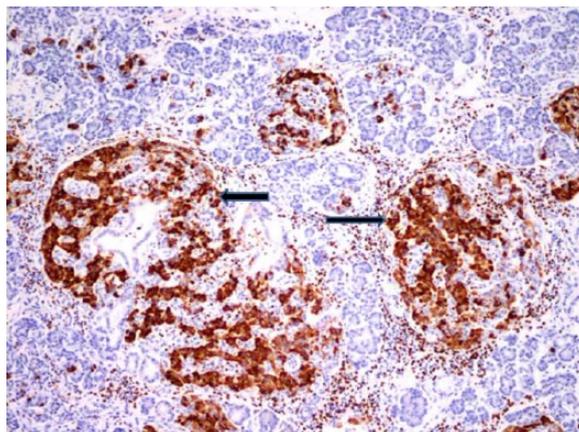
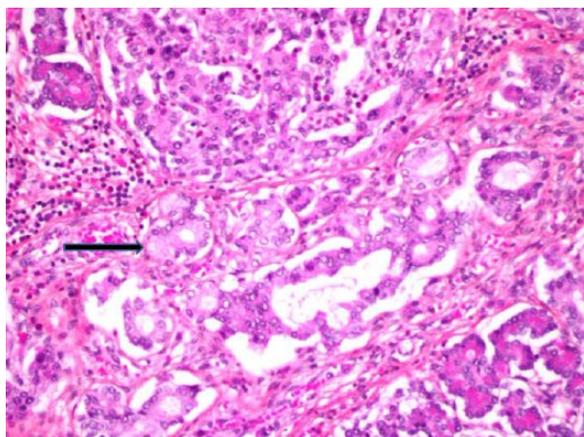
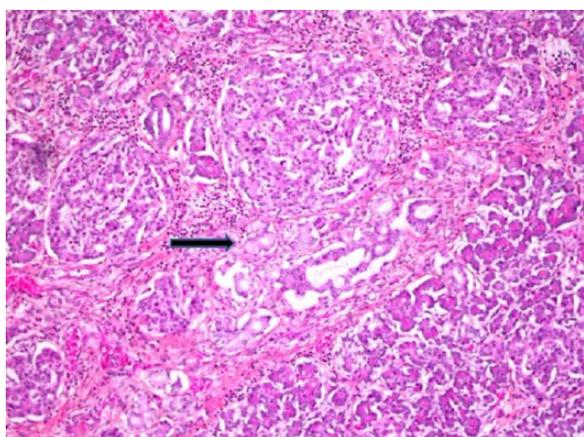


Figure 28. Liver needle biopsy of premature infant with trisomy 21. Status post duodenal atresia repair, clinically suspicious for primary atresia. CK7 highlighting absent bile duct proliferation at the interface along with preserved interlobular ducts in portal tracts in keeping with TPN induced changes (CK7 x100).



Figures 31 and 32. Islets of Langerhans in pancreas of neonate with diabetic fetopathy. Note markedly hyperplastic and hypertrophied islets (Insulin x200 and x400).



Figures 29 and 30. Nesidioblastosis. Pancreas of neonate with diabetic fetopathy (HE x200 and x400)

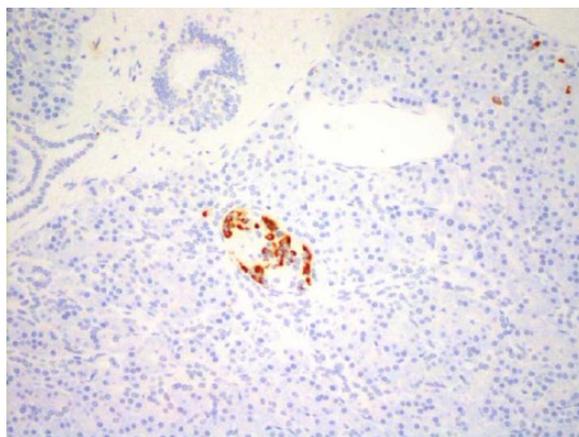


Figure 33. Normal pancreatic islets of Langerhans (Insulin x200).

