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On the implementation of an automated acoustic output optimization algorithm for subharmonic aided pressure estimation

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Abstract

Incident acoustic output (IAO) dependent subharmonic signal amplitudes from ultrasound contrast agents can be categorized into occurrence, growth or saturation stages. Subharmonic aided pressure estimation (SHAPE) is a technique that utilizes growth stage subharmonic signal amplitudes for hydrostatic pressure estimation. In this study, we developed an automated IAO optimization algorithm to identify the IAO level eliciting growth stage subharmonic signals and also studied the effect of pulse length on SHAPE. This approach may help eliminate the problems of acquiring and analyzing the data offline at all IAO levels as was done in previous studies and thus, pave the way for real-time clinical pressure monitoring applications. The IAO optimization algorithm was implemented on a Logiq 9 (GE Healthcare, Milwaukee, WI) scanner interfaced with a computer. The optimization algorithm stepped the ultrasound scanner from 0 to 100 % IAO. A logistic equation fitting function was applied with the criterion of minimum least squared error between the fitted subharmonic amplitudes and the measured subharmonic amplitudes as a function of the IAO levels and the optimum IAO level was chosen corresponding to the inflection point calculated from the fitted data. The efficacy of the optimum IAO level was investigated for *in vivo* SHAPE to monitor portal vein (PV) pressures in 5 canines and was compared with the performance of IAO levels, below and above the optimum IAO level, for 4, 8 and 16 transmit cycles. The canines received a continuous infusion of Sonazoid microbubbles (1.5 μ l/kg/min; GE Healthcare, Oslo, Norway). PV pressures were obtained using a surgically introduced pressure catheter (Millar Instruments, Inc., Houston, TX) and were recorded before and after increasing PV pressures. The experiments showed that optimum IAO levels for SHAPE in the canines ranged from 6 to 40 %. The best correlation between changes in PV pressures and in subharmonic amplitudes ($r = -0.76$; $p = 0.24$), and between the absolute PV pressures and the subharmonic

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amplitudes ($r = -0.89$; $p < 0.01$) were obtained for the optimized IAO and 4 transmit cycles. Only for the optimized IAO and 4 transmit cycles did the subharmonic amplitudes differ significantly ($p < 0.01$) before and after increasing PV pressures. A new algorithm to identify optimum IAO levels for SHAPE has been developed and validated with the best results being obtained for 4 transmit cycles. The work presented in this study may pave the way for real-time clinical applications of estimating pressures using the subharmonic signals from ultrasound contrast agents.

Keywords

noninvasive pressure estimation; ultrasound contrast agents; incident acoustic output optimization; portal hypertension; subharmonic ultrasound signals

1. Introduction

The goal of this study was to develop, implement and validate an algorithm to automatically determine optimum incident acoustic output (IAO; i.e., the acoustic output from ultrasound transducer incident on the region of interest) for subharmonic aided pressure estimation (SHAPE). A secondary goal of this study was to compare SHAPE's performance with 4, 8 and 16 transmit cycles. The clinical relevance and *in vivo* applications of SHAPE have been documented [1-4]. The current study builds on previous *in vitro* and *in vivo* SHAPE studies [1-7] and solves the problem of determining optimum IAO levels to insonate the ultrasound contrast agents (UCAs) for SHAPE applications. If successful, this approach may help eliminate the problems of acquiring and analyzing the data offline at all IAO levels as was done previously [1-3, 6] and, thus, pave the way for real-time clinical pressure monitoring applications.

1.1. Ambient pressure estimation using ultrasound contrast agents

A review of techniques to estimate ambient pressures using microbubbles has been provided in this section. Techniques to estimate ambient hydrostatic pressures using UCAs have been proposed [8-13]. Amongst these, techniques based on utilizing a shift in resonance frequency [9], amplitude of single bubble echoes [10], dual frequency excitations to calculate ambient pressure modulated size changes [12] and onset of ambient pressure modulated cavitations [11] have been tested with free microbubbles (mostly comprising of air) and errors in the range of 10 – 15 mmHg with respect to true pressure values were reported.

