Chronic Liver Disease: Noninvasive Subharmonic Aided Pressure Estimation of Hepatic Venous Pressure Gradient¹

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Purpose:

To compare subharmonic aided pressure estimation (SHAPE) with pressure catheter-based measurements in human patients with chronic liver disease undergoing transjugular liver biopsy.

Materials and Methods:

This HIPAA-compliant study had U.S. Food and Drug Administration and institutional review board approval, and written informed consent was obtained from all participants. Forty-five patients completed this study between December 2010 and December 2011. A clinical ultrasonography (US) scanner was modified to obtain SHAPE data. After transjugular liver biopsy with pressure measurements as part of the standard of care, 45 patients received an infusion of a microbubble US contrast agent and saline. During infusion, SHAPE data were collected from a portal and hepatic vein and were compared with invasive measurements. Correlations between data sets were determined by using the Pearson correlation coefficient, and statistical significance between groups was determined by using the Student *t* test.

Results:-

The 45 study patients included 27 men and 18 women (age range, 19–71 years; average age, 55.8 years). The SHAPE gradient between the portal and hepatic veins was in good overall agreement with the hepatic venous pressure gradient (HVPG) ($R = 0.82$). Patients at increased risk for variceal hemorrhage (HVPG ≥ 12 mm Hg) had a significantly higher mean subharmonic gradient than patients with lower HVPGs ($1.93 \text{ dB} \pm 0.61$ [standard deviation] vs $-1.47 \text{ dB} \pm 0.29$, $P < .001$), with a sensitivity of 100% and a specificity of 81%, indicating that SHAPE may be a useful tool for the diagnosis of clinically important portal hypertension.

Conclusion:

Preliminary results show SHAPE to be an accurate noninvasive technique for estimating portal hypertension.

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Supplemental material: <http://radiology.rsna.org/lookup/suppl/doi:10.1148/radiol.13121769/-/DC1>

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Early diagnosis of portal hypertension is difficult, as symptoms rarely manifest until the later stages of liver disease (1). The need to accurately evaluate portal hypertension through the hepatic venous pressure gradient (HVPG) for assessment and monitoring has been well documented (2). Patients with an HVPG of 10 mm Hg or greater are at increased risk of developing varices, while patients with an HVPG of 12 mm Hg or greater are at risk for variceal bleeding, with mortality rates of 15%–20% (2–6). Currently, the reference standard method of HVPG measurement is determined as the gradient in pressure readings between wedged and free hepatic vein catheter positions obtained by using a transvenous (typically transjugular) approach. While this measurement is simple to acquire, the technique is invasive, is moderately costly, and can be associated with complications (although these are rare) (5). Thus, an accurate, noninvasive method for the measurement of the HVPG would improve patient care by simplifying data acquisition, lowering costs, and limiting invasiveness.

Ultrasonographic (US) contrast agents are gas microbubbles encapsulated by an outer shell for stability (7).

Advances in Knowledge

- Noninvasive subharmonic aided pressure estimation (SHAPE) measurements of portal vein pressures in patients with chronic liver disease were in good correlation with catheter-based transjugular hepatic venous pressure gradient (HVPG) measurements ($R = 0.82$).
- Patients at increased risk for variceal hemorrhage (HVPG ≥ 12 mm Hg) had significantly higher subharmonic gradients than patients with lower HVPGs ($1.93 \text{ dB} \pm 0.61$ [standard deviation] vs $-1.47 \text{ dB} \pm 0.29$, $P < .001$) with a sensitivity of 100% and a specificity of 81%, indicating that SHAPE may be a useful tool for the diagnosis of clinically important portal hypertension.

When exposed to an ultrasound beam at adequate acoustic pressures, these agents demonstrate nonlinear behavior. Previously, it has been shown that the subharmonic amplitude (at half the transmitting frequency) decreases linearly with increases in ambient fluid pressure and that this change is more than three times greater than either the fundamental or the second harmonic peak fluctuation (8–11). This response forms the basis of a technique referred to as subharmonic aided pressure estimation (SHAPE).

