The HepQuant SHUNT Liver Diagnostic Kit for Likelihood of Large Esophageal Varices: The SHUNT-V Study

SHORT TITLE: The SHUNT-V Study for Varices

Protocol Number: HQ-US-SHUNT-1801

National Clinical Trial (NCT) Identified Number: NCT03583996

Principal Investigator: TBD

IDE Sponsor: HepQuant LLC

Funded by: HepQuant LLC

Version Number: 2.4

6 October 2020

Summary of Changes from Previous Version 2.3 to new Version 2.4:

Affected Section(s)	Summary of Revisions Made	Rationale
All	Updated Headers and Footers with current revision and date	Protocol Version 2.4 introduction
1.1, 9	Definition of False Negative Rate (FNR) in the protocol clarified	FNR was defined contextually but not explicitly, added explicit definition for sake of clarity, as well as a discussion of differences in terminology for FNR between the literature and FDA guidance.
1.1, 3, 4.1.3, 4.3.2, 9.4.3, 9.6.2	Addition of "miss rate" to the list of results that will be analyzed and reported for the study	To address discrepancies in calculation of FNR between published literature and FDA guidance.

1.1, 3, 9, 9.1.1, 9.5	**Performance criteria for sensitivity added to primary endpoint**	**Ensuring that the FNR is not unacceptably high when NLR < 0.52**
1.3, 1.4, 4.1.3, 8.1	**Updated to indicate that MELD and CP scores are calculated automatically from lab results**	**Clarification that study site personnel are not required to manually calculate MELD or CP scores.**
1.3	**Clarification that Visit 3 physical exam is symptom-directed**	**Consistency with rest of protocol**
1.4, 8.1	**Added instruction to upload EGD reports and images into EDC system**	**Consistency with design of EDC system**
4.1.1, 5	**Changed total number of subjects from "A total of 420" to "Up to 420"**	**Updates related to introduction of interim analysis**
4.1.3	**Description of interim analysis added**	**Introduction of interim analysis**
5	**Number of sites updated**	**Reference missed in earlier updates to protocol when site number was increased from 20 to 40**
5.5	**Study duration updated**	**Updated to reflect actual length of study recruitment**
6.6	**Removed reference implying HepQuant would be blinded to DSI results**	**HepQuant cannot be blinded to DSI results as HepQuant's laboratory is the only facility capable of analyzing subject samples**
8.1	**References to Karnofsky performance status removed**	**Correction. Requirement was removed in an early edit to the protocol but these references were missed in that update.**
8.1	**Updated description of scheduling status of EGD to include subjects in the process of being scheduled for EGD.**	**Consistency with remainder of protocol**
9.2.1	**Description of simulation study added**	**Simulation study performed to demonstrate adequate power**

9.2.1	**Reference to check of prevalence after 300 subjects removed**	**Obviated by introduction of Interim Analysis.**
9.2.1	**Added reference to interim analysis at 250 subjects**	**Introduction of Interim Analysis necessitated change**
9.2.2	**Description of analysis of secondary endpoints updated to reflect potential for early termination**	**Introduction of Interim Analysis necessitated change**
9.3	**Description of analysis population updated to include references to 250 subjects minimum**	**Introduction of Interim Analysis necessitated change**
9.4.2	**Completely rewritten to describe the analysis of the primary endpoint with the addition of the interim analysis and the performance criteria for sensitivity**	**Introduction of Interim Analysis necessitated change**
9.4.3	**Significant rewriting and moving content to better support description of interim analysis**	**Introduction of Interim Analysis necessitated change**
9.5.1	**New section to describe interim analysis, including initial and final goals and decision criteria (including a binding futility assessment), as well as the processes, controls, and rationale for the introduction of the interim analysis.**	**Introduction of Interim Analysis necessitated change**
9.6.1.2	**Minimum size for subgroup analysis changed from 100 to 50 subjects**	**To account for potential for smaller subsets due to introduction of interim analysis**
9.6.2	**STAT performance goal updated**	**Accounting for change in statistical methodology due to introduction of interim analysis**
10.1.2.2	**Conditions under which study will be stopped updated to include a finding of futility at the interim analysis**	**Acknowledgement that the assessment for futility described in Section 9.5.1 is binding.**

10.2.1	**Composition of committee membership updated**	**Adjusting for changes in staffing**

Table of Contents

STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with the applicable United States (US) laws under the Food, Drug, and Cosmetic Act and implementing regulations under Title 21 of the Code of Federal Regulations (CFR).

The Principal Investigator will sign an investigator's agreement, written per FDA guidelines (**Appendix A**), that no deviation from, or changes to the protocol will take place without prior agreement from the Investigational Device Exemption (IDE) Sponsor and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants.

The protocol, informed consent form, recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the proposed changes are implemented in the study. All changes to the consent form will be IRB approved; a determination will be made by the IRB regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 PROTOCOL SUMMARY

1.1 Synopsis

Title: The HepQuant SHUNT Liver Diagnostic Kit for Likelihood of Large Esophageal Varices: The SHUNT-V Study

Study Description:

The **intended population** for the HepQuant SHUNT Liver Diagnostic Kit (test) is the population with chronic liver disease (CLD) characterized by having either biopsy-proven or clinical cirrhosis (Child-Pugh (CP) A or B), suspected cirrhosis based on surrogate tests for cirrhosis, or, advanced fibrosis with low platelet count, who are selected for future esophagogastroduodenoscopy (EGD) to screen for varices. The **intended use** is to provide the clinician with the likelihood of finding large esophageal varices if the EGD were to be performed. This information may aid the clinician in the decision to proceed with or avoid the EGD.

The purpose of this study is to validate the Disease Severity Index (DSI) from the HepQuant SHUNT Test for likelihood of large esophageal varices. Our **HALT-C Training dataset** demonstrated that the DSI 18.3 had sensitivity 95%, specificity 54%, positive predictive value (PPV) 19%, negative predictive value (NPV) 99%, negative likelihood ratio (NLR; equals ((1-sensitivity)/specificity)) 0.0926, and false negative rate (FNR = 1-NPV[1]) 1% for large esophageal varices. This diagnostic performance compares favorably with published results from other tests or combinations of tests, such as FibroScan plus platelet count.

To validate DSI ≤ 18.3 as a cutoff for cases not likely to have large esophageal varices, we will enroll 420 subjects with chronic liver disease (CLD) of mixed etiologies from no more than 40 USA clinical centers (**CLD Validation dataset**). These will be subjects who have already been selected for variceal screening or surveillance, but have not yet undergone the procedure. The targeted expected prevalence of large esophageal varices within the enrolled

[1] NOTE: There is some disagreement in the literature regarding the calculation of the False Negative Rate (FNR) for a test. FNR has been defined by the U.S. Food and Drug Administration in a disease-centric manner as 1 - Sensitivity ((Σ False negative) / (Σ Condition positive)), while being defined in much of the clinical literature in a test-centric manner as 1- NPV ((Σ False negative) / (Σ Predicted condition negative)). To compare to published results from the literature, in this protocol, the test-centric definition for FNR (1-NPV) has been used, and the term "miss rate" has been used to express 1-Sensitivity.

population is 20%. Each subject will have been selected to undergo a future EGD for variceal screening or surveillance according to standard criteria used in current clinical practice.

Enrolled subjects will undergo standard clinical assessment, laboratory tests, and the HepQuant SHUNT Test. The previously scheduled EGD will be performed no more than 6 weeks following the HepQuant SHUNT Test.

The relationship of DSI to large varices will be analyzed by AUROC and the diagnostic performance of the DSI cutoff ≤ 18.3 will be defined by sensitivity, specificity, PPV, NPV, NLR, PLR, FNR and miss rate[2]. The validated DSI cutoff will aid the clinician in the decision to proceed with or avoid the EGD.

Objectives:

The **primary objective** of this study is to validate DSI ≤18.3 for identifying subjects with CLD selected for future EGD for variceal screening or surveillance who are not likely to have large esophageal varices if the EGD were to be performed ("Rule Out").

The Secondary objective is to define the relationship of DSI to probability for large varices over the full range of DSI.

Exploratory objective 1 is to examine the diagnostic performance of DSI for large varices over the full range of DSI. We will generate and evaluate the AUROC (c-statistic) for DSI in likelihood of large esophageal varices. The AUROC for DSI in likelihood of large varices will be compared to the c-statistic 0.76 reported by Abrades, et al, for LSM plus platelet count (8).

In addition, we will examine the diagnostic performance (sensitivity, specificity, PPV, NPV, NLR, PLR, FNR) of DSI to define the optimal DSI for "Rule Out" – highest sensitivity and NPV with lowest FNR. We will also compare the diagnostic performance of this "optimum" DSI to DSI 18.3. The purpose of this analysis is to provide upside and downside performance of DSI around the validated cutoff of DSI 18.3.

This analysis will yield a table of sensitivities, specificities, NPVs, PPVs, NLRs, PLRs, FNRs, and miss rates[2] over the given range of DSI.

Exploratory Objective 2 (DSI for Small or Any Size of Esophageal Varices) is evaluation of the diagnostic performance of DSI for small esophageal varices and for esophageal varices of any size. The analyses listed above for the primary and secondary objectives and exploratory objective 1 will be repeated using small varices in one set of analyses and any size of varices in another set of analyses.

Exploratory Objective 3 is evaluation of the diagnostic performance of a single 60 minute time point (STAT) from the clearance curve of the orally administered d4-cholate. We will evaluate STAT's diagnostic performance for large, small, and any size of esophageal varices.

Endpoints:

The **endpoint** for the **primary objective** is the negative likelihood ratio (NLR) for large esophageal varices. The performance goal for validation of DSI ≤18.3 is based on two requirements: (a) the upper limit of the confidence interval (CI) for NLR must rule out NLR > 0.52 and (b) the observed sensitivity must be > 0.85. The confidence level

[2] In this protocol, FNR is defined as 1-NPV and miss rate is defined as 1-Sensitivity. See the footnote in Section 1.1 for a discussion of these two measures.

for the confidence interval for NLR will be adjusted in order to maintain a one-sided type I error rate of 0.025 as described in section 9.5.1.

The **endpoint for the secondary objective** is the plot of predicted probability of large varices versus DSI using logistic regression analysis and affiliated statistical tests of the significance of the association between the continuous DSI score and risk of large varices. These analytical results will be evaluated using a calibration table in which the prevalence of large varices is tabulated by DSI quartile.

The **endpoint for exploratory objective 1** is the AUROC (c-statistic) and parameters for diagnostic performance of DSI in likelihood of large esophageal varices. The **endpoints** for **exploratory objective 2** are the c-statistics for the AUROCs for DSI in the likelihood of small or any size of esophageal varices. The **endpoints** for **exploratory objective 3** are the c-statistics for the AUROCs for STAT in the likelihood of large, small, or any size of esophageal varices. The c-statistics for STAT will be compared to the c-statistics for DSI.

Study Population:

This is a US multicenter study. Participants will have been diagnosed with CLD and have been scheduled for EGD for the indication of variceal screening or surveillance. The study will include both men and women, ages ≥18 years, and all races and ethnicities; and, will comprise a broad clinical spectrum of CLD to include noncirrhotic F3 stage of fibrosis with low platelet count, compensated cirrhosis (Child-Pugh class A), and moderately decompensated cirrhosis (Child-Pugh (CP) class B cirrhosis). Child-Pugh (CP) class C cases and cases with prior variceal hemorrhage, refractory ascites, or refractory encephalopathy are excluded. The latter cases are at high risk for large esophageal varices and EGD screening for varices is strongly recommended without the need for additional testing.

Phase: Device, Pivotal Study

Description of Sites/Facilities Enrolling Participants:

This study will be conducted at not more than 40 USA clinical centers. The participating centers will utilize either local or central Institutional Review Boards (IRBs), have extensive experience in the care and management of patients with liver disease, and have effectively managed clinical trials.

Description of Study Intervention:

The HepQuant SHUNT Liver Diagnostic Kit contains one 20 mL vial of 25% human serum albumin, one 30 mL vial containing 10 mL of 40 mg cholate labeled with 4 deuterium atoms (d4-cholate) in sodium bicarbonate solution for oral dosing, one 10 mL val containing 5.5 mL of 22 mg cholate labeled with carbon-13 (13C-cholate) in sodium bicarbonate solution for intravenous injection (5.0 mL, 20 mg is injected),), a 3-way stopcock, pipettes, transfer tubes, instructions for use, labels, and materials for mailing serum to HepQuant's reference laboratory. Cholates and albumin are natural endogenous compounds of the human body. The labels, d4 and 13C, are cold stable labels and are NOT RADIOACTIVE. Deuterium and carbon-13 are naturally abundant in the human body – deuterium represents approximately 0.0156% of all hydrogen atoms and carbon-13 represents 1.1% of all carbon atoms. There is no significant increase in the body's pools of deuterium or carbon-13 from administration of doses of d4- and 13C-cholates used in the HepQuant SHUNT test.

The HepQuant SHUNT test measures liver function (cholate uptake), and physiology (hepatic and portal inflows and portal-systemic shunting) from simultaneous clearance of both the orally and intravenously administered d4- and 13C-cholates. Six timed blood samples (3 mL each) are obtained at 0, 5±1, 20±2, 45±5, 60±5, and 90±5 minutes. Concentrations of d4- and 13C-cholate are measured by LC/MS, clearances defined, and hepatic filtration rates, SHUNT, STAT, and DSI calculated. DSI has a range from 0 to 50 based upon results from over 1600 tests in over 700 persons.

Study's Duration:

18-24 months

Participant's Duration:

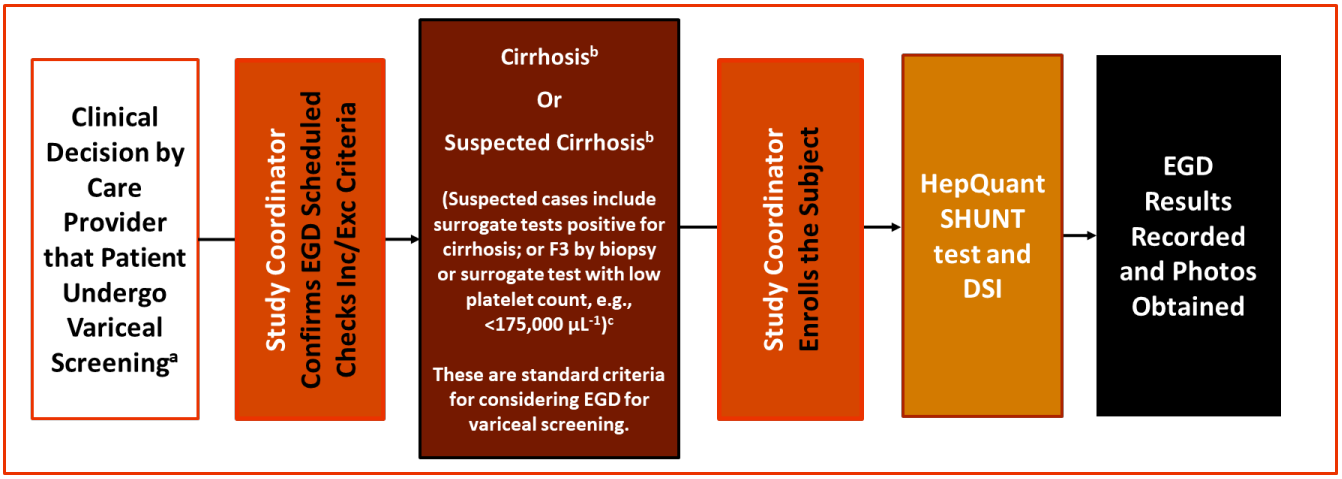
From 3 to 100 days

Sample Size and Power:

The study will enroll no more than 420 subjects, to provide 80% probability (power) that the upper limit of the 95% CI for the observed NLR_0 is <0.52 for the DSI cutoff of 18.3 in identifying subjects unlikely to have large esophageal varices.

1.2 Study Flow Diagram

Figure 1: Study Schematic



[a] The patient's care provider has determined the patient needs an EGD for variceal screening or surveillance. The decision to perform this EGD is not protocol-driven; it is the independent decision of the patient's care provider.

[b] The **intended population** for the HepQuant SHUNT Liver Diagnostic Kit (test) is persons with chronic liver disease (CLD) with cirrhosis (Child-Pugh (CP) A or B but without a history of variceal hemorrhage, refractory ascites, or refractory encephalopathy), suspected cirrhosis (positive surrogate tests for cirrhosis), or, advanced fibrosis with low platelet count, who are selected for future esophagogastroduodenoscopy (EGD) to screen for varices. The **intended use** is to provide the clinician with the likelihood of finding large esophageal varices if the EGD is performed. This information may aid the clinician in the decision to proceed with or avoid the EGD. Cirrhosis may be diagnosed histologically, biochemically, clinically, radiologically, or by liver stiffness measurements.

[c] Subjects with F3 stage of fibrosis on liver biopsy, or F3 by a surrogate test, who also have a low platelet count, <175,000 μL^{-1}, may have undetected cirrhosis, portal hypertension, and varices.

1.3 Visit Schedule and Procedures

Visit 0, Screening ≤ 28 days prior to Enrollment Day -28 to Day 1	Sign Informed Consent. Verify Inclusion and Exclusion Criteria and order for EGD. Lab: complete blood count, chemistry, urine pregnancy test, and ECG. MELD Calculated from labs. If available within 1 year of enrollment, collect reports for liver imaging (US, CT, or MRI), liver biopsy, elastography (FibroScan, ARFI, Shear-Wave or MRE), serum or blood fibrosis panel or biomarker, and HVPG. Complete eCRFs.
Visit 1, Enrollment Day 1	Verify subject eligibility. Record concomitant medications. Medical History, Vital Signs, and Physical exam. Record height and weight, CP score calculated. Schedule the HepQuant SHUNT Test. Complete eCRFs.
Visit 2, HepQuant SHUNT Test ≤ 28 days after Enrollment Day 1 to Day 29	Confirm overnight fast or fast of 5 hours or more. Obtain Vital Signs. If applicable, verify hold of morning dose of ACE or ARB inhibitors, or nonselective β-blocker. Administer the HepQuant SHUNT Test per the Instructions for Use. Complete the Test Requisition Form (TRF) and the "Self-Reported Subject Tolerability" survey. Record AEs or SAEs. Complete eCRFs.
Visit 3, Post-Test Follow-Up 2-30 days after Visit 2 Day 3 to Day 59	Perform an Interim history and symptom-directed physical exam and check vital signs. Draw complete blood count and chemistry. Complete the "Self-Reported Subject Tolerability" survey. Record AEs and SAEs. Complete eCRFs. NOTE: This visit can occur at time of EGD, if EGD falls within the 28 d window.
Collection of EGD Data 0 to 42 days after Visit 2 Day 1 to Day 71	EGD is performed after the HepQuant SHUNT Test and within 42 days of the SHUNT Test. Obtain de-identified EGD report and photos and record EGD results. Record EGD-related AEs and SAEs. Complete eCRFs. The plan of treatment in response to the EGD findings will also be recorded.

1.4 Schedule of Activities (SoA)

Visit 0, Screening (Up to 28 days prior to enrollment)

The subject will meet with the study coordinator to obtain informed consent, review study protocol and procedures, address questions or concerns, and determine eligibility. The study coordinator will ensure that subject satisfies all inclusion criteria and does not meet any of the exclusion criteria, that the subject satisfies CLD criteria and etiology, and that the EGD procedure is scheduled or in the process of being scheduled.

The following tests will be obtained at the screening visit: complete blood count, blood chemistry profile, urine pregnancy test for women of child-bearing potential, and electrocardiogram. MELD score will be calculated automatically from screening lab results.

The coordinator will also collect reports of the following tests, if performed within a year of enrollment, and the results will be captured in the eCRF:

- Hepatic imaging by either ultrasonography (US), computed tomography (CT), or magnetic resonance imaging (MRI)
    - Record hepatic masses – record LiRAD score 1 to 5 if given in report
    - Record patency or occlusion of main portal vein
- Liver biopsy
    - Scoring system (METAVIR, ISHAK, NASH-CRN, other)
    - Fibrosis stage
    - Necro-inflammatory grade
    - Steatosis grade
- Elastography
    - Type of technology used for elastography measurement
    - kPa and associated stage of fibrosis
    - CAP, or similar score, and grade of steatosis
- Blood Panel or Blood Biomarkers
    - Type of test (ELF, other)
    - Fibrosis stage
    - Necro-inflammatory grade
    - Steatosis grade
- HVPG
    - Free hepatic vein pressure (FHVP)
    - Wedged hepatic vein pressure (WHVP)
    - HVPG matches WHVP – FHVP

The above tests are not a requirement for enrollment and are not analyzed for EGD endpoints. The results are collected only to further characterize the CLD study population.

Visit 1, Enrollment (Day 1)

The study coordinator will verify the eligibility of the study subject by review of results from the screening visit. *Note that the Screening and Enrollment visits can be conducted on the same day if results from screening labs are available.* Concomitant medications, demographic information, including gender, race/ethnicity, age (date of birth), height, and weight will be recorded. BMI will be calculated from height and weight. The PI will complete a medical history and physical examination. Child-Pugh (CP) score will be calculated from the screening laboratory tests and the results of the history and physical examination. The coordinator will schedule the subject for the HepQuant SHUNT Test which must be completed within 28 days of Visit 1.

Visit 2, HepQuant SHUNT Test Administration (Up to 28 days after Enrollment Visit)

Visit 1 (Enrollment) and Visit 2 (SHUNT Test) may occur on the same day. The HepQuant SHUNT test must be completed within 28 days of Visit 1 and **prior to the performance of the EGD**. Prior to Test administration the coordinator will record Vital Signs. The HepQuant SHUNT Test will be administered according to the Instructions for Use and the required information will be recorded in the Test Requisition Form (TRF). The coordinator will oversee and confirm all procedures related to receipt, administration, and sample handling for the HepQuant SHUNT test, ensure completion of the "Self-Reported Subject Tolerability" survey, and record any and all AEs and SAEs.

Visit 3, Post-Test Follow-Up (Between 2 and 30 days after SHUNT administration at Visit 2)

The post-Test follow-up visit must be done at least 2 days after the HepQuant SHUNT Test administration, but must be completed within 30 days of SHUNT Test administration. The PI will perform an interim history and physical exam with a focus on any expected and unexpected AEs and SAEs. Vital signs will be obtained. Fasting blood will be obtained for complete blood count, and serum chemistry. This visit can occur at the time of EGD, if EGD falls within the 2-30 day window between Visit 2 and Visit 3.

Collection of EGD Data (EGD must occur within 42 days of SHUNT test at Visit 2)

The EGD must be performed **after** the HepQuant SHUNT Test and within (not more than) 42 days after the HepQuant SHUNT test.

The time interval of 6 weeks, or 42 days, between the HepQuant SHUNT test and EGD allows for logistical complexities in scheduling or rescheduling. Also, the time interval of 6 weeks is short, relative to the generally slow progression from no varices to varices or from small to large varices. From the known rates of progression, varices are unlikely to either develop or change in size within the 6 weeks between SHUNT test and EGD. **Section 2.2.2**, page 16, defines and discusses the rates of development or progression of varices in reference to the time interval of up to 6 weeks between SHUNT test and EGD. Given the study design with 0 to 42 days between SHUNT test and EGD, it is highly likely that the average time interval between SHUNT test and EGD will be far less than 6 weeks.

If convenient for the patient, the EGD can be done on the same day as the HepQuant SHUNT test (Visit 2); but, if this occurs, the EGD must be done after the HepQuant SHUNT test. The study coordinator will obtain de-identified EGD report and photos and record the EGD results, and upload the EGD report and associated photos into the EDC system. We will also record the plan of treatment in response to the EGD findings. EGD findings are NOT recorded as adverse events.

All required eCRFs will be completed at each of the study visits.

1.5 Flow Chart of Procedures

Procedures	Visit 0, Screening ≤28 days before Enrollment	Enrollment Visit 1, Day 1	SHUNT Administration, Study Visit 2 ≤28 days after Enrollment	SHUNT Follow-up, Study Visit 3 2-30 days after SHUNT test	EGD Data Collection 0-42 days after SHUNT test
Informed consent	X				
Confirm CLD criteria	X				
Confirm CLD etiology	X				
Confirm EGD Scheduled	X				
Demographics		X			
Medical history		X			
Interim History				X	
Record results (if done within 1 year and available) for:					
Liver US, CT, and MRI	X				
Liver Biopsy	X				
Liver Elastography	X				
Blood Panel or Biomarker	X				
HVPG	X				
Concomitant Medications		X			
Physical exam		X		X	
Vital signs		X	X	X	
Height		X			
Weight		X			
Complete Blood Count	X			X	
INR	X				
Serum Chemistry [a]	X			X	
MELD Score	X				
CP Score		X			
Pregnancy test [b]	X				
ECG	X				
HepQuant SHUNT Test			**X**		
EGD Performed					**X**
AEs and SAEs			X	X	X
Subject Tolerability Survey			X	X	
Complete CRFs	X	X	X	X	X

[an] Electrolytes, BUN, Creatinine, AST, ALT, Alk Phos, Bilirubin-total, Albumin

[b] Urine pregnancy test for β-HCG for women of child-bearing potential.

2 INTRODUCTION

2.1 Study Rationale

2.1.1 STATEMENT OF PROBLEM AND FILLING AN UNMET NEED

Patients with chronic liver disease (CLD), particularly advanced fibrosis or cirrhosis, are at-risk for esophageal varices. Large esophageal varices are at-risk for hemorrhage and are defined as high-risk varices (HRVs), or varices needing treatment (VNT). Throughout the remainder of this document, the terms large varices, HRVs, and VNTs are used interchangeably.

Hemorrhage from large esophageal varices often precipitates additional complications, including infection, hepatic decompensation, and death. Large esophageal varices are an independent risk factor for mortality. Esophagogastroduodenoscopy (EGD) to detect large esophageal varices is recommended for patients at time of diagnosis of cirrhosis and every 1 to 3 years thereafter, depending on the initial endoscopic findings (i.e., no, small, or large varices) and the severity and course of the CLD (2,3). Once large esophageal varices are diagnosed, therapy should be instituted immediately (1-3).

Many patients referred for EGD, based on the diagnosis of cirrhosis by biopsy or surrogates for hepatic fibrosis, will either have no varices, or small varices which are not clinically significant. In general, small varices are not at risk for hemorrhage. EGD may be considered unnecessary if the yield from EGD is no varices or clinically-irrelevant small varices - avoiding the expense and risk of unnecessary EGD is desirable.

As single tests, INR, platelet count, APRI, FIB-4, Lok score, Forns score, liver or spleen stiffness by transient elastography, computed tomography (CT), and video capsule endoscopy (VCE) have lacked adequate diagnostic performance for exclusion of large varices (4). The most accepted screening strategy to exclude large varices, the BAVENO VI criteria, recommends the combination of liver stiffness measurement (LSM) <20 kPa (transient elastography) plus platelet count >150,000 μL^{-1}.

Based upon the cases with no or small varices meeting these criteria who underwent EGD, approximately 20% of unnecessary EGDs might have been avoided (4,5). However, despite the positive effect of modest reduction in unnecessary EGDs, there is a significant false negative rate (1-negative predictive value) or miss rate (missed cases with large varices/total cases with large varices). In a recent review of 10 studies with 11% prevalence of large varices, the average false negative rate (FNR) was 3.3% and the average miss rate was 6.4 ± 8.3% (6). Four of these studies had over 50 cases with large esophageal varices – the FNRs were 5.3%, 5.8%, 18.2%, and 0.0% (7-11). The miss rates varied considerably across the studies – but were as high as 21.4%.

There is a need for a non- or minimally invasive test with improved diagnostic performance to further reduce the number of unnecessary EGDs and to lower the FNR and miss rate for large esophageal varices.

Unmet Need and the HepQuant SHUNT Test. In **Section 4.2**, the analysis of the **HALT-C Training dataset** found that the Disease Severity Index (DSI) from the HepQuant SHUNT test had a cutoff, DSI ≤18.3 with negative likelihood ratio (NLR) 0.09. If HALT-C subjects with DSI ≤18.3 were excluded, the study could have reduced the number of unnecessary EGDs by 49%. Applying the HALT-C DSI cutoff ≤18.3 to the published studies using the BAVENO VI criteria would have eliminated 52% of unnecessary EGDs. Also, in the HALT-C Training dataset the FNR was 1% and only one case of large varices was missed, 1/22 (4.5%). Overall, the results using the DSI cutoff 18.3 compare favorably with current strategies and indicate that the HepQuant SHUNT Test could fill the unmet need for a minimally invasive test to “Rule Out” large esophageal varices and avoid unnecessary EGD.

2.1.2 STUDY POPULATION AND DISEASE

The **CLD Validation dataset** will consist of subjects with CLD who have been selected for EGD to screen or undergo surveillance for esophageal varices.

Definition of CLD. CLD is defined as a disease process of 6 months or more in duration, characterized by ongoing liver injury and progression of fibrosis, culminating in its most severe form as cirrhosis with clinical complications (12). The SHUNT-V study is focused on CLD cases with F3 or F4 (cirrhosis) stages of disease. Etiologies of CLD include chronic viral hepatitis (hepatitis B and C), alcoholic liver disease, non-alcoholic fatty liver disease (NAFLD) or steatohepatitis (NASH), cholestatic liver disease (primary biliary cholangitis, primary sclerosing cholangitis), and genetic disorders such as hemochromatosis, Wilson's disease, and alpha-1-antitrypsin deficiency.

Prevalence of CLD. CLD is highly prevalent and global in reach. Up to 30% of the general adult population has steatosis and 6 to 7% has liver fibrosis, mostly associated with NAFLD, prompting suggestions for population screening (13-15). A European study of LSM measurement in over 3000 healthy asymptomatic persons without known liver disease found that 3.6% have "silent" significant fibrosis (fibrosis stages F2 to F4 (cirrhosis)) defined by transient elastography of kPa >9.0. Liver biopsy was performed in 92 cases and 16% had F3 or F4 fibrosis (16).

CLD Stage and Risk for Varices. Patients with F3 fibrosis have a pre-cirrhotic stage of fibrosis but are at risk for portal hypertension and varices. Results from the whole HALT-C study demonstrated that 2% of subjects with bridging fibrosis (13/598) and 11% of those with cirrhosis (48/418) had large varices. Twenty one percent (13/61) of the large varices occurred in subjects with bridging fibrosis (17). Platelet count >150,000 μL^{-1} excluded nearly all the cases with large varices.

In the **HALT-C Training dataset**, 4% of subjects with bridging fibrosis (5/124) and 18% of those with cirrhosis (17/93) had large varices. 23% percent of the large varices occurred in subjects with bridging fibrosis. Platelet count >175,000 μL^{-1} excluded all the cases with large varices.

Both F3 and F4 cases are included in the SHUNT-V study, but a requirement for subjects with non-cirrhotic F3 disease is a platelet count <175,000 μL^{-1}. SHUNT-V does not have a platelet count requirement for F4 cases, since diagnosis of cirrhosis is a risk factor for large varices independent of platelet count.

Esophageal Varices. All CLD, regardless of etiology, progresses via inflammation and fibrosis to alter portal blood flow, which may ultimately lead to portal hypertension and portal-systemic shunts (18). Esophageal varices are portal-systemic shunts and the HepQuant SHUNT Test quantifies portal-systemic shunting.

2.1.3 PITFALLS OF CURRENT PRACTICE

Liver biopsy and fibrosis staging. The gold standard for staging fibrosis and diagnosing cirrhosis, particularly in NAFLD, is liver biopsy, but liver biopsy is painful, risky, labor intensive, expensive, prone to sampling error, and requires special expertise (19). Risk of bleeding from liver biopsy in F3/F4 disease is 0.6%, and risk is greatest in cases with platelet count less than 50,000 to 60,000 μL^{-1} (20,21). Liver biopsy is not well tolerated or embraced by patients. Liver biopsy for staging fibrosis is rapidly being replaced by non- or minimally-invasive surrogates for fibrosis.

Non- or minimally invasive surrogates for fibrosis staging. The most widely used non- or minimally-invasive surrogates for staging fibrosis are liver stiffness measurement (LSM) by elastography and blood biomarkers (22-25). Although these tests have gained increasing use their accuracy is questioned, particularly in patients with NAFLD or NASH (26,27). Elastography requires specialized equipment or use of MR facilities, and guidelines suggest that transient elastography should be performed twice on two separate days after overnight fasting to improve the accuracy of the results. Repeat testing increases resource utilization, costs, and patient inconvenience. Despite these concerns LSM has gained wide clinical acceptance for distinguishing cirrhotic from non-cirrhotic stages of disease.

