Pathophysiology and Diseases of the Proximal Pathways of the Biliary System

Yukihiro Nakanishi, MD, PhD; Romil Saxena, MBBS, FRCPath

- Context.—Diseases of the proximal pathways of the biliary system can be divided into those that affect the interlobular bile ducts and those that affect the bile canaliculi. The former include primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), graft-versus-host disease (GVHD), and drug-induced liver injury. The latter include progressive familial intrahepatic cholestasis, benign recurrent intrahepatic cholestasis, intrahepatic cholestasis of pregnancy, and drug-induced liver injury.

- Objective.—To summarize the current state of knowledge of diseases of the proximal pathways of the biliary system, with special emphasis on clinical presentation, pathological features, and differential diagnosis.

- Data Sources.—Clinicopathological information was extracted from pertinent published literature.

- Conclusions.—Care of the patient with cholestasis hinges on identifying the etiology. Diagnostic steps in cholestatic conditions comprise a thorough patient history, abdominal imaging, distinct serological studies, and liver biopsy. Primary biliary cirrhosis is characterized by distinctive serological and histological findings. The small-duct variant of primary sclerosing cholangitis is very rare and difficult to diagnose; imaging of the bile ducts is not helpful. Graft-versus-host disease is characterized by damage and loss of intrahepatic bile ducts. Drugs can cause injury variably at the level of bile canaliculus or the interlobular bile duct. Loss of bile ducts may be seen with primary biliary cirrhosis, primary sclerosing cholangitis, graft-versus-host disease, and drug-induced liver injury. Progressive familial intrahepatic cholestasis and progressive familial intrahepatic cholestasis represent 2 extreme ends of the spectrum of abnormalities in transporters responsible for bile formation. Intrahepatic cholestasis of pregnancy has a variable incidence in different parts of the world and may be due to abnormalities in transporter molecules.


Diseases of the proximal pathways of the biliary system include (1) those that affect the interlobular bile ducts and (2) those that affect the bile canaliculi. The former include primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), graft-versus-host disease (GVHD), and drug-induced liver injury (DILI). The latter include progressive familial intrahepatic cholestasis (PFIC), benign recurrent intrahepatic cholestasis (BRIC), intrahepatic cholestasis of pregnancy (ICP), and DILI. We will focus on the current state of knowledge of these, with special emphasis on clinical presentation, pathological features, and differential diagnosis.

ANATOMY AND PHYSIOLOGY OF THE INTRAHEPATIC BILIARY SYSTEM

The intrahepatic course of the biliary tree consists of 7 to 10 orders of cholangiographically visible bile ducts. The first-order septal ducts are represented by the right and left hepatic ducts, and the third-order septal ducts correspond to the smallest cholangiographically visible ducts. Septal ducts measure more than 100 μm in diameter and converge to form segmental ducts, which measure 400 to 800 μm in diameter. The smallest bile ducts, the interlobular bile ducts, which measure less than 100 μm in diameter, are not visualized radiographically. These are the smallest portion of the biliary tree that can be readily visualized on a routine hematoxylin-eosin (H&E) stain of a normal liver biopsy, where they appear as round structures with a central lumen lined by cuboidal cholangiocytes with round, evenly spaced nuclei (Figure 1, A and B).

Bile produced by hepatocytes drains first into bile canaliculi, which thus represent the smallest pathway of bile flow, but these are not readily visible on an H&E stain. Bile canaliculi, trenchlike depressions lined by microvilli and sealed by tight junctions, are tiny tissue spaces 1 to 2 μm wide formed by the apical membranes of adjacent hepatocytes. Not normally visualized on H&E stain, they become visible when distended by bile or contrasted by pale cytoplasmic staining of hepatocytes. Bile canaliculi can also be highlighted by polyclonal carcinoembryonic antigen and CD10 immunostains, which reveal a delicate framework of linear and branching structures weaving between hepatocytes, creating the so-called canalicular pattern of staining (Figure 2). This canalicular pattern is diagnostic of hepato-
Figure 1. A terminal portal tract containing an interlobular bile duct (arrow) accompanied by a terminal hepatic arteriole (arrowhead). The portal venule is marked by an asterisk. A, Hematoxylin-eosin, original magnification ×200. B, Masson trichrome, original magnification ×200.

