Pathology in Patients with Chronic Liver Disease

A Practical Approach to Liver Biopsy Interpretation in Patients with Acute and Chronic Liver Diseases

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KEYWORDS

• Chronic liver disease • Pathology • Acute liver disease • Liver biopsy • Treatment • Staging

KEY POINTS

- Liver biopsy and histologic examination are the mainstay for the diagnosis of liver diseases, despite recent robust advances in imaging and molecular procedures.
- Liver biopsy can provide useful information regarding the structural integrity and type and degree of injury, disease activity, response to treatment, progression of disease and degree/staging of fibrosis.
- The caveat for liver biopsy is the limited size, possibly representing one 50,000th of whole organ, leading to sampling error.
- However, this issue is obviated to some extent by using ultrasound and computed tomography-guided biopsies.
- Liver biopsies are performed to evaluate both acute and chronic liver diseases, in addition to mass-forming lesions.

INTRODUCTION

Normal Liver Histology

The human liver is a complex organ performing a multitude of different functions that include specialized metabolic, synthetic, and secretory nature as well as uptake and detoxification of xenobiotics. Based on vascular anatomy and portal vein distribution liver is divided into 8 functionally independent segments. Each segment has its own vascular pedicle consisting of branches of portal vein, hepatic artery, and bile duct.

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Histologically, the liver cell organization is referred to as classical lobule or Rappaport's liver acinus. The classical lobule, which is considered a structural unit, is hexagonal, with terminal hepatic vein (central vein) in the center and portal triads, containing branches of hepatic artery, portal vein, and bile duct, are at the periphery (Fig. 1). In contrast with the classic lobule, Rappaport's acinus is based on functional microcirculatory pattern and contains portal triads in the center and hepatic vein radicals at the periphery. The liver acinus is arbitrarily subdivided into zones 1, 2, and 3 corresponding to periportal, mid portion, and perihepatic venular areas, respectively. Hepatocytes in zone 1 (periportal area) receives blood rich in oxygen and nutrients and cells in zones 2 and 3 receive blood that is progressively depleted of energy resources, making hepatocytes in zone 3 particularly vulnerable to circulatory insults. In the lobule, hepatocytes are arranged in rows of single cell cords separated by sinusoids. Although under light microscopy all the hepatocytes in the lobule seem to be homogeneous, it is well-recognized that cells in different zones exhibit structural and functional heterogeneity.

ADEQUACY OF LIVER BIOPSY

Irrespective of the route by which liver biopsy is performed, the goal is to obtain a fragment(s) of tissue measuring 1 to 2 cm in length, preferably using a broader gauge needle so that a minimum of 10 to 20 portal areas are included for proper evaluation of rejection, biliary tract diseases, and acute and chronic liver diseases.[1–3] With an adequate liver biopsy specimen, it is the responsibility of the pathologist to systematically analyze both the lobules and portal areas for parenchymal diseases, biliary tract diseases, sinusoidal changes, and mass lesions.

USEFULNESS OF SPECIAL STAINS

Throughout the years, the use of special stains in histopathologic evaluation of liver biopsies has been refined to accommodate a varied set of diagnostic problems in liver function and disease state. Special stains help to increase the diagnostic accuracy of the grading and staging of chronic hepatitis; diagnosing cirrhosis and biliary tract disorders; and assessing fatty liver disease, iron and copper overload, and neoplastic process.[4] Although the exact methods vary between institutions, many hospitals have a standardized set of stains for each liver biopsy. These stains include hematoxylin and eosin, trichrome, reticulin, iron, periodic acid-Schiff (PAS), and diastase pretreated PAS.[4]

Masson trichrome staining helps to identify fibrosis and is the standard method for demonstrating the extent of fibrosis including cirrhosis (Fig. 2). Pathologist should be

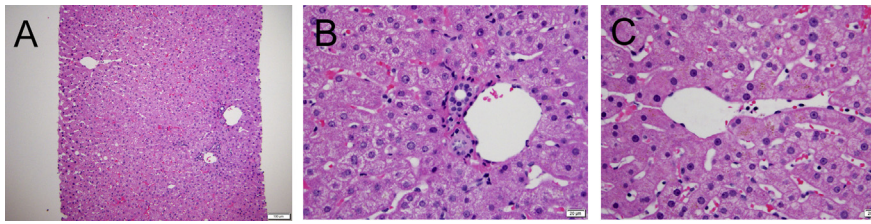

Fig. 1. Morphology of the liver. (*A*) Liver biopsy with normal architecture of central vein to portal triads, (*B*) normal portal tract showing interlobular bile duct, arteriole and portal-vein branch, and (*C*) normal central vein and liver parenchyma.

