Correlation and Comparison of the HepQuant® Disease Severity Index (DSI) With Hepatic Venous Pressure Gradient (HVPG)

NCT02523196

Protocol Version: August 11, 2015

COMIRB Protocol
COLORADO MULTIPLE INSTITUTIONAL REVIEW BOARD
CAMPUS BOX F-490 TELEPHONE: 303-724-1055 Fax: 303-724-0990

Protocol #: 15-0520
Project Title: Correlation and comparison of the HepQuant® Disease Severity Index (DSI) with Hepatic Venous Pressure Gradient (HVPG)
Principal Investigator: Amanda Wieland, M.D.
Version Date: August 11th, 2015

I. Hypotheses and Specific Aims:

Hypothesis

The HepQuant® (HQ)-SHUNT test is an appropriate alternative to Hepatic Venous Pressure Gradient (HVPG) testing.

Specific Aims

HQ-SHUNT is safe, simple to administer, noninvasive, cost-effective, and well tolerated by patients. In order to prove that the HQ-SHUNT test is an appropriate alternative to measurement of HVPG we will conduct a head-to-head comparison of HQ-SHUNT to HVPG. In this study, 100 consecutive patients with various etiologies of liver disease who have undergone technically successful HVPG testing will subsequently undergo HQ-SHUNT testing.

Primary Aim 1: Create the ROC curve of the Disease Severity Index (DSI) from HQ-SHUNT for defining patients with (HVPG ≥6 mmHg) and without (HVPG <6 mmHg) portal hypertension. From the ROC, define the optimal cutoff of DSI and the sensitivity, specificity, and positive and negative predictive values.

Primary Aim 2: In patients with portal hypertension (HVPG ≥6 mmHg), define the optimal relationship of DSI to HVPG.

Exploratory Aims

Approximately 90% of patients undergoing HVPG will have liver biopsy performed as part of the procedure. In addition, most of the patients undergoing HVPG will have undergone or will undergo upper gastrointestinal endoscopy (EGD) to evaluate for varices and variceal treatment. This allows a head-to-head comparison of DSI from HQ-SHUNT with HVPG in identifying patients with cirrhosis and patients with varices. Finally, we will also compare the tolerability of both HVPG and HQ-SHUNT, since tolerability of procedures is critical for patient acceptance and the ability to perform serial testing.

Exploratory Aim 1: Compare the cutoffs of DSI 16.5 and HVPG 6 mmHg in identifying patients with cirrhosis.

Exploratory Aim 2: Compare the cutoffs of DSI 20 and HVPG 10 mm Hg in identifying patients with varices.

Exploratory Aim 3: Survey patients on their experience of having both HQ-SHUNT and HVPG procedures.

II. Background and Significance:

The Clinical Problem in Liver Disease Assessment and Liver Disease Drug Development

HQ-SHUNT, invented at CU by Dr. Gregory T. Everson, MD, fulfills an unmet need in liver drug development and in clinical trials of liver disease – a noninvasive, accurate, safe, easy-to-administer, and cost-effective liver function test [1]. In this study we will define the relationships of the Disease Severity Index (DSI) and other parameters from the HQ-SHUNT test to HVPG.

Hepatic Venous Pressure Gradient (HVPG) has been used in clinic trials, research studies, and clinical medicine to quantify liver disease severity and progression. HVPG >6mmHg signifies portal hypertension and HVPG >10 mmHg increases risk for ascites and variceal hemorrhage. Portal hypertension is a consequence of advanced liver disease and HVPG quantifies risk for clinical outcomes. Measuring HVPG is technically challenging, requires specialized radiologic expertise and equipment, not embraced by patients, and is risky and expensive. Despite the latter concerns, the FDA accepts HVPG as an endpoint in clinical trials of treatments for chronic liver disease. A noninvasive accurate alternative to HVPG is needed.

The HQ-SHUNT test is not FDA approved but over 1000 tests have been done in over 500 subjects or patients without any safety concerns. Outputs from the HQ-SHUNT test include clearances of intravenous (Systemic Hepatic Filtration Rate (HFR)) and oral (Portal HFR) cholate, SHUNT (ratio of Systemic HFR to Portal HFR), and Disease Severity Index (DSI). Healthy controls have DSI <10; DSI >20 is associated with risk for cirrhosis with varices. DSI outperformed liver biopsy and standard laboratory tests in predicting risk for future clinical outcomes. Like HVPG, DSI from HQ-SHUNT is heavily weighted to measure changes in the portal circulation – but, compared to HVPG, HQ-SHUNT is safe, noninvasive, simple to administer, well tolerated by patients, requires no specialized expertise or equipment, and is relatively inexpensive. HQ-SHUNT and DSI may be the noninvasive accurate alternative to HVPG.