Figure 2. Immunohistochemical stain for CD10 (original magnification ×200) shows a delicate framework of bile canaliculi, which appear as linear and branching structures, creating the so-called canalicular pattern of staining.

Figure 3. Immunohistochemical stain for K7 shows an interlobular bile duct (long arrow), bile ductules (arrowheads), and canals of Hering (short arrows), which appear as single or small groups of cells (hematoxylin-eosin, original magnification ×200).

Figure 4. Florid duct lesion in primary biliary cirrhosis comprising an epithelioid cell granuloma centered on a bile duct, which shows evidence of epithelial damage (granulomatous cholangitis) (hematoxylin-eosin, original magnification ×200).

Figure 5. A bile duct showing concentric periductal fibrosis (onion skin fibrosis) and biliary epithelial damage in primary sclerosing cholangitis (hematoxylin-eosin, original magnification ×200).
cellular differentiation (eg, in identifying a tumor as being of hepatocellular origin). At the outer third of the lobule, bile canalicular drain into the canals of Hering, which are lined on one side by hepatocytes and on the other by cholangiocytes. Not normally visible on an H&E stain, they are highlighted by immunohistochemical stains for the biliary keratins K7 and K19, where they appear as small groups or strings of cuboidal cells in the perilobular regions (Figure 3). The canals of Hering drain into bile ductules, which are larger channels with a circumferential lining of cholangiocytes, which in turn drain into interlobular bile ducts. The canals of Hering, representing the de facto hepatobiliary interface, are believed to contain the proliferative cellular compartment of the liver and may be responsible for the ductular reaction seen in chronic biliary diseases. As the most proximal part of the biliary system with a cholangiocytic component, the canals of Hering are targets of the autoimmune process that primarily attacks and destroys small bile ducts in PBC and of hepatic drug toxicity caused by methotrexate.

Bile formation by hepatocytes involves the active transport of bile salts, phospholipids, cholesterol, and other organic solutes from the sinusoidal blood into the biliary canalicular, which is mediated by various active transporters. Hepatic uptake of organic solutes from the substrate-rich portal blood is facilitated by transport systems located on the basolateral membrane. These can be classified into sodium-dependent and sodium-independent transport systems. The transporters responsible for bile formation are located on the canalicular membrane of hepatocytes. Their function requires significant energy expenditure in the form of adenosine triphosphate (ATP), and most transporters belong to the ATP-binding cassette (ABC) transporter superfamily such as ATPB1 (ATPase class I type B1), ABCB11 (ABC, subfamily B, member 11), and ABCB4 (ABC, subfamily B, member 4), all of which are responsible for 3 types of PFIC, respectively. A number of other transporters facilitate the biliary excretion of specific substances. Copper is excreted in bile, and copper accumulation occurs in the liver in chronic cholestasis of any etiology. Copper is bound with proteins and typically accumulates in the lysosomes of peribiliary hepatocytes. It can be demonstrated by rhodamine stain (positive orange granules) or indirectly by orcein stain, which highlights copper-binding protein. ATP7B, an intracellular transporter of copper, is well known to be defective in Wilson disease.

**DISEASES OF TERMINAL BILE DUCTS**

**Primary Biliary Cirrhosis**

Primary biliary cirrhosis is an organ-specific autoimmune disease of the liver characterized by the presence of serum anti-mitochondrial antibodies (AMAs) and chronic nonsuppurative destructive cholangitis involving small-sized and medium-sized bile ducts with progressive fibrosis, leading to cirrhosis. It is the most common cause of chronic cholestatic liver disease in adults.

