Liver Histology in the Diagnosis and Prognosis of Drug-Induced Liver Injury

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Drug-induced liver injury (DILI) presents unique challenges to the clinician as well as the pathologist. Unlike the more prevalent liver diseases of chronic viral hepatitis and steatohepatitis, there is neither a clearly defined role nor professional guidelines for the use of liver biopsy in DILI. In addition, the results of liver histology in DILI patients are not formally incorporated into current causality-assessment instruments or scoring algorithms. Nevertheless, some clinical circumstances wherein liver biopsy may prove useful can be articulated. As in other liver diseases, the liver biopsy can fulfill two main purposes in patients with suspected DILI. A liver biopsy can help confirm or exclude a diagnosis of DILI by careful attention to the histological pattern of injury and correlation with the patient's presenting symptoms, signs, laboratory data, imaging tests, and medication history. Liver biopsy can also help define the severity of the injury, as well as provide mechanistic insight by identifying the cells and tissues (hepatocytes, bile ducts, and vessels) involved in the injury.

Biopsy Evaluation: Pattern of Injury in DILI

When a liver biopsy comes to the pathologist, the first and most important task is to define the pattern of injury. Most organs have a limited set of stereotypical ways of responding to injury, and modern textbooks of pathology are frequently written in a style that takes advantage of this fact (see Saxena et al¹). The words used for some diseases have become synonymous with the pattern of injury most often seen in the disease. For example, chronic hepatitis is a pattern of injury characterized by portal area-dominant lymphocytic inflammation often associated with fibrosis progression that leads to cirrhosis, and it is the most common injury pattern seen in chronic viral infections with hepatitis B and C. It is important for the pathologist to also

be aware of the pattern variations seen with particular diseases. Viral hepatitis may also show a pattern of acute hepatitis either during the acute phase of infection or during a flare of chronic disease, and cholestatic hepatitis (acute hepatitis with cholestasis) can be seen during acute infection. The authors and others²⁻⁴ have advocated analyzing cases of suspected DILI by first defining the pattern of injury. The pattern of injury determines the histological differential diagnosis and permits correlation with known patterns of injury associated with particular drugs. Figure 1 outlines this approach to cases of suspected DILI.

In the landmark article on DILI,⁵ Popper et al characterized seven histological patterns of liver injury observed in 155 cases of DILI and toxic liver injury. The seven patterns were: zonal necrosis, acute cholestasis, hepatitis without cholestasis, hepatitis with cholestasis (cholestatic hepatitis), acute hepatitis, nonspecific reactive hepatitis, and steatosis. Popper et al observed that individual drugs tended to cause particular patterns of injury. Not all of the patterns were equally represented. Cases of acute hepatitis were most common (39%)—followed by cases of cholestatic hepatitis (32%). The US Drug-Induced Liver Injury Network (DILIN) has incorporated the systematic analysis of liver biopsies obtained from enrolled patients from its inception. Eighteen patterns of liver injury were predefined, and biopsies with suspected DILI were blindly evaluated by a single expert hepatopathologist.⁶ As in Popper et al's case series, some patterns were more common than others. Table 1 shows the five most common patterns observed, along with the histological definitions used to classify the patterns. There was no difference in the distribution of injury pattern between those cases that were later confirmed to have DILI and those cases for whom non-DILI etiologies were thought to be more likely. Examples of these common injury patterns

Abbreviations: DILI, drug-induced liver injury; DILIN, Drug-Induced Liver Injury Network.

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Figure 1 Algorithm for biopsy evaluation in suspected drug-induced liver injury.

TABLE 1 Common Histological Patterns of Liver Injury Observed in the DILIN Prospective Study of 249 Consecutive Patients

Histological Pattern	% Observed	Study Definition
Acute hepatitis	21%	Lobular predominant inflammation and apoptosis with lobular disarray in more severe cases. Areas of confluent necrosis allowed.
Chronic hepatitis	14%	Portal predominant inflammation with generally mild to moderate lobular inflammation. Fibrosis not required.
Acute cholestasis	9%	Hepatocellular or canalicular bile with little to no portal or lobular inflammation.
Chronic cholestasis	10%	Definite cholestasis or copper accumulation associated with a chronic hepatitic pattern of inflammation and bile duct injury or loss
Cholestatic hepatitis	29%	Combination pattern with visible hepatocellular or canalicular bile (any degree) with inflammation that was more than minimal. Bile duct injury or loss could be present, but chronic cholestatic changes not yet seen.