Modern UCAs are encapsulated microbubbles containing gases such as C_4F_{10} or SF_6 that have relatively low diffusivity relative to air (coefficient of diffusivity of air, C_4F_{10} and SF_6 in water are $2.05 \times 10^{-9} \text{ m}^2/\text{s}$, $0.69 \times 10^{-9} \text{ m}^2/\text{s}$ and $1.20 \times 10^{-9} \text{ m}^2/\text{s}$, respectively [14]) [15]. These UCAs have diameters less than $8 \mu\text{m}$ and can traverse the entire vasculature including the capillaries [15]. *In vivo* the UCAs provide a source of acoustic impedance mismatch relative to blood and a difference in compressibility between the gas contained within these microbubbles and the blood resulting in relatively strong backscattered signals (about 10-30 dB enhancement) [15]. Apart from a backscattered signal at the insonation frequency (f_0), these UCAs also backscatter signals at the subharmonic ($f_0/2$), harmonic ($n \cdot f_0$; $n \in \mathbb{N}$) and ultraharmonic ($((2n-1)/2) \cdot f_0$; $n \in \mathbb{N}$ & $n > 1$) frequencies [15].

Two techniques to utilize these modern UCAs for ambient pressure estimation have been proposed; one based on dissolution time of free microbubbles following rupture of encapsulated microbubbles [8] and the second one based on ambient pressure modulated subharmonic signal amplitude referred to as SHAPE [13]. However, techniques based on dissolution time of free microbubbles yielded errors as high as 50 mmHg [8], which are not

clinically practical. In clinical practice errors less than 5 mmHg are desirable for most applications.

1.2. Subharmonic aided pressure estimation (SHAPE)

In this section, the theory and previous studies of SHAPE have been summarized. The subharmonic signal amplitude from UCAs exhibits a sigmoidal relationship with IAO showing three distinct stages – occurrence, growth and saturation [13, 16, 17]. The growth stage subharmonic signal amplitudes were shown to be sensitive to ambient pressures and the subharmonic signal amplitudes in the growth stage decreased linearly with an increase in ambient pressures [13]. Other independent studies have also reported on ambient pressure modulated subharmonic amplitudes [18-23].

A proof-of-concept *in vivo* study showed that SHAPE predicted canine aortic pressures with a maximum standard error of 5.4 mmHg compared to pressure catheter measurements (the reference standard), but the experimental setup used in that study was not suitable for clinical implementation [4]. Subsequently, another *in vitro* study undertaken to compare the performance of different UCAs for SHAPE showed that subharmonic signals from Sonazoid microbubbles (GE Healthcare, Oslo, Norway) were most sensitive to ambient pressure changes; the sensitivity of Sonazoid microbubbles to ambient pressure changes peaked at an insonation frequency of 2.5 MHz and 0.35 MPa IAO [7]. Sonazoid microbubbles contain a perfluorobutane gas encapsulated in a membrane of hydrogenated egg phosphatidyl serine, have a volume median diameter of $2.6 \pm 0.1 \mu\text{m}$ and contain about 1.2×10^9 microbubbles per ml [24]. Sonazoid microbubbles are commercially available, have a proven safety profile [25] and are approved for clinical use [26]. *In vivo* SHAPE documented errors in the range of 0.0 to 3.5 mmHg when estimating cardiac pressures in canines [2, 3]. Further, data obtained from a previous *in vivo* study geared towards tracking portal vein (PV) pressures in canines using SHAPE, indicated that out of 2, 3 and 4 transmit cycles, SHAPE worked best with 4 transmit cycles [1].

A major limitation in the above studies [1-4, 7] impeding real-time clinical applications is the lack of knowledge of the IAO levels on the Sonazoid microbubbles *in vivo*. The IAO levels may be known at the transducer focal point based on *in vitro* measurements using a hydrophone, but these IAO levels will differ *in vivo* based on the scanned anatomy and patient body habitus. The IAO levels determine the stage of the subharmonic signal amplitude including the growth stage in which the subharmonic signal amplitudes are sensitive to ambient pressures. Hence, a failure to elicit subharmonic signals in the growth stage will result in erroneous pressure estimation when using SHAPE.