**Clinical Features.**—Primary biliary cirrhosis most commonly affects middle-aged women, with a female to male ratio ranging from 9 to 14 women to every 1 man. The disease is more common in individuals with family history of PBC or other autoimmune disorders. The prevalence among first-degree relatives is 5% to 6%. The incidence is higher in the United States and northern Europe and is low in Africa and the Indian subcontinent. The most common associations of autoimmune diseases are Sjögren syndrome, CREST (calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia) syndrome, scleroderma, and autoimmune thyroiditis. The characteristic liver enzyme abnormalities include elevated alkaline phosphatase level with normal to moderately elevated aspartate aminotransferase and alanine aminotransferase levels. Elevated IgM is also characteristic. Anti-mitochondrial antibodies are the serological hallmark of PBC and can be detected even before the appearance of clinical symptoms or biochemical abnormalities. Nine types of AMAs (M1–M9) are known, with M2 type being highly specific for PBC. Anti-mitochondrial antibodies are absent in 5% to 10% of patients with clinical and pathological features of PBC. This condition has been named AMA-negative PBC. Anti-nuclear antibodies are present in about 30% of PBC cases. Autoantibodies, including smooth muscle antibody, anti-actin antibody, rheumatoid factor, and anti-thyroid antibody, can also be present in patients with PBC. Progressive fibrosis leading to cirrhosis occurs during a 15-year to 20-year period. Progression to portal hypertension and liver failure occurs in 10% to 20% and 25% among symptomatic patients, respectively, compared with less than 5% in asymptomatic patients. A meta-analysis revealed that PBC is closely associated with a greater risk of overall cancer and hepatocellular carcinoma but not with other cancers.

**Pathological Features.**—Nonsuppurative cholangitis is the hallmark of PBC. Primary biliary cirrhosis typically affects interlobular bile ducts that are less than 100 μm in diameter. The pathognomonic lesion called the florid duct lesion is an epithelioid cell granuloma centered on a bile duct, which shows evidence of epithelial damage (granulomatous cholangitis) (Figure 4). This lesion tends to be focal and may not always be present in a biopsy. Lymphocytic cholangitis with bile duct damage is not uncommon in clinically well-characterized cases of PBC. Bile ductular reaction, induced by the bile duct injury, occurs with neutrophilic response, which does not signify infection. Septal and larger bile ducts may show inflammation and epithelial injury but are usually not lost. Bile duct loss and ductopenia primarily involve the small intrahepatic ducts. Bile duct injury is accompanied by portal inflammation composed primarily of lymphocytes and plasma cells. The latter can be numerous and do not necessarily indicate autoimmune hepatitis (AIH). Lymphoid follicles can be seen, occasionally with germinal centers. Hepatic cellular damage is typically minimal, and acidophilic bodies are few or absent. Interface activity can be prominent, mimicking viral or autoimmune hepatitis. Progressive ductular reaction, cholate stasis (bile acid accumulation causing foamy appearance of hepatocytes with wispy cytoplasmic threads [feathery degeneration]), and accumulation of copper and copper-binding protein, representing the morphological features of chronic cholestasis, are accompanied by fibrosis, which eventually leads to micronodular cirrhosis.

**Differential Diagnosis.**—The differential diagnosis includes the following.

**Mechanical Large Bile Duct Obstruction.**—Cholestasis is generally present in a centriflobular distribution in acute mechanical bile duct obstruction. However, cholestasis is not seen in early PBC. Both ultrasonography and computed tomography can readily identify extrahepatic biliary obstruction. Anti-mitochondrial antibodies are negative in obstructive biliary disease.
Primary Sclerosing Cholangitis.—Periductal fibrosis and fibroinflammatory obliteration of medium-sized and large-sized bile ducts are characteristic findings in PSC. Granulomas may be seen and are usually associated with sites of bile duct rupture and bile leak. History of inflammatory bowel disease (IBD) strongly favors PSC. Autoantibodies may be present in PSC, but AMAs are negative. The gold standard for the diagnosis of PSC is the typical “beaded” appearance of the biliary tree on cholangiography caused by alternate stricturing and proximal dilatation of the bile ducts.

Drug-Induced Liver Injury.—Florid duct lesion has rarely been reported in cholestatic DILI. However, AMAs are negative. The most common drugs associated with cholestasis are estrogens and androgenic steroids, phenothiazines, antibiotics such as erythromycin and amoxicillin-clavulanate, anticonvulsants, and tricyclic antidepressants.