Drug companies are investing billions of dollars in developing new anti-fibrotic drugs to treat the ~30 million Americans with liver fibrosis/cirrhosis, but these efforts are hindered because the best method to quantify liver disease progression and the efficacy of new drugs is HVPG, which is invasive, painful, difficult to perform, and very expensive (~$7000 - $9000/test). In contrast, HQ-SHUNT is noninvasive, safe, relatively painless, easy to administer, cost-effective, and can outperform HVPG, and would have great value by allowing the rapid and accurate evaluation of new liver drugs and therapies. Drug companies have expressed interest and supported some small pilot studies of HQ-SHUNT but have asked how well this technology compares to HVPG. The proposed project has been approved for funding with a Colorado Bioscience Discovery and Evaluation Grant awarded to Dr. Everson, and will be a proof-of-concept study to directly compare HQ-SHUNT testing to HVPG testing.

<u>Monitoring Disease Progression in Chronic Liver Disease</u>

Chronic liver disease is caused by alcohol, hepatic steatosis (fatty liver), autoimmune conditions, or viruses, and progresses through common pathological mechanisms (Fig. 1). Inflammation and necrosis lead to fibrosis/cirrhosis, which impedes blood flow causing portal hypertension. Portal blood then is shunted systemically via collateral vessels. All these processes alter the hepatic systemic and portal circulations which causes the serious clinical outcomes of liver disease. Under pressure, collateral vessels swell and some, like varices, bleed. Abdominal fluid accumulates (ascites). Toxins shunt systemically to cause mental impairment (encephalopathy). Biopsy semi-quantitatively assesses inflammation grade and fibrosis/cirrhosis stage, while HVPG provides a quantitative estimation of portal pressure. These painful, risky, difficult, expensive invasive tests can assess individual mechanisms but safe, easy, cost-effective HQ-SHUNT testing can quantify physiologic change over the entire spectrum of liver disease from the earliest to the latest stages. HVPG (Fig. 2A) involves sedating the patient and threading a probe under radiological guidance down their jugular vein to the liver where it is wedged in the hepatic vein to measure the estimated portal pressure. HQ-SHUNT (Fig. 2B), which was invented by Dr. Gregory T. Everson, MD, and licensed through the TTO from the University of Colorado to HepQuant, LLC (US Patent #8,613,904 issued on 12/24/2013 “Methods for Diagnosis and Intervention of Hepatic Disorders”) is much easier and more informative. The patient receives simultaneously an oral dose of d4-Cholate and an IV dose of ^{13}C-Cholate, which are stable isotopes; there is no radioactivity or radiation exposure. Five peripheral venous blood samples are obtained over 1 ½ hours and the patient returns to normal activities, while the blood samples are quantified by LCMS validated to strict FDA guidelines for accuracy,

Fig. 1. Progression of Chronic Liver Disease

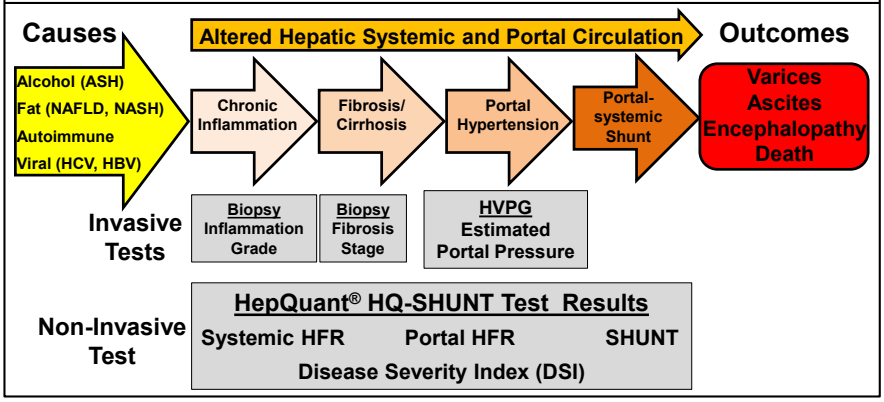
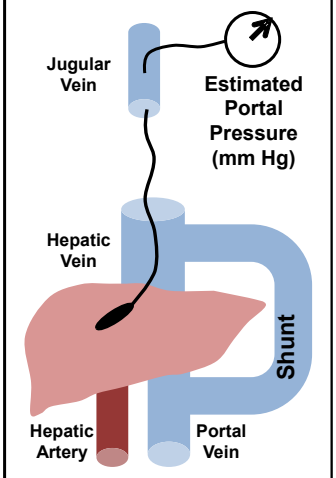
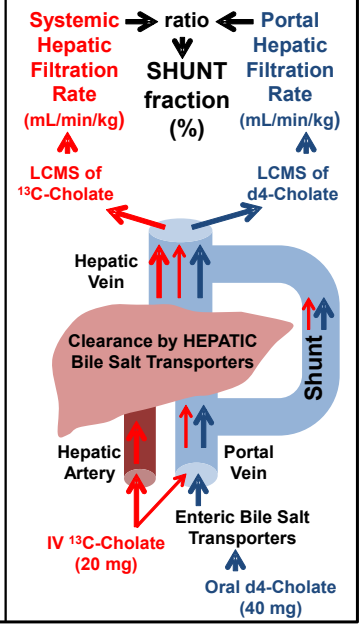


