Primary Sclerosing Cholangitis

Detailed Histologic Assessment and Integration Using Bioinformatics Highlights Arterial Fibrointimal Hyperplasia as a Novel Feature

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ABSTRACT

Objectives: Liver biopsy diagnosis of primary sclerosing cholangitis (PSC) is difficult. We performed a detailed histologic analysis of PSC cases using novel bioinformatics analysis to identify histologic features that may be useful in its diagnosis.

Methods: PSC liver explants were examined and compared with primary biliary cirrhosis and hepatitis C explants to act as controls. Demographic, macroscopic, and histologic variables were analyzed using both conventional statistics and an integrative bioinformatics approach, significance analysis of microarrays (SAM), and hierarchical clustering analysis (HCA).

Results: The PSC group was younger and had distinctive PSC features, including bile duct scars, onion-skin fibrosis, and arterial fibrointimal hyperplasia. SAM allowed the integration of variables by comparing PSC and control groups, whereas HCA was able to correctly categorize each group.

Conclusions: This study demonstrates characteristic PSC histology as well as arterial hyperplasia to be distinctive features that may aid in PSC diagnosis and be confirmed by bioinformatics.

Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease of unknown etiology characterized by the ongoing inflammation, destruction, and fibrosis of intra- and extrahepatic bile ducts (BDs) that leads to progressive alternating BD stricturing and dilatation and eventually cirrhosis requiring liver transplantation.1 Its incidence ranges from 0 to 1.3 per 100,000 persons/year.2,3 PSC occurs mainly in young men and is frequently associated with inflammatory bowel disease, and it should be considered in the differential diagnosis of cholestatic liver disease.4,5 Cholangiography shows localized or multifocal strictures with intervening segments of normal or dilated BDs and is considered the “gold standard” for the diagnosis,6 with a sensitivity and specificity of 89% and 80%, respectively.7 The typical findings on needle liver biopsy are found in only a minority of cases, reaching only 13.9% in some series.6 Thus, liver biopsy is performed not to diagnose PSC but to stage the disease, rule out coexisting liver diseases,5 and diagnose the small-duct variant of PSC, which is characterized by similar clinical, histopathologic, and biochemical features as PSC but normal cholangiography.8,9

The aim of our study was to perform a detailed histologic analysis using a novel integrative bioinformatics approach to identify specific histologic features of PSC to subsequently aid in the definitive diagnosis of PSC. We chose to study liver explants in which the diagnosis of PSC was already well established to ensure the presence of an adequate tissue specimen and number of portal tracts and BDs. For this approach, we used significance analysis of microarrays (SAM), a multiple testing method typically used in genomic research to analyze and integrate demographic, macroscopic, and histologic variables.
Materials and Methods

Clinical Samples

This study was approved by the Mount Sinai Institutional Review Board. We identified 36 PSC liver explants from the archives of the Pathology Department, Mount Sinai School of Medicine and cross-referenced the diagnosis with the Recanati/Miller Transplantation Institute liver transplant database. All patients were adults and had been previously diagnosed by cholangiography with PSC. Cases of the small-duct variant of PSC and cases with cholangiocarcinoma and/or hepatocellular carcinoma were excluded.8 No cases of secondary biliary cirrhosis were included. Ten primary biliary cirrhosis (PBC) and 10 chronic hepatitis C virus (HCV) liver explants were used as controls. At least three H&E-stained slides per case were used for histologic evaluation.

Variables Studied

Variables were divided into demographic, macroscopic, and histologic groups. Demographic variables included age and sex and were obtained from pathology reports. Variables from the macroscopic examination included liver weight, presence of cirrhosis, atrophy or hypertrophy of any liver lobe and/or segment(s), and the presence of hepatolithiasis. H&E- and trichrome-stained slides were evaluated by two hepatopathologists (G.C. and M.I.F.) for the following groups of histologic variables. A summary of these variables is provided in Table II.

1. Vascular changes. These included the presence or absence of arterial fibrointimal hyperplasia (defined as fibrous thickening of the subendothelial layer and/or smooth muscle hyperplasia), nodular regenerative hyperplasia (NRH), phlebosclerosis (defined as perivenular fibrosis with partial or complete obliteration of the portal vein lumen), phlebitis, endotheliitis, intra- and extrahepatic portal vein thrombosis, and foamy macrophages lining the vessel lumen.

