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Novel histological scoring for predicting disease outcome in primary sclerosing cholangitis

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Background: Primary sclerosing cholangitis (PSC) is a progressive cholestatic liver disease that may lead to liver cirrhosis or cholangiocarcinoma. Liver histology and fibrosis stage are predictive markers of disease progression, and histological cirrhosis is defined as a significant endpoint. PSC-specific histological scoring methods are lacking at present. We aimed to develop a tailored classification system for PSC, the PSC histoscore, based on histological features associated with disease progression.

Methods: In total, 300 PSC patients diagnosed between 1988 and 2018 were enrolled; their data were collected from the PSC registry (Helsinki University Hospital), and liver specimens were obtained from the Biobank of Helsinki. Five histological features included in the adapted Nakanuma scoring system and three additional parameters typical for PSC histology were evaluated and compared with the clinical and laboratory data. A compound endpoint consisting of liver transplantation, development of cholangiocarcinoma, or death was used as outcome measurement.

Results: Stage (fibrosis, bile duct loss, ductular reaction, and chronic cholestasis) and grade (portal inflammation, portal edema, hepatitis activity, and cholangitis activity) parameters were found to be independent predictive risk factors for the compound endpoint ($P < 0.001$). High disease grade (2–6) and stage (2–4) better correlated with clinical endpoints when evaluated with the PSC histoscore system compared to the adapted Nakanuma classification. The risk for disease progression in sequential endoscopic retrograde cholangiography (ERC) examinations was increased with elevated total PSC histoscores.

Conclusion: The PSC histoscore is a novel histological classification system for PSC. Our findings support the applicability of liver histology as a marker for disease progression.

Keywords: cholestatic liver disease, liver histology, prognostic tools, Nakanuma classification, PSC histoscore

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Johanna Arola and Martti Färkkilä are contributed equally to this work.

Abbreviations: CCA, Cholangiocarcinoma; CD, Crohn's disease; EASL, European Association for the Study of the Liver; ERC, Endoscopic retrograde cholangiography; ERCtwAUC, Endoscopic retrograde cholangiography time-weighted area under the curve; HUH, Helsinki University Central Hospital; IBD, Inflammatory bowel disease; LT, liver transplantation; MRCP, Magnetic resonance cholangiopancreatography; P-ALP, Plasma alkaline phosphatase; PBC, Primary biliary cholangitis; PSC, Primary sclerosing cholangitis; S-Bil, Serum bilirubin; SDPSC, Small duct primary sclerosing cholangitis; UC, Ulcerative colitis; UDCA, Ursodeoxycholic acid; WSI, Whole-slide image.

Introduction

Primary sclerosing cholangitis (PSC) is a progressive cholestatic liver disease that eventually evolves to strictures of the intra- and extrahepatic bile ducts and cirrhosis, due to chronic fibrosing inflammation of the bile ducts.[1] The prevalence of PSC ranges from 0–16.2 per 100,000 inhabitants, and[2] the median age at the time of diagnosis is usually between 30–40 years.[3] Lifetime risk for malignancies is increased, especially cholangiocarcinoma (CCA).[4-6]

PSC is strongly associated with inflammatory bowel disease (IBD).[7-12] The exact pathogenesis remains to be clarified,[9,11,12] which creates an imminent obstacle in the development of risk stratification tools to evaluate disease progression. Some patients have an indolent disease course.[13] Stratifying patients into discrete categories based on their progression rate for surveillance purposes is challenging, when optimal prognostic tools are lacking.[14,15]

Thus far, only a few viable therapeutic options are available. Liver transplantation (LT) is the only curative treatment for endstage disease.[9] Despite the wide use of ursodeoxycholic acid (UDCA) in the treatment of PSC, its impact on disease progression is not evident.[16] Several drug trials are ongoing, but a lack of proper surrogate endpoints and prognostic tools are hindering the development.[14,15,17]

The internationally recommended protocols for disease surveillance include combinations of several parameters.[18,19] The clinical biomarker plasma alkaline phosphatase (ALP) and imaging modalities, such as ERC (endoscopic retrograde cholangiography) and MRCP (magnetic resonance cholangiopancreatography) are well established.[18,19] Higher age, altered serum bilirubin (S-Bil), and plasma ALP, higher histological disease stage, spleno- and hepatomegaly, and the presence of IBD are independent predictors of poor prognosis in PSC.[20-23] The DNA methylation signature in the peripheral blood, Amsterdam-Oxford-Model, Enhanced liver fibrosis test, and PreSto are examples of noninvasive and indirect predictors of disease stage and progression.[24-27]

The histological findings in PSC are not specific, but in advanced disease bile duct loss, portal, and periductal fibrosis and chronic cholestasis are typically evident.[28,29] Chronic cholestasis and ductular reaction can be visualized by cytokeratin 7 (K7) immunohistochemical staining.[28-31]

At present, there are no validated histological scoring protocols specifically designed for PSC liver specimens. The adapted Nakanuma scoring system was originally developed for primary biliary cholangitis (PBC). The adapted Nakanuma stage and grade[28,29,32] had prognostic value in PSC.[33] Findings were validated in an international cohort study in which histological stage had a significant impact on outcome.[34]

