Immunostaining of Plasma Cells in Primary Biliary Cirrhosis

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Abstract

Primary biliary cirrhosis (PBC) is a chronic cholestatic liver disease characterized by inflammatory destruction of the intrahepatic bile ducts. The differential diagnosis for PBC often includes autoimmune hepatitis (AIH). Both diseases can show prominent plasma cells and other overlapping histologic features. It is interesting that both diseases can involve elevated levels of serum immunoglobulins, with IgM elevations typical of PBC and IgG elevations typical of AIH. We investigated whether this difference could be useful histologically by immunostaining plasma cells in liver biopsy specimens for IgG and IgM. We examined 56 cases: 18 of PBC and 38 of AIH. In PBC, plasma cells in the portal tracts were predominantly IgM+, whereas in AIH, plasma cells were predominately IgG+ (P < .0001). Immunostaining for IgM and IgG can be helpful in differentiating PBC from AIH.

Primary biliary cirrhosis (PBC) is a chronic liver disease of unknown cause that is defined by a constellation of clinical, laboratory, and histologic findings. Patients typically have chronic, often cholestatic, liver disease and have high titers of antimitochondrial antibodies (AMAs). Classically, the liver biopsy specimen shows predominantly a portal hepatitis with bile duct lymphocytosis and injury. At times, the injured and inflamed bile ducts will be associated with granulomatous inflammation (termed florid duct lesions). However, the histologic pattern may be heterogeneous, and granulomas are not always identified.

The clinical and histologic differential diagnosis for patients with PBC often includes autoimmune hepatitis (AIH). Plasma cells can be prominent in both diseases, and there can be other areas of histologic overlap that can make the distinction between PBC and AIH difficult. In PBC, serum levels of IgM are typically elevated, whereas in AIH, serum levels of IgG are typically elevated. Thus, histologic immunostaining of plasma cells for IgM and IgG makes an attractive target that may be useful as a diagnostic aid.

Materials and Methods

After we received institutional review board approval, we searched the pathology archives for cases of PBC and AIH for the interval 1987 to 2007. Cases were selected in which the histologic findings, serologic findings, and clinical findings all were consistent with AIH or PBC. Exclusion criteria included cases with missing slides or blocks, cases in which another hepatic disease was present, biopsy specimens with minimal chronic inflammation without appreciable plasma...
cells, posttransplant biopsy specimens, and cases in which patients were undergoing treatment for PBC or AIH. Because of limited numbers of cases, we also did not study cases of AMA-negative PBC.

A total of 56 cases were identified: 18 of PBC and 38 of AIH. Medical records were reviewed for clinical manifestations and serologic findings, including test results for anti-nuclear antibody (ANA), AMA, anti–smooth muscle antibody (ASMA), and anti–liver kidney microsomal antibody (LKM). Test results were also recorded for aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total protein, albumin, total bilirubin, estimated gammaglobulin fraction, and serum immunoglobulins (IgA, IgM, and IgG).

For laboratory values, data closest to the time of liver biopsy were recorded.

With the exception of 1 wedge biopsy, all remaining biopsies were needle biopsies. All cases were “blinded” for laboratory findings and original diagnosis before analysis. Routine H&E- and trichrome-stained sections were evaluated for inflammation and fibrosis with the Modified Histological Activity Index (MHAH, also referred to as the Ishak system). To isolate and compare the inflammatory component of each disease, necrosis was separated and not added into the total MHAH score. A single biopsy specimen of AIH was inadequate for staging owing to small size. Cases were also evaluated for histologic stage according to the Ludwig stage, which uses the inflammatory findings and the degree of fibrosis in the assignment of stage. This scoring system is specifically designed for PBC, so, after “unblinding” the cases, the Ludwig stage was retained only for PBC cases. The presence of granulomas was recorded, and macrovesicular steatosis was estimated as none, minimal (1%-5%), mild (6%-30%), moderate (31%-60%), and marked (>61%).

Immunohistochemical staining was performed on 5-µm sections from formalin-fixed, paraffin-embedded tissue samples. In most cases, immunostains were available from when the biopsy was originally examined for diagnostic purposes. Polyclonal rabbit antibodies against IgA, IgM, and IgG (DAKO, Carpinteria, CA) were used at dilutions of 1:8,000, 1:25,000, and 1:20,000, respectively, following heat antigen retrieval. Immunostains for IgA were also performed, allowing further comparisons between the groups because serum levels of IgA were available in many cases.

Immunohistochemical labeling for IgA, IgM, and IgG was semiquantitatively graded based on the portal tract with the maximum number of plasma cells on a scale of 0 to 4, corresponding to none, minimal, mild, moderate, and marked. There were no appreciable differences in the staining of portal plasma cells within a given biopsy specimen, so for scoring purposes, the portal tract with the most inflammation was scored. The presence or absence of florid duct lesions in PBC did not seem to influence the plasma cell proportions.

