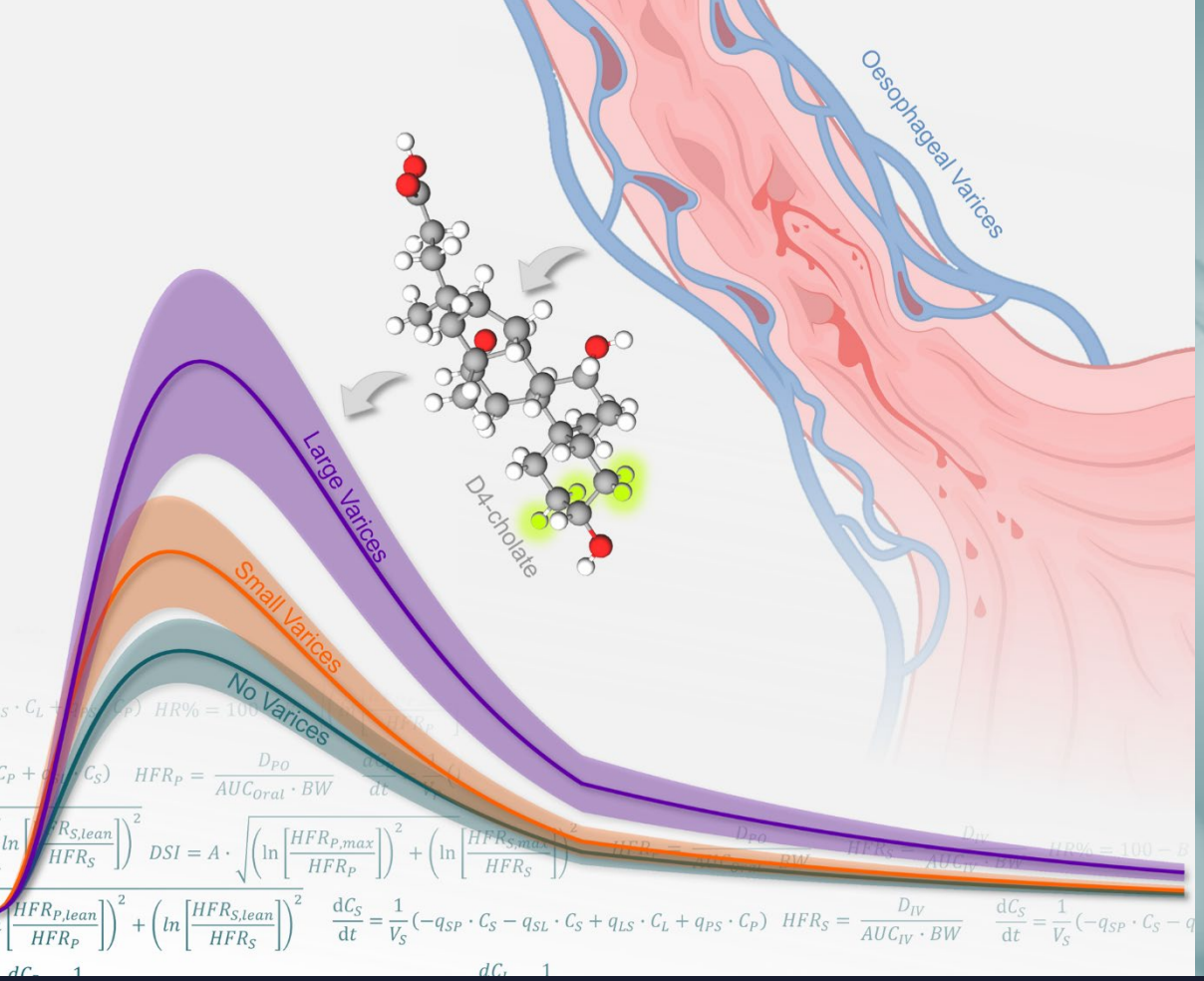

Noninvasive Cholate Challenge (HepQuant DuO™) for Primary Sclerosing Cholangitis

Gregory T. Everson, MD

PSC Forum
November 6, 2025

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Disclosures

- Dr. Gregory T. Everson, MD, is an Emeritus Professor of Medicine at the University of Colorado School of Medicine and Chief Executive Officer of HepQuant LLC.
- Dr. Everson is the inventor of the HepQuant technology, founder of HepQuant LLC, and holds intellectual property rights to HepQuant technology. He is a paid employee of HepQuant LLC.