The role of liver histology in PSC surveillance is controversial, even though the stage in liver biopsy has predictive value in estimating disease outcome.[35] Liver biopsy is an invasive procedure including complication risk and the likelihood of sampling error.[28,35] Moreover, the morphological evaluation of liver histology is always subjective and prone to interobserver variation; however, according to a consensus of an international validation study, the reproducibility of the adapted Nakanuma classification is adequate.[33]

Liver biopsy is no longer recommended for diagnosing large-duct PSC because noninvasive imaging modalities are more accurate[18] and histological findings are generally patchy.[29] Thus, a biopsy might fail to detect the typical changes of PSC.[36] Nevertheless, liver histology is used to rule out overlapping syndromes and small-duct PSC.[18,19,28] It provides an opportunity to detect early biliary changes and gain information on disease stage and inflammation activity.[28] Liver histology is commonly used as a primary or secondary endpoint in clinical trials for drug development.[15,18,19]

The objective of this study was to develop a novel disease-specific histological scoring system, namely, the PSC histoscore, comprising histological features that best predict disease outcome and to compare it to the adapted Nakanuma classification. We also investigated liver histology as a potential predictive marker of disease progression compared to traditionally used clinical markers. The need for optimal surrogate endpoint markers from a clinical point of view and in drug development is obvious in PSC, since new treatment options become available. Thus, we investigated whether liver histology could offer an option for monitoring different treatment options.

Materials and methods

THE PSC COHORT

The cohort in this study (n = 300) comprised patients diagnosed with PSC between 1988–2018 in the Helsinki University Hospital (HUH) area. Our PSC registry data includes ERC and MRCP scores, cytology and histology reports, laboratory parameters, and clinical data. The diagnosis of PSC (the 'large duct' type) was made according to the European

Table 1. Clinical characteristics and laboratory values for the patients based on the compound endpoint

Variable	Compound endpoint reached		
	No *N* = 263	Yes *N* = 34	*P*-value
Female, *n* (%)	153 (58)	25 (74)	0.086
Age at end of follow-up, mean (SD)	44 (14)	47 (12)	0.24
Age at PSC diagnosis, mean (SD)	35 (13)	34 (15)	0.49
IBD present, *n* (%)	189 (71)	29 (85)	0.10
UC	138	23 (79)	
CD	48	5 (17)	
ID	3	1	
FIB4, median (IQR)	0.92 (0.65, 1.30)	1.58 (1.11, 3.23)	<0.001
APRI, median (IQR)	0.36 (0.24, 0.67)	0.79 (0.48, 1.65)	<0.001
B-platelets 10E9, median (IQR)	270 (224, 321)	267 (182, 303)	0.13
P-AST, U/l, median (IQR)	38 (28, 60)	90 (48, 121)	<0.001
P-ALT, U/l, median (IQR)	51 (26, 93)	74 (48, 195)	0.002
P-ALP, U/l, median (IQR)	131 (98, 222)	269(162, 389)	<0.001
P-GT, U/l, median (IQR)	123 (39, 304)	244 (98, 397)	0.014
P-Bil, total, μmol/l, median (IQR)	5.0 (2.0, 9.5)	16.0 (7.0, 30.0)	<0.001
P-Alb, g/l, median (IQR)	39.0 (36.0, 40.8)	35.5 (31.5, 38.6)	<0.001
P-TT, %, median (IQR)	105 (91, 120)	98 (87, 111)	0.059
P-IgG, median (IQR)	12.1 (10.2, 14.4)	14.4 (11.2, 17.2)	0.031
S-IgG4, median (IQR)	0.59 (0.24, 1.10)	0.88 (0.45, 1.47)	0.14
S-CA19_9, median (IQR)	5.00 (3.00, 11.00)	7.00 (4.00, 20.00)	0.075
ERC score at baseline, median (IQR)	4 (2, 7)	8 (6, 10)	<0.001
ERC score at the end of follow-up*, median (IQR)	4 (2, 6)	10 (6, 12)	<0.001

APRI, AST to platelet ratio index; CD, Crohn's disease; ERC, endoscopic retrograde cholangiography; FIB4, fibrosis-4 index; IBD, inflammatory bowel disease; ID, inditermined disease/colitis; P-Alb, plasma albumin; P-ALP, plasma alkaline phosphatase; P-ALT, plasma alanine aminotransferase; P-AST, plasma aspartate aminotransferase; P-Bil, plasma bilirubin; P-GT, plasma gamma-glutamyl transferase; P-IgG, plasma immunoglobulin G; PSC, primary sclerosing cholangitis; P-TT, plasma thromboplastin time; S-IgG4, serum immunoglobulin G4; UC, ulcerative colitis.

*The end of the follow-up was the 30th of November 2020 or the date of the reached endpoint. The parameters were obtained from the PSC registry ±3 months related to the biopsy date.

Association for the Study of the Liver (EASL) Clinical Practice Guidelines.[37] ERC was performed to confirm the diagnosis and for individual risk stratification. The detailed demography is provided in Table 1.

Written informed consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in *a priori* approval by the institution's human research committee. The study protocol was accepted by HUH Ethical Committee IV HUS/1566/2020.

