Portal Tract Eosinophils and Hepatocyte Cytokeratin 7 Immunoreactivity Helps Distinguish Early-Stage, Mildly Active Primary Biliary Cirrhosis and Autoimmune Hepatitis

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Abstract
We studied nondiagnostic liver biopsy specimens from 20 patients with definite primary biliary cirrhosis (PBC) and 18 with definite autoimmune hepatitis (AIH) to identify distinguishing features. All patients had early-stage disease; biopsy specimens were devoid of granulomas or diagnostic features of PBC or AIH. Diagnoses were based on serologic and clinical variables. Sixteen specimens from each group were immunostained with cytokeratin 7. The density of portal tract eosinophils and number with cytokeratin 7–reactive periportal hepatocytes were quantified. Sixteen of 18 patients with AIH and 13 of 20 with PBC had no or minimal bile duct injury. Histologic activity index scores were 5.8 in AIH and 5.7 in PBC. The mean portal eosinophil score was greater in PBC than in AIH. Cytokeratin 7 identified many central bile ducts that were obscured by portal inflammation. The mean periportal cytokeratin 7–reactive hepatocyte score was greater in PBC than in AIH. Portal eosinophils and cytokeratin 7 reactivity in periportal hepatocytes are supportive of PBC rather than AIH. No morphologic features were supportive of AIH. Cytokeratin 7 reactivity in periportal hepatocytes may be an early response to PBC-induced biliary obstruction in other regions of the liver.

Primary biliary cirrhosis (PBC) and autoimmune hepatitis (AIH) can be difficult to distinguish.1-3 Patients with mildly active, early-stage PBC or AIH can have vague, nondescript symptoms. Serum antibody titers and liver function tests can be insufficiently elevated to be strongly supportive of either disease, and multiple serum antibody titers can be elevated, creating a nondiagnostic antibody panel. The distinction between the diseases is important. Corticosteroids, which are the mainstay of AIH therapy, usually are contraindicated in PBC. When the clinical findings and laboratory tests are inconclusive, the diagnosis can rest primarily on the morphologic features of the liver biopsy specimen. Unfortunately, liver biopsy specimens from patients with early-stage, mildly active PBC or AIH also can be nondiagnostic because they lack the characteristic morphologic features of either disease.

Normal biliary epithelium is strongly cytokeratin 7 reactive.4-6 Mature, uninjured hepatocytes are cytokeratin 7 nonreactive.4,7 One author recently suggested that cytokeratin 7 immunohistochemical analysis could be helpful in the distinction of PBC and chronic hepatitis.1,8 Many of the liver biopsy specimens in this study had late-stage changes with marked bridging fibrosis or cirrhosis and characteristic morphologic features.8 The extent to which cytokeratin 7 immunohistochemical analysis could be diagnostically helpful in liver biopsy specimens from patients with early stage, mildly active PBC or AIH has not been explored fully.

Three studies during the 1990s found an increased number of portal tract eosinophils in liver biopsy specimens from patients with PBC compared with patients with chronic hepatitis.9-11 The majority of studied biopsies had late-stage changes, with marked bridging fibrosis or cirrhosis, and they...
also had characteristic morphologic features. The usefulness of portal tract eosinophils in liver biopsy specimens from early-stage, mildly active PBC and AIH also has not been studied.

The goal of the present study was to evaluate whether portal tract eosinophils and cytokeratin 7 immunohistochemical analysis could be helpful in the morphologic distinction between mildly active, early-stage PBC and AIH, especially in biopsy specimens that are devoid of characteristic morphologic features.