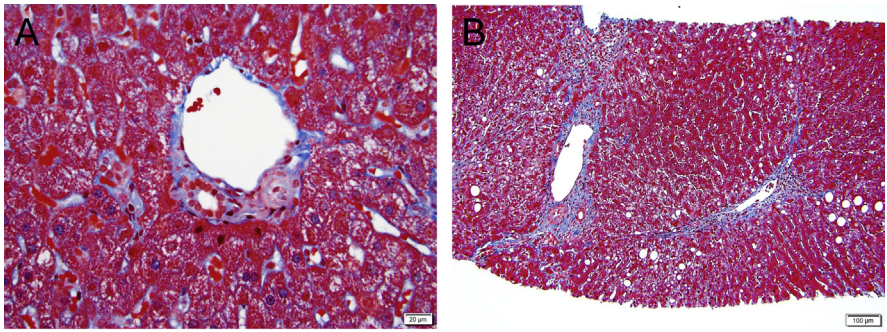

Fig. 2. Masson trichrome stain. (*A*) Normal portal tract and (*B*) portal fibrosis with thin fibrous bridging.

very cautious in interpreting trichrome stain, because collapse owing to necrosis may be interpreted as fibrosis. It is always important to compare the intensity of staining in the portal areas and other areas (see **Fig. 2**A). True fibrosis should show same dark blue stain (see **Fig. 2**B), whereas collapsed areas show a pale blue staining pattern. Reticulin stain is useful in evaluating architecture, collapse, necrosis, fibrosis, and thickness of hepatic plates. Normally, hepatic plates are 1 cell thick (and, rarely, 2 cells thick; **Fig. 3**A), whereas in regeneration and nodular regenerative hyperplasia, the plates are usually 2 to 3 cells thick and in neoplastic lesions they are more than 3 cells thick. In hepatocellular carcinomas, the reticulin fibers are markedly decreased or completely lost (**Fig. 3**B).

PAS stain demonstrates hepatocellular glycogen stores (**Fig. 4**A). Decreased PAS staining can be seen in ischemic damage or necrosis of hepatocytes that have lost their glycogen stores (**Fig. 4**B). Diastase pretreated PAS stains nonglycogen glycoproteins, which has strong usefulness in the identification of phagocytic material within histiocytes (**Fig. 4**C), alpha-1-antritrypsin globules in hepatocytes (**Fig. 4**D), and basement membrane surrounding the bile ducts and ductules.

Iron stain (Perls' Prussian blue stain) identifies hemosiderin and is useful in assessing the amount of iron in both hepatocytes, bile duct epithelium, histiocytes, and Kupffer cells (**Fig. 5**). Hemosiderin with Perls' stain appear as bluish granular material, whereas ferritin appears as a faint blue blush and should not be interpreted as iron.

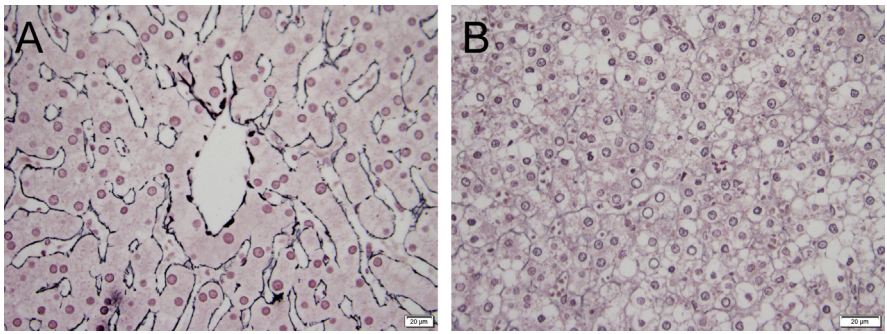

Fig. 3. Reticulin stain. (*A*) Reticulin-highlighted hepatocyte plate in normal liver. (*B*) Well-differentiated hepatocellular carcinoma with loss of reticuline fiber.

