scientific reports

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# Reference equations for pulse wave velocity, augmentation index, amplitude of forward and backward wave in a European general adult population

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**Pulsatile hemodynamics have been shown to be independent predictors of cardiovascular events. The aim of the current study was to describe four pulsatile hemodynamic markers in a large, well-established, population-based cohort and to provide reference equations for sex- and age-based standardization of these measurements. 6828 adult participants from the Austrian LEAD (Lung, hEart, sociAl, boDy) cohort study, who were free from overt cardiovascular disease, non-diabetic based on blood test results, and had no history of pharmacological treatment for hypertension, dyslipidemia, and diabetes, comprised the "reference population". Carotid-femoral pulse wave velocity (cfPWV), augmentation index (AIx), amplitude of forward wave (Pf), and backward wave (Pb) were described in different age categories for both sexes. Sex-specific reference equations for cfPWV, AIx, Pf, and Pb with age as the predictive variable were created using the Lambda-Mu-Sigma (LMS) method. All four parameters increased with age. CfPWV and Pf were higher in males than females, especially in young and middle-age groups ($P < 0.001$). AIx was higher in females than males in all age categories ($P < 0.001$). Pb was also higher in females than males in age groups older than 40 years ($P < 0.01$). Reference equations for the skewness (Lambda), median (Mu), and coefficient of variation (Sigma) values were determined, enabling the calculation of sex- and age-standardized values (z-scores) for each individual's pulsatile hemodynamic measurement, and an online application was developed. Reference equations derived from a large population-based dataset constitute a suitable tool for the standardization of pulsatile hemodynamics and for the accurate interpretation of vascular aging.**

**Keywords** Pulse wave velocity, Augmentation index, Amplitude of forward wave, Backward wave, Reference equation, Z-score

Cardiovascular disease (CVD) remains the leading cause of morbidity and mortality worldwide and its prevalence is still rising. Arterial stiffness has proven to be a valuable parameter for the assessment of cardiovascular (CV) risk[1,2]. Carotid-femoral pulse wave velocity (cfPWV) is considered as the gold standard method[3] for measuring arterial stiffness non-invasively, as illustrated by their inclusion in the European Society of Hypertension/European Society of Cardiology (ESH/ESC) guidelines for the management of hypertension since 2007[4] up to the latest ESH version 2023[5]. In addition, pulse wave analysis (PWA) and wave separation analysis (WSA) based parameters such as augmentation index (AIx), amplitude of forward wave (Pf) and backward wave (Pb) are also independent predictors of major cardiovascular events[6].

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Population-based reference values for cfPWV, AIx, Pf, and Pb, obtained in a single and large sample from the general population, are only sparsely available[7,8]. Moreover, while reference values for pulsatile hemodynamics in healthy populations and their associated factors have been reported, an age- and sex-adjusted reference equation for pulsatile hemodynamics derived from a general population-based database is lacking. We aimed to calculate reference equations, derived from the Austrian Lung, hEart, sociAl, boDy (LEAD) study, a large and carefully characterized cohort of individuals living in Austria[9].

Therefore, the objectives of the present study were to (i) establish 'normal' values of cfPWV, AIx, Pf, and Pb in different age bins in a reference population cohort from the LEAD Study according to a commonly used definition[10], and (ii) to provide age-and sex-related percentile curves to facilitate quantification of vascular age, based on these four main metrics of pulsatile hemodynamics.

## Methods

### Study design

The data for our study were collected within the Austrian LEAD study, a longitudinal, observational, population-based cohort. The design and methodological details have been published earlier[9]. Briefly, the LEAD study is one of major research projects to examine the respiratory, cardiovascular, and metabolic health and relevant comorbidities in Austria. The recruitment and data collection for the first visit started in February 2012. A maximum of three personalized letters of invitation per person was sent to a random sample of inhabitants of Vienna (urban population) and six villages of Lower Austria (rural population), stratified by age, gender and residential area. In order to assess the representativeness of the study population in comparison to the Austrian population, the LEAD study team performed extensive external validity testing[9].

Finally, 11,423 participants, aged 6–82, were recruited between 2012 and 2016 and completed the measurements of visit 1, including the assessment of pulsatile hemodynamics by measuring cfPWV, AIx, Pf, and Pb. Other cardiovascular measurements assessed were electrocardiograms and blood pressure (BP) measurements at both upper and lower limbs. Besides that, the study participants completed a questionnaire on the history of cardiovascular diseases (CVD) and other comorbidities. Habits and lifestyle were also recorded by using validated questionnaires. Smoking status was categorized as never smoker, and ever smoker, with smoking defined as at least 20 packs overall or at least 1 cigarette per day for 1 year or at least 2 cigarettes per day for 6 months. Venous blood samples were collected after at least eight hours overnight fasting for measurement of total, LDL- and HDL-cholesterol, triglycerides, fasting blood glucose (FBG), HbA1c, and high sensitive CRP, using standard laboratory methods (Siemens Dimension™ VISTA 1500). All the measurements were performed at the LEAD study center of the Ludwig Boltzmann Institute at the clinic Penzing in Vienna, Austria.

The LEAD study was performed according to the principles of the Declaration of Helsinki, and was approved by the local Ethics committee of Vienna (protocol number: EK-11-117-0711). All the participants signed an informed consent.

### Subject's selection (reference population)

We defined a 'reference population' as adult participants (aged ≥ 18 years) of both sexes, with no history of overt CVD and no history of pharmacological treatment for hypertension, dyslipidemia, and diabetes. Those who were diagnosed as diabetic according to the blood test, were also excluded from the analysis. Diabetes was defined as fasting blood glucose ≥ 126 mg/dL (7 mmol/l) and/or HbA1c ≥ 6.5% (≥ 48 mmol/mol) in the blood test.

Finally, a total of 6,828 participants comprised the "reference population "– Fig. 1.

### Pulsatile hemodynamics measurements

*Blood pressure and pulse wave velocity*

Brachial blood pressure (BP) was measured in the sitting position using the auscultatory method according to international standards[4]. Carotid-femoral PWV was measured in the supine position for each participant by trained personnel using sequential, ECG gated tonometry (Sphygmocor CP, Model EM3, AtCor medical, West Ryde, New South Wales, Australia) at the carotid and femoral site, respectively. The footpoint of the wave was identified automatically with the intersecting tangents method. Travel distance (TD) was assessed on body surface, using the subtracted method: TD = suprasternal notch – femoral site minus suprasternal notch—carotid site. To facilitate comparison with a similar population in the Reference Values Project[10], "direct" travel distance × 0.8 was calculated according to the formula TDdirect = (0.45 × TD subtracted + 0.21 × height + 0.08) × 0.8[10], and used for the main analysis , where cfPWV was calculated as $TD_{direct\ x\ 0.8}$/transit time (labelled as cfPWV throughout this article). For additional analysis, cfPWV using the originally measured subtracted distance method [$CfPWV_{subtracted\ distance}$ = (suprasternal notch to femoral distance – suprasternal notch to carotid distance) / transit time] was used as well and presented as supplementary materials. As there was only one measurement of cfPWV available for each study participant, strict quality control was applied post hoc in cooperation with PB and RMB: First, a learning population from the Paris cohort (7441 cfPWV measurements) was selected. The coefficient of variation for cfPWV within acquisition (CV-PWV) was considered as an index of quality. Outliers (data screening, n = 224) were defined as a CV-PWV > 16%. The determinants of CV-PWV (log regression, c-stat 0.79) were: tonometric pulse amplitude at carotid and femoral site, ECG amplitude, pulse duration and baseline stability of femoral tonometry. From these 5 variables, cutoff-values were derived and a score (from 0 (good quality) to 5 (bad quality)) were created. Adequate quality data were defined as CV-PWV < 0.16 and a quality score < 4.

