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Impedance plethysmography-based method in the assessment of subclinical atherosclerosis

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ABSTRACT

Background and aims: The aim of this study was to examine an association of individual and combined pulse waveform parameters derived from bioimpedance measurements, that is pulse waves from a distal impedance plethysmographic (IPG), a whole-body impedance cardiographic (ICG) and transformed distal impedance plethysmographic (tIPG) signals, with markers of subclinical atherosclerosis, i.e. carotid intima-media thickness (cIMT), brachial artery flow-mediated dilation (FMD) and carotid artery distensibility (Cdist). The level of the association was also compared for arterial pulse wave velocity (PWV) and cIMT, FMD, and Cdist.

Methods: IPG, ICG, tIPG signals were measured from 1741 Finnish adults aged 30–45 years. The association between pulse wave parameters and cIMT, FMD and Cdist was studied using bootstrapped stepwise Akaike's Information Criterion method resulting in selection of parameters other than PWV, i.e. parameters having stronger association with cIMT, FMD and Cdist than PWV, in the model. Then risk scores were calculated from the selected pulse wave parameters and their association between cIMT, FMD and Cdist was studied with multivariable linear regression analysis.

Results: The risk score was found to be the third strongest predictor of subclinical atherosclerosis as indicated by cIMT measurement, the second strongest predictor of FMD and the strongest predictor of Cdist. These findings show that several individual pulse wave parameters were associated more strongly with cIMT, FMD, and Cdist than PWV when adjusted with clinical risk factors.

Conclusions: Impedance based pulse waveform analysis provides a useful tool for assessing cardiovascular risk and estimating presence of structural changes in the vasculature.

1. Introduction

Cardiovascular diseases (CVDs) are the leading cause of death globally [1]. CVDs take decades to develop and early diagnostic and monitoring methods are therefore needed for efficient preventive strategies against them. Current non-invasive methods, such as carotid

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artery intima-media thickness (cIMT), brachial artery flow-mediated dilation (FMD), and carotid artery distensibility (Cdist) are based on structural and functional measurements that provide information on the status of the systemic vasculature [2,3]. These parameters can be seen as markers of subclinical atherosclerosis. However, the use of these methods is currently limited in clinical practice due to their high costs, requirement of a skilled operator, a lack of standardized measurement protocols, and often, due to lack of suitable ultrasound equipment. In addition, because of the manually performed estimation, the results of cIMT, FMD and Cdist have been shown to vary between operators [4–6].

Currently, there are no clear limits defined for normal and pathological values of cIMT, FMD, or Cdist. cIMT presents advanced structural changes, but its clinical significance is limited [7]. FMD is a surrogate marker of endothelial function, i.e. the body's local nitric oxide (NO) bioavailability in the endothelium. Thus, impairment in FMD in pre-clinical subjects is related to a number of risk factors [3], and a reduction in FMD is predictive for a cardiovascular disease [8]. However, clear additional prognostic value for endothelial function has not been found [9]. Cdist reflects the ability of the carotid artery to expand as a response to change in arterial pressure during the cardiac cycle [10] and it especially reflects the current blood pressure. It describes the arterial stiffness and thus reduced Cdist is considered as a marker of vascular dysfunction. However, Cdist is a complicated parameter because, as said, it is dependent on the instantaneous blood pressure. If the instantaneous blood pressure is momentarily high, the distension of the artery is close to its maximum also during the diastole and due to its non-linearity the artery cannot distend anymore during the systole, which results in a decreased value of Cdist compared with the situation at normal blood pressure.