Autoimmune Hepatitis.—Plasma cells can be numerous in both AIH and PBC. The presence of prominent interface hepatitis with hepatocellular injury and intact bile ducts is most helpful in distinguishing AIH from PBC. Elevation of serum IgG rather than IgM is typically seen in AIH. The typical autoantibody profile in type 1 AIH is anti-nuclear antibody positive, smooth muscle antibody positive, and AMA negative. However, AMAs may be seen in up to 20% of cases, usually in a low titer, whereas anti-nuclear antibodies and smooth muscle antibodies may be present in low titers in PBC. A PBC-AIH overlap syndrome has been reported. It has been proposed that the diagnosis of PBC-AIH overlap syndrome should be made if 2 of 3 characteristic features of PBC (alkaline phosphatase elevation 5 times above normal, presence of serum AMA, and florid duct lesion) and AIH (alanine aminotransferase elevation 5 times above normal, IgG elevation twice normal or presence of anti-smooth muscle antibody, and moderate to severe lymphocytic interface activity) are present. Based on these criteria, approximately 10% of patients with PBC have overlapping features of AIH.

Viral Hepatitis.—Bile duct epithelial injury with cytoplasmic swelling, vacuolation and acidophilia, nuclear pleomorphism, and loss of nuclear polarity has been described in approximately 30% of patients with chronic hepatitis C. Granulomas in portal tracts can also occur in chronic hepatitis C, especially in patients on interferon therapy. Positive viral serology helps establish the diagnosis of viral hepatitis.

Granulomatous Conditions.—In PBC, well-formed epithelioid granulomas can also occur in the parenchyma and portal tracts away from the bile ducts, creating confusion with other granulomatous disorders such as infections, drugs, and sarcoidosis. However, these conditions are AMA negative, and bile duct injury is rarely seen.

Primary Sclerosing Cholangitis

Primary sclerosing cholangitis is a chronic inflammatory disorder causing obliterative fibrosis and ectasia of the intrahepatic biliary tree, the extrahepatic biliary tree, or both. The common form of PSC affects the larger bile ducts, both extrahepatic and intrahepatic, whereas about 5% of cases comprise the small-duct variant, which affects the interlobular bile ducts. However, PSC is discussed herein because, even in the usual form that affects large bile ducts, smaller bile ducts show changes as a consequence of large-duct involvement. The etiology of PSC is not fully understood, which explains the lack of effective medical therapy for this condition. Primary sclerosing cholangitis is the second most common cause of chronic cholestatic liver disease in adults.

Clinical Features.—Up to 80% of patients with PSC have IBD, predominantly ulcerative colitis, and approximately 4% of patients with ulcerative colitis have coexisting PSC. In a recent cohort (2003–2007), most patients were diagnosed with PSC first; in an earlier cohort (1993–1997), most patients were diagnosed with IBD first. Patients are often diagnosed incidentally, and almost 50% are asymptomatic. Unlike other autoimmune diseases, PSC is characterized by poor or no response to immunosuppression, male preponderance, and poor correlation of serum antibodies with clinical and laboratory parameters of the disease. Primary sclerosing cholangitis predominantly affects men, with a 2:1 male to female ratio, and typically presents in the fourth and fifth decades of life. It is more common in Caucasians and northern Europeans than in southern Europeans, Asians, or Africans. Laboratory tests typically show increased levels of serum alkaline phosphatase and gamma-glutamyl-transferase more than those of alanine aminotransferase or aspartate aminotransferase levels. Serum IgM levels tend not to be raised. Primary sclerosing cholangitis is associated with autoantibodies, most frequently perinuclear anti-neutrophil cytoplasmic antibody. Characteristic findings on endoscopic retrograde cholangiopancreatography or magnetic resonance cholangiopancreatography include multifocal strictures giving rise to a typical “beading” appearance of large intrahepatic and extrahepatic bile ducts. As the disease advances and loss of ducts occurs, the biliary tree assumes an appearance like a “pruned tree.” The clinical course is progressive with development of biliary cirrhosis. When patients are symptomatic on presentation, the median time of survival from diagnosis to death or transplantation is reported to be 8 to 9 years. The severity of PSC does not correlate with the severity of the associated IBD, and treatment of one has little effect on the other. Patients with both PSC and IBD are at an increased risk of developing colorectal cancer (odds ratio 5.00; 95% confidence interval, 2.80–8.95 compared with patients without PSC) and cholangiocarcinoma (odds ratio, 55.31; 95% confidence interval, 22.20–137.60 compared with patients without PSC). The prevalence of cholangiocarcinoma in patients with PSC is between 7% and 13%. Occasionally, patients present with histological features of PSC in the setting of chronic colitis but have normal cholangiograms. These patients are considered to have small-duct PSC and comprise approximately 5% of all PSC patients. An estimated 20% of subjects with small-duct PSC develop large-duct PSC during a period of 7 to 10 years.

