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ORIGINAL RESEARCH—CLINICAL

The Oral Cholate Challenge Test Quantifies Risk for Liver-Related Clinical Outcomes in Primary Sclerosing Cholangitis

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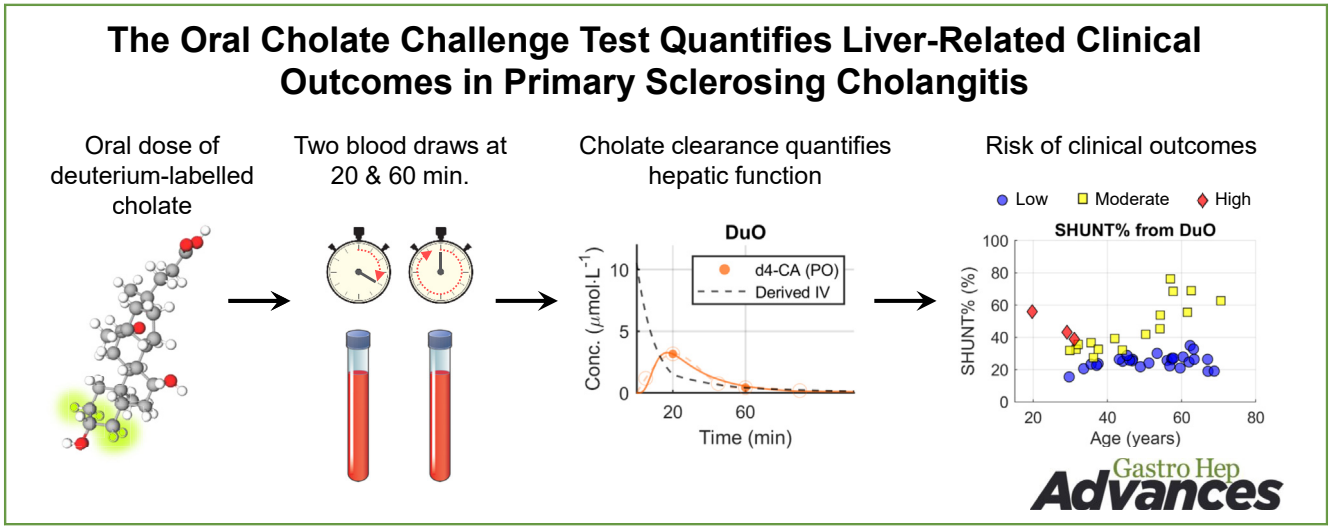

BACKGROUND AND AIMS: We quantified hepatic functional impairment using quantitative function tests and linked severity of functional impairment to liver-related complications and outcome in primary sclerosing cholangitis. **METHODS:** Forty-seven patients had baseline testing, and 40 were retested after 1 year. For each test, cholates labeled with cold, nonradioactive isotopes were administered orally (DuO, SHUNT tests) and intravenously (SHUNT test), and blood was analyzed at 20 and 60 minutes (DuO), or 0, 5, 20, 45, 60, and 90 minutes (SHUNT). Disease severity index (DSI), hepatic reserve (HR%), and portal-systemic shunting (SHUNT%) were calculated. **RESULTS:** Three subgroups with low, moderate, and high disease severity were defined from the age-adjusted results for DSI, HR%, and SHUNT%. Standard laboratory tests, clinical scores, cytokine levels, and clinical outcome correlated with these subgroups. In univariate analysis of baseline tests, SHUNT% was a strong predictor of clinical outcome (n = 13 of 47; areas under the receiver operating characteristic curve, 0.84_{DuO}, 0.90_{SHUNT}). A model combining SHUNT%, DSI (or HR %), platelet count, and changes from baseline was most predictive of outcome (n = 10 of 40; areas under the receiver operating characteristic curve, 0.95_{DuO}, 0.96_{SHUNT}). **CONCLUSION:** DSI, HR%, and SHUNT% identified subgroups of primary sclerosing cholangitis based on the age-related severity of hepatic impairment that predicted risk for liver-related clinical outcome. Further study is warranted to confirm and validate these intriguing findings both in studies of natural progression of primary sclerosing cholangitis and in clinical trials. DuO enhances the utility of quantitative liver function testing.

Keywords: Cholate Clearance; Portal Hypertension; Portal-Systemic Shunting; Quantitative Liver Function Test

Introduction

Primary sclerosing cholangitis (PSC) is a rare chronic liver disease characterized by inflammation and obliterative fibrosis of the biliary tree, leading to cholestasis,

Abbreviations used in this paper: **13C-CA, carbon-13-labeled cholate; AUROC, areas under the receiver operating characteristic curve; CP, Child Pugh; d4-CA, deuterium-labeled cholate; DSI, disease severity index; HFR, hepatic filtration rate; IBD, inflammatory bowel disease; MELD, model for end-stage liver disease; MRCP, magnetic resonance cholangiopancreatography; PSC, primary sclerosing cholangitis; SHUNT, the HepQuant SHUNT test; SHUNT%, the percent of orally administered d4-cholate spilling over into the systemic circulation; UDCA, ursodeoxycholic acid; VCTE, vibration-controlled transient elastography.**

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recurrent cholangitis, portal fibrosis, cirrhosis, and end-stage liver disease.[1] Staging liver disease severity and monitoring disease progression are backbones of clinical management. However, staging and monitoring in PSC by current laboratory tests, imaging, histology, and biomarkers has been challenging, due, in part, to concomitant cholestasis and biliary complications.[2] The most common noninvasive monitoring by vibration-controlled transient elastography (VCTE) has linked liver stiffness to patient outcomes in some studies,[3] but not others.[4]

Drug development in PSC has been challenging owing to the heterogeneity of the disease, inability to adequately stage patients, and the lack of appropriate endpoints, especially for early phase studies.[5] Clearly, there is a need for new quantitative tools for disease staging and monitoring the drug treatments, both for efficacy and toxicity.