**Materials and Methods**

The goal was to retrospectively obtain a group of nondiagnostic liver biopsy specimens from patients with early-stage, mildly active PBC or AIH who later had definite, unequivocal diagnoses of AIH or PBC. The studied liver biopsy specimens were selected from a large group of patients with PBC or AIH treated by one of us (S.C.G.) during the period January 1, 1993, through July 1, 2000. At the time of the retrospective review, all patients with AIH in the group had “definite AIH” according to the International Autoimmune Hepatitis Scoring System (serum alanine transaminase level at least 5 times the upper limit of normal, serum IgG level at least 2 times the upper limit of normal, positive anti–smooth muscle antibodies, and a liver biopsy specimen with moderate or severe periportal or periseptal lymphoplasmacytic piecemeal necrosis), and all patients with PBC had definite PBC (positive serum antimitochondrial antibodies, serum alkaline phosphatase level >2 times the upper limit of normal, serum glutamyltranspeptidase level at least 5 times the upper limit of normal, and a liver biopsy specimen with florid duct lesion).12,13 We were not interested in characterizing these features in liver biopsy specimens with the characteristic morphologic features of PBC or AIH. Liver biopsy slides and pathology reports from the PBC and AIH patient pool were initially reviewed, and the biopsy specimens were excluded if they had extensive bridging fibrosis, cirrhosis, or characteristic morphologic features of PBC (moderate bile duct injury, bile duct–centered lymphoplasmacytic inflammation, peri–bile duct lymphoid aggregates, onion skin–type peri–bile duct fibrosis, portal or parenchymal granulomas, and isolated foreign body giant cells) or AIH (a low-magnification impression of parenchymal-centered inflammation; diffuse, moderate interface hepatitis; and moderate parenchymal inflammation, extensive hepatocyte necrosis, numerous foci of spotty inflammation with acidophilic bodies).3,12

The clinical records of patients also were reviewed, and biopsy specimens were excluded if the patient was receiving corticosteroids or ursodeoxycholic acid at the time of biopsy, had additional liver diseases including B or C viral hepatitis (at the time of liver biopsy or subsequently diagnosed), had a subsequent diagnosis other than definite PBC or AIH, or was lost to follow-up before a definitive diagnosis could be established. Included biopsy specimens were from patients who were diagnosed with definite PBC or AIH at the time of the liver biopsy or after the biopsy.12,14 None of the patients in the study had an overlap syndrome with moderate to markedly active features of both diseases.13,15-18

The final study group included nondiagnostic liver biopsy specimens from 38 patients with definite PBC or AIH. Twenty biopsy specimens were from patients with PBC, and 18 were from patients with AIH. The mean age at biopsy for patients with AIH was 42 years and for patients with PBC, 47 years.

All specimens from patients with PBC were predominantly stage 1 or 2.4 Twenty (20%) had rare portal-portal fibrous bridges. None had extensive bridging fibrosis or cirrhosis. Thirteen patients with PBC had incorrect, nonspecific, or possible PBC diagnoses at the time of the liver biopsy; all biopsies were performed while under the care of nonauthor physicians. These patients came under the care of one of us (S.C.G.), and their disease was reclassified as probable or definite PBC 2.5 to 39 months after the liver biopsy. The other 7 patients with PBC had probable or definite PBC diagnoses made by one us (S.C.G.) at the time of liver biopsy.

The majority of portal tracts in biopsy specimens from patients with AIH had portal fibrosis that was predominantly mild, and less commonly moderate. Ten biopsy specimens (56%) had rare, thin portal-portal fibrous septa. None had extensive bridging fibrosis or cirrhosis. Fourteen of 18 patients with AIH had incorrect or nonspecific hepatitis diagnoses at the time of the liver biopsy, and 3 had probable AIH. Ten patients underwent a second liver biopsy that showed characteristic AIH. A definitive AIH diagnosis was made by one of us (S.C.G.) 1 to 22 months after the initial liver biopsy.

**Specimen Processing and Staining**

All liver biopsy specimens were processed in a similar manner. Blank sections were serially cut after formalin fixation and sequentially stained with H&E, Masson trichrome, reticulin, periodic acid–Schiff without diastase, periodic acid–Schiff with diastase, Prussian blue, orcein, and H&E again.

