

# **Benign hepatocellular tumors: Problem diagnoses and applications of new pathomolecular classifications**

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## **Introduction**

Benign hepatocellular tumors gather 2 separate groups of lesions according to their pathogenesis. Such classification has been successfully achieved owing to molecular studies, especially assessing their biological behaviour. Therefore, one distinguishes polyclonal disorders that concern regenerative non-neoplastic processes, such as focal nodular hyperplasia (FNH), and monoclonal lesions that are consistent with neoplastic tumors, including liver cell adenomas (1, 2). Both lesions are predominantly encountered in young women, usually in the context of oral contraception use.

## **Focal Nodular Hyperplasia**

Focal nodular hyperplasia accounts for the second most common benign liver process. It is a benign tumor-like condition considered as a hyperplastic reaction resulting from arterial malformation (3). FNH displays a typical pathological pattern for both radiologists and pathologists. Grossly, FNH is a well-circumscribed, unencapsulated, usually solitary mass, characterized by a central fibrous scar that radiates into the liver parenchyma. Histologically, FNH is composed of benign-appearing hepatocytes arranged in nodules that are delineated by fibrous septa originating from the central scar. In the fibrous septa, large and dystrophic vessels, ductular proliferation and inflammatory cells are observed. The hepatocytes are hyperplastic, arranged in liver plates of normal or slightly increased thickness.

Besides this classic form of FNH, several variant lesions are described with increased frequency, and commonly classified as “non-typical FNH” by radiologists. This group is somehow heterogeneous, including FNH without central fibrous scar, lesions displaying telangiectatic or steatotic changes (4). On histological examination, FNH without macroscopic central fibrous scar exhibits all the pathological elementary features of classic FNH. However, molecular studies demonstrated that in this group of atypical FNH, lesions displaying telangiectatic changes, initially so-called “telangiectatic form of FNH”, are clonal processes and should be regarded rather as variant form of liver cell adenoma than truly FNH (5). They will be further described in the group of liver cell adenomas.

Complications of FNH such as rupture or bleeding are exceptional. No malignant transformation of FNH has been reported. Therefore, whatever the size and the number of lesions, no treatment is required for asymptomatic FNH when the diagnosis is firmly established.

### **Liver cell adenomas: A heterogeneous group of lesions**

Liver cell adenoma is a rare, benign liver neoplasm that is strongly associated with oral contraceptive use and androgen steroid therapy. Hepatocellular adenoma can also occur spontaneously or be associated with underlying metabolic diseases, including type 1 glycogen storage disease, iron overload related to betathalassemia and diabetes mellitus (6).

Hepatocellular adenoma is usually solitary, sometimes pedunculated, with a diameter that can reach 30 cm. Large subcapsular vessels are commonly found on macroscopic examination. On cut sections, the tumor is well-delineated, sometimes encapsulated, of fleshy appearance ranging in color from white to brown. Adenoma frequently displays heterogeneous areas of necrosis and/or hemorrhage. Histologically, hepatocellular adenoma consists of a proliferation of benign hepatocytes arranged in a trabecular pattern. Small thin vessels are

usually found throughout the tumor. Hepatocytes may have intracellular fat or increased glycogen.

It has been recently proposed that liver cell adenomas are heterogeneous, with regard to their morphological pattern, with steatotic, telangiectatic or inflammatory adenomas and adenomas with cell atypias. Interestingly, specific gene alterations have been found to be associated with some of these variants (see below) (7). The latter form of adenomas may especially be encountered in patients who have taken steroids for many years. In that context, differential diagnosis with a hepatocellular carcinoma may be difficult.

Finally, it is now admitted that some atypical forms of FNH, i.e. “telangiectatic” and “mixed hyperplastic and adenomatous FNH” should be regarded as hepatocellular adenomas, as already discussed. Macroscopically, so-called “telangiectatic FNH” are well-delineated, unencapsulated and showed significant areas of vascular changes, without any fibrous scar. They were initially described by Wanless et al. as multiple nodules and were labelled as multiple FNH syndrome (8). Microscopic examination of these lesions shows transitional morphological features between adenoma and FNH. In all cases, there is no central fibrous scar. Few and short fibrous septa containing several small vessels without significant ductular proliferation are observed throughout the tumor which displays significant vascular changes of telangiectatic type. The “hyperplastic and adenomatous form of FNH” is histologically characterized by two alternating aspects: one resembling telangiectatic type, the other simulating adenoma. Taken together, these forms represent less than 20% of atypical FNH in large surgical series (9). It is of note that both lesions are now recognized as variant forms of hepatocellular adenomas and may be managed as adenomas. At last, a small group of adenomas do not display any specific morphological features.

Several complications may occur, such as bleeding, rupture and malignant transformation. The risk of malignant transformation of adenoma is in the order

of 10%. This risk appears to be higher in males and patients with large adenoma. Most cases of hepatocellular carcinoma develop at the site of the liver cell adenoma and malignancy is most often discovered on the analysis of the specimen.

### **Molecular diagnosis of benign hepatocellular tumors**

Several changes in gene expression have been described in FNH, especially regarding molecules involved in vasculature remodelling, such as angiopoietins (ANGPT1 and ANGPT 2 (10). Such results certainly reinforced the hypothesis of vascular disorder in the pathogenesis of this lesion. A  $\beta$ -catenin activation, without associated mutation of this gene or axin, was recently reported in FNH (11).

In the last few years, very important progress based on molecular studies has been made in the field of liver cell adenomas that enabled to define different subtypes of adenomas associated with specific clinical and pathological features. Therefore, at least 4 subtypes of adenomas may be described. HNF1- $\alpha$  mutated adenomas, which account for approximately for 40% of liver cell adenomas, are characterized by a prominent steatosis, usually of marked intensity. These adenomas may be multiple and observed in the setting of adenomatosis (12).  $\beta$ -catenin mutated adenomas, morphologically characterized by the presence of cellular atypias, are preferentially encountered in male patients. It has been shown that these adenomas display a higher risk of malignant transformation (7). Very recently, a marked activation of interleukin 6 signalling pathway, related to mutations in the IL6ST gene encoding the signalling co-receptor gp130, has been described in the group of telangiectatic (or “inflammatory”) adenomas (13). Finally, a small group of adenomas without any specific clinical, morphological or genetic characteristics is still encountered.

