Role of Special Stains in Diagnostic Liver Pathology

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Diagnostic evaluation of liver tissue is largely based on a thorough examination of sections stained with hematoxylin and eosin (H&E). Additional special stains may be used to highlight or identify features that are not easily seen on an H&E stain. The choice of stain or panel of stains depends on the findings on initial assessment, the clinical context, and the preference of the pathologist. For example, allograft biopsies performed soon after transplantation to assess for rejection usually do not need additional stains. On the contrary, biopsies performed on a native liver to evaluate for abnormal liver enzymes or chronic liver disease may require several special stains for optimal assessment.[1,2] The intent of this review is to discuss the common stains used in the evaluation of nonneoplastic liver specimens and their importance in providing information of clinical relevance.

Trichrome Stain

Masson's trichrome stain is among the most common special stains applied to liver specimens. The stain imparts a blue color to collagen against a red background of hepatocytes and other structures. It stains type 1 collagen that is normally present in the portal tracts and vessel walls, but also highlights the presence and distribution of reactive fibrosis as a result of liver injury. It is used for staging of chronic liver diseases, and helps to delineate patterns of injury, such as the perisinusoidal fibrosis associated with steatohepatitis and periductal fibrosis in primary sclerosing cholangitis[3] (Fig. 1).

Reticulin Stain

Reticulin stain uses silver impregnation to detect reticulin fibers, which are made of type 3 collagen. The fibers appear black against a gray to light pink background. In the liver, such fibers are present as part of the extracellular matrix in the space of Disse. By highlighting these fibers, the stain helps in the assessment of the architecture of the hepatic plates, such as expansion in regenerative and neoplastic conditions, compression of plates in nodular regenerative hyperplasia,[4] and collapse of the reticulin framework in necrosis (Fig. 2). The stain is also used to evaluate such fibers in other tissues, such as bone marrow and kidney.

Iron Stain

The Perl's iron stain (Prussian blue reaction) is a common and reliable stain for detecting iron. Iron is stored in the hepatocytes as a soluble form (ferritin) and an insoluble form (hemosiderin). With the H&E stain, the latter is seen as coarsely granular brown refractile granules in the cytoplasm, whereas ferritin is not seen. Pearl's stain highlights hemosiderin as coarse blue granules, while ferritin is seen as a faint blue cytoplasmic blush (Fig. 3). In hemochromatosis, iron accumulates primarily in the cytoplasm and initially in the periportal hepatocytes. In secondary iron overload, the accumulation is mainly in the Kupffer cells. When a large quantity of iron is present or if there is concurrent active hepatocellular injury, the distribution of iron may become mixed, both in hepatocytes and Kupffer cells.[5] The staining method also allows unstained lipochrome and bile pigments to be seen against the pale counterstain for comparison. Iron stain may be supplemented with tissue iron quantitation in the appropriate clinicopathologic setting. This can be performed on routinely processed paraffin-embedded tissue.

Periodic Acid-Schiff Stain

The periodic acid-Schiff (PAS) stain is useful for identifying glycogen, but removing glycogen with diastase digestion enhances detection of nondigested material, including the alpha-1-antitrypsin globules, basement membrane, debris within macrophages, and fungal organisms. Using PAS with and without digestion confirms the presence of glycogen deposition, such as in the various glycogen storage diseases.

Abbreviations: H&E, hematoxylin and eosin; PAS, periodic acid-Schiff.

