Università degli studi di Torino
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TESI DI DOTTORATO

CENTRAL HAEMODYNAMIC IMPACT ON ASCENDING AORTA REMODELING

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XXXIII CICLO

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“Dici che i tuoi fiori si sono rovinati, non hai abilità
Questa nazione brutta ti fa sentire asciutta, senza volontà
E gioca a fare Dio manipolando il tuo DNA
Così se vuoi cambiare invece resti uguale per l'eternità

Ma non c'è niente che sia per sempre
Perciò se è da un po' che stai così male
se il tuo diploma è un fallimento, c’è la laurea per reagire”
…

Non è per sempre, Afterhours

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1. INTRODUCTION:

Arterial hypertension (IA) is the main cardiovascular (CV) risk factor in the industrialized countries[1]. Furthermore, we know that in general population as well as in subjects suffering from arterial hypertension, central arterial pressure (aortic and carotid) showed a primary role in describing pathophysiology of the cardiovascular system with respect to brachial pressure alone [2,3].

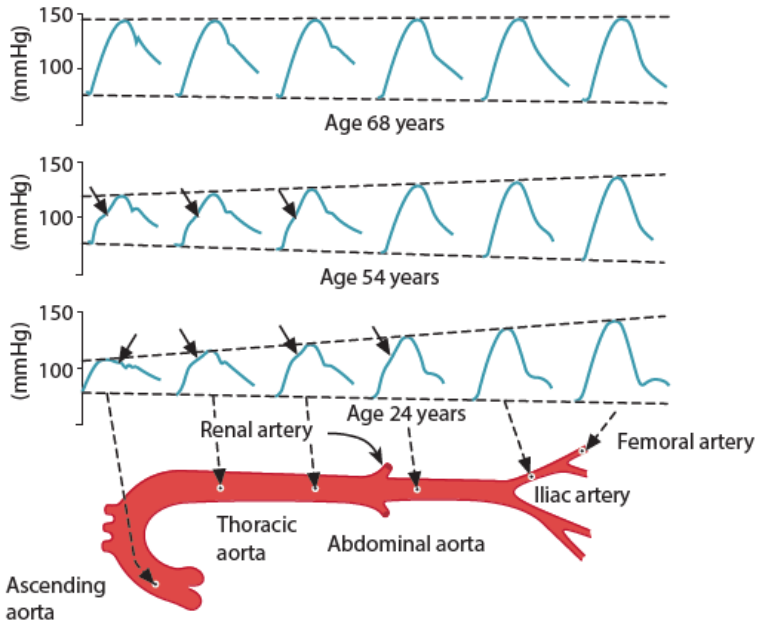


Figure 1. Amplification of the pulse wave along the arterial tree. The change in waveform and degree of amplification in relation to age is illustrated[4].

One of the least studied hypertension-mediated organ damage is undoubtedly the proximal thoracic aorta [5]. Proximal aorta dilation at the level of sinus of Valsalva is a predictor of CV events regardless of left ventricular hypertrophy and other common confounding factors [6]. It is relatively frequent condition in hypertensive patients, with an estimated prevalence up to 16.9% depending on the definitions adopted [7,8]. Focusing on the ascending aorta diameter (AA), it is significantly related to changes in cardiac morphology, central hemodynamics, and arterial stiffness [9]. Currently there are nomograms [10,11] which allow to estimate the expected aortic diameter based on age, gender and body size, but it is not known which anthropometric, hemodynamic and clinical factors have the greatest influence on aortic diameter and aortic dilation rate (Figure 2).

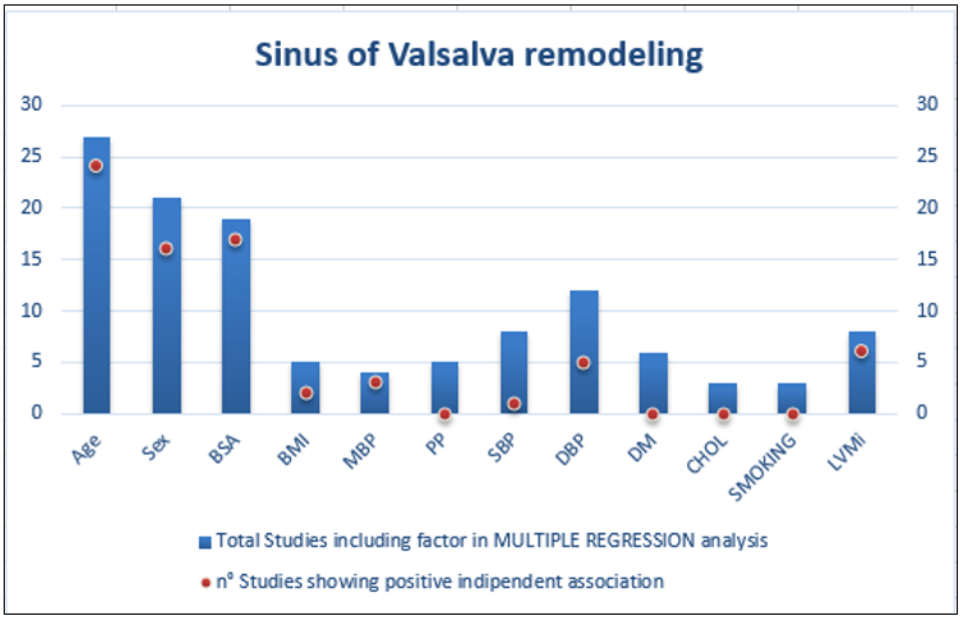


Figure 2. Determinants of sinus of Valsalva remodeling[12]

The size of the proximal aorta alone is an inadequate predictor of the development of acute aortic disease. From previous statistical analyzes, it was found that up to 59% of patients undergo aortic dissection with an aortic diameter <55 mm [13], which is currently the threshold for elective cardiac surgery, suggesting that current surgical guidelines may be inadequate to avoid a significant number of events. For this reason, many studies proposed lowering the threshold for elective aortic repair in special population groups. Although intuitively the risk of acute aortic syndrome is higher in patients with severely dilated thoracic aorta, the rate of cardiovascular events in mild to moderate aortic dilatation and the influence of hypertension on aortic growth need further clarification. Previous studies focused on the study of the mechanical properties of the aortic walls have reported an exponential increase in the risk of rupture for diameters exceeding 60 mm, although the absolute risk is always relatively low [0.6, 1.6 and 4% in patients aged 40, 60 and 80 years, respectively, for baseline diameters of 55 mm [14]]. For this reason we focused on the elastic properties of the vessel walls to predict the evolution of the aortic diameter. Currently the gold standard for the non-invasive assessment of regional aortic stiffness is magnetic resonance imaging (MRI), with the known limits of high costs and reduced availability [15]. In this context, carotid-femoral Pulse Wave Velocity (cfPWV) represents a strategic parameter in the assessment of arterial stiffness as a secondary hypertension mediated organ damage, commonly used for risk stratification [16].

2. AIMS:

2.1 The "ATHOS"project

Currently, there are several devices commercially available and largely used worldwide for non-invasive evaluation of cfPWV [17]. The first introduced was the Complior system, but Sphygmocor was also among the first used in clinical practice and research, becoming the reference device[18], but cost and technically challenging use limit its diffusion in clinical practice. For this reason we are completing the patent registration of a new device for the non-invasive evaluation of cfPWV, ATHOS (Arterial sTiffness faitHful tOol aSessment, patent protocol number P3640IT00, 2020-025, dated 11/20/2020 (14)), designed in collaboration with the Politecnico di Torino and compared with the SphygmoCor. Its simplicity and manageability could help the spread of PWV assessment with the aim of obtaining a better stratification of cardiovascular risk.[19].

2.2 Local *aortic strain* analysis

We proposed a new and simple technique for the non-invasive assessment of the biomechanical properties of the ascending aorta by analyzing transverse vessel strain and its association with well-validated arterial stiffness indices and determinants.

In a second step, we focused our attention on the association between stiffness and aortic remodeling. The goal was to evaluate the possible impairment of the elastic properties of the ascending aorta by a new analysis of the vessel strain, expressed as *β2 Stiffness Index*, in hypertensive patients with different degrees of dilation of the ascending aorta. As a second objective we evaluated the association between mechanical properties of the ascending aorta and indices of cardiac and vascular organ damage.

2.3 The "RECALL"project

The aim of our work was to evaluate the evolution of aortic diameter in a cohort of hypertensive patients with known mild to moderate aortic ectasia at the sinus of Valsalva (SOV) level. In this specific population, we explored the growth rate and analyzed the factors that could help predict its medium-term evolution.

Finally, we evaluated the prognostic impact of ascending aorta dilation as a predictor of cardiovascular events in a population with essential hypertension by clinical and echocardiographic follow-up.

3. The “ATHOS” project

3.1 BACKGROUND

Arterial stiffness is a recognized risk factor for the development of cardiovascular disease. The gold standard for non-invasive measurement of arterial stiffness is PWV recorded between the carotid and femoral sites: a higher cfPWV value means higher arterial stiffness. In the latest guidelines for the management of arterial hypertension [20] a cfPWV value greater than 10 m/s has been indicated as an index of hypertension mediated organ damage (HMOD), leading to an increase in overall cardiovascular risk. Furthermore, cfPWV showed independent predictive value for fatal and non-fatal cardiovascular events, thus allowing for better reclassification of intermediate risk patients.

To implement the use of PWV in clinical practice, it is necessary to have accurate and easy to use tools validated according to current guidelines. At present, there are a number of commercially available devices for evaluating aPWV that use different technologies, such as applanation tonometry (PulsePen (DiaTecne, Milan, Italy), SphygmoCor (AtCor Medical) and the new SphygmoCor Excel), transducers piezoelectric (Complior (Alam Medical, Paris) and Aortic (Exxer, Argentina)) and oscillometric sensors (Mobil-O-graph (IEM, Germany), Arteriograph (TensioMed, Hungary) and Vicorder (Skidmore Medical))[21]. SphygmoCor is currently considered the gold standard for the non-invasive evaluation of cfPWV due to its widespread use in prognostic studies and its proven high repeatability (Figure 3).

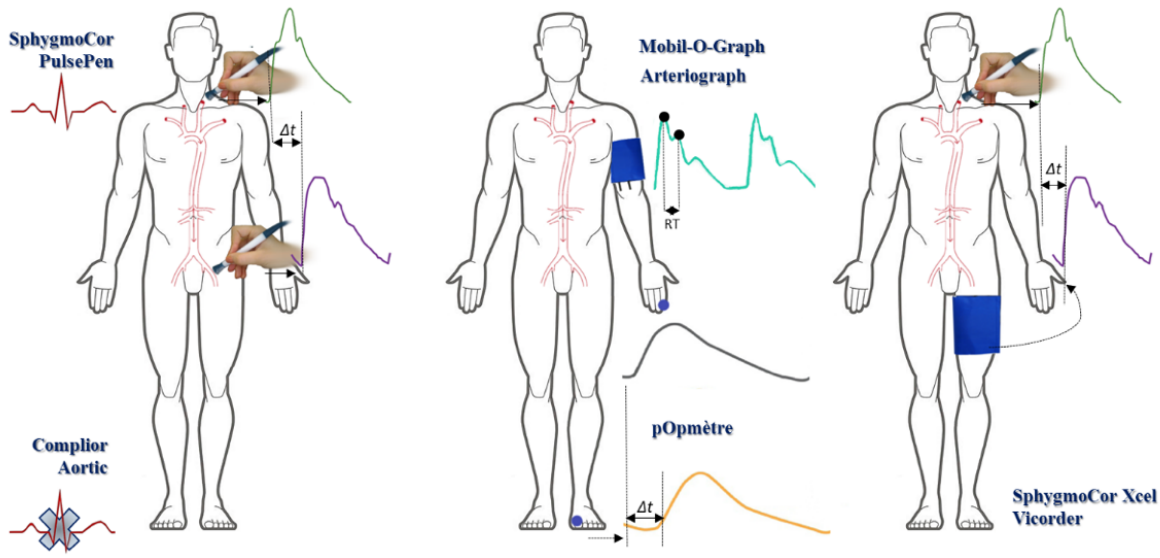


Figure 3. Schematic representation of the main tools currently on the market for the non-invasive measurement of PWV[21]**.**

In this study, a new device for the non-invasive evaluation of cfPWV, ATHOS [Arterial sTiffness faitHful tOol aSessment, patent protocol number P3640IT00, 2020-025, dated 11/20/2020 (14)], was designed in collaboration with the Politecnico di Torino. In a second moment, it was compared with the SphygmoCor [19].

