Collagen proportionate area correlates with histological stage and predicts clinical events in primary sclerosing cholangitis

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Word count: 3,414

Tables: 5

Figures: 3

List of abbreviations:

PSC = primary sclerosing cholangitis

LT = liver transplantation

CPA = collagen proportionate area

DIA = computer-assisted digital image analysis

NAFLD = non-alcoholic fatty liver disease

MRS = Mayo risk score

AOS = Amsterdam-Oxford score

IQR = interquartile range

HR = Hazard ratio

CI = confidence interval

ALP = alkaline phosphatase

PLTs = platelet count

Disclosures:

Conflict of interest: All the authors have no conflict of interest with respect to this work.

Financial support: MdK was supported by a research grant from Takeda.

Abstract

Background & Aims: Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease in need of accurate biomarkers for stratification and as surrogates for clinical endpoints in trials. Quantitative liver fibrosis assessment by collagen proportionate area (CPA) measurement has been demonstrated to correlate with clinical outcomes in chronic hepatitis C, alcohol-related and non-alcoholic fatty liver disease. We aimed to investigate the ability of CPA to quantify liver fibrosis and predict clinical events in PSC.

Methods: Biopsies from 101 PSC patients from two European centers were retrospectively assessed by two expert pathologists in tandem, using grading (Ishak and Nakanuma) and staging (Ishak, Nakanuma, Ludwig) systems recently validated to predict clinical events in PSC. CPA was determined by image analysis of picro-Sirius red-stained sections following a standard protocol. We assessed the correlations between CPA, staging and grading and their associations with three outcomes: (1) time to PSC-related death, liver transplant or primary liver cancer; (2) liver transplant-free survival, (3) occurrence of cirrhosis-related symptoms.

Results: CPA correlated strongly with histological stage determined by each scoring system ($P<0.001$) and was significantly associated with the three endpoints. Median time to endpoint-1, endpoint-2, and endpoint-3 were shorter in patients with higher CPA, on Kaplan-Meier analyses ($P=0.011$, $P=0.034$ and $P=0.001$, respectively).

Conclusion: Quantitative fibrosis assessment by CPA has utility in PSC. It correlates with established histological staging systems and predicts clinical events. CPA may be a useful tool for staging fibrosis and for risk stratification in PSC and should be evaluated further within prospective clinical trials.

Keywords: Primary sclerosing cholangitis; Digital image analysis; Collagen proportionate area; Quantitative fibrosis assessment; Risk-stratification.

Lay Summary

Collagen proportionate area (CPA), a quantitative method to measure the proportion of liver fibrosis on a liver biopsy sample using a digital image analysis, correlates with the quantitative histological scoring systems and the clinical scores validated in primary sclerosing cholangitis (PSC), and predicts clinical outcomes. Adding CPA to traditional histology can offer additional useful information for risk-stratification and prediction of clinical outcomes in patients with PSC. CPA may be particularly useful for the assessment of treatment response and fibrosis regression in clinical trials in PSC, also allowing objective comparison across trials.

INTRODUCTION

Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease characterized by immune mediated damage to the intra- and extrahepatic bile ducts which results in progressive hepatic fibrosis with a biliary pattern and frequently progresses to cirrhosis[1]. Currently, the only effective treatment for advanced disease is liver transplantation (LT)[1,2]. Liver histology is no longer considered essential to establish a diagnosis of large duct PSC[3], but remains crucial when small duct PSC or features of autoimmune hepatitis are suspected, and within clinical trials[3,4]. The distribution of the disease in the hepatic parenchyma is typically patchy, which may result in sampling variability on histological assessment.[5]

The course of the disease is variable and unpredictable, and there is much interest in validating non-invasive predictors of prognosis and surrogate endpoints for clinical outcomes[6,7]. Given the prolonged natural history of PSC, it is difficult to achieve studies adequately powered to obtain robust clinical endpoints for the evaluation of treatment efficacy in clinical trials. Hence, the expanding clinical trial activity in PSC has prompted the development and validation of surrogates of clinical endpoints[8]. Along these lines, the role of liver histology in PSC has been revisited and is now considered essential in the regulatory approval process for new therapeutic strategies[8,9]. This is based on recent data showing that three commonly employed histological scoring systems, namely those described by Ludwig in 1978[10], Ishak in 1995 (mainly developed for chronic viral hepatitis)[11] and Nakanuma in 2010 (originally created to evaluate disease grade and stage in primary biliary cholangitis)[12] are reproducible predictors of disease progression and major clinical outcomes in PSC[13,14].

The measurement of Collagen Proportionate Area (CPA) is a quantitative method to

measure liver fibrosis using computer-assisted digital image analysis (DIA) of the proportion of collagen in liver tissue stained by the picro-Sirius red technique[15,16]. CPA measurement has been demonstrated to correlate with clinical outcomes in pre- and post-transplant chronic hepatitis C and, more recently, in non-alcoholic fatty liver disease (NAFLD) and alcohol-related liver disease[15,17-22]. CPA has been demonstrated to accurately measure fibrosis and is a strong independent predictor of clinical decompensation. Furthermore, allowing a continuous stratification of fibrosis and associated risk, CPA has demonstrated the possibility of further sub-classifying cirrhosis and its associated prognosis where there is a ceiling effect with histological stage evaluated using semi-quantitative methods.[21,23] We aimed to investigate the ability of CPA to quantify liver fibrosis and predict clinical events in a cohort of PSC patients from two European tertiary centers, in comparison with the above-mentioned histological scoring systems.

METHODS

Patients

The study was conducted at the Royal Free Hospital, London, UK, and Amsterdam UMC, University of Amsterdam, The Netherlands. We included 51 PSC patients from a previously described Dutch cohort who had a liver biopsy between 2008 and 2011[14] and 50 PSC patients who underwent histological assessment at the Royal Free between 1991 and 2015. Diagnosis of PSC, small duct PSC and PSC with features of autoimmune hepatitis were made according to the currently accepted recommendations[3,7]. Biochemical values closest to the time of the liver biopsy (±1 month) were retrieved from hospital databases and Mayo risk score (MRS)[24] and the Amsterdam-Oxford score (AOS)[25] were calculated. Since assays varied between

hospitals and over time, laboratory results are expressed in times x upper or lower limit of normal. Clinical information and follow-up data were retrospectively obtained from patients' clinical records.