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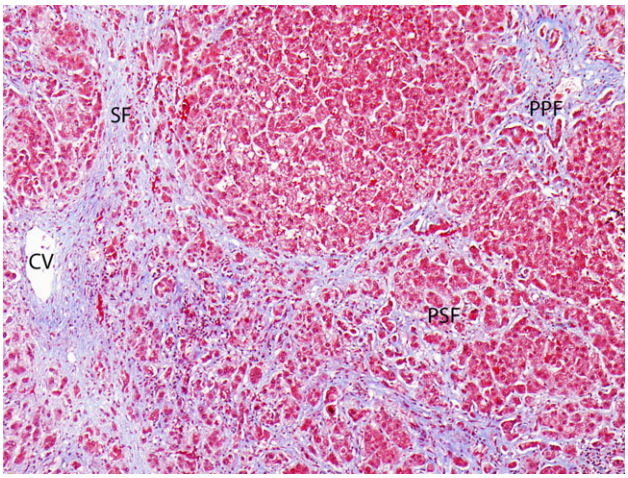

FIGURE 1. Trichrome stain showing characteristic pattern of advanced fibrosis secondary to steatohepatitis. There is scarring around the central vein (CV), perisinusoidal fibrosis (PSF) separating the hepatocyte cords, formation of a fibrous septum (SF), and periportal fibrosis (PPF) (magnification ×100).

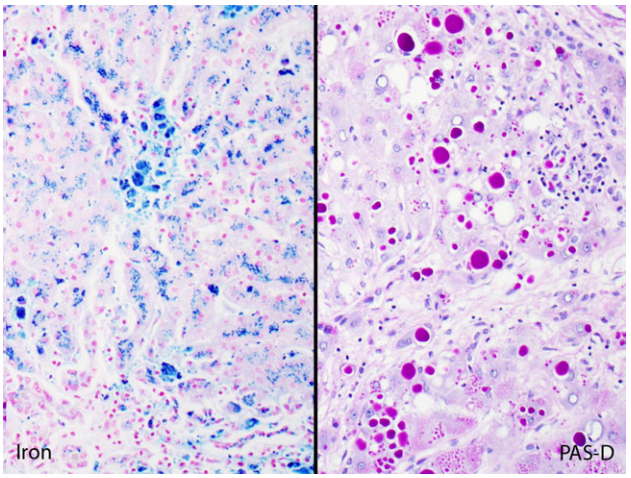

FIGURE 3. Left: Iron stain showing marked iron deposition with a mixed distribution in hepatocytes and Kupffer cells (magnification ×100). Right: PAS stain after diastase digestion showing globules consistent with alpha-1-antitrypsin (magnification ×200).

In patients with alpha-1-antitrypsin deficiency, accumulation is seen as bright magenta globules, typically in periportal hepatocytes, with larger and more numerous globules in homozygous disease[6] (Fig. 3).

Other Histochemical Stains

Congo red stain is used to assess amyloid deposition and is combined with polarization microscopy to demonstrate the characteristic apple-green birefringence (Fig. 4). Oil Red O stain is used to highlight the presence of fat globules, with its most common application being evaluation of pretransplantation donor liver biopsies (Fig. 5). The stain is performed on frozen sections of the liver tissue. Rhodanine stain is used to detect copper-binding protein and is thus used to evaluate for Wilson disease. Copper is excreted in bile and accumulates in the liver in chronic biliary diseases. The stain can therefore also be used in the differential diagnosis of biliary (versus nonbiliary) disease. More commonly, however, copper is evaluated by direct quantitation on routinely processed tissue, similar to iron. Other stains that are currently not in widespread use include orcein, Victoria blue, aldehyde

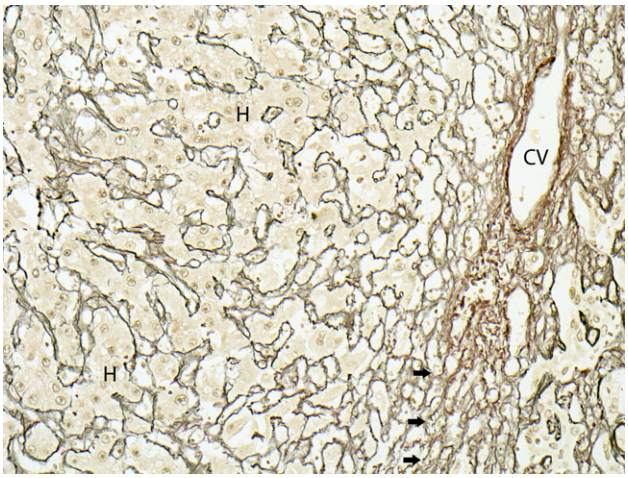

FIGURE 2. Reticulin stain highlighting the reticulin pattern in acute hepatic necrosis. The hepatocyte cords are focally expanded (H), and a band-like area of reticulin collapse (arrows) near the central vein (CV) highlights the necrosis (magnification ×200).

