MINI REVIEW

The role of the pathologist in diagnosing and grading biliary diseases

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Summary Pathological features of primary biliary cirrhosis (PBC) are reviewed. Immune-mediated, non-suppurative cholangitis is the initial lesion and is followed by the gradual and extensive destruction of bile ducts and development of chronic cholestasis. Simultaneously, necro-inflammatory activities of the hepatic parenchyma and limiting plates of milder form develop not infrequently. Eventually, liver fibrosis and cirrhosis develop. A new system applicable to needle liver biopsies in which staging is evaluated using a combination of three factors (fibrosis, cholestasis, and bile duct loss) and necro-inflammatory activities of the bile duct and hepatic parenchyma are graded, is proposed. The clinical and therapeutic evaluation of PBC using this system is warranted.

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Introduction

There are many kinds of biliary diseases affecting various anatomical levels of the biliary tree, and each presents characteristic clinical and pathologic features [1–3]. In this minireview, the roles of pathologists in diagnosing and grading biliary diseases, especially primary biliary cirrhosis (PBC), are described. First, the anatomy of the biliary tree is briefly reviewed.

The biliary tree is composed of intrahepatic bile ducts, right and left hepatic ducts, and the extrahepatic biliary system. While there is no sharp delineation of the various segments, the intrahepatic bile ducts are classifiable into large and small intrahepatic bile ducts [1,4]. The former consist of the first to third branches of the right and left hepatic ducts, and are accompanied by peribiliary glands. They are lined by a tall columnar epithelium on a collagenous duct wall. The latter are classified into septal and interlobular bile ducts. The septal ducts (>100 μm in diameter) are lined by a tall columnar epithelium on a collagenous duct wall. The latter are classified into septal and interlobular bile ducts. The septal ducts (>100 μm in diameter) are lined by tall columnar cells and are surrounded by the duct wall. In contrast, the interlobular bile ducts are lined by a low columnar and cuboidal epithelium. The interlobular bile ducts are connected to bile canaliculi by bile ductules and canals of Hering.

Each segment of the biliary tree has unique anatomical and physiologic characteristics. The bile ducts are lined by biliary epithelial cells (BECs) or cholangiocytes. BECs play
a number of roles in the biliary system; participating in bile acid reabsorption and drug metabolism, contributing to 30–40% of total bile secretion and mediating immune responses [4].

Clinical and immunological features of PBC

PBC is an autoimmune liver disease which predominantly affects middle-aged to elderly women [5]. Serologically, the most helpful diagnostic test is the demonstration of antimitochondrial antibodies (AMAs), which are found in more than 95% of patients, and serum IgM levels are usually elevated. The main features are usually pruritus and lethargy with skin pigmentation and eventually cholestatic jaundice, although icterus may not develop for years after the initial symptoms. PBC is not infrequently associated with extrahepatic autoimmune diseases such as Sjögren syndrome and chronic thyroiditis. PBC patients may remain asymptomatic for some years and experience a normal life expectancy. Liver function tests show a normal or mildly elevated bilirubin level at early stages, accompanied by a striking and disproportionate elevation of serum alkaline phosphatase. Its natural history is presumed to be about 20 years. Death is usually due to hepatocellular failure, with bleeding from oesophageal varices in approximately 30% of cases.

Following antigen cloning, the major autoantigens which AMAs recognize are now identified as components of the 2-oxo-acid dehydrogenase complex (2-OADC) which is located on the mammalian inner mitochondrial membranes [6]. The occurrence of ANA of the centromere type and autoantibodies against gp210 is also characteristic of PBC [7].

PBC may be a multifactorial disease related to genetic and environmental factors [8]. Genetic factors are suggested by a familial predisposition and an increased prevalence of autoantibodies in relatives of PBC patients. Invernizzi et al. reported that DRB1*08 and DRB1*02 were significantly associated with PBC [9]. The association of PBC with environmental factors has been suggested, and the formation of AMAs during the pathogenesis of PBC has been assumed to be triggered by these environmental factors. The formation of granuloma formation is part of the early bile-duct damage in PBC. Harada et al. have identified several indigenous bacteria, among them Propionibacterium acnes (P. acnes) as a major clone, in granulomas of PBC, suggesting that P. acnes and other enteric bacteria are involved in the pathogenesis [10]. Halogenated xenobiotics actively metabolized in the liver can also modify self-molecules, rendering them immunogenic and resulting in the development of PBC [11].

