NONINVASIVE SUBHARMONIC AIDED PRESSURE ESTIMATION OF PORTAL HYPERTENSION

Version 3.1 – July 21, 2014; submitted April 19, 2021

NCT02489045

PROTOCOL

TITLE: NONINVASIVE SUBHARMONIC AIDED PRESSURE ESTIMATION OF PORTAL HYPERTENSION

VERSION & DATE: Version 3.1 – July 21, 2014

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STUDY DURATION: 3 years

Contents

SYNOPSIS

Protocol Title: Noninvasive Subharmonic Aided Pressure Estimation of Portal Hypertension

Trial Objectives: The primary objectives of this trial are:

- Evaluate the use of *in vivo* subharmonic aided pressure estimation (SHAPE) as a first qualitative screening modality for determining the presence of portal hypertension in patients undergoing a transjugular liver biopsy compared to catheter based pressure measurements (as the reference standard)
- Determine if SHAPE measurements can provide a quantitative, noninvasive measurement of hepatic venous pressure gradient (HVPG) to monitor disease progression or treatment response in patients identified with portal hypertension by comparing the results to repeat biopsies and/or clinical outcomes

Trial Design: This is an open-label, non-randomized trial that will be conducted at two clinical sites, Thomas Jefferson University (TJU) and the Hospital of the University of Pennsylvania (HUP). Enrolled patients undergoing trans-jugular liver biopsy with hepatic vein pressure gradient (HVPG) measurements will receive a continuous infusion of Sonazoid® (GE Healthcare, Oslo, Norway) co-infused with 0.9% NaCl solution over a 5-10 minute time period using the setup and infusion rates described in GE Healthcare (GEHC) Protocol Supplement provided in Appendix B. Ultrasound imaging will be performed using a Logiq 9 scanner with a 4C transducer (GE Healthcare, Milwaukee, WI) and the SHAPE algorithm will be used to measure pressure values in the hepatic and portal veins. Data will be stored on a PC and compared to pressure-catheter measurements, Subjects identified in the initial examination as having portal hypertension (by HVPG results) will be monitored by SHAPE for up to 18 months. These subjects typically have surveillance CT or MRI scans every 6 months to screen for liver cancer, and at those times a repeat SHAPE examination will be performed (ideally within 1 month of their clinically indicated imaging follow up appointment). In patients who undergo more frequent screening (generally 3 month intervals), SHAPE exams will be performed at 6 month intervals. Any repeat trans-jugular liver biopsies performed in this population will also trigger a repeat SHAPE study. Results of blood test evaluations (performed every 3 months in this population), medication, concomitant imaging study or procedure (including endoscopies) will be noted (all blood tests and imaging are clinically indicated only and are not required by this protocol). The end point for this part of the study will be any one new complication (e.g., liver cancer) or a marked worsening in any complication, liver

transplantation, death, or the end of this clinical trial (after 3 years). We expect these patients will be monitored three times during the course of this clinical trial. The time to reach the end point will be noted if a new complication or a marked worsening in any complication occurs.

Trial Population: This trial will consist of up to 300 adults (21 years of age or older) undergoing trans-jugular liver biopsy with HVPG measurement at TJU or at HUP.

Trial Procedures: Subjects eligible for trial enrollment will be identified by the co-investigators, Dr. Fenkel (at TJU) and Dr. Soulen (at HUP) from their patient population of subjects scheduled for a trans-jugular liver biopsy. The research coordinators for this study will explain the study to the patients at the respective clinical sites. The patient will be given time to consider the risks and benefits of the study and ask questions about participation. The coordinator will review the consent form with the patient and then the patient will be given the form to review. The patient, coordinator, and a study investigator will all sign the consent form. The patient will be given a copy of the signed consent form for their records.

After consenting to participate in this study, a full demographic profile, known drug allergies or intolerances, and review of the subject's medical/surgical history will be recorded. Patients with known cardiac shunts will be excluded from the study. If the subject is a woman of childbearing potential, she will have a urine pregnancy test (the results of which will be made available to the subject prior to study initiation).

Whenever a subject undergoes a trans-jugular liver biopsy with transcatheter free and wedged hepatic venous pressure measurements as part of their standard clinical care and agrees to participate in the study, we will perform the corresponding SHAPE measurement within the 3 hours of post-recovery observation to ease transportation for patients and ensure that hydration status and other variables, which could potentially affect portal pressures, are equivalent. Administration of Sonazoid will be performed under

direct supervision of a medical doctor. Additionally, resuscitation equipment will be in immediate proximity to the patient during Sonazoid infusion. Three vials (6 ml) of Sonazoid microbubbles (16 µl per vial when reconstituted) will be prepared for each subject by resuspending each vial in 2 ml of water for injection, supplied by GEHC, as described in the instructions provided by GEHC (Appendix B). Infusions will be performed as described in Appendix B. All materials and supplies used for the infusion procedure will be identical to those described in GE Healthcare's current Sonazoid IND. Briefly, anintravenous cannula will be placed in a vein in the subject's arm. A 0.9% NaCl solution will be started and used to fill up the connecting tubes before being connected to a 3-way stopcock. The stopcock will then be connected to the extension tubing leading to the cannula. All three vials of suspended Sonazoid will be drawn into a 10 ml syringe, placed in a syringe pump at the same level or below the patient, and connected directly to the stopcock. After the stopcock to the 0.9% NaCl solution has been opened, the saline solution will be infused at a rate of at least 2 ml/min. Then, Sonazoid will be co-infused at the target rate of 0.024 µl/kg body weight/minute (suspension infusion rate of 0.18 ml/kg/hour), but the rate may be reduced if necessary to achieve optimal levels of contrast. This infusion rate was selected based on our group's previous experiences with Sonazoid infusion in human subjects in general [Halpern et al. 2002; Landmark et al. 2008] and in SHAPE portal hypertension subjects in particular [Eisenbrey et al. 2013]. This dosing scheme is designed to avoid (or limit) the number of cases that has to be discarded due to insignificant enhancement in the hepatic veins (as in our pilot study; Eisenbrey et al. 2013). The dosage is based off the GEHC provided, study specific resuspension and infusion instructions (Appendix B) and the total Sonazoid dose given over a maximum of 15 minutes (0.36 µl/kg) will be well below the maximal tested dose which was still found safe in Phase I clinical trials (2.7 µl of microbubbles/kg in PBI 0001 as detailed in the Sonazoid Investigator's Brochure). The duration of contrast agent infusion will range from 5 to 10 minutes.

After beginning the contrast infusion, a modified Logiq 9 scanner (GE Healthcare) with a broad bandwidth curvi-linear array (model 4C) will be used to obtain RF data (for SHAPE measurements) within the hepatic and portal veins. Both the experimental machine and software will be labeled as an

investigational device with a GRC provided label prior to beginning any clinical experiments (21 CRF 812.5). Once the infusion of Sonazoid has started, ultrasound imaging will be performed with the 4C curved linear array to guide SHAPE ROI placement into the portal vein (while also visualizing a hepatic vein). The SHAPE optimization algorithm will be activated ([Dave et al. 2013]) and the acoustic power will be adjusted to produce the maximum change in subharmonic amplitudes (i.e., maximizing the sensitivity of SHAPE). Then the ROI will be increased to visualize the hepatic and the portal veins, and the corresponding subharmonic data will be acquired at the optimal acoustic power setting in 5 s segments during the infusion of the Sonazoid suspension. Ultrasound imaging will be performed at a transmit frequency of 2.5 MHz and the subharmonic obtained at 1.25 MHz. All measurements will be repeated three times. The depths, sizes, and locations of the portal and hepatic veins will be determined by the sonographer and saved in a reference image.

The proposed agent for the study, Sonazoid (GE Healthcare,), is a sterile non-pyrogenic suspension of lipid stabilized perfluorobutane microbubbles for contrast-enhancement, with a median diameter between 2.4 and 3.5 μm (Forsberg et al. 2008). Previous *in vitro* results have shown Sonazoid to be superior for SHAPE measurements relative to other ultrasound contrast agents (Halldorsdottir et al. 2011). The United States Food and Drug Administration (FDA) has yet to approve Sonazoid for human use in this country. Consequently, we intend to apply to the FDA for an investigator initiated IND (investigational new drug) to cover the use of Sonazoid in patients undergoing a trans-jugular liver biopsy. It should be pointed out that the safety of Sonazoid has already been established in numerous animal studies and in human studies for the evaluation of the liver and heart both in this country and in Europe (Landmark et al. 2008). Sonazoid has been approved for use in patients with liver lesions in Japan and has been marketed in that country since early 2007, and has been approved more recently in South Korea (Bouakaz et al. 2007; Jang et al. 2013. Finally, no adverse reactions attributable to contrast agent infusions were observed in our pilot study (Eisenbrey et al. 2013).

All examinations will be recorded. During the examination, the subharmonic pressure estimates will be

displayed on the Logiq 9 scanner, these estimates will be noted down and the data will be stored on a PC. Data will be compared to the transcatheter portal pressure measurements, which comprise the free and wedged hepatic venous pressure (FHVP and WHVP, respectively) and the hepatic vein pressure gradient (HVPG; measured as the difference between the WHVP and FHVP. Emergency services (e.g., a crash cart) will be available within the hospital in case of any acute adverse reactions. Subjects will be monitored for adverse reactions 2 hours post biopsy (roughly 1 hour post Sonazoid injection), again as a standard of care procedure. Patients will be monitored for a minimum of one hour post Sonazoid administration. During this time a trained CPR personnel and resuscitation equipment will be available. SHAPE data will later be compared to all clinical variables obtained from the patient to determine potential correlations to liver dysfunction.

