Bile Acid Profiles in Primary Sclerosing Cholangitis and their Ability to Predict Hepatic Decompensation

Omar Y. Mousa[1,2*], Brian D. Juran[2*], Bryan M. McCauley[3,], Mette N. Vesterhus[4,5], Trine Folseraas[4,6], Coleman T. Turgeon[7], Ahmad H. Ali[2], Erik M. Schlicht[2], Elizabeth J. Atkinson[3], Chang Hu[8], Denise Harnois[9], Elizabeth J. Carey[10], Andrea A. Gossard[2], Devin Oglesbee[7], John E. Eaton[2], Nicholas F. LaRusso[2], Gregory J. Gores[2], Tom H. Karlsen[4,6], Konstantinos N. Lazaridis[2]

[1]Division of Gastroenterology and Hepatology, Mayo Clinic, Mankato, MN; [2]Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN; [3]Division of Biomedical Statistics and Informatics, Mayo Clinic, Rochester, MN; [4]Norwegian PSC Research Center, Department of Transplantation Medicine, Division of Surgery, Inflammatory Medicine and Transplantation, Oslo University Hospital Rikshospitalet, Oslo, Norway; [5]Department of Clinical Science, University of Bergen, Norway; [6]Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway; [7]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN; [8]University of Illinois Urbana-Champagne, IL; [9]Division of Gastroenterology and Hepatology, Mayo Clinic, Jacksonville, FL; [10]Division of Gastroenterology and Hepatology, Mayo Clinic, Scottsdale, AZ.

*These authors contributed equally to the manuscript

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Corresponding Author: Konstantinos N. Lazaridis, M.D.

200 First Street SW, Rochester, MN 55905

E-mail: lazaridis.konstantinos@mayo.edu

Telephone: 1-507-538-4877, Fax: 1-507-284-0762

List of Abbreviations: Primary sclerosing cholangitis (PSC), inflammatory bowel disease (IBD), cholangiocarcinoma (CCA), ursodeoxycholic acid (UDCA), hepatic decompensation (HD), orthotopic liver transplantation (OLT), bile acid (BA), cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), lithocholic acid (LCA), hyodeoxycholic acid (HDCA), autoimmune hepatitis (AIH), liquid chromatography-tandem mass spectrometry (LC-MS), glycocholic acid (GCA), taurocholic acid (TCA), glycochenodeoxycholic acid (GCDCA), taurochenodeoxycholic acid (TCDCA), glycodeoxycholic acid (GDCA), taurodeoxycholic acid (TDCA), glycolithocholic acid (GLCA), taurolithocholic acid (TLCA), glycoursodeoxycholic acid (GUDCA), tauroursodeoxycholic acid (TUDCA), glycohyodeoxycholic acid (GHDCA), and taurohyodeoxycholic acid (THDCA), total bile acid (TBA), interquartile range (IQR), gradient boosting machines (GBM), confidence intervals (CIs), ulcerative colitis (UC), Crohn's Disease (CD), PSC bile acid profile score (PSC-BAP)

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ABSTRACT

Background & Aims: Altered bile acid (BA) homeostasis is an intrinsic facet of cholestic liver diseases, but clinical usefulness of plasma BA assesment in primary sclerosing cholangitis (PSC) remains understudied. We performed BA profiling in a large retrospective cohort of PSC patients and matched healthy controls, hypothesizing that plasma BA profiles vary among patients and have clinical utility. **Approach & Results**: Plasma BA profiling was performed in the Clinical Biochemical Genetics Laboratory at Mayo Clinic using a mass spectrometry based assay. Cox proportional hazard (univariate) and gradient boosting machines (multivariable) models were used to evaluate whether BA variables predict 5-year risk of hepatic decompensation (HD: defined as ascites, variceal hemorrhage or encephalopathy). There were 400 PSC patients and 302 controls in the derivation cohort (Mayo Clinic) and 108 PSC patients in the validation cohort (Norwegian PSC Research Center). PSC patients had increased BA levels, conjugated fraction and primary-to-secondary BA ratios relative to controls. UDCA increased total plasma BA level while lowering cholic acid (CA) and chenodeoxycholic acid (CDCA) concentrations. Patients without inflammatory bowel disease (IBD) had primary-to-secondary BA ratios between those of controls and patients with ulcerative colitis. HD risk was associated with increased concentration and conjugated fraction of many BA, whereas higher G:T conjugation ratios were protective. The machine learning model, PSC-BAP (bile acid profile) score (C-statistic, 0.95), predicted HD better than individual measures including alkaline phosphatase and performed well in validation (C-statistic, 0.86). **Conclusions**: PSC patients demonstrated alterations of plasma BA consistent with known mechanisms of

cholestasis, UDCA treatment and IBD. Notably, BA profiles predicted future HD, establishing the clinical potential of BA profiling, which may be suited for use in clinical trials.

Primary sclerosing cholangitis (PSC) is a rare, progressive cholestatic liver disease, which leads to stricturing fibrosis of the intra- and extra-hepatic bile ducts and decompensated cirrhosis in the majority of patients over time (1). PSC is highly comorbid with inflammatory bowel disease (IBD) and is associated with increased risk of cholangiocarcinoma (CCA) and colorectal cancer (1). There remain no reliable biomarkers or effective medical therapy for PSC. However, a significant subset of patients are treated with ursodeoxycholic acid (UDCA), a safe and efficacious therapy in improving the liver biochemistries, with no significant impact on hepatic decompensation (HD) or mortality (2). The majority of PSC patients with cirrhosis and HD require orthotopic liver transplantation (OLT) (1).

PSC pathogenesis is complex, involves hereditary and environmental factors, and remains poorly understood. Factors including bile acid (BA) toxicity (3), intestinal dysbiosis (3) and abberant immune activation (4) have been shown to contribute to PSC pathogenesis. Genome-wide association studies suggest the genetic component of PSC lies primarily in immune-inflammatory processes, with little evidence for genetically determined alterations to mechanisms enforcing BA homeostasis (5, 6), However, increasing BA toxicity during the course of progressive cholestasis most certainly impacts disease, and therapeutics focused on altering the BA pool in PSC patients are promising (7).

Bile acids act as signaling molecules regulating hepatic metabolism and contribute to maintaining intestinal microbiota homeostasis. The two primary BA, cholic acid (CA) and

chenodeoxycholic acid (CDCA) are synthesized and conjugated with glycine or taurine in the liver. Following secretion into the small intestine, BA are deconjugated and converted to secondary bile acids by the intestinal microbiota. Specifically, CA is converted to deoxycholic acid (DCA) and CDCA can ultimately be converted to UDCA, lithocholic acid (LCA) or hyodeoxycholic acid (HDCA). The majority of BA are reabsorbed in the ileum and undergo enterohepatic circulation that maintains the BA pool (8). In cholestasis, bile flow is compromised and potentially toxic BA accumulate, stimulating mechanisms to reduce BA concentrations in the hepatocyte including increased excretion into the systemic circulation for removal by the kidneys. However, these compensatory mechanisms are not adequate to overcome progressive liver damage in PSC (9).

Recent advancements have brought inexpensive and non-invasive clinical assays to assess circluting BA profiles. Such tests offer promise for better clinical management of patients with cholestatic liver diseases and are of particular interest in evaluating clinical trials of anti-cholestatic drugs. However, there have been no large-scale studies reporting use of these assays in PSC. In this study, we aim to evaluate the clinical utility of BA profiling in PSC using traditional and machine-learning approaches.

PATIENTS AND METHODS

Patients

Patients and healthy controls comprising the derivation cohort were selected from the PSC Scientific Community Resource, an extension of the PSC Resource of Genetic

Risk, Environment and Synergy Studies (PROGRESS) resource (10), which actively collects biospecimens and clinical data for use in PSC research. PSC diagnoses were confirmed by qualified physicians using standard criteria (11) and controls were excluded if they had history of chronic liver disease. Clinical records were reviewed and pertinent information such as IBD status, PSC subtype including small duct disease and overlap with autoimmune hepatitis (AIH), UDCA treatment and progression to clinical endpoints was documented. Cirrhotic-stage PSC was defined by the presence of histological stage IV according to the Ludwig staging system (12), hepatic parenchymal changes on cross-sectional imaging consistent with cirrhosis (13) and/or presence of portal hypertension manifested as gastroesophageal varices or splenomegaly. Cirrhosis with HD was defined as one or more clinical episodes of ascites, variceal hemorrhage or hepatic encephalopathy. Patients lacking clinical follow-up or with history of OLT, diagnosis of CCA or HD prior to sample collection were excluded. Controls were selected to proportionally match to patients based on sex and to minimize age differences between the groups to the extent possible. We collaborated with the Norwegian PSC Research Center to obtain a validation cohort of patients, selected using identical criteria, to attempt validation of the BA predictive model. The study was carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants and the studies were approved by the Mayo Clinic Institutional Review Board and the Regional Committee for Medical and Health Research Ethics of South-Eastern Norway.

Bile Acid Profiling

Plasma samples had been previously collected in EDTA tubes under fasting conditions and stored at -80^{o}C. Bile acid profiling was performed in the Clinical Biochemical Genetics Laboratory at Mayo Clinic using the BAPS (Bile Acid Profile, Serum) assay. This method is based on liquid chromatography-tandem mass spectrometry (LC-MS) incorporating isotopically labeled internal standards and is well-calibrated to evaluate bile acid levels in EDTA plasma in the research setting. The assay provides quantitation of primary BA: CA and CDCA; secondary BA: DCA, LCA, UDCA and HDCA; and their glyco- and tauro-conjugates: glycocholic acid (GCA), taurocholic acid (TCA), glycochenodeoxycholic acid (GCDCA), taurochenodeoxycholic acid (TCDCA), glycodeoxycholic acid (GDCA), taurodeoxycholic acid (TDCA), glycolithocholic acid (GLCA), taurolithocholic acid (TLCA), glycoursodeoxycholic acid (GUDCA), tauroursodeoxycholic acid (TUDCA), glycohyodeoxycholic acid (GHDCA), and taurohyodeoxycholic acid (THDCA). Additional information about the BAPS assay is provided in **Supplemental Methods**. The total bile acid (TBA) value is calculated by summing the values of all evaluated bile acid forms. Bile acid “family” concentrations were calculated by adding the values of unconjugated and conjugated forms (e.g., CA+GCA+TCA). Conjugated fraction was calculated as the sum of the conjugated forms divided by the total (e.g., (GCA+TCA) / (CA+GCA+TCA)); value was set to blank if the denominator was equal to zero. G:T conjugation ratios were calculated by dividing the the glycine conjugated form by the taurine conjugated form (e.g., GCA / TCA); value was set to blank if either form was equal to zero. Finally, ratios of CA:CDCA, CA:DCA and CDCA:(LCA+HDCA+UDCA) were calculated using the BA family values.

Statistical analyses

Continuous variables were expressed as median and interquartile range (IQR) using the Kruskal-Wallis rank sum test, unless otherwise specified. Categorical variables were compared using the Pearson's chi-squared test. Cox proportional hazard models were run for univariate variables to evaluate their ability to predict risk of HD (defined as ascites, variceal hemorrhage or encephalopathy) within a 5-year time period. Censoring was made at the time of OLT, CCA diagnosis, or at the time of last clinical encounter or death. Probability of HD was estimated using Aalen-Johansen curves based on quartiles of variable values, accounting for death and liver transplantation as competing risks. Hazard ratios were calculated using IQR-normalized values and thus reflect risk of HD in individuals at the 75^{th} percentile relative to the 25^{th} percentile of the variable. The Harrell's concordance statistic (C-statistic) was used to measure discrimination ability of the variables in the univariate analysis. Correlation between individual BA was evaluated using the Spearman's rank correlation coefficient. Principle components analyses was performed using concentrations of the 18 individual bile acids.

Gradient boosting machines (GBM) was used to build a multivariable model to predict development of HD in a 5-year time window, utilizing the generalized boosted model package available in the R software environment (R Foundation for Statistical Computing, Vienna, Austria) (14). GBM utilizes an ensemble of weak prediction models, in this case decision trees, building the model in a step-wise, iterative process involving recursive partitioning. Each decision tree is likely to have relatively poor predictive performance; however, when merged in the final model prediction is significantly

improved. Censoring was as per the univariate analyses. Discrimination was assessed using the C-statistic and 95% confidence intervals (CIs) were created using bootstrapping. Calibration was assessed by comparing observed and expected values in subjects with low, medium, and high predicted risk as determined by the tertiles of the model's risk score distribution.

RESULTS

Patient characteristics

Patient characteristics are presented in **Table 1**. There were 400 PSC patients and 302 controls in the derivation cohort and 108 PSC patients in the validation cohort. Patients and controls in the derivation cohort were well matched on sex and race, with the patients being somewhat younger than controls. The two patient cohorts were similar in terms of race, age at PSC diagnosis and IBD status. Compared to the derivation cohort, the validation group was younger, included fewer female patients and had shorter disease duration before sample collection. In the 5-year post-sample collection observation period there were 51 HD events in the derivation cohort and 8 HD events in the validation cohort. Censoring in time-to-event analyses for both cohorts was primarily based on patients having no clinical event at time of last clinical encounter.

Bile acid profiles in PSC patients and controls

We compared concentrations, conjugated fractions and G:T conjugation ratio for each BA family and for TBA, as well as concentrations of the individual BA, between PSC patients and controls of the derivation group (**Figure 1 and Supplemental Table 1**).

PSC patients demonstrated markedly increased level of TBA (median (IQR) 16.34 (6.04-44.31) μmol/L) compared to controls (median (IQR) 1.90 (1.02-3.52) μmol/L), which was accompanied by significant increases in CA, CDCA, LCA and UDCA. Notably, levels of the secondary bile acid DCA did not significantly differ. The conjugated fraction of TBA was elevated in PSC patients (median (IQR) 0.91 (0.74-0.98)) relative to controls (median (IQR) 0.64 (0.49-0.79)), with similar increases in all of the other BA families save for HDCA. The G:T conjugation ratio of TBA was similar between PSC patients and controls. However, G:T conjugation ratios of CDCA and DCA were significantly lower, and UDCA significantly higher in PSC patients. Differences in concentration of individual BA broadly reflected that of the BA family, except for DCA, in which the unconjugated form was found to be significantly decreased in patients compared to controls, whereas the taurine-conjugated form (TDCA) was slightly increased in patients.Finally, age and sex were not found to have substantial impact on BA concentrations, conjugated fractions or G:T conjugation ratios in both PSC patients and controls (**Supplemental Figures 1 and 2**).

Variabiltiy in BA concentrations could lead to significant alterations to the composition of plasma BA in PSC patients compared to controls. We find that PSC patients have increased proportions of CA and UDCA and decreased proportions of CDCA, DCA, LCA and HDCA relative to controls (**Figure 2A and Supplemental Table 1**). While the findings suggest that PSC is characterized by increased size and altered compostion of plasma BA, there may be PSC patients with BA profiles similar to controls. To begin addressing this possibility, we performed principle components analysis of the individual

BA concentrations. In the plot of the first two principle components it is clear that the controls form a relatively tight cluster intermingled with a subset of the PSC patients (**Figure 2B**).

Many PSC patients are treated with UDCA, which may account for increases in TBA and the proportion of UDCA in PSC patients. To begin understanding the potential effect of UDCA treatment on plasma BA, we first performed a retrospective review of clinical records to establish whether our PSC patients were taking UDCA at or around the time of sample collection. For 246 of the 400 PSC patients (61.5%) we were able to establish UDCA treatment status, with 85 of these 246 patients (34.6%) found to be prescribed UDCA. We next looked at TBA concentration, UDCA concentration and UDCA as a fraction of TBA in light of the review-determined UDCA status (**Figure 2C**). Some of the patients from the “No UDCA” group had very high UDCA levels and were likely on UDCA at the time of sample collection. Conversely, a subset of the “Yes UDCA” group had very low UDCA levels, suggesting they were not taking the therapy or the UDCA had limited bioavailability. In order to include more patients and better compare groups accounting for high BA levels and likely UDCA therapy, we split the patients into 4 groups based on TBA (Normal/High) and predicted UDCA therapy (Yes/No) using the highest levels found in controls for TBA concentration (17.79 µmol/L), UDCA concentration (2.15 µmol/L) and UDCA fraction of TBA (0.39) (**Figure 2C**). We required UDCA concentration and fraction of TBA to be geater or less than the control cut-offs, so a small subset of patients were classified as “no call” for UDCA therapy. Visualiztion of the groups (NN: normal TBA, no UDCA; NY: normal TBA, yes

UDCA; HN: high TBA, no UDCA; and HY: high TBA, yes UDCA) by UDCA and TBA concentration shows the clear separation (**Figure 2D**), with a relatively small number of patients near the demarcation lines, suggesting misclassification effects in comparative analyses should be minimal. Finally, the NN (36.4%) and HY (30.6%) groups were the most prevalent, with HN (16.8%) and NY (16.2%) making up smaller proportions of the PSC patient population (**Figure 2E**).

Bile acid profiles considering TBA/UDCA groups

We compared BA data between various TBA/UDCA determined groups to better understand differences compared to controls (control vs. NN), the effect of UDCA therapy (NN vs. NY and HN vs. HY) and the effect of abnormally high levels of BA (NN vs. HN and NY vs. HY). The results of these analyses are presented in **Figure 3 and Supplemental Tables 2-6**. Compared to controls, the NN PSC group had higher concentration, increased conjugated fraction and similar G:T conjugation ratio of TBA (**Figure 3A**), consistent with the global case-control analysis. However, unlike the broader analysis, UDCA concentration was very similar between NN patients and controls, with concentrations of primary BA (CA and CDCA) significantly increased and concentrations of secondary BA (DCA, LCA and HDCA) significantly decreased in the NN PSC patients (**Supplemental Table 2**). UDCA treated groups (NY and HY) had higher concentration and G:T conjugation ratio and lower conjugated fraction of TBA than comparable non-UDCA treated groups (NN and HN). Changes in conjugated fraction and G:T conjugation ratios were consistent across the individual BA in these groups, with variability in concentrations of individual BA and increased concentration of

TBA primarily coming from UDCA (**Supplemental Tables 3 and 4**). Notably, while TBA was increased in UDCA-treated groups, the non-UDCA component of TBA was significantly reduced in UDCA-treated compared to non-treated groups; median (IQR) of TBA-excluding UDCA: 2.93 (1.87-4.28) μmol/L in NY vs. 4.54 (2.37-7.83) μmol/L in NN, $p<0.001$ and 14.63 (7.75-36.40) μmol/L in HY vs. 38.02 (22.96-62.69) μmol/L in HN, $p<0.001$). In addition to higher TBA concentration, the high BA groups (HN and HY) had increased conjugated fraction and decreased G:T conjugation ratio relative to their comparable normal BA groups (NN and NY) (**Figure 3A and Supplemental Tables 5 and 6**).

The NN PSC patient group demonstrated increased fractions of CA and CDCA and decreased fractions of LCA, DCA, HDCA and UDCA compared to controls (**Figure 3B**), consistent with the observed differences in BA concentrations. As expected, plasma BA of UDCA-treated patient groups (NY and HY) were significantly enriched for UDCA compared to non-treated groups (NN and HN) (**Figure 3B**). The increased UDCA fraction was reflected by reduced representation of CA and CDCA in UDCA-treated patient groups, although the fraction of DCA and HDCA remained similar despite treatment. Notably, while only a small fraction of TBA, the LCA fraction was significantly increased in the UDCA-treated groups (**Figure 3B**), suggesting that supplemental UDCA may be available for conversion to LCA. Removal of UDCA from the analyses of NN:NY and HN:HY differences in BA fraction of TBA were mostly consistent with the UDCA-included results, but with DCA comprising an increased fraction of the non-UDCA TBA in UDCA treated vs. non-treated groups (**Supplemental Figure 3**).

Compared to controls, plasma BA of the NN PSC patient group had increases in CA:CDCA ratio, CA:DCA ratio and CDCA:(LCA+HDCA+UDCA) ratio, all of which were further increased in the comparable high BA group (i.e., control < NN < HN) (**Figure 3C**). Coupled with observed differences in BA concentrations, these findings suggest that alterations in BA metabolism, such as deficiency in conversion of primary to secondary bile acids, increased BA synthesis or decreased BA excretion may play a role in PSC. Comparisons between the two UDCA-treated groups (NY and HY) did not show a difference in CA:CDCA ratio but did demonstrate an increased CA:DCA ratio, supporting the finding in the non-treated groups. Notably, in the high BA groups UDCA treatment lowered both the CA:CDCA and CA:DCA ratios (HN vs. HY, **Supplemental Table 4**); however, this UDCA effect was not noted in the normal BA groups (NN vs. NY, **Supplemental Table 3**).

Comorbid inflammatory bowel disease and bile acid profiles

IBD frequently co-occurs with PSC and has been shown to affect the BA pool through disruption of homeostatic enterohepatic circulation. Thus, we next accounted for IBD in our analysis. First, we performed a principle components analysis of BA levels using only the PSC patients, which shows that patients do not cluster based on presence of IBD or the clinical IBD subtype (**Figure 4A**). We next evaluated plasma BA compositions of PSC patients with comorbid IBD relative to PSC patients without history or evidence of IBD (**Figure 4B and Supplemental Tables 7-10**). Patients with ulcerative colitis (UC) had significantly increased fraction of CA and decreased fractions of LCA, DCA and HDCA, whereas patients with Crohn's Disease (CD) and

Indeterminate IBD had increased fraction of CDCA, relative to the no-IBD patients. G:T conjugation ratios of TBA and the individual BA did not differ based on IBD status. Patients without IBD had similar concentration and conjugated fraction of TBA relative to patients with UC and Indeterminate IBD, but increased compared to patients with CD (**Figure 4C**). Finally, CA:CDCA and CA:DCA ratios were increased in no-IBD PSC patients compared to controls and further increased in PSC patients with UC compared to the no-IBD patients (**Figure 4D**). This suggests that changes to plasma BA in PSC are not dependant on, but are exasperated by, UC comorbidity.

Bile acid profiles in cirrhotic patients and those with AIH overlap or small duct PSC

Comparison of BA variables between PSC patients with compensated cirrhosis and non-cirrhotic patients is presented in **Supplemental Table 11** and **Supplemental Figure 4**. Not surprisingly, cirrhotic patients demonstrated increase concentrations of BA including CA, CDCA, LCA and TBA; although, some cirrhotic patients did have relatively low BA levels. Conjugated fraction of BA tended to be increased while G:T conjugation ratios were decreased in cirrhotic compared to non-cirrhotic patients. As well, CA:DCA and CDCA:(LCA+HDCA+UDCA) ratios were increased in cirrhotic patients. However, the CA:CDCA ratio was similar.

Patients with AIH overlap did not demonstrate significant differences in BA variables compared to non-overlap PSC (**Supplemental Table 12**). However, the number of AIH overlap patients was quite small, limiting our ability to detect differences. Patients with

small duct PSC were younger than patients with traditional large duct disease and only demonstrated significant differences in a few BA variables including having lower concentrations of CA and lower CA:CDCA and CA:DCA ratios (**Supplemental Table 13**). As with AIH, there were a limited number of small duct PSC patients.

Bile acids as potential biomarkers of future hepatic decompensation

To evaluate whether BA have utility in prediction of disease complications we performed univariate time-to-event analyses of phenotypic and BA parameters. Hepatic decompensation was selected as the clinical event instead of liver transplantation due to broad variation in listing and transplant criteria and wait times across the United States and the world (15). Results of these analyses are presented in **Figure 5 and Supplemental Tables 14 and 15**. PSC patients with compensated cirrhosis had elevated risk of HD relative to non-cirrhotic patients (hazard ratio (95% CI) = 5.29 (3.02-9.25), $p < 0.001$). However, cirrhosis status was not among the most predictive variables with a C-statistic of 0.66. Concentration of TBA and 10 of 18 individually measured BA (LCA, UDCA, GCA, GCDCA, GLCA, GUDCA, TCA, TCDCA, TLCA and TUDCA) were significantly associated with development of HD. Conjugation fraction of TBA, CA, CDCA and UDCA were also associated with development of HD; whereas G:T conjugation ratios generally demonstrated significant protective effects. The four parameters with highest C-statistics were TCDCA (0.83), TCA (0.82), TBA (0.80) and TBA with UDCA removed (0.80). These variables outperformed alkaline phosphatase (expressed as times upper limit of normal) and total bilirubin, commonly used biochemical measures to evaluate PSC, which had C-statistics of 0.70 and 0.77,

respectively (**Figure 5**). Notably, there is a complex correlation structure in plasma BA that is somewhat altered in PSC patients compared to controls (**Supplemental Figure 5**) and the top BA in the time-to-HD analysis are highly correlated in PSC: Spearman correlation of TCDCA-TCA = 0.94, TCDCA-TBA = 0.76 and TCA-TBA = 0.72.

Machine learning of bile acid profile to predict HD events: PSC-bile acid profile score

We next used GBM to investigate 40 potential bile acid based model covariates (**Supplemental Table 16**) to determine whether the overall BA profile could better predict future HD in PSC than individual BA. A total of 651 decision trees were utilized, decision tree depth was optimized at 3 and the shrinkage parameter was optimized at 0.005. Six covariates were retained in the final model to predict 5-year probability of HD (**Figure 6A**). The resulting PSC-BAP (bile acid profile) score was median (IQR) 7.1% (5.0-22.3%) in the derivation cohort, performed well in predicting HD (C-statistic = 0.95, 95%CI, 0.92-0.97), and was well calibrated (**Figure 6B**). Notably, these covariates were also among the top variables in alternate GBM models run to test model sensitivity to removal of PSC patients with small duct disease and/or AIH overlap and addition of variables including cirrhosis status, bilirubin and alkaline phosphatase (**Supplemental Figure 6**). The validation cohort had similar TBA concentration as the derivation group, but there were notable differences in BA composition and the validation cohort demonstrated higher conjugation fractions and lower G:T ratios than the derivation cohort (**Supplemental Table 17**). In the validation cohort, median (IQR) of the PSC-BAP score was 7.3% (5.6-23.8) and despite BA differences performed relatively well (C-

statistic = 0.86, 95%CI, 0.74-0.96). Moreover, the score was well-calibrated but tended to overestimate risk, particularly in the high-risk tertile (**Figure 6C**). This overestimation could be a consequence of significantly increased TCDCA and TCA concentrations (**Supplemental Table 17**), both important components of the model, in the validation relative to derivation cohort. Finally, PREsTo did slightly outperform the PSC-BAP score in the validation cohort (PREsTo C-statistic 0.94, 95% CI 0.87-0.98), suggesting PREsTo may be better calibrated across a wide range of patients.

DISCUSSION

We report the first ever large-scale study of plasma BA profiles focused on PSC patients and healthy controls using a non-invasive LC-MS based BA profiling assay. We found that PSC patients have alterations in plasma BA that are consistent with known mechanisms of cholestasis, UDCA treatment and IBD; suggesting that plasma BA are reflective of the overall BA pool. Risk of HD was positively influenced by increased concentration and conjugated fraction of many BA, whereas higher G:T conjugation ratios were generally protective. We developed the PSC-BAP (bile acid profile) score using machine learning of BA variables to create a model capable of predicting future HD events better than individual measures. This model was well-calibrated and validated in an independent cohort of PSC patients; although, overall performance was similar to that of the recently reported PREsTo score (10). Of note, the univariate analyses and GBM model indicate that high levels of taurine-conjugated BA seem to be most predictive of future HD events in PSC. This study provides a solid foundation for

persuing BA profiles as a clinical measure in PSC, which may be particularly well suited to use in clinical trials.

The use of UDCA as a therapy for PSC remains controversial, with American and European medical associations offering conflicting advice towards its use (16). It is generally well-tolerated and has demonstrated efficacy in lowering serum liver enzyme levels (17). However, there is no evidence that UDCA improves outcomes in PSC and a study of high-dose UDCA found an increased rate of adverse events in the treated group (18). In our study, we assign patients to UDCA “yes” or “no” treatment groups using a calculation based on maximum values for UDCA concentration and fraction of the BA pool found in controls, as we found medical record review to be an incomplete and somewhat inaccurate means to determine past UDCA treatment. These groups are further stratified by “normal” and “high” levels of TBA, again based on the maximum value seen in controls. While this approach is artificial and leaves room for potential misclassification, it does separate patients into groups with strong evidence for or against UDCA enrichment. UDCA treated groups had increased TBA level compared to non-treated groups, suggesting that therapy may be directly expanding the BA pool or otherwise facilitating transfer of BA from the enterohepatic to systemic circulation. Increased TBA levels in treated groups were driven by increases in UDCA, with concentrations of more-toxic CA and CDCA (conjugated and unconjugated forms) being significantly reduced with treatment. However, UDCA treated groups also had significantly increased concentrations of the toxic BA LCA, a finding that is consistent with a previous study of 56 PSC patients who had received high-dose UDCA and

showed marked UDCA enrichment in serum with a significant increase in LCA concentration compared to the placebo group (19). Together these studies highlight the assertion that supplemental UDCA may be available for conversion to LCA in the gut. While hepatotoxic, LCA and other secondary bile acids may have have anti-inflammatory effects mediated through the BA membrane receptor TGR5 (20). Finally, UDCA treatment lowered the conjugated fraction and increased the G:T conjugation ratio of most bile acids. Notably, many of the BA changes observed in the UDCA treatment groups were consistent with beneficial effects in the univariate time-to-HD analysis. However, predicted UDCA treatment as a variable was not found to be protective against or a risk factor for future HD.

IBD is common in PSC, affecting some 70% of patients. Dysbiosis of the gut microbiota is a typical characteristic of IBD and effects the BA pool by disrupted enterohepatic circulation and diminished capacity for conversion of primary to secondary bile acids (21). Recent studies have demonstrated that intestinal dysbiosis is also common in PSC, with effects independent of concomitant IBD (22). However, we are not aware of any studies that evaluate BA in the context of IBD in PSC. The ratio of primary to secondary bile acids was found to be significantly increased in PSC patients compared to controls. As intestinal dysbiosis has been shown to drive similar observations in IBD and IBD is a common comorbidity in PSC, we evaluated whether the observation was due solely to concomitant IBD, focusing on the CA:DCA ratio to avoid inflation in secondary bile acids due to UDCA treatment. Notably, the no-IBD PSC patients had significantly elevated CA:DCA ratio compared to controls, with UC-PSC patients having

even greater CA:DCA ratio, suggesting that the increased fraction of primary bile acids is an independent phenomenon in PSC that is further exasperated by IBD. A similar pattern is seen in the CA:CDCA ratio, which has been reported to be increased in intrahepatic cholestasis of preganancy (23) and in animal models of cholestasis (24). This observation highlights the importance of the gut-liver axis in perpetuating cholestasis in PSC.

Modulation of the BA pool forms the basis of several therapeutic approaches to cholestatic liver disease. A number of studies evaluating compounds, which directly- or indirectly-influence BA pathways, have been reported or proposed (25). The majority of these trials rely on using alkaline phosphatase reductions, which are not highly reliable (26), as the primary endpoint and would benefit from using biomarkers that more directly assess the BA changes these therapies attempt to achieve. Our machine learning model, PSC-BAP, includes six BA variables and significantly improved HD prediction relative to individual variables. Notably, four of the six variables included in PSC-BAP are taurine-conjugated bile acids (TUDCA, TCDCA, TCA and TLCA). Levels of TCA are known to be increased in cirrhotic patients and may directly promote liver damage through upregulation of TLR4 and activation of hepatic stellate cells (27). As well, TCDCA and TLCA have been long known to be hepatotoxic (28, 29). However, TUDCA, which is the strongest contributor to the PSC-BAP model, is thought to be hepatoprotective and potentially therapeutic outside of heptatobiliary disease (30). Of note, TUDCA levels are highly correlated with TBA levels in our PSC patients. Thus, the importance of TUDCA in the model could be reflective of metabolic changes driven by

high overall bile acid levels and does not neccessarilly suggest a hepatotoxic effect of UDCA. The PSC-BAP model performed quite well in the derivation and validation cohorts, although the model was over-predicitve in validation, due likely to differences in BA pool compositions between the groups, most notably significant increases in TCA, TCDCA and TLCA concentrations in the validation compared to derivation cohort. Comparison between PSC-BAP and PREsTo, another machine-learning based tool for predicting future HD in PSC, showed performance of the two scores to be similar. Unfortunately, we were not able to compare PSC-BAP to the enhanced liver fibrosis score, which is another non-invasive test that has recently been shown to have prognostic value in PSC (31).