Adapted from Kleiner et al.⁶

in confirmed DILI cases are shown in Figures 2–4. Table 2 highlights the injury patterns observed in some commonly used agents. Because the DILIN study only enrolls patients with clinically significant liver injury who are recruited within 6 months of DILI onset, some patterns of injury such as steatohepatitis or low-grade vascular injury are less commonly encountered. Figure 5 shows injury patterns not

Figure 2 Acute and chronic hepatitis patterns in DILI. A and B show acute hepatitis due to isoniazid. The portal area (A) shows mild inflammation but near circumferential disruption of the limiting plate by interface hepatitis. The bile duct (arrowhead) is not damaged. In contrast, the parenchyma (B) shows loss of the normal sinusoidal architecture due to numerous foci of lobular inflammation, hepatocyte dropout, and rosette formation. These changes can be compared to C and D, which show a chronic hepatitis pattern due to minocycline. The portal area (C) shows a large lymphoid aggregate surrounding a bile duct (arrowhead) that is similar to what is seen in chronic hepatitis C. Interface hepatitis is also present. The parenchyma (D) shows only scattered foci of lobular inflammation (arrow).

Figure 3 Acute and chronic cholestasis patterns in DILI. (A–B) Acute cholestasis due to a body-building supplement. (A) Portal area shows only minimal inflammation without interface hepatitis. The bile ducts (arrowheads) show no evidence of injury in this example. (B) The perivenular parenchyma with prominent canalicular cholestasis (arrows). There is little lobular inflammation. (C–D) In contrast, ductopenic chronic cholestatic injury due to amoxicillin-clavulanate. The portal area (C) shows a mild lymphocytic infiltrate with interface hepatitis, but the bile duct is missing. The arrowhead indicated a small scar that may mark the position of the missing duct. (D) Parenchyma near a portal area. As in the chronic hepatitis example, there are scattered foci of lobular inflammation (arrow). (C–D) Hepatocytes at the portal–parenchymal interface are pale and vacuolated—a typical change in chronic cholestasis called cholestasis or pseudoxanthomatous change.

Figure 4 Cholestatic hepatitis pattern in DILI. This case of trimethoprim-sulfamethoxazole injury shows cholestatic hepatitis, an injury pattern combining features of hepatitis (acute or chronic) with acute cholestasis. (A) Mild lymphocytic portal infiltrate with focal interface hepatitis. (B) Parenchymal injury with an infiltrate of lymphocytes and macrophages, along with canalicular cholestasis (arrows). (C) This case also had several poorly formed granulomas, which may be seen in cases of immuno-allergic DILI.

TABLE 2 Injury Patterns of Selected Drugs in Common Use

Drug	Pattern of Injury
Acetaminophen	Zone 3 necrosis (with little additional inflammation)
Amiodarone	Steatohepatitis-like, with numerous Mallory-Denk bodies and fibrosis
Amoxicillin-clavulanate	Cholestatic hepatitis (with prominent duct injury)
Anabolic steroids	Acute cholestasis to cholestatic hepatitis with little duct injury
Azithromycin	Variable: Hepatitis with or without cholestasis
Diclofenac	Zone 3 necrosis with lymphocytic inflammation
Isoniazid	Acute or chronic hepatitis
Methotrexate	Steatosis and fibrosis, sometimes steatohepatitis-like
Minocycline	Acute or chronic hepatitis
Nitrofurantoin	Acute or chronic hepatitis
Oxaliplatin	Hepatoportal sclerosis, sinusoidal dilation, nodular regenerative hyperplasia

observed frequently in DILIN that are caused by some of the commonly used agents. Pathologists should be aware that essentially any pattern of liver injury, including neoplasms, may be associated with DILI and should therefore fully characterize the pathology observed.

