A Retrospective Method for Pulse-Wave Velocity Measurement in the Mouse

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Abstract —**The pulse-wave velocity (PWV) is inversely related to arterial compliance, and provides a useful measure of vascular function. In this study, the PWV was measured non-invasively in the mouse carotid artery using the time-delay (TD) and flow-area (QA) methods. The TD technique determines the distributed PWV from the time-delay between Doppler-derived upstrokes at two locations a known distance apart. The QA method estimates the local PWV as the ratio between the change in volume flow and the change in cross-sectional area during the reflection-free period of the cardiac cycle. Our new QA approach measures the cross-sectional area and flow through the vessel using a high-frame-rate retrospective colour flow imaging (RCFI) technique. The cross-sectional area is determined by integrating over the region of flow in each frame of the RCFI dataset, while the volume flow is calculated by averaging the velocities over the vessel in each frame and multiplying by the corresponding area. The TD method was compared with the flow-area method in the carotid artery of 7 young CD-1 mice, anesthetized with isoflurane. The average TD PWV was found to be 3.03±0.17 m/s. The average QA PWV was found to be 2.97±0.18 m/s. The TD method was found to correlate well with the QA method ($r=0.91$, $p<0.001$). The mean difference between the TD method and the QA method was 0.06±0.08 m/s, and 95% of the differences fell within ±0.41±0.20 m/s of the mean difference. These results indicate that the QA method should be capable of distinguishing between changes to PWV caused by vascular disease. It was found that the QA method permits the measurement of the local PWV. The TD method offered superior reliability to the QA method for PWV determination in the mouse carotid artery because it was affected by fewer contextual factors. However, the QA method is useful for situations in which the TD method is unsuitable due to the geometry of the vessel.**

I. Introduction

Diseases such as hypertension and atherosclerosis can induce abnormalities in vascular function through alteration of arterial compliance. To study such diseases, transgenic mice have been created which develop abnormal vasculature (e.g. atherosclerosis in ApoE mice [1]). This has engendered a need to develop techniques capable of quantifying vascular function *in vivo*. The PWV is inversely related to arterial compliance, and provides a measurable index of vascular function.

The PWV was measured in mice by Hartley *et al.* [2] without image guidance. They favourably compared the TD method with tonometry and found the PWV varied from 2.20 to 8.50 m/s in the aorta, under a range of vasoactive anesthetics. Mitchell *et al.* [3] measured the PWV in the rat, comparing the TD method with manometry in the aorta. They found the PWV ranged from 5.09 to 6.00 m/s. Rabben *et al.* [4] developed a novel technique for determination of the local PWV, known as the flow-area (QA) method. In a study of humans and canines, they found that the QA method correlated well and was generally in good agreement with estimates based on the Bramwell-Hill equation, however, their results had a large degree of variability.

In this study, we compared a QA method of measuring the PWV with an image-guided TD method in the carotid artery of seven CD-1 mice. The TD technique was used to measure a distributed PWV over the carotid artery from the aortic arch to the bifurcation. The QA method was applied to determine the local PWV at the midsection of the carotid. The objective of this study was to assess the potential of the QA method against the TD method and to demonstrate a simple image-based methodology for performing PWV measurements. Determination of the most reliable PWV estimation method will be useful in a future longitudinal study of vascular development in transgenic mice.

II. Materials and Methods

A. Time-Delay (TD) Method

The TD method estimates the PWV from the pressure wave transit time, Δt, between two measurement locations a known distance, Δd, apart [5]:

$$c = \frac{\Delta d}{\Delta t} \tag{1}$$

The transit time was determined by performing Doppler velocity measurements at the distal and proximal locations while simultaneously recording the ECG signal. The transit time was found by subtracting the distal delay between the ECG R-wave and the foot of the velocity upstroke from the similarly determined proximal delay. The foot of the velocity upstroke is commonly used as a marker because the velocity peak may be altered by reflected waves. The foot is unlikely to be affected by reflected pressure waves [2]. For the carotid artery, the distance between the locations was determined from a B-mode image encompassing both the distal and proximal locations.