Sixteen biopsy specimens from each disease group with the greatest amounts of residual tissue in the blocks were used for cytokeratin 7 immunohistochemical staining. A 3-μm-thick section from each case was placed on a charged slide. Sections were deparaffinized using sequential immersions in 2 xylene baths, 3 baths of decreasing alcohol concentrations, and 2 water baths, followed by a 1-minute
wash in water. Slides were immersed in EDTA buffer (pH 7.0) and put into a commercial vegetable steamer at 95°C for 30 minutes. The slides were allowed to cool on the counter, remaining immersed in the heated EDTA buffer–filled containers for 5 minutes, followed by a 2-minute rinse with water while remaining in the containers. The slides were transferred into tris(hydroxymethyl)aminomethane–filled containers (pH 7.0) and allowed to undergo an additional 10 minutes of cooling on the countertop. They then were transferred to a commercial immunohistochemical autostainer (DAKO, Carpinteria, CA) and first were washed with buffer, followed by a hydrogen peroxide incubation. The latter was rinsed off, and the primary antibody was applied. Cytokeratin 7 (clone OV-TL-12/30; 1:800 dilution; DAKO) antibody was incubated over the sections for 20 minutes at room temperature. After the primary antibody was washed off, the components of the Envision-plus (DAKO) detection system were applied, including an antimouse polymer, 2 distilled-water washes, and a final diaminobenzidine incubation for 4 minutes. Sections were counterstained with hematoxylin, and cover glasses were applied. A positive control slide containing known cytokeratin-reactive tissues was stained with each batch of simultaneously stained slides. The chromogen system used was a 2-step technique that uses a horseradish peroxidase polymer conjugated with secondary antibodies. The labeled polymer does not contain avidin or biotin, and, therefore, nonspecific staining of hepatocytes from endogenous biotin is avoided.

**Evaluated Features**

The following features were evaluated in each biopsy specimen:

- **Activity scores.** Most of the liver biopsy specimens had a range of activity; therefore, a “highest activity score” was given based on the activity within the most active lobule in addition to the histologic activity index score.

- **Number of portal tract eosinophils.** The number was quantified on a scale of 0 to 2+: 0, no eosinophils; 1+, eosinophils in less than one third of the portal tracts; 2+, eosinophils in more than one third of the portal tracts.

- **Number of portal tracts available for evaluation.**

- **Degree of central bile duct injury.** The degree was scored as none, minimal, or mild. Minimal bile duct injury was a few intraepithelial lymphocytes unassociated with biliary epithelium injury; mild bile duct injury was slight lymphocyte infiltration of the duct with biliary epithelium cytoplasmic eosinophilia and nuclear irregularities.

- **Number of cytokeratin 7–reactive hepatocytes.** This feature was scored on a scale of 0 to 3+: 0, no cytokeratin 7–reactive hepatocytes; 1+, single or clusters of cytokeratin 7–reactive periportal hepatocytes located around fewer than 10% of the portal tracts; 2+, single or clusters of cytokeratin 7–reactive periportal hepatocytes around 10% to 50% of the portal tracts; 3+, reactivity around more than 50% of the portal tracts.

The biopsy specimens were examined in a blinded fashion without knowledge of whether the patient had PBC or AIH, and a numeric score for each feature was recorded. Subsequently, the biopsy specimens from all patients with PBC followed by patients with AIH were grouped together and reviewed, and descriptions and qualitative characteristics of each feature were recorded.

**Results**

**Inflammation**

The morphologic features of the PBC and AIH liver biopsy specimens were similar because of the restrictive study inclusion criteria. All specimens had portal tract and parenchymal (lobular) mixed lymphoplasmacytic inflammation that was not plasma cell predominant. The distribution of the inflammation was equal between portal tracts and lobules when mild and portal predominant when moderate. Most biopsy specimens had mild or mild to moderate portal inflammation, and a minority had moderate portal inflammation. The majority of portal tracts had no or mild piecemeal necrosis (interface hepatitis), and a minority had mild to moderate or moderate piecemeal necrosis. Parenchymal inflammation and injury was predominantly mild and focally moderate.

The mean and range for the histologic activity index scores for the liver biopsy specimens from patients with PBC or AIH were 5.8 (range, 3-7) and 5.7 (range, 2-8), respectively. The mean of the greatest regional activity of the PBC biopsy specimens was 7.4 (range, 6-8); for AIH biopsy specimens, it was 8.7 (range, 7-9).

