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**Arterial stiffness assessment by shear wave elastography and ultrafast pulse wave
imaging: comparison with reference techniques in normotensives and hypertensives**

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Abstract (150 words)

Shear wave elastography and ultrafast imaging of the carotid artery pulse wave were performed in 27 normotensive subjects (NTs) and 29 age and sex matched patients with essential hypertension (HTs), and compared to reference techniques: carotid-femoral pulse wave velocity (cf-PWV) determined via arterial tonometry and carotid stiffness (carPWV) determined via echotracking. Shear wave speed in the carotid anterior (a-SWS) and posterior (p-SWS) walls were assessed throughout the cardiac cycle. Ultrafast PWV was measured in early systole (ufPWV-FW) and in end-systole (dicrotic notch, ufPWV-DN). a-SWS appeared as the best candidate to evaluate arterial stiffness from ultrafast imaging. In univariate analysis, a-SWS was associated with carPWV ($r=0.56$, $p=0.003$) and cfPWV ($r=0.66$, $p<0.001$). In multivariate analysis, a-SWS was independently associated with age ($R^2=0.14$, $p=0.02$) and blood pressure (BP) ($R^2=0.21$, $p=0.004$). Moreover, a-SWS increased with BP throughout the cardiac cycle and was not different between NTs and HTs when compared at similar BP values.

Keywords

Arterial stiffness, Pulse wave velocity, Vascular ultrasound, Ultrafast imaging, Shear wave elastography, Echotracking, Hypertension

Introduction

Arterial stiffness has been shown to be an independent risk factor of cardiovascular (CV) diseases and mortality (Ben-Shlomo et al. 2014; Laurent et al. 2006; Vlachopoulos et al. 2010). Its measurement has been integrated into the European guidelines for the management of arterial hypertension (Mancia et al. 2013). Indeed, arterial stiffness increases with age and hypertension, leading to an increased risk of CV events such as stroke, myocardial infarction or heart failure. Arterial stiffness can be regarded as a tissue biomarker which integrates the cumulative influence of CV risk factors with time (Laurent et al. 2016). Therefore, early detection of elevated arterial stiffness would enable more accurate risk prediction to better target preventive therapy.

Several indices and methods have been introduced to estimate arterial stiffness non-invasively *in vivo* at the regional or local level. The measurement of the pulse wave velocity (PWV) is currently regarded as the reference method to assess regional arterial stiffness because of its relative simplicity and reproducibility (Laurent et al. 2006). This technique consists of a direct measurement of the time of propagation of the pulse wave, generated by cardiac ejection, between two sites in the arterial tree, usually the carotid and the femoral arteries. PWV is related to the elastic properties of the arterial wall through the Moens-Korteweg equation, used to derive the Young's modulus E of the arterial wall as a function of its thickness h , radius R , PWV and blood density ρ :

$$PWV = \sqrt{\frac{Eh}{2R\rho}}, \quad (1)$$

Although widely validated in epidemiology research, the measurement of carotid-to-femoral PWV (cfPWV) suffers from limits due to imprecisions in the distance measurement (Van Bortel et al. 2012), timing algorithms (Millasseau et al. 2005) and complexity due to arterial path and pressure dependency (Fung 1993; Nichols et al. 2011). Therefore, recent efforts have been directed to the measurement of local arterial stiffness, mainly through high-

resolution ultrasound techniques (Laurent et al. 2016) allowing measurement of distension together with local pulse pressure and calculating distensibility. Indeed, the Bramwell-Hill equation (derived from the Moens-Korteweg equation) relates the PWV to the arterial distensibility Dist, defined as the relative ratio of the change in arterial volume dV or area dA (if change in length is neglected) to the change in pressure dP driving the change in volume (V) or area (A):

$$PWV = \sqrt{\frac{1}{\rho Dist}} = \sqrt{\frac{VdP}{\rho dV}} \approx \sqrt{\frac{AdP}{\rho dA}} \quad (2)$$

This method was shown to be of predictive value for CV events (Laurent et al. 2006; van Sloten et al. 2014). When pressure and diameter waveforms are available, this method also allows the construction of the pressure-diameter curve and calculation of arterial stiffness for any given level of blood pressure (BP) (Bussy et al. 2000). With echotracking, we demonstrated that the increase in stiffness of the common carotid artery (CCA) observed in patients with essential hypertension was primarily due to the increased level of BP and not to hypertension-induced structural modifications of the artery (Bussy et al. 2000; Laurent et al. 1994). However, BP is present in formulas for arterial stiffness calculation (such as equation (2)), which creates intrinsic tautologies when considering the influence of BP and the difference between hypertensive (HT) and normotensive (NT) subjects. Moreover, local BP is estimated through applanation tonometry which has its own limits. This issue highlights the need to develop methods independent of BP in order to clarify the relationship between hypertrophic wall remodelling, increased arterial pressure and arterial stiffness in essential hypertension.

Ultrafast ultrasound imaging is a recent technique which enables the visualization of very short events due to an ultra-high imaging frame rate (up to 10 000 Hz) allowed by plane wave acquisitions (Tanter et al. 2002). Its ability to measure pulse wave transit time along a short arterial pathway at early and late systole has been demonstrated (Couade et al. 2011;

Mirault et al. 2015). In addition, this very high frame rate also allows for imaging of the propagation of artificial shear waves generated remotely by an acoustic radiation force (ultrasonic push) (Bercoff et al. 2004). The propagation speed of these extrinsic shear waves is directly related to elastic (shear) modulus of the tissue (Royer and Dieulesaint 2000). Shear wave elastography can be repeated along the cardiac cycle to assess the BP related variations of arterial stiffness (Couade et al. 2010). Shear wave elastography appears as a promising technique to measure arterial stiffness in vivo independently of BP (Messas et al. 2013). However, it has never been tested against reference techniques.