3.2 MATERIALS AND METHODS

For this study 90 voluntary healthy subjects were recruited. Patients aged > 18 years and without any known CV diseases or antihypertensive therapy were enrolled. Study subjects were then classified into 3 groups based on age: < 30, 30-59, ≥ 60 years old. All underwent measurement of anthropometric parameters such as weight, height and abdominal circumference. Smoking habit, daily alcohol consumption and weekly physical activity hours were assessed. Family history of arterial hypertension, type 2 diabetes mellitus, acute coronary syndrome, ischemic or hemorrhagic stroke, atrial fibrillation, valvulopathies, aortic pathology were evaluated as well. Pulse Wave Analysis (PWA), cfPWV (by reference instrument and ATHOS prototype) and Transthoracic echocardiography (TTE) were assessed on the same day. The exam took place at the Molinette Hospital, AOU City of Health and Science of Turin, Internal Medicine Department, Echocardiography Laboratory. The study was approved by the local bioethics committee of the University of Turin (protocol number 155412 of 12/04/2018). All the recruited subjects provided a written informed consent.

PULSE WAVE VELOCITY (PWV)

cfPWV assessment by both validated SphygmoCor (SphygmoCor System, Atcor Medical, Sydney, Australia) reference instrument, and by ATHOS (Politecnico di Torino, Turin, Italy) were performed. After illustrating how to acquire the cfPWV with the two devices, each volunteer lied supine for about 15 minutes in a quiet room. During this period, the arterial pulse was palpated at the carotid and femoral level, marking with the dermographic marker the points considered the most appropriate based on the operators' experience.

For each subject, 3 measurements by ATHOS and 3 measurements by SphygmoCor were performed. The device-operator alternation was performed respecting the indications provided by the ARTERY Society guidelines for the validation of non-invasive tools for estimating the PWV [22].

Subject 1	Subject 2
1) Device 1, operator A	1) Device 2, operator A
2) Device 2, operator B	2) Device 1, operator B
3) Device 1, operator A	3) Device 2, operator A
4) Device 2, operator B	4) Device 1, operator B
5) Device 1, operator A	5) Device 2, operator A
6) Device 2, operator B	6) Device 1, operator B

Table 1. Device-operator alternation according to the ARTERY guidelines

PWV by SphygmoCor

SphygmoCor System is a validated instrument equipped with a transcutaneous applanation tonometer on a pen holder. Being equipped with a single sensor, cfPWV recording require 2 sequential 10- to 20-second readings: first the pulse profile at the carotid level is acquired, followed by the registration at the level of femoral artery. Since the sampling is not simultaneous, electrocardiographic trace (ECG) is taken, with the R wave used as a reference point [Figure 4 [23]].

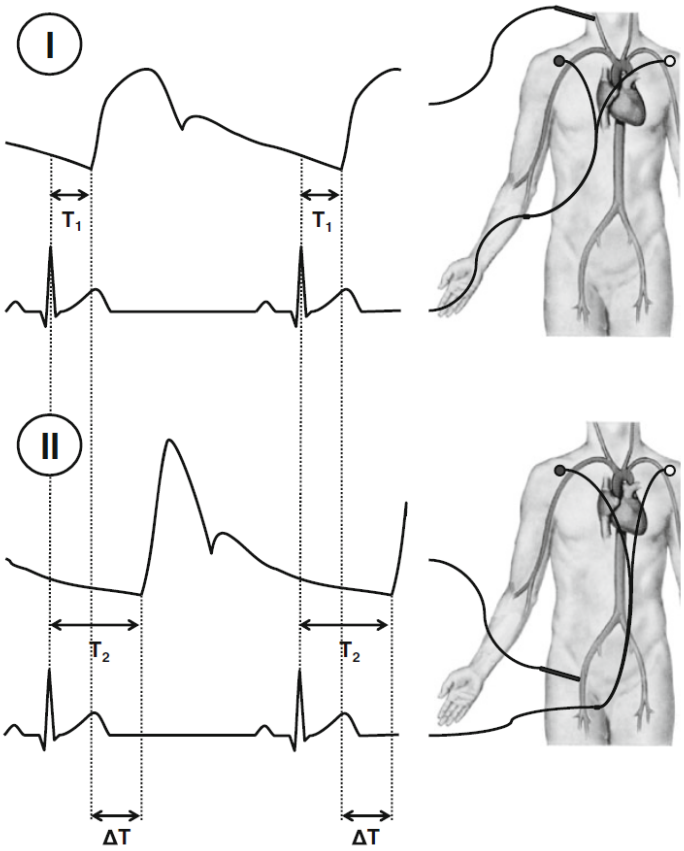


Figure 4. Schematic representation of the two-time cf-PWV recording. (I) recording of the carotid pulse wave; (II) recording of the femoral pulse wave. Two-stage acquisition requires ECG tracing[24].

The foot of the wave was obtained using the intersecting tangent method (ITM) algorithm. Average time delay between the two waves foots (pulse transit time, PTT) is then calculated (Figure 5).

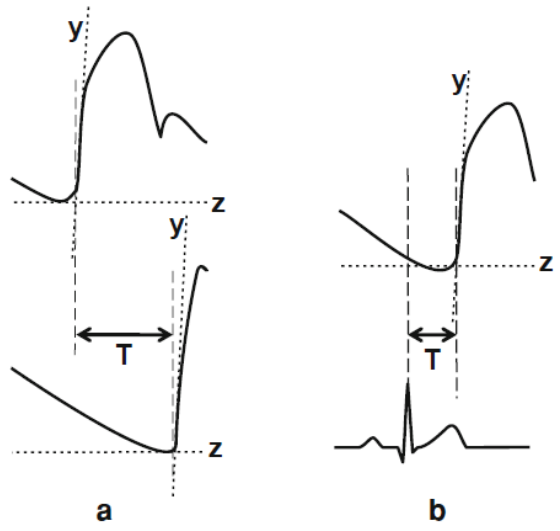


Figure 5. Measurement of the transit time (T) a) of the peripheral wave (below) in relation to the central wave (above); b) determination of the foot of the wave and the time (T) in relation to the QRS complex.

The inputted distance between recording sites *d* can be estimated by superficial measurement and calculated with the "80% method" (direct carotid-femoral distance multiplied by a corrective factor of 0.8) as underlined in international guidelines. The cfPWV is then calculated as follows:

cfPWV (m/s) = (d / PTT)

If the percentage standard deviation (SD, %) of the acquisition was greater than 10, a further measurement was carried out (always by the same operator), discarding the previous one. After each measurement, blood pressure and heart rate were measured with a validated semiautomatic sphygmomanometer (Omron Matsusaka, Kyoto, Japan), to verify the hemodynamic stability of the test subject. In the statistical analysis, the average of the 3 measurements was considered.

PWV by ATHOS

The development of the new device [25] was the result of numerous preliminary tests to determine: a) the most accurate sensor; b) the correct shape of the supports for the tonometers in terms of ergonomics; c) the correct pressure to be exerted. The purpose was to obtain a facilitated and simultaneous acquisition of an accurate and stable signal. ATHOS is a research device compliant with the European regulation for the safety of medical devices (IEC 60601).

As shown in Figure 6, the device is composed of a main unit that collects the signals from two tonometric sensors (developed by STMicroelectronics, Agrate, Italy), capable of detecting changes in surface tension, and an external diagnostic device for the acquisition of the electrocardiographic

signal. These signals, after being acquired synchronously, are sent via Bluetooth to a laptop, where a Graphic User Interface (GUI) allows their processing and display.

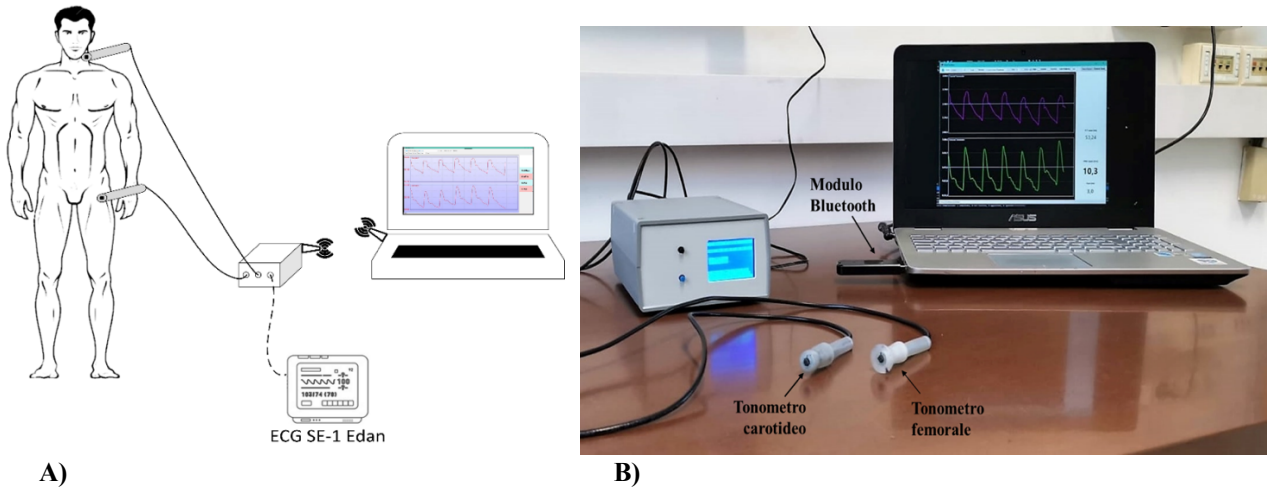


Figure 6 – Athos device summering scheme (A) and iconographic representation (B). It is composed by the main device unit (that collects the two pulse waves), the electrocardiogram, and the data processing software interface, running on the operator laptop. The two tonometers allow the simultaneous acquisition of the pulse waves at the carotid and femoral level. The device and the computer are connected by low energy v4.1 Bluetooth.

To facilitate their handling and use, both sensors have been inserted into two distinct supports made using a 3D printer with biocompatible resin. The probes (Figure 7) have different shapes to detect pulse waves in the two different sites, at the carotid and femoral arteries levels respectively.

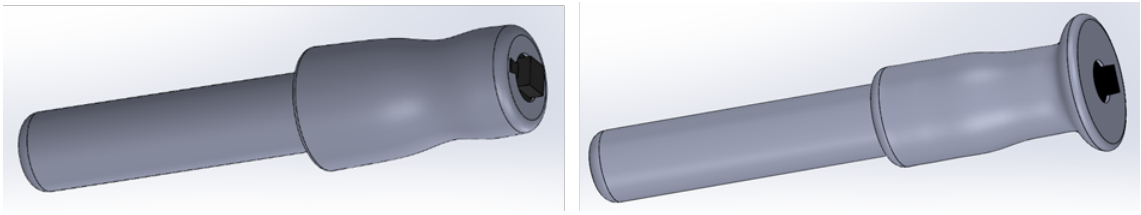


Figure 7 - Graphic model of the tonometers 3D printed pen supports for the MEMS sensor on which the tonometers have been installed. They show a particular handle to facilitate use and stabilize the signal. Left: carotid tonometer; right: femoral tonometer

Eventually, after identifying the sites for the pulse wave detection and positioning the two sensors, the operator verifies the quality of the signals acquired by the GUI. An immediate and real-time feedback allows both repositioning and modulation of the pressure to be exerted on the probes.

After the identification of a stable signal with an appropriate quality, the signal can be acquired by pressing the space bar on the control console. The final report will display the two pulse signal tracks (one for each site of acquisition), recorded in the last ten seconds, individual PTTs obtained for each beat (through the implementation of the ITM) and the final cfPWV value, achieved by applying a discard criterion to the values extracted in the ten seconds considered.

PULSE WAVE ANALYSIS

PWA was recorded radially with a validated instrument equipped with a transcutaneous applanation tonometer (SphygmoCor System, Atcor Medical, Sydney, Australia) after about 15 minutes of supine rest. Two consecutive recordings were made (Figure 8). The average value of the two measurements was used in the statistical analysis. If one of the two measurements did not meet the accuracy standards (see below), a third measurement was performed, and the two acquisitions meeting quality standard were considered in the statistical analysis.