Non- or minimally invasive surrogates for varices diagnosis. By extension of its performance in diagnosing cirrhosis, LSM has also been evaluated for likelihood of large esophageal varices and esophageal varices of any size (3-11). As a standalone test, LSM performs less satisfactorily – likely due to the highly indirect relationship of LSM to portal pressure, i.e., stiffness as surrogate for cirrhosis, which is only a moderate predictor for likelihood of large esophageal varices. The most current guidelines have focused on the use of LSM with platelet count to improve the diagnostic performance for large esophageal varices.

There is a need to identify the CLD patient at low or no likelihood of having large esophageal varices to reduce the performance of unnecessary EGDs (4-11, 28-38); but, only if the FNR or miss rate is acceptably low. A recent review highlighted the pitfalls of current non- or minimally-invasive strategies. The authors concluded that there remains a need for further research and that the noninvasive diagnosis of large varices, HREV, or VNT will be significantly improved by new innovations and strategies (4).

2.1.4 REASONS FOR CONDUCTING THIS STUDY

Our proposed prospective study with prespecified endpoints offers several advantages over the published studies. The SHUNT-V study includes:

- A prospective study design with prespecified objectives, endpoints, and analytical plan;
- Multiple clinical centers within the United States (USA);
- Adequate sample size to ensure a sufficient number of cases with large esophageal varices to achieve statistical validity;
- Short time interval between SHUNT test and EGD;
- Documentation of EGD findings;
- Defined sequence of testing with SHUNT test always preceding EGD;
- Broad spectrum of disease severity of CLD;
- Broad spectrum of etiologies of CLD;
- Capture of the post-EGD plan of clinical management based on the findings at EGD;
- Clinically important primary efficacy endpoint – validation of DSI ≤18.3 to identify the subjects unlikely to have large esophageal varices (HRVs or VNTs) to avoid unnecessary EGD;
- Evaluation of a key secondary objective – defining the probability of large varices for a given DSI over the full range of DSI;
- Exploratory objectives to evaluate the AUROC for DSI in likelihood of large varices and compare DSI 18.3 to other potential DSI cutoffs, to examine the diagnostic performance of DSI for likelihood of small or any esophageal varices; and, to examine a simplified, single point version (STAT) as an alternative to DSI in likelihood of large, small, or any esophageal varices – for potential future development of the STAT test.

Varices develop at certain thresholds of physiologic impairment. In the case of portal pressure, the threshold for large varices is approximately 10 or 12 mmHg (1-3,39). This threshold defines clinically significant portal hypertension, since it is associated with large varices and risk for variceal hemorrhage.

The same concept of development of large varices related to degree of physiologic impairment holds true for the HepQuant SHUNT test (40). Esophageal varices are portal-systemic shunts and the HepQuant SHUNT Test measures portal-systemic shunt. Research studies have examined DSI in reference to liver disease (41-48). These studies correlate with hepatic venous pressure gradient (HVPG) (49), and direct measurement of portal pressure (50), likelihood of varices and size of varices (42,44,46). Our results from the HALT-C Training Dataset indicate that below the threshold of DSI 18.3, there is very low to no risk of large esophageal varices or the complication of variceal hemorrhage. Above a DSI of 18.3, the likelihood of large varices and variceal hemorrhage increases with increasing DSI. Since the Test results are linked pathophysiologically to portal-systemic shunting and clinical

complications, it is reasonable to consider use of the HepQuant SHUNT Test for likelihood of large esophageal varices.

2.1.5 THE URGENT NEED

The emerging wave of cases of advanced liver disease from NAFLD/NASH is creating an urgent need for accurate minimally-invasive tests for likelihood of large esophageal varices.

The incidence of liver disease is dramatically increasing owing to the obesity/diabetes epidemic and NAFLD. Changes in the dietary and lifestyle habits of Americans are leading to a rapid expansion in liver disease – for example, it is currently estimated that over 30 million Americans have NAFLD and over 5 million have the more severe or progressive NASH (51-54). The current estimate of the prevalence of cirrhosis in the USA is 0.27% or 633,323 cases with most related to NAFLD (55). As previously noted, a recent EU study using liver stiffness measurement in over 3000 otherwise healthy individuals observed a prevalence of 3.6% with kPa >9.0, consistent with F2 to F4 fibrosis (16). Limited biopsy information suggested that nearly all cases with F2 to F4 fibrosis were due to NAFLD/NASH and 16% of the cases with LSM >9.0 kPa had F3/F4 disease.

The fear is that many individuals will escape detection and progress silently to cirrhosis, portal hypertension, and esophageal varices. In fact, the rise in liver disease has been occurring over the past two decades, unnoticed. A study using data from The National Inpatient Sample database indicated that the number of hospitalizations for cirrhosis in the United States nearly doubled from 371,000 in 2001 to 659,000 in 2011, and inpatient costs increased 2-fold ($4.8 billion to $9.8 billion) (56).

This emerging crisis related to the surge in liver disease urgently calls for improved liver testing to identify high risk patients. This need is further amplified by the recent flurry in drug development for NASH and lack of suitable efficacy endpoints.

There is an increasingly urgent need for an accurate and minimally invasive test for assessing likelihood of large esophageal varices. Existing blood tests and surrogates for fibrosis or cirrhosis, inadequately assess large esophageal varices (4-11). As a result, there remains an unmet need for a minimally invasive test for likelihood of large esophageal varices.

Other pitfalls of currently available methods are the need for specialized equipment (elastography devices – FibroScan, SuperSonic, MR elastography), or specialized expertise (gastroenterology or hepatology or interventional radiology). Available methods are either invasive and risky (biopsy, HVPG) (19,39), or relatively insensitive (blood tests, radiologic imaging) (23-25, 57-60). In addition, many are expensive and impractical for broad application (liver biopsy, HVPG, MR, CT, US). Others require purchase of expensive equipment (Transient Elastography, BreathID). None of the tests has demonstrated adequate diagnostic performance for detecting the likelihood of large esophageal varices. These pitfalls highlight the need for a new innovative approach.

The HepQuant SHUNT Test addresses a large unmet medical need. Beyond the US, there are over 500 million additional cases of CLD worldwide (61). Accurate diagnostic testing to determine likelihood of large esophageal varices and need to perform or potential to avoid EGD is key to successful case management. The SHUNT Test does not require specialized equipment or expertise. Validating its DSI cutoff of 18.3 in the identification of subjects who are unlikely to have large esophageal varices could improve patient management. Using DSI for ruling out the likelihood of large esophageal varices may reduce costs, not only due to reduction in EGDs but also due to reduction in the need for less accurate, inconvenient, costly, and potentially risky staging procedures.

2.2 Background

2.2.1 CHRONIC LIVER DISEASE (CLD)

With time, CLD may progress to F3 fibrosis, F4 fibrosis/cirrhosis, and clinical complications, including liver transplantation and death. According to recent USA statistics published in May 2017 (62), CLD ranks 4th overall as a leading cause of death in the age range 45 to 65, and 6th in the age range 25 to 44. CLD is one of the leading causes of loss of potential years of life prior to age 75. Males are more likely than females to die of CLD. As a cause of death, CLD ranks 10th in white males, 4th in American Indian males, and 6th in Hispanic males (42).

Early Stages of CLD and hepatic impairment. At early stages of CLD, hepatocyte functions measured by standard blood tests (bilirubin, albumin, INR) are normal or nearly normal. But, even at this early stage, there is ongoing hepatic injury, inflammation, and fibrosis which may alter sinusoidal perfusion via vasoactive mediators of injury and inflammation and by peri-portal or sinusoidal fibrosis (18). The HepQuant SHUNT Test, which quantifies systemic and portal inflow to the liver, as well as portal-systemic spillover, is sensitive to the changes in sinusoidal perfusion and hepatocellular dysfunction that occur during all stages of liver disease, including the earliest stages (40). In the HALT-C Training dataset, DSI was higher in non-cirrhotic subjects with varices compared to non-cirrhotic subjects without varices.

Compensated Advanced CLD or Compensated Cirrhosis. Progression of chronic liver disease is characterized by progressive fibrosis, development of cirrhosis and, ultimately, clinical complications such as varices, ascites, jaundice, and encephalopathy. With progression to cirrhosis hepatocyte function, as measured by standard blood tests (bilirubin, albumin, INR), may still be normal or nearly normal. But as progression continues, the impairment in sinusoidal perfusion increasingly worsens with evolution to dense fibrosis, and the portal circulation is increasingly altered, leading to portal hypertension and portal-systemic shunting. Extra-hepatic portal-systemic collaterals or shunts, including esophageal varices, emerge. The HepQuant SHUNT Test worsens as the portal circulatory derangement progresses – changes that occur prior to any overt clinical complication.

2.2.2 ESOPHAGEAL VARICES

A primary goal of clinical management of the patient with CLD is to determine the likelihood of large esophageal varices. The patient at low likelihood for large esophageal varices may not need EGD screening or surveillance. The patient at high likelihood of large esophageal varices may need EGD to avoid variceal hemorrhage and risk for other complications, including death.

Development of Varices and Risk for Hemorrhage. The rate of development of varices in patients with cirrhosis is approximately 3 to 5% per year (63,64) and the rate of progression from small to large varices is approximately 12 to 15% per year (64-69). Endoscopically-defined large variceal size and clinically significant portal hypertension, ≥ 10 to 12 mmHg, are the major risk factors for variceal hemorrhage (1-3, 70-73). Endoscopic findings of red wale marks over varices or concomitant gastric varices may amplify risk within a given category of variceal size. Patients without varices or who have small varices are at no risk or low risk, respectively, for variceal hemorrhage.

Size Classification and Risk for Hemorrhage. Esophageal varices are classified as small (< 5 mm in diameter) or large (≥ 5 mm in diameter). Large varices may also be designated as medium, large or grade 2 or higher by endoscopists. Guidance from AASLD, EASL, and published literature favors simplification to two categories - either large or small. Additional endoscopic features which may amplify risk for hemorrhage and need for treatment are red wale signs, and concomitant presence of gastric varices.

If untreated, large varices may spontaneously rupture; the resulting acute variceal hemorrhage is associated with significant morbidity, mortality, and may necessitate admission to the intensive care unit and prolonged hospitalization. The 6-week mortality rate from variceal hemorrhage is related to severity of underlying liver disease (CP C > CP B > CP A) and ranges from 10 to 20% (1-3, 70-73). When diagnosed, large esophageal varices need immediate treatment. Small varices do not need immediate treatment but require clinical or endoscopic follow-up to detect evolution to large varices.

Cost of Variceal hemorrhage. In 2011, in the USA there were over 658,000 hospitalizations and $9.8 billion dollars in costs related to admissions for cirrhosis (56). Sixteen percent of these admissions were related to variceal hemorrhage. A recent USA study focused on trends in hospital admissions for upper gastrointestinal hemorrhage over the 20 years from 1989 to 2009 (74). Incident hospitalizations for variceal hemorrhage (VH) dropped from 2.9 to 1.3 per 100,000 USA population, but the cost per hospitalization rose from $14,197 in 1989 to $25,802 in 2009. The total economic burden of UGIH increased over the two decades and the increase was the greatest for VH. A 2008 USA study found average cost ($23,207) and length of stay (15.2 days) to be 4-fold higher for VH with complications compared to UGIH with complications (75). Once discharged, 32% of patients are readmitted within 30 days, mainly for complications of portal hypertension (76). Annualized hospitalization costs for cases with 30-day readmission were $73,252.

2.2.3 SELECTING CLD PATIENTS FOR ENDOSCOPY (EGD) FOR DETECTION OF LARGE ESOPHAGEAL VARICES

The patient with CLD at-risk for large esophageal varices may have noncirrhotic F3 stage of fibrosis, compensated cirrhosis, or decompensated cirrhosis. Noncirrhotic patients with F3 fibrosis and patients with compensated cirrhosis lack overt manifestations of hepatic dysfunction or portal hypertension and have not experienced clinical complications of liver disease. Patients with clinically decompensated cirrhosis are defined as having experienced variceal hemorrhage, ascites, spontaneous bacterial peritonitis, and/or hepatic encephalopathy. Generally, they are classified as Child-Pugh C and are not candidates for the SHUNT-V study.

Risk for large varices increases across this spectrum of disease. In the case of severe hepatic decompensation, tests for likelihood of varices will not influence the clinical decision to perform EGD screening for varices. But, for the less severe cases, encompassing the spectrum from noncirrhotic advanced fibrosis (fibrosis stage 3), compensated cirrhosis, and early decompensated cirrhosis, tests of likelihood of large varices may aid in the decision to avoid or perform EGD (3-11,28-30,38).

Staging by Liver Biopsy. Establishing a diagnosis of cirrhosis or advanced CLD is the standard of practice for determining which patients with CLD should undergo EGD to check for large esophageal varices. The gold standard for diagnosis of cirrhosis or advanced CLD is histologic assessment of liver biopsy. But, liver biopsy is a complex procedure, prone to sampling error, and requires specialized equipment and expertise. Liver biopsy is costly, risky, and not embraced by patients (20,21).

Staging by Non- or Minimally-invasive surrogates. Because liver biopsy is poorly tolerated, non- or minimally-invasive surrogates have been increasingly accepted for diagnosis of cirrhosis. These surrogates include biomarkers, blood test panels, and liver stiffness measurement (LSM) by a variety of methods (transient elastography (TE), acoustic radiation force impulse imaging (ARFI), 2-dimensional shear wave elastography (2D-SWE), and magnetic resonance elastography (MRE). The diagnostic accuracy of these surrogates for cirrhosis varies considerably, and they are even less accurate in determining likelihood for large esophageal varices because none of these surrogates directly measures hepatic fibrosis, portal pressure or the portal circulation and they are prone to interference by non-hepatic factors, such as diet, exercise, medications, renal dysfunction, hemolysis, body size, hepatic steatosis, and hepatic inflammation.

2.2.4 LOW RISK PATIENTS DEFINED BY SURROGATES FOR FIBROSIS

BAVENO VI Criteria. The BAVENO VI conference provided guidelines for use of non- or minimally-invasive tests to determine likelihood of large esophageal varices in patients with CLD (3). The guidelines depend heavily upon LSM by TE. But, accuracy of TE for this purpose is questioned within the same document. To improve accuracy, the authors recommended two measurements on different days in fasting condition and also that invasive tests, such as liver biopsy or HVPG might be needed to confirm suspicion for cirrhosis or clinically significant portal hypertension (CSPH). HVPG was considered the gold standard for this assessment. They further concluded that the

diagnostic value of TE for CSPH remained to be ascertained across the spectrum of etiologies of CLD and that there was specific concern that TE would be less accurate in NAFLD and NASH.

Given the pitfalls of LSM alone, the BAVENO VI guidelines emphasized use of LSM with platelet count, specifically to use LSM <20 kPa and with a platelet count >150,000 μL^{-1}, to select cases with a very low risk of having large esophageal varices, the varices needing treatment (3).

Table 1: Selected Characteristics of Subjects in 10 Published Studies[1] using LSM plus Platelet Count for Likelihood of Large Esophageal Varices[2] Compared to the Subjects in the HALT-C Training Dataset

	Type of		Etiology (%)							Criteria	
Study	Study	N	EtOH	Viral	NAFLD	Male (%)	Age (yr)	CP A	t, LSM-EGD	LSM	Pltl
Abraldes	Retro	379	14	70	6	NA	58	100	NA	25	150000
Ahmed	Retro	478	36	33	21	NA	54	NA	<1yr	20	150000
Chang	Retro	173	NA	55	NA	62	56	100	<1yr	20	150000
Ding	Retro	271	12	69	8	70	58	100	3.4 mo	25	100000
Maurice	Retro	310	13	62	14	67	58	89	<1yr	20	150000
Paternostro	Retro	92	30	47	NA	73	53	100	NA	20	150000
Puigvehi	Pro	368	0	100	0	63	57	100	NA	25	100000
Silva	Retro	112	7	83	NA	77	54	87	<1yr	20	150000
Thabut	Pro	649	0	100	0	73	56	100	NA	20	150000
Tosetti	Retro	146	0	100	0	NA	NA	100	NA	20	150000
HALT-C	**Pro**	**217**	**0**	**100**	**0**	**76**	**50**	**100**	**2 wk, 6 mo, 18 mo**	**DSI 18.3**	

Abbreviations: Retro, retrospective study; Pro, prospective study; N, number of subjects; EtOH, alcohol-related liver disease; Viral, chronic viral hepatitis due to either HBV or HCV; NAFLD, non-alcoholic fatty liver disease; CP A, Child Pugh class A cirrhosis; LSM, liver stiffness measurement; EGD, esophagogastroduodenoscopy (endoscopy); t, LSM-EGD, time interval between performance of the LSM and performance of the EGD; Pltl, platelet count in μL^{-1}. [1]From Marot, et al (6). All studies used LSM and platelet count with cutoffs similar to BAVENO VI criteria. The sample size of the HALT-C Training dataset was similar to the sample sizes of the individual studies and also similar in terms of etiology of CLD, male dominance, mean age, Child-Pugh class, and time interval between performance of the test and EGD. [2]Large esophageal varices are varices of diameter 5 cm or more, or described as medium or large, or graded as 2 or higher. Large esophageal varices are considered high risk for variceal hemorrhage and its consequent morbidity and mortality. Large esophageal varices are varices that need treatment.

Published studies evaluating the BAVENO VI Criteria. Ten published studies, encompassing 2977 cases, using LSM and platelet count and criteria similar to BAVENO VI have focused on the need to identify the CLD patient at low likelihood of having large esophageal varices (3-11,28-30,38) (**Table 1**). In these reports, viral hepatitis was the most common etiology, followed by alcohol and NAFLD/NASH. Most were men with a mean age in the mid-50s. All had a diagnosis of cirrhosis and nearly all had Child-Pugh class A disease severity. These reports do not include subjects with either noncirrhotic fibrosis or Child-Pugh class B cirrhosis. The cases from the published literature have selected characteristics that are similar to the subjects in the HALT-C Training Dataset (**Table 1**).

Avoiding unnecessary EGD is a goal of screening. The percentage of unnecessary EGDs is defined by the number of cases with no or small varices that underwent EGD that would have avoided EGD by application of selection criteria. A meta-analysis of the 10 published studies suggested that application of BAVENO VI criteria might reduce screening or surveillance EGDs by 7% to 30% (6). Each of the 10 studies applied different combinations of cutoffs for platelet count (>100,000 µL-1, >120,000 μL^{-1} or >150,000 μL^{-1}) and LSM (<20 or <25 kPa). The individual study results were pooled, and the pooled estimates were sensitivity 93%, specificity 30%, negative predictive value (NPV) 97%, positive predictive value (PPV) 14%, negative likelihood ratio (NLR) 0.22, positive likelihood ratio (PLR) 1.33 (Table 2), and false negative rate (FNR) 3%. The analysis also revealed that 21 of 319 (6.5%) large varices needing treatment were missed (6). Another review further highlighted the relatively low rate of reduction in EGDs (20% overall) and unacceptably high FNR and rate of missed large varices or varices needing treatment (4).

Table 2: Diagnostic Performance of LSM + Platelet Count versus DSI for Likelihood of Large Esophageal Varices

Study	Prevalence	TP	FN	FP	TN	Sens	Spec	PPV	NPV	FNR	PLR	NLR
Abraldes	0.15	54	3	238	84	0.95	0.26	0.18	0.97	0.03	1.28	0.20
Ahmed	0.11	49	3	318	108	0.94	0.25	0.13	0.97	0.03	1.26	0.23
Chang	0.08	11	3	128	31	0.79	0.19	0.08	0.91	0.09	0.98	1.10
Ding	0.10	26	0	138	107	1.00	0.44	0.16	1.00	0.00	1.78	0.00
Maurice	0.05	13	2	195	100	0.87	0.34	0.06	0.98	0.02	1.31	0.39
Paternostro	0.21	19	0	61	10	1.00	0.14	0.24	1.00	0.00	1.16	0.00
Puigvehi	0.15	45	10	174	139	0.82	0.44	0.21	0.93	0.07	1.47	0.41
Silva	0.18	20	0	80	12	1.00	0.13	0.20	1.00	0.00	1.15	0.00
Thabut	0.08	49	0	445	156	1.00	0.26	0.10	1.00	0.00	1.35	0.00
Tosetti	0.08	12	0	95	39	1.00	0.29	0.11	1.00	0.00	1.41	0.00
Pooled Totals	**0.11**	**298**	**21**	**1872**	**786**	**0.93**	**0.30**	**0.14**	**0.97**	**0.03**	**1.33**	**0.22**
Means of Studies	**0.12**	**30**	**2**	**187**	**79**	**0.94**	**0.28**	**0.15**	**0.98**	**0.02**	**1.32**	**0.23**
SD of Studies	**0.05**	**17**	**3**	**119**	**52**	**0.08**	**0.11**	**0.06**	**0.03**	**0.03**	**0.21**	**0.35**
HALT-C	**0.10**	**21**	**1**	**89**	**106**	**0.95**	**0.54**	**0.19**	**0.99**	**0.01**	**2.09**	**0.08**

Abbreviations: LSM: liver stiffness measurement by transient elastography; DSI, disease severity index from the HepQuant SHUNT test; TP, true positives; FN, false negatives; FP, false positives; TN, true negatives; Sens, sensitivity; Spec, specificity; PPV, positive predictive value; NPV, negative predictive value; FNR, false negative rate; PLR, positive likelihood ratio; NLR, negative likelihood ratio; HALT-C, the NIH-sponsored Hepatitis C Antiviral Treatment to Prevent Cirrhosis Trial.

NLR is a statistical endpoint that encompasses both sensitivity and specificity in its calculation and is not dependent upon prevalence. In these 10 published studies NLR ranged from 0 to 1.10 – a wide variation due to lack of pre-specified study design or criteria and a range for sensitivity that varied from 79% to 100%.

The lower the NLR the greater the strength of "Rule Out" of likelihood of large esophageal varices. The NLR of 0.09 using DSI 18.3 from our HALT-C Training Dataset is favorable, compared to either the pooled results or mean (±SD) of individual studies using LSM plus platelet count displayed in **Table 2**.

In the ANTICIPATE study, 30% of EGDs could have been avoided but 5% of varices needing treatment would have been undetected (29). Most recently, the ANTICIPATE cohort was combined with two other single center cohorts (total N=925) (30). LSM <25 and platelet count >110,000 μL^{-1} were evaluated for exclusion of EGD and suggested that 40% of EGDs might have been avoided, but 6.5% (6/92) of the large varices or VNT were missed. A further modification, the addition of MELD=6 to platelets >100,000 μL^{-1} and LSM <25 kPa, would have spared 46% of EGDs, but 7.6 % (7/92) of the large varices or VNT were missed.

Limitations of Existing Data. The studies using LSM and platelets were limited by referral and selection bias, retrospective analyses, differences in criteria for EGD, and use of different cutoffs for LSM and platelet count. For example, one of the largest studies screened over 12,000 LSM measurements to select 310 patients and the time between the screening tests and EGD was up to 12 months (28). In the meta-analysis only 15 of 419 references were selected and there were nearly 3 times as many cases above cutoffs compared to the cases below cutoffs (6). The 95% confidence intervals for NLR from these studies were extremely wide. In the study of Expanded BAVENO VI Criteria, three cohorts with very different results were combined – the percentage of EGDs spared were 32%, 41%, and 52% (30). In addition, the prevalence of large esophageal varices or varices needing treatment varied greatly between publications. For example, the prevalence of large varices or VNT ranged from 0% to 21% in the 10 studies with data for large varices in Marot's meta-analysis (6). And, it is not clear in all studies that the tests used for "selection" of EGD were actually performed prior to EGD. Accordingly, a prospective study with prespecified endpoints and statistical plan, as proposed herein for DSI, is critically needed.

The HepQuant SHUNT Approach. DSI from the HepQuant SHUNT Test has shown promise as a potential tool to aid in the decision to avoid or perform EGD for large esophageal varices. In our analyses of the HALT-C Training dataset,

we found that the DSI cutoff 18.3 demonstrated a negative likelihood ratio (NLR) 0.09 for large esophageal varices (**Table 2**) – a result that would have avoided 49% of EGDs. If the DSI cutoff ≤18.3 were applied to the cases comprising the published studies of transient elastography plus platelet count described above, there would have been a reduction in EGDs of 52%. These results compare favorably with the reported 7 to 30% reduction (6), or 20% overall reduction (4), in EGDs for LSM plus platelet count. In addition, the false negative rate is very low with DSI ≤18.3 – NPV >99%, FNR <1% with only 1 of 22 large varices missed. The potentially greater reduction in unneeded surveillance EGDs (52% vs 20%) and low number of false negatives favor the use of the DSI. A reduction in number of unnecessary screening or surveillance EGDs by approximately 50% is highly clinically relevant.

There may be additional advantages of the HepQuant SHUNT test. The test is done once, while TE is recommended to be done at least twice on two separate days. Both tests require fasting. The NLR of 0.09 for DSI ≤18.3, compared to published NLRs, suggests that the DSI cutoff may be more accurate in negative likelihood of large esophageal varices compared with the current practice using LSM with or without platelet count.

High Risk Patients defined by Cirrhosis or Advanced CLD

In the ANTICIPATE Study (29) liver stiffness, platelet count, and spleen diameter were evaluated in 518 patients with CLD from five centers in Europe and Canada. Liver stiffness measurement [LSM] by transient elastography [TE], platelet count, and spleen diameter (with calculation of liver stiffness to spleen/platelet score [LSPS] score and platelet-spleen ratio [PSR]) were compared in their ability to predict risk of large esophageal varices ("varices needing treatment, VNT"). The AUROCs were 0.67 for TE alone, 0.73 for TE plus platelet count, 0.74 for PSR, and 0.79 for LSPS.

Furthermore, a diagnosis of cirrhosis, whether by biopsy or noninvasive means, does not provide an unequivocal link to varices or need for endoscopy. Many patients with cirrhosis lack clinically significant portal hypertension and do not have varices. Care providers don't necessarily link cirrhosis, per se, to need for endoscopy. The latter is exemplified by a study of 4230 veterans with HCV cirrhosis with median followup of 6.1 years. Only 33.8% of patients meeting AASLD or Bueno VI guidelines had an EGD performed for variceal screening (77). Diagnosis of cirrhosis fails to trigger care providers to refer patients for endoscopic variceal screening.

The HepQuant SHUNT Approach. The HALT-C Training Dataset defined an AUROC 0.82 for DSI in likelihood of Large Esophageal Varices. This compares favorably with AUROCs measured in the Anticipate Study – ranging from 0.67 for TE alone, to 0.79 for LSPS. The performance of HepQuant SHUNT and DSI for likelihood of Large Esophageal Varices is due to the fact that the HepQuant SHUNT test directly measures portal-systemic shunting, linking it directly to variceal pathophysiology. As such, a HepQuant SHUNT test result may provide a more direct link to varices resulting in an appropriate referral for endoscopy.

2.2.5 HIGH RISK PATIENTS DEFINED BY MEASUREMENT OF THE PORTAL CIRCULATION

HVPG. Portal hypertension, the cause of esophageal varices, can be indirectly measured by percutaneous trans-jugular catheterization of the hepatic venous system to quantify the hepatic venous pressure gradient (HVPG) (39,78-82). HVPG correlates well with the direct measurement of portal pressure. Clinically significant portal hypertension is defined by HVPG ≥ 10 mmHg (some studies suggest ≥ 12 mmHg) and correlates well with risk for varices and variceal hemorrhage. In the US, most HVPG studies are performed in specialized radiology departments. Measurement of HVPG is technically challenging, time consuming, and performed in specialized units; the procedure is invasive, poorly tolerated, risky, and not embraced by patients. Also, the reproducibility of HVPG is questionable, especially if performed only sporadically in less experienced units. Even in experienced centers, HVPG reproducibility is of concern. For example, in a study of patients with HCV and underlying portal hypertension, the mean (±SD, CV%) difference in repeated measurements of HVPG was 0.5 ± 2.52 mmHg (82).

The HepQuant SHUNT Approach. HepQuant SHUNT measures portal-systemic shunting and could be an alternative to HVPG to define groups with or without risk for large esophageal varices. The HepQuant SHUNT Test

satisfies the unmet medical need for a simple-to-administer, well-tolerated, relatively inexpensive, safe, and minimally-invasive test. The overall hypothesis for the SHUNT-V study is that the HepQuant SHUNT Liver Diagnostic Test can measure the liver and portal circulation in patients with CLD and identify those patients who are or are not likely to have large esophageal varices.

2.2.6 FULFILLING AN UNMET NEED

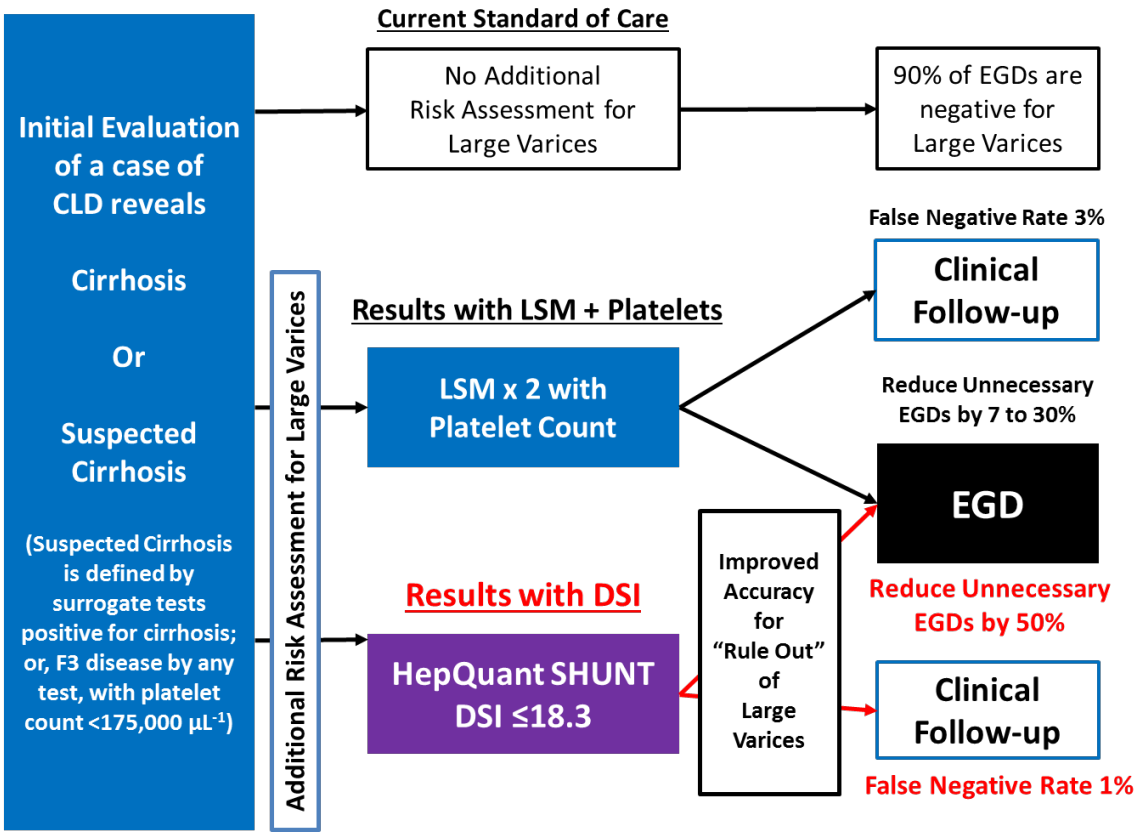
The results from the above studies demonstrate the need for new innovative, non- or minimally-invasive tests to replace the invasive tests and to improve the accuracy of current non- or minimally-invasive tests. The data suggest that the HepQuant SHUNT Test can potentially fulfill this unmet need. Our proposed validation study is a prospective multi-center study of sufficient power to define the cutoff for DSI for both ruling in and ruling out large esophageal varices.

2.2.7 INTEGRATING THE HEPQUANT SHUNT TEST INTO CLINICAL PRACTICE

The current and proposed paradigms for assessing a person with CLD for varices are shown in **Figure 2**.

Current Practice. A physician or care provider initiates a workup to include a medical history, physical examination, and standard laboratory tests (CBC, AST, ALT, Alkaline phosphatase, bilirubin, albumin). The results of this initial assessment may raise suspicion that a patient has chronic liver disease, including cirrhosis. Typically, the care provider would test for etiology of liver disease, such as viral hepatitis (HCV-Ab, HBsAg, HBcAb, others), iron overload (iron, TIBC, ferritin), autoimmune disease (ANA, others), and assess the liver by imaging with a standard ultrasonographic exam (echogenicity as surrogate for fatty liver or fibrosis). Additional tests to stage disease severity and need for EGD screening for varices could include elastography, liver biopsy, radiologic imaging, measuring HVPG, or combinations of these tests. The most commonly accepted noninvasive approach is the combination of LSM plus platelet count. **Figure 2** compares the results with no additional risk assessment, risk assessment by LSM plus platelets from published literature, to risk assessment using DSI based on results from the HALT-C Training dataset. The goal is to better inform the decision to avoid or perform EGD screening for varices.

