European Radiology (2019) 29:1507–1517
https://doi.org/10.1007/s00330-018-5705-7

ULTRASOUND

Carotid stiffness and atherosclerotic risk: non-invasive quantification with ultrafast ultrasound pulse wave velocity

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Received: 12 April 2018 / Revised: 25 July 2018 / Accepted: 6 August 2018 / Published online: 5 September 2018

Abstract

Objectives To evaluate the value of ultrafast pulse wave velocity (ufPWV) for the quantitative assessment of carotid stiffness and its associated with atherosclerosis (AS) risk.

Methods The present study included 233 patients with hyperlipoidaemia (AS risk group) and 114 healthy adults as the control group. The carotid (n = 694) intima-media thickness (cIMT), pulse wave velocity-beginning of systole (PWV-BS) and pulse wave velocity-end of systole (PWV-ES) were measured on sample images. Differences, distributive characteristics and correlation evaluation were assessed in patients (ages 18–29, 30–39, 40–49, 50–59, 60–69 and ≥70) and carotids (control group vs AS risk group).

Results The cIMT, PWV-BS and PWV-ES increased with age; PWV-ES and cIMT showed an early significant increase in the 30–39 years group, whereas PWV-BS displayed a significant increase at 40–49 years compared with the 18- to 29-years group. Besides, PWV-ES correlated well with age compared with PWV-BS and cIMT. For carotid level, cIMT, PWV-BS and PWV-ES measurements were higher in the AS risk group compared with control. To compare the value of ufPWV and cIMT in early AS assessment, we subdivided groups into cIMT subgroups using a cut-off thickness of 0.050 cm. PWV-ES measurements were higher in the AS risk group compared with the control in the 0.040–0.050 cm (not thickened) and 0.051–0.060 cm (thickened) cIMT subgroups.

Conclusions Carotid ufPWV measurement at PWV-ES is a novel modality for the early diagnosis and quantitative assessment of arterial stiffness associated with atherosclerotic risk.

Key Points

- *ufPWV technique is real-time and well repeatable for assessing carotid stiffness*
- *ufPWV measurements increase and correlate well with age*
- *PWV-ES is a quantitative predictor for the early assessment of AS*

Keywords Atherosclerosis · Pulse wave velocity · Carotid intima-media thickness · Carotid arteries · Arterial stiffness

Abbreviations

AS	Atherosclerosis
CCA	Common carotid artery
cfPWV	Carotid-femoral pulse wave velocity
cIMT	Carotid intima-media thickness
DBP	Diastolic blood pressure

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00330-018-5705-7) contains supplementary material, which is available to authorized users.

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HDL	High-density lipoprotein
HL	Hyperlipoidaemia
HT	Hypertension
LDL	Low-density lipoprotein
LOAs	Limits of agreement
PWV	Pulse wave velocity
PWV-BS	Pulse wave velocity-beginning of systole
PWV-ES	Pulse wave velocity-end of systole
SBP	Systolic blood pressure
TC	Total cholesterol
TG	Triglyceride
ufPWV	Ultrafast pulse wave velocity

Introduction

Atherosclerosis (AS) is a key pathophysiological alteration in cardiovascular disease that threatens the general population [1]. Timely detection and dynamic evaluation of AS can ensure efficient early prevention and greatly affect management and prognosis of patients with and without clinical cardiovascular events [1]. Carotid intima-media thickness (cIMT) is a non-invasive ultrasound biomarker of early AS. A positive association exists between cIMT and the risk of subsequent cardiovascular events, independent of all other major risk factors [1]. This correlation has augmented the utility of cIMT in animal studies and clinical trials, in which the role of cIMT has shifted from an imaging indicator to a surrogate of risk for AS and cardiovascular events [2]. Many studies have used cIMT progression to calculate absolute yearly rate of progression and tested the effects of interventions on AS risk [3–5]. However, recent systematic investigation of a large-scale (n = 36,984) study showed that the association between cIMT progression assessed from ultrasonography and cardiovascular risk in the general population remained unproven [6]. Moreover, AS is a lifelong process and could accelerate with accumulation of risk factors [7]. The cIMT positively and individually correlates with age [1]. Thus, in view of the large variability of cIMT values, it is not regarded as a promising tool for evaluating AS.

Pulse wave velocity (PWV) technique is a novel modality for the non-invasive evaluation of aortic stiffness and has been shown to correlate well with atherosclerotic risk and cardiovascular mortality [8, 9]. European guidelines (2013) considered PWV as the "gold standard" for measuring arterial stiffness, which has greatly affected the hypertension (HT) management [10, 11]. However, the conventional PWV methods, such as carotid-femoral PWV (cfPWV), rely on the measurement of the transit time of the pulse wave between carotid and femoral arteries. The accuracy of cfPWV is limited by errors made at this distance [12, 13] and the timing assessment [14], even though efforts at standardisation have been made [15, 16]. This context has fostered the development of new technologies to accurately evaluate arterial stiffness [17].

Ultrafast pulse wave velocity (ufPWV) has recently emerged as a unique PWV technique, enabling visualisation of carotid stiffness with a good repeatability [18, 19]. The ufPWV sampling rate is over 2,000 frames/s and allows tracking of the pulse wave in real time, thereby avoiding the major drawbacks of all previous PWV techniques [19]. Although ufPWV is a currently used modality for the evaluation of age-induced increases of arterial stiffening in healthy adults [20], its role in AS assessment is still unknown.

Therefore, the aim of the present study was to evaluate the role of ufPWV for early detection and quantitative assessment of AS in clinical and ultrasonography findings.

Materials and methods

Our study was approved by the ethics committee of the Affiliated Hospital of Nanjing University of Chinese Medicine, and the methods were carried out in accordance with the relevant guidelines. Written informed consent was obtained from all patients.

Patients

Two prospective patient cohorts were used in our study. The first cohort was included to determine intra-operator reproducibility (n = 10) of ufPWV relative to the measurement variability and inter-operator reproducibility (30 patients, with 60 carotids) between two ufPWV operators (H.H. and Y.L., from February 2015 to April 2015). We included 40 consecutive patients that were scheduled to undergo a conventional carotid ultrasound and ufPWV examination.