HepQuant DuO and SHUNT tests are reproducible, minimally invasive, blood-based, and quantify liver function and portal-systemic shunting through the clearance of cholate from systemic and portal circulations.[6,7] In parenchymal diseases, test parameters correlate with stage of fibrosis,[8] portal pressure,[9] varices,[6,7] and clinical outcome[10,11] and can detect changes in these parameters with treatment.[12] In this pilot exploratory study of PSC, we used the HepQuant DuO and SHUNT tests to characterize liver disease severity, progression of disease, and the link of liver function to risk for liver-related clinical outcome.

Methods

This pilot study of the spectrum and progression of PSC was approved by the Colorado Multi-Institutional Review Board and conducted between 2011 to 2017. Cholate labeled with cold nonradioactive isotopes were studied under Investigational New Drug 65121 ([24-^{13}C]cholate, carbon-13-labeled cholate [13C-CA]) and Investigational New Drug 65123 ([2,2,4,4-^{2}H] cholate, deuterium-labeled cholate [d4-CA]). All subjects received routine clinical care within the hepatology clinics at University of Colorado Hospital. All coauthors had access to the study data and have reviewed and approved the final manuscript.

PSC was diagnosed by clinical, laboratory, and cholangiographic criteria. The time interval from PSC diagnosis to enrollment ranged from 4 to 528 months, with an average of 114 $\pm$ 115 months. The patients represented a spectrum of PSC from relatively stable, well-compensated disease to later stage disease with clinical manifestations. Thirty-five had model for end-stage liver disease (MELD) $<$10, 7 had MELD 10-15, and 5 had MELD $>$15. Patients were excluded if they had advanced or unstable cardiopulmonary or renal disease, prior liver transplantation, known hepatic or biliary malignancy, or advanced PSC with likelihood of death within 1 year.

Forty-seven subjects with PSC were enrolled, 46 had a second baseline test to assess the within-individual reproducibility, and 40 had repeat testing after a year of clinical follow-up. Clinical outcomes were identified from patient histories and chart reviews and defined as hepatic decompensation (variceal hemorrhage, ascites, encephalopathy), liver-related death, or liver transplantation occurring after the baseline test. In subjects with prior hepatic decompensation, clinical outcomes were defined by an additional decompensation event—either recurrence of ascites/encephalopathy or liver transplantation, or liver-related death.

The baseline screening procedures included medical history, physical examination, standard laboratory tests, alpha-fetoprotein, CA19-9, CEA, and clinical scores (APRI, FIB4, Child Pugh [CP], MELD, MELD-Na, and Mayo PSC Risk score). Procedures after 1 year of follow-up included a targeted medical history for clinical outcomes and repeat of blood tests to quantify changes.

Inflammatory biomarkers GM-CSF, IFN-γ, IL-10, IL-12 p70, IL-1β, IL-2, IL-6, IL-8, and TNF-α levels were measured (Human ProInflammatory 9-Plex Ultra-Sensitive Kit, Meso Scale Diagnostics, LLC, Gaithersburg, MD, USA, Catalog No. K15007C) in serum samples from a subset of subjects and healthy controls.

Technicians analyzing samples from the HepQuant tests were blinded and did not have access to any of the subjects' laboratory results or clinical information.

HepQuant DuO and SHUNT Tests

The test administration procedures have been described in detail.[10-12] All tests were performed after an overnight (or $>$5 h) fast, and morning medications were held until after completion of testing. HepQuant DuO parameters were calculated from the d4-CA (oral dose) serum concentrations at 20 and 60 minutes.[13] HepQuant SHUNT V1.0 ("SHUNT") parameters were calculated from d4-CA (oral dose) and 13C-CA (intravenous dose) concentrations at 5, 20, 45, 60, and 90 minutes.[7] Results for 2 other SHUNT test versions, V1.1 and V2.0, can be found in Supplemental Materials. Reference ranges in healthy controls for all test parameters and all test versions are provided in Table A1. The parameters for all test versions were as follows:

- **Systemic hepatic filtration rate** (**HFR**) – 13C-CA clearance adjusted for body weight
- **Portal HFR** – apparent d4-CA clearance adjusted for body weight
- **Disease severity index** (**DSI**) – score of disease severity based on indexing a patient's HFRs against maximum HFRs (0, no disease; 50 end-stage disease)
- **Hepatic reserve %** (**HR%**) – percentage based on indexing a patient's HFRs against the HFRs of healthy persons with lean body mass (100%, normal reserve; 0%, no reserve)
- **SHUNT%** – percentage of cholate shunting from portal to systemic circulation

Statistical Analysis

The projected sample size for detecting progression in PSC was based on an absolute increase of 4%–5% in SHUNT% using SD of 10% and 13%, alpha 0.05, and power 80%. This projection was based on a 4%–7% difference in SHUNT% among Ishak fibrosis stages[8] and an increase of 3.2% in SHUNT% over 1–2 years in the training set of patients with active chronic hepatitis C.[11] The calculated sample sizes ranged from 34 to 85.