Since its implementation on two modified commercial US scanners, SHAPE has been used to track dynamic pressures in vitro (12), to monitor interstitial fluid pressures in a swine melanoma model (13), and to separately measure left ventricular pressures and changes in portal pressures in canine models (14,15).

Our purpose was to compare SHAPE measurements with pressure catheter-based measurements in human patients with chronic liver disease undergoing transjugular liver biopsy.

Materials and Methods

The study was approved by the institutional review board of Thomas Jefferson University, as well as by the U.S. Food and Drug Administration (Investigational New Drug number 110083), and was compliant with the Health Insurance Portability and Accountability Act. Written informed consent was provided by all participants. The US contrast agent was provided by GE Healthcare (Oslo, Norway). S.P., S.D., C.L.C., and K.E.T. were employed by GE Healthcare at the time of the study and aided in equipment and experimental design but did not have any control over the data published (J.R.E.,

Implication for Patient Care

- The presented noninvasive pressure estimation technique may potentially replace transjugular catheter-based measurements of portal vein pressures, reducing both cost and risk to the patient while also allowing more frequent measurements.

J.K.D., V.G.H., D.A.M., C.M., J.M.G., P.M., C.E.K., J.P.B., D.B.B., V.N., and F.F. were nonindustry authors who had control of the data). Inclusion criteria were consecutive adults scheduled for transjugular liver biopsy as part of their clinical care between December 2010 and December 2011. Exclusion criteria are shown in Appendix E1 (online). After the enrollment process shown in Figure 1, 45 patients participated in this study.

Procedures

As part of the scheduled transjugular liver biopsy procedures, HVPG measurements were obtained in triplicate by using a pressure catheter and techniques described in the literature (16). After pressure measurement acquisition, transjugular liver biopsy was performed, and three or four samples were obtained. Results from these procedures, as well as the patients' age, race, body mass index (BMI), and transplant status, were then retrieved from their medical records and were compared with experimental data.

Published online before print

10.1148/radiol.13121769

Content codes: GI US

Radiology 2013; 268:581–588

Abbreviations:

BMI = body mass index
CI = confidence interval
HVPG = hepatic venous pressure gradient
MELD = Model for End-Stage Liver Disease
ROC = receiver operating characteristic
ROI = region of interest
SHAPE = subharmonic aided pressure estimation

Author contributions:

Guarantors of integrity of entire study, J.R.E., J.M.G., F.F.; study concepts/study design or data acquisition or data analysis/interpretation, all authors; manuscript drafting or manuscript revision for important intellectual content, all authors; manuscript final version approval, all authors; literature research, J.R.E., J.K.D., J.M.G., S.D., K.E.T., D.B.B., V.N.; clinical studies, J.R.E., J.K.D., V.G.H., C.M., J.M.G., P.M., D.B.B., V.N., F.F.; experimental studies, J.R.E., J.K.D., D.A.M., J.M.G., P.M., S.P., S.D., C.E.K., K.E.T., D.B.B., V.N.; statistical analysis, J.R.E., J.M.G., C.E.K., V.N., F.F.; and manuscript editing, J.R.E., J.K.D., V.G.H., D.A.M., J.M.G., S.D., C.L.C., J.P.B., K.E.T., D.B.B., V.N., F.F.

Funding:

This research was supported by the National Institutes of Health (grants RC1 DK087365 and R21 HL081892).

Conflicts of interest are listed at the end of this article.

All liver biopsy specimens were interpreted for fibrosis score at our institution as part of clinical care. The specimens were evaluated by a single hepatopathologist (J.P.B., with 4 years of experience) for degree of fibrosis according to the Batts-Ludwig scoring system (17), a five-point scale on which a score of 0 indicates no fibrosis and a score of 4 indicates full cirrhosis.