2. Materials and Methods

2.1. Animal preparation

This research study was approved by the Institutional Animal Care and Use Committee of Thomas Jefferson University and conducted in accordance with the guidelines provided by the National Institutes of Health. Five canines were used in this study (mean weight: $22.4 \pm 1.72 \text{ kg}$). Proof of the value offered by the SHAPE technique for PV pressure monitoring was already elsewhere [1]. In this study, the efficacy and function of the automated IAO optimization algorithm was evaluated and thus, only five canines were used. The canines were fasted for a period of 12 hours prior to the experiments, to reduce post-prandial effects on the flow and, thus, the pressures in the PV [27]. An intravenous injection of Propofol (Abbott Laboratories, Chicago, IL; dose 7 ml/kg) was used as the initial anesthetic. During the course of the experiments, the animals were intubated and anesthesia was maintained with 0.5 to 2.0 % Isoflurane (Iso-thesia; Abbott Laboratories) via an endotracheal tube. The canines were placed on a warming blanket to maintain normal body temperature. An 18

gauge catheter was placed in a forelimb vein for Sonazoid infusion (1.5 μ l/kg/min with 0.9 % saline administered at 2 ml/min). The canines' respiration, ventilation, oxygenation, electrocardiogram, temperature and anesthesia were monitored by certified veterinary technicians throughout the study.

A midline abdominal incision was created to provide access to the main PV. A 5F pressure catheter (SPR 350S/SPR 350, Millar Instruments, Inc., Houston, TX) was surgically introduced into the main PV to provide the reference PV pressures. An additional surgical inlet to the main PV was also created to induce portal hypertension (PH; increase in PV pressures). Pathophysiologically, an increase in intra-hepatic vascular resistance contributes to PH [28-30]. Gelfoam (Ethicon, Somerville, NJ) is used for embolization of liver circulation; it has been used previously in canines with no inflammatory response or foreign body reactions [31, 32]. Also, an acute model of PH induced with Gelfoam was used previously and this model was relatively straightforward to implement with a success rate greater than 90 % in canines [1, 33]. Thus, Gelfoam was selected to induce PH in canines.

A sterile sheet of Gelfoam (100 cm²) was cut into small pieces and diluted with saline. The resulting mixture was introduced into the PV via the additional surgical inlet to induce PH. The PV pressures were continuously monitored via the Millar pressure catheter. Following the experiments, the canines were sacrificed by intravenous injection of Beuthanasia (0.25 mg/kg).

2.2. Ultrasound scanner operation

The experimental setup is shown in Fig. 1. A Logiq 9 scanner (GE Healthcare, Milwaukee, WI) with a curved array 4C probe was modified to operate in a dual imaging mode i.e., grayscale and pulse inversion subharmonic imaging modes [34]. The transmit and receive frequencies were 2.5 MHz and 1.25 MHz, respectively, based on our previous SHAPE studies [1-3, 6, 7, 33]. A prior study also indicated that out of 2, 3 and 4 transmit cycles used for SHAPE, 4 transmit cycles tracked PV pressure changes and absolute PV pressures the best [1]. Thus in this study, 4 transmit cycles were compared to 8 and 16 transmit cycles to more completely assess the effect of the number of transmit cycles on SHAPE's performance. All scanning were performed by a sonographer or by a radiologist with more than 10 years of experience, and data were collected after Sonazoid infusion and visual verification of Sonazoid microbubbles in the PV.

2.3. Automatic IAO optimization

A crossover cable was connected for real-time data transfer between the Logiq 9 scanner and a lab computer running Matlab (Version 7.8.0-R2009a, The Mathworks, Inc., Natick, MA; for optimum IAO determination; Fig. 1). In order to determine the optimum IAO for SHAPE a region of interest (ROI) was placed inside the PV as shown in Fig. 2. The left side of Fig. 2 depicts the grayscale imaging mode, whereas the right side is configured to show subharmonic data (at 1.25 MHz with 1 MHz bandwidth) in the selected ROI [34]. The automatic IAO optimization program was initiated from the lab computer. The flowchart of the IAO optimization program is presented in Fig. 3. First, the scanner was set to 0 % (0 MPa) IAO level (Fig. 2: green arrow) and the corresponding data from the subharmonic ROI were collected over 3 frames. A 50 % threshold mask was applied to the subharmonic data to remove relatively low subharmonic signals e.g., from low Sonazoid microbubble concentrations or from the surrounding tissue (Fig. 3). Then the mean subharmonic amplitude was stored on the PC. This process was repeated for all 28 discrete IAO levels up to 100 % IAO. The incident acoustic pressures, corresponding to 0 to 100 % IAO, were measured *in vitro* at the focus of the 4C transducer using a calibrated 0.2 mm needle hydrophone (Precision Acoustics, Dorchester, Dorset, UK; sensitivity of 57.1 mV/MPa at

2.5 MHz) and a standard water bath approach. The focal point was determined by finding the point of maximum acoustic pressure using a semi-automated electronic x-y-z positioning system (Newport Corporation, Irvine, CA) and all measurements were performed in triplicate. The measured incident acoustic pressures ranged from 0 to 3.34 MPa_{peak-to-peak}.