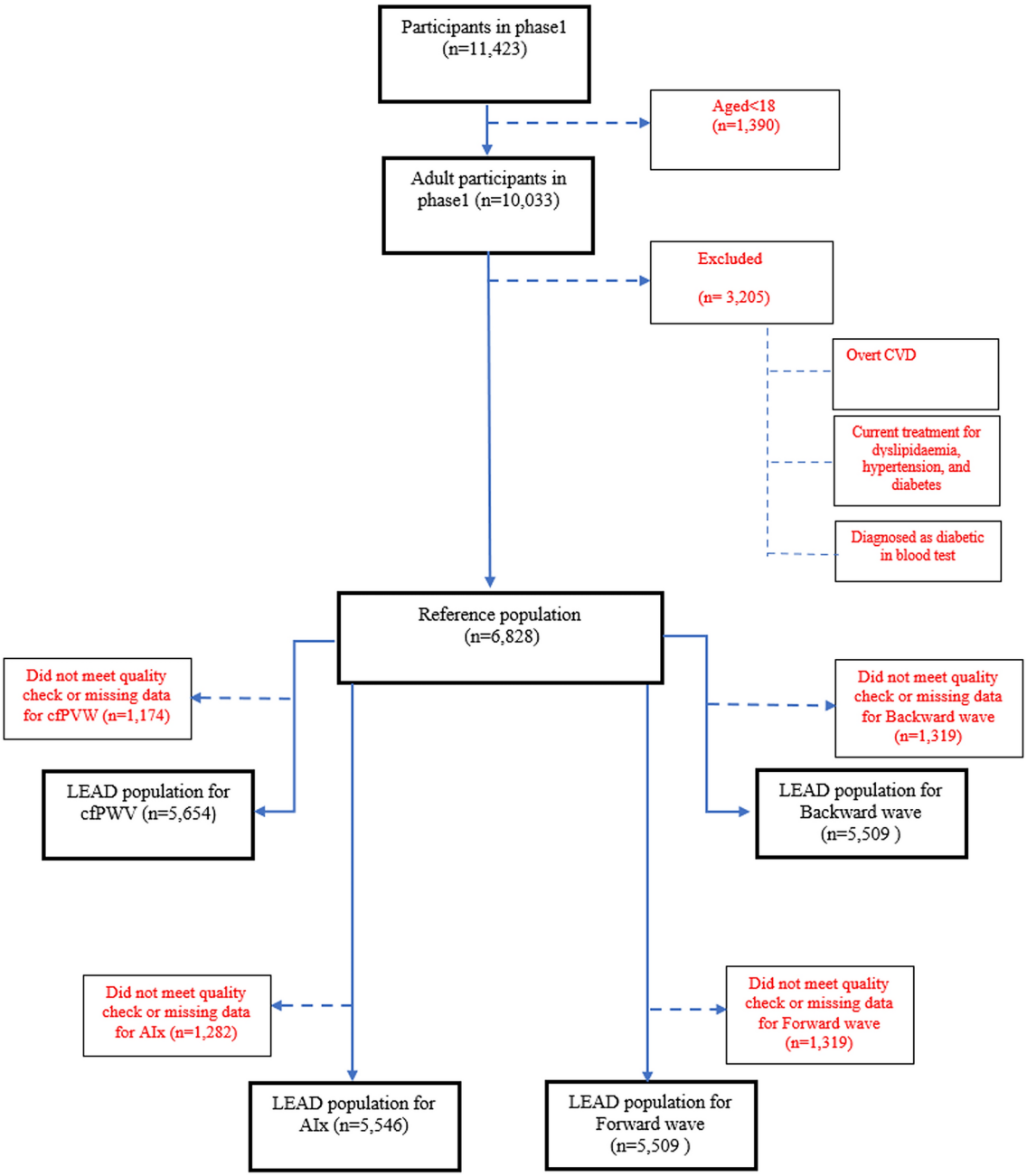


**Fig. 1**. Flowchart describing the selection and categorization of subjects from the LEAD database for the present analysis.

## Antegrade and backward waves

For assessment of antegrade and backward waves, radial pressure waveforms were recorded at the wrist, using applanation tonometry with a high-fidelity micromanometer (Millar Instruments, Houston, Tx, USA) embedded in the SphygmoCor device, and calibrated with brachial systolic and diastolic BP. After 20 sequential waveforms had been acquired, a validated generalized transfer function was used to generate the corresponding central aortic pressure waveform (SphygmoCor device, AtCor medical). AIx was derived from central waveforms with PWA[11]: the merging point of the incident and the reflected wave (the inflection point) was identified on the generated aortic pressure waveform. Augmentation pressure (AP) was the pressure at the second shoulder minus pressure at the inflection point. The AIx was defined as the AP divided by pulse pressure and expressed as a percentage.

The amplitudes of the forward (antegrade) and the backward (reflected) pressure waves were quantified using Wave Separation Analysis (WSA) with the validated ARCSolver method[12–14]: briefly, aortic pressure curves were derived from the SphygmoCor system. Aortic flow curves were estimated from these curves, based on three element Windkessel models, where the outflow of the left ventricle is described as a dynamic system of second

order. Windkessel equations are formulated as an isoperimetric problem with a constraint to minimize hydraulic work, and mathematical solutions, pressure waveform area fitting, and a second-order linear delay element lead to the final flow shape[12]. Then, utilizing pressure and flow waveforms, WSA is performed in the frequency domain. Participants with SphygmoCor quality index < 80 were excluded from the analysis[15]. All waveform recordings measurements were obtained in the supine position in a quiet, temperature-controlled room (22 + /- 1 °C) after a brief period (at least 5 min) of rest, by trained technicians.