Pathological Features.—Primary sclerosing cholangitis involves large bile ducts, and cholangiographic findings of large-duct damage form the mainstay of diagnosis. Typically, a liver biopsy is not required to diagnose PSC unless small-duct PSC is suspected. Primary sclerosing cholangitis is characterized by inflammatory destruction of the biliary tree with concentric “onion skin” periductal fibrosis (Figure 5) and eventual loss of bile ducts, with complete replacement of bile ducts by fibrous scars. Portal biliary fibrosis evolves into periportal and bridging fibrosis, which eventually leads to cirrhosis. The changes are uneven in distribution and chronology. Concentric onion skin periductal fibrosis is typically observed in medium-sized and large-sized bile ducts and may or may not be observed in...
core needle liver biopsy specimens. Bile duct ulceration with bile extravasation and xanthogranuloma formation may be seen in large hilar bile ducts. There may be heavy infiltration of adjacent vascular structures (eg, portal vein phlebitis). The combination of bile duct and vascular obstruction manifests as areas of parenchymal atrophy, fibrosis, and nodular regenerative hyperplasia. Needle biopsy samples often show changes secondary to injury of major bile ducts. Early indirect portal changes include mild portal fibrosis and edema, with a minimal mixed inflammatory cell infiltrate and increased ductular profiles. At later stage, biliary interface activity becomes more prominent, with florid ductular reaction and overt cholate stasis with liver cell ballooning, Mallory hyaline formation, and accumulation of copper and copper-binding protein. Canaliculal cholestasis is usually evident in end-stage livers. Its presence may indicate a dominant stricture, cholangiocarcinoma, or decompenensation.

Differential Diagnosis.—The differential diagnosis includes the following.

Primary Biliary Cirrhosis.—The distinction between PSC and PBC is described in the differential diagnosis of PBC. However, this distinction may be difficult on liver biopsy specimens.

Autoimmune Hepatitis.—Parenchymal collapse or dense lymphoplasmacytic inflammation could suggest the possibility of AIH. On the other hand, copper-associated protein deposition and ductular reaction could suggest PSC. The distinction relies on clinical, laboratory, serological, and imaging findings as described above. An AIH-PSC overlap is well recognized, particularly in children and young adults. The AIH-PSC overlap syndrome is found in 6% to 8% of patients with PSC or AIH.29

IgG4-Related Sclerosing Cholangitis.—IgG4-positive plasma cells are sparse in the affected bile ducts of PSC. Fibrosis is more dense and old in PSC. IgG4-related sclerosing cholangitis is frequently associated with sclerosing pancreatitis (autoimmune pancreatitis). Because (unlike PSC) IgG4-related sclerosing cholangitis responds well to steroid therapy, its diagnosis and distinction from PSC are important.

Hepatolithiasis and Recurrent Pyogenic Cholangitis.—Hepatolithiasis can be complicated by superimposed bacterial infection and supplicative cholangitis. These may show similarities to PSC.

Secondary Sclerosing Cholangitis.—Primary sclerosing cholangitis may be indistinguishable from secondary sclerosing cholangitis clinically, radiologically, and histologically. In adults, exclusion of secondary causes is requisite to establishing a diagnosis of PSC; these include trauma, ischemia, surgery, chronic biliary infection in patients with primary or acquired immunodeficiency, rupture of hydatid cyst, irradiation, tumor, or portal vein pathology such as portal cavernoma with biliaryopathy.

