

Pulse Wave Imaging in Murine Abdominal Aortas

A Feasibility Study

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Abstract—One of the most crucial aspects of abdominal aortic aneurysm (AAA) diagnosis lies in the early detection of the aneurysm and its propensity for rupture. This study aims at determining whether the estimation of the aortic wall stiffness of the natural mechanical, pulsating motion of the aorta is feasible using high-resolution and high-frame-rate imaging in a murine model. Twelve wild-type (WT) mice were anesthetized, and underwent laparotomy. The abdominal aortas of five normal mice were scanned using a high-resolution (30 MHz) Vevo 770 (Visualsonics, Inc., Ontario, Canada) system and acquiring the RF signals. A composite frame rate of 8 kHz was achieved through ECG triggering on the RF acquisition over several cardiac cycles, which increased the tracking ability of the elastographic technique. Crosscorrelation techniques using windows of 150-micron size and 90% overlap were applied to estimate motions on the order of tens of microns between successive frames. The strain and the velocity of the pulsive wave in the aorta were estimated and imaged using the gradient and phase shift estimation techniques, respectively. Finally, the Young's modulus of the aortic wall was derived using the pulsive wave velocity based on the Moens-Korteweg equation. The pulsive wave during pulsatile flow was imaged by mapping the wall displacements consecutively in a ciné-loop format. High wall displacements were observed 10.3 ms after the R-wave peak of the ECG and a pulsive wave was initiated traveling from the heart's side along the aortic wall in less than 3 ms within the image view. The phase velocity of the pulse wave was computed at the frequency of 200 Hz and a velocity of 2.60 ± 0.57 m/s was found. The radius of the aorta and the wall thickness (measured on the B-mode image) were found equal to $R=0.47$ mm and $h=0.12$ mm, respectively. Using these measured parameters, the Young's modulus of the aortic wall was found equal to $E = 50.9 \pm 20.0$ kPa. This value is in agreement with previously reported Young's moduli in the adventitia layer of the porcine descending aorta. In this study, we demonstrated that pulse wave imaging is feasible and that it can be used for mapping the propagation of the pulsive wave along the wall of the abdominal aorta in a murine model in order to determine its elasticity. Ongoing studies aim at determining the potential for this technique to be used in the detection of aneurysms based on their associated change of mechanical properties of the aortic wall.

Keywords; abdominal aortic aneurysm (AAA), high-frame-rate, displacement, high-resolution, pulse-wave velocity, Young's modulus

I. INTRODUCTION

Aortic stiffness has been indicated as an early predictor of cardiovascular mortality, primary coronary events, and fatal stroke [1,2,3]. In aortic aneurysm cases, disruption of the medial elastic fibers may produce aortic stiffening, which

amplifies the aneurysmal process [4]. Pulse-wave velocity (PWV) is widely used for estimating the stiffness of an artery [5]. Although diameter measurement is currently used clinically as a rupture predictor of AAA, increasing compliance of aneurysmal wall at the maximum diameter point over the time course has been discussed as a better rupture predictor.

Non-invasive ways to evaluate the wall in the case of AAA have been investigated by several groups [6,7]. A non-invasive and easy way of detecting local PWV in the aorta may contribute a future screening test to reduce the overall morbidity and mortality. Ultrasound allows non-invasive measurement of PWV, and has been an area of recent interest [8,9,10]. Kanai et al. [11] demonstrated high frame-rate, coarse lateral resolution (16 beams) ultrasound imaging is a technique to capture very fast propagation of pulsive wave generated by the valve vibration in the heart. The axial displacements within tissues could be estimated from the radio-frequency (RF) signals before and after compression [12]. In addition to axial displacements and strain, myocardial elastography has been shown capable of obtaining lateral displacements and strains [13].

We developed an ultrafast data acquisition system operating on a high-resolution Vevo 770 system (VisualSonics Inc., Toronto, ON, Canada) combined with an ECG-gating technique [14], which allowed us to acquire RF data and ECG signals simultaneously. The RF images of the mouse myocardium and aorta were obtained at an extremely high frame rate, up to 8 kHz, and the RF data corresponding to a complete cardiac cycle were then gated using the ECG to reconstruct full-view 2-D images. This technique allowed us to estimate the small displacement of the murine myocardium and abdominal aorta, which indicated the mechanical wave propagation along a cardiac cycle.

As mentioned above, PWV is a surrogate measure of stiffness of the aortic wall, which may vary with the type and severity of disease. In this paper, the same setting as in our previous study [14] was used, including the Vevo 770 system, and the RF data and ECG acquisition protocol. The temporal resolution of our system was equal to 0.125 ms, i.e. the frame rate up to 8 kHz. The system also displayed color-coded motion to allow us to visually diagnose the wave propagation pattern.