Statistical Methodology: This project is a large clinical trial to further develop SHAPE and to employ this method to conduct a large scale human clinical trial to conclusively establish the accuracy of SHAPE for the assessment of portal hypertension. Our statistical analysis will address following major questions:

1. Can patients with HVPG of 10 mmHg or greater be identified using SHAPE? The fundamental hypothesis is that a sensitivity of 89% and a specificity of 81 % can be achieved by using SHAPE measurements to identify patients with HVPGs of 10 mmHg or greater.
2. Can portal vein pressures (in the form of HVPGs) in patients with liver disease be quantified accurately using SHAPE? The fundamental hypothesis is that a correlation coefficient of 0.82 can be achieved between in vivo SHAPE and HVPG measurements.
3. Can SHAPE accurately monitor disease progression or treatment response in patients identified with portal hypertension? The fundamental hypothesis is that correlation coefficients above 0.90 can be achieved between SHAPE and repeat HVPG results and that SHAPE will significantly differentiate responders from non-responders (using clinical outcomes as the reference standard).

The findings of SHAPE will be correlated to the invasive catheter based measurements of portal vein blood pressure (i.e., HVPG) using least squares, multiple linear regression analysis with robust standard errors. Comparisons of sensitivities, specificities and accuracies for diagnosing portal hypertension with SHAPE will be conducted with an unpaired Student's t-test (using HVPG measurements as the reference standard). Receiver operating characteristic (ROC) curves will be computed using a continuous scale

with HVPG cutoff levels of 10 and 12 mmHg. The optimal operating point (OOP) will be determined as the point closest to '0,1' on the ROC curve (representing 100% sensitivity and 100% specificity). All clinical variables (e.g., concomitant imaging results, presence of cancer or ascites, blood tests, fibrosis and MELD scores etc.) will also be compared to the SHAPE values. When both types of variables are ordinal or continuous, correlations will be calculated. When one type of variable is nominal and one continuous non-parametric rank order tests such as Mann-Whitney U-tests or Kruskal-Wallis tests will be performed [Rosner 1990]. All of the statistical analyses proposed for the human clinical trial will be repeated split by racial and ethnic groups to determine if clinically important race/ethnicity differences exist in the ability of SHAPE to diagnose portal hypertension.

All analyses and computations will be performed using NCSS/PASS 2008 and Stata 12.0 (Stata Corporation, College Station, TX), while the study database will be designed and implemented in Filemaker Pro 10.0 (Filemaker Inc, Santa Clara, CA). This database will contain all patient information (except names and other identifiers), including results of biopsies, HVPG and SHAPE measurements as well as the clinical variables.

1. INTRODUCTION

There is clearly a well-established clinical need to accurately measure the hepatic vein pressure gradient (HVPG) (Garcia-Tsao 1985). Unfortunately, current techniques are either invasive, such as catheter based pressure transducers, which have to be introduced directly into the hepatic vasculature, or noninvasive but indirect and qualitative, such as using CT, MRI or ultrasound imaging to diagnose liver cirrhosis (Kudo et al. 2008). However, the diagnostic accuracy of CT, MRI and ultrasound for grading cirrhosis into 3 categories (chronic hepatitis, cirrhosis and pre-cirrhosis) was only between 64 and 70% in a recent multi-center study (Kudo et al. 2008) and this is still only a very indirect measure of HVPG.

Our group has proposed and patented a novel and innovative technique called subharmonic-aided pressure estimation (SHAPE) for noninvasive pressure measurements (Forsberg et al. 2005; Shi et al. 1999; 2001). We have shown hydrostatic pressure can be measured through variations of the subharmonic amplitude of ultrasound contrast agents during insonation. Our group has just completed a first-in-humans clinical trial of SHAPE in 45 patients with chronic liver disease supported by RC1 DK087365 [Eisenbrey et al. 2013]. The SHAPE gradient between the portal and hepatic veins was in good overall agreement with the HVPG (R = 0.82) indicating SHAPE may be a useful tool for the diagnosis of portal hypertension. This project will serve as a large clinical trial to further develop SHAPE and to employ this method to conduct a large scale human clinical trial to conclusively establish the accuracy of SHAPE for the assessment of portal hypertension.

1.1 Background

Portal hypertension is defined as an increase in the pressure gradient between the portal vein and hepatic veins or the inferior vena cava (IVC) exceeding 5 mmHg (Sanyal et al. 2008). Cirrhosis (prevalence of 2-10%) accounts for at least 90% of cases of portal hypertension in the developed world. From a pathophysiological perspective, the increase in portal venous pressure results from a sequence of events that begins with an increase in resistance to portal outflow, into the liver (Navarro et al. 2007). This is the result of two factors; architectural distortion of the liver parenchyma, as occurs in the accumulation of

fibrosis, and vasoconstriction of the hepatic microcirculation. The rise in portal pressure is largely asymptomatic until the body mounts a homeostatic response to systemic arterial vasodilatation. Specifically, systemic vasodilatation yields predictable physiological consequences, including systemic arterial hypotension, retention of salt and water and the formation of ascites, and renal insufficiency. In addition, up to 90% of the portal flow can be diverted into the systemic circulation through portosystemic collaterals (Sanyal et al. 2008).

Cirrhosis due to most causes, without the clinical manifestations of portal hypertension, has a small effect on mortality. However, it is the manifestations of portal hypertension which greatly impact survival. Common complications of portal hypertension include gastroesophageal varices, ascites, and portosystemic encephalopathy. Patients who develop ascites have a 50% two year mortality and those who develop spontaneous infection of the ascites fluid carry a 70% one year mortality. Patients with cirrhosis have a 5-10% yearly incidence of gastroesophageal variceal formation, and a 4-15% yearly incidence of bleeding, with larger varices having a greater risk for bleeding (D'Amico et al. 2006). Although the mortality associated with each bleeding event has fallen significantly due to endoscopic, pharmacological, and clinical care interventions, each bleeding episode continues to carry up to a 20% risk of death (D'Amico et al. 2006; Navarro et al. 2007; Sanyal et al. 2008).

In patients with chronic liver disease, transcatheter portal pressure measurement represents the "gold standard", albeit indirect, for assessment of portal hypertension. The wedge hepatic venous pressure (WHVP) reflects values of portal venous pressure in cirrhosis (Sanyal et al. 2008). Another useful parameter is the gradient between the portal vein and the hepatic vein pressure, the hepatic vein pressure gradient (HVPG). The value of HVPG in assessing and monitoring several of the complications of portal hypertension is well established (D'Amico et al. 2006; Sanyal et al. 2008). It has also been found that an HVPG above 10 mmHg predicts the presence of varices, while an HVPG of 12 mmHg or more is required for varices to bleed (Garcia-Tsao 1985).

Variceal hemorrhage represents one of the most dramatic and immediately life threatening complications of portal hypertension and while the mortality rate has declined over the last decade it still ranges from 15 to 20% (Sanyal et al. 2008). Clinical management depends upon preventing an initial bleed when varices are present, intervening to stop active bleeding, and providing measures to prevent repeat bleeding. A sustained reduction in the HVPG to below 12 mmHg is the best predictor of successful reduction in the risk of bleeding with non-selective β-blockers. Likewise, a reduction in the risk of re-bleeding from varices is associated with a reduction in the HVPG of more than 20% from baseline or to less than 12 mmHg. Finally, the HVPG has been evaluated as a prognostic marker for disease progression, survival and mortality (Kalambokis et al. 2007). Most studies found that the HVPG, when added to other measures such as the Child Pugh or MELD scores, increased the ability to predict survival.

Consequently, our group has proposed and patented a novel and innovative technique called subharmonic-aided pressure estimation (SHAPE) for noninvasive pressure measurements (Forsberg et al. 2005; Shi et al. 1999; 2001). An accurate marker for portal hypertension would impact literally millions of Americans with liver disease. Such a marker could be used to predict the presence of portal hypertension and its complications (in particular varices) and might also be used to predict morbidity and mortality. Moreover, the value of HVPG measurements in the management of hepatocellular cancer (HCC) has been demonstrated. The noninvasive and accurate measurement of HVPG would also be clinically useful for trans-jugular intrahepatic portosystemic shunt (TIPS) procedures. TIPS are frequently performed for patients with intractable ascites not responding to medications or variceal bleeding which does not respond to pharmacological or endoscopic means. Patients with suspected variceal bleeding routinely undergo ultrasound examination to evaluate patency of the portal venous branches and determine whether TIPS placement is feasible. Getting a reliable estimate of HVPG in these patients with SHAPE would expedite therapy. SHAPE may also assist in the evaluation of ultrasound findings at TIPS follow-up studies, which can be technically challenging.

The proposed agent for the current study, Sonazoid® (GE Healthcare, Oslo, Norway), is a sterile non-pyrogenic suspension of lipid stabilized perfluorobutane microbubbles for contrast-enhancement, with a median diameter between 2.4 and 3.5 μm (Forsberg et al. 2008). Previous *in vitro* results have shown that Sonazoid is superior for SHAPE measurements relative to other ultrasound contrast agents (Halldorsdottir et al. 2011). The FDA has yet to approve Sonazoid for human use in this country. Consequently, we intend to apply to the FDA for an investigator initiated IND to cover the use of Sonazoid in patients undergoing a trans-jugular liver biopsy. It should be pointed out that the safety of Sonazoid has already been established in numerous animal studies and in human studies for the evaluation of the liver and heart both in this country and in Europe (Landmark et al. 2008). Sonazoid has been approved for use in patients with liver lesions in Japan and has been marketed in that country since early 2007 and more recently in South Korea (Bouakaz et al. 2007; Jang et al. 2013). Finally, no adverse reactions attributable to contrast agent infusions were observed in our pilot study (Eisenbrey et al. 2013).

Sonazoid Clinical Safety

Sonazoid was administered in clinical trials in 1699 patients. In these patients 404 (23.8%) reported at least one adverse event, while 12 of 62 (19.4%) of patients receiving a placebo reported adverse events.

Forty-three (2.5 %, 43/1699) subjects experienced serious adverse events, including 13 deaths. None of these events were considered by the investigators to be related to Sonazoid. All the serious adverse events were considered to be caused by the underlying disease or related treatment. In addition, there were no clinically significant trends in laboratory tests, vital signs, ECGs, or physical examination findings.