### Statistical analysis

The characteristics of the study population and reference population were described as mean and standard deviation for continuous variables, and number and proportional frequency for categorical variables. The distribution of the pulsatile hemodynamics (cfPWV, AIx, Pf, Pb) within the reference population was checked, and extreme outliers—data points more extreme than Q1 − (3 × IQR) or Q3 + (3 × IQR)—were excluded from the analysis. Pulsatile hemodynamics (cfPWV, AIx, Pf, Pb) were reported as mean (standard deviation) and median (10th to 90th percentile) for males and females at different age categories. Comparison of these values between male and female were performed using non-parametric Mann–Whitney U test. The sex-specific reference equations for cfPWV, AIx, Pf and Pb with age, as the predictive variable, were created using Lambda-Mu-Sigma (LMS) method as described earlier by Cole and Green[16]. Given a variable of interest $Y$ with median μ and a power transformation so that $Y^{\lambda}$ (or $\log(Y)$ if λ = 0) is normally distributed, the transformed variable is considered as:

$$x = \begin{cases} \frac{(Y/\mu)^{\lambda}-1}{\lambda} if \lambda \neq 0, \\ \log(\frac{Y}{\mu}) if \lambda = 0, \end{cases}$$

based on the Box-Cox transformation. In fact, the transformation maps the median μ of $Y$ to $z = 0$. For λ = 1 the SD of $x$ is the coefficient of variation (CV) of $y$. This fact remains approximately true for all moderate values of λ, Therefore,

$$z = \frac{x}{\sigma} = \begin{cases} \frac{\left(\frac{Y}{\mu}\right)^{\lambda}-1}{\lambda\sigma} & if \lambda \neq 0, \\ \frac{\log\left(\frac{Y}{\mu}\right)}{\sigma} & if \lambda = 0, \end{cases}$$

is the z-score of $Y$, where $\sigma$ is the SD of $x$. Assume that the distribution of $Y$ varies with age and.

that $L_{Age}$, $M_{Age}$, and $S_{Age}$ are smooth curves with respect to age, denoting λ, μ, and $\sigma$, respectively. Then the z-scores of the target variable adjusted based on the age is given by:

$$z = \begin{cases} \frac{\left(Y/M_{Age}\right)^{L_{Age}}-1}{L_{Age}S_{Age}} & if L_{Age} \neq 0, \\ \frac{\log\left(\frac{Y}{M_{Age}}\right)}{S_{Age}} & if L_{Age} = 0. \end{cases}$$

LMS methodology is implemented in generalized additive model of location, scale, and shape (GAMLSS) package in R (version 4.3.1). GAMLSS is a semiparametric regression type of statistical model, which is highly flexible as it relaxes the traditional distributional assumptions about normality to include even highly skewed and kurtotic distributions[17]. As AIx contains negative values, + 100 units were added to each value to ensure that all values are positive. Reference equations derived from LMS models summarize the distribution of the four pulsatile hemodynamics measurements, based on three parameters: L(x) which indicates the power in the Box-Cox transformation for the skewness adjustment, M(x) the median, and S(x) the generalized coefficient of variation for each age x. The best model was selected to achieve the lowest Bayesian Information Criterion (BIC). To examine whether the distribution of the Z-scores corresponds to a standard Gaussian distribution, Kolmogorov–Smirnov test was used. $P < 0.05$ was considered to be statistically significant.

## Results

### Population characteristics

Out of the 11,423 participants, 10,033 aged ≥ 18 years and formed our "study population". Participants who received treatment for hypertension, dyslipidemia, and diabetes, as well as those who were diagnosed as diabetic in the blood test, were excluded from the database. Finally, 6,828 subjects were defined as the "reference population". Demographic parameters and clinical characteristics of both the "study population" and the "reference population" are summarized in Table 1.

Carotid-femoral PWV, AIx, Pf, and Pb were calculated for those reference populations that had valid data for each measurement and met the quality check (Fig. 1).

Mean ± 2SD and median (10–90th percentile) for cfPWV, AIx, Pf, and Pb are summarized for male and females at each age category (Table 2).

Average cfPWV increased by age in both sexes, and it was significantly higher in males than females in the first four age categories ($p < 0.001$) (Table 2; Fig. 2). Likewise, average $cfPWV_{subtracted\ distance}$ increased by age in both sexes, and it was significantly higher in males than females in the first four age categories ($p < 0.001$) (Supplementary Table S1 and Fig. S1 online).

| Characteristic* | LEAD study population | | | LEAD reference population | | |
|---|---|---|---|---|---|---|
| | Overall, N = 10,033 | Male, N = 4678 | Female, N = 5355 | Overall, N = 6828 | Male, N = 3036 | Female, N = 3792 |
| Age (years) | 49.0 (16.4) | 49.0 (16.5) | 49.0 (16.3) | 43.1 (14.7) | 42.2 (14.4) | 43.9 (14.9) |
| Weight (kg) | 75.7 (16.2) | 84.5 (14.2) | 68.1 (13.8) | 72.8 (15.0) | 81.9 (13.0) | 65.6 (12.3) |
| Height (cm) | 170.4 (9.5) | 177.5 (7.0) | 164.2 (6.6) | 171.0 (9.4) | 178.5 (6.8) | 165.0 (6.4) |
| Systolic BP (mm Hg) | 131.6 (20.6) | 135.7 (19.6) | 128.0 (20.8) | 126.3 (18.0) | 131.0 (17.2) | 122.6 (17.7) |
| Diastolic BP (mm Hg) | 78.2 (11.3) | 80.5 (11.1) | 76.2 (11.1) | 76.4 (10.7) | 79.0 (10.5) | 74.3 (10.4) |
| Total cholesterol (mmol/L) | 5.4 (1.1) | 5.3 (1.1) | 5.5 (1.1) | 5.4 (1.1) | 5.3 (1.1) | 5.4 (1.1) |
| HDL cholesterol (mmol/L) | 1.7 (0.5) | 1.5 (0.4) | 1.9 (0.5) | 1.7 (0.5) | 1.5 (0.4) | 1.9 (0.5) |
| LDL cholesterol (mmol/L) | 3.2 (1.0) | 3.2 (1.0) | 3.2 (1.0) | 3.2 (1.0) | 3.3 (1.0) | 3.1 (1.0) |
| Triglycerides (mmol/L) | 1.3 (0.8) | 1.4 (1.0) | 1.1 (0.6) | 1.1 (0.7) | 1.3 (0.9) | 1.0 (0.5) |
| Glucose (mg/dl) | 94.1 (17.3) | 97.2 (19.3) | 91.4 (14.8) | 89.9 (8.8) | 92.0 (9.0) | 88.2 (8.3) |
| Smoking status | | | | | | |
| Never smoker | 4411 (44.0%) | 1783 (38.1%) | 2628 (49.1%) | 3138 (46.0%) | 1269 (41.8%) | 1869 (49.3%) |
| Ever smoker | 5619 (56.0%) | 2894 (61.9%) | 2725 (50.9%) | 3689 (54.0%) | 1766 (58.2%) | 1923 (50.7%) |
| Dyslipidaemia | 6966 (71.5%) | 3391 (74.2%) | 3575 (69.0%) | 4635 (68.2%) | 2166 (71.7%) | 2469 (65.4%) |
| Hypertension | 2037 (20.3%) | 1160 (24.8%) | 877 (16.4%) | 875 (12.8%) | 512 (16.9%) | 363 (9.6%) |
| Overt CVD | 1704 (17.0%) | 923 (19.7%) | 781 (14.6%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| Treated diabetes mellitus | 475 (4.7%) | 293 (6.3%) | 182 (3.4%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| Treated hypertension | 2295 (22.9%) | 1205 (25.8%) | 1090 (20.4%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| Treated Dyslipidemia | 1072 (10.7%) | 583 (12.5%) | 489 (9.1%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| Diabetes mellitus | 603 (6.2%) | 391 (8.5%) | 212 (4.1%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |

**Table 1**. Description of general clinical parameters of the study population and the reference population. *Data summarized as mean (Standard Deviation) for continuous and n (%) for categorical variables.