ERC

The indications for ERC were (i) documentation of PSC diagnosis due to elevated or fluctuating S-ALP levels

Table 2. Scoring protocol for histological features in liver tissue. The deposition of orcein granules is replaced with the quantity of K7-positive hepatocytes as an indicator of chronic cholestasis

Features in adapted Nakanuma classification	Score	Included in Nakanuma Stage	Included in Nakanuma Grade	Included in PSC Histoscore Stage	Included in PSC Histoscore Grade
Fibrosis	0–3	X		X	
Bile duct loss	0–3	X		X	
Chronic cholestasis (deposition of periportal K7-positive hepatocytes)	0–3	X		X	
Hepatitis activity	0–3		X		X
Cholangitis activity	0–3		X		X
Additional parameters included in PSC-histoscore					
Portal inflammation	0–3				X
Portal Edema	0–2				X
Ductular reaction	0–2			X	

in conjunction with IBD, (ii) MRCP findings or liver histology suggestive of PSC, or (iii) surveillance of disease progression and/or biliary dysplasia (EASL). ERC was performed using the balloon occlusion technique to ensure adequate and standardized filling of the intra- and extrahepatic bile ducts Images were evaluated using the Helsinki Score (modified Amsterdam score, mERC score).[38] ERC load, namely, the time-weighted area under the curve (ERCtwAUC), was determined by calculating the AUC from the curve, whereby all patients' ERC scores were added and divided by the ERC procedures' total time interval.

CLINICAL PARAMETERS AND ENDPOINTS

The clinical characteristics and laboratory variables of the patients in the study are presented in Table 1.

Due to the limited number of clinical endpoints (n = 34), we used a compound endpoint including LT (n = 21), cholangiocarcinoma (n = 12), and/or liver-related death (n = 1). Patients with an endpoint within a year of the diagnosis were not included. Additionally, if a patient was not eligible for LT or the patient had an overlap-syndrome, they were excluded.

Indications for LT were suspicion or documented high-grade biliary dysplasia in brush cytology (n = 5), symptoms (n = 4), or endstage liver disease (n = 12). The follow-up time for patients without a reached endpoint (n = 266) was completed on the 30th of October 2020.

Cirrhosis was determined clinically, since the biopsy specimens were obtained from most patients at the time of diagnosis. The patients with clinical cirrhosis had either esophageal varices or cirrhosis evident in imaging.

LIVER BIOPSY SPECIMENS

In our center, a core needle liver biopsy specimen is acquired from patients with PSC to verify cases with mild intrahepatic disease and to exclude overlapping diseases

SAMPLE PREPARATION

Herovici- and K7-stained sections were prepared for analysis due to their ability to highlight the quantity of collagen and chronic cholestasis, respectively. The staining protocol is presented in Appendix C. K7-positivity in cholestatic hepatocytes in our slides was mainly membranous and ductular reaction had an intensive cytoplasmic staining pattern.[39]

HISTOPATHOLOGY/SCORING

Two independent liver pathologists (S.B. and N.S.) analyzed the digitalized slides. Only biopsy specimens with over six portal tracts were included. All parameters included in the adapted Nakanuma system and