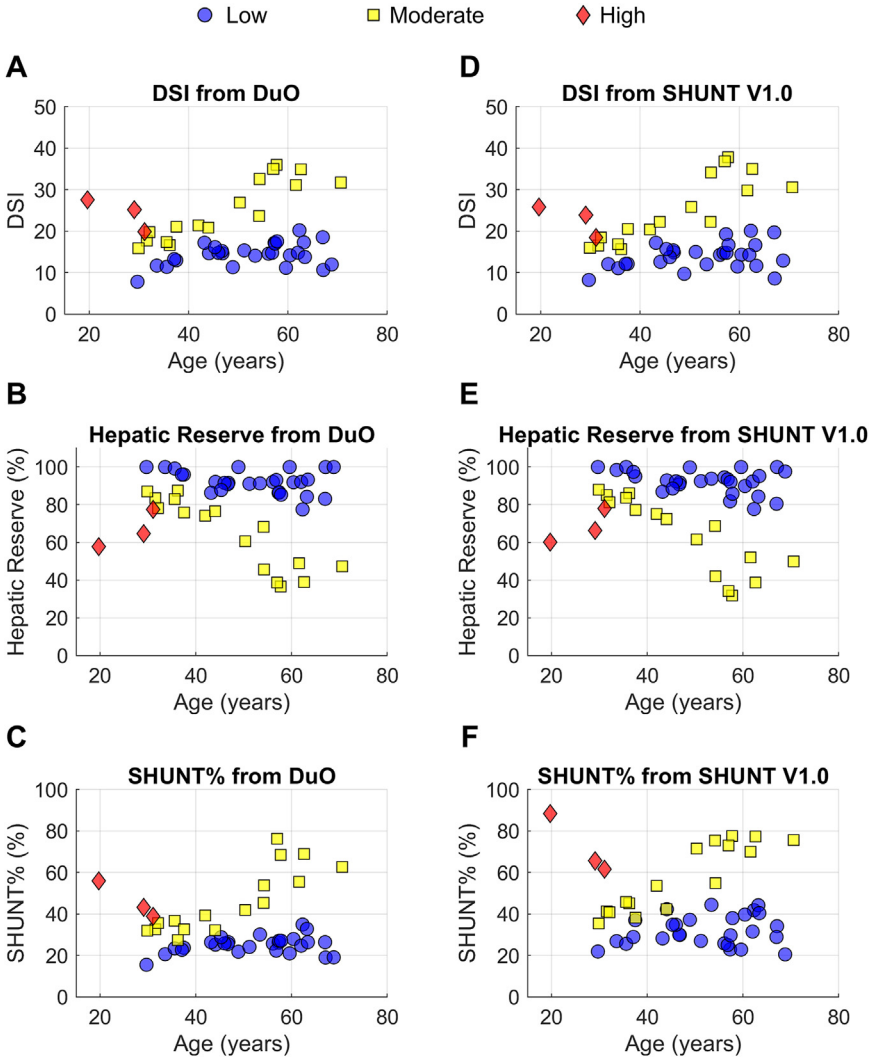


Figure 1. HepQuant Test parameters from test versions DuO and SHUNT. Test parameters are plotted vs age and indicated by subgroup; (A) DSI from DuO, (B) Hepatic Reserve from DuO, (C) SHUNT% from DuO, (D) DSI from SHUNT, (E) Hepatic Reserve from SHUNT, and (F) SHUNT% from SHUNT.

Our actual sample size of 40 with both baseline and follow-up testing was near the lower limit.

Subgroups based on severity of impairment of DSI, HR%, and SHUNT% (low, moderate, and high) were defined from plots of HepQuant test results vs age. Differences between subgroups were analyzed by t-tests and analysis of variance. Associations with varices and clinical outcomes were analyzed by logistic regression and area under the receiver operating characteristic curve (AUROC). Effects of ursodeoxycholic acid (UDCA) and inflammatory bowel disease (IBD) drug therapy on changes in DSI and SHUNT% from baseline to follow-up testing were evaluated by t-test.

Results

Characteristics of the Study Population

The study population characteristics were White:African American:Hispanic 43:3:1, M:F 36:11, age 48.8 ± 12.9 years, weight 81.3 ± 14.9 kg, and body mass index 26.2 ± 3.9 kg/m^2. Thirty-three (70%) had IBD, 25 of whom had ulcerative colitis (76%). Eight subjects had a history of variceal bleed, diuretic-responsive ascites, or treatment-responsive encephalopathy. Twenty-three had varices, splenomegaly from radiology reports, or platelet counts less than 140,000 μL^{-1}. Nine (19%) had a history of jaundice, 18 (38%) had a history of bacterial cholangitis, and 28 of 39 patients with ERCP reports (72%) had stricture dilation, stenting, or both. Thirty-one (66%) were taking UDCA (average dose 14.6 ± 3.9 mg/kg), and 17 of the 33 with IBD (52%) were taking IBD medications.

Higher DSI and SHUNT% and lower HR% at baseline correlated with clinical features of hepatic disease and not biliary or intestinal disease. DSI and SHUNT% were significantly increased and HR% decreased in patients who had varices, indirect evidence for portal hypertension, or a prior history of hepatic decompensation (Table A2). In contrast, DSI, SHUNT%, and HR% were similar between patients with or without a history of bacterial cholangitis (n = 18) or inflammatory bowel disease (n = 33).

Subgroups of Hepatic Impairment

Heterogeneity of hepatic dysfunction was evident from the plots of baseline DSI, HR, and SHUNT% vs age (Figure 1)

Table 1. Comparison of HepQuant Results, Laboratory Tests, and Clinical Models by Subgroups of Hepatic Impairment.