The purpose of the present study was therefore to evaluate the agreement between arterial stiffness measured by shear wave elastography and the reference techniques: local stiffness measured by echotracking and aortic stiffness measured by applanation tonometry. In addition, a secondary aim was to study the variations of arterial stiffness during the cardiac cycle for comparing arterial stiffness in HT patients and NT subjects at similar levels of BP. Finally, the opportunity was also taken to test ultrafast imaging of the natural pulse wave in HT patients.

Materials and methods

Study population

This observational case-control study included fifty-six subjects. The HT group consisted of 29 patients aged between 25 and 71 years, equally distributed by sex, with never treated essential hypertension or under antihypertensive treatment but with insufficiently controlled BP. The NT group consisted in 27 age and sex matched healthy volunteers. In both groups, subjects were equally distributed by age group (25-39, 40-54, 55-71 years). HT patients were recruited from the Hypertension and the Pharmacology Units of Georges Pompidou European Hospital (HEGP). Essential hypertension was defined by elevated BP (systolic BP [SBP] > 140 mmHg and diastolic BP [DBP] > 90 mmHg) and the verified absence of secondary hypertension. Healthy volunteers were recruited by the Center for

Clinical Investigation of HEGP with a supine SBP < 140 mmHg and DBP < 90 mmHg and no history of cardiovascular disease or additional risk factors. The study was approved by the institutional medical ethics committee of St Germain en Laye hospital and all participants gave informed consent. The study is registered at ClinicalTrials.gov (identifier NCT01743352).

Arterial Measurements

Investigations were performed in a controlled environment after 10 minutes of recumbent rest. Brachial BP was recorded using an automated oscillometric cuff (Colin BP-880, Colin Medical, TX, USA) every 3 minutes. Right CCA pressure waveform was determined non-invasively via applanation tonometry using the Sphygmocor© device (AtCor Medical, Australia) (Boutouyrie et al. 1999) and calibrated using DBP and mean BP (MBP) from radial tonometry. cfPWV was also determined via applanation tonometry using the Sphygmocor© device. The intersecting tangent algorithm was used to assess the foot-to-foot transit time. The direct distance between the two sites was determined with a tape measure and corrected by a 0.8 factor according to recommendations (Van Bortel et al. 2012).

Right CCA internal diameter, distension and wall intima-media thickness (IMT) were measured using a high-resolution echotracking device (MyLab™ 70, ART.LAB, Esaote, Italy) consisting of a 128 radio-frequency line multiarray covering a 4-cm arterial segment (Paini et al. 2007). Circumferential wall stress was calculated according to Lamé's equation as $\sigma_{\text{circ}} = \text{MBP} \cdot d / (2 \cdot \text{IMT})$, where d is the diastolic internal diameter. Cross-sectional distensibility was calculated as $\text{Dist} = \Delta A / (A \cdot \Delta P)$, where A is the diastolic lumen area, ΔA is the stroke change in lumen area and ΔP is the carotid pulse pressure (PP), i.e. the difference between SBP and DBP. Incremental elastic modulus was calculated as $E_{\text{inc}} = [3(1 + A/\text{WCSA})] / \text{Dist}$, where WCSA is the diastolic wall cross-sectional area (Bussy et al. 2000). Local CCA PWV (carPWV) was determined from the distensibility coefficient using equation (2) above.

Shear wave elastography and ultrafast imaging measurements were performed with the commercial ultrasound scanner Aixplorer® (SuperSonic Imagine, France) equipped with a linear array probe (128 elements, 8 MHz, pitch 0.2 mm, 28 mm elevation focus). This system is programmable per channel both in receive (128 channels) and in transmit (256 channels). Plane wave transmit enables the reconstruction of a complete frame from a single transmit/receive event. In this study, images were obtained by applying receive-only dynamic beamforming to backscattered echoes and coherent compounding of three plane waves with different inclinations (-5° , 0° and 5°) (Montaldo et al. 2009). The supersonic shear imaging (SSI) technique was used for shear wave elastography measurements (Bercoff et al. 2004). Shear waves were created by a supersonic push which is composed of three consecutive pushes of 100 μ s located at three depths 5mm apart along the center line of a region of interest (ROI) manually positioned by the sonographer in order to include the anterior and posterior CCA walls. The propagation of the shear waves was then imaged during 5 ms with a frame rate of 8000 images/s. A sequence of 8 consecutive supersonic pushes at intervals of 200 ms was triggered on the R-wave peak (maximum) of the ECG. This acquisition sequence was repeated three times for reproducibility assessment. The propagation of the natural pulse wave along the right CCA was imaged in immediate succession with the probe maintained in the same position. A sequence of 1000 images acquired with a frame rate of 2000 images/s was triggered on the R-wave peak through the ECG-coupled imaging system. Measurements were performed three times to assess reproducibility. The in-phase and quadrature (IQ) data were exported, stored and processed a posteriori in a blind manner using MATLAB R2014a (The MathWorks Inc., USA).