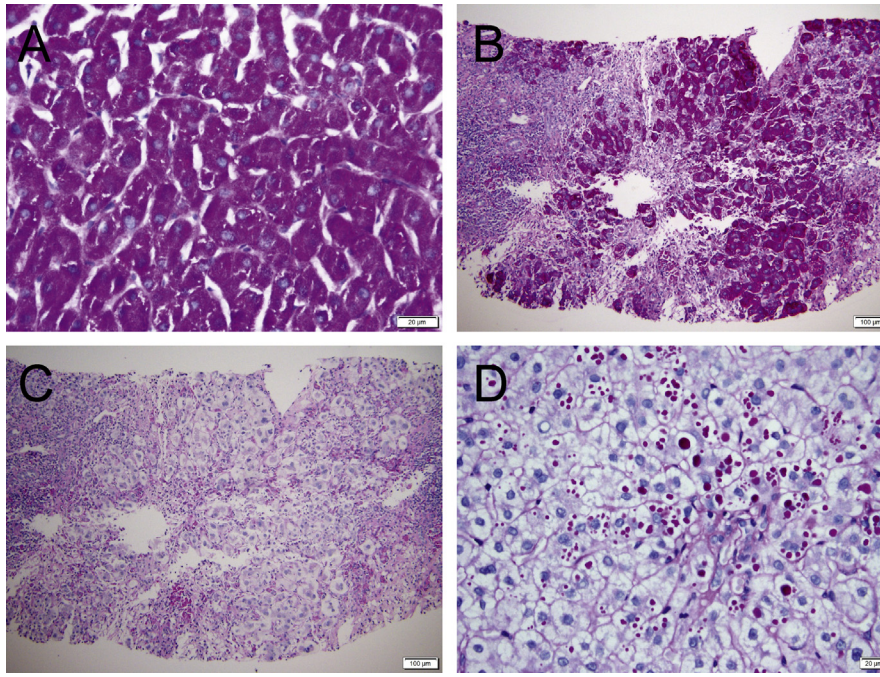

Fig. 4. PAS and diastase pretreated PAS (DPAS) stains. (*A*) PAS-highlighted normal glycogen-rich hepatocytes, (*B*) Liver with bridging necrosis and PAS-highlighted hepatocytes, (*C*) DPAS highlighted clusters of histocytes surrounding necrotic hepatocytes, (*D*) DPAS highlighted alpha-1-antritrypsin globules in hepatocytes.

Iron stain is also helpful in differentiating hemosiderin from other brown or tan staining pigments seen on hematoxylin and eosin, particularly lipofuscin.

PIGMENTS IN LIVER

Commonly observed pigments in the liver include lipofuscin, bile, and hemosiderin. Other rare pigments include Dubin-Johnson pigment and black granules of gold in patients receiving gold chloride for rheumatoid arthritis. Lipofuscin pigment is usually present in perivenular areas and increases with advancing age (Fig. 6A) and usually stains with diastase PAS stain and Ziehl-Neelsen stain. A ground glass hepatocyte is a hepatocyte with a flat, hazy, and uniformly dull appearing cytoplasm, commonly owing to either glycogene accumulation or hepatitis viral B surface antigen inclusion (Fig. 6B). Hemosiderin is usually first accumulates in zone 1 (see Fig. 5C, D) and gradually extends to zones 2 and 3 (see Fig. 5A, B). Hemosiderin is a golden brown refractile pigment and easily differentiated from other pigments by Perls' stain (see Fig. 5A). Bile pigment is yellow or green and present in hepatocyte cytoplasm and in the canaliculi (Fig. 6C, D). Bland cholestasis that is associated with anabolic steroid or estrogen therapy is mostly confined to the perivenular area (Fig. 6E, F). Similarly, in the early stages of bile duct obstruction, cholestasis is mostly confined to the perivenular area. The pigment in hepatocytes in Dubin-Johnson syndrome is granular dark brown lipomelanin and stained with Ziehl-Neelsen stain.

