

# UNIVERSITY OF TWENTE.

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## Radboudumc

MASTER'S THESIS Local pulse wave velocity imaging as a cardiovascular biomarker

P. van Lochem

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**Examination committee:** Technical committee and chairman: Prof.dr.ir. C.L. de Korte

Medical committee: Dr. M.C. Warlé

Process committee: Drs. P.A. van Katwijk

External committee: Dr. E. Groot Jebbink



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## 1 Abbreviations

CCA	Common Carotid Artery
cfPWV	carotid-femoral Pulse Wave Velocity
CoV	Coefficient of Variation
CV	Cardiovascular
DN	Dicrotic Notch
IMT	Intima Media Thickness
LPWV	Local Pulse Wave Velocity
PWV	Pulse Wave Velocity
RF	Radiofrequency
SD	Standard Deviation
SF	Systolic Foot
SNR	Signal-to-Noise Ratio

## 2 Abstract

Increased arterial stiffness in the cardiovascular (CV) system is associated with CV diseases and events. Due to lack of reliable methods, however, conventional arterial stiffness methods have not resulted in widespread application in the current clinical setting. Therefore, a new method is developed to measure the local pulse wave velocity by plane wave ultrasound imaging. The local pulse wave velocity can be measured accurately at the systolic foot (LPWV<sub>SF</sub>) and the dicrotic notch (LPWV<sub>DN</sub>) by using the radial acceleration values of the common carotid artery wall. The reproducibility of both velocity methods is determined using multiple measurements obtained from young healthy volunteers (n=12) and CV patients (n=19) with similar blood pressures and heart rates, but with significant differences in other CV risk factors. A significantly higher pulse wave velocity is found in the CV patient group in comparison with the healthy volunteer group (LPWV<sub>DN</sub>: 7.1  $\pm$  1.4 m/s versus 5.4  $\pm$  1.3 m/s, p < 0.001). The LPWV<sub>DN</sub> method appears to outperform the LPWV<sub>SF</sub> method, demonstrating more stable performance within this study population. However, the reproducibility within subjects with both methods is rather low, with an average coefficient of variation of approximately 20%. Consequently, the reproducibility first needs to be improved before the predictive value of the LPWV<sub>DN</sub> method can be investigated as a biomarker for CV diseases.

*Key words:* Plane wave imaging, Pulse wave velocity, Carotid artery, Arterial stiffness, Ultrasound.

## 3 Introduction

#### 3.1 Background

In the Netherlands, 1.4 million people suffer from a cardiovascular (CV) disease and 750 CV patients are hospitalized each day.<sup>1</sup> Additionally, CV diseases accounted for the loss of 863.100 healthy life years in 2015.

Before manifestation of a CV disease, functional and morphological changes already take place in the arterial wall and CV system. Classic CV risk factors are age, hypertension, diabetes mellitus, dyslipidemia and smoking. These factors are used in the Framingham Risk Score, a tool for estimating a patient's 10-year risk of developing a CV disease or event. Besides classic risk factors, CV biomarkers are also used to further stratify patients' risk at an individual level. In an early stage, biomarkers can reclassify patients, monitor the effect of drug therapy and monitor disease progression. At a later stage, they can aid in making decisions for complex cases (e.g., intervention versus wait-and-see policy) to minimize life-threatening events. Particular biomarkers are therefore recommended by international scientific societies for the improvement of CV risk stratification.<sup>2,3</sup>

Aging and hypertension are the main causes of the arterial stiffening process.<sup>4,5</sup> The stiffening of the arterial wall, also called arteriosclerosis, results from a loss of elastin content, increasing levels of type 1 and 3 collagen and the formation of cross-links between collagen.<sup>6</sup> This degenerative process can lead to atherosclerosis, a specific type of arteriosclerosis, which is a disease of the buildup of atheromatous plaques in the inner layer of an artery and is involved in many CV diseases.<sup>7,8</sup> Therefore, arterial stiffness might be a good candidate as a biomarker for CV diseases.

The relationship between arterial stiffness and hypertension is rather complex because they are, in a certain way, dependent on each other. For arteries, this relationship is non-linear and therefore, vessels become stiffer at higher blood pressures (see Figure 1).<sup>7</sup> Traditional antihypertensive drugs take advantage of this non-linear behavior by decreasing blood pressure and thereby indirectly decreasing the stiffness. However, most of these drugs do not solve the ongoing process of arteriosclerosis, as mentioned in the previous paragraph. Therefore, drug therapies that reduce arterial stiffness via direct effects on large arteries possess great potential for the treatment of CV diseases.<sup>7</sup>



Figure 1: The stress-strain relationship of the human aorta is non-linear and therefore, the elastic modulus depends on the stress at which it is measured.<sup>7</sup>