Graft-Versus-Host Disease

The clinical differential diagnosis of GVHD is frequently delayed because early symptoms are often nonspecific. Many of the clinical signs of GVHD such as refractory diarrhea and abdominal pain may also be seen with cytomegalovirus infection.35 Hepatic GVHD is histopathologically characterized by damage and loss of intrahepatic bile ducts (LIBD) and is a frequent complication of hematopoietic stem cell transplantation. Graft-versus-host-disease has historically been divided into acute and chronic GVHD using day 100 after transplantation as the cutoff point between the 2 types. However, the histological findings in a liver biopsy cannot distinguish acute and chronic GVHD, and they are not specific for GVHD. Extensive bile duct damage with relatively mild portal inflammation is the most characteristic feature of GVHD, mostly affecting the interlobular bile ducts (Figure 6). They are seen during the intermediate period, 35 to 90 days following hematopoietic stem cell transplantation. Segmental disruption of bile ducts eventually culminates in LIBD. Cholestasis is present in most cases showing bile duct damage. Ductular reaction is not a prominent feature in GVHD. Endothelitis is an infrequent but relatively specific finding, generally seen in more severe cases of GVHD. Lobular inflammation may be present.

Drug-Induced Liver Injury

Approximately 30% of cases with DILI are cholestatic.31 It has been reported that there is limited correlation between the biochemical categorization and the pathological pattern of injury.32 For example, an injury that would be classified as cholestatic on biochemical grounds could show acute or chronic hepatitis without evidence of bile accumulation.32 Histologically, a cholestatic pattern of liver injury can be classified into acute and chronic injury, of which acute cholestasis is much more common.33,34 Cholestasis caused by drugs commonly resolves without significant consequences after termination of drug intake. In some instances, liver injury becomes chronic, causing sclerosis and loss of bile ducts, portal cholate stasis, portal fibrosis, and copper accumulation, which may mimic PSC or PBC. Fibrosis may occasionally progress to biliary cirrhosis. Some of the histological features seen in acute cholestatic DILI may overlap with chronic cholestatic DILI but marked bile duct injury is the hallmark of chronic cholestatic DILI. Drugs known to cause LIBD include neuroleptic agents (chlorpromazine, imipramine, carbamazepine, amitriptyline, haloperidol, cyproheptadine, and phenoxytoin), antibiotics (amoxicillin, fluocoxacin, quinolones, clindamycin, makrolides, and tetracyclines), complementary and alternative medicines (ajmaline and glycyrrhizin), nonsteroidal anti-inflammatory drugs (diclofenac and ibuprofen), amiodarone, cimetidine, thiabendazole, and zonisamide, among others.33,34 Of the whole list, chlorpromazine, ajmaline, and fluocoxacin are the commonly reported drugs associated with LIBD.35 Primary sclerosing cholangitis–like injury is a relatively uncommon pattern that is mainly seen with infusion of chemotherapy agents into the hepatic artery such as fluorouracil and fluorouracil to treat metastatic colorectal cancer to the liver, as well as hepatic arterial chemoembolization in hepatocellular carcinoma.36,37 The injury has been attributed to indirect bile duct damage secondary to drug-caused vascular injury.38 Primary biliary cirrhosis–like injury is rare and has resulted from the use of herbal drugs or statins.39,40

Loss of Intrahepatic Bile Ducts

Loss of intrahepatic bile ducts or ductopenia is usually designated when bile ducts are absent in at least 50% of portal tracts provided that at least 10 portal tracts are available for assessment. The mechanisms involved in the loss of bile ducts include immune-mediated destruction of bile ducts and ischemic damage to the biliary tree. Diseases frequently associated with LIBD include PBC, PSC, chronic liver graft rejection, GVHD, ischemic cholangitis, drug-
induced bile duct loss, sarcoidosis, and idiopathic adulthood ductopenia. Almost all (96%) hepatic arteries run in parallel with a bile duct. The extent of bile duct loss could therefore be determined by the number of “unpaired arterioles” (Figure 7). Arterialization of central zone that shows small arteries in scarred centrizonal areas accompanied by a mild ductular reaction, as well as the portal-like structures such as seen in focal nodular hyperplasia or hepatic adenoma, presents pitfalls in determining LIBD. Loss of intrahepatic bile duct is more prominent in PSC than in PBC. The finding of bile duct loss is important in differentiating PBC from hepatitis C, which shows bile duct damage without actual loss of bile ducts. Ischemic cholangitis or ischemic cholangiopathy is an additional cause of LIBD. The histological features of ischemic biliary damage at the affected sites, which commonly are the extrahepatic and larger intrahepatic ducts, include epithelial atrophy, erosion, granulation tissue, and necrosis of the bile duct wall. Bacterial colonization may be present. The histological findings in needle biopsies reflect the upstream effects of bile flow obstruction in the larger affected ducts and represent features of large-duct obstruction. The smaller portal tracts show portal edema, mild portal inflammation, ductular reaction, and cholestasis. In rare cases of sarcoidosis, bile ducts damage, including LIBD and ductopenia, may histologically resemble PBC and PSC. Nonsuppurative cholangitis and periductal fibrosis may be seen in the remaining bile ducts with ductular reaction. Loss of intrahepatic bile duct can be present at the early stage of chronic allograft rejection and may be diffuse. Progression to late-stage chronic allograft rejection shows increasing widespread LIBD, leading to the so-called “burnt out” or scarred, mildly fibrotic portal tracts that are devoid of inflammation, bile ducts, and ductules. Some portal tracts may also show concomitant loss of hepatic artery tributaries, adding to the difficulties in recognizing portal tracts and assessing the severity of LIBD.