PBC is characterized by a breakdown in self-tolerance of T and B cells to the conserved mitochondrial self antigen PDC-E2, and then the occurrence of autoreactive T and B cells against PDC-E2 [12]. The hypothesis of molecular mimicry implies that foreign pathogens with homology to self-protein or modified self-protein can break the tolerance.

Autoreactive T cells, recognizing the PDC-E2 component, may be involved in the pathogenesis of bile duct injuries in PBC. In PBC, CD4+ T cells have been shown to recognize amino acids 159–167 of PDC-E2 and there is a corresponding marked increase of CD4+ CD28− T cells in PBC livers [12]. Furthermore, CD8+ cytotoxic T lymphocytes that recognize components of amino acids 159–167 of PDC-E2 have an effector role in the bile duct injury.

Pathology of PBC

The interlobular bile ducts are affected at an early stage in PBC [2]. Parenchymal necro-inflammatory changes involving lobular and periportal areas are not infrequent, though they are usually mild [13]. In time, the cholestatic and necro-inflammatory processes progress and are followed by a progressive fibrosis and eventually cirrhosis.

Bile duct damages

The early lesion of PBC is characterized by injuries to interlobular bile ducts, which eventually undergo destruction. These bile-duct lesions are called chronic non-suppurative destructive cholangitis (CNSDC) or florid duct lesions [14,15]. Epithelial cells of the bile ducts are variably swollen or show an eosinophilic shrunken appearance with pyknotic nuclei. The key pathologic process is immune-mediated damage to bile ducts with cellular senescence of BECs undergoing poor regeneration and eventual apoptotic loss [16–19]. The majority of lymphocytes in the portal tracts are CD4+ (Th1 subset) and CD8+ T cells. Plasma cells and eosinophils can be conspicuous in the early stages. Aggregates of epithelioid cells and a few epithelioid cells are characteristically seen in the vicinity of the bile ducts in the early stages.

Serial sections disclose the segmental disappearance of interlobular bile ducts [2,20]. In contrast, the septal and large intrahepatic ducts, although they may show some inflammation in their walls, are preserved even at an advanced stage of the disease.

Parenchymal and interfacial changes

In the early stages, the bile-duct injury and associated inflammation remain confined within the portal tract boundaries. In the parenchyma, single hepatocellular necrosis, Kupffer-cell hyperplasia and sinusoidal infiltration with scattered lymphocytes and pigment-laden macrophages are found, though their degree is usually mild [21]. Subsequently, an extension of the necro-inflammatory process to the periportal parenchyma may eventually develop. This may take either or both of two forms; chronic cholestatic (biliary interface activity) and necro-inflammatory changes (lymphocytic interface activity) [21,22].

Biliary interface activity

Biliary interface activity becomes the main feature as the duct loss progress. In common with other cholestatic disorders, atypical bile ductular proliferation may be a striking feature [1,23]. Another form is cholate stasis showing hydropic hepatoctytic swelling and the deposition of copper or copper-associated protein granules, and this may result from the "toxic" effect of hydrophobic bile acids. Mallory bodies and intracellular or canalicular bile pigment dominate the interface at an advanced stage.
Pathologic progression of biliary diseases

**Lymphocytic interface activity**
Lymphocytic interface activity resembling autoimmune hepatitis (AIH) is involved. In some cases, this lesion seems to reflect an extension to adjacent hepatocytes of the same immunological process in CNSDC, while in other cases, this activity may be apparently independent of CNSDC. Lymphocytic interface activity is present in a substantial number of cases, usually in a milder form. In less than 10% of cases, it may even dominate with variable lobular activities and be associated with clinical features of AIH, so-called synchronous PBC-AIH overlap syndrome [24–26].