The study protocol was in accordance with the Declaration of Helsinki and its latest amendments. Patient data were treated anonymously and participation by individual centers was appropriately approved at a local level [central Committee for Research Ethics in Utrecht and all 44 local ethics committees of the participating hospitals in the Netherlands (Trialregister.nl number NTR2813), for the Dutch cohort; 07/Q0501/50 granted by the National Research Ethics Service, for the UK cohort].

Liver biopsy samples

The original haematoxylin and eosin, connective tissue, and orcein-stained liver sections were collected. If staining was not well preserved, additional histochemical staining was carried out. Liver biopsy sections containing fewer than six evaluable portal tracts were excluded. Specimens were retrospectively assessed in tandem, using a multi-headed microscope with the intention to reach consensus, by two expert liver pathologists (J.V. and S.G.H) blinded to clinical and laboratory information, who scored histological grade and stage according to Ishak, Nakanuma and Ludwig systems (see **supplementary material**)[10-12]. Due to the relatively small number of patients for each grade and stage, subgroups were generated for grade and stage of each system, maintaining the severity order, as previously described[14]. CPA was determined by image analysis of picro-Sirius red stained sections according to our protocol[15]**.** The equipment used was a digital camera (Canon Powershot A640 attached to a close-up copy-stand with backlighting) connected to a compatible personal computer. After whole section digital image capture, CPA was measured

with a custom-made script for Zeiss Axiovision v4.8.2.0 image analysis software. The CPA measurement included editing steps to eliminate image artefacts and structural collagen such as large portal tracts, liver capsule and blood vessel walls, which do not represent disease-related liver fibrosis. An operator dependent segmentation was then used to detect areas of stained collagen and the whole biopsy area, each computed as an area in pixels. CPA was calculated as a proportion of the collagenous area on the whole parenchyma and expressed as a percentage.

Endpoints

As in the recent histological studies in PSC, three main endpoints were chosen for the time-to-event analyses[13,14]. Endpoint-1 was defined as PSC-related death, LT or presentation with primary liver cancer (cholangiocarcinoma or hepatocellular carcinoma). Endpoint-2, which focuses on progression of liver disease only, was time to LT or cirrhosis-related death, excluding cholangiocarcinoma-related deaths. Endpoint-3 was the occurrence of cirrhosis-related symptoms, defined as the first occurrence of any of the following: endoscopic evidence of gastro-esophageal varices or variceal bleeding, clinically or radiologically detectable ascites, splenomegaly (spleen length >120mm) or hepatic encephalopathy.

Statistical analysis

Dichotomous variables were expressed as frequencies and percentage and continuous variables as mean ± standard deviation or median and interquartile range (IQR), as appropriate (Kolmogorov-Smirnov test). Correlations were assessed by Spearman's ranking test. The prognostic value of CPA, histology scores, clinical and biochemical variables was assessed using univariable Cox proportional hazards model. Potential

multicollinearity between variables was checked by Spearman's rank correlation coefficient (Rho). The predictive value of histology scores and CPA for the three endpoints was studied by separate multivariable analyses. Hazard ratio (HR) and 95% confidence interval (CI) were calculated. Kaplan-Meier plots with patients subdivided according to CPA tertiles and to histological stages were generated for each endpoint; the associations of CPA and the histologic scoring systems with the different clinical endpoints were estimated using Wilcoxon log-rank test. Patients were censored at the time of the development of an endpoint or last follow-up. Statistical analysis was performed using SPSS (v.20.0; IMB® SPSS®, Inc., Chicago, IL). Significance testing was 2-sided and set to <0.05.

RESULTS

Patients and Distribution of grade and stage

After the exclusion of the biopsies with insufficient material or patients with incomplete data, 101 patients [median follow-up post-biopsy 91.5 (0-367) months] were included in the analysis (50 from the UK cohort and 51 from The Netherland cohort). Characteristics of the study population are summarized in **Table 1**.

Liver specimens had a median of 15 (IQR 11) portal tracts. Consensus about grading and staging according to the three histologic scoring systems was reached in 100% of cases by the two in tandem scoring pathologists.

According to the Ishak system, the median grade of the interface hepatitis component was 1 (range 0-2). Confluent necrosis was present only in two cases, resulting in a median score of 0 (0-1). Lobular inflammation and portal inflammation had a median grade of 1 (0-4) and 1 (0-3), respectively. Both cholangitis activity [median grade 0 (0-3)] and hepatitis activity [median grade 0 (0-2)] components of the Nakanuma

grading system were absent in most of the biopsies. Median stage scored using the Ishak system was 2 (range 0-6). The Nakanuma stage, derived from the components fibrosis, bile duct loss and deposition of orcein positive granules, had a median value of 3 (1-4). Finally, the median stage according to Ludwig was 2 (0-4). The distribution of Nakanuma and Ishak grade and the fibrosis stage according to Ishak, Nakanuma and Ludwig systems, are shown in **Supplementary Figure 1**.

The median CPA was 8.4% (0.8-63.9, IQR 8.8).

Correlation between CPA and histological grading and staging

The Nakanuma, Ishak and Ludwig staging systems were highly correlated (Spearman's correlation coefficient for Nakanuma and Ishak 0.62, Nakanuma and Ludwig 0.67, Ishak and Ludwig 0.88, P<0.001 in all cases). CPA strongly correlated with histological stage determined by Ishak and Ludwig systems (P<0.001); the correlation with the Nakanuma stage was weaker (there was a good correlation with component fibrosis, a slight correlation with deposition of orcein positive granules, no correlation with the bile duct loss component; **Table 2**). Bile duct loss was present in 68/101 (67%) patients, with only 10 scoring ≥2. Mean CPA values for each histological stage according to Ishak, Nakanuma and Ludwig score are depicted in **Figure 1**. The correlations between CPA and histology grade according to Ishak and Nakanuma are reported in **Supplementary Table 1**.

MRS at the time of the biopsy correlated with CPA (Rho 0.34, P=0.019) and Nakanuma stage (Rho 0.45, P=0.012). A weaker but still significant correlation with MRS was found for Ishak and Ludwig stages (Rho 0.29, P=0.041 and 0.27, P=0.024, respectively). AOS at time of the biopsy correlated well with CPA (Rho 0.44, P<0.001) and all the histology stages, with similar Rho coefficients (Rho 0.47,

P<0.001; 0.40, P<0.002; 0.46, P<0.001 for Nakanuma, Ishak and Ludwig stage, respectively).