Fig. 2. A. HVPG



B. HQ-SHUNT



precision, and specificity. Oral cholate is delivered by specific enteric bile salt transporters directly into the portal blood flow where hepatic bile salt transporters take it up with high first pass efficiency quantifying the Portal Hepatic Filtration Rate (Portal HFR, mL/min/kg). The hepatic transporters also take up the IV dose of 13C-cholate from the systemic circulation, quantifying the Systemic HFR (mL/min/kg). These HFRs of the liver are analogous to the standard assessment of the kidney, the GFR. The ratio of the Systemic HFR to the Portal HFR determines the portal-systemic shunt fraction (SHUNT). The Portal HFR, Systemic HFR, and SHUNT together model the disease severity index (DSI).

III. **Preliminary Studies/Progress Report:**

Predicting Clinical Outcomes

The strongest measure of a liver test's clinical utility is its ability to predict future outcomes. The HQ-SHUNT test was shown to predict outcomes in 220 compensated HCV patients with advanced fibrosis or cirrhosis in the HALT-C Trial [2]. This data was used to develop a single composite score, the disease severity index (DSI), which was superior to the individual test results and even to biopsy fibrosis score for predicting outcomes [3]. An HVPG study followed 213 compensated cirrhotic patients, 62 had outcomes (29%), and the ROC c-statistic for predicting outcomes was 0.71 and the NPV was 90% for an HVPG <10 mm HG [4]. In 94 compensated cirrhotic patients in HALT-C, 26 had outcomes (28%), and the ROC c-statistic for predicting outcomes was 0.77 for DSI and the NPV was 95% for a DSI < 19. These data suggest that HQ-SHUNT appears to be equivalent or better at predicting outcomes than HVPG.

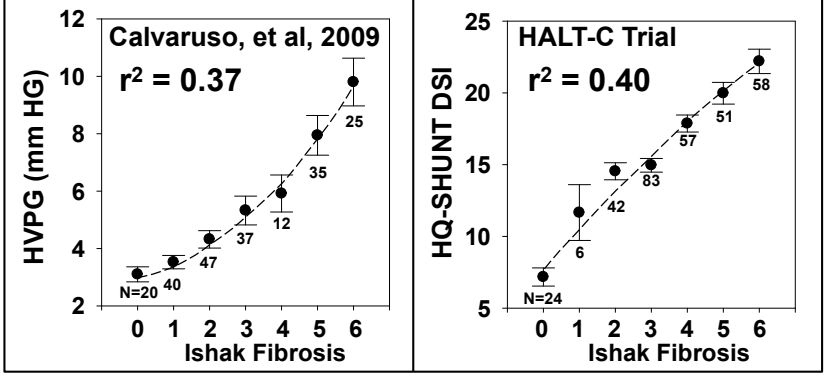
Monitoring Treatment Efficacy

Monitoring treatment efficacy can be evaluated using the cure of HCV as a model system. Patients cured of HCV had a 26% reduction in HVPG [5], a 25% reduction in SHUNT [6], a 10% reduction in DSI, and a 30% increase in Portal HFR, showing that HQ-SHUNT would be comparable at monitoring new liver drug efficacy but also provide more detailed information about drug impacts on function.

Correlations with Fibrosis and Varices

Both HVPG and HQ-SHUNT results correlate with fibrosis stage, prevalence of cirrhosis, and the prevalence and size of varices [7], [8], [9]. DSI appears to have a better and more linear correlation with fibrosis, and more sensitivity at lower fibrosis stages than HVPG (Fig. 3). However all the results summarized above were from separate studies and the two methods have never before been compared in a head-to-head fashion.

Fig. 3. HQ-SHUNT DSI has a Better Correlation with Fibrosis, a More Linear Correlation, and More Sensitivity at Lower Fibrosis Stages than HVPG



IV. Research Methods

A. Outcome Measure(s):

The following outcome measures will be assessed for each Specific Aim:

Primary Aim 1. Two measurements will be compared, HVPG and DSI. The HVPG test results will define the patients with portal hypertension (HVPG ≥ 6 mmHg). DSI will be determined from the HepQuant (HQ)-SHUNT test and the DSI cutoff for portal hypertension will be defined from ROC analysis.