Arterial pulse wave velocity (PWV) reflects arterial stiffness [11]. Few researchers have shown association between PWV and Cdist [12,13]. However, an association of PWV with brachial FMD or carotid cIMT has been found to be inconsistent [12,14–17]. However, it is unclear if segmental arterial stiffening in its early stages reflects the atherosclerotic process or an alternative pathology of the arterial wall [12,18,19]. Due to the conflicting results and complexity of ultrasound measurement methods, there is a need for new methods for the assessment of pre-clinical vascular condition that would provide consistent results in a large study population. Pulse wave (PW) morphology analysis provides an interesting and fresh approach for characterizing vascular health. While the formation of PW shape has been extensively studied and is currently well understood, it is difficult to make analytical conclusions about certain features in the wave shape that would disclose information on sub-clinical arterial changes. Therefore, data-driven approaches with a large, representative dataset are preferred for finding parameters that have association between cIMT, brachial FMD and Cdist.

Previously, our group has developed methods for computing morphology-based volume pulse wave parameters and studied their use in assessing the condition of vasculature of atherosclerotic patients [20,21]. The objective of the present study is to examine the association of individual and combined waveform morphology parameters derived from IPG, ICG and tIPG pulse waves with cIMT, FMD or Cdist. The measurement of these impedance signals does not require skilled operators and parameters derived from these signals could therefore be made more repeatable and easier to attain.

2. Materials and methods

2.1. Participants

The data analyzed in the study has been collected as part of the Cardiovascular Risk in Young Finns Study [22] in 2007. At that time, the test participants were aged between 30 and 45 years. The study has been reviewed by local ethics committees of the participating centers and the study has been conducted according to the guidelines of the Declaration of Helsinki. All the volunteer participants have given their informed consents in written form. The data that support the findings of this study are available from the corresponding author upon reasonable request.

In total, the dataset consists of 1853 volunteer participants. In the present study, 112 participants were excluded due to following reasons: low signal-to-noise ratio of distal impedance plethysmogram or whole-body impedance cardiogram ($n = 16$), incomplete cardiovascular risk factor data ($n = 47$), necessary PW parameters impossible to determine because of unsuitable shape of the obtained pulse waves ($n = 26$), or participant being pregnant during the measurement ($n = 23$). Thus, 1741 participants were included in the present analysis. From these 1741 participants, 118 (6.8%) were on antihypertensive, 16 (0.9%) on antidiabetic and 37 (2.1%) on lipid lowering medication.

2.2. Clinical characteristics

Weight and height were measured and body mass index (BMI, kg/m^2) calculated during the data collection. The blood pressure was measured using random-zero sphygmomanometer, and smoking behavior data collected through questionnaire [12]. High-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, insulin, glucose, and C-reactive protein (CRP) were measured during the data collection as described in more detail in previous studies [23,24].

2.3. Ultrasound imaging

The measurement of ultrasound variables (cIMT, FMD and Cdist) was performed during the data collection with high-resolution B-mode ultrasound device [12]. cIMT was calculated as a mean of at least four measurements from the left carotid artery. FMD was determined by measuring the diameter of brachial artery both at rest and during reactive hyperemia, which was induced by inflation of a pneumatic tourniquet placed around the forearm to a pressure of 250 mmHg for 4.5 min, followed by a release [25]. Carotid artery ultrasound and concomitant brachial blood pressure measurements were used to calculate carotid artery distensibility: Cdist (%/10 mmHg) = $([D_s - D_d]/D_d)/(P_s - P_d)$, where D_s is the systolic diameter, D_d is the diastolic diameter, P_s is the systolic blood pressure and P_d is the diastolic blood pressure [26].