## **Impact of molecular data on pathological diagnosis**

Based upon the molecular abnormalities described, a set of several antibodies may be used in the sub-typing of benign hepatocellular tumors with a high sensitivity and specificity, especially in liver cell adenomas (14). For instance, steatotic adenomas displaying HNF1- $\alpha$  mutations do not express L-FABP, a protein involved in the fatty acid trafficking normally expressed by normal hepatocytes. In addition,  $\beta$ -catenin mutated adenomas display glutamine synthetase overexpression and express  $\beta$ -catenin both in the cytoplasm and the nucleus of the tumoral hepatocytes. At last, telangiectatic adenomas may show positive immunostaining with markers of the acute-phase inflammatory response, including serum amyloid A and C reactive protein.

**In conclusion**, molecular studies undoubtedly gained further insights into the understanding of benign hepatocellular tumors, and especially in the group of liver cell adenomas. Whether diagnosis and sub-typing of those lesions may rely on relevant and specific morphological features in most cases, additional studies using molecular markers may be helpful, in cases of atypical lesions and on biopsy samples.

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## The Hepatic Vasculature: Missed Lesions, Missed Diagnoses

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### Introduction

The possibility of overlooking hepatic vascular disease occurs both in evaluation of liver biopsies, and examining macroscopic specimens of the liver, surgical or autopsy. The standard approach to evaluating a liver biopsy involves assessment of portal tract architecture, bile duct features, and the hepatic parenchyma – sinusoids included. Giving specific attention to evaluating the hepatic microvasculature (portal veins, hepatic arteries, terminal hepatic veins) is mandatory. However, interpreting vascular lesions so identified is problematic.

The clinical indication for a percutaneous liver biopsy usually involves some version of “hepatitis”, “bile duct disease”, “fibrosis/cirrhosis”, or “neoplasia”. “Vascular disorder of the liver” is rarely in the clinical differential diagnosis, and will not be in the pathologist’s differential diagnosis either unless s/he thinks of it. Yet, vascular disorders can present clinically in a manner similar to the usual diagnostic entities above. Even more importantly, the liver biopsy is a very imperfect mechanism to diagnose vascular disorders. Identification of a hepatic vascular disorder as the etiology for a clinical condition is both challenging, and rewarding when demonstrated to be correct.

Table 1 gives disorders which merit at least cursory consideration in the differential diagnosis during histopathologic evaluation of hepatic tissue. This table is organized according to the pathophysiology of the vasculature. The differential diagnosis is less easily organized, since vascular disease can mimic essentially any of the more common hepatic conditions.

Table 1. Vascular Disorders of the Liver, to be considered in histopathologic evaluation of hepatic biopsies

Vascular Disorder	in the differential diagnosis of:
Portal Vein Obstruction To include: Hepatoportal Sclerosis	Fibrosis/Cirrhosis
Arterial Disease Arteritis (e.g., Polyarteritis Nodosa) Hepatic artery obstruction	Cholestasis, Hepatitis
Sinusoidal Disease (other than cirrhosis) Veno-occlusive Disease (Sinusoidal Obstruction Syndrome) Amyloidosis and light chain deposition disease Disseminated Intravascular Coagulation Sinusoidal spread of metastatic tumor	Hepatitis, Fibrosis/Cirrhosis
Hepatic Venous Outflow Obstruction (HVOO) Passive congestion and centrilobular necrosis Budd-Chiari Disease Congestive Heart Failure	Fibrosis/Cirrhosis
Nodular Regenerative Hyperplasia (NRH) Focal Nodular Hyperplasia (FNH)	Fibrosis/Cirrhosis Isolated hepatic neoplasm

It also is important to note that *vascular disease is inherent to essentially every hepatic disorder*. No one has articulated this perspective better than Dr. Ian Wanless (vis. reference 1), based on his meticulous histopathologic analysis of hepatic tissue in hepatitis, cirrhosis, and benign nodular disorders of the liver. I invite any pathologist to focus on the hepatic vasculature – portal venous, hepatic arterial, sinusoidal, and hepatic venous – in any hepatic disease of choice. Vascular lesions will be present.

Thus, the challenge is, first, to determine whether there is vascular pathology in a liver biopsy. If identified, the pathologist must then decide whether the vascular lesions are part-and-parcel of a more conventional hepatic disease, or represent what might be considered a “primary” vascular disorder of the

liver. Failure to recognize vascular disorders of the liver places the medical team at risk for subjecting the patient to a protracted course of ineffective diagnostic evaluation and intervention.

This discussion will *not* address hepatic transplantation pathology, in which hepatic arterial compromise, especially, constitutes a major diagnostic category. The differential diagnosis that includes Focal Nodular Hyperplasia is covered elsewhere in this symposium. Rather, this discussion will focus on the grab-bag of hepatitis, fibrosis/cirrhosis, and bile duct disease, in non-transplant patients.

### **Portal Vein Disease**

Blockage of the extrahepatic portal vein may be insidious and well tolerated or may be a catastrophic and potentially lethal event; most cases fall somewhere in between. Occlusive disease of the portal vein or its major radicles typically produces abdominal pain and, in most instances, ascites and other manifestations of portal hypertension, principally esophageal varices which are prone to rupture. Hence, this condition falls under the differential diagnosis of cirrhosis/portal hypertension.

*Extrahepatic portal vein obstruction* may arise from the following conditions, but in about half of cases, no cause can be implicated.:

- *Banti syndrome*, in which subclinical occlusion of the portal vein (as from neonatal umbilical sepsis or umbilical vein catheterization) presents as variceal bleeding and ascites years later
- Intra-abdominal sepsis, for example, acute diverticulitis or appendicitis leading to *pylephlebitis* in the splanchnic circulation
- Thrombogenic disorders, including postsurgical thromboses, perinatal exchange transfusion (through the umbilical vein)
- Trauma
- Pancreatitis that initiates splenic vein thrombosis, which propagates into the portal vein

Ideally, portal vein occlusion is identified in a living patient by radiographic mechanisms. Involvement of a pathologist is usually at the time of post-mortem examination. In a deceased patient exhibiting ascites, intestinal congestion, splenomegaly, or other features of portal hypertension, *examination of the porta hepatis for portal vein thrombosis is mandatory*. Causes of portal vein thrombosis include cirrhosis, hepatocellular carcinoma, pancreatitis, and even intraabdominal sepsis.

