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iCONCEPTS

CONCEPTS ON THE VERGE OF TRANSLATION

Noninvasive LV Pressure Estimation Using Subharmonic Emissions From Microbubbles

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To develop a new noninvasive approach to quantify left ventricular (LV) pressures using subharmonic emissions from microbubbles, an ultrasound scanner was used in pulse inversion grayscale mode; unprocessed radiofrequency data were obtained with pulsed wave Doppler from the aorta and/or LV during Sonazoid infusion. Subharmonic data (in dB) were extracted and processed. Calibration factor (mm Hg/dB) from the aortic pressure was used to estimate LV pressures. Errors ranged from 0.19 to 2.50 mm Hg when estimating pressures using the aortic calibration factor, and were higher (0.64 to 8.98 mm Hg) using a mean aortic calibration factor. Subharmonic emissions from ultrasound contrast agents have the potential to noninvasively monitor LV pressures. (J Am Coll Cardiol Img 2012;5:87–92) © 2012 by the American College of Cardiology Foundation

Knowledge of left ventricular (LV) pressures is important for the diagnosis, treatment, and management of patients with several cardiac abnormalities (1,2). Invasive hemodynamic monitoring with right heart catheterization has remained the clinical standard of care for these patients (1). However, the potential complications associated with this invasive technique (1) underscore the need for reliable noninvasive methods to measure intracardiac pressures (2).

Microbubble-based ultrasound contrast agents (UCA) were approved in the United States for LV opacification studies but have the potential to be applied much more broadly. A promising approach to monitoring pressure changes with UCA relies on the fact that these gas bubbles exposed to pressure changes exhibit volume pulsations. Furthermore, the difference in compressibility between the shell-encapsulated microbubbles and the surrounding medium (i.e., blood) enhances the backscattered/reflected signals from these microbubbles. Thus, when microbubbles are imaged by conventional ultrasound machines, they reflect

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sound waves over a range of frequencies with the greatest amplitude at the insonation frequency. Additional peaks of reflectance occur at multiples (harmonics) of that frequency, as well as at intermediate harmonics, the largest of which is the subharmonic frequency at one-half the insonation frequency. We have found that the amplitude of this subharmonic signal is sensitive to changes in pressure and have proposed a technique that uses subharmonic signals for pressure estimation called subharmonic aided pressure estimation (SHAPE) (3). This technique is based on the principle that the subharmonic amplitude in the received signal spectrum is linearly ($r^2 = 0.96$) and inversely related to an increase in ambient pressures (3). Previously, the use of SHAPE to monitor pressure changes in the aorta of open-chest canines using single-element transducers was reported (4). However, the use of single-element transducers (without imaging capability) and open-chest measurements preclude clinical investigations. Therefore, the goal of this pilot study was to assess the potential of SHAPE to determine LV pressures noninvasively, with a commercially available ultrasound scanner.

ABBREVIATIONS AND ACRONYMS

LV = left ventricular

LVD_{min} = minimum left ventricular diastolic pressure

LVEDP = left ventricular end diastolic pressure

LVPSP = left ventricular peak systolic pressure

M-LVDP = mean left ventricular diastolic pressure

SHAPE = subharmonic aided pressure estimation

UCA = ultrasound contrast agents

This research study was approved by the Institutional Animal Care and Use Committee of Thomas Jefferson University and conducted in accordance with National Institutes of Health guidelines. Four mongrel dogs weighing 22.5 ± 1.00 kg were studied. Intravenous injection of Propofol (Abbott Laboratories, Chicago, Illinois; dose 7 ml/kg) was used as initial anesthetic, while a facemask with isoflurane 2% to 4% (Isothesia; Abbott Laboratories) maintained sedation. The canines were placed on a warming blanket to maintain normal body temperature. Based on previous studies, Sonazoid microbubbles (GE Healthcare, Oslo, Norway) were selected for use in this study (5). An 18-gauge catheter was placed in a forelimb vein for Sonazoid infusion (0.015 μl/kg/min). A 5-F solid-state catheter tip manometer (SPR-350; Millar Instruments, Inc., Houston, Texas) was used as the reference standard and was introduced at the site for pressure measurements under ultrasound guidance. Poststudy, the canines were sacrificed by using an intravenous injection of Beuthanasia (Schering-Plough Animal Health, Kenilworth, New Jersey; 0.25 mg/kg).