The Model for End-Stage Liver Disease (MELD) score incorporates serum bilirubin, creatinine, and international normalized ratio to help assess the severity of chronic liver disease and help predict mortality in patients after transjugular intrahepatic portosystemic shunt placement (18). It is used to prioritize candidates for liver transplantation on the basis of its reliability as a measure of mortality risk in this population (19). Although the MELD score has not been shown to be useful for the characterization of patients without cirrhosis, in the present study it was applied to the entire patient population to determine if a noninvasive measurement of liver disease severity could be used to predict portal hypertension.

A US scanner (Logiq 9; GE Healthcare, Milwaukee, Wis) with a 4C curvilinear probe was modified to acquire radiofrequency data within a selected region of interest (ROI) during scanning. Scanning was performed by D.A.M. (a sonographer with 26 years of scanning experience) or P.M. (a radiologist with 10 years of experience). The SHAPE mode was set to transmit four-cycle pulses at 2.5 MHz and to receive subharmonic signals at 1.25 MHz, as determined during previous optimization of the technique (11). In addition to acquiring SHAPE data, this unit also provided the ability to simultaneously display B-mode US images (at 4.0 MHz and a mechanical index of 0.32, intermittently transmitting between SHAPE pulses) and subharmonic US images (generated from the time domain of the SHAPE data) for easier US navigation and identification of the vascular structures of interest; namely, the portal and hepatic veins (20).

Within 2 hours after biopsy (after complete recovery from all sedation),

Figure 1

74 Screened for enrollment

23 Excluded

- 13 Declined to participate
- 10 Did not meet inclusion criteria (Due to unstable cardio-pulmonary conditions)

51 Enrolled

- 2 Dropped out post enrollment
- 2 Removed due to biopsy complications
 - 1 Experienced post biopsy abdominal pain and was taken for follow up imaging
 - HVPg measurements during biopsy were not successfully obtained for 1 patient
- 1 Removed due to co-primorbidity deterioration (Renal failure prior to biopsy)
- 1 Removed due to equipment failure

45 Underwent SHAPE examination

Figure 1: Flowchart of enrollment procedures in this pilot study. Values are numbers of patients.

patients received a co-infusion of a microbubble US contrast agent (Sonazoid; GE Healthcare, Oslo, Norway) at 0.72 μ L microbubbles per kilogram of body weight per hour and saline at 120 mL per hour. The microbubble contrast agent was reconstituted with 2 mL of water for injection according to the manufacturer's recommendations before being co-infused with saline by means of a split line through an existing intravenous port in the ante-cubital vein that had been placed for sedation during biopsy. The syringe pump was placed below the patient to avoid loss of contrast agent because of buoyancy. Five minutes after the start of infusion, an ROI within the portal vein was selected and an automated power control algorithm was initiated to determine the optimal acoustic output power for maximum SHAPE sensitivity. Briefly, the automated program acquires data over 3 frames at each acoustic output level, and the extracted subharmonic amplitude is plotted as a function of acoustic output. A logistic curve is fit to the data and the inflection point selected (previously shown to be the point of greatest SHAPE sensitivity [8]). Figure 2 shows an example of this process, which was performed once for each patient to account for individual variations in depth and signal attenuation levels.

After power optimization, the ROI was enlarged to collect radiofrequency data simultaneously from the portal and hepatic veins over 5 seconds in triplicate, and findings were averaged

after processing. The depths, sizes, and locations of the portal and hepatic veins were determined by the sonographer, and a reference image was saved. Patients were monitored for 1 hour after infusion for adverse events before being discharged from the hospital.