Biopsy Evaluation: Severity and Prognosis

In addition to defining the pattern of injury, the pathology report should contain information about the severity of

the injury as well as the anatomic elements that are affected. Because the pathologic changes in DILI span the full breadth of liver pathology, there is no single staging and grading system for general use. The DILIN histopathology study⁶ created a structured biopsy assessment that examined 48 separate histological features but generally used accepted scales of severity, such as the Ishak method for grading inflammation and staging fibrosis,⁷ whenever possible. Information about disease severity can help guide clinical decision making, even if the pattern of injury does not help to confirm or exclude a particular etiology of DILI.

Several histological features have also been associated with outcome in DILI. The degree of necrosis and the presence of ductular reaction have both been associated with an increased risk of liver failure and death in fulminant acute hepatitis (not necessarily related to DILI).⁸ In this study, ductular reaction was associated with the loss of at least one-half of the parenchyma through necrosis and hepatocyte replicative failure, leading to an inadequate progenitor cell response. Bjornsson et al⁹ evaluated 570 case reports of DILI and found an association between reported histological eosinophilia and a lower risk of mortality, while also confirming that necrosis in DILI was associated with a poor

Figure 5 Other patterns of injury from common agents. (A) Acetaminophen injury with zone 3 necrosis around central veins. (B) Marked sinusoidal dilation is seen in this example of oxaliplatin injury. The liver also showed nodular regenerative hyperplasia. (C) Methotrexate injury with steatosis, mild portal, and lobular inflammation. (D) Amiodarone injury with numerous Mallory-Denk bodies, three of which are indicated by the arrowheads.

outcome. The study of 249 suspected DILI cases referred to DILIN⁶ was able to confirm and extend these observations in a prospectively collected cohort. In that series, the severity of hepatic necrosis, fibrosis, microvesicular steatosis, ductular reaction, cholangiolar cholestasis, and portal venopathy were associated with clinical acute liver failure and/or liver transplantation or death within 6 months of DILI onset, whereas granulomas and eosinophils were associated with milder clinical injury.

Summary

Although liver biopsy is not used routinely in the evaluation of suspected DILI, it does offer the opportunity to define the pattern of injury, which may help to exclude or confirm DILI, as well as to characterize the severity and distribution of injury within the liver in ways that are not

currently available through noninvasive tests. It may be the case that complete confirmation of DILI will require following the patient long past the point of the liver biopsy. Nevertheless, the pathology report should contain information about the pattern of injury, the severity of injury, and a detailed histological differential diagnosis correlating the changes with the reported changes of suspected drugs or other agents. Going forward, an anthology of the liver histology of well-phenotyped cases enrolled in the DILIN prospective protocol will be posted on the LiverTox Web site (see <http://livertox.nih.gov/>).

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References

1. Saxena R, editor. *Practical Hepatic Pathology: A Diagnostic Approach*. 1st ed. Philadelphia, PA: Elsevier Saunders; 2011.
2. Goodman ZD. Drug hepatotoxicity. *Clin Liver Dis* 2002;6:381–397.
3. Kleiner DE. The pathology of drug-induced liver injury. *Semin Liver Dis* 2009;29:364–372.
4. Ramachandran R, Kakar S. Histological patterns in drug-induced liver disease. *J Clin Pathol* 2009;62:481–492.
5. Popper H, Rubin E, Cardiol D, Schaffner F, Paronetto F. Drug-Induced Liver Disease: a Penalty for Progress. *Arch Intern Med* 1965;115:128–136.
6. Kleiner DE, Chalasani NP, Lee WM, Fontana RJ, Bonkovsky HL, Watkins PB, Hayashi PH, et al. Hepatic histological findings in suspected drug-induced liver injury: systematic evaluation and clinical associations. *Hepatology* 2014; 59:661–670.
7. Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, Denk H, et al. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995;22: 696–699.
8. Katoonizadeh A, Nevens F, Verslype C, Pirenne J, Roskams T. Liver regeneration in acute severe liver impairment: a clinicopathological correlation study. *Liver Int* 2006;26:1225–1233.
9. Björnsson E, Kalaitzakis E, Olsson R. The impact of eosinophilia and hepatic necrosis on prognosis in patients with drug-induced liver injury. *Aliment Pharmacol Ther* 2007;25:1411–1421.