A commercial Sonix RP ultrasound scanner with PA4-2 phased array probe (2.5-MHz center frequency; Ultrasonix Medical Corporation, Richmond, British Columbia, Canada) was operated with pulse inversion grayscale imaging to scan the canines (closed-chest). The accumulated signal from 2 ultrasound pulses (with a phase difference of 180°) was acquired from the pulsed wave Doppler gate placed at the region of interest (i.e., in the LV or aorta) (Fig. 1). A synchronization signal from the ultrasound scanner triggered an oscilloscope (9350 AM; LeCroy Corporation, Chestnut Ridge, New York) for simultaneous acquisition of the pressure catheter data on a computer through LabVIEW (version 8.0; National Instruments Corporation, Austin, Texas). The subharmonic response from the UCA and their ability to track ambient pressures depend on insonation frequency and incident acoustic pressure (5). Thus, transmit frequency was maintained at 2.5 MHz (5), while incident acoustic output from the scanner was varied from −8 to 0 dB. Ultrasound radiofre-

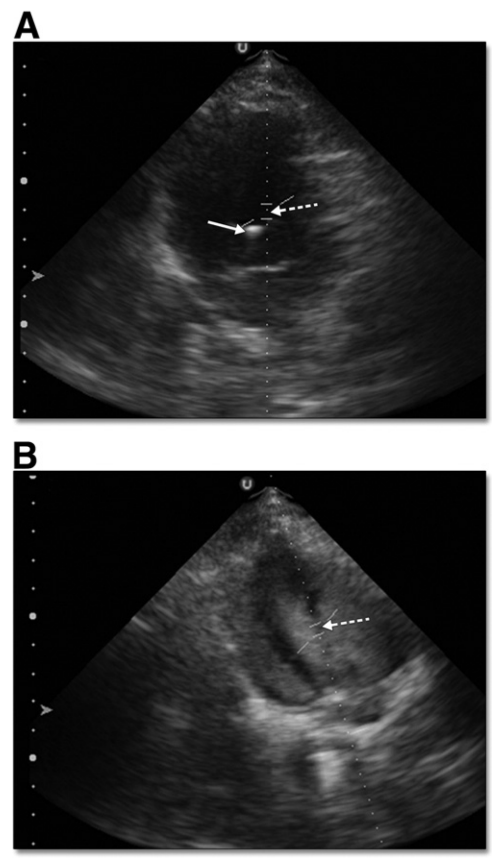

Figure 1. Ultrasound Data Acquisition

Grayscale ultrasound image of the left ventricle **(A)** without and **(B)** with ultrasound contrast agents. **Solid arrow** in **(A)** indicates the pressure catheter; **dotted arrow** in **(A and B)** indicates the pulsed wave Doppler gate used to acquire the ultrasound data.

quency data were acquired for 5 s (n = 3) simultaneously with the pressure catheter data from the aorta of 2 canines and the LV of 4 canines, cumulating at 90 acquisitions.

Data processing was performed offline using MATLAB (version 7.8.0; The MathWorks, Inc., Natick, Massachusetts). Unprocessed radiofrequency data for each accumulated pulse (Fig. 2A) were transformed to the Fourier domain, and the subharmonic signal amplitude in decibels (Fig. 2B) was extracted as the average signal in a 40% bandwidth around the subharmonic frequency (i.e., 1.25 MHz). These data were processed using a median filter to eliminate noise spikes. The range of the subharmonic signal (i.e., maximum minus minimum subharmonic amplitude) was compared from each pulse contour (after eliminating noisy pulses) for each incident acoustic pressure. The incident acoustic pressure with maximum stable subharmonic range was selected for LV pressure tracking (Fig. 2C) to avoid loss of microbubbles' pressure sensitivity due to bubble collapse at high-incident acoustic pressures or lack of subharmonic signals at low acoustic pressures. The calibration factor (in millimeters of mercury per decibel) was calculated using least square regression analyses from the aortic data (using both subharmonic data and pressure values). This calibration factor was applied to the subharmonic data from the LV of the individual canine to determine the mean left ventricular diastolic pressure (M-LVDP), minimum left ventricular diastolic pressure (LVD_{min}), left ventricular end diastolic pressure (LVEDP), and left ventricular peak systolic pressure (LVPSP). For 2 other canines, LV pressure estimates were obtained with the mean calibration factor from the first 2 canines. The subharmonic estimates were compared with the manometer pressures. Two-tailed paired *t* tests were conducted for each LV pressure parameter obtained from SHAPE and the reference standard (p values <0.05 were considered significant).