Both the fundamental data (B-mode data at 4 MHz) and the subharmonic data (transmitted at 2.5 MHz, received at 1.25 MHz) were analyzed offline by using Matlab (version 7.8; Mathworks, Natick, Mass). Regions within the hepatic and portal veins previously identified by the sonographer were selected on maximum intensity projection B-mode images (compiled from reconstructed images from the radiofrequency data) and were fixed throughout the 5-second acquisition (approximately 70–90 frames). In patients with excessive motion ($n = 5$), regions were selected on a frame-by-frame basis from the B-mode images. The subharmonic gradient was computed as the difference in average subharmonic amplitude between the hepatic and portal veins obtained from both the time domain signal (the subharmonic intensity displayed during scanning, with ROIs determined from maximum intensity projections of the screen-captured images) and the radiofrequency data acquired by using a 0.5-MHz filter centered at 1.25 MHz. This analysis was repeated for the obtained B-mode data, with a 0.5-MHz filter centered at the fundamental frequency (4.0 MHz). Data points with a gradient below -4 dB ($n = 9$) were removed because this phenomenon

Figure 2

Figure 2: (a) US image in 56-year-old man with ascites shows the dual-imaging display mode, with the subharmonic ROI (yellow box) placed within the portal vein (PV) for acoustic output calibration. The hepatic vein (HV), the portal vein, and the inferior vena cava (IVC) are marked. (b) Graph shows subharmonic amplitudes as a function of acoustic output power. Red dot = selected acoustic output after optimization, where the change in subharmonic amplitude is greatest (as determined by the automatic power control program).

was attributed to a lack of microbubble contrast agent detection in the hepatic vein. Correlations between data from these inadequate studies and HVPG, BMI, hepatic vein depth, hepatic vein diameter, and disease status were investigated. Subharmonic gradients were then compared with the patient's HVPG, MELD score, and histologic fibrosis score.

Statistical Analysis

Correlations were determined by using the Pearson correlation coefficient. For discussion purposes, $R < 0.1$ was considered to indicate no correlation, $R = 0.1$ – 0.5 was considered to indicate moderate correlation, and $R > 0.5$ was considered to indicate high correlation. Significant variation between correlations was determined by using the Fisher z test. Statistical significance between groups was determined by using an unpaired Student t test. Receiver operating characteristic (ROC) curves were computed by using a continuous scale with HVPG cutoff levels of 10 and 12 mm Hg. The optimal operating point was determined as the point closest to 0,1 on the ROC curve (representing 100% sensitivity and 100% specificity).

From this analysis, sensitivity and specificity were both calculated, along with 95% confidence intervals (CIs). Statistical tests were performed by using Prism, version 5 (GraphPad Software, San Diego, Calif), with $P < .05$ considered to indicate a statistically significant difference.

Results

Of the 45 patients who completed this study, 27 (60%) were men and 18 (40%) were women. Thirty-nine patients (87%) were white, and the remaining six (13%) were black. Thirty-three patients (73%) had not previously undergone liver transplantation, while the remaining 12 patients (27%) had previously undergone successful transplantation. Disease etiology consisted of hepatitis C in 21 patients (47%), nonalcoholic steatohepatitis in 13 patients (29%), hepatitis B in three patients (7%), cryptogenic cirrhosis in three patients (7%), amyloidosis in one patient (2%), alcoholic hepatitis in one patient (2%), venous outflow obstruction with possible autoimmune hepatitis in one patient (2%), adenocarcinoma from primary breast cancer in one

patient (2%), and primary sclerosing cholangitis in one patient (2%).

Subharmonic signals from the portal vein were successfully obtained in all 45 patients, while signals from the hepatic vein were obtained in 42 patients because of scanning difficulties in three larger individuals (with BMIs of 37, 40, and 51 kg/m²). Data from an additional nine patients were removed because of inadequate signal within the hepatic vein (signal gradient between the hepatic and portal veins < -4 dB). No correlations were established among excluded patients in terms of HVPG, BMI, hepatic vein depth or diameter, or disease status ($P > .24$). Participant demographic data and selected clinical data are shown in the Table. No adverse reactions were observed as a result of the contrast agent infusions.