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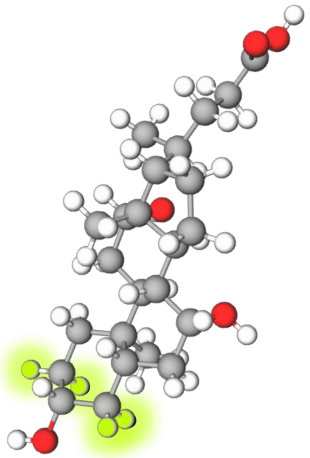
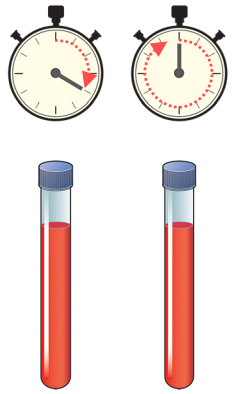
Test Administration

The HepQuant DuO Test

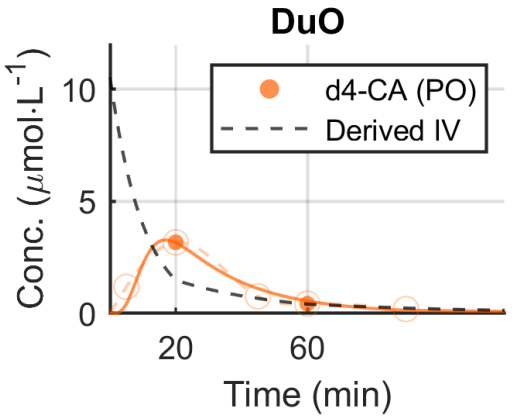
Oral dose of d4-cholate



Two blood draws at 20 & 60 min



Measure cholate clearance by LC-MS/MS



Measures of Liver Health

Disease Severity Index (DSI)

Hepatic Filtration Rates

SHUNT%

Hepatic Reserve

RISK ACE

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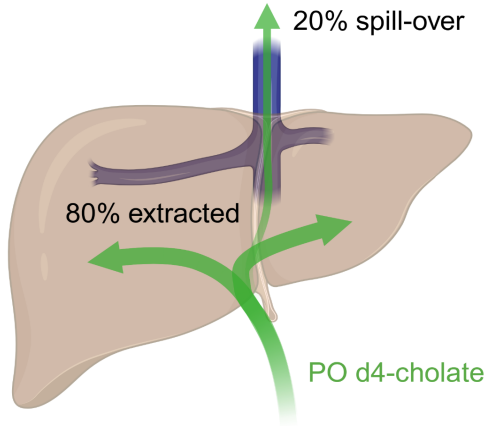
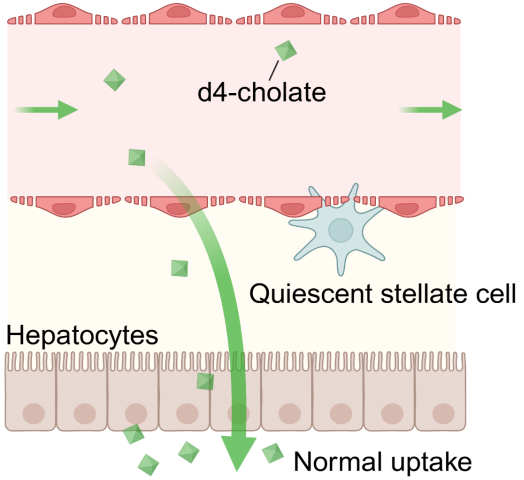
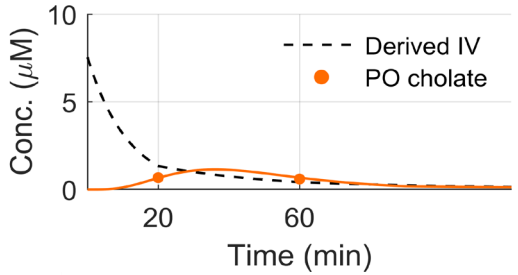
What Hepatic Functions and Physiology are Assessed?