A second cohort was included to determine the value of ufPWV for the quantification of carotid stiffness and AS. Between May 2015 and June 2017, 347 individuals with 694 carotids were enrolled in this study. Among these, 233 were newly diagnosed with hyperlipoidaemia (HL) (AS risk group, Table 1) and 114 were healthy adults with no previous history of HL or HT, and had normal examination findings, blood pressure and no current smoking habit (control group, Table 1). In the present study, HL was defined by elevated serum low-density lipoprotein (LDL) levels (≥ 3.37 mmol/L), and/or total cholesterol (TC) levels (≥ 5.18 mmol/L), and/or triglyceride (TG) levels (≥ 1.70 mmol/L) according to the guidelines [21]. The exclusion criteria (both for control and AS groups) was as follows: (1) abnormal haemoglobin findings; (2) previous history of stroke or cardiovascular events within the last 6 months; (3) cancer, diabetes, thyroid dysfunction, chronic liver and

Table 1 Summary of clinical characteristics

	Control	AS risk	Total	*p* value
No. of patients	114	233	347	—
No. of carotids	228	466	694	—
Age (years)				0.068
Average	46.5 ± 13.1	49.4 ± 14.2	48.4 ± 13.8	
Range	23-79	18-86		
Sex				0.994
Male	66	135	201	
Female	48	98	146	
Weight (kg)				<0.001
Average	59.1 ± 7.5	66.2 ± 11.0	63.9 ± 9.9	
Range	43-87	43-108.5		
BMI (kg/m^2)				<0.001
Average	21.6 ± 2.0	23.9 ± 3.1	23.1 ± 2.7	
Range	16.9-25.7	17.8-37.1		
Current smoking, *n* (%)	0 (0)	26 (11.2)	26 (7.5)	<0.001
Laboratory findings				
C-reactive protein, mg/L	1.03 ± 1.25	1.22 ± 1.54	1.16 ± 1.44	0.253
Haemoglobin, g/L	141.4 ± 8.0	140.1 ± 7.3	140.5 ± 7.5	0.132
Fasting blood glucose, mmol/L	4.82 ± 0.55	4.91 ± 0.62	4.88 ± 0.60	0.189
Serum creatinine, μmol/L	66.5 ± 13.3	68.8 ± 16.5	68.0 ± 15.4	0.196
TC, mmol/L	4.53 ± 0.61	4.83 ± 1.15	4.66 ± 0.97	0.009
LDL-cholesterol, mmol/L	2.41 ± 0.48	2.63 ± 0.81	2.56 ± 0.70	0.008
HDL-cholesterol, mmol/L	1.53 ± 0.31	1.34 ± 0.42	1.40 ± 0.42	<0.001
TG, mmol/L	0.85 ± 0.47	1.46 ± 0.91	1.26 ± 0.77	<0.001
Blood pressure, mmHg				
SBP	117.3 ± 14.3	128.3 ± 18.2	124.7 ± 16.9	<0.001
DBP	71.3 ± 9.9	73.3 ± 11.2	72.6 ± 10.8	0.106
ΔP	46.0 ± 10.3	55.0 ± 14.2	52.0 ± 12.9	<0.001

Data are shown as mean value ± standard deviation. Numbers in parentheses are percentages

TC total cholesterol, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *TG* triglyceride, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *ΔP* = SBP - DBP

kidney disease or autoimmunity diseases; (4) could not undergo the ultrasonography examination of both sides of the carotid, such as torticollis, or failure to lay down, such as heart failure; (5) failure to obtain a stable ufPWV-gram or successful measurement. Laboratory tests were performed to obtain C-reactive protein, haemoglobin, fasting blood glucose, TC, TG, high-density lipoprotein (HDL)-cholesterol, LDL, and serum creatinine. All individuals (n = 347) underwent conventional ultrasonography and ufPWV on both sides of the carotid (n = 694), which was performed by two experienced operators separately (one single operator for one subject, H.H. and Y.L., 191 of 347 vs 156 of 347, both with 10 years of ultrasound experience). Then the ufPWV data were independently reviewed frame by frame on the scanner by two additional experienced investigators (final results together with consensus), blinded to the patient groups and clinical findings.

Conventional ultrasonography and cIMT measurement

Each patient was evaluated using ultrasonography at baseline according to the standard carotid ultrasound examination protocol, which was performed with a 2- to 10-MHz linear array transducer SL10-2 (Aixplorer; Supersonic Imagine, Aix-en-Provence, France). Normal grey-scale imaging was performed with a probe frequency of 4–8 MHz and a dynamic range of 55 dB. All carotid cIMT acquisitions were performed using a width ≥ 1 cm box (Fig. 1a and b), placed at the longitudinal plane and posterior wall of the common carotid artery (CCA), avoiding the atherosclerotic plaque area. To avoid user-dependency, a major limitation in cIMT measurement, we used the Aixplorer cIMT measure system (Fig. 1a and b), which automatically tracked and recorded the

intima and media line of the CCA with two dotted lines, and calculated the mean cIMT of the region. The length of the successfully recorded region divided by the width of the observed box formed a coincidence ratio named "Fit" (Fig. 1a and b). In our study, Fit ≥ 70% was considered a valid cIMT measurement. Three successful evaluations were recorded. The median value of the cIMT measurements was used for carotid-level analysis, and the mean cIMT value of the two sides of the CCA was used for patient-level analysis.

ufPWV procedure

An Aixplorer ultrasound ufPWV system (Supersonic Imagine) with a linear array probe (SL10-2) was utilised for the present study. All ufPWV acquisitions were performed at the longitudinal plane of the CCA [17, 22], avoiding the area of atherosclerotic plaque (Fig. 1c and d). Patients were instructed to hold their breath for 5 s while the ufPWV measurement was being performed. After a stable ufPWV-gram formed, the region of interest (ROI) box (3.0 × 3.0 cm) was moved to cover the anterior and posterior walls of the CCA using two red lines that automatically tracked and recorded the measurements (Fig. 1c and d). The mean PWV-beginning of systole (PWV-BS) and the PWV-end of systole (PWV-ES) in the ROIs were calculated. Measurement variance at the PWV-BS or the PWV-ES in the ROIs was recorded and named as "Δ±" (Fig. 1c and d). In the present study, all Δ± ≤ 1.0 m/s was considered a successful ufPWV measurement.