	Low impairment (N = 28)	Moderate/High impairment (N = 19)	Group t-test (*P* value)
HepQuant DuO			
SHUNT% (%)	25.02 ± 4.09	46.27 ± 14.73	**<.001**
DSI	14.43 ± 2.73	25.01 ± 6.81	**<.001**
Hepatic reserve (%)	92.12 ± 6.03	64.74 ± 17.53	**<.001**
Portal HFR (mL/min/kg)	18.46 ± 4.79	8.18 ± 4.06	**<.001**
Systemic HFR (mL/min/kg)	4.45 ± 0.48	3.27 ± 0.87	**<.001**
HepQuant SHUNT			
SHUNT% (%)	31.94 ± 7.13	59.66 ± 16.56	**<.001**
DSI	13.96 ± 3.04	24.58 ± 7.43	**<.001**
Hepatic reserve (%)	92.34 ± 6.17	64.86 ± 18.54	**<.001**
Portal HFR (mL/min/kg)	17.98 ± 4.16	7.91 ± 4.02	**<.001**
Systemic HFR (mL/min/kg)	5.62 ± 1.34	4.20 ± 1.44	**.0013**
Laboratory tests, clinical models, and clinical findings			
Alkaline phosphatase (IU/L)	128 ± 83	387 ± 353	**.0006**
GGT (IU/L)	153 ± 141	341 ± 343	**.0152**
AST (IU/L)	40 ± 15	88 ± 51	**.0000**
ALT (IU/L)	46 ± 25	80 ± 59	**.0086**
Bilirubin (mg/dL)	1.16 ± 0.46	2.99 ± 2.91	**.0023**
Bili >1.5	7 (25%)	12 (63%)	-
INR	1.05 ± 0.28	1.29 ± 0.80	.15
Albumin (g/dL)	3.87 ± 0.31	3.31 ± 0.42	**<.0001**
Creatinine (mg/dL)	0.95 ± 0.17	0.82 ± 0.22	**.0448**
Platelets (nL^{-1})	201 ± 78	160 ± 103	.13
Platelet <140 (nL^{-1})	4 (14%)	9 (47%)	-
APRI	0.58 ± 0.31	1.92 ± 1.52	**.0001**
FIB4	1.91 ± 1.16	4.05 ± 3.17	**.0029**
MELD score	8.11 ± 2.23	11.58 ± 5.44	**.0039**
MELD-Na score	10.11 ± 2.39	13.58 ± 6.01	**.0074**
≤ 10	26 (93%)	9 (47%)	-
11 to 15	1 (3.5%)	6 (32%)	-
> 15	1 (3.5%)	4 (21%)	-
Child Pugh score	5.25 ± 0.59	7.1 ± 1.8	**<.0001**
CP A	26 (93%)	9 (47%)	-
CP B	2 (7%)	8 (42%)	-
CP C	0 (0%)	2 (11%)	-
Mayo PSC risk score	0.314 ± 0.517	1.313 ± 0.941	**<.0001**
Splenomegaly	4 (14%)	13 (68%)	**.0002**
Varices	3 (11%)	12 (63%)	**.0002**
Decompensation	1 (3.5%)	7 (37%)	**.0030**

Values are reported as mean ± SD or number of subjects (%). Bold values indicate statistical significance ($p < 0.05$).
ALT, alanine transaminase; AST, aspartate transaminase; CP, Child Pugh; GGT, gamma glutamyl-transferase; MELD, model for end-stage liver disease; PSC, primary sclerosing cholangitis.

indicating subgroups of low, moderate, and high degree of hepatic impairment. The average DSI per year of age for the 3 subgroups were 0.28 ± 0.06, 0.53 ± 0.07, and 0.91 ± 0.37 $year^{-1}$, respectively ($P < .001$ for differences between groups, analysis of variance). Subjects in the high subgroup had high DSI, low HR%, and high SHUNT% at early age. Across all ages, those in the moderate subgroup had higher DSI, lower HR%, and greater SHUNT% compared to subjects in the low subgroup.

Subjects in the high or moderate subgroups had clinical and laboratory evidence of more severe liver disease (Table 1). Compared to subjects with a low degree of impairment, they had higher mean bilirubin levels, lower albumin levels, lower platelet counts, worse APRI, FIB4, MELD, MELD-Na, CP, and Mayo PSC Risk Scores, and elevations in IL-6, IL-8, and GM-CSF (Table A5). Also, they were more likely to have splenomegaly on prior imaging, varices on prior endoscopy, and a history of clinical decompensation.

Prediction of Clinical Outcome

Of the baseline clinical variables, varices had the greatest association with development of clinical outcomes (AUROC 0.76, $P = .0008$).

Standard laboratory tests were poorly predictive of clinical outcome. Baseline alkaline phosphatase levels were significantly higher in those who experienced clinical outcome (363 ± 387 vs 183 ± 181 IU/L, $P = .034$). However, follow-up alkaline phosphatase levels (284 ± 232 vs

Table 2. Diagnostic Performance of Baseline Tests (N = 47) in Prediction of Clinical Outcome by Univariate Logistic Regression in Terms of AUROC (95% CI)

	HepQuant SHUNT		HepQuant DuO	
Variable	AUROC	95% CI[a]	AUROC	95% CI[a]
SHUNT%	0.90	0.78–0.97	0.84	0.71–0.93
HFR_P	0.85	0.71–0.94	0.83	0.70–0.93
Hepatic reserve	0.84	0.71–0.93	0.83	0.69–0.93
DSI	0.83	0.69–0.92	0.83	0.69–0.92
Mayo	0.79	0.65–0.90	-	-
Alk. Phos.	0.75	0.60–0.87	-	-
RCA	0.73	0.58–0.85	0.80	0.66–0.90
FIB4	0.72	0.57–0.84	-	-
Bilirubin	0.70	0.55–0.82	-	-
CP	0.69	0.54–0.82	-	-
APRI	0.69	0.54–0.82	-	-
MELD	0.68	0.52–0.81	-	-
HFR_S	0.66	0.51–0.79	0.78	0.64–0.89
PLT	0.60	0.43–0.74	-	-

[a]Binomial exact.

167 ± 169 IU/L, $P = .09$) and changes from baseline (−58 ± 195 vs 11 ± 41, $P = .07$) were not significantly different between those with vs those without clinical outcomes. Baseline alkaline phosphatase had an AUROC 0.75 (0.60–0.87) for predicting clinical outcome (Table 2).