Association of CPA and histology stages with the endpoints

The median follow-up from PSC diagnosis until reaching an endpoint or date of last follow-up was 58 months (0-367, IQR 120). Twenty-nine (28.7%) patients met the composite endpoint-1 and 22 (21.7%) met the endpoint-2, at a median time of 73 months (0-367, IQR 131) from the time of biopsy. Forty-three (42.6%) patients developed enpoint-3 (**Table 1**) at a median time of 58 months (0-367, IQR 116). Kaplan-Meier estimates showed a significant association of staging measured by all three histological scoring systems with endpoint-1 (Nakanuma P<0.001, Ishak P<0.001 ~~P=0.015~~, Ludwig P=0.002), endpoint-2 (P<0.001 for all) and with endpoint-3 (P<0.001 for Ishak and Ludwig stages, P=0.001 for Nakanuma stage). ~~Nakanuma and Ludwig (P<0.001) staging, but not Ishak (P=0.063), were significantly predictive of endpoint-2~~ (**Supplementary Figures 2-4**). Median time to endpoint-1, endpoint-2 and endpoint-3 were significantly shorter in the higher CPA tertile compared to the middle and lower tertiles (P=0.011, P=0.034 and P=0.001, respectively) (**Figure 2A-C**).

The potential associations of clinical, biochemical, histological variables and CPA with the three endpoints were identified by univariable Cox proportional hazard analyses (**Supplementary Table 2**). The predictive value of each staging system and CPA for the three endpoints was studied by separate multivariable analyses, as multicollinearity precluded their inclusion in the same multivariable model. Since MRS and AOS significantly correlated with bilirubin and albumin and were not available in all the patients, they were not included in the multivariable analyses.

ENDPOINT-1: PSC-related death, liver transplantation or primary liver cancer

CPA, Nakanuma stage and all its three sub-components, Ishak stage, Ludwig stage, Nakanuma grade hepatitis activity component, Ishak grade interface hepatitis component, alkaline phosphatase (ALP), bilirubin, albumin, platelet count (PLTs), MRS and AOS were predictors of endpoint-1 on univariable Cox regression analysis (**Supplementary Table 2**). Separate multivariable analyses showed that Nakanuma (HR 9.14 [95%CI 3.08-27.09], P<0.001), Ishak (HR1.60 ~~1.67~~ [95%CI 1.17-2.19 ~~1.03-2.70~~], P=0.003 ~~P=0.037~~) and Ludwig stage (HR 2.45 [95%CI 1.31-4.55], P=0.005), bilirubin and ALP, were all independently associated with endpoint-1 (**Table 3**). The Cox multivariable regression model including CPA revealed that also CPA was an independent predictor of endpoint-1. The HR per 5% increase of the CPA value was 1.21 (95%CI 1.05-1.39), P=0.009. Bilirubin (HR 1.13 [95%CI 1.04-1.23], P=0.003) and ALP (HR 1.25 [95%CI 1.03-1.51], P=0.023), also remained significantly associated with the outcome (**Table 3**). A higher HR was obtained for CPA when the Cox regression model was performed substituting CPA with its tertiles (HR 2.59 [95%CI 1.31-5.11], P=0.006) (**Table 3**). Results did not change substantially when the models were adjusted for sex, age, age at diagnosis and concomitant inflammatory bowel disease.

ENDPOINT-2: Transplant-free survival

CPA, Nakanuma stage and its two sub-components fibrosis and orcein deposition, Ishak stage, Ludwig stage, ALP, bilirubin, albumin, PLTs, MRS and AOS were predictors of endpoint-2 on univariable Cox regression analysis (**Supplementary Table 2**). On multivariable analyses, Nakanuma (HR 9.24 [95%CI 2.50-34.32],

P=0.001), Ishak (HR 1.49 [95%CI 1.02-2.19], P=0.040) and Ludwig stage (HR 2.97 [95%CI 1.40-6.29], P=0.005), bilirubin and ALP~~, but not Ishak stage,~~ remained independently associated with transplant-free survival (**Table 4**).

CPA (HR 1.28 [95%CI 1.09-1.50], P=0.002, per 5% CPA increment), bilirubin (HR 1.19 [95%CI 1.09-1.31], P<0.001) and ALP (HR 1.33 [95%CI 1.07-1.65], P=0.011) remained all significantly associated with endpoint-2 in the multivariable model including CPA (**Table 4**). CPA demonstrated a higher HR when the Cox regression model was performed substituting the continuous variable with its tertiles (HR 2.45 [95%CI 1.11-5.42], P=0.026) (**Table 4**). Results did not change significantly whether or not platelet count (PLTs) was included in the multivariable models.

ENDPOINT-3: Occurrence of cirrhosis-related symptoms

On univariable Cox regression, CPA, Nakanuma grade hepatitis activity component, Nakanuma stage and its components fibrosis and orcein deposition, Ishak stage, Ludwig stage, bilirubin, MRS, AOS and PLTs were found to be significantly associated with the occurrence of cirrhosis-related symptoms (**Supplementary Table 2**). Even if not significant on univariable, ALP was included in the multivariable models. On multivariable analyses, Ishak (HR 1.90 ~~2.12~~ [95%CI 1.41-2.58 ~~1.36-3.32~~], P<0.001 ~~P=0.001~~) and Ludwig stage (HR 2.38 [95%CI 1.29-4.38], P=0.005) were found to be independent predictors of cirrhosis-related symptoms. In the model including Nakanuma stage, the only factor independently associated with endpoint-3 was PLTs (HR 0.56 [95%CI 0.33-0.93], P=0.026) (**Table 5**). In the Cox multivariable model including CPA, CPA (HR 1.19 [95%CI 1.03-1.37], P=0.019, per 5% CPA increase) and PLTs (HR 0.58 [95%CI 0.34-0.99], P=0.045) were independently associated with endpoint-3 (**Table 5**). CPA showed a higher HR when CPA tertiles

were included in the Cox regression analysis (HR 2.04 [95%CI 1.15-3.62], P=0.015) (**Table 5**).

Median CPA values were significantly higher in patients who reached an endpoint at 5 and 10 years from the liver biopsy, compared to patients who did not (**Figure 3**).