Two typical images from SHAPE acquisitions at their respective optimal acoustic outputs are shown in Figure 3. Overall, there was only a limited correlation ($R = 0.35$) between HVPG and the SHAPE gradient in the time domain signal displayed onscreen. Differences in subharmonic signals between the hepatic and portal veins were also calculated from

Figure 3

Figure 3: SHAPE acquisitions (obtained at their respective optimal acoustic outputs) in two patients. **(a)** Image in 56-year-old man with an HVP of 5 mm Hg insonated at an acoustic output of 10%. (This patient had a fibrosis score of 2, a hemoglobin level of 9.4 g/dL [94 g/L], a platelet count of $327 \times 10^9/L$, an albumin level of 2.7 g/dL [27 g/L], a creatinine level of 4.8 mg/dL [424.32 $\mu\text{mol/L}$], a bilirubin level of 0.2 mg/dL [3.42 $\mu\text{mol/L}$], and an international normalized ratio of 1.27 seconds.) Strong subharmonic signal in the portal vein (PV) is seen within the color box, while very limited signal is observed within the hepatic vein (HV). **(b)** Image in 60-year-old woman with an HVP of 23 mm Hg insonated at an acoustic output of 70%. (This patient had a fibrosis score of 4, a hemoglobin level of 10.9 g/dL [109 g/L], a platelet count of $219 \times 10^9/L$, an albumin level of 2.6 g/dL [26 g/L], a creatinine level of 1.1 mg/dL [97.24 $\mu\text{mol/L}$], a bilirubin level of 7.4 mg/dL [126.54 $\mu\text{mol/L}$], and an international normalized ratio of 1.61 second.) In this patient, subharmonic signals are greater in the hepatic vein (HV) than in the portal vein (PV). In patients like this one with elevated HVPs, hydrostatic pressure suppresses the subharmonic signal within the portal vein, lowering its signal intensity relative to the signal intensity of the hepatic vein.

Study Participant Statistics and Transjugular Liver Biopsy Findings

Parameter	Mean $\pm$ Standard Deviation	Interquartile Range	Range
Age (y)	55.8 $\pm$ 10.4	12.0	19–71
BMI (kg/m ²)	31.4 $\pm$ 8.6	10.7	17.2–57.2
HVP (mm Hg)	7.4 $\pm$ 6.4	7.5	0.5–24.6
Free pressure (mm Hg)	12.7 $\pm$ 5.2	6.6	2.7–25.7
Wedge pressure (mm Hg)	20.7 $\pm$ 8.3	7.7	7.0–45.7
Fibrosis score	2.1 $\pm$ 1.5	2.6	0–4
MELD score	10.6 $\pm$ 5.8	7.3	1–23
Bilirubin level (mg/dL)	2.9 $\pm$ 5.1	0.7	0.2–28.0
Creatinine level (mg/dL)	1.4 $\pm$ 1.4	0	0.7–7.6
International normalized ratio (sec)	1.20 $\pm$ 0.17	0.21	0.94–1.63
Platelet count ($\times 10^9/L$)	151 $\pm$ 81	131	35–336
Hemoglobin level (g/dL)	12.1 $\pm$ 1.9	2.5	8.7–17
Albumin level (g/dL)	3.7 $\pm$ 0.5	0.7	2.4–4.4

Note.—To convert bilirubin levels to Système International (SI) units (micromoles per liter), multiply by 17.1. To convert creatinine levels to SI units (micromoles per liter), multiply by 88.4. To convert hemoglobin and albumin levels to SI units (grams per liter), multiply by 10.0.

the radiofrequency data and were compared with the patient's HVP. Patients at higher risk of variceal

bleeding (HVP ≥ 12 mm Hg) showed a significantly higher mean SHAPE gradient than those at lower risk