Shear wave elastography post-processing

First, the arterial walls were localized on the IQ images. Because of the high acquisition frame rate (8000 Hz), the contrast of the images was insufficient to use automatic segmentation and tracking algorithms. Instead we used the standard ultrasound image

recorded at the beginning of the acquisition sequence to manually determine the anterior and posterior wall position for the first push using three points and a second order polynomial interpolation (Fig. 1a). We assumed that the wall did not move significantly during the 5 ms of imaging following the push so that a tracking operation was not needed. However, the time between two consecutive pushes (200 ms) was long enough with regard to the cardiac cycle to lead to an important displacement of the walls. To determine the new positions of the walls for the seven subsequent pushes, we used the diameter values measured by echotracking. The mean cycles of CCA diameter measured by echotracking and CCA BP measured by applanation tonometry were aligned temporally (Fig. 1b). The time delay between the R-wave peak of the ECG (trigger for 1st push) and the foot of the pressure wave ($\Delta T_{R\text{-peak foot}}$) was extracted from the applanation tonometry measurement. It enabled identification of the time point corresponding to the 1st push and the subsequent 7 pushes every 200ms (Fig. 1b). As shown in Figure 1c, the contrast of the IQ images was sufficient to evaluate the position of the posterior wall but not the anterior wall. Therefore, the change in position of the posterior wall **corresponding to the time points of** the different pushes was manually evaluated by comparing the first IQ image of the consecutive pushes. The position of the anterior wall **at the time of each push** was then automatically determined using the **lumen diameter measured by echotracking** (Fig. 1c). In order to cover the entire wall thickness and in case of small shifts due to manual positioning, five adjacent position lines covering 1 mm, centred on the previously determined position line, were analysed (Fig. 1c).

Secondly, a frame-to-frame IQ cross-correlation algorithm was performed to determine the radial velocity of the whole image (Pernot et al. 2007). The velocity values corresponding to the longitudinal elements of the anterior and posterior walls were extracted to obtain space-time representations of the arterial wall velocities (5 for each wall corresponding to the 5 position lines) (Fig. 1d). These graphs displayed two main diagonal wavefronts corresponding to the propagation of the shear wave in the anterograde and

retrograde direction. The shear wave propagation speed (SWS) was derived by calculating the group velocity (infinite medium assumption neglecting dispersion). For this, we computed the Radon transform of the velocity map, which represents the sum of all the pixel values over straight lines in the image, oriented at different angles (Maksuti et al. 2016). We then selected the maximum of the Radon transform, which corresponds to the angle of the most significant wavefront of the map. This angle is directly related to the slope of the wavefront and therefore to the SWS (Fig. 1d). The median value of SWS for the 5 position lines was retrieved and the anterograde and retrograde SWS were averaged to obtain a single SWS value for the anterior wall (a-SWS) and posterior wall (p-SWS). Moreover, a mean SWS value for each push was calculated by averaging the different acquisitions and the inter-acquisitions variability was assessed using the coefficient of variation (CV). Finally, a mean SWS value over the cardiac cycle was calculated for each patient, for both walls, by averaging the 8 pushes.

Pulse wave imaging post-processing

The first two steps of pulse wave imaging post-processing are the same as for shear wave elastography processing, i.e. arterial wall localization and frame-to-frame IQ cross-correlation to retrieve the radial velocity map. With a 2000Hz frame rate, the contrast of the images was better than previously and enabled to use semi-automatic segmentation and tracking algorithms. First, the sonographer manually segmented a longitudinal section where the arterial walls appeared with a satisfactory quality (in plane and lumen/intima interface visible) on the first frame. Then an automatic segmentation of the anterior and posterior wall was performed and repeated every 50 frames (tradeoff between accuracy of wall localization and computing time). The velocity values corresponding to the longitudinal elements of the anterior and posterior wall were extracted and subtracted from each other to remove the global motion of the artery. Thus, we obtained a space-time representation of the arterial wall velocity (Fig. 2a). By averaging the velocity signal for all longitudinal positions and integrating the mean velocity signal with respect to time we came up with an arterial wall

distension signal exhibiting the expected shape of a pressure waveform with a clear systolic upstroke delimiting the “foot of the wave” (FW), a systolic peak and a dicrotic notch (DN) during late systole and before diastolic decay (Fig. 2b). Then, to identify the time-reference points corresponding to the foot of the wave and the dicrotic notch, we used the classical maximum of the second derivative method (Hermeling et al. 2009). Therefore, we calculated the acceleration of the wall using a 2nd order Kalman filter (Shumway and Stoffer 1982) (Fig. 2c). The arterial wall acceleration exhibited the expected two peaks corresponding to two forward-propagating waves: the first wave corresponds to the beginning of systole (foot of the wave), after aortic valve opening, while the second wave corresponds to the end of systole (or dicrotic notch), after aortic valve closure. However, we observed that these peaks were usually surrounded by other acceleration maxima or minima, which form visible wavefronts in the space-time representation of arterial wall acceleration. Therefore, to ensure an accurate comparison between subjects, we introduced a coding system of the different wavefronts (Fig. 2d):

- At the foot of the wave: the latest maximum before systolic rise was numbered 0, the following minimum was numbered 1, the preceding minimum -1 and, when a preceding second maximum was visible, it as numbered -2.
- At the dicrotic notch: the first maximum after the systolic peak was numbered 0, the preceding and following minima were respectively numbered -1 and 1 and, when a following second maximum was visible, it was numbered 2.

For each identified wavefront, a robust linear regression of the spatiotemporal coordinates of the acceleration maxima (or minima) was performed (Fig. 2e). The slope of the regression lines gave the corresponding wave velocities and the correlation coefficient (r^2) was reported. Then a quality analysis was performed by two independent reviewers which had not been involved in data processing. Based on the graphs showing the regression lines and correlation coefficients, they assigned a score between 0 (mediocre) and 4 (very good) to every wavefront.