**Table 1**

*Morphologic Features in Liver Biopsy Specimens From Patients With Primary Biliary Cirrhosis (PBC) or Autoimmune Hepatitis (AIH)*

<table>
<thead>
<tr>
<th>Feature</th>
<th>PBC (n = 20)</th>
<th>AIH (n = 18)</th>
</tr>
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<tbody>
<tr>
<td>Portal eosinophil score (mean)</td>
<td>1.20</td>
<td>0.42</td>
</tr>
<tr>
<td>Portal eosinophil score of 0 or 1</td>
<td>9.0 (45)</td>
<td>12.0 (67)</td>
</tr>
<tr>
<td>Periporal cytokeratin 7–reactive hepatocyte score (mean)</td>
<td>2.13</td>
<td>0.13</td>
</tr>
<tr>
<td>Periporal cytokeratin 7–reactive hepatocyte score of 0 or 1</td>
<td>70 (35)</td>
<td>18.0 (100)</td>
</tr>
<tr>
<td>Minimal or mild bile duct damage</td>
<td>16.0 (80)</td>
<td>13.0 (72)</td>
</tr>
<tr>
<td>Bile ducts/portal tract</td>
<td>1.10</td>
<td>1.35</td>
</tr>
<tr>
<td>Histologic activity index score</td>
<td>5.8</td>
<td>5.7</td>
</tr>
</tbody>
</table>

* Data are given as number (percentage) unless otherwise indicated.
Portal tract eosinophils were appreciably greater in liver biopsy specimens from patients with PBC than from patients with AIH. The majority of portal tracts in the biopsy specimens of patients with PBC with mild to moderate or moderate inflammation had several eosinophils centered around the bile duct. Portal tracts in PBC biopsy specimens with no or mild inflammation usually had no eosinophils. In contrast with the PBC liver biopsy specimens, moderately inflamed portal tracts from AIH specimens usually had no eosinophils or a rare eosinophil. The mean portal eosinophil scores in biopsy specimens from patients with PBC were 1.20 (range, 0-2) and from specimens from patients with AIH, 0.42 (range, 0-2). Despite the difference in mean scores, the percentages of biopsies with portal eosinophil scores of 0 or 1 were similar between the groups, constituting 45% (9/20) of the PBC biopsy specimens and 67% (12/18) of the AIH biopsy specimens.

**Portal Tracts, Bile Duct Number and Injury, and Bile Ductular Proliferation**

The mean number of portal tracts in PBC and AIH biopsy specimens was 12.6 (range, 7-24) and 10.2 (range, 6-19), respectively. The mean number of central bile ducts per portal tract in PBC and AIH biopsy specimens quantified on H&E-stained slides was 0.8 and 0.6, respectively. These values increased to 1.1 and 1.3, respectively, when the cytokeratin 7–stained slides were used to quantify the number of central bile ducts. Many central bile ducts that were obscured by portal inflammation on the H&E-stained slides became evident on the cytokeratin 7–stained slides. Approximately two thirds of the central bile ducts from both patient groups were round or oval, with an obvious central lumen. Some were partially flattened, producing a lumen of decreased diameter, and rare ducts were slit-shaped and devoid of a central lumen.

Minimal and mild bile duct injuries were common in portal tracts with moderate inflammation in biopsy specimens from patients with PBC or AIH. The liver biopsy specimens from 16 patients with PBC (80%) and 13 patients with AIH (72%) had minimal bile duct damage. Ten biopsy specimens from patients with PBC (50%) and 4 from patients with AIH (22%) had mild bile duct damage.

There was a bile ductular proliferation around portal tracts that were widened by fibrosis in PBC and AIH biopsy specimens. The proliferation tended to involve one third to one half of the portal tract circumference in PBC specimens, whereas it remained less than one third of the circumference in AIH biopsy specimens.