2. Biliary changes. These comprised the percentage of medium-size and small (terminal)–size bile duct (BD) loss (defined as number of missing BDs in 25 portal tracts) and the presence or absence of acute cholangitis (defined as neutrophilic infiltration of the BD wall and epithelium) of different BD sizes, lymphocytic cholangitis, BD scars, BD atrophy, periductal onion-skin fibrosis of medium- and small-size (terminal) BDs, and florid duct lesions. In cirrhotic livers, where “classic” portal tracts may not be evident, we defined them as the presence of the portal vein and hepatic artery, with or without BD.

3. Cholestatic changes, which included the presence or absence of bile plugs identified when inspissated bile is present within BDs, ductules, and canalicular lumina; hepatocytic cholestasis (defined as presence of bile in the hepatocyte’s cytoplasm); rosetting; bile infarcts; cholestasis inducing a halo effect (defined as feathery degeneration of perisepal hepatocytes); Mallory-Denk hyaline; and ductular reaction, which was semiquantitatively graded from 0 to 3 (none or minimal, mild, moderate, and severe, respectively).

4. Inflammatory changes. The presence or absence of mononuclear portal/septal infiltrate and interface hepatitis, both also semiquantitatively graded from 0 to 3 (none or minimal, mild, moderate, and severe, respectively); presence or absence of lymphoid aggregates; and features of overlap syndrome with autoimmune hepatitis (ie, portal and lobular/parenchymal plasma cells with single cell and confluent hepatocytic necrosis).

5. Fibrosis. The stage of fibrosis was assessed using both the modified Knodell criteria by Ishak et al10 and PSC fibrosis staging system described by Ludwig et al11 and Silveira and Lindor,12 both graded semiquantitatively from 0 to 6 and 0 to 4, respectively.10,13

![Table II: Histologic Variables Assessed](https://example.com/table-ii)
6. Additional variables when noted included ballooning degeneration, single cell or confluent necrosis, granulomas, and steatosis.

Statistical Analysis, Bioinformatics, and Integration Approaches

We applied a software-based analysis using MultiExperiment Viewer v.4.8.1 (Dana-Farber Cancer Institute, Boston, MA)\(^1\)\(^-\)\(^1\)\(^6\) to perform statistical analysis and integration of data. A t test (significant if \(P < .05\)) was applied to compare PSC with the control group (PBC + HCV). One-way analysis of variance (ANOVA) was used to find significant differences across the three groups (PSC vs PBC vs HCV). The results of both tests were expressed as means and SDs of the mean. The multiple hypotheses testing bias, which determines that the more tests performed on a set of data, the more likely the null hypothesis can be rejected when it is true (type I error), resulting in an increased false-positive rate, was corrected using the adjusted Bonferroni correction\(^17\) and the false discovery rate (FDR), which is the expected proportion of false positives among the declared significant results.\(^18\)\(^-\)\(^21\) Integration was done by SAM (significant if median FDR = 0), which is a multiple testing approach method that has been applied extensively in genomic research.\(^22\) Although SAM was originally designed for comparison of gene expression patterns (continuous variables) between two groups of interest, herein we adapted it for discontinuous variables (presence or absence) as previously described,\(^23\) so that we were able to combine and integrate demographic, macroscopic, and histologic variables. Finally, we carried out an unsupervised hierarchical clustering analysis (HCA; Euclidean distance, complete linkage) to explore the reliability of self-classification of PSC, PBC, and HCV groups. HCA is another genomic method that has been applied previously to complementary DNA microarrays and tissue microarray/immunohistochemistry data in several tumors, including liver malignancies.\(^24\)\(^,\)\(^25\) This method allows the self or unsupervised clustering of the cases included based on their characteristics (studied variables, gene expression, etc).

Results

The findings from the comparison between PSC and control (PBC + HCV) groups by t test are as follows; significant differences are shown in Table 2.