(HVP < 12 mm Hg) ($1.93 \text{ dB} \pm 0.61$ [standard deviation] vs $-1.47 \text{ dB} \pm 0.29$; $P < .001$; Fig 4a). This difference was also present when we separated patients into two groups according to whether their HVP was less than or was equal to or greater than 10 mm Hg ($1.37 \text{ dB} \pm 0.59$ vs $-1.68 \text{ dB} \pm 0.27$, respectively; $P < .001$). On the basis of cutoffs of 12 and 10 mm Hg, the areas under the ROC curve were 0.94 and 0.90, respectively (Fig 4b). From these curves, the optimal operating point was determined to be greater than -0.57 dB for separating patients on the basis of either portal hypertension or increased risk of variceal bleeding. In the identification of patients with HVPs of 10 mm Hg or greater, SHAPE had a sensitivity of 89% (95% CI: 52%, 100%) and a specificity of 88% (95% CI: 68%, 97%). In the identification of patients with HVPs of 12 mm Hg or greater, SHAPE achieved a sensitivity of 100%

(95% CI: 54%, 100%) and a specificity of 81% (95% CI: 62%, 94%).

When we examined the linear relationship between the SHAPE gradient and the HVPG over the entire data set, a strong positive correlation was observed between the SHAPE pressure estimate and the HVPG ($R = 0.82$, Fig 5a). Analysis of the B-mode data (4.0 MHz transmitting and receiving) showed no correlation between the signal gradient between the hepatic and portal veins and HVPG in either the time or the frequency domain ($R < 0.11$). The correlation of the SHAPE gradient with the HVPG in the subpopulation of patients with HVPGs of 12 mm Hg or greater (Fig 5b) showed a strong correlation with HVPG ($R = 0.97$); however, the sample size was only six patients.

Only moderate correlation was observed between the SHAPE gradient and both the patients' MELD scores ($R = 0.38$) and their fibrosis scores ($R = 0.13$). Moreover, these correlations were significantly weaker than the correlation observed with HVPG ($P < .004$). The correlations between HVPG and MELD score ($R = 0.24$) and HVPG and fibrosis score ($R = 0.19$) were also significantly weaker than the correlation of HVPG with SHAPE gradient ($P < .002$). No correlation was observed between a patient's MELD score and his or her fibrosis score ($R = 0.08$).

Discussion

In this study, we correlated SHAPE with the HVPG and found good overall agreement, indicating that this noninvasive technique may be a useful screening tool to predict the presence of clinically important portal hypertension in patients undergoing transjugular liver biopsy. Despite the large variation in SHAPE values in patients without portal hypertension, SHAPE was able to help identify patients with portal hypertension (HVPG ≥ 10 mm Hg) and those at increased risk of variceal bleeding (HVPG ≥ 12 mm Hg). While weaker correlation was observed for SHAPE in patients with HVPGs less than 12 mm Hg, this correlation strengthened as pressure values increased. The lack

Figure 4

Figure 4: (a) Box plot shows the subharmonic gradient—the average subharmonic amplitude in the hepatic vein (HV) minus that in the portal vein (PV)—in patients at risk for variceal bleeding (HVPG ≥ 12 mm Hg) and in those at lower risk (HVPG < 12 mm Hg). (b) The ROC curves demonstrate the ability to use SHAPE to identify patients with portal hypertension (HVPG ≥ 10 mm Hg) and those at risk for variceal bleeding (HVPG ≥ 12 mm Hg). Az = area under the ROC curve.

Figure 5

Figure 5: (a) Graph shows correlation between the noninvasively determined subharmonic gradient—the average subharmonic amplitude in the hepatic vein (HV) minus that in the portal vein (PV)—and the corresponding HVPG, which is measured by using a pressure catheter during biopsy. (b) Graph shows correlation between the subharmonic gradient and the corresponding HVPG measured in patients at higher risk of variceal bleeding (HVPG ≥ 12 mm Hg).

of correlation observed between HVPG and the obtained fundamental B-mode frequency data ($R < 0.11$) is consistent with findings in the literature that indicate that the subharmonic frequency component is a better indicator of hydrostatic pressures than the fundamental response (8).