**Cytokeratin 7–Reactive Parenchymal Cells**

Three different-shaped cells could be seen with the cytokeratin 7 stain in addition to portal bile ducts and ductule cells, parenchymal spindle or stellate cells, small oval cells, and hepatocyte-like cells. Parenchymal spindle and stellate cells that usually were strongly cytokeratin 7 reactive were most numerous in areas of spotty parenchymal inflammation and, to a lesser degree, piecemeal necrosis. Oval cells that were approximately half the size of hepatocytes and devoid of prominent nuclei usually had weak to moderate cytokeratin staining. These cells were found most
often in the periportal parenchyma adjacent to active piece-meal necrosis. The number of these 2 cell types seemed to be similar in PBC and AIH biopsy specimens and was not explored further.

Only cytokeratin 7 reactivity in periportal hepatocyte-like cells was quantified. Images 4 and 5. Twelve (75%) of 16 PBC biopsy specimens had cytokeratin 7–reactive periportal hepatocytes. Cytokeratin 7–reactive periportal hepatocytes were present around fewer than 10% of the portal tracts in 2 (12%) of 16 AIH biopsy specimens.

The mean cytokeratin 7–reactive periportal hepatocyte score was 2.13 (range, 0-3) in PBC biopsy specimens and 0.13 (range, 0-1) in AIH biopsy specimens. Despite the differences in the mean values, 7 (35%) of 20 PBC biopsy specimens and 18 (100%) of 18 AIH biopsy specimens had periportal cytokeratin 7 hepatocyte scores of 0 or 1.

Discussion

The liver biopsy specimens from patients with early-stage, mildly active PBC or AIH can be morphologically similar. Liver biopsy specimens from patients with PBC can have mild, AIH-like piecemeal and parenchymal lymphoplasmacytic inflammation; and the portal tracts can be devoid of granulomas, lack bile duct–centered inflammation, have only mild bile duct injury, and contain a minimal bile ductular proliferation. Liver biopsy specimens from patients with early-stage, mildly active AIH can have portal tract–centered inflammation that is of mild activity with mild bile duct damage and a slight bile ductular proliferation. Dense portal inflammation can obscure bile ducts in either disease. Some authors have classified biopsy specimens with morphologic features that could not be classified as AIH or PBC as nondiagnostic.

Two studies from the 1990s reported that portal tracts from PBC biopsy specimens had increased eosinophils
compared with those of chronic hepatitis.\textsuperscript{9,10} Little attention was given to these observations until a more recent study confirmed that PBC biopsy specimens had an overall greater number of portal eosinophils and a greater number of activated portal eosinophils than biopsy specimens from patients with chronic hepatitis.\textsuperscript{11} This study also found that ursodeoxycholic acid suppressed the number of activated circulating and intrahepatic eosinophils. The authors concluded that eosinophils were an active component of the inflammatory process in PBC, and the clinical effects of ursodeoxycholic acid in patients with PBC was through alterations in eosinophil degranulation.\textsuperscript{11}

We also found that portal eosinophils were increased in early-stage, mildly active PBC biopsy specimens compared with AIH biopsy specimens, supporting the findings of previous studies. However, portal eosinophils seemed to be a useful diagnostic morphologic feature of PBC only when most of the portal tracts had eosinophils. Approximately 50\% of PBC and AIH biopsy specimens had a limited number of eosinophils in a minority of portal tracts.

Decreased portal bile ducts is a characteristic morphologic feature of PBC.\textsuperscript{1,8} Cytokeratin 7 immunohistochemical analysis facilitated the identification of bile ducts in portal tracts with dense inflammation where the bile duct was obscured. Although the H&E-stained sections and the recut sections used for cytokeratin 7 stains were not always similar, the mean number of bile ducts per portal tract increased approximately 37\% when quantified on the cytokeratin 7 immunohistochemical section compared with the number identified on the H&E-stained slide. These values are similar to those of a study by Rubio.\textsuperscript{1} Rubio\textsuperscript{8} recommended that cytokeratin 7 stain be used whenever the evaluation of bile ducts is an important component of the diagnosis. We second this recommendation.