We hypothesize that this technique of detecting displacement, pulse-wave propagation and elasticity is reproducible and that it may contribute to a non-invasive

modality of evaluating the mechanical properties of the aorta, both at the absence and presence of diseases. We evaluated the possibility of detecting these parameters of the abdominal aortic wall in mice using the extremely high frame-rate ultrasound system. The pulse-wave was imaged during the propagation, the PWV was measured, and the stiffness of the abdominal aortic wall was estimated by calculating the Young's modulus. The purpose of this study was to develop a reproducible technique and to demonstrate the feasibility of analyzing aortic wall stress non-invasively.

II. METHODS

A. *Selecting a Template (Heading 2)*

All procedures used in this study were approved by the institutional animal committee (IACUC) of Columbia University. Twelve wild-type mice (three to four months old) were anaesthetized with 125 mg/kg tribromoethanol, and the abdominal hair was removed using potassium thioglycolate. The mice were placed in the supine position on the heating stage (VisualSonics, Toronto, Ontario, Canada) in order to maintain constant body temperature. ECG was obtained from the extremities.

A longitudinal view of the abdominal aorta was obtained non-invasively with ultrasound at 30 MHz with Vevo 770 system using degassed ultrasound gel (Aquasonic 100, Parker Laboratories Inc., Fairfield, NJ, USA) as a coupling medium. The focal depth of the ultrasound transducer was 12.7 mm from the surface of the transducer, the field of view was 12-mm \times 12-mm, the axial resolution was equal to 50 μm , and the lateral resolution was equal to 100 μm . The ultrasound probe was placed on the animal's abdomen in the parasternal position to obtain a longitudinal (long axis) view of the abdominal aorta. Real-time 2D images were acquired at a frame rate of up to 60 Hz.

A two-channel, 200 MS/s, 14 Bit Waveform Digitizer for PCI Bus Bus (CompuScope 14200, Gage Applied Technologies, Inc., Lachine, QC, Canada) was used to synchronously acquire the RF signals of the ultrasound scanner and the associated ECG, with the ECG allowing the gating of the RF signals. The aortic wall displacements were accurately estimated using 1D cross-correlation (window size = 0.240 mm, window shift = 0.024 mm). The ECG was recorded simultaneously, which enabled the triggering based on the R-wave peaks. Two TTL outputs were used to trigger the digitizer on the 2D frames, which allowed the real-time acquisition of more than one thousand frames with 2D RF data. During acquisition, the RF and ECG signals were digitized synchronously and transferred to the computer in real-time. After acquisition, the data was processed off-line, and the RF-lines from different cardiac cycles were synchronized based on a standardized cardiac cycle, with adjusted R-R intervals. With such a technique similar to EKV reconstruction, the complete set of 2D ultrasound RF data was reconstructed at an extremely high frame rate of 8 kHz for a complete heart cycle [14]. The pulse wave generated by the pulsatile blood flow in the abdominal aorta was imaged, and the resulting displacement of the aortic wall and the velocity of the pulse wave along the aortic wall were calculated, as described in the next section

using signal processing techniques built in Matlab-based software (MathWorks, Inc., Natick, MA, USA) [14].

The axial displacements of the tissue were estimated off-line using a 1-D cross correlation technique on the consecutive RF signals obtained [13]. The window size was 240 μm , with 90% overlap.

The endothelial and epithelial sides of the abdominal aortic walls were manually traced on a B mode image by an operator. The maximum displacement of the aortic wall was thus found detected on each RF line in the ROI. The distance from the proximal side of the aorta displayed on the screen was plotted as a function of the timing of maximum displacement of each RF line, which was measured from the peak of the R-wave of the ECG. The best-fit linear line was obtained, and the slope was defined as indicative of the PWV. The correlation coefficient was also calculated to evaluate the reliability of the PWV acquisition.

Five wild-type mice, three to four months old, were anesthetized, and underwent laparotomy. The abdominal aortas of the mice were exposed to 0.5 mol/L CaCl_2 . After four weeks of the operation, the abdominal aorta was scanned using Vevo 770 system; the 2D images, RF data and ECG signals were acquired.

III. RESULTS

The pulse wave propagating along the murine aortic wall was clearly detected in the forward direction using color-coded cine-loop. The time course of the pulse wave propagation was clearly visualized along the aortic wall by color-coded cine-loop with a frame rate of 8 kHz. Figure 1 displays eight frames every 1.25 ms.