The most commonly noted adverse events were headache (3.6%, 62/1699), chest pain (2.3%, 39/1699), abdominal pain (1.5%, 25/1699), diarrhea (1.5%, 25/1699), and nausea (1.6%, 28/1699). The majority of adverse events were mild to moderate in severity (92.6%, 652/704).

Table 1.
Selected Adverse Events Reported in ≥ 0.5% of the Subjects who Received Sonazoid in Controlled Clinical Studies (% of 1699)

No. of Patients Exposed to Sonazoid	1699	
No. of Patients Reporting an Adverse Event	404	(23.8%)
Application Site Disorders	9	(0.5%)
Body as a Whole	91	(5.4%)
Back Pain	10	(0.6%)
Chest Pain	39	(2.3%)
Cardiovascular System, General	45	(2.6%)
ECG Abnormal Specific	15	(0.9%)
Hypotention	9	(0.5%)
Nervous	94	(5.5%)
Dizziness	18	(1.1%)
Headache	62	(3.6%)
Gastrointestinal	126	(7.4%)
Abdominal Pain	25	(1.5%)
Diarrhea	25	(1.5%)
Nausea	28	(1.6%)
Vomiting	21	(1.2%)
Hear Rate & Rhythm	55	(3.2%)
Liver and Billiary System	22	(1.3%)
SGOT/AST increased	9	(0.5%)
Metabolic & Nutritional	42	(2.5%)
Increased LDH	17	(1.0%)
Musculo-Skeletal System	18	(1.1%)
Myo-, Endo-, Pericardial & Valve	29	(1.7%)
Angina Pectoris	14	(0.8%)
Platelet, Bleeding & Clotting	21	(1.2%)
Psychiatric	19	(1.1%)
Respiratory System	58	(3.4%)
Dyspnoea	19	(1.1%)
Skin & Appendages	18	(1.1%)
Urinary System	31	(1.8%)
Albuminuria	17	(1.0%)
White Cell & RES	9	(0.5%)

Additional information concerning pre-clinical and clinical experience with Sonazoid, including the dosing levels and reported subject complaints, can be found in the Sonazoid investigational drug brochure (Version 7, October 4th 2002).

1.2 Rationale

The quantitative aspect of this project is based on the assumption that the magnitude of subharmonic signals from insonated contrast agents depend on the hydrostatic pressure of the surrounding media. SHAPE estimates internal pressure variations by transmitting at one frequency, receiving at its subharmonic frequency and then monitoring the subharmonic amplitude variations from the contrast bubbles. The optimal sensitivity for SHAPE was determined *in vitro* to be at a transmit frequency of 2.5 MHz (i.e., receiving at 1.25 MHz) at a 0.35 MPa acoustic pressure using the contrast agent Sonazoid (GE Healthcare, Oslo, Norway), which declined approximately 14.4 dB over a 0 to 186 mmHg pressure range.

We propose a large clinical trial at two sites to further develop SHAPE and to employ this method to conduct a large scale human clinical trial to conclusively establish the accuracy of SHAPE for the assessment of portal hypertension. The purpose of this study is to evaluate if SHAPE measurements may be used as a first qualitative screening modality for determining the presence of portal hypertension in patients undergoing a transjugular liver biopsy and determine if SHAPE measurements may be used to monitor disease progression or treatment response for patients diagnosed with portal hypertension.

2. TRIAL OBJECTIVES

Trial Objectives: The primary objectives of this trial are:

- Evaluate the use of *in vivo* subharmonic aided pressure estimation (SHAPE) as a first qualitative screening modality for determining the presence of portal hypertension in patients undergoing a transjugular liver biopsy compared to catheter based pressure measurements (as the reference standard)
- Determine if SHAPE measurements can provide a quantitative, noninvasive measurement of hepatic venous pressure gradient (HVPG) to monitor disease progression or treatment response in patients identified with portal hypertension by comparing the results to repeat biopsies and/or clinical outcomes.

3. TRIAL DESIGN

This is an open-label, non-randomized trial that will be conducted at two clinical sites, Thomas Jefferson

University (TJU) and the Hospital of the University of Pennsylvania (HUP). Enrolled patients undergoing trans-jugular liver biopsy will receive a continuous infusion of Sonazoid® (GE Healthcare, Oslo, Norway) co-infused with 0.9% NaCl solution over a 5-10 minute time period using the setup and infusion rates described in GE Healthcare (GEHC) Protocol Supplement provided in Appendix B. Ultrasound imaging will be performed using a Logiq 9 scanner with a 4C transducer (GE Healthcare, Milwaukee, WI) and the SHAPE algorithm will be used to measure pressure values in the hepatic and portal veins. Data will be stored on a PC and compared to pressure-catheter measurements, which are taken as part of the standard of care for all patients undergoing a trans-jugular biopsy. Subjects identified in the initial examination as having portal hypertension (by HVPG results) will be monitored by SHAPE for up to 18 months. These subjects typically have surveillance CT or MRI scans every 6 months to check for liver cancer and at those times a repeat SHAPE examination will be performed (ideally within 2 weeks). Any repeat transjugular biopsies performed in this population will also trigger a repeat SHAPE study. Results of blood test evaluations (performed every 3 months in this population), medication, concomitant imaging study or procedure (including endoscopies) done as standard of care will be noted. The end point for this part of the study will be any one new complication (e.g., liver cancer) or a marked worsening in any complication, liver transplantation, death, or the end of the clinical trial participation (up to 18 months). On an average, these patients will be monitored three times during the course of this clinical trial.

A full demographic profile, known drug allergies or intolerances, and review of the subject's medical/surgical history will be recorded. If the subject is a woman of childbearing potential, she will have a urine pregnancy test (the results of which will be made available to the subject prior to study initiation).

3.1 Trial Duration

Individual participation in this trial will be limited to ultrasound imaging studies within the two hour period following a trans-jugular liver biopsy (during which their hepatic wedge pressure will be measured

with a pressure catheter as part of the standard of care at TJU and HUP). Patients are monitored for two hours (1 hour post Sonazoid injection). The entire ultrasound imaging protocol will require approximately one hour. Subjects identified in the initial examination as having portal hypertension (by HVPG results) will be monitored by SHAPE for up to 18 months. These subjects typically have surveillance CT or MRI scans every 6 months to check for liver cancer and at those times a repeat SHAPE examination will be performed. Any repeat trans-jugular biopsies performed in this population will also trigger a repeat SHAPE study. Moreover, these patients will have a complete blood test every 3 months and these clinical variables (platelets, albumin, MELD, etc.) as well as any medication will be recorded. Finally, most subjects with portal hypertension will undergo an endoscopy examination every 1 to 2 years [de Franchis 2005; Merkel et al. 2004]. Results of any concomitant imaging study or procedure (including endoscopies) will be noted. The end point for this part of the study will be any one new complication (e.g., liver cancer) or a marked worsening in any complication (e.g., MELD scores), liver transplantation, death, or the end of this clinical trial (after 3 years). On an average, these patients will be monitored three times during the course of this clinical trial. The time to reach the end point will be noted if a new complication or a marked worsening in any complication occurs.

Subject recruitment is expected to last 3 years (April, 2014 – January, 2017). This includes monitoring disease progression and/or treatment response. Analysis and publication of results are expected to take an additional 2 months (January– March, 2017). Volunteer Registry Database forms will be submitted to the National Institute of Health's Office of Regulatory Compliance and Quality at the completion of the research study.

4. TRIAL POPULATION

Trial Population: This trial will consist of up to 300 adults (21 years of age or older) undergoing trans-jugular liver biopsy with HVPG measurement at TJU or at HUP.

Subjects eligible for trial enrollment will be identified by the co-investigators, Dr. Fenkel (at TJU) and Dr. Soulen (at HUP) from their patient population of subjects scheduled for a trans-jugular liver biopsy. The research coordinators for this study will explain the study to the patients at the respective clinical sites. The patient will be given time to consider the risks and benefits of the study and ask questions about participation. The coordinator will review the consent form with the patient and then the patient will be given the form to review. The patient, coordinator, and a study investigator will all sign the consent form. The patient will be given a copy of the signed consent form for their records.

4.1 Inclusion Criteria

All subjects accepted for this trial must fulfill all the following criteria:

- Be scheduled for trans-jugular liver biopsy the day of the ultrasound procedure.
- Be at least 21 years of age.
- Be medically stable.
- If a female of child-bearing potential, must have a negative pregnancy test.
- Be conscious and able to comply with study procedures.
- Have read and signed the IRB-approved Informed Consent form for participating in the study.

4.2 Exclusion Criteria

Subjects who fulfill any of the following conditions or who have had the following procedures will be excluded from this trial:

- Females who are pregnant or nursing.
- Patients not scheduled for trans-jugular liver biopsy
- Patients who have received an investigational drug in the 30 days before study drug administration, or will receive one within 72 h afterwards,.
- Patients with known or suspected right-to-left, bi-directional, or transient right-to-left cardiac shunts
- Patients with pulmonary hypertension or unstable cardiopulmonary conditions
- Patients currently on chemotherapy or with other primary cancers requiring systemic or hepatic loco-regional treatment.
- Patients who are medically unstable, patients who are seriously or terminally ill, and patients whose clinical course is unpredictable. For example:
  - Patients on life support or in a critical care unit.
  - Patients with unstable occlusive disease (e.g., crescendo angina)
  - Patients with clinically unstable cardiac arrhythmias, such as recurrent ventricular tachycardia.
  - Patients with uncontrolled congestive heart failure (NYHA Class IV)
  - Patients with recent cerebral hemorrhage.
  - Patients who have undergone surgery within 24 hours prior to the study sonographic examination.
- Patients with a history of anaphylactic allergy to eggs or egg products, manifested by one or more

of the following symptoms: generalized urticaria, difficulty in breathing, swelling of the mouth and throat, hypotension, or shock. (Subjects with nonanaphylactic allergies to eggs or egg products may be enrolled in the study, but must be watched carefully for 1 h following the administration of SONAZOID).