**Progression of bile duct and hepatic lesions**
Histologically, interlobular bile ducts are selectively affected, presenting characteristic findings such as CNSDC, and those affected eventually disappear from the liver. This pathology may evolve over many years without any obvious morphological cholestasis or bilirubinostasis, and this period of evolution is gradually associated with characteristic changes at the interface between the portal tracts and parenchyma—periportal cholate stasis and biliary interface activity and fibrosis. At the same time, hepatitic activities of hepatic lobules and perportal areas are not infrequent, though they are usually mild. Biliary type fibrosis is dense, scar-like in the deeper portions of the septa, but oedematous at the periphery. The second hepatitic activities may also be involved in the progression of PBC, and some cases of PBC with rather prominent hepatitic activities and interface activities may present some features of post-necrotic cirrhosis. Chronic cholestatic and hepatitis activities in various combinations may be responsible for progressive hepatocellular damage and fibrosis in a majority of cases, and liver cirrhosis and hepatic failure may eventually develop [22,23].

**Histopathologic diagnosis of PBC**

**At early and relatively early stages of PBC**
Pathologic lesions strongly suggestive of PBC
A florid duct lesion or CNSDC of the interlobular bile duct, is pathognomonic of PBC [15]. Epithelioid cell granulomas around damaged bile ducts, well-formed granulomas or loosely arranged epithelioid cells, are also a characteristic finding of PBC [26]. However, these pathological features strongly suggestive of PBC are distributed heterogeneously within the liver, and might not be sampled by liver biopsy needles.

Bile duct loss or ductopenia which follows chronic destructive cholangitis, is also a characteristic finding of PBC. This ductopenic lesion may develop and progress in the early stages and the more extensive in advanced stages [27]. The presence of arteries unaccompanied by ducts is a useful, yet rough, marker of bile-duct loss [28]. Immunostaining of a biliary cytokeratin such as K19 or K7 is very useful for the detection or identification of bile ducts in portal tracts.

Pathologic lesions suggestive of PBC
Mild chronic cholangitis (lymphocytic or pleomorphic) and variable biliary epithelial damage are frequently found and suggestive of PBC [29], but not diagnostic [30]. Elevated levels of IgM and predominant infiltration by IgM+ plasma cells in portal tracts are typical of PBC [31]. Epithelioid granulomas in the hepatic lobules suggest a diagnosis of PBC. Atypical ductular reactions and the focal deposition of copper or copper-binding proteins are also found in PBC.

**Pathological changes raising the possibility of PBC**
Small cell changes of hepatocytes in zone 1 and 2 provide us a hint of chronic cholestatic liver disease, particularly PBC [32]. Vague nodularity of the hepatic parenchyma in similar zones is also found in PBC [33]. Prominent eosinophilic infiltration in portal tracts is evident in some cases [34].

**Diagnosis of PBC at relatively advanced and advanced stages of PBC**
At advanced stages, characteristic features suggestive of PBC are rare, but prominent chronic cholestasis, extensive fibrosis and regenerative nodules are found. PBC should be suspected based on the following features [2,14]:
- a virtual absence of interlobular bile ducts;
- focal lymphocytic aggregates seem to replace the missing bile duct(s);
- peripheral cholate stasis or cholestasis with Mallory bodies and extensive copper deposition;
- a biliary pattern of fibrosis;
- partial or focal preservation of the normal architecture in otherwise established cirrhosis.

**New staging and grading system of PBC**
Progression of PBC eventually leading to cirrhosis has been evaluated histologically using classical staging systems proposed by Scheuer [14] and Ludwig et al. [35]. In Scheuer's staging system, PBC is histologically classified into four stages by using characteristic histologic features: stage 1 is characterized by florid duct lesions or CNSDC, and in stage 2, there is a characteristic proliferation of bile ductules. Stage 3 is characterized by fibrosis or scarring and stage 4, by cirrhosis. Ludwig’s system applies the histologic features used for the staging of chronic active hepatitis to the staging of PBC: portal hepatitis (stage 1), periportal interface hepatitis (stage 2), bridging necrosis or bridging fibrosis (stage 3), and cirrhosis (stage 4). Histological changes of PBC are, however, heterogeneous in whole liver and sampling errors occur in needle liver biopsies of PBC. Furthermore, as Scheuer pointed out, stage 1 and/or stage 2 lesions are found in stage 3 and stage 4 [36]. In Ludwig's system, bile ductal lesions or cholestatic changes which are very important features of PBC were not evaluated at all. Furthermore, the grading of necro-inflammatory activities of the bile ducts and hepatocytes to reflect the autoimmune-mediated pathogenesis of PBC is not reflected in these staging systems.

