Estimation of local pulse wave velocity using arterial diameter waveforms: Experimental validation in sheep

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Abstract. Increased arterial stiffness is associated with an increased risk of cardiovascular events. Estimation of arterial stiffness using local pulse wave velocity (PWV) promises to be very useful for noninvasive diagnosis of arteriosclerosis. In this work we estimated in an instrumented sheep, the local aortic pulse wave velocity using two sonomicrometry diameter sensors (separated 7.5 cm) according to the transit time method (PWV\textsubscript{TT}) with a sampling rate of 4 KHz. We simultaneously measured aortic pressure in order to determine from pressure-diameter loops (PWV\textsubscript{PDLoop}), the “true” local aortic pulse wave velocity. A pneumatic cuff occluder was implanted in the aorta in order to compare both methods under a wide range of pressure levels. Mean pressure values ranged from 47 to 101 mmHg and mean proximal diameter values from 12.5 to 15.2 mm. There were no significant differences between PWV\textsubscript{TT} and PWV\textsubscript{PDLoop} values (451±43 vs. 447±48 cm/s, p=ns, paired t-test). Both methods correlated significantly (R=0.81, p<0.05). The mean difference between both methods was only -4±29 cm/s, whereas the range of the limits of agreement (mean ± 2 standard deviation) was -61 to +53 cm/s, showing no trend. In conclusion, the diameter waveforms transit time method was found to allow an accurate and precise estimation of the local aortic PWV.

1. Introduction
Cardiovascular disease is one of the most important causes of death around the world [1]. Increased arterial stiffness is associated with an increased risk of cardiovascular events [2]. Measurement of the changes in arterial diameter and pressure at the same site allows direct measurements of arterial stiffness [3, 4]. However, in clinical practice this is not easy to achieve. Alternative methods have emerged, being the pulse wave velocity (PWV) one of the most accepted [5].

From the Moens Korteweg relation, it is possible to relate the square of PWV, directly to the product of the elastic modulus and wall thickness, and inversely with the product of the arterial diameter and the density of blood [3]. PWV is commonly assessed over a given arterial segment length, such as carotid-femoral, carotid-radial or brachial-radial [5]. Many of these measurements are based upon simultaneous measurements of pressure or flow at two sites in the arteries and the determination of the time it takes a wave to propagate from one site to the other (transit time method) [5]. These measurements represent the average wave speed over the distance between the measurement sites. However, due to the non elastic and non geometric uniformity of the arterial tree, arterial stiffness is different in proximal and elastic aortas compared with distal and muscular arteries. Therefore, “local” PWV measurements, at specific arterial sites are desirable.
In clinical practice, ultrasound is frequently used to non-invasively assess blood velocity, wall thickness and diameter waveforms [6]. Therefore, recently several methods emerged for the estimation of local PWV using ultrasound principles. Among others: a) the flow and area method (QA), in which the local PWV is estimated during a reflection free period of the cardiac cycle, as the ratio between the change in flow and the change in cross sectional area [7, 8], b) the 2D distension waveforms method, in which multiple adjacent distension waveforms are determined simultaneously along a short arterial segment, using a single 2D-vessel wall tracking system with a high frame rate (651 Hz) [9], c) tissue Doppler imaging method, in which a relatively new color Doppler modality for measuring tissue motion offers multiple recording sites along the artery [10]. However, none of these methods includes an exhaustive in vivo validation.

In this work we propose to estimate in an instrumented sheep, the local aortic pulse wave velocity using two sonomicrometry diameter sensors according to the transit time method. We simultaneously measured aortic pressure in order to determine from pressure-diameter loops (PDLoop method considered as gold standard) [4], the “true” local aortic pulse wave velocity. A pneumatic cuff occluder was implanted in the aorta in order to compare both methods under a wide range of pressure levels.