The carotid artery was chosen as a measurement target because it was easily accessible, and offered a uniform and branch-free pathway over approximately 10mm between the aortic arch and carotid bifurcation. Proximal measurements were taken 1 mm from the aortic arch. Distal measurements were taken 1.5 mm from the bifurcation.

TD measurements were performed using a VisualSonics Vevo770 ultrasound biomicroscope. Doppler data were acquired at 30 MHz, with a 50 kHz pulse-repetition frequency (PRF). B-mode data were acquired at 40 MHz, using a transducer with a focal depth of 6 mm and f# 2. Results were processed using VisualSonics measurement software and Matlab.

B. Flow-Area (QA) Method

With the QA method introduced by Rabben *et al.*[4], the PWV in an artery is estimated as the ratio between the change in flow and the change in cross-sectional area during the reflection-free period of the cardiac cycle. From the wave equation for arterial propagation and the characteristic impedance relation, it can be shown that:

$$c = \frac{\mathrm{d}Q}{\mathrm{d}A} \qquad (2)$$

where Q is the volume flow through the vessel and A is the cross-sectional area of the vessel. In most humans, the early systolic wave, associated with the ECG QR complex, is reflection free [5]. We have assumed the same to be true in the mouse.

In the work of Rabben *et al.* [4], the cross-sectional area and volume flow were independently determined. To find the cross-sectional area, M-mode data were collected and the area was found by assuming the vessel to be circular. The flow was found by recording a Doppler velocity measurement at the same location as the M-mode acquisition, and the flow was extrapolated from the peak Doppler velocities using a modified form of Womersley's theory of pulsatile flow in rigid tubes [4]. The PWV was found by plotting the flow with respect to the area. During the brief time that the flow and area are linearly related, the PWV is equal to the associated slope.

The main source of error in the method of Rabben *et al.* arises from the possibility of temporal misalignment between the flow and area curves, since they are independently measured. The effect of misalignment can be minimized by averaging repeated measurements of the area and flow. Their method is also prone to error because of the flow estimation method. Womersley's theory of pulsatile flow in rigid tubes is an idealized method that requires the flow to be laminar and the vessel to be uniform. Also, the angle at which the vessel is imaged can affect the results, since it can lead to skewing of the area.

In the QA method that we have developed, the area and flow are determined directly from colour-flow data (Fig. 1). Using the ECG-gated, EKV retrospective imaging mode [6] implemented by VisualSonics, slow-motion B-mode and colour flow data were simultaneously acquired at 40 MHz with a 1000 frames-per-second frame-rate. The carotid vessel was then segmented in the EKV M-mode data, and was used as a mask

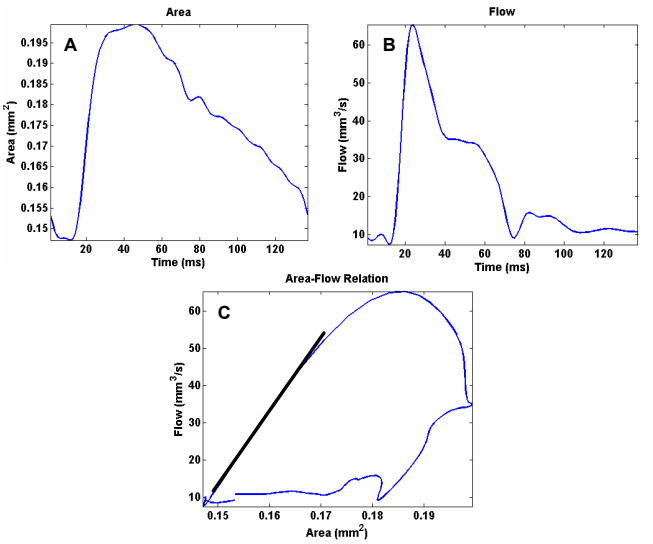

Figure 1. Typical PWV flow-area (QA) results. (A) Cross-sectional area of the carotid artery during one cardiac cycle. (B) Flow over one cardiac cycle in the carotid artery. (C) The PWV is equal to the slope of the flow-area relation during the early systolic phase of the cardiac cycle.

to extract the colour flow data within the vessel. The velocities were estimated using a 1-D velocity estimation algorithm developed by VisualSonics. Clutter-filter segmentation of the vessel was not feasible, because the clutter filter introduced artifacts when the wall velocity exceeded the cut-off velocity. Using a higher cut-off velocity caused slow flow around the edges of the vessel to be excluded. The cross-sectional area was found by integrating over the region of flow in each frame of the RCFI dataset, while the volume flow was calculated by averaging the velocities over the vessel in each frame and multiplying by the corresponding area (Fig. 2). The area and flow were then plotted against one another to determine the PWV.

