Introduction

The histologic evaluation of cholestasis presents a diagnostic challenge not only because of the many potential anatomic sites of interrupted bile flow, but to some degree due to the relative non-specificity of certain morphologic features as seen down the microscope. Nevertheless, a clear mandate for pathologists is to attempt to distinguish between obstructive diseases affecting the large bile ducts (which may be amenable to surgical or endoscopic intervention) vs. those causes of cholestasis which reside in the liver and require other types of therapy. This session will approach the diagnostic problems of cholestasis first by identifying the “pattern sets” of features on liver histology which are characteristic to specific types of cholestatic disease and second by illustrating the easily (or in some cases not so easily) missed diagnoses. Lastly, those hepatic disorders which are not related to cholestasis per se but which may be mistaken for cholestatic or biliary disease will be addressed.

Histologic pattern set of “acute” cholestasis

The histologic responses to acute cholestasis (days to weeks duration) can be categorized as 4 types:

Pattern 1 (Classical type): This is the characteristic pattern seen with obstruction of large bile ducts, including extrahepatic bile ducts and perihilar ducts. The type 1 pattern comprises parenchymal cholestasis plus a distinctive triad of portal tract changes which includes edema, proliferation of bile ductular structures and infiltrates of neutrophils (sometimes with admixed lymphocytes and/or plasma cells, since in many instances the obstruction has been present for weeks or longer). This classic portal reaction was described nearly 40 years ago by Christoffersen and Poulsen. The significance of the flattened tubular structures resembling bile ductules as a stereotypic reaction derived from periportal hepatic progenitor/stem cells (HPSCs) has more recently led to the term “ductular reaction” for this process. Seen in more contemporary light, the ductular reaction reflects the local interplay of various paracrine and autocrine mediators, including biliary pressure and cytokine release. Neutrophils within the edematous portal stroma are in part related to active cytokine secretion (e.g. interleukin-8) by the ductular cells. Bile pigment in pattern 1, as with most histologic cholestasis, predominates in centrilobular regions where it is seen within hepatocytes and bile canaliculi and sometimes within Kupffer cells. The ductular reaction may be present in the absence of cholestasis if there is incomplete obstruction (i.e., if a segmental or other conducting bile duct is obstructed but perihilar bile ducts and bile secretion remain intact) or if the obstruction has recently been relieved by biliary stenting. Unless specific
Changes referable to primary sclerosing cholangitis (PSC) are present (e.g., periductal “onion-skin” fibrosis, fibro-obliterative cholangitis), the anatomic site of biliary obstruction is uncertain without further radiologic evaluation of the biliary tree.

**Pattern 2 (pure cholestasis):** in which centrilobular cholestasis within hepatocytes and bile canaliculi is the predominant finding, without significant portal tract pathology. This pattern is exemplified by the intrahepatic cholestasis of sepsis, toxicity of certain drugs, functional bile flow impairment (ischemia/reperfusion preservation injury) and mutations of pericanalicular bile salt transport proteins.

**Pattern 3 (intrahepatic bile duct disease):** this is exemplified by primary biliary cirrhosis in which immune cell (lymphocytes, plasma cells, eosinophils) targeting of interlobular and septal bile ducts results in damage comprising intra-epithelial inflammatory cells, epithelial apoptosis, vacuolization and degeneration, with reactive stratification. Similar features may be seen in drug-induced cholangiopathies such as that produced by amoxicillin-clavulanic acid (augmentin) and in acute cellular rejection following liver transplantation. This pattern of cholestatic injury also includes ductopenic diseases (colloquially sometimes referred to as “vanishing bile duct syndrome”). The disorders which may lead to loss of 50% or more of bile ducts from portal tracts (i.e., ductopenia) include primary biliary cirrhosis, primary sclerosing cholangitis, ductopenic rejection following liver transplantation and drug-induced cholangiopathy. Other ductopenic conditions include idiopathic adulthood ductopenia and the pediatric intrahepatic bile duct paucity conditions (Alagille syndrome or syndromic paucity of intrahepatic bile ducts, and non-syndromic paucity of intrahepatic bile ducts). In order to recognize pattern 3 cholestatic disease, it should be emphasized that routine liver histologic examination requires surveillance for the presence of interlobular bile ducts of similar caliber to accompanying hepatic arterioles.

**Pattern 4 (Hepatitis with cholestasis):** this pattern is usually recognized by the distinctive changes of hepatitis which affect the liver parenchyma (lobular disarray, hepatocyte ballooning and apoptosis, intrasinusoidal lymphocytes and ceroid-laden Kupffer cells) and portal tracts (inflammation). Cholestasis may be inconspicuous in many cases of viral hepatitis, although a predilection for histologic cholestasis is seen with HAV and HEV infections. For idiosyncratic drug hepatotoxicity, the aforementioned parenchymal changes may be accompanied by cholestasis. It should also be noted that variable damage (or even destruction) of bile ducts may accompany hepatitis, particularly drug-induced hepatitis.