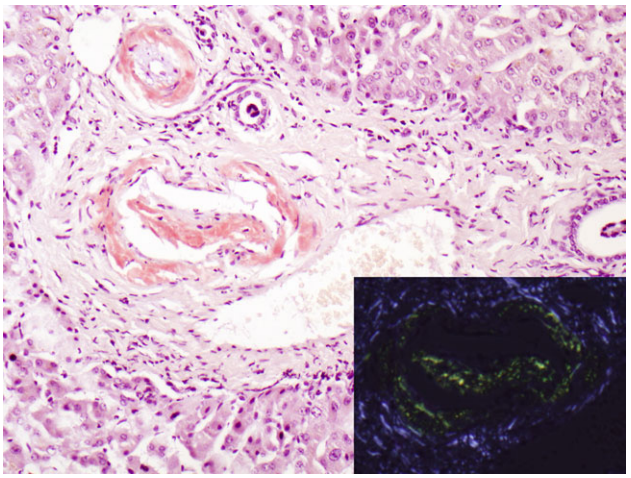

FIGURE 4. Congo red stain showing orange-staining vascular amyloid deposition, positive for the characteristic apple-green birefringence under polarized microscopy (inset) (both magnification ×400).

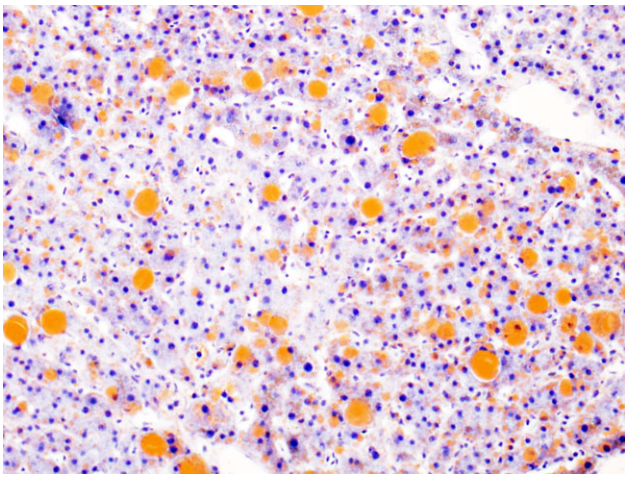

FIGURE 5. Oil Red O stain highlighting fat globules in a frozen section of the liver. Note the presence of large (macrovesicular) and small (microvesicular) globules (magnification ×20).

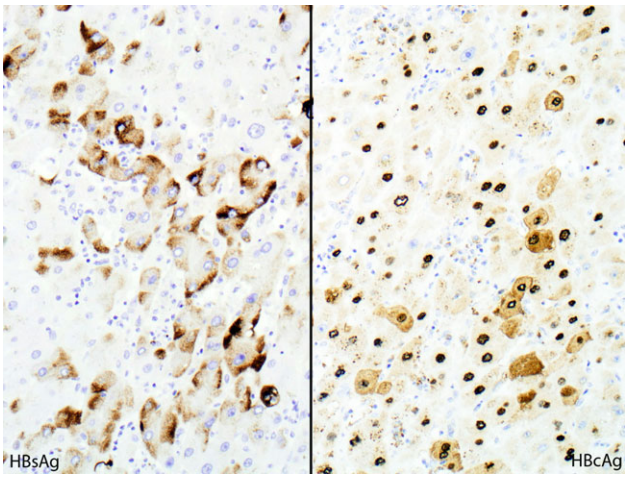

FIGURE 6. Immunostains showing hepatitis B surface (right) and core (left) antigens. Surface antigen is present in the cytoplasm, and core antigen is present in both the nucleus and the cytoplasm (magnification ×200).