In addition to semiquantitative scoring, immunohistochemical stains for each case were also reviewed back-to-back to assess if IgM+ and IgG+ plasma cells were approximately equal in number or whether one was distinctly a grade above or below that of the other.

Results

Clinical and Biochemical Findings

The PBC cases included 15 women and 3 men with a mean ± SD age of 56 ± 9 years (range, 38-71 years) Table II. The AIH cases included 30 women and 8 men with a mean ± SD age of 49 ± 17 years (range, 20-87 years).

Other autoimmune conditions were associated with PBC and AIH. In PBC cases, the diseases included 1 case each of systemic lupus erythematosus, scleroderma/CREST (calcinosis cutis, Raynaud phenomenon, esophageal dysfunction, sclerodactyly, and telangectasia) syndrome, Sjögren syndrome, and rheumatoid arthritis. Similar autoimmune conditions were seen in AIH cases, including systemic lupus erythematosus (3 cases), scleroderma/CREST syndrome (2 cases), and Sjögren syndrome (1 case).

Biochemical laboratory tests were available in 55 (98%) of 56 cases, 40 (73%) of which were within a month of biopsy. The predominant biochemical abnormality in PBC was an elevated alkaline phosphatase level, whereas AIH cases showed predominantly elevated aminotransferase levels (Table 1).

Serologic Findings and Gammaglobulin Levels

Serologic studies and serum immunoglobulin levels were available in 51 (91%) and 26 (46%) of 56 cases, respectively, with 39 (76%) of 51 and 17 (65%) of 26 within 6 months of biopsy (Table 1). In 14 PBC cases, AMA testing was available and all were positive. In 13 of 14 cases, titers were also available, and the median titer was 1:320. It is interesting that in 3 of 28 cases of AIH, AMA results were positive with titers of 1:320, 1:40, and 1:320. The 2 AIH cases with high AMA titers had normal serum IgM levels, and the case with a low titer had elevations of all immunoglobulin levels. In all 3 of these AIH cases, the clinical findings, remaining laboratory findings, and histologic findings were those of AIH. Therefore, these cases were classified as AIH with an abnormal AMA result and were not considered to be overlap syndromes.

ANA testing was positive in 33 of 36 AIH cases. Titers were available in 30 cases, with a median titer of 1:320. ASMA testing was positive in 20 of 28 AIH cases with a median titer of 1:320. Of 15 AIH cases, 1 was positive for LKM (titers not available). ANA serologic results were also available in 14 PBC cases with 10 positive cases and titers...
available in 9 of the 10 cases (median titer, 1:160). ASMA testing was positive in 6 of 13 PBC cases, but results were predominantly low with titers ranging from 1:40 to 1:80. Only 3 PBC cases were tested for LKM, and all were negative.

An estimated gammaglobulin fraction can be calculated by subtracting albumin value (the major component of serum protein) from the total protein value, with the difference made up predominantly by gammaglobulins. Values of more than 3.5 g/dL are considered elevated, and elevated values were more common in AIH cases than in PBC cases (Table 1). Overall, a clear elevation of the average serum IgM level was seen in PBC cases and an elevation of serum IgG was seen in AIH cases (Table 1).

**Histologic Findings**

The average length of biopsy specimens in PBC was 24 mm and in AIH was 26 mm (Table 1). PBC cases had an average activity index of 4.1 (range, 1-8) with mild to moderate portal chronic inflammation and minimal to mild lobular chronic inflammation. Overall, PBC showed mild interface activity (Table 1). In contrast, AIH cases had more inflammation with an average activity index of 6.8 (range, 3-11). The inflammation in AIH was lobular and portal with a more brisk interface component (Table 1). In addition, 4 AIH cases showed bridging necrosis, whereas no PBC cases showed this feature. More than half (11/18 [61%]) of PBC cases showed granulomas; no granulomas were seen in AIH cases. Fat

**Image 1** Immunohistochemical grading. Immunohistochemical stains for IgA, IgM, and IgG were graded according to the number of plasma cells that were positive. The relative numbers correspond with minimal (A, grade 1), mild (B, grade 2), moderate (C, grade 3), and marked (D, grade 4) labeling.
accumulation was seen in 2 cases of PBC and 2 cases of AIH, ranging from minimal to mild. In PBC, 5 of 18 cases had no fibrosis, 10 cases had portal fibrosis (stages 1-2), and 3 cases had advanced fibrosis (stages 3-6), leading to an overall average ± SD MHAI fibrosis score of 1.2 ± 1.2 (range, 0-4). For AIH, fibrosis was absent (6/38), portal (7/38), and advanced (24/38). One AIH biopsy specimen was inadequate for staging. In 16 AIH cases, cirrhosis was present. Overall, the AIH cases showed more advanced fibrosis than the PBC cases, with an average ± SD MHAI fibrosis score of 3.6 ± 2.4 (range, 0-6).