Figure 2: DSI as an Aid in the Decision to Avoid or Perform EGD for Screening for Varices



HepQuant Paradigm. In the HepQuant paradigm, a patient with CLD meets criteria for selection for EGD and the HepQuant SHUNT test is performed. DSI ≤18.3, implying low likelihood of large esophageal varices, might aid in the clinician's decision to avoid or proceed with EGD. For DSI >18.3, the higher the DSI the higher the risk for large esophageal varices and the greater the urgency to perform EGD.

If the HepQuant SHUNT Test indicates low risk, e.g, DSI ≤18.3, endoscopy may be avoided, and standard clinical follow-up may be all that is warranted. The results for DSI ≤18.3 from HALT-C (Sensitivity 95%, Specificity 54%, NPV > 99%, NLR 0.09, and FNR < 1.0%) suggest that application of DSI could lower the number of unnecessary EGDs by 50% and reduce the false negative rate by greater than two thirds (**Figure 2**).

With increasing DSI beyond 18.3, the likelihood of large esophageal varices increases – the clinician could incorporate DSI into his decision regarding urgency for endoscopic screening. Endoscopic screening for varices is increasingly warranted as DSI increases.

In other studies of DSI, DSI increases in parallel with the increase in fibrosis and progression to cirrhosis and predicts risk for clinical outcome (ascites, spontaneous bacterial peritonitis, encephalopathy, variceal hemorrhage, and liver-related death) (40-50). In the HALT-C Trial, the prototype for the HepQuant SHUNT test outperformed liver biopsy and standard laboratory tests in likelihood of cirrhosis and varices and in identifying the patients at high risk for clinical outcomes (83-86). With one parameter from the HepQuant SHUNT Test, DSI, a clinic provider could counsel a patient on risk for large esophageal varices to aid in the decision to perform EGD.

2.3 Risk/Benefit Assessment

2.3.1 KNOWN POTENTIAL RISKS

1. Risks from the Test Compounds
    a. Allergic reaction to cholate compounds (theoretical – none yet reported)
    b. Allergic reaction to human serum albumin (HSA)
        Reactions could include:
        i. rash
        ii. having a hard time breathing
        iii. wheezing when you breathe
        iv. sudden drop in blood pressure
        v. swelling around the mouth, throat, or eyes
        vi. fast pulse
        vii. sweating
        viii. severe reactions are very rare but a severe reaction (called anaphylaxis) can lead to profoundly low blood pressure and even death
2. Risks from the Indwelling catheter
    a. Pain with placement of catheter
    b. Thrombosed vein
    c. Hematoma
3. Risk of Phlebotomy
    a. Localized pain
    b. Bruising
    c. Occasional lightheadedness,
    d. Fainting
    e. Infection at the site (rare)
4. Risk of Fasting
    a. Dizziness
    b. Headache
    c. Stomach Discomfort
    d. Fainting

Risk of Test Administration

As stated above, over 1600 HepQuant SHUNT tests have been performed in over 700 subjects without any reports of adverse events, serious adverse events, or serious unexpected serious adverse events.

Cholates, labeled with stable (nonradioactive) isotopes, occur naturally and are not known to have any deleterious or adverse effects when given intravenously or orally in the doses used in HQ tests. The serum cholate concentrations that are achieved by either the intravenous or oral doses are similar to the serum concentrations of bile acids that occur after the ingestion of a fatty meal. Because cholates are naturally occurring with a pool size in humans of 1 to 5 g, the 20 and 40 mg doses of labeled cholates used in the HQ tests are unlikely to be harmful to a fetus. However, the effects of these compounds on the fetus are not definitively known. Possible adverse event associated with the cholate test compounds may include: allergic reaction.

The two cholates used in the HepQuant SHUNT test for this study are labeled with stable (non-radioactive) forms of carbon and hydrogen that are found in nature and can be measured in blood. These forms of cholate have been used with FDA INDs (65121 and 65123) since 2002, and their use in humans has been monitored since that time.

To date, the cholates used in this study have not been associated with any allergic reactions or side effects. However, they are still considered experimental and there may be unknown risks.

Cholate, in a dose of 15 mg kg^{-1} d^{-1}, is FDA-approved for the treatment of pediatric liver diseases, even in neonates as young as 3 weeks. It is safe in these high doses even after several years of administration (87,88).

There are no known immediate or long-term physical, psychological, social, legal, economic, or other risks to participants related to the labeled cholates.

Human serum albumin is mixed with the 13C-cholate for intravenous injection. Some individuals may have a known reaction to serum albumin and will be excluded from this study. Possible adverse event from the HSA may include: allergic reaction (89-93).

Rare Hypersensitivity Reactions have been reported to human serum albumin preparations and include anaphylaxis, severe anaphylaxis or anaphylactoid reactions, fever, chills, rash, urticaria, pruritus, angioneurotic edema, and erythema or flushing. Individuals who are hypersensitive to albumin preparations, any ingredient in the formulation, or components of the containers should be excluded from this study.

Risk of Transmissible Diseases in Plasma-derived Preparations. Because human albumin is prepared from pooled human plasma, there is a potential to pass human viruses (e.g., hepatitis viruses, HIV) to the recipient and may carry a risk of transmitting Creutzfeldt-Jakob disease (CJD) or its variant CJD (vCJD). Through donor plasma screening and specific procedures like pasteurization to eliminate or inactivate any possible causes have reduced, but not entirely eliminated, the risk of transmission of disease causing agents. Risk of transmission of viral disease with plasma-derived human albumin is considered extremely remote. No causes of transmission of HBV, HCV, or HIV have been documented following use of commercially available human albumin. There are no documented cases of CJD or vCJD transmitted through plasma-derived preparations (including plasma-derived human albumin); theoretical risk for transmission of CJD with commercially available human albumin is considered extremely remote.

However, no purification method has been shown to be totally effective in removing the risk of viral infectivity from plasma-derived preparations and because new blood-borne viruses or other disease agents may emerge which may not be removed or inactivated by current manufacturing processes, the risks of human albumin are not entirely known.

Risk of Indwelling Intravenous Catheter. Placing the indwelling intravenous catheter will cause minor pain and discomfort. With any blood draw, there is a small risk of hematoma and a very small risk of a blood clot (1 in 100) or infection (1 in 1000). HepQuant has performed the HepQuant SHUNT test or the prototypical dual cholate research test on over 700 individuals and most had the test multiple times. There has never been a test-related serious adverse event - the risk from the test is very small. Possible adverse event from the placement of an indwelling catheter may include: hematoma at injection site.

Risk of Phlebotomy. Drawing blood from a vein may cause local pain, bruising, occasional lightheadedness, fainting, and very rarely, infection at the site of the blood draw.

Risk of Fasting. Fasting could cause dizziness, headache, stomach discomfort, or fainting.

Pregnancy. Because cholates are naturally occurring with a pool size in humans of 1 to 5 g, the 20 and 40 mg doses of labeled cholates used in the HQ tests are unlikely to be harmful to a fetus. However, the effects of these compounds on the fetus are not definitively known (see **Section 8.4.10**).

Unknown or Unexpected Risks. The two cholates used in the HepQuant SHUNT test have been registered with the FDA since 2002, and their use in humans has been monitored since that time and IND annual reports filed with FDA. Given the endogenous and ubiquitous nature of the cholates, we do not anticipate any unexpected risks. However, the compounds are still considered experimental and there may be unknown risks.

Risk of False Positive Test. The intended population, CLD patients scheduled for EGD for screening for varices, would undergo the EGD independent of a positive DSI test. There is no clinical consequence of a false positive DSI.

Risk of False Negative Test. The study design encompasses a broad spectrum of CLD etiologies and severity of disease. The targets for sensitivity and specificity as defined in Section 9.0 below, for the expected prevalence of large varices of approximately 20%, will ensure an acceptably low FNR when compared to current SOC. Subjects in the clinical trial will not be exposed to this risk, as the physician performing the EGD will be blinded to the results of the HepQuant test, and the EGD will performed regardless of the test outcome.

2.3.2 POTENTIAL COMPLICATING MEDICAL ISSUES

Use of Concomitant Medications. Non-selective beta blockers, ARB and/or ACE inhibitors could affect the blood flow to the liver, and therefore could affect the flow of the HepQuant SHUNT cholate test compounds into the liver as well. If the flow of blood to the liver was altered by these drugs, it could impact the SHUNT test results. Additionally, because individual patients may take different amounts of these drugs, we are unable to correct for the different concentrations and their impact on blood flow to the liver. As such, subjects who are currently taking either a non-selective beta blocker, ARB and/or an ACE inhibitor will be asked to delay taking their normal morning dose the day of their testing and until the 90-minute test is completed. Delaying these medications could cause a temporary elevation in blood pressure but the risk would be minimal, like that of subjects that delay doses of medications in everyday life. Patients will therefore be instructed to immediately take their morning dose after the final 90-minute blood draw has been completed. Possible adverse event from delaying taking ACE or ARB inhibitor and/or Non-Selective Beta Blockers may include: minimal change in blood pressure.

Medical or Surgical Conditions and might interfere with interpretation of test results. Subjects with serious intercurrent medical or surgical illness, such as acute myocardial infarction, acute cerebral hemorrhage, sepsis, or other immediate life-threatening illness are excluded. Subjects with extensive resection of large segments of small intestine (short gut) or severe gastroparesis might not be able to absorb the oral dose of d4-cholate and are excluded.

2.3.3 THE MANNER IN WHICH RISKS WILL BE MINIMIZED

Any subject that does not meet all of the inclusion criteria, and none of the exclusion criteria, will not be enrolled in this study.

All sites coordinators and other staff are trained by HepQuant personnel on the use of the HepQuant SHUNT Kit. Training includes the review of all warnings and precautions. Site personnel record any medications the subject is taking and whether or not specific medications have been withheld per the IFU.

The site personnel administering the HepQuant SHUNT test are trained healthcare professionals with substantial experience with phlebotomy, use of indwelling catheters, and mixing drug substances for patient administration. All sites must undergo training prior to use of the HepQuant SHUNT kit. Training is recorded.

2.3.4 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

Assessment of known potential risks. The potential risks of the administration of the HepQuant SHUNT Test are:

- IV catheter and blood sampling: Standard risk of local pain, hematoma. Rare risk for infection or thrombosis – although none reported to date.

- Albumin: Very rare hypersensitivity reactions reported with risk <1:10,000. No hypersensitivity reactions reported to date with the injectable mixture of albumin:13C-cholate.

There are no known risks associated with cholate, and no known AEs or SAEs associated with the stable isotopes or formulations of cholate employed in the combination product.

The above safety profile supports the conclusion that administration of the HepQuant SHUNT Test exposes subjects to minimal risk. To further minimize risk, the test will be administered in research or infusion centers with appropriate supervision, nursing support and expertise, and availability of emergency care and procedures.

Another potential risk is the risk of inappropriate clinical decisions based upon the results of the HepQuant SHUNT Test. In this study, the decision to perform EGD is made prior to enrollment of the patient by clinicians using standard criteria. The results of the HepQuant SHUNT test are not being used for any clinical decisions in the SHUNT-V study.

As stated above, the criteria for validation of DSI 18.3 for "Rule Out" of large esophageal varices will ensure an acceptably low FNR.

Recording and Reporting of AEs, SAEs, SUSARs. All AEs, SAEs, and SUSARs will be recorded and evaluated for association with the HepQuant SHUNT test. Safety data and any associated regulatory reporting obligations for individual or periodic safety reports will be reported to the appropriate authorities and clinical investigators and applicable IRB/EC, in compliance with all applicable laws and the requirements of the IRB/EC.

In the HALT-C Trial, reporting of AEs, SAEs, and SUSARs was performed by New England Research Institute (NERI) and the DSMB for the Trial. No AEs, SAEs, or SUSARs related to the HepQuant SHUNT research prototype (dual cholate test) were reported.

Justification for conduct of this study. The risks to the individual participant are minimal. However, the information gained from the DSI cutoff has implications for potentially improving the care and management of CLD patients. The main benefit is more accurate assessment of the likelihood for large esophageal varices.

The information regarding the DSI cutoff and negative likelihood of large esophageal varices could assist the trained clinician by providing additional data that could help in the decision to avoid or defer EGD. Of course, the clinician will consider the algorithm associating DSI with varices, incorporate other factors, and use his own judgment, about the decision to avoid or defer EGD.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
The **primary objective** of this study is to validate DSI ≤18.3 for identifying subjects with CLD selected for future EGD for variceal screening or surveillance who are not likely to have large esophageal varices ("Rule Out").	The **endpoint** for the **primary objective** is the negative likelihood ratio (NLR) for large esophageal varices. The performance goal for validation of DSI ≤18.3 is the upper limit of the confidence interval (CI) for NLR to rule out NLR > 0.52, in addition the observed sensitivity must be > 0.85. The confidence level for the confidence interval will be adjusted in order to maintain a one-sided type I error rate of 0.025 as described in section 9.5.1.	**Primary:** The current minimally-invasive practice using liver stiffness measurement (LSM) by transient elastography (TE) plus platelet count, yields NLRs from 0 to 1.1. A critical value (*cv*) of 0.27 for NLR represents the average of results for these studies in ruling out large esophageal varices. The study is designed for superiority over current practice.
Secondary		
The secondary objective is to perform logistic regression analysis of the probability of large esophageal varices for DSI > 18.3. In the HALT-C Training Dataset the probability of large varices increased in proportion to increasing DSI in subjects with DSI above 18.3.	**The secondary endpoint** is the significance of the logistic regression equation for the relationship of DSI >18.3 to probability of large esophageal varices.	**Secondary**: Predicted probability of large varices versus DSI will be analyzed by logistic regression analysis and affiliated statistical tests of the significance of the association between the continuous DSI score and risk of large varices. These analytical results will be evaluated using a calibration table in which the prevalence of large varices is tabulated by DSI quartile. A clinician knowing that a high DSI has a high probability of large varices could enhance his sense of urgency to perform EGD.
Tertiary/Exploratory		
Exploratory objective 1 is to generate and evaluate the AUROC (by c-statistic) for DSI in likelihood of large esophageal varices over the full range of DSI. One goal of this analysis is to define the diagnostic performance (sensitivity, specificity, PPV, NPV, NLR, FNR, miss rate[3]) for DSIs below and above the validated DSI of 18.3. This	The **endpoint for exploratory objective 1** is the AUROC (c-statistic) for DSI in the likelihood of large esophageal varices over the full range of DSI.	**Exploratory 1:** The purpose of this analysis is to provide upside and downside performance of DSI around the validated cutoff of DSI 18.3. This analysis will yield a table of sensitivities, specificities, NPVs, PPVs, NLRs, FNRs, and miss rates over the given range of DSI that

[3] In this protocol, FNR is defined as 1-NPV and miss rate is defined as 1-Sensitivity. See the footnote in Section 1.1 for a discussion of these two measures.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
will yield a more complete description of DSI performance in "Rule Out" of large varices and potentially allow optimization of the DSI cutoff.		could be included in a package insert to help the clinician interpret a given DSI result.
Exploratory objective 2 is the evaluation of the diagnostic performance of DSI for small esophageal varices and for esophageal varices of any size.	The **endpoints for exploratory objective 2** are the c-statistics for the AUROCs for DSI in the likelihood of small or any size of esophageal varices.	**Exploratory 2**: Linking DSI to small or any size of esophageal varices may aid the decision regarding closer clinical followup.
Exploratory Objective 3 is evaluation of the diagnostic performance of a single 60 minute time point (STAT) from the clearance curve of the orally administered d4-cholate. We will evaluate STAT's diagnostic performance for large, small, and any size of esophageal varices.	**The endpoints for exploratory objective 3** are the c-statistics for the AUROCs for STAT in the likelihood of large, small, or any size of esophageal varices. The c-statistics for STAT will be compared to the c-statistics for DSI.	**Exploratory 3:** Linking STAT, which is derived from the 60-minute time point of the orally administered d4-cholate, to large esophageal varices, small varices, or varices of any size could provide a simple, oral only, "drink and draw" test at the point-of-care in the clinic.

4 STUDY DESIGN

4.1 Overall Design

The Study Schematic is shown in Figure 1 in **Section 1.2**.

4.1.1 STUDY POPULATION

All subjects will give informed consent to participate in the study. Potential study subjects will be recruited from the clinical practices at up to 40 participating USA clinical centers. Up to 420 subjects will be enrolled. Study coordinators, principal investigator, or sub-investigators will meet with potential subjects, review the ICF (Informed Consent Form) with the subject, address any questions, and obtain signed informed consent. The coordinator will confirm that the subject meets the protocol-defined definition for CLD, has a protocol-designated etiology for CLD, satisfies all inclusion and no exclusion criteria (see Sections 5.1 and 5.2), and the subject is in the scheduling process for an EGD for the indication of variceal screening or surveillance. Based on the study's inclusion criteria the anticipated prevalence of large esophageal varices in the study population is 20%.

Selected characteristics of the study population are:

- Age in years
- Height and Weight for calculation of BMI (kg/m^2)
- Gender distribution (Male:Female)
- Race and Ethnicity (Non-Hispanic White, Hispanic, Black, Asian, Other)
- Severity of CLD
    - Fibrosis stage F3 with platelet count <175,000 μL^{-1}, defined by at least one of the following additional criteria:
        - F3 by liver biopsy (list staging index; e.g., METAVIR, ISHAK, NASH-CRN, etc.)
        - F3 by elastography (list kPa and type of elastography)

        - F3 by blood panel or biomarker panel (list result and type of test)
    - Child-Pugh (CP) class A (CP score 5 or 6) or B (CP score 7, 8 or 9) but without prior history of variceal hemorrhage, treatment for varices, or refractory ascites or encephalopathy.
    - Subjects with CP class C cirrhosis or uncontrolled ascites or uncontrolled encephalopathy, large esophageal varices, variceal hemorrhage, or treatment of varices are excluded from participation.
- Categories of Etiology of CLD
    - Viral (chronic hepatitis C or B)
    - Fatty liver disease (NAFLD/NASH)
    - Alcoholic liver disease
    - Cholestatic liver disease (primary biliary cholangitis or primary sclerosing cholangitis)
    - Other (includes autoimmune hepatitis, genetic diseases, and miscellaneous conditions causing fibrosis/cirrhosis)
- History of and Current Complications and their treatment will be recorded
    - Variceal hemorrhage (exclusionary)
    - Ascites (refractory ascites is exclusionary)
    - Spontaneous bacterial peritonitis (exclusionary)
    - Hepatic encephalopathy (refractory encephalopathy is exclusionary)
    - Hepatocellular carcinoma (Extensive HCC or invasion of the main portal vein is exclusionary)
- Physical findings of
    - Jaundice
    - Spider telangiectasia
    - Hepatomegaly
    - Splenomegaly
    - Ascites
    - Encephalopathy
- Laboratory Tests
    - Complete blood count (Hgb, WBC, platelet count)
    - Standard chemistry (bilirubin, albumin, creatinine)
    - INR
    - MELD score
    - Child-Pugh (CP) score
- Results of following tests if performed within 1 year of enrollment:
    - Liver imaging (US, CT, or MRI)
    - Liver biopsy (necro-inflammatory grade, fibrosis stage)
    - Elastography (kPa, type of elastography)
    - Blood panel or biomarker panel (record result and type of test)
    - HVPG (free hepatic vein and wedged pressures, mmHg)
    - Prior EGD

The above characteristics will be compared across categories of etiology of CLD and categories of severity of liver disease.

4.1.2 METHODS AND PROCEDURES

Clinical Assessments. Standard clinical assessments will include history, physical examination, complete blood count, chemistry profile, INR, ECG, and pregnancy testing. The coordinator will obtain de-identified copies of available source documents and reports for imaging studies, elastography, liver biopsy, biomarker panels, HVPG, and EGD. Adverse Events (AEs) and Serious Adverse Events (SAEs) related to test administration and any

components of the HepQuant SHUNT Liver Diagnostic Kit will be evaluated on both the day of Test administration, for any immediate safety issues, and again, 2-30 days later for delayed reactions.

The HepQuant SHUNT Test. HepQuant SHUNT Liver Diagnostic kits will be shipped to participating clinical centers, and the HepQuant SHUNT test will be performed within 28 days of enrollment. A detailed Instructions for Use (IFU) is shipped with every kit (**Appendix C**). The SHUNT test is a minimally-invasive, blood-based dual clearance test that assesses the liver specific function of cholate uptake, and quantifies clearance of cholate from the systemic circulation, clearance of cholate from the portal circulation, and portal-systemic shunting. Six blood samples are collected over 90 minutes, the serum is separated, and serum samples shipped to the designated HepQuant laboratory for analysis. Kit components, test administration, sample processing, laboratory analysis, and reporting of results are covered in other sections of this application (see **Section 6** "Study Intervention").

EGD. The EGD findings are the endpoints for the study. The EGD must occur no later than 6 weeks after the HepQuant SHUNT test – most likely the EGD will be done within 1 or 2 weeks of the SHUNT test. However, the time window of 6 weeks allows for sufficient time due to delays in scheduling or rescheduling or should the patient no show for a visit or test for valid reason and still wish to remain in the study. Acceptable reasons for no show might be intercurrent illness, sudden requirement for job-related travel, or other personal or family issue. The rescheduled testing must occur within the 6-weeks of the SHUNT test to be included for analysis.

New varices are unlikely to develop, and small varices are unlikely to become large within 6 weeks. The rate of developing new varices in a cirrhotic patient without varices is approximately 3 to 5%/year (63,64). The rate of small varices enlarging to become large varices is approximately 12-15%/year (64-69). Over 6 weeks these rates would be 0.6% and 1.8%, respectively. Given these slow rates of variceal progression, we conclude that the EGD findings are not expected to change significantly within the 6-week window. Note that the average time interval between the SHUNT test and EGD is likely to be far less than 6 weeks.

The following EGD findings will be recorded:

- Esophageal varices
    - Present or absent; to be categorized as present the varices must protrude into the esophageal lumen during insufflation of the esophagus
    - If varices are present, record size as small (<5 mm diameter, or Grade 1) versus large (5 mm or more in diameter, or Grades 2 or 3)
    - If varices are present, record presence or absence of red wale marks, and whether they are mild, moderate, or severe
- Gastric varices
    - Present or absent
    - If gastric varices are present, record size as small (<5 mm diameter, or Grade 1) versus large (5 mm or more in diameter, or Grades 2 or 3)
    - If gastric varices are present, record presence or absence of red wale marks, and whether they are mild, moderate, or severe
- Portal hypertensive gastropathy
    - Present or absent
    - If present, record whether it is mild, moderate or severe

The primary endoscopic endpoint is large esophageal varices. We will also record and report the endoscopic findings of red wale marks, gastric varices, and portal hypertensive gastropathy, but are not planning any formal analysis of these features as endpoints. Red wale marks may amplify risk for variceal hemorrhage in those with esophageal varices – for this reason we will examine the relationship of DSI to red wale marks.

To ensure consistency across centers, all EGDs will be performed by endoscopists experienced in the evaluation and treatment of esophageal varices. The endoscopists will be identified and designated by the clinical center's PI.

4.1.3 ANALYSES

The relationship of DSI to the primary endoscopic endpoint of large esophageal varices will be analyzed by univariate and multivariate logistic regression analyses, ROC curve analysis, and linear and nonlinear regression and correlation coefficients. The diagnostic performance of DSI cutoff 18.3 will be defined in terms of sensitivity, specificity, PPV, NPV, NLR, FNR, and miss rate[4]. For the primary efficacy endpoint, validation of DSI ≤18.3 as a cutoff for negative likelihood of large esophageal varices will require two that two criteria are satisfied: (a) confidence interval with an upper limit that rules out NLR > .52 and (b) the observed sensitivity > 0.85.

There are no planned stratifications or sub-studies.

A single interim analysis is planned after the trial data set has compete DSI and EGD measurements on approximately 250 participants. The objective of the interim analysis will be to determine whether DSI $\leq$ 18.3 has sufficient sensitivity and specificity to rule out NLR for large varices > 0.52, and that the observed sensitivity demonstrates an acceptable number of false negative results. The interim decision criteria have been chosen using standard group sequential designs as described in Section 9.5.1. The interim analysis will be performed by an independent statistician. The results of the interim analysis will be reviewed and interpreted by the biostatistician, who will determine if the trial should continue to full enrollment or if it can be stopped for either efficacy or futility.

Minimizing Bias: See **Section 6.6**.

4.2 Scientific Rationale for Study Design

This is a USA multi-center, open-label, single-arm study with a point-in-time analysis to validate the DSI cutoff ≤18.3 from the HepQuant SHUNT Test for negative likelihood of large esophageal varices.

The rationale for this study is based upon our analysis of patients with chronic hepatitis C who were enrolled in the HALT-C Trial **(HALT-C Training dataset)**. We examined the relationship of DSI to likelihood of varices in this Trial and found that DSI ≤18.3, had NPV >99%, FNR <1%, and NLR 0.09; indicating that DSI ≤18.3 could potentially be a cutoff to "Rule Out" large esophageal varices.

The **primary objective** is to validate the cutoff of DSI ≤18.3 to "Rule Out" large esophageal varices using a properly powered prospective study of subjects with CLD of various etiologies and spectrum of severity of disease **(CLD Validation dataset)**.

4.2.1 DESCRIPTION OF THE HALT-C TRAINING DATASET

Characteristics of the HALT-C Training Dataset. The prototypical research test used in the HALT-C study was the precursor for the HepQuant SHUNT test (83-86,94-103). Although sampling times, duration of sampling, and laboratory methods have changed, the basic structure of the test has been maintained (83). 13C-cholate (20mg) in sodium bicarbonate solution was and is mixed with 25% human serum albumin (5 mL) for IV injection and administered simultaneously with d4-cholate (40 mg) in sodium bicarbonate solution orally. Timed blood samples are obtained for determination of the clearances of the 13C-cholate and d4-cholate.

[4] In this protocol, FNR is defined as 1-NPV and miss rate is defined as 1-Sensitivity. See the footnote in Section 1.1 for a discussion of these two measures.

To make comparisons between the current HepQuant SHUNT Test and the prior research test, under a Data Use Agreement with NIDDK, we re-processed and re-analyzed all of the HALT-C samples using the HepQuant SHUNT sampling times and laboratory procedures. Only the latter results are reported below.

HALT-C Patients: HALT C patients were adults with advanced fibrosis or compensated cirrhosis due to chronic hepatitis C who had failed prior treatment with an interferon-based regimen (102,103). A total of 1393 patients enrolled in the HALT-C Trial, and 287 (21%) of these enrolled in the Quantitative Liver Function Test (QLFT) ancillary study (83-86); HepQuant SHUNT was one of a battery of QLFTs assessed in the QLFT ancillary study. There were two phases to HALT C, Lead-In and Randomized. During the Lead-In phase, patients were treated with peginterferon/ribavirin (PEG/RBV).

Patients failing to achieve SVR were eligible to enroll into the Randomized phase, where they were randomized to receive either low-dose peginterferon or no treatment. Patients achieving sustained virologic response (SVR), defined by negative HCV RNA 24 weeks after completion of PEG/RBV treatment, were not followed further in the main HALT-C study. Those achieving SVR who were enrolled in the QLFT ancillary study were studied once more with HepQuant SHUNT after achieving SVR.

Randomized Phase Patients. Patients who had positive HCV RNA after 24 weeks of PEG/RBV (Non-responders), and patients who had positive HCV RNA in follow-up after achieving negative HCV RNA during 48 weeks of PEG/RBV (Relapsers), entered the Randomized phase. In addition, a cohort ('Express' patients) with positive HCV RNA who had received at least 24 weeks of PEG/RBV outside HALT-C were also randomized. A total of 1050 patients entered the HALT-C Randomized Phase (44) and 227 (22%) of these were also enrolled in the QLFT ancillary study. The baseline HepQuant SHUNT test was performed at enrollment to the HALT-C Trial – prior to Lead-In in the case of Non-responders, Relapsers, or those achieving SVR; and, just prior to Randomization in the case of Express patients. EGDs were performed just prior to randomization for all groups.

Baseline HepQuant SHUNT Test. The Baseline HepQuant SHUNT test was performed prior to Lead-in in 234 patients and prior to randomization in 53 Express patients. 227 patients had both a baseline HepQuant SHUNT testing either prior to Lead-In or at randomization, and, an EGD at randomization.

Minimal Model for HepQuant SHUNT test. Data from the HALT-C trial were used to generate a minimal model for measuring cholate clearances and shunt in humans (83). The prototypical research test was cumbersome, requiring 14 blood samples collected over 180 minutes. We devised a minimal model using 5 samples of blood collected over 90 minutes. The minimal model is the basis for the HepQuant SHUNT test.

Laboratory Analyses. The original method for quantifying 13C-cholate and d4-cholate from serum used a complex sample preparation, including derivatization to tri-silyl methyl esters, and capillary gas chromatography (GC) – mass spectrometry (MS). In addition, 14 blood samples collected over 3 hours were used to generate both the intravenous and oral clearance curves (83). We obtained a Data Use Agreement with NIH and authorization to re-analyze all the HALT-C samples by our simple sample processing and LC/MS method. Of the 227 original studies in patients with EGDs, 217 had sufficient residual serum to allow for re-analysis by the LC/MS method – this cohort of 217 patients constitutes the HALT-C Training Dataset.

Upper gastrointestinal endoscopy (EGD) was performed just prior to the patient entering the Randomized phase of the HALT-C Trial, and the EGD was always performed after the HepQuant SHUNT test.

Large Variceal Size. In both the HALT-C Training dataset and the CLD Validation dataset, the primary endpoint is endoscopically-defined large varices. Variceal size described as medium, large, Grade 2 or higher, or variceal diameter of 5 mm or more are classified as LARGE. Varices not meeting criteria for large are classified as SMALL.

Any Varices. The endoscopic findings of patients will be defined as either large varices, small varices, or no varices. For the analysis of patients with any varices, patients with large varices will be combined with patients with small varices.

4.2.2 DETERMINING THE DSI CUTOFF 18.3 FROM THE HALT-C TRAINING DATASET

4.2.2.1 LIKELIHOOD OF LARGE ESOPHAGEAL VARICES (HALT-C TRAINING DATASET)

Uni-variable Logistic Regression. Of the 217 patients who had HepQuant SHUNT testing and protocol endoscopy, 22 had large esophageal varices. Univariate logistic regression analysis was performed using DSI as the independent variable and large varices as the outcome (**Table 3**). The relationship was highly statistically significant ($p < 0.0001$).

Table 3: Univariate Analysis of DSI for Large Esophageal Varices

Analysis of Maximum Likelihood Estimates					
Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	-6.4682	1.0014	41.7186	<.0001
DSI_ruln_round	1	0.1947	0.0403	23.3607	<.0001

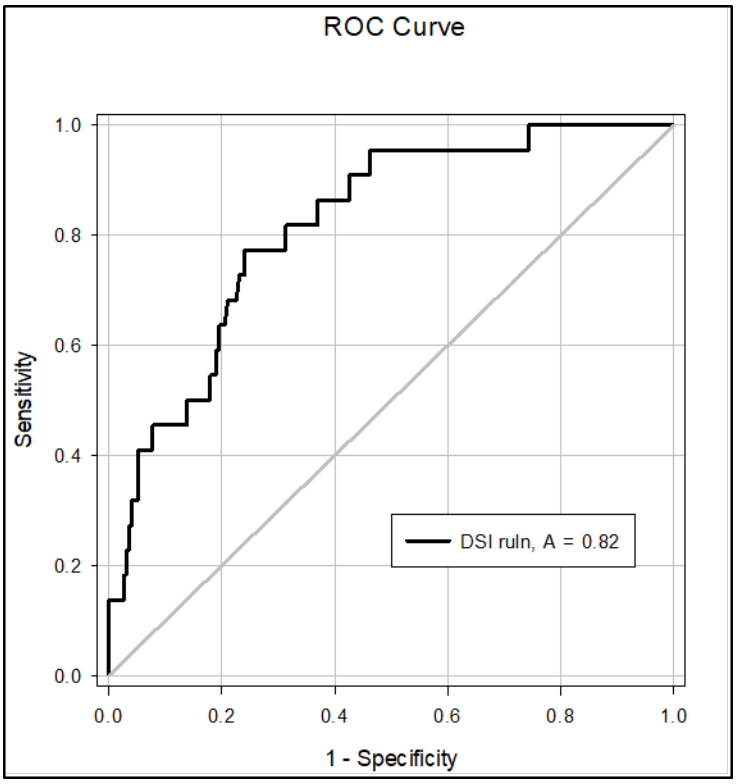
Multi-variable Logistic Regression. As presented above, the gold standard for assessing risk for varices by AASLD and EASL guidelines is a diagnosis of cirrhosis. In BAVENO VI, platelet count is another major modifier of risk for clinically significant portal hypertension and risk for varices. For these reasons, we evaluated the independence of DSI as a predictor after adjusting for diagnosis of cirrhosis or stage of fibrosis and platelet count (**Table 4**).