Invasion of the portal vein system by primary or secondary cancer in the liver can progressively occlude portal inflow to the liver; tongues of hepatocellular carcinoma can even occlude the extrahepatic portal vein. These lesions may be identified radiographically. Identification of vascular invasion by hepatocellular carcinoma is a mandatory part of evaluating resected surgical specimens.

*Idiopathic portal hypertension* is a chronic, generally bland condition of impaired portal vein inflow and noncirrhotic portal hypertension. In those instances in which a cause can be identified, it may be associated with hypercoagulability of the blood, myeloproliferative disorders, peritonitis, chronic exposure to arsenicals, or autoimmune disorders. The presumed cause is a long-standing state of intrahepatic vascular inflammation and fibrosis, predominantly in the portal tree, leading to obliteration of intrahepatic vascular channels and ensuing portal tract fibrosis. The histologic manifestation is termed *hepatoportal sclerosis*, owing to dense fibrosis of intrahepatic portal tracts with obliteration of portal vein channels. Whether this histology represents a late stage of healed liver injury or a primary progressive disorder is unclear. Idiopathic portal hypertension has unusually high incidence in some parts of the world, for instance, up to 25% of the incidence of esophageal variceal bleeding in India are attributed to the presence of idiopathic portal hypertension.

*Suspicion of hepatoportal sclerosis is raised when sclerotic portal tracts are identified in a patient with clinical evidence of portal hypertension, in the absence of histopathological features of cirrhosis*. What constitutes sufficient evidence for hepatoportal sclerosis is a matter of opinion, since it remains unclear whether hepatoportal sclerosis should be viewed as a primary pattern of progressive non-bridging

intrahepatic fibrosis, or a healed state of more severe bridging intrahepatic fibrosis, now partially regressed.

### **Hepatic Artery Disease**

Thrombosis or compression of a large intrahepatic branch of the hepatic artery by embolism or neoplasia may result in a localized intrahepatic infarct. The clinical scenario of a patient with a severe thromboembolic disorder, hepatocellular carcinoma, or disseminated metastatic cancer, is not subtle and the differential diagnosis is not likely to be difficult. The one situation in which the clinical presentation may be exceedingly confusing is polyarteritis nodosa, which may present with a bewildering array of intra-abdominal symptoms, features of “hepatitis”, or other localizing symptomatology (e.g., “cholecystitis”). The pathologist may have the first opportunity to identify the vasculitic lesion of polyarteritis nodosa, on a liver biopsy or resection of hepatic tissue.

Interruption of the main hepatic artery does not always produce ischemic necrosis of the organ, particularly if the liver is otherwise normal. Retrograde arterial flow through accessory vessels, when coupled with the portal venous supply, is usually sufficient to sustain the liver parenchyma. (The one exception is hepatic artery thrombosis in a transplanted liver, which generally leads to infarction of the major ducts of the biliary tree and loss of the organ.)

### **Impaired Blood Flow Through The Liver**

The most common *intrahepatic cause* of blood flow obstruction is *cirrhosis*, for which this audience needs no elaboration. In addition, physical occlusion of the *sinusoids* occurs in a small but striking group of diseases. In *sickle cell disease*, the hepatic sinusoids may become packed with sickled erythrocytes, free in the sinusoids, or phagocytosed by Kupffer cells, leading to panlobular parenchymal necrosis.

*Disseminated intravascular coagulation (DIC)* may occlude sinusoids. This is usually inconsequential except for the spectacular periportal sinusoidal occlusion and parenchymal necrosis that may arise in pregnancy as part of *eclampsia*.

It is surprising the relative frequency with which a diagnosis of *amyloidosis* is first made by the pathologist examining a liver biopsy – and without antecedent clinical history that might raise suspicions of this disorder. In addition to alerting the clinical team of the need to work up the patient for the cause, the pathologist needs to characterize the amyloid deposits by immunohistochemistry.

Metastatic tumor cells (e.g., breast carcinoma, lymphoma, malignant melanoma) may fill the hepatic sinusoids in the absence of a mass lesion. The attendant obstruction to blood flow and massive necrosis of hepatocytes can lead to fulminant hepatic failure. An example of such a process was a patient referred for hepatic transplantation for presumed drug-induced acute hepatic failure. The patient succumbed to hepatic failure in the hours prior to transplantation, and was found at autopsy to have a liver 95% replaced by intrasinusoidal malignant melanoma. The small remainder of hepatocellular parenchyma had, indeed, been compromised by an unintended overdose of acetaminophen owing to the small hepatic reserve remaining, tipping the patient into “drug-induced hepatic failure”.

### **Passive Congestion and Centrilobular Necrosis**

The condition of “congestive hepatopathy”, or “cardiac sclerosis”, is not usually an issue in evaluation of liver biopsies, since the clinical history is usually so striking. However, the pathologist may be called upon to verify that such “cardiac congestion” is the only apparent cause of hepatic compromise. Absence of other features of hepatitis, such as inflammatory portal tract disease, pan-lobular hepatocellular degeneration, or periportal fibrosis, is helpful.

Arguably, passive congestion of the liver occurs in virtually every non-catastrophic natural death, as the heart slowly fails. In such instances, the vascular channels of the liver, including the terminal hepatic vein, remain sharply defined. In the case of a more protracted circulatory compromise of the liver,

usually preterminal, *centrilobular necrosis* (of hepatocytes), without or with hemorrhage of erythrocytes into the hepatic plates, may occur.

It is those patients without a clear cardiac history, but with centrilobular hemorrhage and necrosis, that beg the issue of whether there is a primary hepatic vascular disorder. This situation may arise both in evaluation of a liver biopsy, or a post-mortem liver. The chief differential diagnosis is Sinusoidal Obstruction Syndrome, and other causes of Hepatic Venous Outflow Obstruction.

### ***Sinusoidal Obstruction Syndrome (Veno-Occlusive Disease)***

Originally described in Jamaican drinkers of pyrrolizidine alkaloid-containing bush tea and named veno-occlusive disease, the disease is now called sinusoidal obstruction syndrome, and occurs primarily in the immediate weeks following bone marrow transplantation. The incidence approaches 25% in recipients of allogeneic marrow transplants, usually within the first 3 weeks. Sinusoidal obstruction syndrome can occur in cancer patients receiving chemotherapy, especially with agents such as gemtuzumab and ozagamicin, used in the treatment of acute myeloid leukemia, actinomycin D in the treatment of Wilm's tumors, dacarbazine - a drug that is activated by sinusoidal endothelial cells, and in patients who receive cytotoxic agents such as cyclophosphamide prior to bone marrow transplantation (discussed below). The mortality rates can be over 30%. Although histology is the gold standard for the diagnosis, a diagnosis of sinusoidal obstruction syndrome is frequently made on clinical grounds only (tender hepatomegaly, ascites, weight gain, and jaundice), owing to the high risk of liver biopsy in these patients.