2. Material and methods

2.1. Animal instrumentation

A healthy corrediale sheep of 60 kg and 2 year old was used for the experiment. Surgery instrumentation was performed under general anesthesia premedicated with acepromazine (0.2 mg / kg) induced with thiopental sodium (20 mg/kg) and maintained with isoflurane (2.5% in pure oxygen at 2 L / min) with assisted mechanical ventilation (Neumovent 910). During surgery, blood pressure, electrocardiogram, capnography and heart rate were monitored (Siemens Sirecust 404-1). By two thoracotomies in the fourth and seventh intercostal left spaces, the proximal and distal thirds of the aorta were accessed to implant two pairs of ultrasonic crystals (5 MHz, 4 mm), for subsequent instantaneous diameter determination by sonomicrometry (Triton Technology Inc. San Diego, CA). Each pair of crystals was sutured in two places diametrically opposite in the adventitia of the aorta with 6-0 silk suture, with a separation of 7.5 cm between pairs. The optimal placement of the crystals was verified by the digital oscilloscope screen (Tektronix TOS 220, Tektronix Inc. Beaverton, USA). A pneumatic cuff occluder made from silicon rubber was implanted around the descending thoracic aorta, distal to the ultrasonic crystals. This ensured that no artifacts appeared in diameter measurements during aortic occlusions. After sensors implantation, wires were tunneled to emerge through a small hole in the skin at the back of the sheep, which was then sutured. Finally, the thoracotomies were closed by layers.

2.2. Experimental protocol and recordings

The study comprised the recording of instantaneous aortic pressure and aortic diameter signals. A high-fidelity pressure catheter (Millar Instruments Inc, Texas, USA) connected to an amplifier (Gould 6600 Series Transducer) was used to measure the pressure in the proximal third of the aorta. The catheter was advanced from the left brachial artery through a little incision, and was positioned near the proximal ultrasonic crystals. The pressure transducer had been previously calibrated in vitro in physiological saline at 37 ºC with a pressure caliper (Xcaliber, Vigo-Spectramed, Oxnard. Cal.). The diameter signals were calibrated in millimeters using the 1-mm step calibration facility of the sonomicrometer. Aortic pressure and diameter signals were digitized at 4 KHz on a computer (PC Pentium IV 300 MHz) using an analog to digital converter (National Instruments Lab PC) and digitally stored for later analysis.

The acquisition started with approximately 10 consecutive beats in basal state (CTL) and then the pneumatic occluder was inflated in order to obtain high-pressure values for a couple of seconds (OCL). Finally, the occluder was released in order to obtain low-pressure values (REL). In all cases,
the instantaneous pressure-diameter loops were monitored and registered until stabilization was evidenced. This procedure was repeated several times.

2.3. Data analysis
Signals were processed using a proprietary software developed in our laboratory. Twenty eight pressure-diameter loops, corresponding to baseline, mechanical occlusion and release conditions were considered. For each cardiac cycle, systolic, diastolic and mean (average value computed from numerical integral of the waveform) values from pressure and diameter signals were obtained. Concomitantly, local arterial stiffness indexes were computed with the following indexes.

Local arterial distension was calculated as:

$$\text{Distension} = \left( \frac{D_s - D_d}{D_d} \right) \times 100$$  \hspace{1cm} (1)

where $D_s$ and $D_d$ are systolic and diastolic proximal diameters. Peterson modulus ($E_P$) was calculated as:

$$E_P = \frac{P_s - P_d}{D_s - D_d} \cdot 0.1334$$  \hspace{1cm} (2)

where $P_s$ and $P_d$ are systolic and diastolic blood pressures.

For the assessment of PWW using pressure and proximal diameter waveforms ($PWV_{PDLoop}$) we considered the Bramwell-Hill equation [11],

$$PWV = \sqrt{\frac{V \cdot dP}{dV \cdot \delta}}$$  \hspace{1cm} (3)

where $dV$ represent the change in volume (V) due to a change in pressure (dP), and $\delta$ blood density (1.06 g/cm3). For each cardiac cycle, the diastolic phase was separated from the pressure-diameter loops and the resulting diameter was adjusted to a logarithmic model, according to the following formula:

$$D = \alpha + \beta \cdot \ln P$$  \hspace{1cm} (4)

where $\alpha$ and $\beta$ are two constant determined by the fitting procedure, and $P$ the arterial pressure (See Figure 1). Assuming a circular section for the artery, changes in volume and diameter are related according to [12]:

$$\frac{dV}{V} = 2 \cdot \frac{dD}{D}$$  \hspace{1cm} (5)
Figure 1. Left: Typical example of aortic pressure waveform (top) and aortic proximal (bottom, in black) and distal (bottom, in grey) diameter waveforms. Arrows indicate temporal delay ($\Delta T$) between diameter waveforms. Right: Pulse wave velocity assessed from transit time and from pressure-diameter loop methods.