The d4-Cholate Measured in the Peripheral Venous Blood samples is that which is not cleared by Flow-dependent delivery to and liver-specific uptake by Hepatocytes

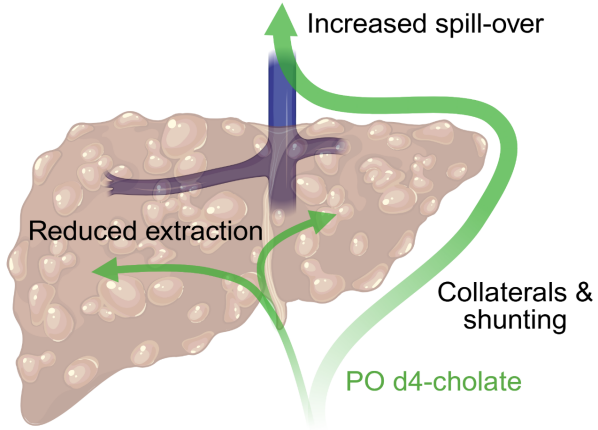
Normal Function

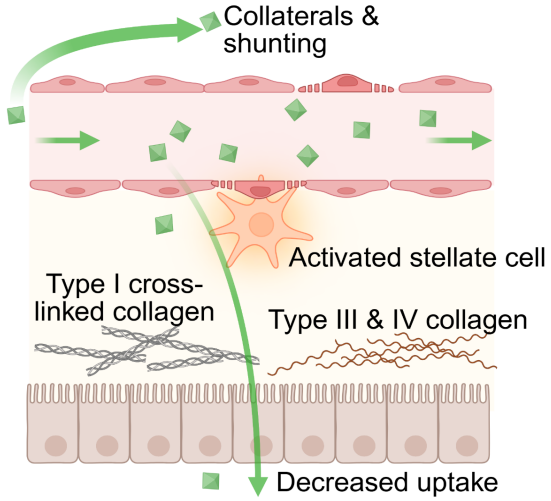


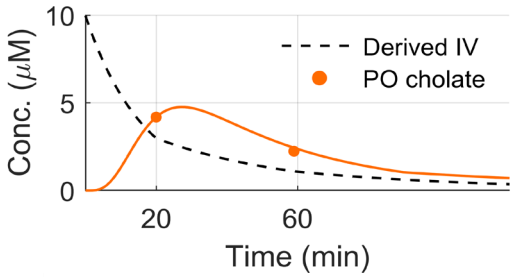




Hepatic impairment







- As PSC progresses there is an increase:
  - [d4-CA] in peripheral venous blood
  - DSI
  - SHUNT%
- And, decrease in:
  - Hepatic Filtration Rate
  - Hepatic Reserve (HR%)

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Unique Attributes of Unconjugated d4-Cholate

Unconjugated Cholate in PSC (drink and draw)

- **Intestinal Absorption** – 100% absorbed from the upper small bowel – natural endogenous compound, no radioactivity, no xenobiotic exposure
  - May be used in patients with IBD
  - Terminal ileal disease or IBAT therapies not likely to affect absorption
  - In our PSC study, 70% had IBD – test predicted varices risk and clinical outcome

- **Hepatic Uptake** – multiple pathways, not dependent on energy or Na cotransport – passive, facilitated diffusion, OATPs, NTCP – unlikely affected by SNP mutations
  - Early stage cholestasis – uptake of ^{75}Se-homocholic acid preserved despite reduced excretion
  - Uptake of cholate more reflective of severity of disease (function/physiology)
  - Oral administration is more sensitive than IV injection in detecting change due to disease

- **Gastric Emptying** – not absorbed from stomach – drugs that slow gastric emptying (e.g. GLP-1 agonists, narcotics) or bind bile acids (e.g. cholestyramine) must be held