- Patients with congenital heart defects.
- Patients with severe emphysema, pulmonary vasculitis, or a history of pulmonary emboli.
- Patients with respiratory distress syndrome
- Patients with thrombosis within the hepatic, portal, or mesenteric veins.

Subject identification will be maintained with a study specific alphanumeric code including the institution (TJU or HUP), patient number (001 and onwards at both the sites) and the patient's initials.

5. MEDICATIONS

Sonazoid will be provided by GEHC (Oslo, Norway). An FDA Sponsor-Investigator IND application will be submitted to the FDA and studies will not commence until regulatory approval has been obtained from the FDA as well as the IRB committee for TJU and HUP.

Sonazoid is a sterile non-pyrogenic suspension of microspheres of lipid stabilized perfluorobutane (PFB) for contrast-enhancement, with a median diameter between 2.4 and 3.5 μm and less than 0.1 % larger than 7 μm. Sonazoid is formulated as a powder for injection consisting of lyophilized sucrose entrapping hydrogenated egg phosphatidyl serine (HEPS) PFB microspheres under a PFB headspace. Each milliliter of Sonazoid contains roughly 1.2 billion microspheres.

Sonazoid is supplied as a dry powder within 10 ml sealed vials. The headspace of the vials contains PFB. Three vials with 48 μl of Sonazoid microbubbles (6 ml) will be prepared for each subject by resuspending each vial in 2 ml of injection grade water supplied by GEHC, as described in Appendix B. Infusions will be performed as described in the Instructions for Use protocol supplement provided by GEHC (Appendix B). All materials and supplies used for the infusion procedure will be identical to those described in GE Healthcare's current Sonazoid IND. A diagram of this infusion setup is shown below in Figure 1.

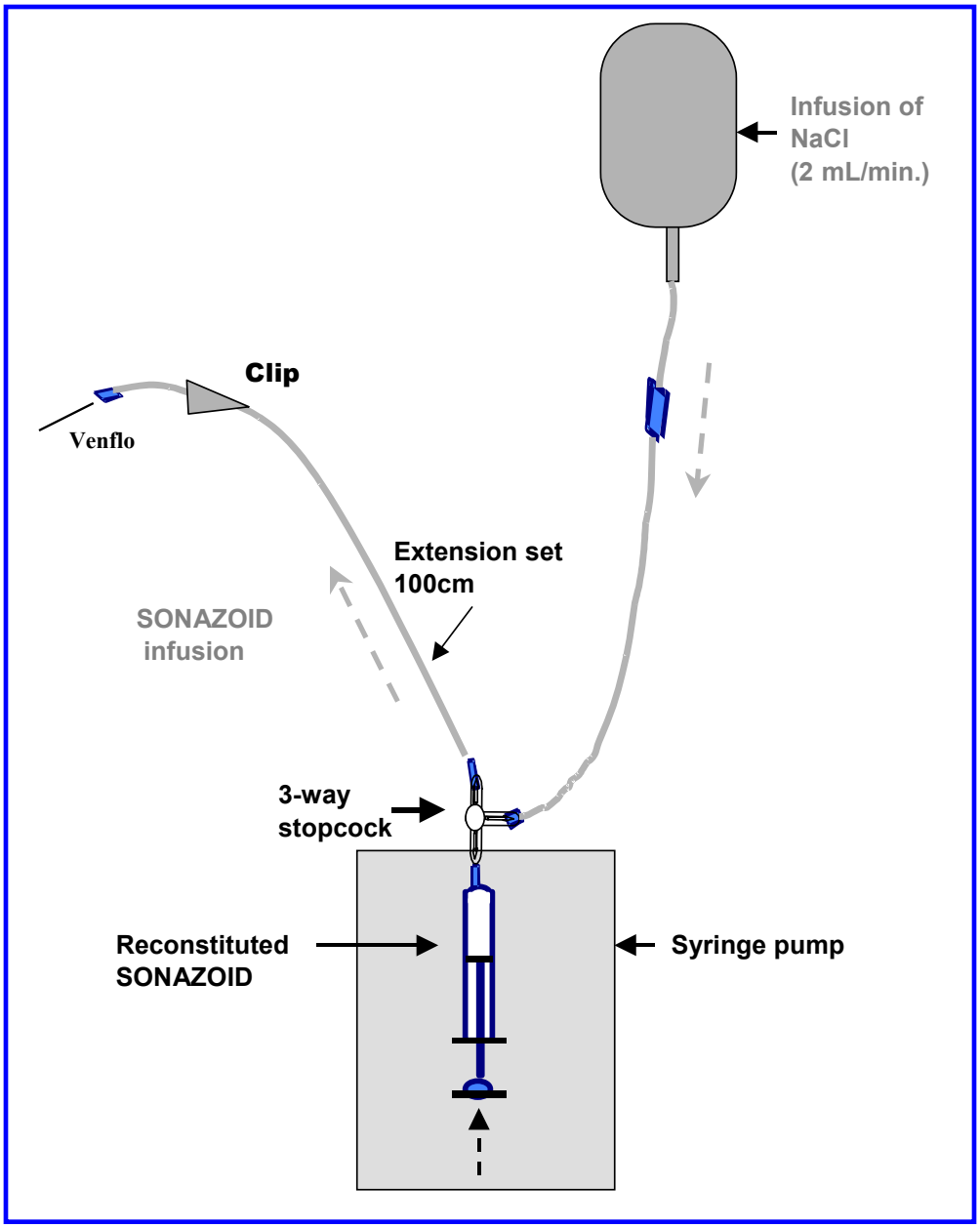

Figure 1: Setup used for Sonazoid infusion for all patients during SHAPE imaging. This setup is discussed briefly in section 5 and in detail in the attached GEHC provided infusion protocol (Appendix B).

Briefly, a 20-22 gauge cannula will be placed in a vein in the subject's right or left arm. A 0.9%NaCl solution will be started and used to fill up the connecting tubes before being connected to a 3-way stopcock. The stopcock will then be connected to the extension tubing leading to the cannula. All three

vials of suspended Sonazoid will be drawn into a 10 ml syringe, placed in a syringe pump at the same level or below the patient, and connected directly to the stopcock. After the stopcock has been opened, the NaCl solution will be infused at a rate of at least 2 ml/min, and Sonazoid will be infused at the desired rate of 0.024 µl/kg body weight/minute (suspension infusion rate of 0.18 ml/kg/hour), but may be reduced if necessary to achieve optimal levels of contrast. This infusion rate was selected based on our group's previous experiences with Sonazoid infusion in human subjects in general [Halpern et al. 2002; Landmark et al. 2008] and in SHAPE portal hypertension subjects in particular [Eisenbrey et al. 2013]. This dosing scheme is designed to avoid (or limit) the number of cases that has to be discarded due to insignificant enhancement in the hepatic veins (as in our pilot study; Eisenbrey et al. 2013). This dosage is based on GEHC's previous experience of dosing Sonazoid by infusion. The maximum amount of microbubbles infused over 15 minutes (0.36 µl/kg) is well below the maximal tested dose which was still found safe in Phase I clinical trials (2.7 µl of microbubbles/kg in PBI 0001 as detailed in the Sonazoid Investigator's Brochure).

Sonazoid will be stored in a secure cabinet, with only the study investigators and research personnel having access. New vials must be prepared for each subject's contrast administration. Unused drug and empty vials will be properly disposed of after reconciling in the log of study drug.

5.1 Contraindications

Sonazoid should not be administered to patients with known or suspected hypersensistivity to egg phosphatidyl serine. Patients with a history of anaphylactic allergy to eggs or egg products will be excluded from the study.

The safety of ultrasound contrast agents in patients with cardiac shunts has recently been called into question. Therefore, patients with known or suspected right-to-left, bi-directional, or transient right-to-left cardiac shunts will be excluded from this study.

The safety of Sonazoid in patients with 1) severe emphysema, pulmonary vasculitis or a history of pulmonary emboli; and 2) respiratory distress syndrome has not been studied. Therefore, patients with any of these conditions will be excluded from participation. Additional exclusion criteria are listed in section 4.2.

5.2 Randomization

This is a non-randomized trial; therefore, no randomization procedure is required.

5.3 Blinding and Unblinding Methods

This is an open-label trial; therefore, no blinding or unblinding procedures for the trial drug are required.

5.4 Storage

Sonazoid vials will be stored in a secure cabinet, with only the study investigators and research personnel having access. The study researchers will be responsible for drug reconstitution and inventory control. Sonazoid is stable for 8 hours after reconstitution. If the agent is suspended and not used within 8 hours it will be discarded.

6. TRIAL PROCEDURES

6.1 Screening Assessments

Screening assessments will be performed within 4 weeks prior to the administration of Sonazoid. All subjects will receive a written consent form and a verbal explanation of the trial by the investigator and/or the research study coordinator and will be asked to sign the written informed consent. Trial participants will have the presence of inclusion criteria and absence of exclusion criteria verified by providing a comprehensive medical history, which will include screening assessments for their scheduled trans-jugular liver biopsy. Patients with cardiac shunts will be excluded from the study.