Sinusoidal obstruction syndrome is characterized by obliteration of hepatic vein radicles by varying amounts of subendothelial swelling and fine reticulated collagen. In acute disease, there is striking centrilobular congestion with hepatocellular necrosis and accumulation of hemosiderin-laden macrophages. As the disease progresses, obliteration of the lumen of the venule is easily identified by using special stains for connective tissue (Fig. 18–48). In chronic or healed sinusoidal obstruction syndrome, dense perivenular fibrosis radiating out into the parenchyma may be present, frequently with total obliteration of the venule; hemosiderin deposition is evident in the scar tissue, and congestion is minimal.

Sinusoidal obstruction syndrome arises from toxic injury to the sinusoidal endothelium<sup>66</sup> which presumably starts with the depolymerization of actin in sinusoidal endothelial cells and increased production of metalloproteinases. Endothelial lining cells round up and slough off the sinusoidal wall, embolizing downstream and obstructing sinusoidal blood flow. This is accompanied by entry of erythrocytes into the space of Disse, necrosis of perivenular hepatocytes and downstream accumulation of cellular debris in the terminal hepatic vein. Proliferation of perisinusoidal stellate cells and subendothelial fibroblasts in the terminal hepatic vein follows, with fibrosis and deposition of extracellular matrix in the sinusoids.

### **Hepatic Venous Outflow Obstruction (other than Sinusoidal Obstruction Syndrome)**

Obstruction of a single main hepatic vein by thrombosis is clinically silent. The obstruction of two or more major hepatic veins produces liver enlargement, pain, and ascites, a condition known as *Budd-Chiari syndrome*. Hepatic damage is the consequence of increased intrahepatic blood pressure, and an inability of the massive hepatic blood flow to shunt around the blocked outflow tract. *Hepatic vein thrombosis* is associated with primary myeloproliferative disorders (including polycythemia vera), inherited disorders of coagulation (e.g., deficiencies in antithrombin, protein S, or protein C, or mutations of factor V), antiphospholipid syndrome, paroxysmal nocturnal hemoglobinuria, and intra-abdominal cancers, particularly hepatocellular carcinoma. The occurrence of hepatic vein thrombosis in the setting of pregnancy or oral contraceptive use is usually through interaction with an underlying thrombogenic disorder. About 10% of cases are idiopathic in origin, presumably unrecognized thrombogenic disorders.

With acutely developing thrombosis of the major hepatic veins or the hepatic portion of the inferior vena cava, the liver is swollen and red-purple and has a tense capsule (Fig. 18–47). Microscopically, the

affected hepatic parenchyma reveals severe centrilobular congestion and necrosis. Centrilobular fibrosis develops in instances in which the thrombosis is more slowly developing. The major veins may contain totally occlusive fresh thrombi, subtotal occlusion, or, in chronic cases, organized adherent thrombi.

The challenge for the pathologist examining a liver biopsy with profound pericentral congestion is to distinguish between a Budd-Chiari syndrome pattern versus a Sinusoidal Obstruction Syndrome pattern. Terminal hepatic vein lumina will be visible in the former, and difficult to identify or absent in the latter.

### **Nodular Regenerative Hyperplasia**

Nodular regenerative hyperplasia is a condition that presents with portal hypertension and its attendant symptomatology. Not uncommonly, the clinical presentation is muddled, in that a clear antecedent cause (such as a sub-clinical vasculitic condition such as rheumatoid arthritis) is not identified. A liver biopsy is obtained to identify a cause of portal hypertension, usually suspected to be "cirrhosis".

Instead, the pathologist observes essentially no intrahepatic inflammation, and a variable pattern of hepatocyte plate atrophy and regeneration, without significant hepatic fibrosis. *It is only identification of a curvilinear pattern to the plate atrophy* that the potential arises of Nodular Regenerative Hyperplasia. A reticulin stain can confirm the impression of curvilinearity. However, a liver biopsy is too limited in sampling, and diameter, to permit a definitive diagnosis of Nodular Regenerative Hyperplasia. Rather, the pathologist raises the possibility, and only then can a clinical history be pieced together that does, or does not, support this interpretation.

Nodular Regenerative Hyperplasia affects the entire liver with roughly spherical nodules, in the absence of fibrosis. Microscopically, plump hepatocytes are surrounded by rims of atrophic cells, confirmed on reticulin staining. The presumed cause is a smoldering subclinical pattern of intrahepatic vascular occlusion; this leads to variable but pan-hepatic atrophy of regions of the parenchyma. Better-vascularized regions then hypertrophy, in the form of nodules. This lesion occurs in association with conditions affecting intrahepatic blood flow, including solid organ (particularly renal) transplantation, bone marrow transplantation, and vasculitic conditions. The common factor in both lesions appears to be heterogeneity in hepatic blood supply, arising from focal obliteration of portal vein radicles with compensatory augmentation of arterial blood supply.

### **Conclusion**

In the end, the hepatic pathologist must grow comfortable with the fact that intravascular thrombosis and occlusion may occur in virtually any condition of hepatitis and as a key component of progressive fibrosis and cirrhosis. It is a heightened suspicion for vascular disease per se that gives opportunity for the pathologist to make a singular contribution to the diagnostic evaluation of a patient with intrinsic vascular disease, and hence their clinical management.

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# Acute and Chronic Hepatitis

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## Acute Hepatitis

### Clinical Features

Classically acute hepatitis is defined clinically as a significant (at least 2X elevation above the upper reference level) elevation of the serum ALT and/or AST levels in a patient without a previous history of liver disease. Serum markers of cholestatic liver injury should not be present. Most patients with acute hepatitis are asymptomatic. The causes of mild elevations in serum ALT or AST are listed in Table 1. In more severe cases fatigue, abdominal pain, nausea and vomiting, muscle aches or jaundice might be present (fulminant acute hepatitis is considered separately below). Serologic tests for hepatitis A, B or C infection and autoimmune hepatitis should be obtained routinely. A careful drug/toxin history must also be sought.