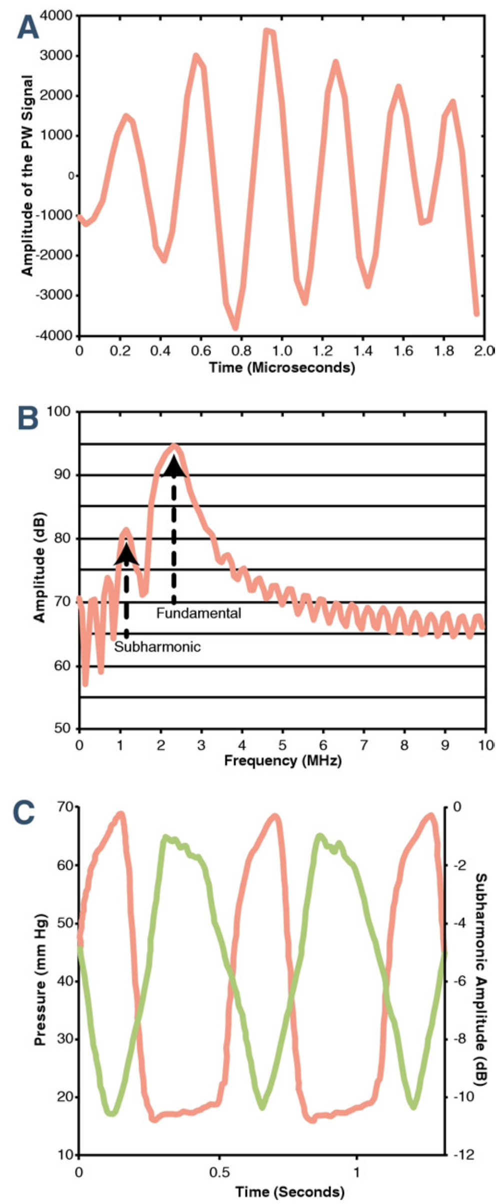

Figure 2. Ultrasound Data Processing

Steps for extracting and processing the subharmonic signal. **(A and B)** Illustrate a typical signal from a pulsed wave Doppler gate and its frequency domain representation. Fundamental and subharmonic signals within the bandwidth of the transducer are labeled **(B)**. **(C)** Illustrates the processed subharmonic signal **(green)** and the pressure catheter data **(pink)**; note the inverse relationship, which is in agreement with documented literature (3–5).

Analysis of the Calibration Factor

The calibration factors obtained from the aorta of 2 canines verified that the subharmonic signal varied inversely with the underlying pressure waveform. The maximum error between the mean aortic pressures recorded by the SHAPE approach and the reference standard was 2.5 mm Hg (Table 1).

For 2 canines in whom the calibration factor was obtained from the aorta, the LV pressure estimates were calculated using the known peak systolic LV pressures. Figure 3 shows a trace of LV pressure obtained using SHAPE and the manometer pressures for canine 1. Overall, errors between the SHAPE approach and the pressure catheter ranged from 0.19 to 2.5 mm Hg (Table 2).

Table 1. Subharmonic-Pressure Calibration Factor From the Aorta

Canine	Calibration Factor in the Aorta (mm Hg/dB)	SE of the Estimates (mm Hg)	Mean Aortic Pressures (mm Hg)	
			SHAPE	Catheter
Canine 1	−4.929	4.32	67.98	68.29
Canine 2	−4.920	7.13	59.61	62.10

Calibration factor calculated from the aorta of 2 canines shown with the SE of the estimates from the linear regression analysis. Mean aortic pressures obtained using subharmonic aided pressure estimation (SHAPE) and the manometer are compared.

For the 2 other canines, the mean calibration factor from the first 2 canines (i.e., −4.9245 mm Hg/dB obtained from Table 1) was used to estimate the LV pressures. For these canines, relatively higher errors between the SHAPE approach and the pressure catheter were reported, ranging from 0.64 to 8.98 mm Hg (Table 3).