Since publication of the latest staging method, that of Ludwig et al. in 1978 [35], much progress has been made in clinical areas, particularly in therapeutic fields. There are now a number of treatments for PBC such as ursodeoxycholic acid (UDCA) and also combined UDCA and corticosteroid therapy for overlapping syndrome [24–26].
A recent study proposed a new histologic staging and grading system for comprehensive analysis of the histological progression of PBC (staging), and also of the immune-mediated necro-inflammatory activities of small bile ducts (chronic cholangitis) and of hepatocytes (interface and lobular hepatitis) [37, 38].

New system for histologic staging and grading of necroinflammatory activities

Staging
Three items (fibrosis, bile duct loss and cholestasis) are chosen for staging. Chronic cholestaticis is evaluated by deposition of copper or copper-binding protein by orcein stain [39]. These three items are scored as follows (Table 1A, B, C). For fibrosis (F), a score of 0 means that there is almost no fibrosis or the fibrosis is confined to the portal tracts. A score of 1 means that the fibrosis extends beyond the portal area occasionally with incomplete fibrous septa, and a score of 2, that there is bridging fibrosis with variable lobular disarray, and a score of 3, cirrhosis. For bile duct loss (B), a score of 0 means portal fibrosis, or fibrosis limited to portal tracts. A score of 1 means that bile ducts were absent in more than 2/3 of portal tracts. A score of 2 and 3 means that bile ducts were absent in more than 2/3 of portal tracts. For chronic cholestasis (C), a score of 0 means no deposition of orcein-positive granules in periportal hepatocytes. A score of 1 indicates deposition in less than one third of periportal hepatocytes of at least one portal tract, and a score of 2, deposition in more than two thirds of periportal hepatocytes along all portal tracts or fibrous septa. A score of 2 is that between 1 and 3. Then, a total score of the three items was obtained: a total score of 0 is stage 1 (no or minimum progression), 1–3 is stage 2 (mild progression), 4–6 is stage 3 (moderate progression), and 7–9 is stage 4 (advanced progression) (Table 2A). If orcein staining is not available, the sum of the scores for fibrosis and bile duct loss is also applicable as shown in Table 2B.

Grading of necroinflammatory activities
As mentioned above, chronic cholangitis and chronic active hepatitis like changes are two representative necroinflammatory activities of PBC, which are evaluated by using scoring systems (Table 3).

Table 1: Scoring for staging of primary biliary cirrhosis.

<table>
<thead>
<tr>
<th>A. Scoring of fibrosis</th>
<th>Score 0</th>
<th>No portal fibrosis, or fibrosis limited to portal tracts</th>
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<tbody>
<tr>
<td>Score 1</td>
<td>Portal fibrosis with periploral fibrosis or incomplete septal fibrosis</td>
<td></td>
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<tr>
<td>Score 2</td>
<td>Bridging fibrosis with variable lobular disarray</td>
<td></td>
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<tr>
<td>Score 3</td>
<td>Liver cirrhosis with regenerative nodules and extensive fibrosis</td>
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<tr>
<th>B. Scoring of bile duct loss</th>
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<tbody>
<tr>
<td>Score 0</td>
</tr>
<tr>
<td>Score 1</td>
</tr>
<tr>
<td>Score 2</td>
</tr>
<tr>
<td>Score 3</td>
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</tbody>
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<table>
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<tr>
<th>C. Scoring of deposition of copper granules or orcein-positive granules</th>
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</thead>
<tbody>
<tr>
<td>Score 0</td>
</tr>
<tr>
<td>Score 1</td>
</tr>
<tr>
<td>Score 2</td>
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<tr>
<td>Score 3</td>
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</tbody>
</table>

We recently proposed a new histologic staging and grading system for comprehensive analysis of the histological progression of PBC (staging), and also of the immune-mediated necro-inflammatory activities of small bile ducts (chronic cholangitis) and of hepatocytes (interface and lobular hepatitis) [37, 38].