| | | Female reference population | | | Male reference population | | | |
|---|---|---|---|---|---|---|---|---|
| | Age category (years) | N | Mean ± 2 SD | Median (10–90%) | N | Mean−+2 SD | Median (10%–90%) | *P*-value |
| cfPWV (m/sec) | 18–30 | 696 | 6.7 (4.7–8.7) | 6.6 (5.6–8) | 625 | 7.1 (4.7–9.4) | 6.9 (5.8–8.6) | < 0.001 |
| | 30–40 | 606 | 7.2 (4.7–9.8) | 7 (5.9–8.8) | 574 | 7.7 (5.2–10.2) | 7.6 (6.2–9.4) | < 0.001 |
| | 40–50 | 767 | 7.8 (5.2–10.4) | 7.6 (6.3–9.4) | 575 | 8.2 (5.3–11.1) | 8 (6.7–10.1) | < 0.001 |
| | 50–60 | 600 | 8.3 (5–11.7) | 8.0 (6.5–10.6) | 445 | 8.7 (5.6–11.8) | 8.5 (6.9–10.8) | < 0.001 |
| | 60–70 | 360 | 9.1 (5.8–12.4) | 8.8 (7.2–11.3) | 203 | 9.4 (5.5–13.2) | 9.2 (7.1–12.2) | 0.2 |
| | > = 70 | 138 | 10.3 (6.2–14.4) | 10.3 (7.6–13.1) | 65 | 10.7 (6.4–15.0) | 10.4 (8.1–13.8) | 0.3 |
| AIx (%) | 18–30 | 606 | 8.2 (− 15.3–31.6) | 9.0 (− 8.0–23.0) | 585 | 0.6 (− 22.3–23.4) | 0.0 (− 14.0–15.6) | < 0.001 |
| | 30–40 | 499 | 18.2 (− 3.9–40.3) | 19.0 (4.0–32.0) | 553 | 8.7 (− 14–31.3) | 9.0 (− 6.0–23.0) | < 0.001 |
| | 40–50 | 736 | 27.7 (7.6–47.8) | 28.0 (14.0–40.0) | 612 | 17.4 (− 5.4–40.2) | 18.0 (2.0 – 31.0) | < 0.001 |
| | 50–60 | 599 | 33.9 (12.6–55.2) | 34.0 (21.0–47.0) | 471 | 22.9 (3.2–42.5) | 23.0 (9.0 – 35.0) | < 0.001 |
| | 60–70 | 392 | 35.2 (17.2–53.2) | 35.0 (25.0–45.0) | 238 | 28.3 (7.8–48.8) | 29.0 (15.0–40.3) | < 0.001 |
| | > = 70 | 158 | 36.1 (16.7–55.5) | 36.5 (23.0–48.0) | 98 | 30.1 (12–48.3) | 30.0 (19.0 – 41.0) | < 0.001 |
| Pf (mmHg) | 18–30 | 604 | 24.1 (13.3–35) | 23.7 (17.5–31.2) | 583 | 26.6 (13.2–40.1) | 25.9 (19–35.8) | < 0.001 |
| | 30–40 | 494 | 23.0 (13.4–32.7) | 22.6 (17.2–28.8) | 551 | 25.3 (12.8–37.8) | 24.3 (18.5–33) | < 0.001 |
| | 40–50 | 731 | 24.3 (13.5–35.2) | 23.9 (17.9–31.3) | 610 | 26 (13.6–38.4) | 25.4 (19.3–34.1) | < 0.001 |
| | 50–60 | 598 | 26.6 (12.9–40.3) | 25.8 (19.1–35) | 463 | 26.5 (14.5–38.5) | 26.2 (19.4–33.8) | 0.8 |
| | 60–70 | 390 | 30.1 (14.5–45.8) | 29.2 (20.9–41.1) | 236 | 30.5 (15–46) | 29.4 (22–41.7) | 0.6 |
| | > = 70 | 156 | 33.8 (16.3–51.3) | 33.1 (23.5–45.1) | 93 | 33.6 (16.7–50.5) | 31.5 (24.9–44) | 0.6 |
| Pb (mmHg) | 18–30 | 604 | 11.3 (5.4–17.1) | 11 (7.8–15.2) | 585 | 11.7 (4.3–19) | 11.1 (7.6–16.2) | 0.3 |
| | 30–40 | 495 | 12.6 (5.6–19.6) | 12.1 (8.7–16.8) | 551 | 12.5 (5.6–19.3) | 12.1 (8.4–17) | 0.5 |
| | 40–50 | 730 | 15.2 (7.1–23.4) | 14.7 (10.4–20.4) | 610 | 14.4 (5.9–22.9) | 13.9 (9.5–19.8) | < 0.001 |
| | 50–60 | 597 | 18.1 (7.5–28.6) | 17.7 (11.8–24.9) | 464 | 15.9 (7.2–24.5) | 15.3 (10.8–21.6) | < 0.001 |
| | 60–70 | 389 | 20.9 (9.2–32.7) | 20.3 (14.1–29.2) | 235 | 19.4 (8.3–30.6) | 18.7 (12.5–27.8) | 0.003 |
| | > = 70 | 156 | 24 (12.1–35.8) | 23.9 (16.6–32.5) | 93 | 22.1 (10.5–33.8) | 20.9 (15.3–29.1) | 0.007 |

**Table 2**. Description of carotid-femoral pulse wave velocity (cfPWV), Augmentation Index (AIx), forward wave amplitude (Pf), and backward wave amplitude (Pb) according to the age category in the female and male reference population. SD: standard deviation; 10 pc: the 10th percentile; 90 pc: the 90th percentile.

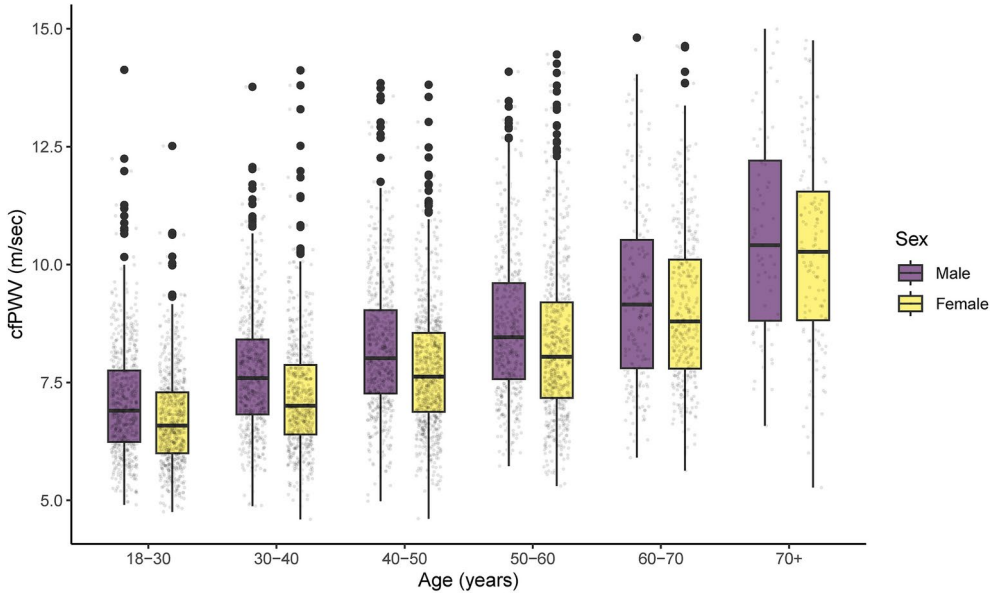


**Fig. 2**. Distribution of carotid-femoral pulse wave velocity (cfPWV) according to the age in females (n = 3167) and males (n = 2487).