Table 4: Multi-Variable Analysis of DSI for Large Esophageal Varices

Analysis of Maximum Likelihood Estimates					
Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	-3.8601	1.5311	6.3560	0.0117
DSI_ruln_round	1	0.1375	0.0454	9.1718	0.0025
PLT	1	-0.0117	0.00605	3.7174	0.0538
cirrho_yn	1	0.3511	0.6240	0.3166	0.5737

In these analyses, both a diagnosis of cirrhosis and platelet count were no longer statistically significant. The results demonstrate that DSI was an independent predictor of large varices and a better predictor than cirrhosis and platelet count.

Figure 3: ROC Analysis of DSI for Large Esophageal Varices



Area Under the Receiver Operator Curve (AUROC). The diagnostic performance of DSI for predicting large varices was evaluated by Area under the Receiver Operating Curve (AUROC) (**Figure 3**). The c-statistic for the AUROC was 0.82. This compares favorably with an Area Under the Curve (AUC) of 0.67 (range of 0.59 to 0.72 by boot-strapping) for transient elastography that was found in the European study of Abraldes (8).

Probability and Calibration Plots. The plot of predicted probability of large varices versus DSI is shown along with 95% confidence intervals in **Figure 4**. The number of subjects within each group is shown as is the average predicted probability for each group. The number of patients with large varices within each group is shown as is the actual prevalence for each group. The probability of large varices reached 50% at a DSI = 33.2.

Figure 4: Probability of Large Varices for a Given DSI

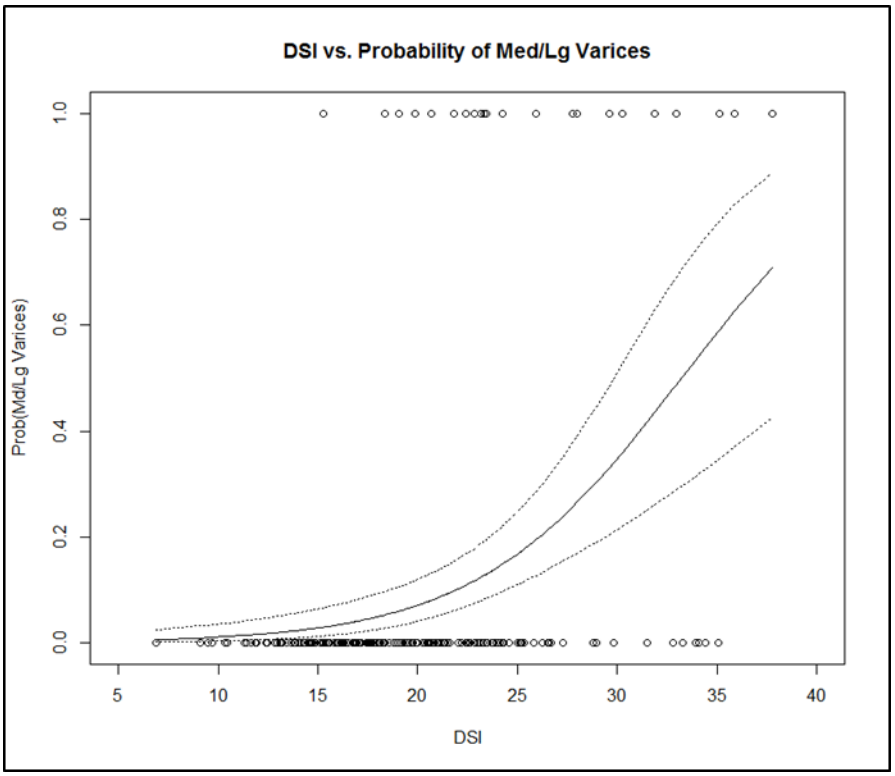
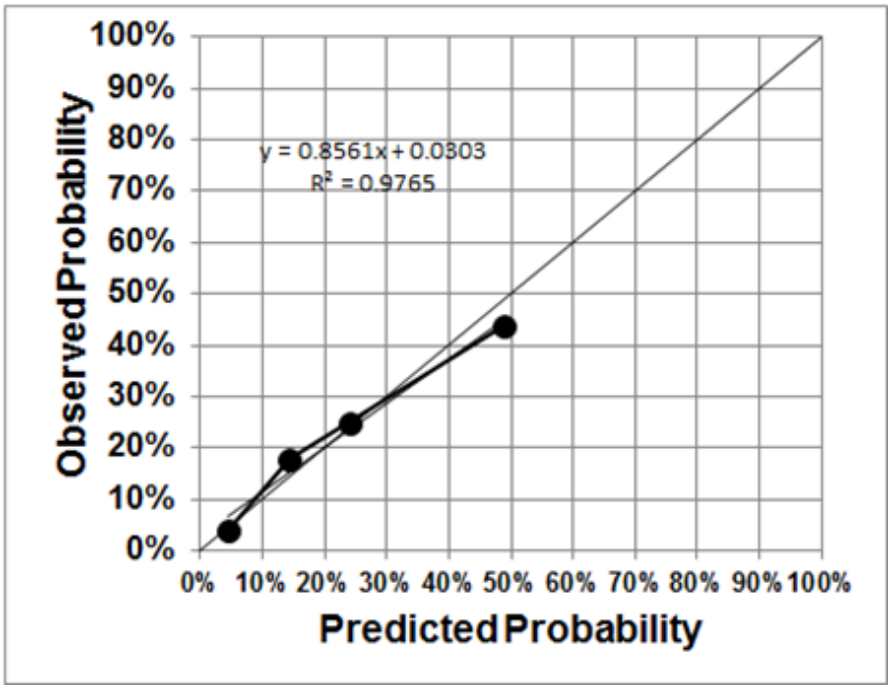


Figure 5: Probability Calibration Curve: Observed versus Expected Probabilities for Large Varices



A calibration plot was constructed (**Figure 5**). There were only 4 patients in the 0.30 to <0.40 decile, only 4 in the 0.40 to <0.50 decile, only 6 in the 0.50 to <0.60 decile, only 1 in the 0.60 to <0.70 decile, and only 1 in the 0.70 to <0.80 decile, and so these 16 patients were consolidated into a single group with predicted probabilities from 0.30 to <0.80 as shown. The number of patients within each group is shown as is the average predicted probability for each group. The number of patients with large varices within each group is shown as is the actual prevalence for each group.

The prevalence or observed probability of large varices was plotted against the average predicted probability to generate the calibration curve. Although the number of groups is small, this plot also shows good agreement between the predicted probabilities for each group and the actual observed prevalence. In this calibration plot the patients are grouped by decile of predicted probability which corresponds with data in **Table 5**. As previously noted, deciles with few patients were combined into single groups to have enough patients to assess prevalence.

Table 5: Distribution of Patients across Probability Categories for Large Varices

Predicted Probability of Large Varices Groups (Deciles*)	Average Predicted Probability of Large Varices of Patients within each Group	No. of Patients within each Group	No. of Patients within each Group with Large Varices	Actual Prevalence of Large Varices
0.30 to <0.80	49.0%	16	7	43.8%
0.20 to <0.30	24.0%	8	2	25.0%
0.10 to <0.20	14.3%	39	7	17.9%
0.00 to <0.10	4.4%	154	6	3.9%

4.2.2.2 LIKELIHOOD OF ESOPHAGEAL VARICES OF ANY SIZE (HALT-C TRAINING DATASET)

Uni-variable Logistic Regression Analysis. Of the 217 patients who had HepQuant SHUNT testing and protocol endoscopy, 74 had varices of any size (52 small, 22 large).

Table 6: Univariate Analysis of DSI for Varices of Any Size

Analysis of Maximum Likelihood Estimates					
Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	-3.4685	0.6113	32.1957	<.0001
DSI_ruln_round	1	0.1405	0.0292	23.1138	<.0001

Univariate logistic regression analysis was performed using DSI as the independent variable and any size of esophageal varices as the outcome (**Table 6**). The relationship was highly significant (p<0.0001).

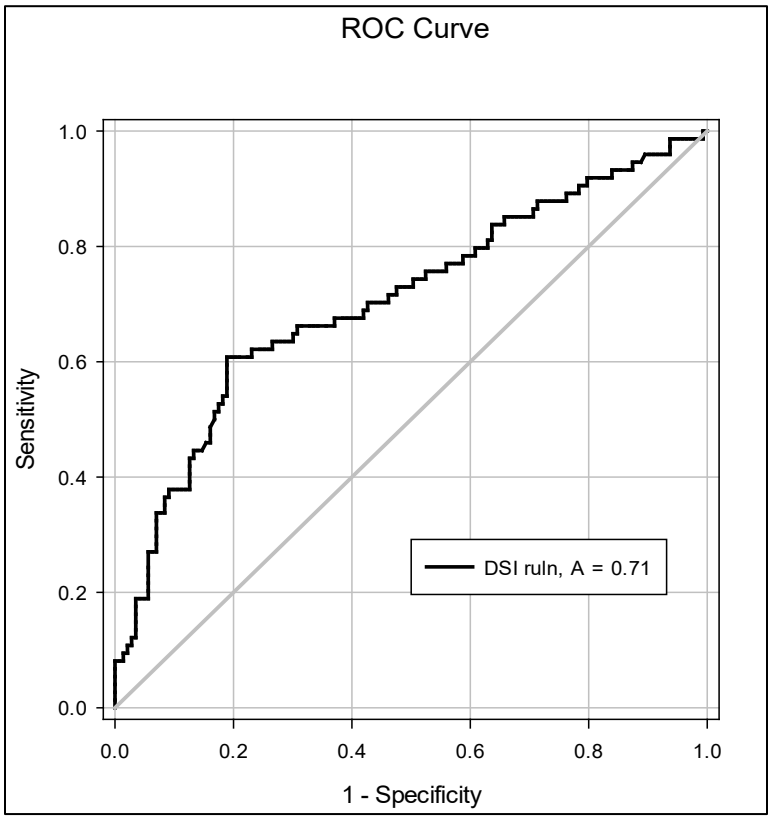
Multi-Variable Logistic Regression. The independence of DSI from the HepQuant SHUNT test in defining likelihood for large esophageal varices was tested in multi-variable analyses including biopsy-diagnosis of cirrhosis and platelet count. In predicting any varices, the diagnosis of cirrhosis dropped from significance. Based on the regression results, platelet count still reached statistical significance but was not as strong a predictor as DSI (**Table 7**).

Table 7: Multi-variable Analysis of DSI for Varices of Any Size

Analysis of Maximum Likelihood Estimates					
Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	-1.7083	0.9265	3.3998	0.0652
DSI_ruln_round	1	0.0902	0.0326	7.6589	0.0056
PLT	1	-0.00647	0.00299	4.6743	0.0306
cirrho_yn	1	0.5225	0.3583	2.1265	0.1448

These results demonstrate that DSI was an independent predictor of likelihood of large varices and a better predictor than cirrhosis and platelet count.

Figure 6: ROC Analysis of DSI for Varices of Any Size



Area Under the Receiver Operator Characteristics Curve (AUROC). The diagnostic performance of DSI for predicting any size of varices was evaluated by Area Under the Receiver Operating Curve (AUROC) (**Figure 6**). The c-statistic was 0.71 for DSI predicting the likelihood of any size varices. This was identical to the AUROC of 0.71 (range of 0.67 to 0.74 by boot-strapping) for transient elastography in the European study published by Abraldes JG, et al (24).

Figure 7: Probability of Varices of any size across range of DSI

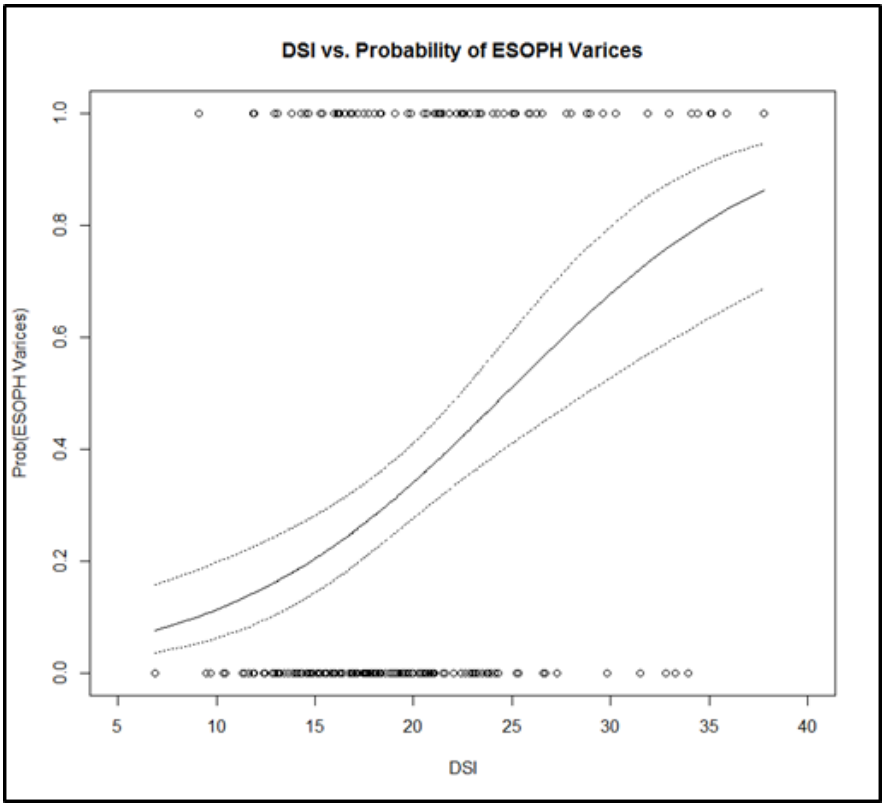
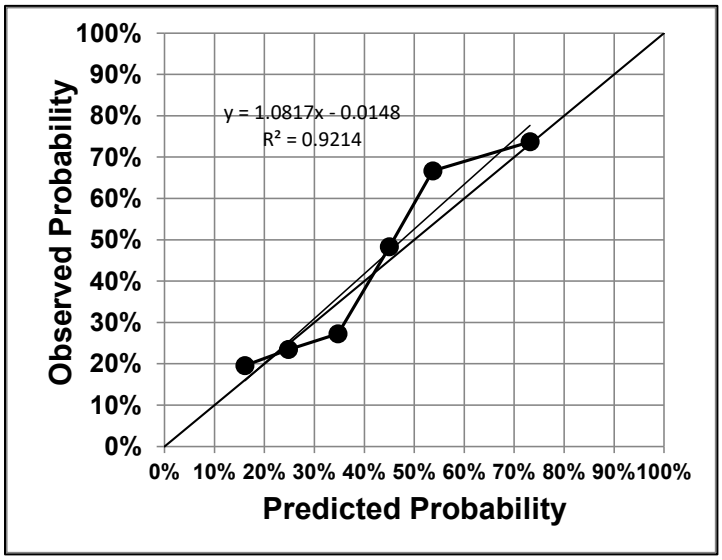


Figure 8: Probability Calibration Curve: Observed versus Expected Probability



Probability and Calibration Curves. The plot of predicted probability versus DSI is shown along with 95% confidence intervals (**Figure 7**). The number of subjects within each group is shown as is the average predicted probability for each group. The number of patients with any varices within each group is shown as is the actual prevalence for each group. The probability of varices reached 50% at a DSI = 24.7.

In order to assess the accuracy of the probability prediction, a calibration curve was constructed by dividing the patients into groups by decile of predicted probability. There were only 7 patients in the 0.60 to <0.70 decile, only 8 in the 0.70 to <0.80 decile, and only 4 in the 0.80 to <0.90 decile, and so these 19 patients were consolidated into a single group with predicted probabilities from 0.60 to <0.90 as shown in **Table 8**. There were no patients in the 0.90 to <1.00 decile. Likewise, there was only 1 patient in the 0.00 to <0.10 decile and this patient was combined with the 45 patients in the 0.10 to <0.20 decile to form a group of 46 patients with predicted probabilities from 0.00 to <0.20.

The prevalence or observed probability was plotted against the average predicted probability to generate the calibration curve (**Figure 8**). This plot shows good agreement between the predicted probabilities for each group and the actual prevalence or observed probability. In this calibration plot the patients are grouped by decile of predicted probability which corresponds with data in **Table 8**. As previously noted, deciles with few patients were combined into single groups to have enough patients to assess prevalence.

Table 8: Distribution of Patients across Probability Categories for Any Varices

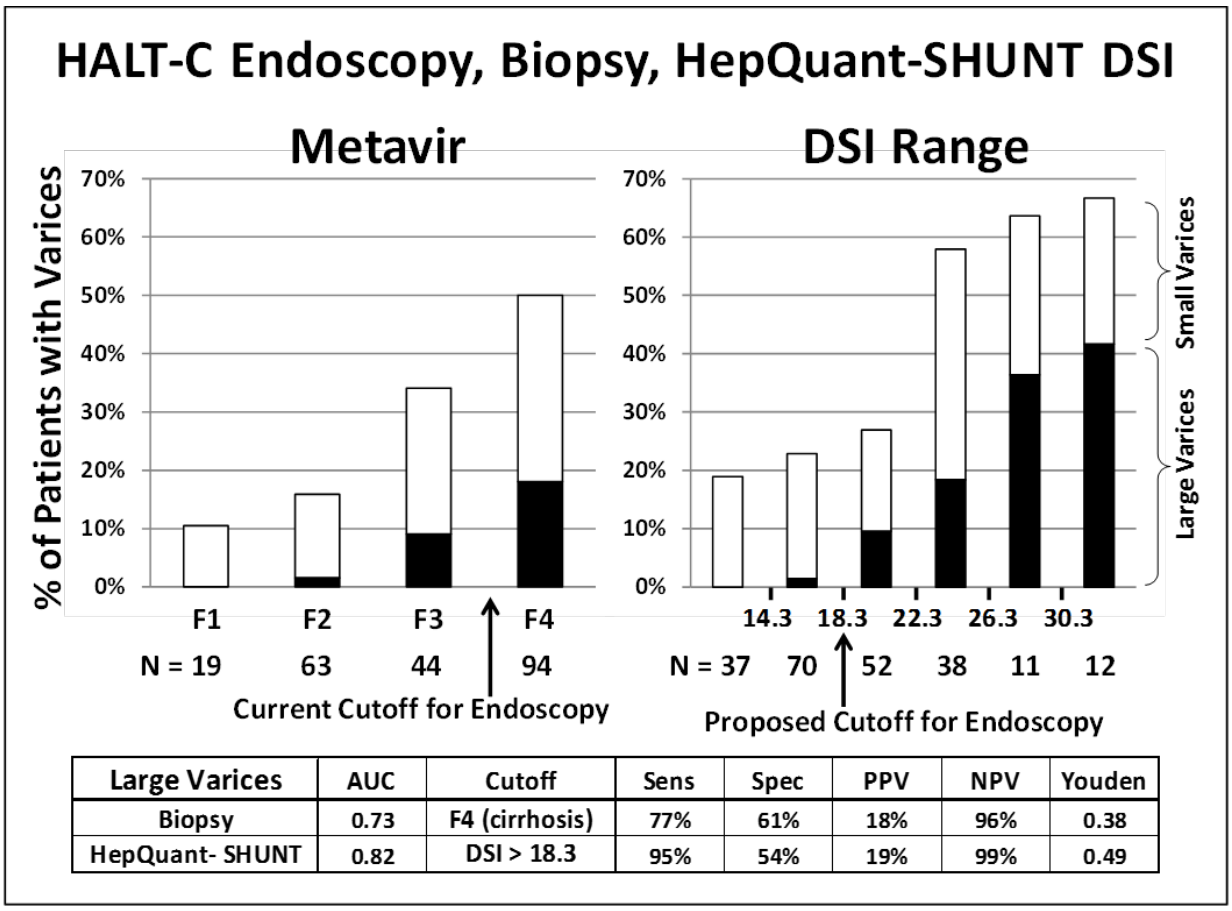
Predicted Probability of Varices Groups (Deciles*)	Average Predicted Probability of Varices of Patients within each Group	No. of Patients within each Group	No. of Patients within each Group with Varices	Actual Prevalence of Varices
0.60 to <0.90	73.2%	19	14	73.7%
0.50 to <0.60	53.7%	15	10	66.7%
0.40 to <0.50	45.0%	29	14	48.3%
0.30 to <0.40	34.7%	44	12	27.3%
0.20 to <0.30	24.8%	64	15	23.4%
0.00 to <0.20	16.1%	46	9	19.6%

4.2.3 DSI 18.3 FROM THE HALT-C TRAINING DATASET IS A CLINICALLY MEANINGFUL CUTOFF

As stated at the introduction to this application, the primary objective of this study is to validate DSI ≤18.3 as a cutoff for "Rule Out" of large esophageal varices. In HALT-C, DSI ≤18.3 had NPV >99%, NLR 0.0926, and FNR 1%. A person with DSI ≤18.3 may be able to avoid EGD; reducing healthcare costs, improving clinical management, and enhancing the person's experience.

Patients with large esophageal varices (>5 mm diameter) are at immediate risk for variceal hemorrhage and need urgent treatment. The HALT-C Training dataset indicated that likelihood for large esophageal varices or varices of any size is proportional to DSI, as DSI increases likelihood for both large varices and varices of any size increases. DSI >18.3 had sensitivity 95% and PLR 2.09; "Ruling In" large esophageal varices. A patient with DSI >18.3 may be a candidate for EGD. Performing EGD and initiating treatment due to early detection of large esophageal varices can also reduce health care costs and improve patient management by preventing subsequent variceal hemorrhage and the associated morbidity and mortality.

Figure 9: Comparison of DSI with Liver Biopsy



Large Varices	AUC	Cutoff	Sens	Spec	PPV	NPV	Youden
Biopsy	0.73	F4 (cirrhosis)	77%	61%	18%	96%	0.38
HepQuant- SHUNT	0.82	DSI > 18.3	95%	54%	19%	99%	0.49

Both fibrosis stage by METAVIR and the range of DSI by HepQuant SHUNT test correlate with risk for any but especially large varices. The DSI cutoff 18.3 has improved diagnostic performance for large varices, compared to histologic diagnosis of cirrhosis.

The cutoff of DSI 18.3 corresponding to sensitivity of 95% (CI 93% to 98%) for large varices compares favorably with the gold standard of biopsy-diagnosed-cirrhosis which has a sensitivity of only 77% (**Figure 9)** and, as noted previously, the current noninvasive practice using LSM plus platelet count. Therefore, fewer cases of dangerous large varices are missed when using DSI.

DSI cutoff of 18.3 also identified the patients with any size varices (52 small and 22 large) with 69% sensitivity (CI 57% to 79%), specificity 58%, PPV of 46%, and NPV of 78%.

Of the 52 patients with small varices, 30 (58%) had baseline DSI >18.3 - 19/30 (63%) experienced clinical outcomes an average of 3.1 ± 1.6 years later, including 10/30 (33%) with liver related death. The remaining 22 (42%) with small varices had DSI ≤18.3 - 4/22 (18%) experienced clinical outcomes an average of 5.5 ± 1.1 years later, and only 1/22 (4.5%) experienced liver-related death. No patient with small varices at baseline experienced variceal hemorrhage.

The DSI cutoff of 18.3 appears to separate the patients with small varices who are at low risk for clinical outcomes (DSI ≤18.3) from the patients with small varices who have higher risk for clinical outcomes (DSI >18.3). Given the benign long-term clinical course of subjects with small varices and DSI ≤18.3 (see also **Appendix B**, Investigator's Brochure), we speculate that these cases may only need serial clinical assessment. That assessment might also include periodic HepQuant SHUNT testing, every 2 to 3 years, and if DSI exceeds 18.3, EGD might be warranted.

4.3 Validating DSI Cutoff 18.3 in the CLD Validation Dataset

4.3.1 DESCRIPTION OF THE CLD VALIDATION DATASET

Characteristics of CLD Patients: The goal is to recruit a population of patients with chronic liver disease (CLD) that spans the spectrum of severity and etiologies of CLD to encompass all CLD patients meeting criteria for EGD to screen for varices. The inclusion and exclusion criteria were selected to encompass severity of CLD ranging from non-cirrhotic fibrosis with low platelet count to moderately compensated Child-Pugh B cirrhosis and to achieve an overall prevalence of large esophageal varices of ≥20%. Enrolled subjects must satisfy 4 main criteria:

- Meets the protocol-defined definition for chronic liver disease (CLD).
- For patients with CLD with non-cirrhotic F3 disease, platelet count must be <175,000 μL^{-1}. The BAVENO VI criteria used platelet count cutoff of 150,000 μL^{-1} to enhance the diagnostic performance of LSM. Others have used lower platelet counts of 100,000, 110,000, and 120,000 μL^{-1}. The lower the platelet count the higher the likelihood of large varices or varices of any size. But, as the cutoff for platelet count decreases the number of missed cases of large varices increases.

  The goal of the DSI cutoff ≤18.3 is exclusion of all cases with large esophageal varices. In the HALT-C Training dataset, the prevalence of large varices was 18.3% (17/93) in the cases with F4 fibrosis (cirrhosis), and similar, at 21.8% (17/78), in F4 cases with platelet count <175,000 μL^{-1}. I n contrast, the prevalence of large varices was 9% (4/44) in all cases with F3 fibrosis, but much higher, at 16% (4/25), in the F3 cases with platelet count <175,000 μL^{-1}. There was no case of large varices in the cases with ≤ F3 fibrosis and platelet count >175,000 μL^{-1}.

  Applying this platelet count requirement to cirrhotic cases would not enhance the prevalence of large varices in cirrhotic cases but would increase prevalence of large varices in F3 fibrosis. In addition, AASLD and EASL guidelines state that cirrhosis, independent of platelet count, is a risk factor for large esophageal varices. For these reasons, our study only requires a platelet count <175,000 μL^{-1} for non-cirrhotic F3 cases. There is no platelet count requirement for enrollment of cases with cirrhosis.
- Meets one of the protocol-defined etiologies for CLD.
- Is scheduled or in the process of being scheduled for EGD.

Selecting Subjects Scheduled for EGD. The target population for application of the HepQuant SHUNT Test is the clinical population with CLD encompassed by our inclusion and exclusion criteria who are selected for EGD for variceal screening.

Categories of Etiology of CLD. We will compare the baseline characteristics of the study population across 5 general etiologic categories: viral, NAFLD/NASH, EtOH, cholestatic disease (PBC, PSC), and other miscellaneous etiologies.

Severity of CLD. The baseline characteristics of three broad categories will be compared: non-cirrhotic F3 fibrosis with platelet count <175,000 μL^{-1}, compensated cirrhosis (Child-Pugh class A), and mildly to moderately decompensated cirrhosis (Child-Pugh class B).

HepQuant SHUNT Test. The study design is relatively straightforward and simple – one HepQuant SHUNT test is linked to the findings of one EGD performed within 6 weeks of the SHUNT Test. The primary independent variable is DSI from the HepQuant SHUNT Test. The primary dependent variable or endpoint is large esophageal varices on EGD.

The analysis of the CLD Validation Dataset will be similar to the analysis described above for the HALT-C Training Dataset. The minimal model for the HepQuant SHUNT Test will be used and the laboratory analyses have been described elsewhere in this application (see **Section 6**).

Large and Small Esophageal Varices (EGD finding). The primary endpoint is endoscopically-defined large esophageal varices. The presence of varices is defined as visible venous channels protruding into the esophageal lumen during air insufflation of the esophagus. Large varices are defined as medium, large, Grade 2 or 3, or variceal diameter of 5 mm or more. Varices not meeting criteria for large varices will be classified as small varices.

Any Varices (EGD finding). The endoscopic findings of patients will be defined as either large varices, small varices, or no varices. For the analysis of patients with any varices, patients with large varices will be combined with patients with small varices.

4.3.2 LIKELIHOOD OF LARGE ESOPHAGEAL VARICES (CLD VALIDATION DATASET)

The analytical and statistical methods described in this section apply to the **primary and secondary objectives**. The steps in this analysis are similar to those used for analysis of the HALT-C Training Dataset:

1. **Uni-variable Logistic Regression.** Univariate logistic regression analysis will be performed using DSI as the independent variable and large varices as the outcome. (see **Table 3** for example of the output from this analysis).
2. **Multi-variable Logistic Regression.** As presented above, the gold standard for varices risk by AASLD guidelines is a liver biopsy diagnosis of cirrhosis. In BAVENO VI, platelet count was another major modifier of risk for clinically significant portal hypertension and risk for varices. Risk for large varices also correlates with CP class. For these reasons, we will evaluate the independence of DSI as a predictor after taking these variables into account. (see **Table 4** for example of the output from this analysis).
3. **Receiver Operator Curve (ROC) analysis.** The diagnostic performance of DSI for predicting large varices will be evaluated by Area under the Receiver Operating Curve (AUROC) and c-statistic. The optimum performance characteristics will be defined by Youden Index and sensitivity, specificity, PPV, NPV, NLR,

FNR, miss rate[5] and 95% CIs will be defined over the spectrum of the findings (see **Figure 3** for an example of output from this analysis).

4. **Probability and Calibration Curves.** The plot of predicted probability of large varices versus DSI will be analyzed by linear regression and correlation coefficients and displayed along with 95% confidence intervals (see **Figure 4** and **5** and **Table 5** for example of the output from this analysis).

The diagnostic performance (sensitivity, specificity, PPV, NPV, NLR, FNR, miss rate[5]) of DSI cutoff 18.3 will be evaluated for large esophageal varices. In addition, we will define the relationship of DSI to the probability of large esophageal varices over the full range of DSI.

An exploratory objective is to examine the diagnostic performance of DSI cutoff 18.3 for large varices compared to the diagnostic performance of a range of DSI cutoffs bracketing DSI 18.3. We will examine, in 0.5 increments of DSI, DSI's diagnostic performance (sensitivity, specificity, PPV, NPV, NLR, FNR) from 13 to 23 to define the Optimal DSI for "Rule Out" – highest sensitivity and NPV with lowest FNR. We will also compare the diagnostic performance of this "optimum" DSI to the DSI chosen for validation, DSI 18.3. The purpose of this analysis is to provide upside and downside performance of DSI around the validated cutoff of DSI 18.3. This analysis will yield a table of sensitivities, specificities, NPVs, PPVs, NLRs, and FNRs over the given range of DSI that could be included in a package insert to help the clinician interpret a given DSI result., as defined from AUROC analysis and Youden Index.

4.3.3 LIKELIHOOD OF SMALL VARICES OR VARICES OF ANY SIZE (CLD VALIDATION DATASET)

The analytical and statistical methods described below apply to the **exploratory objectives**. The steps in this analysis are similar to those used in the analysis above for large esophageal varices:

1. **Uni-variable Logistic Regression.** Univariate logistic regression analysis will be performed using DSI as the independent variable and varices of any size as the outcome. (see Table **6** for example of the output from this analysis).
2. **Multi-variable Logistic Regression.** As presented above, the gold standard for varices risk by AASLD guidelines is a liver biopsy diagnosis of cirrhosis. In BAVENO VI, platelet count is another major modifier of risk for clinically significant portal hypertension and risk for varices. For these reasons, we will evaluate the independence of DSI as a predictor after taking these variables into account. (see **Table 7** for example of the output from this analysis).
3. **Receiver Operator Curve (ROC) analysis.** The diagnostic performance of DSI for predicting varices of any size will be evaluated by Area under the Receiver Operating Curve (AUROC) and c-statistic. The optimum performance characteristics will be defined by Youden Index and sensitivity, specificity, PPV, NPV, NLR, PLR and 95% CIs will be defined over the spectrum of the findings (see **Figure 6** for an example of output from this analysis).
4. **Probability and Calibration Curves.** The plot of predicted probability of varices of any size versus DSI will be analyzed by linear regression and correlation coefficients and displayed along with 95% confidence intervals (see **Figure 7** and **8** and **Table 8** for example of the output from this analysis).

The diagnostic performance (sensitivity, specificity, PPV, NPV, NLR, PLR) of DSI cutoff 18.3 will be evaluated for esophageal varices of any size. The diagnostic performance of DSI cutoff 18.3 will be compared to the diagnostic performance of a range of DSI cutoffs, as defined from AUROC analysis and Youden Index.

[5] In this protocol, FNR is defined as 1-NPV and miss rate is defined as 1-Sensitivity. See the footnote in Section 1.1 for a discussion of these two measures.

Although defining likelihood of small varices is not as clinically relevant as defining likelihood of large varices, small varices can progress to large varices and risk for variceal hemorrhage. We would speculate that the small varices with DSI >18.3 are more likely at risk to progress to large varices, than small varices with DSI ≤18.3. Diagnosing these small varices with EGD generally provides justification for continued EGD surveillance in 1 to 3 year intervals. We speculate that the undetected small varices with DSI ≤18.3 would likely be the lowest risk varices to either enlarge to large varices or to result in variceal hemorrhage.