The resulting subharmonic amplitudes were plotted as a function of IAO. A logistic equation fitting function was applied to this data set to identify the parameters (of the fitting function) that lead to minimum least squared error between the fitted data and the measured subharmonic amplitudes as a function of IAO. Additional details about the fitting function are provided elsewhere [35]. The resulting point of inflection i.e., the point where the second derivative of the fitting function is zero, was identified. Based on the value of this inflection point, the IAO level showing the least squared error with respect to the inflection point was chosen as the optimum IAO level for SHAPE. The scanner was configured to acquire further data for SHAPE at that level.

2.4. Data acquisition and processing for SHAPE

As shown in Fig. 1, a synchronization signal from the Logiq 9 scanner was connected to an oscilloscope (Model 9350 AM, LeCroy, Chestnut Ridge, NY) set to acquire the PV pressures from the pressure catheter and the transducer control unit (TCB 500, Millar Instruments). The synchronization signal enabled simultaneous and synchronous ultrasound and catheter data acquisition. The oscilloscope transferred the pressure catheter data to a lab computer via a GPIB interface (Driver Version 2.7.0.49152) through LabVIEW (Version 8.0, National Instruments Corp., Austin, TX).

Under baseline conditions i.e., with normal PV pressures (6 to 12 mmHg; before inducing PH) during Sonazoid infusion, the ultrasound data and the pressure catheter data were acquired synchronously with 4, 8 and 16 transmit cycles at the optimum IAO level, at an IAO level below the optimum IAO level and then at an IAO level above the optimum IAO level (thus, three consecutive IAO levels were used). Each acquisition consisted of a 5 s run (based on previous observations of PV pressures as a function of time [1, 33]) and acquisitions at each IAO level were repeated in triplicate. The order of data acquisition was randomly varied after acquiring 3 data sets at a given configuration (combination of IAO levels and transmit cycles). Next, the Sonazoid infusion was stopped and PH was induced by administering Gelfoam into the PV. After observing PH as increased pressure values on the oscilloscope (greater than 5 mmHg as compared to baseline PV pressures for each canine [33]), Sonazoid infusion was resumed and data were again acquired for all combinations of IAO levels and transmit cycles (in the same order as during baseline conditions). Thus, a total of 54 data sets per canine were to be acquired, 27 before inducing PH and 27 after inducing PH. Each data set consisted of the pressure catheter data and the corresponding synchronous RF data. The data were transferred to a computer for offline analyses in Matlab to extract and evaluate the best parameter set for PV pressure estimation.

Since the ultrasound data from each acquisition were saved as a DICOM file, the subharmonic data were extracted using a proprietary software 'GE Raw RF data extraction facility' (GE Global Research, Niskayuna NY). The proprietary software only provided access to the RF data for subharmonic signal amplitude extraction; the algorithm proposed in this work is based on acquired RF data and may be implemented on any ultrasound scanner provided that RF data is accessible. This RF data were DC filtered. The subharmonic data for SHAPE from the ROI shown in Fig. 2 were extracted using a filter centered at 1.25 MHz with 0.25 MHz bandwidth. These filter parameters were selected based on previous results [1, 5]. The mean subharmonic signal amplitude was then calculated from all the frames in the acquired DICOM file. This process was repeated for all acquired data.

2.5. Statistical analyses

For all statistical analyses p -values below 0.05 were considered significant. The hypothesis was that SHAPE's ability to estimate PV pressures and PV pressure changes would be best at the optimum IAO level identified by the automatic IAO optimization program and at 4 transmit cycles. The subharmonic signal amplitudes and PV pressures before and after inducing PH were compared using paired t -tests. Linear correlation analyses were performed to identify the relationship between change in the PV pressures and change in the subharmonic signal amplitude, and between absolute PV pressures and absolute subharmonic signal amplitudes. The values are reported as mean $\pm$ standard deviation where applicable. All analyses were performed using IBM SPSS Statistics (Release 19.0.0; IBM Corporation, Armonk, NY).