Although other groups (21,22) have explored the relationship between fluid pressure and the subharmonic amplitude of US contrast agents in compression-only behavior, to our knowledge, this work has thus far been limited to in vitro results in ideal conditions. A variety of alternative US techniques have been explored for the estimation of

portal pressures, although none to date have been proven to be robust enough for clinical use. Portal vein diameter, the presence of ascites, and splenic size have all been investigated, but all suffer from relatively low sensitivity (generally $< 60\%$) because of physiologic variability (23–25). Doppler US has been used to measure flow parameters within the portal and splenic veins such as flow velocity, resistive indexes, and pulsatility, but with limited correlation with HVPG (23,26,27). Using US contrast agents, research groups have investigated the transit time (time from contrast agent injection to contrast agent arrival in the hepatic vein) for noninvasive estimation

of liver disease, but the majority of work has focused on successful identification of cirrhosis (28–30). Maruyama et al (31) used a similar approach with the same microbubble contrast agent to differentiate eight patients with idiopathic portal hypertension (identified by esophageal varices, hypersplenism, or ascites) from 47 patients with cirrhosis and 36 control subjects. Their group found no significant differences in transit times to the hepatic artery and no significant differences in portal vein transit times between the group of patients with portal hypertension and the group of patients with cirrhosis, but they did see a significant difference in portal vein transit times between the two patient groups and the control subjects ($P < .0014$) (31). Zhang and colleagues (32) compared the hepatic-vein-to-hepatic-artery transit time with free portal pressures measured surgically and showed a significant difference ($P < .001$) between six patients who were undergoing gastrointestinal tumor resection and 25 patients with portal hypertension who were undergoing surgical disconnection therapy, but HVPG was not investigated and portal pressures were quite high (approximately 23–39 mm Hg).

While SHAPE measurements correlated well with HVPG measurements in our study, correlations between SHAPE measurements and MELD or fibrosis scores were less robust. This finding is not surprising in light of the close correlation with HVPG, which was also not predictive of MELD or fibrosis score in this data set. MELD scores are an important indicator of liver disease severity and short-term survival and are obtained with noninvasive blood tests (18,19), but they are generally not applicable to patients without cirrhosis. In our study, MELD scores were calculated for the entire study population to determine if a noninvasive measure would be predictive of HVPG.

Patients enrolled in this study were also referred for liver biopsy and thus would still require transjugular catheterization. Although SHAPE is not a reliable indicator of fibrosis, it is still expected to be clinically beneficial in patients

who are not simultaneously undergoing biopsy. Results from this cross-sectional study indicate that SHAPE may be useful as a first qualitative screening modality for determining the presence of portal hypertension, followed by a quantitative measurement of HVPG to monitor disease progression or treatment response at higher pressure levels (where SHAPE has improved accuracy). This could potentially improve patient outcomes and satisfaction by avoiding repeated transjugular catheterization while also providing clinicians with more easily repeatable HVPG estimations.

While the results of this study are promising, there were several limitations. Correlations and conclusions were based on a limited sample size ($n = 33$ after the removal of data points), with only nine patients with portal hypertension and only six patients with HVPGs of 12 mm Hg or greater. Thus, correlations were somewhat dominated by patients with higher HVPGs, and a larger, more robust population should be investigated before making final conclusions. Data from several patients were removed because of an inability to simultaneously image the portal and hepatic veins in three larger patients (with BMIs of 37, 40, and 51 kg/m²), although data were collected successfully in even larger patients. (Patients in whom data were successfully obtained had a mean BMI of 30.7 kg/m² $\pm$ 8.2, with a range of 17.2–57.2 kg/m².) Nonetheless, this inability to collect data in these patients demonstrates the well-known shortcomings of US during scanning at greater depths. Data in an additional nine patients were removed because of inadequate signal within the hepatic vein (with signal gradient between the hepatic and portal veins < -4 dB). No obvious correlations (eg, those involving HVPG, BMI, hepatic vein depth or diameter, or disease status) were established among excluded patients. While the reason for this contrast agent insufficiency is unknown, the use of a higher contrast agent infusion rate may more adequately fill the hepatic veins with contrast agent and potentially reduce the number of discarded cases in the future.