The Ludwig stage for PBC takes into account the inflammatory component and the degree of fibrosis. Most of the PBC cases were early Ludwig stage (mean ± SD, 1.5 ± 0.9; range, 0-4). Immunophenotyping of Plasma Cells

In most cases, the numbers of IgM+ plasma cells in PBC cases were approximately equal to or slightly more than the numbers of IgG+ plasma cells in AIH cases. In contrast, AIH cases typically showed moderate to marked IgG staining and consistently showed more IgG than IgM immunostaining on direct comparison. IgA immunostaining was slightly more prominent in AIH cases, which correlated with serum levels that tended to be higher (Table 1). By back-to-back comparison of IgM and IgG stains with regard to any other histologic or clinical feature, we classified each case into 1 of 2 groups: (1) cases with more IgG+ plasma cells than IgM+ plasma cells and (2) cases with equal or slightly more IgM+ plasma cells than IgG+ plasma cells. These 2 groups strongly correlated with disease category, with...
PBC showing equal or more IgM staining than IgG staining and AIH always showing more IgG staining than IgM staining \( P < .0001; \) Fisher exact test). These findings seemed to be consistent across varying degrees of fibrosis, although a formal subset analysis could not be performed because of the small number of PBC cases with advanced fibrosis.

**Discussion**

The histologic findings are an important part of the diagnoses of PBC and AIH. Although both diseases are autoimmune, correctly separating the 2 entities is important because of differences in the natural history and treatment. In this study, we demonstrated that immunophenotyping of plasma cells can help differentiate PBC and AIH. It is interesting that early studies from the 1960s reported that immunofluorescence for immunoglobulin was increased in hepatitis\(^5,6\) and PBC\(^6\) and that immunofluorescence for IgM and IgG could separate PBC from “chronic active hepatitis and cirrhosis exhibiting piecemeal necrosis.”\(^7\) However, these observations were not further pursued for diagnostic purposes, and, in this study, we extended these observations and demonstrated that immunophenotyping plasma cells correlates strongly with the diagnoses of PBC and AIH.
The histologic features of AIH and PBC may be sufficiently distinct in many biopsy specimens to allow separation of these entities by experienced pathologists. In these cases, immunophenotyping of plasma cells may not add significantly more information than available from the routine stains. However, immunostaining may be a useful diagnostic aid in many cases. As can be seen from the data in this study, overreliance on cholestatic features as a marker of PBC or interface activity and abundant plasma cells as being strongly suggestive of AIH may lead to diagnostic errors. In this study, immunostains for IgG and IgM were not compared with an external standard but were compared back-to-back on the same case and within the same portal tracts. This approach reduces the effects of any staining variability that may result from different numbers of plasma cells.

It is unclear why cases of PBC are strongly linked to IgM elevations in the serum and increased IgM+ plasma cells in the portal tracts. There is no evidence that we are aware of that the

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<th>Table 2</th>
<th>Immunohistochemical Findings in 18 Cases of PBC and 38 Cases of AIH*</th>
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<td>IgM ≥ IgG</td>
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<td>PBC</td>
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*AIH, autoimmune hepatitis; PBC, primary biliary cirrhosis. *P < .0001; Fisher exact test.
IgM elevations are directly involved in damage to the biliary tree. It is interesting that IgM is secreted in bile, suggesting that the etiologic agent(s) of PBC or the inflammatory response may be stimulating the overproduction of IgM antibodies.

The predominance of IgG+ portal plasma cells in AIH parallels the typically increased IgG gammaglobulin levels in the serum. However, it has been our anecdotal experience that other liver diseases with occasionally prominent plasma cells, such as a drug reaction, can also show strong IgG staining similar to that of AIH. Thus, the use of IgG and IgM immunostains on liver biopsy specimens is most useful for the differential diagnosis of PBC vs AIH.

Serologic results are critical tools in the diagnosis of PBC and AIH. However, serologic findings are not always straightforward because some cases of PBC can have detectable ANA titers and some cases with AIH can have detectable AMA titers, as illustrated by cases included in this study. It is interesting that low-titer ANA or ASMA can be seen in up to one third of patients with PBC and in people with fatty liver disease and chronic hepatitis. Thus, the presence of low-level ANA and ASMA should not preclude the diagnosis of PBC in the context of AMA positivity and compatible histologic findings. In turn, low levels of AMA antibodies are also reported to occur in up to 25% of people with AIH. Whether such cases should be called overlap syndromes based solely on serologic findings is debatable, but it has been our practice to reserve the term overlap syndrome for cases in which serologic and histologic findings demonstrate sufficient overlap that the 2 cannot be differentiated with available pertinent clinical, laboratory, and histologic findings. With this more restrictive definition, cases of overlap syndrome are extremely rare. Because of their rarity in our files and the rarity of AMA-negative PBC, such cases were not included in this study.

The portal tracts show increased numbers of IgM+ plasma cells in PBC, whereas they show increased numbers of IgG+ plasma cells in AIH. When combined with serologic, clinical, and routine histologic findings, immunohistochemical stains for IgG and IgM can be useful aids in identifying cases of PBC and AIH.

References


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