3. Results and discussion

3.1. Portal vein pressures in canines

For one canine, technical failure with the surgical procedure precluded data acquisition. The mean baseline PV pressures were 11.9 ± 3.9 mmHg, which are similar to the mean PV pressure values reported previously [1, 36-40]. For the 4 canines there was a statistically significant difference of 15.93 ± 6.15 mmHg between the PV pressures before and after inducing PH (95 % Confidence Interval: 6.15 to 25.71 mmHg; $p = 0.014$; Table 1).

3.2. Analyses of IAO optimization program output

Figure 4 represents a sample output of the IAO optimization program. The optimum IAO selected by the program for the case shown in Fig. 2 was 20 % ($0.56 \text{ MPa}_{\text{peak-to-peak}}$) (Fig. 4). As seen in Fig. 4, the subharmonic signal amplitude follows a sigmoidal relationship with IAO as reported previously [7, 13, 16, 17]; the initial occurrence stage, the growth stage and the gradual saturation at IAO above 60 % ($1.19 \text{ MPa}_{\text{peak-to-peak}}$) are seen. The fitting function shown in Fig. 4 compensates for motion artifacts, mainly due to respiration, during the data collection phase for determining the optimum IAO levels. Fig. 5 illustrates the subharmonic ROI signal at 5 % ($0.18 \text{ MPa}_{\text{peak-to-peak}}$; beginning of the growth stage; Fig. 5(a)), 20 % ($0.56 \text{ MPa}_{\text{peak-to-peak}}$; at the center of the growth stage; Fig. 5(b)), 60 % ($1.19 \text{ MPa}_{\text{peak-to-peak}}$; the end of the growth stage; Fig. 5(c)) and 100 % ($3.34 \text{ MPa}_{\text{peak-to-peak}}$; saturation of the subharmonic signal; Fig. 5(d)) IAO levels. The occurrence, growth and saturation stages were also seen in the program output obtained from the automatic IAO optimization program for the other canines. The optimum IAO levels computed by the automatic IAO optimization program were 6 %, 28 %, 40 % and 32 % (range: 0.2 to $0.9 \text{ MPa}_{\text{peak-to-peak}}$), respectively, for the 4 canines.

3.3. SHAPE's performance for estimating PV pressures and PH

The correlation coefficient relating the change in PV pressures to the change in subharmonic signal amplitude, before and after inducing PH, and relating the absolute values of PV pressures and subharmonic signal amplitude are presented in Table 2. For 2 canines the ultrasound data were not acquired after PH induction at IAO levels that were higher than the optimized IAO levels due to timing constraints. Fig. 6(a)-(c) represent the data obtained with 4, 8 and 16 transmit cycles at the optimized IAO level. The best correlation ($r = -0.76$) for changes in PV pressures and the subharmonic signal amplitude was obtained at the optimized IAO level with 4 transmit cycles (Table 2 and Fig. 6(a)-(c)). This demonstrates that the optimized IAO level selected by the IAO optimization program and 4 transmit cycles is best suited for SHAPE applications. Also, the data acquired with optimized IAO and 4 transmit cycles shows that the increases in PV pressures were associated with a greater reduction in subharmonic signal amplitude consistent with previously published reports [1-7, 13, 18-20, 23].

An established theoretical explanation for the decrease in subharmonic signal amplitude due to increase in ambient pressure has not been provided in the literature. It was surmised that the decrease in growth stage subharmonic signal amplitude as a function of ambient pressure may be due to damping of nonlinear oscillations by a rise in ambient pressure [6]. An experimental evaluation complemented by simulation results indicated that the decrease (or increase) in subharmonic response of microbubbles as a function of ambient pressure variations may be dependent on the size distribution of the microbubbles [21]. Based on single bubble oscillations, a different study postulated that the ratio of the excitation and the bubbles' natural resonance frequency affect the subharmonic signal amplitude and the bubbles' natural resonance frequency may be affected by changes in ambient pressure [41], ultimately resulting in decreased subharmonic amplitude for increased ambient pressure. Hopefully future simulation studies using individual encapsulated microbubbles and then an aggregate of encapsulated microbubbles may provide further insight into this phenomenon.