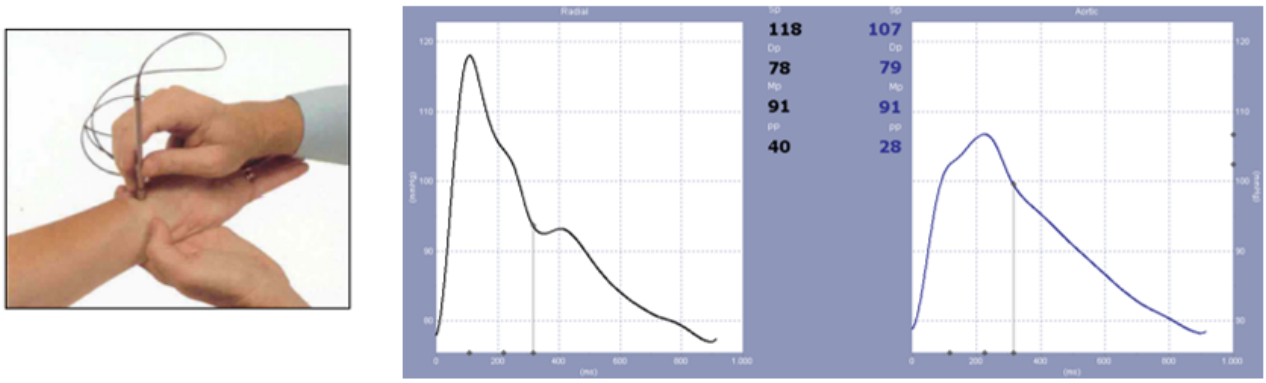


Figure 8. Evaluation of radial waveform (left and center) by PWA and generation of aortic waveform through the transfer function (personal data)

After each acquisition, blood pressure and heart rate were measured with a validated semiautomatic sphygmomanometer (Omron Matsusaka, Kyoto, Japan), equipped with an adequately sized cuff and operated by a healthcare professional. The central blood pressure values (systolic - SBPc; diastolic - DBPc; mean - MBPc; pulse pressure - PPc) were obtained from the pulse wave profile at the radial level (Figure 9).

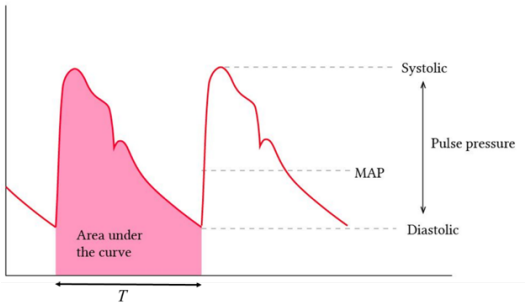


Figure 9. Time course of the pressure wave P (t). T: cardiac period

The central augmentation index (AIx) was calculated as follows:

AIx = (augmentation pressure / (SBP - DBP)) X 100

where SBP and DBP are the systolic and diastolic blood pressure values respectively, while augmentation pressure is the increase in pressure due to the reflex component of the PW (corresponds to the wave profile from the inflection point up to the maximum value systolic, Figure 10).

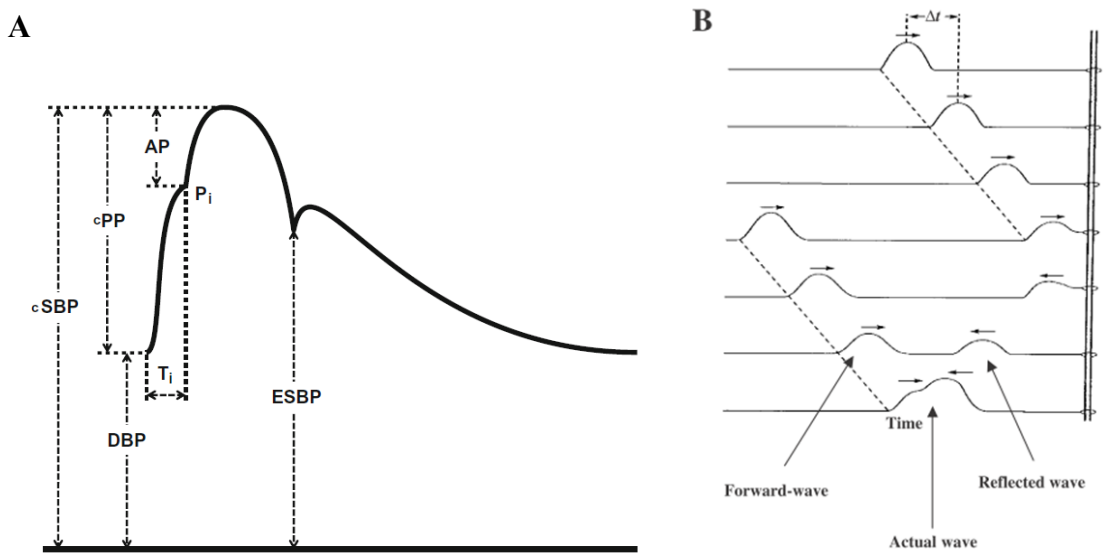


Figure 10. Central pulse wave and main parameters. On the left (A) illustration of the pulse wave recorded centrally, that derives from the sum of the antegrade wave and the reflected wave (B). SBPc: systolic pressure; DBP: diastolic pressure; PP: pulse pressure; ESBP: end systole pressure; AP: augmented pressure; Pi: point of inflection; Ti: wave reflection time [24,26].

The pulse pressure amplification index (PPA) was calculated as:

PPA = ((PPp - PPc) / PPc) x 100

where pulse pressure is the difference between the systolic and diastolic blood pressure values, measured at the brachial (periferal pulse pressure, PPp) or central level by PWA (central pulse pressure, PPc).

Echocardiography

A complete two-dimensional echocardiogram (TTE) was performed by commercially available ultrasound systems equipped with tissue Doppler imaging software (iE33, Philips Medical System, Andover, Massachusetts). Multiple frequency phased array transducers (2–4 MHz) were used. The TTE was performed by EACVI (European Association of Cardiovascular Imaging) accredited personnel. Patients were examined at rest in left lateral decubitus, with ECG monitoring and continuous respirometer. Standard 2D and Doppler images were acquired and archived in a continuous loop format (cine-loop), and measurements were performed offline. Measurements of the heart chambers, left ventricular mass, systolic and diastolic function were performed according to current international recommendations[27].

Briefly, left ventricular mass (LVM) was estimated from left ventricular telediastolic internal diameter (LVIDd), inter ventricular septum (IVS), and inferolateral wall thickness (ILW) and was indexed by BSA. The relative wall thickness (RWT) was calculated as (2ILW) / LVIDd. Left ventricular hypertrophy (LVH) was defined as a BSA-indexed left ventricular mass greater than 95 g/m2 in women or greater than 115 g/m2 in men. BSA was calculated using the Dubois and Dubois formula: BSA = 0.20247 [weight 0.425 x (height / 100) 0.725].

The size of the aorta was measured using both one-dimensional Motion-mode (M mode) 3 cm above the aortic valve, and two-dimensional echocardiography at the SOV and ASC level from a parasternal long axis standard view, as the maximum distance between the two upper edges of the anterior and posterior root walls at the end of diastole ("leading edge to leading edge" approach), thus considering the thickness of one wall and excluding the other (Figure 11). The first operative definition of proximal aortic dilation adopted in our study was based on absolute gender-specific measurements [28]. However, in a second moment we used the aortic diameter indexed for BSA in normal body size and indexed for height in extreme body size (obese patients) as underlined in the last guidelines recommendation [29]. Eventually, in consideration of the updated nomograms for aortic size, we began to follow patients with aortic size larger than expected for gender, age and BSA in a given reference population. Therefore, these new findings were adopted in our clinical practice and the z score of the aortic dimensions was calculated, applying a new definition of aortic dilation to the study population. The z score expresses the aortic diameter in terms of standard deviations from the mean value of the variable considered in the general population. Therefore, dilated patients are those with aortic diameters that exceed the expected for gender, sex and BSA by more than two standard deviations, as in previously published models [30]. Therefore, patients with a z score of less than 2

were used as control population, consisting of hypertensive patients with a SOV diameter within the expected in general population.

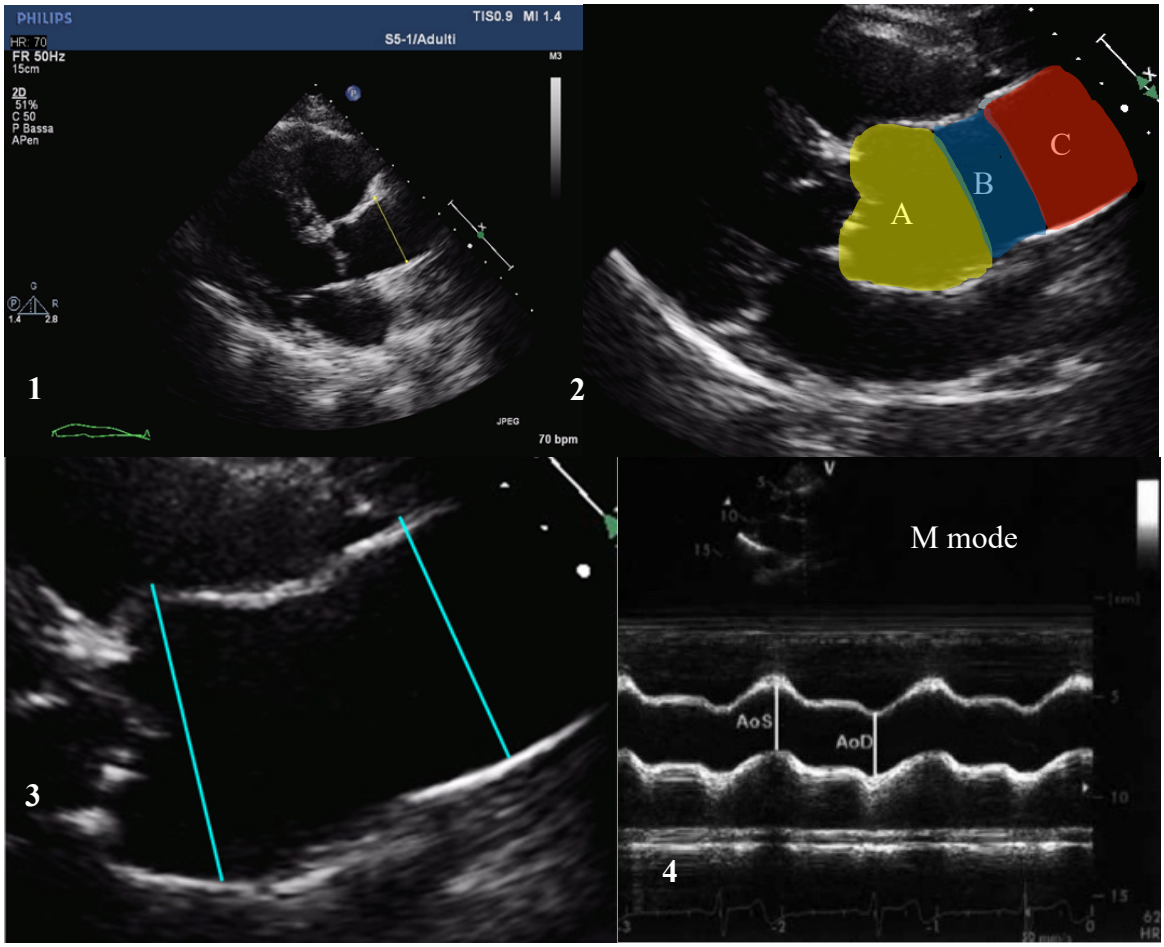


Figure 11. Evaluation of proximal thoracic aorta by transthoracic echocardiography (TTE) 1) Parasternal long axis view 2) various segments of the proximal aorta A) sinus of Valsalva; B) sinotubular junction; C) ascending aorta 3) leading edge to leading edge approach 4) M mode on ascending aorta [31]. Aortic systolic diameter (AoS) was measured at the maximum anterior motion of the aorta, and aortic diastolic diameter (AoD) was measured at the peak of the QRS complex on the recorded electrocardiogram, simultaneously

Statistical analysis

The statistical analysis was performed with dedicated software (SPSS - Statistical Package for the Social Sciences, v22 for Microsoft Windows, SPSS Inc. Chicago, Illinois, USA). The normal distribution of the variables was verified by graphical evaluation and Shapiro-Wilk test. Descriptive statistics are reported as "mean ± standard deviation". The categorical variables are reported as "frequency (percentage)". A two-sided Student's *t* test for continuous variables was performed to verify presence of a significant difference with a threshold of $P < 0.05$. The groups of subjects were compared by ANOVA, while the post-hoc analyzes were performed by Bonferroni tests. For the analysis of the correlation between cfPWV and anthropometric and hemodynamic parameters, the average of 3 measurements performed for each subject was used. The accuracy of the instrument being validated was assessed by Bland-Altman plot and linear regression analysis. The correlation coefficient was evaluated by Pearson correlation coefficient, using a cut off value of > 0.8 for identifying a strong correlation.