2.4. Impedance plethysmography

Impedance plethysmography (IPG) is a non-invasive and continuous method for detecting changes in the conductivity of the measurement target caused by changes in the absolute and relative volumes of the tissues with different conductivities. The IPG signal mainly originates from the changes in the volume of blood in the arteries. A pressure pulse affects the diameter of an artery and thus the blood volume in the artery; when the diameter of the artery increases, the impedance decreases. The changes in the bioimpedance are caused by the conductivity of the blood being greater than the other tissues [27]. Distal IPG and whole-body impedance cardiogram (ICG) were measured with a non-invasive whole-body impedance cardiography device (CircMon, JR Medical Ltd, Tallinn, Estonia). The device enables to measure a tetrapolar (4-lead, 1-channel) whole-body impedance cardiogram, a bipolar (2-lead) distal impedance plethysmogram and a bipolar electrocardiogram (ECG) [12]. The whole-body ICG was measured with parallel current feeding and voltage measuring electrodes placed at both ankles and wrists (8 electrodes in total) with the distance between current feeding and voltage sensing electrodes being 5 cm [28]. The current feeding electrodes inject an alternating current (30 kHz ± 2%, 0.7 mA), and the voltage sensing electrodes measure the voltage drop generated by the applied electrical current [29]. In the distal IPG, one electrode was placed on the lateral side of the knee joint and the other one on the calf [30]. The distance between the electrodes was approximately 20 cm [30]. The ECG was measured with one electrode placed under the right

clavicle and the other was placed at the location of the chest V5 electrode of the basic 12-lead system. More details on the standard electrode configuration [28], the validation of the method as well as reference values, and repeatability and reproducibility evaluation of the method have been published earlier in Refs. [30–32].

2.5. Calculating the pulse wave parameters

Firstly, R-peak from the ECG was recognized to find the starting point of the PW, which is defined as the local minimum after each R-peak and before the rising edge of the PW [33,34]. After recognizing the PWs, the parameters were calculated from all the recognized individual waves, and median parameter values were computed from each approximately 13-min time series. The waveform of the ICG signal resembles conventional peripheral blood pressure or volume PW, so the feature extraction algorithms, originally proposed for the analysis of pressure and volume PWs [20,21], were implemented directly to the ICG-based pulse waveforms. It was observed that in many cases the IPG signals resemble the first derivative of the conventional peripheral PW. For this reason, the IPG signals were transformed with a trapezoidal numerical integration method as we hypothesized that the algorithms perform better with a pulse waveform that resembles conventional peripheral waveform. The feature extraction algorithms were however also implemented for the raw IPG signals without integral transformation. The PW parameters were thus calculated from IPG signals, ICG signals, and transformed IPG (tIPG) signals. Correct operation of the PW parameter extraction algorithms was verified by visual inspection.

Fig. 1 illustrates and example pulse wave with its essential fiducial points used in the PW parameter calculation. The implemented feature extraction algorithms for parameters T_2, and T_3 utilized in this study are originally proposed for the analysis of peripheral blood pressure or volume PWs [20]. T_2 is the time delay between the early systolic peak P_1 and the diastolic peak B whereas T_3 is a time delay between the late systolic peak P_2 and the diastolic peak B [20]. An augmentation index was defined as the ratio between late systolic peak and early systolic peak: P_2/P_1 [35]. The second derivative of the PW, which consists of four waves in systole: a, b, c and d and one wave in diastole: e, was calculated in order to calculate an ageing index, which is defined as $(b\text{-}c\text{-}d\text{-}e)/a$ [36]. Fast Fourier Transform (FFT) was computed by removing the mean of each individual pulse waveform and adding 2^{12} zeros to the end of each signal vector containing the samples of each individual pulse waveform in order to improve the spectral resolution and to calculate the ratio between the amplitudes of the first two

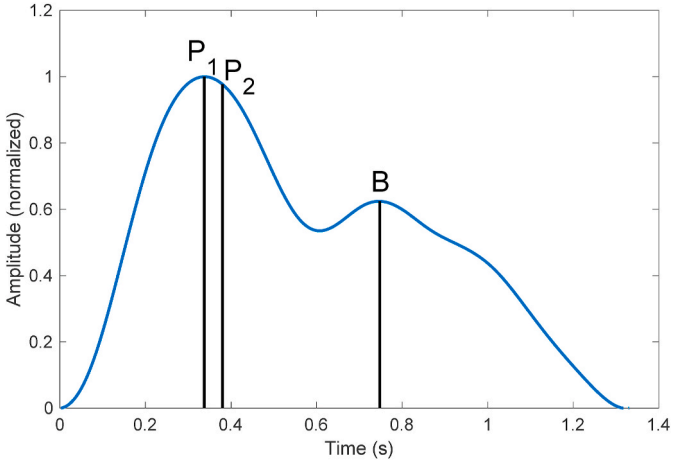

Fig. 1. Fiducial points of pulse wave for the calculation of time delay T_2, which is the time delay between the early systolic peak P_1 and the diastolic peak B, and T_3, which is the time delay between the late systolic peak P_2 and the diastolic peak B.