PWV estimates were accepted for further evaluation if the scores of both reviewers were above 1. A mean PWV value per wavefront for each patient was calculated by averaging the different acquisitions and the inter-acquisitions variability was assessed using the coefficient of variation (CV). Finally, we calculated the difference between PWV measured at the foot of the wave and at the dicrotic notch (Δ PWV), as well as the ratio Δ PWV/carotid PP (Mirault et al. 2015).

Statistical analysis

Data were compared between the two groups (NT and HT) using the non-parametric Mann-Whitney test. Because of the small sample size, all data sets were assumed to be non-normal. Correlations between parameters measured by the different techniques were analyzed by computing the Spearman-rank correlation coefficients. Multivariate models with robust regression (Huber's method) were also built to study the relationships between arterial stiffness, age and BP. The proportion of the variance of arterial stiffness explained by age and BP was evaluated through the partial coefficient of determination R^2 . Moreover, to study the variations of arterial stiffness with BP during the cardiac cycle, we ranked the 8 pushes in ascending order of the BP values corresponding to the push time (1 in diastole to 8 in systole). We then used linear mixed models including the SWS, the age, the group (NT or HT), the BP and the rank of the push. We tested each variable as well as the interaction group*rank. Indeed, if significant, this interaction shows that the relationship between SWS and the level of BP (given by the rank) is different in NT and HT subjects. In addition, we compared SWS between groups for a common level of BP, 100 mmHg, by performing a quadratic fitting of the BP-SWS data of the 8 pushes per patient. Statistical analyses were completed using NCSS 11 Statistical Software (2016) (NCSS, LLC. Kaysville, Utah, USA) and a p-value < 0.05 was considered significant.

Results

Study population

The clinical characteristics of the population are presented in Table 1 and the arterial parameters measured on the right carotid are reported in Table 2. The two groups were comparable in terms of age, sex ratio, and heart rate. The HT group included more smokers and presented a higher BMI than the NT group. Brachial SBP, DBP and PP and carotid SBP and DBP were significantly higher in HT than in NT subjects. Note that 41% of HT patients were under antihypertensive treatment and that their mean SBP/DBP values, 143/81 mmHg were relatively low in view of the threshold values defining hypertension (140/90 mmHg). HT patients exhibited a significant increase in carotid diameter and wall cross-sectional area. Their IMT was also higher than for NT subjects, although not significant. These morphological changes are in accordance with previously observed remodeling of large arteries in hypertension (Laurent and Boutouyrie 2015). Moreover, we observed a significant reduction of carotid distensibility and a significant increase in Young's elastic modulus and circumferential wall stress for HT patients (Table 2). Note that these changes in mechanical parameters are also consistent with previous studies (Bussy et al. 2000). In addition, aortic and carotid arterial stiffness evaluated by the reference techniques (applanation tonometry and echotracking) were found significantly higher in the HT group.

Shear wave elastography, agreement with reference techniques and determinants

Shear wave elastography acquisitions were available for only 25 **of the** subjects **because appropriate device settings were under consideration for the first few patients.** Feasibility and reproducibility data are detailed in Supplementary materials. The main clinical characteristics and arterial parameters of this sub-population are presented in Supplementary Table 1. Note that the sub-population is slightly younger than the full study population (mean age 46 and 45 years for NTs and HTs vs. 49 and 50 years) but has very similar characteristics in terms of BP, carotid morphological and mechanical parameters. In addition, no significant difference was observed between this group and the rest of the study population in terms of clinical characteristics. We therefore considered this group as representative of the full study

population. Mean values of SWS over the cardiac cycle (for the 8 pushes) are compared between groups in Table 2. No difference was observed for p-SWS, whereas a-SWS was significantly higher for HTs than for NTs. Note that the average of the 8 pressure values corresponding to the 8 pushes was strongly correlated with carotid MBP ($r=0.99$, $p<10^{-6}$) and brachial MBP ($r=0.92$, $p<10^{-6}$), showing the adequacy of the sampling frequency with respect to the cardiac cycle duration.

We assessed the agreement between a-SWS, p-SWS and the reference techniques by using Spearman correlation tests considering the whole population (Table 3). The corresponding scatter plots are presented in Figure 3. We observed a significant positive correlation between aortic and carotid stiffness measured by the reference techniques ($r=0.39$, $p=0.003$). a-SWS was strongly correlated with cfPWV ($r=0.66$, $p<0.001$) and carPWV ($r=0.56$, $p=0.003$). However, we did not find any correlation between p-SWS and cfPWV nor carPWV.

Then we studied the relationship between SWS and the two main determinants of arterial stiffness, age and BP, using a multivariate model (Table 4). Age and MBP were independently associated with aortic and carotid stiffness, explaining together 44% and 36% of their respective variance. Age and MBP appeared to be independent determinants of a-SWS, explaining respectively 14% and 21% of its variance. However, only age was significantly associated with p-SWS with 18% of explained variance.

Variations of shear wave speed with BP during the cardiac cycle

To study the relationship between SWS and BP over the different pushes, we used mixed models including the rank of the pushes in ascending order of corresponding BP, the age and the group (NT or HT). a-SWS was significantly associated with BP level (rank of the push) ($p<10^{-6}$) but not p-SWS ($p=0.19$). Moreover, the association between a-SWS and the group was close to significance level ($p=0.052$) but the interaction group*rank was not significant ($p=0.89$). This shows that the relationship between a-SWS and BP does not differ

between the NT and HT group. These observations are illustrated in Figure 4a where the mean SWS values are presented for the two groups. a-SWS increases with the push rank. Although mean a-SWS values seem higher for HTs than NTs, the evolution with pressure is similar for both groups. By contrast, for p-SWS, no clear tendency was observed nor any difference seen between groups. The analysis of the mixed model showed that there was a significant increase in a-SWS compared with rank 1 from rank 6 onwards ($p=0.01$) and even greater for rank 7 ($p<0.001$) and 8 ($p<0.0001$). However, the difference in p-SWS compared with rank 1 was not significant until last rank (8) which was close to significance level ($p=0.08$).