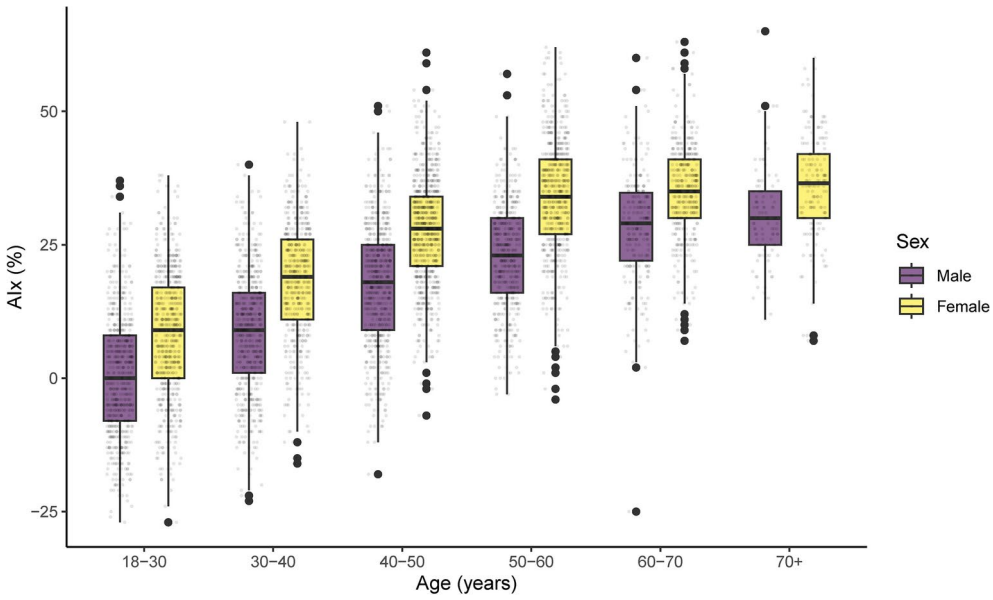


**Fig. 3**. Distribution of AIx according to age in females (n = 2989) and males (n = 2557).

Average AIx increased by age in both sexes, and was significantly higher in females than males in all age categories ($p < 0.001$) (Table 2; Fig. 3). Above the age of 60 years, the curves flattened in both sexes.

Average Pf decreased from first (18–30 years) to second (30–40 years) age category and then increased by age in both sexes with significantly higher values in males than females in 18–30, 30–40 and 40–50 age categories ($p < 0.001$) (Table 2; Fig. 4).

Average Pb increased by age in both sexes, with no plateau in older age, and was significantly higher in females than males in 40–50 and older age categories ($p < 0.01$) (Table 2; Fig. 5).

Since the distribution of cfPWV, AIx, Pf, and Pb differed significantly between males and females across most age categories, separate reference equations were generated for men and women.

## Standardized pulsatile hemodynamics measurements

The LMS fitting procedure used for the transformation of cfPWV, AIx, Pf, and Pb measurements of the reference subjects to z-scores. Generated z-scores for four pulsatile hemodynamic measurements resulted in a normal

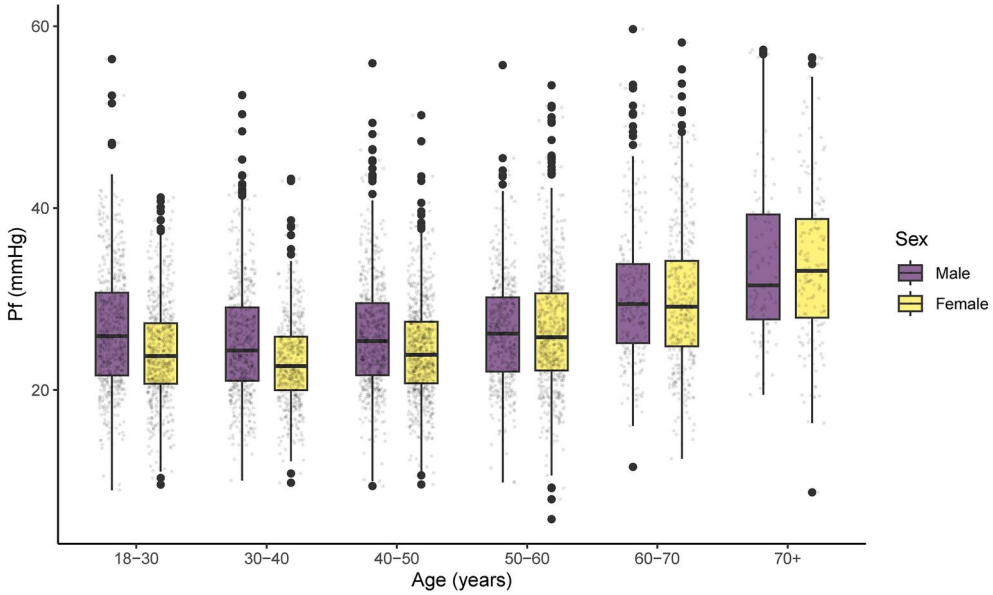


**Fig. 4**. Distribution of Forward wave amplitude (Pf) according to age in females (n = 2973) and males (n = 2536).

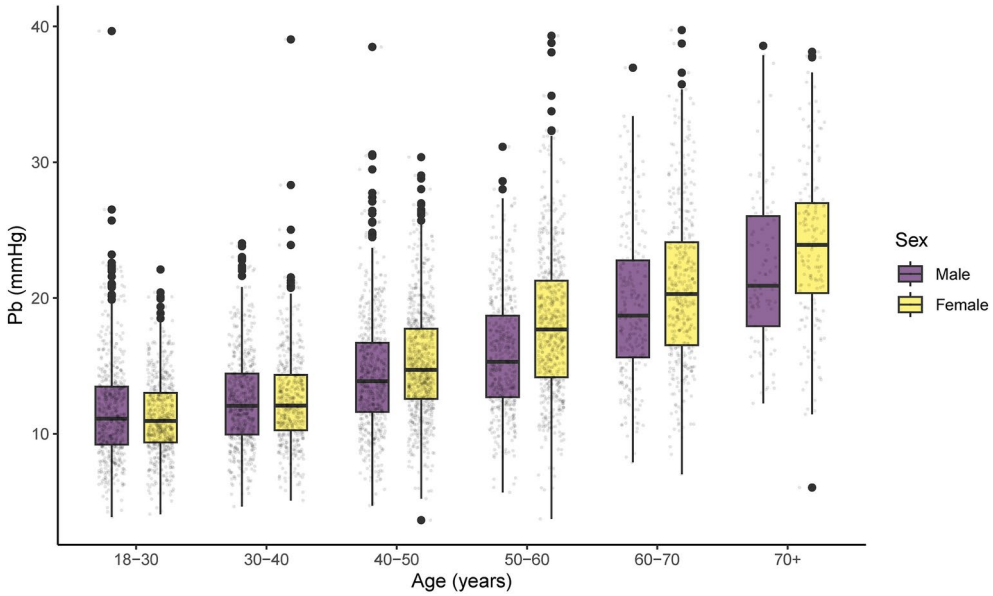


**Fig. 5**. Distribution of Backward wave amplitude (Pb) according to the age in females (n = 2971) and males (n = 2538).

distribution with mean ± standard deviation of 0 ± 1 for all measurements in male and female reference population, indicating effective normalization of the original data. Kolmogorov–Smirnov test confirmed the normality of distribution for all the measurements ($p > 0.05$).