DISCUSSION

This is the first study assessing the utility of quantitative assessment of liver fibrosis by CPA in PSC, in comparison with traditional semi-quantitative scoring methods. In this retrospective evaluation of PSC patients from two European tertiary centres, we demonstrated that CPA has a very good correlation with histological staging using Nakanuma, Ishak and Ludwig systems, which have prognostic value in PSC[13,14]. Furthermore, we showed that CPA itself predicts outcomes in PSC and can be a useful tool to identify patients at higher risk of experiencing clinical events.

Liver biopsy is not routinely performed in PSC. However, the results of various studies indicate that histology can adequately assess progression and regression of grade and stage[26,27] and the histological degree of parenchymal damage and fibrosis has important prognostic value in the disease[14,28]. Accordingly, histological changes have been included as an endpoint in several clinical trials in PSC[14,29-31]. Histological scoring systems provide a semi-quantitative score reflecting the extension and distribution of liver fibrosis based on the pathologist's analysis according to standard descriptors. Although these scores show sufficient reproducibility and can be used to predict clinical outcomes[13,14], they lack absolute objectivity and the possibility of stratifying quantitatively the fibrotic evolution occurring in liver cirrhosis, a condition considered an evolutive end-stage. Therefore, systems able to overcome this ceiling

effect are urgently needed.

Computer-assisted DIA of histological sections is based on the picro-Sirius red staining histochemical technique. Picro-Sirius red staining identifies tissue fibrillar collagens and allows to assess the topographic distribution of fibrosis. DIA uses segmentation of digital images to measure the area of collagen and of tissue, producing a "fibrosis ratio" or CPA.[16,32,33] It has recently been shown that CPA using picro-Sirius red stained biopsies is more accurate than the trichrome staining for quantifying hepatic collagen[34], and has been shown to be a robust and reproducible method for quantifying liver fibrosis[18,21,35], with the clear advantage of drastically reducing inter-observer variability. The assessment of CPA is characterized by a relatively short learning curve and can be undertaken by a skilled pathology technician with a solid understanding of liver histology. Hence, it is scalable and has the potential for widespread use. Unlike the semi-quantitative scoring systems, which express fibrosis in stage categories with no quantitative relation with each other[21], CPA offers a continuous numerical scale that allows a more accurate measurement of fibrosis, thus providing additional useful information e.g. for the evaluation of fibrosis regression and sub-classification of cirrhosis[23]. For all these reasons, quantitative assessment of liver fibrosis by CPA may potentially function as a valuable tool for risk stratification and evaluation of treatment response in patients with PSC, in particular in the context of clinical trials.

In the present study, we confirmed that liver fibrosis stage assessed by the Nakanuma, Ishak and Ludwig system correlated significantly with all the relevant clinical outcomes. CPA correlated with histology stage scored by Ishak and Ludwig systems. As expected, CPA correlated less well with Nakanuma stage, as this incorporates elements such as bile duct loss and deposition of orcein positive granules, which are

not indices of fibrosis but, as sensitive markers of chronic cholestasis, are strongly associated with long-term clinical outcomes in PSC[13,36].

It has been demonstrated that, in hepatitis C cirrhosis, CPA allows identification of patients at higher likelihood of having clinically significant portal hypertension and higher risk of decompensation[20]. When evaluated in patients with recurrent HCV cirrhosis after liver transplantation, CPA correlated better than Ishak score with portal hypertension measured by hepatic venous pressure gradient, and was a strong predictor of cirrhosis decompensation[15,18]. The utility of CPA for quantifying fibrosis and predicting clinical outcomes has been recently shown also in alcohol-related liver disease and NAFLD[21,22]. Our results demonstrate that, despite the possibility of histological sample variability ~~intrinsic~~ related to the patchy distribution of fibrosis in PSC, CPA can be ~~used as~~ a useful tool to stratify patients and predict outcomes. It could be argued that the possible increased variability of histological staging and CPA in PSC, could potentially lead to a reduction of the sensitivity/specificity assessment to real change in fibrosis as compared, for instance, to serum based tests. It has to be underlined that, despite the intrinsic variability of fibrosis in PSC, the prognostic value of both histology and elastography has been validated in the disease[13,14,37]. Furthermore, in a study assessing the distribution of fibrosis between the right and the left lobe in explants with cirrhosis of various aetiologies by means of CPA, a difference between the two lobes was detected in all the underlying aetiologies. Importantly, this difference was not statistically significant in any of the aetiologies, including PSC[35]. Several clinical and serological markers have been evaluated in PSC. FibroTest, APRI and FIB-4 have shown relatively low sensitivities in diagnosing advanced fibrosis and cirrhosis, and were unable to identify those patients at increased risk of developing cirrhosis or liver related events[38,39]. On the other hand, in

the recent years, ELF score has gained a role as a good predictor of outcomes in PSC[40,41]. However, most of the studies on non-invasive markers in PSC are cross-sectional, and data with biomarkers measured in serial serum samples for the prospective assessment of liver fibrosis stage are limited. Upon evaluation of the association of the inter- and intra-individual variation over time of ALP and ELF score with fibrosis progression and outcomes, an analysis of the delta values was not performed[38]. Therefore, despite the ELF score at the single time points was significantly associated with the endpoints, the value of its variation over time remains unclear.

The seemingly weaker performance of CPA (lower HRs) compared to the histology scoring systems is most likely secondary to the smaller percentage changes in collagen measured by CPA, against the ordinal scales used by the traditional semi-quantitative scores to define stages, which estimate larger transitions. This hypothesis is supported by the fact, that when we performed Cox regression analyses with CPA as a categorised variable (tertiles), the HRs for each outcome were similar or even better to those of the Ishak and Ludwig scores. The Nakanuma score showed a higher HR for endpoint-1 and endpoint-2, although the 95%CI interval was much wider and, therefore, less precise. Notably, for each percentage point increase in the CPA there was a 4-5% increased hazard of reaching an endpoint.

We did not define specific CPA cut-offs for the various endpoints because, given the relatively small cohort, these could be inaccurate. However, from our results it appears clear that a CPA higher than 11% (higher tertile) is associated with faster disease progression and unfavorable prognosis in PSC.

