A Spectrum of Histopathologic Findings in Autoimmune Liver Disease

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Key Words: Autoimmune liver disease; Primary biliary cirrhosis; Autoimmune hepatitis; Overlap syndrome; Autoimmune cholangitis

Abstract

We retrospectively studied 42 liver biopsy specimens from 39 patients who met serologic and histologic criteria of autoimmune liver diseases. We found 10 cases of overlap syndrome (OLS), 10 autoimmune cholangitis (AIC), 10 primary biliary cirrhosis (PBC), and 9 autoimmune hepatitis (AIH) type 1. The following results were obtained: (1) Granulomas and biliary duct lesions were more prominent in PBC and AIC than in OLS and AIH. (2) Bile duct loss was not observed in AIH cases. (3) Features of hepatocellular damage such as piecemeal necrosis, spotty lobular necrosis, and confluent necrosis, were much more prevalent in OLS and AIH than in PBC and AIC. (4) HLA-DR antigen expression by hepatocytes was more frequent in AIH and OLS, whereas the expression of the same antigen by the bile duct epithelium was more frequent in PBC and AIC. We conclude there is a morphologic spectrum in autoimmune liver diseases, in which PBC forms one end of the spectrum, AIH the other, OLS the middle but closer clinically and histologically to AIH than to PBC, and AIC, which seems to be an antimitochondrial antibody–negative subtype of PBC.

Primary biliary cirrhosis (PBC) and autoimmune hepatitis (AIH) type 1 are 2 well-characterized autoimmune liver diseases with different clinicopathologic features. PBC typically affects middle-aged women and is defined by a progressive destruction of intrahepatic bile ducts and high titers of antimitochondrial antibodies (AMAs).1-3 In contrast, AIH type 1 occurs most often in young or middle-aged women and is characterized morphologically by prominent piecemeal and confluent necrosis and serologically by strong positive tests for serum antinuclear antibodies (ANAs), smooth muscle antibodies, or both.4-6 Additional entities of autoimmune liver disease have been described showing an overlap of features, such as the “overlap syndrome PBC/AIH” (OLS)5,7-11 and autoimmune cholangitis (AIC), which also is referred to as “immunocholangitis” or “autoimmune cholangiopathy.”12-22 The OLS includes patients who have clinical, serologic, and histologic features of both PBC and AIH. AIC is a PBC-like chronic nonsuppurative destructive cholangitis associated with high titers of serum ANAs and lacking AMAs.

The primary aim of the present study was to characterize the clinicopathologic and immunohistochemical features of OLS and AIC in comparison with PBC and AIH. The second aim was to describe the spectrum of morphologic findings in autoimmune liver diseases that may exist between PBC and AIH.

Material and Methods

A retrospective study was conducted. The medical records and the pathology reports from the University of Basel, Basel, Switzerland, and the University of Naples,
Naples, Italy, were available for review. For the study, we used the records of patients fulfilling the serologic and histologic parameters of autoimmune liver disease who underwent liver biopsy before any therapy was given. A total of 39 patients with 42 liver biopsy specimens met the criteria.

Formalin-fixed paraffin-embedded sections were stained with H&E, periodic acid–Schiff after diastase, chromotrope-aniline blue, Perls Prussian blue, orcein, and Sirius red. Paraffin-embedded tissue blocks were analyzed by immunohistochemistry using the avidin-biotin-peroxidase complex technique (Vectastain ABC Elite, Vector, Burlingame, CA) as described by the manufacturer. The following antibodies were used for immunohistochemistry: LU-5, cytokeratin 19 (CK19), CD3, CD45RO, CD20, and HLA-DR. A semiquantitative 4-grade system, (absent, minimal, moderate, and severe) was used to assess histologic parameters such as portal inflammation, lymphocytic aggregates in portal tracts, piecemeal necrosis, and spotty lobular necrosis and to analyze the immunohistochemical findings. The results obtained were further divided into 2 categories: (1) absent or minimal, (2) moderate or severe. Fibrosis was graded as portal, bridging, or cirrhosis. Other findings such as confluent necrosis, bile duct lesions (“biliary” and “hepatitic” type, as previously defined), bile duct loss, as dotted pattern,

Results

Patients with AIH were considerably younger than patients with PBC (44.3 vs 66.4 years), whereas those with PBC and OLS were almost the same age (66.4 vs 71.7 years). In addition, the mean age of patients with AIC was less than that for patients with PBC. Patients with OLS showed a marked elevation of alanine aminotransferase, whereas a slight elevation of aminotransferase levels was observed in patients with AIC. Clinical and laboratory data are given in Table 2.