Despite the significance of our findings, we recognize the limitations of the study. This is a retrospective, cross sectional study of BA measurements and PSC outcomes. Potentially important BA species, such as sulfated and glucuronidated forms, were not evaluated due to limitations of our profiling assay. Moreover, our assay is quite sensitive but not fully capable of quantifying extremely low BA levels. Thus, interpretation of observed differences in low-concentration BA such as HDCA and LCA should be done with caution. Underlying processes in PSC, which may significantly alter BA levels of individual patients, such as presence of dominant strictures, variation in IBD activity or specific disease location were not accounted for. We assign patients to UDCA therapy groups using a calculation, so some patients may be misclassified. While this is not likely to have a major impact on the findings, an important aspect of BA metabolism, particularly limited UDCA bioavailability as a potential treatment failure is not addressed.

Finally, we are not able to directly compare plasma BA profiles to those present in the enterohepatic circulation due to the invasive nature of collecting BA from the liver or gallbladder. Furure prospective and longitutunal studies of BA are needed to further address the limitations of this work.

In conclusion, plasma BA profiles were able to predict future HD in PSC patients, establishing the clinical potential of the PSC-BAP scoring system. Further exploration of BA profiles in PSC, particularly their use in clinical management of patients and as end-points in clinical trials is warranted.

ACKNOWLEDEMENTS

We are indebted to all the patients and controls who participated in this study.

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FIGURE LEGENDS

Fig. 1. Bile acids in PSC patients and controls. Comparisons of (A) Concentration (μmol/L), (B) Conjugated fraction and (C) G:T conjugation ratio of total bile acids (TBA) and each bile acid family (e.g., the CA family includes CA (unconjugated), GCA and TCA) between PSC patients and controls. *Significance level*: *p<0.05, **p≤0.001; Kruskal-Wallis rank sum test.

Fig. 2. PSC patient subgrouping based on total bile acid levels and UDCA treatment. (A) Plasma bile acid (BA) composition by BA family type in PSC patients and controls, (B) Principle components analysis of individual bile acid concentrations in PSC patients and controls, (C) Concentrations of total bile acids (TBA), UDCA and fraction of UDCA in TBA (UDCA/TBA) of PSC patients separated by chart-reviewed determination of UDCA treatment and controls. Values shown are maximum cut-offs observed in controls and were used to establish PSC patient subgroups based on TBA (normal: ≤ 17.79, high: > 17.79 μmol/L) and predicted UDCA treatment (no: UDCA conc. ≤ 2.15 and fraction ≤ 0.39, yes: UDCA conc. > 2.15 and fraction > 0.39). (D) UDCA and TBA concentrations in PSC patients and controls colored by TBA/UDCA groups, (E) proportion of PSC patients in each of the TBA/UDCA groups.

Fig. 3. Bile acids in controls and PSC patient TBA/UDCA subgroups. Comparisons of (A) Concentration (μmol/L), Conjugated fraction and G:T conjugation ratio of total bile acids (TBA) and (B) Concentration of each BA family as fraction of TBA between (1) controls and NN PSC patients, (2) NN and NY PSC patients and (3) HN and HY PSC

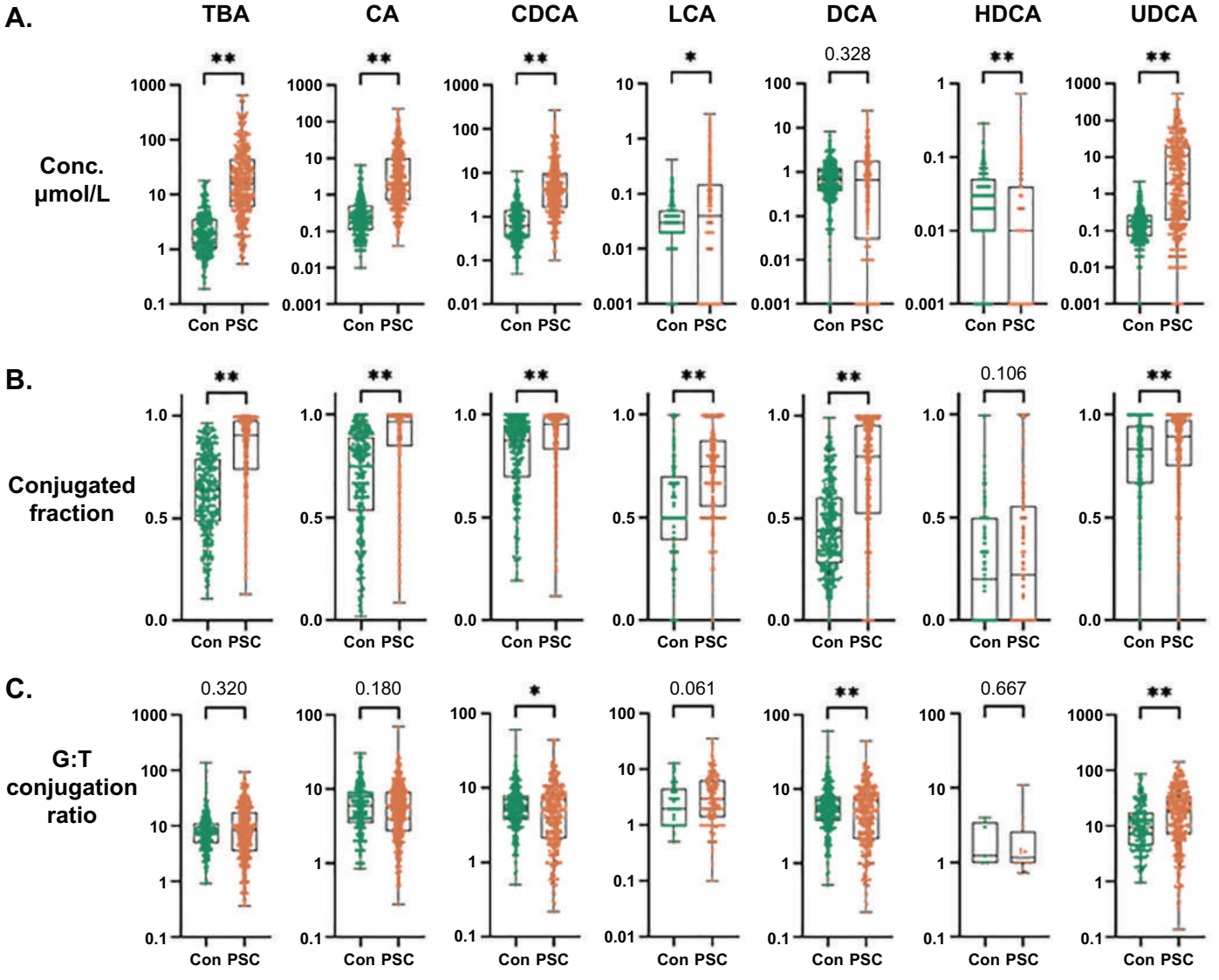
patients. (C) Comparison of CA:CDCA, CA:DCA and CDCA:LCA+HDCA+UDCA ratios between (1) controls and NN PSC patients, (2) NN and HN PSC patients and (3) NY and HY PSC patients. *Significance level*: *p<0.05, **p≤0.001; Kruskal-Wallis rank sum test.

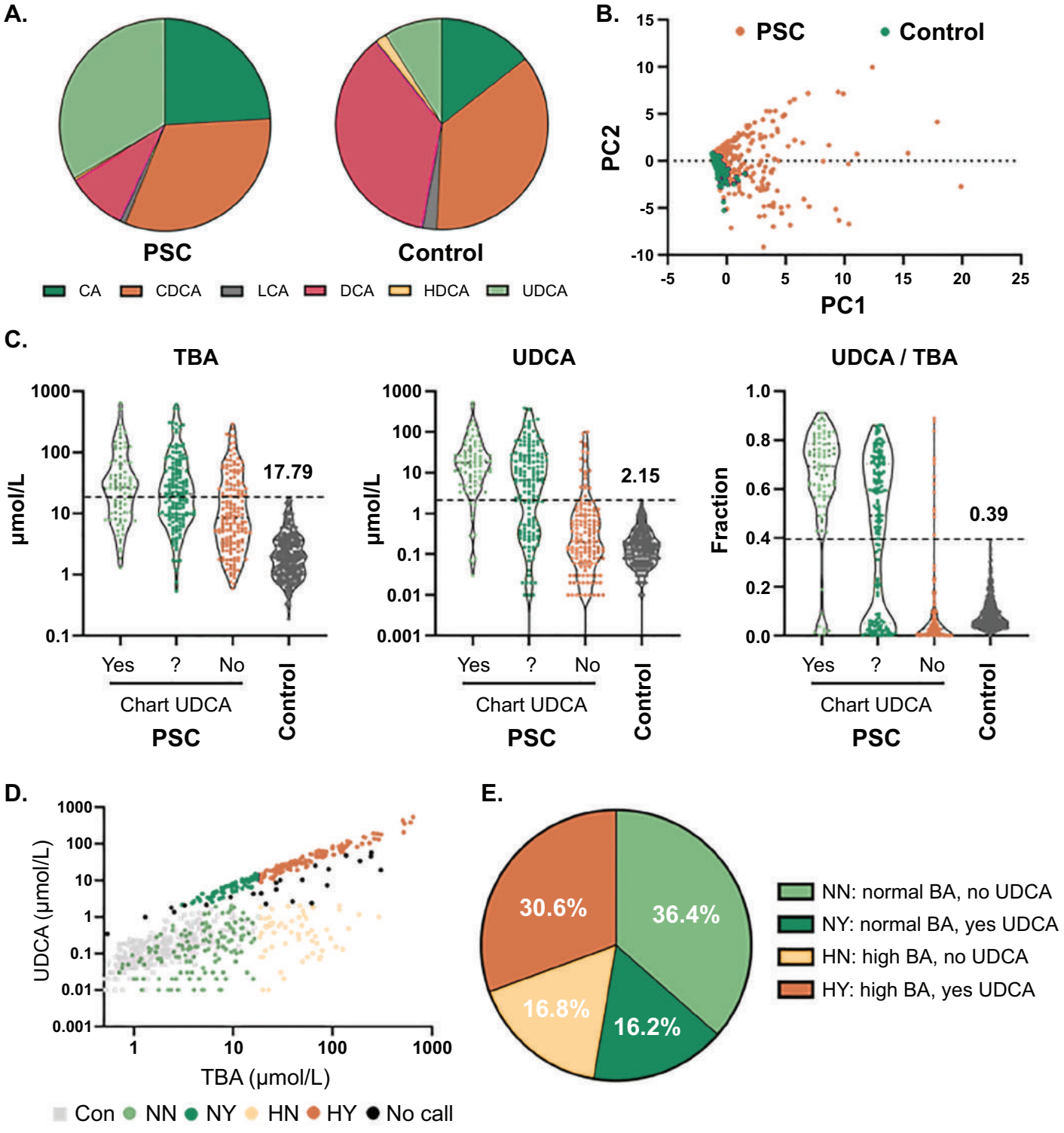
Fig. 4. Inflammatory bowel disease and bile acids in PSC. (A) Principle components analysis (PCA) of individual BA concentrations in PSC patients with No IBD, ulcerative colitis (UC), Crohn's Disease (CD) or indeterminate IBD (Ind IBD); (B) Plasma BA composition by BA family type in PSC patients by IBD group; (C) Comparison of concentration (μmol/L) and conjugated fraction of total bile acids (TBA) between PSC patients with No IBD and PSC patients with Ind IBD, CD or UC; (D) Comparison of CA:CDCA and CA:DCA ratios between (1) Controls and PSC patients with No IBD and (2) PSC patients with No IBD and PSC patients with Ind IBD, CD or UC. *Significance level*: *p<0.05, **p≤0.001; Kruskal-Wallis rank sum test. Non-significant comparisons (p≥0.05) are not highlighted in this figure.

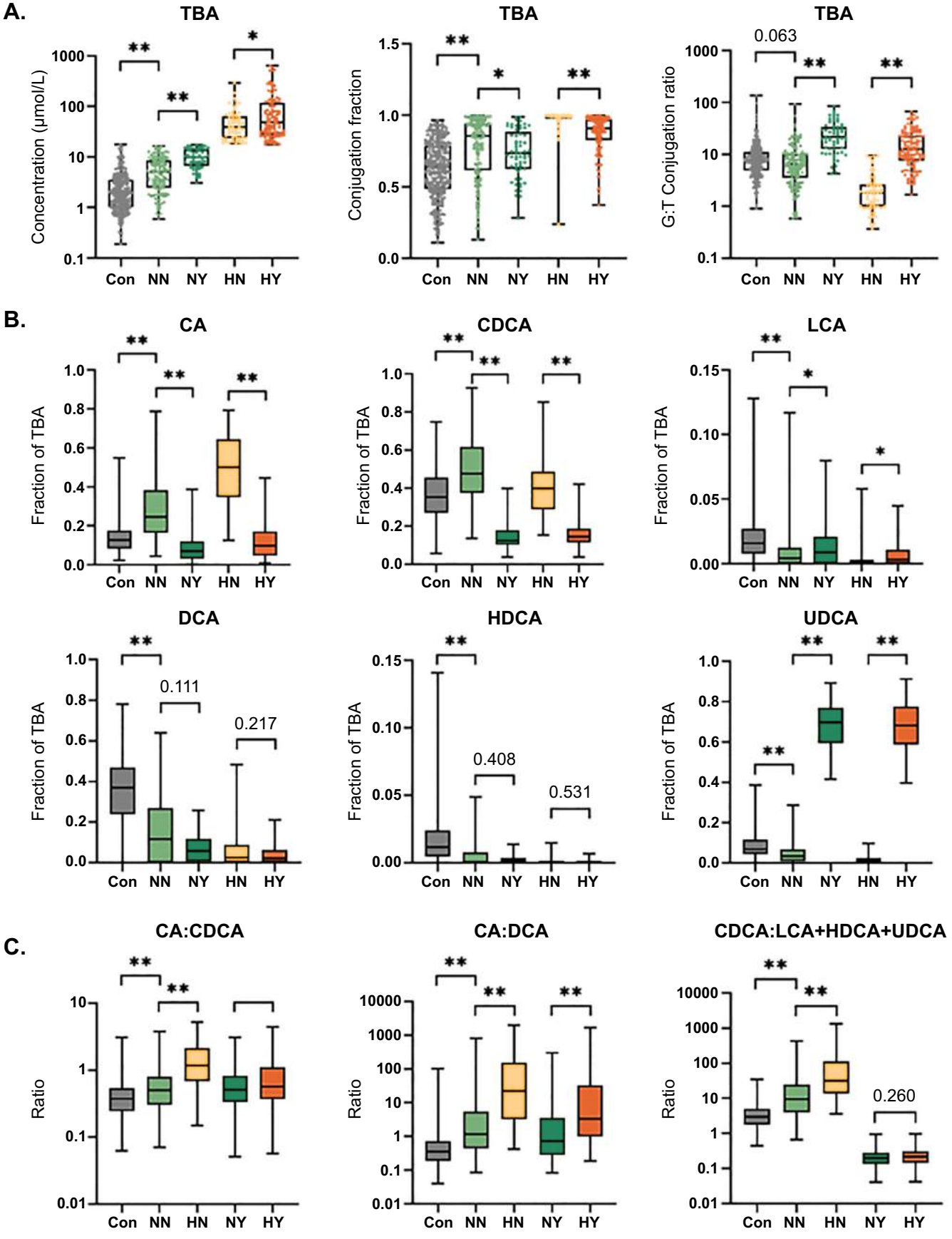
Fig. 5. Univariate analysis of bile acid variables for time-to-event (hepatic decompensation) in PSC. (A) TCDCA, (B) TCA and (C) total bile acids (TBA) were the top 3 variables capable of predicting hepatic decompensation (HD; defined as ascites, esophageal varices or encephalopathy) in PSC over a 5 year window. (D) TBA with UDCA and it's conjugates subtracted performed similar to TBA. These variables outperformed (E) Total bilirubin and (F) alkaline phosphatase expressed as times upper limit of normal (ALPxULN). Cox proportional hazard models were used to evaluate risk

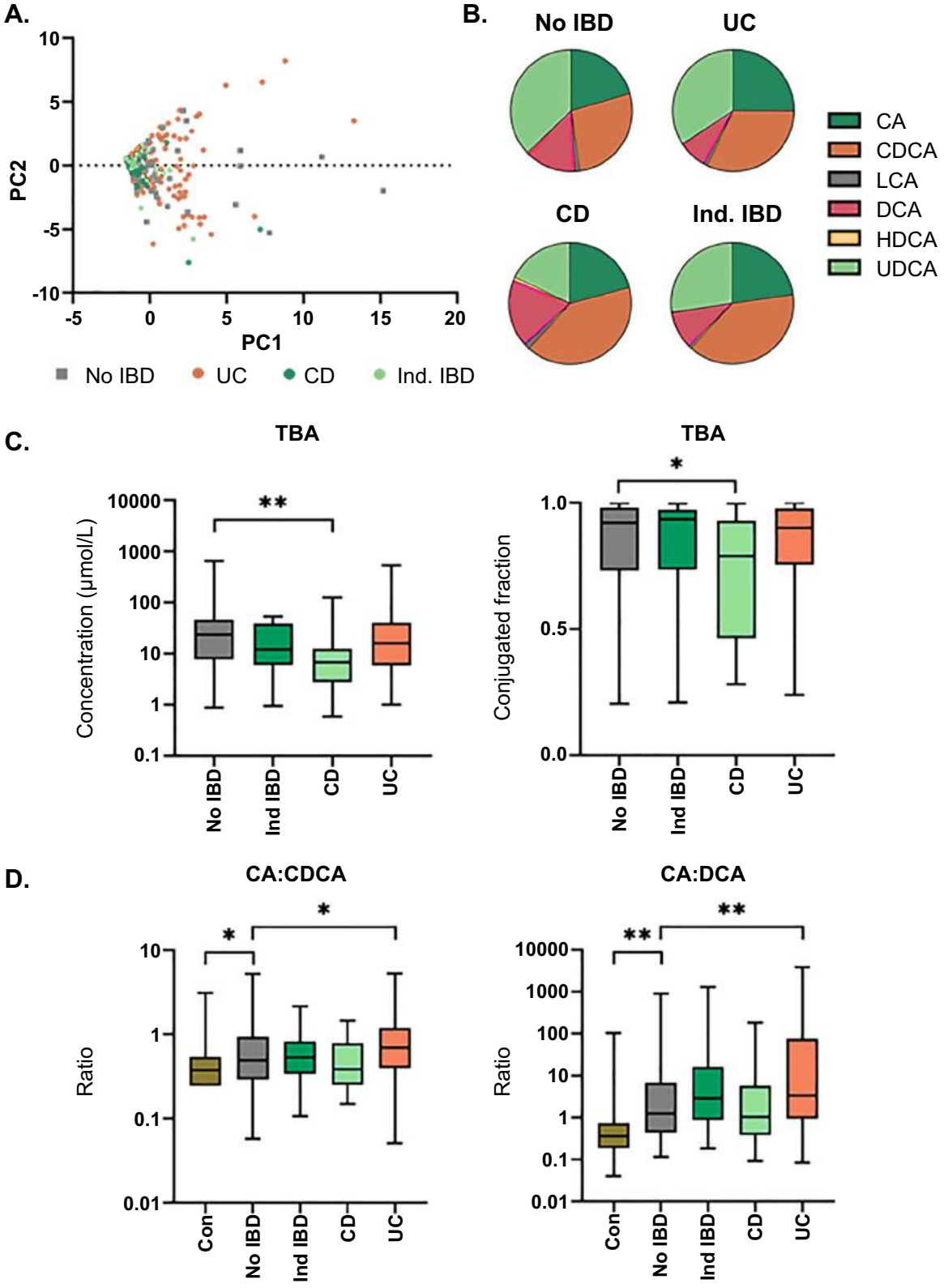
of HD ($p<0.001$ all variables). The Harrell's concordance statistic (c-stat) was used to measure discrimination ability of the variables. Data is presented using Aalen-Johansen curves based on quartiles of variable values, accounting for death and liver transplantation as competing risks.

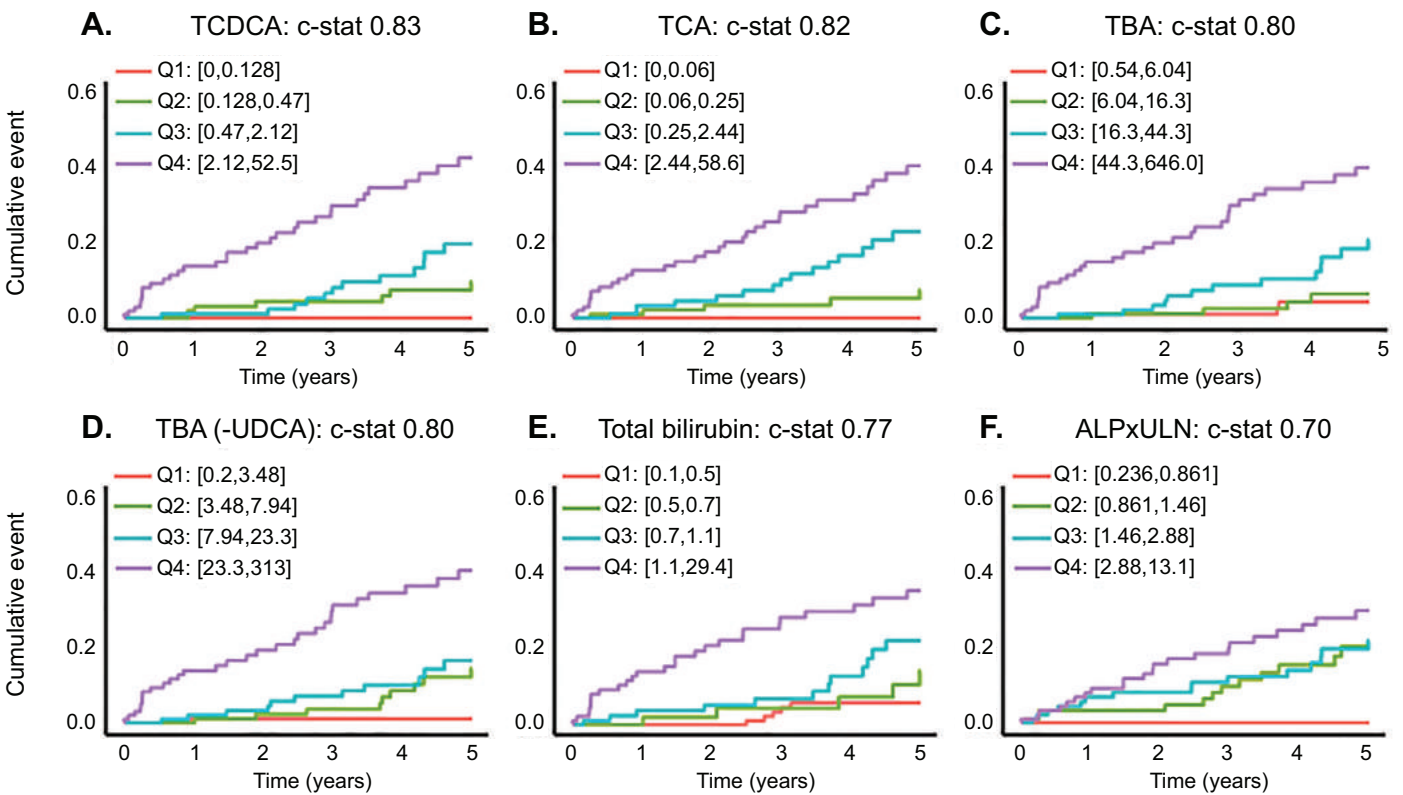
Fig. 6. Predictive model for hepatic decompensation in PSC using machine learning and the bile acid profile: PSC-BAP score. (A) Variables included in the model and their relative importance, (B) derivation cohort and (C) validation cohort model calibration by tertiles (low, medium, high) of predicted risk.

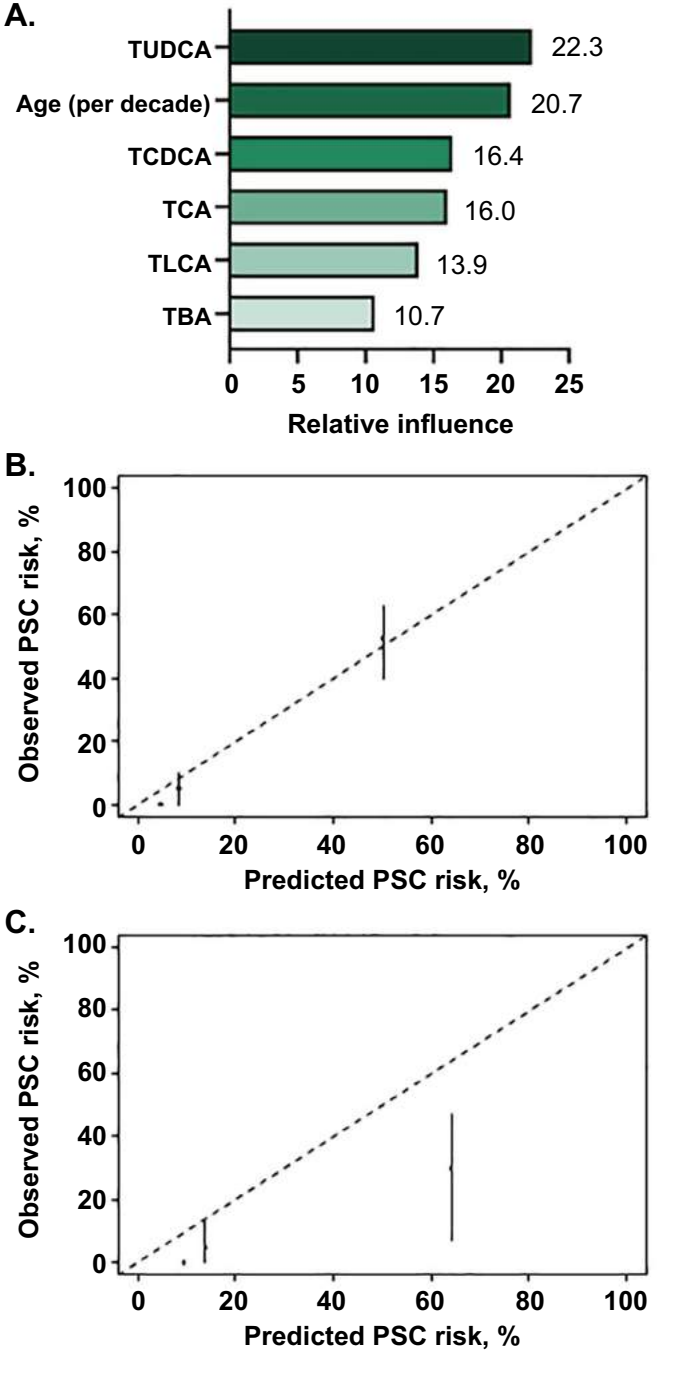

A.
TBA
CA
CDCA
LCA
DCA
HDCA
UDCA
Conc. µmol/L
**
*
0.328
Con PSC
B.
Conjugated fraction
0.106
C.
G:T conjugation ratio
0.320
0.180
0.061
0.667


A.
PSC
Control
CA
CDCA
LCA
DCA
HDCA
UDCA
B.
PSC
Control
PC2
PC1
C.
TBA
UDCA
UDCA / TBA
µmol/L
Fraction
17.79
2.15
0.39
Yes
?
No
Control
Chart UDCA
PSC
D.
UDCA (µmol/L)
TBA (µmol/L)
Con
NN
NY
HN
HY
No call
E.
30.6%
36.4%
16.8%
16.2%
NN: normal BA, no UDCA
NY: normal BA, yes UDCA
HN: high BA, no UDCA
HY: high BA, yes UDCA


A.
TBA
Concentration (μmol/L)
1000
100
10
1
0.1
**
**
*
Con NN NY HN HY
TBA
Conjugation fraction
1.5
1.0
0.5
0.0
**
*
**
Con NN NY HN HY
TBA
G:T Conjugation ratio
1000
100
10
1
0.1
0.063
**
**
Con NN NY HN HY
B.
CA
Fraction of TBA
1.0
0.8
0.6
0.4
0.2
0.0
**
**
**
Con NN NY HN HY
CDCA
Fraction of TBA
1.0
0.8
0.6
0.4
0.2
0.0
**
**
**
Con NN NY HN HY
LCA
Fraction of TBA
0.15
0.10
0.05
0.00
**
*
*
Con NN NY HN HY
DCA
Fraction of TBA
1.0
0.8
0.6
0.4
0.2
0.0
**
0.111
0.217
Con NN NY HN HY
HDCA
Fraction of TBA
0.15
0.10
0.05
0.00
**
0.408
0.531
Con NN NY HN HY
UDCA
Fraction of TBA
1.0
0.8
0.6
0.4
0.2
0.0
**
**
**
Con NN NY HN HY
C.
CA:CDCA
Ratio
10
1
0.1
0.01
**
**
Con NN HN NY HY
CA:DCA
Ratio
10000
1000
100
10
1
0.1
0.01
**
**
**
Con NN HN NY HY
CDCA:LCA+HDCA+UDCA
Ratio
10000
1000
100
10
1
0.1
0.01
**
**
0.260
Con NN HN NY HY


A.
PC2
PC1
No IBD
UC
CD
Ind. IBD
B.
No IBD
UC
CD
Ind. IBD
CA
CDCA
LCA
DCA
HDCA
UDCA
C.
TBA
Concentration (µmol/L)
No IBD
Ind IBD
CD
UC
TBA
Conjugated fraction
D.
CA:CDCA
Ratio
Con
CA:DCA


A.
TCDCA: c-stat 0.83
Q1: [0,0.128]
Q2: [0.128,0.47]
Q3: [0.47,2.12]
Q4: [2.12,52.5]
B.
TCA: c-stat 0.82
Q1: [0,0.06]
Q2: [0.06,0.25]
Q3: [0.25,2.44]
Q4: [2.44,58.6]
C.
TBA: c-stat 0.80
Q1: [0.54,6.04]
Q2: [6.04,16.3]
Q3: [16.3,44.3]
Q4: [44.3,646.0]
D.
TBA (-UDCA): c-stat 0.80
Q1: [0.2,3.48]
Q2: [3.48,7.94]
Q3: [7.94,23.3]
Q4: [23.3,313]
E.
Total bilirubin: c-stat 0.77
Q1: [0.1,0.5]
Q2: [0.5,0.7]
Q3: [0.7,1.1]
Q4: [1.1,29.4]
F.
ALPxULN: c-stat 0.70
Q1: [0.236,0.861]
Q2: [0.861,1.46]
Q3: [1.46,2.88]
Q4: [2.88,13.1]
Cumulative event
0.0
0.2
0.4
0.6
0
1
2
3
4
5
Time (years)