Primary Aim 2. Two measurements will be compared, HVPG and DSI. The relationship between these measurements, in the patients with portal hypertension, will be analyzed by linear and nonlinear regression.

Exploratory Aims:

Exploratory Aim 1. The outcome measure is cirrhotic fibrosis score on liver biopsy. Prevalence of cirrhosis will be determined for cutoffs of DSI 20 and HVPG 6 mmHg.

Exploratory Aim 2. The outcome measure is medium to large varices at EGD. Prevalence of varices will be determined for cutoffs of DSI 20 and HVPG 10 mmHg.

Exploratory Aim 3. The outcome measure is patient tolerability for testing procedures defined by the Patient Survey.

B. Description of Population to be Enrolled:

Inclusion Criteria

- Liver disease patient scheduled to have an Hepatic Venous Pressure Gradient (HVPG) procedure
- At time of enrollment, being between the ages of 18 and 75

Exclusion Criteria

- Concomitant treatment with both a beta blocker and an ACE inhibitor
- Concurrent hepatic malignancy. Patients with a history of treated HCC can be included if there is no evidence of recurrent disease at the time of this study.
- Unstable angina or history of myocardial infarction or congestive heart failure within 6 months prior to enrollment into this study
- Renal insufficiency with chronic kidney disease stage 4 or 5 (GFR < 30 mL/min/1.73m^2)
- Crohn's disease or any active intestinal inflammatory condition
- Having had an ileal resection
- Diabetic Gastroparesis
- Pregnancy or intent to become pregnant. Urine pregnancy tests will be performed prior to HQ SHUNT testing.
- Inability to consent for one's self

Stopping Criteria

- Decision by subject to withdraw from study for any reason
- Development of any of the above-mentioned exclusion criteria (being diagnosed with a hepatic malignancy, becoming or intending to become pregnant, clinical decompensation, development of unstable coronary disease, congestive heart failure or renal insufficiency with GFR < 30mL/min/1.73m^2
- Adverse reaction to test compounds

C. Study Design and Research Methods

Patient Selection

The HVPG procedure costs ~$8500 and is currently ordered on a case-by-case basis as part of the standard of care (SOC) for liver disease. Patients already scheduled to have an HVPG measurement will be recruited to participate in this study of "Correlation and Comparison of HQ-SHUNT with HVPG". The HVPG procedure is quite uncomfortable, requires sedation and commitment of an entire day. Therefore, patients will not undergo HQ-SHUNT testing on the day of their HVPG, but rather within a window of ±60 days of their HVPG. Liver disease progresses slowly over years so that HQ-SHUNT® and HVPG results will represent the patient's current status. Patients will be censored in the analysis if clinical decompensation occurs between the HQ-SHUNT and HVPG tests.

We will enroll 110 patients into the study. Over 90% of all HVPG tests performed by UCH Radiology are ordered through the Hepatology clinic at UCH. Patients and care providers in the Hepatology clinic will be made aware of the "Correlation and Comparison of HQ-SHUNT with HVPG" study via direct communications. Patients may be enrolled within 60days following the performance of the HVPG procedure. HQ-SHUNT tests will only be performed in the patients who had a technically successful HVPG test as determined by the radiologist performing the procedure. All HQ-SHUNT tests will be performed within the 60 days following the HVPG test. HQ-SHUNT will only be performed in patients whose clinical status is stable and who have not had any major intervening procedures such TIPS or surgical shunt. Elective EGD and banding of varices, in an otherwise clinically stable patient is an allowable intervention.

PROCEDURES AND METHODS

HVPG

This will be done according to the standard of care of the Radiology department performing the procedure. All HVPG (mmHg) measurements will be recorded. In addition, if liver biopsy is obtained, the reading of the biopsy will be the standard of care reading by the Clinical Pathology department. The pathological diagnosis, inflammation score, steatosis score, and fibrosis score will be recorded.