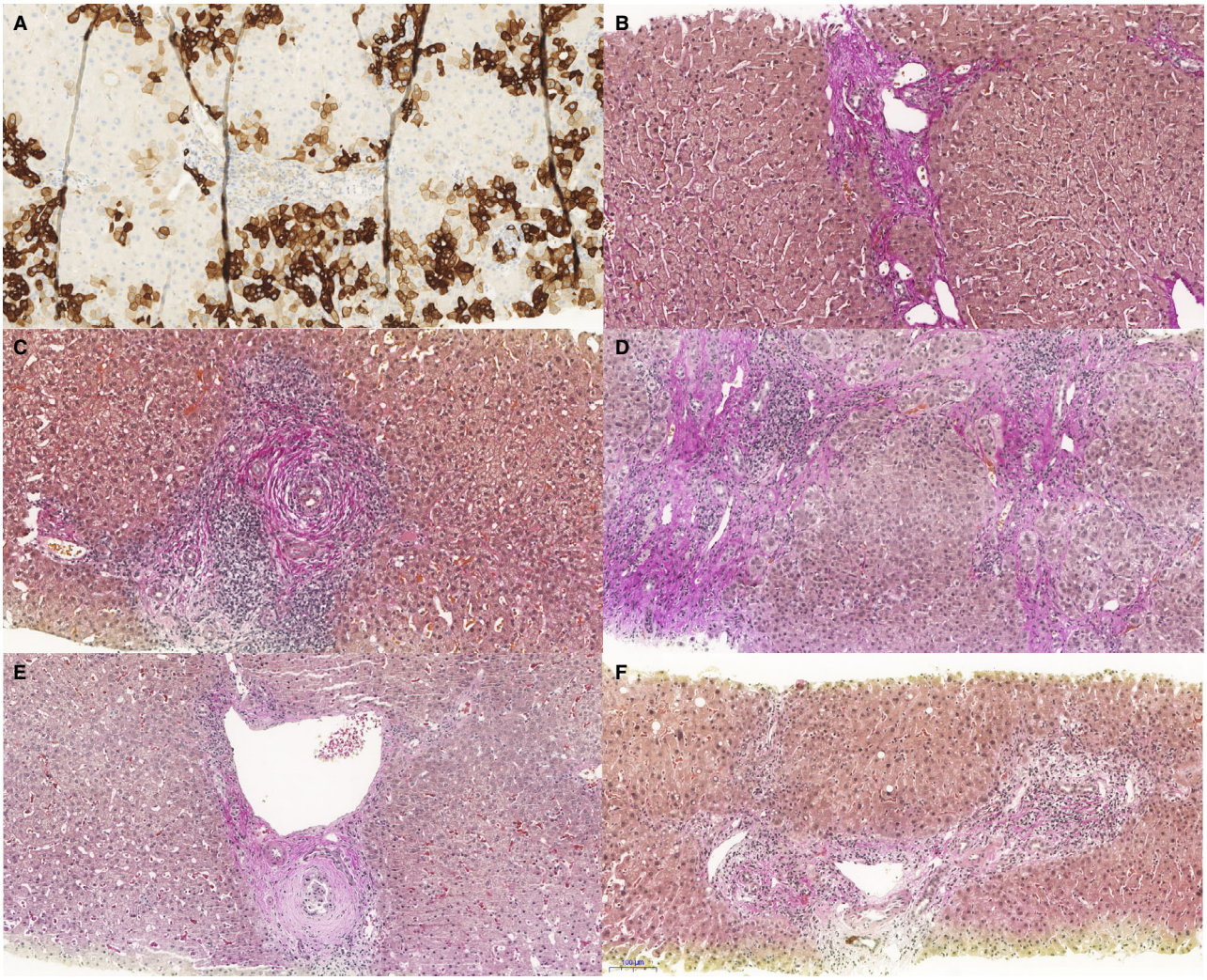


Figure 1. Histological findings for PSC demonstrated by features in the PSC histoscore system. **A**, Chronic cholestasis is illustrated by K7-positive hepatocytes in the periportal area. Bile duct loss is also evident, and there is ductular reaction surrounding the portal area in the middle. The bile duct epithelial cells are highlighted by K7 staining. **B**, Bridging (stage 2) fibrosis is highlighted by Herovici staining between separate portal areas. There are also inflammatory cells in the bile duct epithelium illustrating cholangitis. **C**, The larger bile duct is strongly inflamed, indicating cholangitis. There is also mild pericholangitis and portal inflammation. **D**, Bridging fibrosis advanced to stage 3, forming nodular structures among the liver parenchyma. Portal inflammation and reactive ductular reaction are evident. **E**, Disease-specific histological findings at an advanced stage of PSC. There is concentric onion-like periductal fibrosis surrounding the bile duct, as well as inflammation within the bile duct epithelium, indicating cholangitis. Mild periductal inflammation can be seen around the fibrotic area. **F**, Moderate portal inflammation in addition to mild interface hepatitis activity and edema are evident. There is also moderate (stage 2) bridging fibrosis reaching from one portal area to another.

three additional features (Table 2) were scored. For the histological findings classified in each category, see Figure 1.

In the adapted Nakanuma classification, the amount of chronic cholestasis was evaluated from orcein-stained slides.[32] As presented in Appendix A, we used the quantity of K7-positive hepatocytes to evaluate chronic cholestasis. The total scores for inflammation grade and disease stage were calculated as presented in Table 3.

STATISTICAL ANALYSIS

The data were expressed as means with standard deviations (SD), as medians with interquartile range (IQR), or as counts with percentages. Statistical

Table 3. Protocol for determining the total grade and total stage in the PSC histoscore and adapted Nakanuma system

PSC-histoscore GRADE 0–6	PSC-histoscore STAGE 1–4
0 = 0	0–1 = 1
1–2 = 1	2–4 = 2
3–4 = 2	5–7 = 3
5–6 = 3	8–11 = 4
7–8 = 4	
9–10 = 5	
11 = 6	
Nakanuma GRADE 0–6	Nakanuma STAGE 1–4
0 = 0	0 = 1
1 = 1	1–3 = 2
2 = 2	4–6 = 3
3 = 3	7–9 = 4
4 = 4	
5 = 5	
6 = 6	

comparison between the groups was performed by *t*-test, Mann–Whitney test, Chi-square test, or logistic models, when appropriate. The Kaplan–Meier method was applied to estimate the cumulative survival for the compound endpoint. Cox proportional hazards regression was used to estimate the adjusted hazard ratios (HR) and their 95% confidence intervals (CIs). Age, gender, and duration of PSC years were used as covariates in these models. The proportional-hazards assumption was evaluated by Schoenfeld residuals and log–log plots.

We calculated areas under the curve (AUC) with the trapezoidal method in terms of longitudinal ERC-score. AUC was divided by the total time of study and the results are depicted in time-weighted mean scores (ERCtwAUC). The statistical significance for the hypothesis for linearity across GRADE-score in ERCtwAUC was determined by bootstrap type analysis of covariance (ANCOVA) with an appropriate contrast (orthogonal polynomial). A possible nonlinear relationship between the combined endpoint and the PSC- and Nakanuma-scores were assessed by using 3-knot restricted cubic spline Cox regression models. The length of the distribution of knots were located at the 10th, 50th, and 90th percentiles. For restricted cubic splines, also known as natural splines, knot locations are based on Harrell's recommended percentiles.[40]