Demographic Data

Patients with PSC were significantly younger at the time of transplantation than those in the control group (45.4 vs 56.2 years; \(P = .020\); FDR = .002) (Table 2). There was a male predominance in PSC (55.6%) and HCV (70%) cases, whereas there was a female predominance in the PBC (80%) group. These results are similar to prior population-based studies.\(^2\)

Macroscopic Features

PSC liver explants had a mean weight of 1,657 g, which was not significantly different from the control group (1,362 g). Eleven (31%) of 36 PSC explants did not show fully developed cirrhosis, while all PBC and HCV explants were grossly cirrhotic (\(P < .02\); FDR = .002). Atrophy and/or hypertrophy of any of the liver lobes and/or segments were seen in seven (19%) of the PSC explants, in none of the PBC explants, and in only one (10%) of the HCV explants (not significant).
Hepatolithiasis was present in 14 (39%) cases in the PSC group and none in the control group ($P = .002$; FDR = .00039).

**Histologic Features**

**Vascular Changes**

Arterial fibrointimal hyperplasia was the only vascular finding that was significantly more frequent in PSC compared with controls (75% vs 30%; $P = .012$; FDR = .001).

**Image II** Examples of histologic features evaluated and described in primary sclerosing cholangitis. **A**, Portal tract with fibrosis and severe bile duct (BD) atrophy; feathery degeneration of some periportal hepatocytes; Mallory-Denk hyalines (arrow) is also present (H&E, ×400). **B**, Small-size BD with periductal onion-skin fibrosis, mild inflammation, and epithelial damage (H&E, ×400). **C**, Example of fibrointimal hyperplasia in an artery (H&E, ×400). **D**, BD plug with mild chronic inflammation and foreign-body giant cell reaction (H&E, ×200).

Medium-size BD loss, BD scars, and onion-skin fibrosis of terminal BDs were the most distinguishing features of PSC compared with control cases (48.4% vs 11.4%, 89% vs 5%, and 78% vs 20%, respectively), being highly significant ($P < .0001$; FDR < .00001). Large-size BD cholangitis and medium-size BD onion-skin fibrosis were also more frequently seen in PSC compared with controls (33% vs 0%, $P = .01$, FDR = .001 and 92% vs 45%, $P = .024$, FDR = .002, respectively). The frequency of medium- and small-size BD cholangitis, small-size BD loss, BD atrophy, and lymphocytic cholangitis was not significantly different between the groups.

**Biliary Changes**

Medium-size BD loss, BD scars, and onion-skin fibrosis of terminal BDs were the most distinguishing features of PSC compared with control cases (48.4% vs 11.4%, 89% vs 5%, and 78% vs 20%, respectively), being highly significant ($P < .0001$; FDR < .00001). Large-size BD cholangitis and medium-size BD onion-skin fibrosis were also more frequently seen in PSC compared with controls (33% vs 0%, $P = .01$, FDR = .001 and 92% vs 45%, $P = .024$, FDR = .002, respectively). The frequency of medium- and small-size BD cholangitis, small-size BD loss, BD atrophy, and lymphocytic cholangitis was not significantly different between the groups.
Cholestatic Changes

Bile plugs and bile infarcts were more frequently seen in PSC explants compared with controls (28% vs 0%, \( P = 0.039 \), FDR = 0.003 and 47% vs 5%, \( P = 0.004 \), FDR = 0.00058, respectively), whereas no differences were found regarding ductular, hepatocytic, and canalicular cholestasis; rosetting; ductular reaction; periseptal “halo effect”; copper deposition; or the presence of Mallory-Denk hyaline.

Inflammatory Changes

The PSC group showed significantly less inflammatory activity compared with the controls, represented by less mononuclear portal/septal inflammation (mean score 1.5 vs 2.5, \( P = 0.004 \), FDR = 0.00062), less interface hepatitis (mean score 1.03 vs 2.25, \( P = 0.0086 \), FDR = 0.00022), and lower frequency of lymphoid aggregates (17% vs 75%, \( P = 0.001 \), FDR = 0.00024). None of the cases showed an overlap syndrome with autoimmune hepatitis (presence of portal/septal and lobular/parenchymal plasma cells and single cell and confluent hepatic necrosis).

Fibrosis

The PSC group showed less fibrosis than controls, represented by a lower Ludwig PSC fibrosis stage score (3.69 vs 4, \( P = 0.02 \), FDR = 0.002). There was no significant difference in fibrosis when using the Ishak fibrosis staging (5.25 vs 5.85).