A.
TUDCA
Age (per decade)
TCDCA
TCA
TLCA
TBA
22.3
20.7
16.4
16.0
13.9
10.7
0
5
10
15
20
25
Relative influence
B.
Observed PSC risk, %
Predicted PSC risk, %
C.
Observed PSC risk, %
Predicted PSC risk, %

TABLES

Table 1. Patient Characteristics					
	Derivation			**Validation**	
	Controls	**PSC Patients**		**PSC Patients**	
	(n=302)	(n=400)	**p-value[a]**	(n=108)	**p-value[b]**
Age (yrs), median (IQR)	56.4 (48.5-65.3)	48.4 (33.0-59.7)	<0.001	41.2 (33.3-52.0)	0.006
Sex, %Male	58.3	57.8	0.888	76.9	<0.001
Caucasian Race, %	99.0	96.9	0.398	100	0.495
ALP x ULN, median (IQR)	0.6 (0.5-0.7)	1.5 (0.9-2.9)	<0.001	2.2 (1.2-3.4)	<0.001
Total Bilirubin, median (IQR)	NA	0.7 (0.5-1.1)	-	0.2 (0.1-0.4)	<0.001
Age PSC Diagnosis (yrs), median (IQR)	NA	40.0 (26.8-51.5)	-	34.7 (26.9-47.1)	0.113
PSC Duration (yrs), median (IQR)	NA	4.8 (1.8-9.7)	-	2.2 (0.1-7.9)	<0.001
PSC subtype, n		400		108	
Small duct, n (%)	NA	26 (6.5)	-	0	0.007
AIH overlap, n (%)	NA	14 (3.5)	-	0	0.049
IBD status, n		347		108	
Ulcerative colitis, n (%)	NA	231 (66.6)	-	69 (63.9)	0.615
Crohn's disease, n (%)		26 (7.5)		10 (9.3)	
Indeterminate, n (%)		25 (7.2)		6 (5.6)	
None, n (%)		65 (18.7)		23 (21.3)	
HD Events, n		51		8	
Ascites, n (%)	NA	42 (82.4)	-	8 (100)	0.435
Encephalopathy, n (%)		4 (7.8)		0	
Variceal bleeding, n (%)		5 (9.8)		0	
Censoring Events, n		349		100	
Last encounter, n (%)	NA	321 (92.0)	-	70 (70.0)	<0.001

CCA, n (%)		10 (2.9)		11 (11.0)	
OLT, n (%)		15 (4.3)		19 (19.0)	
Death, n (%)		3 (0.9)		0	

[a]Controls vs. Derivation PSC, [b]Derivation PSC vs. Validation PSC; IQR: interquartile range, ALP x ULN: alkaline phosphatase times upper limit of normal

Abbreviations: PSC: primary sclerosing cholangitis; AIH: autoimmune hepatitis; CCA: cholangiocarcinoma; OLT: orthotopic liver transplantation; IBD: inflammatory bowel disease; HD: hepatic decompensation

Supplementary Material

Bile Acid Profiles in Primary Sclerosing Cholangitis and their Ability to Predict Hepatic Decompensation

Omar Y. Mousa, Brian D. Juran, Bryan M. McCauley, Mette N. Vesterhus, Trine Folseraas, Coleman T. Turgeon, Ahmad H. Ali, Erik M. Schlicht, Elizabeth J. Atkinson, Chang Hu, Denise Harnois, Elizabeth J. Carey, Andrea A. Gossard, Devin Oglesbee, John E. Eaton, Nicholas F. LaRusso, Gregory J. Gores, Tom H. Karlsen, Konstantinos N. Lazaridis

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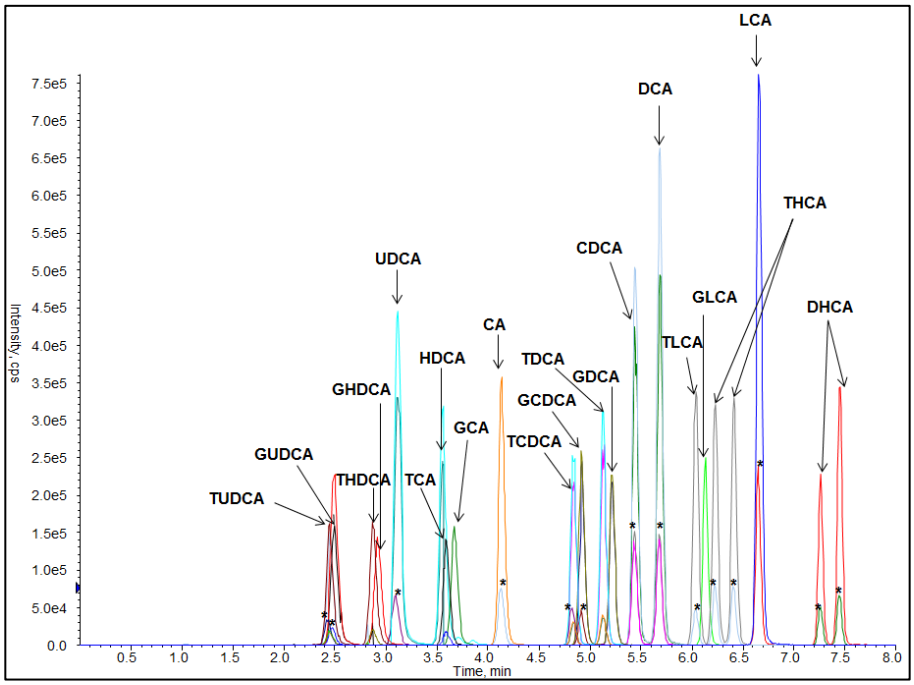
Supplemental Methods: Bile acid profile method and validation

BA Profiling Method

Samples were prepared by precipitating matrix proteins with an acetonitrile/water (50/50) mixture containing deuterated internal standards at appropriate concentrations. Protein precipitate was removed by filtering through an AcroPrep 0.45 µm GHP filter plate. The filtrate was diluted into water/methanol (80/20) solvent for injection into the LC-MS/MS system (ABSciex API 3200 LC-MS/MS with TurboIonSpray source, LEAP HTC Pal Autosampler, and a Shimadzu LC-20 AB Binary Pump) with an inline Poroshell 120 EC-C18, 2.1x50 mm, 2.7 micron column. Reverse-phase liquid chromatography was initiated with 42% mobile phase A (10 mM Ammonium Acetate in 95%Water/5% Methanol) and 58% mobile phase B (10 mM Ammonium Acetate in 99 % Methanol/ 1% Water) at 0.3 ml/min rate with a binary flow over 13 min. The MS/MS instrument collected 37 scheduled MRMs in negative ion mode with a 60 sec target window and a 0.8 sec scan time. Declustering potential (DP) ranged from -75 volts to -115 volts, collision energy potential (CEP) ranged from -20 to -30 volts, collision energy (CE) from -20 to -125 volts, and collision cell exit potential (CXP) ranged from -5 to -45 volts across the method. An example chromatogram is shown in **Methods Figure 1**. Analyte concentrations were derived from calculations in Analyst 1.6.2 and peak area ratios with internal labeled standards.



Methods Figure 1. A chromatogram with each measured BA analyte. Cholic Acid (CA), Chenodeoxycholic Acid (CDCA), Deoxycholic Acid (DCA), Ursodeoxycholic Acid (UDCA), Hyodeoxycholic Acid (HDCA), Lithocholic Acid (LCA), Glycocholic Acid (GCA), Glycochenodeoxycholic Acid (GCDCA), Glycodeoxycholic Acid (GDCA), Glycoursodeoxycholic Acid (GUDCA), Glycohyodeoxycholic Acid (GHDCA), Glycolithocholic Acid (GLCA), Taurocholic Acid (TCA), Taurochenodeoxycholic Acid (TCDCA), Taurodeoxycholic Acid (TDCA), Tauroursodeoxycholic Acid (TUDCA), Taurohyodeoxycholic Acid (THDCA), Taurolithocholic Acid (TLCA), Dihydroxycholestanoic Acid (DHCA), Trihydroxycholestanoic Acid (THCA). The asterisks (*) indicate isotopically labeled internal standards for each analyte with the same retention time. This includes d4-Cholic Acid (CA-d4), d4-Chenodeoxycholic Acid (CDCA-d4), d4-Deoxycholic Acid (DCA-d4), d5-Ursodeoxycholic Acid (UDCA-d5), d4-Lithocholic Acid (LCA-d4), d4-Glycochenodeoxycholic Acid (GCDCA-d4), d5-Glycourosdeoxycholic Acid (GUDCA-d5), d5-Taurochenodeoxycholic Acid (TCDCA-d5),

d5-Taurourosdeoxycholic Acid (TUDCA-d5), d3-Dihydroxycholestanoic Acid (DHCA-d3), and d3-Trihydroxycholestanoic Acid (THCA-d3).

Plasma and Serum Specimen Equivalency

Equivalency between serum and plasma BA analytical performance was shown previously. **Reference**: Max Scherer, Carsten Gnewuch, Gerd Schmitz, Gerhard Liebisch. Rapid quantification of bile acids and their conjugates in serum by liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2009 Nov 15;877(3):3920-3925. PMID: 19819765.

Bile Acid Profile Imprecision

Intra-assay imprecision of BA assay was performed with quality control specimens in matrix at low BA concentrations (QC 1) and high concentrations (QC 2) over 20 replicates in a single batch. Inter-assay imprecision was evaluated by preparing and analyzing a low (QC1) and high (QC2) control across 20 separate runs. The calculated coefficient of variation and mean concentrations for each analyte detected by these methods is shown in **Methods Table 1**.

Methods Table 1. BA profile Intra-assay and Inter-assay Imprecision

	Intra-assay imprecision (N = 20 Specimens)		**Inter-assay imprecision (N = 20 Runs)**	
	QC1	QC2	QC1	QC2
Analyte	CV (mean conc., µM)	CV (mean conc., µM)	CV (mean conc., µM)	CV (mean conc., µM)
LCA	4.8% (0.80)	4.2% (7.69)	9.6% (0.81)	4.5% (7.51)
CDCA	5.8% (1.18)	4.2% (7.90)	21.0 % (1.20)	6.4% (7.53)
DCA	5.1% (1.60)	5.4% (8.43)	11.8% (1.59)	8.2% (8.15)
UDCA	5.0% (0.79)	3.6% (7.07)	9.0% (0.79)	8.4% (7.05)
HDCA	18.5% (0.26)	10.1% (2.50)	23.0% (0.29)	25.8% (2.64)
CA	4.9% (1.07)	3.7% (7.41)	7.4% (1.08)	4.5% (7.37)
GLCA	11.1% (0.31)	11.8% (3.15)	22.8% (0.38)	22.6% (3.79)
GCDCA	5.5% (3.16)	4.5% (9.72)	10.3% (3.22)	8.6% (9.56)
GDCA	11.7% (0.88)	9.4% (5.61)	15.6% (0.91)	17.0% (5.76)
GUDCA	7.0% (0.99)	4.6% (7.44)	9.1% (1.00)	6.9% (7.11)
GHDCA	11.8% (0.31)	11.5% (2.76)	29.2% (0.30)	32.7% (2.54)
GCA	6.2% (1.85)	10.5% (9.32)	10.6% (1.78)	14.2% (8.92)
TLCA	18.6% (0.34)	16.8% (3.80)	41.1% (0.45)	52.1% (5.31)
TCDCA	4.2% (1.21)	4.3% (7.64)	7.6% (1.20)	6.6% (7.39)
TDCA	10.2% (0.41)	9.4% (4.68)	29.1% (0.45)	28.0% (4.73)
TUDCA	4.8% (0.72)	4.2% (6.86)	4.2% (0.72)	5.3% (6.65)
THDCA	11.0% (0.56)	8.4% (6.26)	18.2% (0.49)	27.0% (5.51)
TCA	6.2% (0.92)	11.2% (7.68)	14.7% (0.89)	23.5% (7.40)
DHCA	6.9% (0.82)	4.5% (7.66)	22.2% (0.90)	9.3% (7.11)
THCA	9.0% (1.42)	4.6% (8.24)	12.0% (1.47)	4.8% (8.23)

BA Profile Limit of Detection, Biological Limit of Detection and Linearity

The limit of detection (LOD) was determined by the analysis of 12 reagent blanks. The biological limit of detection (BLD) was determined by the analysis (N = 6) of a single serum specimen spiked with BA analyte concentrations near the LOD and measured in triplicate on two separate runs. Linearity was

assessed by the analysis of 12-point analyte calibration curves (0.00, 0.05, 0.10, 0.20, 0.50, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0, 100.0 µM), 5 times, inter-assay. An 11-point calibration curve with each analytical batch was necessary for BA quantitation as a few conjugated bile acids demonstrated correlation coefficients of 0.96-0.98. The LOD and BLD values and linearity results are shown in **Methods Table 2**.

Methods Table 2. Analytical Sensitivity and Linearity

Analyte	**LOD** (µM)	**BLD** (µM)	**Curve 1** Correlation Coefficient	**Curve 2** Correlation Coefficient	**Curve 3** Correlation Coefficient	**Curve 4** Correlation Coefficient	**Curve 5** Correlation Coefficient
LCA	0.00	0.01	0.99714	0.99910	0.99944	0.99790	0.99819
CDCA	0.00	0.02	0.99861	0.99961	0.99939	0.99925	0.99866
DCA	0.00	0.02	0.99776	0.99819	0.99788	0.99748	0.99828
UDCA	0.01	0.02	0.99974	0.99977	0.99918	0.99958	0.99954
HDCA	0.00	0.01	0.98951	0.99365	0.99310	0.99533	0.99575
CA	0.00	0.00	0.99793	0.99895	0.99870	0.99770	0.99783
GLCA	0.00	0.02	0.99115	0.99709	0.99449	0.99407	0.99757
GCDCA	0.00	0.01	0.99968	0.99974	0.99948	0.99924	0.99972
GDCA	0.00	0.02	0.98578	0.99065	0.98901	0.98844	0.99669
GUDCA	0.02	0.03	0.99950	0.99927	0.99765	0.99896	0.99715
GHDCA	0.00	0.00	0.98449	0.98087	0.98442	0.98278	0.99136
GCA	0.00	0.02	0.99602	0.99848	0.99804	0.99748	0.99924
TLCA	0.01	0.01	0.98667	0.99129	0.98845	0.98842	0.98399
TCDCA	0.00	0.03	0.99796	0.99775	0.99790	0.99726	0.99822
TDCA	0.00	0.03	0.98190	0.99064	0.96426	0.96745	0.98078
TUDCA	0.01	0.03	0.99955	0.99908	0.99938	0.99847	0.99962
THDCA	0.01	0.02	0.99761	0.99440	0.99238	0.99298	0.99269
TCA	0.00	0.03	0.99771	0.99760	0.99499	0.99735	0.99879
DHCA	0.00	0.01	0.97576	0.98129	0.97869	0.97720	0.97848
THCA	0.00	0.23	0.97297	0.98018	0.98044	0.97546	0.97733

BA Profile Specimen Dilution Limit

As patient specimens with BA concentrations above the analytical measurement range (AMR) of 100 µM were expected for certain clinical conditions, dilutions limits for each BA analyte were determined by measuring 2 serum specimens spiked to an approximate concentration of 15 µM for each bile acid. The spiked serum specimens were then diluted 2x, 5x, 10x, 20x, 25x, 40x, and 50x with water, and diluted solutions were carried through the analytical procedure. At a 2-fold dilution, the resulting %-difference from expected concentration was near, or less than, 20%. Beyond a 2-fold dilution, various conjugated bile acids resulted in significant overestimation (greater than 25%) of their original concentration. Therefore, it was concluded that only a 2x specimen dilution would yield acceptable BA calculated concentrations, which increased the effective AMR of the assay to 200 µM for most BAs when completing a specimen dilution.

Use of BA Profile Concentration Values in This Study

As our study is broadly based on comparing BA concentrations between relatively large groups we used the actual reported values for BA concentrations even when they were below the previously detemined LOD or above the AMR. Low values approximate a concentration of zero, which could affect results of

low-abundance bile acids such as the LCAs and HDCAs. Values above the AMR were only found for GUDCA in 4 of the 400 PSC patients and likely do not significantly impact our findings.

Supplementary Table 1. Bile acids in PSC patients and controls.

	Control	PSC	p-value	Con (n)	PSC (n)
Age in years					
Median (IQR)	56.40 (48.49-65.29)	48.44 (32.99-59.65)	<0.001	302	400
Range	19.28-86.12	10.13-80.48			
Sex (% Male)	58.3	57.8	0.888	302	400
Total BA					
Median (IQR)	1.90 (1.02-3.52)	16.34 (6.04-44.31)	<0.001	302	400
Range	0.19-17.79	0.54-646.47			
CA (total)					
Median (IQR)	0.22 (0.11-0.49)	2.09 (0.73-10.01)	<0.001	302	400
Range	0.01-6.43	0.04-224.36			
CA (unconjugated)					
Median (IQR)	0.04 (0.02-0.12)	0.06 (0.03-0.22)	<0.001	302	400
Range	0.00-5.23	0.00-10.65			
GCA					
Median (IQR)	0.12 (0.05-0.25)	1.60 (0.44-6.12)	<0.001	302	400
Range	0.01-5.84	0.01-165.50			
TCA					
Median (IQR)	0.02 (0.01-0.04)	0.25 (0.06-2.44)	<0.001	302	400
Range	0.00-1.50	0.00-58.61			
CDCA (total)					
Median (IQR)	0.63 (0.33-1.39)	4.09 (1.61-9.81)	<0.001	302	400
Range	0.05-10.77	0.10-268.56			
CDCA (unconjugated)					
Median (IQR)	0.07 (0.03-0.23)	0.15 (0.05-0.46)	<0.001	302	400
Range	0.00-5.28	0.00-14.78			
GCDCA					
Median (IQR)	0.42 (0.21-0.88)	2.87 (1.10-6.90)	<0.001	302	400
Range	0.02-5.38	0.02-214.48			
TCDCA					
Median (IQR)	0.05 (0.03-0.11)	0.47 (0.13-2.12)	<0.001	302	400
Range	0.00-2.49	0.00-52.46			
DCA (total)					
Median (IQR)	0.65 (0.36-1.21)	0.66 (0.03-1.76)	0.328	302	400
Range	0.00-8.26)	0.00-24.36			

DCA (unconjugated)					
Median (IQR)	0.33 (0.19-0.58)	0.10 (0.01-0.44)	<0.001	302	400
Range	0.00-4.49	0.00-5.99			

	Control	**PSC**	**p-value**	**Con (n)**	**PSC (n)**
GDCA					
Median (IQR)	0.21 (0.10-0.46)	0.28 (0.02-0.96)	0.339	302	400
Range	0.00-5.94	0.00-15.49			
TDCA					
Median (IQR)	0.04 (0.02-0.08)	0.06 (0.00-0.27)	0.013	302	400
Range	0.00-1.13	0.00-8.55			
LCA (total)					
Median (IQR)	0.03 (0.02-0.05)	0.04 (0.00-0.15)	0.037	302	400
Range	0.00-0.42	0.00-2.85			
LCA (unconjugated)					
Median (IQR)	0.01 (0.01-0.02)	0.01 (0.00-0.04)	0.512	302	400
Range	0.00-0.21	0.00-0.96			
GLCA					
Median (IQR)	0.01 (0.01-0.02)	0.02 (0.00-0.09)	0.002	302	400
Range	0.00-0.19	0.00-2.14			
TLCA					
Median (IQR)	0.00 (0.00-0.01)	0.00 (0.00-0.02)	<0.001	302	400
Range	0.00-0.07	0.00-0.56)			
HDCA (total)					
Median (IQR)	0.03 (0.01-0.05)	0.01 (0.00-0.04)	<0.001	302	400
Range	0.00-0.29	0.00-0.73			
HDCA (unconjugated)					
Median (IQR)	0.02 (0.01-0.03)	0.01 (0.00-0.02)	<0.001	302	400
Range	0.00-0.11	0.00-0.24			
GHDCA					
Median (IQR)	0.00 (0.00-0.01)	0.00 (0.00-0.01)	<0.001	302	400
Range	0.00-0.11	0.00-0.73			
THDCA					
Median (IQR)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.007	302	400
Range	0.00-0.09	0.00-0.07			
UDCA (total)					
Median (IQR)	0.13 (0.07-0.27)	1.95 (0.19-18.27)	<0.001	302	400
Range	0.00-2.15	0.00-537.71			
UDCA (unconjugated)					
Median (IQR)	0.02 (0.01-0.06)	0.17 (0.01-2.02)	<0.001	302	400

Range	0.00-0.92	0.00-52.33			
GUDCA					
Median (IQR)	0.09 (0.05-0.19)	1.53 (0.12-13.52)	<0.001	302	400
Range	0.00-1.78	0.00-472.47			

	Control	PSC	p-value	Con (n)	PSC (n)
TUDCA					
Median (IQR)	0.01 (0.00-0.01)	0.09 (0.01-0.52)	<0.001	302	400
Range	0.00-0.25	0.00-51.06			
Total BA (conj. frac.)					
Median (IQR)	0.64 (0.49-0.79)	0.91 (0.74-0.98)	<0.001	302	400
Range	0.11-0.97	0.13-1.00			
CA (conj. frac.)					
Median (IQR)	0.75 (0.53-0.89)	0.97 (0.85-0.99)	<0.001	302	400
Range	0.21-1.00	0.09-1.00			
CDCA (conj. frac.)					
Median (IQR)	0.87 (0.70-0.94)	0.95 (0.83-0.99)	<0.001	302	400
Range	0.19-1.00	0.12-1.00			
DCA (conj. frac.)					
Median (IQR)	0.44 (0.29-0.60)	0.80 (0.52-0.95)	<0.001	299	345
Range	0.00-1.00	0.00-1.00			
LCA (conj. frac.)					
Median (IQR)	0.50 (0.40-0.69)	0.75 (0.56-0.87)	<0.001	274	286
Range	0.00-1.00	0.00-1.00			
HDCA (conj. frac.)					
Median (IQR)	0.20 (0.00-0.50)	1.22 (0.00-0.55)	0.106	261	231
Range	0.00-1.00	0.00-1.00			
UDCA (conj. frac.)					
Median (IQR)	0.83 (0.67-0.94)	0.90 (0.75-0.97)	0.001	301	392
Range	0.00-1.00	0.00-1.00			
Total BA (G:T ratio)					
Median (IQR)	7.66 (4.89-11.17)	8.36 (3.57-17.44)	0.320	300	399
Range	0.91-139.00	0.37-93.11			
CA (G:T ratio)					
Median (IQR)	6.00 (3.50-9.00)	5.56 (2.72-9.23)	0.180	263	393
Range	0.83-31.00	0.27-70.33			
CDCA (G:T ratio)					
Median (IQR)	8.00 (5.33-12.14)	5.76 (2.80-10.30)	<0.001	298	399
Range	1.00-100.00	0.36-92.17			
DCA (G:T ratio)					

Median (IQR)	5.50 (3.74-8.00)	4.90 (2.13-8.26)	0.007	272	292
Range	0.50-61.00	0.22-45.00			
LCA (G:T ratio)					
Median (IQR)	2.00 (1.00-4.25)	3.00 (1.40-6.22)	0.061	79	169
Range	0.50-13.00	0.10-36.00			

	Control	**PSC**	**p-value**	**Con (n)**	**PSC (n)**
HDCA (G:T ratio)					
Median (IQR)	1.22 (1.00-3.00)	1.17 (1.00-1.88)	0.666	5	12
Range	1.00-4.00	0.71-11.00			
UDCA (G:T ratio)					
Median (IQR)	10.00 (5.00-18.00)	18.40 (7.25-35.75)	<0.001	177	330
Range	1.00-95.00	0.14-145.00			
CA (frac. BA pool)					
Median (IQR)	0.13 (0.08-0.18)	0.18 (0.08-0.36)	<0.001	302	400
Range	0.02-0.55	0.01-0.79			
CDCA (frac. BA pool)					
Median (IQR)	0.35 (0.27-0.45)	0.26 (0.15-0.46)	<0.001	302	400
Range	0.06-0.75	0.04-0.93			
DCA (frac. BA pool)					
Median (IQR)	0.37 (0.24-0.47)	0.04 (0.01-0.14)	<0.001	302	400
Range	0.00-0.78	0.00-0.64			
LCA (frac. BA pool)					
Median (IQR)	0.02 (0.01-0.03)	0.01 (0.00-0.01)	<0.001	302	400
Range	0.00-0.13	0.00-0.12			
HDCA (frac. BA pool)					
Median (IQR)	0.01 (0.01-0.02)	0.00 (0.00-0.01)	<0.001	302	400
Range	0.00-0.14	0.00-0.05			
UDCA (frac. BA pool)					
Median (IQR)	0.07 (0.04-0.12)	0.18 (0.02-0.66)	<0.001	302	400
Range	0.00-0.39	0.00-0.91			
CA:CDCA ratio					
Median (IQR)	0.38 (0.24-0.54)	0.60 (0.35-1.12)	<0.001	302	400
Range	0.06-3.10	0.05-5.26			
CA:DCA ratio					
Median (IQR)	0.36 (0.19-0.73)	2.60 (0.79-26.43)	<0.001	299	345
Range	0.04-103.00	0.09-3796.67			
CDCA:LCA+HDCA+UDCA					
Median (IQR)	3.00 (1.78-5.07)	1.57 (0.22-13.81)	0.001	302	392
Range	0.44-35.00	0.04-1310.00			

Supplementary Table 2. Bile acids in NN PSC patients and controls.

	Control	NN PSC	p-value	Con (n)	NN PSC (n)
Age in years					
Median (IQR)	56.40 (48.49-65.29)	51.14 (32.76-61.38)	<0.001	302	137
Range	19.28-86.12	11.82-78.53			
Sex (% Male)	58.3	59.1	0.868	302	137
Total BA					
Median (IQR)	1.90 (1.02-3.52)	4.98 (2.45-8.50)	<0.001	302	137
Range	0.19-17.79	0.59-16.35			
CA (total)					
Median (IQR)	0.22 (0.11-0.49)	1.00 (0.46-2.12)	<0.001	302	137
Range	0.01-6.43	0.04-10.09			
CA (unconjugated)					
Median (IQR)	0.04 (0.02-0.12)	0.07 (0.03-0.23)	<0.001	302	137
Range	0.00-5.23	0.00-2.99			
GCA					
Median (IQR)	0.12 (0.05-0.25)	0.52 (0.27-1.44)	<0.001	302	137
Range	0.01-5.84	0.02-6.74			
TCA					
Median (IQR)	0.02 (0.01-0.04)	0.09 (0.04-0.34)	<0.001	302	137
Range	0.00-1.50	0.00-3.67			
CDCA (total)					
Median (IQR)	0.63 (0.33-1.39)	2.14 (1.22-4.11)	<0.001	302	137
Range	0.05-10.77	0.16-10.18			
CDCA (unconjugated)					
Median (IQR)	0.07 (0.03-0.23)	0.14 (0.05-0.46)	<0.001	302	137
Range	0.00-5.28	0.00-5.23			
GCDCA					
Median (IQR)	0.42 (0.21-0.88)	1.47 (0.69-2.44)	<0.001	302	137
Range	0.02-5.38	0.10-8.29			
TCDCA					
Median (IQR)	0.05 (0.03-0.11)	0.20 (0.08-0.61)	<0.001	302	137
Range	0.00-2.49	0.00-3.25			
DCA (total)					
Median (IQR)	0.65 (0.36-1.21)	0.39 (0.00-1.26)	<0.001	302	137
Range	0.00-8.26)	0.00-7.81			
DCA (unconjugated)					
Median (IQR)	0.33 (0.19-0.58)	0.09 (0.00-0.40)	<0.001	302	137
Range	0.00-4.49	0.00-5.99			

	Control	NN PSC	p-value	Con (n)	NN PSC (n)
GDCA					
Median (IQR)	0.21 (0.10-0.46)	0.13 (0.00-0.56)	0.004	302	137
Range	0.00-5.94	0.00-2.41			
TDCA					
Median (IQR)	0.04 (0.02-0.08)	0.03 (0.00-0.12)	0.067	302	137
Range	0.00-1.13	0.00-1.35			
LCA (total)					
Median (IQR)	0.03 (0.02-0.05)	0.02 (0.00-0.06)	<0.001	302	137
Range	0.00-0.42	0.00-1.18			
LCA (unconjugated)					
Median (IQR)	0.01 (0.01-0.02)	0.00 (0.00-0.02)	<0.001	302	137
Range	0.00-0.21	0.00-0.41			
GLCA					
Median (IQR)	0.01 (0.01-0.02)	0.01 (0.00-0.04)	0.022	302	137
Range	0.00-0.19	0.00-0.71			
TLCA					
Median (IQR)	0.00 (0.00-0.01)	0.00 (0.00-0.00)	0.367	302	137
Range	0.00-0.07	0.00-0.06			
HDCA (total)					
Median (IQR)	0.03 (0.01-0.05)	0.01 (0.00-0.04)	<0.001	302	137
Range	0.00-0.29	0.00-0.41			
HDCA (unconjugated)					
Median (IQR)	0.02 (0.01-0.03)	0.01 (0.00-0.02)	<0.001	302	137
Range	0.00-0.11	0.00-0.24			
GHDCA					
Median (IQR)	0.00 (0.00-0.01)	0.00 (0.00-0.01)	0.002	302	137
Range	0.00-0.11	0.00-0.17			
THDCA					
Median (IQR)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.158	302	137
Range	0.00-0.09	0.00-0.02			
UDCA (total)					
Median (IQR)	0.13 (0.07-0.27)	0.13 (0.03-0.33)	0.280	302	137
Range	0.00-2.15	0.00-1.98			
UDCA (unconjugated)					
Median (IQR)	0.02 (0.01-0.06)	0.01 (0.00-0.06)	0.106	302	137
Range	0.00-0.92	0.00-0.83			
GUDCA					
Median (IQR)	0.09 (0.05-0.19)	0.09 (0.03-0.22)	0.234	302	137

Range	0.00-1.78	0.00-1.71			
	Control	**NN PSC**	**p-value**	**Con (n)**	**NN PSC (n)**
TUDCA					
Median (IQR)	0.01 (0.00-0.01)	0.01 (0.00-0.02)	0.742	302	137
Range	0.00-0.25	0.00-0.17			
Total BA (conj. frac.)					
Median (IQR)	0.64 (0.49-0.79)	0.86 (0.62-0.94)	<0.001	302	137
Range	0.11-0.97	0.13-1.00			
CA (conj. frac.)					
Median (IQR)	0.75 (0.53-0.89)	0.89 (0.67-0.97)	<0.001	302	137
Range	0.02-1.00	0.09-1.00			
CDCA (conj. frac.)					
Median (IQR)	0.87 (0.70-0.94)	0.93 (0.76-0.98)	0.003	302	137
Range	0.19-1.00	0.12-1.00			
DCA (conj. frac.)					
Median (IQR)	0.44 (0.29-0.60)	0.66 (0.38-0.83)	<0.001	299	102
Range	0.00-1.00	0.00-1.00			
LCA (conj. frac.)					
Median (IQR)	0.50 (0.40-0.69)	0.70 (0.50-0.86)	<0.001	274	80
Range	0.00-1.00	0.00-1.00			
HDCA (conj. frac.)					
Median (IQR)	0.20 (0.00-0.50)	0.26 (0.00-0.50)	0.582	261	76
Range	0.00-1.00	0.00-1.00			
UDCA (conj. frac.)					
Median (IQR)	0.83 (0.67-0.94)	0.88 (0.69-0.97)	0.239	301	129
Range	0.00-1.00	0.00-1.00			
Total BA (G:T ratio)					
Median (IQR)	7.66 (4.89-11.17)	6.60 (3.59-10.24)	0.063	300	136
Range	0.91-139.00	0.58-93.11			
CA (G:T ratio)					
Median (IQR)	6.00 (3.50-9.00)	6.00 (3.60-9.38)	0.851	263	135
Range	0.83-31.00	0.50-70.33			
CDCA (G:T ratio)					
Median (IQR)	8.00 (5.33-12.13)	6.53 (3.34-11.17)	0.012	298	136
Range	1.00-100.00	0.38-92.17			
DCA (G:T ratio)					
Median (IQR)	5.50 (3.74-8.00)	5.09 (2.54-8.00)	0.133	272	83
Range	0.50-61.00	0.30-44.50			
LCA (G:T ratio)					