Among the semi-quantitative scoring systems, both Ishak and Ludwig scores were ~~and was the only one~~ independently correlated with all the endpoints, although the

HRs were smaller for the Ishak stage ~~did not predict LT-free survival~~. Nakanuma stage was the strongest predictor of endpoint-1 and endpoint-2, in accordance with previous results[13,14], but was not an independent predictor of endpoint-3, likely because of its composite nature including factors not related to fibrosis stage. Importantly, CPA remained significantly related to all three endpoints. It is also important to underline the fact that the Nakanuma system is currently available only in a limited number of hepatology centres, as it requires specialized expertise in liver pathology, and is not widely used routinely.

Notably, all three histology scoring systems and CPA strongly correlated with the MRS, one of the most widely accepted prognostic scores in PSC[24], as well as with the newly proposed AOS[25], as a further confirmation that the degree of liver fibrosis can adequately predict PSC progression.

CPA and histological semi-quantitative scores share the same limitations in routine clinical practice in PSC. Indeed, the invasiveness and the notorious sampling variability in cholestatic disease have limited the use of liver biopsy in PSC, favoring the development of surrogate non-invasive markers[37,40,42]. However, in the absence of validated non-invasive surrogates, histological assessment of disease severity and evaluation of histological progression of fibrosis is the mainstay of phase III clinical trials in PSC[8] and allows the exploration of other potential surrogates in comparison[43]. In this context, the quantitative scale measure offered by CPA provides a more objective tool, offering the possibility of accurate comparisons across trials. As such, CPA has already been included in the protocol of ongoing clinical trials in PSC which include histological endpoints (NCT03872921).

Our study is limited by its retrospective design and the subsequent possible incomplete data collection. The occurrence of cirrhosis-related symptoms had to rely

on medical records, therefore leading to a possible underestimation of endpoint-3. Furthermore, a selection bias of patients cannot be excluded, since liver biopsy is not performed systematically in PSC. For the same reason, in a number of cases liver biopsy was performed at different time-points than that of diagnosis and for different indications.

In conclusion, in this study we demonstrate that quantitative liver fibrosis assessment by CPA correlates with established histological staging systems, as well as with validated prognostic clinical scores in PSC. CPA predicts clinical events and may be used to stage fibrosis and for risk stratification in these patients, as well as a surrogate endpoint in clinical trials.

Even though in our study CPA did not generally show a prognostic superiority compared with the semi-quantitative scores of liver fibrosis in PSC, we wish to highlight its utility and advantages, which include the ability to detect small changes in liver fibrosis (progression or regression) and therefore perform a more granular assessment, as well as its objectiveness and reproducibility, which do not necessarily require advanced expertise in liver pathology.

Nevertheless, consolidation of the role of CPA as both diagnostic and prognostic tool in PSC should be evaluated further within larger and preferably prospective studies.

REFERENCES

1. Hirschfield GM, Karlsen TH, Lindor KD, Adams DH. Primary sclerosing cholangitis. *Lancet* 2013; **382**(9904): 1587-99.
2. Saffioti F, Gurusamy KS, Hawkins N, et al. Pharmacological interventions for primary sclerosing cholangitis: an attempted network meta-analysis. *Cochrane Database Syst Rev* 2017; **3**: CD011343.

3. European Association for the Study of the L. EASL Clinical Practice Guidelines: management of cholestatic liver diseases. *J Hepatol* 2009; **51**(2): 237-67.
4. Ponsioen CY, Chapman RW, Chazouilleres O, et al. Surrogate endpoints for clinical trials in primary sclerosing cholangitis: Review and results from an International PSC Study Group consensus process. *Hepatology* 2016; **63**(4): 1357-67.
5. Portmann B, Zen Y. Inflammatory disease of the bile ducts-cholangiopathies: liver biopsy challenge and clinicopathological correlation. *Histopathology* 2012; **60**(2): 236-48.
6. Thorburn D. Prognostic scores and non-invasive markers in primary sclerosing cholangitis: good for patients or for papers? *Lancet Gastroenterol Hepatol* 2017; **2**(11): 774-6.
7. Karlsen TH, Folseraas T, Thorburn D, Vesterhus M. Primary sclerosing cholangitis - a comprehensive review. *J Hepatol* 2017; **67**(6): 1298-323.
8. Ponsioen CY, Lindor KD, Mehta R, Dimick-Santos L. Design and Endpoints for Clinical Trials in Primary Sclerosing Cholangitis. *Hepatology* 2018.
9. Gessel L, Alcorn J. Variants of varices: is it all "downhill" from here? *Dig Dis Sci* 2015; **60**(2): 316-9.
10. Ludwig J, Dickson ER, McDonald GS. Staging of chronic nonsuppurative destructive cholangitis (syndrome of primary biliary cirrhosis). *Virchows Arch A Pathol Anat Histol* 1978; **379**(2): 103-12.
11. Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995; **22**(6): 696-9.
12. Nakanuma Y, Zen Y, Harada K, et al. Application of a new histological staging and grading system for primary biliary cirrhosis to liver biopsy specimens: Interobserver agreement. *Pathol Int* 2010; **60**(3): 167-74.
13. de Vries EM, de Krijger M, Farkkila M, et al. Validation of the prognostic value of histologic scoring systems in primary sclerosing cholangitis: An international cohort study. *Hepatology* 2017; **65**(3): 907-19.
14. de Vries EM, Verheij J, Hubscher SG, et al. Applicability and prognostic value of histologic scoring systems in primary sclerosing cholangitis. *J Hepatol* 2015; **63**(5): 1212-9.
15. Calvaruso V, Burroughs AK, Standish R, et al. Computer-assisted image analysis of liver collagen: relationship to Ishak scoring and hepatic venous pressure gradient. *Hepatology* 2009; **49**(4): 1236-44.
16. Standish RA, Cholongitas E, Dhillon A, Burroughs AK, Dhillon AP. An appraisal of the histopathological assessment of liver fibrosis. *Gut* 2006; **55**(4): 569-78.
17. Manousou P, Dhillon AP, Isgro G, et al. Digital image analysis of liver collagen predicts clinical outcome of recurrent hepatitis C virus 1 year after liver transplantation. *Liver Transpl* 2011; **17**(2): 178-88.
18. Calvaruso V, Dhillon AP, Tsochatzis E, et al. Liver collagen proportionate area predicts decompensation in patients with recurrent hepatitis C virus cirrhosis after liver transplantation. *J Gastroenterol Hepatol* 2012; **27**(7): 1227-32.
19. Manousou P, Burroughs AK, Tsochatzis E, et al. Digital image analysis of collagen assessment of progression of fibrosis in recurrent HCV after liver transplantation. *J Hepatol* 2013; **58**(5): 962-8.