Table 1: Cause of Mild Elevations of Serum AST or ALT

Hepatic: predominantly ALT
Acute viral hepatitis (A-E, EBV, CMV)
Chronic hepatitis B & C
Autoimmune hepatitis
Medications/Herbal preparations/Dietary supplements
Toxins
Steatosis and Steatohepatitis
Genetic hemochromatosis
Alpha-1-antitrypsin deficiency
Wilson's disease
Celiac disease
Hepatic: predominantly AST
Alcoholic steatosis and steatohepatitis
Cirrhosis of any cause
Non-hepatic
Hemolysis
Myopathy
Thyroid disease
Strenuous exercise
Macro-AST

Adapted from Green R, Flamm S. AGA medical position statement. Gastroenterology 2002.

## Histologic Features

Biopsy provides little useful information in most cases of acute hepatitis, and therefore is rarely performed by knowledgeable hepatologists. Biopsies are indicated in the following clinical situations:

- 1) When there is clinical suspicion of a second independent hepatic insult (i.e., an underlying chronic liver disease).
- 2) In immunocompromised patients, where an unusual infectious process is possible
- 3) To ascertain the degree of hepatocyte necrosis in a patient with possible submassive hepatic necrosis, who may need to be listed for liver transplantation

The defining histologic pattern for all forms of acute hepatitis is encompassed by the term “**lobular disarray**”. The following features characterize this pattern:

- Hepatocyte ballooning degeneration
- Lobular and sinusoidal inflammatory cell infiltrates
- Scattered acidophil bodies
- Foci of individual hepatocyte dropout
- Kupffer cell hyperplasia

The portal tracts changes in acute hepatitis are generally inconspicuous, consisting of sparse mononuclear cell infiltrates. Steatosis is not a histologic feature of any form of acute hepatitis, and when present suggests steatohepatitis or both acute hepatitis and a separate steatotic process. Biliary changes (bile duct proliferation or bile duct loss, cholate stasis, or prominent cholestasis should also suggest another process.

The diagnosis of acute hepatitis is usually apparent in the H&E stained section. However, special stains can be very helpful in confirming the initial impression. Histologic evidence of acute hepatitis in the resolving phase can be quite subtle and is easily overlooked if a detailed clinical history is not provided. A PAS with diastase stain is very useful in such cases because it highlights the presence of cellular debris in Kupffer cells (a good sign of previous hepatocyte necrosis/apoptosis). A reticulin stain may also highlight foci of individual hepatocyte dropout. In more severe cases the reticulin confirms the presence of zonal or bridging necrosis. No fibrosis should be evident in the trichrome stained section.

## Acute Hepatitis C

**Clinical:** Acute HCV hepatitis is almost always entirely asymptomatic, and therefore is rarely recognized clinically or biopsied. The most common symptom is mild fatigue. A diagnosis is usually made only when an exposure event is recognized (e.g., accidental needle stick). There is no clinical reason to perform a biopsy in a known case, except to rule out a second contributing cause of liver dysfunction.

**Histologic features:** Biopsies usually exhibit nonspecific features of acute hepatitis, although mild ductular proliferation and cholestasis have also been described in biopsies obtained immediately after infection (see *Johnson K et al Am J Surg Pathol 2007*).

## Acute Hepatitis B

**Clinical features:** Acute HBV hepatitis is usually asymptomatic, but some patients present with a flu-like illness, jaundice, or lethargy. Serologic testing for acute HBV hepatitis is quite accurate, so there is no role for biopsy in suspected cases.

**Histologic features:** There are no distinctive histologic features of acute HBV hepatitis. Ground glass hepatocytes are never present in *acute* HBV hepatitis. Likewise, immunohistologic stains with HBsAg and HBcAg antibodies are negative in acute HBV hepatitis.

## Acute Hepatitis A

**Clinical features:** Acute HAV hepatitis is usually asymptomatic in children, but can produce jaundice or a flu-like syndrome in adults. Serologic testing for anti-HAV IgM is diagnostic of acute infection. Although there is no chronic form of HAV hepatitis, there is a relapsing form with a prolonged course (over 6 months), which sometimes takes a cholestatic form. Biopsy is often performed in these cases because of this unusual course, and to rule out any possible superimposed disorder.

Histologic features: Acute HAV hepatitis is usually indistinguishable from other types of acute hepatitis. However, on occasion there is more portal inflammation than is typical, and the infiltrate may include a prominent component of plasma cells. These features can result in an appearance easily confused with autoimmune hepatitis. Thus, the possibility of acute HAV hepatitis should always be considered before making a diagnosis of serologically negative autoimmune hepatitis (unless fibrosis is present).

## Acute Autoimmune Hepatitis

Clinical features: Generally autoimmune hepatitis causes significant symptoms, including fatigue, nausea and vomiting, jaundice and muscle aches. Clinical distinction between acute and chronic AIH is usually not possible, and many believe there is no acute form of the disease (i.e., AIH begins with an asymptomatic phase and when diagnosed is by definition “chronic”).

Histologic features: Because chronic AIH often exhibits severe interface and lobular necroinflammatory activity (including bridging *necrosis* in some cases) it is difficult to distinguish from acute AIH. The difficulty in distinguishing between true fibrosis (an undeniable feature of chronic disease) and periportal or bridging necrosis with resultant reticulin collapse (which could occur in either acute or chronic AIH) makes it even harder to histologically separate acute and chronic AIH. Moreover, a prominent component of plasma cells in the portal infiltrates is usually present in acute AIH. The most reliable feature for the diagnosis of *acute* AIH is the presence of a degree of lobular disarray in excess of that expected by the degree of portal inflammation and interface activity. In practical terms histologic distinction between acute and chronic AIH has little importance, since the same treatment with steroids will be instituted regardless. The most important task of the surgical pathologist is to recognize the possibility of AIH and to not overestimate the degree of fibrosis (i.e., avoid confusion with reticulin collapse due to periportal necrosis). In a small subset of cases of acute AIH centrilobular necroinflammatory activity predominates, with relatively inconspicuous portal inflammation.