4.3.4 STAT EXPLORATORY ANALYSES

The statistical and analytical methods described above for DSI will be applied to the analysis of STAT. The HALT-C Training dataset will be used to define a STAT cutoff for likelihood of large esophageal varices and esophageal varices of any size. The diagnostic performance of the STAT cutoff will then be analyzed in the CLD Validation dataset. Linking STAT to likelihood for large esophageal varices or esophageal varices of any size would provide justification for future development of STAT for this indication.

4.4 Justification for Dose

Dose of 13C-cholate for intravenous injection: 20 mg of 13C-cholate in 5 mL of 8.4% sodium bicarbonate is mixed with 5 mL of 25% human serum albumin prior to injection, and then injected intravenously over 1 minute. The cholate concentration of the initial cholate solution, 4 mg/mL or 10mM, and after mixing with albumin, 2 mg/mL or 5 mM, is below the critical micellar concentration for cholate of 15 mM (104-106).

Albumin binding of cholate further reduces free cholate concentration. Upon mixing, nearly all the cholate binds immediately to albumin which has 7 hydrophobic binding sites for cholate (107). 20 mg cholate equals 50 µmol cholate; and, 5mL of 25% albumin (Mol Wt. 66,500) contains 1.25 g albumin, or 19 µmol albumin. Given 7 binding sites for cholate per albumin molecule, 19 µmol albumin has the capacity to bind 133 µmol cholate – nearly three times the amount of cholate in the injectate. Pre-binding of the 13C-cholate to albumin reduces free cholate concentrations within the injectate and reduces risk of any adverse detergent effect of cholate on the vein or surrounding tissues.

Dose of d4-cholate for oral administration: 40 mg of d4-cholate in 10 mL of 8.4% sodium bicarbonate is mixed with 10 to 20 mL flavored juice or drink and taken orally. Published studies of intestinal absorption of cholate indicate that absorption approaches 100% when given as a solution (108).

Cholate Blood Concentrations. The blood concentrations achieved after administration of 20 mg of 13C-cholate and 40 mg of d4-cholate in the HepQuant SHUNT test are in the physiologic range. In a mixed population of healthy controls and patients with fibrosis or cirrhosis due to chronic hepatitis C, the peak serum concentrations at 60 minutes after oral administration of 40 mg d4-cholate were 1.2±0.5 µM; peak concentration at 5 minutes after IV administration of 20 mg 13C-cholate were 5.1±1.7 µM (83). These serum concentrations are in the range of the serum concentrations of total bile acids and unconjugated cholate in healthy persons 2 hours after ingestion of a lipid meal: 9.9±0.8 µM and 0.9±0.4 µM, respectively (109). Specific intestinal and hepatic transporters are involved with intestinal absorption and hepatic uptake of cholate (110-112) (see **Appendix B**, Investigator's Brochure).

Maximum Doses:	13C-cholate	Fixed Dose, Intravenous	20 mg
	d4-cholate	Fixed Dose, Oral	40 mg

Study Intervention: None

Control Product: None

4.5 End of Study Definition

A **single participant is considered to have completed the study** if he or she has completed all phases of the study including the last visit or the last scheduled procedure shown in the Schedule of Activities (SoA), **Section 1.4**.

The **end of all patient participation** in the study is defined as the last enrolled patient completing the last visit or procedure shown in the SoA in the trial. The **end of the study** is defined as the completion of all data entry, cleaning of data, analysis of results, presentation of results, and publication of results.

5 STUDY POPULATION

Target Study Population. We plan to enroll up to 420 Adult patients with chronic liver disease from up to 40 clinical centers throughout the United States. The expected composition of our study population is based on a recent publication of USA trends in the burden of healthcare related to chronic liver disease and cirrhosis (56). The expected characteristics of the study population are: gender distribution (M:F) 3:2; mean age 58 years; race/ethnicity with non-Hispanic white 65%, black 12%, Hispanic 17%, and Asian/Pacific Islander 2%; and, etiology of liver disease as viral 15%, Alcohol 34%, and non-alcohol/non-viral 50%.

CLD etiologies. The enrolled CLD patients will be representative of clinical practice in that the cases will span the spectrum of both etiology and severity of liver disease. Etiologies will include chronic hepatitis C (HCV) and B (HBV), alcoholic liver disease, non-alcoholic fatty liver (NAFLD) or steatohepatitis (NASH), primary sclerosing cholangitis (PSC), primary biliary cirrhosis (PBC) and others.

Range of Disease Severity. Severity of disease will encompass noncirrhotic fibrosis stage F3 (with platelet count <175,000 μL^{-1}), compensated cirrhosis (CP class A), and Child-Pugh class B cirrhosis with exclusion of cases with refractory ascites (continued ascites despite treatment) or refractory encephalopathy (continued encephalopathy despite treatment).

Like HVPG, the HepQuant SHUNT test uniquely targets the portal circulation. For this reason, HepQuant SHUNT could be the minimally invasive alternative to HVPG. This study is critical for defining the performance of DSI in defining likelihood of large varices over an appropriate spectrum of etiology and range of severity of liver disease.

5.1 Inclusion Criteria

The main criteria for enrollment are:

1. CLD diagnosis, based upon at least one of the protocol-defined inclusion criteria for CLD (see below),
2. CLD etiology, from the list of acceptable protocol-defined etiologies (see below), and
3. EGD for the indication of either screening or surveillance for esophageal varices is scheduled or in the process of being scheduled.

The Inclusion Criteria are:

- Signed and dated informed consent
- Stated willingness to comply with all study procedures and availability for the duration of the study
- Adult male or female, age at entry at least 18 years
- Adequate peripheral venous access for intravenous catheter
- Ability to take the oral dose of d4-cholate
- Ability to hold morning doses of medications (Non-Selective Beta Blocker, ARB and/or Ace Inhibitor) for the 90 minute duration of the HepQuant SHUNT Test
- Meets at least one of the following criteria for CLD:
    - Abnormal liver enzymes (AST, ALT, or alkaline phosphatase), or, abnormal direct bilirubin, albumin, or INR (liver-related) of greater than 6 months duration. These subjects must also have platelet count <175,000 μL^{-1}
    - Fibrosis stage 3 (F3) or higher by liver biopsy (by METAVIR, Batts-Ludwig, or Brunt-Kleiner). Patients with F3 fibrosis must have platelet count <175,000 μL^{-1}
    - Fibrosis stage 3 or higher by elastography (FibroScan, SuperSonic, or MRE). Patients with F3 fibrosis must have platelet count <175,000 μL^{-1}
    - Fibrosis stage 3 or higher by FDA-approved laboratory panels. Patients with F3 fibrosis must have platelet count <175,000 μL^{-1}
    - Child-Pugh class A or B cirrhosis

  - HVPG of 6 mmHg or higher
  - Radiologic imaging consistent with either cirrhosis or portal hypertension
- Has one of the following diagnoses of CLD:
  - Hepatitis C virus (HCV) infection with documentation of a prior positive HCV RNA. Patients who achieved virologic cure are eligible to participate as long as they have CLD as defined above
  - Hepatitis B virus (HBV) infection defined by positive HBsAg
  - Non-alcoholic steatohepatitis (NASH)
  - Cryptogenic cirrhosis
  - Alcoholic liver disease
  - Cholestatic liver disease
    - Primary biliary cholangitis (PBC)
    - Primary sclerosing cholangitis (PSC)
  - Autoimmune hepatitis
  - Inherited disorders causing CLD
    - Hereditary hemochromatosis
    - Alpha-1-antitrypsin deficiency
    - Wilson disease
- Scheduled, or in the process of being scheduled for EGD

Indications for EGD. A key inclusion criteria is that the subject must have been selected and scheduled or in the process of being scheduled for EGD for the indication of either screening or surveillance for esophageal varices. Patients who are known to have had large esophageal varices on prior endoscopy or who have undergone treatment for varices, either radiologically (TIPS), surgically (Portal-Systemic Shunt), or endoscopically (banding or sclerotherapy) are excluded. Patients with prior endoscopic diagnosis of small esophageal varices who are undergoing EGD for surveillance of varices may be included.

5.2 Exclusion Criteria

The primary objective is to validate DSI 18.3 to "Rule Out" large esophageal varices. Our prior studies of the distribution of DSI by stage of liver disease have demonstrated increasing DSI with increasing stage of fibrosis and increasing CP class of cirrhosis (unpublished data). Patients with CP class C cirrhosis have up to an 85% prevalence of esophageal varices, and clear indications for EGD to evaluate for varices, independent of DSI result. For these reasons, CP class C cirrhosis is an exclusion to participation. The performance of DSI in CP class C cirrhosis might be a focus for future study and consideration.

The primary endpoint for this study is the endoscopic finding of large esophageal varices. Given this endpoint, a prior known endoscopic diagnosis of large esophageal varices, variceal hemorrhage, or treatment of varices are exclusions to participation.

The Exclusion Criteria are:

- Unable to give informed consent
- Unable to obtain venous access for administration of intravenous cholate
- Unable to absorb orally-administered cholate (examples could include underlying severe gastroparesis or having undergone extensive small bowel resection, conditions or post-operative state that could impair the absorption of the orally administered d4-cholate)
- Known hypersensitivity to human serum albumin
- Known hypersensitivity to any of the components of the HepQuant SHUNT Liver Diagnostic Kit
- Acute hepatitis, Acute Liver Failure, or Acute on Chronic Liver Failure
- Acute drug-induced liver disease (DILI)
- Noncirrhotic causes for portal hypertension and varices

- Ongoing active alcoholic hepatitis
- Child-Pugh class C defined by Child-Pugh score 10 or higher
- Dialysis
- Active infection or febrile illness within the last month
- Documented history of esophageal or gastric variceal hemorrhage
- Documented history of treatment of varices
- Documented history of endoscopic findings of large esophageal varices
- Hepatocellular carcinoma beyond Milan or UCSF criteria
- Thrombosis of main portal vein
- Liver transplant recipient
- Pregnancy
- Women who are breast-feeding
- Serious intercurrent medical or surgical illness, such as acute myocardial infarction, acute cerebral hemorrhage, sepsis, or other immediate life-threatening illness
- NPO (nothing through the mouth) status

5.3 Lifestyle Considerations

There are no restrictions regarding smoking or use of tobacco or marijuana products, concomitant drugs, over-the-counter products, medications, or ongoing alcohol use, except for the case of alcoholic hepatitis (see below). There are no dietary restrictions or restrictions on daily activity.

Patients with acute hepatitis, acute liver failure, or those with ongoing active alcoholic hepatitis are excluded to avoid confounding the relationship of DSI to varices risk by including patients with severe hepatocellular dysfunction.

5.4 Screen Failures

Screen failures are defined as participants who consent to participate in the study but do not subsequently undergo either the HepQuant SHUNT Test or the EGD, or for some other reason are not entered into the study (due to travel, medical illness, death, or other reason). HepQuant will comply with collecting a minimal set of screen failure information which is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. The minimal information will include demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial because of a travel, family emergency, or other modifiable reason may be rescreened. In case of Rescreening, the number for the original screening should not be re-used. Create a NEW subject ID number for any rescreened subject.

5.5 Strategies for Recruitment and Retention

Anticipated Accrual Rate: Up to 40 clinical sites enrolling 5 to 10 patients per month
Overall, the study should be completely enrolled over 20 to 24 months

Source of participants: Outpatient clinics
Patients will be identified by their care provider by:

- Diagnosis of CLD,
- Etiology of CLD, and
- In process of scheduling for EGD

The study coordinator will be notified by the care team.

The study coordinator, principal investigator, or subinvestigators will then meet with the patient for consent.

Recruitment venues: Communications with referring clinics and care providers
Posted notices in clinics

Recruitment Strategies: Notices to care providers
Posted notices in GI and Liver Clinics
Brochures to patient advocacy groups
Local advertisement
Social Media

Long-term Participation: Not applicable

Recruitment of women and minorities: Because CLD is more common in men, we expect a greater proportion of men to be enrolled. However, women are not restricted in terms of enrollment – in the USA about 40% of cases of CLD are women. Likewise, the study is not restrictive of race or ethnicity. We expect enrollment to reflect the distribution of gender and race/ethnicity as shown above.

Inclusion of Vulnerable participants: Not applicable.

Compensation and Incentives: A single payment will be made to the participant for completion of all procedures and visits. There are 3 unique study-related visits (excluding the EGD that is considered to have been ordered as part of the patients non study-related care). Payments will be prorated for participants by the number of study visits completed.

6 STUDY INTERVENTION

6.1 The HepQuant SHUNT Liver Diagnostic Kit (HepQuant SHUNT Test)

The HepQuant SHUNT Liver Diagnostic Kit contains two forms of cholate labeled with stable isotopes as molecular probes to quantify global liver-specific function and the portal circulation. A complete description can be found in the Instructions for Use (IFU) in **Appendix C** and Investigator's Brochure in **Appendix B**.

Cholate as a Molecular Probe. Cholate has several characteristics that favor its use as a molecular probe of the hepatic and portal circulations:

- Cholate is efficiently absorbed from the intestine.
- Cholate has a 70 to 80% first-pass hepatic extraction.
- Cholate is not metabolized, nor does it affect metabolism in the doses used in the HepQuant SHUNT test.
- Cholate is endogenous to the human body and, therefore, has none of the risks associated with xenobiotic exposure.
- Cholate in a dose of 15 mg kg^{-1} d^{-1} is FDA-approved for therapy of rare forms of pediatric liver disease.
- Clinical Trials Grade and GMP Grade Cholate are commercially available for purchase as a single carbon-13 label, [24-^{13}C]cholate (13C-cholate), and as a tetra-deuterium label, [2,2,4,4-^{2}H]cholate (d4-cholate).

- The deuterium-tagged internal standard is commercially available for use in the laboratory as an internal standard for quantification of cholate species by LC/MS.

The HepQuant SHUNT Liver Diagnostic Kit. The HepQuant SHUNT test is provided as test kits that are shipped to a testing center. At the testing center, the test compounds are administered and blood samples collected over 90 minutes. The sera from the timed blood samples are separated and shipped to a qualified laboratory for quantifying cholate concentrations, calculating test parameters, and reporting results. The kit contents include:

- Sealed vial of sterile solution of d4-cholate (40 mg in 10 mL sodium bicarbonate) for oral use
- Sealed vial of sterile solution of 13C-cholate (22 mg in 5.5 mL sodium bicarbonate) for intravenous use
- Sealed vial of 20 mL of 25% human serum albumin for intravenous use – 5 mL is added to 5 mL of the 13C-CA solution prior to intravenous injection
- Pipettes
- 6 transfer tubes (for transport of serum to HepQuant designated lab)
- 3-way stopcock
- Labels
- Instructions for Use (IFU)
- Test Requisition Form (TRF)Mailer

6.2 Test Outputs

6.2.1 SYSTEMIC HFR

The intravenous clearance (Cl_{iv}, mL min^{-1}) is defined as the dose/AUC for 13C-cholate. The Systemic Hepatic Filtration Rate (Systemic HFR) is defined as the Cl_{iv} per kg of body weight and is expressed as mL min^{-1} kg^{-1}.

6.2.2 PORTAL HFR

The apparent oral clearance (Cl_{oral}, mL min^{-1}) is defined as the dose/AUC for d4-cholate. The Portal Hepatic Filtration Rate (Portal HFR) is defined as the Cl_{oral} per kg of body weight and is also expressed as mL min^{-1} kg^{-1}.

6.2.3 SHUNT

SHUNT, the portal-systemic shunt fraction, is calculated as the ratio Systemic HFR/Portal HFR x 100%.

6.2.4 DSI

The calculation for Disease Severity Index is a proprietary formula derived from Systemic HFR and Portal HFR. DSI 0 implies no hepatic impairment and is based on the means (+3 SDs) for Systemic and Portal HFRs of normal weight healthy volunteers. DSI 50 represents severe hepatic impairment as measured in terminally ill CP (Child-Pugh) C cases with clinically advanced liver disease. Subjects with intermediate severity of liver disease have intermediate DSI scores.

6.2.5 STAT

STAT is defined as the 60-minute 4D-CA concentration normalized to an ideal body weight of 75 kg. STAT was found in previous studies to correlate closely with DSI (r2 = 0.88). Utilizing this single time point will also be evaluated as a simplified testing approach.

6.3 Documents, Instructions, and Brochures

Investigator Agreement: See **Appendix A**.
Investigator Brochure: See **Appendix B**.
Instructions for Use: See **Appendix C**.
Proposed Labeling: See **Appendix D**.
Material Safety Data Sheet: See **Appendix E**.
Informed Consent Form: See **Appendix F.**
Self-Reported Subject Tolerability Survey: See **Appendix G**.

Risk classification: Significant risk device, Class III

Commercial availability: For investigational use only.
Not for commercial sale or distribution.

Device size: 9 x 6.5 x 2.75 inches

Device model: SHUNT

Components: See IFU and Labels, **Appendices C** & **D**, Respectively

Duration of exposure: In our studies, we have measured the clearance rates for IV 13C-cholate removal from the blood. The k_{elim} of the first phase of clearance of IV 13C-cholate ranges from approximately 0.10 min^{-1} in healthy persons or persons with minimal liver disease, to approximately 0.05 min^{-1} in persons with advanced liver disease. There is a second slower phase of clearance, which contributes little to overall clearance in healthy persons. But, in persons with liver disease, the second phase contributes increasingly to overall clearance as liver disease worsens due to impaired hepatic uptake of cholate and portal-systemic shunting. In healthy persons or persons with minimal liver disease, the half-life of clearance of cholate from the blood is 7 to 10 minutes; cholate is completely eliminated from the blood within 35 to 50 minutes. In advanced liver disease, complete elimination of cholate from blood may take hours.

The 13C- and d4-cholates enter the body's cholate pool and are cleared based on turnover rate of the cholate pool. The turnover of the cholate pool is approximately 0.3 d^{-1} or $t_{1/2}$ of 2.3 days. The cholates are completely removed from the body after 12 days (5 half-lives).

Frequency of exposure: Once

6.4 Dosing and Administration

Time of Day: The HepQuant SHUNT Test is usually administered in the morning after an overnight fast. The Test may also be administered anytime during the day if the subject has fasted for at least 5 hours.

Duration of Administration of Cholates. The administration of the 13C-cholate intravenously in combination with the d4-cholate orally takes 1 minute.

Routes of Administration:	13C-cholate	Intravenously
	d4-cholate	Orally

Test administration is described in the Instructions for Use (IFU, **Appendix C**). A winged (or "butterfly") infusion or blood collection set or an intravenous catheter will be placed in one arm for administration of the 13C cholate, then removed following administration. An indwelling intravenous catheter is placed in the opposite arm for blood sampling. For subjects in which access cannot be obtained in both arms, the administration and sampling can occur in through a single indwelling catheter, provided adequate flushing is performed post-administration per the IFU. 13C-cholate, 20 mg (fixed dose), in sterile sodium bicarbonate solution is mixed with 5 mL of 25% human serum albumin prior to injection, and then injected intravenously over one minute. Simultaneously, d4-cholate, 40 mg

(fixed dose), in sterile sodium bicarbonate solution, is administered orally. Blood is sampled from the peripheral venous catheter at baseline and subsequently at 5±1, 20±2, 45±5, 60±5, and 90±5 minutes post-dosing. The clearances of both 13C- and d4-cholate are measured from the timed blood samples.

Minimum Doses:	13C-cholate	Fixed Dose, Intravenous	20 mg
	d4-cholate	Fixed Dose, Oral	40 mg
Maximum Doses:	13C-cholate	Fixed Dose, Intravenous	20 mg
	d4-cholate	Fixed Dose, Oral	40 mg

Dose Escalation: NA

Hold Times: The HepQuant SHUNT Liver Diagnostic Kits are held or stored at ambient temperature. Once the 13C-cholate has been mixed with the 25% human serum albumin or the d4-cholate vial has been opened the Test must be administered within 4 hours.

6.5 Preparation/Handling/Storage/Accountability

6.5.1 ACQUISITION AND ACCOUNTABILITY

How Kits will be Provided to the Investigator. The HepQuant SHUNT Liver Diagnostic Kits will be shipped in sealed containers. Within each container is a temperature indicator that ensures the temperature of the internal contents of the shipping container have been appropriately controlled.

Distribution of Kits. The HepQuant SHUNT Liver Diagnostics Kits are assembled by Prospect Life Sciences, Westminster, CO, who ships the containers with the Kits directly to clinical testing centers. The Kits will be logged-in when received and stored in secure environment under lock and key. In some centers, the Kits will be sent to a research pharmacist who will be responsible for receiving and securing the Kits. In others, the Kits will be sent to the study coordinator who will be responsible for receiving and securing the Kits. The details of acquisition and storage are delineated in the IFU (**Appendix C**).

6.5.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

NOTE: Additional details may be found in **Appendix B** (Investigator's Brochure), **Appendix C** (Instructions for Use), and **Appendix D** (Labeling).

Formulations. Sterile formulations of the labeled cholates, both 13C- and d4-cholate are supplied in clear glass vials. The labeled cholates are purchased in powder form and dissolved in 1M sodium bicarbonate solution (USP grade) to achieve concentrations of 4 mg/mL. The solutions are passed through 0.22-micron filters and undergo sterility and pyrogen testing. The absolute concentrations of d4-cholate and 13C-cholate are defined by LCMS methods – concentrations typically range from 3.5 to 4.2 mg/mL. Each vial of d4-cholate contains 40 mg in 10 mL 1M $NaHCO_3$ and each vial of 13C-cholate contains 22 mg in 5.5 mL 1M $NaHCO_3$. The vials are distributed in a HepQuant SHUNT Liver Diagnostic Kit and stored at ambient temperature.

A sterile glass vial of human serum albumin (25%, 20 mL, Grifols BLA STN 102478) is also contained within the HepQuant SHUNT Liver Diagnostic Kit.

NOTE: Although, the d4-cholate vial contains the exact volume, 10 mL, and amount of d4-cholate, 40 mg, the 13C-cholate vial for intravenous injection contains an excess volume, 5.5 mL, and amount, 22 mg, of 13C-cholate. The

excess is intentional, to provide sufficient "dead space" volume to allow for aspiration of exactly 5 mL of the 13C-cholate solution into a syringe. Accurate testing requires administration of the entire 10 mL of d4-cholate oral solution; and, exactly 5 mL of 13C-cholate intravenous solution.

Appearance:


Packaging: There is a 9 x 6 1/2 x 2 ¾" outer kit box that contains a foil pouch envelope, which contains an ambient gel wrap holding the interior kit contents (test solutions, supplies). The kit boxes are shipped within a sealed cooler that is packaged within an outer shipping box.

Labeling: Each cooler box has a HepQuant identification label. Each kit has an outer label containing the contents, Use by Date, caution warnings of investigational use only and storage instructions. Tube labels are pre-filled for the site's convenience with places for protocol, subject ID numbers, date and timepoint of test. UN3373 Biological Substance Category B labels are included within each kit for return of samples.

Manufacturers: The 13C- and d4-cholate compounds raw materials used in the HepQuant SHUNT Liver Diagnostic Kit are manufactured by Sigma-Aldrich-Isotec (P/N's W3713 and W6829, respectively). The formulations of 13C- and d4-cholate in 8.4% sodium bicarbonate (Hospira, 80098EV) are manufactured by PINE Pharmaceuticals (P/Ns HQ-13C-ECG and HQ-d4-ECG), and the 25% human serum albumin was purchased from Grifols (BLA 102478), all other materials in the kit are sourced from commercial suppliers.

6.5.3 PRODUCT STORAGE AND STABILITY

Storage: The HepQuant SHUNT Liver Diagnostic Kit is stored at ambient temperature until ready for use in a room temperature environment. The product is securely stored at the clinical testing site.

Stability: The stability of the 13C- and d4-cholate solutions at ambient temperature has been verified by appearance, pH, isotope qualification, confirmation of sterility, pyrogen testing, particle analysis, color analysis, and purity checks by HPLC. The vial of 25% human serum albumin has a shelf-life at ambient temperature of 3 years.

Once a vial has been opened or solution removed from the vial, the solution must be used within 4 hours. If not used within 4 hours, it should be discarded.

6.5.4 PREPARATION

Upon opening the HepQuant SHUNT Liver Diagnostic Kit, the person administering the test will confirm clarity of all solutions and integrity of the Test Kit and its contents.

The HepQuant SHUNT test is performed after an overnight fast or after at least 5 hours of fasting during the daytime. Morning doses of ACE or ARB inhibitors, non-selective β-blockers (nadolol, timolol, propranolol), or other investigational drugs are held until after completion of the 90 minute HepQuant SHUNT test. Medications may be administered immediately following the drawing of the 90 minute blood sample. In these patients or subjects, or

in persons with known hypertension the blood pressure should be checked prior to test administration. The test should not be administered if the diastolic BP is 110 mmHg or higher. The test can be rescheduled once the blood pressure is controlled. The HepQuant SHUNT test requires peripheral venous access via a standard indwelling intravenous catheter. The injectable solution of 13C-cholate, 20 mg, dissolved in sodium bicarbonate and mixed with 25% human serum albumin (HSA), is administered over 1 minute. Simultaneously (during the 1 minute), the oral solution of d4-cholate, 40 mg, dissolved in sodium bicarbonate, is given as a quick bolus. The oral solution is mixed with flavoring or juice (apple or grape) for administration. A three-way stopcock is provided in the Kit to facilitate the mixing of 5 mL of 25% HSA with 5 mL of the 13C-cholate solution (20 mg 13C-cholate per 5 mL) in preparation for intravenous injection.

Albumin has 7 hydrophobic binding sites for cholate (107). The intravenous 13C-cholate solution is pre-mixed with human serum albumin (HSA) just prior to intravenous injection to bind cholate, maintain 13C-cholate in the intravascular space, and reduce risk of thrombophlebitis or phlebothrombosis at the injection site.

Within four hours of injection, exactly 5 mL of the 13C-cholate intravenous solution is mixed with 5 ml of the albumin solution (25% w/v human serum albumin (HSA), Albutein ®-25 from Grifols Therapeutic, Inc, BLA 102478). The vials of 25% HSA provided in the HepQuant SHUNT Liver Diagnostic Test Kit have a total volume of 20 mL; but, ONLY 5 mL is pre-mixed with the 13C-cholate in the syringe, 15 mL is discarded. The 13C-cholate/HSA mixture is injected intravenously through a winged (or “butterfly”) infusion or blood collection set or an indwelling intravenous catheter over 1 minute as the d4-cholate solution is taken orally.

Blood samples are obtained via an intravenous catheter in the opposite arm at baseline and at 5±1, 20±2, 45±5, 60±5, and 90±5 minutes after dosing. The indwelling catheter is flushed with saline after each blood sample is obtained. The serum is separated and sent to the HepQuant laboratory for quantification of concentrations of endogenous cholate, d4-cholate and 13C-cholate.

Detailed Instructions For Use of the HepQuant SHUNT Test are provided in **Appendix C**.

6.6 Measures to Minimize Bias: Randomization and Blinding

Randomization: Not a randomized trial

Assignment to study groups: Single Arm study

Blinding: Triple-blind of HepQuant SHUNT Test results

- Patient
- Investigator and clinical center
- HepQuant

Single-blind of EGD results – HepQuant

Minimizing Bias: In the study the HepQuant SHUNT Test will be done prior to EGD, a sequence that is true for the clinic. The use of the HepQuant SHUNT test in this study mimics its intended use – i.e., a CLD patient meets sufficient clinical criteria to warrant EGD for screening or surveillance of varices. In the study, the enrolled subjects are scheduled for EGD, and meet these standard clinical criteria. In both the study, and for its intended use in the clinic, the HepQuant SHUNT test is administered after the clinical decision to perform EGD is made but prior to the performance of the EGD. The study results will be used to inform the clinician performing the EGD about the likelihood of large varices to aid in the decision to proceed or avoid the EGD.

Additionally, bias will be further minimized by blinding of patient, investigator and clinical center to the HepQuant SHUNT and DSI results; and, blinding of HepQuant to the EGD results.

Missing Data: Patients who have a DSI score but the EGD results are missing will be classified as "missing data". These patients are censored from the primary analysis which will only include the patients with both EGD results and DSI scores.

6.7 Study Intervention Compliance

Assessment of adherence to Protocol: Adherence to the study protocol will be defined by the following processes and procedures:

- Monitoring by the CRO with ongoing review of eCRFs, observed versus expected
- Receipt of correct blood samples for standard laboratory tests
- Receipt of correct blood samples, dates, times for the samples used for the laboratory analysis of the HepQuant SHUNT Test
- Review of results of laboratory tests, dates, times
- Review of the TRF (Test Requisition Form)
- Review of the appearance and disappearance curves for 13C- and d4-cholate after their quantification – to determine if sample times may have been switched
- Review of the completion of all study visits and procedures

The calculation of study intervention compliance will be determined by the formula:

% Compliance = (Observed DSI-EGD pairs) / (Expected DSI-EGD pairs) x 100%

6.8 Concomitant Therapy

Concomitant Medications: For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the Case Report Form (CRF) are concomitant prescription medications, over-the-counter medications and supplements.

Cholate Uptake and Drug Metabolism: Cholate is absorbed from the intestine and cleared from the blood by the liver via specific transporters (108-112). Many drugs are removed from the body via hepatic metabolism primarily via hepatic cytochrome P450 reactions. Cholate, especially in the low doses used in the HepQuant SHUNT test, does not interfere with hepatic metabolism or influence cytochrome P450 regulation. Given these considerations there would be no suspected interaction between drugs that undergo hepatic metabolism and cholate.

There are no known interactions of drugs or medications with the cholates used in the HepQuant SHUNT test. Non-selective β-blockers, ARB and/or ACE inhibitors could affect the blood flow to the liver, so subjects who are currently taking a non-selective beta blocker, an ARB/ACE inhibitor, or any combination will be asked to delay taking their normal dose the morning of their testing. They can take the morning dose of these medications immediately after the 90 minute sample for the HepQuant SHUNT test is obtained.

Interference with Cholate Measurements: Use of concomitant medications could theoretically present potential interference in serum cholate measurement. The liquid chromatography-mass spectrometry (LCMS) technique used to measure cholate levels was validated according to FDA guidelines [66] for selectivity, accuracy, precision, recovery, stability, and freedom from interferences by serum components or medications. Freedom from interference was tested on blanks and at the LLOQ for each analyte with a number of metabolites and medications including: Bilirubin, Cholesterol, Carbamazepine, Oxazepam, Diazepam, Nordiazepam, Lorazepam, Temazepam, Flunitrazepam, Nitrazepam, Clonazepam, Alprazolam, Ephedrine, Codeine, Diphenhydramine, Nortriptyline,

Propoxyphene, d-Amphetamine, d-Methamphetamine, Phenylpropanolamine, Phenmetrazine, Caffeine, Phencyclidine, Imipramine, Spironolactone, Furosemide, and dl-Propranolol. There was no interference at the concentrations tested which were above those usually observed in patient serum samples.

7 DISCONTINUATION AND WITHDRAWAL

7.1 Discontinuation of Study Intervention

Discontinuation related to administration of the HepQuant SHUNT Test, either prior to or during the Test administration, does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified (including, but not limited to changes from baseline) after enrollment, the investigator or qualified designee will determine if any change in participant management is needed. Any new clinically relevant finding (aside from the findings from the EGD procedure) will be reported as an adverse event (AE).

The data to be collected will include the following:

- Reason for discontinuation
- Protocol-specified safety follow-up to capture adverse events (AE), serious adverse events (SAE), and unanticipated problems (UPs).

7.2 Participant Discontinuation/Withdrawal from the Study

Participants are free to withdraw from participation in the study at any time upon request. An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Pregnancy
- Significant study intervention non-compliance
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- Disease progression which requires discontinuation of the study intervention
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- Participant unable to receive the HepQuant SHUNT Test
- Participant unable to receive the EGD within 42 days of the HepQuant SHUNT Tests

The reason for participant discontinuation or withdrawal from the study will be recorded on the "Withdrawal from Study" Case Report Form (CRF). Subjects who sign the informed consent form and are enrolled but do not receive the HepQuant SHUNT Test may be replaced. Subjects who signed the informed consent form, and are enrolled and received the HepQuant SHUNT Test but the EGD was not performed within 6 weeks of the HepQuant SHUNT Test will be replaced. The HepQuant SHUNT test results from these subjects will be censored and not included in the analysis of the study.

7.3 Lost to Follow-Up

A participant will be considered lost to follow-up if he or she fails to return for either the HepQuant SHUNT test or the post-test visit 2-30 days after the HepQuant SHUNT test and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within the studies pre-defined windows for each study visit and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to

the participant's last known mailing address or local equivalent methods). These contact attempts will be documented in the participant's medical record or study file.

- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 Efficacy Assessments

Independent Variables: DSI (for primary and secondary objectives)

Dependent Variables: Large Esophageal Varices diagnosed by EGD, or
Esophageal Varices of Any Size diagnosed by EGD

Screening (≤ 28 days prior to Enrollment): The timeline for screening may be relatively short since subjects enrolled in this study will have been scheduled for EGD for the indication of variceal screening or surveillance. This study protocol must accommodate the potentially short time period between ordering, scheduling, and performing the EGD.