**DISEASES OF BILE CANALICULI**

**Progressive Familial Intrahepatic Cholestasis**

Progressive familial intrahepatic cholestasis is a clinical syndrome of intrahepatic cholestasis that presents in infancy or early childhood, progresses rapidly to fibrosis and end-stage liver disease, and results in death in the absence of liver transplantation. The diagnosis of PFIC is established by a combination of clinical, radiological, laboratory, and biopsy findings. The first and most important step is to rule out biliary obstruction. Despite the name of familial intrahepatic cholestasis, most cases of PFIC are not familial. Genetic analysis of patients with PFIC has identified mutations in 3 genes, including \( \text{ATP8B1} \) (ATPase class I type 8B1), \( \text{ABCB11} \) (ABC, subfamily B, member 11), and \( \text{ABCB4} \) (ABC, subfamily B, member 4), that encode 3 key canalicular proteins namely FIC1, BSEP, and MDR3, respectively. Disorders caused by absence or severe dysfunction of these proteins are now known as PFIC-1 or \( \text{ATP8B1} \) disease, PFIC-2 or \( \text{ABCB11} \) disease, and PFIC-3 or \( \text{ABCB4} \) disease, respectively.

**PFIC-1** or **ATP8B1 Disease.**—PFIC-1 or severe ATP8B1 disease is an autosomal recessive disorder. PFIC-1 results from biallelic mutations in ATP8B1. ATP8B1 encodes a P-type ATPase, FIC1, which is an aminophospholipid flippase that flips phosphatidylserine from the outer plasma leaflet of the canalicular membrane into the inner one. How

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*Figure 6.* Graft-versus-host disease showing extensive bile duct damage (arrows) with relatively mild portal inflammation. Arrowhead points to the hepatic arteriole (hematoxylin-eosin, original magnification ×200).

*Figure 7.* An unpaired arteriole (arrow) unaccompanied by its bile duct in graft-versus-host disease indicating loss of bile ducts (hematoxylin-eosin, original magnification ×200).

*Figure 8.* Extensive canalicular cholestasis with formation of biliary rosettes in progressive familial intrahepatic cholestasis 1 (hematoxylin-eosin, original magnification ×200).
deficiency of FIC1 leads to cholestasis is not fully understood. Patients with PFIC-1 present in early infancy with jaundice, foul-smelling stools, and failure to thrive. Hemorrhage potentiated by vitamin K deficiency is an occasional first manifestation. The histological features in PFIC-1 are those of bland cholestasis. Centrilobular canaliculal cholestasis with acinar or pseudos rosette formation is the predominant finding (Figure 8). The bile in PFIC-1 is pale with scant hepatocellular cholestasis. At initial biopsy, the interlobular bile ducts appear hypoplastic and threadlike. Centrilobular hepatocyte loss and perivenular and periporal fibrosis ensue. Progression to portal-portal and portal-central bridging fibrosis leads ultimately to micronodular cirrhosis. Fibrosis progresses in the absence of significant inflammation and ductular reaction.