## Drug Induced Acute Hepatitis

Clinical features: Elevation of serum ALT and ALP after institution almost any medication should strongly suggest the possibility of drug induced hepatitis. Unfortunately the temporal relationship between the onset of liver chemistry test abnormalities (or symptoms) and the start of the drug therapy is not of much use (particularly if it is the patient's first exposure to the agent). A Medline search for reports of acute hepatitis caused by the medications the patient is taking is helpful, but no drug can completely be excluded on the basis of lack of prior reports of hepatotoxicity. Antibiotics and anti-epileptics are the most common classes of medications that cause clinically significant liver dysfunction. Of course, the possibility of toxicity due to an herbal preparation or dietary supplement must always be sought assiduously in any case of unexplained hepatotoxicity. Many toxins can also produce acute hepatitis, and again a careful clinical history is key to arriving at the proper diagnosis.

Histologic features: The presence of more than rare scattered eosinophils is very unusual in acute HAV, HBV, or HCV hepatitis, and should raise the possibility of drug toxicity. Unfortunately, eosinophils are not prominent in most cases of drug-induced acute hepatitis. Autoimmune hepatitis is sometimes triggered by a drug exposure, and this possibility should be kept in mind when eosinophils are prominent in a biopsy that otherwise exhibits typical histologic features of AIH. Many toxins produce a distinct centrilobular pattern of hepatocyte injury.

## Massive and Submassive Hepatocyte Necrosis

Clinical features: The common causes of fulminant hepatic failure include: acute HAV hepatitis, acute HBV infection, acute autoimmune hepatitis, drug and toxin induced injury, ischemic injury (due to decreased portal vein or hepatic vein flow), and Wilson's disease. The later two are not regarded as forms of acute hepatitis. Fulminant HCV hepatitis is extraordinarily rare. Fulminant herpes simplex virus or adenovirus infection can cause massive hepatic necrosis in immunocompromised hosts. Rarely massive infiltration of the liver by tumor (melanoma, leukemia) can cause liver failure. Patients present with signs and symptoms of liver failure. Symptoms include lethargy, encephalopathy, and coma. Laboratory evaluation may reveal AST and ALT levels of 1000 or more, jaundice, coagulopathy, and high serum

ammonia. In many cases of fulminant hepatic failure the etiology is never identified.

Histologic features: Biopsy is not needed in most cases, and is contraindicated in the face of severe coagulopathy. Biopsy is performed in less severe cases as part of the assessment for the need to list the patient for possible liver transplantation. Acute necrosis of more than 30-40% of the parenchyma suggests that the injury may not be recoverable. Marked periportal or panlobular necrosis results in prominent bile ductular proliferation, which should not be misinterpreted as evidence of biliary tract disease. These proliferating ductules, which represent a regenerative response, can transform into hepatocytes to repopulate the liver, assuming that the degree of hepatocyte loss doesn't lead to death before the transformation can occur.

Not infrequently large areas of parenchymal collapse and extinction with subsequent nodular hepatic regeneration can simulate cirrhosis both radiographically and histologically. The deep blue color of true fibrosis in a trichrome stained section can easily be confused with the lighter blue color produced by condensation of reticulin fibers in massive hepatic necrosis, compounding the confusion with cirrhosis. An orcein or reticulin stain is very helpful in such cases.

## Histologic Differential Diagnosis

The histologic pattern of lobular disarray in acute hepatitis is distinctive and is not often confused with other entities. Lobular inflammation is present in EBV hepatitis, but in this disorder there is little hepatocellular injury. CMV hepatitis in heavily immunocompromised hosts is characterized by lobular neutrophilic microabscesses but no lobular disarray. In more immunocompetent patients the appearance can be similar to EBV hepatitis. HSV and adenovirus hepatitis produces randomly distributed foci of hepatocyte necrosis. Viral inclusions are usually evident at the edges of the necrotic zones. Severe sudden hepatic ischemia (e.g., "shock liver") causes a distinct centrilobular pattern of hepatocyte ballooning and necrosis. Bacterial infections of the liver (e.g. listeria) produce focal neutrophilic infiltrates with localized foci of hepatocyte necrosis. The presence of

cholestatic hepatitis raises an entirely distinct set of diagnostic considerations, which is beyond the scope of this discussion.

## The Bottom Line – how to sign out the case

The term “acute hepatitis” is an acceptable diagnosis to describe the changes in biopsies thought or known to be due to HAV, HBV, HCV, AIH or drug induced injury. A descriptor of severity can also be added (mild, severe) to highlight the degree of hepatocyte injury. The presence of bridging necrosis should be mentioned in the diagnosis, if present, since it may indicate a greater risk for the later development of cirrhosis. In cases of massive hepatic necrosis a rough estimate of the percentage of hepatocytes that are necrotic or have dropped out is useful to the clinician. The appearance of remaining viable hepatocytes should also be mentioned. In many cases of acute hepatitis an exact etiology is never determined.

## Chronic Hepatitis

**Clinical features:** Many patients with chronic hepatitis are completely asymptomatic, even when cirrhosis is present. The most common symptom is fatigue. Symptoms of decompensated liver failure may develop in some patients with end-stage cirrhosis, often precipitated by an intercurrent illness. Variceal bleeding and hepatic encephalopathy may develop. Physical signs of cirrhosis may also be manifested (edema and ascites, spider angioma, palmar erythema, gynecomastia and testicular atrophy in men etc.). Patients with cirrhosis are at particular risk of hepatocellular carcinoma, especially those with cirrhosis due to chronic HBV and HCV hepatitis.

**Histologic features:** The typical histologic features of chronic hepatitis include portal inflammation, interface activity, spotty lobular inflammation, and scattered acidophil bodies or foci of individual hepatocyte dropout. The severity of each of the changes obviously varies from case to case, and may be absent in some. Prominent lobular disarray, which defines the histologic pattern of acute hepatitis, resolves as conversion to chronic hepatitis develops. Acidophil body formation generally does not result in an increase in the serum AST or ALT levels. Of course the sine qua non of chronic hepatitis is the presence of portal fibrosis, which can ultimately lead to the development of macronodular cirrhosis.

## Chronic Hepatitis C

**Clinical features:** A large majority of patients with chronic HCV hepatitis are asymptomatic. About 85% of patients infected by the HCV infection go on to develop chronic HCV hepatitis. Cirrhosis will develop in about 20-35% of these patients, generally taking more than 20 years to develop. Cirrhosis is a much more likely outcome in patients who become infected as adults.