There are different methods for determining the arterial stiffness process and the most commonly and non-invasively used are intima media thickness (IMT) and carotid-femoral Pulse Wave Velocity (cfPWV). The IMT is measured locally by high resolution B-mode ultrasound and it is the most widely accepted non-invasive marker of subclinical atherosclerosis.<sup>9,10</sup> Changes in thickness can depend on multiple factors, but they do not necessarily reflect the atherosclerotic development and progression.<sup>11</sup> Moreover, a meta-analysis including 41 studies showed that regression or slowed progression of carotid IMT, induced by CV drug therapies, does not reflect reduction in CV events.<sup>12</sup>

Another method, the carotid-femoral Pulse Wave Velocity (cfPWV) measurement method, is considered to be the gold standard of general arterial stiffness as a CV biomarker because of its simplicity, non-invasive application and reproducibility (see Figure 2). It possesses the largest amount of clinical evidence with a quite high predictive value of CV events: the risk increases by 47% if the cfPWV increases by one standard deviation.<sup>13</sup> The cfPWV value provides a measurement of the average stiffness over a long trajectory without discrimination between the difference of muscular and elastic arteries. However, these two types of arteries may respond differently to aging and disease.<sup>7</sup> Change in the treatment of CV diseases by the use of the cfPWV value as a biomarker remains debatable, principally because of unavailable data concerning the effect of early drug therapy on the "de-stiffening" characteristics.<sup>14</sup> This can be caused by inaccuracies of the method due to opposite pulse wave propagation, distance assessment errors and the inability to discriminate between different segments (e.g., muscular and elastic segments) within the trajectory.<sup>15</sup>



Figure 2: The golden standard for arterial stiffening estimation is the cfPWV method. Two pulse sensors are placed at the carotid and femoralis artery. The estimated distance between the sensors divided by the time delay of the pulse waves provides the cfPWV.

All arterial stiffness methods possess theoretical, technical and practical limitations as a biomarker, and therefore have not resulted in widespread application to improve CV risk stratification in the current clinical setting.<sup>8,16</sup> Therefore, there is an urgent demand for an accurate CV biomarker with minor limitations. Requirements for a new method are that it needs to be non-invasive and able to detect local changes in stiffening at an early stage. Furthermore, the method needs to be stable and reproducible under different circumstances (i.e., with the ability to correct for variating blood pressures, aging and other risk factors). In addition, this method requires a high predictive value related to CV diseases and events or the ability to detect local changes in the wall characteristics induced by, for example, drug therapies.

#### 3.2 Problem solution

A new method, local pulse wave velocity (LPWV) using plane wave ultrasound imaging, appears to overcome most of these aforementioned limitations with conventional stiffness methods. This method can measure the propagation of the pulse wave of an artery locally instead of over a longer trajectory with an average over different segments with the cfPWV method, and it might serve as a new biomarker for CV diseases. Increased arterial stiffness correlates with an increased velocity of the pulse wave. The LPWV is measured at the common carotid artery (CCA) due to similar elastic characteristics with the aorta and its easy accessibility (see Figure 3).<sup>17</sup> Furthermore, the method is also applicable at other superficial arteries. A plane wave ultrasound image with ultrafast imaging is created from one single insonification and is able to achieve up to 15000 frames per second (see Figure 4).<sup>18</sup> This high frame rate contrasts the conventional ultrasound, where the trade-off is the number of scan lines with the frame rate (see Figure 5), which makes it possible to locally track the pulse wave of a few meters per second.



Figure 3: The position of transducer is placed longitudinal to the common carotid artery.



Figure 4: A plane wave is sent by a linear transducer and insonifies the whole region of interest, resulting in an instantaneously acquired image.<sup>18</sup>



Figure 5: Conventional ultrasound where every image line is subsequently acquired with a focused beam, resulting in the order tenths of a millisecond to acquire a full image, which restricts the maximum frame rate.<sup>18</sup>

The aim of this study is as follows:

To determine the reproducibility of the local pulse wave velocity method combined with plane wave imaging in a pilot setting within a variating CV population.

In addition to the aim of this study, the following sub-questions are investigated:

- Is it possible to find different velocities between young healthy volunteers versus CV patients?
- What are the requirements for the local pulse wave velocity method before implementation in the clinic?

## 4 Materials and methods

#### 4.1 Data acquisition

This study utilized a Vantage256 ultrafast ultrasound research imaging system, which was developed by Verasonics Inc. (Kirkland, WA, USA). Data was acquired with a linear array transducer (ATL L12-5 38 mm, Bothell, WA, USA) and acquisition scripts that were developed in MATLAB R2015b (The MathWorks, Natick, WA, USA). A total of 128 of the 192 scan lines of the transducer were used, with a total image width of 25 mm. A frame rate of 2 kHz was used to be able to track LPWVs up to 16 meters per second. The transmitted ultrasound pulse had an effective center frequency of 8.9286 MHz and the received ultrasound signal was sampled at 35.7144 MHz. The elevation focus of the used transducer was approximately 15 mm and pitch of the elements was 0.1979 mm. This Vantage256 system is programmable per channel, both in receiving (128 channels) as well as in transmitting (128 channels).