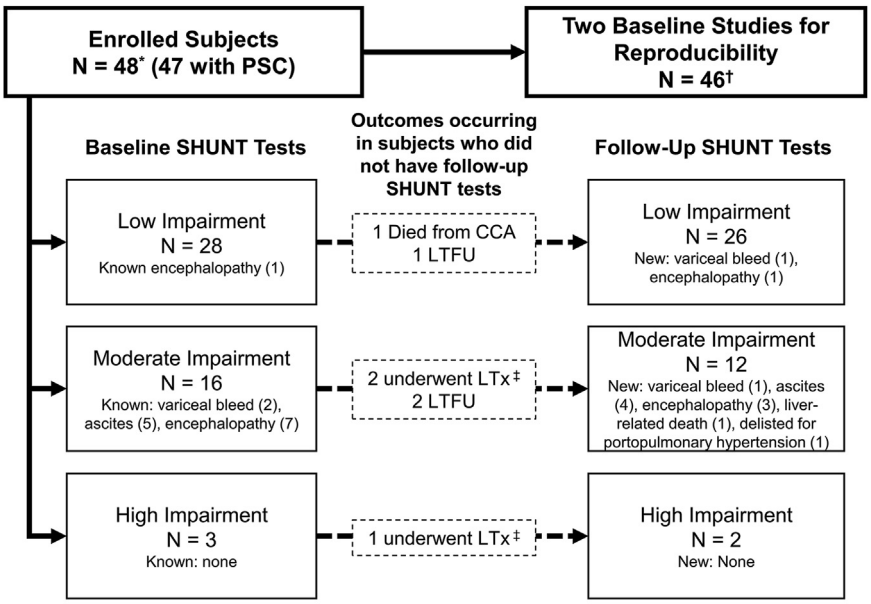
Subgroup, defined by degree of hepatic impairment, correlated with risk for clinical outcome (Figure 2). Two of 28 (7%) in the low subgroup experienced clinical outcomes, stomal variceal hemorrhage in one and encephalopathy in the other. Eleven of the 19 (58%) moderate and high subgroups experienced the following clinical outcomes: ascites (3), encephalopathy (2), ascites and encephalopathy (1), uncomplicated liver transplantation (3), death post-TIPS for varices and portal vein thrombosis (1), and listing for liver transplantation with subsequent delisting due to severe portopulmonary hypertension (1).

Baseline HepQuant results predicted risk for clinical outcome. DSI, HR%, and SHUNT% highly correlated with varices (Figures A1 and A2) and were significantly higher in the 13 who experienced clinical outcome (Table A3). SHUNT % was the strongest baseline predictor of clinical outcome with AUROCs 0.84 (0.71–0.93) for DuO and 0.90 (0.78–0.97) for SHUNT test versions (Table 2).

Overall, in the 40 patients who had follow-up HepQuant tests, there were nonsignificant increases in SHUNT% (DuO: 1.3 ± 7.0%; SHUNT: 2.4 ± 9.4%) and DSI (DuO: 0.3 ± 3.6; SHUNT: 0.5 ± 3.5). This unanticipated slow overall rate of progression was due to the high percentage of cases with MELD ≤10, brief period of follow-up (391 ± 39 days), and heterogeneity in the rate of disease progression.

Ten of the 40 subjects who had follow-up experienced clinical outcome and 30 remained clinically stable. As shown in Table 3, baseline and follow-up SHUNT% and DSI were significantly higher in subjects who experienced outcomes. DSI increased significantly between baseline and follow-up in the subjects experiencing outcomes (2.4 ± 4.2 vs −0.2 ± 3.0, $P = .039$ for DuO; 2.3 ± 4.2 vs −0.4 ± 3.2, $P = .042$ for SHUNT). There was a nonsignificant trend in the mean increase in SHUNT% (DuO: 4.5%, $P = .10$; SHUNT: 4.2%, $P = .48$).

Of note was a sole case of an acute flare of biopsy-proven chronic active hepatitis complicating the PSC course (fatigue,



*One of the 48 enrolled had primary biliary cholangitis and was excluded, leaving 47 subjects with primary sclerosing cholangitis (PSC).
† One PSC subject only had a single baseline test.
‡Liver transplantation (LTx) occurred after baseline testing but before their scheduled follow-up test.

Figure 2. Disposition of subjects and clinical outcomes.

Table 3. SHUNT% and DSI in the 40 PSC Subjects With HepQuant Tests at Baseline and Follow-Up (391 ± 39 Days)

	SHUNT% (%)			DSI		
	Baseline	Follow-up	ΔSHUNT%	Baseline	Follow-up	ΔDSI
HepQuant DuO						
No clinical outcome (N = 30)	28.9 ± 10.6	29.2 ± 12.7	0.2 ± 5.5	16.5 ± 5.7	16.1 ± 6.5	−0.4 ± 3.2
Decomp, death, or transplant (N = 10)	44.2 ± 16.5	48.7 ± 17.5	4.5 ± 9.9	23.5 ± 7.6	25.8 ± 7.9	2.3 ± 4.2
P value	**.0016**	**.0005**	.1000	**.0036**	**.0004**	**.0417**
HepQuant SHUNT						
No clinical outcome (N = 30)	36.2 ± 13.6	38.0 ± 19.2	1.8 ± 9.5	16.1 ± 6.0	15.9 ± 6.4	−0.2 ± 3.0
Decomp, death, or transplant (N = 10)	58.9 ± 15.6	63.1 ± 13.7	4.2 ± 9.3	22.8 ± 8.1	25.3 ± 8.3	2.4 ± 4.2
P value	<**.0001**	**.0005**	.4800	**.0008**	**.0007**	**.0386**

Changes in SHUNT% and DSI are compared between the subjects who did or did not experience clinical outcomes. Values are mean ± SD. Bold values indicate statistical significance ($p < 0.05$).

jaundice, and pruritus, with elevations of alanine transaminase, aspartate transaminase, alkaline phosphatase, and bilirubin). All functional parameters worsened during the flare and recovered with successful prednisone therapy (Figure A4).

In multivariable regression analysis, a model including SHUNT%, DSI, platelet count, and change in these parameters from baseline had AUROCs 0.95 (DuO) and 0.96 (SHUNT) for clinical outcomes. The AUROC for this model, whether by DuO ($P = .005$) or SHUNT ($P = .001$), significantly outperformed the model of baseline alkaline phosphatase plus change in alkaline phosphatase which had an AUROC 0.60 (Figure 3).