6.2 Treatment Administration

Administration of Sonazoid will be performed under direct supervision of a medical doctor. Emergency services (e.g., a crash cart) will be available within the hospital in case of any acute adverse reactions. Additionally, resuscitation equipment will be in immediate proximity to the patient during Sonazoid infusion. Patients will be monitored for AEs post Sonazoid treatment for a minimum of one hour. Trained CPR personnel and resuscitation equipment will be in attendance during this monitoring period. A dose of three vials with 48 µl of Sonazoid microbubbles (6 ml total after resuspension) will be prepared for each subject by resuspending each vial in 2 ml of injection grade water supplied by GEHC. Infusions will be performed as described in the GEHC protocol supplement (Appendix B), and shown in Figure 1. All materials and supplies used for the infusion procedure will be identical to those described in GE Healthcare's current Sonazoid IND. Briefly, an IV cannula will be placed in a vein in the subject's right arm. A 0.9%NaCl solution will be started and used to fill up the connecting tubes before being connected to a 3-way stopcock. The stopcock will then be connected to the extension tubing leading to the stopcock the cannula. All three vials of suspended Sonazoid will be drawn into a 10 ml syringe, placed in a syringe pump at the same level or below the patient, and connected directly to the stopcock. After the stopcock has been opened, the NaCl solution will be infused at a rate of at least 2 ml/min, and Sonazoid will be infused at the desired rate of 0.024 µl/ kg body weight/ minute (suspension infusion rate of 0.18 ml/kg/hour), but may be reduced if necessary to achieve optimal levels of contrast . This infusion rate was selected based on our group's previous experiences with Sonazoid infusion in human subjects in general [Halpern et al. 2002; Landmark et al. 2008] and in SHAPE portal hypertension subjects in particular [Eisenbrey et al. 2013]. This dosing scheme is designed to avoid (or limit) the number of cases that has to be discarded due to insignificant enhancement in the hepatic veins (as in our pilot study; Eisenbrey et al. 2013). This dosage is based off the GEHC instructions for storage and well below the maximal tested dose which was still found safe in Phase I clinical trials (2.7 µl of microbubbles/kg in PBI 0001 as detailed in the Sonazoid Investigator's Brochure).

6.3 Trial Assessments

6.3.1 Medical History

A full demographic profile, known drug allergies or intolerances, and a review of the subject's medical/surgical history will be recorded. If the subject is a woman of childbearing potential, she will have a urine pregnancy test (the results of which will be made available to the subject prior to study initiation). Emergency services (e.g., a crash cart) will be available within the hospital in case of any acute adverse reactions. Adverse events will be monitored during the entire procedure.

6.3.2 Ultrasound Imaging

The ultrasound examinations will be performed by a qualified sonographer. Procedures and equipment for this trial will be used in accordance with typical clinical procedures. All trial procedures will be conducted in accordance with Good Clinical Practice. A modified Logiq 9 scanner (GE Healthcare, Milwaukee, WI) with a broad bandwidth curvi-linear array will be used in to obtain RF data (for SHAPE measurements) within the hepatic and portal veins. Both the experimental machine and software will be labeled as an investigational device with a GE provided label prior to beginning any clinical experiments (21 CRF 812.5). Ultrasound imaging will be performed at a transmit frequency of 2.5 MHz and the subharmonic obtained at 1.25 MHz. Using this setup, acoustic pressure amplitudes will all be below 1.21 MPa peak negative pressure, 2.19 MPa peak positive pressure. The SHAPE algorithm will be activated and the acoustic power will be adjusted to produce the maximum change in subharmonic amplitudes (i.e., maximizing the sensitivity of SHAPE). Subharmonic data from the microbubbles (i.e., SHAPE) will be acquired in 5 s segments during the infusion of the Sonazoid suspension. All measurements will be repeated three times.

During the examination, the subharmonic pressure estimates will be displayed on the Logiq 9 scanner, these estimates will be noted and the data will be stored on a PC. Data will be compared to the

transcatheter portal pressure measurements, which comprise the free and wedged hepatic venous pressure (FHVP and WHVP, respectively) and the hepatic vein pressure gradient (HVPG; measured as the difference between the WHVP and FHVP), which are acquired as part of standard of care for all patients undergoing trans-jugular liver biopsy at TJU and HUP.

6.4 Efficacy Assessments

The findings of SHAPE will be correlated to the invasive catheter based measurements of portal vein blood pressure (i.e., HVPG) using least squares, multiple linear regression analysis with robust standard errors. Comparisons of sensitivities, specificities and accuracies for diagnosing portal hypertension with SHAPE will be conducted with an unpaired Student's t-test (using HVPG measurements as the reference standard). Receiver operating characteristic (ROC) curves will be computed using a continuous scale with HVPG cutoff levels of 10 and 12 mmHg. The optimal operating point (OOP) will be determined as the point closest to '0,1' on the ROC curve (representing 100% sensitivity and 100% specificity). All clinical variables (e.g., concomitant imaging results, presence of cancer or ascites, blood tests, fibrosis and MELD scores etc.) will also be compared to the SHAPE values.

6.5 Safety Assessments

Emergency services (e.g., a crash cart) will be available within the hospital in case of any acute adverse reactions. Adverse events will be monitored during the entire procedure. Specifically, the patient will be monitored with non-leading questions to monitor the patient for the occurrence of transient side effects that are described below.

6.5.1 Risks/Benefits Assessment.

The known risks from the administration of Sonazoid are minimal. Transient side effects that have been described as possibly related to Sonazoid administration include headache (3.6%, 62/1699), chest pain (2.3%, 39/1699), abdominal pain (1.5%, 25/1699), diarrhea (1.5%, 25/1699), and nausea (1.6%, 28/1699). The majority of adverse events were mild to moderate in severity (92.6%, 652/704).

The use of an intravenous needle and the fluids given through the needle may cause minor discomfort, bleeding under the skin (bruise), and possible infection at the site of needle insertion.

Clinically significant adverse effects from the administration of Sonazoid are unlikely. The use of contrast with the new ultrasound imaging techniques is expected to provide significantly more information than conventional ultrasound techniques. This may lead to a non-invasive method for measurement of hepatic pressure.

To minimize and/or eliminate risks a nurse will be present during the entire procedure. Emergency services (e.g., a crash cart) will be available within the hospital in case of any acute adverse reactions. Adverse events will be monitored during the entire procedure.

The risk benefit ratio is low. Based on the available nonclinical and clinical safety data and the anticipated dose levels of Sonazoid that will be used in this study, safety concerns are minimal. The potential side effects related to Sonazoid administration are described above and listed in greater detail in the investigator's brochure.

6.6.2 Adverse Events (AE)

An AE includes any condition that was not present prior to trial treatment, but appeared following initiation of trial medication; any condition that was present prior to trial treatment, but worsened during trial medication; or any condition, of which the subject has a history, that was not present prior to trial medication initiation but reappeared following administration of Sonazoid. This would include conditions that are likely to be associated with an underlying or intermittent disease (e.g., angina, flu, etc.).

The subjects will be monitored for AEs during the entire procedure. All AEs, including observed or

volunteered problems, complaints, signs or symptoms, and diagnoses, occurring from the initiation of Sonazoid dosing until the completion of the Sonazoid administration will be recorded on a serious or non-serious AE data form, whether or not associated with the use of the trial medication. GEHC will be informed of all AEs. In addition all unexpected and serious adverse events (SAEs) are reported to the TJU or HUP IRB (as applicable) and to the FDA. The investigator is required to submit all unexpected and serious adverse events to the TJU or HUP IRB (as applicable) within 48 hours, and to GEHC. Fatal adverse events related to treatment which are unexpected must be reported within 24 hours to the TJU or HUP IRB (as applicable) and to GEHC. Fatalities not related to the study drug/device must be reported within 5 days. The PI of the study has previous experience running ultrasound clinical trials and will serve as the study sponsor. He will be responsible for ensuring all FDA requirements are met and all AE are properly reported.

The AE forms will include: subject identification number and initials; subject's date of birth, gender, and ethnicity; name of the institution, date of Sonazoid administration; signs/symptoms and severity; date of onset; date of resolution or death; relationship to the study drug; action taken; concomitant medication(s) including dose, and route and duration of treatment.

Whenever possible, the AE will be evaluated and reported as a diagnosis rather than individual signs and symptoms. If a definitive diagnosis is not possible, the individual signs and symptoms will be recorded. The investigator(s) will evaluate and note the duration, intensity, and relationship to (association with) the Sonazoid administration, the action taken, and the determination of seriousness for each AE.

INTENSITY OF AEs

The intensity of the AE will be characterized as mild, moderate, or severe.

Mild AEs are usually transient, require no special treatment, and do not interfere with the subject's daily activities.

Moderate AEs traditionally introduce a low level of inconvenience or concern to the subject and may

interfere with daily activities but are usually ameliorated by simple therapeutic measures.

Severe AEs interrupt a subject's usual daily activity and typically require systemic drug therapy or other treatment.

When the intensity of the AE changes over time, the maximum intensity will be recorded.

RELATIONSHIP TO SONAZOID ADMINISTRATION

The relationship or association of the AE to the Sonazoid administration will be characterized as "unlikely," "possible," or "probable." A relationship assessment will be performed by the investigator to determine if an AE is attributable to Sonazoid and will be recorded on a data form. The investigator will refer to the Sonazoid investigator brochure for assistance in determining AE relationship.

An "unlikely" relationship indicates that there is little or no chance that Sonazoid caused the reported AE; other conditions, including concurrent illnesses, progression or expression of the disease state, or a reaction to a concurrent medication, appear to explain the reported AE.

A "possible" relationship indicates that the association of the AE with Sonazoid is unknown. However, the AE is not reasonably attributed to any other condition.

A "probable" relationship indicates that a reasonable temporal association exists between the AE and Sonazoid administration and, based upon the investigator's clinical experience, the association of the event with the trial medication seems likely.

SERIOUS ADVERSE EVENTS

A "serious" AE (SAE) is defined as a significant clinical hazard, contraindication, or precaution that:

- Results in death
- Is life-threatening (In the opinion of the investigator, there is an immediate risk of death from the AE as it occurred. This does not include an AE that had it occurred in a more serious form may have caused death.)