The time course of the displacement transition of aortic wall of each RF line was tracked for one cardiac cycle, from one R-wave peak to the next. The maximum displacement that occurred at every depth across the entire field-of-view (Fig. 1) can be plotted against the time, at which the maximum displacement occurred. This yields an image that clearly shows the linear propagation of the pulse wave in the case of a normal aorta (Fig. 2a) and the non-linear propagation in the case of a AAA aorta (Fig. 2b). The top line of Figure 2 is acquired from the RF segment on the most proximal side of the full image; the bottom line is acquired from the RF segment on the most distal side. The displacement of the abdominal aortic wall corresponds to the pulse excitation. In wild-type mice, the displacement propagated from the proximal to the distal side along the abdominal aortic wall, which was translated to a pulse-wave propagation along the aortic wall. The time coordinate of the image (horizontal axis of Figure 2) had a resolution of 125 μs : the distance coordinate of the image (vertical axis of Figure 2) was with resolution of 50 μm . The signal amplifier gain was adjusted so that the wave amplitude signals were digitized between ± 8 kHz.

The relative timing of maximum displacement on the aortic wall segment was plotted as a function of the distance from

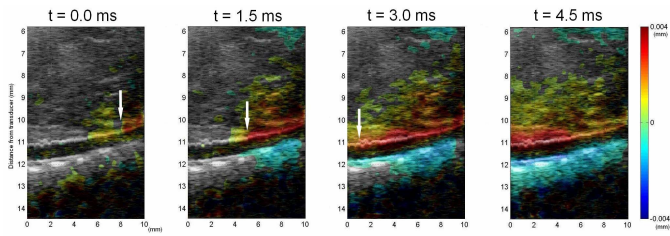


Fig. 1. Displacement image, every 1.5 ms, along abdominal aortic wall of WT mouse.

the proximal edge of the ultrasound full-image. The wave velocity was 2.60 ± 0.57 m/s ($r^2 = 0.82 \pm 0.07$, $n=12$).

The average wall thickness of the wall was found equal to 0.13 ± 0.01 mm. Because the wall thickness was so close to the lower limitation of the measurement accuracy, we applied the average value for the Young's modulus calculation to avoid dispersion cause by the measurement variance. The average Young's modulus of the aorta was calculated as 50.9 ± 20.0 kPa ($n=12$).

The relative timing of maximum displacement on the aortic wall segment was plotted as a function of the distance from the proximal edge of the ultrasound full-image (Fig. 3a, 3b). The wave velocity of WT mice was 2.60 ± 0.57 m/s ($r^2 = 0.82 \pm 0.07$, $n=12$). The inner diameter and the wall thickness of the abdominal aorta were measured using standard B-mode method. The average wall thickness was found equal to 0.13 ± 0.01 mm. The Young's modulus calculation to avoid dispersion cause by the measurement variance. The average Young's modulus of the aorta was calculated as 50.9 ± 20.0 kPa ($n=12$). PWVs along the AAA wall were also estimated using the slope of the best linear fit line in each wild-type mouse case. The wave velocity was 2.95 ± 0.90 m/s ($r^2 = 0.51 \pm 0.22$, $n=5$). The correlations established statistical significance between the two groups ($p < 0.01$).

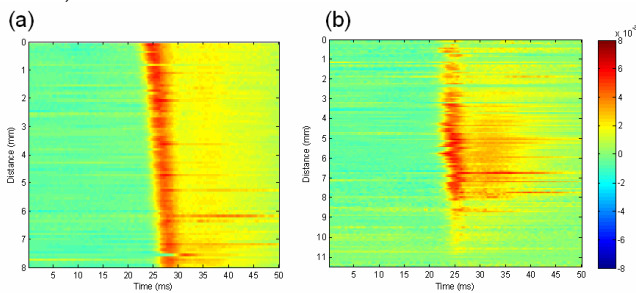


Fig.2. Displacement amount transition of a segment from each RF line. (a) WT, (b)AAA

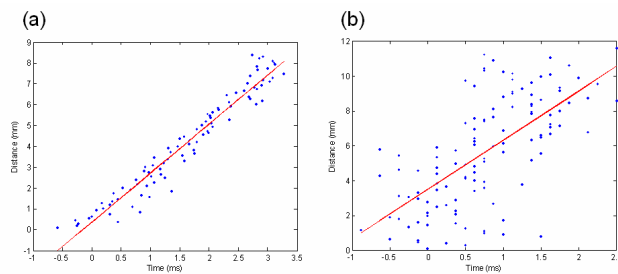


Fig. 3. The relative timing of maximum displacement on the aortic wall segment. (a) WT, (b)AAA

IV. CONCLUSION AND DISCUSSION

This study demonstrated a state-of-the-art technique to image pulse-wave propagation *in vivo* along the abdominal aortic wall of mice using extremely high frame-rate. The high axial resolution, as small as $0.05 \mu\text{m}$, enabled speckle tracking of the very thin abdominal aortic wall with great precision. This technique allows non-invasive estimation of stiffness on arteries *in vivo*, which may be useful in assessing the processes of various diseases. In future clinical applications, it might contribute in detecting the early phase of the degeneration of arterial wall caused by various diseases. This early detection will allow early initiation of necessary treatment to prevent the disease to further deteriorate, which may consequently decrease its mortality among high-risk patients.

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