The plane wave preview mode with a framerate of 30 Hz was utilized to locate the common carotid artery. Start and end depth were adjusted to conform the depth of the CCA with an end depth variating from 24 to 37 mm. For the end depth, a margin below the vessel was used to fulfil to the geometry of the radiofrequency (RF) backscatter waves.

Subjects were lying in supine position for four minutes of rest before the first measurement. By contra rotation of the head, the CCA presents itself as most optimal for the measurement. However, it is still unknown whether this rotation and possible twisting of the CCA could influence the PWV. To avoid interferences of reflection waves, the transducer was located as far away as possible from the bifurcation of the carotid artery (see Figure 6). When a longitudinal view of the CCA at its maximum diameter was precisely aligned with the transducer, the subject was instructed to hold his or her breath and the ultrafast acquisition was performed for few seconds. During and after the acquisition, the system was frozen due to calculations without giving any feedback. Moreover, ten seconds of acquisition time required twenty minutes of saving time. During the acquisition, the backscattered echoes were beamformed into two-dimensional IQ data images.



Figure 6: At every bifurcation, the reflected pulse wave (right) propagates in the opposite direction of the incident pulse wave (left) with a comparable velocity and so can interfere. To illustrate, the difference between the two pulse waves (illustrated as yellow) becomes smaller when getting closer to the bifurcation of the carotid artery (lower element numbers).

Both carotid arteries of the healthy volunteers (n=12) were scanned at three different moments in time with a minimum of four cardiac cycles each. For the CV patients (n=19), only three consecutive measurements at one side were performed, consisting of a minimum of three cardiac cycles each. Just before and after an acquisition, the blood pressure was measured at the brachial artery with an automatic clinical sphygmomanometer. The average blood pressure per subject was used for further analysis.

#### 4.2 Local pulse wave velocity estimation

The signal processing and analyzing part was performed in MATLAB R2014b (The MathWorks, Natick, WA, USA). During a cardiac cycle, two pulse waves with a certain velocity along the arterial vessel wall are generated when the aortic valve opens (systolic foot) and closes (dicrotic notch). The systolic foot (SF) and the dicrotic notch (DN) refer as time-points by the local maxima appearing in the acceleration waveform of the wall to estimate the LPWV (see Figure 7).<sup>19</sup>



Figure 7: One cardiac cycle with the SF (triangle) and the DN (circle) locations in the distension, velocity and acceleration waveforms of the vessel from left to right, respectively. The mean waveform (in red) is visualized in the distension and acceleration graph.

The pulse wave of the SF propagates at the end-diastolic pressure, whereas the DN propagates near the mean arterial pressure.<sup>20</sup> To determine the time moments of the SF and the DN, a quick and easy method was used. By manual wall segmentation of the anterior and posterior wall, which is fixed over time (Figure 8), the axial displacement velocity of every element line was determined with inter-frame displacements by a phase difference algorithm. Taking the average of all scan lines of the acceleration waveforms, the time-points of all cardiac cycles can be precisely located (see Figure 9).

For a more precise method of LPWV estimation, new wall segmentations were performed: one for the SF phase and one for the DN phase (see Figure 8). Thereby, we assume that consecutive SFs and DNs within a measurement were located at the same position within the image plane and therefore, the same wall segmentation was used.



Figure 8: B-mode image of the ultrasound IQ data with the manual segmented anterior (upper) and posterior (lower) wall in red. Therefore, the two red lines closest to the lumen were manually selected, and twenty pixel samples (accounting for 0.87 mm) above and below these lines provide the total wall segmentation.



Figure 9: The mean acceleration waveform of all included scan lines. Five cardiac cycles with precisely the time-point references for every SF and DN pulse wave.

For the next step, small time periods before and after the SF and DN time-points were included for axial displacement estimations by, again, a phase difference algorithm followed by a pixel displacement tracking algorithm. The distension waveforms of every included scan line were obtained by subtracting the mean samples of the posterior wall from the anterior wall. The second derivative provides the acceleration waveforms and by using a low-pass fourth order Butterworth filter with a cutoff frequency variating from 60 to 120 Hz for filtering the high frequency noise. The linear regression slope through the peaks of all scan lines provides the LPWV. All these steps are illustrated in Figure 10 and Figure 11 for the SF and DN method, respectively. Furthermore, the coefficient of determination ( $r^2$ ) was estimated to evaluate the quality of the pulse wave velocity estimation.