Illustrative Case

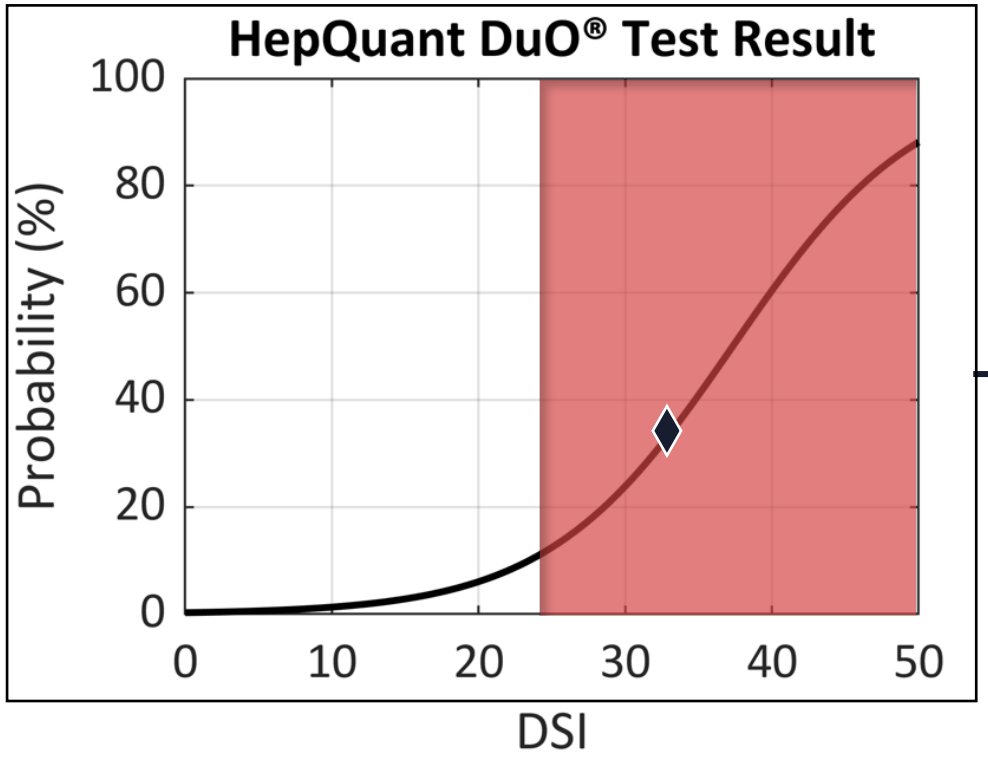
Defining Disease Severity to Aid the Transplant Decision

48 yo patient with PSC and bacterial cholangitis treated w antibiotics and stricture dilation. BMI 22.3. MELD: 11. AST: 164; ALT: 132; T.Bili: 2.2; Albumin: 3.7; Alk Phos: 513; GGT: 437. Prior EGDs - small varices.

HepQuant DuO ordered to quantify disease severity.

Standard of Care

Based on current assessment, would you:
1. **Repeat EGD?**
2. **Evaluate for LDLT?**



DSI 33.5
35.3% risk for LEV
SHUNT 75.5%,
HR 42.2%

Impact of HepQuant DuO

CARE PLAN: FORGO EGD

- DSI SHUNT% and HR suggest high risk for LEVs and portal hypertension, and support need for EGD.
- EGD showed LEV that were banded
- Based on HepQuant DuO results she has severe liver dysfunction despite low MELD score
- She was evaluated and underwent LDLT

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PSC – the dual disease

- **Biliary** – obstruction/infection – stricture management
  - Anatomic measurements – MRCP, LiverMultiScan MRCP+, ERCP, other
  - Additional imaging – MRI, CT, US

- **Hepatic** – decompensation – liver transplantation
  - Standard laboratory tests, clinical models
  - Elastography – gadexotate (MRI)
  - **HepQuant** tests – SHUNT and **DuO** (portal-systemic shunt)