fuchsin (copper-associated protein, elastic fibers, hepatitis B surface antigen), sirius red (collagen, reticulin), and aniline blue (collagen). Stains commonly used for microorganisms include the Ziehl-Neelsen stain for acid fast bacteria, Gomori methenamine silver (or PAS) stain for fungus, and Gram stain for bacteria.[7,8]

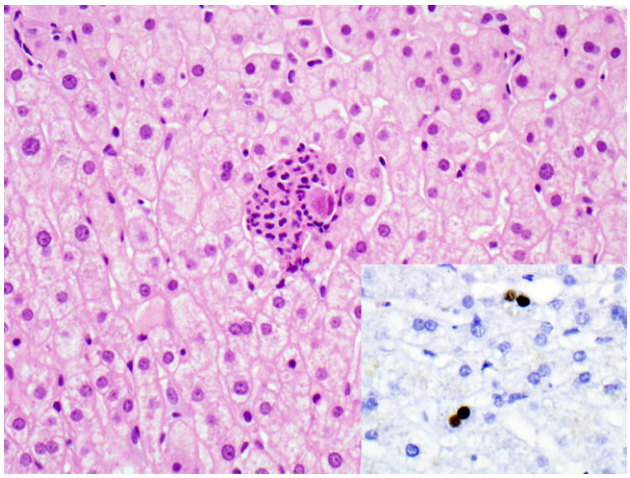

FIGURE 7. Cytomegalovirus hepatitis. H&E stain shows a typical microabscess containing a cell with nuclear and cytoplasmic inclusions. The inset shows immunostaining for cytomegalovirus (both magnification ×400).

Immunohistochemical Stains

Immunohistochemical stains are much less commonly used in the diagnostic evaluation of nonneoplastic liver diseases compared with the histochemical stains described above. The most commonly used stains are those for diagnosing or confirming viral infections involving the liver, including hepatitis B virus (Fig. 6), cytomegalovirus virus (Fig. 7), and herpes simplex virus, although the latter can also be detected via *in situ* hybridization. These infections may be suggested by cytopathic changes present on the H&E stain, such as ground-glass cytoplasm in hepatitis B infection, nuclear and/or cytoplasmic inclusions in cytomegalovirus infection, and nuclear inclusion in herpes infection.[7,8]

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References

1. Bravo AA, Sheth SG, Chopra S. Liver biopsy. N Eng J Med 2001;344:495–500.
2. Bateman AC. Patterns of histological change in liver disease: my approach to "medical" liver biopsy reporting. Histopathology 2007;51:585–596.
3. Lefkowitch JH. Special stains in diagnostic liver pathology. Sem in Diag Pathol 2006;23:190–198.
4. Hartleb M, Gutkowski K, Milkiewicz P. Nodular regenerative hyperplasia: evolving concepts on underdiagnosed cause of portal hypertension. World J Gatroenterol 2011;17:1400–1409.
5. Batts KP. Iron overload syndromes and the liver. Mod Pathol 2007;20: S31–S39.
6. Fairbanks KD, Tavill AS. Liver disease in alpha 1-antitrypsin deficiency: a review. Am J Gastroenterol 2008;103:2136–2141.
7. Roskams T, Desmet VJ, Verslype C. Development, structure and function of the liver. In: Burt AD, Portmann BC, Ferrell LD, eds. MacSween's Pathology of the Liver. 5th ed. Edinburgh, UK: Churchill Livingstone/Elsevier; 2007:1–74.
8. Lefkowitch JH. Scheuer's Liver Biopsy Interpretation. 8th ed. Saunders/Elsevier; 2010.