HQ-SHUNT

On the day of HQ-SHUNT testing patients will come to the UCH CTRC and receive a physical examination and a review of their medical history. Prior to testing, blood samples will be taken for CBC, complete chemistry profile, and INR. From these clinical and laboratory data the MELD, CTP, and D'Amico scores will be determined from the following:

1. MELD (formula): MELD score = 3.8ln(bili) + 11.2ln(INR) + 9.6ln(creat) + 6
2. CTP (table):

	1 Point	2 Points	3 Points
Bilirubin, total (mg/dL)	<2	2 to 3	>3
PBC/PSC	<4	4 to 10	>10
INR	<1.7	1.7 to 2.3	>2.3
Albumin (g/dL)	>3.5	2.8 to 3.5	<2.8
Ascites	None	Responsive	Unresponsive
Encephalopathy	None	Responsive	Unresponsive

NOTE: CTP class A: 5 or 6; CTP class B: 7 to 9; CTP class C: 10 to 15

3. Cirrhosis Stage [10]:
    a. 1 = Compensated cirrhosis without varices or ascites
    b. 2 = Compensated cirrhosis with varices
    c. 3 = Bleeding varices without other disease complications
    d. 4 = First nonbleeding decompensating event
    e. 5 = Any second decompensating event

HQ-SHUNT Testing

The cholate testing compounds will be purchased from Sigma-Aldrich Isotec. The test compound 2,2,4,4-2H-cholate (d4-Cholate, product #614149) will be studied under FDA Investigational New Drug (IND) application 65,123. The test compound 24-13C-cholate (13C-Cholate, product #605883) will be studied under FDA IND 65,121. The 13C-Cholate powder will be dissolved at 4 mg/ml in 1.0 meq/ml sodium bicarbonate (injection solution, Hospira) and passed through a 0.22 micron filter. Aliquots of 5 ml will be transferred to sterile glass vials and stored frozen. Sterility and absence of pyrogens will be confirmed prior to use. The University of Colorado Hospital Investigational Drug Service will perform the sterile filtering, prepare sterile aliquots, and be responsible for pyrogens and sterility testing. An aliquot of 13C-Cholate will be thawed, mixed with 5 ml of 25% w/v human serum albumin (Plasbumin®-25, Talecris), and administered to the patient intravenously through an indwelling intravenous catheter over 1 minute by the nurse administering the test. A plastic vial containing the oral dose of 40 mg of d4-Cholate powder and 600 mg of sodium bicarbonate powder (USP, Fisher) will be prepared by a lab technician. The nurse administering the test will add water to the vial to dissolve the powder overnight and then mix the solution with juice for the subject to drink at the same time as the intravenous injection. Peripheral venous blood samples will be drawn through the indwelling catheter and obtained prior to (0 min) and at 5, 20, 45, 60, and 90 minutes after administration of cholate isotopes, and processed to serum in the standard manner. Serum will be stored frozen at -80 degrees until analysis. An aliquot of each serum sample (0.5 ml) will be dispensed and 2,2,3,4,4-2H-cholate (d5-Cholate) will be added as internal standard. Cholates will be isolated from the serum samples using C18 solid phase extraction cartridges. The eluates will be concentrated, acidified with concentrated HCl and extracted with diethyl ether. The extracted cholates will be dissolved in 10 mM ammonium acetate / 60% methanol and subjected to LCMS. Selected ion monitoring (m/z 407, 408, 411, 412) will be used to quantify the labeled cholates by the isotope dilution method. The serum concentration of each labeled cholate as a function of time will be modeled as a spline curve in order to calculate the area under curve (AUC). The IV Clearance (mL/min) is defined as the 13C-Cholate dose / 13C-Cholate AUC and the Systemic HFR is the IV clearance normalized to patient weight (mL/min/kg). The Oral Clearance (mL/min) is defined as

the d4-Cholate dose / d4-Cholate AUC and the Portal HFR is the Oral Clearance normalized to patient weight (mL/min/kg). The concentration of d4-Cholate (μM) at 60 min was found in previous studies to exhibit a reasonable correlation (r2 = 0.88) with Portal HFR so this single blood sample will also be evaluated as a simplified testing approach. The cholate shunt fraction is calculated as 100% x Systemic HFR / Portal HFR. The DSI is calculated as 5.34 (SHUNT) – 6.65 (Lne Portal HFR) - 8.57 (Lne Systemic HFR) + 44.66, and varies from ~0 in the healthiest liver to ~50 at liver failure.

SURVEY OF PATIENT TOLERABILITY

After undergoing both procedures, at the completion of the HQ-SHUNT test (always the last test), the patients would then be given two surveys, one for HVPG and another for HQ-SHUNT. The purpose of these surveys is to have the patient record their experiences with each procedure as regards their pain, discomfort, inconvenience, and willingness to undergo the procedures again. The two surveys are very similar but are nuanced for the type of testing.

D. Description, Risks and Justification of Procedures and Data Collection Tools:

Only patients who are already scheduled to have an HVPG procedure as part of their standard-of-care will be recruited to participate in this study. Most of these patients will also have liver biopsy either prior to or at the time of the HVPG procedure. In addition, these patients often undergo esophageal endoscopy procedures as part of their standard-of-care. While we will collect the data from these procedures, the subjects will not be exposed to any additional risks from these procedures beyond those associated with these standard-of-care procedures. Neither biopsy nor EGD are requirements for enrollment into this study of HVPG and HQ-SHUNT. The only additional risks will be those incurred from the HQ-SHUNT test.