Associations of Use of UDCA or Treatments for IBD with Changes in DSI and SHUNT%

Thirty-one subjects (66%) were taking UDCA at baseline (14.4 ± 3.9 mg kg^{-1} d^{-1}), including 28 who had follow-up at 1 year. At baseline, there was no difference in either SHUNT% (41.8% ± 17.0% vs 42.6% ± 17.9%) or DSI (17.50 ± 7.82 vs 18.22 ± 5.01) among subjects taking UDCA vs not taking UDCA. However, in follow-up DSI increased (1.91 ± 3.88 vs −1.54 ± 3.44, $P = .011$) in subjects taking UDCA.

Nineteen of 33 with IBD (58%) were taking IBD medications at baseline, including 14 who had follow-up at 1 year. At baseline, there was no difference in either SHUNT% (44.8% ± 20.5% vs 41.5% ± 16.8%) or DSI (18.2 ± 7.8 vs 18.4 ± 6.7) among IBD subjects taking vs not taking these medications. However, in follow-up SHUNT% increased (6.2% ± 10.2% vs −1.8% ± 9.7%, $P = .043$) in IBD subjects exposed to IBD medications. The DSI and SHUNT% results for IBD subjects not taking IBD medications were similar to the results for subjects without IBD.

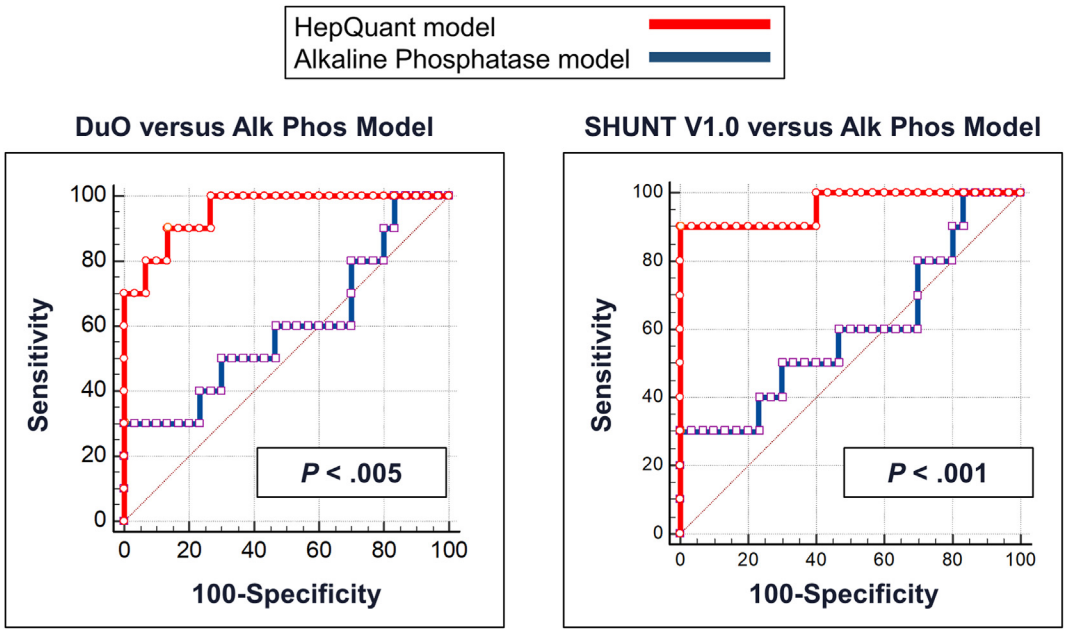


Figure 3. Comparison of HepQuant and alkaline phosphatase models (baseline and change in alkaline phosphatase) in predicting clinical outcome. The HepQuant DuO (left panel) and SHUNT (right panel) models included DSI, SHUNT%, platelet count, and changes from baseline.

Discussion

Our study cohort spanned the spectrum of disease in patients with PSC. In contrast to subjects in the simtuzumab trial, we did include patients who had recovered and stabilized after a prior clinical decompensation event. In addition, at baseline our cohort had higher bilirubin and international normalized ratio and lower albumin compared to the simtuzumab cohort. These differences likely explain the higher rate of hepatic decompensation, 25% over 18 months vs 9% over 24 months.[4]

The top 4 predictors of clinical outcome were SHUNT%, Portal HFR, HR%, and DSI with AUROCs exceeding those of alkaline phosphatase, APRI, FIB4, MELD score, CP score, and Mayo PSC score. These comparisons suggest that quantifying liver function and physiology could allow for a more precise determination of disease severity and risk for clinical outcome compared to current standard assessments.

We identified subgroups of PSC patients based on low, moderate, or high degree of hepatic functional impairment as quantified by DSI, HR%, and SHUNT%. These functional subgroups correlated with clinical manifestations of liver disease, laboratory tests, clinical scores, and inflammatory cytokines. Others have noted age-related differences in severity of PSC disease,[14] but age-related degree of hepatic functional impairment to classify risk for disease progression is a unique attribute of this study.

Age-adjusted measures of disease severity have been used in other studies, such as fibrosis stage in HCV patients, to identify progressor groups.[15] In PSC, biopsy is not routinely performed, and at the time of our study, other disease severity indicators (enhanced liver fibrosis, elastography, magnetic resonance cholangiopancreatography [MRCP]) were not standard assessments. For these reasons, the main indicators of disease severity, HepQuant functional parameters, were plotted against age – the 3 subgroups were defined and the differences in DSI per year of life were statistically significant. Phenotyping of PSC patients by age-related degree of functional impairment may help to target clinical management to those at greatest risk for progression and identify those in need of more urgent liver transplantation. In therapeutic drug development, characterization of subjects into subgroups of low, moderate, and high degree of hepatic impairment may aid investigations into the genetic and molecular mechanisms of disease progression and allow for the development of more precise strategies for the treatment and follow-up of these patients. Subjects with the highest risk for liver disease progression could be targeted for participation in clinical outcome trials. This intriguing observation will require confirmation in other studies.