**Histologic pattern set of chronic cholestasis**

Chronic biliary obstruction, whether due to large bile duct disease, destruction of widespread intrahepatic bile ducts in PBC or extra- and intra-hepatic PSC, often lead to similar late features. Cholestasis which predominated in acute cholestasis in centrilobular regions frequently progresses to a panlobular distribution and periportal hepatocytes acquire changes including pallor and swelling (pseudoxanthomatous change or “cholate stasis” due to chronic bile salt retention), copper and copper-binding protein and Mallory-
Denk bodies (MDBs). The specific diagnosis in late cholestatic/biliary disease therefore often resides in observing the specific portal/periportal changes as outlined in 3 patterns below.

**Pattern 1 (Classic portal/periportal fibrotic):** The major causes of chronic large bile duct obstruction result in expansion of portal tracts by fibrosis, often in a regular manner of thick fibrous septa which ultimately bridge between portal tracts. A vigorous ductular reaction is often apparent, and the native interlobular bile duct is preserved. Prototypes of this pattern include large bile duct obstruction seen with pancreatic carcinoma, biliary stricture and extrahepatic biliary atresia.

**Pattern 2 (Fibrosing periductal or duct abnormality type):** In this pattern, specific changes affecting the bile ducts are seen within portal tracts. In large and/or small duct PSC, for example, periductal “onion-skin” fibrosis or fibro-obliterative lesions are diagnostically helpful (though the former does not definitively exclude secondary sclerosing cholangitis due to other obstructive disorders). Bile duct epithelium may become dystrophic, with simplified ducts containing fewer than normal cells and individual cellular dysmorphism.

**Pattern 3 (Ductopenic and atypical types):** This type of late cholestatic disease is characterized by loss of bile ducts from variable numbers of portal tracts with or without ductular reaction and fibrosis resembling type 1 pattern. The absence of typical thick fibrous septa, edema and vigorous ductular reaction in atypical types of bile duct destruction (e.g., drug-induced cholangiopathies) may lead to problems in diagnostic classification. Formation of portal lymphoid aggregates are an additional feature seen in PBC perhaps more commonly than PSC.

**Easily (or not so easily) missed cholestatic diseases**

**Primary biliary cirrhosis (PBC):** Probably one of the most common disorders to go unrecognized on liver biopsy or other histologic specimens, PBC presents several diagnostic disadvantages: the focality of the bile duct lesions; the associated chronic lymphoplasmacytic infiltrates (often with interface hepatitis) which may readily confuse the diagnosis with chronic hepatitis; cases with granulomas may be dismissed as PBC when the granulomas have other diagnostic features; late in PBC the absence of bile ducts and formation of portal tract lymphoid aggregates may be mimicked by primary sclerosing cholangitis (PSC).

**Helpful diagnostic points:**

1. Serial and deeper H&E sections may demonstrate florid bile duct lesions in suspected cases.
2. Certain cases of PBC are accompanied by abundant portal eosinophils, which may help distinguish the diagnosis from chronic hepatitis where only a few scattered eosinophils are usually present.
3. Granulomas in PBC are characteristically centered within portal tracts around damaged bile ducts; lobular granulomas, if present, are distinctly few.

4. Late PBC is typically accompanied by the features of chronic cholestasis, including periportal pseudoxanthomatous change of hepatocytes, stainable copper and copper-binding protein, and sometimes Mallory-Denk bodies. Therefore, rhodanine and orcein/Victoria blue/DPAS stains may help in establishing the chronic cholestatic nature of the condition.

**Late chronic cholestatic disease, such as PBC or PSC:** This not infrequent problem arises when diffuse chronic portal inflammation with periportal and portal-to-portal bridging fibrosis are present, and bile ducts are missing. One obvious reason for missing these diagnoses is not registering that native bile ducts are missing. Moreover, periportal pseudoxanthomatous change may fail to be identified and special stains such as rhodanine or orcein/Victoria blue may not be standard methods at the given institution. While the ductular reaction is usually a feature of both late ductopenic PBC and PSC, this may vary considerably from case to case and may not be an entirely reliable finding.