C. Method Comparison

The PWV method comparison was carried out according to [7]. The criteria for determining if the flow-area method was of sufficient accuracy to determine the PWV in healthy versus diseased mice are based on the results of Hartley et al. [2]. They determined that the precision of the TD method was sufficient to detect realistic changes in the PWV, and simulated the effect of vascular disease by using vasoactive anesthetics. Using Nembutal, a vasodilation agent, they found the PWV to be 3.12±0.57 m/s (mean ± SD). With ketamine, a vasoconstriction agent, the PWV was found to be 4.59±0.83 m/s. In this study, we have assumed that if the QA methods are sensitive enough to distinguish between these extremes, then they should be sufficient to consistently differentiate between healthy and diseased mice.

D. Experimental Protocols

Animal experiments were performed under a protocol approved by the Animal Care Committee of Sunnybrook and Women's College Health Sciences Centre. Seven CD-1 female mice (Charles River Laboratories, St. Constant, QC, Canada) were examined at 5-6 months of age. The mice were anesthetized with isoflurane (2% oxygen) and positioned on a

TABLE 1
PULSE-WAVE VELOCITIES

Mouse	Time-Delay (m/s)	Flow-Area (m/s)
1	3.62	3.33
2	2.44	2.11
3	2.97	3.02
4	3.29	3.07
5	2.48	2.59
6	3.01	3.14
7	3.37	3.55

mouse imaging stage that provided heart rate monitoring (THM100, Indus Instruments, Houston, TX, USA). Depilatory cream (Nair™, Carter-Horner, Mississauga, ON, Canada) was used to remove fur from the throat, and ultrasound gel (Aquasonic 100, Parker Laboratories, Fairfield, NJ, USA) was used as a coupling fluid between the RMV probe and the skin. Using B-mode imaging on the Vevo770 system, the probe was positioned to provide either a longitudinal section or cross section of the mouse carotid artery, with the region of interest located in the focal region of the transducer.

III. RESULTS AND DISCUSSION

A. Time-Delay Method

The PWV's measured with the TD method are listed in Table 1. The average PWV was found to be 3.03±0.17 m/s. Repeated measurements in the same mouse showed that the distal delay was 19.55±0.08 ms and the proximal delay was 16.79±0.08 ms. Repeated measurements of the distal-to-proximal length in the same mouse produced an average value of 8.27±0.01 mm. These results indicate that the TD method is highly repeatable.

It was found that this technique was relatively insensitive to artifacts caused by the Doppler angle, since the angle did not significantly affect the relative time of the upstroke. It can be difficult to estimate the distance if the vessel is tortuous or if the estimation length exceeds the field-of-view, neither of which was found to be a problem in the carotid artery. The TD method is limited by the temporal resolution of the Doppler acquisition: the shorter the propagation distance, the higher the required PRF. For example, with a time-delay of 3 ms and 50 kHz PRF, the sampling period is 20 µs, allowing approximately 150 samples between the distal and proximal upstroke events.

B. Flow-Area Method

The PWV's measured with the QA method are listed in Table 1. The average PWV was found to be 2.97±0.18 m/s

The QA method that we developed is not sensitive to temporal misalignment errors because both the area and flow are derived from the same data. The main sources of error in our QA method relate to velocity estimation and area segmentation. The velocity correction angle could not be determined when imaging the vessel in cross-section, so the velocity was scaled to agree with the velocity observed at the same location from a longitudinal perspective, where the correction angle could be easily observed. The segmentation of the vessel was also problematic. It was found that it was easier to segment the RCFI dataset in M-mode form than in B-mode form.