Moreover, we analyzed with a mixed model the relationship between the actual (tonometry) BP values corresponding to each push, the push rank and the group (Fig. 4b). By construction, we observed a strong correlation of BP with the push rank ($p<10^{-6}$) and with the group ($p<0.001$). Nonetheless, the interaction push rank*group was not significant ($p=0.5$), showing that the relationship between BP and push rank was not different for NTs and HTs. Thus we could represent the relationship between SWS and BP for each group (Fig. 4c). We observed again that a-SWS increases with BP. The curve of the HT group is shifted towards higher BP values but not higher a-SWS values. Indeed, the curves of both groups are overlapping in the range of common BP values. Regarding p-SWS, as noted previously, no association was observed with BP nor difference between groups.

To further test our hypothesis regarding the absence of difference in intrinsic wall stiffness between HTs and NTs, we fitted the BP-(a-SWS) relationship with a second order polynomial function for each subject individually. We then interpolated the a-SWS value calculated by the model for a pressure of 100 mmHg and compared it between the two groups (Table 2). We did not find any difference between NTs and HTs ($p=0.23$) for a-SWS at a common pressure of 100 mmHg. Furthermore, we observed that in multivariate analysis

a-SWS at 100 mmHg was significantly correlated with age ($p=0.002$) independently of the mean BP of the patient.

Ultrafast imaging of the pulse wave

PWV values measured by ultrafast echography in diastole at the foot of the wave (ufPWV-FW) and in end systole at the aortic notch (ufPWV-DN) are compared between the two groups in Table 2 (see Supplementary materials for feasibility and reproducibility data). No significant differences were found between the two groups for any parameter measured by ultrafast imaging of the pulse wave. However, carPWV and cfPWV were significantly higher in the HT group even when considering only the subjects having had ultrafast imaging or shear wave elastography (data not shown).

We evaluated the agreement between PWV measured by ultrafast imaging and the reference techniques by using Spearman correlation tests considering the whole population (Table 3). The corresponding scatter plots are presented in Figure 3. ufPWV-DN appeared significantly correlated with carotid stiffness measured by echotracking ($r=0.49$, $p=0.001$) but not with aortic stiffness. Note that no correlation was observed with ufPWV-FW.

We then studied the relationship between arterial stiffness and its two main determinants, age and BP, using a multivariate model (Table 4). We observed that ufPWV-FW and ufPWV-DN were not associated with age nor with MBP. Finally, we did not find any association with age of the difference between PWV measured by ultrafast imaging at the foot of the wave and at the aortic notch ($r=0.10$, $p=0.59$) as well as this difference normalized by carotid PP ($r=0.11$, $p=0.56$), even when adjusted for BP. Note that when considering the NT subjects only, ufPWV-DN was associated with age, independently of MBP, with 39% of explained variance (see Supplementary Table 3).

Discussion

We have implemented two methods based on ultrafast echography to measure carotid arterial stiffness of hypertensive patients and normotensive subjects: first by measuring the

speed of propagation of artificial shear waves generated along the cardiac cycle in the anterior and posterior carotid wall, second through carotid PWV by ultrafast imaging of the pulse wave propagation. The main results were that shear wave speed on the anterior wall is strongly associated with reference techniques, as well as with age and BP independently. Moreover, with anterior wall shear wave analysis, we demonstrate that normotensives and hypertensives exhibit similar mechanical behavior of carotid wall, when studied at the same pressure. By contrast, shear wave speed on the posterior wall showed a significant association with age independently of BP but no association with BP nor with reference techniques. PWV at the dicrotic notch was associated with carotid stiffness only and PWV at the foot of the wave showed no association at all.

Agreement with reference techniques

We have shown that a-SWS is correlated with carotid and aortic PWV while carotid PWV measured by ultrafast imaging of the pulse wave at end systole (at the dicrotic notch) is correlated with carotid PWV measured by echotracking only. In contrast, we did not find any association with the reference techniques of carotid p-SWS nor ufPWV-FW. Regarding carotid ufPWV-FW, previous publications have shown that local PWV measurement at the “foot of the wave” could be disturbed by early wave reflections and associated with a lower reproducibility than measurement at the dicrotic notch (20% variation coefficient instead of 10%) (Hermeling et al. 2008; Hermeling et al. 2009). In the studies by Hermeling, PWV was calculated by linear regression after identifying the foot of the wave (or the dicrotic notch) for 14 ultrasound lines every 1.26 mm with a frame rate around 600 Hz. The low distance combined with the frame rate might partly explain these results. In any case, we observed comparable results in the present study by increasing both the distance (40 mm) and the frame rate (2 kHz). Therefore, the obtained results confirm the important variability of local PWV measurement at the foot of the wave and the better quality of data obtained at the dicrotic notch. The explanation provided by Hermeling (2008; 2009) was the presence of early wave

reflections which make the analysis of the foot of the wave complex. Indeed, the wavefronts observed at the foot of the wave are very complex and sometimes difficult to identify, although we paid particular attention in their identification. Contrary to late reflections which can be observed as wave travelling in opposite directions, early reflected waves are superimposed with forward waves and cannot be individualized.