The reproducibility was assessed as coefficient of repeatability (1.96 × standard deviation of differences of the measurements), while the within-subject coefficient of variation was calculated as the square root of the mean standard deviation/average of the measurements. Significant results were considered with p value <0.05.

Intra-observer agreement for cfPWV for SphygmoCor compared to ATHOS was analyzed by intraclass correlation coefficients (ICC) estimates and their 95% confidence interval, based on a single-rating, absolute-agreement, 2-way mixed-effects model.

In the same group, 10 patients were randomly selected and measurements of two independent blinded observers were compared. Inter-observer agreement for PWV_{ATHOS} was analyzed by ICC based on a mean-rating ($k = 2$), absolute-agreement, 2-way mixed-effects model. Values less than 0.5 are indicative of poor reliability, values between 0.5 and 0.75 indicate moderate reliability, values between 0.75 and 0.9 indicate good reliability, and values greater than 0.90 indicate excellent reliability[32].

Any difference between the measurements obtained with the gold standard technique (Sphigmocor) and experimental approach tested in the present study (ATHOS) was considered as an error; independent variables that could be associated with such an error were searched for and used to perform a multivariate linear regression analysis.

3.3 RESULTS

Study population

90 healthy voluntaries were involved in the study. The clinical and anamnestic characteristics of the subjects are summarized in Table 2, while echocardiographic parameters in Table 3. Population's mean age was 45.6 ± 17.8 years, ranging from 18 to 86 years. Subjects were divided into 3 groups depending on age: age < 30, 30 to 59, and ≥ 60 years old (mean of 24.5 ± 2.8, 47.3 ± 8.3, 65.1 ± 6.5 years, respectively).

Variable (Mean±Sd)	General population (n=90)	Group <30 (n=30)	Group 30-59 (n=30)	Group ≥ 60 (n=30)	p value ANOVA
Age	45.6 ±17.8	24.5 ± 2.8$^{\#}$	47.3 ±8.2§	65.1 ± 6.5*	< 0.001
Gender (male, %)	48 (53.3%)	17 (56.7%)	15 (50%)	16 (53.3%)	0.878
Weight (kg)	68.2 ±13.6	65.2 ±10.5	68.3 ± 15.2	70.9 ± 14.5	0.271
height (m)	1.70 ±0.1	1.71 ±0.1	1.71 ± 0.1	1.68 ± 0.1	0.480
BMI (kg/m^2)	23.4 ±3.5	22.1 ± 1.8	23.2 ± 4	24.8 ± 3.9*	0.007
Waist (cm)	87.1±11.4	80.5 ± 7.9	87 ± 10.9§	94.1 ± 10.9*	<0.001
BSA (m^2)	1.79±0.2	1.77 ± 0.2	1.79 ± 0.23	1.80 ± 0.21	0.778
SBP (mmHg)	116 ± 13	113 ± 12	114 ± 13§	120 ± 13*	0.053
DBP (mmHg)	72 ±8	68 ±7$^{\#}$	73.1 ± 9	75 ± 8*	0.004
PP (mmHg)	44 ± 9	45 ± 8$^{\#}$	41 ± 9§	46 ± 9	0.039
MAP (mmHg)	86.6 ± 9.0	83.2 ± 8.0	86.6 ± 9.2	89.9 ± 8.7*	0.014
HR (rpm)	66 ±12	68 ± 12	65.5 ± 11	66 ± 12	0.674
Smoke	27 (30%)	4 (13.3%)$^{\#}$	11 (36.7%)	12 (40%)*	0.049
Alcool	25 (27.8%)	1 (3.3%)$^{\#}$	11 (36.7%)	13 (43.3%)*	0.001
Sport	68 (75.6%)	19 (63.3%)	25 (83.3%)	24 (80%)	0.159
Fam_CV	53 (58.9%)	22 (73.3%)	18 (60%)	13 (43.3%)*	0.061

Table 2. Anthropometric and anamnestic parameters of the study population (whole and age-based groups). *BMI: body mass index; BSA: body surface area; Waist: abdominal circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure; PP: pressure pulse; HR: heart rate; Fam_CV: family history for cardiovascular diseases. #: p <0.05 between group <30 and group 30-59; §: p <0.05 between group 30-59 and group ≥ 60; *: p <0.05 between group <30 and group ≥ 60*

Echographic Variable (Mean±Sd)	General population (n=90)	Group <30 (n=30)	Group 30-59 (n=30)	Group ≥ 60 (n=30)	p value ANOVA
SoV	31.6 ± 4.1	29.2 ± 3.0#	32.1 ± 4.1	33.5 ± 3.8*	< 0.001
Asc	29.9 ± 4.7	25.4 ± 1.7#	30.5 ± 4.0§	33.5 ± 3.8*	< 0.001
EF	63.3 ± 5.2	62.2 ± 4.6	64 ± 5.9	63.6 ± 5.1	0.374
E/A	1.36 ± 0.54	1.84 ± 0.46#	1.3 ± 0.44§	0.9 ± 0.19*	< 0.001
E/e'	6.5 ± 1.8	5.3 ± 0.9#	6.3 ± 1.4§	7.8 ± 1.9*	< 0.001
LAVi (mm3/m2)	20.1 ± 6.6	18 ± 5.6	19.5 ± 5	22.6 ± 8.2*	0.022
RWT	0.38 ± 0.08	0.37 ± 0.07	0.38 ± 0.08	0.4 ± 0.09	0.536
LVM (g)	121.5 ± 37.1	116.2 ± 32.9	113.7 ± 30.3§	134.6 ± 44.2	0.057
LVMi (g/m²)	67.3 ± 16.2	65.3 ± 14.7	62.9 ± 12.1§	73.8 ± 19.3	0.021
LVR (%)	18 (20%)	7 (23.3%)	4 (13.3%)	7 (23.3%)	0.490
LVH (%)	1 (1.1%)	0 (%)	0 (%)	1 (3.3%)	0.302

Table 3. Echocardiographic parameters of the study population (whole and age-based groups). *SoV: Sinus of Valsalva; Asc: Ascending aorta; EF: ejection fraction; E: E wave on Transmitralic Doppler ; A: A wave on Transmitralic Doppler ; e': mean tissutal doppler E wave; E/A: E wave on A wave ratio; E/e': E wave on e' wave ratio; LAVi: Left Atrium Volume Indexed for BSA; RWT: relative wall thickness; LVM: left ventricular mass; LVMi: LVM indexed for BSA; LVR: left ventricular remodeling; LVH: left ventricular hypertrophy. #: p <0.05 between group <30 and group 30-59; §: p <0.05 between group 30-59 and group ≥ 60; *: p <0.05 between group <30 and group ≥ 60*

Validation of the ATHOS instrument

The PWV and PTT values of the examinated population, measured with the reference instrument SphygmoCor and with ATHOS are summarized in Table 4.

Variable (Mean ± SD)	General population (n=90)	Group <30 (n=30)	Group 30-59 (n=30)	Group ≥ 60 (n=30)	p value ANOVA
PTT_{ATHOS} (ms)	64.99 ± 13.6	77.40 ± 9.65#	64.58 ± 8.39§	53.00 ± 9.65*	< 0.001
PWV_{ATHOS} (m/s)	7.88 ± 1.96	6.30 ± 0.96#	7.79 ± 1.10§	9.54 ± 2.06*	< 0.001
PTT_S (ms)	66.4 ± 14.77	80.13 ± 12.24#	64.5 ± 8.89§	54.57 ± 9.83*	< 0.001
PWV_S (m/s)	7.73 ± 1.95	6.12 ± 1.04#	7.81 ± 1.11§	9.25 ± 2.07*	< 0.001
ΔPWV (m/s)	0.15 ± 0.56	0.18 ± 0.48	-0.02 ± 0.58	0.29 ± 0.59	0.104
ΔPTT (ms)	-1.40 ± 5.56	-2.73 ± 6.93	0.09 ± 5.13	- 1.57 ± 4.08	0.143

Table 4. Pulse wave velocity parameters of the study population (whole and age-based groups).. *PTT: pulse transition time; PWV pulse wave velocity; PTT_{ATHOS}: PTT by ATHOS; PWV_{ATHOS} : PWV by ATHOS; PTT_S: PTT by Sphigmocor; PWV_S : PWV by Sphigmocor; ΔPWV: difference between PTT_{ATHOS} and PTT_S; ΔPTT: difference between PWV_{ATHOS} and PWV_S. #: p <0.05 between group <30 and group 30-59; §: p <0.05 between group 30-59 and group ≥ 60; *: p <0.05 between group <30 and group ≥ 60*

The average cfPWV measured with ATHOS (PWV_{ATHOS}) and with SphygmoCor ($PWV_{SphygmoCor}$) was 7.88 ± 1.96 m/s and 7.72 ± 1.95 m/s respectively (p= 0.013, Figure 12).

The correlation between the two measurements showed a R = 0.959 (p <0.001). The mean difference was 0.15 ± 0.56 m/s.

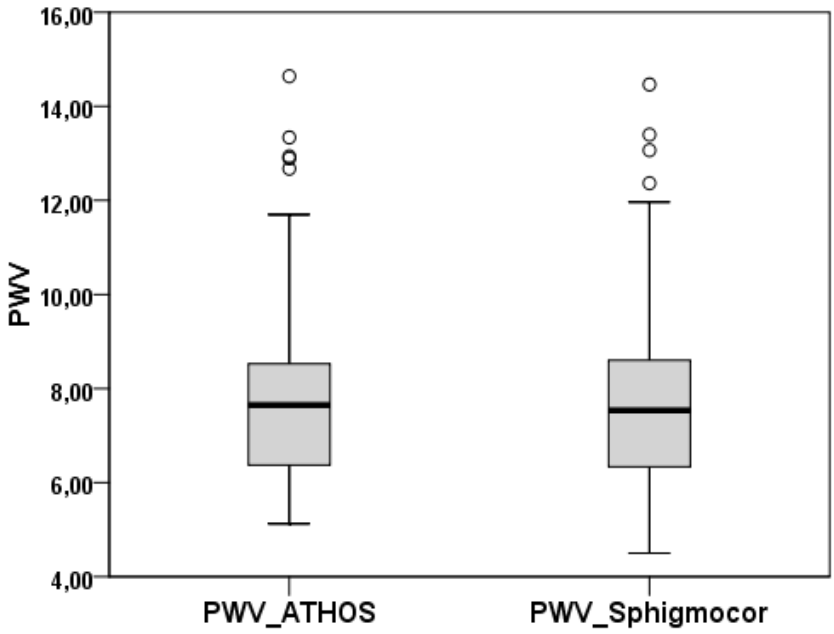


Figure 12. Box-plot comparison of PWV from SphygmoCor device (PWV_SphygmoCor) and the new ATHOS device (PWV_ATHOS). An excellent correlation was observed between PWV_ATHOS and PWV_SphygmoCor (7.88 ± 1.96 m/s and 7.72 ± 1.95 m/s respectively, r = 0.959, p = 0.013)

The coefficients of repeatability for ATHOS and SphygmoCor were 0.96 and 1.04 m/s respectively, while the coefficient of variation for ATHOS was significantly lower than SphygmoCor (3.5 % vs 4.3 % respectively, p = 0.01). Analyzing the intra-observer agreement between the evaluations with the same device, ICC were 96.5% (95.0 – 97.5) and 95.7 (94.0-97.0) for ATHOS and Sphygmocor respectively.