Granulomas were present in 8 (80%), 7 (70%), and 5 (45%) of PBC, AIC, and OLS liver biopsy specimens, respectively. In contrast, only 1 case (9%) with AIH had histologic evidence of granuloma. Histologically, 2 types of granulomas were seen. The first type was well-defined granuloma, which consisted of epithelioid cells surrounded by a rim of lymphocytes, and it was mainly in close contact with a damaged interlobular bile duct. The second type was poorly defined granuloma, which consisted of aggregates of epithelioid cells and was mainly lobular. No giant cell granuloma was seen.

Biliary lesions were more prominent in PBC and AIC than in OLS and AIH. The affected bile duct epithelium was hyperplastic and infiltrated by lymphocytes, and sometimes it showed reactive dysplastic changes. The basal membrane quite often was disrupted, and the loss of bile ducts was replaced by fibrosis. Granulomas

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Sources and Dilution of Markers</th>
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<tbody>
<tr>
<td>Marker</td>
<td>Source</td>
</tr>
<tr>
<td>LU-5</td>
<td>Roche, Basel, Switzerland</td>
</tr>
<tr>
<td>Cytokeratin 19</td>
<td>Readysysteme, Bad Zurzach, Switzerland</td>
</tr>
<tr>
<td>CD3</td>
<td>Dakopatts, Glostrup, Denmark</td>
</tr>
<tr>
<td>CD45RO</td>
<td>Dakopatts</td>
</tr>
<tr>
<td>CD20</td>
<td>Dakopatts</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>Behring, München, Germany</td>
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</tbody>
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<table>
<thead>
<tr>
<th>Table 2</th>
<th>Clinical and Laboratory Findings</th>
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<tbody>
<tr>
<td>Sex Ratio</td>
<td>Age (y)</td>
</tr>
<tr>
<td>-----------</td>
<td>---------</td>
</tr>
<tr>
<td>PBC</td>
<td>66.4</td>
</tr>
<tr>
<td>AIC</td>
<td>57.7</td>
</tr>
<tr>
<td>OLS</td>
<td>71.7</td>
</tr>
<tr>
<td>AIH</td>
<td>44.3</td>
</tr>
</tbody>
</table>

AIC, autoimmune cholangitis; AIH, autoimmune hepatitis; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMA, antimitochondrial antibodies; ANA, antinuclear antibodies; GGT, gamma-glutamyltransferase; OLS, overlap syndrome; PBC, primary biliary cirrhosis.

1. Data are given as mean or median (median) unless otherwise indicated. Laboratory values are given in traditional units. Systeme International (SI) units are the same for ALT, GGT, and ALP. To convert values for IgG to SI units (grams per liter), multiply by 0.01; to convert bilirubin values to SI units (micromoles per liter), multiply by 17.1.

2. Dotted pattern.

3. Homogeneous pattern.
and biliary duct lesions (of the biliary type) also could be found in OLS and AIH, but not as a predominant histologic finding. In addition, bile duct loss was not observed in AIH cases.

Features of hepatocellular damage, such as piece-meal necrosis and spotty lobular necrosis, were seen frequently in AIH and OLS (Table 3) but were found in one third or fewer of the PBC and AIC cases Image 3 and Image 4. Confluent necrosis was observed in 5 (45%) of the AIH cases but was absent or rare in PBC and AIC Image 5.

AIC cases showed a consistent low degree of fibrosis, with only 1 case showing liver cirrhosis.

The expression of HLA-DR by the hepatocytes was more frequent in AIH and OLS, whereas the expression of the same antigen by the bile duct epithelium was more frequent in PBC and AIC Table 4.

In all biopsy specimens, monoclonal antikeratin antibody LU-5 was diffusely positive in zone 1 and zone 3 hepatocytes. In addition, zone 2 showed an increase in intensity of LU-5 in half or more of the cases. However, the difference among the 3 zones was not remarkable. Expression of CK19 was much stronger in zone 1 hepatocytes in OLS and AIH cases in comparison with expression in PBC and AIC cases. The composition of cellular infiltrates was similar in all 4 groups where CD3+ and UCHL1-positive cells were the most numerous mononuclear cells infiltrating portal tracts and lobules.