Because this was a pilot study, no blinding was performed, introducing potential selection bias. However, this bias is believed to be minimal, as ROIs were selected on B-mode images (the data of which showed no correlation with HVPG), and minimal correlation with the time domain subharmonic signal (displayed in real time during imaging) was observed. US data were obtained up to 2 hours after biopsy, which may have induced errors due to changes in portal pressures over this time period. While performing scanning during or immediately prior to biopsy would have been optimal, postbiopsy scanning was selected to minimize patient anesthesia (during biopsy) and to avoid giving an investigational drug (the microbubble US contrast agent Sonazoid) immediately before a surgical procedure. However, the portal pressures over this time are still believed to be relatively stable. Despite its excellent safety profile and daily clinical use in Japan since 2007 (33,34), the U.S. Food and Drug Administration (FDA) has yet to approve this contrast agent for clinical use. In our study, we chose to use this contrast agent because previous in vitro work showed its subharmonic amplitude to be the most sensitive to hydrostatic pressures of all commercially available agents (11). However, SHAPE has also been shown to be feasible when performed with other FDA-approved contrast agents (9,11,13), and the usage of such agents could potentially result in more rapid clinical translation of SHAPE.

In conclusion, SHAPE measurements appear to correlate well with transjugular HVPG measurements. The technique allows identification of patients with portal hypertension and those at elevated risk of variceal bleeding from among the general patient population, albeit on the basis of a limited data set.

Acknowledgments: The authors acknowledge GE Healthcare for contrast agent support. We also thank Laura Pino, PA, Monica Wellings, PA, and Marge Keuny-Beck, PA, for assistance with patient recruitment; Norman Alexander, BS, RDMS, for cardiac-echo screening; and Robin Miller, PA, and John Furlong, RN, for nursing support.

Disclosures of Conflicts of Interest: J.R.E. No relevant conflicts of interest to disclose. J.K.D. No relevant conflicts of interest to disclose. V.G.H. No relevant conflicts of interest to disclose. D.A.M. Financial activities related to the present article: none to disclose. Financial activities not related to the present article: is a consultant for GE Healthcare. Other relationships: none to disclose. C.M. No relevant conflicts of interest to disclose. J.M.G. No relevant conflicts of interest to disclose. P.M. No relevant conflicts of interest to disclose. S.P. Financial activities related to the present article: is an employee of GE Healthcare; is an employee of Samsung Electronics. Financial activities not related to the present article: none to disclose. Other relationships: none to disclose. S.D. Financial activities related to the present article: was an employee of GE Healthcare; owns stock in GE Healthcare as part of 401(k). Financial activities not related to the present article: is an employee of Philips Healthcare. Other relationships: none to disclose. C.L.C. Financial activities related to the present article: is an employee of GE Healthcare. Financial activities not related to the present article: none to disclose. Other relationships: none to disclose. C.E.K. No relevant conflicts of interest to disclose. J.P.B. No relevant conflicts of interest to disclose. K.E.T. Financial activities related to the present article: is an employee of GE Healthcare. Financial activities not related to the present article: none to disclose. Other relationships: none to disclose. D.B.B. Financial activities related to the present article: none to disclose. Financial activities not related to the present article: is a consultant for Cook Medical and for Biocompatibles. Other relationships: none to disclose. V.N. No relevant conflicts of interest to disclose. E.F. Financial activities related to the present article: GE Healthcare provided institution with the US contrast agent used in the study. Financial activities not related to the present article: is a member of a clinical advisory board to Toshiba America Medical Systems; has given expert testimony for the firm of Kirkland and Ellis; institution has grants or grants pending with the Department of Defense; has been paid for development of educational presentations by the North American Center for Continuing Medical Education. Other relationships: none to disclose.

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