Median (IQR)	2.00 (1.00-4.25)	2.58 (1.08-6.75)	0.363	79	30
Range	0.50-13.00	0.50-17.00			

	Control	**NN PSC**	**p-value**	**Con (n)**	**NN PSC (n)**
HDCA (G:T ratio)					
Median (IQR)	1.22 (1.00-3.00)	3.50 (3.25-3.75)	0.232	5	2
Range	1.00-4.00	3.00-4.00			
UDCA (G:T ratio)					
Median (IQR)	10.00 (5.00-18.00)	10.50 (5.00-22.00)	0.833	177	73
Range	1.00-95.00	0.71-124.00			
CA (frac. BA pool)					
Median (IQR)	0.13 (0.08-0.17)	0.25 (0.17-0.38)	<0.001	302	137
Range	0.02-0.55	0.04-0.79			
CDCA (frac. BA pool)					
Median (IQR)	0.35 (0.27-0.45)	0.48 (0.38-0.61)	<0.001	302	137
Range	0.06-0.75	0.14-0.93			
DCA (frac. BA pool)					
Median (IQR)	0.37 (0.24-0.47)	0.11 (0.00-0.26)	<0.001	302	137
Range	0.00-0.78	0.00-0.64			
LCA (frac. BA pool)					
Median (IQR)	0.02 (0.01-0.03)	0.00 (0.00-0.01)	<0.001	302	137
Range	0.00-0.13	0.00-0.12			
HDCA (frac. BA pool)					
Median (IQR)	0.01 (0.01-0.02)	0.00 (0.00-0.01)	<0.001	302	137
Range	0.00-0.14	0.00-0.05			
UDCA (frac. BA pool)					
Median (IQR)	0.07 (0.04-0.12)	0.03 (0.01-0.07)	<0.001	302	137
Range	0.00-0.39	0.00-0.29			
CA:CDCA ratio					
Median (IQR)	0.38 (0.24-0.54)	0.50 (0.31-0.80)	<0.001	302	137
Range	0.06-3.10	0.07-3.77			
CA:DCA ratio					
Median (IQR)	0.36 (0.19-0.73)	1.16 (0.45-5.25)	<0.001	299	102
Range	0.04-103.00	0.09-822.00			
CDCA:LCA+HDCA+UDCA					
Median (IQR)	3.00 (1.78-5.07)	9.33 (4.00-23.00)	<0.001	302	129
Range	0.44-35.00	0.66-422.00			
Total BA*					
Median (IQR)	1.72 (0.94-3.24)	4.54 (2.37-7.83)	<0.001	302	137
Range	0.19-16.79	0.59-16.10			

Total BA (conj. frac.)*					
Median (IQR)	0.62 (0.47-0.78)	0.85 (0.61-0.94)	<0.001	302	137
Range	0.10-0.97	0.12-1.00			
	Control	**NN PSC**	**p-value**	**Con (n)**	**NN PSC (n)**
Total BA (G:T ratio)*					
Median (IQR)	7.26 (4.62-10.56)	6.43 (3.48-10.00)	0.105	300	136
Range	0.89-119.00	0.58-90.89			
CA (frac. BA pool)*					
Median (IQR)	0.14 (0.09-0.19)	0.25 (0.18-0.39)	<0.001	302	137
Range	0.03-0.56	0.04-0.79			
CDCA (frac. BA pool)*					
Median (IQR)	0.38 (0.29-0.49)	0.51 (0.40-0.65)	<0.001	302	137
Range	0.06-0.91	0.14-0.93			
DCA (frac. BA pool)*					
Median (IQR)	0.41 (0.27-0.53)	0.12 (0.00-0.29)	<0.001	302	137
Range	0.00-0.81	0.00-0.65			
LCA (frac. BA pool)*					
Median (IQR)	0.02 (0.01-0.03)	0.00 (0.00-0.01)	<0.001	302	137
Range	0.00-0.13	0.00-0.12			
HDCA (frac. BA pool)*					
Median (IQR)	0.01 (0.01-0.03)	0.00 (0.00-0.01)	<0.001	302	137
Range	0.00-0.16	0.00-0.06			

*Values with UDCA and its conjugated forms (GUDCA and TUDCA) removed from the analysis;
NN PSC: Normal total bile acids, No UDCA treatment

Supplementary Table 3. Bile acids in NN and NY PSC patients.

	NN PSC	NY PSC	p-value	NN PSC (n)	NY PSC (n)
Age in years					
Median (IQR)	51.14 (32.76-61.38)	43.92 (33.74-54.55)	0.058	137	61
Range	11.82-78.53	10.13-72.71			
Sex (% Male)	59.1	50.8	0.276	137	61
Total BA					
Median (IQR)	4.98 (2.45-8.50)	9.79 (6.73-14.02)	<0.001	137	61
Range	0.59-16.35	3.06-17.51			
CA (total)					
Median (IQR)	1.00 (0.46-2.12)	0.60 (0.31-1.28)	0.002	137	61
Range	0.04-10.09	0.04-5.47			
CA (unconjugated)					
Median (IQR)	0.07 (0.03-0.23)	0.04 (0.02-0.15)	0.018	137	61
Range	0.00-2.99	0.00-0.90			
GCA					
Median (IQR)	0.52 (0.27-1.44)	0.35 (0.18-0.88)	0.032	137	61
Range	0.02-6.74	0.03-4.77			
TCA					
Median (IQR)	0.09 (0.04-0.34)	0.05 (0.02-0.13)	<0.001	137	61
Range	0.00-3.67	0.00-0.66			
CDCA (total)					
Median (IQR)	2.14 (1.22-4.11)	1.19 (0.73-1.92)	<0.001	137	61
Range	0.16-10.18	0.18-4.58			
CDCA (unconjugated)					
Median (IQR)	0.14 (0.05-0.46)	0.17 (0.07-0.44)	0.688	137	61
Range	0.00-5.23	0.01-1.73			
GCDCA					
Median (IQR)	1.47 (0.69-2.44)	0.85 (0.50-1.24)	<0.001	137	61
Range	0.10-8.29	0.10-3.39			
TCDCA					
Median (IQR)	0.20 (0.08-0.61)	0.07 (0.04-0.18)	<0.001	137	61
Range	0.00-3.25	0.01-0.79			
DCA (total)					
Median (IQR)	0.39 (0.00-1.26)	0.62 (0.09-1.22)	0.353	137	61
Range	0.00-7.81	0.00-3.35			
DCA (unconjugated)					
Median (IQR)	0.09 (0.00-0.40)	0.28 (0.04-0.56)	0.031	137	61
Range	0.00-5.99	0.00-1.32			

	NN PSC	NY PSC	p-value	NN PSC (n)	NY PSC (n)
GDCA					
Median (IQR)	0.13 (0.00-0.56)	0.19 (0.04-0.50)	0.526	137	61
Range	0.00-2.41	0.00-2.10			
TDCA					
Median (IQR)	0.03 (0.00-0.12)	0.02 (0.00-0.08)	0.514	137	61
Range	0.00-1.35	0.00-0.50			
LCA (total)					
Median (IQR)	0.02 (0.00-0.06)	0.09 (0.00-0.19)	<0.001	137	61
Range	0.00-1.18	0.00-0.53			
LCA (unconjugated)					
Median (IQR)	0.00 (0.00-0.02)	0.03 (0.00-0.07)	<0.001	137	61
Range	0.00-0.41	0.00-0.23			
GLCA					
Median (IQR)	0.01 (0.00-0.04)	0.04 (0.00-0.10)	<0.001	137	61
Range	0.00-0.71	0.00-0.28			
TLCA					
Median (IQR)	0.00 (0.00-0.00)	0.00 (0.00-0.01)	0.010	137	61
Range	0.00-0.06	0.00-0.07			
HDCA (total)					
Median (IQR)	0.01 (0.00-0.04)	0.02 (0.00-0.05)	0.316	137	61
Range	0.00-0.41	0.00-0.14			
HDCA (unconjugated)					
Median (IQR)	0.01 (0.00-0.02)	0.01 (0.00-0.03)	0.501	137	61
Range	0.00-0.24	0.00-0.07			
GHDCA					
Median (IQR)	0.00 (0.00-0.01)	0.00 (0.00-0.01)	0.292	137	61
Range	0.00-0.17	0.00-0.10			
THDCA					
Median (IQR)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.098	137	61
Range	0.00-0.02	0.00-0.00			
UDCA (total)					
Median (IQR)	0.13 (0.03-0.33)	6.35 (4.51-9.24)	<0.001	137	61
Range	0.00-1.98	2.15-14.82			
UDCA (unconjugated)					
Median (IQR)	0.01 (0.00-0.06)	1.18 (0.60-2.54)	<0.001	137	61
Range	0.00-0.83	0.07-8.58			
GUDCA					
Median (IQR)	0.09 (0.03-0.22)	4.82 (2.79-6.50)	<0.001	137	61
Range	0.00-1.71	1.36-9.59			

	NN PSC	NY PSC	p-value	NN PSC (n)	NY PSC (n)
TUDCA					
Median (IQR)	0.01 (0.00-0.02)	0.09 (0.06-0.18)	<0.001	137	61
Range	0.00-0.17	0.02-0.92			
Total BA (conj. frac.)					
Median (IQR)	0.86 (0.62-0.94)	0.74 (0.63-0.87)	0.015	137	61
Range	0.13-1.00	0.28-0.99			
CA (conj. frac.)					
Median (IQR)	0.89 (0.67-0.97)	0.90 (0.65-0.98)	0.803	137	61
Range	0.09-1.00	0.27-1.00			
CDCA (conj. frac.)					
Median (IQR)	0.93 (0.76-0.98)	0.82 (0.69-0.94)	0.015	137	61
Range	0.12-1.00	0.37-0.99			
DCA (conj. frac.)					
Median (IQR)	0.66 (0.38-0.83)	0.45 (0.29-0.63)	0.006	102	64
Range	0.00-1.00	0.00-1.00			
LCA (conj. frac.)					
Median (IQR)	0.70 (0.50-0.86)	0.58 (0.49-0.75)	0.026	80	44
Range	0.00-1.00	0.25-1.00			
HDCA (conj. frac.)					
Median (IQR)	0.26 (0.00-0.50)	0.22 (0.00-0.57)	0.659	76	36
Range	0.00-1.00	0.00-1.00			
UDCA (conj. frac.)					
Median (IQR)	0.88 (0.69-0.97)	0.77 (0.65-0.88)	0.007	129	61
Range	0.00-1.00	0.27-0.99			
Total BA (G:T ratio)					
Median (IQR)	6.60 (3.59-10.24)	21.59 (12.74-33.80)	<0.001	136	61
Range	0.58-93.11	4.30-85.82			
CA (G:T ratio)					
Median (IQR)	6.00 (3.60-9.38)	8.62 (5.64-12.70)	<0.001	135	57
Range	0.50-70.33	2.59-26.00			
CDCA (G:T ratio)					
Median (IQR)	6.53 (3.34-11.17)	9.17 (6.35-13.78)	0.002	136	61
Range	0.38-92.17	1.58-35.11			
DCA (G:T ratio)					
Median (IQR)	5.09 (2.54-8.00)	8.31 (5.00-10.81)	<0.001	83	44
Range	0.30-44.50	2.00-45.00			
LCA (G:T ratio)					
Median (IQR)	2.58 (1.08-6.75)	5.60 (4.18-8.67)	0.035	30	23
Range	0.50-17.00	0.67-22.00			

	NN PSC	NY PSC	p-value	NN PSC (n)	NY PSC (n)
HDCA (G:T ratio)					
Median (IQR)	3.50 (3.25-3.75)	na	na	2	0
Range	3.00-4.00	na			
UDCA (G:T ratio)					
Median (IQR)	10.50 (5.00-22.00)	46.45 (29.94-64.75)	<0.001	73	61
Range	0.71-124.00	7.09-145.00			
CA (frac. BA pool)					
Median (IQR)	0.25 (0.17-0.38)	0.07 (0.03-0.12)	<0.001	137	61
Range	0.04-0.79	0.00-0.39			
CDCA (frac. BA pool)					
Median (IQR)	0.48 (0.38-0.61)	0.12 (0.10-0.17)	<0.001	137	61
Range	0.14-0.93	0.04-0.40			
DCA (frac. BA pool)					
Median (IQR)	0.11 (0.00-0.26)	0.06 (0.01-0.12)	0.111	137	61
Range	0.00-0.64	0.00-0.26			
LCA (frac. BA pool)					
Median (IQR)	0.00 (0.00-0.01)	0.01 (0.00-0.02)	0.026	137	61
Range	0.00-0.12	0.00-0.08			
HDCA (frac. BA pool)					
Median (IQR)	0.00 (0.00-0.01)	0.00 (0.00-0.00)	0.408	137	61
Range	0.00-0.05	0.00-0.01			
UDCA (frac. BA pool)					
Median (IQR)	0.03 (0.01-0.07)	0.70 (0.59-0.77)	<0.001	137	61
Range	0.00-0.29	0.42-0.89			
CA:CDCA ratio					
Median (IQR)	0.50 (0.31-0.80)	0.51 (0.33-0.75)	0.913	137	61
Range	0.07-3.77	0.05-3.09			
CA:DCA ratio					
Median (IQR)	1.16 (0.45-5.25)	0.73 (0.29-3.27)	0.092	102	54
Range	0.09-822.00	0.09-305.00			
CDCA:LCA+HDCA+UDCA					
Median (IQR)	9.33 (4.00-23.00)	0.20 (0.14-0.27)	<0.001	129	61
Range	0.66-422.00	0.04-0.94			
Total BA*					
Median (IQR)	4.54 (2.37-7.83)	2.93 (1.87-4.28)	0.001	137	61
Range	0.59-16.10	0.61-8.92			
Total BA (conj. frac.)*					
Median (IQR)	0.85 (0.61-0.94)	0.71 (0.54-0.86)	0.005	137	61
Range	0.12-1.00	0.29-0.99			

	Control	NN PSC	p-value	NN PSC (n)	NY PSC (n)
Total BA (G:T ratio)*					
Median (IQR)	6.43 (3.48-10.00)	9.33 (6.65-13.92)	<0.001	136	61
Range	0.58-90.89	2.54-36.50			
CA (frac. BA pool)*					
Median (IQR)	0.25 (0.18-0.39)	0.24 (0.12-0.32)	0.131	136	61
Range	0.04-0.79	0.02-0.73			
CDCA (frac. BA pool)*					
Median (IQR)	0.51 (0.40-0.65)	0.44 (0.30-0.56)	0.010	136	61
Range	0.14-0.93	0.16-0.86			
DCA (frac. BA pool)*					
Median (IQR)	0.12 (0.00-0.29)	0.24 (0.03-0.41)	0.008	136	61
Range	0.00-0.65	0.00-0.58			
LCA (frac. BA pool)*					
Median (IQR)	0.00 (0.00-0.01)	0.03 (0.00-0.06)	<0.001	136	61
Range	0.00-0.12	0.00-0.28)			
HDCA (frac. BA pool)*					
Median (IQR)	0.00 (0.00-0.01)	0.01 (0.00-0.01)	0.098	136	61
Range	0.00-0.06	0.00-0.05			

*Values with UDCA and its conjugated forms (GUDCA and TUDCA) removed from the analysis;
NN PSC: Normal total bile acid, No UDCA treatment; NY PSC: Normal total bile acid, Yes UDCA treatment

Supplementary Table 4. Bile acids in HN and HY PSC patients.

	HN PSC	HY PSC	p-value	HN PSC (n)	HY PSC (n)
Age in years					
Median (IQR)	47.50 (32.21-59.69)	49.13 (34.11-60.88)	0.758	63	115
Range	20.31-79.68	10.74-77.56			
Sex (% Male)	54.0	60.9	0.372	63	115
Total BA					
Median (IQR)	38.73 (23.61-63.57)	48.69 (28.84-116.70)	0.022	63	115
Range	18.53-291.62	17.81-646.47			
CA (total)					
Median (IQR)	18.65 (10.26-32.42)	4.20 (1.89-13.54)	<0.001	63	115
Range	3.65-224.36	0.34-192.53			
CA (unconjugated)					
Median (IQR)	0.07 (0.04-0.17)	0.07 (0.03-0.22)	0.701	63	115
Range	0.01-10.65	0.00-5.72			
GCA					
Median (IQR)	11.67 (5.27-17.57)	3.33 (1.56-10.12)	<0.001	63	115
Range	2.44-165.50	0.19-151.29			
TCA					
Median (IQR)	7.60 (2.74-16.16)	0.53 (0.15-2.94)	<0.001	63	115
Range	0.47-58.61	0.00-41.19			
CDCA (total)					
Median (IQR)	14.21 (9.36-24.92)	6.22 (3.96-16.37)	<0.001	63	115
Range	3.53-71.89	1.05-141.73			
CDCA (unconjugated)					
Median (IQR)	0.07 (0.03-0.21)	0.25 (0.08-0.66)	<0.001	63	115
Range	0.00-14.78	0.01-7.27			
GCDCA					
Median (IQR)	7.80 (5.56-16.44)	4.73 (3.04-13.17)	<0.001	63	115
Range	2.16-39.30	0.85-119.44			
TCDCA					
Median (IQR)	5.22 (3.09-10.93)	0.98 (0.35-3.01)	<0.001	63	115
Range	0.35-42.03	0.04-27.78			
DCA (total)					
Median (IQR)	0.87 (0.18-3.19)	1.15 (0.22-2.71)	0.995	63	115
Range	0.00-24.36	0.00-18.32			
DCA (unconjugated)					
Median (IQR)	0.03 (0.01-0.24)	0.13 (0.01-0.51)	0.006	63	115

Range	0.00-2.63	0.00-2.92			
	HN PSC	**HY PSC**	**p-value**	**HN PSC (n)**	**HY PSC (n)**
GDCA					
Median (IQR)	0.40 (0.08-1.84)	0.78 (0.14-1.82)	0.458	63	115
Range	0.00-14.96	0.00-15.49			
TDCA					
Median (IQR)	0.34 (0.08-1.08)	0.15 (0.02-0.34)	0.001	63	115
Range	0.00-8.55	0.00-3.41			
LCA (total)					
Median (IQR)	0.04 (0.02-0.12)	0.18 (0.02-0.66)	0.002	63	115
Range	0.00-1.15	0.00-2.85			
LCA (unconjugated)					
Median (IQR)	0.01 (0.00-0.02)	0.05 (0.00-0.15)	<0.001	63	115
Range	0.00-0.67	0.00-0.96			
GLCA					
Median (IQR)	0.02 (0.01-0.06)	0.09 (0.01-0.36)	<0.001	63	115
Range	0.00-0.31	0.00-2.14			
TLCA					
Median (IQR)	0.02 (0.01-0.04)	0.01 (0.00-0.08)	0.951	63	115
Range	0.00-0.17	0.00-0.56			
HDCA (total)					
Median (IQR)	0.01 (0.00-0.03)	0.01 (0.00-0.06)	0.358	63	115
Range	0.00-0.28	0.00-0.73			
HDCA (unconjugated)					
Median (IQR)	0.01 (0.00-0.01)	0.00 (0.00-0.03)	0.328	63	115
Range	0.00-0.11	0.00-0.15			
GHDCA					
Median (IQR)	0.00 (0.00-0.01)	0.00 (0.00-0.01)	0.662	63	115
Range	0.00-0.17	0.00-0.73			
THDCA					
Median (IQR)	0.00 (0.00-0.00)	0.00-0.00	0.100	63	115
Range	0.00-0.07	0.00-0.03			
UDCA (total)					
Median (IQR)	0.45 (0.15-0.91)	31.10 (19.71-63.93)	<0.001	63	115
Range	0.01-2.03	8.87-537.71			
UDCA (unconjugated)					
Median (IQR)	0.01 (0.00-0.03)	3.87 (1.56-6.83)	<0.001	63	115
Range	0.00-0.36	0.24-52.33			
GUDCA					

Median (IQR)	0.29 (0.08-0.73)	27.12 (14.82-52.66)	<0.001	63	115
Range	0.01-1.71	2.72-472.47			

	HN PSC	HY PSC	p-value	HN PSC (n)	HY PSC (n)
TUDCA					
Median (IQR)	0.07 (0.02-0.20)	1.20 (0.49-3.75)	<0.001	63	115
Range	0.00-0.68	0.09-51.06			
Total BA (conj. frac.)					
Median (IQR)	0.99 (0.98-1.00)	0.91 (0.82-0.97)	<0.001	63	115
Range	0.24-1.00	0.37-1.00			
CA (conj. frac.)					
Median (IQR)	1.00 (0.99-1.00)	0.99 (0.91-1.00)	<0.001	63	115
Range	0.24-1.00	0.18-1.00			
CDCA (conj. frac.)					
Median (IQR)	1.00 (0.99-1.00)	0.97 (0.90-0.99)	<0.001	63	115
Range	0.24-1.00	0.39-1.00			
DCA (conj. frac.)					
Median (IQR)	0.97 (0.93-0.99)	0.85 (0.69-0.94)	<0.001	61	107
Range	0.67-1.00	0.00-1.00			
LCA (conj. frac.)					
Median (IQR)	0.87 (0.80-1.00)	0.74 (0.63-0.84)	<0.001	55	93
Range	0.33-1.00	0.00-1.00			
HDCA (conj. frac.)					
Median (IQR)	0.41 (0.00-0.67)	0.11 (0.00-0.61)	0.516	37	71
Range	0.00-1.00	0.00-1.00			
UDCA (conj. frac.)					
Median (IQR)	0.98 (0.92-1.00)	0.90 (0.79-0.97)	<0.001	63	115
Range	0.42-1.00	0.28-1.00			
Total BA (G:T ratio)					
Median (IQR)	1.81 (0.99-2.62)	12.52 (7.62-23.15)	<0.001	63	115
Range	0.37-9.61	1.67-67.52			
CA (G:T ratio)					
Median (IQR)	1.69 (0.96-2.80)	6.25 (3.93-10.48)	<0.001	63	114
Range	0.27-9.23	0.62-30.00			
CDCA (G:T ratio)					
Median (IQR)	1.76 (0.99-2.83)	6.28 (4.05-10.21)	<0.001	63	115
Range	0.36-10.69	0.69-22.86			
DCA (G:T ratio)					
Median (IQR)	1.27 (0.71-2.13)	6.02 (3.45-9.35)	<0.001	55	94
Range	0.22-11.33	0.59-22.00			

LCA (G:T ratio)					
Median (IQR)	1.00 (0.68-2.94)	4.25 (2.40-7.80)	<0.001	42	65
Range	0.10-11.00	0.89-36.00			

	HN PSC	**HY PSC**	**p-value**	**HN PSC (n)**	**HY PSC (n)**
HDCA (G:T ratio)					
Median (IQR)	1.00 (0.81-1.25)	6.25 (3.88-8.62)	0.044	6	2
Range	0.71-1.40	1.50-11.00			
UDCA (G:T ratio)					
Median (IQR)	3.0 (1.76-6.82)	23.01 (13.57-38.67)	<0.001	58	115
Range	0.14-26.67	3.22-102.82			
CA (frac. BA pool)					
Median (IQR)	0.50 (0.35-0.65)	0.10 (0.05-0.17)	<0.001	63	115
Range	0.13-0.79	0.01-0.44			
CDCA (frac. BA pool)					
Median (IQR)	0.40 (0.29-0.49)	0.14 (0.11-0.19)	<0.001	63	115
Range	0.15-0.85	0.04-0.42			
DCA (frac. BA pool)					
Median (IQR)	0.03 (0.00-0.09)	0.02 (0.00-0.06)	0.217	63	115
Range	0.00-0.48	0.00-0.21			
LCA (frac. BA pool)					
Median (IQR)	0.00 (0.00-0.00)	0.00 (0.00-0.01)	0.021	63	115
Range	0.00-0.06	0.00-0.04			
HDCA (frac. BA pool)					
Median (IQR)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.531	63	115
Range	0.00-0.02	0.00-0.01			
UDCA (frac. BA pool)					
Median (IQR)	0.01 (0.00-0.02)	0.68 (0.59-0.77)	<0.001	63	115
Range	0.00-0.10	0.40-0.91			
CA:CDCA ratio					
Median (IQR)	1.17 (0.69-2.10)	0.57 (0.38-1.09)	<0.001	63	115
Range	0.15-5.17	0.06-4.43			
CA:DCA ratio					
Median (IQR)	21.96 (3.34-129.27)	3.37 (1.03-31.84)	<0.001	61	107
Range	0.42-2002.00	0.19-1681.00			
CDCA:LCA+HDCA+UDCA					
Median (IQR)	31.84 (13.96-109.23)	0.21 (0.15-0.31)	<0.001	63	115
Range	3.62-1310.00	0.004-0.97			
Total BA*					
Median (IQR)	38.02 (22.96-62.69)	14.63 (7.75-36.40)	<0.001	63	115

Range	17.10-290.60	2.41-313.39			
Total BA (conj. frac.)*					
Median (IQR)	0.99 (0.98-1.00)	0.94 (0.86-0.99)	<0.001	63	115
Range	0.24-1.00	0.32-1.00			
	Control	**NN PSC**	**p-value**	**HN PSC (n)**	**HY PSC (n)**
Total BA (G:T ratio)*					
Median (IQR)	1.79 (0.98-2.59)	6.49 (3.96-10.44)	<0.001	63	115
Range	0.37-9.31	0.67-23.86			
CA (frac. BA pool)*					
Median (IQR)	0.50 (0.35-0.65)	0.31 (0.21-0.45)	<0.001	63	115
Range	0.13-0.81	0.05-0.81			
CDCA (frac. BA pool)*					
Median (IQR)	0.41 (0.30-0.50)	0.47 (0.38-0.64)	0.002	63	115
Range	0.16-0.87	0.18-0.93			
DCA (frac. BA pool)*					
Median (IQR)	0.03 (0.00-0.10)	0.09 (0.01-0.23)	0.026	63	115
Range	0.00-0.50	0.00-0.51			
LCA (frac. BA pool)*					
Median (IQR)	0.00 (0.00-0.00)	0.01 (0.00-0.03)	<0.001	63	115
Range	0.00-0.06	0.00-0.20			
HDCA (frac. BA pool)*					
Median (IQR)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.017	63	115
Range	0.00-0.02	0.00-0.0			

*Values with UDCA and its conjugated forms (GUDCA and TUDCA) removed from the analysis;
HN PSC: High total bile acid, No UDCA treatment; HY PSC: High total bile acid, Yes UDCA treatment

Supplementary Table 5. Bile acids in NN and HN PSC patients.