Three successful ufPWV measurements from both sides of the CCA were recorded, and the median value of PWV-BS and PWV-ES of each side was used for carotid analysis and the mean value of the two sides was

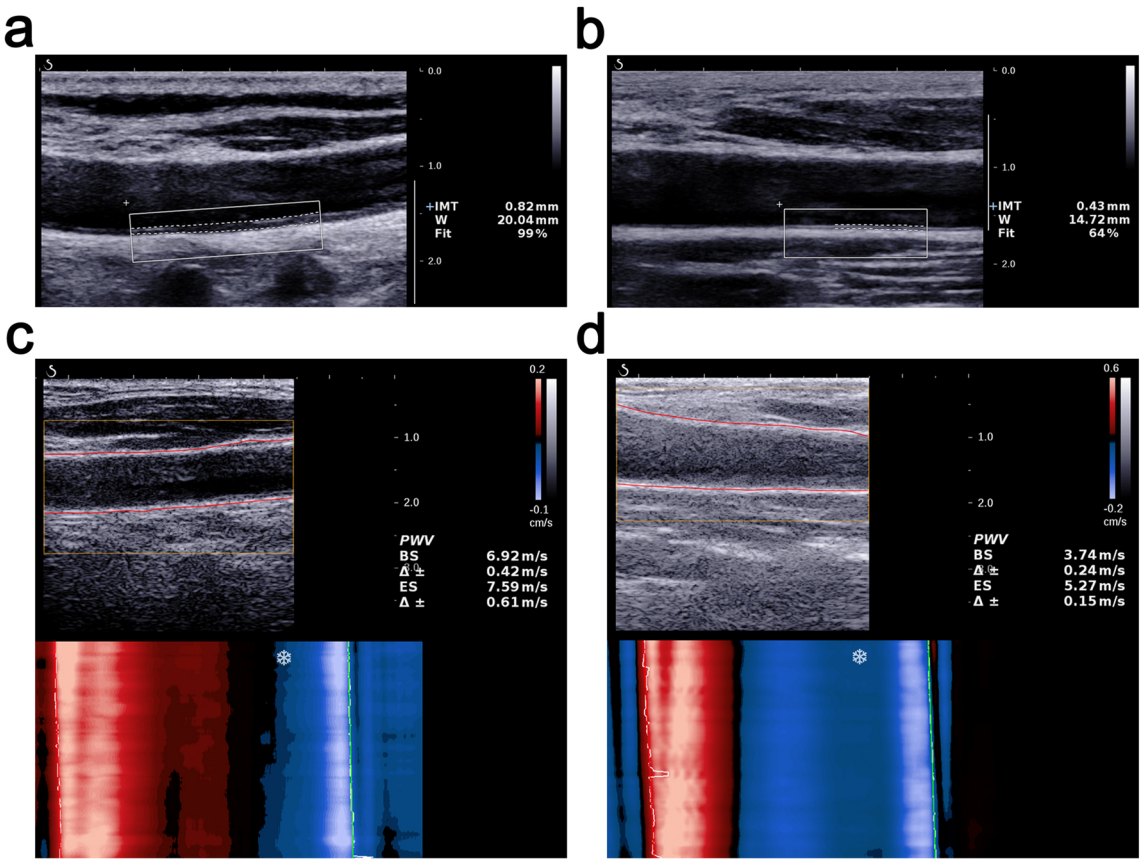

Fig. 1 Representative images showing carotid intima-media thickness (cIMT) and ultrafast pulse wave velocity (ufPWV) measurements in an region of interest (ROI). To obtain the cIMT measurements, a *white box* was drawn around the ROI at the posterior wall of the common carotid artery (CCA); the intima and media line of the CCA was recorded with *two dotted lines*, and the mean cIMT of the ROI was obtained. (**a**) Measurement of cIMT with Fit of 99% and (**c**) ufPWV mapping of a carotid in a 65-year-old patient. (**b**) Measurement of cIMT measurement with Fit of 64% (< 70%) and (**d**) ufPWV mapping of a carotid in a 28-year-old patient. For ufPWV measurements, the *yellow box* indicates the ROI of the CCA, and the *red line* shows the automatically tracking of the anterior and posterior wall of the CCA. The mean pulse wave velocity-beginning of systole (PWV-BS) and pulse wave velocity-end of systole (PWV-ES) were calculated in the ROI. Abscissa in the colour graphs below the images represents time (s), and vertical axis represents the anterior CCA wall (mm); *red* shows movements toward the probe and *blue* shows movements away from the probe. *Colour brightness* (red or blue) signifies the speed of the movements. The brightest line indicates the fastest pulse wave. Pulse wave velocity (PWV) was calculated as follows: ufPWV (m/s) =Δlength (m)/Δtime (s)

used for patient analysis. The entire examination was saved in a Digital Imaging and Communications in Medicine format and transmitted to a workstation for further analysis.

Statistical analysis

All statistical analyses were carried out using SPSS statistics version 18 software (IBM). In the first cohort, intra-operator reproducibility of ufPWV was performed by repeated-measures analysis of variance. Mauchly sphericity test was performed, and if the sphericity index could be assumed, the Greenhouse-Geisser corrected p value was reported. The PWV-BS and PWV-ES measurements were obtained three times for each of the ten patients in the first cohort. Bland-Altman analysis [23] was performed to test the inter-operator reproducibility of ufPWV. In the second cohort, classifiable variables were compared using the chi-squared test. Continuous variables were compared using Student's t-test. Histograms and line charts were performed to compare cIMT or ufPWV between groups. For all analyses, two tailed p values <0.05 were considered statistically significant.

Results

Clinical characteristics

For the first cohort of 40 patients used to measure intra- and inter-operator reproducibility, the median patient age was 40.5 years old (range, 23–68 years old). For the second cohort, the clinical characteristics of 347 individuals with 694 carotids are listed in Table 1. Among these, 114 were healthy adults (control group) and 233 were HL patients (AS risk group). AS disease prevalence is based on pathological changes and arterial calcification, which is evident in 10–30% of individuals aged 20–29 years old, and by age 65, 80–97% of the population shows evidence of arterial calcification [24, 25]. The case/total ratio (233/347 = 67.1%) in the second cohort was very close to the average prevalence above (up to 63.5%). There was no significant difference between control and AS risk groups with regard to age, sex or C-reactive protein, haemoglobin, fasting blood glucose, serum creatinine levels or diastolic blood pressure (DBP; all $p > 0.05$). The AS risk group had a significantly higher weight, BMI, smoking prevalence, TG, systolic blood pressure (SBP), ΔP (ΔP = SBP - DBP) (all $p < 0.001$), LDL ($p = 0.008$) and TC ($p = 0.009$) compared with the control group. In addition, a significantly lower HDL level was observed in the AS risk group compared with the control group ($p < 0.001$).