Additional Variables

The frequency of granulomas, steatosis, ballooning degeneration, and necrosis was not significantly different between the groups.

When the data were analyzed using one-way ANOVA comparing all three groups, only arterial fibrointimal hyperplasia, medium-size BD loss, presence of BD scars, and onion-skin fibrosis of terminal and medium-size BDs significantly characterized PSC. The test also confirmed PSC’s lower inflammatory activity (ie, mononuclear portal/septal inflammation, interface hepatitis, and presence of lymphoid follicles) compared with the PBC and HCV groups, with HCV cases showing the greatest inflammatory activity and PBC showing an intermediate level of it

Bioinformatics Approach: Integration of Demographic, Macroscopic, and Histologic Variables

SAM

We used SAM to compare the PSC and control (PBC + HCV) groups. SAM showed all the tested variables in a graphic representation, demonstrating the strength of each variable; all the variables outside the dashed lines in Figure 1 are significantly more frequent in one group compared with the other group. The PSC group is characterized, in descending order of significance, by the presence of BD scars, onion-skin fibrosis of terminal BDs, medium-size BD loss, onion-skin fibrosis of medium-size BDs, arterial fibrointimal hyperplasia, presence of bile infarcts, hepatolithiasis, acute cholangitis of large BDs, and presence of bile plugs. On the other hand, the control group is characterized, also in descending order of significance, by the presence of lymphoid aggregates, interface hepatitis, mononuclear portal/septal inflammation, older age at the time of transplantation, ballooning degeneration, iron deposition, presence of florid duct lesions, higher Ludwig PSC fibrosis stage, macroscopic cirrhosis, and higher Ishak fibrosis stage.
To explore the possibility that this integrated approach was also able to self-classify PSC, PBC, and HCV cases, we carried out an unsupervised HCA. 

**Figure 2** HCA produced a dendrogram showing two main groups: the PBC + HCV group and the PSC group. The PBC + HCV group was formed by two subgroups, A and B, the first one formed mainly by HCV cases and the second one by PBC, HCV, and PSC cases sharing demographic, macroscopic, and histologic features, demonstrating the heterogeneity of these entities. The PSC group was subdivided into two subgroups (C and D). Group C was characterized by advanced fibrosis compared with group D and was subsequently characterized by the presence of NRH and lymphoid aggregates (comparison was made using SAM, not shown).

**Discussion**

We performed a detailed histologic analysis of 36 PSC liver explants. Ten PBC and 10 HCV cases were used as controls, with the former serving as a cholestatic...
Unsupervised hierarchical clustering analysis (Euclidean distance and complete linkage) of 36 primary sclerosing cholangitis (PSC), 10 primary biliary cirrhosis (PBC), and 10 hepatitis C virus (HCV) cases was based on integration of demographic, macroscopic, and histologic features. Two main groups were formed: the PBC + HCV group and the PSC group, each formed by two subgroups (A, B, C, and D). “A” is formed mainly by HCV cases; “B” has roughly the same proportion of PBC, HCV, and PSC cases; both “C” and “D” are formed almost exclusively by PSC cases; “C” is characterized by advanced fibrosis compared with “D,” which is characterized by the presence of nodular regenerative hyperplasia and lymphoid aggregates.

autoimmune liver disease affecting small intrahepatic BDs and the latter a chronic hepatitis process. HCV is known to damage and even destroy BDs, and it is also the most common etiology of liver disease leading to liver transplantation, contributing to approximately 26% of all the liver transplants in United States. In this study, we found that vascular changes such as arterial fibrointimal hyperplasia and biliary changes, particularly medium-size BD loss, BD scars, and onion-skin fibrosis of medium and terminal BDs, were the findings most specific for PSC cases compared with the combined control group (PBC + HCV) as well as when both PBC and HCV groups were separated. We also showed that PSC has less inflammatory activity compared with the control group, either together or separately. In addition to the classic biostatistics tools, we performed a bioinformatics analysis that allows the analysis of large amounts of data and the integration (systems pathology approach) of different types of variables in a visual or schematic way; these showed the most relevant variables differentiating the PSC from the control (PBC + HCV) group (SAM) and also identified distinct subgroups based on their specific features (HCA).