None of the differences between SHAPE and the reference standard for all the estimates of LV pressures were significant ($p > 0.17$) (Table 4). The mean absolute errors for M-LVDP, LVD_{min}, LVEDP, and LVPSP were 1.58 ± 1.01 mm Hg, 1.35 ± 0.92 mm Hg, 4.90 ± 3.26 mm Hg, and 1.75 ± 0.98 mm Hg, respectively. Heart rate obtained from the frequency domain representation of SHAPE data and pressure catheter data was also in good agreement with maximum absolute error of 4.39 beats/min (Tables 2 and 3). These results, based on estimates from all 4 canines, indicate that SHAPE is able to noninvasively track in vivo pressures in the LV if the pulse pressures in the aorta are known.

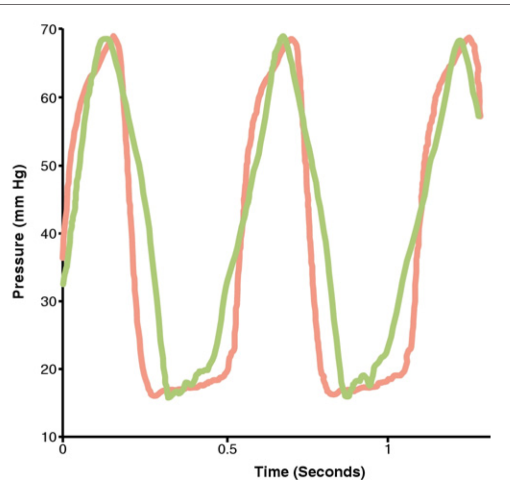

Figure 3. LV Pressure Tracking

Left ventricular (LV) pressure waveform obtained using the manometer **(pink)** and the corresponding subharmonic aided pressure estimation results **(green)**.

Discussion

The use of microbubbles to quantify ambient pressures based on shift in resonance frequency, detection of single bubble echoes, excitation using dual frequencies, monitoring cavitation onset, or dissolution time of microbubbles has been tested in vitro, with errors ranging from 10 to 15 mm Hg under ideal conditions. None of these techniques have documented in vivo results. Conversely, Shi et al. (3) verified empirically that subharmonic signal amplitude from UCA show ambient pressure sensitivity. This technique was used in vivo to investigate its efficacy (4), though invasively, and finally studies were performed to identify optimum parameters for noninvasive application of SHAPE (5), which were used in this study (i.e., Sonazoid microbubbles and 2.5-MHz insonation frequency). The incident acoustic pressure required to elicit a subharmonic response sensitive to in vivo pressures will vary on a case-by-case basis depending on attenuation of the incident beam and the scanning depth. Therefore, acoustic output power on the scanner was varied, and the level showing maximum stable subharmonic range was selected for pressure monitoring.

To the best of our knowledge, this is the first in vivo study demonstrating the application of subharmonic emissions from microbubbles for noninvasive quantification of LV pressures. First, it was hypothesized that the subharmonic emissions from microbubbles can be used to quantify in vivo LV pressures noninvasively. The resultant pressure errors between SHAPE and the pressure catheter did not exceed 2.50 mm Hg when the calibration factor from the aorta of the individual canine was used in the pressure derivation stage (Table 2). In addition, there were no statistically significant differences between pressures recorded by SHAPE and the pressure catheter ($p > 0.17$) (Table 4). Second, it was hypothesized that a mean calibration factor may be used across all subjects. The errors increased to 8.98 mm Hg under this hypothesis (Table 3).

Table 2. LV Pressure Measurements With Aortic Calibration Factors

Measurement	Canine 1				Canine 2			
	SHAPE	Catheter	Error (mm Hg)	Percent Error	SHAPE	Catheter	Error (mm Hg)	Percent Error
M-LVDP	20.11	17.61	2.50	14	14.21	13.42	0.79	6
LVD_{min}	15.88	15.69	0.19	1	7.53	8.89	−1.36	−15
LVEDP	22.07	19.74	2.33	12	19.11	16.89	2.22	13
LVPSP	70.23	68.81	1.42	2	83.84	82.11	1.73	2
Heart rate (beats/min)	109.77	109.86	NA	NA	105.47	109.86	NA	NA

LV pressures from 2 canines (in whom data were also acquired from the aorta) using SHAPE compared with the reference standard (manometer catheter). Errors are indicated as reference standard subtracted from the SHAPE data. Percent errors are calculated using the reference standard.
LV = left ventricular; LVD_{min} = minimum left ventricular diastolic pressure; LVEDP = left ventricular end diastolic pressure; LVPSP = left ventricular peak systolic pressure; M-LVDP = mean left ventricular diastolic pressure; other abbreviation as in Table 1.