Although evaluation of the biliary tree by magnetic resonance imaging/MRCP is currently in PSC guidelines,[16,17] at the time of our study magnetic resonance imaging/MRCP was performed primarily for assessment of biliary complications or detection of cancer. As opposed to focusing on the biliary tree, this exploratory study was focused on the linkage of hepatic functional impairment to severity of liver disease and liver-related clinical outcomes.

Our finding that the change in alkaline phosphatase in combination with baseline alkaline phosphatase was poorly predictive of clinical outcome may be considered surprising. However, similar results were reported in the simtuzumab clinical trial where baseline alkaline phosphatase had an AUROC 0.71 for clinical outcome, and the combination of baseline alkaline phosphatase plus change in alkaline phosphatase had an AUROC 0.68.[4] Furthermore, a secondary analysis of the same study reported lack of association of alkaline phosphatase with disease progression.[18]

In contrast to the findings with alkaline phosphatase, DSI or SHUNT% alone or in combination (Table 2 and Figure 3) were strongly predictive of clinical outcomes. Progression to clinical outcome was associated with a significant worsening of DSI. These data imply that DSI and SHUNT% could be useful tools to augment current standard of care in the management of patients with PSC.

HepQuant tests measure the unconjugated cholate remaining in the blood that has not been hepatically cleared, thereby reflecting the cholate uptake process. Although data are limited, that which exists suggests that cholate uptake as measured by HepQuant would not be altered by cholestasis *per se*.[19] Jazrawi, et al, used the radionuclide, ^{75}Se-homocholic acid, and radioscintigraphy in controls, PSC, and PBC to probe the 3 phases of movement of bile acid from sinusoidal blood to bile – uptake into hepatocytes, residence within hepatocytes, and biliary excretion. Despite prolonged residence time and impaired excretion, uptake of the cholate homologue was normal in PSC and PBC. UDCA treatment improved residence time and excretion but had no effect on uptake of the cholate homologue. These findings support the conclusion that the cholestasis associated with PSC and PBC does not interfere with cholate uptake.

However, with development of fibrosis sinusoidal blood flow is compromised, impairing hepatocyte uptake. Studies using gadobenate– or gadexetate–magnetic resonance imaging have shown that impaired uptake correlates with stage of fibrosis (F0-F2 vs F3-F4) and severity of disease as assessed by Mayo risk score, MELD, and liver biochemistries.[20] These findings are consistent with the results of our study showing direct correlation of SHUNT and DuO test parameters with severity of disease and risk for clinical outcome.

Herein we demonstrated that HepQuant tests were linked to features of parenchymal disease (portal hypertension, decompensation, and varices) and not to history of cholangitis events or underlying inflammatory bowel disease. In our cohort we observed a PSC case with an AIH flare where DSI, SHUNT%, and HR % paralleled the severity of hepatic dysfunction and recovery.

Other routine laboratory tests, clinical models, transient elastography, cholangiography, and histology have all been proposed as surrogates for clinical outcome.[2-5,21-23] Of the noninvasive tests, transient elastography, enhanced liver fibrosis test, and MRCP+ have been the most recently

studied. Corpechot, in a study of 168 PSC patients undergoing VCTE, found that both baseline liver stiffness and change in stiffness were associated with clinical outcome.[3] In the simtuzumab study, a subset of 58 patients had VCTE; baseline VCTE but not change in VCTE correlated with clinical outcomes.[4] In a study of 210 PSC patients, Ehlken reported AUROC 0.72 for VCTE and 0.76 for spleen length in prediction of clinical outcome.[24] In the simtuzumab trial baseline enhanced liver fibrosis score had an AUROC 0.71 for clinical outcome.[4] In the Lemoinne study of the link of MRCP + to clinical outcome, an internal cohort had AUROCs 0.89 and 0.76, and an external cohort had AUROCs 0.76 and 0.73, for MRCP + studies done without and with gadolinium, respectively.[25] DSI, SHUNT% and HR % compare favorably with these results.

We examined DSI and SHUNT% in subjects taking UDCA to treat PSC and subjects using drug therapy to treat IBD. Baseline DSI, SHUNT%, and HR% were similar between those taking and not taking UDCA, but DSI appeared to worsen in association with ongoing chronic use of UDCA. The latter finding might be consistent with data suggesting potential hepatic toxicity of UDCA in PSC.[26] In contrast, SHUNT% worsened in the sicker IBD subjects requiring IBD treatment, vs untreated IBD subjects. This worsening of SHUNT% in those on IBD treatment suggests linkage of severity of intestinal disease to the portal fibroinflammatory process in PSC.

Baseline parameters from DuO and SHUNT, with AUROCs ranging from 0.83 to 0.90, compared favorably to the other noninvasive tests in predicting clinical outcome. Our model of DSI, SHUNT%, and platelet count, incorporating both baseline and follow-up testing, was highly predictive of clinical outcome (DuO test: AUROC 0.95, Sensitivity 90%, Specificity 87%; SHUNT test: AUROC 0.96, Sensitivity 90%; Specificity 100%).

The ability to identify the subpopulation of PSC patients at risk for clinical outcome is further evidence of the practical application of DSI and SHUNT%, not just for clinical management but also in design of drug trials. As indicated in Table 3, a trial enrolling patients with low DSI and low SHUNT% might never reach a clinical outcome endpoint. In contrast, enriching studies in high DSI and high SHUNT% cases could potentially enhance the chances to reach a clinical endpoint in a reasonable period of time and with a limited sample size.

The HepQuant DuO and SHUNT tests can be easily performed in the clinic, infusion center, or hospital. The DuO test involves oral ingestion of d4-CA followed by collection of 2-timed blood samples via direct venipuncture, with no requirements for specialized equipment or expertise for test collection. The SHUNT test involves placement of indwelling peripheral venous catheters in each arm for sampling and for intravenous administration of 13C-CA (in addition to the oral d4-CA dose), with blood samples collected before dosing and 5, 20, 45, 60, and 90 minutes after dosing. The completed test collection kits are then shipped to the HepQuant Laboratory (Denver, CO, USA) for analysis by LC-MS/MS, and the results are transmitted back to the ordering providers along with guides for interpreting the test results and conveying the information to patients.