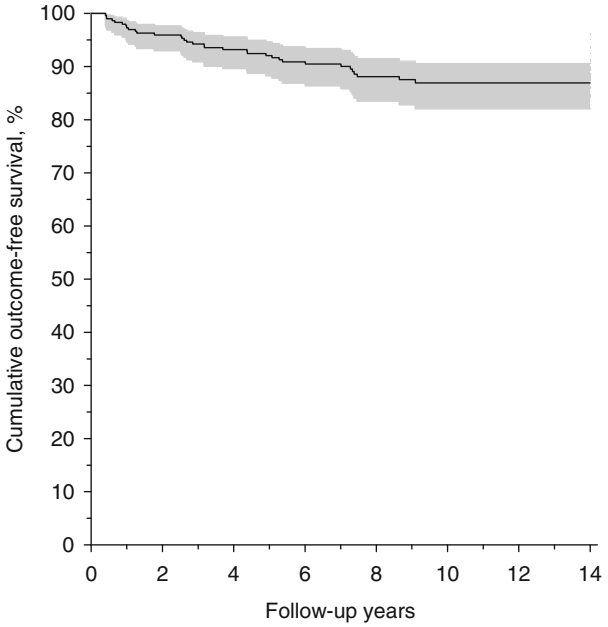


Figure 2. Outcome-free survival for the compound endpoint. The endpoint was reached if the patient underwent transplantation (due to endstage liver disease, symptoms, or biliary dysplasia), was diagnosed with cholangiocarcinoma or died of the disease. The 95% confidence intervals are denoted by the gray area.

The scoring agreement between the two independent pathologists (S.B. and N.S.), was determined by the kappa statistic (κ), with ordinal weights. The level of agreement is considered almost perfect if kappa is >0.80[40] (Appendix B). The normality of variables was evaluated graphically and by using the Shapiro–Wilk W test. Stata 16.0 (StataCorp, College Station, TX, USA) was used for the statistical analyses.

Results

CLINICAL DATA AND CORRELATION WITH DISEASE OUTCOME

Of 300 patients, 12.3% (n = 34) reached at least one of the compound endpoints during the median follow-up time of 9.0 (IQR 5.8, 11.9) years. The outcome-free survival, namely, the survival time without reaching the compound endpoint, of our study cohort is illustrated in Figure 2. The 5-year survival rates were 92.1% (95% CI: 88.3–94.7; 10-year survival rates were 86.9% (95% CI: 82.1–90.6).

All biochemical results, including noninvasive markers of disease progression, of the cohort

Table 4. Histological variables of the study population based on the compound endpoint

PSC-histoscore	Endpoint reached		
	No	Yes	*P* value
No. of portal tracts, median (IQR)	9 (7, 13)	12 (8, 15)	0.066
Grade:			
Hepatitis activity, *n* (%)*			<0.001
0	204 (78)	12 (35)	
1	36 (14)	7 (21)	
2	14 (5)	9 (26)	
3	9 (3)	6 (18)	
Cholangitis activity, *n* (%)			<0.001
0	90 (34)	4 (12)	
1	61 (23)	4 (12)	
2	90 (34)	19 (56)	
3	22 (8)	7 (21)	
Portal inflammation, *n* (%)			<0.001
0	95 (36)	2 (68)	
1	111 (42)	13 (38)	
2	42 (16)	10 (29)	
3	15 (6)	9 (26)	
Portal edema, *n* (%)			<0.001
0	216 (82)	18 (53)	
1	39 (15)	12 (35)	
2	8 (3)	4 (12)	
Stage:			
Fibrosis, *n* (%)			<0.001
0	84 (32)	3 (9)	
1	87 (33)	4 (12)	
2	84 (32)	21 (62)	
3	8 (3)	6 (18)	
Bile duct loss, *n* (%)			<0.001
0	234(89)	21 (62)	
1	20(8)	9 (26)	
2	9(3)	4 (12)	
3	0 (0)	0 (0)	
Ductular reaction, *n* (%)			<0.001
0	118 (45)	3 (9)	
1	118 (45)	16 (47)	
2	27 (10)	15 (44)	
Chronic cholestasis/cytokeratin 7-positive hepatocytes, *n* (%)			<0.001
0	155 (59)	5 (15)	
1	47 (18)	6 (18)	
2	33 (13)	6 (18)	
3	28 (11)	17 (50)	

*Patients with overlap syndromes, such as autoimmune hepatitis, were clinically excluded from the analysis.

evaluated in this study are presented in Table 1. The groups did not significantly differ regarding gender or the presence of IBD. All liver enzymes and surrogate markers of liver fibrosis were elevated in patients who reached any of the endpoints.

HISTOLOGICAL FEATURES AND CLINICAL ENDPOINTS

Altogether, eight histological features were scored (six of them are included in the adapted Nakanuma system). All these features showed a statistically significant correlation with the likelihood of reaching an endpoint ($P < 0.001$) (Table 4). These eight variables were chosen as components of the final PSC histoscore. Cholangitis and hepatitis are features indicating inflammatory activity, i.e. the grade of the disease; both are included in the PSC histoscore and the adapted Nakanuma classification. Additional parameters indicating disease grade by the PSC histoscore were portal inflammation and portal edema.

Concordance between the pathologists varied between 0.83–0.98 in different histological parameters. Please see Appendix B for parameter-specific values.

Parameters of stage in the adapted Nakanuma classification include fibrosis, bile duct loss, and chronic cholestasis. For the PSC histoscore, we also included ductular reaction for the assessment of disease stage.