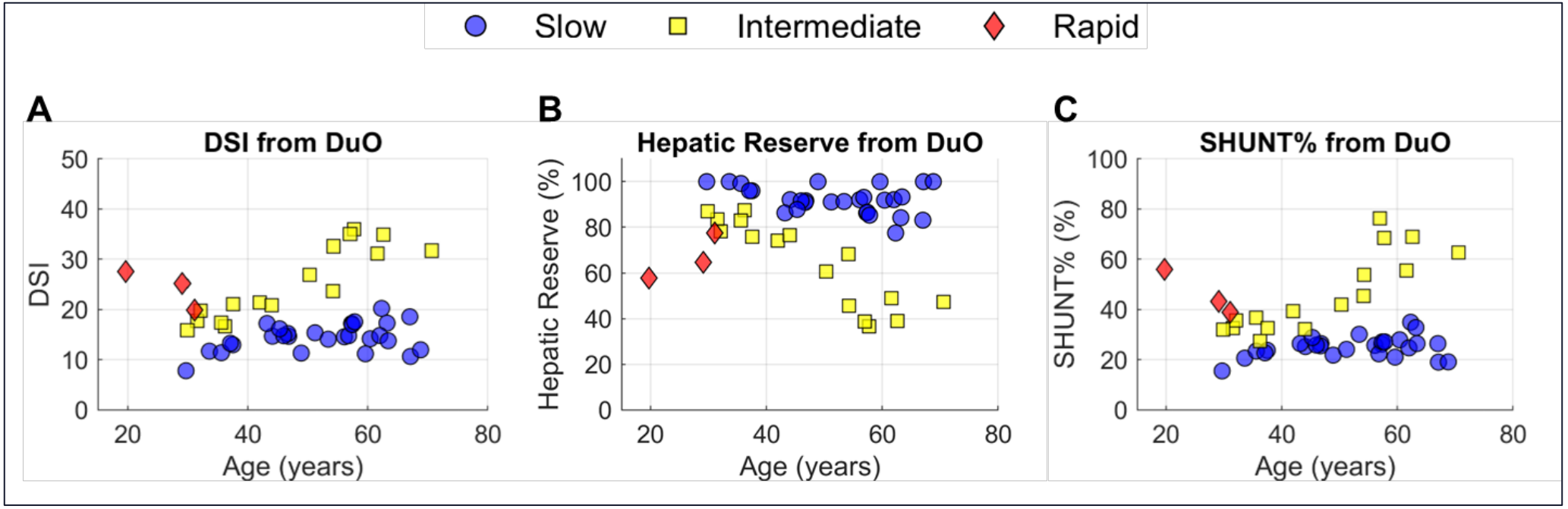
Key Findings from HepQuant's PSC Study

HEPQuant™ DuO

Helmke S, et al. The Oral Cholate Challenge Test Quantifies Risk for Liver-Related Clinical Outcomes in Primary Sclerosing Cholangitis. Gastro Hep Advances 2024;3:944-953.

Progressor Category

Progressor Status – Functional Impairment by Age



Progressor Status		
Slow	0.28	ΔDSI / year
Intermediate	0.53	ΔDSI / year
Rapid	0.91	ΔDSI / year

$p < 0.001$ for differences between groups by ANOVA

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HepQuant DuO Parameters in Intermediate/Rapid Progressors

Test Parameter	Slow Progressors (N=28)	Intermediate and Rapid Progressors (N=19)	p value
DuO			
SHUNT% (%)	25.02 ± 4.09	46.27 ± 14.73	**<0.001**
DSI	14.43 ± 2.73	25.01 ± 6.81	**<0.001**
Hepatic Reserve (%)	92.12 ± 6.03	64.74 ± 17.53	**<0.001**
Portal HFR (mL min^{-1} kg^{-1})	18.46 ± 4.79	8.18 ± 4.06	**<0.001**
Systemic HFR (mL min^{-1} kg^{-1})	4.45 ± 0.48	3.27 ± 0.87	**<0.001**

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Intermediate/Rapid Progressors also showed significant Impairment in Standard Laboratory Tests and Clinical Scores

	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	**<0.001**
DSI	14.43 ± 2.73	25.01 ± 6.81	**<0.001**
Hepatic Reserve (%)	92.12 ± 6.03	64.74 ± 17.53	**<0.001**
Portal HFR (mL/min/kg)	18.46 ± 4.79	8.18 ± 4.06	**<0.001**
Systemic HFR (mL/min/kg)	4.45 ± 0.48	3.27 ± 0.87	**<0.001**
Lab Tests and Clinical Models			
Alkaline Phosphatase (IU/L)	128 ± 83	387 ± 353	**0.0006**
Bilirubin (mg/dL)	1.16 ± 0.46	2.99 ± 2.91	**0.0023**
Platelets (nL^{-1})	201 ± 78	160 ± 103	0.13
APRI	0.58 ± 0.31	1.92 ± 1.52	**0.0001**
FIB4	1.91 ± 1.16	4.05 ± 3.17	**0.0029**
MELD-Na Score	10.11 ± 2.39	13.58 ± 6.01	**0.0074**
≤ 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	**<0.0001**
Mayo PSC Risk Score	0.314 ± 0.517	1.313 ± 0.941	**<0.0001**