ufPWV technique validation

In the first cohort, both PWV-BS and PWV-ES differed significantly between patients relative to measurement variability, as assessed by repeated-measures analysis of variance (all $p < 0.001$, Mauchly sphericity test; $p = 0.223$ for PWV-BS; $p = 0.454$ for PWV-ES; no correction was need) (Fig. 2a and b). The inter-patient variation of PWV-BS (3.44–9.17 m/s) and PWV-ES (4.00–13.45 m/s) cannot be solely interpreted by measurement variability or instability (average measurement, 0.716 ± 0.293 m/s for PWV-BS, 0.787 ± 0.448 m/s for PWV-ES). Moreover, both PWV-BS and PWV-ES were similar between repeated measures ($p = 0.936$ for PWV-BS, $p = 0.897$ for PWV-ES, no correction was need) (Fig. 2a and b). Bland-Altman analysis showed a mean difference of -0.359 m/s for PWV-BS and -0.256 m/s for PWV-ES inter-operator comparison, with a 95% limits of agreement (LOAs) of -2.309, 1.570 m/s and -2.135, 1.622 m/s, respectively (Fig. 2c and d). Inter-operator reproducibility for PWV-BS and PWV-ES measurements showed no significant bias (Fig. 2c and d). These results demonstrated that ufPWV measures had satisfactory intra- and inter-operator reproducibility.

In the second cohort, different sides of the carotid artery demonstrated no significant difference in cIMT and PWV-ES (all $p > 0.05$ compared in control, AS risk and total analysis) (Fig. 2e and g), but not in PWV-BS (all $p < 0.05$) (Fig. 2f). This illustrated that further data analysis should be performed at the patient and carotid levels respectively.

ufPWV and cIMT in patients

The results of ufPWV and cIMT measurements between the control and AS risk groups per patient and per carotid are shown in Table 2. AS risk group showed increased cIMT and PWV-ES (all $p < 0.001$) measurements at the patient level ($n = 347$) compared with control (Table 2). AS risk group had significantly higher cIMT ($p < 0.001$), PWV-BS ($p = 0.025$) and PWV-ES ($p < 0.001$) measurements compared with the control group at the carotid level ($n = 694$).

Age is a strong independent risk factor for AS. HDL and ΔP levels were significantly different in patient's aged 50–59 years old compared with 18- to 29-year-old adults (both $p < 0.05$; Fig. 3b and e). LDL, TG, and TC levels showed a notable increase early in 30- to 39-year-old patients compared with 18- to 29-year-old adults (all $p < 0.05$; Fig. 3a, c and d). Moreover, cIMT, PWV-BS, and PWV-ES all progressively increased with age. PWV-ES levels significantly increased earlier in 30- to 39-year-old patients ($p < 0.001$) compared with cIMT and PWV-BS levels, which increased in the 40- to 49-year-old patient group ($p = 0.003$ and $p = 0.009$; Fig. 3f, g and h). Besides, PWV-ES significantly correlated with age ($r = 0.710$, $p < 0.001$) compared with PWV-BS ($r = 0.304$, $p < 0.001$) and cIMT ($r = 0.594$, $p < 0.001$) (Fig. 4a, b and c).