Congenital hyperinsulinism (formerly, nesidioblastosis) is a developmental rather than a neoplastic condition. It consists of budding islands of epithelial cells off pancreatic endocrine ducts and islets of Langerhans, present in septa (Figures 29 and 30).

These aberrant nests of epithelial cells come about as a result of β -cell hypertrophy and can be highlighted by proinsulin (Figures 31 and 32) compared with normal islets (Figure 33), neuron-specific enolase, chromogranin, and synaptophysin²⁹ (Table 2).

Table 2. Immunohistochemical stains in nonneoplastic pediatric hepatobiliary and pancreatic conditions.

DISEASE/LESION	IHC STAINS	RESULTS
Bile duct proliferation	CK7, CK20	Interlobular bile ducts
Bile duct paucity	CK7, CK20	Interlobular bile ducts
Biliary atresia Alagille syndrome	CK7, CK20 CD56, CD16, CD48	Interlobular bile ducts NK cells around damaged interlobular bile ducts
Choledochal cyst	CK7	Biliary epithelium lining the cyst
Pancreatic (pseudo) cyst	CK7, vimentin	Absent epithelial lining (negative IHC staining)
Congenital hyperinsulinemia (nesidioblastosis)	Proinsulin, chromogranin, synaptophysin, NSE	Aberrant ductuloinsular proliferation of insulin-producing β cells

Abbreviations: NK, natural killer; NSE, neuron-specific enolase.

Author Contributions

Joseph de Nanassy, Lead Author; Dina El Demellawy, Senior Author.