For other combinations of transmit parameters (except 4 transmit cycles and optimum IAO level) the correlations between changes in subharmonic signal amplitude and changes in PV pressures were low and ranged from 0.04 to 0.31 (Table 2). Table 2 also presents the correlation coefficient between absolute values of the subharmonic signal amplitudes and PV. Again, the best (and only significant) correlation coefficient ($r = -0.89$, $p < 0.01$) was obtained for a combination of the optimized IAO level and 4 transmit cycles (Fig. 6(d)). For the other sets of transmit parameters, the absolute correlation between subharmonic signal amplitude and PV pressures ranged from -0.18 to -0.65, with the exception of 4 transmit cycles where correlations of -0.72 at IAO below the optimized IAO and -0.73 at IAO above the optimized IAO were observed (Table 2). These correlation coefficients of -0.72 and -0.73 may stem from the fact that the subharmonic signal amplitudes were in the growth stage of subharmonic emissions even above and below the optimum IAO; but these correlations were still less than -0.89 observed at optimum IAO. Also the correlation of -0.73 was obtained with 4 data points only. The results obtained here also agree with results obtained previously where the sensitivity of Sonazoid microbubbles to ambient pressures was maintained throughout the growth stage of subharmonic emissions, but peaked at optimum insonification [7]. The decrease in axial resolution and/or possible microbubble destruction by using higher transmit cycles (8 and 16) may have resulted in the relatively lower observed correlation coefficient for the changes in PV pressures and subharmonic signal amplitudes, and the absolute PV pressures and subharmonic signal amplitudes. While increasing the number of transmit cycles may increase the subharmonic signal received from the UCAs and may be useful for subharmonic imaging, for SHAPE the IAO levels are selected to elicit the subharmonic signal in the growth stage (where the subharmonic signal amplitude is below the amplitude in the saturation stage).

The slope of the best fit line that denotes the sensitivity of Sonazoid microbubbles to ambient pressures was computed to be -1.62 mmHg/dB. Previously, simulation studies have reported this sensitivity to be -6.58 mmHg/dB [19], *in vitro* studies have found sensitivity values in the range of -13.98 mmHg/dB to -4.55 mmHg/dB [6, 7] and *in vivo*, a range of -4.92 mmHg/dB to -1.44 mmHg/dB was observed [1-4]. In contrast to the above studies [1-4, 6, 7, 19], a different study [22] reported an in-phase relationship between the subharmonic signal amplitudes and the ambient pressures; in that study specifically engineered phospholipid-shell microbubbles with "optimized" initial surface tension values were used and an ambient pressure sensitivity of the subharmonic signal amplitude was reported as 6.22 mmHg/dB at incident acoustic pressure of 50 kPa_{peak negative} [22]. However, such prototype microbubbles will require further research and safety studies prior to their potential clinical use. Conversely, in this study, Sonazoid microbubbles that are commercially available and that have a proven safety profile [25] and that are approved for clinical use [26] have been used. Also, with Sonazoid microbubbles an in-phase relationship

between ambient pressures and pressure modulated subharmonics has not been observed and at incident acoustic pressures of 50 kPa_{peak negative} (used in [22]) the subharmonic signals from Sonazoid microbubbles were too low to be separated from the ambient noise level. Another study that exploits the results presented in [22], shows that pressure modulated subharmonic signal amplitude may be useful for subharmonic imaging because higher subharmonic signal amplitude from the microbubbles may be induced by varying ambient pressures [21], but *in vivo* implementation of this concept has not been provided.

In the current work subharmonic signal amplitude was characterized in 3 distinct stages – occurrence, growth and saturation. However, separate *in vitro* studies [22, 42], have shown that subharmonic signal amplitude may be characterized in 5 distinct stages – occurrence, growth, stabilization, regrowth and saturation; the subharmonic signal amplitude in the current study in the growth stage corresponding to the subharmonic signal amplitude in the regrowth stage in the *in vitro* studies [22, 42]. Possible reasons for this observation (of 5 stages of subharmonic signal amplitude) as part of the *in vitro* studies [22, 42] may be due to different types of UCAs used or due to the fact that the current study was conducted *in vivo* where conditions are markedly different from an idealized *in vitro* setup. Further, explicit analysis with Sonazoid microbubbles (UCA used in the current study) *in vitro* with an idealized experimental setup described elsewhere [7] did not reveal the presence of 5 distinct stages of subharmonic signal amplitude; instead the subharmonic signal amplitude only revealed 3 stages as was observed previously (e.g., [13] using Levovist microbubbles) and in the current study.