We do not believe the effects of the oral or intravenous cholate are harmful to a fetus, however the effects are not definitively known. Because of this, we will not enroll participants into this study who are or are planning to become pregnant. We recommend using a reliable form of birth control throughout the study. Hormonal birth control pills, intrauterine device (IUD), DepoProvera, Norplant, barrier methods (condom or diaphragm) plus a spermicidal agent, surgical sterilization, and complete abstinence are examples of reliable methods of birth control. All women of child-bearing potential will be given a urine pregnancy test prior to HepQuant testing to ensure the subject is not pregnant.

Beta blockers or ACE inhibitors could affect the blood flow to the liver, so patients who are currently taking either a beta blocker or an ACE inhibitor will be asked to delay taking their normal dose on the morning of their test until their test is completed. Merely delaying the dose of a beta blockers or ACE inhibitor for a few hours would only minimally increase risk to subjects, similar to that of patients who miss doses of medications in everyday life. To minimize risk, patients taking both a beta blocker and an ACE inhibitor will be excluded. To further minimize risk, patients with a history of ongoing or unstable angina, or recent myocardial infarction, or congestive heart failure (within 6 months of enrollment) will also be excluded.

Placing the indwelling intravenous catheter for the HQ-SHUNT test 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). As part of numerous COMIRB approved studies, on healthy controls and liver disease patients with HCV, PSC, and NASH, we have performed the HQ SHUNT test over 1000 times in over 500 individuals. Most subjects had the test multiple times. There has never been an adverse event so we believe the risk from the test is very small.

Study data will be collected and managed using REDCap (Research Electronic Data Capture). REDCap is a secure, web application designed to support data capture for research studies, providing user-friendly web-based case report forms, real-time data entry validation (e.g. for data types and range checks), audit trails and a de-identified data export mechanism to common statistical packages (SPSS, SAS, Stata, R/S-Plus). The system was developed by a multi-institutional consortium which includes University of Colorado Denver and was initiated at Vanderbilt University. REDCap is HIPAA compliant and can be configured to be Part 11 compliant.

E. Potential Scientific Problems:

Concomitant medication use 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 [11] 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.

The circulatory changes seen in medical conditions, including right and left-sided heart failure or severe renal failure, could affect IV and oral cholate clearances. Therefore, patients with ongoing heart failure or with GFR < 30 mL/min/1.73m2 corresponding to chronic kidney disease stage 4 or 5 will be excluded.

F. Data Analysis Plan:

Power and Sample size

Primary Aim 1: Assuming that there are 50 patients with and 50 patients without portal hypertension in the study, a sample size of 50 from the portal hypertension group and 50 from the no portal hypertension group achieve 80% power to detect a difference of 0.14 between the area under the ROC curve (AUC) for DSI under the null hypothesis of 0.65 and an AUC under the alternative hypothesis of 0.79 using a two-sided z-test at a significance level of 0.05. The data are continuous responses. The AUC is computed between false positive rates of 0 and 1. The ratio of the standard deviation of the responses in the negative group to the standard deviation of the responses in the positive group is 1.

The following table shows the range of AUC's that can be detected based on alternative scenarios for the null hypothesis AUC using HQ-SHUNT:

Numeric Results for Testing AUC0 = AUC1 with Continuous Data
Test Type = Two-Sided. False positive rate1 = 0.000. False positive rate2 = 1.000. B (ratio of SDs) = 1.000.

Power	N+	N-	AUC0	AUC1	Difference	Alpha	Beta
0.80000	50	50	0.6500	0.7945	0.1445	0.05000	0.20000
0.80000	50	50	0.6600	0.8029	0.1429	0.05000	0.20000
0.80000	50	50	0.6700	0.8113	0.1413	0.05000	0.20000
0.80000	50	50	0.6800	0.8196	0.1396	0.05000	0.20000
0.80000	50	50	0.6900	0.8278	0.1378	0.05000	0.20000
0.80000	50	50	0.7000	0.8359	0.1359	0.05000	0.20000
0.80000	50	50	0.7100	0.8439	0.1339	0.05000	0.20000
0.80000	50	50	0.7200	0.8518	0.1318	0.05000	0.20000
0.80000	50	50	0.7300	0.8597	0.1297	0.05000	0.20000
0.80000	50	50	0.7400	0.8674	0.1274	0.05000	0.20000
0.80000	50	50	0.7500	0.8751	0.1251	0.05000	0.20000
0.80000	50	50	0.7600	0.8826	0.1226	0.05000	0.20000
0.80000	50	50	0.7700	0.8901	0.1201	0.05000	0.20000
0.80000	50	50	0.7800	0.8975	0.1175	0.05000	0.20000
0.80000	50	50	0.7900	0.9047	0.1147	0.05000	0.20000
0.80000	50	50	0.8000	0.9119	0.1119	0.05000	0.20000