This finding suggests that the addition of aortic measurements to obtain a calibration factor from each individual subject allows LV pressure estimation with less error. The calibration factor relates the change in subharmonic signal amplitude to change in pressure value; this relationship extends across the full range of clinical pressures (5). In cases presented with disease states such as aortic stenosis, in which LVPSP may differ from the peak cuff-based pressure estimate, the Doppler-based LV–aortic pressure gradient may be additionally incorporated for LVPSP determination. Thus, we have shown that SHAPE is in good agreement with the reference standard (maximum mean absolute error 2.40 ± 2.22 mm Hg) based on data from 4 canines across all pressure estimates.

Study limitations. A new version of the subharmonic extraction and processing algorithm will permit real-time pressure estimation in future studies, unlike the off-line data processing of this study. In addition, the SHAPE technique is dependent on incident acoustic pressures. In this study, discrete increments (of 2 dB [i.e., 25% relative increase]) for the incident acoustic pressures (fixed by the ultrasound scanner) were used. Thus, if the most "sensitive" incident acoustic pressure falls within the range of 25%, then the SHAPE pressure sensing will not be "optimum," thereby contributing to the errors attained in the LV pressure estimates.

The small sample size (4 canines) may obscure any statistically significant difference between SHAPE estimates and the manometer pressures.

For potential clinical use, the accuracy of this technique with cuff-based pressure measurements remains to be investigated. Lastly, the hemodynamics in the canines were not altered. However, as evident in Figure 3, relative changes occurred in the subharmonic amplitude of the signal in response to changes in the ambient pressures, and thus real-time pressure tracking is feasible (to monitor instantaneous hemodynamic changes as well).

Conclusions

Clinical implications. A single underlying technique to quantify LV pressures noninvasively may aid patient management in addition to the existing indices, which are mostly applicable in specific subsets of cardiac pathologies. The absolute LV pressures obtained using the SHAPE approach may be applicable to all clinical populations without the added risks and costs involved in right heart catheterization. As UCA are already approved in the United States for LV opacification, the supplemen-

Table 3. LV Pressure Measurements Without Aortic Calibration Factors

Measurement	Canine 3				Canine 4			
	SHAPE	Catheter	Error (mm Hg)	Percent Error	SHAPE	Catheter	Error (mm Hg)	Percent Error
M-LVDP	13.61	14.25	−0.64	−4	15.43	13.02	2.41	19
LVD_{min}	5.82	7.23	−1.41	−20	12.04	9.59	2.45	26
LVEDP	12.06	18.13	−6.07	−33	21.99	13.01	8.98	69
LVPSP	82.66	79.57	3.09	4	83.84	84.59	−0.75	1
Heart rate (beats/min)	99.98	100.71	N/A	N/A	94.79	91.55	N/A	N/A

LV pressures from 2 canines (in whom data were acquired from the left ventricle only) using SHAPE compared with the reference standard (manometer catheter). The errors are indicated as in Table 2.
Abbreviations as in Tables 1 and 2.

Table 4. Paired Comparisons of SHAPE in the LV and the Manometer Pressures for All Canines

	Difference (mm Hg) (SHAPE − manometer pressure)			95% CI of the Difference (mm Hg)		
Measurement	Mean	SD	SEM	Lower	Upper	Significance (2-tailed)
M-LVDP	1.26	1.49	0.75	−1.11	3.64	0.18
LVD_{min}	−0.03	1.81	0.91	−2.92	2.85	0.97
LVEDP	1.86	6.16	3.08	−7.94	11.67	0.59
LVPSP	1.37	1.59	0.79	−1.16	3.90	0.18

CI = confidence interval; other abbreviations as in Tables 1 and 2.

tary pressure values obtained using SHAPE may provide a quantitative parameter for the diagnosis of a myriad of cardiac pathologies. Moreover, these pressures can be obtained with the same contrast administration dosage because the subharmonic signal amplitude reduction was negligible even when the concentration of the bubbles was varied by a factor of 3 (3). Therefore, we concluded that the ability of subharmonic emissions from UCA to noninvasively monitor LV pressures should now be investigated in clinical populations to verify ultimate applicability.

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Key Words: contrast echocardiography ■ noninvasive pressure estimation ■ subharmonic microbubble signals.