**Helpful diagnostic points:**
1. Special stains (rhodanine; orcein/Victoria blue) help to identify periportal hepatocellular copper and copper-binding protein. DPAS is a fairly standard staining methods to use as an alternative to orcein/Victoria blue if the latter are not available in the lab’s repertory.
2. Evaluation for additional bile duct lesions, periductal “onion-skin” fibrosis or fibroobliterative scars can help identify the diagnosis of PBC or PSC.
3. Formation of portal lymphoid aggregates at sites of prior duct destruction (PBC) or of fibrous obliteration (PSC) is a useful feature (though this may be confused with chronic hepatitis due to HCV, HBV or autoimmune hepatitis). The absence of lobular changes of chronic hepatitis such as apoptotic bodies, hepatocyte ballooning and necroinflammatory foci favors chronic biliary disease.

**Premature ductopenic PBC:** This unusual variant of PBC is characterized by accelerated diffuse bile duct destruction prior to the progressive fibrosis and nodularity which are usually associated with generalized loss of bile ducts in the later stages of PBC\(^2\). This condition may show substantial cholestasis in addition to widespread bile duct disappearance and chronic portal inflammation, with little ductular reaction or portal/periportal fibrosis.

**Helpful diagnostic points:**
1. Mild or moderate cholestasis (or worse) is NOT a typical feature of PBC in the early stages (Stage 1-2) of the disease, so if this is present in association with widespread bile duct loss, consider premature ductopenic PBC—or drug-induced cholangiopathy as a possible alternative etiology.
2. CK7 (or CK19) immunostaining can further confirm the extent of the bile duct loss.
Inconspicuous cholestasis: Cholestasis is often overlooked if mild, and while its presence in other hepatic disorders may be a histological footnote, it may occasionally be a major clue as to the diagnosis of a condition such as drug hepatotoxicity. Cholestasis may be overlooked or masked by the intensity of eosin staining, the thickness of the histologic section or to overlying changes in centrilobular regions. Examples of settings in which cholestasis is overlooked include postmortem liver where the centrilobular necrosis of pre-terminal cardiac failure masks the patchy mild accompanying cholestasis; centrilobular ballooning and/or necrosis following preservation injury in allograft livers; older-aged individuals with increased hepatocellular lipofuscin.

Helpful diagnostic points:
1. Iron stain is useful to identify mild cholestasis by virtue of the less intense nuclear fast red counterstain. Alternatively, Hall’s bile stain may be used.
2. In attempting to distinguish lipofuscin intracellular pigment from cholestasis, finding bile plugs within canaliculi is useful in centrilobular regions.

Biliary disease with limited ductular reaction: In certain cases, evidence of biliary tract disorders is only manifested by a mild ductular reaction. Changes such as periductal fibrosis or bile duct damage by which to more directly diagnose PSC and PBC, respectively, may be absent. In such cases the pathologist may unfortunately be unable to provide more than just the suggestion that biliary disease is present, with recommendations for further visualization of the biliary tree by radiology and evaluation of serum anti-mitochondrial antibody and pANCA. Obviously, elevated serum alkaline phosphatase level as the only significant liver function test abnormality in such patients would also point to biliary tract disease.

Helpful diagnostic points:
1. Deeper levels and/or CK7 immunostaining, to further confirm the presence of the ductular reaction.

Neonatal hepatitis with bile duct paucity: Liver disease in neonates and infants often is accompanied by many histologic changes (e.g., multinucleated giant hepatocytes, cholestasis, extramedullary hematopoiesis—both within portal tracts, at their edges and within sinusoids--, inflammation and variable ductular reaction) which distract attention from key features such as bile duct paucity. Special stains often are unhelpful, such as DPAS which in adults may demonstrate alpha-1-antitrypsin (AAT) globules in AAT deficiency but which may be insufficiently developed in neonatal liver tissue to be diagnostic. Careful attention to the status of interlobular bile ducts is therefore mandatory in handling this material, including the presence of duct destructive lesions as may be seen with hepatitis (e.g. CMV hepatitis) and certain metabolic disorders (e.g., AAT deficiency). It is also important to distinguish between a well-developed ductular reaction (which is the pathognomonic feature of extrahepatic biliary atresia) vs. the absent or minimal reaction seen in other conditions, including intrahepatic bile duct.
Some helpful histologic guidelines were reviewed in a recent single-topic conference sponsored by the American Association for the Study of Liver Diseases.25

**Helpful diagnostic points:**
1. Check for normal (or inflamed/absent) interlobular bile ducts in all neonatal/infant liver specimens.
2. CK7 immunostaining is very helpful in identifying native bile ducts and the ductular reaction (vs. limited ductular reaction/intermediate hepatobiliary cells).
3. Visually “subtract out” extramedullary hematopoiesis (EMH) from portal/periportal regions and sinusoids in order to determine whether or not a real hepatitis is present (inflammation other than EMH; hepatocellular apoptosis; giant cells).
4. With neonatal cholestasis, if the portal/periportal features do not support extrahepatic biliary atresia, then consider the broad categories of neonatal giant cell hepatitis, bile duct paucity conditions (both Alagille’s syndrome and non-syndromatic types), metabolic diseases (e.g., AAT deficiency), bile salt synthesis deficiency states and bile salt transport protein mutations.