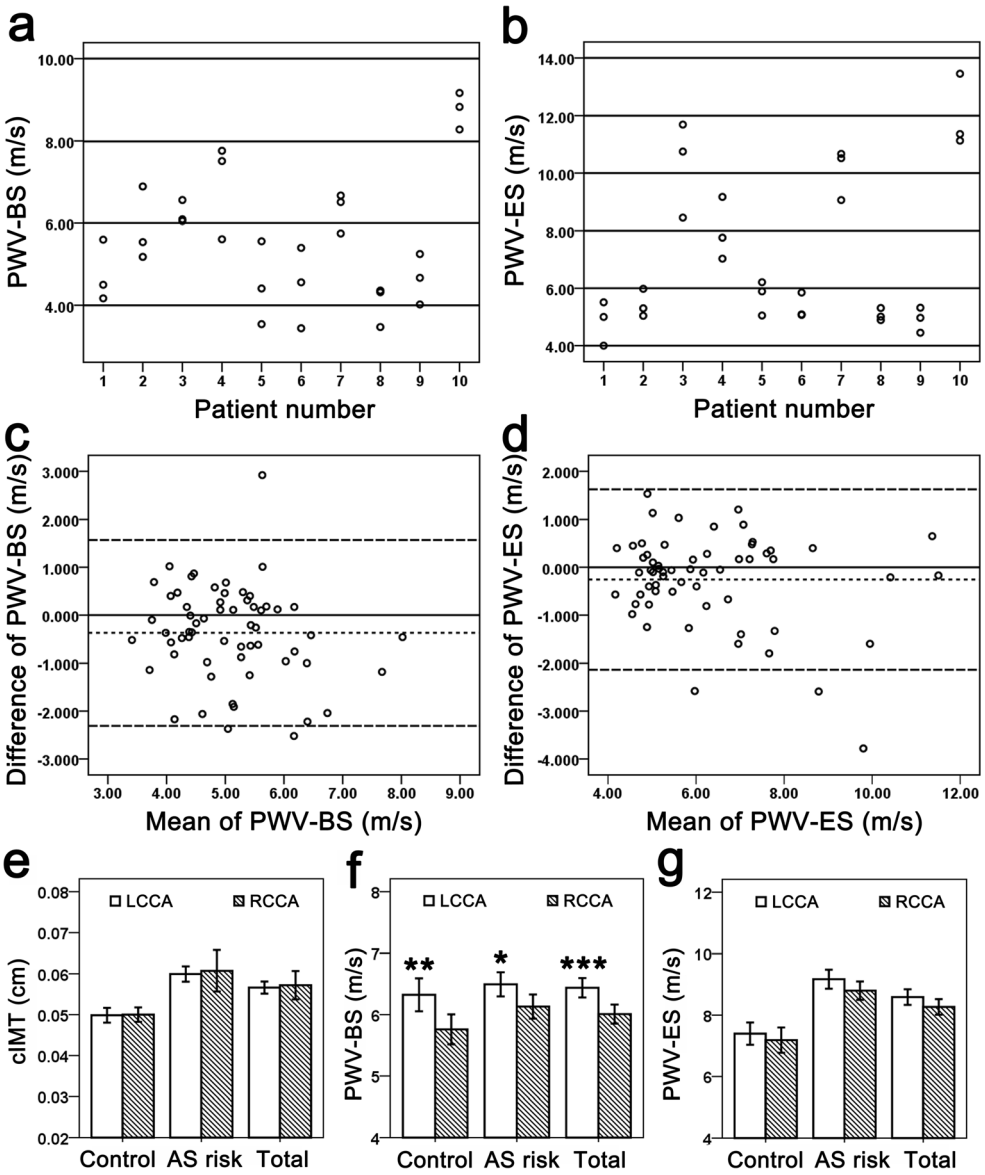

Fig. 2 Technique validations of ufPWV. **a**-**d** Intra-operator and inter-operator reproducibility of ufPWV measurements by repeated-measures analysis of variance and Bland-Altman plotting in the first patient cohort. Shown are triplicate measurements of (**a**) PWV-BS and (**b**) PWV-ES in ten patients. The *black circles* represent the individual measurements, the vertical axis shows the PWV-BS and PWV-ES measurements, and the horizontal axis shows the patient number. Inter-operator reproducibility (n = 30 patients with 60 carotids) of (**c**) PWV-BS (bias: -0.359 m/s; 95% limits of agreement (LOAs): -2.309, 1.570 m/s) and (**d**) PWV-ES (bias: -0.256 m/s; 95% LOAs: -2.135, 1.622 m/s). (**e**-**g**) cIMT and ufPWV measurements in the left and right sides of the carotid arteries in the second patient cohort. Shown are comparison measurements (n = 347 patients with 694 carotids) of (**e**) cIMT, (**f**) PWV-BS and (**g**) PWV-ES between left common carotid artery (LCCA) and right common carotid artery (RCCA) in control, AS risk and total analysis, respectively. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

In our study, 27% (63/233) of individuals in the AS risk group showed HL plus HT and 73% (170/233) showed HL without HT. To assess the role of HT involved in AS patients with HL, we further subdivided the AS risk group into two subgroups (HL and HL + HT, n = 170 and 63) compared with the control group. We detected no significant difference in SBP and DBP in the HL group compared with the control group (all $p > 0.05$); however, the HL + HT group showed a notably higher SBP and DBP than the control and HL groups (all $p < 0.001$) (Fig. 5). We observed no difference in PWV-BS between subgroups (all $p > 0.05$), whereas PWV-ES demonstrated significant differences between each subgroup (all $p < 0.001$) (Fig. 5d and e). In addition, we found that cIMT was significantly higher in the HL and HL + HT groups than in the control group (all $p < 0.001$) (Fig. 5c).

Distribution analysis of cIMT

To avoid the major basis of user-dependency, cIMT measurement in our study was carried out by automatically tracking, recording and calculation of fixed-size ROIs with the Aixplorer cIMT system, not by measuring with conventional manual ultrasound. Therefore, the conventional cIMT cut-off is not suited to this study. Currently, no recognised cut-off has been reported for this novel cIMT measurement system. To evaluate a reliable cut-off for classifying whether a cIMT measurement is "thickened" or "not thickened" in further carotid analysis, we did a distribution analysis of the pooled cIMT measurements (n = 694). In the 694 carotids measured, the value of cIMT ranged from 0.043 to 0.620 cm (Table 2). The highest cIMT measurement and proportion was 0.043 cm, with 37 (37/466, 7.9%) carotids in the AS risk group and 63

Table 2 Measurements of cIMT, PWV-BS and PWV-ES per patient and per carotid

Performance level	Values	Control[a]	AS risk[b]	Total	*p* value
Per patient[c]	cIMT (cm)	0.0499 ± 0.0088	0.0603 ± 0.0238	0.0569 ± 0.0208	<0.001
	Range	0.043 – 0.084	0.043 – 0.370	0.043 – 0.370	
	PWV-BS (m/s)	6.042 ± 1.153	6.313 ± 1.261	6.224 ± 1.231	0.054
	Range	3.37 – 9.78	3.50 – 10.44	3.37 – 10.44	
	PWV-ES (m/s)	7.292 ± 1.861	8.985 ± 2.043	8.429 ± 2.136	<0.001
	Range	3.84 – 12.65	4.06 – 14.23	3.84 – 14.23	
Per carotid	cIMT (cm)	0.0499 ± 0.0096	0.0603 ± 0.0294	0.0569 ± 0.0252	<0.001
	Range	0.043 – 0.097	0.043 – 0.620	0.043 – 0.620	
	PWV-BS (m/s)	6.042 ± 1.409	6.313 ± 1.536	6.224 ± 1.500	0.025
	Range	3.12 – 10.26	3.14 – 11.10	3.12 – 11.10	
	PWV-ES (m/s)	7.292 ± 2.092	8.985 ± 2.362	8.429 ± 2.410	<0.001
	Range	3.12 – 13.52	3.15 – 14.95	3.12 – 14.95	

Data are shown as mean value ± standard deviation

cIMT carotid intima-media thickness, *PWV-BS* pulse wave velocity-beginning of systole, *PWV-ES* pulse wave velocity-end of systole

[a] For patient level $n = 114$; for carotid level $n = 228$

[b] For patient level $n = 233$; for carotid level $n = 466$

[c] Patient values are the mean value of the left and right side of the carotid

(63/228, 27.6%) carotids in the control group (Fig. 6a). Moreover, distribution analysis showed that carotid numbers and percentages all gradually diminished from 0.043 cm to 0.620 cm in both control and AS risk groups (Fig. 6a). No significant boundary was observed between the control and AS risk groups in the distribution analysis of carotid numbers. Interestingly, a larger proportion of the carotids in the control group decreased at cIMT 0.051 cm and the proportion remained consistent from 0.051 cm to 0.620 cm compared with the AS risk group (Fig. 6a). Therefore, our results determined that a range of 0.043 – 0.050 cm cIMT was considered normal and, consequently, ≥ 0.051 cm was considered thickened for further carotid analysis.