The data in Table 3 represent the results of paired comparisons for subharmonic signal amplitude before and after inducing PH. A statistically significant difference between subharmonic signal amplitude, before and after inducing PH, was only noted for data acquired with 4 transmit cycles and at optimized IAO level. Thus, based on data presented in Tables 2 and 3, and Fig. 6, the hypothesis that SHAPE's performance would be best at optimum IAO level identified by the automatic IAO optimization program and at 4 transmit cycles was accepted. These results also demonstrate the need to optimize IAO levels for SHAPE applications.

The results presented in this study were based on 180 data sets acquired from 4 canines. However, a small sample size of 4 canines used in this study remains a limitation. Since ultrasound scanning and data acquisition were performed by placing the transducer through the open abdominal cavity created to gain access to the PV, the backscattered signals had less attenuation relative to an intact abdomen.

3.4. Clinical relevance

Clinically, PV pressures cannot be obtained without creating explicit access to the PV, which is an invasive process. Hepatic venous pressure gradient (HVPG) is a clinical standard to assess PV pressures [43]. The technique involves guiding a diagnostic catheter from the jugular vein to the hepatic vein and measuring the difference between wedged and free hepatic venous pressures [43]. This HVPG measurement process is invasive, requires fluoroscopic guidance and is mostly performed at the time of transjugular liver biopsy [43, 44]. On the other hand, the progression of normal liver to cirrhotic liver is marked by increases in PV pressures and is characterized by a prolonged pre-clinical phase and a short clinical phase where symptoms manifest and can be fatal [29]. Thus, early identification of elevated PV pressures may help curb the associated morbidity and mortality.

Some noninvasive techniques are being developed to assess portal hypertension [45-51]. A noninvasive technique to monitor liver stiffness as an indirect indicator of HVPG showed weak correlation between liver stiffness and HVPG for patients with severe PH and these

measurements are only applicable in patients with body mass index < 35 and without ascites (common complication of PH) and nonalcoholic fatty liver diseases [51]. The use of grayscale and Doppler ultrasound to study PV, hepatic vein or hepatic artery characteristics such as diameter and flow patterns, as a predictor for elevated PV pressures suffer due to differential development of portosystemic shunts and collateral circulation as a result of PH [46, 52]. The use of Doppler ultrasound waveforms of the hepatic vein and some Doppler derived indices to monitor PV pressures indirectly has also been studied, but the approach has yielded mixed results [45, 48, 49]. Another limitation of using Doppler derived indices lies in using Doppler measurements that are known to be operator dependent. The current work documents that SHAPE technique may be used to estimate PV pressures and PV pressure changes; implementation of the automated IAO optimization program may assist in real-time PV pressure estimation in the future.

4. Conclusion

A novel automated IAO optimization program was developed to determine optimum acoustic pressure levels to insonate the UCAs for SHAPE applications. The approach was validated to estimate and monitor PV pressures in canines using SHAPE. The results demonstrated that SHAPE functions best when 4 transmit cycles were used to insonate the UCAs at the optimum IAO level determined by the IAO optimization program. The performance of SHAPE was sub-optimal when IAO levels below- or above- the optimized levels were used. Thus, the need to identify the optimum IAP level for SHAPE applications is justified and the approach presented here is validated.

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Highlights

- Tested subharmonic aided pressure estimation (SHAPE) for portal vein (PV) pressures
- Proposed automated algorithm for optimum incident acoustic output (IAO) for SHAPE
- Investigated the effect of 4, 8 and 16 transmit cycles on SHAPE
- 4 transmit cycles and optimum IAO represented the best combination for SHAPE
- SHAPE at optimum IAO tracked PV pressures; real time SHAPE may be feasible now

Fig. 1.
Schematic of the experimental setup.

Fig. 2.

The dual grayscale (left) and pulse inversion subharmonic (right: in the region of interest (ROI)) imaging modes. The portal vein (PV) and inferior vena cava (IVC) are marked. At the start of the IAO optimization function, the acoustic output corresponding to the subharmonic mode is set to 0 % (green arrow); due to no insonation, noise artifacts are seen in the subharmonic ROI.

Fig. 3.
Flowchart of the incident acoustic output optimization function.