**PFIC-2 or ABCB11 Disease.**—PFIC-2 or severe ABCB11 disease is an autosomal recessive disorder. PFIC-2 results from ABCB11 biallelic mutations.45 ABCB11 encodes an ABC transporter, BSEP (bile salt export pump), which is the principal transporter of amidated bile acids from hepatocytes into the biliary canaliculus against a powerful concentration gradient. The resulting failure of bile salts from hepatocytes into bile canaliculi leads to bile salt accumulation in hepatocytes, causing severe hepatocellular damage, and impaired bile flow because of the lack of bile salt secretion in bile. Patients with PFIC-2, like patients with PFIC-1, present in early infancy with jaundice, foul-smelling stools, failure to thrive, and hemorrhage potentiated by vitamin K deficiency. PFIC-2 progresses to end-stage liver disease more rapidly, with cirrhosis, liver failure, and death in the first decade if liver transplantation is not performed. Patients with PFIC-2 have a disproportionately high risk of hemorrhage potentiated by vitamin K deficiency. PFIC-2 progresses to end-stage liver disease, respectively.50,51 Patients may present at any age, but most are seen in the first 2 decades of life. Episodes are precipitated by hormonal milieu perturbations such as pregnancy and administration of oral contraceptives, as well as by infections. Centrilobular canaliculal cholestasis with formation of biliary rosettes is seen during episodes of illness. Hepatocellular cholestasis may also be present. The cholestasis is unaccompanied by inflammation, duct damage, duct loss, or ductular reaction. A BRIC diagnosis can be confirmed only after exclusion of other possible causes of intrahepatic cholestasis.

**Intrahepatic Cholestasis of Pregnancy**

Intrahepatic cholestasis of pregnancy manifests in the third trimester of pregnancy as skin itching, especially on the palms and soles, and as an elevation of the serum levels of bile acids and liver enzymes.52,53 The incidence varies across countries from 0.5% to as high as 28% among the Araucanos Indians of Chile.4,55 Intrahepatic cholestasis of pregnancy is thought to be the result of an insufficient liver capacity to metabolize large amounts of placental hormones during pregnancy in patients with an underlying genetic disorder in bile production.56 Many cases of ICP are associated with mutations in the ABCB4 and ABCB11 genes.57,58 Pruitus spontaneously resolves, and liver function test results typically normalize within 4 weeks after delivery. Thus, ICP is a self-limiting condition for the mother but increases the risk of preterm birth, fetal distress during labor, and intrauterine death.59,60 Intrahepatic cholestasis of pregnancy also confers a significant risk of later hepatobiliary disease, particularly gallstone and hepatitis C infection.61 A liver biopsy is not indicated in ICP. If performed, it shows findings similar to those in BRIC such as centrilobular canalicular cholestasis without inflammation, duct damage, duct loss, or ductular reaction.

**Drug-Induced Liver Injury**

Acute intrahepatic cholestasis is characterized by the accumulation of bile within canaliculi (canalicular cholestasis) and hepatocytes (hepatocellular cholestasis). In most cases of drug-induced cholestasis, both hepatocellular cholestasis and canalicular cholestasis are present, although the anabolic and oral contraceptive steroids notably produce only canalicular cholestasis (Figure 10, A).62 The acute intrahepatic cholestasis induced by drugs can be classified into pure (bland) cholestasis and cholestatic hepatitis.33,34 In pure cholestasis, bile plugs are seen in canaliculi and/or hepatocytes. No inflammation, hepatocyte necrosis, or bile duct damage is identified. In cholestatic hepatitis ( hypersensitivity cholestasis), accumulation of bile in the liver tissue is accompanied by inflammation and hepatocellular injury. Eosinophils may be present, which indicates a better prognosis.63 In cholestatic hepatitis, cholestasis is most
prominent in zone 3. Therefore, sepsis and acute large-duct obstruction, which also show isolated zone 3 cholestasis, should be excluded. A mild to moderate degree of lobular and/or portal inflammation, as well as duct injury, may also be present (Figure 10, B). Various drugs have been associated with cholestatic hepatitis (eg, erythromycin and chlorpromazine).33

CONCLUSIONS

This article reviewed the key clinical and pathological features of the diseases of the proximal pathways of the biliary system. The characteristic pathological findings in liver biopsy can usually provide sufficient information to guide patient management. Additionally, diagnostic steps in cholestatic conditions require a thorough patient history, imaging, serological studies, and liver biopsy.

References
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