The Bland-Altman plot and the linear regression for PWV and PTT are showed in Figure 13 and 14 respectively.

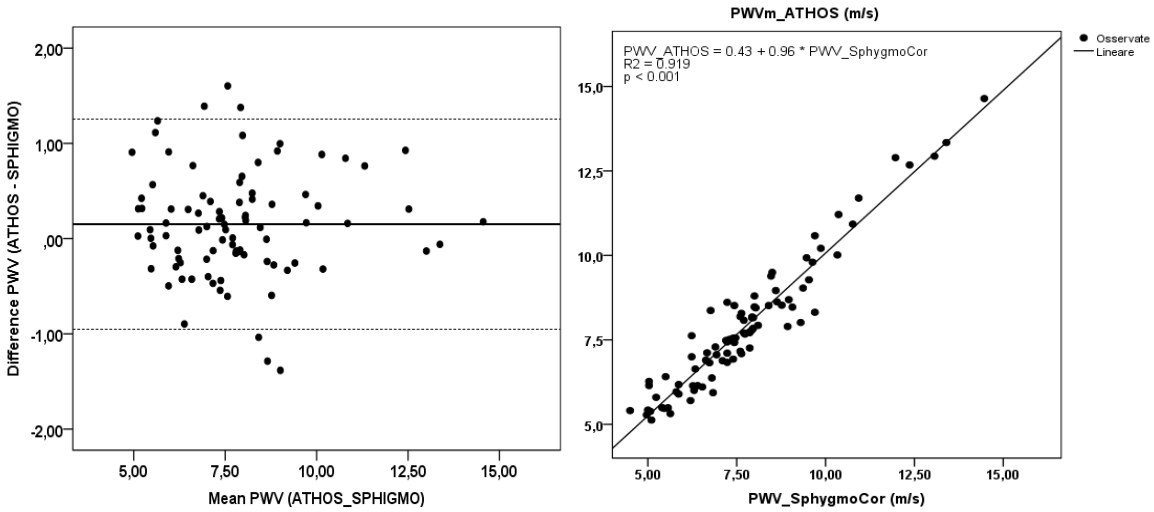


Figure 13 Comparison of PWV from SphygmoCor device and the new ATHOS device. A) Bland Altman plot of the difference. B) Scatter plot with linear regression (solide line)

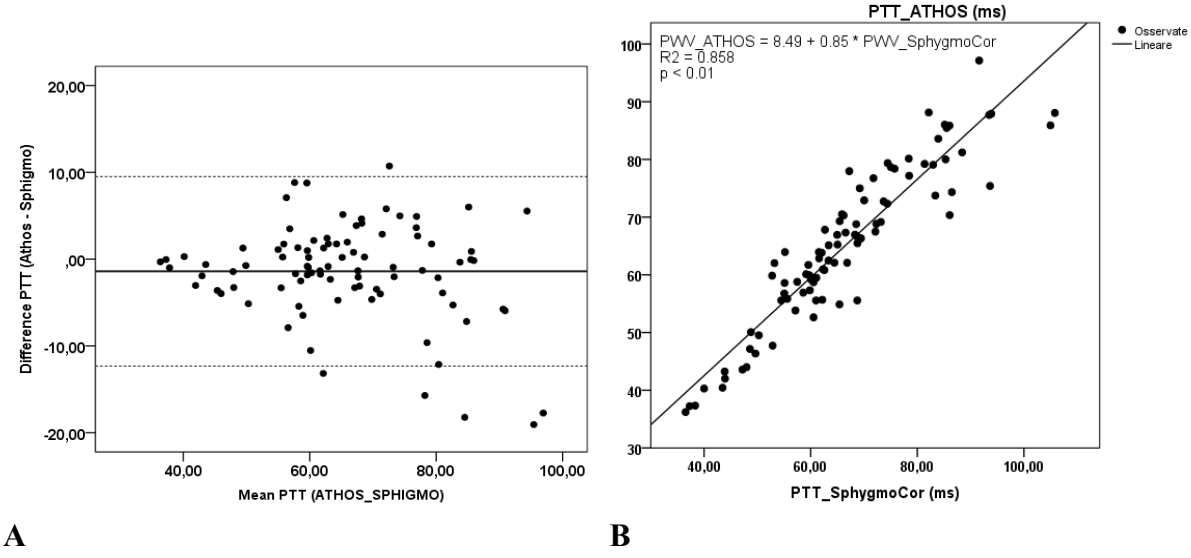


Figure 14. Comparison of PTT from SphygmoCor device and the new ATHOS device. A) Bland Altman plot of the difference. B) Scatter plot with linear regression (solide line)

Considering the cases with PWV ≥ 8 m/s (30 subjects), a difference between the measured PWV values of 0.1 ± 0.63 m/s was demonstrated, while considering the cases with PWV ≥ 9 m/s (18 subjects) the difference was 0.04 ± 0.67 m/s.

There was no statistically significant difference between the mean differences for both PWV and PTT in the 3 groups (p = 0.104 and 0.143 respectively). Considering the 3 groups separately, the two

measurements correlated significantly in each group (r = 0.889, p <0.001; r = 0.857, p <0.001; r = 0.959, p <0.001 respectively).

Analyzing possible variables related to the difference between $PWV_{SphygmoCor}$ and PWV_{ATHOS}, no anatomic, demographic, echocardiographic or hemodynamic variables resulted to be significant predictors of such a discrepancy (data not shown).

Reproducibility

Reproducibility of results between two operators using ATHOS was excellent, with ICC of 98% (91-99). Furthermore, the averages of the acquisitions made by the two operators were 6.61 ± 1.1 m/s and 6.68 ± 1.16 m/s respectively, with no statistically significant difference (p = 0.397).

Pulse Wave Analysis

The PWA values of the total population and the 3 age groups are shown in Table 5.

$cfPWV_{ATHOS}$ direct correlation to AIx was present (r = 0.611; p <0.001) and showed a significant inverse linear correlation with PPA (r = - 0.610; p <0.001). Moreover, $cfPWV_{ATHOS}$ was significantly related to central emodynamic parameters (SBPc (r = 0.688; p <0.001); DBPc (r = 0.357; p <0.001); MBPc (r = 0.552; p <0.001); PPc (r = 0.650; p <0.001)).

Variable (Mean ± SD)	General population (n=90)	Group <30 (n=30)	Group 30-59 (n=30)	Group ≥ 60 (n=30)	p value
SBP_P(mmHg)	118 ± 13	113 ± 12	117 ± 13§	124 ± 11*	0.003
DBP_P (mmHg)	73 ± 7	69 ± 6$^{\#}$	73 ± 8	76 ± 6*	0.001
SBP_C (mmHg)	106 ± 13	98 ± 9$^{\#}$	108 ± 12§	115 ± 11*	< 0.001
DBP_C (mmHg)	73 ± 8	69 ± 6$^{\#}$	74 ± 8	77 ± 6*	0.001
MAP_C (mmHg)	85 ± 9	79 ± 6$^{\#}$	86 ± 9	89 ± 7*	<0.001
PP_C (mmHg)	33 ± 9	28 ± 8$^{\#}$	34 ± 8§	38 ± 10*	<0.001
AIx (%)	19.31 ± 15.8	4.58 ± 10.98$^{\#}$	23.73 ± 10.52	29.60 ± 13.42*	<0.001
PPA	139.83 ± 21.02	28.21 ± 7.80$^{\#}$	132.90 ± 15.51	127.10 ± 16.14*	<0.001

Table 5. Pulse wave analysis parameters of the study population (whole and age-based groups). *SBP_P: periferal Systolic Blood Pressure; SBP_C: central Systolic Blood Pressure ; DBP_P: periferal Diastolic Blood Pressure; DBP_C: central Diastolic Blood Pressure; MBP_C: central Mean Blood Pressure; PP_C: central Pulse Pressure; Aix (%): Augmentation Index; PPA: Pulse Pressure Amplification #: p <0.05 between group <30 and group 30-59; §: p <0.05 between group 30-59 and group ≥ 60; *: p <0.05 between group <30 and group ≥ 60*

3.4 DISCUSSION

In this study, a new ATHOS instrument for the non-invasive evaluation of arterial stiffness and its correlation with SphygmoCor were tested. The ATHOS device was born from the collaboration with Politecnico di Torino and STMicroelectronics. It showed an excellent level of agreement with *Sphygmo*Cor, even at high PWV values, with a good reproducibility.

Compared to currently available devices, ATHOS features numerous advantages and technologic innovations, reaching the same performance of other commercial devices at lower cost. In particular, compared to Complior which uses a piezoelectric mechanical transducer, ATHOS uses standard electronics and a modified commercial MEMS pressure sensor instead of traditional force sensors. Both determine the foot of the wave through ITM. While Complior has a sampling rate of 1 kHz, in ATHOS device the MCU sampling frequency is set at 680 Hz, to better synchronize both the digital output of MEMS pressure sensors and the analog ECG signal which ensures a temporal resolution of 1.5 ms. It allows the simultaneous acquisition of two impulse waves, the real-time display of the acquired signals, the instant cfPWV parameter and the quality factors to improve their estimation, as better explained in the technical paper by Buraioli et al[25].

To facilitate their handling and use, both sensors have been inserted into two distinct pen-shaped supports specifically created using a 3D printer with biocompatible resin. They have different shapes that leads to a better positioning and the best signal-to-noise ratio, in order to better detect pulse waves in the two different sites, femoral and carotid. The probes (figure 7) are ergonomically designed to have the best performance for their application site. Lastly, dimensions of the final device are strongly reduced leading to an improved portability.

Our population included healthy normotensive individuals, within a wide age range, an equal distribution between genders and a wide range of PWV values. We did observe a significant difference in mean cfPWV values between the two devices, probably due to the different way it was assessed. While SphygmoCor use sequential recordings of the waveform with ECG gating, ATHOS allows the non-invasive recording of the pulse wave simultaneously at the level of the carotid and femoral sites, providing a real-time acquired PWV value (obtained from the last 10 cardiac cycles recorded). Nevertheless, there was a strong correlation between the measurements ($r=0.959$, $p<0.001$) and furthermore this difference did not hinder the excellent accuracy of the ATHOS readings.

The ARTERY Society guidelines for the validation of tools for PWV measurements defines three classes of accuracy (poor, acceptable and excellent) based on the mean difference and the corresponding standard deviation [22]. An excellent accuracy is defined as mean difference <0.5 m/s and standard deviation ≤ 0.8 m/s. In our study we found an excellent level of accuracy, with an

average difference of 0.15 ± 0.56 m/s: in fact, ATHOS slightly overestimated the values compared to the SphygmoCor. In addition, the accuracy between the two methods remained “excellent” also considering the different age groups (<30, 30-59,≥ 60 years, Table 4).

In a recent review, comparing validation studies of devices for the non-invasive measurement of PWV, it was shown that accuracy between the methods under examination significantly decreased for cfPWV values > 8 m/s [21]. In our cohort, the average difference between SphygmoCor and ATHOS remained in the “excellent” range even for cfPW > 8 m/sec.

Excellent reproducibility was found with the ATHOS instrument, even slightly better than for SphygmoCor (coefficient of variation was 3.5 % with ATHOS and 4.3 % with SphygmoCor). ATHOS allows the simultaneous acquisition of the carotid and femoral pulse waves, while with SphygmoCor the acquisition is sequential, which represents a potential source of measurement variability, although available data in literature are controversial. Under perfectly controlled hemodynamic conditions it has been demonstrated that the simultaneity acquisitions or lack thereof does not affect the reproducibility of the measurement [33]. Despite this, in a validation study that compared SphygmoCor and Complior Analyze (which allows simultaneous acquisition), a slightly greater variability was found in the measurements performed with SphygmoCor [34]. Moreover, in a study conducted to evaluate the short-term repeatability of 6 devices, simultaneous acquisition did not prove to be a source of greater repeatability [35]. In our study, hemodynamic conditions were correctly monitored and controlled, to reduce possible sources of variability.

Other sources of variability can be identified in the method used to identify the foot of the pressure wave and measure the carotid-femoral distance to be used in the calculation. The same algorithm (ITM) for identification of the foot of the pressure wave identification was used in both devices in the present study, basically removing this issue. The ITM algorithm, in fact, has been considered the most accurate and least dependent on changes in reflection of waveform [36] , and for this reason its use is recommended by the Artery Society for PTT calculation [22].