Table 5. Histological grade and stage in the groups according to the adapted Nakanuma system and PSC histoscore

	Endpoint reached		
	No	Yes	*P*-value
PSC-histoscore			
Total score, median (IQR)	4 (2, 9)	12 (9, 15)	<0.001
Grade, median (IQR)	2 (1, 4)	6 (3, 8)	<0.001
Stage, median (IQR)	2 (1, 4)	6 (5, 7)	<0.001
Nakanuma			
Total score, median (IQR)	1 (0, 3)	5 (4, 6)	<0.001
Grade, median (IQR)	1 (0, 2)	3 (2, 5)	<0.001
Stage, median (IQR)	1 (1, 2)	3 (1, 3)	<0.001

CLINICAL ENDPOINTS AND HISTOLOGICAL STAGE AND GRADE

The disease stage evaluated by either scoring method was significantly higher in patients who reached the compound endpoint ($P < 0.001$). In addition, the probability of reaching the endpoint increased with the stage of disease (Table 5). Furthermore, an increase in total disease grade increased the probability of reaching an endpoint ($P < 0.001$) (Table 5).

The hazard ratios of total histological grade and stage for both scoring methods for the compound endpoint are illustrated in Figure 3A,B. The *P* value for linearity for grade and stage according to both scoring methods was <0.001.

The grade assigned correlates with the increasing ERC load assessed by ERCtwAUC (Figure 4A). When comparing the two scoring systems and their total stage and total grade of disease (Table 5), both correlated with the clinical endpoints. Moreover, the histological results of these two methods correlated highly (Figure 3B).

COMPARISON OF BIOMARKERS AND PSC HISTOSCORE IN PREDICTING DISEASE OUTCOME

Histology was then compared with P-ALP, P-GT, and transaminases, and the noninvasive fibrosis markers FIB4 and APRI, as well as with ERC scores, in predicting the compound endpoint. ERCtwAUC and liver histology were the best predictive markers. The PSC histoscore showed the highest predictive value for the compound endpoint, whereas P-ALP had very low predictive value (Figure 5). The risk for biliary strictures assessed by need of first-time dilatation increased with higher disease grade of inflammation evaluated by either the PSC histoscore or adapted Nakanuma (Figure 4B). The *P* value for linearity for both scoring methods was <0.001 (adjusted age, sex, and duration of PSC in years).

Discussion

The adapted Nakanuma classification, originally developed for evaluation of PBC histology,[32] has value in evaluating PSC and histological fibrosis stage and grade correlated with prognosis.[33] We aimed to build a disease-specific histological scoring system containing the most relevant histological prognostic features for PSC, and the adapted Nakanuma system served as the comparator for this novel scoring method. Additional parameters such as portal inflammation, portal edema, and ductular reaction were analyzed to evaluate their potential value compared to the adapted Nakanuma classification.

The histological disease stage serves as an independent marker of prognosis[33,34] and our results support this conclusion. Overall, the stage of fibrosis in histological liver specimens correlates with reaching endpoints.

We also showed that inflammatory activity and disease grade have a significant value in predicting disease progression and biliary changes. The stage of fibrosis is, in fact, a secondary change to the inflammatory process of the biliary tree. The correlation of liver histology with sequential ERC-examinations, the ERCtwAUC, has not previously been studied. The disease grade correlated with ERC changes, which is, from a clinical and therapeutic point of view, more relevant than the late endpoints, such as CCA, LT, and death, that are commonly used in many prognostic models. Additionally, the total grade based on the PSC histoscore or single histological features indicating inflammatory activity correlated well with the clinical endpoints and served as independent predictors for disease progression. The results were similar regardless of classification: the PSC histoscore or the adapted Nakanuma system.

The total PSC histoscore exhibited the most significant predictive value for the clinical endpoints compared with conventional markers or the adapted Nakanuma score (Figure 5). The adapted Nakanuma classification and PSC histoscore results were almost identical because the latter includes all the