Reference equation for L, M, and S values were determined and age adjusted percentile boundaries were calculated and plotted for each sex category (Table 3 and Fig. 6).

Reference equations and age-adjusted percentile curves for $cfPWV_{\text{subtracted distance}}$ in males and females, are presented in Supplementary Table S2 and Fig. S2 online.

The standardized value (z- score) for each individual's pulsatile hemodynamic measurement (with respect to age and sex) can be calculated using the following formula:

$$Z = \frac{\left[\left(\frac{Y}{M_{Age}}\right)^{L_{Age}}\right] - 1}{S_{Age} * L_{Age}} \quad (1)$$

| Variable | Sex | M (Median) | S (Variability around the Median) | L (Skewness) |
|---|---|---|---|---|
| cfPWV (m/sec) | Female | Exp (1.705 + 0.0073 * Age) | Exp (− 2.140 + 0.0074 * Age) | − 0.7200 |
| | Male | Exp (1.769 + 0.0070 * Age) | Exp (− 2.046 + 0.0058 * Age) | − 0.6307 |
| AIx[1] (%) | Female | Exp (3.976 + 0.2266 * log (Age)) | Exp (− 1.273–0.3239 * log (Age)) | 1.6943 |
| | Male | Exp (3.815 + 0.2485 * log (Age)) | Exp (− 1.025 − 0.3617 * log (Age)) | 1.0469 |
| Pf (mmHg) | Female | Exp (2.980 + 0.00548 * Age) | Exp (− 1.576 + 0.0035 * Age) | 0.3488 |
| | Male | Exp (3.133 + 0.0030 * Age) | Exp (− 1.382–0.0003* Age) | 0.1310 |
| Pb (mmHg) | Female | Exp (1.998 + 0.0157 * Age) | Exp (− 1.371 + 0.0016 * Age) | 0.3678 |
| | Male | Exp (2.078 + 0.01258 * Age) | Exp (− 1.200 − 0.0011* Age) | 0.1720 |

**Table 3**. The age-adjusted equations for predicted values of the median (M), the variability around the median (S) and the skewness (L) for carotid-femoral pulse wave velocity (cfPWV), Augmentation Index (AIx), forward wave amplitude (Pf), and backward wave amplitude (Pb) in females and males, extracted by LMS method. [1]AIx reference equations are extracted after shifting all values + 100 units to ensure all values of AIx are positive. y + 100 must be used instead of y in the formula of LMS for calculating z scores for AIx (Eq. 1).

where Z is the standardized (sex and age adjusted) value of a pulsatile hemodynamic measurement, Y is the individual's pulsatile hemodynamic measurement (exception: Y + 100 for individual's AIX), and L(Age), M(Age), and S(Age) are the specific values of L, M, and S for each sex that can be calculated using the formula presented in the Table 3, considering the individual's age. An application was published in R-shiny to facilitate the calculation of z-scores, for each pulsatile hemodynamic marker by inputting the measured value, age, and sex of the individual. The **link** is available on the LEAD website at the following address: https://leadstudy.at/. Figure 7 presents a snapshot of the dashboard designed for the calculation of z-scores for cfPWV, AIx, Pf, and Pb.

## Discussion

In the current study, we determine reference values for relevant parameters of pulsatile hemodynamics (cfPWV,AIx, Pf, and Pb) in adult individuals in the LEAD study, a longitudinal, observational, population-based Austrian cohort[9]. In addition, we provide age-and sex-specific reference equations for these parameters, allowing easy calculation of z-scores and, thus, quantification of vascular age, at least for Caucasian individuals.

Participants included in the analysis were free from previous cardiovascular disease, non-diabetic, and not taking medications for hypertension and dyslipidemia. Average BP was 126/76 mmHg, and average LDL-cholesterol was 3.2 mmol/L. Thus, the cohort can be considered as apparently healthy. Furthermore, the LEAD-cohort was randomly chosen from the general Austrian population[9], suggesting that the derived reference values should be generalizable, when used to separate healthy from unhealthy individuals, at least until outcome-based thresholds become available.

It is well-known that not only brachial BP, but also measures of pulsatile hemodynamics increase with aging[8]. This is the basis of the increasingly popular concept of vascular aging (VA)[18], where normal or healthy VA is contrasted with premature or early VA. Later on, extremely healthy or supernormal VA was added to the concept[19]. In any case, the classification of VA into normal, early or supernormal is not straightforward[20]. As all possible measures of pulsatile hemodynamics in question as classifiers for VA[21] show age-related changes, a single age-independent threshold value (which still is used to diagnose hypertension in adults, dyslipidemia or diabetes[5]), is unlikely the best option. The derivation of reference values from large population-based samples of apparently healthy individuals, and a classification of VA based on percentiles (commonly age-stratified 90th or 95th percentiles are used) is much more appealing, and already used for the diagnosis of hypertension in children[22]. Our approach has been used for cfPWV in the so-called "Reference Values Project[10]", with a definition for the reference population similar to our work. In a broader sense, it is worth mentioning that the use of z-scores to define thresholds for age-related clinical measurements is common, typical examples are lung function[23] and body composition parameters[24] or bone density.

Carotid-femoral PWV, often seen as the gold-standard measurement for large artery stiffness[3], is an accepted indicator for hypertension-mediated organ damage[5] and an independent predictor of cardiovascular events[2]. Carotid-femoral PWV increases with age in cross-sectional[7,10] and longitudinal[25] studies. Its absolute value depends on the method for assessing the travel distance of the pulse wave[8]. To facilitate comparability, we transformed our cfPWV values, which were initially assessed with the "subtracted" method, to the "direct distance × 0.8" method, which was the main method in the Reference Value Project[10]. It appears that our values are very similar to those from the Reference Values Project, specifically in the high-normal BP range (comparable to our cohort). Interestingly, and similar to the Reference Values Project, we observed higher cfPWVs in men, as compared to women. This is probably related to the (slightly) higher BP in men in both studies.

Wave Separation Analysis, calculated from simultaneous assessment of pressure and flow waves, can be used to derive the Pf and Pb in the arterial system[15]. Both are major determinants of BP and its changes with age[26,27]. Whereas the acquisition of pressure waves is straightforward, the high-quality recording of flow waves is more time consuming. Thus, although large studies have successfully recorded pressure and flow waves for WSA[28,29], methods to replace measured with derived (from circulatory models) flow waves have been developed, validated[12], and used in the setting of large-scale clinical studies[30].