- Results in a persistent or significant temporary disability/incapacity defined as a substantial disruption of a person's ability to conduct normal life functions
- Results in or prolongs an existing in-patient hospitalization (an overnight stay in the hospital, regardless of length) [Note: A hospitalization for an elective procedure or treatment which is not associated with an AE, hospitalization for a pre-existing condition which did not worsen, and hospitalization for reasons of convenience or observation, do not constitute an SAE.]
- Is a congenital anomaly/birth defect (in offspring of a subject taking the trial medication, regardless of time to diagnosis)
- Is an important medical event that may not result in death, be life-threatening, or require hospitalization but based upon the appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed for the definition of a serious adverse experience.

The designated medical monitor will review all serious and unexpected adverse events associated with the protocol and provide an unbiased written report of the event within 10 calendar days of the initial report. At a minimum, the medical monitor will comment on the outcomes of the adverse event and relationship of the event to the Sonazoid administration. The medical monitor will also indicate whether he concurs with the details of the report provided by the principal investigator.

A copy of the SAE will be retained on file with the respective subject's data forms.

6.7 End-of-Treatment and End-of-Trial Evaluations

6.7.1 Discontinuation of Subjects

Subjects will be free to discontinue trial participation at any time. The investigator will also discontinue any subject from the trial if, in the investigator's opinion, it is not safe for the subject to continue. The date the subject is withdrawn from a treatment and/or from the trial and the reason for discontinuation will be recorded on the CRF. For portal hypertension patients that will be monitored by SHAPE for up to 18 months, the end point will be any one new complication (e.g., liver cancer) or a marked worsening in any complication (e.g., MELD scores) or the end of their clinical trial participation (up to 18 months). The time to reach the end point will be noted if a new complication or a marked worsening in any complication occurs. The time to reach the end point will be noted.

Trial participation will be considered completed if the subject has met all of the following trial

requirements:

- Has received at least one infusion of Sonazoid
- Has undergone the complete ultrasound imaging study as described in this protocol

If a subject's participation in the trial is interrupted for any reason (e.g., because of an AE or if the subject is lost to follow-up) and the subject has met the criteria described above for completing the trial, the subject's trial participation will be considered completed. If a subject's trial participation is interrupted for any reason by the subject's or investigator's choice and the subject has not met all of the criteria listed above, then the subject will be considered a discontinued subject.

7. DATA MANAGEMENT AND STATISTICAL ANALYSES

7.1 Data Management

Data forms will be completed for all subjects enrolled in the trial. The patient study files will be stored in a secure file cabinet and maintained by the research study coordinators at each institution (TJU and HUP). Patient study files will be kept for 7 years after the completion of the study. The PI of the study has previous experience running ultrasound clinical trials and will serve as the study sponsor. He will be responsible for ensuring all FDA requirements are met and all AE are properly reported.

The final data will be entered into a database. The investigator will be responsible for management of the database. The database will be maintained within an organized and secure directory system.

7.2 Statistical Analyses

7.2.1 Hypotheses

H_1: Can patients with HVPG of 10 mmHg or greater be identified using SHAPE? The fundamental hypothesis is that a sensitivity of 89% and a specificity of 81 % can be achieved by using SHAPE measurements to identify patients with HVPGs of 10 mmHg or greater.

H_2: A correlation coefficient of 0.82 will be achieved between in vivo SHAPE and HVPG measurements.

H_3: A correlation coefficients above 0.90 will be achieved between SHAPE and repeat HVPG results.

H_4: SHAPE will significantly differentiate responders from non-responders using clinical outcomes as the reference standard.

7.2.2 Analysis of Results

The findings of SHAPE will be correlated to the invasive catheter based measurements of portal vein blood pressure (i.e., HVPG) using least squares, multiple linear regression analysis with robust standard errors. Receiver operating characteristic (ROC) curves will be computed using a continuous scale with HVPG cutoff levels of 10 and 12 mmHg. The optimal operating point (OOP) will be determined as the point closest to '0,1' on the ROC curve (representing 100% sensitivity and 100% specificity). All clinical variables (e.g., concomitant imaging results, presence of cancer or ascites, blood tests, fibrosis and MELD scores etc.) will also be compared to the SHAPE values. When both types of variables are ordinal or continuous, correlations will be calculated. When one type of variable is nominal and one continuous non-parametric rank order tests such as Mann-Whitney U-tests or Kruskal-Wallis tests will be performed [Rosner 1990]. All of the statistical analyses proposed for the human clinical trial will be repeated split by racial and ethnic groups to determine if clinically important race/ethnicity differences exist in the ability of SHAPE to diagnose portal hypertension.

All analyses and computations will be performed using NCSS/PASS 2008 and Stata 12.0 (Stata Corporation, College Station, TX), while the study database will be designed and implemented in Filemaker Pro 10.0 (Filemaker Inc, Santa Clara, CA). This database will contain all patient information (except names and other identifiers), including results of biopsies, HVPG and SHAPE measurements as well as the clinical variables.

7.2.3 Sample Size Calculation

As the most clinically important aim, the sample size analysis is based on specific aim 1 where each patient represents an independent entity. Based on our pilot study, the sensitivity and specificity of

SHAPE for the diagnosis of portal hypertension (i.e., HVPG > 10 mmHg) is 89% and 88%, respectively [Eisenbrey et al. 2013]. However, the confidence intervals achieved in the pilot study were (due to the limited sample size) very wide (worst case scenario: 52–100%). Hence, a power analysis was performed using NCSS/PASS 2008 (NCSS, East Kaysville, UT) to estimate the number of cases required to produce clinically acceptable 95% two-sided confidence intervals (for one correlation) [Newcombe 1998]. Table 2 below illustrates sample size calculations for the number of independent observations needed in the study population based upon 95% two-sided confidence intervals ranging from 0.06 to 0.10 for one correlation. A sample size between 259 and 278 subjects will provide 95% confidence intervals from 0.84 to 0.92 (i.e., a width of 0.08). A similar analysis was conducted based on the correlation between SHAPE and HVPG of 0.82 obtained in our pilot study [Eisenbrey et al. 2012a]. In that case to demonstrate that SHAPE can accurately measure HVPG values with a correlation coefficient of 0.82 requires between 264 and 343 patients (for confidence interval widths of 0.08 and 0.07, respectively). Hence, we selected a sample size for this study of 300 subjects.

Table 2: Sample size calculations for different 95% confidence intervals in the human clinical trial.

Target width	Proportion (R)	Lower limit	Upper limit	Sample size (N)
0.06	0.88	0.848	0.908	483
0.06	0.89	0.857	0.917	450
0.07	0.88	0.842	0.912	359
0.07	0.89	0.851	0.921	335
0.08	0.88	0.836	0.916	278
0.08	0.89	0.845	0.925	259
0.09	0.88	0.830	0.920	222
0.09	0.89	0.839	0.929	207
0.10	0.88	0.824	0.923	181
0.10	0.89	0.833	0.933	170

The Interventional Radiology Divisions at Thomas Jefferson University (TJU) and the Hospital of the University of Pennsylvania (HUP) performs approximately 85 and 100 transjugular liver biopsy procedures with pressure measurements per year, respectively. The patient population of this project will reflect the population demographics found at major American urban academic health centers. The overall hospital demographics for TJU and HUP include 66% Caucasian, 30% African American, and 4% Asian, with 10% representing Hispanic patients. The goal is to enroll 300 patients over the last 3 years of this project, which means we are aiming for a recruitment rate of approximately 54%.

7.2.4 Disposition of Software

Investigational software containing the modified SHAPE algorithm will be removed from the Logiq 9 scanner upon completion of the study to avoid the investigational device being used for any unapproved investigations. At the conclusion of the study, the developed software will be saved on CD for future experimentation. These investigations may ultimately further the development of SHAPE technology as a pressure monitoring system. However, under no circumstances with the software be used in a clinical environment (i.e., for human studies) without GE's consent. This will be ensured by the use of a GE controlled password for installation of experimental software which is currently in use and will expire at the conclusion of the study.

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APPENDIX A - INVESTIGATOR OBLIGATIONS

A. Institutional Review Board (IRB) and Human Subjects Research Review Board (HSRRB) Review/Approval

The protocol and informed consent for this study must be reviewed and approved by an appropriate IRB and HSRRB prior to enrollment of participants in the study. It is the responsibility of the investigator to assure that all aspects of the ethical review are conducted in accordance with FDA Regulations 21 CFR Part 56. A letter documenting the IRB and HSRRB approval which specifically identifies the study/protocol must be obtained by the investigator prior to initiation of the study. Amendments to the protocol will be subject to the same requirements as the original protocol. The HSRRB must review and approve each modification to the study prior to implementation.

A progress report with a request for re-evaluation and re-approval will be submitted by the investigator to the IRB and HSRRB at intervals required by the IRB, and not less than annually.

After completion or termination of the study, the investigator will submit a final report to the IRB. This report should include: deviations from the protocol, the number and types of participants evaluated, the number of participants who discontinued (with reasons), results of the study, if known, and all AEs, including deaths.

B. Informed Consent

Signed, written informed consent which conforms to FDA Regulation 21 CFR Part 50, must be obtained from each participant prior to entering the study. Each participant will be provided a written consent form and verbal information in an understandable manner which describes the nature and duration of the study. The research study coordinator or the investigator will conduct the informed consent interview in a private examination room. The potential subject will be allowed to discuss the study with the investigator, research study coordinator, or any persons who may have accompanied the potential subject. Additionally, the participant must be allowed adequate time to consider the potential risks and benefits associated with his participation in the study. The research study coordinator will sign the informed consent as the person conducting the consent interview.

C. Data Reporting and Data Forms

Data reflecting participant's experiences with the study will be recorded on CRFs by the investigator.

D. Records Retention

All records pertaining to the conduct of the clinical study, including CRFs, informed consent forms, source documents, and other study documentation must be retained for seven (7) years after the end of the study.

Other study documentation includes all protocols and amendments, drug supply receipt, dispensing and final disposition records, IRB correspondence and approvals, signed consent forms, a blank copy of study consent forms, Form 1572, curriculum vitae or biosketches of members of the research team including the medical monitor, HSRRB correspondence and approval, and Statement of Investigator forms.