As for the carotid-femoral distance, two methods are currently recommended by the guidelines, a “subtraction method” (distance from the femoral site to the sternal notch - distance from the carotid site to the sternal notch), and the “80% method” (direct carotid to femoral distance × 0.8) as they both demonstrated a high level of correlation with the invasive method in a study conducted in 915 patients[37]. However, the former requires two separate measurements, thus increasing the level of inaccuracy. Moreover, the “80% method”, which involves a single measurement, demonstrated the best correlation with the measurement of the aortic length performed by magnetic resonance imaging[38] and it was the method used in studies that identified the cfPWV 10 m/s cut-off for the

management and treatment of high blood pressure [20] . For the above reasons, the latter method was preferred in our study. Furthermore, distance is measured superficially and therefore may not be representative of the true aortic length. For this, to further reduce the possibility of error, the acquisition sites were marked on the skin of the voluntary subjects after careful palpation of the pulse by expert operators.

In our study, AIx and PPA were also measured with the validated SphygmoCor instrument as additional parameters for the measurement of arterial stiffness [39]. The linear regression analysis showed a significant correlation between the AIx and PPA values obtained from the pulse wave analysis recorded radially with SphygmoCor and the cfPWV values measured with ATHOS. Although AIx and PPA have shown a limited predictive value in terms of cardiovascular events or mortality compared to cfPWV measurement [40], the correlation with $cfPWV_{ATHOS}$ represents an added value in the evaluation of the accuracy of the new instrument for the viscoelastic arterial vessels properties assessment.

3.5 Limits of the study

This study has some limitations. Firstly, the ATHOS instrument was validated against the SphygmoCor, an instrument that allows a transcutaneous, non-invasive assessment, while the current gold standard is represented by the invasive measurement of the PWV. The intrinsic characteristics of the invasive measurement, however, preclude its applicability. In addition, the SphygmoCor tool is considered by the guidelines to be an alternative gold standard in validation studies and has recently been invasively validated in a very large number of subjects[37].

The BMI value represents a potential confounding in the surface measurement of PWV. The guidelines recommend exclusion from validation studies for subjects with BMI> 30 kg/m^2 [22]. In our study, 4 subjects with BMI greater than 30 kg/m^2 were included. Despite the increased BMI, the physical constitution did not prevent an accurate path length measurement between the two sites, and therefore they were considered in the final statistical analysis. Although this could represent a possible limitation of our study, the comparison of the transit times in these 4 subjects (which are not affected by the distance measurement) proved to be comparable with the two devices.

4. Local ascending aorta strain analysis

4.1 Summary

The advent of echocardiography software for strain analysis provided new tools for the dynamic assessment of cardiovascular structures deformation. Strain analysis focused on proximal aorta could be another non-invasive approach to evaluate vessel walls biomechanical properties, but so far it has been only experimentally applied for the evaluation of aortic function in few studies [41].

In order to verify the association between local aortic mechanic properties and aortic remodeling, we tested the usefulness aortic strain analysis, by echocardiographic technology of speckle tracking (ST) [42]. We focused our attention on the ascending aorta level, which showed to be more related to hypertension mediated organ damage [8].

Then we tested aortic strain assessment in 100 hypertensive outpatients with increasing dilation of the proximal thoracic aorta. β2 Stiffness Index (β2 SI) increases exponentially with the size of the AA, resulting in correlation with cardiovascular organ damage in terms of left ventricular mass and PWV [43].

4.2 PART ONE:

A new technique for non-invasive evaluation of ascending aorta biomechanical properties

4.2.1 MATERIALS AND METHODS

Peak ascending aorta strain (PaAS)

Measurements of the proximal aorta were performed in 2D TTE images in a parasternal long axis view. The region of interest was the section with the maximum measurable transverse diameter at ascending aorta level.

Aortic strain analysis is based on the identification of *spekles*, acoustic pixels in a gray scale. They are followed frame by frame during the cardiac cycle, allowing the calculation of their reciprocal movement (deformation). Peak ascending aorta strain (PaAS) was defined as maximum deformation of the proximal ascending aorta during a complete cardiac cycle (Fig. 15).

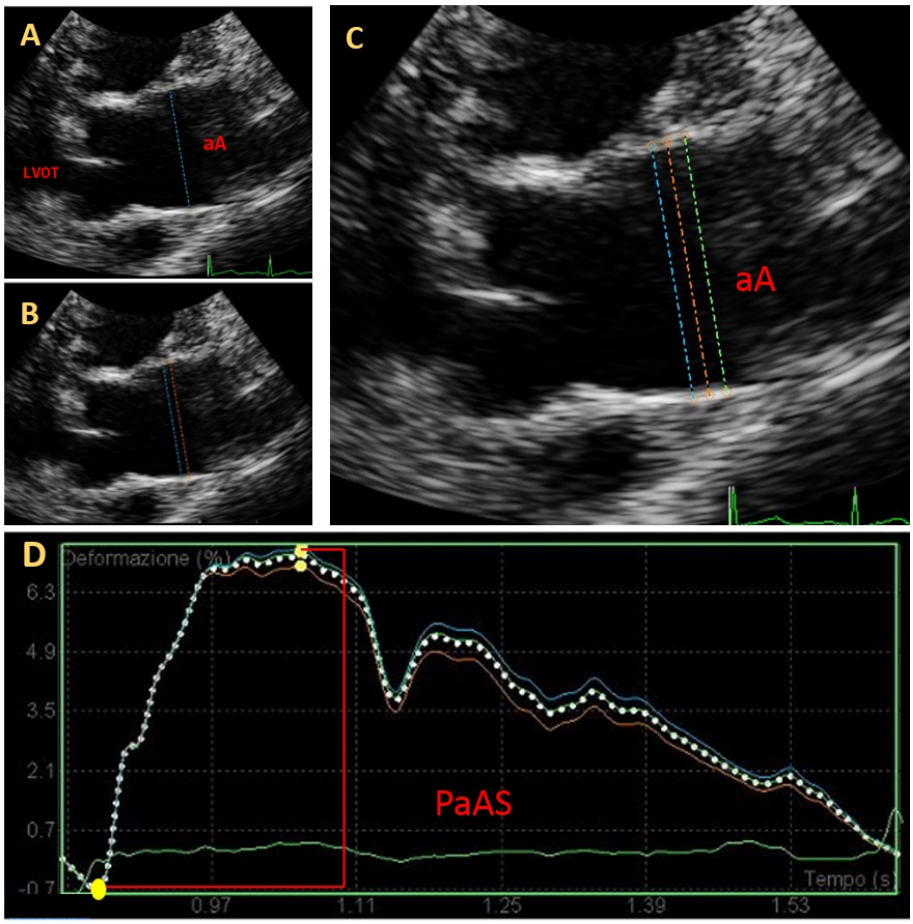


Figure 15: Simplified method for evaluating peak ascending aorta strain (PaAS). Three pairs of speckles are identified on the anterior and posterior walls of the ascending aorta and connected with 3 segments of different colors that define the cross section of the vase (panels A-C). The deformation of each segment during the cardiac cycle is calculated and plotted as a function of time (panel D). The software provides the average deformation of the three segments (dashed line in panel D). PaAS was obtained as the average of the maximum deformation of the 3 segments during the cardiac cycle.

The analysis was performed offline with dedicated software (QLAB v8.1 software, Philips, The Netherlands). Three pairs of speckles were identified within the anterior and posterior aortic walls and transverse segments connecting each pair were traced. Each segment was represented with a different color and its deformation was represented graphically as a function of time during the cardiac cycle, starting from the onset of the QRS complex on the ECG traces. The maximum deformation of each segment was calculated as a percentage deformation:

$$\Delta L = (Lmax - L0) / L0,$$

where L0 is the initial length at the beginning of the QRS complex.
The PaAS was then calculated as the average of the maximum percentage deformation of the three segments.
The aortic stiffness index (β2 Stiffness Index) was subsequently derived, as previously proposed:

$$\beta 2 \text{ stiffness Index} = 100 \times Ln (SBP / DBP) / PaAS$$

where SBP and DBP are the systolic and diastolic blood pressure, respectively, while PaAS is the peak ascending aorta strain.

4.2.2 RESULTS

A total of 60 normotensive patients with aortic diameters in the normal range were considered for the present study. All echocardiographic images were reviewed for quality and suitability for aortic root analysis. Suboptimal quality images were present in 17 studies, therefore the final study population consisted of 43 patients, whose clinical and echocardiographic characteristics are summarized in Table 6.

Variable (Mean ± SD)	
Age (years)	63.2 (42.8 – 69.8)
Gender (male, %)	25 (58.1%)
Weight (kg)	71.6 ± 12.5
Height (m)	1.70 ± 0.1
BMI (kg/m^2)	25.7 ± 3.3
BSA (m^2)	1.8 ± 0.2
SBP (mmHg)	132.5 ± 16.5
DBP (mmHg)	73.8 ± 6.7
HR (bpm)	69.7 ± 14.1
cfPWV (m/s)	8 ± 1.8
EF (%)	60.7 ± 4.7
LVM (g)	154.9 ± 46.2
LVMi (g/m2)	86.1 ± 23.8
Stroke volume (ml)	52.5 ± 16.2

Table 6: characteristics of general population. BMI: body mass index; BSA: body surface area; SBP: systolic blood pressure; DBP diastolic blood pressure; HR: heart rate; cfPWV: carotid-femoral pulse wave velocity; EF: ejection fraction, LVM: left ventricular mass, LVMi :left ventricular mass indexed to BSA

Study patients were predominantly male (n = 25, 58,1%), the mean age was 63.2 [42.8-69.8] years old. Aortic diameters were measured both at sinus of Valsalva (33.8 ± 3.6 mm) and ascending aorta (aA 32.3 ± 5.3 mm) level. Mean PaAS was 5.5% [3.7–8.6], with no significant differences between males and females (6.6% [4.4–10.4] vs 4.5% [3.3 –8.4] respectively, p = 0.334). PaAS showed significant inverse correlation with AA diameter (r = - 0.446, p = 0.003) as well as with patients' age (r = - 0.647, p <0.001), heart rate (r = - 0.497 , p = 0.001) and cfPWV (r = - 0.417, p = 0.01). No significant association was found between aortic root and standard hemodynamic parameters (systolic blood pressure, mean diastolic or pulse pressure), except for stroke volume (r = - 0.416, p = 0.01).

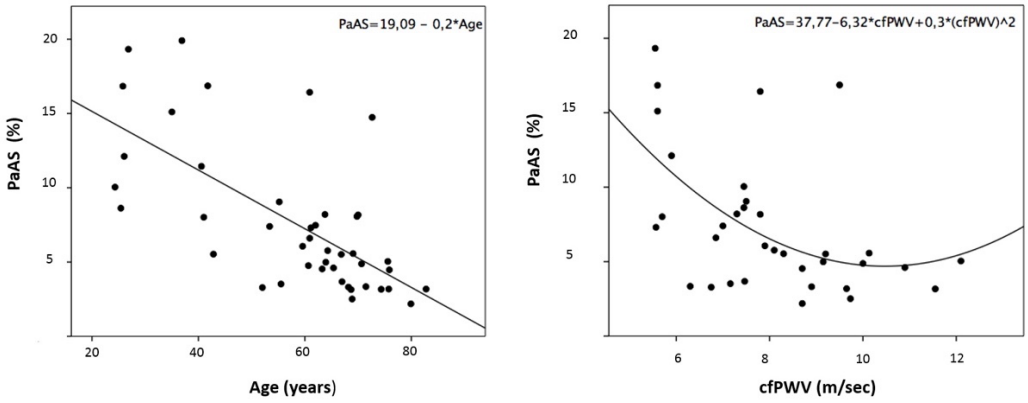


Figure 16: Correlation between PaAS and age (left panel), and cfPWV (right panel)

In a multivariate linear regression analysis (Table 7) after adjustment for gender, BMI, aortic size, systolic volume, and cfPWV, age remained the only independent determinant of PaAS. The model explained 42% of the variability of the deformation.