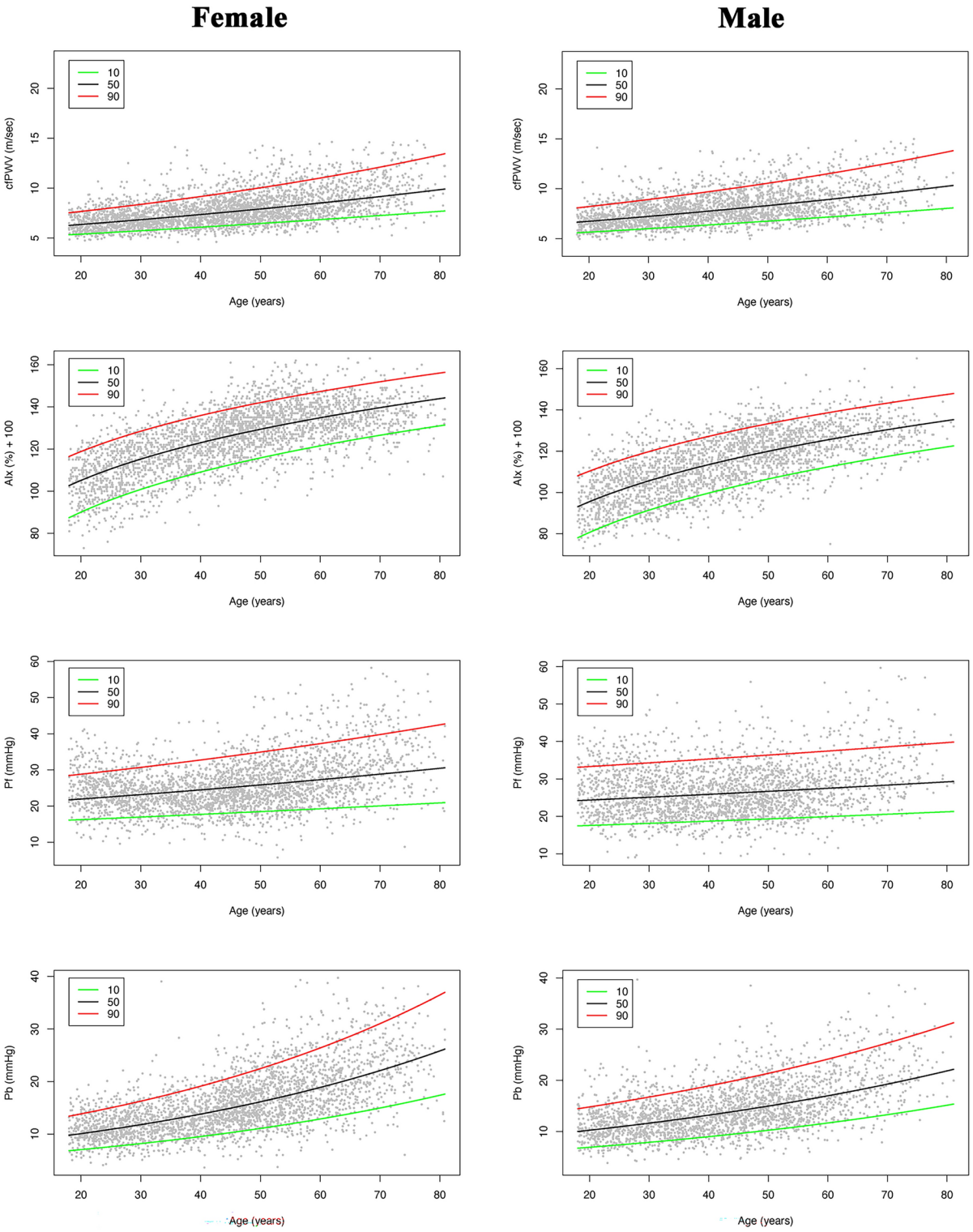


**Fig. 6**. Adjusted-age percentile curves for carotid-femoral pulse wave velocity (cfPWV), Augmentation Index (AIx), forward wave amplitude (Pf), and backward wave amplitude (Pb) in female and male participants in the reference population. Green, blue, and red lines indicate 10th, 50th, and 90th percentile, respectively.

In our cohort, Pf is stable or even slightly decreasing from 18 to 50 years, and increases thereafter. This resembles the results from the Heinz Nixdorf Recall and MultiGeneration study, which have been published earlier[8]. Pf is slightly higher in men than women up to middle age (again in line with findings from a previous study[8]), with women catching up and passing men in the oldest age group. The strong increase in Pf in older age, particularly in women, has been reported in the Framingham study as well[29]. Pf has been independently