Source documents include all original records of observations, results, and activities necessary to reconstruct and evaluate the study. Source documents include but are not limited to laboratory reports, electrocardiogram tracings, X-ray films, ultrasound images, subject diaries, subject progress notes, hospital charts, appointment books, radiologic reports or pharmacy records, and any other records or reports of procedures performed during the study. Source documents also may include copies of the CRF or sponsor supplied worksheets when original information is recorded directly onto these forms.

Whenever possible, an original recording of an observation should be retained as the source document. However, a photocopy of a record is acceptable provided it is legible and is a verified copy of the original document.

E. Deviation from the Protocol

The investigator will not deviate from the protocol without prior written approval from the IRB and the HSRRB. In medical emergencies, the investigator will use medical judgment and remove the participant from immediate hazard. The HSRRB and the IRB will be notified regarding the type of emergency and course of action taken. Any other changes to or deviations from the protocol will be made as an amendment to the protocol. The amendment must be submitted for review and approval to the local IRB and the HSRRB for review and approval.

F. Roles and Responsibilities of Study Personnel

Flemming Forsberg, Ph.D., Professor of Radiology and Director of Ultrasound Physics, will serve as Principal Investigator on this project. He will be responsible for the scientific goals of the project. Dr. Forsberg will oversee patient recruitment, informed consent, ultrasound studies, and the data entry and statistical analyses. He will also supervise the SHI data acquisition from patients. Dr. Forsberg will also prepare any manuscript(s) resulting from this grant.

Jonathan Fenkel, M.D., Professor of Medicine, Pharmacology And Experimental Therapeutics Division of Gastroenterology and Hepatology will assist with the patient recruitment, interpret hepatic pressure results and advise on clinical issues.

Collete Shaw, M.D., Research Professor of Radiology will assist with the patient recruitment, interpret ultrasound images and advise on clinical issues.

John Eisenbrey, Ph.D., Assistant Professor of Radiology will aid in patient recruitment, informed consent, ultrasound studies, and the data entry and statistical analyses.

Michael Soulen, M.D., Professor of Radiology at the Hospital of the University of Pennsylvania (site #2), Principal Investigator, will oversee all aspects of project at site #2.

Chandra Sehgal, Ph.D., Professor and Director of Ultrasound Research, Site #2 Investigator, will oversee all Ultrasound activities.

Stephen Hunt, M.D., Ph.D., Radiology Clinical Instructor, Site #2 Investigator, who will assist the PI with clinical procedures and project oversight.

Susan Schultz, R.D.M.S., Radiology research sonographer who will perform ultrasound studies.

Suzanne Sweeney, B.S.N., R.N., Investigator/nurse manager, will perform and manage IV infusions.

Bradford Dungan, B.S.N., R.N., Investigator/nurse will perform and manage IV infusions under Suzanne Sweeney.

Evelyn Stainthorpe, B.S., C.C.R.C., will coordinate all aspects of the project under Dr. Soulen including IRB submission and reporting, recruitment, consent and data collection and submission.

Signature of PI: _____
Flemming Forsberg, PhD

APPENDIX B - GE PROVIDED STUDY SPECIFIC PROTOCOL SUPPLEMENT ON SONAZOID SUSPENSION AND INFUSION

INSTRUCTION FOR STORAGE AND USE OF SONAZOID™ POWDER FOR INJECTION, 16 µL MICROBUBBLES PER VIAL

Emergency drugs and equipment should be present in or nearby the examination room for immediate treatment, should a serious adverse event occur.

1 CONTENTS OF PACKAGE

- SONAZOID™ Powder for Injection, 16 µL microbubbles per vial;
- Sterile water for reconstitution of Sonazoid™
- Sterile NaCl 0.9 % for i.v. infusion, Baxter
- 1 Plastic syringe 10 mL for reconstituted product, Becton Dickinson
- 5 Plastic syringes 2 mL with hypodermic needles
- Venflon™ i.v. cannula 22G (Luer-Lock)
- CODAN Chemospike (contains 0.20 µm air filter and 5 µm liquid filter), 5 spikes
- Connecta™ Plus3, 3-way stopcock , Becton Dickinson
- Original Perfusor®-Leitung MR, B. Braun, 150 cm
- Administration set, R87 plus, Ohmeda GmbH & Co, 180cm

2 STORAGE OF SONAZOID™

2 - 30°C / 35.6 - 86°F protected from light.

3 RECONSTITUTION OF PRODUCT

An illustration of the reconstitution procedure is shown in Figure 1.

3.1 Perforate the stopper of the SONAZOID™ vial with the provided *CODAN Chemospike.*
3.2 Remove protective cap from the syringe port of the *Chemospike.*
3.3 Using a 2-mL syringe, add **2 mL** s*terile water* through the *Chemospike.*
3.4 With the syringe remaining attached to the *Chemospike*, **immediately** shake the product for 1 minute to ensure a homogeneous product.
3.5 Withdraw the product into the syringe and re-inject the product back into the vial again. This is to avoid dilution of the product due to the dead-space volume in the *Chemospike.*
3.6 Remove the syringe from the syringe port and reattach the protective cap. The concentration of the reconstituted product is 8 µL microbubbles/mL.
3.7 Repeat the reconstitution procedure for the necessary number of vials as stated in Table 1.

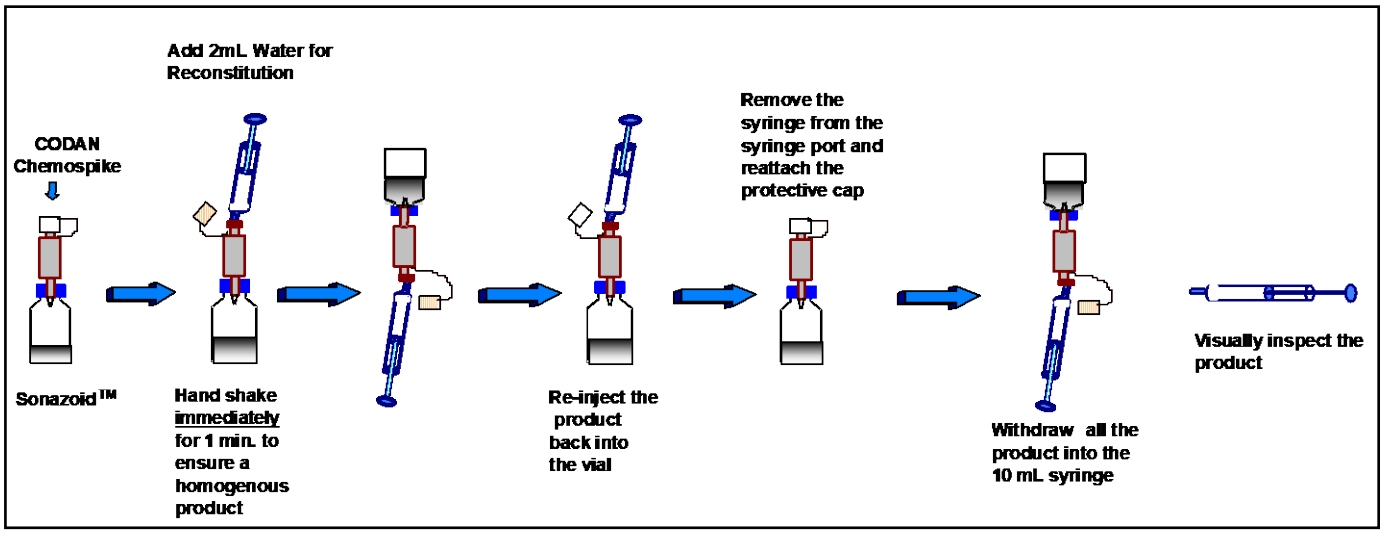

Figure 1 Reconstitution procedure for Sonazoid™

4 APPEARANCE OF RECONSTITUTED PRODUCT

The reconstituted product appears as a milky white dispersion.

5 STORAGE OF RECONSTITUTED PRODUCT

If the reconstituted product is not used immediately, leave the vial at rest in an upright position. Store between 15-25°C.

NOTE - The infusion procedure must be started **within 1 hour** from reconstitution of the product.

NOTE - Always shake the product for 10-15 seconds before withdrawal from the vial.

6 ADMINISTRATION PROCEDURE

6.1 Infusion set up

The infusion set up and procedure is illustrated in Figures 2 and 3.

6.1 Place the cannula in a vein in the subjects right upper limb (preferably), as the venous circulation of the left arm might be compromised during imaging procedures if the subject is placed in the left decubitus position.

7.1 Connect the 3-way stopcock to the 150 cm extension tube, and connect it to the administration set.

NOTE - The stopper of the third port on the 3-way stopcock should not be removed yet.

8.1 Put the 3-way stopcock in the right position (Figure 3A) and start the infusion of 0.9% NaCl, fill up the tubes and connect the outlet to the Venflon™ cannula. **Set the Infusion rate**: NLT 2.0 mL/min (= NLT 40 drops/min with the submitted infusion set).

9.1 Close the stopcock as shown in Figure 3B in order to temporarily stop the infusion.

10.1 **Place the syringe pump in a vertical position so that the syringe is at the same level or below the patient.** This is important in order to maintain a homogenous product during infusion.

6.2 Infusion procedure:

For each subject, reconstitute the number of vials of Sonazoid™ given in Table 1.

6.2.1 Shake the reconstituted product **immediately before** product withdrawal.

6.2.2 Just before start of infusion, draw reconstituted Sonazoid™ from all three vials into the 10 mL syringe via the *CODAN Chemospike.*

6.2.3 Remove air from the 3-way stopcock by removing the stopper of the third port (Figure 3C). Attach the 10 mL syringe containing the homogenous contrast agent to the 3-way stopcock according to Figure 1 and Figure 3D.

6.2.4 **Open the 3-way stopcock** (Figure 3E) so that all ports are open and check the saline infusion rate (NLT 40 drops/min).