	NN PSC	HN PSC	p-value	NN PSC (n)	HN PSC (n)
Age in years					
Median (IQR)	51.14 (32.76-61.38)	47.50 (32.21-59.69)	0.741	137	63
Range	11.82-78.53	20.31-79.68			
Sex (% Male)	59.1	54.0	0.493	137	63
Total BA					
Median (IQR)	4.98 (2.45-8.50)	38.73 (23.61-63.57)	<0.001	137	63
Range	0.59-16.35	18.53-291.62			
CA (total)					
Median (IQR)	1.00 (0.46-2.12)	18.65 (10.26-32.42)	<0.001	137	63
Range	0.04-10.09	3.65-224.36)			
CA (unconjugated)					
Median (IQR)	0.07 (0.03-0.23)	0.07 (0.04-0.17)	0.937	137	63
Range	0.00-2.99	0.01-10.65			
GCA					
Median (IQR)	0.52 (0.27-1.44)	11.67 (5.27-17.57)	<0.001	137	63
Range	0.02-6.74	2.44-165.50			
TCA					
Median (IQR)	0.09 (0.04-0.34)	7.60 (2.74-16.16)	<0.001	137	63
Range	0.00-3.67	0.47-58.61			
CDCA (total)					
Median (IQR)	2.14 (1.22-4.11)	14.21 (9.36-24.92)	<0.001	137	63
Range	0.16-10.18	3.53-71.89			
CDCA (unconjugated)					
Median (IQR)	0.14 (0.05-0.46)	0.07 (0.03-0.21)	0.025	137	63
Range	0.00-5.23	0.00-14.78			
GCDCA					
Median (IQR)	1.47 (0.69-2.44)	7.80 (5.56-16.44)	<0.001	137	63
Range	0.10-8.29	2.16-39.30			
TCDCA					
Median (IQR)	0.20 (0.08-0.61)	5.22 (3.09-10.93)	<0.001	137	63
Range	0.00-3.25	0.35-42.03			
DCA (total)					
Median (IQR)	0.39 (0.00-1.26)	0.87 (0.18-3.19)	<0.001	137	63
Range	0.00-7.81	0.00-24.36			
DCA (unconjugated)					
Median (IQR)	0.09 (0.00-0.40)	0.03 (0.01-0.24)	0.237	137	63
Range	0.00-5.99	0.00-2.63			

	NN PSC	HN PSC	p-value	NN PSC (n)	HN PSC (n)
GDCA					
Median (IQR)	0.13 (0.00-0.56)	0.40 (0.08-1.84)	<0.001	137	63
Range	0.00-2.41	0.00-14.96			
TDCA					
Median (IQR)	0.03 (0.00-0.12)	0.34 (0.08-1.08)	<0.001	137	63
Range	0.00-1.35	0.00-8.55			
LCA (total)					
Median (IQR)	0.02 (0.00-0.06)	0.04 (0.02-0.12)	<0.001	137	63
Range	0.00-1.18	0.00-1.15			
LCA (unconjugated)					
Median (IQR)	0.00 (0.00-0.02)	0.01 (0.00-0.02)	0.521	137	63
Range	0.00-0.41	0.00-0.67			
GLCA					
Median (IQR)	0.01 (0.00-0.04)	0.02 (0.01-0.06)	0.005	137	63
Range	0.00-0.71	0.00-0.31			
TLCA					
Median (IQR)	0.00 (0.00-0.00)	0.02 (0.01-0.04)	<0.001	137	63
Range	0.00-0.06	0.00-0.17			
HDCA (total)					
Median (IQR)	0.01 (0.00-0.04)	0.01 (0.00-0.03)	0.996	137	63
Range	0.00-0.41	0.00-0.28			
HDCA (unconjugated)					
Median (IQR)	0.01 (0.00-0.02)	0.01 (0.00-0.01)	0.460	137	63
Range	0.00-0.24	0.00-0.11			
GHDCA					
Median (IQR)	0.00 (0.00-0.01)	0.00 (0.00-0.01)	0.747	137	63
Range	0.00-0.17	0.00-0.17			
THDCA					
Median (IQR)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.012	137	63
Range	0.00-0.02	0.00-0.07			
UDCA (total)					
Median (IQR)	0.13 (0.03-0.33)	0.45 (0.15-0.91)	<0.001	137	63
Range	0.00-1.98	0.01-2.03			
UDCA (unconjugated)					
Median (IQR)	0.01 (0.00-0.06)	0.01 (0.00-0.03)	0.080	137	63
Range	0.00-0.83	0.00-0.36			
GUDCA					
Median (IQR)	0.09 (0.03-0.22)	0.29 (0.08-0.73)	<0.001	137	63
Range	0.00-1.71	0.01-1.71			

	NN PSC	HN PSC	p-value	NN PSC (n)	HN PSC (n)
TUDCA					
Median (IQR)	0.01 (0.00-0.02)	0.07 (0.02-0.20)	<0.001	137	63
Range	0.00-0.17	0.00-0.68			
Total BA (conj. frac.)					
Median (IQR)	0.86 (0.62-0.94)	0.99 (0.98-1.00)	<0.001	137	63
Range	0.13-1.00	0.24-1.00			
CA (conj. frac.)					
Median (IQR)	0.89 (0.67-0.97)	1.00 (0.99-1.00)	<0.001	137	63
Range	0.09-1.00	0.24-1.00			
CDCA (conj. frac.)					
Median (IQR)	0.93 (0.76-0.98)	1.00 (0.99-1.00)	<0.001	137	63
Range	0.12-1.00	0.24-1.00			
DCA (conj. frac.)					
Median (IQR)	0.66 (0.38-0.83)	0.97 (0.93-0.99)	<0.001	102	61
Range	0.00-1.00	0.67-1.00			
LCA (conj. frac.)					
Median (IQR)	0.70 (0.50-0.86)	0.87 (0.80-1.00)	<0.001	80	55
Range	0.00-1.00	0.33-1.00			
HDCA (conj. frac.)					
Median (IQR)	0.26 (0.00-0.50)	0.41 (0.00-0.67)	0.137	76	37
Range	0.00-1.00	0.00-1.00			
UDCA (conj. frac.)					
Median (IQR)	0.88 (0.69-0.97)	0.98 (0.92-1.00)	<0.001	129	63
Range	0.00-1.00	0.42-1.00			
Total BA (G:T ratio)					
Median (IQR)	6.60 (3.59-10.24)	1.81 (0.99-2.62)	<0.001	136	63
Range	0.58-93.11	0.37-9.61			
CA (G:T ratio)					
Median (IQR)	6.00 (3.60-9.38)	1.69 (0.96-2.80)	<0.001	135	63
Range	0.50-70.33	0.27-9.23			
CDCA (G:T ratio)					
Median (IQR)	6.53 (3.34-11.17)	1.76 (0.99-2.83)	<0.001	136	63
Range	0.38-92.17	0.36-10.69			
DCA (G:T ratio)					
Median (IQR)	5.09 (2.54-8.00)	1.27 (0.71-2.13)	<0.001	83	55
Range	0.30-44.50	0.22-11.33			
LCA (G:T ratio)					
Median (IQR)	2.58 (1.08-6.75)	1.00 (0.68-2.94)	0.004	30	42
Range	0.50-17.00	0.10-11.00			

	NN PSC	HN PSC	p-value	NN PSC (n)	HN PSC (n)
HDCA (G:T ratio)					
Median (IQR)	3.50 (3.25-3.75)	1.00 (0.81-1.25)	0.044	2	6
Range	3.00-4.00	0.71-1.40			
UDCA (G:T ratio)					
Median (IQR)	10.50 (5.00-22.00)	3.00 (1.76-6.82)	<0.001	73	58
Range	0.71-124.00	0.14-26.67			
CA (frac. BA pool)					
Median (IQR)	0.25 (0.17-0.38)	0.50 (0.35-0.64)	<0.001	137	63
Range	0.04-0.79	0.13-0.79			
CDCA (frac. BA pool)					
Median (IQR)	0.48 (0.38-0.61)	0.40 (0.29-0.49)	<0.001	137	63
Range	0.14-0.93	0.15-0.85			
DCA (frac. BA pool)					
Median (IQR)	0.11 (0.00-0.26)	0.03 (0.00-0.09)	0.111	137	63
Range	0.00-0.64	0.00-0.48			
LCA (frac. BA pool)					
Median (IQR)	0.00 (0.00-0.01)	0.00 (0.00-0.00)	0.167	137	63
Range	0.00-0.12	0.00-0.06			
HDCA (frac. BA pool)					
Median (IQR)	0.00 (0.00-0.01)	0.00 (0.00-0.00)	0.005	137	63
Range	0.00-0.05	0.00-0.02			
UDCA (frac. BA pool)					
Median (IQR)	0.03 (0.01-0.07)	0.01 (0.00-0.02)	<0.001	137	63
Range	0.00-0.29	0.00-0.10			
CA:CDCA ratio					
Median (IQR)	0.50 (0.31-0.80)	1.17 (0.69-2.10)	<0.001	137	63
Range	0.07-3.77	0.15-5.17			
CA:DCA ratio					
Median (IQR)	1.16 (0.45-5.25)	21.96 (3.34-129.27)	<0.001	102	61
Range	0.09-822.00	0.42-2002.00			
CDCA:LCA+HDCA+UDCA					
Median (IQR)	9.33 (4.00-23.00)	31.84 (13.96-109.2)	<0.001	129	63
Range	0.66-422.00	3.62-1310.00			

NN PSC: Normal total bile acids, No UDCA treatment; HN PSC: High total bile acids, No UDCA treatment

Supplementary Table 6. Bile acids in NY and HY PSC patients.

	NY PSC	HY PSC	p-value	NY PSC (n)	HY PSC (n)
Age in years					
Median (IQR)	43.92 (33.74-54.55)	49.13 (34.11-60.88)	0.088	61	115
Range	10.13-72.71	10.74-77.56			
Sex (% Male)	50.8	60.9	0.199	61	115
Total BA					
Median (IQR)	9.79 (6.73-14.02)	48.69 (28.84-116.7)	<0.001	61	115
Range	3.06-17.51	17.81-646.47			
CA (total)					
Median (IQR)	0.60 (0.31-1.28)	4.20 (1.89-13.54)	<0.001	61	115
Range	0.04-5.47	0.34-192.53			
CA (unconjugated)					
Median (IQR)	0.04 (0.02-0.15)	0.07 (0.03-0.22)	0.061	61	115
Range	0.00-0.90	0.00-5.72			
GCA					
Median (IQR)	0.35 (0.18-0.88)	3.33 (1.56-10.12)	<0.001	61	115
Range	0.03-4.77	0.19-151.29			
TCA					
Median (IQR)	0.05 (0.02-0.13)	0.53 (0.15-2.94)	<0.001	61	115
Range	0.00-0.66	0.00-41.19			
CDCA (total)					
Median (IQR)	1.19 (0.73-1.92)	6.22 (3.96-16.37)	<0.001	61	115
Range	0.18-4.58	1.05-141.73			
CDCA (unconjugated)					
Median (IQR)	0.17 (0.07-0.44)	0.25 (0.08-0.66)	0.109	61	115
Range	0.01-1.73	0.01-7.27			
GCDCA					
Median (IQR)	0.85 (0.50-1.24)	4.73 (3.04-13.17)	<0.001	61	115
Range	0.10-3.39	0.85-119.44			
TCDCA					
Median (IQR)	0.07 (0.04-0.18)	0.98 (0.35-3.01)	<0.001	61	115
Range	0.01-0.79	0.04-27.78			
DCA (total)					
Median (IQR)	0.62 (0.09-1.22)	1.15 (0.22-2.71)	<0.001	61	115
Range	0.00-3.35	0.00-18.32			
DCA (unconjugated)					
Median (IQR)	0.28 (0.04-0.56)	0.13 (0.01-0.51)	0.331	61	115
Range	0.00-1.32	0.00-2.92			

	NY PSC	HY PSC	p-value	NY PSC (n)	HY PSC (n)
GDCA					
Median (IQR)	0.19 (0.04-0.50)	0.78 (0.14-1.82)	<0.001	61	115
Range	0.00-2.10	0.00-15.49			
TDCA					
Median (IQR)	0.02 (0.00-0.08)	0.15 (0.02-0.34)	<0.001	61	115
Range	0.00-0.50	0.00-3.41			
LCA (total)					
Median (IQR)	0.09 (0.00-0.19)	0.18 (0.02-0.66)	0.005	61	115
Range	0.00-0.53	0.00-2.85			
LCA (unconjugated)					
Median (IQR)	0.03 (0.00-0.07)	0.05 (0.00-0.15)	0.060	61	115
Range	0.00-0.23	0.00-0.96			
GLCA					
Median (IQR)	0.04 (0.00-0.10)	0.09 (0.01-0.36)	0.004	61	115
Range	0.00-0.28	0.00-2.14			
TLCA					
Median (IQR)	0.00 (0.00-0.01)	0.01 (0.00-0.08)	<0.001	61	115
Range	0.00-0.07	0.00-0.56			
HDCA (total)					
Median (IQR)	0.02 (0.00-0.05)	0.01 (0.00-0.06)	0.841	61	115
Range	0.00-0.14	0.00-0.73			
HDCA (unconjugated)					
Median (IQR)	0.01 (0.00-0.03)	0.00 (0.00-0.03)	0.978	61	115
Range	0.00-0.07	0.00-0.15			
GHDCA					
Median (IQR)	0.00 (0.00-0.01)	0.00 (0.00-0.01)	0.237	61	115
Range	0.00-0.10	0.00-0.73			
THDCA					
Median (IQR)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.036	61	115
Range	0.00-0.00	0.00-0.03			
UDCA (total)					
Median (IQR)	6.35 (4.51-9.24)	31.10 (19.71-63.93)	<0.001	61	115
Range	2.15-14.82	8.87-537.71			
UDCA (unconjugated)					
Median (IQR)	1.18 (0.60-2.54)	3.87 (1.56-6.83)	<0.001	61	115
Range	0.07-8.58	0.24-52.33			
GUDCA					
Median (IQR)	4.84 (2.79-6.50)	27.12 (14.82-52.66)	<0.001	61	115
Range	1.36-9.59	2.72-472.47			

	NY PSC	HY PSC	p-value	NY PSC (n)	HY PSC (n)
TUDCA					
Median (IQR)	0.09 (0.06-0.18)	1.20 (0.49-3.75)	<0.001	61	115
Range	0.02-0.92	0.09-51.06			
Total BA (conj. frac.)					
Median (IQR)	0.74 (0.63-0.87)	0.91 (0.82-0.97)	<0.001	61	115
Range	0.28-0.99	0.37-1.00			
CA (conj. frac.)					
Median (IQR)	0.90 (0.65-0.98)	0.99 (0.91-1.00)	<0.001	61	115
Range	0.27-1.00	0.18-1.00			
CDCA (conj. frac.)					
Median (IQR)	0.82 (0.69-0.94)	0.97 (0.90-0.99)	<0.001	61	115
Range	0.37-0.99	0.39-1.00			
DCA (conj. frac.)					
Median (IQR)	0.45 (0.29-0.63)	0.85 (0.69-0.94)	<0.001	54	107
Range	0.00-1.00	0.00-1.00			
LCA (conj. frac.)					
Median (IQR)	0.58 (0.49-0.75)	0.74 (0.63-0.84)	<0.001	44	93
Range	0.25-1.00	0.00-1.00			
HDCA (conj. frac.)					
Median (IQR)	0.22 (0.00-0.57)	0.11 (0.00-0.61)	0.986	36	71
Range	0.00-1.00	0.00-1.00			
UDCA (conj. frac.)					
Median (IQR)	0.77 (0.65-0.88)	0.90 (0.79-0.97)	<0.001	61	115
Range	0.27-0.99	0.28-1.00			
Total BA (G:T ratio)					
Median (IQR)	21.59 (12.74-33.80)	12.52 (7.62-23.15)	<0.001	61	115
Range	4.30-85.82	1.67-67.52			
CA (G:T ratio)					
Median (IQR)	8.62 (5.64-12.70)	6.25 (3.93-10.48)	0.016	57	114
Range	2.59-26.00	0.62-30.00			
CDCA (G:T ratio)					
Median (IQR)	9.17 (6.35-13.78)	6.28 (4.05-10.21)	<0.001	61	115
Range	1.58-35.11	0.69-22.86			
DCA (G:T ratio)					
Median (IQR)	8.31 (5.00-10.81)	6.02 (3.45-9.35)	0.011	44	94
Range	2.00-45.00	0.59-22.00			
LCA (G:T ratio)					
Median (IQR)	5.60 (4.18-8.67)	4.25 (2.40-7.80)	0.277	23	65
Range	0.67-22.00	0.89-36.00			

	NY PSC	HY PSC	p-value	NY PSC (n)	HY PSC (n)
HDCA (G:T ratio)					
Median (IQR)	na	6.25 (3.88-8.62)	na	0	2
Range	na	1.50-11.00			
UDCA (G:T ratio)					
Median (IQR)	46.45 (29.94-64.75)	23.01 (13.57-38.67)	<0.001	61	115
Range	7.09-145.00	3.22-102.82			
CA (frac. BA pool)					
Median (IQR)	0.07 (0.03-0.12)	0.10 (0.05-0.17)	0.034	61	115
Range	0.00-0.39	0.01-0.44			
CDCA (frac. BA pool)					
Median (IQR)	0.12 (0.10-0.17)	0.14 (0.11-0.19)	0.186	61	115
Range	0.04-0.40	0.04-0.42			
DCA (frac. BA pool)					
Median (IQR)	0.06 (0.01-0.12)	0.02 (0.00-0.06)	0.003	61	115
Range	0.00-0.26	0.00-0.21			
LCA (frac. BA pool)					
Median (IQR)	0.01 (0.00-0.02)	0.00 (0.00-0.01)	0.034	61	115
Range	0.00-0.08	0.00-0.04			
HDCA (frac. BA pool)					
Median (IQR)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.002	61	115
Range	0.00-0.01	0.00-0.01			
UDCA (frac. BA pool)					
Median (IQR)	0.70 (0.59-0.77)	0.68 (0.59-0.77)	0.873	61	115
Range	0.42-0.89	0.40-0.91			
CA:CDCA ratio					
Median (IQR)	0.51 (0.33-0.75)	0.57 (0.38-1.09)	0.068	61	115
Range	0.05-3.09	0.06-4.43			
CA:DCA ratio					
Median (IQR)	0.73 (0.29-3.27)	3.37 (1.03-31.84)	<0.001	54	107
Range	0.09-305.00	0.19-1681.00			
CDCA:LCA+HDCA+UDCA					
Median (IQR)	0.20 (0.14-0.27)	0.21 (0.15-0.31)	0.260	61	115
Range	0.04-0.94	0.04-0.97			

NY PSC: Normal total bile acids, Yes UDCA treatment; HY PSC: High total bile acids, Yes UDCA treatment

Supplementary Table 7. Bile acids in Control and No IBD PSC patients.

	Control	No IBD PSC	p-value	Con (n)	No IBD PSC (n)
Age in years					
Median (IQR)	56.40 (48.49-65.29)	55.82 (43.32-61.82)	0.032	302	65
Range	19.28-86.12	14.25-79.68			
Sex (% Male)	58.3	49.2	0.182	302	65
Tot BA / UDCA Group					
NN - # (%)	na	19 (29.2)	na	na	65
NY - # (%)	na	10 (15.4)			
HN - # (%)	na	13 (20.0)			
HY - # (%)	na	23 (35.4)			
Total BA					
Median (IQR)	1.90 (1.02-3.52)	23.57 (7.71-45.45)	<0.001	302	65
Range	0.19-17.79	0.88-646.47			
CA (total)					
Median (IQR)	0.22 (0.11-0.49)	1.84 (0.86-8.72)	<0.001	302	65
Range	0.01-6.43	0.13-68.19			
CA (unconjugated)					
Median (IQR)	0.04 (0.02-0.12)	0.05 (0.03-0.12)	0.169	302	65
Range	0.00-5.23	0.00-4.02			
GCA					
Median (IQR)	0.12 (0.05-0.25)	1.11 (0.45-5.13)	<0.001	302	65
Range	0.01-5.84	0.06-53.99			
TCA					
Median (IQR)	0.02 (0.01-0.04)	0.30 (0.07-2.09)	<0.001	302	65
Range	0.00-1.50	0.00-30.29			
CDCA (total)					
Median (IQR)	0.63 (0.33-1.39)	3.85 (2.14-9.80)	<0.001	302	65
Range	0.05-10.77	0.30-141.73			
CDCA (unconjugated)					
Median (IQR)	0.07 (0.03-0.23)	0.16 (0.05-0.47)	<0.001	302	65
Range	0.00-5.28	0.01-7.27			
GCDCA					
Median (IQR)	0.42 (0.21-0.88)	2.99 (1.32-6.33)	<0.001	302	65
Range	0.02-5.38	0.18-119.44			
TCDCA					
Median (IQR)	0.05 (0.03-0.11)	0.56 (0.16-2.77)	<0.001	302	65
Range	0.00-2.49	0.02-42.03			

	Control	No IBD PSC	p-value	Con (n)	No IBD PSC (n)
DCA (total)					
Median (IQR)	0.65 (0.36-1.21)	1.43 (0.76-2.23)	<0.001	302	65
Range	0.00-8.26	0.00-24.36			
DCA (unconjugated)					
Median (IQR)	0.33 (0.19-0.58)	0.29 (0.09-0.65)	0.263	302	65
Range	0.00-4.49	0.00-3.63			
GDCA					
Median (IQR)	0.21 (0.10-0.46)	0.64 (0.35-1.07)	<0.001	302	65
Range	0.00-5.94	0.00-15.49			
TDCA					
Median (IQR)	0.04 (0.02-0.08)	0.15 (0.05-0.31)	<0.001	302	65
Range	0.00-1.13	0.00-8.55			
LCA (total)					
Median (IQR)	0.03 (0.02-0.05)	0.10 (0.06-0.21)	<0.001	302	65
Range	0.00-0.42	0.00-2.85			
LCA (unconjugated)					
Median (IQR)	0.01 (0.01-0.02)	0.03 (0.01-0.07)	<0.001	302	65
Range	0.00-0.21	0.00-0.29			
GLCA					
Median (IQR)	0.01 (0.01-0.02)	0.05 (0.02-0.13)	<0.001	302	65
Range	0.00-0.19	0.00-2.14			
TLCA					
Median (IQR)	0.00 (0.00-0.01)	0.01 (0.00-0.03)	<0.001	302	65
Range	0.00-0.07	0.00-0.42			
HDCA (total)					
Median (IQR)	0.03 (0.01-0.05)	0.02 (0.00-0.05)	0.322	302	65
Range	0.00-0.29	0.00-0.17			
HDCA (unconjugated)					
Median (IQR)	0.02 (0.01-0.03)	0.02 (0.00-0.04)	0.448	302	65
Range	0.00-0.11	0.00-0.13			
GHDCA					
Median (IQR)	0.00 (0.00-0.01)	0.00 (0.00-0.00)	0.003	302	65
Range	0.00-0.11	0.00-0.06			
THDCA					
Median (IQR)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	<0.001	302	65
Range	0.00-0.09	0.00-0.07			
UDCA (total)					
Median (IQR)	0.13 (0.07-0.27)	3.33 (0.29-18.37)	<0.001	302	65

Range	0.00-2.15	0.02-537.71			
	Control	**No IBD PSC**	**p-value**	**Con (n)**	**No IBD PSC (n)**
UDCA (unconjugated)					
Median (IQR)	0.02 (0.01-0.06)	0.12 (0.02-2.29)	<0.001	302	65
Range	0.00-0.92	0.00-52.33			
GUDCA					
Median (IQR)	0.09 (0.05-0.19)	1.73 (0.24-15.78)	<0.001	302	65
Range	0.00-1.78	0.01-472.47)			
TUDCA					
Median (IQR)	0.01 (0.00-0.01)	0.14 (0.03-0.52)	<0.001	302	65
Range	0.00-0.25	0.00-35.47			
Total BA (conj. frac.)					
Median (IQR)	0.64 (0.49-0.79)	0.92 (0.74-0.98)	<0.001	302	65
Range	0.11-0.97	0.20-1.00			
CA (conj. frac.)					
Median (IQR)	0.75 (0.53-0.89)	0.97 (0.83-1.00)	<0.001	302	65
Range	0.02-1.00	0.09-1.00			
CDCA (conj. frac.)					
Median (IQR)	0.87 (0.70-0.94)	0.96 (0.79-0.99)	<0.001	302	65
Range	0.19-1.00	0.29-1.00			
DCA (conj. frac.)					
Median (IQR)	0.44 (0.29-0.60)	0.78 (0.52-0.93)	<0.001	299	63
Range	0.00-1.00	0.18-1.00			
LCA (conj. frac.)					
Median (IQR)	0.50 (0.40-0.69)	0.71 (0.54-0.80)	<0.001	274	62
Range	0.00-1.00	0.00-1.00			
HDCA (conj. frac.)					
Median (IQR)	0.20 (0.00-0.50)	0.00 (0.00-0.41)	0.275	261	48
Range	0.00-1.00	0.00-1.00			
UDCA (conj. frac.)					
Median (IQR)	0.83 (0.67-0.94)	0.90 (0.75-0.97)	0.068	301	65
Range	0.00-1.00	0.28-1.00			
Total BA (G:T ratio)					
Median (IQR)	7.66 (4.89-11.17)	8.09 (2.80-18.65)	0.959	300	65
Range	0.91-139.00	0.62-69.38			
CA (G:T ratio)					
Median (IQR)	6.00 (3.50-9.00)	5.12 (2.33-9.54)	0.171	263	64
Range	0.83-31.00	0.50-27.00			
CDCA (G:T ratio)					

Median (IQR) Range	8.00 (5.33-12.13) 1.00-100.00	5.43 (2.37-9.74) 0.67-35.50	<0.001	298	65

	Control	No IBD PSC	p-value	Con (n)	No IBD PSC (n)
DCA (G:T ratio) Median (IQR) Range	5.50 (3.74-8.00) 0.50-61.00	5.09 (2.17-8.60) 0.50-45.00	0.263	272	61
LCA (G:T ratio) Median (IQR) Range	2.00 (1.00-4.25) 0.50-13.00	3.00 (1.50-7.00) 0.50-36.00	0.062	79	37
HDCA (G:T ratio) Median (IQR) Range	1.22 (1.00-3.00) 1.00-4.00	0.71 (0.71-0.71) 0.71-0.71	0.137	5	1
UDCA (G:T ratio) Median (IQR) Range	10.00 (5.00-18.00) 1.00-95.00	13.81 (6.47-31.96) 0.14-101.14	0.073	177	59
CA (frac. BA pool) Median (IQR) Range	0.13 (0.08-0.17) 0.02-0.55	0.12 (0.05-0.28) 0.01-0.79	0.691	302	65
CDCA (frac. BA pool) Median (IQR) Range	0.35 (0.27-0.45) 0.06-0.75	0.23 (0.13-0.38) 0.04-0.79	<0.001	302	65
DCA (frac. BA pool) Median (IQR) Range	0.37 (0.24-0.47) 0.00-0.78	0.08 (0.03-0.19) 0.00-0.64	<0.001	302	65
LCA (frac. BA pool) Median (IQR) Range	0.02 (0.01-0.03) 0.00-0.13	0.01 (0.00-0.01) 0.00-0.04	<0.001	302	65
HDCA (frac. BA pool) Median (IQR) Range	0.01 (0.01-0.02) 0.00-0.14	0.00 (0.00-0.00) 0.00-0.03	<0.001	302	65
UDCA (frac. BA pool) Median (IQR) Range	0.07 (0.04-0.12) 0.00-0.39	0.43 (0.02-0.70) 0.00-0.89	0.024	302	65
CA:CDCA ratio Median (IQR) Range	0.38 (0.24-0.54) 0.06-3.10	0.51 (0.30-0.91) 0.06-5.17	0.002	302	65
CA:DCA ratio Median (IQR) Range	0.36 (0.19-0.73) 0.04-103.00	1.18 (0.44-5.94) 0.12-698.25	<0.001	299	63

CDCA:LCA+HDCA+UDCA					
Median (IQR)	3.00 (1.78-5.07)	0.48 (0.19-11.13)	0.031	302	65
Range	0.44-35.00	0.05-414.18			

Supplementary Table 8. Bile acids in No IBD and UC PSC patients.

	No IBD PSC	**UC PSC**	**p-value**	**No IBD PSC (n)**	**UC PSC (n)**
Age in years					
Median (IQR)	55.82 (43.32-61.82)	47.61 (31.69-61.33)	0.044	65	231
Range	14.25-79.68	10.74-77.56			
Sex (% Male)	49.2	61.9	0.066	65	231
Tot BA / UDCA Group					
NN - # (%)	19 (29.2)	83 (35.9)	0.706	65	231
NY - # (%)	10 (15.4)	38 (16.5)			
HN - # (%)	13 (20.0)	37 (16.0)			
HY - # (%)	23 (35.4)	73 (31.6)			
Total BA					
Median (IQR)	23.57 (7.71-45.45)	15.93 (5.90-39.39)	0.206	65	231
Range	0.88-646.47	1.00-530.95			
CA (total)					
Median (IQR)	1.84 (0.86-8.72)	2.21 (0.81-8.82)	0.691	65	231
Range	0.13-68.19	0.04-224.36			
CA (unconjugated)					
Median (IQR)	0.05 (0.03-0.12)	0.07 (0.03-0.24)	0.244	65	231
Range	0.00-4.02	0.00-10.65			
GCA					
Median (IQR)	1.11 (0.45-5.13)	1.74 (0.51-5.36)	0.682	65	231
Range	0.06-53.99	0.03-165.50			
TCA					
Median (IQR)	0.30 (0.07-2.09)	0.25 (0.07-1.67)	0.957	65	231
Range	0.00-30.29	0.00-58.61			
CDCA (total)					
Median (IQR)	3.85 (2.14-9.80)	3.91 (1.52-9.02)	0.426	65	231
Range	0.30-141.73	0.29-120.63)			
CDCA (unconjugated)					
Median (IQR)	0.16 (0.05-0.47)	0.14 (0.06-0.38)	0.539	65	231
Range	0.01-7.27	0.00-14.78			
GCDCA					
Median (IQR)	2.99 (1.32-6.33)	2.71 (1.14-6.53)	0.648	65	231
Range	0.18-119.44	0.21-92.80			
TCDCA					

Median (IQR)	0.56 (0.16-2.77)	0.45 (0.13-1.71)	0.360	65	231
Range	0.02-42.03	0.01-35.85			

	No IBD PSC	UC PSC	p-value	No IBD PSC (n)	UC PSC (n)
DCA (total)					
Median (IQR)	1.43 (0.76-2.23)	0.48 (0.01-1.54)	<0.001	65	231
Range	0.00-24.36	0.00-16.23			
DCA (unconjugated)					
Median (IQR)	0.29 (0.09-0.65)	0.06 (0.00-0.41)	<0.001	65	231
Range	0.00-3.63	0.00-5.99			
GDCA					
Median (IQR)	0.64 (0.35-1.07)	0.19 (0.01-0.84)	<0.001	65	231
Range	0.00-15.49	0.00-10.84			
TDCA					
Median (IQR)	0.15 (0.05-0.31)	0.04 (0.00-0.18)	<0.001	65	231
Range	0.00-8.55	0.00-4.02			
LCA (total)					
Median (IQR)	0.10 (0.06-0.21)	0.03 (0.00-0.14)	<0.001	65	231
Range	0.00-2.85	0.00-2.18			
LCA (unconjugated)					
Median (IQR)	0.03 (0.01-0.07)	0.01 (0.00-0.04)	<0.001	65	231
Range	0.00-0.29	0.00-0.88			
GLCA					
Median (IQR)	0.05 (0.02-0.13)	0.01 (0.00-0.08)	<0.001	65	231
Range	0.00-2.14	0.00-1.63			
TLCA					
Median (IQR)	0.01 (0.00-0.03)	0.00 (0.00-0.01)	0.009	65	231
Range	0.00-0.42	0.00-0.43			
HDCA (total)					
Median (IQR)	0.02 (0.00-0.05)	0.01 (0.00-0.04)	0.006	65	231
Range	0.00-0.17	0.00-0.28			
HDCA (unconjugated)					
Median (IQR)	0.02 (0.00-0.04)	0.00 (0.00-0.02)	0.001	65	231
Range	0.00-0.13	0.00-0.14			
GHDCA					
Median (IQR)	0.00 (0.00-0.00)	0.00 (0.00-0.01)	0.372	65	231
Range	0.00-0.06	0.00-0.17			
THDCA					
Median (IQR)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.051	65	231

Range	0.00-0.07	0.00-0.05			
UDCA (total)					
Median (IQR)	3.33 (0.29-18.37)	1.82 (0.13-18.98)	0.163	65	231
Range	0.02-537.71	0.00-355.15			

	No IBD PSC	**UC PSC**	**p-value**	**No IBD PSC (n)**	**UC PSC (n)**
UDCA (unconjugated)					
Median (IQR)	0.12 (0.02-2.29)	0.17 (0.01-2.25)	0.328	65	231
Range	0.00-52.33	0.00-19.26			
GUDCA					
Median (IQR)	1.73 (0.24-15.78)	1.31 (0.09-13.86)	0.200	65	231
Range	0.01-472.47)	0.00-297.47			
TUDCA					
Median (IQR)	0.14 (0.03-0.52)	0.08 (0.01-0.53)	0.072	65	231
Range	0.00-35.47	0.00-51.06			
Total BA (conj. frac.)					
Median (IQR)	0.92 (0.74-0.98)	0.90 (0.76-0.98)	0.855	65	231
Range	0.20-1.00	0.24-1.00			
CA (conj. frac.)					
Median (IQR)	0.97 (0.83-1.00)	0.97 (0.85-0.99)	0.652	65	231
Range	0.09-1.00	0.18-1.00			
CDCA (conj. frac.)					
Median (IQR)	0.96 (0.79-0.99)	0.95 (0.85-0.99)	0.961	65	231
Range	0.29-1.00	0.24-1.00			
DCA (conj. frac.)					
Median (IQR)	0.78 (0.52-0.93)	0.81 (0.55-0.96)	0.307	63	187
Range	0.18-1.00	0.00-1.00			
LCA (conj. frac.)					
Median (IQR)	0.71 (0.54-0.80)	0.75 (0.57-0.87)	0.089	62	146
Range	0.00-1.00	0.00-1.00			
HDCA (conj. frac.)					
Median (IQR)	0.00 (0.00-0.41)	0.33 (0.00-0.61)	0.020	48	121
Range	0.00-1.00	0.00-1.00			
UDCA (conj. frac.)					
Median (IQR)	0.90 (0.75-0.97)	0.90 (0.77-0.98)	0.790	65	225
Range	0.28-1.00	0.00-1.00			
Total BA (G:T ratio)					
Median (IQR)	8.09 (2.80-18.65)	9.01 (4.08-18.02)	0.578	65	231
Range	0.62-69.38	0.37-67.52			
CA (G:T ratio)					

Median (IQR) Range	5.12 (2.33-9.54) 0.50-27.00	6.00 (3.08-9.56) 0.27-30.00	0.311	64	230
CDCA (G:T ratio) Median (IQR) Range	5.43 (2.37-9.74) 0.67-35.50	6.28 (2.93-10.32) 0.36-51.00	0.247	65	231

	No IBD PSC	**UC PSC**	**p-value**	**No IBD PSC (n)**	**UC PSC (n)**
DCA (G:T ratio) Median (IQR) Range	5.09 (2.17-8.60) 0.50-45.00	4.69 (1.96-9.00) 0.29-44.50	0.648	61	153
LCA (G:T ratio) Median (IQR) Range	3.00 (1.50-7.00) 0.50-36.00	3.00 (1.80-7.00) 0.10-25.00	0.881	37	91
HDCA (G:T ratio) Median (IQR) Range	0.71 (0.71-0.71) 0.71-0.71	1.40 (1.00-4.00) 0.75-11.00	0.143	1	5
UDCA (G:T ratio) Median (IQR) Range	13.81 (6.47-31.96) 0.14-101.14	20.23 (7.58-37.57) 0.33-124.00	0.324	59	185
CA (frac. BA pool) Median (IQR) Range	0.12 (0.05-0.28) 0.01-0.79	0.19 (0.10-0.38) 0.00-0.79	0.022	65	231
CDCA (frac. BA pool) Median (IQR) Range	0.23 (0.13-0.38) 0.04-0.79	0.28 (0.14-0.47) 0.04-0.83	0.211	65	231
DCA (frac. BA pool) Median (IQR) Range	0.08 (0.03-0.19) 0.00-0.64	0.02 (0.00-0.12) 0.00-0.56	<0.001	65	231
LCA (frac. BA pool) Median (IQR) Range	0.01 (0.00-0.01) 0.00-0.04	0.00 (0.00-0.01) 0.00-0.08	<0.001	65	231
HDCA (frac. BA pool) Median (IQR) Range	0.00 (0.00-0.00) 0.00-0.03	0.00 (0.00-0.00) 0.00-0.04	0.005	65	231
UDCA (frac. BA pool) Median (IQR) Range	0.43 (0.02-0.70) 0.00-0.89	0.18 (0.01-0.67) 0.00-0.91	0.226	65	231
CA:CDCA ratio Median (IQR) Range	0.51 (0.30-0.91) 0.06-5.17	0.67 (0.40-1.16) 0.05-4.43	0.012	65	231

CA:DCA ratio					
Median (IQR)	1.18 (0.44-5.94)	3.12 (0.90-60.92)	<0.001	63	187
Range	0.12-698.25	0.09-2002.00			
CDCA:LCA+HDCA+UDCA					
Median (IQR)	0.48 (0.19-11.13)	1.44 (0.21-18.25)	0.317	65	225
Range	0.05-414.18	0.04-1310.00			

Supplementary Table 9. Bile acids in No IBD and CD PSC patients.