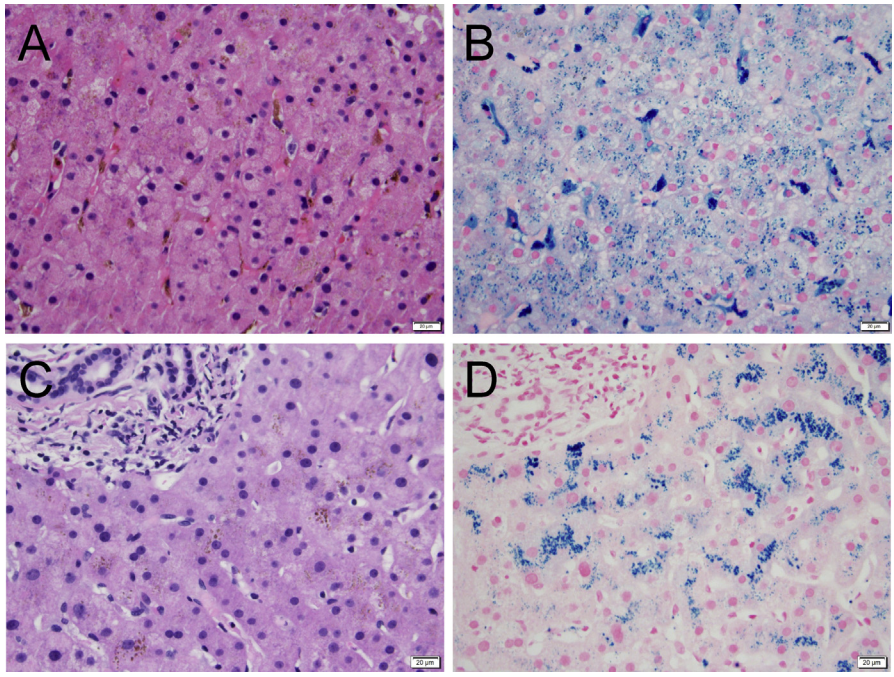

Fig. 5. Perls' iron stain. (*A*) Liver with hemosiderin laden Kupffer cells in the sinusoid space, (*B*) intensely iron-stained Kupffer cells and iron deposition in the hepatocytes, (*C*) hematoxylin and eosin stain showing cytoplasmic hemosiderin granules, and (*D*) iron deposition in the hepatocytes.

COMMON MORPHOLOGIC CHANGES: CLUES TO DISEASE RECOGNITION

Some of the commonly used buzz words that are diagnostically and clinically relevant include macrovesicular fatty change, microvascular fatty change, ballooning change of hepatocytes, lobular disarray, and interface hepatitis.

Macrovesicular Fatty Change

Cytoplasmic fat accumulation in the liver is in the form of either a macrovesicular or microvesicular type. Macrovesicular fatty change can be either in the form of single large fat droplet or several small droplets (Fig. 7A). Macrovesicular fatty change is the most common type and represents an excess accumulation of triglycerides in the hepatocytes secondary to diverse etiologies, including alcohol and the metabolic syndrome. Morphologically, the large droplet type is characterized by a large single vacuole in the cytoplasm displacing the nucleus to the periphery. Small droplet fatty change appears as multiple small droplets with centrally located nucleus. Fatty change is (grading of steatosis) scored as mild (6%–33%), moderate (34%–66%), or marked (>67%).[5–7] Less than 5% is consider as normal. Scoring should be on a low power objective (4× or 10×). Pathologists are often asked to evaluate the percentage of fat in the donor liver and should report only the amount of macrovesicular fat.

Microvesicular Fatty Change

In this category, cytoplasm is replete with tiny vesicles appearing foamy and nucleus retained in the center. Sometimes, it is difficult to identify microvesicular fatty change