Variable	t	p
PaAS		
Gender	− 1.475	0.154
Age (years)	− 2.20	0.038
BMI (Kg/m^2)	− 0.266	0.792
aA (mm)	0.405	0.689
SV (ml)	1.05	0.302
cfPWV (m/s)	0.01	0.992
F 4.46 (p = 0.003), adjusted R^2 = 0.42		

Table 7: Multivariate regression analyzes for PaAS. BMI body mass index; aA: ascending aorta diameter; SV stroke volume; cfPWV: carotid-femoral pulse wave velocity;

The β2 stiffness index (β2 Stiffness Index, β2 SI) was found to be 10.8 [5.9-15.0], with no significant gender difference. It showed significant direct correlation with age (r = 0.608, p <0.001), pulse pressure (r = 0.426, p = 0.004), diameter aA (r = 0.429, p 0.004) and cfPWV (r = 0.370, p 0.03). There was no correlation with any of the echocardiographic parameters describing morphology or function of the left ventricle (particularly with left ventricular mass, systolic volume, or ejection fraction). The β2 SI index was not associated with anthropometric descriptors (i.e. BMI or BSA). In a multiple linear regression analysis that considered variables showing significant correlation on univariate analysis, the variables independently associated with β2 SI were pulse pressure and age, with the model accounting for 40% of its variability (Table 8).

Variable	t	p
β2 Stiffness index		
Age (years)	2.986	0.004
aA (mm)	− 0.779	0.650
cfPWV (m/s)	0.428	0.926
PP (mmHg)	2.171	0.038
F 6.5 ($p < 0.001$), adjusted $R^2 = 0.40$		

Table 8: Multivariate regression analyzes for β2 stiffness index. aA: ascending aorta diameter; cfPWV: carotid-femoral pulse wave velocity; PP pulse pressure

The inter-observer and intra-observer variability, assessed with the Interclass Correlation Coefficient (ICC) on 5 randomly selected patients, was 95% (confidence interval 0.59-0.99) and 97% (confidence interval 0.71–0.99).

4.3 SECOND PART: Impaired aortic deformation in dilation of the ascending aorta (aA)

4.3.1 RESULTS

A total of 148 hypertensive patients were enrolled in this second part of the study, but only 100 (68%) were included in the final analysis after the exclusion of patients with suboptimal images. In addition, a cohort of 55 healthy subjects was used to define the reference values of β2 SI. Considering a threshold value at the 95th percentile, median β2 SI in this population was 9 [7 - 14].

Clinical and echocardiographic characteristics of the 2 populations are shown in Table 9. Hypertensive patients were mostly male (74%), mean age was 65.8 ± 10.5 years, with a fair blood pressure control (SBP 141 ± 18, DBP 79 ± 11 and PP 62 ± 17 mmHg; 84 % of patients with BP < 140/90, 72 % with BP < 130/80). Mean LVMi was 104 ± 37 g/m2, while mean PWV was 9.32 ± 2.54 m/s. Mean aA was 40.7 ± 5.7 mm.

Variable	Normotensives (n=55)	Hypertensives (n=100)	p value ANOVA
Age	44.9 ± 16.6	65.8 ± 10.5	< 0.001
Gender (male, %)	31 (56%)	74 (74%)	0.025
Weight (kg)	70.7 ± 13.8	80.7 ± 14.2	< 0.001
Height (m)	1.71 ± 0.10	1.69 ± 0.1	0.445
BMI (kg/m^2)	24.2 ± 3.8	28.1 ± 3.7	< 0.001
BSA (m^2)	1.82 ± 0.21	1.92 ± 0.2	<0.001
SBP (mmHg)	122 ± 14	141 ± 18	<0.001
DBP (mmHg)	73 ± 8	79 ± 11	<0.001
PP (mmHg)	49 ± 11	62 ± 17	<0.001
HR (bpm)	72 ± 11	70 ± 13	0.288
aA (mm)	29.8 ± 4.5	40.78 ± 5.7	<0.001
EF (%)	63 ± 5	62 ± 7	0.469
LVMi (g/m2)	68 ± 15	104 ± 37	<0.001
SV (ml)	52 ± 17	53 ± 16	0.760
LAVi (ml/m2)	21 ± 7	30 ± 12	<0.001
PWV (m/s)	7.5 ± 1.6	9.32 ± 2.54	<0.001
Beta-stiffness index	9 (7–14)	21 (14–39)	<0.001

Table 9: Clinical and echocardiographic variables of the two populations, normotensives (n = 55) vs hypertensives (n = 100) aA, ascending aorta; EF, ejection fraction; HR: heart rate; LAVi, left atrial volume indexed to BSA; LVMi, left ventricular mass indexed to BSA; PP, pulse pressure; PWV, pulse wave velocity; SV, stroke volume.

β2 SI in hypertensive patients with ascending aorta dilatation

Dividing our population into three groups based on the ascending aorta size, 44 patients were in the first group (aA <40 mm), 31 patients in the second (aA 40 - 45 mm) and 25 patients in the third group (aA > 45 mm). The clinical and echocardiographic characteristics of the three groups and the comparison between the groups are summarized in Table 10. β2 SI, BMI, BSA and percentage of males were significantly different in the three groups (P <0.05).

	Normotensives	Hypertensives			
Variable	**(n = 55)**	**aA<40mm (n=44)**	**aA 40–45mm (n=31)**	**aA >45mm (n=25)**	**p value ANOVA**
Age	44.9 ± 16.6	64.0 ± 11.0	65.3±10.5	69.7±9.1	0.094
Gender (male, %)	31 (56 %)	27 (61%)	26 (84%)	21 (84%)	0.038
Weight (kg)	70.7 ± 13.8	75.9 ± 13.2	84.5 ± 10.6	84.6 ± 17.4	0.010
Height (m)	1.71 ± 0.10	1.67 ± 0.12	1.72 ± 0.07	1.70 ± 0.95	0.227
BMI (kg/m^2)	24.2 ± 3.8	26.9 ± 3.1	28.6 ± 3.0	29.39 ± 5.0	0.022
BSA (m^2)	1.82 ± 0.21	1.85 ± 0.22	1.97 ± 0.14	1.95 ± 0.22	0.017
SBP (mmHg)	122 ± 14	139 ± 18	141 ± 17	145 ± 18	0.429
DBP (mmHg)	73 ±8	77 ± 8	81 ± 12	81± 14	0.337
HR (rpm)	72 ±11	72 ± 14	70 ± 10	67 ± 13	0.306
aA (mm)	29.8 ± 4.5	35.5 ± 3.2	42.2 ± 1.5	47.8 ± 2.8	< 0.001
EF (%)	63 ± 5	63 ± 7	62 ± 7	60 ± 7	0.268
LVMi (g/m2)	68 ± 15	95 ± 24	107 ± 49	115 ± 38	0.089
SV (ml)	52 ± 17	51 ± 16	54 ± 19	53 ± 14	0.757
LAVi (ml/m2)	21 ± 7	30 ± 12	27 ± 10	32 ± 15	0.365
PWV (m/s)	7.5 ± 1.6	8.93 ± 2.03	9.15 ± 2.48	10.14 ± 3.17	0.169
Beta-stiffness index	9 (7-.14)	14 (10-21)	23 (15-36)	49 (8-86)	< 0.001

Table 10: Clinical and echocardiographic variables of the two populations, normotensive (n = 55) vs hypertensive (n = 100) patients divided into 3 groups based on aortic size: <40 mm, 40-45,> 45 mm. aA, ascending aorta; EF, ejection fraction; HR: heart rate; LAVi, left atrial volume indexed to BSA; LVMi, left ventricular mass indexed to BSA; PP, pulse pressure; PWV, pulse wave velocity; SV, stroke volume

Hypertensive drugs and comorbidities of our study population are shown in Table 11. No difference in antihypertensive therapy or clinical comorbidities was observed between patients with varying degrees of dilation of the ascending aorta.

Hypertensive patients					
Variable	**General population (n = 100)**	**aA<40mm (n=44)**	**aA 40–45mm (n=31)**	**aA >45mm (n=25)**	**p value ANOVA**
Antihypertensive therapy					
Active therapy (n, %)	88 (88 %)	35 (79%)	29 (94%)	24 (96%)	0.067
Number of drugs	2.0 (1.0–3.0)	2.0 (1.0-2.75)	2.0 (1.0–3.0)	2.0 (1.5-3.0)	0.052
> 3 drugs (n, %)	35 (35%)	11 (25%)	12 (39%)	12 (48%)	0.137
ACEI/ARB (n, %)	80 (80%)	33 (75%)	24 (77%)	23 (92%)	0.216
Vasodilators (n, %)	51 (51%)	18 (41%)	18 (58%)	15 (60%)	0.315
Diuretics (n, %)	33 (33%)	10 (23%)	12 (39%)	11 (44%)	0.140
Beta blockers (n, %)	33 (33%)	12 (27%)	11 (36%)	10 (40%)	0.524
Comorbidities					
Obesity (n, %)	19 (19%)	6 (14%)	5 (16%)	8 (32%)	0.155
Vascular pathology (n, %)	27 (27%)	9 (20%)	6 (19%)	12 (48%)	0.053
CKD (n, %)	4 (4%)	0 (0%)	2 (6%)	2 (8%)	0.186
DM (n, %)	9 (9%)	4 (9%)	2 (6%)	3 (12%)	0.771
Dyslipidemia (n, %)	50 (50%)	20 (45%)	15 (48%)	15 (60%)	0.498

Table 11: Antihypertensive therapy and comorbidities of hypertensive patients divided by aortic size. ACE-I, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; CKD, chronic kidney disease; DM: diabetes mellitus; Vasodilators, calcium channel blockers, alfa1-adrenergic receptor blockers, nitrates

Local mechanical characteristics of the ascending aorta

β2 SI had a broad distribution in our clinical population of hypertensive patients, with a median value of 21 (IQR 14–39). In patients with aA dilatation, transverse β2 SI was significantly higher than in patients with normal aA diameter [14 (10 - 20) vs 33 (20 - 53), respectively, P <0.001]. The β2 SI was found to increase with increasing size of the ascending aorta, with an exponential model that better explains their relationship (P <0.001, r = 0.60, R2 = 0.363) compared to a standard linear mode (P = 0.003, r = 0.295, R^2 = 0.087). Consequently, the stiffness of the local ascending aorta was

significantly different between the three patient groups, with increasing β2 SI values as aA diameters enlarge [14 (10-21) vs 23 (15-36) vs 49 (28-86), respectively, P <0.05, Figure 17].

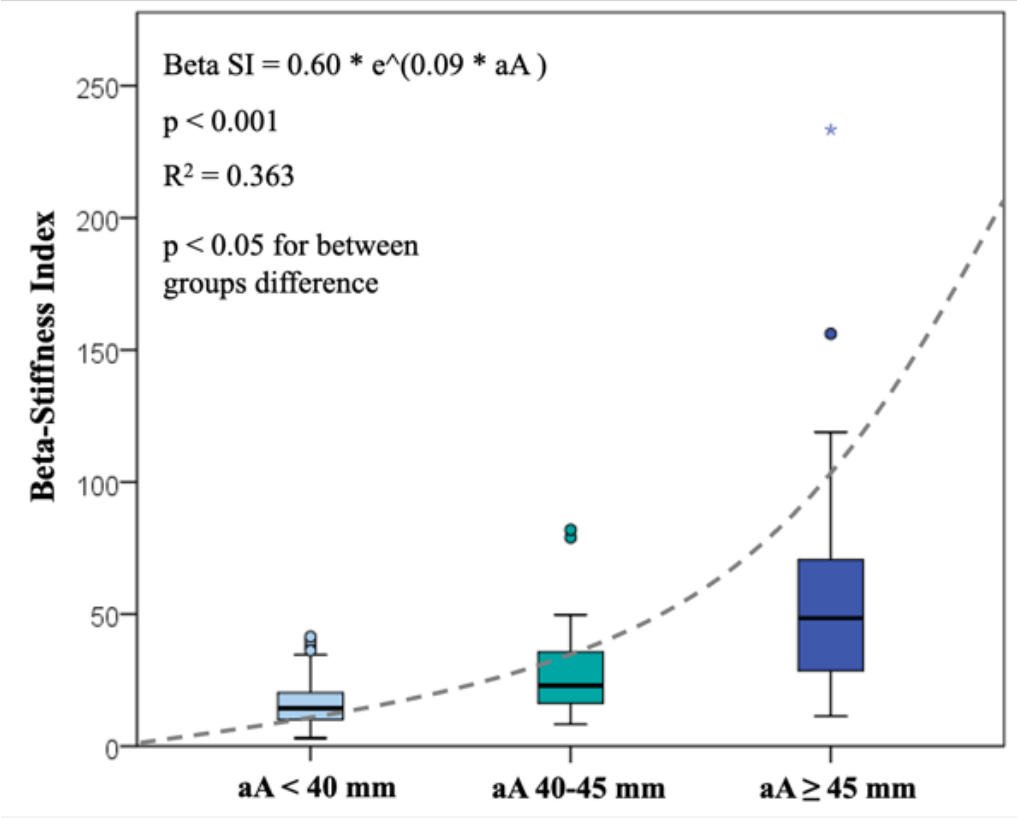


Figure 17: Exponential relationship between β2 stiffness index (SI) and aortic size. Data are displayed as a combination of box plot and regression curve. aA, ascending aorta; Beta-SI, β2 Stiffness Index, Stiffness Index β2.