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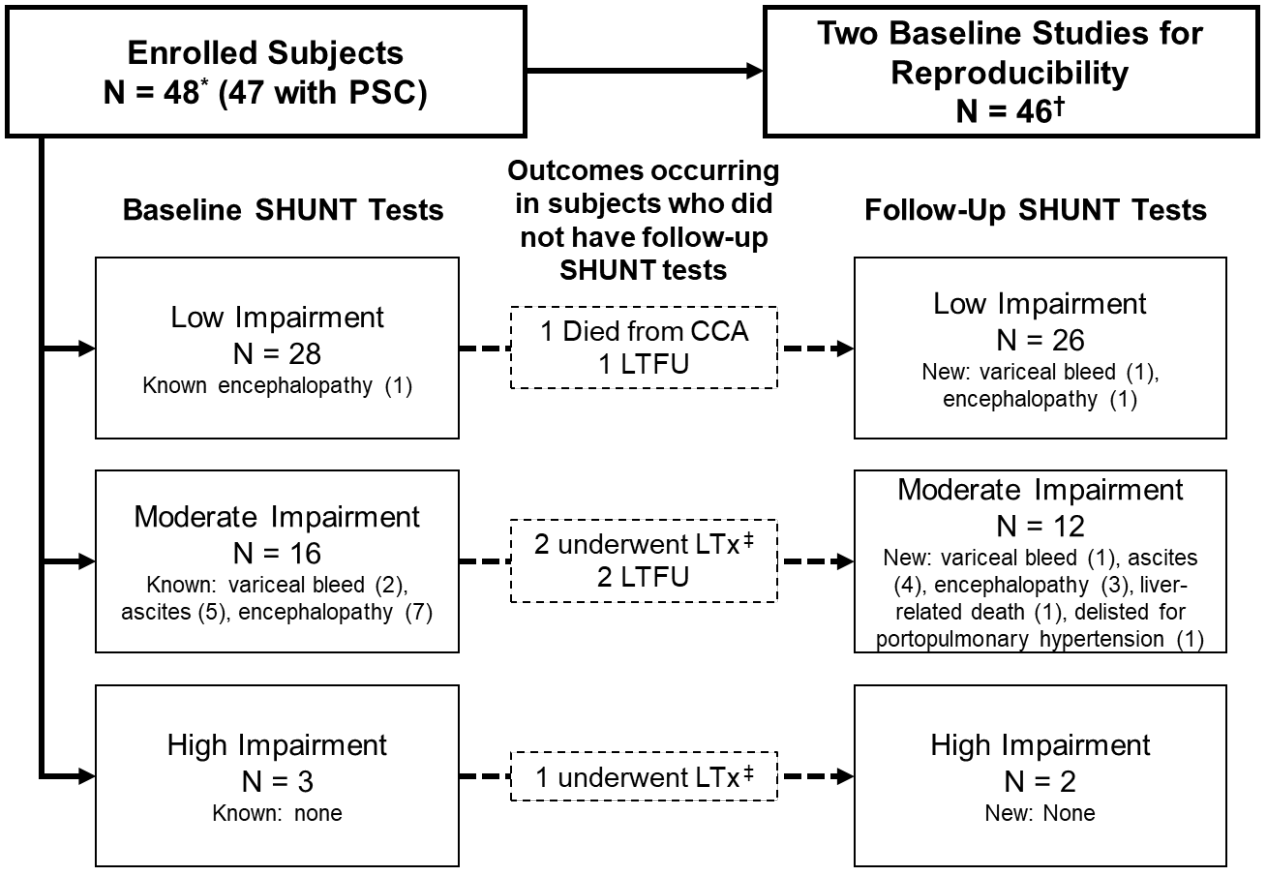
Study Flow Chart by Progressor Status



*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.