6.2.5 **Place the syringe in the vertically positioned pump in an upright position (syringe outlet up**), **and start infusion immediately** (as described below).

NOTE - Make sure that the 3-way stopcock is open.

NOTE - Check that the correct syringe is recognized by the pump (10 mL BD Plastipak).

NOTE - Ensure that the tubing has no bends/kinks that may cause obstruction.

6.2.6 Enter the syringe pump rate according to the subject's b.w., see Table 1.

6.2.7 START PUMP.

NOTE - Maximum infusion time is **15 minutes**. The syringe contains a calculated overage of product in order to ensure homogeneity. The overage should be discarded after infusion is finished.

NOTE - The infusion rate of NaCl should always be NLT 2 mL/min (= 40 drops/min) during Sonazoid™ infusion, to ensure a homogeneous infusion of Sonazoid.

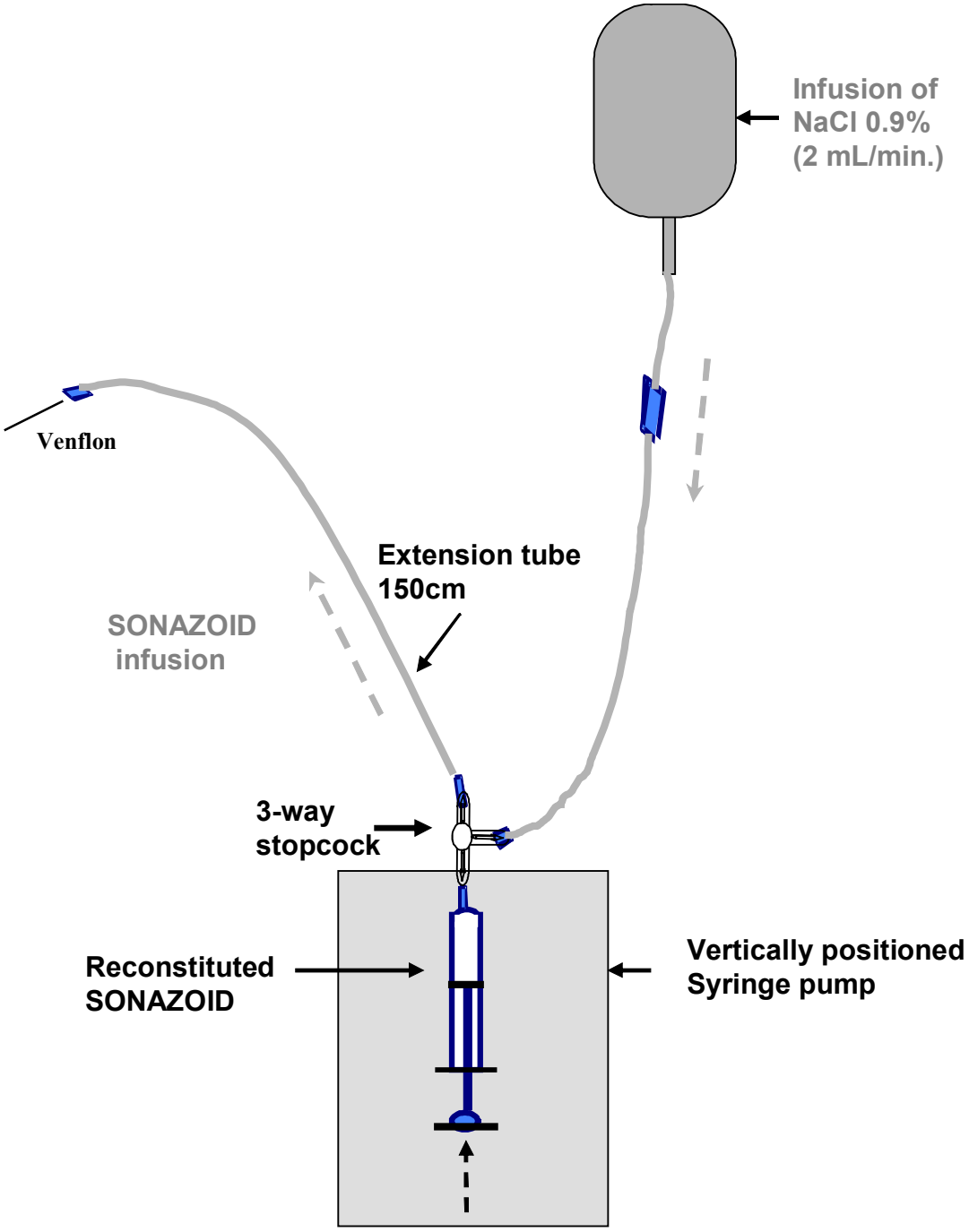

Figure 2 The infusion set up

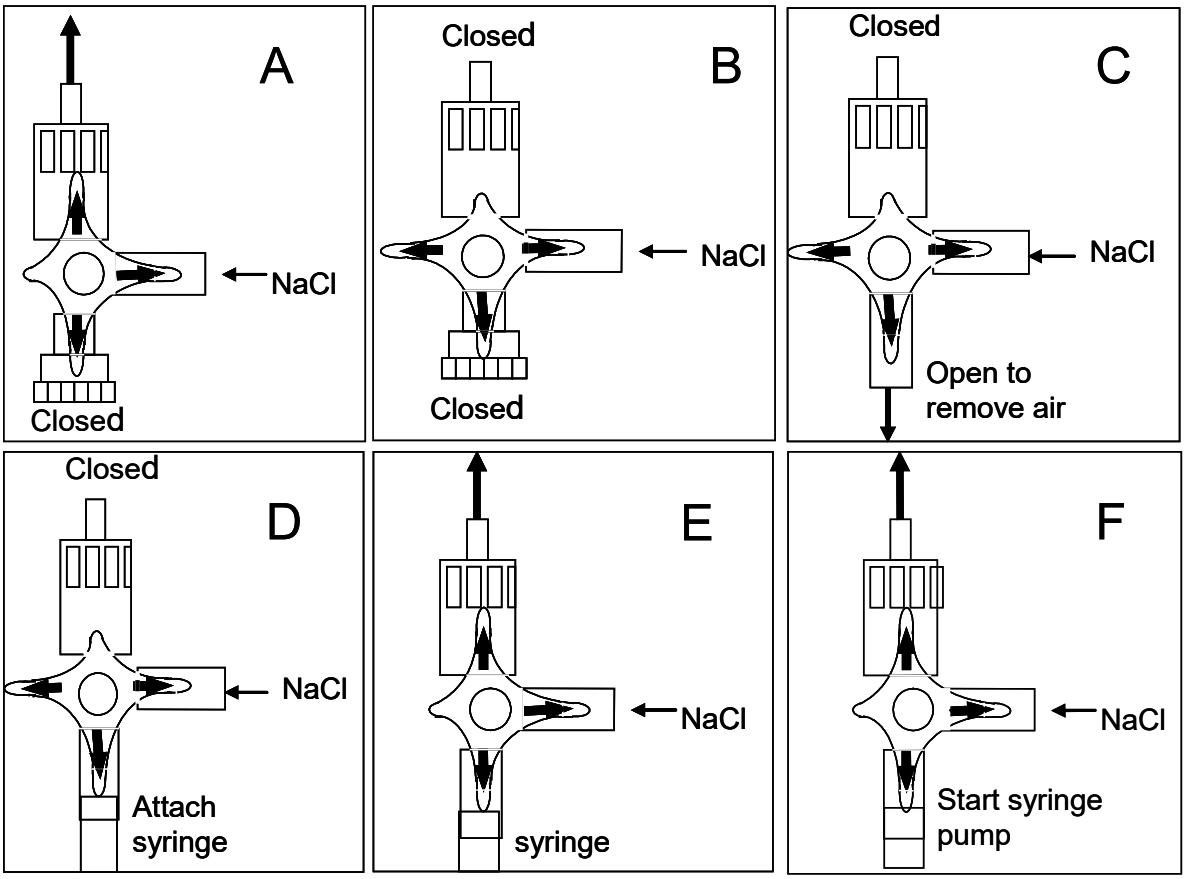

Figure 3 Positions of the 3-way stopcock as described in the text

Table 1 Infusion rate of reconstituted Sonazoid™ 8 µL microbubbles/mL.

BODY WEIGHT			INFUSION RATE = SYRINGE PUMP RATE (mL/hour)
Kg	Lbs.	Number of Sonazoid™ vials to be withdrawn into the 10 mL syringe[1]	
40	88	3	3.6 mL/hour
45	99	3	4.1 mL/hour
50	110	3	4.5 mL/hour
55	121	3	5.0 mL/hour
60	132	3	5.4 mL/hour
65	143	3	5.9 mL/hour
70	154	3	6.3 mL/hour
75	165	3	6.8 mL/hour
80	176	3	7.2 mL/hour
85	187	3	7.7 mL/hour
90	198	3	8.1 mL/hour
95	209	3	8.6 mL/hour
100	220	3	9.0 mL/hour
105	231	3	9.5 mL/hour
110	242	3	9.9 mL/hour
115	253	3	10.4 mL/hour
120	264	3	10.8 mL/hour
125	275	3	11.3 mL/hour
130	286	3	11.8 mL/hour
135	297	3	12.2 mL/hour
140	308	3	12.6 mL/hour
145	319	3	13.1 mL/hour
150	330	3	13.5 mL/hour

Calculation of infusion rate:

Table 1 lists the infusion rate per hour, which corresponds to the syringe pump rate for a body weight range of 40-130 kg.

Dose of 0.012 µL microbubbles/kg bw/minute corresponds to 0.72 µL microbubbles/kg bw/hour:

$$\text{Infusion rate (mL/hour)} = \frac{0.090 \times \text{body weight in kg}}{}$$

[1]The calculation of number of vials includes a necessary overage of minimum 2mL. The maximum extractable volume from each vial should be drawn into the 10 mL syringe.