20. Calvaruso V, Di Marco V, Bavetta MG, et al. Quantification of fibrosis by collagen proportionate area predicts hepatic decompensation in hepatitis C cirrhosis. *Aliment Pharmacol Ther* 2015; **41**(5): 477-86.
21. Buzzetti E, Hall A, Ekstedt M, et al. Collagen proportionate area is an independent predictor of long-term outcome in patients with non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2019.
22. Authors/Task Force M, Ryden L, Grant PJ, et al. ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD: the Task Force on diabetes, pre-diabetes, and cardiovascular diseases of the European Society of Cardiology (ESC) and developed in collaboration with the European Association for the Study of Diabetes (EASD). *Eur Heart J* 2013; **34**(39): 3035-87.
23. Tsochatzis E, Bruno S, Isgro G, et al. Collagen proportionate area is superior to other histological methods for sub-classifying cirrhosis and determining prognosis. *J Hepatol* 2014; **60**(5): 948-54.
24. Kim WR, Therneau TM, Wiesner RH, et al. A revised natural history model for primary sclerosing cholangitis. *Mayo Clin Proc* 2000; **75**(7): 688-94.
25. de Vries EM, Wang J, Williamson KD, et al. A novel prognostic model for transplant-free survival in primary sclerosing cholangitis. *Gut* 2018; **67**(10): 1864-9.
26. Mitchell SA, Bansi DS, Hunt N, Von Bergmann K, Fleming KA, Chapman RW. A preliminary trial of high-dose ursodeoxycholic acid in primary sclerosing cholangitis. *Gastroenterology* 2001; **121**(4): 900-7.
27. Angulo P, Batts KP, Jorgensen RA, LaRusso NA, Lindor KD. Oral budesonide in the treatment of primary sclerosing cholangitis. *Am J Gastroenterol* 2000; **95**(9): 2333-7.
28. Ruiz A, Lemoinne S, Carrat F, Corpechot C, Chazouilleres O, Arrive L. Radiologic course of primary sclerosing cholangitis: assessment by three-dimensional magnetic resonance cholangiography and predictive features of progression. *Hepatology* 2014; **59**(1): 242-50.
29. Lindor KD, Kowdley KV, Luketic VA, et al. High-dose ursodeoxycholic acid for the treatment of primary sclerosing cholangitis. *Hepatology* 2009; **50**(3): 808-14.
30. Farkkila M, Karvonen AL, Nurmi H, et al. Metronidazole and ursodeoxycholic acid for primary sclerosing cholangitis: a randomized placebo-controlled trial. *Hepatology* 2004; **40**(6): 1379-86.
31. Hommes DW, Erkelens W, Ponsioen C, et al. A double-blind, placebo-controlled, randomized study of infliximab in primary sclerosing cholangitis. *J Clin Gastroenterol* 2008; **42**(5): 522-6.
32. Bedossa P, Dargere D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. *Hepatology* 2003; **38**(6): 1449-57.
33. Rojkind M, Ponce-Noyola P. The extracellular matrix of the liver. *Coll Relat Res* 1982; **2**(2): 151-75.
34. Huang Y, de Boer WB, Adams LA, et al. Image analysis of liver collagen using sirius red is more accurate and correlates better with serum fibrosis markers than trichrome. *Liver Int* 2013; **33**(8): 1249-56.
35. Hall A, Germani G, Isgro G, Burroughs AK, Dhillon AP. Fibrosis distribution in explanted cirrhotic livers. *Histopathology* 2012; **60**(2): 270-7.

36. Nakanuma Y, Karino T, Ohta G. Orcein positive granules in the hepatocytes in chronic intrahepatic cholestasis. Morphological, histochemical and electron X-ray microanalytical examination. *Virchows Arch A Pathol Anat Histol* 1979; **382**(1): 21-30.
37. Corpechot C, Gaouar F, El Naggar A, et al. Baseline values and changes in liver stiffness measured by transient elastography are associated with severity of fibrosis and outcomes of patients with primary sclerosing cholangitis. *Gastroenterology* 2014; **146**(4): 970-9; quiz e15-6.
38. Trivedi PJ, Muir AJ, Levy C, et al. Inter- and Intra-individual Variation, and Limited Prognostic Utility, of Serum Alkaline Phosphatase in a Trial of Patients With Primary Sclerosing Cholangitis. *Clin Gastroenterol Hepatol* 2020.
39. Irvine KM, Wockner LF, Shanker M, et al. The Enhanced liver fibrosis score is associated with clinical outcomes and disease progression in patients with chronic liver disease. *Liver Int* 2016; **36**(3): 370-7.
40. Vesterhus M, Hov JR, Holm A, et al. Enhanced liver fibrosis score predicts transplant-free survival in primary sclerosing cholangitis. *Hepatology* 2015; **62**(1): 188-97.
41. de Vries EMG, Farkkila M, Milkiewicz P, et al. Enhanced liver fibrosis test predicts transplant-free survival in primary sclerosing cholangitis, a multi-centre study. *Liver Int* 2017; **37**(10): 1554-61.
42. Nielsen MJ, Thorburn D, Leeming DJ, et al. Serological markers of extracellular matrix remodeling predict transplant-free survival in primary sclerosing cholangitis. *Aliment Pharmacol Ther* 2018; **48**(2): 179-89.
43. Trivedi PJ, Corpechot C, Pares A, Hirschfield GM. Risk stratification in autoimmune cholestatic liver diseases: Opportunities for clinicians and trialists. *Hepatology* 2016; **63**(2): 644-59.

Table 1. Characteristics of patients.