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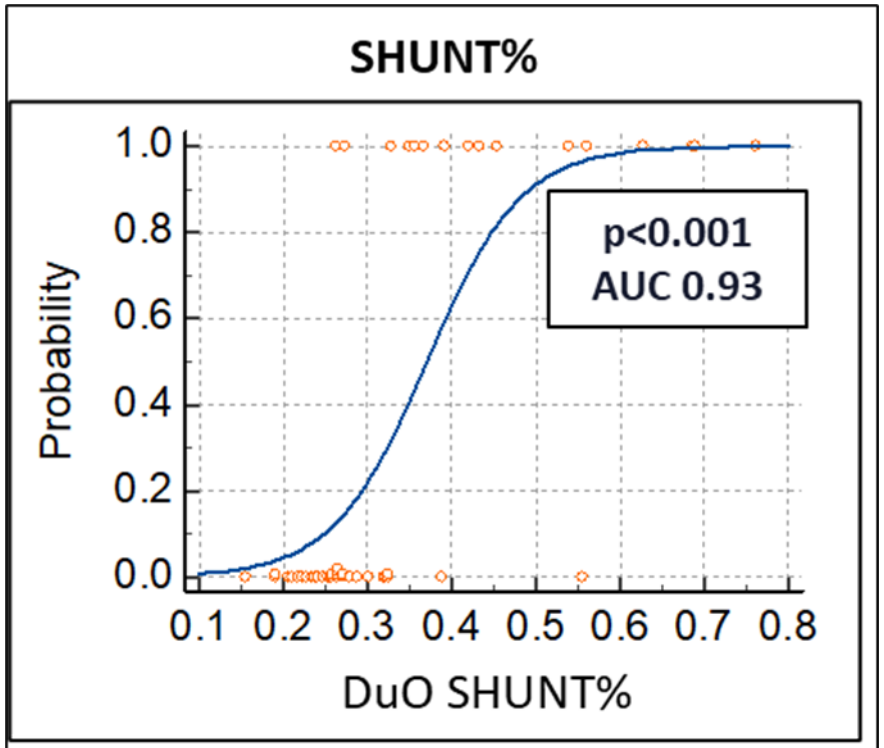
Link to Clinical Outcome

A Higher SHUNT% at Baseline was Associated with Greater Probability of Diagnosing Varices at Prior Endoscopy



Sample size	47
Positive cases [a]	16 (34.04%)
Negative cases [b]	31 (65.96%)

Variable	Coeff.	SE	P
DuO SHUNT%	17.9765	5.7954	0.0019
Constant	-6.6578	1.9052	0.0005

A Higher SHUNT% at Baseline was Associated with Greater Probability of Future Clinical Outcome

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.

Baseline Test Parameters: Comparison of Subjects Experiencing Clinical Outcome to Subjects who Remained Clinically Stable

	Patients who Experienced Clinical Outcomes (N=13)	Patients who Remained Clinically Stable (N=34)	Difference in the Means	95% CI	*P* [a]
SHUNT%	**46.0% (16.6%)**	**28.9% (10.2%)**	17.2%	9.1 to 25.1%	**.0001**
DSI	**24.58 (7.54%)**	**16.46 (5.51%)**	8.12	4.10 to 12.14	**.0002**
HR	**65.9 (19.5%)**	**86.9 (13.8%)**	-21.0	-31.19 to -10.78	**.0001**