Table 3

<table>
<thead>
<tr>
<th>Histologic Findings</th>
<th>PBC (n = 10)</th>
<th>AIC (n = 10)</th>
<th>OLS (n = 11)</th>
<th>AIH (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Periportal orcein-positive granules†</td>
<td>7 (70)</td>
<td>6 (60)</td>
<td>3 (27)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Bile duct lesions, biliary type†</td>
<td>4 (40)</td>
<td>4 (40)</td>
<td>3 (27)</td>
<td>1 (9)</td>
</tr>
<tr>
<td>Bile duct loss†</td>
<td>6 (60)</td>
<td>6 (60)</td>
<td>3 (27)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Bile duct lesions, hepatic type†</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (9)</td>
</tr>
<tr>
<td>Portal inflammation</td>
<td>8 (80)</td>
<td>9 (90)</td>
<td>11 (100)</td>
<td>9 (82)</td>
</tr>
<tr>
<td>Lymphocytic aggregates†</td>
<td>3 (30)</td>
<td>3 (30)</td>
<td>2 (18)</td>
<td>3 (27)</td>
</tr>
<tr>
<td>Spotty lobular necrosis</td>
<td>2 (20)</td>
<td>2 (20)</td>
<td>7 (64)</td>
<td>6 (73)</td>
</tr>
<tr>
<td>Confluent necrosis†</td>
<td>0 (0)</td>
<td>1 (10)</td>
<td>2 (18)</td>
<td>5 (45)</td>
</tr>
<tr>
<td>Piece-meal necrosis</td>
<td>3 (30)</td>
<td>3 (30)</td>
<td>9 (82)</td>
<td>9 (82)</td>
</tr>
<tr>
<td>Portal fibrosis</td>
<td>9 (90)</td>
<td>5 (50)</td>
<td>9 (82)</td>
<td>11 (100)</td>
</tr>
<tr>
<td>Bridging fibrosis</td>
<td>8 (80)</td>
<td>2 (20)</td>
<td>9 (82)</td>
<td>5 (55)</td>
</tr>
<tr>
<td>Cirrhosis†</td>
<td>7 (70)</td>
<td>1 (10)</td>
<td>7 (64)</td>
<td>5 (45)</td>
</tr>
<tr>
<td>Granulomas†</td>
<td>8 (80)</td>
<td>7 (70)</td>
<td>5 (45)</td>
<td>1 (9)</td>
</tr>
</tbody>
</table>

AIC, autoimmune cholangitis; AIH, autoimmune hepatitis; OLS, overlap syndrome; PBC, primary biliary cirrhosis.
† Data are given as number (percentage) of biopsy specimens with moderate and/or severe lesions.
* Present or absent.
The differential diagnosis between PBC and AIH remains a challenging problem. Advances in biochemistry, immunology, and pathology since 1970 have, in many cases, facilitated the distinction between these diseases. Therefore, AMA and ANA titers exceeding 1:80 have long been used as clinical markers for the diagnosis of PBC and AIH, respectively.\(^3\)\(^5\)\(^{25-28}\) PBC is a chronic progressive liver disease of unknown cause that affects mainly middle-aged women and is characterized by the destruction of small intrahepatic bile ducts. Laboratory studies reveal an increase in alkaline phosphatase and IgM with AMA titers exceeding 1:80 in more than 90% of the patients.\(^{26-28}\) Nine AMA types (anti-M1 to anti-M9) with different clinical significance have been described so far.\(^{25-29}\) Four AMA types (anti-M2, anti-M4, anti-M8, and anti-M9) are typical for PBC.\(^3\)\(^3\)\(^{25-29}\) However, the literature has shown that the AMA titer is not a specific test for autoimmune liver diseases, as it can be seen in nonhepatic diseases and conditions such as syphilis, collagen diseases, heart disease, and drug-induced disorders.\(^{30-33}\)

In 1987, Brunner and Klinge\(^13\) reported 3 AMA-negative women with histologic features of PBC, such as bile duct lesions of the biliary type, granulomas, and ductopenia, as well as serologic features of AIH, such as high ANA titers; they called this condition immunocholangitis. The patients described showed a favorable clinical response to immunosuppressive therapy (azathioprine and prednisone). Since the first description, several cases with similar histologic and clinical features have been reported and referred to as primary autoimmune cholangitis or autoimmune cholangiopathy.\(^{12-14,22}\) However, these subsequent reports failed to demonstrate a lasting and/or clear-cut positive response to immunosuppressive therapy.

Our data confirm recent observations\(^{16,19,22,34}\) showing the lack of substantial clinical and histologic differences between AIC and classic PBC. In our study, the only relevant differences between AIC and PBC were the lower grade of fibrosis and the younger patients in AIC. The incidence of AMA-negative PBC cases reported is 5% to 10%.\(^27\) and

**Discussion**

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ANAs can be detected in 25% of PBC cases. These ANAs frequently display unique immunofluorescence patterns, such as nuclear dots or a nuclear ring-like pattern. All of our AIC cases displayed ANAs with a dotted pattern. These data lead us to believe that AIC may represent an AMA-negative subtype of PBC.