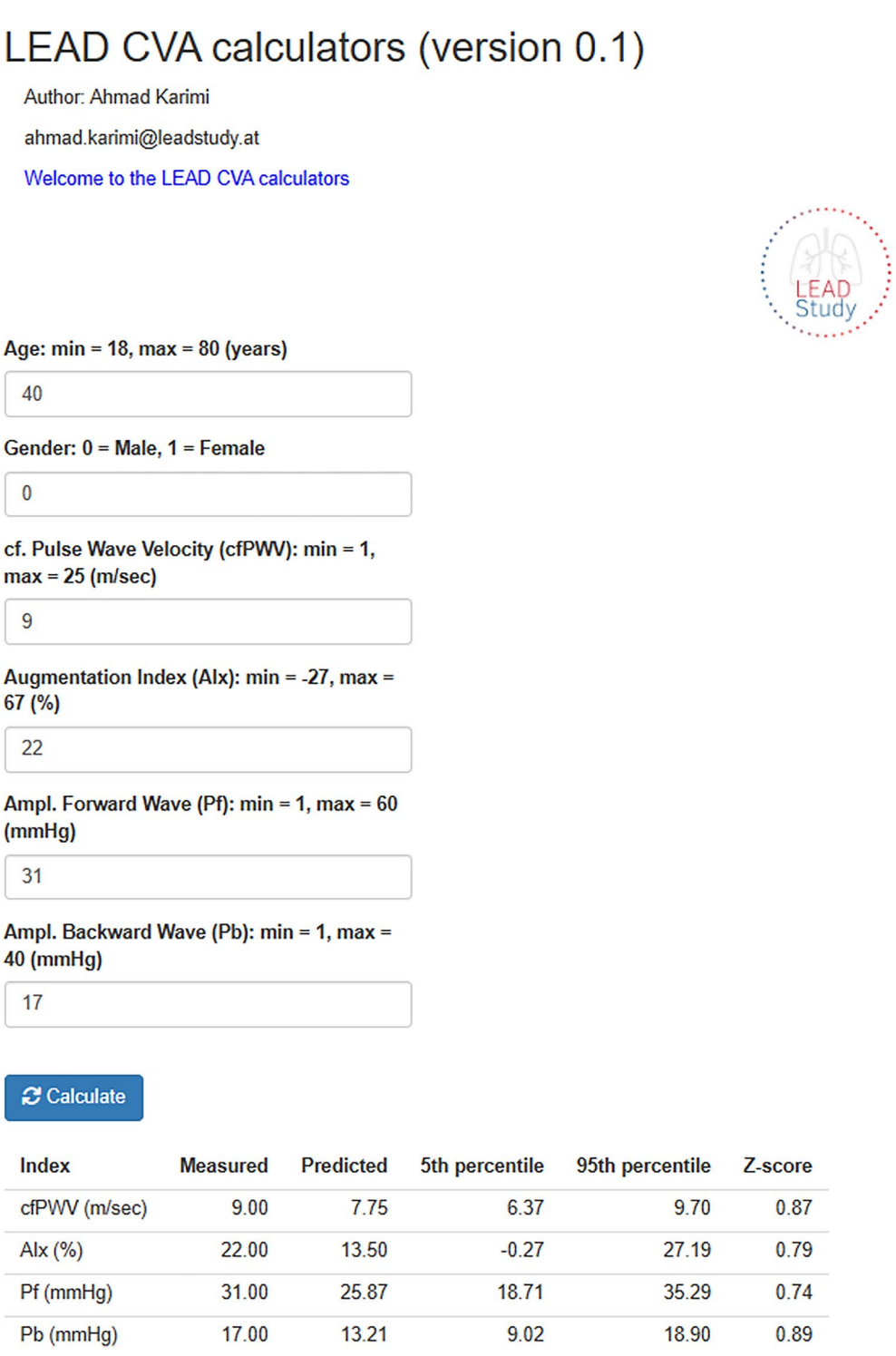


**Fig. 7**. A snapshot of the dashboard designed for the calculation of z-scores for four parameters: carotid-femoral pulse wave velocity (cfPWV), Augmentation Index (AIx), forward wave amplitude (Pf), and backward wave amplitude (Pb). The entered data are hypothetical and pertain to a 40-year-old man, with cfPWV = 9 m/s, AIx = 22%, Pf = 31 mmHg, and Pb = 17 mmHg. The corresponding z-scores can be found in the table below.

associated with cardiovascular events in the large population-based CARTaGENE cohort in Canada[30] and in the Framingham study[28], and with all-cause and cardiovascular mortality in a study from Taiwan[6].

Backward Wave amplitude showed an increase across all age groups, was similar in men and women in younger age groups, and higher in women from 40 years on in our cohort. The unattenuated increase with age is in line with previous results from the MultiGeneration and Heinz Nixdorf Recall studies[8], where the sex-differences were somewhat smaller. In the Framingham study[29], sex-differences were similar to our results up to

60 years of age, with a plateau and then a decrease of Pb only in women thereafter. Smaller sample size and lower BP may have contributed to these differences. Pb has been associated independently with cardiovascular events in the large population-based CARTaGENE cohort[30] in Canada, with all-cause and cardiovascular mortality in the Multi-Ethnic Study of Atherosclerosis (MESA)[31] in the US and in a study from Taiwan[6].

The AIx is probably the most popular waveform parameter. While initially thought to characterize the influence of wave reflection on the central pressure waveform – which is true to some amount -, it has been shown that the AIx is determined by other factors as well, such as heart rate[32], left ventricular systolic function[33], peripheral resistance[33], and sex[7,29]. In our cohort, we observed an increase in AIx up to middle-age and a plateau thereafter in both sexes, and higher values in women throughout the adult lifespan. This is relatively similar to the seminal study in the UK[7], where the plateau occurred later in life, and in slight and partial contrast to data from the Framingham study[29], where the AIx decreased in women above the age of 50. Despite the fact that the AIx is a "composite" measure with several determinants, it has been shown to be an independent predictor of cardiovascular events in several populations, including the MESA study[34], the CARTaGENE cohort[30], patients with resistant hypertension in Brazil[35], and others, which have been recently summarized in a meta-analysis[36].

It needs to be pointed out that the measures of pulsatile hemodynamics investigated here differ not only by age, but also by sex. This clearly implicates the need for age- and sex-specific thresholds, as presented here. One important related clinical scenario is the observation that measures of wave reflection tend to be higher in women, as compared to men. Given the facts that increased (and premature) wave reflections—by increasing late systolic load on the left ventricle—are closely related to diastolic (dys)function[37], and are therefore particularly important for the development of heart failure[38], a major hemodynamic cause, ie increased wave reflections, for the female exhibit predominantly heart failure with preserved ejection fraction[39].

A potential limitation of our study is the subjects in the underlying cohort for development of the ARCSolver models for wave separation were predominantly male, and that this could be a limitation of the model. However, in our analysis of covariates for both Pf and Pb based either on measured or modelled flow, sex was not a significant contributor in any case[13]. Another potential limitation is the cross-sectional design of the present study. Indeed, in longitudinal studies, which provide a true measure of aging, similarities and differences can be seen. The age-associated increase in cfPWV is observed in longitudinal data as well[25]. Moreover, the decrease of Pf in young adulthood in both sexes, and the increase in Pf in older individuals is observed in longitudinal studies as well[25]. In contrast to findings in our cohort and other cross-sectional studies, Pb decreases in longitudinal analysis in both sexes from the age of 35 years onwards. The reason for this difference is currently not well understood, but further longitudinal data, coming from studies like LEAD may shed some light on this issue. Moreover, our findings were obtained in a Middle-European and Caucasian population (though information about ethnicity was not collected, according to Austrian policy for epidemiological studies), and may not be applicable in other regions of the world.

The potential clinical usefulness of the app, ie. the knowledge of a person´s vascular age, has yet to be proven. One may expect a better adherence to antihypertensive and lipid-lowering drugs by patients, but also a way to overcome physician´s inertia when treating hypertension and hyperlipidemia. It has already been shown that ultrasound-based pictorial presentation of subclinical carotid atherosclerosis lowers risk factor burden in asymptomatic people[40], with an effect lasting over at least 3 years[41]. Furthermore, informing patients about their CV risk expressed as "Heart Age" tool resulted in a reduction in their CV risk[42].

## Conclusions

In summary, we present reference equations for relevant measures of pulsatile hemodynamics in a large, population-based cohort, and communicate the findings in an app allowing individual calculation of vascular age. The information may be used for individuals in primary prevention, and in further studies to refine assessment of vascular age and its determinants. The next steps might include longitudinal analyses, when the next study phases get available.

## Data availability

De-identified participant data and may be shared, after publication, on a collaborative basis upon reasonable request made to Prof. Marie-Kathrin Breyer (Marie-Kathrin.Breyer@lunghealth.lbg.ac.at). Requesting researchers will be required to submit an analysis plan.

Received: 27 June 2024; Accepted: 24 September 2024
Published online: 05 October 2024

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## Acknowledgements

The authors thank all participants for their willingness to contribute to medical research, and all field workers for their daily work.

## Author contributions

S.H., O.C.B., and T.W. conceived the study. M.A., and A.K. accessed and verified the data. P.B. and R.M.B. performed quality control for cfPWV. BH and S.W. performed analysis of pulsatile hemodynamics, using ARC-Solver algorithms. A.K. and M.A. were responsible for statistical analysis/interpretation. M.A. and T.W. prepared

the manuscript. All authors provided critical revision of the manuscript, as well as read and approved the final manuscript.

## Funding
The Austrian LEAD Study is supported by Ludwig Boltzmann Gesellschaft, the Municipal Department of Health and Environment of Vienna, the Federal State Governmental Department of Health of Lower Austria, and unrestricted scientific grants from AstraZeneca, Chiesi Farmaceutici, GlaxoSmithKline, and Menarini Pharma. The supporting parties had no participation in data collection or interpretation, nor did they contribute to the design or the content of the manuscript.

## Competing interests
The authors declare no competing interests.

## Ethics approval
The LEAD study was approved by the Ethics committee of Vienna (protocol number: EK-11-117-0711). Written informed consent was obtained from all included participants.

## Additional information
**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1038/s41598-024-74162-5.

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