Finally, after deriving equation (4) respect to pressure ($dD/dP$), using (5) and replacing in (3), pulse wave velocity was computed as:

$$PWV_{PLoop} = \frac{P_d \cdot D_d}{\beta \cdot 2 \cdot \delta}$$

(6)

In the same cardiac cycle, PWW was assessed using the transit time method ($PWV_{TT}$), computed as the ratio of the distance between the two diameter sensors (7.5 cm) and the temporal delay ($\Delta T$) between the two arterial diameter waves (see figure 1), as:

$$PWV_{TT} = \frac{7.5 \, cm}{\Delta T}$$

(7)

We considered the foot (point of the onset of the wave) on each waveform as the reference point, and was determined using the intersecting tangent algorithm [13]. Pulse wave velocity computed using these reference points correspond to the diastolic PWV.

Data are expressed as mean ± standard deviation. Comparisons between variables were analyzed by paired Student-t test or ANOVA as appropriate. A value of $p<0.05$ was considered statistically significant.

3. Results
Mean pressure values ranged from 47 to 101 mmHg and mean proximal diameter values from 12.5 to 15.2 mm. Figure 2 shows mean pressure values plotted against mean diameter values during CTL, OCL, and REL conditions.
Figure 2. Mean aortic pressure values plotted against mean aortic proximal diameter values during control (CTL), cuff occlusion (OCL) and cuff release (REL) conditions with their respective mean (squares box) and standard deviation, and the non linear regression curve.

Values of aortic pressure, aortic proximal diameter and aortic stiffness indexes during control, occlusion and release conditions are given in Table 1.

Table 1. Hemodynamic parameters during control (CTL), cuff occlusion (OCL) and cuff release (REL) conditions. $E_p$: Peterson modulus. Values are expressed as mean ± standard deviation. $^a$ p<0.05 vs. CTL; $^b$ p<0.05 vs. REL; ANOVA and Neuman-Keuls post hoc tests.

|                | CTL (n=14) | REL (n=8) | OCL (n=6) |
|----------------|------------|-----------|-----------|
| **Aortic Pressure** |            |           |           |
| Systolic, mmHg  | 69 ± 2     | 53 ± 8    | 94 ± 5 $^a_{ab}$ |
| Diastolic, mmHg | 57 ± 3     | 41 ± 9 $^a$ | 82 ± 6 $^a$ |
| Mean, mmHg      | 63 ± 2     | 47 ± 9 $^a$ | 88 ± 6 $^a$ |
| Pulse, mmHg     | 11.7 ± 2.9 | 12.4 ± 1.9 | 11.5 ± 1.5 |
| **Aortic Proximal Diameter** |            |           |           |
| Systolic, mm    | 14.01 ± 0.23 | 13.31 ± 0.47 | 14.75 ± 0.52 $^a_{ab}$ |
| Diastolic, mm   | 13.52 ± 0.28 | 12.74 ± 0.56 $^a$ | 14.38 ± 0.58 $^a$ |
| Mean, mm        | 13.74 ± 0.24 | 13.00 ± 0.50 $^a$ | 14.57 ± 0.54 $^a$ |
| Pulse, mm       | 0.48 ± 0.11 | 0.57 ± 0.12 | 0.37 ± 0.07 $^a_{ab}$ |
| **Local Stiffness Indexes** |            |           |           |
| Distension, %   | 4 ± 1      | 5 ± 1 $^a$ | 3 ± 1 $^b$ |
| $E_p$, kPa      | 37 ± 3     | 32 ± 3 $^a$ | 52 ± 7 $^a_{ab}$ |
| PWV$_{TT}$, cm/s | 450 ± 27  | 417 ± 34 $^a$ | 498 ± 45 $^a_{ab}$ |
| PWV$_{PDLloop}$, cm/s | 442 ± 15 | 402 ± 35 $^a$ | 518 ± 25 $^a_{ab}$ |