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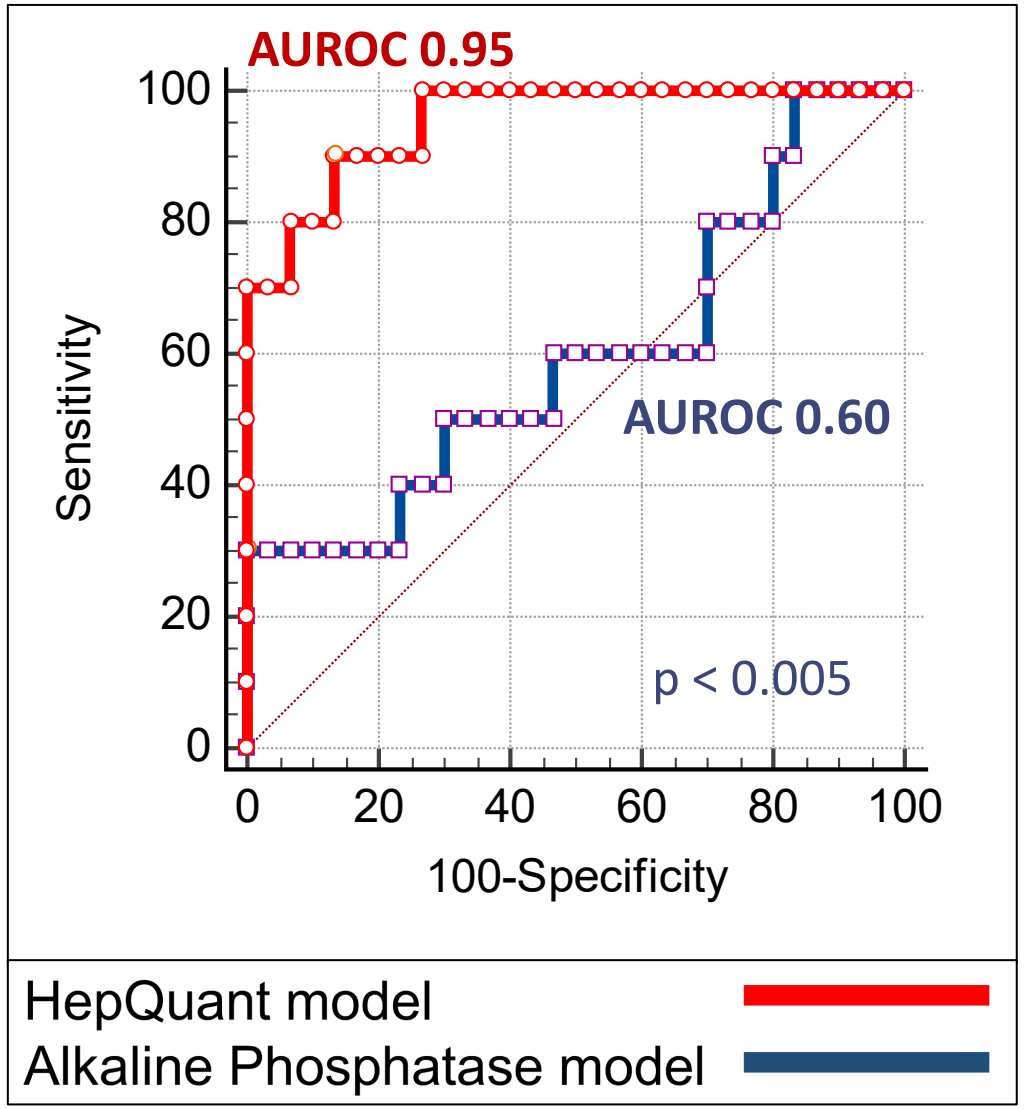
Changes in SHUNT% and DSI Predict Risk for Clinical Outcome

(Change in Alkaline Phosphatase did not Predict Clinical Outcome)

	SHUNT% (%)		
	Baseline	Follow-up	ΔSHUNT%
HepQuant DuO			
No clinical outcome (N = 30)	28.9 ± 10.6	29.2 ± 12.7	0.2 ± 5.5
Decomp, death, or transplant (N = 10)	44.2 ± 16.5	48.7 ± 17.5	4.5 ± 9.9
P value	**.0016**	**.0005**	.1000

	DSI		
	Baseline	Follow-up	ΔDSI
HepQuant DuO			
No clinical outcome (N = 30)	16.5 ± 5.7	16.1 ± 6.5	−0.4 ± 3.2
Decomp, death, or transplant (N = 10)	23.5 ± 7.6	25.8 ± 7.9	2.3 ± 4.2
P value	**.0036**	**.0004**	**.0417**



Summary

Summary of Key Findings with HepQuant DuO

- **Identified Functional Progressor Subtypes** linked to clinical outcome that could influence frequency of clinic visits, monitoring, or even selection for liver transplantation
- **Outperformed alkaline phosphatase** in prediction of clinical outcome
- **Defined baseline functional heterogeneity**
- For drug developers, functional phenotyping may be useful to more precisely classify patient groups and reduce heterogeneity between treatment arms
- Within individual reproducibility was established
- Further validation by inclusion in PSC trials is warranted

McRae et al. 2024. Clin Transl Sci.
Helmke S et al. 2024. Gastro Hep Advances

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