	No IBD PSC	CD PSC	p-value	No IBD PSC (n)	CD PSC (n)
Age in years					
Median (IQR)	55.82 (43.32-61.82)	48.17 (38.38-55.93)	0.150	65	26
Range	14.25-79.68	10.13-71.10			
Sex (% Male)	49.2	38.5	0.352	65	26
Tot BA / UDCA Group					
NN - # (%)	19 (29.2)	17 (65.4)	0.003	65	26
NY - # (%)	10 (15.4)	5 (19.2)			
HN - # (%)	13 (20.0)	3 (11.5)			
HY - # (%)	23 (35.4)	1 (3.8)			
Total BA					
Median (IQR)	23.57 (7.71-45.45)	6.79 (3.08-12.15)	<0.001	65	26
Range	0.88-646.47	0.59-124.14			
CA (total)					
Median (IQR)	1.84 (0.86-8.72)	0.98 (0.41-1.54)	0.015	65	26
Range	0.13-68.19	0.04-31.62			
CA (unconjugated)					
Median (IQR)	0.05 (0.03-0.12)	0.12 (0.03-0.28)	0.127	65	26
Range	0.00-4.02	0.01-0.76			
GCA					
Median (IQR)	1.11 (0.45-5.13)	0.54 (0.16-1.24)	0.009	65	26
Range	0.06-53.99	0.02-19.29			
TCA					
Median (IQR)	0.30 (0.07-2.09)	0.07 (0.02-0.22)	0.010	65	26
Range	0.00-30.29	0.00-20.10			
CDCA (total)					
Median (IQR)	3.85 (2.14-9.80)	2.21 (0.91-4.99)	0.021	65	26
Range	0.30-141.73	0.16-24.66			
CDCA (unconjugated)					
Median (IQR)	0.16 (0.05-0.47)	0.31 (0.08-0.63)	0.318	65	26
Range	0.01-7.27	0.00-5.23			
GCDCA					
Median (IQR)	2.99 (1.32-6.33)	1.35 (0.37-3.32)	0.007	65	26

Range	0.18-119.44	0.10-17.57			
TCDCA					
Median (IQR)	0.56 (0.16-2.77)	0.12 (0.04-0.69)	0.008	65	26
Range	0.02-42.03	0.00-15.94			

	No IBD PSC	CD PSC	p-value	No IBD PSC (n)	CD PSC (n)
DCA (total)					
Median (IQR)	1.43 (0.76-2.23)	0.81 (0.15-2.20)	0.063	65	26
Range	0.00-24.36	0.00-11.70			
DCA (unconjugated)					
Median (IQR)	0.29 (0.09-0.65)	0.37 (0.03-0.66)	0.598	65	26
Range	0.00-3.63	0.00-3.67			
GDCA					
Median (IQR)	0.64 (0.35-1.07)	0.17 (0.05-0.85)	0.009	65	26
Range	0.00-15.49	0.00-7.51			
TDCA					
Median (IQR)	0.15 (0.05-0.31)	0.02 (0.00-0.25)	0.014	65	26
Range	0.00-8.55	0.00-3.41			
LCA (total)					
Median (IQR)	0.10 (0.06-0.21)	0.03 (0.01-0.09)	0.001	65	26
Range	0.00-2.85	0.00-1.44			
LCA (unconjugated)					
Median (IQR)	0.03 (0.01-0.07)	0.01 (0.00-0.02)	0.001	65	26
Range	0.00-0.29	0.00-0.41			
GLCA					
Median (IQR)	0.05 (0.02-0.13)	0.01 (0.01-0.06)	0.005	65	26
Range	0.00-2.14	0.00-0.71			
TLCA					
Median (IQR)	0.01 (0.00-0.03)	0.00 (0.00-0.01)	0.017	65	26
Range	0.00-0.42	0.00-0.56			
HDCA (total)					
Median (IQR)	0.02 (0.00-0.05)	0.01 (0.00-0.05)	0.701	65	26
Range	0.00-0.17	0.00-0.41			
HDCA (unconjugated)					
Median (IQR)	0.02 (0.00-0.04)	0.01 (0.00-0.03)	0.719	65	26
Range	0.00-0.13	0.00-0.24			
GHDCA					
Median (IQR)	0.00 (0.00-0.00)	0.00 (0.00-0.01)	0.311	65	26
Range	0.00-0.06	0.00-0.17			

THDCA					
Median (IQR)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.291	65	26
Range	0.00-0.07	0.00-0.01			
UDCA (total)					
Median (IQR)	3.33 (0.29-18.37)	0.49 (0.12-1.15)	0.005	65	26
Range	0.02-537.71	0.00-60.88			

	No IBD PSC	**CD PSC**	**p-value**	**No IBD PSC (n)**	**CD PSC (n)**
UDCA (unconjugated)					
Median (IQR)	0.12 (0.02-2.29)	0.06 (0.01-0.40)	0.135	65	26
Range	0.00-52.33	0.00-6.51			
GUDCA					
Median (IQR)	1.73 (0.24-15.78)	0.24 (0.06-0.73)	0.003	65	26
Range	0.01-472.47)	0.00-54.41			
TUDCA					
Median (IQR)	0.14 (0.03-0.52)	0.02 (0.00-0.05)	<0.001	65	26
Range	0.00-35.47	0.00-4.77			
Total BA (conj. frac.)					
Median (IQR)	0.92 (0.74-0.98)	0.75 (0.47-0.91)	0.014	65	26
Range	0.20-1.00	0.28-1.00			
CA (conj. frac.)					
Median (IQR)	0.97 (0.83-1.00)	0.85 (0.55-0.96)	0.006	65	26
Range	0.09-1.00	0.25-1.00			
CDCA (conj. frac.)					
Median (IQR)	0.96 (0.79-0.99)	0.87 (0.59-0.93)	0.015	65	26
Range	0.29-1.00	0.24-1.00			
DCA (conj. frac.)					
Median (IQR)	0.78 (0.52-0.93)	0.58 (0.23-0.80)	0.042	63	24
Range	0.18-1.00	0.00-1.00			
LCA (conj. frac.)					
Median (IQR)	0.71 (0.54-0.80)	0.75 (0.50-0.89)	0.386	62	21
Range	0.00-1.00	0.33-1.00			
HDCA (conj. frac.)					
Median (IQR)	0.00 (0.00-0.41)	0.00 (0.00-0.41)	0.737	48	17
Range	0.00-1.00	0.00-0.67			
UDCA (conj. frac.)					
Median (IQR)	0.90 (0.75-0.97)	0.81 (0.60-0.92)	0.032	65	24
Range	0.28-1.00	0.27-1.00			
Total BA (G:T ratio)					
Median (IQR)	8.09 (2.80-18.65)	8.19 (4.70-20.57)	0.818	65	25
Range	0.62-69.38	0.46-85.82			

CA (G:T ratio)					
Median (IQR)	5.12 (2.33-9.54)	6.29 (3.07-10.25)	0.453	64	23
Range	0.50-27.00	0.57-22.80			
CDCA (G:T ratio)					
Median (IQR)	5.43 (2.37-9.74)	7.85 (3.81-13.20)	0.126	65	25
Range	0.67-35.50	0.36-67.00			
	No IBD PSC	**CD PSC**	**p-value**	**No IBD PSC (n)**	**CD PSC (n)**
DCA (G:T ratio)					
Median (IQR)	5.09 (2.17-8.60)	6.86 (5.09-8.60)	0.456	61	19
Range	0.50-45.00	0.22-23.50			
LCA (G:T ratio)					
Median (IQR)	3.00 (1.50-7.00)	4.25 (0.79-8.21)	0.733	37	8
Range	0.50-36.00	0.20-13.00			
HDCA (G:T ratio)					
Median (IQR)	0.71 (0.71-0.71)	3.00 (3.00-3.00)	0.317	1	1
Range	0.71-0.71	3.00-3.00			
UDCA (G:T ratio)					
Median (IQR)	13.81 (6.47-31.96)	21.00 (11.41-51.25)	0.310	59	17
Range	0.14-101.14	0.42-145.00			
CA (frac. BA pool)					
Median (IQR)	0.12 (0.05-0.28)	0.17 (0.12-0.27)	0.404	65	26
Range	0.01-0.79	0.01-0.56			
CDCA (frac. BA pool)					
Median (IQR)	0.23 (0.13-0.38)	0.39 (0.25-0.56)	0.004	65	26
Range	0.04-0.79	0.08-0.85			
DCA (frac. BA pool)					
Median (IQR)	0.08 (0.03-0.19)	0.12 (0.04-0.22)	0.404	65	26
Range	0.00-0.64	0.00-0.61			
LCA (frac. BA pool)					
Median (IQR)	0.01 (0.00-0.01)	0.01 (0.00-0.01)	0.626	65	26
Range	0.00-0.04	0.00-0.12			
HDCA (frac. BA pool)					
Median (IQR)	0.00 (0.00-0.00)	0.00 (0.00-0.01)	0.463	65	26
Range	0.00-0.03	0.00-0.05			
UDCA (frac. BA pool)					
Median (IQR)	0.43 (0.02-0.70)	0.05 (0.04-0.20)	0.150	65	26
Range	0.00-0.89	0.00-0.83			
CA:CDCA ratio					
Median (IQR)	0.51 (0.30-0.91)	0.38 (0.26-0.71)	0.366	65	26
Range	0.06-5.17	0.15-1.45			

CA:DCA ratio					
Median (IQR)	1.18 (0.44-5.94)	1.03 (0.40-5.40)	0.812	63	24
Range	0.12-698.25	0.09-182.50			
CDCA:LCA+HDCA+UDCA					
Median (IQR)	0.48 (0.19-11.13)	4.27 (0.87-11.00)	0.189	65	24
Range	0.05-414.18	0.09-50.65			

Supplementary Table 10. Bile acids in No IBD and Indeterminate IBD PSC patients.

	No IBD PSC	Ind IBD PSC	p-value	No IBD PSC (n)	Ind IBD PSC (n)
Age in years					
Median (IQR)	55.82 (43.32-61.82)	47.03 (29.80-59.11)	0.187	65	25
Range	14.25-79.68	18.90-78.53			
Sex (% Male)	49.2	52.0	0.814	65	25
Tot BA / UDCA Group					
NN - # (%)	19 (29.2)	11 (44.0)	0.465	65	25
NY - # (%)	10 (15.4)	4 (16.0)			
HN - # (%)	13 (20.0)	5 (20.0)			
HY - # (%)	23 (35.4)	5 (20.0)			
Total BA					
Median (IQR)	23.57 (7.71-45.45)	12.06 (6.73-38.40)	0.184	65	25
Range	0.88-646.47	0.96-53.68			
CA (total)					
Median (IQR)	1.84 (0.86-8.72)	1.85 (0.60-7.10)	0.564	65	25
Range	0.13-68.19	0.16-25.90			
CA (unconjugated)					
Median (IQR)	0.05 (0.03-0.12)	0.04 (0.03-0.14)	0.910	65	25
Range	0.00-4.02	0.01-2.65			
GCA					
Median (IQR)	1.11 (0.45-5.13)	1.26 (0.33-4.17)	0.525	65	25
Range	0.06-53.99	0.07-12.90			
TCA					
Median (IQR)	0.30 (0.07-2.09)	0.15 (0.03-2.10)	0.452	65	25
Range	0.00-30.29	0.01-12.74			
CDCA (total)					
Median (IQR)	3.85 (2.14-9.80)	4.68 (1.48-8.18)	0.675	65	25
Range	0.30-141.73	0.70-24.40			
CDCA (unconjugated)					
Median (IQR)	0.16 (0.05-0.47)	0.14 (0.04-0.56)	0.708	65	25
Range	0.01-7.27	0.01-3.68			
GCDCA					

Median (IQR)	2.99 (1.32-6.33)	3.14 (1.00-5.53)	0.656	65	25
Range	0.18-119.44	0.36-16.22			
TCDCA					
Median (IQR)	0.56 (0.16-2.77)	0.42 (0.13-2.06)	0.441	65	25
Range	0.02-42.03	0.03-8.40			

	No IBD PSC	**Ind IBD PSC**	**p-value**	**No IBD PSC (n)**	**Ind IBD PSC (n)**
DCA (total)					
Median (IQR)	1.43 (0.76-2.23)	0.61 (0.24-2.34)	0.073	65	25
Range	0.00-24.36	0.00-6.13			
DCA (unconjugated)					
Median (IQR)	0.29 (0.09-0.65)	0.12 (0.02-0.39)	0.054	65	25
Range	0.00-3.63	0.00-3.06			
GDCA					
Median (IQR)	0.64 (0.35-1.07)	0.33 (0.08-1.27)	0.091	65	25
Range	0.00-15.49	0.00 – 4.71			
TDCA					
Median (IQR)	0.15 (0.05-0.31)	0.08 (0.01-0.32)	0.239	65	25
Range	0.00-8.55	0.00-1.35			
LCA (total)					
Median (IQR)	0.10 (0.06-0.21)	0.04 (0.01-0.11)	0.005	65	25
Range	0.00-2.85	0.00-1.13			
LCA (unconjugated)					
Median (IQR)	0.03 (0.01-0.07)	0.01 (0.00-0.03)	0.002	65	25
Range	0.00-0.29	0.00-0.96			
GLCA					
Median (IQR)	0.05 (0.02-0.13)	0.01 (0.00-0.05)	0.008	65	25
Range	0.00-2.14	0.00-0.30			
TLCA					
Median (IQR)	0.01 (0.00-0.03)	0.00 (0.00-0.03)	0.237	65	25
Range	0.00-0.42	0.00-0.11			
HDCA (total)					
Median (IQR)	0.02 (0.00-0.05)	0.01 (0.00-0.04)	0.224	65	25
Range	0.00-0.17	0.00-0.15			
HDCA (unconjugated)					
Median (IQR)	0.02 (0.00-0.04)	0.01 (0.00-0.03)	0.178	65	25
Range	0.00-0.13	0.00-0.15			
GHDCA					
Median (IQR)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.850	65	25

Range	0.00-0.06	0.00-0.06			
THDCA					
Median (IQR)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.723	65	25
Range	0.00-0.07	0.00-0.02			
UDCA (total)					
Median (IQR)	3.33 (0.29-18.37)	0.67 (0.21-7.33)	0.254	65	25
Range	0.02-537.71	0.01-44.31			
	No IBD PSC	**Ind IBD PSC**	**p-value**	**No IBD PSC (n)**	**Ind IBD PSC (n)**
UDCA (unconjugated)					
Median (IQR)	0.12 (0.02-2.29)	0.06 (0.01-0.76)	0.238	65	25
Range	0.00-52.33	0.00-5.65			
GUDCA					
Median (IQR)	1.73 (0.24-15.78)	0.49 (0.20-4.89)	0.266	65	25
Range	0.01-472.47)	0.01-39.65			
TUDCA					
Median (IQR)	0.14 (0.03-0.52)	0.07 (0.02-0.17)	0.161	65	25
Range	0.00-35.47	0.00-2.63			
Total BA (conj. frac.)					
Median (IQR)	0.92 (0.74-0.98)	0.94 (0.78-0.97)	0.811	65	25
Range	0.20-1.00	0.21-1.00			
CA (conj. frac.)					
Median (IQR)	0.97 (0.83-1.00)	0.95 (0.88-0.99)	0.488	65	25
Range	0.09-1.00	0.15-1.00			
CDCA (conj. frac.)					
Median (IQR)	0.96 (0.79-0.99)	0.97 (0.84-0.99)	0.853	65	25
Range	0.29-1.00	0.24-1.00			
DCA (conj. frac.)					
Median (IQR)	0.78 (0.52-0.93)	0.83 (0.59-0.94)	0.565	63	23
Range	0.18-1.00	0.20-1.00			
LCA (conj. frac.)					
Median (IQR)	0.71 (0.54-0.80)	0.75 (0.58-0.96)	0.151	62	19
Range	0.00-1.00	0.15-1.00			
HDCA (conj. frac.)					
Median (IQR)	0.00 (0.00-0.41)	0.00 (0.00-0.53)	0.558	48	15
Range	0.00-1.00	0.00-1.00			
UDCA (conj. frac.)					
Median (IQR)	0.90 (0.75-0.97)	0.92 (0.78-0.98)	0.766	65	25
Range	0.28-1.00	0.44-1.00			
Total BA (G:T ratio)					

Median (IQR)	8.09 (2.80-18.65)	8.47 (2.41-22.54)	0.989	65	25
Range	0.62-69.38	0.62-93.11			
CA (G:T ratio)					
Median (IQR)	5.12 (2.33-9.54)	5.19 (2.87-9.27)	0.671	64	25
Range	0.50-27.00	0.66-70.33			
CDCA (G:T ratio)					
Median (IQR)	5.43 (2.37-9.74)	4.54 (2.33-12.08)	0.715	65	25
Range	0.67-35.50	0.56-92.17			

	No IBD PSC	**Ind IBD PSC**	**p-value**	**No IBD PSC (n)**	**Ind IBD PSC (n)**
DCA (G:T ratio)					
Median (IQR)	5.09 (2.17-8.60)	4.05 (2.00-7.74)	0.462	61	18
Range	0.50-45.00	0.59-14.00			
LCA (G:T ratio)					
Median (IQR)	3.00 (1.50-7.00)	1.45 (1.00-5.00)	0.131	37	9
Range	0.50-36.00	0.33-8.00			
HDCA (G:T ratio)					
Median (IQR)	0.71 (0.71-0.71)	1.50 (1.50-1.50)	0.317	1	1
Range	0.71-0.71	1.50-1.50			
UDCA (G:T ratio)					
Median (IQR)	13.81 (6.47-31.96)	15.37 (4.33-29.00)	0.840	59	21
Range	0.14-101.14	2.41-87.17			
CA (frac. BA pool)					
Median (IQR)	0.12 (0.05-0.28)	0.23 (0.10-0.33)	0.240	65	25
Range	0.01-0.79	0.02-0.67			
CDCA (frac. BA pool)					
Median (IQR)	0.23 (0.13-0.38)	0.43 (0.18-0.57)	0.023	65	25
Range	0.04-0.79	0.08-0.74			
DCA (frac. BA pool)					
Median (IQR)	0.08 (0.03-0.19)	0.07 (0.01-0.12)	0.240	65	25
Range	0.00-0.64	0.00-0.55			
LCA (frac. BA pool)					
Median (IQR)	0.01 (0.00-0.01)	0.00 (0.00-0.01)	0.010	65	25
Range	0.00-0.04	0.00-0.04			
HDCA (frac. BA pool)					
Median (IQR)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.317	65	25
Range	0.00-0.03	0.00-0.01			
UDCA (frac. BA pool)					
Median (IQR)	0.43 (0.02-0.70)	0.06 (0.02-0.59)	0.479	65	25
Range	0.00-0.89	0.01-0.89			

CA:CDCA ratio					
Median (IQR)	0.51 (0.30-0.91)	0.53 (0.34-0.72)	0.960	65	25
Range	0.06-5.17	0.11-2.14			
CA:DCA ratio					
Median (IQR)	1.18 (0.44-5.94)	2.71 (0.89-16.38)	0.139	63	23
Range	0.12-698.25	0.18-1295.00			
CDCA:LCA+HDCA+UDCA					
Median (IQR)	0.48 (0.19-11.13)	7.78 (0.27-18.80)	0.075	65	25
Range	0.05-414.18	0.09-75.00			

Supplementary Table 11. Bile acids in cirrhotic and non-cirrhotic PSC patients.

	Non-cirrhotic	**Cirrhotic**	**p-value**	**NC (n)**	**Cir (n)**
Age in years					
Median (IQR)	47.99 (32.95-59.36)	55.37 (33.51-61.41)	0.143	340	60
Range	10.13-80.48	17.27-79.68			
Sex (% Male)	58.5	53.3	0.453	340	60
Total BA					
Median (IQR)	13.50 (5.38-32.27)	48.84 (22.67-119.16)	<0.001	340	60
Range	0.54-291.62	1.86-646.47			
CA (total)					
Median (IQR)	1.80 (0.64-6.38)	13.34 (3.04-32.12)	<0.001	340	60
Range	0.04-224.36	0.46-192.53			
CA (unconjugated)					
Median (IQR)	0.06 (0.03-0.20)	0.08 (0.03-0.26)	0.766	340	60
Range	0.00-10.65	0.01-9.85			
GCA					
Median (IQR)	1.33 (0.41-4.53)	8.07 (1.96-18.62)	<0.001	340	60
Range	0.01-165.50	0.18-151.29			
TCA					
Median (IQR)	0.18 (0.05-1.23)	4.19 (0.49-11.65)	<0.001	340	60
Range	0.00-58.61	0.01-41.19			
CDCA (total)					
Median (IQR)	3.53 (1.37-80.3)	14.80 (4.69-42.63)	<0.001	340	60
Range	0.10-71.89	0.73-268.56			
CDCA (unconjugated)					
Median (IQR)	0.16 (0.05-0.46)	0.11 (0.05-0.46)	0.823	340	60
Range	0.00-14.78	0.01-9.35			
GCDCA					
Median (IQR)	2.39 (0.97-5.39)	7.88 (2.95-23.59)	<0.001	340	60
Range	0.02-47.74	0.51-214.48			

TCDCA					
Median (IQR)	0.36 (0.11-1.32)	5.07 (1.11-11.79)	<0.001	340	60
Range	0.00-35.85	0.01-52.46			
DCA (total)					
Median (IQR)	0.66 (0.02-1.78)	0.55 (0.17-1.57)	0.540	340	60
Range	0.00-24.36	0.00-9.94			
DCA (unconjugated)					
Median (IQR)	0.13 (0.01-0.46)	0.03 (0.01-0.19)	0.032	340	60
Range	0.00-5.99	0.00-3.63			

	Non-cirrhotic	**Cirrhotic**	**p-value**	**NC (n)**	**Cir (n)**
GDCA					
Median (IQR)	0.27 (0.01-1.03)	0.31 (0.08-0.83)	0.483	340	60
Range	0.00-15.49	0.00-8.20			
TDCA					
Median (IQR)	0.05 (0.00-0.25)	0.17 (0.04-0.32)	0.007	340	60
Range	0.00-8.55	0.00-3.44			
LCA (total)					
Median (IQR)	0.04 (0.00-0.13)	0.06 (0.02-0.26)	0.006	340	60
Range	0.00-2.18	0.00-2.85			
LCA (unconjugated)					
Median (IQR)	0.01 (0.00-0.04)	0.01 (0.00-0.07)	0.151	340	60
Range	0.00-0.96	0.00-0.70			
GLCA					
Median (IQR)	0.02 (0.00-0.08)	0.02 (0.01-0.14)	0.070	340	60
Range	0.00-1.63	0.00-2.14			
TLCA					
Median (IQR)	0.00 (0.00-0.02)	0.01 (0.00-0.06)	<0.001	340	60
Range	0.00-0.56	0.00-0.42			
HDCA (total)					
Median (IQR)	0.01 (0.00-0.04)	0.01 (0.00-0.03)	0.310	340	60
Range	0.00-0.41	0.00-0.73			
HDCA (unconjugated)					
Median (IQR)	0.01 (0.00-0.03)	0.00 (0.00-0.01)	0.125	340	60
Range	0.00-0.24	0.00-0.13			
GHDCA					
Median (IQR)	0.00 (0.00-0.01)	0.00 (0.00-0.00)	0.204	340	60
Range	0.00-0.17	0.00-0.73			
THDCA					
Median (IQR)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.018	340	60
Range	0.00-0.07	0.00-0.01			

UDCA (total)					
Median (IQR)	2.00 (0.19-14.29)	1.09 (0.22-33.79)	0.207	340	60
Range	0.00-194.12	0.00-537.71			
UDCA (unconjugated)					
Median (IQR)	0.17 (0.01-2.00)	0.14 (0.01-2.33)	0.784	340	60
Range	0.00-26.13	0.00-52.33			
GUDCA					
Median (IQR)	1.62 (0.12-12.06)	0.88 (0.09-30.72)	0.239	340	60
Range	0.00-175.83	0.00-472.47			

	Non-cirrhotic	**Cirrhotic**	**p-value**	**NC (n)**	**Cir (n)**
TUDCA					
Median (IQR)	0.08 (0.01-0.47)	0.17 (0.04-2.63)	<0.001	340	60
Range	0.00-18.60	0.00-51.06			
Total BA (conj. frac.)					
Median (IQR)	0.89 (0.72-0.97)	0.98 (0.88-0.99)	<0.001	340	60
Range	0.13-1.00	0.20-1.00			
CA (conj. frac.)					
Median (IQR)	0.96 (0.82-0.99)	0.99 (0.96-1.00)	<0.001	340	60
Range	0.09-1.00	0.09-1.00			
CDCA (conj. frac.)					
Median (IQR)	0.94 (0.82-0.99)	0.99 (0.93-1.00)	<0.001	340	60
Range	0.09-1.00	0.24-1.00			
DCA (conj. frac.)					
Median (IQR)	0.77 (0.50-0.93)	0.95 (0.80-0.99)	<0.001	286	59
Range	0.00-1.00	0.18-1.00			
LCA (conj. frac.)					
Median (IQR)	0.75 (0.55-0.86)	0.79 (0.57-1.00)	0.081	231	55
Range	0.00-1.00	0.00-1.00			
HDCA (conj. frac.)					
Median (IQR)	0.20 (0.00-0.52)	0.33 (0.00-0.67)	0.314	199	32
Range	0.00-1.00	0.00-1.00			
UDCA (conj. frac.)					
Median (IQR)	0.88 (0.74-0.97)	0.97 (0.86-0.99)	0.002	333	59
Range	0.00-1.00	0.46-1.00			
Total BA (G:T ratio)					
Median (IQR)	9.04 (4.44-18.55)	3.80 (1.77-9.92)	<0.001	339	60
Range	0.47-93.11	0.37-55.50			
CA (G:T ratio)					
Median (IQR)	6.00 (3.38-9.67)	2.44 (1.42-5.80)	<0.001	333	60
Range	0.47-70.33	0.27-22.96			

CDCA (G:T ratio)					
Median (IQR)	6.46 (3.27-10.71)	2.52 (1.74-5.60)	<0.001	339	60
Range	0.38-92.17	0.36-51.00			
DCA (G:T ratio)					
Median (IQR)	5.47 (2.61-8.75)	2.17 (1.29-3.98)	<0.001	241	51
Range	0.29-45.00	0.22-38.50			
LCA (G:T ratio)					
Median (IQR)	3.82 (2.00-7.00)	1.45 (1.00-2.85)	<0.001	132	37
Range	0.50-36.00	0.10-12.00			

	Non-cirrhotic	**Cirrhotic**	**p-value**	**NC (n)**	**Cir (n)**
HDCA (G:T ratio)					
Median (IQR)	1.37 (1.00-2.62)	1.00 (1.00-1.00)	0.382	10	2
Range	0.71-11.00	1.00-1.00			
UDCA (G:T ratio)					
Median (IQR)	21.00 (8.88-38.21)	7.55 (4.25-18.05)	<0.001	276	54
Range	0.14-145.00	0.33-89.15			
CA (frac. BA pool)					
Median (IQR)	0.17 (0.08-0.34)	0.26 (0.12-0.43)	0.008	340	60
Range	0.00-0.79	0.01-0.76			
CDCA (frac. BA pool)					
Median (IQR)	0.26 (0.14-0.45)	0.31 (0.20-0.50)	0.136	340	60
Range	0.04-0.93	0.06-0.88			
DCA (frac. BA pool)					
Median (IQR)	0.05 (0.00-0.14)	0.01 (0.00-0.04)	0.026	340	60
Range	0.00-0.64	0.00-0.46			
LCA (frac. BA pool)					
Median (IQR)	0.00 (0.00-0.01)	0.00 (0.00-0.01)	0.992	340	60
Range	0.00-0.12	0.00-0.06			
HDCA (frac. BA pool)					
Median (IQR)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.039	340	60
Range	0.00-0.05	0.00-0.02			
UDCA (frac. BA pool)					
Median (IQR)	0.21 (0.03-0.68)	0.08 (0.01-0.60)	0.029	340	60
Range	0.00-0.97	0.00-0.88			
CA:CDCA ratio					
Median (IQR)	0.59 (0.34-1.07)	0.66 (0.44-1.45)	0.106	340	60
Range	0.05-5.26	0.06-4.43			
CA:DCA ratio					
Median (IQR)	1.91 (0.59-17.09)	19.69 (2.81-162.95)	<0.001	286	59
Range	0.09-3796.67	0.36-2002.00			

CDCA:LCA+HDCA+UDCA					
Median (IQR)	1.36 (0.21-11.07)	5.27 (0.30-36.10)	0.017	333	59
Range	0.04-1310.00	0.06-422.00			
Bile acid / UDCA group			<0.001	319	57
NN	127 (39.8%)	10 (17.5%)			
NY	60 (18.8%)	1 (1.8%)			
HN	41 (12.9%)	22 (38.6%)			
HY	91 (28.5%)	24 (42.1%)			

Supplementary Table 12. Bile acids in autoimmune hepatitis (AIH) overlap and non-overlap large duct PSC patients.