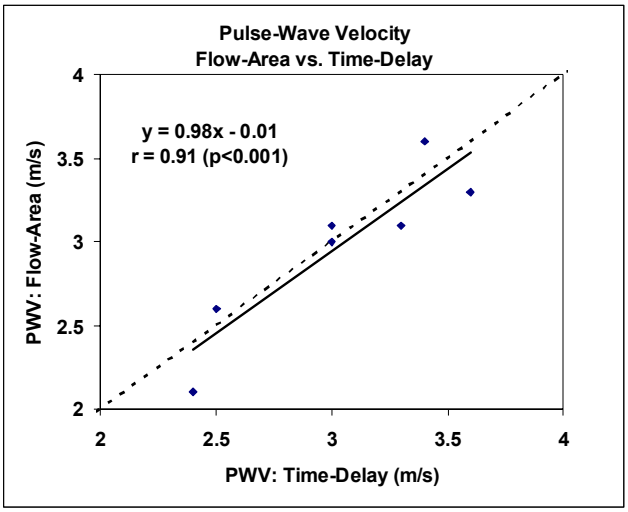

Figure 2. The QA method is compared with the TD method in the mouse carotid artery.

C. Method Comparison

Correlation and bias plots showing how the QA PWV measurement method compared with the TD method are shown in Figs. 2 and 3. The upper and lower limits of agreement (bias ± 2SD) represent the bounds within which 95% of the data can be expected to be found. The TD method was found to correlate well with the QA method ($r=0.91$, $p<0.001$), with a mean difference between methods of 0.06±0.08 m/s. The limits of agreement for the QA method were ±0.41±0.20 m/s around the mean difference. The large errors on the upper and lower limits of agreement resulted from the limited sample size. The QA method nearly satisfied the comparison criteria, for which 95% of the differences must lie within ±0.5 m/s of the mean difference for the methods to be useful for pre-clinical research. Studies of the repeatability of the QA method

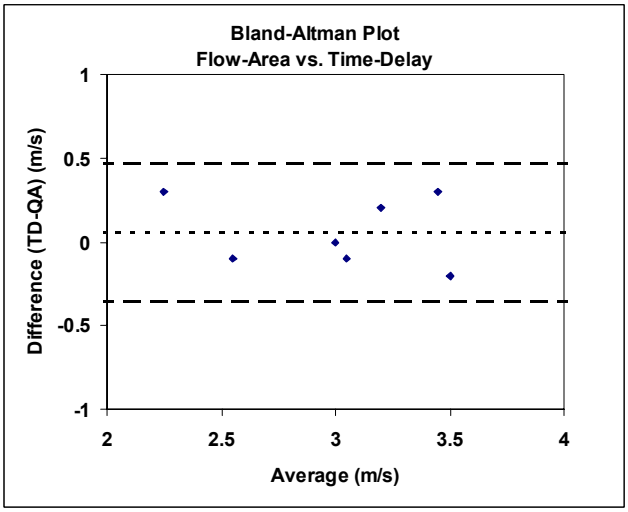

Figure 3. A Bland-Altman plot of the difference between the flow-area and time-delay methods, against the average of the two. The upper and lower dashed lines are ±2SD from the mean.

would further aid in determining the degree of agreement with the TD method.

IV. Conclusions

This study demonstrated two image-guided approaches to measure PWV in mice. A distributed method based on time delays was compared to a local measurement based on flow and area measurements. Good agreement with previous non-image-guided results was obtained. The QA method was successfully applied in the mouse using a straight-forward retrospective imaging technique. The TD method provided a distributed PWV over the carotid artery, and this value was found to agree on average with the local QA PWV observed at the carotid midsection. The TD method was found to be the most reliable method to determine the PWV, largely because the QA method was affected by a larger number of parameters than the TD method. For a longitudinal study of vascular development in transgenic mice, the TD method would seem to be the most accurate and precise non-invasive measurement technique currently available. However, the QA method is applicable in situations where the TD method is unsuitable due to the vessel geometry.

Acknowledgments

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