**Histologic features:** Before serologic tests were developed the histologic triad of portal lymphoid aggregates, macrovesicular steatosis, and lymphocytic infiltration of bile duct epithelium were identified as features that defined “non-A, non-B” hepatitis. Bile duct loss and granulomas are not features of HCV hepatitis. Steatosis is present in about 50% of cases, and steatohepatitis in about 10%. The presence of steatohepatitis is often overlooked by pathologists, and called simple steatosis instead.

Centrilobular sinusoidal fibrosis, centrilobular hepatocyte ballooning change, and Mallory bodies are not features of chronic HCV hepatitis, and their presence should prompt a diagnosis of superimposed steatohepatitis. Fatty change occurs due to either viral or host factors. Genotype 3 of the HCV virus has been shown to directly cause steatosis, and effective anti-viral treatment will result in resolution of steatosis. In patients infected by other genotypes of the virus host factors are responsible for steatosis and steatohepatitis (e.g., obesity, diabetes, ethanol use, medications, insulin resistance). The presence of steatohepatitis or significant steatosis (>33%) diminishes the efficacy of anti-viral therapy, and therefore these features should be mentioned in the report if present.

The degree of necroinflammatory activity in chronic HCV hepatitis is almost always quite mild (grade 1 or 2). The presence of grade 3 or 4 necro-inflammatory activity should strongly suggest the possibility of another superimposed condition (most often autoimmune hepatitis or drug induced hepatotoxicity). There is some data to suggest that the presence of hemosiderosis may decrease the probability of treatment response and/or increase the risk for subsequent fibrosis. Therefore, a comment regarding the degree of iron deposition should be included in the report.

## Chronic Hepatitis B

Clinical features: Chronic HBV hepatitis occurs in 70-90% of patients infected as infants or in childhood, but the majority of adults infected resolve their acute infection and become immune. In 30% of infected patients an identifiable risk factor is not evident. Serologic testing for HBsAg is diagnostic of active infection. A chronic inactive carrier state is defined by an asymptomatic patient with normal serum AST and ALT with positive serum HBsAg and anti-HBeAg tests and negative HBV DNA test.

Histologic features: Ground glass hepatocytes are the most diagnostic feature of chronic HCV hepatitis, but are seen in only about 50% of biopsies. Ground glass hepatocytes have finely granular acidophilic cytoplasm, often with a clear halo at the plasma membrane. This appearance is created by a proliferation of the endoplasmic reticulum that contains abundant viral particles. The hepatocyte nucleus is usually pushed to the periphery by the inclusion. A “sanded” appearance of the nucleus may occur rarely. Scattered individual or small clusters of ground glass hepatocytes are usually randomly distributed throughout the lobules. In cirrhotic livers there may be many ground glass hepatocytes in some of the regenerative nodules and none in others. Hepatocytes with oncocytic change can appear very similar, but this change often involves larger clusters of hepatocytes and does not produce a peripheral halo or push the nucleus to the side. Immunohistologic studies can be used to demonstrate that the ground glass cells are reactive with the HBsAg antibody (many other hepatocytes are usually also positive). The HBcAg antibody reveals nuclear reactivity in hepatocytes in which there is ongoing active viral replication. However, the availability of highly accurate serologic tests makes immunohistologic demonstration of HBsAg or HBcAg unnecessary except in specific and very rare situations.

In a chronic inactive carrier there is usually no or very minimal portal or lobular inflammation, but ground glass hepatocytes may be numerous. In patients with active chronic HBV hepatitis there is a variable degree of portal inflammation, interface activity, and lobular necroinflammatory changes. Steatosis is uncommon. At presentation grade 3 necroinflammatory changes are frequently seen. An acute flare of disease may also result in prominent necroinflammatory changes. The possibility of superinfection by hepatitis delta virus should be considered in such patients.

## Chronic Autoimmune Hepatitis

Clinical features: Females are much more likely than males to be affected by chronic AIH. There is a bimodal age distribution, with peaks in the teenage years and in the fourth and fifth decades. Although AIH is not completely excluded if serologic tests are negative, a significant elevation of the titer of anti-nuclear antibody (ANA), anti-smooth muscle antibody (ASMA) or anti-liver/kidney microsomal antibody (anti-LKM) is very helpful in making the diagnosis. The anti-LKM antibody is elevated in less than 5% of patients. In borderline case application of an international consensus scoring system can be utilized. As mentioned previously, AIH can be triggered by exposure to a drug, and this possibility should be kept in mind clinically since withdrawal of the drug can be curative in a small proportion of cases. Minocycline, an antibiotic used in the treatment of acne, is a common drug trigger of chronic AIH. Treatment with high dose steroids is usually rapidly effective in inducing remission, and treatment response actually is useful in confirming the diagnosis. Imuran is then typically introduced as a steroid-sparing agent. Re-biopsy is often performed after several years of tapering treatment to assess the degree of ongoing necroinflammatory activity. If minimal activity is evident histologically an attempt to completely halt steroid therapy may be considered.

Histologic features: In the initial diagnostic biopsy AIH usually exhibits striking necroinflammatory activity (grade 3 or 4). The presence of bridging necrosis should suggest the possibility of AIH. Clusters of plasma cells are often a prominent component of the portal tract inflammatory cell infiltrates, but are not required for the diagnosis. Pseudoacinar formations (hepatocyte rosettes) are a common lobular histologic feature. In a small subset of patients giant cell transformation occurs, but there is no particular clinical significance of this feature. Significant zone 3 inflammation and hepatocyte necrosis or dropout is evident in some patients with autoimmune hepatitis.

Assessment for the presence of fibrosis should be approached cautiously in the initial AIH biopsy, because periportal or bridging necrosis leads to collapse of the reticulin framework. In the trichrome stain the condensed reticulin fibers will produce a grayish (steel) blue staining that can easily be

confused with true (bright blue) fibrosis. The presence of more than rare eosinophils in the portal and lobular inflammatory cell infiltrates should raise the possibility of drug-induced AIH.

## Drug-induced Chronic Hepatitis

Clinical features: While several agents are known to trigger autoimmune hepatitis, it is uncommon for a drug to produce a simple chronic hepatitis pattern (See Table 2).