SAM is a method extensively used for expression profile analysis to compare the expression of thousands of genes between study groups. This method can be adapted to other types of experimental data such as survival time or tumor stage. SAM allows the integration of continuous/quantitative, semiquantitative, and discontinuous variables, including demographic, macroscopic, and histologic variables, as previously described. SAM is a supervised analysis where two groups are compared, giving a sequence of all tested variables in a graphical representation showing the strength of variables in each group. Thus, SAM analysis showed that BD scars, onion-skin fibrosis of terminal and medium-size BDs, medium-size BD loss, and arterial fibrointimal hyperplasia were the findings more specifically characterizing...
PSC. Taking only the presence of BD scars, its sensitivity and specificity for the histologic diagnosis of PSC compared with PBC and HCV were 91% and 95%, respectively; when onion-skin fibrosis of terminal BDs was added, the sensitivity and specificity reached 100% and 95%, respectively.

Although these features have been previously described in PSC, it is important to highlight two features: (1) the presence of onion-skin fibrosis even in small-size BDs and (2) identification of arterial fibrointimal hyperplasia. It is a well-known fact that periductal onion-skin fibrosis is often seen around medium- and large-size BDs, but our results demonstrate that this can also be seen affecting small-size BDs and may be a distinctive feature that may aid in the diagnosis of PSC in needle liver biopsy specimens. Fibrointimal hyperplasia of medium- and small-size arteries was a frequent and distinguishing feature of PSC (found in 75% of the cases); interestingly, this feature has not been shown before and may also be helpful in suggesting the diagnosis of PSC when evaluating liver needle biopsy specimens. In addition, this finding may, at least in part, explain why some end-stage PSC livers show atrophy and compensatory hypertrophy of segments and/or lobes. This further raises the possibility of a primary or secondary vascular pathophysiology in PSC, in which the initial and/or subsequent damage is in the BD vascular supply, producing a secondary BD injury with alternating areas of strictures and dilatations and small-size BD loss.

HCA is a method used for expression profiling analysis. As opposed to SAM, HCA is an unsupervised method that allows an exploratory data analysis, providing rough maps and suggesting directions for further study in a concise, visually effective way, such as a tree or dendrogram to identify candidate subgroups in complex data. In our data, HCA produced two main groups, one formed mainly by PSC cases and the other by PBC and HCV cases; the PSC group is formed by two subgroups, one characterized by end-stage fibrosis and the other by precirrhotic fibrosis stage and NRH. The presence of NRH in precirrhotic (stages 1-3) PSC cases has been previously described and may be related to the vascular changes, as demonstrated in this study, in which arterial fibrointimal hyperplasia is significant and distinctive. Finally, an interesting subgroup was found in the PBC and HCV group, formed by roughly the same proportion of PBC, HCV, and PSC cases, demonstrating the heterogeneity of these diseases and how they may share demographic, macroscopic, and histologic features contributing to the difficulty of diagnosis based on pathologic evaluation alone.

In summary, our findings show that PSC is characterized by younger age at time of transplantation, presence of BD scars, onion-skin fibrosis of terminal BDs, medium-size BD loss, onion-skin fibrosis of medium-size BDs, and arterial fibrointimal hyperplasia. The two most relevant features are the presence of BD scars and onion-skin fibrosis of terminal BDs. The former two histologic variables are not unexpected findings from our analysis. However, the finding of arterial fibrointimal hyperplasia is not part of the well-categorized histologic changes noted in PSC. In fact, there is little known about microscopic vascular changes in patients with PSC. We chose to use liver explants in our study to first establish “proof of principle” regarding the specific histologic variables to ensure an adequate tissue specimen and number of portal tracts and BDs with which to apply this novel and robust bioinformatics analysis. This method was used as a tool to analyze a large amount of data to identify unbiased specific variables, but it does not replace a well-trained liver pathologist at the time of the diagnosis. We hope to now apply a similar histologic review to needle liver biopsy specimens in patients with well-characterized cholangiographic and clinical PSC. The ultimate goal is to allow the pathologist to more reliably diagnose PSC on needle biopsy specimens.

References