Figure 10: The (a) distension, (b) velocity, (c) unfiltered acceleration and (d) filtered acceleration waveforms of all included scan lines for the SF phase. The linear regression slope through the peaks in the (e) spatio-temporal image of the unfiltered acceleration waveforms results in a LPWV<sub>SF</sub> of 4.0 m/s with a  $r^2$  of 0.99.



Figure 11: The (a) distension, (b) velocity, (c) unfiltered acceleration and (d) filtered acceleration waveforms of all included scan lines for the DN phase. The linear regression slope through the peaks in the (e) spatio-temporal image of the unfiltered acceleration waveforms results in a LPWV<sub>DN</sub> of 4.7 m/s with a  $r^2$  of 0.99.

#### 4.3 Subjects

The *in vivo* reproducibility study was performed with 12 healthy volunteers and 19 patients diagnosed with a CV disease (e.g., peripheral arterial occlusive disease, heart attack, aneurysm). Patient selection was performed at the vascular diagnostic laboratory. Patients that underwent carotid vascular surgery, visible or known carotid plaques or intervention at both sides were excluded. Unfortunately, CV patients are more difficult to measure due to stiffer systems and, therefore, higher pulse wave velocities, decreased distension and more complex physiologies, which results in more noise at the distension waveform.<sup>21</sup> Therefore, it is important to determine the reproducibility in this complex CV population with the current method. Furthermore, differences in LPWVs between groups and risk factors can provide insights into how to interpret the pulse wave velocity values.

The medical history of each subject was acquired by way of oral questionnaire, which recorded the type of CV disease, CV risk factors (i.e., diabetes, hypertension, smoking, dyslipidemia and family history of CV disease), height, weight, gender and age. Risk factors were defined when hypertension or hypercholesterolemia was diagnosed (with or without treatment) and when family history included a mother, father, brother or sister that was diagnosed with a CV disease before 65 years of age. All volunteers were free of any CV diseases, risk factors and visible carotid plaque. Furthermore, multiple blood pressures and heart rates were recorded. The study was approved by the local ethical committee and all subjects provided written informed consent before performing the ultrafast scanning.

#### 4.4 Performance evaluation

With a minimum of four cardiac cycles per measurement in volunteers (n=12), the variation within the measurement can be explored. By measuring at three different times of the day, insight can be gained concerning the influence of external factors. Measuring both sides could provide insights between both carotid arteries, which are expected to possess the same kind of physiology and stiffness and thereby, comparable pulse wave velocities. For the CV patients (n=19), only one side was consecutively measured three times with a minimum of three cardiac cycles. The reason for different measurements between groups is because of technical and logistic reasons and achieved insights from the volunteer measurements. With 12 volunteers, 19 CV patients and over 9 LPWVs per participant, the reproducibility of the technique in this pilot setting can be determined among a variating CV population for the LPWV<sub>SF</sub> and LPWV<sub>DN</sub> method.

LPWV<sub>SF</sub> and LPWV<sub>DN</sub> that exceed the range between 2 and 16 m/s or a corresponding  $r^2$  value of < 0.8 were considered unreliable and were therefore rejected. A rejection rate was defined for each artery as the percentage of rejected LPWVs. Arteries with a rejection rate below 30% were excluded and the average of the remaining LPWVs of each artery was determined. Furthermore, the coefficient of variation (CoV), defined as the standard deviation divided by the mean LPWV, was estimated to evaluate the precision of the method. For both groups, all CoV values were averaged to determine the overall CoV within a group. Because of the unknown *in vivo* ground truth of the LPWV, it is still impossible to determine the accuracy of this method.

Statistical differences between groups were analyzed with a non-parametric Wilcoxon ranksum test. However, dichotomous variables were analyzed by binary logistic regression. A pvalue of 0.01 was considered indicative of statistical significance. Values were reported as mean  $\pm$  standard deviation (SD).

## 5 Results

#### 5.1 Analysis of filters

Because the method is still under development, different settings and parameters can be used to optimize it. The one with the largest influence at the LPWV is the low pass frequency filter. Low pass frequencies from 60 to 120 Hz in steps of 10 Hz were investigated, see Figure 12 for an example. Therefore, to determine the best filter, a certain weight is given by the following criteria: the cutoff frequency with the smallest standard deviation (SD) obtains two points and for every SD within a range of 0.05 m/s, obtains one point. This is only applied to the healthy volunteer group and a minimum of four LPWV values are required. According to these criteria, the best cutoff frequency for the low-pass filter is 60 and 120 Hz for the LPWV<sub>SF</sub> and LPWV<sub>DN</sub> methods, respectively (see Figure 13).



Figure 12: The mean and SD of all LPWV<sub>DN</sub> of all three times of the day of the right carotid artery of a healthy volunteer per low-pass cutoff frequency filter. A minimum of four LPWV values are required for a mean and SD.