The first step in screening is to ensure that the subject meets all inclusion and exclusion criteria as outlined in Sections 5.1 and 5.2. The study coordinator, principal investigator, or co-investigators will obtain informed consent, review study protocol and procedures, address questions or concerns, obtain written informed consent, and determine eligibility. The study coordinator will ensure that subject satisfies all inclusion criteria and lacks exclusion criteria.

The second step in screening is to ensure the subject meets three over-riding criteria:

1. Verification that subject satisfies pre-specified criteria for CLD;
2. Verification that subject has a confirmed diagnosis associated with CLD, e.g., chronic viral hepatitis or NASH; and,
3. Verification that the subject is in the process of scheduling an EGD.

The study coordinator will verify that the EGD procedure has been ordered and the subject is either scheduled or to be scheduled for the EGD.

Results of hepatic imaging, either US, CT or MR will be captured, if performed within one year prior to enrollment. Only de-identified data will be entered into a CRF, and the source document will stay at the site. The reason for the imaging study is to exclude extensive HCC or HCC with thrombosis of the main portal vein. Subjects can have possible, probable or definite hepatocellular carcinoma (HCC); but, the stage of the HCC cannot exceed Milan or UCSF criteria for candidacy for liver transplantation. A chart review will be conducted to record dates and results for hepatic imaging (US, CT, or MR). The de-identified data or results of these tests will be entered into a CRF from the study's database and the source document will remain at the clinical center.

The results of the following tests will also be recorded if they have been done within one year of enrollment:

1. Liver biopsy (grade of steatosis, activity or inflammation score, fibrosis score)
2. Elastography (type of elastography (transient elastography, ARFI, MRE, other), and kPa results)
3. Blood-based biomarkers or panels (type of blood test, and result)
4. HVPG (free HV pressure, wedged HV pressure in mmHg).

None of these tests are a requirement for enrollment.

The following additional tests will be obtained during the screening visit: complete blood count, blood chemistry profile, prothrombin time/INR, urine pregnancy test for women of childbearing potential, and electrocardiogram. MELD score will be calculated and recorded automatically from screening lab results.

Study Visit 1 (Enrollment, Day 1). The study coordinator will verify the eligibility of the study subject by review of screening visit results and enroll the patient. The subject will undergo a complete history and physical examination and recording of all concomitant medications. Demographic information, including gender, race/ethnicity, age (month and year of birth), height, weight, and BMI will be recorded. The Child-Pugh score will be calculated and recorded from screening lab results. The coordinator will schedule the subject for the HepQuant SHUNT Test which must be completed within 28 days of Visit 1.

NOTE: The HepQuant SHUNT Test (Study Visit 2) can be done on the same day as Study Visit 1.

Study Visit 2 (HepQuant SHUNT Test Administration, ≤28 days after enrollment). The time between Study Visit 1 and Study Visit 2 is from 0 to 28 days. The subjects enrolled in this study will have been scheduled or in the process of being scheduled for EGD and this protocol must accommodate the potentially short time period between ordering, scheduling, and performing the EGD. At the same time, some subjects may require flexibility in scheduling the SHUNT test, therefore up to 4 weeks may elapse between enrollment and SHUNT test administration.

The HepQuant SHUNT Test will be administered according to the Instructions for Use and required information recorded in the Test Requisition Form (TRF). The coordinator will oversee and confirm:

- Receipt of the HepQuant SHUNT Liver Diagnostic Kit
- Verify integrity of the shipping boxes and kits and kit contents
- Verify recording of the temperature log
- Mixing the 25% human serum albumin with the 13C-cholate for intravenous injection
- Administration of the albumin:13C-cholate mixture by intravenous injection over 1 minute
- Administration of the d4-cholate solution orally after addition of flavoring
- Blood sampling at the specified time points
- Serum separation and transfer to transport tubes
- Placement of serum transport tubes into mailer and document shipping
- Recording of any AEs or SAEs related to test administration or procedures
- Completion of the Self-Reported Subject Tolerability Survey

Study Visit 3 (Post-Test Follow-up, 2-30 days after SHUNT test). This post-Test follow-up visit must be done within 30 days after the HepQuant SHUNT Test administration to evaluate the subject for any AEs or SAEs that occurred following Test administration. At least two days must pass between SHUNT test administration and the follow-up visit to allow time for any non-immediate AEs that may occur. The site's PI will perform history and physical exam with a focus on any expected and unexpected AEs and SAEs. Vital signs will be. Blood will be obtained for complete blood count, and serum chemistry. All AEs and SAEs will be recorded.

Collection of EGD Data. The only stipulations regarding the performance of EGD are that it be done after the HepQuant SHUNT Test and that it be done within 42 days after the HepQuant SHUNT test. If convenient for the patient, the EGD can be done on the same day as the HepQuant SHUNT test; but, if this occurs, the EGD must be done after the HepQuant SHUNT test. The coordinator will record concomitant medications, obtain de-identified EGD report and photos, upload the report and photos to the EDC system. and record the EGD results. The findings of the EGD procedure are NOT recorded as Adverse Events.

Defining Large Varices from Endoscopy Reports. The diagnosis of varices and determination of variceal size, as either small or large, will be determined from the source documents reporting the results of the EGD. This same technique was used for varices diagnosis and variceal size determination in the HALT-C Training Dataset. Originally in HALT-C, the varices were recorded as small, medium, or large. But, the category of medium introduced more

subjectivity in interpretation and greater variance between observers. In analyses of the HALT-C Training dataset medium and large were combined into one category as "Large". Our decision to combine medium and large varices into one category of large is consistent with current guidelines recommended by professional societies for Gastroenterology, Hepatology, and Gastrointestinal Endoscopy (2,3). Thus, in our analyses of the CLD Validation Dataset we will define esophageal varices as either small or large.

Published literature regarding use of endoscopy to define variceal size indicates that there is excellent inter-observer agreement in distinguishing large from small varices. In the study by Merkel (67,72), the kappa statistic for inter-observer agreement between endoscopists in distinguishing large from small varices was 0.71. Results were similar from several other reports (113-116). Our inclusion of all patients undergoing endoscopy, which will include endoscopic findings of all types of varices and no varices, minimizes the risk for verification bias.

Other endoscopic features that may further define high-risk varices (HRV) or varices needing treatment (VNT) include:

1. Esophageal varices associated with gastric varices
2. Esophageal varices with moderate to severe red wale signs.

We will capture this information and will record their occurrence but are not planning any formal analysis of these endoscopic features since none of the current AASLD or EASL guidelines include these in decision-making algorithms.

8.2 Chart Review and Data Collection

HepQuant will retain a CRO to conduct and monitor this study. Subject ID and data from all the individual subjects enrolled in this study will be collected and recorded via an Electronic Data Collection system (EDC) with electronic Case Report Forms (eCRFs) and site monitoring.

The data collected will include demographic information (age, gender, weight, height, body mass index, race/ethnicity), criteria for CLD, CLD etiology or liver disease diagnosis, medical and surgical history, complications of liver disease, physical examination, standard blood tests, and liver imaging within 1 year of enrollment. Reports of liver biopsy (where available), elastography, blood biomarkers, and HVPG (where available) will also be de-identified and recorded.

The endoscopy data will include EGD date, description of EGD findings relative to varices, e.g. none or present, variceal size, and other variceal features (such as concomitant gastric varices and "red wale signs"). Data will also be collected regarding whether treatment of varices was performed at time of EGD, and what type of treatment was given, e.g., β-blocker and/or ACE inhibitor medical treatment, variceal band ligation, variceal sclerotherapy, transjugular intrahepatic portal-systemic shunt (TIPS), or surgical shunt.

The HepQuant SHUNT results will include DSI, SHUNT, STAT, Portal HFR, and Systemic HFR.

NOTE: For participants that may discontinue or withdraw early, we will capture the rationale for discontinuation or withdrawal during the final visit. See **Section 7, Study Intervention Discontinuation and Participant Discontinuation/Withdrawal**.

8.3 Safety and Other Assessments

The safety measurements are primarily the recording of AEs and SAEs at Visits 2 and 3, and the patient reported outcomes from the Self-Reported Subject Tolerability survey at Visits 2 and 3.

The primary safety issues with the HepQuant SHUNT Test could potentially be:

1. Pain, hematoma, bruising at site of intravenous catheter
2. Thrombosis of peripheral vein at intravenous catheter sites
3. Allergic reaction to any component of the HepQuant SHUNT Liver Diagnostic Kit

a. Cholate labeled with 13C or d4
b. Human serum albumin, 25%
c. Other kit components – there is no latex product in the kit

4. Other unknown late consequences of IV or Oral cholate, albumin, test kit components

In addition to monitoring injection site and for any systemic reactions, the subjects will complete a Self-Reported Subject Tolerability survey on the day of administration of the HepQuant SHUNT Test (Visit 2) and at the Post-Test Follow-up visit (Visit 3). The purpose of the survey is to assess the patient's experience with the testing procedures to define ways to improve test procedures if needed.

Screening: No safety procedures are planned at screening.

Study Visit 1 (Enrollment). No safety procedures are planned at Visit 1.

Study Visit 2 (HepQuant SHUNT Test Administration). Safety procedures will include:

1. Recording of AEs and SAEs
2. Administration of the Self-Reported Subject Tolerability Survey

Study Visit 3 (Post-Test Follow-up). Safety procedures will include:

1. Recording of AEs and SAEs
2. Administration of the Self-Reported Subject Tolerability Survey

Collection of EGD Data. AEs and SAEs related to performance of the EGD will be recorded. Findings of the EGD are NOT recorded as Adverse Events.

8.4 Adverse Events and Serious Adverse Events

8.4.1 DEFINITION OF ADVERSE EVENTS (AE)

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)). As EGD findings are part of the primary endpoint of this study, findings of the EGD procedure should NOT be recorded as Adverse Events.

8.4.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias, or convulsions that do not result in inpatient hospitalization.

8.4.3 CLASSIFICATION OF AN ADVERSE EVENT

8.4.3.1 SEVERITY OF EVENT

For adverse events (AEs) not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

8.4.3.2 RELATIONSHIP TO STUDY INTERVENTION

All adverse events (AEs) must have their relationship to HepQuant SHUNT Liver Diagnostic Kit assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to HepQuant SHUNT Test administration and cannot be explained by concurrent disease or other drugs or chemicals. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the HepQuant SHUNT Test, is unlikely to be attributed to concurrent disease or other drugs or chemicals. Rechallenge information is not required to fulfill this definition.
- **Potentially Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the HepQuant SHUNT Test). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.
- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to HepQuant SHUNT Test administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Not Related** – The AE is completely independent of the HepQuant SHUNT Test administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

8.4.3.3 EXPECTEDNESS

Expected adverse reactions are AEs that are known to occur for the study intervention being studied and will be collected in a standard, systematic format using the WHO grading scale based on functional assessment or magnitude of reaction. The expected adverse reactions as listed in the IB for the HepQuant SHUNT Liver Diagnostic Kit are:

An AE or suspected adverse reaction is considered "unexpected" if it is not listed in the IB or is not listed at the specificity or severity that has been observed; or, is not consistent with the risk information described in the protocol.

Investigators and the Sponsor will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention. Unanticipated adverse device effects (UADEs) will be reported as per FDA Guidance and as outlined below.

8.4.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All AEs, including local and systemic reactions not meeting the criteria for SAEs, will be captured in the source and on the appropriate case report form (CRF) within 7 days of awareness. Information to be collected includes event description, date and time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and date and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Findings of the EGD procedure are part of the primary endpoint of the study, and should not be recorded as Adverse Events.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

Site study staff will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

8.4.5 ADVERSE EVENT REPORTING

Any Grade 4 AE is to be reported within 3 days of awareness to:

Ring Central
800-793-8534

AND

Andrea Herman
Manager of Clinical Accounts and Quality
Andrea.herman@hepquant.com
Phone: 303-923-2210

Mobile: 720-314-2777
HepQuant, LLC
5251 DTC Parkway, Suite 300
Greenwood Village, CO 80111

All SAEs assessed to be possibly, probably or definitely related to the SHUNT procedure or compounds will be captured in the source and on the appropriate case report form (CRF) within 3 days of awareness. Information to be collected includes event description, date and time of onset, clinician's assessment of severity, relationship to study product (assessed only by PI or Sub-investigator), and date and time of resolution/stabilization of the event. All SAEs occurring while on study must be documented appropriately within the source documents and EDC. All SAEs will be followed to adequate resolution.

A Serious Adverse Event is to be reported within 3 days of site's awareness to:

Ring Central
800-793-8534

AND

Andrea Herman
Manager of Clinical Accounts and Quality
Andrea.herman@hepquant.com
Phone: 303-923-2210
Mobile: 720-314-2777
HepQuant, LLC
5251 DTC Parkway, Suite 300
Greenwood Village, CO 80111

8.4.6 SERIOUS ADVERSE EVENT REPORTING

The study investigator shall complete an Unanticipated Adverse Device Effect Form and submit to the HepQuant LLC and to the reviewing Institutional Review Board (IRB) as soon as possible, but in no event later than 10 working days after the investigator first learns of the effect. HepQuant LLC is responsible for conducting an evaluation of an unanticipated adverse device effect and shall report the results of such evaluation to the Food and Drug Administration (FDA) and to all reviewing IRBs and participating investigators within 10 working days after the sponsor first receives notice of the effect. Thereafter, HepQuant LLC shall submit such additional reports concerning the effect as FDA requests.

8.4.7 REPORTING EVENTS TO PARTICIPANTS

AEs and SAEs related to the HepQuant SHUNT Liver Diagnostic Kit that are associated with device malfunction or test administration will be reported to PIs, IRBs, and through them to study participants.

Amended ICF. Unexpected AEs or SAEs may require amendment to the Informed Consent Form (ICF). Any amendments to the ICF will be distributed to all clinical site for review and approval by the appropriate regulatory bodies, ie, IRB, etc.

Study-related results will not be distributed to participants.

Incidental findings of potential clinical relevance to the participant will be conveyed to the PI and study coordinator with documentation that the findings were discussed with the participant and a follow-up plan was established.

8.4.8 EVENTS OF SPECIAL INTEREST

Any medical device incident, including defects in the HepQuant SHUNT Liver Diagnostic Kit and malfunctions with the device are considered reportable events. The clinical testing site will inform HepQuant of any of these events or situations by contacting:

Ring Central
1-800-793-8534.

8.4.9 REPORTING OF PREGNANCY

Given that HepQuant will exclude any patient with a positive pregnancy test at screening, it is unlikely that there will be any pregnancies occurring within the relatively short time period of the participant's involvement with the study. Nonetheless, should the patient test positive for pregnancy during the study, HepQuant will report the pregnancy to study leadership, IRB, and regulatory agencies. In addition, HepQuant will request to follow pregnant women to pregnancy outcome. HepQuant expects the clinical testing site to forward appropriate source and EDC documentation.

8.4.10 PREGNANCY AND CHOLATE

Because cholates are naturally occurring with a pool size in humans of 1 to 5 g, the 20 and 40 mg doses of labeled cholates used in the HQ tests are unlikely to be harmful to a fetus. Cholate in doses of 15 mg kg^{-1} d^{-1} are used for treatment of liver disease in neonates and children (87,88). The 13C- and d4-cholates used in this study have been used in pregnancy to study bile acid and biliary lipid composition in women during different phases of the ovulatory cycle and in pregnancy (97-100).

However, without formal safety studies, the effects of these compounds on the fetus are not definitely known. Therefore, to be enrolled, women of child-bearing potential must have a negative pregnancy test to be enrolled in the study. Study participants will be asked to comply with pregnancy precautions, and use of approved contraceptive methods.

8.5 Unanticipated Problems

8.5.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

Device: HepQuant SHUNT Liver Diagnostic Kit.

HepQuant LLC will use the Office for Human Research Protections (OHRP) definition of unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets all the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and

- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

This definition could include an unanticipated adverse device effect, any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects (21 CFR 812.3(s)).

8.5.2 UNANTICIPATED PROBLEM REPORTING

An investigator shall submit to HepQuant LLC and to the reviewing Institutional Review Board (IRB) a report of any unanticipated adverse device effect with the HepQuant SHUNT Liver Diagnostic Kit as soon as possible, but in no event later than 10 working days after the investigator first learns of the effect (21 CFR 812.150(a)(1)). HepQuant LLC, who will conduct an evaluation of an unanticipated adverse device effect under 812.46(b) shall report the results of such evaluation to the Food and Drug Administration (FDA) and to all reviewing IRB's and participating investigators within 10 working days after HepQuant LLC first receives notice of the effect. Thereafter HepQuant LLC shall submit such additional reports concerning the effect as FDA requests (21 CFR 812.150(b)(1)).

8.5.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

Unanticipated problems with the HepQuant SHUNT Liver Diagnostic Kit that are associated with device malfunction, SUSARs, or other unexpected events that could potentially place participants at safety risk will be reported to PIs, IRBs, and through them to study participants.

9 STATISTICAL CONSIDERATIONS

The accuracy of the diagnostic test is commonly measured by its sensitivity and specificity or a measure that combines these quantities, such as the negative likelihood ratio: NLR = [(1-sensitivity)/specificity]. One of the primary performance goals in this trial is formulated using NLR as a primary statistical endpoint. The pre-test odds of large esophageal varices is given by [π/(1-π)], where π is prevalence of large varices. The post-test odds that there are no large esophageal varices is given by: NLR x [π/(1-π)]. The definitions of the various measures of diagnostic accuracy and their variance are given in the equations in **Table 9**.

An approximate critical value (cv) for NLR can be calculated using equation E such that with N=400 participants the upper limit of the 95% confidence interval will rule out NLR > 0.52 if the observed NLR is smaller than the critical value. Graphically, we display the approximate critical value (*cv*) for NLR as a line on the ROC plot of sensitivity versus (1-specificity) as shown in **figure 10** below , which illustrates the acceptable limits for validation of the DSI cutoff. The other performance objective is that the observed sensitivity be greater than or equal to 0.85, which is shown as the lower limit of for sensitivity and serves to ensure an acceptable lower limit for the False Negative Rate (FNR). **Table 11** shows how this critical value defines the acceptable limits for sensitivity and specificity.

The evaluable sample size is N=400[6]. The total sample size of N=420 is the evaluable sample size plus an additional 20 subjects to adjust for a 5% dropout rate. For all the efficacy endpoints the **time period** between the HepQuant SHUNT test and the performance of EGD is from 0 to 42 days.

Table 9: Definitions for measures of diagnostic accuracy

Equation label	Statistic	
A	Prior odds of large varices	$Odds = \frac{\pi}{1-\pi}$
B	Negative likelihood ratio	$NLR = \frac{1-sens}{spec}$
C	Positive likelihood ratio	$PLR = \frac{sens}{1-spec}$
D	False negative rate = (1-NPV)	$FNR = 1 - \left[1 + NLR\left(\frac{\pi}{1-\pi}\right)\right]^{-1}$
E	Variance of natural logarithm of estimated NLR	$V_{nlr} = \left[\frac{sens}{(1-sens)V}\right] + \left[\frac{1-spec}{spec(N-V)}\right]$
F	Variance of natural logarithm of estimate PLR	$V_{plr} = \left[\frac{1-sens}{(sens)V}\right] + \left[\frac{spec}{(1-spec)(N-V)}\right]$
G	Miss Rate	$Miss\ Rate = 1 - sens$

Symbols: π = prevalence of large varices; *sens* = sensitivity, *spec* = specificity, *N = trial sample size, V = number of subjects with large varices (V = N*π).

9.1 Statistical Hypotheses

9.1.1 PRIMARY EFFICACY ENDPOINT (DSI ≤18.3 "RULES OUT" LARGE ESOPHAGEAL VARICES).

For each subject, the primary endpoint is the presence of large varices based on EGD examination. The primary estimands are NLR and sensitivity which then define the standard for diagnostic accuracy of DSi as a rule-out test for large varices. A primary objective is to determine whether the NLR for DSI ≤18.3 is an improvement over the average value for NLR from 10 published studies of 2977 cases of the use of LSM plus platelet count in likelihood of large esophageal varices. Improvement will be decided if the confidence interval for the observed NLR rules out NLR > 0.52, which corresponds to the observed NLR being smaller than the approximate critical value of cv= 0.27. With this partition of the ROC outcome space, the study will conclude that the diagnostic accuracy of DSI < 18.3 is acceptable if the observed NLR is smaller than that observed in most previously published studies (**Figure 10**). The null hypothesis of NLR = 0.52 is chosen to demonstrate improvement over the diagnostic accuracy of tests evaluated in the 10 published studies of 2977 cases.

[6] A single interim analysis is planned after the trial data set has compete DSI and EGD measurements on a minimum of 250 participants as described in Section 9.5.1.

9.1.2 SECONDARY EFFICACY ENDPOINT

Secondary efficacy endpoint (Relationship between DSI score and risk of large esophageal varices): Logistic regression will define the relationship of the probability of large esophageal varices to DSI score over the entire range of DSI. The DSI coefficient in the logistic regression model is hypothesized to be significantly larger than 0, which would confirm that the odds of large varices increases with DSI. The probability of large esophageal varices will also be measured across categories of DSI, i.e., quartiles, deciles, or other. The observed probability will be compared to the expected probability from the logistic regression analysis.

9.2 Sample Size Determination

9.2.1 PRIMARY EFFICACY ENDPOINT: SAMPLE SIZE AND POWER

Based on NLR

The trial sample size was evaluated and selected to assure adequate statistical power that the upper limit of the 95% CI for NLR will be smaller than 0.52. For design purposes we evaluate power and sample size using the variance from Table 9 equation E under the hypothesis that NLR = 0.20 with sensitivity = 0.90, specificity = 0.50, and 20% prevalence of large varices (i.e., 80 subjects with large varices = 0.2x400). A sample size of 400 participants (80 with large varices, 320 without) was selected to provide adequate power based on the following power characteristics:

		Power for various sample sizes			
NLR	**FNR**	**380**	**400**	**420**	**440**
0.20	0.048	0.78	0.80	0.82	0.84
0.19	0.045	0.82	0.84	0.86	0.87
0.18	0.043	0.86	0.88	0.89	0.91
0.17	0.041	0.89	0.91	0.92	0.93
0.16	0.038	0.92	0.93	0.94	0.95
0.15	0.036	0.95	0.96	0.96	0.97

Table 10: Initial Power Analysis for Selection of N-400

In this analysis 400 subjects provide over 80% probability/power of rejecting the rule-out hypothesis of NLR > 0.52 when the true NLR is smaller than 0.20. From Table 9 equation D, NLR = 0.2 corresponds to FNR = 0.048; thus, the trial has more than 80% power for FNR smaller than 5%. With 400 subjects, the upper limit of the 95% confidence will exclude NLR > 0.52 if the NLR in the trial is smaller than 0.27; that is, the threshold (critical value) for rejecting the NLR null hypothesis is NLR≤0.27 (corresponding to FNR smaller than 6.5%). This evaluation is based on the large-sample variance of equation E and is adequate for selecting the total sample size for the trial. A complete power evaluation of trial power with and without an interim analysis requires a simulation study as described in section 9.5.1.

Based on Predictive Value of a Negative Test (NPV)

NPV is the probability that a subject with DSI ≤18.3 does not have varices, and the false negative rate is defined from NPV according to equation D in **Table 9**. The goal is to ensure that *NPV is suitably large and FNR* is suitably small. NLR can also be written in terms of NPV. From equation D, *NLR* <0.27 corresponds to FNR <6.5% and a NPV over 0.937. Similarly, the critical value rules out NLR > 0.52, which corresponds to NPV > 0.885 (FNR < 11.5%). The study sample size gives 80% power under the hypothesis that *NLR* = 0.2, which corresponds to FNR < 5%.

Impact of Dropout, Withdrawal, or Missing Data on Study Power: This is a very short-term study with maximum duration of a participant's participation of 100 days and a minimum participation of 3 days. We expect a low dropout rate – probably less than 5%. Nonetheless, we will recruit an excess of 5% of cases, N=420 total, to ensure enrollment of 400 evaluable cases.

Planned Interim Analysis: A single interim analysis is planned after the trial data set has compete DSI and EGD measurements on 250 participants. Details of the interim analysis can be found in Section 9.5.1.

9.2.2 SECONDARY EFFICACY ENDPOINTS: SAMPLE SIZE AND POWER

The sample size of 400 evaluable subjects is determined by the statistical power requirements for the primary efficacy endpoint. The secondary efficacy endpoints will use the same subjects as the primary analysis. With 20% prevalence, the study should have a sufficient number of subjects with large esophageal varices. The number of varices and total sample size will provide adequate power for evaluation of the secondary and exploratory endpoints.

9.3 Populations for Analyses

Population for Analysis: All enrolled subjects who underwent the HepQuant SHUNT Test; a maximum of N=420 and minimum of N=400 will be analyzed (the interim analysis will be conducted when DSI and EGD exam results are available in the analysis dataset for approximately N=250 participants).

Safety Analysis Dataset: All enrolled subjects who underwent the HepQuant SHUNT Test; a maximum of N=420 and minimum of N=250 will be analyzed.

- **Per-Protocol Analysis:** All enrolled subjects who completed the entire protocol
- **Other Datasets that may be used for sensitivity analyses:** None

9.4 Statistical Analyses

9.4.1 GENERAL APPROACH & DESCRIPTIVE STATISTICS

Selected characteristics will be analyzed for the total enrolled CLD population, and in subgroups by categories of etiology of liver disease (viral, NASH, EtOH, and cholestatic disease), and disease severity (noncirrhotic fibrosis, compensated cirrhosis (CP-A), decompensated cirrhosis (CP-B)). For descriptive statistics, categorical and continuous data will be presented as percentages, means with standard deviations, median, and range. Statistical significance will be defined by two-sided p-values <0.05 and 95% confidence intervals will be used to summarize uncertainty in estimated measures of diagnostic accuracy or differences between groups.

Appropriate covariates are pre-specified in the sections below.

9.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

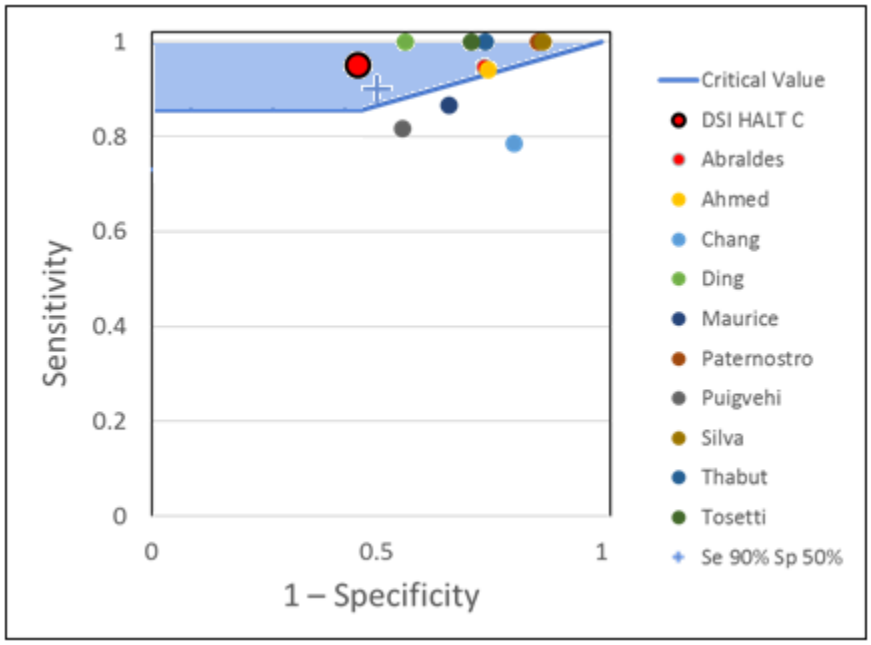
Primary efficacy endpoint (DSI ≤18.3 "Rules Out" Large Esophageal Varices): The independent variable is DSI and the dependent variable is the presence/absence of large esophageal varices diagnosed by EGD within 42 days of the HepQuant SHUNT test. The sample size evaluation of section 9.2.1 is based on the large-sample variance of **Table 9** equation E. This equation cannot be used for data analysis if sensitivity is close to 1.0 (Marril, et al [118]). The following methods will be used to evaluate whether the trial results satisfy the performance goals for NLR and sensitivity:

Rule out NLR > 0.52: This objective will be satisfied if the upper limit of the confidence interval for NLR is smaller than 0.52. The score-based confidence interval that is derived by inverting the score test (see Marril, et al. [118]) will be used to calculate the NLR confidence interval. The confidence level will be chosen to assure an overall one-sided type-I error rate of 0.025 as described in section 9.5.1.

Sensitivity >= 0.85: This objective will be satisfied if the observed sensitivity is larger than 0.85. The corresponding 95% confidence interval will also be reported and will be calculated using the exact binomial distribution.

Evaluation of standard for the rule-out hypothesis (DSI ≤18.3 "Rules Out" large esophageal varices): The two performance goals can be evaluated graphically and compared with previously published studies of the diagnostic accuracy of existing tests for ruling out the presence of large varices. NLR is a function of sensitivity and specificity and can be plotted on a graph of sensitivity versus one minus specificity in a manner similar to the ROC curve. **Figure 10** shows the approximate critical value for ruling out NLR > 0.52 plotted in the ROC plane, along with the critical value for sensitivity of 0.85. The shaded region of the plot represents acceptable performance. The plot includes results from the 10 previously published studies of LSM plus platelets. The plot demonstrates that the HALT-C study using DSI and half of the studies using LSM plus platelets would satisfy the standards for improved NLR in the proposed trial. We conclude that the null hypothesis (NLR >0.52) is empirically consistent with the diagnostic tests that are currently used in clinical practice, and therefore the proposed standard (ruling out NLR >0.52) is a clinically meaningful basis for the design of the SHUNT-V trial to evaluate whether DSI meets a valid standard for clinical diagnosis of large esophageal varices in the CLD population.

Figure 10: Plot of NLR Critical Value (blue line) for Improved NLR for Large Varices



Abbreviations: NLR, negative likelihood ratio; cv, critical value; Se, sensitivity; Sp, specificity. DSI 18.3 is validated for "Rule Out" large esophageal varices if trial results fall in the shaded region above the blue line. The approximate critical value (NLR = 0.27) is calculated using **Table 9** equation E. The published results using LSM and platelet count are shown by their individual colored markers with the first author of the respective publication listed. The red marker with black border represents the results of DSI 18.3 from the HALT-C training dataset with sensitivity 95% and specificity 54%. The high sensitivity and higher specificity of DSI 18.3 from the HALT-C Trial demonstrated favorable diagnostic performance compared to the studies of LSM plus platelet count.

The HALT-C Training dataset (red marker with black border) showed that DSI 18.3 had sensitivity 0.95 and specificity 0.54 yielding a NLR of 0.0926, well within the shaded blue region. The upper limit of the 95% CI for NLR was 0.143, well below the upper limit of the 95% CI for the *cv* of 0.52. We anticipate that diagnostic performance of DSI 18.3 in the CLD Validation dataset will equal or exceed its performance in the HALT-C Training dataset, with sensitivity >95%. If that is the case, then specificity may drop below 0.50 as long as the observed NLR is <0.27. The mean value for specificity from the 10 published studies of LSM plus platelet count was 0.28 with a minimum of 0.13 and maximum of 0.44. Specificity ≥0.28 will be considered acceptable, as long as the confidence interval rules out NLR > 0.52.

The following table shows specific values of sensitivity and specificity with 95% confidence intervals that are on the critical value line in **Figure 10**. The table shows the ranges of sensitivity and specificity that would be ruled out if the trial results are on the critical value line.

Specificity (95% CI)		Sensitivity (95% CI)	
0.25	(0.203, 0.297)	0.932	(0.878, 0.987)
0.30	(0.250, 0.350)	0.919	(0.859, 0.979)
0.35	(0.298, 0.402)	0.906	(0.841, 0.970)
0.40	(0.346, 0.454)	0.892	(0.824, 0.960)
0.45	(0.395, 0.505)	0.878	(0.807, 0.950)
0.50	(0.445, 0.555)	0.865	(0.790, 0.940)
0.55	(0.495, 0.605)	0.851	(0.774, 0.929)

Table 11: Values of sensitivity and specificity that are on the critical value line.