The similarity between AIC and PBC also was confirmed by clinical response to treatment. Both groups of patients were treated for at least 18 months with ursodiol given in a dose of 15 mg/kg of body weight. Response to therapy was assessed by a liver chemistry profile, such as alkaline phosphatase, bilirubin, and alanine aminotransferase, which failed to show a significant difference between patients with AIC and patients with PBC. These results are in agreement with those of previous studies showing a similar clinical spectrum and outcome for both entities.

The OLS was first described in 1997 by Klöppel et al. It is an uncommon chronic liver disease with morphologic, serologic, and clinical features of both PBC and AIH. The term OLS has been confusing, mainly for 2 reasons: first, it is generally accepted that about 20% of patients with typical type 1 AIH have AMA, and second, ANAs with a dotted pattern can be detected in 25% of PBCs, as we and others found. In our opinion, PBC cases with a dotted ANA pattern are best interpreted as “severe active PBC,” because they often show severe lobular and piecemeal necrosis, in addition to the typical biliary features of PBC (unpublished data).

Our OLS cases were characterized serologically by AMA and ANA positivity with a homogeneous ANA pattern and histologically by features of PBC and AIH. While moderate and severe biliary duct lesions and bile duct loss were detected in only 27% (3 cases) of OLS, granulomas were seen in 45% (5 cases), and the typical hepatic features, such as spotty lobular necrosis, piecemeal necrosis, and confluent necrosis were found in 64% (7 cases), 82% (9 cases), and 18% (2 cases), respectively. These data suggest that the histologic features of OLS are closer to AIH than to PBC. Controversy exists whether OLS represents a distinct entity with peculiar histologic and clinical features or whether it is a coincidence of 2 different diseases (eg, PBC and AIH). It is conceivable that chronic liver diseases with overlapping features of AIH and PBC could be a unique autoimmune defect resulting in damage to bile ducts and hepatocytes. Recently, Lohse et al described a subset of patients with autoimmune liver disease with clinical and/or histologic findings of AIH and PBC. The authors suggested that OLS might manifest as a hepatitic form of PBC in genetically susceptible people. However, 2 important features for the diagnosis of OLS were not mentioned in their study: the pattern of ANAs (homogeneous or dotted) and the frequency of confluent necrosis. It is possible, therefore, that some cases of the hepatic form of PBC reported in the study by Lohse et al could be consistent with our form of severe active PBC.

With the exception of anti–HLA-DR, all immunohistochemical antibodies used in the present study failed to demonstrate a significant difference among the 4 groups. These findings are consistent with previous reports. The pattern of biliary and hepatocellular HLA-DR expression showed a distribution that was similar to the observed histologic features. HLA-DR expression on bile duct epithelial cells was much more evident in PBC and AIC, whereas a hepatocellular expression was observed mainly in OLS and AIH cases. Although the expression of HLA-DR...
antigens by bile duct epithelium and hepatocytes may be a nonspecific phenomenon of cell damage, it remains intriguing that PBC and AIC on the one hand and OLS and AIH type 1 on the other hand show a similar pattern of expression.

CK19 expression by zone 1 hepatocytes was more strongly positive in OLS and AIH than in PBC and AIC, and it was independent of the fibrosis staging. This finding may indicate that recruitment of periportal hepatocytes for ductular proliferation occurs earlier in OLS and AIH than in PBC and AIC. The extended LU-5 positivity in all cases was not surprising, given the cholestatic features of these 4 diseases as described previously.

Based on the histologic and immunohistochemical data from the present study, we were able to define a histologic spectrum of autoimmune liver disease, in which PBC forms one end of the spectrum, AIH the other end, and OLS the middle but clinically and histologically closer to AIH than to PBC and AIC. This conclusion has important implications for the concept of etiopathogenesis in PBC. It is possible that the clinical and histologic pattern seen in PBC can occur despite the absence of AMAs, which contradicts the current theory suggesting that AMAs may have a role in the damage of the bile duct epithelium through their action against the pyruvate dehydrogenase E-2.

The group of autoimmune liver diseases is complex and not a well-explored area. In our experience, the combination of the morphologic features with strict serologic criteria should be the first step to resolving some of the difficulties that face pathologists when making the final diagnosis and, therefore, giving the clinicians better options for treating patients.

References

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