In this study, we demonstrated equivalency in diagnostic performance between DuO and SHUNT tests for clinical decompensation, varices, and risk for clinical outcomes in patients with PSC. Recent publications verify equivalency[13] and reproducibility in other populations.[27] By simplifying test administration (oral dose only), reducing blood samples (from 5 to 2), and shortening the period of testing (from 90 to 60 minutes), the DuO test enhances the clinical utility of quantifying liver function and physiology.

Admittedly, our study has limitations of single-center experience, relatively small sample size, and short period of clinical follow-up. More data, including long-term follow-up and larger cohorts, are needed to verify the findings of this important study. The predictive models presented herein demonstrate proof-of-concept requiring validation in a second, larger cohort. Additionally, alignment of DuO and SHUNT results with clinical outcomes in larger trials would further support the use of DSI, HR%, and SHUNT% as surrogates likely to predict clinical outcome.

PSC-associated cholestasis may raise serum bile acid levels, but, as discussed previously, this alone may not affect the hepatic uptake of the administered cholates of the DuO and SHUNT tests. There are at least 5 separate mechanisms of uptake of unconjugated cholate by the liver including passive diffusion, facilitated transport, and active transport.[28,29] In PSC, OATP-C mRNA was decreased 50%,[30] while OATP-A mRNA was up 230%.[31] Others have shown that, despite reduction in biliary secretion, the hepatic uptake of an intravenous-administered labeled cholate derivative was not significantly different between normal individuals and PSC patients.[32] These findings suggest that cholestasis, per se, will not affect the results of the HepQuant SHUNT test.

Endogenous cholate levels also do not affect the accuracy of the SHUNT test (HepQuant internal validation data). In advanced PSC with marked cholestasis, endogenous cholate levels may be elevated eight-fold.[19,33,34] Our validated liquid chromatography/mass spectrometry methods separate and selectively quantify the administered 13C-CA and d4-CA even in the presence of high bile acid and cholate concentrations.

Because cholate is absorbed in the upper small bowel by passive or facilitated diffusion,[35-37] ileal disease, ileal resection, or drugs affecting the ileal bile salt transporter will not alter the intestinal absorption of d4-CA. Findings from our study are consistent with absorption in the upper small bowel. The d4-CA was detected in peripheral venous blood within 5 minutes of its oral administration, and the peak of the d4-CA clearance curve was typically between 20 and 45 minutes.

Some medications or over-the-counter supplements may affect the HepQuant DuO and SHUNT test results. To mitigate any potential impact on test results, patients should withhold the following for the appropriate amount of time prior to testing: prescription bile acid sequestrants, over-the-counter fiber supplements, and cyclodextrin products

may affect absorption of the oral d4-CA dose and should be withheld for at least 24 hours prior to testing; glucagon-like-peptide-1 receptor agonists may potentially slow gastric emptying and should be withheld at least 5 days prior to testing; β-blockers, ACE inhibitors, or ARBs could affect blood flow to the liver and should be withheld on the morning of testing; UDCA, cholic acid, or other bile acids alter intestinal absorption and affect results and should be withheld on the morning of testing.

In conclusion, HepQuant DuO and SHUNT are novel and potentially useful tests for quantifying liver function and physiology that may improve our understanding of the underlying pathophysiological basis for clinical heterogeneity of PSC. By identifying functional subgroups, clinicians may more accurately determine frequency of follow-up visits, appropriate use of laboratory tests, and identify those in need of closer clinical follow-up or earlier evaluation for liver transplant. For drug developers, functional phenotyping may be useful to more precisely classify patient groups and reduce heterogeneity between treatment arms. Due to ease of administration, DuO is ideally suited for identifying more advanced patients appropriate for enrollment into longer-term clinical outcome studies. Further investigation into the clinical role of HepQuant DuO and SHUNT tests in PSC is warranted.

Supplementary Materials

Material associated with this article can be found, in the online version, at https://doi.org/10.1016/j.gastha.2024.07.005.

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Authors' Contributions:
Gregory T. Everson conceived the study concept and design. Steve Helmke and Gregory T. Everson analyzed results, interpreted data, conducted statistical analyses, and drafted and reviewed the manuscript. John Kittelson was a statistical consultant. Joanne C. Imperial assisted in interpretation of the data and original draft preparation, reviewing, and editing the manuscript. Steve Helmke performed all cholate analyses. Michael P. McRae performed data analysis, prepared figures and tables, and drafted and reviewed the manuscript. The study sponsors had no role in study design, conduct, and interpretation of findings. All authors participated in contributing to writing the manuscript, including revisions and edits. The authors take responsibility for the accuracy of the analysis, manuscript preparation, and the decision to submit the manuscript for publication.

Conflicts of Interest:
The authors disclose the following: Gregory T. Everson, Steve Helmke, and University of Colorado Denver have several issued and pending patents relevant to the analytical procedures used in this study. Gregory T. Everson is an equity owner/member and CEO of HepQuant LLC and Steve Helmke is an employee of HepQuant LLC. John Kittelson, Joanne C. Imperial, and Michael P. McRae are paid consultants to HepQuant LLC. Gregory T. Everson, Steve Helmke, and Michael P. McRae have patent applications pending related to the oral cholate test (HepQuant DuO) and simplified versions of the SHUNT test.

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Ethical Statement:
This study was conducted according to the Declaration of Helsinki and approved by the Colorado Multi-Institutional Review Board. All subjects gave written informed consent. Cholate labeled with cold nonradioactive isotopes were studied under FDA IND 65121 ([24-^{13}C]cholate, 13C-CA) and IND 65123 ([2,2,4,4-^{2}H]cholate, d4-CA).

Data Transparency Statement:
Individual participant data will not be shared.

Reporting Guidelines:
None.