Mean pressure increased by 40 % during OCL and decreased by 26 % during REL, compared to control values. Concomitantly, mean proximal diameter increased by 6% during OCL and decreased
by 5% during REL. Aortic distension decreased by 25% during OCL and increased by 25% during REL. $E_p$ increased by 41% during OCL and decreased by 14% during REL compared to CTL. PWV increased by 17% for PWV$_{PDLoop}$ and 12% for PWV$_{TT}$ during occlusion, and decreased 9% for PWV$_{PDLoop}$ and 7% for PWV$_{TT}$ during cuff release, compared to control values. All these changes were statically significant ($p<0.05$).

There were no significant differences between PWV$_{TT}$ and PWV$_{PDLoop}$ values ($451\pm43$ vs. $447\pm48$, $p=ns$, paired t-test). Figure 3 (left panel) shows PWV estimated by the transit time method plotted against PWV estimated by the PDLoop method. The methods correlated significantly ($R=0.81$, $p<0.05$). In figure 3 (right panel), the difference between the two methods is plotted against the average of the two methods. The mean difference between both methods was only $-4\pm29$ cm/s, whereas the range of the limits of agreement (mean $\pm$ 2 standard deviation) was $-61$ to $+53$ cm/s, showing no trend.

**Figure 3** Left: PWV by the transit time method plotted against PWV by the PDLoop method. Right: The difference between the two methods plotted against the average of the two methods. The dotted line is the mean difference and the dashed lines represent the mean $\pm$ two times SD.

Considering systolic pressure and systolic proximal diameter values in equation 6, we found that PWV$_{PDLoop}$ was $13\pm5$ % higher ($p<0.05$) than the corresponding diastolic PWV$_{PDLoop}$, assessed with diastolic pressure and diameter values. A linear regression analysis showed that PWV$_{TT}$ was related to mean pressure ($R=0.73$; $p<0.05$), mean diameter ($R=0.80$; $p<0.05$), Peterson modulus ($R=0.72$; $p<0.05$) and distension ($R=-0.78$; $p<0.05$). Similarly, PWV$_{PDLoop}$ was related to mean pressure ($R=0.95$; $p<0.05$), mean diameter ($R=0.92$; $p<0.05$), Peterson modulus ($R=0.86$ $p<0.05$) and distension ($R=-0.82$; $p<0.05$).

**4. Discussion**

In this study we demonstrated the feasibility of estimating local PWV from two diameter waveforms. Undoubtedly, for a complete characterization of the arterial wall mechanics, a simultaneous assessment of arterial pressure and arterial diameter is required [4]. Therefore, we instrumented the aorta of a sheep with a pressure sensor and two diameter sensors. The use of a pneumatic cuff occluder, allowed a variation of the mean aortic pressure of $\sim56$ mmHg. This is essential for comparison due to the pressure dependence of the mechanical properties of arteries. Figure 2 shows the typical nonlinear behavior of the arterial wall.
The procedure to determine PWV from pressure-diameter loops is based on the Bramwell-Hill equation. We assumed a circular section for the artery and that changes in volume and diameter are related according to equation (5). This simplification is allowed because in unimpeded arteries length is constant so that change in volume during the cardiac cycle is caused by a change in luminal cross-sectional area alone [12]. For practical purpose this relation can be expressed as a function of diameter. This is allowed if it is assumed that the artery lumen is circular in cross-section and that the change in diameter (dD) is small relative to the diameter. For the transit time method, considering a separation distance of 7.5 cm between sensors, a maximum PWV value of 556 cm/s and a sampling frequency of 4 KHz, the uncertainty in determining the time delay was less than 2%.

We found no significant differences between PWV\textsubscript{TT} and PWV\textsubscript{PDLoop} methods. The mean difference was only 4 cm/s, and 95% of the differences were less than two standard deviations, showing no trend. By other side, using the systolic and diastolic pressure and diameter values in equation (6), we determined a systo-diastolic variation of PWV of 13% during the cardiac cycle.