Evaluation of trial power: The initial power evaluation for selection of the total sample size (**Table 10**) uses the large sample variance approximation of **Table 9** equation E. A simulation study was designed and conducted to provide a full evaluation of trial power including the planned interim analysis. Power is a function of sensitivity, specificity as determined by NLR (specificity = (1-sensitivity)/NLR), and the prevalence of large varices. The simulation study calculated power as the proportion of 10000 simulated trials that satisfy the two performance goals (upper CI limit for NLR < 0.52 and sensitivity > 0.85). Key results of the simulation study are summarized in **Table 12.**

	Without Interim Analysis			With Interim Analysis		
			20% prevalence of large varices			
NLR	Sensitivity = 0.85	Sensitivity = 0.90	Sensitivity = 0.95	Sensitivity = 0.85	Sensitivity = 0.90	Sensitivity = 0.95
0.52	0.020	0.017	0.011	0.020	0.015	0.007
0.40	0.146	0.088	0.041	0.131	0.077	0.032
0.30	0.502	0.380	0.157	0.417	0.348	0.124
0.20	0.524	0.916	0.583	0.520	0.855	0.545
0.15	NA	0.930	0.910	NA	0.880	0.890
			25% prevalence of large varices			
0.52	0.021	0.021	0.012	0.019	0.017	0.010
0.40	0.170	0.107	0.054	0.151	0.100	0.041
0.30	0.515	0.462	0.193	0.450	0.422	0.172
0.20	0.515	0.944	0.696	0.523	0.888	0.668
0.15	NA	0.945	0.956	NA	0.902	0.951
	NA denotes combinations that produce specificity = 1.0 and results that cannot occur in a trial					

Table 12: Power for the SHUNT-V trial with and without interim analyses. The type I error rate is the power when NLR = 0.52 and is always smaller than the target one-sided 0.025 alpha level.

9.4.3 STATISTICAL TESTS AND STEPS

The initial **statistical tests and steps** in the analysis of the **CLD Validation dataset** are:

Uni-variable Logistic Regression. The relationship of DSI (independent variable) to large esophageal varices (dependent variable) will first be evaluated by univariate logistic regression.

Multi-variable Logistic Regression. Independence of DSI as a predictor of large esophageal varices will be evaluated in multi-variable logistic regression analyses including other risk factors for varices. The current clinical gold standard for likelihood of varices and recommendation for EGD is a diagnosis of cirrhosis - by either liver biopsy or other criteria. Likelihood of large varices is further increased by increase in CP class. In BAVENO VI, platelet count was a major modifier of risk. For these reasons, we will evaluate the independence of DSI as a predictor of large esophageal varices adjusting for demographic variables, diagnosis of cirrhosis, stage of cirrhosis, and platelet count.

Area Under the Receiver Operator Curve (AUROC). The diagnostic performance of DSI for predicting large esophageal varices will be evaluated by Area under the Receiver Operating Curve (AUROC). The c-statistic for DSI in likelihood of large esophageal varices will be defined. The performance characteristics (sensitivity, specificity, PPV, NPV, NLR, PLR, FNR, miss rate[7] and their 95% CIs) will be defined for DSI cutoff 18.3.

[7] In this protocol, FNR is defined as 1-NPV and miss rate is defined as 1-Sensitivity. See the footnote in Section 1.1 for a discussion of these two measures.

Secondary efficacy endpoint (probability and calibration curves). We will evaluate the relationship of predicted probability of large varices versus DSI using logistic regression analysis and affiliated statistical tests of the significance of the association between the continuous DSI score and risk of large varices. These analytical results will be further evaluated using a calibration table to compare the observed probabilities of large varices in quartiles, deciles, or other categories of DSI to the expected probabilities from the logistic regression equation.

9.5 Summary and Implications of Validating the Primary Endpoint

We anticipate test performance to be similar between the HALT-C Training dataset and the CLD Validation datasets. In the HALT-C Training dataset with prevalence of large varices of 10%, DSI ≤18.3 yielded sensitivity 95%, specificity 54%, NLR 0.0926, NPV 99%, and FNR 1%. Therefore, we expect the NLR and upper limit of the 95% CI for NLR to meet the validation criteria: upper limit of 95% CI $<NLR_0$, or <0.52, and observed sensitivity > 0.85. There are several clinical implications of this validation.

DSI ≤18.3 improves the diagnostic performance to "Rule Out" Large Varices. The high sensitivity and moderate specificity of DSI ≤18.3, compared to the lower sensitivity and specificity of publication of LSM plus platelet count, suggest that the DSI cutoff might perform well in identifying the cases free of large varices who might avoid screening EGD. The HALT-C Training dataset results suggested that up to 50% of screening EGDs might be avoided. In contrast, the data with LSM plus platelet count suggested that only 20% of screening EGDs might be avoided.

DSI ≤18.3 lowers the FNR for Large Varices. The pre-test odds of large esophageal varices is given by $[\pi/(1-\pi)]$, where π is prevalence of large varices. For prevalence of 10%, the pre-test odds (0.1/0.9) for large esophageal varices is 0.11. The post-test odds of large esophageal varices is given by: NLR x $[\pi/(1-\pi)]$. For NLR of 0.0926, the post-test odds of large esophageal varices for DSI ≤18.3 was: 0.0926 x (0.1/0.9) = 0.0102, or a pre- to post-test reduction in odds for large varices of 11.0 – a highly significant reduction based on DSI.

In contrast, for an average NLR of 0.27 and prevalence of 11% (pre-test odds (0.11/0.89) = 0.12) from the 10 published studies of LSM plus platelet count, the post-test odds of large esophageal varices was 0.27 x (0.11/0.89) = 0.033, or odds ratio of 3.6. This comparison suggests that DSI has better diagnostic performance.

Applying these results for DSI to the CLD Validation dataset where the prevalence of large esophageal varices is projected to be 20% yields a post-test odds for large varices of 0.09 x (0.2/0.8) = 0.0225. In comparison, LSM plus platelets would yield a post-test odds for large varices of 0.27 x (0.2/0.8) = 0.0675. The data suggests that DSI has a lower FNR than LSM plus platelet count over the prevalence range of 10 to 20% for large varices.

DSI ≤18.3 lowers the Miss Rate for Large Varices. Miss rate for large varices is defined by the number of cases with large varices below cutoff divided by the total number of cases with large varices. For the HALT-C Training dataset the Miss Rate was 1/22 = 4.5%. For the pooled results from the 10 published studies of LSM plus platelet count the Miss Rate was 21/319 = 6.6%. For individual published studies of LSM plus platelet count, the Miss Rate ranged from 0.0 to 18.2%.

In addition, the AUROC for DSI in likelihood of large varices was c-statistic = 0.82 which compares favorably to c-statistic = 0.76 for LSM plus platelet count, i.e., an improvement in overall diagnostic performance.

9.5.1 PLANNED INTERIM ANALYSIS

One interim analysis is planned after the analysis data sets contain complete results (DSI measurements and EGD examination results) on at least 250 participants. The objective of the interim analysis will be to evaluate whether the interim results would allow the trial to be stopped for either efficacy or futility; i.e., because the performance goals for DSI < 18.3 as a test for ruling out large varices has been satisfied (efficacy) or because it is futile to continue because sensitivity is too low and NLR is too large.

Process for preparing and reviewing interim analysis: The trial database is maintained by an independent CRO. Trial investigators do not have access to DSI results, and the Sponsor does not have access to EGD findings. The interim analysis will therefore be performed by a statistician independent from both the sponsor and the CRO. The results of the interim analysis will be reviewed and interpreted by the independent biostatistician using the interim decision criteria to make a recommendation to HepQuant to either continue or stop the trial based on the interim results. If the determination is made to continue the trial, no further analyses will be conducted until the full recruitment is reached, and no blinded information will be provided to the sponsor, the CRO, or any investigator. If the determination is made to stop the trial, analysis of all endpoints will be performed on the interim data (N=250) according to the trial SAP.

Decision criteria for the interim analysis: The interim decision criteria are based on standard methods for group sequential clinical trial design as published by Emerson, et. al. [117] as implemented in the software package RCTdesign. With complete results on 250 subjects the independent unblinded statistician will convey one of three conclusions based on the results of the data analysis:

i. *The efficacy decision criteria have been satisfied:* This conclusion is reached if:
    a. The upper limit of the 97.64% score-based confidence interval for NLR is less than 0.52, and
    b. the observed sensitivity greater than or equal to 0.85.
ii. *The futility decision criteria have been satisfied:* This conclusion is reached if:
    a. The upper limit of the 51.41% score-based confidence interval for NLR is larger than 0.52, and
    b. the observed sensitivity less than 0.85.
iii. *Neither the efficacy nor the futility decision criteria have been satisfied.*

In the case of the second conclusion, i.e., futility, the trial will be stopped; the assessment of futility represents a binding assessment.

In the case of the third conclusion, the trial will continue to the full sample of 400 subjects with complete data. If the study is not stopped early, then the full-sample (N=400) analysis will reach an efficacy conclusion if the upper limit of the 96.07% confidence interval is smaller than 0.52 and the observed sensitivity is greater than or equal to 0.85.

If the efficacy or futility decision criteria are satisfied, then HepQuant will request the analysis data set and analysis report from the independent statistician. HepQuant will reproduce the analysis from the independent statistician and initiate the trial termination processes. If neither the efficacy nor futility criteria are satisfied, then HepQuant will remain blinded and will not receive the results of the interim analysis. The independent statistician will archive the analysis and the interim analysis data set for reference upon trial completion.

The power and type I error rate using the above interim decision criteria have been evaluated in a simulation study as described in section 9.4.2 and summarized in **Table 12**. The simulation study also calculates the probability that the trial will satisfy the interim decision criteria. **Table 13** summarizes the chance of satisfying the interim efficacy and futility decision criteria as a function of the true NLR when true sensitivity is 90%.

	Decisions at N=250		Decisions at N=400		Total power
NLR	Futility	Efficacy	Futilty	Efficacy	for Efficacy
	20% prevalence of large varices				
0.52	0.744	0.004	0.241	0.011	0.015
0.40	0.521	0.022	0.403	0.055	0.077
0.30	0.220	0.124	0.433	0.223	0.348
0.20	0.118	0.652	0.027	0.203	0.855
0.15	0.120	0.880	0.000	0.001	0.880
	25% prevalence of large varices				
0.52	0.747	0.005	0.236	0.012	0.017
0.40	0.484	0.032	0.416	0.069	0.100
0.30	0.184	0.166	0.394	0.256	0.422
0.20	0.100	0.760	0.013	0.128	0.888
0.15	0.098	0.902	0.000	0.000	0.902

Table 13: Probability of reaching the futility and efficacy decisions at the interim (N=250) and full sample (N=400) analyses. Probabilities from simulations for the indicated NLR when sensitivity = 0.90.

The interim analysis improves the efficiency of the trial by allowing early stopping for efficacy if the DSI test has NLR=0.15 (88% chance of stopping at the interim analysis with 20% large varices) or for futility under the null hypothesis of NLR=0.52 (77% chance of stopping at the interim analysis with 20% prevalence of large varices).

Statistical analyses and reporting of results: Although the confidence levels for NLR in the interim decision criteria have been adjusted to maintain the one-sided level 0.025 type I error rate, study results will be reported using 95% confidence intervals as described in section 9.4.1. Exact methods for proportions and the score-based confidence intervals for ratios of proportions will be used for 95% confidence intervals when reporting results for NLR, sensitivity, and other measures of diagnostic accuracy.

9.5.2 SAFETY ANALYSES

The known potential adverse events associated with the HepQuant SHUNT Test are primarily related to the indwelling intravenous catheter and the very rare risk for allergic reaction to human serum albumin. These are delineated in **Section 2.3.1**. AEs and SAEs are evaluated and recorded at Study Visits 2 and 3. While EGD findings are not reported, AEs and SAEs associated with the EGD itself are recorded. The Self-Reported Subject Tolerability survey is completed at Study Visits 2 and 3.

Safety events will be analyzed as summary statistics. AEs and SAEs will be coded according to the Medical Dictionary for Regulatory Activities (MedDRA). The incidence and frequency will be calculated by counting each AE once only for a given participant. Severity and frequency of AEs, and the relationship of AEs to study intervention will be presented by System Organ Class (SOC) and preferred term groupings. The following additional information will be reported about each AE: start date, stop date, severity, relationship, expectedness, outcome, and duration. Adverse events leading to premature discontinuation from the study intervention and serious treatment-emergent AEs will be presented in a table. The information will be consistent with the information contained within **Section 8.3,** Safety and Other Assessments.

9.5.2.1 ADVERSE EVENTS (AES)

Adverse events will be tabulated by subject by study visit – Visits 2, 3, and EGD-related.

9.5.2.2 SERIOUS ADVERSE EVENTS (SAES AND SUSARS)

Serious adverse events will be tabulated by subject by study visit – Visits 2, 3, and EGD-related.

9.6 BASELINE DESCRIPTIVE STATISTICS

Descriptive statistics will characterize:

- The total enrolled CLD population
- Subgroups of etiology of CLD
    - NASH and Cryptogenic cirrhosis
    - Viral (HCV and HBV)
    - Alcohol
    - Cholestatic (PBC and PSC)
- Subgroups of severity of liver disease
    - F3 Fibrosis stage with platelets <175,000 μL^{-1}
    - Compensated cirrhosis (Child-Pugh class A cirrhosis)
    - Decompensated Cirrhosis (Child-Pugh class B cirrhosis)

For descriptive statistics, categorical and continuous data will be presented as percentages, means with standard deviations, median, and range. For inferential tests, in comparing the characteristic of the two subgroups, p-value <0.05 (two-sided) and 95% confidence intervals will be required for statistical significance (Type I error).

9.6.1 SUB-GROUP ANALYSES

9.6.1.1 PRIMARY ENDPOINT

The target population for the HepQuant SHUNT Test will include adults, age 18 or higher, both men and women, all races or ethnicities, and all etiologies of CLD. The Test measures the flow-dependent clearance of cholate from both systemic and portal circulations – measurements that are related to the common pathophysiology of progression of all liver disease. Because the pathophysiology is similar across age, gender, and race or ethnicity, we do not anticipate any differences in the relationship of DSI cutoff to likelihood for large esophageal varices related to these variables.

There could be differences in the relationship of DSI cutoff to likelihood for large esophageal varices related to the etiology of liver disease. Liver diseases, especially at earliest stages differ in the lobular target or locus of the necroinflammatory and fibrotic process, i.e., periportal (zone 1), sinusoidal or transitional (zone 2), or pericentral (zone 3). Although necro-inflammation overlaps between zones for all liver diseases, the primary target for cholestatic liver disease is zone 1, viral liver diseases zones 1 and 2, NASH zone 2, and alcohol zone 3. With progression to end-stage decompensated cirrhosis these distinctions disappear.

Performance of the DSI test (NLR, FPR, sensitivity and specificity) will be reported separately in subgroups defined by age, sex, race, and disease etiology. These results will be presented as forest plots in order to identify any subgroups in which DSI test performance differs from the overall performance.

9.6.1.2 SECONDARY ENDPOINTS

Secondary efficacy endpoint (Probability and Calibration Curves): Regression analyses will be performed for DSI versus probability of large varices for individual etiologies of disease with over 50 subjects and for the categories of cholestatic and non-cholestatic liver diseases.

9.6.1.3 TABULATION OF INDIVIDUAL PARTICIPANT DATA

Individual participant data will not be listed.

9.6.2 EXPLORATORY ANALYSES

Exploratory 1 (AUROC of DSI for large esophageal varices): The c-statistic for the AUROC for DSI in likelihood of large esophageal varices will be compared to the c-statistic for the AUROC of LSM plus platelet count in likelihood of large esophageal varices. The most complete study with publication of AUROCs for LSM plus platelet count is from Abraldes, et al (8) where the AUROC was 0.76 for varices needing treatment (large varices or small varices with red signs). Given this benchmark the performance goal is an AUROC ≥0.76 for DSI in likelihood of large esophageal varices.

In addition, we will examine the diagnostic performance (sensitivity, specificity, PPV, NPV, NLR, PLR, FNR, miss rate[8]) of DSI over the full range of DSI to define the Optimal DSI for "Rule Out" – highest sensitivity and NPV with lowest FNR. We will also compare the diagnostic performance of this "optimum" DSI to DSI 18.3. The purpose of this analysis is to provide upside and downside performance of DSI around the validated cutoff of DSI 18.3.

This analysis will yield a table of sensitivities, specificities, NPVs, PPVs, NLRs, PLRs, FNRs, and miss rates[8] over the given range of DSI that could be included in a package insert to help the clinician interpret a given DSI result.

AUROCs of DSI in likelihood of large varices will be constructed for individual etiologies of disease with over 100 subjects and for the categories of cholestatic and non-cholestatic liver diseases.

Exploratory 2 (DSI for Small or Any Size of Esophageal Varices). The analyses listed above for primary objective, secondary objective 1, and secondary objective 2 will be repeated using small varices in one set of analyses and any size of varices in another set of analyses.

Exploratory 3 (STAT). The serum concentration of d4-cholate from the 60-minute time point (STAT) of the HepQuant SHUNT test correlates well with DSI (r^2=0.88). The STAT value represents a simplified, single point version of DSI. For this reason, we propose to evaluate the diagnostic performance of a cutoff for STAT defined from the HALT-C Training dataset for large esophageal varices and esophageal varices of any size.

The NLR performance goals for the STAT cutoff applied to the CLD Validation dataset are identical to the goals explained above for DSI: that the upper limit of the 95% Confidence Interval for NLR rules out NLR > 0.52. The sample size considerations with DSI for the primary efficacy endpoint and secondary efficacy endpoint 1 are also applicable STAT – the same limits for sensitivity and specificity. We will include age, gender, race, and ethnicity in multi-variable logistic regression of the association of STAT with large esophageal varices.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

[8] In this protocol, FNR is defined as 1-NPV and miss rate is defined as 1-Sensitivity. See the footnote in Section 1.1 for a discussion of these two measures.

10.1 Regulatory, Ethical, and Study Oversight Considerations

10.1.1 INFORMED CONSENT PROCESS

In obtaining and documenting informed consent, the investigator must comply with applicable regulatory requirements (e.g., 45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56). Prior to the beginning of the trial, the investigator should have the IRB's written approval for the protocol and the written informed consent form(s) and any other written information to be provided to the participants.

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study intervention. The ICF for this study is in **Appendix F**.

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

The process of Informed Consent will be initiated prior to the individual's agreeing to participate in the study and will continue throughout the individual's study participation. Consent forms will be Institutional Review Board (IRB)-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise.

A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate.

The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records.

The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to the study participants, investigators, Investigational Device Exemption (IDE) Sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping

- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or Food and Drug Administration (FDA).

10.1.2.1 CRITERIA FOR WHEN AN INDIVIDUAL WIILL STOP THEIR PARTICIPATION

Any patient with a hypersensitivity reaction to albumin or any component of the HepQuant SHUNT kit will be withdrawn from the study and will not undergo any further testing with the HepQuant SHUNT Kit.

10.1.2.2 CRITERIA FOR WHEN THE WHOLE HEPQUANT STUDY WILL BE STOPPED

The whole study will be stopped if 2 or more device related unanticipated moderate to severe adverse events occur or a device related serious adverse event such as a death occurs, or If a determination of futility is made at the interim analysis described in Section 9.5.1.

10.1.3 CONFIDENTIALITY AND PRIVACY

Participants in this study, the study doctor and his/her study team will look at the personal health information and collect only the information needed for the study. Personal health information is any information that could be used to identify the subject and includes his or her: name, address, date of birth, new or existing medical records; or types, dates, and results of medical tests or procedures.

As part of this consent procedure the subject will be asked to read and confirm their agreement to the Health Insurance Portability and Accountability Act (HIPAA), which is designed to protect the confidentiality of personal health information.

The information that is collected for the study will be safeguarded in line with accepted industry standards. Only the study team or the people or groups listed below will be allowed to look at the records. The subject's participation in this study may also be recorded in the medical record at the study site.

The following people or entities may be granted access to study records and personal health information to check that the information collected for the study is correct and to make sure the study followed proper laws and guidelines: HepQuant (the Sponsor), or its partner companies or representatives, representatives of [Institution] or its Institutional Review Board (IRB), or representatives of the FDA or other regulatory authorities.

All information collected during this study, including personal health information, will be kept confidential and will not be released to anyone outside the study unless required by law. Any information about the subject that is sent out of the clinic will have a code (Subject ID number – constructed as protocol number, site number, and subject-assigned ID number, e.g., 1801-00023-001) and will not show name or address, or any information that directly identifies the subject.

The subject will not be named in any reports, publications, or presentations that may come from this study. The Sponsor may use the study information (subject data) and share it with national and international regulatory agencies to get approval to sell their product, to engage in research or develop other studies, or for research related to this study.

De-identified personal information about the subject may be transferred to third parties for processing in the conduct of the study or as otherwise set forth in the Informed Consent Form.

The subject has the right to obtain updated information about what data is recorded as well as the right to request corrections of errors according to applicable laws and procedures. A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by the U.S. Law.

If the subject decides to leave the study, the information that was collected prior to the subject leaving the study will still be used. No new information will be collected without the permission of the subject, except to follow up on safety events that might have happened as part of the study.

The subject can cancel their permission at any time. It must be done in writing to the study doctor at the address on the front of the consent form.

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

Data collected for this study will be analyzed and stored electronically within HepQuant's secure electronic storage environment which is CFR Part 11 compliant. After the study is completed, the de-identified, archived data will be transmitted to and stored in the same location

With the participant's approval and as approved by local Institutional Review Boards (IRBs), de-identified biological samples will be stored at the HepQuant laboratory. These samples could be used to research other applications or modifications to the HepQuant technology or applications related to chronic liver disease, its complications and treatment. The HepQuant laboratory will also be provided with a code-link that will allow linking the biological specimens with other data from each participant, maintaining the blinding of the identity of the participant.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to biosample storage may not be possible after the study is completed.

When the study is completed, access to study data and/or samples will be provided through the HepQuant LLC.

10.2 Key Roles and Study Governance

TBD according to the listings below**.**

Principal Investigator	**Medical Monitor**
Name, degree, title	*Name, degree, title*
Institution Name	*Institution Name*
Address	*Address*
Phone Number	*Phone Number*
Email	*Email*

10.2.1 KEY COMMITTEES FOR THE SHUNT-V STUDY

- Executive Committee
    - Principal investigator - TBD
    - HepQuant CMO – Gregory T. Everson, MD
    - HepQuant Data management representative – Steve Helmke, PhD
    - HepQuant COO – Sean Bundy
- Steering Committee

  - Principal investigator - TBD
  - HepQuant CMO – Gregory T. Everson, MD
  - Study coordinator from each clinical site – Nominated by Site
  - Investigator from each clinical site – Nominated by Site
  - HepQuant Data management representative – Sean Bundy
- Publications Committee
  - HepQuant CMO – Gregory T. Everson, MD
  - HepQuant CSO – Steve Helmke PhD
  - HepQuant Data management representatives – Sean Bundy, Elyse Handley
  - HepQuant Biostatistician – John Kittelson, PhD
  - One investigator or sub-investigator from each clinical site – Nominated by Site
  - Principal investigator - TBD
- Sponsor's SHUNT-V Study Committee – focus on optimizing operations related to study
  - COO – Sean Bundy, Chair
  - CSO – Steve Helmke PhD
  - Manager of Clinical Accounts – Andrea Herman, RN
  - Recruitment Specialist – Elyse Handley
  - Supply Chain – Lisa Goggin
- Data, Safety and Monitoring Board
  - Medical Monitor and Safety Monitor for safety monitoring as described in section 10.2.2 below
  - An independent biostatistician will evaluate the results of the interim analysis without revealing blinded information to investigators or HepQuant

10.2.2 SAFETY OVERSIGHT

Per the FDA Guidance for Establishment and Operation of Clinical Trial Data Monitoring Committees, safety oversight will include a Safety Monitor with the appropriate expertise, including hepatology expertise, and expertise in clinical trials. The Safety Monitor will communicate closely with the CRO-appointed Medical Monitor for the study. The Safety Monitor will be independent from the study conduct and free of conflict of interest. The Safety Monitor will review all safety data from the study at the time points specified in the study protocol but no less than semiannually to assess safety data for the study. The Safety Monitor will operate under the rules of an approved charter that will be written and reviewed at the organizational meeting for the study. At this time, each data element that the Safety Monitor needs to assess will be clearly defined. The Safety Monitor will provide recommendations to HepQuant LLC.

The CRO for the study will be conducting the primary clinical site monitoring, including for complaints, adverse events, and site monitoring. The PI at each clinical site is responsible for monitoring the conduct of the trial at the clinical site and overseeing study coordinators and staff. The PI or his staff will contact HepQuant LLC, via the complaint line or email to: *steve.helmke@hepquant.com* or *andrea.herman@hepquant.com* for complaints related to the HepQuant product. Information received via these avenues, or others such as face-to-face or phone conversations, will be logged into the HepQuant complaint handling system and investigated accordingly.

HepQuant utilizes a complaint handling procedure specifically for monitoring activities associated with any use of the product. Complaint investigation and reporting occur as needed to close out the complaint. If necessary, the CAPA system is utilized to better understand and / or mitigate the problem associated with the complaint.

HepQuant includes the complaint phone number with all HepQuant labeling. HepQuant will monitor the study for safety concerns related to the device during the period of administration and while the patient is at the clinic. Any complaints received via the complaint phone number, or via any other method, will be investigated by HepQuant using the complaint handling SOP. Any complaints received by HepQuant regarding the Study via the complaint phone number, or via any other method, will be immediately communicated to appropriate regulatory bodies for inclusion in their records.

If a clinical site were to receive a complaint regarding the HepQuant product, for instance between study visits, the site's study staff will notify HepQuant directly (via email or phone). HepQuant will investigate complaints received by the site using the complaint handling procedure. The results of any HepQuant complaint investigation associated with the Study will be provided to regulatory bodies for inclusion in their records.

As the Sponsor of this Study and IDE, HepQuant will take on all IDE monitoring requirements set out in the FDA guidance "Oversight of clinical investigations – a risk-based approach to monitoring," August 2013. Monitoring is specific to HepQuant related events only, as identified by the principal investigator, study staff, and / or HepQuant employees.

10.2.3 CLINICAL SITE MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment, and with applicable regulatory requirement(s).

- Clinical Monitoring will include a combination of on-site and remote monitoring. Extent of source document verification will be clearly defined in the Clinical Monitoring Plan (CMP) after careful consideration and before monitoring begins. Considerations will include a focus on importance of the following data for all subjects: ICFs, inclusion/exclusion criteria, primary endpoints (endoscopy data) and safety (SAEs at a minimum)
- Final approved monitoring visit reports will be provided to the sponsor within 15 business days following the last date of the visit.
- Details of clinical site monitoring are documented in a Clinical Monitoring Plan (CMP). The CMP describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.

10.2.4 QUALITY ASSURANCE AND QUALITY CONTROL

Each clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol and applicable regulatory requirements.

The investigational sites will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

10.2.5 DATA HANDLING AND RECORD KEEPING

Each participating site will maintain appropriate medical and research records for this trial, in compliance with regulatory and institutional requirements for the protection of confidentiality of participants.

As part an FDA regulated study, each site will permit authorized representatives of the IDE Sponsor, and regulatory agencies to examine (and when permitted by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety, progress, and data validity.

The persons with access to these records will be listed in the site delegation log.

10.2.5.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into an EDC (TBD). The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

10.2.5.2 STUDY RECORDS RETENTION

Study documents will be retained by study clinical centers for a minimum of 2 years after the last approval of a marketing application and until there are no pending or contemplated marketing applications or until at least 2 years have elapsed since the formal discontinuation of clinical development of the HepQuant SHUNT Test. These documents will be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of HepQuant LLC, if applicable. It is the responsibility of HepQuant LLC to inform the investigator when these documents no longer need to be retained.

10.2.6 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol. The noncompliance may be either on the part of the participant, the investigator, or the study site staff.

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation, or within 1 working day of the scheduled protocol-required activity. All deviations must be addressed in study source documents, reported to IRB, Sponsor, and FDA Program Official and Safety Monitor, as appropriate. Protocol deviations must be sent to the reviewing IRB per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB requirements.

10.2.7 PUBLICATION AND DATA SHARING POLICY

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

- Publications via policies of the Publications Committee for the study
- Data sharing only as delineated by contracts or pre-specified agreements

This trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. Data from this study may be requested from other researchers many years after the completion of the primary endpoint by contacting HepQuant LLC.

10.2.8 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry or device company, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed.

Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the Sponsor, Participating Research Centers, and FDA has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

All study investigators, site staff, coordinators will sign a financial disclosure and conflict of interest form.

10.3 Additional Considerations

Additional Institutional Requirements: None

Additional IRB Requirements: FDA-issued Investigational Device Exemption (IDE)
This will be provided to each clinical site with the protocol

10.4 Abbreviations

β-hCG	β-human chorionic gonadotropin
ACE	Angiotensin Converting Enzyme
AE	Adverse event
AH	Alcoholic hepatitis
ALD	Alcoholic liver disease
ALT	Alanine aminotransferase (also SGPT)
ANC	Absolute neutrophil count
ANCOVA	Analysis of Covariance
ARB	Angiotensin Receptor Blocker
AST	Aspartate aminotransferase (also SGOT)
BLQ	Below the limit of quantitation
BMI	Body mass index
BW	Body weight
CFR	Code of Federal Regulations
CI	Confidence Interval
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CLD	Chronic liver disease
CP	Child-Pugh (for clinical/laboratory classification of cirrhosis)
CrCL	Creatinine clearance
CRF	Case Report Form
Cr	Serum creatinine
CRF	Case report form(s)
CT	Computed Tomography
cv	Critical value
CYP	Cytochrome P450
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
dL	Deciliter
DNA	Deoxyribonucleic acid
DSI	Disease Severity Index (from HepQuant SHUNT test)
DSMB	Data Safety Monitoring Board
DRE	Disease-Related Event
DSPH	Drug Safety and Public Health
EC	Ethics Committee
ECG	Electrocardiogram
eCRF	Electronic case report form(s)
EGD	Esophagogastroduodenoscopy (endoscopy)
EOT	End of Treatment
EU	European Union
FDA	(United States) Food and Drug Administration
FFR	Federal Financial Report
GCP	Good Clinical Practice
GGT	Gamma glutamyl transferase
GI	Gastrointestinal
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices

GT	Genotype (viral)
GWAS	Genome-Wide Association Studies
HBcAb	Hepatitis B core antibody
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HCV Ab	Hepatitis C virus antibody
HFR	Hepatic Filtration Rate
Hgb	Hemoglobin
HgbA1c	Hemoglobin A1c
HIPAA	Health Insurance Portability and Accountability Act
HREV	High Risk Esophageal Varices
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ISO	International Organization for Standardization
IB	IB Investigator Brochure
IEC	IEC independent ethics committee
IFU	IFU Instructions for Use
IMP	IMP Investigational Medicinal Product
IND	IND Investigational New Drug (Application)
INR	INR International Normalized Ratio of prothrombin time
IRB	IRB institutional review board
ITT	Intention-To-Treat
ISHAK	Fibrosis staging system for liver histology
IUD	IUD intrauterine device
IU	IU international units
IV	IV Intravenous (intravenously administered)
IWRS	IWRS interactive web response system
Kg	Kilogram
kPa	KiloPascals (units of measurement of liver stiffness)
L	Liter
LLN	Lower limit of the normal range
LLOQ	Lower limit of quantification
LSM	Liver Stiffness Measurement
LSMEANS	Least-squares Means
MedDRA	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
MSDS	Material Safety Data Sheet
MCV	Mean corpuscular volume or mean cell volume
MedDRA	Medical Dictionary for Regulatory Activities
METAVIR	Fibrosis staging system in liver histology
µM	Micromolar
µg	Microgram
mg	Milligram
mm	Millimeter

mM	Millimolar
MH	Mantel-Haenszel
mL	Milliliter
min	Minute
mmHg	Millimeters mercury (as a measurement of pressure)
NAFLD	NAFLD Non-Alcoholic Fatty Liver Disease
NASH	Non-Alcoholic Steatohepatitis
NASH-CRN	NASH Clinical Research Network; also a system for staging liver biopsy
NCT	National Clinical Trial
NIH	National Institutes of Health
NLR	Negative Likelihood Ratio
NPV	Negative Predictive Value
OHRP	Office for Human Research Protections
PBC	Primary biliary cholangitis
P-gp	P-glycoprotein
PH	Portal hypertension
PI	Principal Investigator
PO	By mouth (orally administered)
PK	Pharmacokinetic
PLR	Positive Likelihood Ratio
PPV	Positive Predictive Value
PSC	Primary sclerosing cholangitis
PT	Prothrombin time
QA	Quality Assurance
QD	Once daily (use only in tablets)
QC	Quality Control
RBC	Red blood cell count
RBV	Ribavirin
RNA	Ribonucleic acid
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard deviation
SMC	Safety Monitoring Committee
SMV	Olysio/Simeprevir
SOA	Schedule of Activities
SOC	System Organ Class
SOF	Sovaldi/Sofosbuvir
SOP	Standard operating procedure
STAT	60 minute d4-cholate sample reading obtained from the SHUNT raw data
SUSAR	Suspected Unexpected Serious Adverse Reaction
SVR	Sustained Virologic Response
UP	Unanticipated Problem
US	Ultrasonography or ultrasound
USA	United States
VNT	Varices needing treatment
WBC	White blood cell count

11 REFERENCES

1. Sanyal AJ, Bosch J, Blei A, Arroyo V. Portal hypertension and its complications. Gastroenterology 2008;134-1715-1728.
2. Garcia-Tsao G, Sanyal AJ, Grace ND, Carey W and the Practice Guidelines Committee of the American Association for the Study of Liver Diseases, the Practice Parameters Committee of the American College of Gastroenterology. Prevention and Management of Gastroesophageal Varices and Variceal Hemorrhage in Cirrhosis. Hepatology 2007;46:922-938.