spectral peaks. The length of the amplitude-normalized individual PW curves was approximated by determining the distance of the successive discrete sample points with the Pythagorean theorem implemented for the vertical and horizontal differences of their coordinates and summing the individual distances. In addition, the ratio of the areas under the ICG PW and IPG PW curves was calculated. PWs were also decomposed in individual components using a non-linear iterative Levenberg-Marquardt optimization algorithm. A model consisting of five lognormal basis functions or components (Supplementary Fig. S1) or one Gaussian basis function and four lognormal basis functions (Supplementary Fig. S2) was created for modeling the superposition of the heartbeat induced wave (the highest peak in Supplementary Fig. S1 and S2) and its reflections (the second and the third highest peaks in Supplementary Fig. S1 and S2). Then decomposition-based parameters were defined from both decomposition models for the components having the highest and the second highest amplitudes as well as the highest and the third highest amplitudes, the time differences of the peak locations of the individual components, and the ratios of the areas under the individual components. In total, this yielded 6 PW decomposition parameters for both decomposition models. All the PW parameters were calculated with MATLAB 2018a (MathWorks, Natick, MA, USA).

2.6. Statistical methods

The data was analyzed with R Statistics version 4.0.0. (R Development Core Team, Vienna, Austria). Mean and standard deviation were calculated for normally distributed descriptives and median and interquartile range were calculated for non-normally distributed descriptives. Comparison between continuous variables between the groups were calculated with two sample t-test for normally distributed characteristics and with Mann-Whitney U test for non-normally distributed characteristics. Categorical variables were compared with chi-square tests between the groups.

For further analysis, all the PW parameters and continuous covariates except age were subjected to inverse normal transformation to convert all variables normally distributed. Usage of inverse normal transformation makes β-values of variables comparable with each other, minimizes the incidence of type I errors and reduces the impact of outliers [37]. After converting PW parameters, linear regression was utilized to test association between an outcome (cIMT, FMD, Cdist) and each PW parameter adjusted with traditional risk factors (systolic blood pressure, HDL cholesterol, LDL cholesterol, BMI, triglycerides, glucose, insulin and CRP) (see Supplementary Table S1a, b and c for multivariate cIMT, FMD, and Cdist, respectively).

To find out the most significant PW parameters that are independently associated with the outcome, bootstrapped stepwise Akaike's Information Criterion (bootstepAIC) was utilized with 1000 Bootstrap samples. The lower model consisted of traditional risk factors, thus forcing the variables into the minimum model, and the upper model consisted of all PW parameters including PWV. The PW parameters left in the final model were tested for excess covariance using Variable Inflation Factor (VIF), which had to be lower than 5. See Supplementary Table S2a, b and c for the found parameters.

The β coefficients of pulse wave variables in the final bootstepAIC model were used to calculate a weighted PW risk score for each outcome. The risk score for each outcome was constructed by multiplying each individual's PW parameter by its β coefficient, and then these multiplied values were summed up for each individual. After calculating the risk scores, they were inverse normal transformed to make them comparable with the other traditional risk factors.

The risk score for each outcome was tested in a linear regression model per outcome and adjusted with traditional risk factors. To minimize the effect of overfitting of the risk scores, standard deviations and p-values for each risk score were bootstrapped 1000 times. Sex by age by risk score interactions were evaluated in linear regression model. There

Table 1
Clinical characteristics of the test participants.