Fig. 4.

Output of the incident acoustic output (IAO) optimization function (dots). The abscissa corresponds to log transformation of the IAO indicated on the scanner in percentage. The fitting function compensates for motion artifacts (mostly observed due to respiration) during IAO optimization. The optimum IAO was calculated as the IAO level with the maximum slope. In this case, the optimum IAO level (arrow) was 20 % (0.56 MPa_{peak-to-peak}) which corresponds to 1.30 on the abscissa.

Fig 5.

Representation of the subharmonic signal at varying incident acoustic output (IAO) levels (green arrows) used in the optimization function. (a) At 5 % IAO level the subharmonic signal begins to appear and resides at the beginning of the growth stage. (b) At 20 % IAO level the subharmonic signal resides in the linear portion of growth stage (ambient pressure sensitive stage). (c) At 60 % IAO level the subharmonic saturation begins, possibly with the occurrence of bubble destruction. (d) At 100 % IAO level the subharmonic signal is not that different from (c) indicating saturation.

Fig. 6.

(a), (b) and (c) depict the relationship between change in portal vein pressures and change in subharmonic signal amplitude at optimized incident acoustic output levels with 4, 8 and 16 transmit cycles – the best fit line is shown (dashed) with correlation coefficient. The best correlation was obtained with 4 transmit cycles and optimized incident acoustic output level (a). (d) The relationship between absolute portal vein pressures and subharmonic signal amplitude is shown with the best fit line (dashed) and the correlation coefficient is indicated.

Table 1

Portal vein pressures (pressures from 4 canines indicated with standard deviation)

	Minimum Pressure (mmHg)	Mean Pressure (mmHg)	Maximum Pressure (mmHg)
Baseline conditions	8.45	11.88 ± 3.89	15.77
Induced portal hypertension	17.21	27.81 ± 7.15	32.22

Correlation coefficient relating change in subharmonic signal amplitude to change in portal vein pressures (r_{change}) and relating absolute subharmonic signal amplitude to absolute portal vein pressures (r_{absolute})

Table 2

Incident acoustic output (IAO) level	Number of transmit cycles		Correlation results			
	n	r_{change}	p-value	n	r_{absolute}	p-value
Optimized	4	-.76	0.24	8	-.89	< 0.01
	8	.30	0.70	8	-.54	0.16
	16	.10	0.90	8	-.59	0.12
Below optimized	4	.04	0.95	8	-.72	0.04
	8	.15	0.85	8	-.29	0.49
	16	.31	0.69	8	-.65	0.08
Above optimized	4	N/A ^a	N/A ^a	4	-.73	0.27
	8	N/A ^a	N/A ^a	4	-.61	0.39
	16	N/A ^a	N/A ^a	4	-.18	0.82

^aN/A: not applicable as the number of data points was insufficient, because data from 2 canines were not acquired at higher IAO level

Table 3

Difference between subharmonic signal amplitudes before (SH_{before}) and after (SH_{after}) inducing portal hypertension (mean differences presented as: $SH_{\text{after}} - SH_{\text{before}}$)

Incident acoustic output (IAO) level	Number of transmit cycles	n	Results of paired differences test			p-value
			Mean Difference $\pm$ Standard Deviation (dB)	95 % Confidence Interval (dB)		
				Lower	Upper	
Optimized	4	4	-8.35 $\pm$ 2.45	-12.26	-4.44	<0.01
	8	4	-3.99 $\pm$ 6.19	-13.85	5.86	0.29
	16	4	-3.61 $\pm$ 4.48	-10.74	3.51	0.21
Below optimized	4	4	-4.51 $\pm$ 3.81	-10.57	-1.55	0.10
	8	4	-1.67 $\pm$ 6.01	-11.23	7.89	0.62
	16	4	-5.02 $\pm$ 4.42	-12.04	-2.01	0.11
Above optimized	4	2 ^a	-7.57 $\pm$ 5.57	N/A ^b	N/A ^b	0.31
	8	2 ^a	-6.85 $\pm$ 7.12	N/A ^b	N/A ^b	0.40
	16	2 ^a	-2.52 $\pm$ 5.01	N/A ^b	N/A ^b	0.61

^aShould be interpreted with caution as data from 2 canines at higher IAO level were not acquired due to timing constraints (data provided here for reference)

^bN/A: not applicable