Figure 13: The tallied results for the LPWV<sub>SF</sub> (left) and the LPWV<sub>DN</sub> method (right) for the best cutoff low-pass frequency filter.

#### 5.2 Analysis of accepted measurements

According to the criteria in section 4.4, the rejected measurements are listed in Table 1. Within the healthy volunteer group, none of the measurements were rejected. However, the CV patient group resulted in a relatively large amount of rejections for the LPWV<sub>SF</sub> method. Hereby, the LPWV<sub>DN</sub> method appears to outperform the LPWV<sub>SF</sub> method in stability with 16% versus 47% of the rejected arteries. The rejected measurements are highly dependent on the chosen  $r^2$  cutoff value. This is the tradeoff of the quality for the linear regression slope and the number of rejected measurements.

	Healthy volunteer	Cardivascular patient	
	group (n=24)	group (n=19)	
Systolic foot (%)	0 (0%)	9 (47%)	
Dicrotic notch (%)	0 (0%)	3 (16%)	

Table 1: Number of rejected arteries for the healthy and CV patient group.

The patient characteristics can provide insight into the likely causes for the rejection of a measurement (see Table 2). However, due to the small number of patients, statistical analysis cannot be performed and the upcoming potential explanations cannot be supported based on evidence. All rejected measurements for the LPWV<sub>DN</sub> method were also rejected for the LPWV<sub>SF</sub> method.

Parameter	Accepted SF (n=10)	Rejected SF (n=9)	Accepted DN (n=16)	Rejected DN (n=3)
Age (year)	63 ± 9	71 ± 6	67 ± 9	68 ± 6
Sex (male/female)	8/2	7/2	12/4	3/0
Body mass index (kg/m^2)	25.9 ± 5.8	27.8 ± 5.4	26.5 ± 5.7	28.4 ± 4.7
Familiar CV disease history	5 (50%)	5 (56%)	10 (63%)	1 (33%)
Hypertension	3 (30%)	7 (78%)	12 (75%)	2 (67%)
Dyslipidemia	6 (60%)	5 (56%)	9 (56%)	2 (67%)
Smoker	10 (100%)	7 (78%)	15 (94%)	2 (67%)
Diabetes	3 (33%)	2 (22%)	5 (56%)	0 (0%)
Heart rate (bpm)	70 ± 14	64 ± 10	67 ± 13	65 ± 4
Diastolic blood pressure (mmHg)	71 ± 6	72 ± 9	71 ± 7	74 ± 9
Systolic blood pressure (mmHg)	123 ± 11	129 ± 21	127 ± 18	119 ± 8
Pulse pressure (mmHg)	53 ± 10	57 ± 19	57 ± 15	45 ± 6

Table 2: Patient characteristics for the LPWV<sub>SF</sub> and LPWV<sub>DN</sub> method according to the rejection criteria.

In both rejected groups, the BMI values are slightly higher compared with the accepted groups. Higher BMI values can result in more deeply located arteries and therefore, a decreased image quality, which may cause rejected measurements. Furthermore, the age is slightly increased in the LPWV<sub>SF</sub> rejected group. Aging is associated with stiffer systems and therefore leads to higher pulse wave velocities, which results in more unreliable measurements.<sup>22</sup>

The image quality of two of the three rejected LPWV<sub>DN</sub> measurements was rather poor, which can be the cause for their rejection. By contrast, the residual LPWV<sub>DN</sub> measurement shows an excellent image quality, but with a high level of noise around the DN peaks in the mean acceleration waveform. This high level of noise can influence the reliability of the measurement, as discussed in the upcoming first paragraph of section 6.3. Besides that, this patient is the only one with a CV history of an abdominal aortic aneurysm graft placement, which may influence the pulse wave in any manner.

#### 5.3 Comparison of groups and methods

Characteristics of the healthy volunteer group, the CV patient group and the performed measurements are summarized in Table 3 and the boxplots of the LPWVs of both groups are illustrated in Figure 14. Between the groups, there are significant differences in age (p < 0.001) and other risk factors. However, no significant differences between the blood pressures and heart rates are found. Therefore, it can be concluded that there is a significant difference between the groups of the LPWV<sub>DN</sub> method (p < 0.001), which is caused by higher velocities due to stiffer CV systems. Because the blood pressures and heart rates are similar for both groups, higher velocities and therefore stiffer arteries cannot be caused by this. Nonetheless, stiffer arteries can be caused by significant higher age and other risk factors or also partially because stiffer arteries are associated with CV diseases. In addition to that, higher velocities are more difficult to measure and will be less accurate, resulting in more rejections in this CV group than in the normal group.<sup>22</sup> This could be the reason why the LPWV<sub>SF</sub> is lowered in the patient group, but this should be further investigated. The reason why the LPWV<sub>DN</sub> demonstrates a significant difference between groups could be that the DN has a better prognostic value than the LPWV<sub>SF</sub> method due to pressure differences. The LPWV<sub>DN</sub> operates near the mean arterial pressure, which may better reflects the effective arterial stiffness over the cardiac cycle.<sup>23</sup> The reproducibility of the method is comparable between groups and methods with a CoV of approximately 20% (see Table 3).