	All (n = 1741)	Women (n = 952)	Men (n = 789)	p-value
Female/male distribution (%)	–	54.7	45.3	<0.001
Age	37.7 ± 5.0	37.8 ± 5.0	37.6 ± 5.1	0.282
Body mass index (kg/m^2)	25.9 ± 4.7	25.4 ± 5.0	26.5 ± 4.1	<0.001
Systolic blood pressure (mmHg)	126 ± 14	120 ± 13	132 ± 12	<0.001
Diastolic blood pressure (mmHg)	77 ± 9	75 ± 9	79 ± 9	<0.001
cIMT (mm)	0.63 ± 0.10	0.61 ± 0.09	0.65 ± 0.11	<0.001
FMD (%)	8.9 ± 4.6	10.0 ± 4.9	7.6 ± 3.8	<0.001
Cdist (%/10 mmHg)	1.9 ± 0.7	2.0 ± 0.7	1.7 ± 0.6	<0.001
Carotid artery diastolic diameter (mm)	5.6 ± 0.5	5.3 ± 0.4	5.9 ± 0.5	<0.001
HDL cholesterol (mmol/L)	1.3 ± 0.3	1.4 ± 0.3	1.2 ± 0.3	<0.001
LDL cholesterol (mmol/L)	3.1 ± 0.8	2.9 ± 0.7	3.3 ± 0.8	<0.001
Triglycerides (mmol/L)	1.15 (0.85–1.56)	0.95 (0.75–1.36)	1.26 (0.95–1.87)	<0.001
Insulin (mmol/L)	6.9 (4.3–10.8)	6.8 (4.0–10.6)	7.2 (4.5–10.9)	0.088
Glucose (mmol/L)	5.2 (4.9–5.6)	5.2 (4.8–5.5)	5.5 (5.2–5.7)	<0.001
C-reactive protein (mg/L)	0.9 (0.4–1.8)	1.0 (0.4–2.1)	0.8 (0.4–1.6)	0.008
Smoker (%)	19.0	15.7	22.9	<0.001
Antihypertensive medication (%)	6.8	6.9	6.6	0.852
Lipid-lowering medication (%)	2.1	1.3	3.2	0.010
Anti-diabetic medication (%)	0.9	0.6	1.3	0.257

Values are mean ± standard deviation or geometric mean (25th–75th percentile) or percentage of participants.

were no statistically significant interactions between the aforementioned variables.

3. Results

3.1. Study population

The clinical characteristics of all test participants are shown in Table 1.

3.2. Results of multivariable linear regression for cIMT

Table 2 presents covariates of the multivariable linear regression model. A risk score for IMT consisted of four different parameters (see Supplementary Table S1a), but PWV was not selected as one of them. The results are presented in order of preference based on the magnitude of normalized β-values. A PW-derived risk score parameter for cIMT, produced as the combination of the PW parameters, was statistically significantly associated with cIMT in multivariable linear regression analysis ($p < 0.001$) (see Supplementary Table S1a).

3.3. Results of multivariable linear regression for FMD

Table 3 presents covariates of multivariable linear regression for FMD. The results are presented in order of preference based on the magnitude of β-value. A PW risk score for FMD (see Supplementary Table S1b) was statistically significantly associated with FMD ($p < 0.001$). In addition, the risk score for FMD was the second most significant variable right after BMI. BootstepAIC chose 11 PW parameters for the risk score of FMD. Again, PWV was not selected into the model (see Supplementary Table S1b).

3.4. Results of multivariable linear regression for Cdist

Table 4 presents covariates of multivariable linear regression for Cdist. The results are presented in order of preference based on the magnitude of β-value. A risk score for Cdist (see Supplementary Table S1c), composed of 17 PW parameters, was determined as the most significant covariate of the multivariable linear regression ($p < 0.001$). Also, in this case PWV was not selected as a parameter for the risk score (see Supplementary Table S1c).