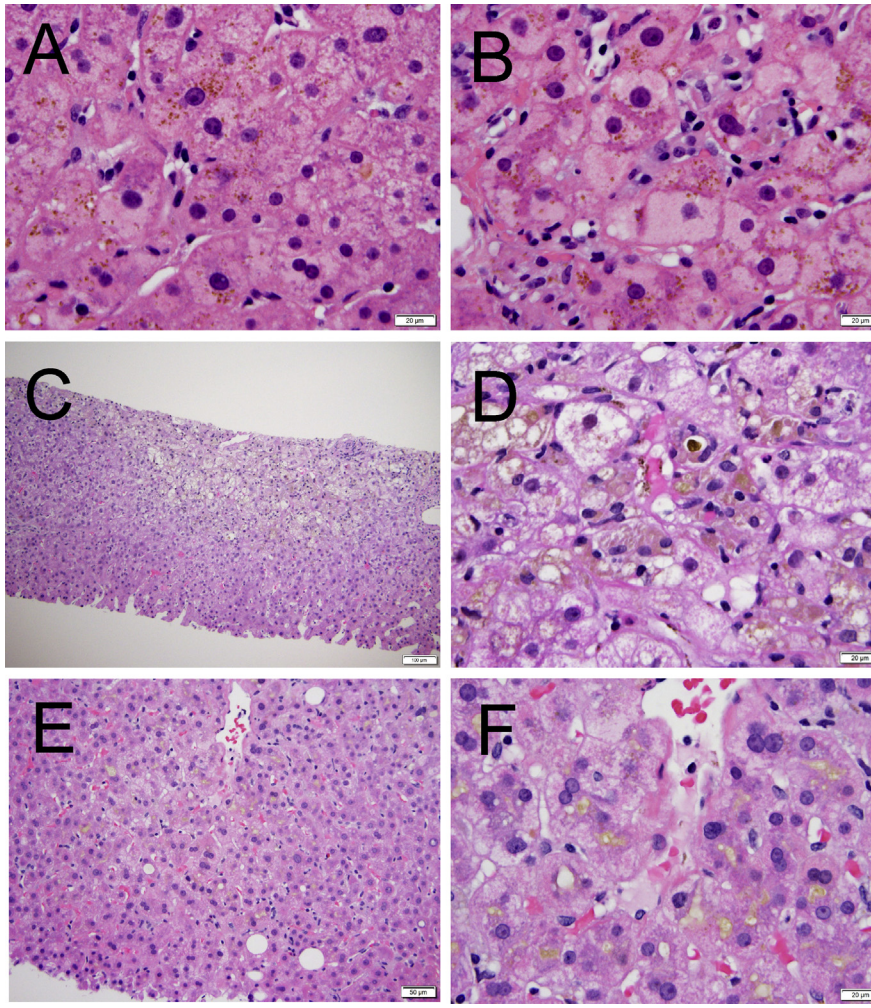

Fig. 6. (*A*) Hepatocytes with abundant lipofuscin. (*B*) Liver with ground-glass hepatocytes and lipofuscin. (*C*) Zone 3 hepatocytes showing ballooning change and cholestasis. (*D*) Canaliculi cholestasis. (*E, F*) Numerous bile plugs are present in canaliculi.

necessitating the use of specific fat stains on frozen sections. Microvesicular fatty change is due to genetic or toxin-induced abnormalities in peroxisomal and mitochondrial fatty acid oxidation.[8–10]

Ballooning Change of Hepatocytes

One of the key histologic components to make a diagnosis of steatohepatitis is the identification of ballooned hepatocytes along with lobular inflammation and pericellular fibrosis.[5–7] Ballooning change is characterized by markedly swollen hepatocytes, 2 to 3 times larger than normal hepatocytes with clear or rarefied cytoplasm secondary to intracellular fluid accumulation (Fig. 7B). Cells without these classic features should not be classified as ballooned cells. In case of uncertainty, ballooned cells can be easily confirmed by demonstration of loss of intermediate filaments, cytokeratins

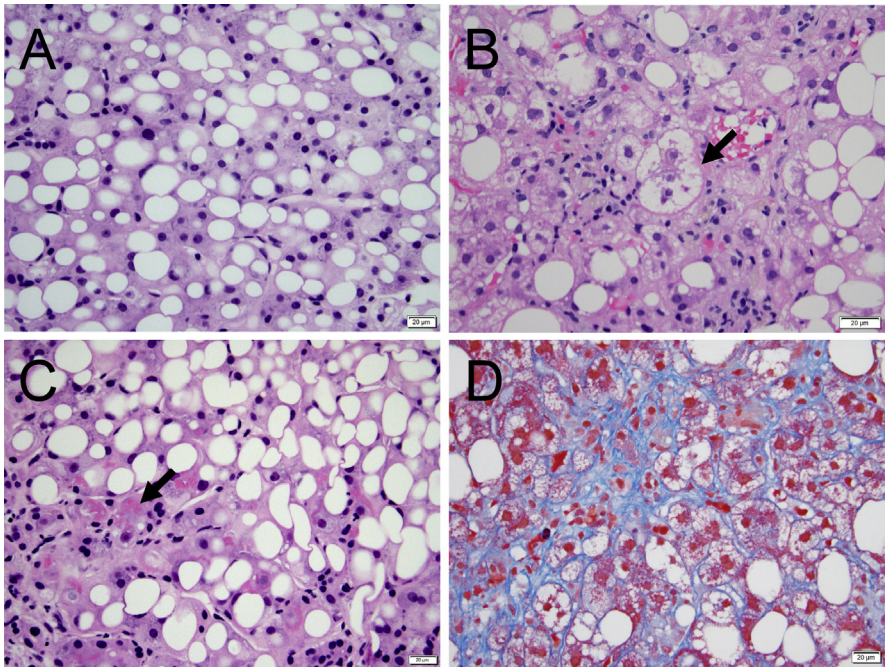

Fig. 7. Steatohepatitis. (*A*) Macrovesicular steatosis, (*B*) macrovesicular steatosis with ballooning hepatocytes (*arrow*), (*C*) macrovesicular steatosis with Mallory body (*arrow*), and (*D*) trichrome stain highlighted pericellular fibrosis.