Parameter	Healthy volunteer group (n=12)	Cardiovascular patient group (n=19)	p value
Age (year)	29 ± 5	67 ± 9	< 0.001
Sex (male/female)	9/3	15/4	0.798
Body mass index (kg/m^2)	23.2 ± 2.0	26.8 ± 5.5	0.039
Familiar CV disease history	0 (0%)	11 (58%)	0.006
Hypertension	0 (0%)	14 (74%)	< 0.001
Dyslipidemia	0 (0%)	11 (58%)	0.001
Medication related to CV diseases	0 (0%)	19 (100%)	< 0.001
Smoker	0 (0%)	17 (89%)	< 0.001
Diabetes	0 (0%)	5 (26%)	0.030
Heart rate (bpm)	63 ± 9	67 ± 12	0.477
Diastolic blood pressure (mmHg)	71 ±7	71 ± 7	0.597
Systolic blood pressure (mmHg)	121 ± 10	126 ± 17	0.320
Pulse pressure (mmHg)	50 ± 6	55 ± 15	0.655
LPWV systolic foot (m/s)	5.3 ± 1.4	4.7 ± 1.2	0.212
LPWV dicrotic notch (m/s)	5.4 ± 1.3	7.1 ± 1.4	< 0.001
CoV systolic foot (%)	$20.4 \pm 10.6$	21.5 ± 9.7	-
CoV dicrotic notch (%)	17.4 ± 9.9	20.8 ± 12.6	-

Table 3: Characteristics of healthy volunteer and CV patient groups with the measured values.

CV = cardiovascular; LPWV = local pulse wave velocity; CoV = coefficient of variation



Figure 14: The boxplots of the LPWVs of every non-rejected artery for each group for the LPWV<sub>SF</sub> (left) and the LPWV<sub>DN</sub> (right) method with a significant difference between groups for the LPWV<sub>DN</sub> method.

## 6 General discussion

A method was developed that can perform LPWV<sub>SF</sub> and LPWV<sub>DN</sub> measurements by plane wave imaging. Hereby, in the current setting, the LPWV<sub>DN</sub> method appears to outperform the LPWV<sub>SF</sub> method in the diagnosis of a stiffer artery with a highly significant difference and higher stability (fewer rejected measurements and slightly lower CoV) (see Table 1 and Table 3). This is also in line with the earlier reported studies.<sup>21,24,25</sup> During the analysis of the healthy volunteer measurements, it became clear that several factors during the data acquisition and post-processing influenced the quality of the results. This is discussed in the upcoming sections.

#### 6.1 Downsides of plane wave imaging at image quality

The downsides of plane wave imaging compared to conventional ultrasound imaging are the decreased lateral resolution and lower signal-to-noise ratio (SNR) due to the use of fully unfocussed beams in transmission (see Figure 15). The axial resolution of plane wave imaging is not affected due to the lack of focusing.<sup>26,27</sup> Nonetheless, the lower SNR appears to hamper the tracking of the wall in axial direction. Furthermore, the low image quality sometimes resulted in difficulties for the manual wall selection. A method to improve this low SNR could be to use coherent spatial compounding, although this reduces the maximum frame rate.<sup>26,27</sup>



Figure 15: Conventional (left) versus plane wave imaging (right) of the same CCA. Note: with the used number of scan lines for the conventional ultrasound, it is impossible to perform a LPWV measurement, but by decreasing the scan lines, it is possible.<sup>23,24</sup> Further note: better post-processing is performed for the conventional image, which improves the image quality in comparison with the plane wave image.

#### 6.2 Influence of filters

The variation of the LPWV values induced by the cutoff frequency filter influences the reproducibility and precision of the measurement is a great deal. Starting with a cutoff frequency of 60 Hz appeared to be too smooth for the measurements, especially for the LPWV<sub>DN</sub> method. Therefore, all frequencies from 60 to 120 Hz in steps of 10 Hz were tested. Tests of a cutoff frequency below 60 Hz resulted in too much information loss by smoothing and above 120 Hz, the results became too noisy and resulted in a decreased  $r^2$ . However, looking at Figure 13, the "best" filters for the SF and DN method are at both ends of the spectrum, which can give a reason to further investigate what is truly the most optimal filter per method. In addition to the cutoff frequency, the order of the Butterworth filter was also investigated. Additionally, the variations between the orders of the filter were negligible and therefore, the fourth order continued to be used. Instead of using low-pass filters for signal processing,

another option can be a least-squares fitting of the distension and acceleration waveforms. This could overcome the issues or insecurities concerning the cutoff frequency of the low-pass filter.