4. Discussion

This study showed that we are able to calculate parameters from the whole-body ICG and distal IPG PWs that are strongly associated with cIMT, FMD, and Cdist. The risk scores calculated as a linear combination of the PW derived parameters were among the three most significant risk factors that predict subclinical atherosclerosis in all three cases with other cardiovascular risk factors in the Finnish population. The

Table 2
Relationship between cardiovascular risk factors and cIMT.

Variable	β	SE	p-value
Body mass index	0.016	$2.75*10^{-3}$	<0.001
Systolic blood pressure	0.015	$2.46*10^{-3}$	<0.001
Score for IMT	$\mathbf{8.60*10^{-3}}$	$\mathbf{2.18*10^{-3}}$	**<0.001**
HDL cholesterol	$-6.13*10^{-3}$	$2.47*10^{-3}$	0.013
Age	$5.84*10^{-3}$	$4.50*10^{-4}$	<0.001
Male sex	$5.50*10^{-3}$	$5.53*10^{-3}$	0.320
LDL cholesterol	$3.58*10^{-3}$	$2.44*10^{-3}$	0.143
Insulin	$2.72*10^{-3}$	$2.78*10^{-3}$	0.328
Triglycerides	$1.47*10^{-3}$	$2.76*10^{-3}$	0.594
C-reactive protein	$-1.03*10^{-3}$	$2.44*10^{-3}$	0.674
Glucose	$-8.1*10^{-4}$	$2.40*10^{-3}$	0.734

Multivariable linear regression including product of risk score for IMT, sex and age, and sex, age, systolic blood pressure, HDL cholesterol, LDL cholesterol, BMI, triglycerides, glucose, insulin and c-reactive protein. SE, standard error.

Table 3
Relationship between cardiovascular risk factors and FMD.

Variable	β	SE	p-value
Body mass index	2.81	0.418	<0.001
Score for FMD	**2.71**	**0.317**	**<0.001**
Male sex	−1.27	0.813	0.118
HDL cholesterol	0.780	0.399	0.051
Glucose	0.667	0.391	0.088
Insulin	−0.453	0.485	0.351
Triglycerides	0.432	0.436	0.321
C-reactive protein	−0.429	0.356	0.228
Systolic blood pressure	−0.290	0.387	0.454
LDL cholesterol	0.179	0.336	0.594
Age	−0.115	0.064	0.073

Multivariable linear regression including product of risk score for percentage of FMD, sex and age, and sex, age, systolic blood pressure, HDL cholesterol, LDL cholesterol, BMI, triglycerides, glucose, insulin and c-reactive protein. SE, standard error.

Table 4
Relationship between cardiovascular risk factors and Cdist.

Variable	β	SE	p-value
Score for Cdist	**0.218**	**0.014**	**<0.001**
Male sex	−0.162	0.032	<0.001
Systolic blood pressure	−0.132	0.017	<0.001
Body mass index	−0.053	0.020	0.006
Insulin	−0.049	0.019	0.010
Age	−0.032	$2.72*10^{-3}$	<0.001
Glucose	−0.016	0.015	0.292
Triglycerides	0.015	0.018	0.399
C-reactive protein	0.012	0.015	0.430
LDL cholesterol	$6.29*10^{-3}$	0.014	0.657
HDL cholesterol	$5.67*10^{-3}$	0.016	0.726

Multivariable linear regression including product of risk score for Cdist, sex and age, and sex, age, systolic blood pressure, HDL cholesterol, LDL cholesterol, BMI, triglycerides, glucose, insulin and c-reactive protein. SE, standard error.

bootstepAIC algorithm did not select PWV as a descriptive parameter whereas PW parameters were chosen into the model.