ufPWV and cIMT in carotids

A thickened cIMT measurement reflects an AS characteristic of an artery and is correlated with the stiffness of the carotid. With the defined cut-off of ≥ 0.050 cm, we subdivided the patients using cIMT measurements into five subgroups as follows: 0.043–0.050 cm ($n = 306$, 44.1%, cIMT subgroup A, control $n = 162$, AS risk $n = 144$), 0.051–0.060 cm ($n = 182$, 26.2%, cIMT subgroup B, control $n = 36$, AS risk $n = 146$), 0.061–0.070 cm ($n = 107$, 15.4%, cIMT subgroup C, control $n = 19$, AS risk $n = 88$), 0.071–0.080 cm ($n = 58$, 8.4%, cIMT subgroup D, control $n = 6$, AS risk $n = 52$) and ≥ 0.081 cm ($n = 41$, 5.9%, cIMT subgroup E, control $n = 5$, AS risk $n = 36$). PWV-ES levels were significantly different between subgroups A and B (both $p < 0.05$), but no significant difference was observed between subgroups C, D, and E (all $p > 0.05$; Fig. 6b). No significant difference in PWV-BS levels was observed in the cIMT control and AS risk subgroups (all $p > 0.05$; Fig. 6c). However, AS risk individuals showed remarkably higher PWV-ES levels compared with healthy control adults in cIMT subgroups A and B (all $p < 0.001$), but not in C, D and E (all $p > 0.05$; Fig. 6d).

Discussion

Assessment of cIMT is a useful and investigational method for AS risk reclassification in clinical practice [26]. A yearly progression of cIMT levels has shown a significant and positive association with risk of stroke [27] and cardiovascular events [28, 29]. Our study revealed that, compared with healthy adults, AS risk individuals displayed thickened cIMT in relation to both patient age and carotid analysis (Table 2). In addition, cIMT correlated with age, similar to arterial stiffness and ufPWV (Fig. 4). Nevertheless, cIMT alterations seem to lag behind ageing and PWV-ES measurements using ufPWV, especially at a younger age (Fig. 3). Using a cut-off of 0.050 cm, 28.9% (66/228) of healthy individuals showed a thickened cIMT and 30.9% of (144/466) of AS risk patients showed a non-thickened cIMT. Moreover, the progression of a thickened cIMT measurement has recently been proven not to indicate cardiovascular risk in a large-scale study ($n = 36{,}984$) [6]. Repeated thickened cIMT measurements may be a reliable way to qualify AS, but insufficient to quantify AS.

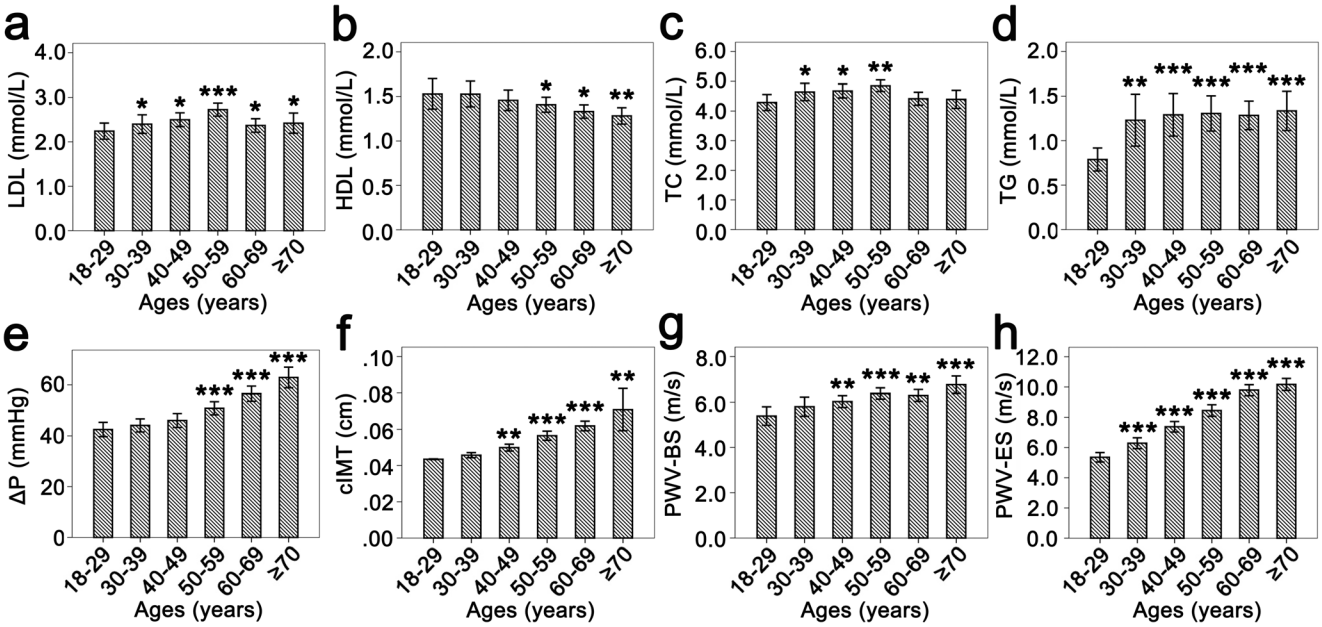

Fig. 3 Box and whisker plot (n = 347) shows alternation of (**a**) low-density lipoprotein (LDL), (**b**) high-density lipoprotein (HDL), (**c**) total cholesterol (TC), (**d**) triglyceride (TG), (**e**) ΔP (ΔP = systolic blood pressure (SBP) - diastolic blood pressure (DBP)), (**f**) cIMT, (**g**) PWV-BS and (**h**) PWV-ES with ageing (*p < 0.05, **p < 0.01, ***p < 0.001; all compared with the 18- to 29-year-old group)

Arterial stiffness is an independent predictor of AS risk and cardiovascular events [30, 31], which can be estimated by measuring PWV, a widely used tool in epidemiological and clinical studies [32]. Previous studies of cfPWV, a well-known PWV technique that relies on complex equations and measurements, showed an increase in arterial stiffness and a decrease in carotid distensibility with increased age [33, 34]. Recently, ufPWV, which is a novel, non-invasive and real-time PWV technique, has been reported to be accurate and reproducible in the assessment of arterial stiffness in mice [35] and humans [20, 22]. In our study, carotid artery stiffness determined by enhanced ufPWV values indicated that AS risk (Table 2) and stiffness increased with age (Fig. 4b and c). The ufPWV values showed a strong correlation between age and arterial stiffness, consistent with previous ufPWV studies, such as by Tristan et al [20] (our PWV-ES, r = 0.710 vs Tristan's PWV-ES, r = 0.682), which was higher than cfPWV values (cfPWV, r = 0.609). We further identified that PWV-ES is a stronger predictor of artery stiffness compared with PWV-BS (r = 0.304); this finding is consistent with a previous study (PWV-BS, r = 0.476) [20]. There could be two reasons [36, 37]: (1) regional PWV-ES can be more accurately estimated than PWV-BS in a technical aspect; (2) PWV-ES represents the arterial stiffness at the systole phase, which is more effective and sensitive to changes in arterial stiffness due to age and other relative diseases.