Table 2

<u>Drug induced AIH</u>	<u>Drug induced CH</u>
Diclofenac	Etretinate
Ecstasy (MDMA)	Lisinopril
Methyldopa	Sulfonamide
Minocycline	Trazodone
Nitrofurantoin	Isoniazid
Propylthiouracil	
Dihydralazine	

Histologic features: Other than the presence of a prominent component of eosinophils, which occurs in only a minority of cases, there are no distinctive histologic features of drug induced chronic hepatitis.

## Differential Diagnosis of Chronic Hepatitis

### Wilson's disease

Clinical features: Wilson's disease is an autosomal recessive disorder caused by a mutation in a copper binding protein involved in the transport of copper from hepatocytes into bile. Patients usually present in childhood or as young adults. The serum level of another protein, ceruloplasmin, is often decreased, but may rise into the normal range in patients with significant hepatic necroinflammatory activity. Hemolysis (Coombs' negative) is common, particularly at the time of menarche in girls. It is thought to be caused by the sudden release of large amounts from the liver due to massive

hepatic necrosis. Keiser-Fleischer rings are present in patients with significant neurologic involvement, but are less common in patients who present primarily with hepatic disease. A 24-hour urine copper level is helpful in establishing the diagnosis. Genetic testing is difficult to perform because of the size of the Wilson's disease gene and the large number of possible mutation sites. Genetic testing is useful to identify affected family members of index patients with known mutations.

**Histologic features:** In childhood a liver biopsy may exhibit only steatosis or mild steatohepatitis. A copper stain (rhodanine) may be negative, but quantitative copper determination can be performed from the paraffin embedded tissue. In older patients the biopsy appearance can be indistinguishable from other forms of chronic hepatitis. One subtle clue is the presence of Mallory bodies in periportal hepatocytes, a feature not present in chronic HBV, HCV or AIH. A copper stain usually reveals deposition in periportal hepatocytes. Often considerable fibrosis, or even cirrhosis, is present at the time of diagnosis. The combination of a clinical presentation of severe acute hepatitis and a biopsy showing cirrhosis in a patient less than 45 years old is almost always due to Wilson's disease or autoimmune hepatitis. In fact, Wilson's disease should be ruled out in any patient less than 45 with unexplained chronic hepatitis. In particular, Wilson's disease should be excluded before a diagnosis of seronegative AIH is made. In cirrhotic patients copper deposition can be patchy, with no deposition at all in some regenerative nodules. Quantitative copper deposition can be helpful in such cases.

## Primary Biliary Cirrhosis (PBC)

**Clinical features:** Primary biliary cirrhosis is primarily a disease of middle-aged women. Clinical confusion with chronic hepatitis is not an issue since there is a reliable serologic marker (anti-mitochondrial antibody, or AMA) and because PBC patients demonstrate a cholestatic pattern of liver chemistry test abnormalities (elevated TB, alk P, GGT), rather than a hepatitic pattern. There are patients with PBC/AIH overlap, but this topic is beyond the scope of this review.

**Histologic features:** If no clinical information is provided histologic confusion between chronic hepatitis and PBC is possible, since both conditions are characterized by dense portal mononuclear cell infiltrates,

including lymphoid aggregates or follicles in some cases of each condition. However, in PBC there are also histologic features of chronic cholestasis, including lymphocytic cholangitis, bile duct loss, and cholate stasis. Mallory bodies may be seen in the periportal zones in advanced PBC. Granulomas are common in PBC and are exceptionally uncommon in most forms of chronic hepatitis.

## Non-specific Portal Inflammation

A few scattered inflammatory cells can be found in some portal tracts in a normal liver. The presence of a slightly increased number of portal mononuclear cells is a not uncommon finding in donor liver biopsies and at autopsy. It is possible that subclinical biliary tract disease (i.e., gallstones) or intestinal inflammation may lead to mild portal inflammation. Certainly mild portal inflammation is common with any form of clinically significant biliary obstruction. The possibility of celiac disease should also be kept in mind when a biopsy reveals unexplained mild portal inflammation. Other autoimmune diseases may also cause mild portal inflammation, and the possibility of other as yet unrecognized viral infections can not be excluded.

## **The Bottom Line – how to sign out the case**

The diagnostic term “chronic hepatitis” is best reserved for cases known or thought to be due to chronic HBV or HCV infection, AIH, Wilson’s disease and rare cases of drug toxicity. If these processes have been ruled out the descriptive term “portal inflammation” is more appropriate.

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# **Cholestasis in Hepatobiliary Diseases: A Practical Guide to Diagnosis** *--including the easily (and not so easily) missed diagnoses*

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## **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<sup>1</sup>. 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<sup>2</sup>. 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<sup>3,4</sup>. Seen in more contemporary light, the ductular reaction reflects the local interplay of various paracrine and autocrine mediators, including biliary pressure and cytokine release<sup>5</sup>. Neutrophils within the edematous portal stroma are in part related to active cytokine secretion (e.g. interleukin-8) by the ductular cells<sup>6</sup>. 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<sup>7</sup>, toxicity of certain drugs, functional bile flow impairment (ischemia/reperfusion preservation injury) and mutations of pericanalicular bile salt transport proteins<sup>8-11</sup>.

*Pattern 3 (intrahepatic bile duct disease):* this is exemplified by primary biliary cirrhosis<sup>12,13</sup> 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)<sup>14-16</sup> and in acute cellular rejection following liver transplantation<sup>17</sup>. This pattern of cholestatic injury also includes *ductopenic diseases* (colloquially sometimes referred to as “vanishing bile duct syndrome”<sup>18</sup>). 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<sup>15</sup>. Other ductopenic conditions include idiopathic adulthood ductopenia<sup>19,20</sup> and the pediatric intrahepatic bile duct paucity conditions (Alagille syndrome or syndromatic paucity of intrahepatic bile ducts, and non-syndromatic paucity of intrahepatic bile ducts)<sup>21</sup>. 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<sup>22</sup>. 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 fibro-obliterative 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<sup>23</sup>. 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.
3. Be aware of the entity of premature ductopenia PBC—though uncommon.

***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<sup>24</sup>) vs. the absent or minimal reaction seen in other conditions, including intrahepatic bile duct

paucity. Some helpful histologic guidelines were reviewed in a recent single-topic conference sponsored by the American Association for the Study of Liver Diseases<sup>25</sup>.

*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-syndromic 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<sup>26</sup>. 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<sup>27</sup>. 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.

*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.

***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<sup>28-31</sup>, 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.

*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.

***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<sup>32</sup>. 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.

*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.

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