#### 6.3 Other factors

It is of great importance to perform a stable and correct measurement, because when the vessel wall is not parallel to the transducer, the waveforms become more noisy and unreliable, which influences the reproducibility. Furthermore, the vessel lumen should be at its maximum diameter in the whole scan field and acquisition time. This will provide the best reflection of the ultrasound waves and therefore, an optimal SNR. In addition, the displacement of the vessel wall is also aligned in that direction. The mean acceleration waveform resulting from the quick and easy method (Figure 9) is a good predictor based on the noise level of the signal for the reliability of the measurement. Overall, the DN curves are more noise-free with steeper acceleration peaks in comparison with the SF.

Where the overall variation of the LPWV within a measurement is rather small, the variation between measurements is occasionally large. Between measurements of healthy subjects, there are long-time intervals that can already cause physiological changes in blood pressure and heart rate. This variation can also be the standard deviation of this technique or this pulse wave velocity itself. However, this variation is still unknown due to the missing *in vivo* ground truth. An example of a factor that influences the variation is the blood pressure. This method also depends on this and the blood pressure itself is not stable over time. Therefore, to perform a correct LPWV, there is a need to correct for the current blood pressure. Nevertheless, how much this correction should be for the LPWV<sub>SF</sub> and LPWV<sub>DN</sub> method separately is still unknown. Hereby, it should be taken into account that measured blood pressure at the brachial artery differs from the carotid artery, especially in younger adults.<sup>28</sup> The variation of blood pressure and heart beat within the CV patient group is minimal because the measurements are within few minutes from each other. Therefore, further investigation of variation between these LPWV measurements can provide additional insights into the methodology-induced variation.

Other influencing factors aside from blood pressure and heart rate can include caffeine products or the state of the subject. Therefore, restrictions for subjects can be introduced for better standardization. Examples of these standardizations can be the restriction of caffeine products, overnight fasting, refrainment from smoking and measuring at the same time of the day. Furthermore, the assumptions that could influence the reproducibility are that the pulse wave velocity propagates linearly over the arterial segment and without attenuation of the pulse wave.

## 7 Recommendations and implementation

#### 7.1 Recommendations

The memory of the workspace and the time to save after the acquisition are limited factors of the current system. The maximum cumulative acquisition time is just over 10 seconds and requires more than 20 minutes of saving time. To overcome these limitations, it is recommended to make use of an ECG-triggered system for the acquisition of only the SF and DN pulse wave phases. Alternatively, multiple measurements with one or two cardiac cycles can be performed, in contrast with the current two or three larger measurements performed here. These two options provide the opportunity to experiment easily with transducer replacements and to determine the induced variation by the method itself on reproducibility among almost the same conditions. When this is explored, the influences of other factors can be investigated.

Increasing the image width can increase the reproducibility of the method. An increased width results in decreasing the CoV and increasing r<sup>2</sup> values, especially by higher PWV.<sup>22,29</sup> The choice for the current small image width of 25 mm has several reasons: the limited workspace, long time to save of the system and the test measurements appeared well enough for the study. However, the results showed that the CoV is rather high. Already, several developments assisted in decreasing the workspace limitation, including a smaller region of interest for the acquisition and changing a setting, resulting in the same quality data being obtained, but with less storage data. By implementing an ECG-triggered acquisition within the system mentioned earlier, the workspace is no longer an issue. The image width of 25 mm is exactly 128 elements, which equals the maximum transmit and receive channels of the system for one segment of the transducer. However, when using two overlapping segments, all 192 elements of the transducer can be used, which accounts for 38 mm, but the data storage will increase by 50%. As a result of the increased image width, be aware of possible influence of the reflection pulse wave closer to the bifurcation (see Figure 6). Since the chosen frame rate correlates with the image width, a decreasing frame rate can be used by an increased image width.

For simplicity, the same wall mask segmentation is used for consecutive LPWV<sub>SF</sub> and LPWV<sub>DN</sub> phases with the assumption that the position of the vessel wall remains the same over every consecutive heartbeat. Within the healthy volunteer group, when the wall position slightly differs with another heartbeat, the results did not variate in quality and reproducibility. Conversely, for the CV patient group, an incorrect mask appeared to influence much more. This can be the cause of the decreased image quality in the CV patient group, which makes it therefore more difficult to perform the method. This issue could be solved by applying individual masks per SF or DN phase or by using a cross-correlation algorithm with the currently used mask.