Artery stiffening is a lifelong process that progresses slowly at a young age and may accelerate with the accumulation of risk factors [7]. In our study, increased PWV-ES levels seemed to continuously synchronise with ageing and the acceleration of HL, even at a young age (Fig. 3). LDL, TG, and TC levels, and also the PWV-ES levels, showed a notable increase early in 30- to 39-year-old patients (Fig. 3). Currently, high LDL, TG, or TC levels are used to characterise high AS risk. The accumulation of risk due to increase in arterial stiffness over weeks and perhaps months, is still unknown but, as our findings show, it may be assessed by PWV-ES measurement. A recent study has shown that HT increases ufPWV values, which is well correlated with the cIMT of the HT patient [38]. Our results demonstrated that the increased PWV-ESmeasurements observed in the AS risk group compared

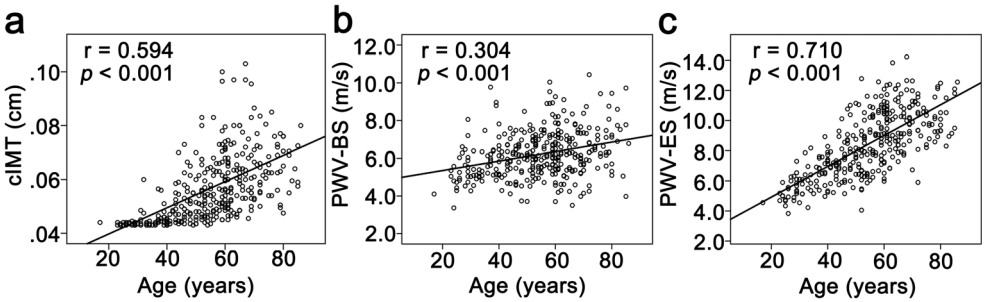

Fig. 4 Scatter plots show correlation between age and cIMT (**a**, n = 346, r = 0.594, p < 0.001), PWV-BS (**b**, n = 347, r = 0.304, p < 0.001) and PWV-ES (**c**, n = 347, r = 0.710, p < 0.001). One extreme value of cIMT (0.370 cm in the AS risk group) had been excluded in (**a**)

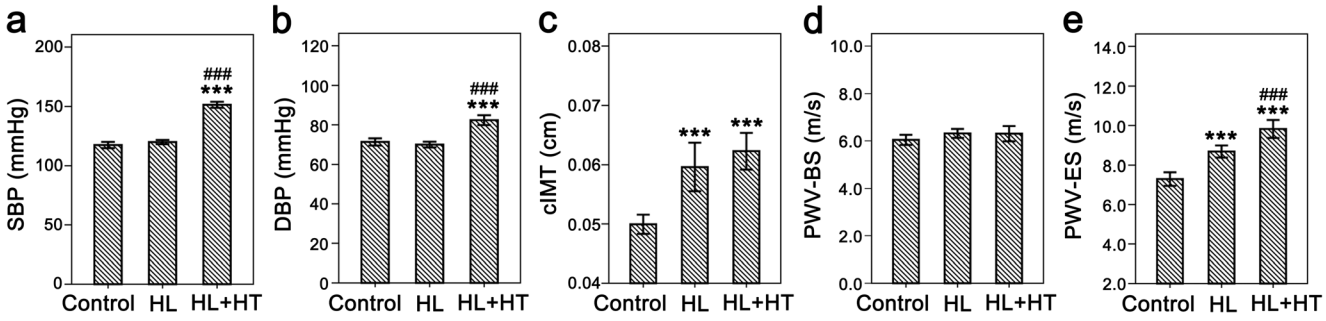

Fig. 5 Box and whisker plot ($n = 347$) showing alternation of (**a**) SBP, (**b**) DBP, (**c**) cIMT, (**d**) PWV-BS, (**e**) PWV-ES between control ($n = 114$), hyperlipoidaemia (HL) ($n = 170$) and HL + hypertension (HT) ($n = 63$) groups ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$: all compared with control; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$: HL + HT compared with HL group)

with the control group could not be solely due to high blood pressure. Without HT, HL can still cause increased ufPWV, which can be strengthened by the addition of HT (Fig. 5). AS is often enhanced by risk factors, and the accumulation of those factors increase the difference of arterial stiffness between healthy and AS individuals. These results suggest that ufPWV, a dynamic and real-time technique, could be an optimal quantitative modality for assessing arterial stiffness and AS.