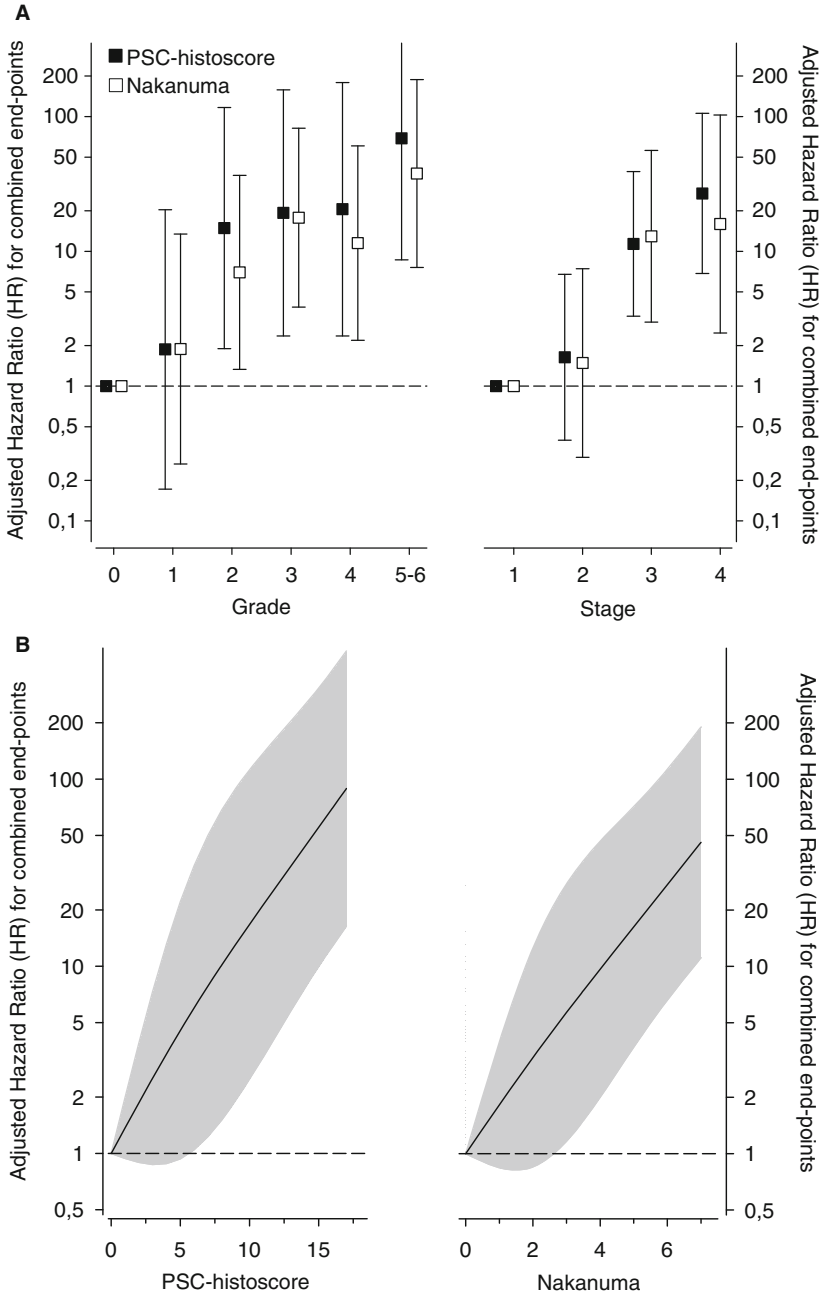
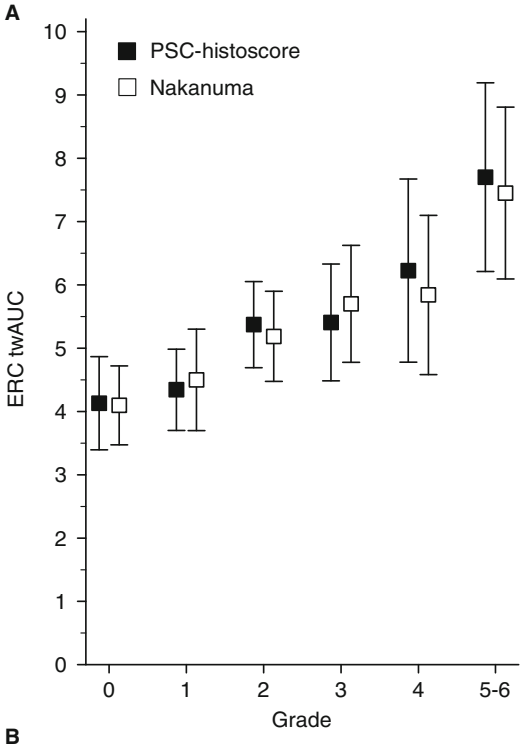


Figure 3. **A**, Adjusted hazard ratios (age, sex, and duration of PSC years) of histological grade and stage for the combined endpoint. The reference value for disease grade was 0; that for stage was 1. Whiskers show 95% confidence intervals. **B**, Adjusted hazard ratios (age, sex, and duration of PSC years) of the continuous histological score on combined endpoints. Hazard ratios were derived from 3-knot restricted cubic spline models, with a PSC histoscore and adapted Nakanuma score of 0 as the reference value. The 95% confidence intervals are denoted by the gray area.

parameters of the former. A high stage and high grade evaluated by the PSC histoscore, however, better predicted disease outcome (compound endpoints). Additionally, disease grade in the PSC histoscore predicted the progression of ERC changes better than the adapted Nakanuma classification.

In general, disease grade can be considered a marker of treatment response in clinical drug trials. Indeed, the European Medicines Agency (EMA) and U.S. Food and Drug Administration (FDA) highly recommend using the histological fibrosis stage and P-ALP values as surrogate markers in drug trials.[14,15]

A



B

Grade (0-6)	Risk according to PSC histoscore grade (pericholangitis excluded)	Risk according to Nakanuma grade
0	1.00 (Reference)	1.00 (Reference)
1	0.93 (0.46 to 1.88)	1.04 (0.50 to 2.17)
2	1.39 (0.70 to 2.74)	1.71 (0.91 to 3.24)
3	1.80 (0.86 to 3.76)	2.29 (1.15 to 4.55)
4	2.13 (0.85 to 5.33)	1.86 (0.77 to 4.49)
5-6	3.07 (1.26 to 7.52)	3.09 (1.36 to 7.02)
p for linearity	0.003	0.002

Figure 4. **A**, Adjusted ERCtwAUC (age, sex and duration of PSC years) according to histological inflammation (grade) score. Whiskers show 95% confidence intervals. **B**, Adjusted hazard ratio to 1st dilatation (age, sex and duration of PSC years).