**Liver diseases which may be mistaken for cholestatic/biliary disease**

**Chronic hepatitis with ductular reaction:** The activation of periportal hepatic progenitor cells in more active forms of chronic hepatitis may result in a prominent and potentially confusing ductular reaction suggesting biliary disease.26 When seen in association with interface hepatitis the admixed features include extension of lymphocytes and plasma cells into the periportal limiting plate region accompanied by scattered neutrophils which are microscopically in proximity to the proliferated ductular structures. The greater the periportal activity in chronic hepatitis, the more likely ductular reaction will be present, regardless of the cause of the chronic hepatitis.

**Helpful diagnostic points:**
1. Chronic hepatitis, rather than biliary/cholestatic disease, is usually associated with lobular necroinflammatory changes, apoptosis and other hepatocellular alterations which help distinguish the real nature of the condition.
2. CK7 immunostaining should confirm the presence of native bile ducts as well as not only the periportal ductular reaction but frequently the presence of intermediate hepatobiliary cells in greater numbers than would be seen in biliary disease alone.
3. Absence of cholestasis in many forms of chronic hepatitis (although this may not be true of autoimmune chronic hepatitis or progressive recurrent chronic hepatitis C after liver transplantation).

**Intermediate and advanced stages of steatohepatitis:** Both alcoholic and nonalcoholic steatohepatitis (ASH and NASH, respectively) evolve from a centrilobular lesion with characteristic hepatocyte ballooning, MDBs and pericellular inflammation with pericellular/perisinusoidal fibrosis to a progressively fibrogenic process in tandem with
ductular reaction\textsuperscript{27}. When steatosis and the centrilobular hepatocellular changes are less conspicuous, the prominent ductular reaction embedded within bridging fibrous septa often results in architectural obscuration, potentially leading to the misdiagnosis of biliary tract disease.

\textit{Helpful diagnostic points:}

1. The most dense, least cellular regions of fibrosis in steatohepatitis are centrilobular regions. It is often helpful to evaluate these regions and the surrounding hepatocytes for residual evidence of steatohepatitis.

2. Combined immunostaining with CK7 and p21 (the latter indicating non-replicating hepatocytes in G1 arrest) may help clarify the genesis of the ductular reaction and the state of impaired hepatocellular replication which activated progenitor cells.

3. Absence of portal/periportal edema is evidence against true biliary obstruction.

\textit{Fibrosing cholestatic hepatitis (FCH) after liver transplantation:} This condition, seen in a minority of individuals with recurrent hepatitis B or C virus infection in the liver allograft after transplantation\textsuperscript{28-31}, is characterized by an extensive periportal ductular reaction accompanied by fibrosis. Parenchymal cholestasis is conspicuous and other hepatocellular damage (apoptosis, ballooning) is often apparent. Since biliary obstruction due to anastomotic stricture must be excluded after liver transplantation, the presence of FCH-like features usually prompts the clinical team to assess the status of patency of the bile ducts.

\textit{Helpful diagnostic points:}

1. The marked lobular changes accompanying FCH are striking (including hepatocyte swelling, cholestasis, apoptosis, necroinflammation, ground-glass inclusions in the case of FCH-hepatitis B) and would not be characteristic of biliary obstruction.

2. CK7 immunostaining to demonstrate the ductular reaction characteristically shows the abundant proliferated ductular structures. The absence of edema in the same regions is against biliary obstruction.

\textit{Focal nodular hyperplasia (FNH) on needle liver biopsy:} Needle liver biopsy for the diagnosis of FNH may present diagnostic problems because of the irregular distribution of many of the hallmark features of the lesion\textsuperscript{32}. Samples of the fibrous septa with ductular reaction, particularly if the specimen is submitted without indicating that the sample is from a liver mass, may prompt consideration of biliary obstruction.

\textit{Helpful diagnostic points:}

1. The distinctive loose stroma accompanying the fibrous septa and ductular structures in FNH differs from typical portal connective tissue edema in biliary obstruction.

2. Assessment of the portal tracts for abnormally thickened arterioles may enable the correct diagnosis.
3. Consider FNH in needle biopsies—particularly when clinical information is lacking in the accompanying specimen requisition.

References