	non-overlap "Large duct" PSC	**AIH overlap**	**p-value**	**No (n)**	**AIH (n)**
Age in years					
Median (IQR)	48.83 (33.42-60.14)	45.84 (25.23-64.63)	0.716	362	14
Range	10.13-80.48	19.44-67.75			
Sex (% Male)	57.2	57.1	0.998	362	14
Total BA					
Median (IQR)	16.34 (5.89-45.87)	25.92 (12.76-55.13)	0.234	362	14
Range	0.54-646.47	2.56-184.74			
CA (total)					
Median (IQR)	2.12 (0.76-10.54)	2.26 (1.12-5.25)	0.894	362	14
Range	0.04-224.36	0.18-110.08			
CA (unconjugated)					
Median (IQR)	0.07 (0.03-0.23)	0.04 (0.02-0.11)	0.347	362	14
Range	0.00-10.65	0.01-0.77			
GCA					
Median (IQR)	1.70 (0.46-6.41)	1.96 (0.69-3.87)	0.975	362	14
Range	0.01-165.50	0.16-51.45			
TCA					
Median (IQR)	0.27 (0.07-2.57)	0.27 (0.08-1.27)	0.911	362	14
Range	0.00-58.57	0.01-58.61			
CDCA (total)					
Median (IQR)	4.09 (1.69-10.26)	4.50 (3.06-8.57)	0.660	362	14
Range	0.10-268.56	0.73-71.89			
CDCA (unconjugated)					
Median (IQR)	0.15 (0.05-0.46)	0.29 (0.14-0.63)	0.170	362	14
Range	0.00-14.78	0.01-1.55			
GCDCA					

Median (IQR)	2.87 (1.10-7.18)	3.59 (1.81-5.57)	0.651	362	14
Range	0.02-214.48	0.40-36.01			
TCDCA					
Median (IQR)	0.49 (0.13-2.25)	0.62 (0.20-2.17)	0.678	362	14
Range	0.00-52.46	0.06-35.85			
DCA (total)					
Median (IQR)	0.65 (0.03-1.73)	1.43 (0.32-2.02)	0.279	362	14
Range	0.00-24.36	0.00-6.45			
DCA (unconjugated)					
Median (IQR)	0.09 (0.01-0.41)	0.38 (0.07-0.54)	0.160	362	14
Range	0.00-5.99	0.00-0.85			

	Non-overlap	AIH overlap	p-value	No (n)	AIH (n)
GDCA					
Median (IQR)	0.28 (0.02-0.93)	0.65 (0.10-1.36)	0.386	362	14
Range	0.00-15.49	0.00-4.16			
TDCA					
Median (IQR)	0.06 (0.00-0.29)	0.11 (0.01-0.39)	0.350	362	14
Range	0.00-8.55	0.00-1.98			
LCA (total)					
Median (IQR)	0.04 (0.00-0.15)	0.10 (0.01-0.24)	0.379	362	14
Range	0.00-2.85	0.00-1.71			
LCA (unconjugated)					
Median (IQR)	0.01 (0.00-0.04)	0.04 (0.00-0.06)	0.160	362	14
Range	0.00-0.96	0.00-0.55			
GLCA					
Median (IQR)	0.02 (0.00-0.09)	0.03 (0.00-0.13)	0.672	362	14
Range	0.00-2.14	0.00-1.03			
TLCA					
Median (IQR)	0.00 (0.00-0.02)	0.01 (0.00-0.02)	0.440	362	14
Range	0.00-0.56	0.00-0.13			
HDCA (total)					
Median (IQR)	0.01 (0.00-0.04)	0.01 (0.00-0.07)	0.341	362	14
Range	0.00-0.73	0.00-0.09			
HDCA (unconjugated)					
Median (IQR)	0.00 (0.00-0.02)	0.01 (0.00-0.03)	0.872	362	14
Range	0.00-0.24	0.00-0.09			
GHDCA					
Median (IQR)	0.00 (0.00-0.01)	0.01 (0.00-0.01)	0.101	362	14
Range	0.00-0.73	0.00-0.05			
THDCA					

Median (IQR)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.894	362	14
Range	0.00-0.07	0.00-0.04			
UDCA (total)					
Median (IQR)	1.80 (0.17-18.69)	8.73 (1.47-24.76)	0.106	362	14
Range	0.00-537.71	0.03-78.99			
UDCA (unconjugated)					
Median (IQR)	0.15 (0.01-2.03)	2.79 (0.14-5.47)	0.060	362	14
Range	0.00-52.33	0.00-16.90			
GUDCA					
Median (IQR)	1.41 (0.11-13.86)	5.80 (1.08-15.36)	0.124	362	14
Range	0.00-472.47	0.02-66.92			

	Non-overlap	**AIH overlap**	**p-value**	**No (n)**	**AIH (n)**
TUDCA					
Median (IQR)	0.08 (0.01-0.53)	0.28 (0.10-0.70)	0.114	362	14
Range	0.00-51.06	0.01-8.13			
Total BA (conj. frac.)					
Median (IQR)	0.91 (0.74-0.98)	0.84 (0.68-0.93)	0.371	362	14
Range	0.13-1.00	0.63-1.00			
CA (conj. frac.)					
Median (IQR)	0.97 (0.84-0.99)	0.98 (0.89-0.99)	0.591	362	14
Range	0.09-1.00	0.44-1.00			
CDCA (conj. frac.)					
Median (IQR)	0.96 (0.83-0.99)	0.91 (0.77-0.98)	0.449	362	14
Range	0.12-1.00	0.57-1.00			
DCA (conj. frac.)					
Median (IQR)	0.81 (0.54-0.96)	0.71 (0.51-0.81)	0.359	313	13
Range	0.00-1.00	0.00-1.00			
LCA (conj. frac.)					
Median (IQR)	0.75 (0.57-0.88)	0.68 (0.33-0.79)	0.099	258	11
Range	0.00-1.00	0.00-1.00			
HDCA (conj. frac.)					
Median (IQR)	0.25 (0.00-0.55)	0.59 (0.06-0.97)	0.111	205	10
Range	0.00-1.00	0.00-1.00			
UDCA (conj. frac.)					
Median (IQR)	0.90 (0.75-0.97)	0.80 (0.74-0.96)	0.394	354	14
Range	0.00-1.00	0.58-1.00			
Total BA (G:T ratio)					
Median (IQR)	8.26 (3.39-17.30)	11.42 (5.03-20.53)	0.612	361	14
Range	0.37-93.11	0.92-47.57			
CA (G:T ratio)					

Median (IQR)	5.53 (2.64-9.00)	5.82 (2.98-11.86)	0.663	356	14
Range	0.27-70.33	0.88-21.30			
CDCA (G:T ratio)					
Median (IQR)	5.68 (2.77-10.33)	5.75 (2.67-9.27)	0.684	361	14
Range	0.36-92.17	0.97-16.33			
DCA (G:T ratio)					
Median (IQR)	4.69 (2.08-8.20)	4.82 (3.07-6.57)	0.900	265	11
Range	0.22-44.50	0.63-18.38			
LCA (G:T ratio)					
Median (IQR)	3.00 (1.45-6.00)	5.05 (1.66-6.25)	0.657	153	8
Range	0.10-36.00	1.00-7.92			

	Non-overlap	**AIH overlap**	**p-value**	**No (n)**	**AIH (n)**
HDCA (G:T ratio)					
Median (IQR)	1.33 (1.00-2.25)	0.75 (0.75-0.75)	0.185	11	1
Range	0.71-11.00	0.75-0.75			
UDCA (G:T ratio)					
Median (IQR)	17.44 (7.00-34.58)	27.54 (8.80-35.44)	0.552	299	14
Range	0.14-145.00	1.11-62.84			
CA (frac. BA pool)					
Median (IQR)	0.19 (0.09-0.37)	0.08 (0.04-0.18)	0.066	362	14
Range	0.00-0.79	0.03-0.60			
CDCA (frac. BA pool)					
Median (IQR)	0.27 (0.15-0.45)	0.17 (0.12-0.36)	0.141	362	14
Range	0.04-0.88	0.06-0.55			
DCA (frac. BA pool)					
Median (IQR)	0.03 (0.00-0.14)	0.06 (0.01-0.10)	0.743	362	14
Range	0.00-0.64	0.00-0.23			
LCA (frac. BA pool)					
Median (IQR)	0.00 (0.00-0.01)	0.00 (0.00-0.01)	0.538	362	14
Range	0.00-0.12	0.00-0.06			
HDCA (frac. BA pool)					
Median (IQR)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.385	362	14
Range	0.00-0.05	0.00-0.01			
UDCA (frac. BA pool)					
Median (IQR)	0.17 (0.02-0.66)	0.65 (0.18-0.72)	0.096	362	14
Range	0.00-0.91	0.00-0.88			
CA:CDCA ratio					
Median (IQR)	0.63 (0.35-1.15)	0.51 (0.34-0.86)	0.374	362	14
Range	0.05-5.26	0.15-1.53			
CA:DCA ratio					

Median (IQR)	2.72 (0.81-27.51)	1.08 (0.76-3.56)	0.448	313	14
Range	0.09-3796.67	0.28-1650.00			
CDCA:LCA+HDCA+UDCA					
Median (IQR)	1.88 (0.22-14.22)	0.27 (0.17-3.66)	0.159	354	14
Range	0.04-1310.00	0.06-462.67			
Bile acid / UDCA group			0.185	342	13
NN	128 (37.4%)	1 (7.7%)			
NY	52 (15.2%)	3 (23.1%)			
HN	58 (17.0%)	3 (23.1%)			
HY	104 (30.4%)	6 (46.2%)			

Supplementary Table 13. Bile acids in small duct (SD) and large duct (LD) PSC patients.

	large duct PSC	small duct PSC	p-value	LD (n)	SD (n)
Age in years					
Median (IQR)	48.83 (33.42-60.14)	38.20 (29.06-50.11)	0.013	362	26
Range	10.13-80.48	11.82-68.15			
Sex (% Male)	57.2	65.4	0.413	362	26
Total BA					
Median (IQR)	16.34 (5.89-45.87)	11.36 (5.95-28.85)	0.184	362	26
Range	0.54-646.47	0.88-60.71			
CA (total)					
Median (IQR)	2.12 (0.76-10.54)	1.18 (0.37-5.82)	0.020	362	26
Range	0.04-224.36	0.30-24.40			
CA (unconjugated)					
Median (IQR)	0.07 (0.03-0.23)	0.04 (0.02-0.08)	0.034	362	26
Range	0.00-10.65	0.01-0.50			
GCA					
Median (IQR)	1.70 (0.46-6.41)	0.92 (0.26-2.66)	0.064	362	26
Range	0.01-165.50	0.06-13.69			
TCA					
Median (IQR)	0.27 (0.07-2.57)	0.12 (0.03-0.44)	0.040	362	26
Range	0.00-58.57	0.00-4.92			
CDCA (total)					
Median (IQR)	4.09 (1.69-10.26)	3.35 (1.19-5.82)	0.114	362	26
Range	0.10-268.56	0.30-24.40			
CDCA (unconjugated)					
Median (IQR)	0.15 (0.05-0.46)	0.13 (0.07-0.38)	0.773	362	26
Range	0.00-14.78	0.02-1.67			
GCDCA					
Median (IQR)	2.87 (1.10-7.18)	2.06 (0.86-4.46)	0.183	362	26

Range	0.02-214.48	0.18-19.54			
TCDCA					
Median (IQR)	0.49 (0.13-2.25)	0.33 (0.07-0.94)	0.117	362	26
Range	0.00-52.46	0.02-8.04			
DCA (total)					
Median (IQR)	0.65 (0.03-1.73)	0.64 (0.01-1.71)	0.565	362	26
Range	0.00-24.36	0.00-5.98			
DCA (unconjugated)					
Median (IQR)	0.09 (0.01-0.41)	0.30 (0.00-0.44)	0.545	362	26
Range	0.00-5.99	0.00-1.23			

	large duct PSC	**small duct PSC**	**p-value**	**LD (n)**	**SD (n)**
GDCA					
Median (IQR)	0.28 (0.02-0.93)	0.23 (0.01-1.02)	0.605	362	26
Range	0.00-15.49	0.00-4.76			
TDCA					
Median (IQR)	0.06 (0.00-0.29)	0.04 (0.00-0.12)	0.160	362	26
Range	0.00-8.55	0.00-0.59			
LCA (total)					
Median (IQR)	0.04 (0.00-0.15)	0.04 (0.00-0.13)	0.752	362	26
Range	0.00-2.85	0.00-0.60			
LCA (unconjugated)					
Median (IQR)	0.01 (0.00-0.04)	0.01 (0.00-0.06)	0.666	362	26
Range	0.00-0.96	0.00-0.23			
GLCA					
Median (IQR)	0.02 (0.00-0.09)	0.01 (0.00-0.07)	0.686	362	26
Range	0.00-2.14	0.00-0.33			
TLCA					
Median (IQR)	0.00 (0.00-0.02)	0.00 (0.00-0.01)	0.300	362	26
Range	0.00-0.56	0.00-0.15			
HDCA (total)					
Median (IQR)	0.01 (0.00-0.04)	0.01 (0.00-0.04)	0.668	362	26
Range	0.00-0.73	0.00-0.12			
HDCA (unconjugated)					
Median (IQR)	0.00 (0.00-0.02)	0.01 (0.00-0.03)	0.310	362	26
Range	0.00-0.24	0.00-0.08			
GHDCA					
Median (IQR)	0.00 (0.00-0.01)	0.00 (0.00-0.00)	0.555	362	26
Range	0.00-0.73	0.00-0.04			
THDCA					
Median (IQR)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.176	362	26

Range	0.00-0.07	0.00-0.00			
UDCA (total)					
Median (IQR)	1.80 (0.17-18.69)	3.56 (0.23-11.21)	0.954	362	26
Range	0.00-537.71	0.02-52.22			
UDCA (unconjugated)					
Median (IQR)	0.15 (0.01-2.03)	0.51 (0.01-1.49)	0.792	362	26
Range	0.00-52.33	0.00-5.84			
GUDCA					
Median (IQR)	1.41 (0.11-13.86)	2.62 (0.19-9.51)	0.869	362	26
Range	0.00-472.47	0.01-44.53			

	large duct PSC	**small duct PSC**	**p-value**	**LD (n)**	**SD (n)**
TUDCA					
Median (IQR)	0.08 (0.01-0.53)	0.10 (0.00-0.29)	0.498	362	26
Range	0.00-51.06	0.00-2.61			
Total BA (conj. frac.)					
Median (IQR)	0.91 (0.74-0.98)	0.87 (0.74-0.93)	0.317	362	26
Range	0.13-1.00	0.51-1.00			
CA (conj. frac.)					
Median (IQR)	0.97 (0.84-0.99)	0.95 (0.88-0.99)	0.811	362	26
Range	0.09-1.00	0.37-1.00			
CDCA (conj. frac.)					
Median (IQR)	0.96 (0.83-0.99)	0.91 (0.81-0.98)	0.164	362	26
Range	0.12-1.00	0.45-1.00			
DCA (conj. frac.)					
Median (IQR)	0.81 (0.54-0.96)	0.63 (0.42-0.83)	0.049	313	20
Range	0.00-1.00	0.00-1.00			
LCA (conj. frac.)					
Median (IQR)	0.75 (0.57-0.88)	0.62 (0.47-0.79)	0.041	258	18
Range	0.00-1.00	0.25-1.00			
HDCA (conj. frac.)					
Median (IQR)	0.25 (0.00-0.55)	0.00 (0.00-0.31)	0.072	205	18
Range	0.00-1.00	0.00-1.00			
UDCA (conj. frac.)					
Median (IQR)	0.90 (0.75-0.97)	0.88 (0.80-0.96)	0.864	354	26
Range	0.00-1.00	0.50-1.00			
Total BA (G:T ratio)					
Median (IQR)	8.26 (3.39-17.30)	10.64 (5.99-17.69)	0.133	361	26
Range	0.37-93.11	2.07-69.38			
CA (G:T ratio)					
Median (IQR)	5.53 (2.64-9.00)	6.00 (4.17-10.56)	0.105	356	25

Range	0.27-70.33	2.08-14.00			
CDCA (G:T ratio)					
Median (IQR)	5.68 (2.77-10.33)	6.79 (4.58-10.42)	0.301	361	26
Range	0.36-92.17	2.02-31.00			
DCA (G:T ratio)					
Median (IQR)	4.69 (2.08-8.20)	8.06 (4.13-10.67)	0.036	265	17
Range	0.22-44.50	1.83-45.00			
LCA (G:T ratio)					
Median (IQR)	3.00 (1.45-6.00)	3.86 (1.40-7.00)	0.583	153	9
Range	0.10-36.00	1.00-14.00			

	large duct PSC	**small duct PSC**	**p-value**	**LD (n)**	**SD (n)**
HDCA (G:T ratio)					
Median (IQR)	1.33 (1.00-2.25)	NA	NA	11	0
Range	0.71-11.00	NA			
UDCA (G:T ratio)					
Median (IQR)	17.44 (7.00-34.58)	29.78 (19.47-44.73)	0.019	299	19
Range	0.14-145.00	5.91-101.14			
CA (frac. BA pool)					
Median (IQR)	0.19 (0.09-0.37)	0.12 (0.06-0.28)	0.054	362	26
Range	0.00-0.79	0.02-0.62			
CDCA (frac. BA pool)					
Median (IQR)	0.27 (0.15-0.45)	0.26 (0.14-0.47)	0.780	362	26
Range	0.04-0.88	0.05-0.93			
DCA (frac. BA pool)					
Median (IQR)	0.03 (0.00-0.14)	0.08 (0.00-0.13)	0.828	362	26
Range	0.00-0.64	0.00-0.39			
LCA (frac. BA pool)					
Median (IQR)	0.00 (0.00-0.01)	0.01 (0.00-0.01)	0.421	362	26
Range	0.00-0.12	0.00-0.04			
HDCA (frac. BA pool)					
Median (IQR)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.232	362	26
Range	0.00-0.05	0.00-0.03			
UDCA (frac. BA pool)					
Median (IQR)	0.17 (0.02-0.66)	0.53 (0.04-0.66)	0.417	362	26
Range	0.00-0.91	0.00-0.86			
CA:CDCA ratio					
Median (IQR)	0.63 (0.35-1.15)	0.48 (0.30-0.64)	0.044	362	26
Range	0.05-5.26	0.07-2.31			
CA:DCA ratio					
Median (IQR)	2.72 (0.81-27.51)	0.80 (0.38-4.20)	0.030	313	20

Range	0.09-3796.67	0.16-520.00			
CDCA:LCA+HDCA+UDCA					
Median (IQR)	1.88 (0.22-14.22)	0.41 (0.22-9.28)	0.504	354	26
Range	0.04-1310.00	0.07-375.67			
Bile acid / UDCA group			0.245	342	23
NN	128 (37.4%)	8 (34.8%)			
NY	52 (15.2%)	7 (30.4%)			
HN	58 (17.0%)	2 (8.7%)			
HY	104 (30.4%)	6 (26.1%			

Supplementary Table 14. Univariate time to event (hepatic decompensation) analyses.

Parameter	HR (95% CI)	p-value	c-stat	n
Age (per 10 years)	1.50 (1.24-1.83)	<0.001	0.69	400
Male sex	0.75 (0.43-1.30)	0.302	0.52	400
IBD type (vs. No IBD)				347
Indeterminate IBD	0.75 (0.21-2.70)	0.66	0.53	
Crohn's Disease (CD)	0.25 (0.03-1.96)	0.19	0.53	
Ulcerative colitis (UC)	0.78 (0.39-1.57)	0.49	0.53	
PSC subtypes (vs. non-subtype)				400
Cirrhotic	5.29 (3.02-9.25)	<0.001	0.66	
Small duct disease	0.24 (0.03-1.75)	0.16	0.53	
AIH overlap	2.94 (1.17-7.40)	0.02	0.53	
Total bile acids				
Total	1.15 (1.11-1.18)	<0.001	0.80	400
Total (excluding UDCA)	1.15 (1.11-1.19)	<0.001	0.80	400
Conjugated fraction	10.90 (3.47-34.24)	<0.001	0.72	400
G:T conjugation ratio	0.47 (0.30-0.72)	<0.001	0.69	399
CA				
Total	1.06 (1.04-1.08)	<0.001	0.80	400
Unconjugated	0.98 (0.90-1.06)	0.60	0.56	400
GCA	1.05 (1.03-1.07)	<0.001	0.79	400
TCA	1.03 (1.02-1.03)	<0.001	0.82	400
Conjugated fraction	42.27 (5.09-351.22)	<0.001	0.77	400
G:T conjugation ratio	0.30 (0.17-0.53)	<0.001	0.72	393
Fraction of bile acid pool	1.31 (1.04-1.65)	0.02	0.55	400
CDCA				
Total	1.16 (1.11-1.20)	<0.001	0.80	400
Unconjugated	0.98 (0.87-1.11)	0.75	0.53	400
GCDCA	1.13 (1.09-1.16)	<0.001	0.78	400

TCDCA	1.09 (1.07-1.11)	<0.001	0.83	400
Conjugated fraction	12.68 (3.30-48.76)	<0.001	0.75	400
G:T conjugation ratio	0.20 (0.10-0.39)	<0.001	0.74	399
Fraction of bile acid pool	0.74 (0.50-1.11)	0.14	0.54	400
DCA				
Total	1.02 (0.91-1.15)	0.71	0.56	400
Unconjugated	0.71 (0.48-1.06)	0.09	0.56	400
GDCA	1.02 (0.94-1.11)	0.58	0.56	400
TDCA	1.03 (0.99-1.07)	0.18	0.70	400
Conjugated fraction	7.35 (3.04-17.73)	<0.001	0.71	345
G:T conjugation ratio	0.24 (0.12-0.45)	<0.001	0.74	292
Fraction of bile acid pool	0.08 (0.02-0.39)	<0.001	0.61	400

Parameter	**HR (95% CI)**	**p-value**	**c-stat**	**n**
LCA				
Total	1.12 (1.07-1.17)	<0.001	0.66	400
Unconjugated	1.12 (1.08-1.15)	<0.001	0.62	400
GLCA	1.06 (1.02-1.10)	0.01	0.62	400
TLCA	1.05 (1.03-1.08)	<0.001	0.75	400
Conjugated fraction	1.08 (0.71-1.65)	0.70	0.53	286
G:T conjugation ratio	0.55 (0.33-0.91)	0.02	0.65	169
Fraction of bile acid pool	1.00 (0.65-1.53)	0.99	0.49	400
HDCA				
Total	0.98 (0.75-1.29)	0.90	0.52	400
Unconjugated	1.06 (0.83-1.36)	0.65	0.49	400
GHDCA	0.94 (0.80-1.11)	0.47	0.52	400
THDCA	na (0.00-na)	Na	na	400
Conjugated fraction	0.81 (0.46-1.42)	0.46	0.52	231
G:T conjugation ratio	na (0.00-na)	Na	na	12
Fraction of bile acid pool	0.17 (0.04-0.76)	0.02	0.59	400
UDCA				
Total	1.04 (1.03-1.06)	<0.001	0.66	400
Unconjugated	1.05 (1.02-1.08)	<0.001	0.57	400
GUDCA	1.04 (1.02-1.05)	<0.001	0.65	400
TUDCA	1.02 (1.01-1.02)	<0.001	0.75	400
Conjugated fraction	4.18 (1.97-8.87)	<0.001	0.66	392
G:T conjugation ratio	0.30 (0.17-0.54)	<0.001	0.72	330
Fraction of bile acid pool	1.22 (0.81-1.83)	0.34	0.50	400
High total BA (>17.79 µmol/L)	8.00 (3.60-17.77)	<0.001	0.72	400
UDCA treatment (predicted)	1.65 (0.94-2.90)	0.08	0.57	376
CA:CDCA Ratio	1.19 (1.05-1.35)	0.01	0.62	400
CA:DCA Ratio	1.00 (1.00-1.00)	0.67	0.71	345
Bilirubin (total)	1.15 (1.09-1.21)	<0.001	0.77	370

Alkaline phosphatase (AP)				
AP x ULN (upper limit of normal)	1.21 (1.10-1.33)	<0.001	0.70	375
AP > 1.0 x ULN	7.93 (2.47-25.51)	<0.001	0.65	375
AP > 1.5 x ULN	2.81 (1.51-5.23)	0.001	0.64	375

Supplementary Table 15. Univariate event rate (hepatic decompensation) by quartiles analyses.

	Quartile of parameter value			
Parameter	**Q1**	**Q2**	**Q3**	**Q4**
Total bile acids				
Total	3.18	4.42	14.56	45.51
Total (excluding UDCA)	1.07	9.56	12.35	45.75
Conjugated fraction	3.20	11.79	15.32	31.71
G:T conjugation ratio	26.13	15.83	14.48	4.29
CA				
Total	1.05	3.27	21.90	40.81
Unconjugated	17.71	17.58	13.66	10.89
GCA	1.04	5.59	20.26	38.52
TCA	0.00	4.43	18.42	44.10
Conjugated fraction	2.05	8.18	15.19	41.70
G:T conjugation ratio	37.62	13.21	8.24	3.35
Fraction of bile acid pool	10.26	17.94	11.83	19.66
CDCA				
Total	4.21	7.55	9.99	47.97
Unconjugated	20.91	9.83	17.19	12.34
GCDCA	3.12	9.83	14.88	38.55
TCDCA	0.00	7.52	13.81	48.07
Conjugated fraction	0.00	14.13	14.85	34.74
G:T conjugation ratio	34.84	20.37	4.31	3.30
Fraction of bile acid pool	16.40	17.64	16.50	9.23
DCA				
Total	4.44	18.72	23.18	14.15
Unconjugated	11.47	30.51	8.68	11.40
GDCA	5.61	18.67	21.63	14.22
TDCA	2.21	5.38	25.99	29.62
Conjugated fraction	3.38	14.82	35.28	9.36
G:T conjugation ratio	36.66	17.59	4.43	3.42
Fraction of bile acid pool	8.11	37.57	14.33	2.27
LCA				
Total	5.53	13.34	13.41	28.74
Unconjugated	10.80	12.59	11.28	25.80
GLCA	8.04	11.84	15.13	25.52
TLCA	1.06	8.21	10.36	44.74
Conjugated fraction	19.95	15.60	19.56	5.43
G:T conjugation ratio	38.75	18.80	3.35	2.22
Fraction of bile acid pool	4.39	30.18	15.04	12.42
HDCA				
Total	11.03	20.91	11.53	16.72
Unconjugated	11.09	16.61	19.70	12.55
GHDCA	14.84	5.29	28.01	12.79

Parameter	Quartile of parameter value Q1	Q2	Q3	Q4
HDCA (cont.)				
THDCA	13.26	18.92	6.87	21.08
Conjugated fraction	15.01	16.07	5.83	23.05
G:T conjugation ratio	29.81	25.55	5.58	3.08
Fraction of bile acid pool	6.72	31.92	15.32	7.79
UDCA				
Total	11.91	10.76	6.51	33.66
Unconjugated	19.19	4.68	11.36	24.87
GUDCA	15.53	8.07	3.25	36.78
TUDCA	4.16	10.69	11.48	37.41
Conjugated fraction	3.11	12.29	24.77	21.43
G:T conjugation ratio	34.27	21.17	5.64	3.07
Fraction of bile acid pool	21.46	4.50	19.11	15.12
CA:CDCA Ratio	6.85	9.69	16.79	26.52
CA:DCA Ratio	2.24	14.33	36.40	9.37

Event rate calculated as Number of Events / Person Years

Supplementary Table 16. Variables and relative influence on GBM Model

Variable	Relative Influence	Influence direction[#]
Age (per decade)	14.684	Increase
TUDCA	14.475	Increase
TCDCA	12.421	Increase
TLCA	8.865	Increase
TCA	8.603	Increase
TBA	4.234	Increase
DCA (fraction of BA pool)	3.606	Decrease
GDCA	3.023	Decrease
CDCA (total)	2.890	Increase
LCA (total)	2.462	Increase
LCA (conjugated fraction)	2.198	Decrease
CA (fraction of BA pool)	1.831	Increase
GCDCA	1.814	Increase
CA (conjugated fraction)	1.775	Increase
GUDCA	1.739	Increase
TDCA	1.421	Decrease
TBA (conjugated fraction)	1.386	Increase
CA (total)	1.356	Increase
DCA (conjugated fraction)	1.207	Increase
UDCA (fraction of BA pool)	1.191	Decrease
UDCA (total)	1.123	Increase
CDCA (conjugated fraction)	1.086	Decrease
GLCA	0.947	Decrease
GCA	0.873	Decrease
UDCA (conjugated fraction)	0.849	Increase
DCA (total)	0.787	Decrease
LCA (fraction of BA pool)	0.659	Increase
CDCA (unconjugated)	0.576	Increase
UDCA (unconjugated)	0.451	Decrease
HDCA (unconjugated)	0.426	Increase
CDCA (fraction of BA pool)	0.361	Decrease
CA (unconjugated)	0.144	Decrease
HDCA (fraction of BA pool)	0.121	Decrease
HDCA (total)	0.102	Increase
Male sex	0.100	Decrease
DCA (unconjugated)	0.070	Decrease
GHDCA	0.038	Increase
HDCA (conjugated fraction)	0.000	Decrease
THDCA	0.000	Decrease
UDCA use (calculated)	0.000	Increase

Increase or decreased risk of hepatic decompensation with increase in value of variable based on visualization of functional form plots. For instance, increased TUDCA concentration increased risk in the model whereas increased GDCA concentration lowered the risk.

Supplementary Table 17. Bile acids in PSC patients: Derivation and Validation cohorts.