The adapted Nakanuma classification, and now the PSC histoscore, bring additional value to the applicability of liver biopsy specimens.[41]

The unique patient cohort and data available in the PSC registry enabled this comprehensive retrospective study. The follow-up periods were long, and the cohort consisted of patients at different stages of disease. In other centers, similar unique ERC surveillance protocols and liver biopsies are not included in routine follow-up programs. Our surveillance program is structured and involves all patients with PSC. Thus, liver samples for almost the entire cohort were available, and we were able to correlate histology to the disease progression. Extensive ERC follow-up data, unavailable in most centers, was for the first time available to assess the role of histological grade on disease progression. As ERC is not the standard procedure elsewhere, MRCP was also performed for all the patients to obtain the most accurate results of the pathognomonic changes in the biliary tree. The heterogeneity in our cohort is minimal, and the large size of the cohort allows us to compare the data with international study populations.

A limitation of the present analysis is that this is a single-center study. Further validation in a larger international cohort is imperative. Moreover, the number of endpoints was low despite the long follow-up period The overall survival of PSC patients in our center is excellent, compared to many international cohorts.[4] Additionally, our scoring method did not include concentric periductal fibrosis, which is considered a diagnostic histological

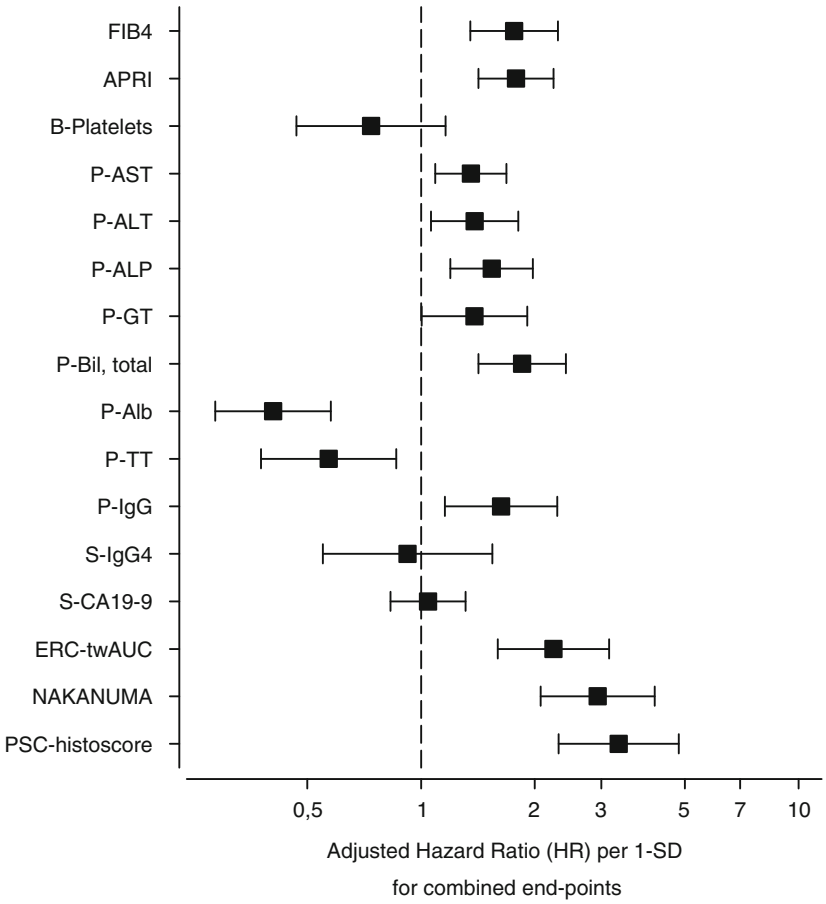


Figure 5. Adjusted (age, sex, and duration of PSC years) univariate hazard ratios of histology, laboratory parameters, and ERCtwAUC for reaching the compound endpoint. Hazard ratios are expressed as a 1-SD increase. Whiskers show 95% confidence intervals.

finding in PSC. However, our aim was not to create a diagnostic tool for PSC, but to build a method to assess disease progression.

According to our results, histological grade and stage and our novel histological scoring method, the PSC histoscore, can be used as surrogate endpoint markers for evaluating both progression and overall survival in PSC. In particular, the disease grade evaluated by the adapted Nakanuma system or the PSC histoscore can serve as a potential assessment tool for treatment response. We promote the use of liver histology as part of the routine diagnostic work-up and recommend considering it as part of the surveillance protocol for PSC patients.

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Author Contributions

Supervision, Resources, and Conceptualization: Färkkilä, M., and Arola, J. Writing, Methodology, and Investigation: Sjöblom, N. and Boyd, S. Formal analysis and Data curation: Kautiainen, H.

Conflict of Interest

The authors certify that they have no affiliations with nor involvement in any organization or entity with any financial interest in the subject matter or materials discussed in this article.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix A. Scoring protocol for liver biopsy specimens.

Appendix B. Concordance values of scoring results.

Appendix C. Sample preparation, scanning and analysis of digitalized liver biopsy specimens.