Concerning p-SWS, we also found the highest inter-acquisitions variability (20.5%). This low reproducibility can be attributed to the lower quality of the distance-time maps of wall velocity obtained for the posterior wall compared to the anterior wall. Due to the posterior wall being deeper in the tissue, the quality of the ultrasound signal could be altered. Another hypothesis is the difference in anatomical location between the anterior and posterior carotid walls. Indeed, the anterior wall is located below the jugular vein. It is attached to the vein wall but is well separated from the other surrounding tissues and can move quite freely. On the other hand, the posterior wall is directly attached to the underneath muscular tissues. This binding could affect the wall movement and stiffness. This influence could be rather limited for the anterior wall but more significant for the posterior wall, explaining the lack of agreement between the local posterior wall stiffness and the reference techniques that take into account the whole vessel distension.

Association with age

As expected, we found independent associations with age and MBP of aortic and carotid stiffness measured by the reference techniques. Even in this small number of subjects these associations were meaningful and comparable to previous larger studies (Engelen et al. 2015; Paini et al. 2006; The Reference Values for Arterial Stiffness' Collaboration 2010). We have shown that a-SWS (and to a lesser extent p-SWS) was associated with age independently of MBP. Yet SWS is a parameter representing the intrinsic stiffness of the arterial wall material, which can be compared to the Young's incremental elastic modulus measured in previous studies. In the present analysis, we have privileged SWS because it is directly

measured, and any manipulation can increase the variability of the parameter by transmission of measurement errors in formulas. It has been shown that ageing was associated with an increase in the Young's incremental elastic modulus (Bussy et al. 2000). Therefore, the present results confirm this relationship with a direct measurement instead of a calculation based on BP.

We showed that ufPWV-DN, ufPWV-FW and their difference were not associated with age. This is at contrast with a recent study which has shown that these parameters were associated with age independently of BP in a population of 102 healthy normotensive subjects (Mirault et al. 2015). For proper comparison, we analyzed the association with age and MBP in the NT group only (Supplementary Table 3). We found that ufPWV-DN was significantly associated with age with 39% of explained variance ($p=0.002$), which is in accordance with Mirault's results. However, ufPWV-FW, Δ PWV and Δ PWV/PP still showed no association with age. We acknowledge that our sample size is low and should be increased in order to ensure a meaningful comparison. In its current form and in our hands, pulse wave imaging seems to lack robustness. Our results highlight the importance of developing more robust analysis tools for the precise quantification of the PWV.

Association with BP

We found that mean a-SWS was significantly higher for the HT group than the NT group and was significantly correlated with MBP, independently of age. Consequently, a-SWS appears to be a very interesting parameter for hypertension management and detection, especially since its measurement does not require BP measurements. Studies on larger and more varied populations should be carried out in order to better understand this new variable. Moreover, we have shown that this index of local intrinsic stiffness of the anterior wall changes during the cardiac cycle and significantly increases with BP. This confirms, in a larger population, the preliminary results obtained by Couade et al. (2010). Furthermore, the fact that the rank*group interaction was not significant demonstrates that the correlation

between a-SWS and BP was not different between the HT and NT group. Therefore, the observed difference in a-SWS between the two groups is essentially explained by the difference in BP. In addition, the (a-SWS)-BP curves of the two groups overlap in the common pressure range and exhibit no upward shift. These results show that the intrinsic stiffness of the arterial wall is not increased in HT patients compared to NT subjects of same age and sex when it is measured at the same BP level. The apparent increase in arterial stiffness of HTs compared to NTs is mainly explained by their higher BP. Hence, our results confirm by a direct method, independent of BP, that the absence of increase in intrinsic arterial stiffness of the arterial wall of HTs (Bussy et al. 2000) is not a tautology caused by BP values in formulas. However, because of the small size of our groups, we were not able to perform analyses according to age groups neither to study the evolution of intrinsic arterial stiffness of young HTs. The absence of increase in arterial stiffness in HTs might be explained by the antihypertensive treatment allowing a partial control of hypertension and an at least partial correction of the underlying mechanical abnormality. However, because of the small sample size we could not test this hypothesis in the present study.

A novel finding from this study is the fact that we observed different results for the anterior and posterior wall. Indeed, p-SWS appeared correlated with age but not with BP and was not different between the HT and NT group. We recall that the quality of the posterior wall data was lower than for the anterior wall and the inter-acquisitions variability of p-SWS was much higher than for a-SWS (20.5% compared to 8.3%). This might be due to the alteration of the ultrasound shear waves and signals with depth. Yet an asymmetric motion of the arterial anterior and posterior walls could explain the observed difference and has previously been observed in rats using high-resolution ultrasound (Nam et al. 2014). Indeed, circumferential stress and strain related to pressure and diameter pulsatility have generally been considered to be equally distributed over the entire circumference of the artery. However, the fact that the stiffness of the posterior wall does not increase with BP could be explained by

a lower stretch on this side compared to the anterior side, which deforms more. In addition, as we previously mentioned, the difference in anatomical location between the anterior wall, in close proximity to the vein, and the posterior wall, in close proximity to the underneath muscular tissues might also account for this discrepancy. Transverse analyses in the circumferential direction would help to further investigate this aspect.