Our models, which are multivariable adjusted, found significant association between proposed risk scores and cIMT, FMD and Cdist without dividing test participants into age-based subgroups like Koivistoinen et al. [12] did when analyzing the association between the three outcomes and PWV. Furthermore, we did not find any age by sex by risk score interaction with cIMT, FMD or Cdist. Thus, age and sex do not affect the risk scores. The risk score for IMT was the third strongest predictor of cIMT after BMI and systolic blood pressure, which indicates that PW parameters provide predictive information on the magnitude of cIMT, thus reflecting the vascular damage. BMI was found to have the highest association with cIMT, which is in line with previous studies [38,39]. BMI could reflect the effect of energy intake and physical exercise to the progression of atherosclerosis [39]. Our method showed that systolic blood pressure is associated with cIMT, which is in agreement with other studies [38,40,41]. Systolic blood pressure might induce higher pressure overload and therefore induce more arterial hypertrophy or hyperplasia, thus making it an important determinant of cIMT [40,41].

In our study, the risk score for FMD was the second strongest predictor for the value of FMD and it was independently associated with FMD. Therefore, the parameters utilized in calculating the risk score have predictive value, and thus they reflect functional atherosclerotic changes in the arterial wall. Our findings showed that BMI has association with FMD, and previously this association has been shown to be curvilinear [25]. However, Schroeder et al. [42] did not find a significant association between impairment of FMD and BMI. In contrast to our study, the test participants in their study were older [42]. Our findings also showed that male sex has an effect on FMD, which has been shown also previously [25]. However, Black et al. [43] concluded that impacts of ageing, fitness and exercise training on FMD were less evident in men than in women.

In our calculations, the risk score for Cdist was independently associated with Cdist and it was the strongest predictor for Cdist. Because Cdist measures the arterial ability to expand and contract [6], the parameters of the risk score correlate with the arterial stiffness. Therefore, these parameters provide information about the atherosclerotic process. Our results showed that Cdist is associated with systolic blood pressure. Moreover, Cdist is sensitive to systolic blood pressure because the relative arterial diameter increases during the systole [44]. We also noted that male sex is associated with Cdist. Previously, van Merode et al. [45] showed that when arterial distensibility is interpreted, sex-dependent differences must be considered.

This study has few limitations. Firstly, Cdist was calculated using brachial artery pulse pressure [12]. Thus, the pressure in the carotid artery might be overestimated because of pulse pressure amplification between central and peripheral arteries [11]. Secondly, all the test participants share ethnic homogeneity; therefore, the results are only generalizable to Caucasian subjects. The results need replication in other cohorts, preferably also with a wider age range of the subjects.

To the best of our knowledge, this is the first study where PW derived parameters are combined as a risk score and used in linear multivariable regression analysis to find associations between PW morphology and subclinical atherosclerosis defined by traditional arterial parameters measured by ultrasonic examination. Regression analyses were adjusted with traditional clinical risk factors and removing the effect of these risk factors did not reduce the association between the calculated risk scores and cIMT, FMD or Cdist. In conclusion, PW derived parameters provide more information about the condition of the artery wall than PWV, which was demonstrated when the bootstepAIC did not include PWV into the final model amongst other PW parameters in association with the markers of subclinical atherosclerosis (cIMT, FMD or Cdist).

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CRediT authorship contribution statement

Mira Haapala: Writing - original draft, Software, Formal analysis, Data curation, Validation. **Leo-Pekka Lyytikäinen:** Formal analysis, Software, Data curation, Methodology, Writing - original draft. **Mikko Peltokangas:** Software, Data curation, Funding acquisition, Writing - review & editing. **Teemu Koivistoinen:** Writing - review & editing. **Nina Hutri-Kähönen:** Writing - review & editing. **Mika-Matti Laurila:** Writing - review & editing. **Matti Mäntysalo:** Funding acquisition, Writing - review & editing. **Olli T. Raitakari:** Resources, Writing - review & editing. **Mika Kähönen:** Resources, Writing - review & editing. **Terho Lehtimäki:** Resources, Writing - review & editing. **Antti Vehkaoja:** Funding acquisition, Writing - review & editing, Supervision. **Niku Oksala:** Funding acquisition, Conceptualization, Writing - review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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