It is well known that PWV is strongly related to blood pressure [3]. An increase in blood pressure level will increase PWV. Our data confirmed this relationship. However, changes in PWV can not be only explained by changes in pressure. For example, comparing CTL and OCL condition, an increase of 40% in mean pressure caused a rise of only 17% in PWV. For the same increase in pressure, the Peterson modulus increased 41%, the mean diameter increased only 6% and the distension decreased 25%. By other side, comparing CTL and REL condition, a decrease of 26% in mean pressure caused a fall of only 9% in PWV.

Finally, further studies involving structural changes in the wall, and not just functional changes, will be necessary to demonstrate that the methodology remains valid.

5. Conclusion
The diameter waveforms transit time method was found to allow an accurate and precise estimation of the local aortic PWV in a wide range of pressure levels.

Acknowledgments
This research was supported in part by grant from PIP No 112-200901-00734 (CONICET) and PICTO 31355 (ANPCyT).

References
[1] Naghavi M, Falk E, Hecht HS, Jamieson MJ, Kaul S, Berman D, et al 2006 SHAPE Task Force. From vulnerable plaque to vulnerable patient- Part III: Executive summary of the Screening for Heart Attack Prevention and Education (SHAPE) Task Force report *Am J Cardiol* 98 2
[2] Mitchell GF, Hwang SJ, Vasan RS, Larson MG, Percina MJ, Hamburg NM, Vita JA, Levy D, Benjamin EJ 2010 Arterial stiffness and cardiovascular events: the Framingham Heart Study *Circulation* 2;121(4) 505-11
[3] Nichols WW, O'Rourke MF. Vascular impedance. In: McDonald's Blood Flow in Arteries: Theoretical, Experimental and Clinical Principles. 4th ed. London, UK: Edward Arnold; 1998:54–97, 243–283, 347–395.
[4] Armentano R L, Barra J G, Levenson J, Simon A and Pichel R 1995 Arterial wall mechanics in conscious dogs: Asessment of viscous, inertial, and elastic moduli to characterize aortic wall behaviour *Circ. Res.* 76(3) 468-78
[5] Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannatasio C, Hayoz D, et al 2006 Expert consensus document on arterial stiffness: methodological issues and clinical applications *Eur Heart J* 2006 22 2588–2605
[6] Reneman RS, Meinders JM, Hoeks AP. Non-invasive ultrasound in arterial wall dynamics in humans: what have we learned and what remains to be solved 2005 *Eur Heart J.* 26(10) 960-966
[7] Hermeling E, Reesink KD, Reneman RS, Hoeks AP. Measurement of local pulse wave velocity:
effects of signal processing on precision 2007 *Ultrasound Med Biol* **33**(5) 774-781

[8] Hermeling E, Reesink KD, Kornmann LM, Reneman RS, Hoeks AP. The dicrotic notch as alternative time-reference point to measure local pulse wave velocity in the carotid artery by means of ultrasonography 2009 *J Hypertens* **27**(10) 2028-2035

[9] Meinders JM, Kornet L, Brands PJ, Hoeks AP 2001 Assessment of local pulse wave velocity in arteries using 2D distension waveforms *Ultrason Imaging* **23**(4) 199-215

[10] Eriksson A, Greiff E, Loupas T, Persson M, Pesque P. Arterial pulse wave velocity with tissue Doppler imaging 2002 *Ultrasound Med Biol* **28**(5) 571-80.

[11] Bramwell JC, Hill AV. The velocity of pulse wave in man 1922 *Proc Soc Exp Biol Med* **93** 298-306

[12] Reneman RS, Hoeks APG, Westerhof N. Non-invasive assessment of artery wall properties in humans: methods and interpretation 1996 *Journal of Vascular Investigation* **2**(2) 53-64

[13] Chiu C Y, Arand PW, Shroff A, Feldman T, Carroll J. Determination of pulse wave velocities with computerized algorithms 1991 *Am Heart J* **121** 1460