Primary Aim 2: Assuming that there are 50 patients with portal hypertension, the following table shows the 95% confidence intervals around correlations ranging from 0.4 to 0.8 with a sample size of 50 patients with portal hypertension:

Numeric Results for Two-Sided Confidence Intervals for One Correlation

Confidence Level	Sample Size (N)	Actual Width	Correlation (R)	Lower Limit	Upper Limit	Width if R = 0.0
0.950	50	0.473	0.400	0.137	0.610	0.557
0.950	50	0.426	0.500	0.257	0.683	0.557
0.950	50	0.367	0.600	0.386	0.753	0.557
0.950	50	0.295	0.700	0.524	0.819	0.557
0.950	50	0.211	0.800	0.671	0.882	0.557

Correlation (R) is the assumed sample correlation.
Width if R = 0.0 is the maximum width for a confidence interval with sample size N.

For the linear regression analysis, a sample size of 50 achieves 80% power to detect a change in slope from 0 under the null hypothesis to 0.50 under the alternative hypothesis when the standard deviation of DSI is 3, the standard deviation of HVPG is 4.00, and the two-sided significance level is 0.05.

General Statistical Analysis

Primary Aim 1. It is expected that both HQ-SHUNT results and HVPG measurements will reflect the extent of liver disease. Higher pressures will in general be associated with lower Systemic HFR and Portal HFR, and with higher SHUNT and DSI. The performance of DSI to identify patients with portal hypertension (HVPG ≥6 mmHg) will be determined by ROC analysis. Optimal DSI cutoff, sensitivity, specificity, PPV, and NPV will be defined.

Primary Aim 2. We will develop models of the relationships of HQ-SHUNT test results to the HVPG test results based on correlations. We expect there to be a linear relationship for HVPG pressures between 6 and 25 mmHg (i.e. in patients with portal hypertension) and will assess the correlation (with 95% confidence intervals) for HVPG vs. DSI in the 50 patients expected to have HVPG pressures ≥6 mmHg. Linear regression will be used to assess the relationship between these variables, taking other covariates into account, and R^2 values will be reported.

Exploratory Aims:

Exploratory Aim 1. It is expected that both HQ-SHUNT results and HVPG measurements will, in general, reflect the extent of liver disease, as measured by the liver fibrosis stage and by the D'Amico cirrhosis stage. Regression models will be developed of the relationships of HQ-SHUNT test results to fibrosis stage and to cirrhosis stage. Likewise regression models will be developed of the relationship of HVPG portal pressure to fibrosis stage and to cirrhosis stage. It is expected that correlations will be found similar to those shown in Fig 3, but now demonstrated in the same patient population.

Exploratory Aim 2. HQ-SHUNT test results will be compared to HVPG test results at identifying patients with all sizes of varices, as well as at identifying just those patients with medium to large varices that require therapeutic intervention, usually banding. These comparisons will be made using ROC curves and their associated c-statistics. The optimum diagnostic cutoffs of each test will be determined from the ROC curves by the Youden index (J statistic). The sensitivity, specificity, PPV, and NPV of each test will be compared at their respective optimum diagnostic cutoffs.

Exploratory Aim 3. For each procedure, HVPG and HQ-SHUNT testing, the patients will report their pain, discomfort, inconvenience, overall experience, and willingness to undergo repeat testing, each on a scale of 1 to 10. The numerical scores of each question will be compared between the HVPG and HQ-SHUNT testing, in terms of mean score, standard deviation, and statistical significance (t-test).

G. Summarize Knowledge to be Gained:

Overall this study will demonstrate whether The HQ-SHUNT test results can generate physiological information on liver disease patients that is as good, or better, than HPVG pressure measurements. A close correlation between HQ-SHUNT test results and HPVG pressure measurements would allow the safer, less invasive, lower cost HQ-SHUNT test to replace HVPG in patient assessment and in clinical trials of new liver disease therapies and therapeutic agents. However the HQ-SHUNT test reports on the systemic clearance, portal clearance, and portal-systemic shunt and provides a more comprehensive picture of the portal circulation than just the portal pressure. For example, the presence of extensive or large collaterals, such as a spleno-renal collateral, should relieve portal pressure, reduce varices, and reduce the HVPG; while, in contrast, HQ-SHUNT would reveal an increase in SHUNT and DSI. In terms of disease severity, HVPG would be falsely low, while HQ-SHUNT would report accurately.