Finally, we have shown that ufPWV-FW and ufPWV-DN were not significantly different between the HT and the NT group. Note that 41% of HT patients were under antihypertensive treatments, which could affect carotid and aortic stiffness adaptation to the actual level of BP. Yet, groups were too small to test the effect of treatments. Nevertheless, carPWV and cfPWV were found to be significantly higher for the HT group than for the NT group. Moreover, the multivariate analysis showed that the reference indices of aortic and carotid stiffness were significantly correlated with MBP independently of age, whereas ufPWV-FW and ufPWV-DN were not associated with MBP. Therefore, PWV measured by ultrafast ultrasound still needs improvement before being proposed for measuring the evolution of arterial stiffness in the presence of hypertension.

Limitations and perspectives

As previously mentioned, the sample size of our population was smaller than foreseen because of technical problems at the beginning of the study. Besides, an important part of included HT patients were under antihypertensive treatment and the pressure values of the HT group are therefore close to the thresholds defining hypertension. Hence, there was no marked contrast between the two groups considered in the present study, which helped for comparison because of the BP overlap, but could underestimate the effect of high, untreated hypertension.

Actual pulse wave propagation wavefronts were complex to decipher. It appeared that some wavefronts were very clear whereas R^2 was low. To limit subjectivity, we used a scoring system rather than a selection based on R^2 . This procedure was only used for selecting the most consistent wavefronts (wavefronts 0). The scoring system helped for

503 **setting the algorithm but does not need to be repeated during up-to-come clinical**
504 **applications.**

505 We measured the group velocity of shear waves by neglecting dispersion phenomena.
506 Yet several studies on arterial phantoms have shown that using the group velocity led to an
507 underestimation of the shear and Young's moduli compared to using the phase velocity
508 (Maksuti et al. 2016; Widman et al. 2016). However, one study on an *ex vivo* porcine carotid
509 artery has shown that the calculation of Young's modulus based on the group velocity and the
510 Moens-Korteweg equation gave very similar results compared to the approach using the phase
511 velocity (Bernal et al. 2011). The calculation of phase velocity requires a more complex
512 processing of acquired data. Although the present study suggests that the group velocity
513 approach is sufficient to retrieve relevant parameters *in vivo*, it would be interesting to
514 evaluate the shear waves phase velocity in our population. Besides, we have evaluated the
515 SWS in the longitudinal direction of the artery only. Yet the arterial mechanical behavior is
516 anisotropic and one *ex vivo* study on a horse aorta has shown that the variations of SWS with
517 deformation were more important in the circumferential direction than in the longitudinal one
518 (Shcherbakova et al. 2014). Hence, it would be interesting to include measurements in the
519 circumferential direction in future studies.

520 The results of the application of shear wave elastography for the measurement of
521 arterial stiffness *in vivo* seem very encouraging and further studies should be carried out to
522 better understand the retrieved indices of stiffness. Acquisitions are simple, however, in its
523 present form, post-processing is very complex because we need to integrate data from
524 tonometry and echotracking. Improvement in hardware and signal processing (ECG-gated
525 sequence of diameter measurement similar to the echotracking) could be integrated into the
526 ultrafast ultrasound scanner.

527 **Conclusion**

The approach proposed in this study allowed for the assessment of carotid arterial stiffness in hypertensive patients and healthy subjects via a new technique using ultrafast ultrasound, that does not depend on BP. Anterior wall SWS appears as the best candidate to evaluate local arterial stiffness because of its good correlation with age, BP and reference methods (aortic stiffness and carotid distensibility). In addition, we have demonstrated with a method not requiring BP that the intrinsic arterial stiffness of HT patients is not increased compared to healthy subjects of the same age when measured at a common BP level. Finally, we have confirmed that SWE is able to capture the increase in arterial stiffness with BP during the cardiac cycle.

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665

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Figure captions list

Fig. 1. The main steps of the shear wave elastography post-processing used to derive the shear wave speed (SWS). (a) Manual delimitation of anterior and posterior wall on the first ultrasound image: red crosses correspond to the manually positioned points (3 for each wall) and blue dotted lines to the entire wall position after second order polynomial interpolation between corresponding points. (b) Temporally aligned mean cycles of CCA diameter measured by echotracking and CCA BP measured by applanation tonometry. The time points corresponding to the 8 pushes were identified knowing the time delay between the R-wave peak of the ECG and the foot of the pressure wave. (c) IQ images of two pushes used to assess the change in position of the CCA walls delimited by 5 position lines. (d) Spatiotemporal maps of wall velocity corresponding to the 5 position lines of anterior and posterior wall. The shear wave speed (SWS) was derived by calculating the slope of the main retrograde and anterograde wavefronts using the maximum of the Radon transform.

Fig. 2. The main steps of the pulse wave imaging post-processing used to derive the pulse wave velocity (PWV) at the foot of the wave (FW) and at the dicrotic notch (DN). (a) Spatiotemporal map of wall velocity (anterior and posterior walls were subtracted from each other). (b) Arterial wall distension curve obtained by integrating the mean velocity signal over the longitudinal distance. (c) Spatiotemporal map of wall acceleration used to identify the wavefronts corresponding to FW and DN. (d) Acceleration trace of the middle of the wall exhibiting characteristic wavefronts and their coding. (e) Corresponding PWV values are given by the slope of the wavefronts calculated by robust linear regression.

691 Fig. 3. Scatter plots evaluating the agreement between PWV measured by shear wave
692 elastography, ultrafast imaging and the reference techniques: (a) carotid PWV measured by
693 echotracking and (b) carotid-femoral PWV measured by applanation tonometry.