REFERENCES

- Matoso A, Allen D, Herzlinger M, et al. Correlation of ALOX15 expression with eosinophilic or reflux esophagitis in a cohort of pediatric patients with esophageal eosinophilia. *Hum Pathol.* 2014;45:1205–1212.
- Dellon ES, Chen X, Miller CR, Woosley JT, Shaheen NJ. Diagnostic utility of major basic protein, eotaxin-3, and leukotriene enzyme staining in eosinophilic esophagitis. *Am J Gastroenterol.* 2012;107:1503–1511.
- Lauwers GY, Mino M, Ban S, et al. Cytokeratins 7 and 20 and mucin core protein expression in esophageal cervical inlet patch. *Am J Surg Pathol.* 2005;29:437–442.
- Mowry SE, Strocker AM, Chan J, et al. Immunohistochemical analysis and Epstein-Barr virus in the tonsils of transplant recipients and healthy controls. *Arch Otolaryngol Head Neck Surg.* 2008;132:936–939.
- Patnayak K, Reddy V, Jena A, Rukmangadha N, Panhasarathy S, Reddy MK. Utility of immunohistochemistry in demonstrating *Helicobacter pylori*. *Oncol Gastroenterol Hepatol.* 2015;4:4–7.
- Ushiku T, Moran CJ, Lauwers GY. Focally enhanced gastritis in newly diagnosed pediatric inflammatory bowel disease. *Am J Surg Pathol.* 2013;37:1882–1888.
- Mubarak A, Spierings E, Wolters VM, Otten HG, ten Kate FJ, Houwen RH. Children with celiac disease and high tTGA are genetically and phenotypically different. *World J Gastroenterol.* 2013;19:7114–7120.
- Villanacci V, Ceppa P, Tavani E, Vindigni C, Volta U. Coeliac disease: the histology report. *Dig Liver Dis.* 2011;43:S385–S395.
- Mubarak A, Wolters VM, Gmelig-Meyling FH, Ten Kate FJ, Houwen RH. Tissue transglutaminase levels above 100 U/mL and celiac disease: a prospective study. *World J Gastroenterol.* 2012;18:4399–4403.
- Alexander JS, Vijay CG, Jordan PA, Witte MH. Gastrointestinal lymphatics in health and disease. *Pathophysiology.* 2010;17:315–335.
- Farstad I, Malavasi F, Haraldsen G, Huitfeldt H, Brandtzaeg P. CD38 is a marker of human lacteals. *Vircow's Arch.* 2002;441:605–613.
- Groisman GM, Amar M, Livne E. CD10: a valuable tool for the light microscopic diagnosis of microvillous inclusion disease (familial microvillous atrophy). *Am J Surg Pathol.* 2002;26:902–907.
- Talmon G, Holzapfel M, DiMaio DJ, Muirhead D. Rab11 is a useful tool for the diagnosis of microvillous inclusion disease. *Int J Surg Pathol.* 2012;20:252–256.
- Ranganathan S, Schmitt LA, Sindhi R. Tufting enteropathy revisited: the utility of MOC31 (EpCAM) immunohistochemistry in diagnosis. *Am J Surg Pathol.* 2014;38:265–272.
- Martin BA, Kerner JA, Hazard FK, Longacre TA. Evaluation of intestinal biopsies for pediatric enteropathy: a proposed immunohistochemical panel approach. *Am J Surg Pathol.* 2014;38:1387–1395.
- Lampasona V, Passerini L, Barzaghi F, et al. Autoantibodies to harmonin and villin are diagnostic markers in children with IPEX syndrome. *PLoS ONE.* 2013;8:e78664.
- Russo P. Enteropathies of infancy and childhood. *Surg Pathol Clin.* 2010;3:603–640.
- Perminow G, Reikvam DH, Lyckander LG, Brandtzaeg P, Vatn MH, Carlsen HS. Increased number and activation of colonic macrophages in pediatric patients with untreated Crohn's disease. *Inflamm Bowel Dis.* 2009;15:1368–1378.
- Bachmann L, Besendörfer M, Carbon R, et al. Immunohistochemical panel for the diagnosis of Hirschsprung's disease using antibodies to MAP2, calretinin, GLUT1 and S100. *Histopathology.* 2015;66:824–835.
- Barshak I, Fridman E, Goldberg I, Chowers Y, Kopolovic J. The loss of calretinin expression indicates aganglionosis in Hirschsprung's disease. *J Clin Pathol.* 2004;57:712–716.
- Singhi AD, Montgomery EA. Gastrointestinal tract langerhans cell histiocytosis: a clinicopathologic study of 12 patients. *Am J Surg Pathol.* 2011;35:305–310.
- Detlefsen S, Fagerberg CR, Ousager LB, et al. Histiocytic disorders of the gastrointestinal tract. *Hum Pathol.* 2013;44:683–696.
- Fisher S, Trivedi PJ, Ward S, Greig PD, Therapondos G, Hirschfield GM. Frequency and significance of IgG4 immunohistochemical staining in liver explants from patients with primary sclerosing cholangitis. *Int J Exp Pathol.* 2014;95:209–215.
- Lee H, Stapp RT, Ormsby AH. The usefulness of IgG and IgM immunostaining of periportal inflammatory cells (plasma cells and lymphocytes) for the distinction of autoimmune hepatitis and primary biliary cirrhosis and their staining pattern in autoimmune hepatitis-primary biliary cirrhosis overlap syndrome. *Am J Clin Pathol.* 2010;133:430–437.
- Wolf DC. Autoimmune hepatitis (ed. Anand BS). *Medscape.* October 31, 2014 emedicine.medscape.com/article/172356-overview. Updated February 18, 2016.
- Moreira RK, Revetta F, Koehler E, Washington MK. Diagnostic utility of IgG and IgM immunohistochemistry in autoimmune liver disease. *World J Gastroenterol.* 2010;16:453–457.
- Trivedi PJ, Hirschfield GM. Review article: overlap syndromes and autoimmune liver disease. *Aliment Pharmacol Ther.* 2012;36:517–533.
- Okamura A, Harada K, Nio M, Nakanuma Y. Participation of natural killer cells in the pathogenesis of bile duct lesions in biliary atresia. *J Clin Pathol.* 2013;66:99–108.
- Barthlen W, Brecht IB, Sipsos B. Pancreatic tumors. In: Schneider DT, Brecht IB, Olson TA, Ferrari A, eds. *Rare Tumors in Children and Adolescents.* New York, NY: Springer; 2012:288.