8/18, by immunohistochemistry. Mallory-Denk bodies, when present, are seen only in ballooned cells (Fig. 7C).

Lobular Disarray

Normally, hepatocytes are arranged in single cell cords surrounded by sinusoids. However, in acute hepatitis, irrespective of etiology, hepatocytes with ballooning change, individual (apoptotic bodies) or focal cell necrosis, regenerating cells and condensed reticulin resulting in disorganized appearance of cords (Fig. 8A). In addition, there is associated inflammatory infiltrate and may be canalicular cholestasis. Recognition of this histologic pattern (lobular disarray) is very helpful in the diagnosis of acute hepatitis.

Interface Hepatitis

Limiting plate or membrane is an imaginary line where the hepatic parenchyma comes in contact with portal mesenchyme. Usually, this stromal–parenchymal interface is well-defined (see Fig. 1B). In the event of acute or chronic hepatitis, this interface is distorted by inflammatory infiltrate with an extension of portal inflammation into the adjacent parenchyma causing hepatocyte loss (apoptosis) and eventually fibrosis.[11] Interface activity can be mild, moderate, or severe and is seen commonly with autoimmune hepatitis (AIH), chronic viral infection, Wilson's disease, and biliary tract diseases (Fig. 8). An appreciation of interface activity is very helpful in the diagnosis of chronic hepatitis.[11–13]

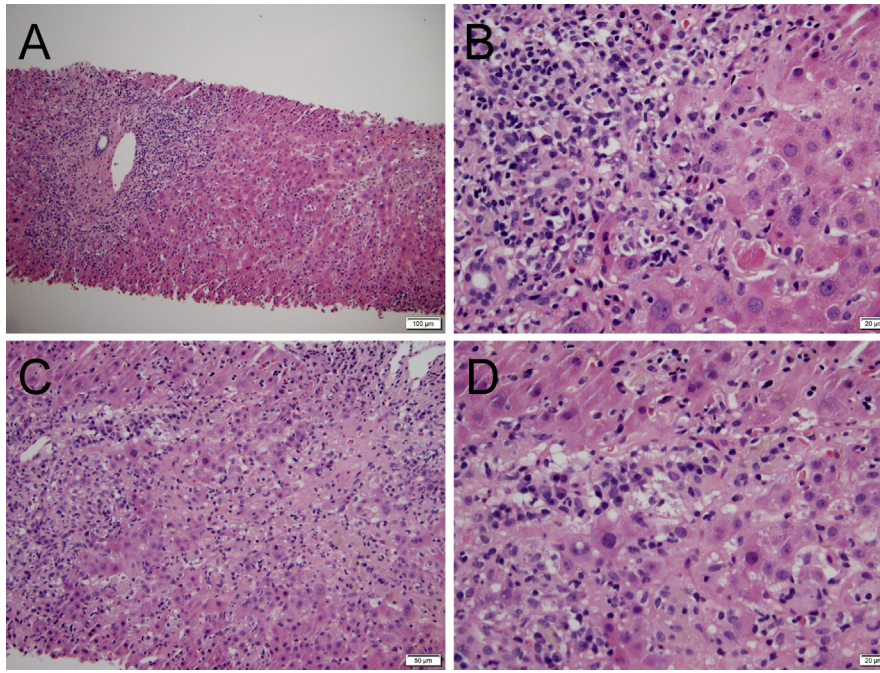

Fig. 8. (*A*) AIH with portal inflammation, minimal central necrosis and lobular disarray, (*B*) portal inflammation with interface activity and numerous plasma cells, (*C*) central and bridging necrosis, and (*D*) bridging necrosis and cluster of plasma cells.

DISEASE PATTERN RECOGNITION

Some novel or specific disease pattern(s) can be recognized morphologically. Particularly, with this background information, we provide clues and guidelines for the diagnosis of most common liver diseases for which biopsies are performed.