3. de Franchis R on behalf of the Baveno VI Faculty. Expanding consensus in portal hypertension Report of the Baveno VI Consensus Workshop: Stratifying risk and individualizing care for portal hypertension. J Hepatology 2015;63:743–752.
4. Pateu E, Oberti F, Cales P. The noninvasive diagnosis of esophageal varices and its application in clinical practice. Clinics and Research in Hepatology and Gastroenterology 2018;42:6-16.
5. Jangouk P, Turco L, De Oliviera A, Schepis F, Villa E, Garcia-Tsao G. Validating, deconstructing, and refining Baveno criteria for ruling out high-risk varices in patients with compensated cirrhosis. Liver Int 2017;37;1177-1183.
6. Marot A, Trepo E, Doerig C, Schoepfer A, Moreno C, Deltenre P. Liver Stiffness and platelet count for identifying patients with compensated liver disease at low risk of variceal bleeding. Liver Int 2017;37:707-716.
7. Chang PEJ, Cheah CCM, Li WJ, et al. Real-world validation of Baveno VI recommendations for screening endoscopy in patients with compensated advanced chronic liver disease. J Hepatol 2016;64:S178.
8. Abraldes JG, Bureau C, Stefanescu H, et al. Non-invasive tools and risk of clinically significant portal hypertension and varices in compensated cirrhosis: the "Anticipate" study. Hepatology 2016;64:2173–2184.
9. Ahmed OF, Forrest EH, Stanley AJ, et al. The utility of Fibroscan and platelet count to identify patients who can omit surveillance endoscopy: validation of the Baveno guidelines in a Scottish cohort. J Hepatol 2016;64:S735.
10. Puigvehi M, Londono MC, Morillas RM, et al. Platelet count and liver stiffness measurement to exclude high-risk gastroesophageal varices in patients with HCV-cirrhosis. A multicentric study. J Hepatol 2016;64:S273.
11. Thabut D, Bureau C, Layese R, et al. New recommendations of Baveno VI conference for the screening of portal hypertension: an independent sequential validation in patients with compensated viral cirrhosis taking into account virological status (ANRS CO12 CIRVIR cohort). J Hepatol 2016;64:S178.
12. Asrani SK, Kamath PS. Natural History of Cirrhosis. Curr Gastroenterol Rep 2013;15:308-314.
13. Gines P, Graupera I, Lammert F, Angeli P, Caballeria L, Krag A, Guha IN, Murad SD, Castera L. Screening for liver fibrosis in the general population: a call for action. Lancet Gastroenterol Hepatol 2016;1:256-260.
14. Poynard T, Peta V, Pollo-Flores P, Munteanu M, Ratzui V. Screening studies of transient elastography and FibroTest in the general population. Lancet Gastroenterol Hepatol 2017;2:246.
15. Gines P, Graupera I, Lammert F, Angeli P, Caballeria L, Krag A, Guha IN, Murad SD, Castera L. Screening studies of transient elastography and FibroTest in the general population – Author's reply. Lancet Gastroenterol Hepatol 2017;2:246-247.
16. Caballería L, Pera G, Arteaga I, Rodríguez L, Alumà A, Morillas RM, de la Ossa N, Díaz A, Expósito C, Miranda D, Sánchez C, Prats RM, Urquizu M, Salgado A, Alemany M, Martinez A, Majeed I, Fabrellas N, Graupera I, Planas R, Ojanguren I, Serra M, Torán P, Caballería J, Ginès P. High Prevalence of Liver Fibrosis Among European Adults With Unknown Liver Disease: A Population-Based Study. Clin Gastroenterol Hepatol 2018; Feb 13. pii: S1542-3565(18)30006-5. doi: 10.1016/j.cgh.2017.12.048. [Epub ahead of print].
17. Sanyal AJ, Fontana RJ, Di Bisceglie AM, Everhart JE, Doherty MC, Everson GT, Donovan JA, Malet PF, Mehta S, Sheikh MY, Reid AE, Ghany MG, Gretch DR, and the HALT-C Trial Group. The prevalence and risk factors associated with esophageal varices in subjects with hepatitis C and advanced fibrosis. Gastrointest Endosc 2006;64:855-864.
18. Iwakiri Y, Shah V, Rockey DC. Vascular pathobiology in chronic liver disease and cirrhosis - current status and future directions. J Hepatol 2014;61:912-24.
19. Rockey, DC, Caldwell, SH, Goodman, ZD, Nelson, RC and Smith, AD. Liver biopsy. Hepatology 2009;49: 1017-1044.
20. Seeff LB, Everson GT, Morgan TR, Curto TM, Lee WM, Ghany MG, Shiffman ML, Fontana RJ, DiBisceglie AM, Bonkovsky HL, Dienstag JL, and the HALT-C Trial Group. Complication Rate of Percutaneous Liver Biopsies Among Persons With Advanced Chronic Liver Disease in the HALT-C Trial. Clin Gastroenterol Hepatol 2010;8:877-883.
21. Caldwell S, Northup PG. Bleeding Complication With Liver Biopsy: Is It Predictable? Clin Gastroenterol Hepatol 2010;8:826-829.
22. Wang J, Li J, Zhou Q, Zhang D, Bi Q, Wu Y, Huang W. Liver Stiffness Measurement Predicted Liver-Related Events and All-Cause Mortality: A Systematic Review and Nonlinear Dose-Response Meta-Analysis. Hepatology Comm 2018;2:467-476.
23. Nguyen D, Talwalkar JA. Noninvasive Assessment of Liver Fibrosis. Hepatology 2011;53:2107-2110.
24. Trautwein C, Friedman SL, Schuppan D, Pinzani M. Hepatic Fibrosis: Concept to Treatment. J Hepatol 2015;62:S15-S24.
25. Torok N, Dranoff JA, Schuppan D, Friedman SL. Strategies and Endpoints of Antifibrotic Drug Trials. Hepatology 2015;62:627-634
26. Chalasani Naga, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, Harrison SA, Brunt EM, Sanyal AJ. The Diagnosis and Management of Nonalcoholic Fatty Liver Disease: Practice Guidelines from the American Association for the Study of Liver Diseases. Hepatology 2018;67:328-357.
27. European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD), and European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. J Hepatol 2016;64:1388-1402.
28. Maurice JB, Brodkin E, Arnold F, Navaratnam A, Paine H, Khawar S, Dhar A, Patch D, O'Beirne J, Mookerjee R, Pinzani M, Tsochatzis E, Westbrook RH. Validation of the Baveno VI criteria to identify low risk cirrhotic patients not requiring endoscopic surveillance for varices. J Hepatology 2016;65:899–905.
29. Abraldes JG, Bureau C, Stefanescu H, Augustin S, Ney M, Blasco H, Procopet B, Bosch J, Genesca J, Berzigotti A for the Anticipate Investigators. Noninvasive Tools and Risk of Clinically Significant Portal Hypertension and Varices in Compensated Cirrhosis: The "Anticipate" Study. Hepatology 2016;64:2173-2184.
30. Augustin S, Pons M, Maurice JB, Bureau C, Stefanescu H, Ney M, Blasco H, Procopet B, Tsochatzis E, Westbrook RH, Bosch J, Berzigotti A, Abraldes JG, Genesca J. Expanding the Baveno VI Criteria for the Screening of Varices in Patients with Compensated Advanced Chronic Liver Disease. Hepatology 2017;66:1980-1988.
31. Bureau C, Metivier S, Peron JM, Selves J, Robic MA, Gourrauds PA, Rouquet O, Dupuis E, Alric L, Vinel JP. Transient elastography accurately predicts presence of significant portal hypertension in patients with chronic liver disease. Aliment Pharmacol Ther 2008;27:1261-1268.
32. Vizzutti F, Arena U, Romanelli RG, Rega L, Foschi M, Colagrande S, Petrarca A, Moscarella S, Belli G, Zignego AL, Marra F, Laffi G, Pinzani M. Liver Stiffness Measurement Predicts Severe Portal Hypertension in Patients with HCV-Related Cirrhosis. Hepatology 2007;45:1290-1297.

33. Castera L, Le Bail B, Roudot-Thoraval F, Bernard P-H, Foucher J, Merrouche W, Couzigou P, de Ledinghem V. Early detection in routine clinical practice of cirrhosis and oesophageal varices in chronic hepatitis C: Comparison of transient elastography (FibroScan) with standard laboratory tests and non-invasive scores. J Hepatol 2009;50:59-68.
34. Qamar AA, Grace ND, Groszmann RJ, Garcia-Tsao G, Bosch J, Burroughs AK, Maurer R, Planas R, Escorell A, Garcia-Pagan J-C, Patch D, Matloff DS, Makuch R, for the Portal Hypertension Collaborative Group. Platelet Count is not a Predictor of the Presence or Development of Gastroesophageal Varices in Cirrhosis. Hepatology 2008;47:153-159.
35. Berzigotti A, Gilabert R, Abraldes JG, Nicolau C, Bru C, Bosch J, Garcia-Pagan J-C. Noninvasive Prediction of Clinically Significant Portal Hypertension and Esophageal Varices in Patients with Compensated Liver Cirrhosis. Am J Gastroenterol 2008;103:1159-1167.
36. D'Amico G, Morabito A. Noninvasive Markers of Esophageal Varices: Another Round, Not the Last. Hepatology 2004;39:30-34.
37. Giannini EG, Savarino V. Noninvasive Assessment of Varices Needing Treatment in Patients With Advanced Chronic Liver Disease: No One Should Be Left Behind. Hepatology 2017;65:2131-2132.
38. Montes-Ramirez ML, Pascual-Pareja JF, Sanchez-Conde M, de la Serna JIB, Vargas FXZ, Miralles P, Castro JM, Ramirez M, Gutierrez I, Gonzalez-Garcia J, Berenguer J, Arribas-Lopez JR. Transient Elastography to rule out esophageal varices and portal hypertensive gastropathy in HIV-infected individuals with liver cirrhosis. AIDS 2012;26:1807-1812.
39. Bosch J, Abraldes JG, Berzigotti A, García-Pagan JC. The clinical use of HVPG measurements in chronic liver disease. Nat Rev Gastroenterol Hepatol 2009;6:573–582.
40. Helmke S, Colmenero J, Everson GT. Noninvasive assessment of liver function. Curr Opin Gastroenterol 2015;31:199-208.
41. Helmke SM, De Santo JL, Herman A, Lauriski S, Everson GT. (2013) A Disease Severity Index based on Dual Cholate Clearances and Shunt Outperforms Biopsy at Predicting Clinical Outcomes in Chronic Hepatitis C. Gastroenterology 144: (5):S951-952.
42. Helmke SM, De Santo J, Herman A, et al. Dual Cholate Liver Function Testing is Better than Fibrosis Stage at Identifying Chronic HCV Patients with Medium/Large Varices and Predicting Future Clinical Outcomes. Hepatology 2013; 58 (S1):1000A.
43. Helmke SM, Wallack A, Herman A, Lauriski S, Everson GT. A Disease Severity Index Based on Dual Cholate Clearances and Shunt Identifies Primary Sclerosing Cholangitis Waiting List Patients at Risk for Clinical Complications. Am J Transplant 2013; 13(S5):513.
44. Helmke SM, Wallack A, Herman A, Isberg H, Brocato M, Bhatt A, Lauriski S, Everson GT. Cholate Testing is Superior to MELD in Assessing Disease Severity in Patients with Primary Sclerosing Cholangitis. Liver Transplantation 2013; 19:S97-98.
45. Helmke SM, Wallack A, Herman A, Isberg H, Brocato M, Bhatt A, Lauriski S, Everson GT. Serial Cholate Testing is Better than ΔMELD in Assessing Disease Progression in Waiting List Patients with Primary Sclerosing Cholangitis. Liver Transplantation 2013; 19:S294.
46. Helmke SM, Marr JD, Cookson MW, DeSanto J, Lauriski S, Trotter JF, Everson GT. Diagnosing NASH and Assessing NASH Disease Severity by a Global Measure of Liver Function, the HepQuant® (HQ)-SHUNT Test. Hepatology 2016; 64 (S1):584A.
47. Helmke SM, DeSanto J, Lauriski S, Everson GT. A New Simple Quantitative Liver Function Test, HepQuant-STAT, Correlates with all Chronic HCV Ishak Fibrosis Stages, but Outperforms Fibrosis at Predicting the Risk of Future Clinical Outcomes. Hepatology 2015; 62(S1): 1111A.
48. Helmke SM, Marr JD, Lauriski S, Cookson M, Desanto J, Trotter JF, Everson GT. Diagnosing NASH and Assessing NASH Disease Severity with HepQuant-STAT, a Simple Quantitative Liver Function Test. Hepatology 2017; 66 (S1):56A.
49. Helmke SM, DeSanto J, Lauriski S, Wieland A, Everson GT. Portal-systemic Shunt Fraction measured by the HepQuant-SHUNT Test Correlates with the Hepatic Venous Pressure Gradient (HVPG). Hepatology 2017; 66 (S1):348A.
50. Etzion O, Ali RO, Koh C, Zhang GY, Townsend EC, Koh C, Levy E, Takyar V, Takyar V, Han MAT, Lingala S, Fryzek N, Haynes-Williams V, Kleiner DE, Everson G, Helmke S, Heller T. Liver Functional Assessment by Dual Cholate Testing Is Strongly Correlated with Direct Portal Pressure and Ishak Fibrosis Stage. Hepatology 2017; 66 (S1):1036A.
51. Fazel Y, Koeniga AB, Sayiner M, Goodman ZD, Younossi ZM. Epidemiology and natural history of non-alcoholic fatty liver disease. Metabolism 2016;65:1017-1025.
52. Harrison S. NASH, From Diagnosis to Treatment: Where Do We Stand? Hepatology 2015;62:1652-1655.
53. Byrne CD, Targher G. NAFLD: A multisystem disease. J Hepatol 2015;62:S47–S64.
54. Rich NE, Oji S, Mufti AR, Browning JD, Parikh ND, Odewole M, Mayo H, Singal AG. Racial and Ethnic Disparities in Nonalcoholic Fatty Liver Disease Prevalence, Severity, and Outcomes in the United States: A Systematic Review and Meta-analysis. Clin Gastroenterol Hepatol 2018;16:198-210.
55. Scaglione S, Kliethermes S, Cao G, Shoham D, Durazo R, Luke A, et al. The epidemiology of cirrhosis in the United States: a population-based study. J Clin Gastroenterol 2015;49:690-696.
56. Allen AM, Kim WR, Moriarity JP, Shah ND, Larson JJ, Kamath PS. Time Trends in the Health Care Burden and Mortality of Acute on Chronic Liver Failure in the United States. Hepatology 2016;64:2165-2172.
57. Frebourg T, Delpech B, Bercoff E, Senant J, Bertrand P, Deugnier Y, Bourreille J. Serum hyaluronate in liver diseases: Study by enzymoimmunological assay. Hepatology 1986;6:392-395.
58. Shaheen AA, Wan AF, Myers RP. Fibrotest and fibroscan for the prediction of hepatitis c-related fibrosis: A systematic review of diagnostic test accuracy. Am J Gastroenterol 2007;102:2589-2600.
59. Tripodi A, Caldwell SH, Hoffman M, Trotter JF, Sanyal AJ. Review article: The prothrombin time test as a measure of bleeding risk and prognosis in liver disease. Aliment Pharmacol Ther 2007;26: 141-148.
60. Castera L. Assessing Liver Fibrosis. Expert Rev Gastroenterol Hepatol 2008;2:541-552.
61. Farrell GC, Wong VW, Chitturi S. NAFLD in Asia – as common and important as in the West. Nat Rev Gastroenterol Hepatol 2013;10:307-318.
62. National Center for Health Statistics. Health, United States, 2016: With Chartbook on Long-term Trends in Health. Hyattsville, MD. 2017.
63. Groszmann RJ, Garcia-Tsao G, Bosch J, Grace ND, Burroughs AK, Planas R, Escorsell A, Garcia-Pagan JC, Patch D, Matloff DS, Gao H, Makuch R, for the Portal Hypertension Collaborative Group. Beta-Blockers to Prevent Gastroesophageal Varices in Patients with Cirrhosis. N Engl J Med 2005;353:2254-2261.
64. Merli M, Nicolini G, Angeloni G, Rinaldi V, De Santis A, Merkel C, Attili AF, Riggio O. Incidence and natural history of small esophageal varices in cirrhotic patients. J Hepatol 2003;38:266-272.

65. Qi X-S, Bao Y-X, Bai M, Xu W-D, Dai J-N, Guo X-Z. Nonselective beta-blockers in cirrhotic patients with no or small varices: A meta-analysis. World J Gastroenterol 2015;21:3100-3108.
66. Sarin SK, Mishra SR, Sharma P, Sharma BC, Kumar A. Early primary prophylaxis with beta-blockers does not prevent the growth of small esophageal varices in cirrhosis: a randomized controlled trial. Hepatol Int 2013;7:248-256.
67. Merkel C, Marin R, Angeli P, Zanella P, Felder M, Bernardinello E, Cavallarin G, Bolognesi M, Donada C, Bellini B, Torboli P, Gatta A, and the Gruppo Triveneto per L'Ipertensione Portale. A Placebo-Controlled Clinical Trial of Nadolol in the Prophylaxis of Growth of Small Esophageal Varices in Cirrhosis. Gastroenterology 2004;127:476-484.
68. Cales P, Oberti F, Payen JL, Naveau S, Guyader D, Blanc P, Abergel A, Bichard P Raymond JM, Canva-Delcambre V, Vetter D, Valla D, Beauchant M, Hadenque A, Champigneulle B, Pascal JP, Poynard T, Lebrec D. Lack of effect of propranolol in the prevention of large oesophageal varices in patients with cirrhosis: a randomized trial. French-Speaking for the Study of Portal Hypertension. Eur J Gastroenterol Hepatol 1999;11:741-745.
69. D'Amico G, Pagliaro L, Bosch J. Pharmacological Treatment of Portal Hypertension: An Evidence-Based Approach. Seminars in Liver Disease 1999;19:475-505.
70. North Italian Endoscopic Club for the Study and Treatment of Esophageal Varices. Prediction of the first variceal hemorrhage in patients with cirrhosis of the liver and esophageal varices. A prospective multicenter study. N Engl J Med 1988;319:983-989.
71. deFranchis R, Pascal JP, Burroughs AK, Henderson JM, Fleig W, Groszmann RJ, et al. Definitions, methodology, and therapeutic strategies in portal hypertension. A consensus development workshop. J Hepatol 1992;15:256-261.
72. Merkel C, Zoli M, Siringo S, van Buuren H, Magalotti D, Angeli P, Sacerdoti D, Bolondi L, Gatta A. Prognostic Indicators of Risk for First Variceal Bleeding in Cirrhosis: A Multicenter Study in 711 Patients to Validate and Improve the North Italian Endoscopic Club (NIEC) Index. Am J Gastroenterol 2000;95:2915-2920.
73. Garcia-Tsao G, Sanyal AJ, Grace ND, Carey W, and Practice Guidelines Committee of the American Association or the Study of Liver Diseases, The Practice Parameters Committee of the American College of Gastroenterology. Prevention and Management of Gastroesophageal Varices and Variceal Hemorrhage in Cirrhosis. Hepatology 2007;46:922-938.
74. Abougergi MS, Travis AC, Saltzman JR. The in-hospital mortality rate for upper GI hemorrhage has decreased over 2 decades in the United States: a nationwide analysis. Gastrointestinal Endoscopy 2015;81:882-888.
75. Viviane A, Alan BN. Estimates of Costs of Hospital Stay for Variceal and Non-variceal Upper Gastrointestinal Bleeding in the United States. ISPOR 2008;11:1-3.
76. Chirapongsthorn S, Krittanawong C, Enders FT, Pendegraft R, Mara KC, Borah BJ, Visscher SL, Loftus CG, Shah VJ, Talwalker JA, Kamath PS. Incidence and Cost Analysis of Hospital Admission and 30-Day Readmission Among Patients with Cirrhosis. Hepatology Communications 2018;2:188-198.
77. Flemming JA, Saxena V, Shen H, Terrault NA, Rongey C. Facility- and Patient-Level Factors Associated with Esophageal Variceal Screening in the USA. Dig Dis Sci 2016;61:62–69.
78. Samonakis, DN, Cholongitas, E, Thalheimer, U, Kalambokis, G, Quaglia, A, Triantos, CK, Mela, M, Manousou, P, Senzolo, M, Dhillon, AP, Patch, D and Burroughs, AK. 2007. Hepatic venous pressure gradient to assess fibrosis and its progression after liver transplantation for hcv cirrhosis. Liver Transpl 2007; 13: 1305-1311.
79. Rincon, D, Ripoll, C, Lo Iacono, O, Salcedo, M, Catalina, MV, Alvarez, E, Nunez, O, Matilla, AM, Clemente, G and Banares, R. 2006. Antiviral therapy decreases hepatic venous pressure gradient in subjects with chronic hepatitis c and advanced fibrosis. Am J Gastroenterology 2006; 101: 2269-2274.
80. Ripoll, C, Groszmann, R, Garcia-Tsao, G, Grace, N, Burroughs, A, Planas, R, Escorsell, A, Garcia-Pagan, JC, Makuch, R, Patch, D, Matloff, DS and Bosch, J. 2007. Hepatic venous pressure gradient predicts clinical decompensation in subjects with compensated cirrhosis. Gastroenterology 2007; 133: 481-488.
81. Lens S, Alvarado-Tapias E, Mariño Z, Londoño M-C, LLop E, Martinez J, Fortea JI, Ibañez L, Ariza X, Baiges A, Gallego A, Bañares R, Puente A, Albillos A, Calleja JL, Torras X, Hernández-Gea V, Bosch J, Villanueva C, Forns X, García-Pagán JC. Effects of All-Oral Anti-Viral Therapy on HVPG and Systemic Hemodynamics in Patients With Hepatitis C Virus-Associated Cirrhosis. Gastroenterology 2017;153:1273–1283.
82. Afdhal N, Everson GT, Calleja JL, McCaughan GW, Bosch J, Brainard DM, McHutchison JG, De-Oertel S, An D, Charlton M, Reddy KR, Asselah T, Gane E, Curry MP, Forns X. Effect of viral suppression on hepatic venous pressure gradient in hepatitis C with cirrhosis and portal hypertension. J Viral Hepat 2017;24:823–831.
83. Everson, GT, Martucci, MA, Shiffman, ML, Sterling, RK, Morgan, TR and Hoefs, JC. Portal-systemic shunting in subjects with fibrosis or cirrhosis due to chronic hepatitis c: The minimal model for measuring cholate clearances and shunt. Aliment Pharmacol Ther 2007;26: 401-410.
84. Everson, GT, Shiffman, ML, Morgan, TR, Hoefs, JC, Sterling, RK, Wagner, DA, Kulig, CC, Curto, TM and Wright, EC. The spectrum of hepatic functional impairment in compensated chronic hepatitis c: Results from the hepatitis c anti-viral long-term treatment against cirrhosis (HALT-C) trial. Aliment Pharmacol Ther 2008; 27:798-809.
85. Everson, GT, Shiffman, ML, Hoefs, JC, Morgan, TR, Sterling, RK, Wagner, DA, Desanto, JL, Curto, TM and Wright, EC. Quantitative tests of liver function measure hepatic improvement after sustained virological response: Results from the hepatitis c anti-viral long-term treatment against cirrhosis (HALT-C) trial. Aliment Pharmacol Ther 2009; 29: 589-601.
86. Everson, GT, Shiffman, ML, Hoefs JC, Morgan, TR, Sterling, RK, Wagner, D, Lauriski S, Curto, TM, Stoddard A, Wright, EC and The HALT-C Trial Group. Quantitative Liver Function Tests Improve the Prediction of Clinical Outcomes in Chronic Hepatitis C: Results from the Hepatitis C Antiviral Long-term Treatment Against Cirrhosis Trial. Hepatology 2012; 55:1019-1029.
87. Gonzales E, Gerhardt MF, Fabre M, Setchell KDR, Davit-Spraul A, Vincent I, Heubi JE, Bernard O, Jacquemin E. 2009. Oral Cholic Acid for Hereditary Defects of Primary Bile Acid Synthesis: A Safe and Effective Long-term Therapy. Gastroenterology 137:1310-1320.
88. Woollett LA, Buckley DD, Yao L, Jones PJH, Granholm NA, Tolley EA, Tso P, Heubi JE. Cholic Acid Supplementation Enhances Cholesterol Absorption in Humans. Gastroenterology 2004;126:724-731.
89. Ring J, Messmer K. Incidence and Severity of Anaphylactoid Reactions to Colloid Volume Substitutes. Lancet 1977 Feb 26;1 (8009):466-469.
90. Kasahara A, Kita K, Tomita E, Toyota J, Imai Y, Kumada H. Repeated administration of recombinant human serum albumin caused no serious allergic reactions in patients with liver cirrhosis: a multicenter clinical study. J Gastroenterol 2008;43:464-472.

91. Santoso S, Kiefel V, Volz H, Mueller-Eckhardt C. Quantification of Soluble HLA Class I Antigen in Human Albumin and Immunoglobulin Preparations for Intravenous Use by Solid-Phase Immunoassay. Vox Sang 1992;62:29-33.
92. Crowder LA, Schonberger LB, Dodd RY, Steele WR. Creutzfeldt-Jakob disease lookback study: 21 years of surveillance for transfusion transmission risk. Transfusion 2017;57;1875–1878.
93. Seed CR, Hewitt PE, Dodd RY, Houston F, Cervenakova L. Creutzfeldt-Jakob disease and blood transfusion safety. Vox Sanguinis (2018) 113, 220–231.
94. Everson, GT. 1993. Hepatic cysts in autosomal dominant polycystic kidney disease. Am J Kidney Dis. 22: 520-525.
95. Shrestha, R, McKinley, C, Showalter, R, Wilner, K, Marsano, L, Vivian, B and Everson, GT. 1997. Quantitative liver function tests define the functional severity of liver disease in early-stage cirrhosis. Liver Transpl Surg 1997; 3: 166-173.
96. Everson GT, Hoefs JC, Niemann CU, Olthoff KM, Dupuis R, Lauriski S, Herman A, Milne N, Gillespie BW, Goodrich NP, Everhart JE. Functional elements associated with hepatic regeneration in living donors after right hepatic lobectomy. Liver Transpl 2013; 19(3):292-304.
97. DeMark, BR, Everson, GT, Klein, PD, Showalter, RB and Kern, F, Jr. 1982. A method for the accurate measurement of isotope ratios of chenodeoxycholic and cholic acids in serum. J Lipid Res 1982; 23: 204-210.
98. Kern, F, Jr., Everson, GT, DeMark, B, McKinley, C, Showalter, R, Braverman, DZ, Szczepanik-Van Leeuwen, P and Klein, PD. Biliary lipids, bile acids, and gallbladder function in the human female: Effects of contraceptive steroids. J Lab Clin Med 1982; 99: 798-805.
99. Everson, GT. Steady-state kinetics of serum bile acids in healthy human subjects: Single and dual isotope techniques using stable isotopes and mass spectrometry. J Lipid Res 1987; 28: 238-252.
100. Everson, GT, Fennessey, P and Kern, F, Jr. Contraceptive steroids alter the steady-state kinetics of bile acids. J Lipid Res 1988; 29: 68-76.
101. Everson, GT, Daggy, BP, McKinley, C and Story, JA. Effects of psyllium hydrophilic mucilloid on ldl-cholesterol and bile acid synthesis in hypercholesterolemic men. J Lipid Res 1982; 33: 1183-1192.
102. Lee, WM, Dienstag, JL, Lindsay, KL, Lok, AS, Bonkovsky, HL, Shiffman, ML, Everson, GT, Di Bisceglie, AM, Morgan, TR, Ghany, MG, Morishima, C, Wright, EC and Everhart, JE. Evolution of the HALT-C trial: Pegylated interferon as maintenance therapy for chronic hepatitis c in previous interferon nonresponders. Control Clin Trials 2004; 25: 472-492.
103. Di Bisceglie, AM, Shiffman, ML, Everson, GT, Lindsay, KL, Everhart, JE, Wright, EC, Lee, WM, Lok, AS, Bonkovsky, HL, Morgan, TR, Ghany, MG, Morishima, C, Snow, KK and Dienstag, JL. Prolonged therapy of advanced chronic hepatitis C with low-dose peginterferon. N Engl J Med 2008; 359: 2429-2441.
104. Subuddhi U, Mishra AK. Micellization of bile salts in aqueous medium: a fluorescence study. Colloids Surf B Biointerfaces. 2007. 57:102-107.
105. Jojart B, Posa M, Fiser B, Szori M, Farkas Z, Viskolcz B. Mixed Micelles of Sodium Cholate and Sodium Dodecylsulphate 1:1 Binary Mixture at Different Temperatures – Experimental and Theoretical Investigations. PLoS ONE 9(7): e102114. Doi:10.1371/journal.pone.0102114.
106. Natalinia B, Sardellaa R, Gioiello A, Iannia F, Di Micheleb A, Marinozzia M. Determination of bile salt critical micellization concentration on the road to drug discovery. Journal of Pharmaceutical and Biomedical Analysis 2014;87:62-81.
107. Meyuhas D, Lichtenberg D. The effect of albumin on the state of aggregation and phase transformations in phosphatidylcholine-sodium cholate mixtures. Biochimica et Biophysica Acta 1995;1234:203-213.
108. Dawson PA, Karpen SJ. Intestinal transport and metabolism of bile acids. J Lipid Research 2015;56:1085-1099.
109. Schmid A, Neumann H, Karrasch T, Liebisch G, Schäffler A. Bile Acid Metabolome after an Oral Lipid Tolerance Test by Liquid Chromatography-Tandem Mass Spectrometry (LCMS/MS). PLoSONE 2016;11(2):e0148869.doi:10.1371/ journal.pone.0148869.)
110. Geier A, Wagner M, Dietrich CG, Trauner M. Principles of hepatic organic anion transporter regulation during cholestasis, inflammation, and liver regeneration. Biochimica et Biophysica Acta 2007;1773:283-308.
111. Baghdasaryan A, Chiba P, Trauner M. Clinical application of transcriptional activators of bile salt transporters. Molecular Aspects of Medicine 2014;37:57-76.
112. Csanaky IL, Lu H, Zhang Y, Ogura K, Choudhuri S, Klaasen CD. Organic Anion-Transporting Polypeptide 1b2 (Oatp1b2) is Important for the Hepatic Uptake of Unconjugated Bile Acids: Studies in Oatp1b2-Null Mice. Hepatology 2011;53:272-281.
113. Winkfield B, Aube C, Burtin P, Cales P. Inter-observer and intra-observer variability in hepatology. Eur J Gastroenterol Hepatol 2003;15:959-966.
114. Oberti F, Burtin P, Maiga M, Valsesia E, Pilette C, Cales P. Gastroesophageal endoscopic signs of cirrhosis: independent diagnostic accuracy, interassociation, and relationship to etiology and hepatic dysfunction. Gastrointest Endosc 1998;48:148-157.
115. Bendtsen F, Skovgaard LT, Sorensen TI, Matzen P. Agreement among multiple observers on endoscopic diagnosis of esophageal varices before bleeding. Hepatology 1990;11:341-347.
116. Cales P, Zabotto B, Meskens C, Caucanas JP, Vinel JP, Desmorat H, Fermanian J, Pascal JP. Gastroenterology 1990;98:156-162.
117. Emerson, S.S., Kittelson, J.M., Gillen, D.L. Frequentist evaluation of group sequential clinical trial designs. Statistics in Medicine, 2007, 26:5047-5080.
118. Marill, K. A., Chang, Y., Wong, K. F., & Friedman, A. B. (2015). Estimating Negative Likelihood Ratio Confidence When Test Sensitivity is 100%: A Bootstrapping Approach. Statistical Methods in Medical Research, 1-16. DOI:/10.1177/0962280215592907.

12 APPENDIX A INVESTIGATOR AGREEMENT

13 APPENDIX B INVESTIGATOR BROCHURE

14 APPENDIX C INSTRUCTIONS FOR USE

15 APPENDIX D LABELING

16 APPENDIX E MATERIAL DATA AND SAFETY SHEETS

17 APPENDIX F INFORMED CONSENT FORM

18 APPENDIX G SELF-REPORTED SUBJECT TOLERABILITY SURVEY