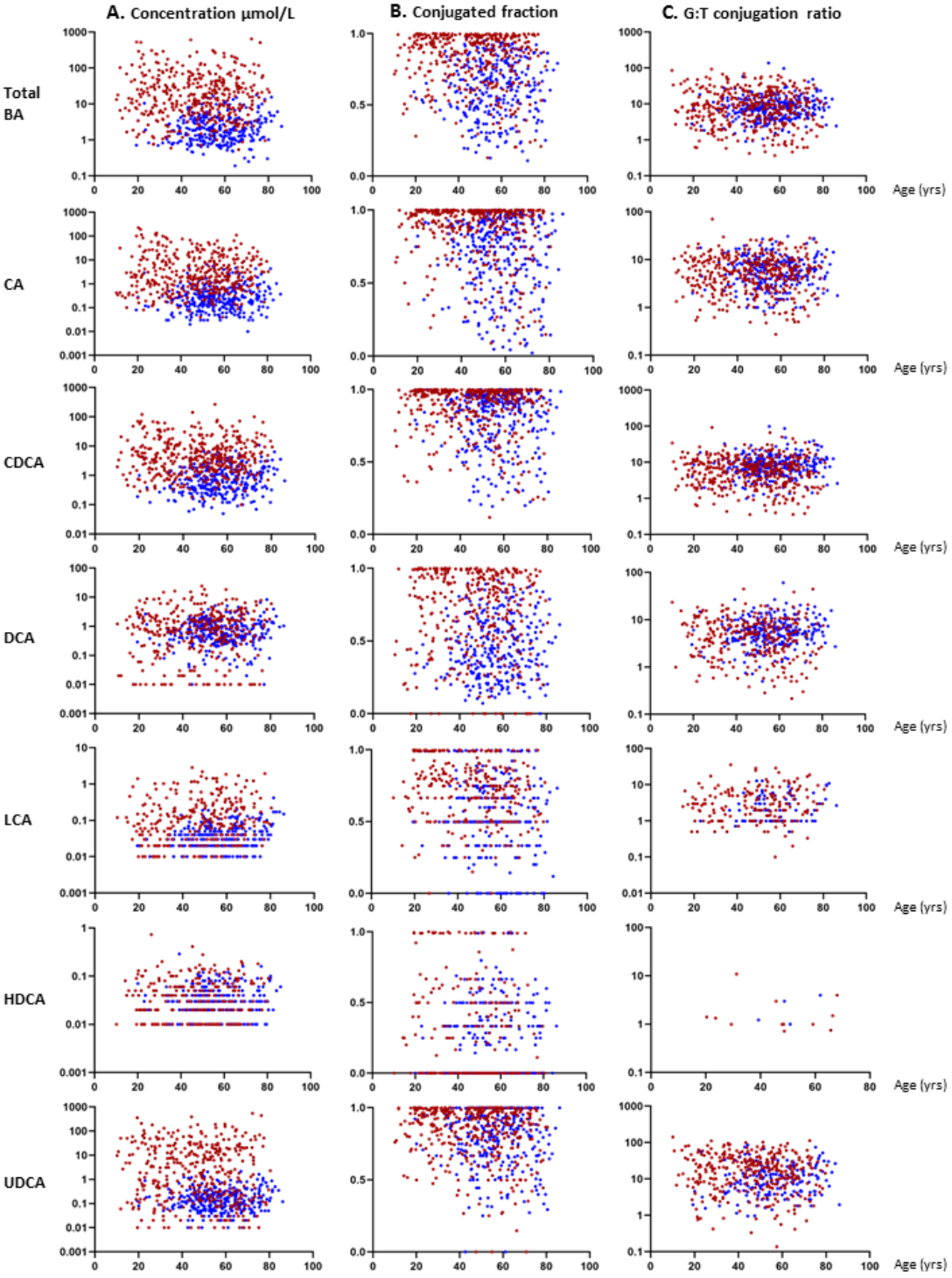
	PSC (derivation)	PSC (validation)	p-value	PSC Der (n)	PSC Val (n)
Total BA					
Median (IQR)	16.34 (6.04-44.31)	14.31 (5.12-59.30)	0.895	400	108
Range	0.54-646.47	0.74-457.45			
CA (total)					
Median (IQR)	2.09 (0.73-10.01)	3.01 (1.26-20.19)	0.019	400	108
Range	0.04-224.36	0.15-251.95			
CA (unconjugated)					
Median (IQR)	0.06 (0.03-0.22)	0.04 (0.02-0.09)	0.003	400	108
Range	0.00-10.65	0.00-3.92			
GCA					
Median (IQR)	1.60 (0.44-6.12)	2.03 (0.98-15.33)	0.010	400	108
Range	0.01-165.50	0.11-192.78			
TCA					
Median (IQR)	0.25 (0.06-2.44)	0.42 (0.14-6.64)	0.003	400	108
Range	0.00-58.61	0.01-149.57			
CDCA (total)					
Median (IQR)	4.09 (1.61-9.81)	4.30 (1.86-12.04)	0.482	400	108
Range	0.10-268.56	0.31-125.26			
CDCA (unconjugated)					
Median (IQR)	0.15 (0.05-0.46)	0.07 (0.02-0.20)	<0.001	400	108
Range	0.00-14.78	0.01-5.13			
GCDCA					
Median (IQR)	2.87 (1.10-6.90)	2.77 (1.30-7.63)	0.672	400	108
Range	0.02-214.48	0.19-87.38			
TCDCA					
Median (IQR)	0.47 (0.13-2.12)	0.92 (0.30-3.98)	0.003	400	108
Range	0.00-52.46	0.02-67.72			
DCA (total)					
Median (IQR)	0.66 (0.03-1.76)	0.61 (0.16-1.79)	0.702	400	108
Range	0.00-24.36	0.00-20.69			
DCA (unconjugated)					
Median (IQR)	0.10 (0.01-0.44)	0.05 (0.00-0.16)	0.025	400	108
Range	0.00-5.99	0.00-2.96			
GDCA					
Median (IQR)	0.28 (0.02-0.96)	0.38 (0.10-1.13)	0.158	400	108
Range	0.00-15.49	0.00-16.97			

	PSC (derivation)	PSC (validation)	p-value	PSC Der (n)	PSC Val (n)
TDCA					
Median (IQR)	0.06 (0.00-0.27)	0.07 (0.01-0.25)	0.266	400	108
Range	0.00-8.55	0.00-3.71			
LCA (total)					
Median (IQR)	0.04 (0.00-0.15)	0.02 (0.00-0.05)	0.010	400	108
Range	0.00-2.85	0.00-1.82			
LCA (unconjugated)					
Median (IQR)	0.01 (0.00-0.04)	0.01 (0.00-0.02)	0.043	400	108
Range	0.00-0.96	0.00-0.23)			
GLCA					
Median (IQR)	0.02 (0.00-0.09)	0.00 (0.00-0.00)	<0.001	400	108
Range	0.00-2.14	0.00-1.38			
TLCA					
Median (IQR)	0.00 (0.00-0.02)	0.01 (0.00-0.02)	0.037	400	108
Range	0.00-0.56)	0.00-0.26			
HDCA (total)					
Median (IQR)	0.01 (0.00-0.04)	0.01 (0.00-0.03)	0.117	400	108
Range	0.00-0.73	0.00-0.18			
HDCA (unconjugated)					
Median (IQR)	0.01 (0.00-0.02)	0.00 (0.00-0.02)	0.063	400	108
Range	0.00-0.24	0.00-0.16			
GHDCA					
Median (IQR)	0.00 (0.00-0.01)	0.00 (0.00-0.00)	0.004	400	108
Range	0.00-0.73	0.00-0.09			
THDCA					
Median (IQR)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.065	400	108
Range	0.00-0.07	0.00-0.10			
UDCA (total)					
Median (IQR)	1.95 (0.19-18.27)	0.36 (0.09-10.56)	0.039	400	108
Range	0.00-537.71	0.00-308.01			
UDCA (unconjugated)					
Median (IQR)	0.17 (0.01-2.02)	0.05 (0.00-0.53)	<0.001	400	108
Range	0.00-52.33	0.00-17.16			
GUDCA					
Median (IQR)	1.53 (0.12-13.52)	0.27 (0.06-9.42)	0.063	400	108
Range	0.00-472.47	0.00-278.90			
TUDCA					
Median (IQR)	0.09 (0.01-0.52)	0.06 (0.01-0.60)	0.428	400	108
Range	0.00-51.06	0.00-44.90			

	PSC (derivation)	PSC (validation)	p-value	PSC Der (n)	PSC Val (n)
Total BA (conj. frac.)					
Median (IQR)	0.91 (0.74-0.98)	0.95 (0.88-0.99)	<0.001	400	108
Range	0.13-1.00	0.23-1.00			
CA (conj. frac.)					
Median (IQR)	0.97 (0.85-0.99)	0.98 (0.94-1.00)	<0.001	400	108
Range	0.09-1.00	0.185-1.00			
CDCA (conj. frac.)					
Median (IQR)	0.95 (0.83-0.99)	0.98 (0.92-1.00)	<0.001	400	108
Range	0.12-1.00	0.23-1.00			
DCA (conj. frac.)					
Median (IQR)	0.80 (0.52-0.95)	0.92 (0.74-0.98)	<0.001	345	95
Range	0.00-1.00	0.00-1.00			
LCA (conj. frac.)					
Median (IQR)	0.75 (0.56-0.87)	0.71 (0.40-0.88)	0.069	286	77
Range	0.00-1.00	0.00-1.00			
HDCA (conj. frac.)					
Median (IQR)	0.22 (0.00-0.55)	0.00 (0.00-0.48)	0.134	231	55
Range	0.00-1.00	0.00-1.00			
UDCA (conj. frac.)					
Median (IQR)	0.90 (0.75-0.97)	0.95 (0.86-1.00)	<0.001	392	105
Range	0.00-1.00	0.23-1.00			
Total BA (G:T ratio)					
Median (IQR)	8.36 (3.57-17.44)	4.70 (2.55-8.21)	<0.001	399	108
Range	0.37-93.11	0.45-57.86			
CA (G:T ratio)					
Median (IQR)	5.56 (2.72-9.23)	4.71 (2.25-8.07)	0.038	393	108
Range	0.27-70.33	0.49-19.00			
CDCA (G:T ratio)					
Median (IQR)	5.76 (2.80-10.30)	3.24 (1.83-5.53)	<0.001	399	108
Range	0.36-92.17	0.37-21.00			
DCA (G:T ratio)					
Median (IQR)	4.90 (2.13-8.26)	4.97 (2.82-9.25)	0.290	292	89
Range	0.22-45.00	0.68-30.29			
LCA (G:T ratio)					
Median (IQR)	3.00 (1.40-6.22)	2.43 (1.00-5.00)	0.174	169	23
Range	0.10-36.00	0.50-11.57			
HDCA (G:T ratio)					
Median (IQR)	1.17 (1.00-1.88)	1.50 (1.50-1.80)	0.336	12	5
Range	0.71-11.00	1.00-2.67			

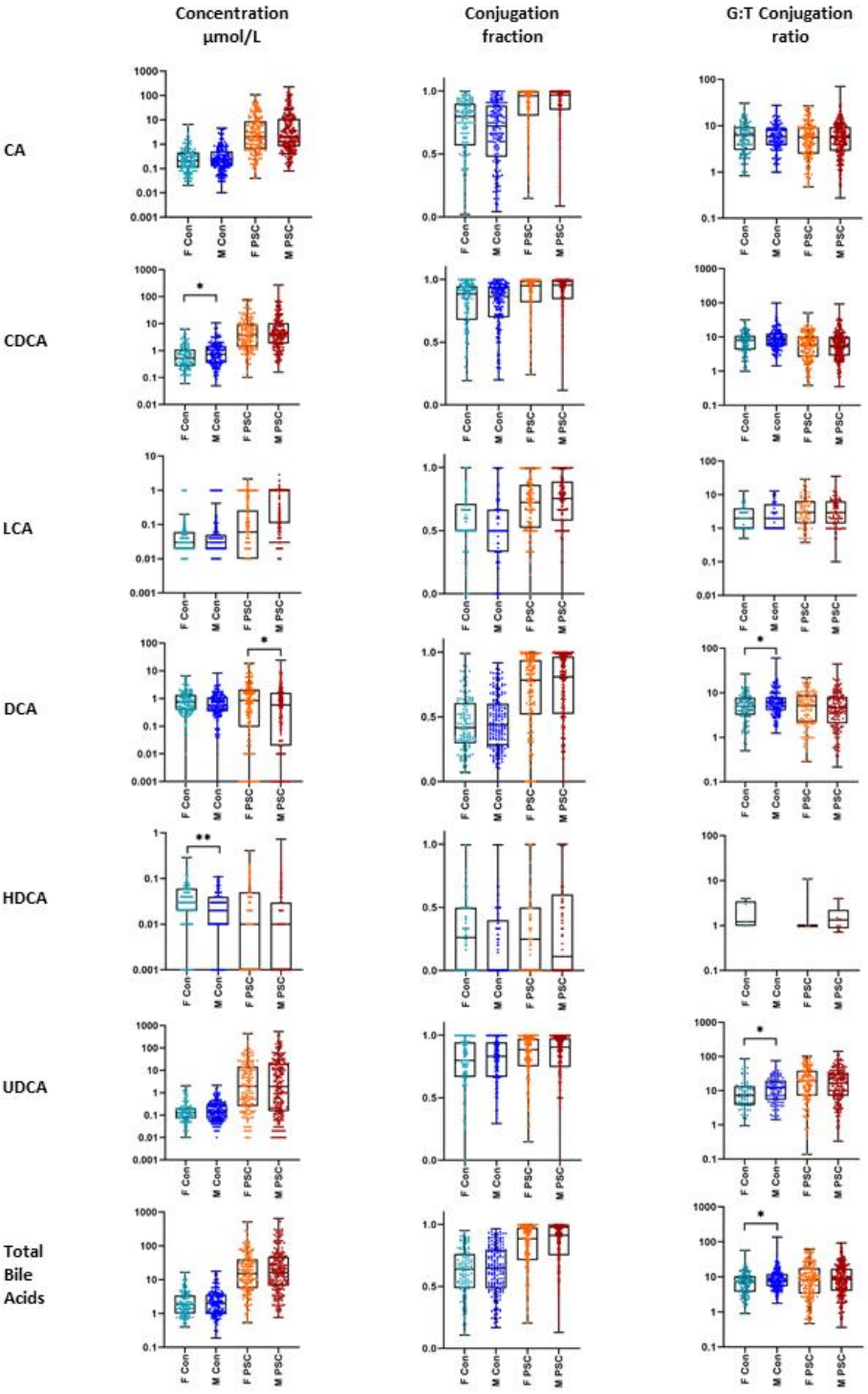
	PSC (derivation)	PSC (validation)	p-value	PSC Der (n)	PSC Val (n)
UDCA (G:T ratio)					
Median (IQR)	18.40 (7.25-35.75)	9.26 (4.45-18.00)	<0.001	330	83
Range	0.14-145.00	0.27-107.33			
CA (frac. BA pool)					
Median (IQR)	0.18 (0.08-0.36)	0.29 (0.18-0.44)	<0.001	400	108
Range	0.01-0.79	0.02-0.87			
CDCA (frac. BA pool)					
Median (IQR)	0.26 (0.15-0.46)	0.32 (0.19-0.46)	0.153	400	108
Range	0.04-0.93	0.09-0.79			
DCA (frac. BA pool)					
Median (IQR)	0.04 (0.01-0.14)	0.03 (0.01-0.15)	0.532	400	108
Range	0.00-0.64	0.00-0.54			
LCA (frac. BA pool)					
Median (IQR)	0.00 (0.00-0.01)	0.00 (0.00-0.00)	0.004	400	108
Range	0.00-0.12	0.00-0.05			
HDCA (frac. BA pool)					
Median (IQR)	0.00 (0.00-0.01)	0.00 (0.00-0.00)	0.064	400	108
Range	0.00-0.05	0.00-0.04			
UDCA (frac. BA pool)					
Median (IQR)	0.18 (0.02-0.66)	0.05 (0.01-0.47)	<0.001	400	108
Range	0.00-0.91	0.00-0.84			
CA:CDCA ratio					
Median (IQR)	0.60 (0.35-1.12)	0.89 (0.49-1.60)	<0.001	400	108
Range	0.05-5.26	0.13-7.15			
CA:DCA ratio					
Median (IQR)	2.60 (0.79-26.43)	4.47 (1.38-32.18)	0.101	345	95
Range	0.09-3796.67	0.09-1683.67			
CDCA:LCA+HDCA+UDCA					
Median (IQR)	1.57 (0.22-13.81)	5.93 (0.38-22.31)	0.002	392	105
Range	0.04-1310.00	0.12-673.13			

Supplementary Figure 1. Bile acids by age in PSC and controls.



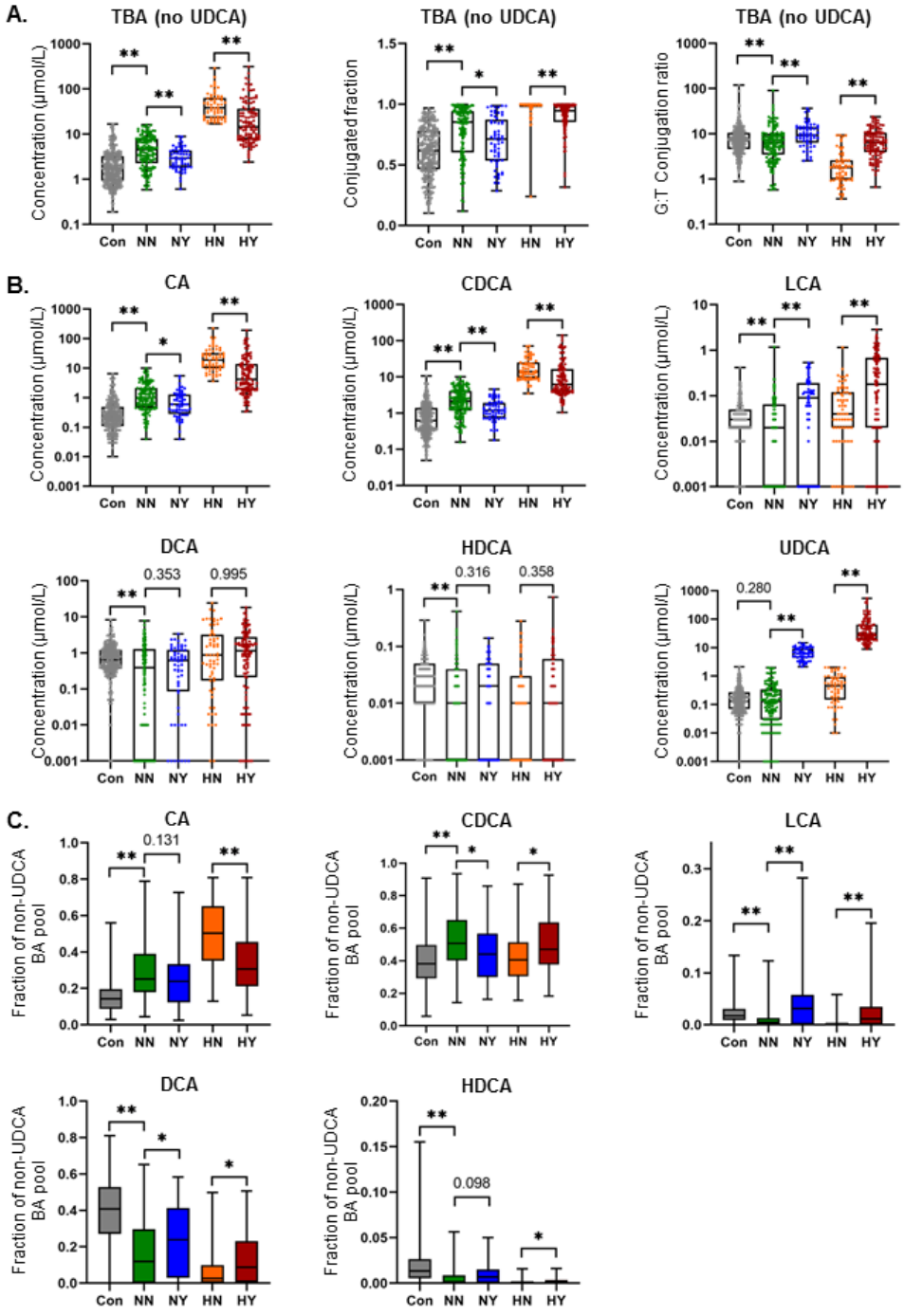
Supplementary Figure 1. Plots showing bile acid (A) concentration, (B) conjugated fraction and (C) G:T conjugation ratio (Y axis) by age in years (X axis). Red dots indicate PSC patients and blue dots indicate controls.

Supplementary Figure 2. Bile acids by sex in PSC and controls.



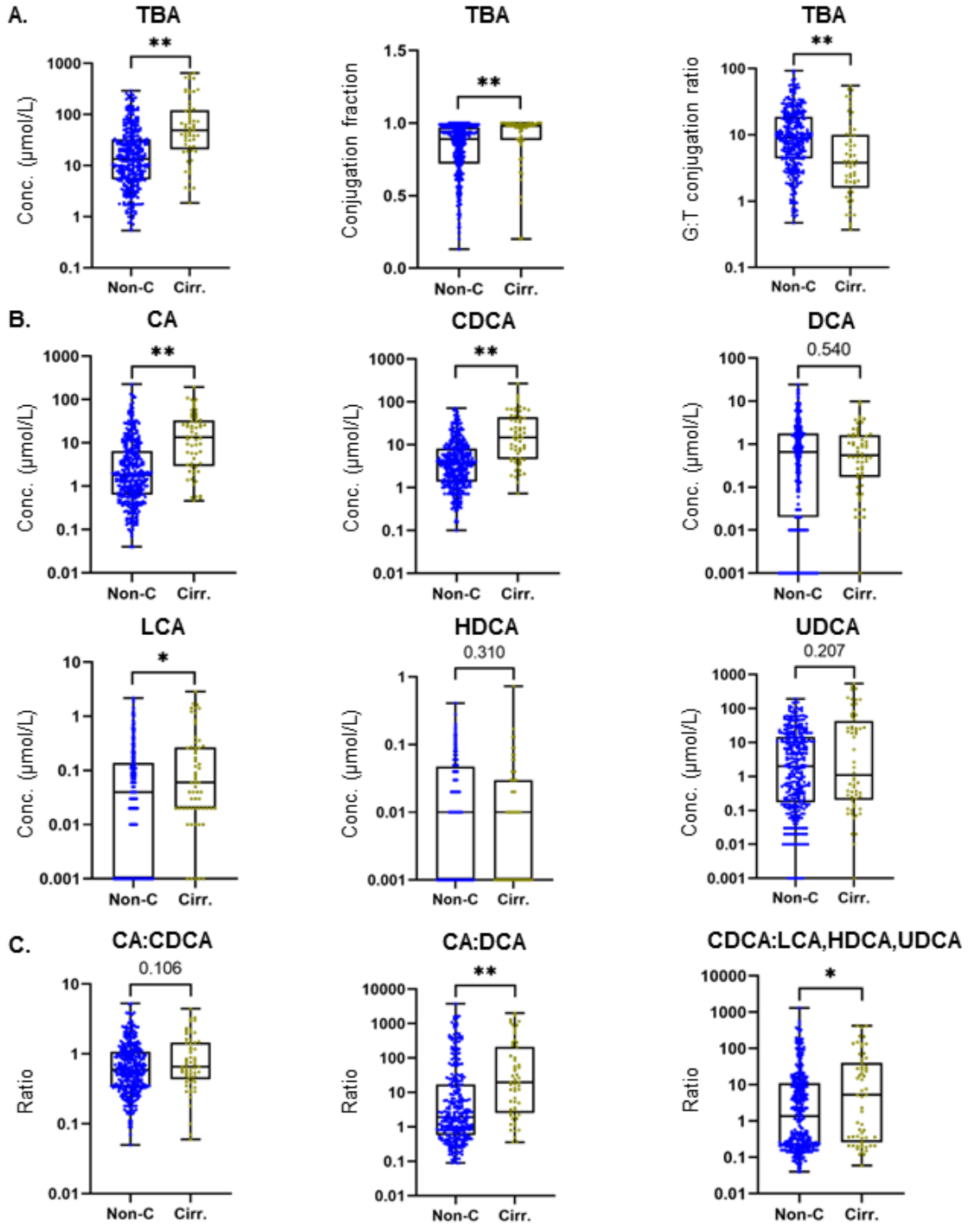
Supplementary Figure 2. Plots showing bile acid (A) concentration, (B) conjugated fraction and (C) G:T conjugation ratio by comparing values in females to males within PSC and control groups. *p<0.05, **p<0.01.

Supplementary Figure 3. Bile acids in controls and PSC patients by bile acid group.



Supplementary Figure 3. Plots showing (A) concentration, conjugation fraction, and G:T conjugation ratio of total bile acids (TBA) with UDCA, GUDCA and TUDCA removed; (B) concentration of bile acid "families" (i.e., the sum of conjugated and unconjugated forms); and (C) bile acid "families" as a fraction of the non-UDCA BA pool in controls (Con) and PSC patients separated by BA group. NN: normal TBA, no UDCA; NY: normal TBA, yes UDCA; HN: high TBA, no UDCA; HY: high TBA, yes UDCA. *p < 0.05, **p < 0.001

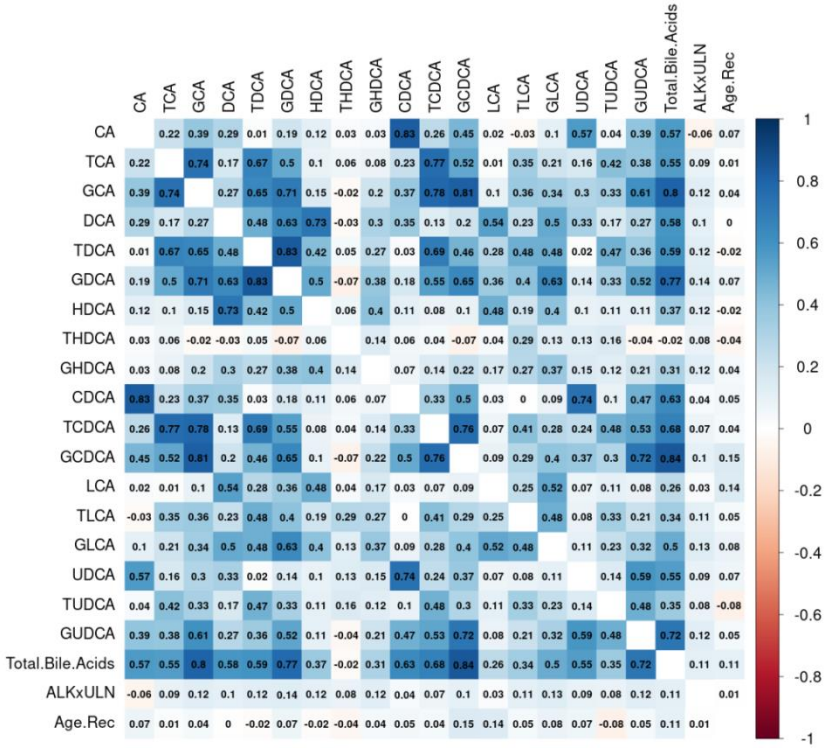
Supplementary Figure 4. Bile acids in cirrhotic and non-cirrhotic PSC patients



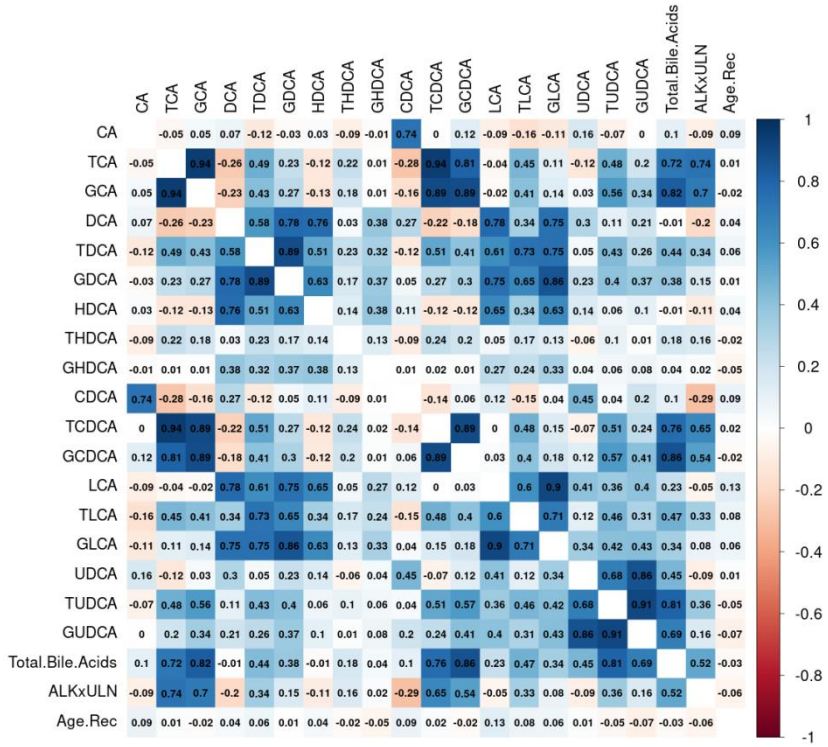
Supplementary Figure 4. Plots showing (A) concentration, conjugation fraction and G:T conjugation ratio of total bile acid (TBA); (B) concentration of bile acid "families" (i.e., total of conjugated and unconjugated forms); and (C) bile acid ratios in PSC patients who were non-cirrhotic (Non-C) or cirrhotic (Cirr.) at time of sample collection. *p < 0.05, **p < 0.001

Supplementary Figure 5. BA correlation plots in PSC and controls.

A. Controls

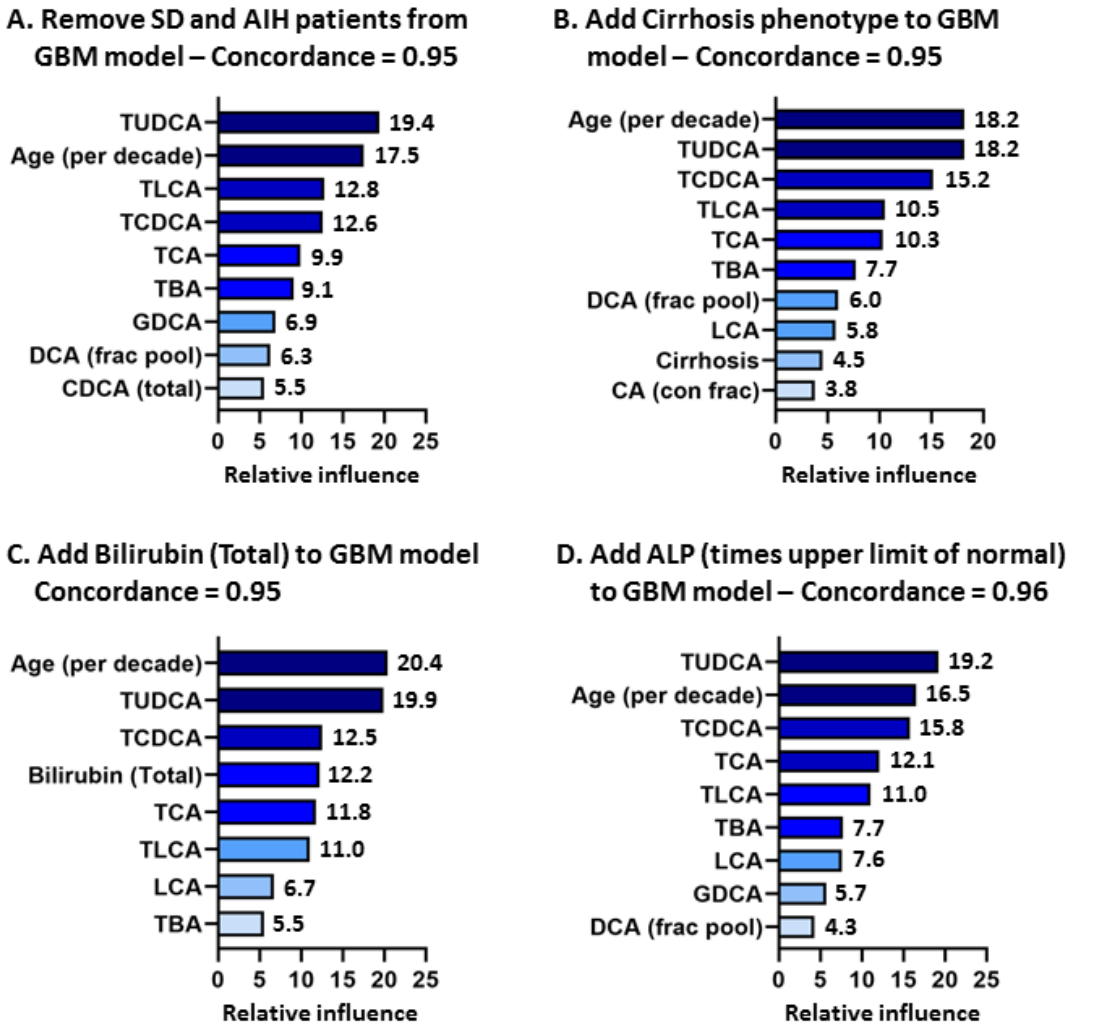


B. PSC



Supplementary Figure 5. Spearman correlation plots of BA concentrations in (A) controls and (B) PSC patients. Dark blue values indicate high correlation whereas red values indicate low correlation.

Supplementary Figure 6. Alternate GBM models using BA profiles



Supplementary Figure 6. Alternate GBM models using BA profiles with (A) PSC patients with small duct (SD) disease and/or having overlapping autoimmune hepatitis (AIH) removed, (B) cirrhosis phenotype (yes/no) added to the model, (C) Bilirubin (total) added to the model, and (D) alkaline phosphatase (ALP) added to the model.