Rubio\textsuperscript{8} also noted from the examination of cytokeratin 7–stained sections that lumens were present in central bile ducts in 27\% of PBC biopsy specimens and in 90\% of AIH biopsy specimens. Our results differed. An equal percentage of PBC and AIH biopsy specimens had central bile ducts with lumens. One plausible explanation for these differences is that we studied only biopsy specimens from early-stage, mildly active disease, whereas some of the biopsy specimens evaluated by Rubio were of higher stage and had greater activity.

Cytokeratin 7–reactive periportal hepatocytes located around the majority of portal tracts were found only in PBC biopsy specimens and, therefore, could be used as a morphologic feature of this disease. However, similar to portal eosinophils, the usefulness of this feature is limited when focal because 35\% of PBC biopsy specimens and all AIH biopsy specimens had no or rare cytokeratin 7 reactive perportal hepatocytes around fewer than 10\% of the portal tracts.

It is difficult to compare the cytokeratin 7 staining results of the present study with those of other studies. No other study used a chromogen system that was independent of avidin and biotin, which is necessary to consider the weak staining of hepatocytes as a reliable result. One study noted that cytokeratin 7–reactive hepatocyte-like cells were found in some areas with moderate to severe parenchymal inflammation in biopsy specimens from patients with chronic viral hepatitis.\textsuperscript{31} A recent study that focused on overlap syndromes and autoimmune liver diseases between the spectral ends of PBC and AIH found that 30\% of PBC biopsy specimens and 100\% of AIH biopsy specimens had cytokeratin 19–reactive periportal hepatocytes.\textsuperscript{18} Most of the biopsy specimens in that study had greater amounts of fibrosis and active inflammation.\textsuperscript{18} Regardless, we are at a loss to explain the disparate results between that study and ours. The recent study by Rubio\textsuperscript{8} raises the question of the specific cells being evaluated by the previous authors. Similar to the results of our study, Rubio noted that cytokeratin 7–reactive periportal hepatocytes were present in PBC biopsy specimens with many peripheral bile ducts. These same cells were nonreactive for cytokeratin 19. After our reading of the studies by these authors, we recut 12 PBC biopsy specimens with the most cytokeratin 7–reactive periportal hepatocytes and stained them with cytokeratin 19. Identical to the findings of Rubio, the cytokeratin 7–reactive periportal hepatocytes were nonreactive for cytokeratin 19.

The mechanism underlying periportal hepatocyte cytokeratin 7 reactivity in PBC biopsy specimens is unknown. One possibility is that it represents an early response by stem cells. These cells increase in the parenchyma immediately adjacent to portal tracts or within bile ducts at the periphery of portal tracts in long-term extrahepatic biliary obstruction. Some authors have speculated that this may be a proliferation of cells that normally reside in or compose the small ducts that connect bile canaliculi to the canals of Hering.\textsuperscript{5,32-39} They can acquire the cytologic appearance of hepatocytes, yet have a bile duct or coexpress a bile duct and hepatocyte immunophenotype.\textsuperscript{31,34,39-41} The existence of cells that possess the ability to undergo dual biliary and hepatocyte differentiation has been used to explain why otherwise typical hepatocytes around the periphery of cirrhotic nodules can have a bile duct–type keratin immunophenotype.\textsuperscript{42-44} Disruption of intrahepatic biliary flow, rather than inflammation and piecemeal necrosis, may induce this reaction because cytokeratin 7 expression in hepatocytes was not seen to any substantial degree in AIH biopsy specimens.

H&E staining underestimated the number of bile ducts compared with cytokeratin 7 staining. Portal tract eosinophils and cytokeratin 7–reactive periportal hepatocytes
are supportive of PBC when present in moderate to extensive degrees. None of the studied features was morphologically supportive of AIH. A liver biopsy specimen with no portal eosinophils and no periportal hepatocytes does not exclude PBC and is not diagnostic of AIH. Pathologists should consider including a cytokeratin 7 stain when evaluating liver biopsy specimens from patients who may have intrahepatic biliary disease.

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References