Number of patients	101
Age at biopsy, years, mean ± SD	41 ± 15
Male sex, n (%)	67 (66.3)
Large duct PSC/small duct PSC, n (%)	89/12 (88.1/11.9)
PSC with features of AIH, n (%)	6 (5.9)
Age at PSC diagnosis, years, mean ± SD	39 ± 15
Inflammatory bowel disease, n (%)	66 (64.4)
Ulcerative colitis, n (%)	47 (46.5)
Crohn's disease, n (%)	15 (14.8)
Indeterminate, n (%)	3 (3.0)
Age at IBD diagnosis, years, mean ± SD	35 ± 15
Portal tracts, median (IQR)	15 (11)
PSC duration at time of biopsy, months, median (IQR)	0 (5)
CPA, median (IQR)	8.4 (8.8)
Biochemistry at the time of biopsy**	
Bilirubin x ULN, median (IQR)	1.0 (1.1)
Albumin x LLN, median (IQR)	1.2 (0.3)
AST x ULN, median (IQR)	1.8 (1.7)
ALT x ULN, median (IQR)	2.2 (2.2)
ALP x ULN, median (IQR)	1.7 (2.4)
PLTs x LLN, median (IQR)	1.6 (1.1)
Mayo risk score at time of biopsy, median (IQR)	0.22 (1.79)
Amsterdam-Oxford score at time of biopsy, mean ± SD	2.06 ± 0.88

Follow-up from biopsy, months, median (IQR)	91 (128)
PSC-related deaths, n (%)	12 (12)
Liver transplantation, n (%)	17 (16.8)
Cholangiocarcinoma, n (%)	7 (6.9)
Hepatocellular carcinoma, n (%)	4 (4)
Cirrhosis-related symptoms, n (%)	43 (42.6)
Ascites, n (%)	16 (15.8)
Esophageal varices, n (%)	27 (26.7)
Variceal bleeding, n (%)	9 (8.9)
Hepatic encephalopathy, n (%)	6 (5.9)
Splenomegaly, n (%)*	35 (41.7)
Pharmacological treatment at the time of biopsy**	
Ursodeoxycholic acid, n (%)	74 (73.3)
Aminosalicylates, n (%)	39 (38.6)
Steroids, n (%)	20 (19.8)
Thiopurine therapy, n (%)	10 (11.9)

**Spleen size was available for 84 patients.*

***Data were available for bilirubin (n=83), albumin (n=65), AST (n=87), ALT (n=83), ALP (n=83), PLTs (n=68), MRS (n=66), Amsterdam-Oxford score (n=59). Ursodeoxycholic acid (n=90), Aminosalicylates (n=91), Corticosteroids (n=90), Thiopurine therapy (n=90).*

SD, standard deviation; PSC, primary sclerosing cholangitis; AIH, autoimmune hepatitis; IBD, inflammatory bowel disease; IQR, interquartile range; CPA, collagen

proportionate area; ULN, upper limit of normal; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; PLTs, platelets.

Table 2. Correlations between CPA and histological staging systems calculated by Spearman's rank correlation test.

	Nakanuma stage			**Ishak stage**	**Ludwig stage**
CPA	*0.47 (P<0.001)*			*0.72 (P<0.001)*	*0.66 (P<0.001)*
	Fibrosis	***Bile duct loss***	***Orcein deposition***		
	0.69 (P<0.001)	*0.13 (P=0.214)*	*0.27 (P=0.048)*		

CPA, collagen proportionate area.

Table 3. Multivariable Cox regression analyses of endpoint-1 (PSC-related death, liver transplantation or presentation with primary liver cancer).

	Univariable		Multivariable							
			Model 1 CPA		Model 2 Nakanuma stage		Model 3 Ishak stage		Model 4 Ludwig stage	
	HR (95%CI)	P	HR (95%CI)	P	HR (95%CI)	P	HR (95%CI)	P	HR (95%CI)	P
CPA†	1.21 (1.08-1.36)	0.001	1.21 (1.05-1.39)	0.009						
CPA tertiles	1.90 (1.18-3.08)	0.009	2.59 (1.31-5.11)	0.006	-	-	-	-	-	-
ALP	1.19 (1.00-1.43)	0.046	1.25 (1.03-1.51) 1.32 (1.07-1.63)*	0.023 0.010*	1.34 (1.07-1.70)	0.010	~~1.22 (1.01-1.50)~~ 1.21 (1.27-1.04)	~~0.042~~ 0.038	1.24 (1.02-1.51)	0.028
Bilirubin	1.15 (1.07-1.23)	<0.001	1.13 (1.04-1.23) 1.15 (1.06-1.25)*	0.003 0.001*	1.10 (1.01-1.21)	0.027	~~1.13 (1.05-1.23)~~ 1.12 (1.02-1.22)	~~0.003~~ 0.016	1.12 (1.02-1.22)	0.011
Albumin	0.01 (0.00-0.26)	0.003	-	-	-	-	-	-	-	-
MRS	1.59 (1.22-2.07)	0.001	-	-	-	-	-	-	-	-
AOS	3.94 (1.99-7.81)	<0.001	-	-	-	-	-	-	-	-
Nakanuma stage	6.25 (3.03-12.92)	<0.001	-	-	9.14 (3.08-27.09)	<0.001	-	-	-	-
Ishak stage	~~1.62 (1.15-2.29)~~ 1.53 (1.20-1.95)	~~0.005~~ 0.001	-	-	-	-	~~1.67 (1.03-2.70)~~ 1.60 (1.17-2.19)	~~0.037~~ 0.003	-	-
Ludwig stage	1.99 (1.28-3.08)	0.002	-	-	-	-	-	-	2.45 (1.31-4.55)	0.005

†*Per 5% increment.* **When the analysis was performed including CPA tertiles.*

EP1, endpoint-1; EP2, endpoint-2; EP3, endpoint-3; CPA, collagen proportionate area; HR, hazard ratio; CI, confidence interval; ALP, alkaline phosphatase; PLTs, platelets; MRS, Mayo risk score; AOS, Amsterdam-Oxford score.

Table 4. Multivariable Cox regression analyses of endpoint-2 (transplant-free survival).