Varices are clinically very serious because they require intervention to prevent internal bleeding. An HVPG > 10 mm HG has been associated with a risk of varices. A DSI > 20 similarly indicates a risk of large varices, ROC c-statistic 0.82, sensitivity 86%, specificity 65%. This study allows a direct comparison in the same patients of the relative ability of HQ-SHUNT and HVPG to identify patients with varices.

Previous studies (Fig. 3) suggest that HQ-SHUNT DSI will have a better, more linear correlation with fibrosis (higher r^2), and more sensitivity at lower fibrosis stages than HVPG. Patients with D'Amico cirrhosis stage 1 (no varices), stage 2 (varices), and stages 3-4 (decompensation) had mean HVPG's of 10, 12, and 17 mm Hg [11] while a study of the PSC patients with no varices, varices, or decompensation found mean HQ-SHUNT DSI's of 14, 20, and 35, suggesting that HQ-SHUNT will be comparable to HVPG at early cirrhosis stages. HVPG leveled off and could not distinguish later cirrhosis stages 3-4 [12], but studies of HQ-SHUNT at Baylor University Medical Center, Dallas, found a wide range of DSI's in decompensated patients, up to a DSI of ~50. These results suggest that HQ-SHUNT will be better than HVPG at assessing and differentiating the later clinical stages of cirrhosis.

It is expected that patients will overwhelmingly prefer HQ-SHUNT as having only a minor needle stick, not requiring sedation, and not interfering much with their routine. Patient acceptance is very important when tests must be repeated over several months to follow the efficacy of a new treatment or drug.

In summary, this study could demonstrate whether HQ-SHUNT could be the safer, less invasive, lower cost, superior alternative to HVPG providing greater accuracy and more information over the entire spectrum of disease severity.

H. References:

1. Helmke S, Colmenero J, Everson GT. Noninvasive assessment of liver function. Current opinion in gastroenterology. 2015 Feb 24. [Epub ahead of print].
2. Everson GT, Shiffman ML, Hoefs JC, Morgan TR, Sterling RK, Wagner DA, Lauriski S, Curto TM, Stoddard A, Wright EC. 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(4):1019-29.
3. Helmke SM, Desanto JL, Herman A, Lauriski S, Everson GT. A Disease Severity Index Based on Dual Cholate Clearances and Shunt Outperforms Biopsy at Predicting Clinical Outcomes in Chronic Hepatitis C Gastroenterology. 2013;144(5):S951-2.
4. Ripoll C. Hepatic venous pressure gradient and outcomes in cirrhosis. Journal of clinical gastroenterology. 2007;41 Suppl 3:S330-5.
5. Rincon D, Ripoll C, Lo Iacono O, Salcedo M, Catalina MV, Alvarez E, Nunez O, Matilla AM, Clemente G, Banares R. Antiviral therapy decreases hepatic venous pressure gradient in patients with chronic hepatitis C and advanced fibrosis. Am J Gastroenterol. 2006;101(10):2269-74.
6. Everson GT, Shiffman ML, Hoefs JC, Morgan TR, Sterling RK, Wagner DA, Desanto JL, Curto TM, Wright EC. Quantitative tests of liver function measure hepatic improvement after sustained virological response: results from the HALT-C trial. Aliment Pharmacol Ther. 2009;29(5):589-601.
7. Bosch J, Abraldes JG, Berzigotti A, Garcia-Pagan JC. The clinical use of HVPG measurements in chronic liver disease. Nat Rev Gastroenterol Hepatol. 2009;6(10):573-82.
8. Everson GT, Martucci MA, Shiffman ML, Sterling RK, Morgan TR, Hoefs JC. Portal-systemic shunting in patients with fibrosis or cirrhosis due to chronic hepatitis C: the minimal model for measuring cholate clearances and shunt. Aliment Pharmacol Ther. 2007;26(3):401-10.
9. Everson GT, Shiffman ML, Morgan TR, Hoefs JC, Sterling RK, Wagner DA, Kulig CC, Curto TM, 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 Trial. Aliment Pharmacol Ther. 2008;27(9):798-809.
10. D'Amico LP et al. Competing risks and prognostic stages of cirrhosis: a 25-year inception cohort of 494 patients. Aliment Pharmacol ther. 2014; 39: 1180-1193.
11. U. S. Department of Health and Human Services Food and Drug Administration. Guidance for Industry: Bioanalytical Method Validation. 2001.
12. Zipprich A, Garcia-Tsao G, Rogowski S, Fleig WE, Seufferlein T, Dollinger MM. Prognostic indicators of survival in patients with compensated and decompensated cirrhosis. Liver international: official journal of the International Association for the Study of the Liver. 2012;32(9):1407-14. PMCID: 3713489.