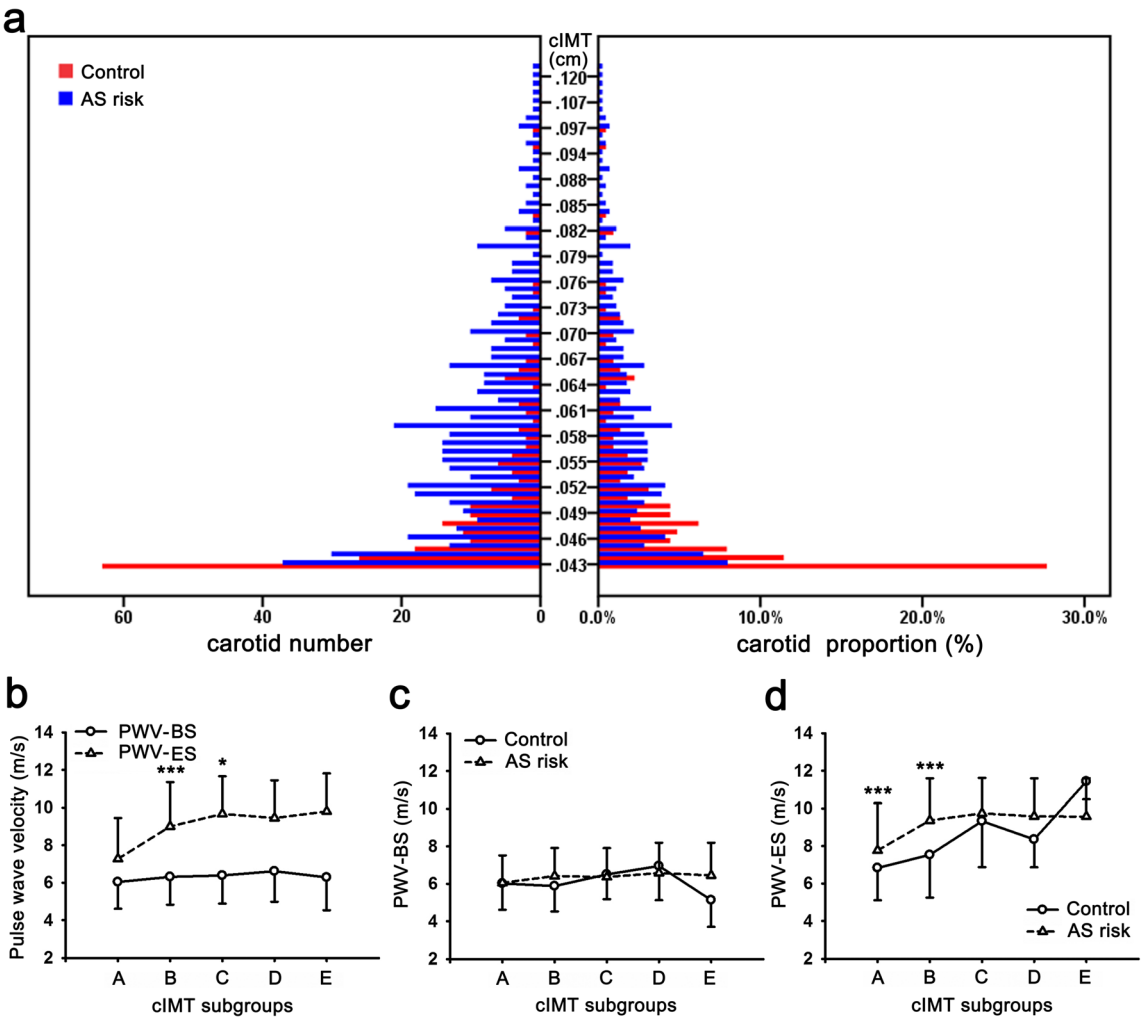

Fig. 6 **a** Distribution analysis of the pooled cIMT ($n = 694$) measurements. The *red* colour represents the cIMT measurement of the control group and *blue* represents the atherosclerosis (AS) risk group. The vertical axis shows the cIMT. The left horizontal axis shows the carotid number, and the right horizontal axis shows the carotid proportion (control, $n = 228$; AS risk, $n = 466$). (**b**) Graph of PWV-BS and PWV-ES measurements compared with the front cIMT subgroup for each other. Graphs of (**c**) PWV-BS and (**d**) PWV-ES measurements of the control group and AS risk group at each cIMT subgroup ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$)

The cIMT is a vital ultrasound marker for early AS and is associated with carotid stiffness [1]. Early AS diagnosis and timely assessment is vital for the management and outcome of cardiovascular disease [26, 39] and stroke [40, 41]. Thus, compared to cIMT, what is the role of ufPWV for early AS assessment? Using the cut-off of 0.050 cm, AS risk individuals showed increased ES levels in the thickened (0.051–0.060 cm) cIMT subgroup and the non-thickened (0.043–0.050 cm) subgroup (Fig. 6d). These results indicate that PWV-ES could be a novel and sensitive modality for the assessment of early AS, possibly earlier than alterations of cIMT values.

However, when both PWV-ES and cIMT measurements were high, there was no evidence that ufPWV values for the AS risk group were significantly higher than those of the controls (Fig. 6c and d). Moreover, an increase in PWV-ES is associated with thickened cIMT, with a possible limitation existing in cIMT subgroup C (0.061–0.070cm; Fig. 6b) ranging from 11.63 to 14.95 cm/s (mean ± standard deviation, 9.64 ± 1.99 m/s; maximum, 14.95 cm/s, pooled subgroup C + D + E). These results illustrate that an increase in arterial stiffness may not unendingly correlate with continuously increasing cIMT. Arterial stiffness is theoretically associated with both the thickness of the vessel wall and PWV. The combination of cIMT and ufPWV could potential be useful to diagnosis arterial stiffness and AS in the future.

Our study had several limitations. First, HL is only one vital AS risk factor. Other factors, such as hyperglycaemia, smoking (Supplementary Fig. S3) or obesity, were not investigated in the present study. Second, four subjects in the control group were not obese but did exhibit an overweight BMI (maximum, 25.7 kg/m^2; Table 1). However, blood examination and blood pressure were normal. These individuals may have slightly affected the results of our study. Third, the success rate and reliability of the ufPWV notably decreased with increase in cIMT and presence of plaque [42] (Supplementary Figs. S1 and S2). Excluding the failure measurements in this study cohort led to a notable absence of plaque condition, and thereby led to a biased result for the effect of plaque on ufPWV. Moreover, one of our purposes was to compare the role of ufPWV and cIMT in early AS assessment, which is not characterised by atherosclerotic plaques. Therefore, the relationship between ufPWV and atherosclerotic plaques was not investigated in the present study and needs further assessment. Fourth, Pan et al [42] have reported that valid ufPWV measurement and a higher intra-observer reproducibility is provided by the SL15-4 transducer than the SL10-2 transducer that was used in the present study; thus, the validity of the ufPWV assessment and cIMT cut-off should be further assessed.

In conclusion, our findings demonstrated that the quantitative parameters of ufPWV might enable the quantification of arterial stiffness correlated with AS.

Funding This work was supported by the Research Project of Jiangsu Province Hospital of TCM (grant number Y17048) and the Research Project of Jiangsu Province of TCM (grant number YB2015020).

Compliance with ethical standards

Guarantor The scientific guarantor of this publication is Hui Huang, MD (Department of Ultrasound, Affiliated Hospital of Nanjing University of CM, Nanjing 210029, China).

Conflict of interest The authors of this manuscript declare no relationships with any companies, whose products or services may be related to the subject matter of the article.

Statistics and biometry Pin Wang, PhD (Department of Endocrinology, Sichuan Academy of Medical Science and Sichuan Provincial People's Hospital, Chengdu, Sichuan, China), kindly provided statistical advice for this manuscript.

Informed consent Written informed consent was obtained from all subjects (patients) in this study.

Ethical approval Institutional Review Board approval was obtained.

Methodology
- prospective
- diagnostic or prognostic study
- performed at one institution

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