	Univariable		Multivariable							
			Model 1 CPA		**Model 2** Nakanuma stage		**Model 3** Ishak stage		**Model 4** Ludwig stage	
	HR (95%CI)	**P**	**HR (95%CI)**	**P**	**HR (95%CI)**	**P**	**HR (95%CI)**	**P**	**HR (95%CI)**	**P**
CPA†	1.24 (1.09-1.40)	0.001	1.28 (1.09-1.49)	0.002	-	-	-	-	-	-
CPA tertiles	1.82 (1.06-1.48)	0.029	2.45 (1.11-5.42)	0.026	-	-	-	-	-	-
ALP	1.23 (1.02-1.48)	0.023	1.33 (1.07-1.65) 1.38 (1.09-1.75)*	0.011 0.008*	1.45 (1.12-1.89)	0.006	~~1.31 (1.04-1.64)~~ 1.33 (1.05-1.67)	~~0.020~~ 0.016	1.36 (1.08-1.72)	0.009
Bilirubin	1.17 (1.09-1.25)	<0.001	1.19 (1.09-1.31) 1.20 (1.10-1.32)*	<0.001 <0.001*	1.15 (1.05-1.26)	0.004	~~1.17 (1.07-1.28)~~ 1.15 (1.04-1.27)	~~0.001~~ 0.005	1.15 (1.05-1.26)	0.003
Albumin	0.01 (0.00-0.26)	0.004	-	-	-	-	-	-	-	-
PLTs	0.34 (0.14-0.85)	0.021	0.48 (0.91-1.19)	0.113	0.46 (0.19-1.12)	0.089	~~0.44 (0.19-1.05)~~ 0.53 (0.24-1.18)	~~0.063~~ 0.12	0.54 (0.22-1.30)	0.166
MRS	1.56 (1.16-2.09)	0.003	-	-	-	-	-	-	-	-
AOS	5.31 (2.23-12.64)	<0.001	-	-	-	-	-	-	-	-
Nakanuma stage	5.42 (2.46-11.95)	<0.001	-	-	9.24 (2.50-34.32)	0.001	-	-	-	-
Ishak stage	~~1.56 (1.06-2.28)~~ 1.56 (1.18-2.04)	~~0.023~~ 0.001	-	-	-	-	~~1.66 (0.92-2.99)~~ 1.49 (1.02-2.19)	~~0.089~~ 0.040	-	-
Ludwig stage	2.29 (1.38-3.79)	0.001	-	-	-	-	-	-	2.97 (1.40-6.29)	0.005

†*Per 5% increment.* ~~*When the analysis was performed including CPA tertiles.*~~

EP1, endpoint-1; EP2, endpoint-2; EP3, endpoint-3; CPA, collagen proportionate area; HR, hazard ratio; CI, confidence interval; ALP, alkaline phosphatase; PLTs, platelets; MRS, Mayo risk score; AOS, Amsterdam-Oxford score.

Table 5. Multivariable Cox regression analyses of endpoint-3 (occurrence of cirrhosis-related symptoms).

	Univariable		Multivariable							
			Model 1 CPA		**Model 2** Nakanuma stage		**Model 3** Ishak stage		**Model 4** Ludwig stage	
	HR (95%CI)	**P**	**HR (95%CI)**	**P**	**HR (95%CI)**	**P**	**HR (95%CI)**	**P**	**HR (95%CI)**	**P**
CPA†	1.25 (1.19-1.38)	<0.001	1.19 (1.03-1.37)	0.019	-	-	-	-	-	-
CPA tertiles	1.91 (1.29-2.81)	0.001	2.04 (1.15-3.62)	0.015	-	-	-	-	-	-
ALP	1.10 (0.95 -1.27)	0.209	1.16 (0.99-1.36) 1.21 (1.01-1.44)*	0.075 0.036*	1.14 (0.96-1.34)	0.128	~~1.19 (0.99-1.42)~~ 1.17 (0.98-1.40)	~~0.063~~ 0.082	1.18 (0.99-1.39)	0.057
Bilirubin	1.08 (1.01-1.16)	0.035	1.04 (0.95-1.13) 1.06 (0.97-1.15)*	0.413 0.184*	1.03 (0.94-1.13)	0.495	~~1.03 (0.95-1.12)~~ 1.01 (0.92-1.11)	~~0.499~~ 0.821	1.02 (0.93-1.12)	0.650
PLTs	0.36 (0.18-0.71)	0.003	0.58 (0.34-0.99) 0.58 (0.33-0.99)*	0.045 0.047*	0.56 (0.33-0.93)	0.026	~~0.60 (0.36-1.02)~~ 1.00 (0.99-1.00)	~~0.057~~ 0.364	0.63 (0.37-1.05)	0.076
AOS	2.62 (1.61-4.25)	<0.001	-	-	-	-	-	-	-	-
Nakanuma stage	3.03 (1.95-4.72)	<0.001	-	-	2.06 (0.90-4.70)	0.086	-	-	-	-
Ishak stage	~~2.08 (1.53-2.82)~~ 1.75 (1.42-2.15)	<0.001	-	-	-	-	~~2.12 (1.36-3.32)~~ 1.90 (1.41-2.58)	~~0.001~~ <0.001	-	-
Ludwig stage	2.29 (1.52-3.43)	<0.001	-	-	-	-	-	-	2.38 (1.29-4.38)	0.005

†*Per 5% increment.* **When the analysis was performed including CPA tertiles.*

EP1, endpoint-1; EP2, endpoint-2; EP3, endpoint-3; CPA, collagen proportionate area; HR, hazard ratio; CI, confidence interval; ALP, alkaline phosphatase; PLTs, platelets; MRS, Mayo risk score; AOS, Amsterdam-Oxford score.

Figure legends

Figure 1: Mean collagen proportionate area values for each histological stage determined by (A) Ishak, (B) Nakanuma and (C) Ludwig scoring systems.

Figure 2. Kaplan-Meier survival curves illustrating prediction of endpoints for primary sclerosing cholangitis (PSC) patients according to collagen proportionate area stratified into tertiles. (A) Endpoint-1: time to PSC-related death, liver transplant or primary liver cancer. (B) Endpoint-2: liver transplant-free survival. (C) Endpoint-3: occurrence of cirrhosis-related symptoms.

Footnotes:

CPA, collagen proportionate area; EP1, endpoint-1 (PSC-related death, liver transplantation or presentation with primary liver cancer); EP2, endpoint-2 (transplant-free survival); EP3, endpoint-3 (cirrhosis-related symptoms).

Figure 3: Median collagen proportionate area values in patients who reached or not an endpoint at 5 and 10 years from liver biopsy.

Footnotes:

CPA, collagen proportionate area; EP1, endpoint-1 (PSC-related death, liver transplantation or presentation with primary liver cancer); CRS, cirrhosis-related symptoms.