694

695 Fig. 4. (a) Relationship between SWS and pressure level represented by push rank for anterior
696 and posterior wall. Data presented are mean values for the NT (n=14) and the HT group
697 (n=11). (b) Mean carotid pressure values corresponding to each push rank for the two groups.
698 (c) Relationship between SWS and carotid pressure over the cardiac cycle for the two groups.
699 Error bars represent standard errors of the mean.

Table 1. Clinical characteristics of the population

Parameters	NT Subjects (n=27)	HT Patients (n=29)	p
Age (y)	49 ± 12	50 ± 12	0.92
Gender ratio (male/female)	15 / 12	14 / 15	0.78
Smoking (yes/no)	1 / 26	16 / 13	< 0.001
Treatment for hypertension (yes/no)	0 / 27	12 / 17	< 0.001
BMI (kg/m ²)	24.2 ± 3.3	27.1 ± 4.2	0.007
Heart rate (bpm)	62 ± 8	66 ± 8	0.09
Brachial BP			
SBP (mmHg)	119 ± 14	143 ± 13	< 0.001
DBP (mmHg)	66 ± 7	82 ± 9	< 0.001
PP (mmHg)	53 ± 9	61 ± 7	0.001
Carotid BP			
SBP (mmHg)	113 ± 20	140 ± 23	< 0.001
DBP (mmHg)	65 ± 9	81 ± 9	< 0.001
PP (mmHg)	49 ± 20	59 ± 20	0.06

BMI: body mass index. Mean ± SD

Table 2. Arterial parameters of the right CCA in NT Subjects and HT Patients

Parameters	NT Subjects (n=27)	HT Patients (n=29)	p
External Diameter (mm)	6.92 ± 0.57	7.59 ± 0.84	0.002
Distension (µm)	443 ± 161	419 ± 129	0.61
IMT (µm)	549 ± 118	595 ± 112	0.08
WCSA (mm ²)	11.0 ± 2.7	13.2 ± 3.4	0.008
Wall to lumen ratio	0.19 ± 0.05	0.19 ± 0.04	0.95
σ _{circ} (kPa)	62.2 ± 14.6	76.9 ± 15.6	0.001
Distensibility (MPa ⁻¹)	28.0 ± 14.4	19.7 ± 10.8	0.015
E _{inc} (kPa)	472 ± 227	667 ± 341	0.01
carPWV (m/s)	6.6 ± 1.7	7.8 ± 2.1	0.015
cfPWV (m/s)	7.7 ± 1.6	9.0 ± 1.3	0.001
Mean a-SWS (m/s) *	5.2 ± 1.0	5.9 ± 0.9	0.03
Mean p-SWS (m/s) *	7.4 ± 2.4	6.8 ± 1.4	0.70
a-SWS at 100mmHg (m/s) *	5.4 ± 1.0	5.9 ± 1.1	0.23
ufPWV-FW 0 (m/s) *	6.4 ± 1.7	6.2 ± 1.8	0.97
ufPWV-DN 0 (m/s) *	7.3 ± 1.4	8.3 ± 3.3	0.38
ΔPWV (m/s) *	0.8 ± 2.1	1.4 ± 2.5	0.57
ΔPWV/carotid PP (m/s/mmHg) *	0.012 ± 0.052	0.023 ± 0.041	0.57

Mean ± SD; * means that the number of subjects is smaller for these parameters.

SWS was obtained in 14 and 11 normotensives and hypertensives respectively, ufPWV-FW was obtained in 21 and 14 normotensives and hypertensives respectively, ufPWV-DN in 24 and 20 normotensives and hypertensives respectively.

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Table 3. Spearman correlation matrix between arterial stiffness measured by the different techniques.

Parameters	carPWV		cfPWV	
	r	p	r	p
cfPWV	0.39	0.003		
Mean a-SWS	0.56	0.003	0.66	< 0.001
Mean p-SWS	0.30	NS	0.20	NS
ufPWV-FW	0.05	NS	0.02	NS
ufPWV-DN	0.49	0.001	0.24	NS

705

Table 4. Multivariate analysis of the association with age and brachial mean blood pressure of aortic and carotid stiffness measured by the different methods.

cfPWV	(n=56)	R coef	95% CL	R²	p
age (10 years)		0.451	[0.193; 0.708]	0.120	0.001
MBP (10 mmHg)		0.574	[0.373; 0.774]	0.321	< 0.001
carPWV	(n=56)				
age (10 years)		0.544	[0.247; 0.840]	0.149	0.001
MBP (10 mmHg)		0.530	[0.289; 0.771]	0.213	< 0.001
a-SWS	(n=25)				
age (10 years)		0.304	[0.022; 0.586]	0.136	0.036
MBP (10 mmHg)		0.257	[0.067; 0.447]	0.214	0.010
p-SWS	(n=25)				
age (10 years)		0.683	[0.053; 1.313]	0.184	0.035
MBP (10 mmHg)		0.056	[-0.403; 0.515]	0.002	0.802
ufPWV-FW	(n=35)				
age (10 years)		0.222	[-0.251; 0.695]	0.028	0.347
MBP (10 mmHg)		-0.034	[-0.404; 0.336]	0.001	0.853
ufPWV-DN	(n=44)				
age (10 years)		0.271	[-0.170; 0.711]	0.034	0.222
MBP (10 mmHg)		0.269	[-0.080; 0.618]	0.054	0.127

a. Standard ultrasound image and manual wall delimitation

b. Diameter and pressure cycles with 8 pushes identified

c. IQ images for two pushes with 5 position lines covering each wall thickness

d. Spatiotemporal wall velocity maps and SWS assessment using Radon transform

a **b**

a **b** **c**