Fatty Liver Disease

Although varied etiologies cause fatty liver, the 2 most common causes in the contemporary period are alcohol and obesity (the metabolic syndrome) referred to as alcoholic and nonalcoholic fatty liver disease, respectively.[5] Both alcoholic and nonalcoholic fatty liver disease cause macrovesicular fatty changes that usually start in zone 3 and extend to the other zones with increasing severity. Fatty change should be reported as mild, moderate, or severe, depending on the percentage of hepatocytes with macrovesicular fat.[6] If there is lobular or portal inflammation, it should be included in the report. Fatty change, irrespective of the grade, is a benign and reversible condition with the cessation of underlying cause(s). However, if the inciting cause persists, steatosis progresses to steatohepatitis. Interestingly, there are no identifiable morphologic features that indicate progression or the underlying cause of steatosis. In addition to macrovesicular fatty change, diagnostic criteria for steatohepatitis are ballooned hepatocytes with or without Mallory-Denk bodies, necroinflammatory changes in the lobules, and pericellular (chicken-wire type) fibrosis (**Fig. 7**D).[6] Inflammatory infiltrate secondary to hepatocyte injury includes lymphocytes, histiocytes, and neutrophils. It is generally believed that the number of neutrophils are greater in alcoholic

steatohepatitis than in nonalcoholic steatohepatitis. However, satellitosis (neutrophils surrounding ballooned cells with Mallory-Denk bodies) is seen in both alcoholic and nonalcoholic steatohepatitis (see **Fig. 7**). To indicate the disease activity and fibrosis, the NASH [Nonalcoholic Steatohepatitis] Clinical Research Network developed a scoring system based on fatty change, ballooning change, lobular inflammation (**Tables 1** and **2**).[6] Although this scoring system was developed for nonalcoholic fatty liver disease, it can be used for scoring both alcoholic and nonalcoholic fatty liver disease.

Autoimmune Hepatitis

AIH is an immune-mediated liver disease that can present either as acute onset hepatitis mimicking acute viral hepatitis or as chronic hepatitis. Patients with a chronic form of AIH may present with nonspecific clinical symptoms, advanced liver disease, or after having been diagnosed during routine laboratory evaluation for liver enzymes.

Morphologic features of acute autoimmune hepatitis

Lobular disarray consisting of plasma cell infiltrate, apoptotic bodies, ballooning change, Kupffer cell hyperplasia, and variable degrees of necrosis, ranging from perivenular areas to submassive necrosis (see **Fig. 8**).[11–14] In addition, portal inflammation with predominant plasma cell infiltrate and interface activity usually seen (see **Fig. 8**B, D). Canalicular cholestasis and hepatocyte resetting can be seen. In true acute AIH, no portal fibrosis is seen.

Morphologic features of chronic autoimmune hepatitis

The key histologic features are portal inflammation with interface activity, hepatocyte rosette formation, emperipolesis, variable lobular necroinflammatory activity and fibrosis.[11–14] Portal inflammation with predominant plasma cell infiltrate (>70%) or clusters of plasma cells (groups of 5–10 plasma cells) with interface activity is a good diagnostic indicator of AIH (see **Fig. 8**B, D). In chronic AIH portal fibrosis is a consistent finding and the degree of fibrosis depends on the duration and severity of disease. Fibrosis should be staged as portal expansion (stage 1), periportal septa (stage 2), bridging septa (stage 3), and obvious cirrhosis (stage 4). Similar morphologic features can be seen in chronic hepatitis caused by several etiologies including viral infection and drugs. A definitive diagnosis of AIH is possible and should be rendered only after correlating with levels of serum IgG, antinuclear antibodies, anti-smooth muscle antibodies, and liver/kidney microsomal antibodies.

Table 1
Nonalcoholic fatty liver disease activity score system from the nonalcoholic steatohepatitis clinical research network

Score	Fat	Ballooning Cells	Lobular Inflammation
0	Minimal (<5%)	None	None
1	6%–33%	Few	<2 foci per 20× field
2	33%–66%	Many	2–4 foci per 20× field
3	>67%	–	>4 foci per 20× field

From Klein DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology.* 2005;41(6):1313-1321; with permission.

Table 2
Nonalcoholic steatohepatitis clinical research network scoring system

Fibrosis Stage	Histologic Findings
0	None
1A	Mild zone 3, perisinusoidal
1B	Moderate zone 3, perisinusoidal
1C	Portal/periportal
2	Perisinusoidal and periportal
3	Bridging fibrosis
4	Cirrhosis

From Klein DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology.* 2005;41(6):1313-1321; with permission.