A summary of all options for improving the method and its reproducibility is provided below:

- Employ coherent spatial compounding
- Optimize current filter setting (or test the least-squares fitting)
- Standardize the state of subjects
- Utilize ECG-triggered measurements
- Increase image width
- Utilize individual wall segmentations

It can be concluded that this pilot setting of the method still lacks reproducibility for studying the method's predictive power. Nonetheless, the impact of the earlier mentioned improvements should first be investigated. To our knowledge, only two studies have used conventional ultrasound techniques for similar LPWV measurements with reduced scanlines and were able to achieve a reproducibility with a CoV of approximately 10%.<sup>23,24</sup> However, one of the studies showed a high rejection rate.<sup>23</sup> The plane wave imaging method needs to be comparable or be an improvement to the reproducibility of the conventional ultrasound method.

Furthermore, there is already a commercial clinical plane wave system available with a pulse wave velocity tool, namely, the Aixplorer by Supersonics (Aixen Provence, France). It can automatically calculate the LPWV for the LPWV<sub>SF</sub> and LPWV<sub>DN</sub> method with a high reproducibility amongst 25 volunteers with an average CoV of 13.8% and 8.3% for the LPWV<sub>SF</sub> and LPWV<sub>DN</sub> method, respectively.<sup>25</sup> With the same system, 10 measurements within one volunteer resulted in a CoV of 23% LPWV<sub>SF</sub> and 13% LPWV<sub>DN</sub>.<sup>30</sup> Furthermore, group comparison studies with this system have already been performed, demonstrating significant differences between groups.<sup>31,32</sup> Within these studies, reproducibility tests consist of three measurements of one cardiac cycle and utilize the median value for further data analysis. This suggests that the reproducibility does not have to be tested because it is already good enough. However, for us, the ins and outs of this system remain unknown. Nevertheless, the system seems very promising. In contrast with the upcoming issue mentioned in section 7.2 concerning the technical expertise for the LPWV method, the Aixplorer does not require a high level of technical expertise and can be performed by any clinician.<sup>31</sup> When the Vantage system has more advantages, it is recommended to continue with this project. Otherwise, it is advised to not put too much effort in the LPWV method with this system. Therefore, the comparison should be made with the Aixplorer system and the Vantage system with our method.

#### 7.2 Clinical opportunities

The major advantage of the LPWV arterial stiffness method is that it is directly determined and not derived from any kind of model or assumption like other (local) stiffness methods.<sup>28</sup> For now, the downside is the high degree of technical expertise that is required with a fully programmable system. Therefore, this is not an easy applicable method for epidemiological studies yet. Nevertheless, when the reproducibility is improved, the current clinical opportunities will lie within the pathophysiology, pharmacology and therapeutic fields, given the fact that these studies require smaller patient groups and shorter follow-up.<sup>28</sup> However, when the system becomes more easily applicable, i.e., when less technical expertise is required, and improved reproducibility and inter-observer variation are achieved, it can also be used for epidemiological studies as an alternative to the current golden standard of cfPWV.

Furthermore, the cfPWV method provides an average stiffness measurement of elastic and muscular arterial segments over a long trajectory. However, there are substantial differences in characteristics between elastic and muscular arteries (e.g., different collagen/elastin ratio) present along this trajectory. Therefore, these differences can result in distinct pathways in developing CV diseases or events.<sup>33</sup> Moreover, arterial stiffness is not uniform along the CV system. In contrast with cfPWV, LPWV makes it possible to measure stiffness locally and thereby to measure both elastic and muscular arteries separately. In the current study, the carotid artery is measured, which is an elastic vessel. In the same way, the method can be applied to the femoral artery, a muscular artery. In addition to the distinct pathways, the ARIC study indicates that carotid stiffness is more associated with cerebrovascular diseases than with coronary heart diseases.<sup>34</sup> The authors of this study suggest that a stiffer elastic artery is associated with increased blood pressures, which leads to extra pressure load on the brain and

causes cerebrovascular diseases. In the same way for muscular arteries, coronary arteries have comparable wall characteristics with femoral arteries and therefore may be more associated with coronary heart disease. Studies about local stiffness associated with CV diseases and events are scarce. Thereby, LPWV by plane wave imaging can play an important role as an innovative stiffness method.

## 8 Conclusion

In this pilot setting, the reproducibility is determined of a variating CV population of the local PWV method by plane wave imaging. Hereby, the LPWV<sub>DN</sub> method appears to outperform the LPWV<sub>SF</sub> method, demonstrating a more stable performance of the current method within this study population. However, the reproducibility within subjects with both methods is rather low, with an average coefficient of variation of approximately 20%. Despite the rather low reproducibility, significant differences were found between healthy volunteer and CV patient groups by stiffer systems due to higher velocities measured with the LPWV<sub>DN</sub> method. Consequently, the reproducibility first needs to be improved before the predictive value of the LPWV<sub>DN</sub> method can be investigated as a CV biomarker.

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