Table 2: Staging by summing for scores of three and two items.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Sum of score</th>
</tr>
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<tbody>
<tr>
<td>Stage 1 (no progression)</td>
<td>0</td>
</tr>
<tr>
<td>Stage 2 (mild progression)</td>
<td>1—3</td>
</tr>
<tr>
<td>Stage 3 (moderate progression)</td>
<td>4—6</td>
</tr>
<tr>
<td>Stage 4 (advanced progression)</td>
<td>7—9</td>
</tr>
</tbody>
</table>

Three items; fibrosis, bile duct loss and deposition of copper granules on orcein-positive granules. Two items; fibrosis and bile duct loss.

Table 3: Grading of activities of cholangitis and hepatitis of primary biliary cirrhosis.

<table>
<thead>
<tr>
<th>A. Activities of cholangitis (CA)</th>
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<tbody>
<tr>
<td>CA 0 (no activities)</td>
</tr>
<tr>
<td>CA 1 (mild activities)</td>
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<tr>
<td>CA 2 (moderate activities)</td>
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<tr>
<td>CA 3 (marked activities)</td>
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<table>
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<tr>
<th>B. Activities of hepatitis (HA)</th>
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<tbody>
<tr>
<td>HA 0 (no activities)</td>
</tr>
<tr>
<td>HA 1 (mild activities)</td>
</tr>
<tr>
<td>HA 2 (moderate activities)</td>
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<tr>
<td>HA 3 (marked activities)</td>
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</tbody>
</table>

CNSDC: chronic non suppurative destructive cholangitis.
Chronic cholangitis activities (CA)

Chronic cholangitis is categorized into four grades. Grade 0 means absent or ambiguous bile duct damage. Mild biliary epithelial damage can also be found in grade 0. In grade 1, at least one damaged bile duct showing CNSDC or florid duct lesion [14,15] is found. Damaged bile ducts with periductal epithelioid granulomas are also included in CNSDC. In grade 1, one damaged bile duct showing evident cholangitis entirely surrounded by mild to moderate, duct-oriented lymphoplasmacytes is found, and this type of cholangitis is also occasionally encountered in chronic viral hepatitis. Interlobular bile ducts which are surrounded by small number of lymphoplasmacytes or adjacent to lymphoid cell infiltration in the portal tract are not regarded as evident chronic cholangitis. In grade 2, more than two bile ducts with evident chronic cholangitis are present, and bile ducts in one to two thirds of portal tracts are variably damaged.

Hepatitis activities (HA)

Interface hepatitis and lobular hepatitis are evaluated in combination and are categorized into four grades. Grade 0 means no interface hepatitis. Grade 1 and 2 mean the presence of interface hepatitis affecting about 10 continuous hepatocytes at the interface of one portal tract or fibrous septa, respectively. Grade 3 means the presence of interface hepatitis affecting more than 20 continuous hepatocytes at the limiting plate of many portal tracts or fibrous septa. While no or minimum lobular hepatitis is found in grade 0, mild to moderate lobular hepatitis is found in grade 1 or 2, and moderate lobular hepatitis in grade 3. Occasional zonal necrosis and bridging necrosis is regarded as grade 3.

Conclusions and perspectives

The diagnosis of PBC is usually made by a constellation of clinical, serological and pathological findings. Pathological changes valuable for the diagnosis of PBC differ according to the phase or stage of the disease, and a combination of several pathological lesions may be needed for an exact diagnosis. A new system applicable to needle liver biopsies in which many of the shortcomings of classical staging systems are overcome, has been proposed and is recommended for the clinical and therapeutic evaluation of PBC patients from a histological standpoint.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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