Drug-Induced Liver Injury

More than 350 drugs have been implicated in causing liver injury, and this number is progressively increasing with the use of immunomodulatory drugs in the treatment of different malignant neoplasms.[8] Drugs can cause acute or chronic liver injury that can simulate any type of liver disease. Usually, drug-induced liver injury is either due to a direct toxic effect (predictable) or an idiosyncratic effect (unpredictable), the latter being the most common type.[8] The direct toxic effect is dose dependent (acetaminophen, some chemotherapeutic drugs) and idiosyncratic through an immunologic idiosyncrasy or an immunoallergic injury (drug hypersensitivity). The pattern of liver injury caused by drugs include zonal necrosis, hepatitis (acute or chronic) with or without cholestasis, granulomas, bland cholestasis, reactive hepatitis, steatosis/steatohepatitis, and sinusoidal obstruction syndrome.[8,9,15] Findings of zonal necrosis or submassive necrosis, predominant eosinophilic infiltrate in the portal areas (**Fig. 9**) and lobules and sinusoidal obstruction syndrome are highly suggestive of drug-induced liver injury. However, in a majority of cases the diagnosis of drug-induced liver injury depends on good clinical history and elimination of viral and autoimmune etiologies.

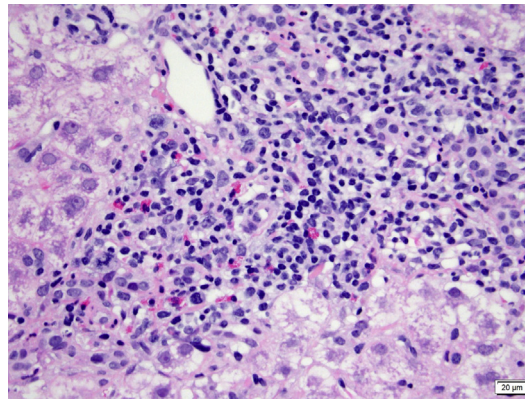

Fig. 9. Drug-induced portal inflammation with dominant lymphocytes and a few of eosinophils.

Immune Checkpoint Inhibitor-Induced Liver Injury

Immune checkpoint inhibitors are currently used in the treatment of different types of malignancies that can cause adverse liver effects. Liver biopsy findings are variable and include lobular hepatitis with or without focal necrosis, sinusoidal macrophage infiltrate with microgranulomas, perivenular hepatitis, portal infiltrate with interface activity, and rarely only bile duct injury with ductular proliferation.[16–18] Lymphoid infiltrates consists of mostly CD3- and CD8-positive cells and fewer CD20- or CD4-positive cells.[17] There are no specific immune checkpoint inhibitor-induced morphologic changes, and requires differentiation from acute and chronic viral diseases and AIH.[16,18]

Sinusoidal Obstruction Syndrome

Sinusoidal obstruction syndrome, previously referred to as veno-occlusive disease, is seen in patients with bone marrow transplantation, hematopoietic stem cell transplantation, and patients receiving chemotherapy for metastatic colorectal carcinoma to the liver.[8,19] Sinusoidal obstruction syndrome is due to toxic injury to sinusoidal endothelial cells and hepatic vein endothelial cells resulting in swelling and the rounding up of endothelial cells. Morphologically, sinusoidal obstruction syndrome is characterized by sinusoidal dilatation, congestion, leakage of blood into space of Disse, atrophy of liver cords, and necrosis of hepatocytes. In severe cases, perisinusoidal fibrosis, nodular regenerative hyperplasia and fibrous obliteration of central vein can be seen.

A PRACTICAL APPROACH TO BIOPSY INTERPRETATION AND CONCLUSION

As discussed elsewhere in this article, there is tremendous overlap in the cellular alterations and morphologic changes caused by virus infection, autoimmunity, drug- and toxin-induced injury, or metabolic diseases. No single morphologic finding can point to a definitive diagnosis. Pathologists should first identify the cellular alterations, pattern of injury, and severity of injury. After a thorough evaluation of the biopsy, and based on histologic findings, a proper differential diagnosis should be formulated. A final diagnosis should be rendered only after evaluating detailed clinical history and laboratory and serologic studies. Without such correlation, the diagnosis will be incomplete or inaccurate.

DISCLOSURE

The authors have disclosed that they have no significant relationships with or financial interest in any commercial companies pertaining to this article.

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