Variability in β2 SI assessment was evaluated. The ICC for intra-observer and inter-observer agreement was 94% [confidence interval (CI) 79-99%] and 91% (CI 69-98%), respectively.

Vascular stiffness increases with aging, therefore our healthy population was divided in two different groups based on age. Considering a threshold value at the 95th percentile, age-specific reference values of β2 SI were estimated: it was 18 in the first group (patients aged < 55 years old, n = 34) and 25 in the second group (patients aged ≥ 55 years, n = 21).

When age-specific β2 SI reference values were used to classify ascending aortic stiffness as normal or increased, a total of 43 (43%) patients had β2 SI above the age-specific threshold with a significantly larger aA (44.3 ± 5 vs 38.0 ± 4 mm, P <0.001). On the other hand, those with aA dilatation were more likely to show greater local stiffness (prevalence of increased β2 SI 64.8 versus 17.4%, P <0.001). In addition, the proportion of patients with abnormal β2 SI was higher in the subgroups of patients with larger aA size [8 (18.2%) vs 15 (48.4%) vs 20 (80.0%) in the first, second and third group based on aA respectively, P <0.05]. Similar results were obtained when aA dilatation

diagnosis was based on the ascending aortic diameter predicted by age, gender and BSA instead of using absolute values.

Considering other indices of aortic elasticity, distensibility and AIx were also compared in patients with normal or increased β2 SI values. Distensibility was significantly lower and the AIx was significantly greater in patients with abnormal β2 SI (P = 0.002 and 0.008, respectively). Distensibility was significantly lower in patients with aA dilation (P = 0.008), but it was similar between patients with normal aA diameter and those with 40–45 mm aA (P = 0.99). There was no difference in AIx between the ascending aorta size groups (P = 0.103).

Antihypertensive therapy and clinical comorbidities of patients with normal and increased β2 SI are shown in Table 12.

		β2 SI		
Variable	**General population (n = 100)**	**Normal (n=57)**	**Increased (n=43)**	**p value ANOVA**
Antihypertensive therapy				
Active therapy (n, %)	88 (88 %)	48 (84%)	40 (93%)	0.179
Number of drugs	2.0 (1.0–3.0)	2.0 (1.0–2.0)	3.0 (2.0–3.0)	< 0.001
> 3 drugs (n, %)	35 (35%)	12 (21%)	23 (53%)	0.001
ACEI/ARB (n, %)	80 (80%)	43 (75%)	37 (86%)	0.189
Vasodilators (n, %)	51 (51%)	20 (35%)	27 (63%)	0.006
Diuretics (n, %)	33 (33%)	14 (25%)	19 (44%)	0.039
Beta blockers (n, %)	33 (33%)	13 (23%)	20 (47%)	0.013
Comorbidity				
Obesity (n, %)	19 (19%)	10 (18%)	9 (21%)	0.669
Vascular pathology (n, %)	27 (27%)	20 (35%)	24 (56%)	0.039
CKD (n, %)	4 (4%)	2 (3.5%)	2 (4.7%)	0.773
DM (n, %)	9 (9%)	4 (7%)	5 (12%)	0.425
Dyslipidemia (n, %)	50 (50%)	26 (46%)	24 (56%)	0.313

Table 12: Antihypertensive therapy and comorbidities of hypertensive patients broken down by β2 SI values (normal vs increased). ACE-I, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; CKD, chronic kidney disease; DM: diabetes mellitus; Vasodilators, calcium channel blockers, alfa1-adrenergic receptor blockers, nitrates

Patients with increased local aortic stiffness (β2 SI) were taking more drugs than those with normal β2 SI (P <0.001), with a higher percentage of patients taking three or more drugs (P = 0.001), and also a higher percentage of patients taking vasodilators, diuretics and beta-blockers (P <0.05). No differences were observed in comorbidities between patients with normal or increased β2 SI.

Predictors of aA dilatation assessment

Multivariate logistic regression was performed to evaluate possible predictors of ascending aortic dilation, including in the model variables that were significantly different between patients with and without ascending aortic dilation as well as possible confounding factors (such as age and gender). An increased β2 SI and weight were found to be the only variables capable of predicting the presence of ascending aorta dilatation. The model that included β2 SI was the best in explaining aA dilatation (R^2 = 0.427, P <0.001), compared to models based on other aortic stiffness indices, such as PWV (R^2 = 0.176), distensibility (R^2 = 0.355) and AIx (R^2 = 0.186). Defining aA dilatation as the diameter exceeding the expected on the base of age, gender and BSA, only β2 SI was able to predict it [11] in a model that included the other indices of aortic stiffness (R^2 = 0,589 , P <0,001).

Aortic strain and cardiovascular organ damage

The association between β2 SI and indexes of organ damage mediated by hypertension was then evaluated. In the study population, β2 SI increased exponentially with increasing LVMi (r = 0.29, P = 0.003), PWV (r = 0.23, P = 0.028), SBP (r = 0.22, P = 0.031) and PP (r = 0.29, P = 0.003). In a subsequent analysis, patients with increased β2 SI had significantly greater LVMi than patients with normal β2 SI (117 ± 47 vs 94 ± 24 g/m2, P = 0.010, Figure 18). Furthermore, the percentage of patients with LVH was significantly higher in patients with impaired β2 SI than in patients with normal aortic stiffness (46.5 vs 26.3%, P = 0.036). Patients with abnormal β2 SI had significantly higher PWV than patients with normal local stiffness of the ascending aorta (10.20 ± 2.99 versus 8.63 ± 1.88 m / s, P = 0.013), with a higher percentage of patients with a PWV > 10 m/s (40.0 vs 19.6%, P = 0.033). Furthermore, SBP and PP were significantly higher in patients with increased β2 SI.

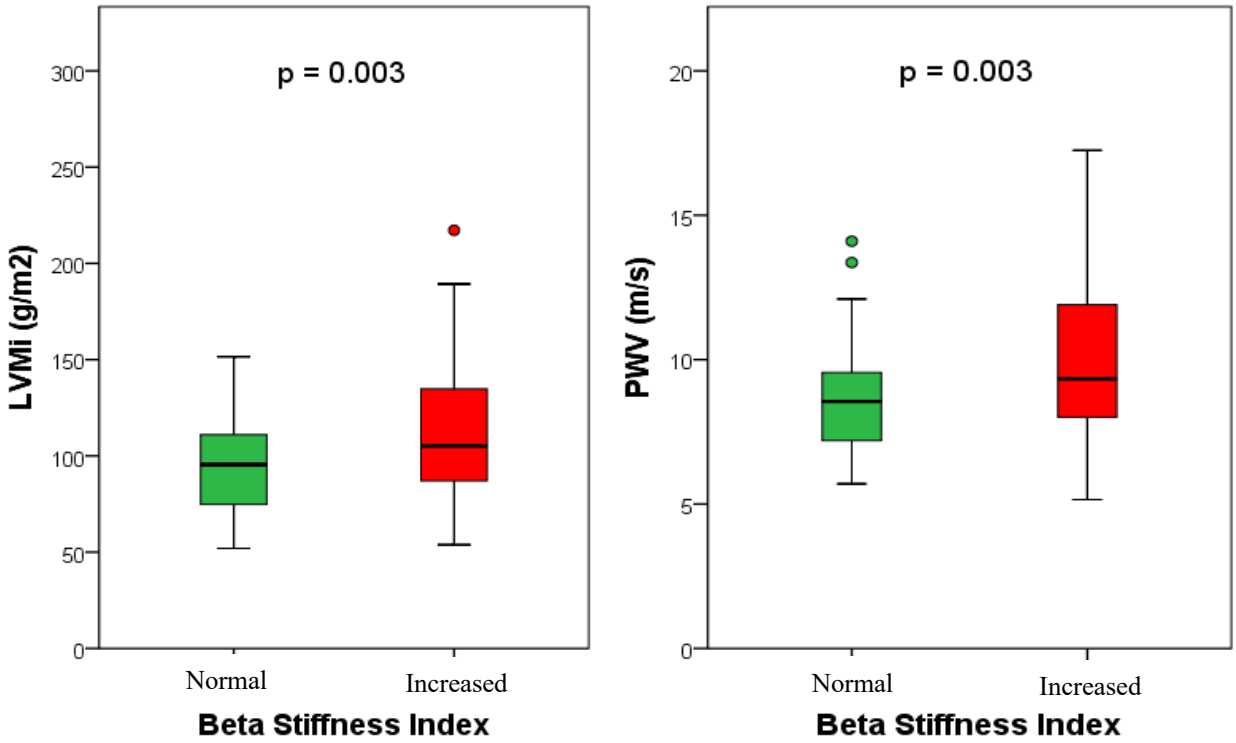


Figure 18 Cardiac (LVMi) and vascular (PWV) organ damage in patients with and without local aortic stiffness increase (β2 Stiffness Index).

4.4 DISCUSSION

Feasibility study, main strain parameters and other parameters of aortic stiffness

The present study demonstrates the feasibility of strain analysis based on a simplified speckle tracking technique for the evaluation of proximal aorta elastic properties. Peak deformation of the ascending aorta (PaAS) and β2 Stiffness Index (β2SI) were significantly correlated with recognized indices of aortic stiffness.

In physics, "stiffness" is defined as the resistance offered by an elastic body to deformation. Stiffness parameters describe the relationships between forces applied to an elastic body, mechanical stress and consequent strain in that body. [44]. Aortic walls elasticity is fundamental for its correct functioning: deformation of the vessel during cardiac systole allows adaptation to the passage of blood flow, storing potential energy that is returned during the next diastole as kinetic energy. In this way, the continuous progression of blood flow is ensured (Figure 19). Therefore, an increase in arterial stiffness, or loss of elasticity, leads to an increase in cardiac afterload, a reduction in coronary perfusion and excessive remodeling of the aortic walls.

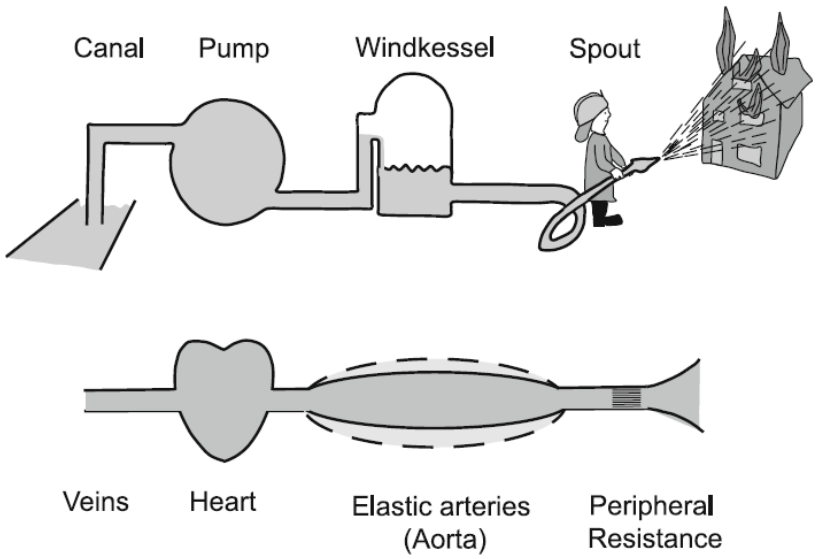
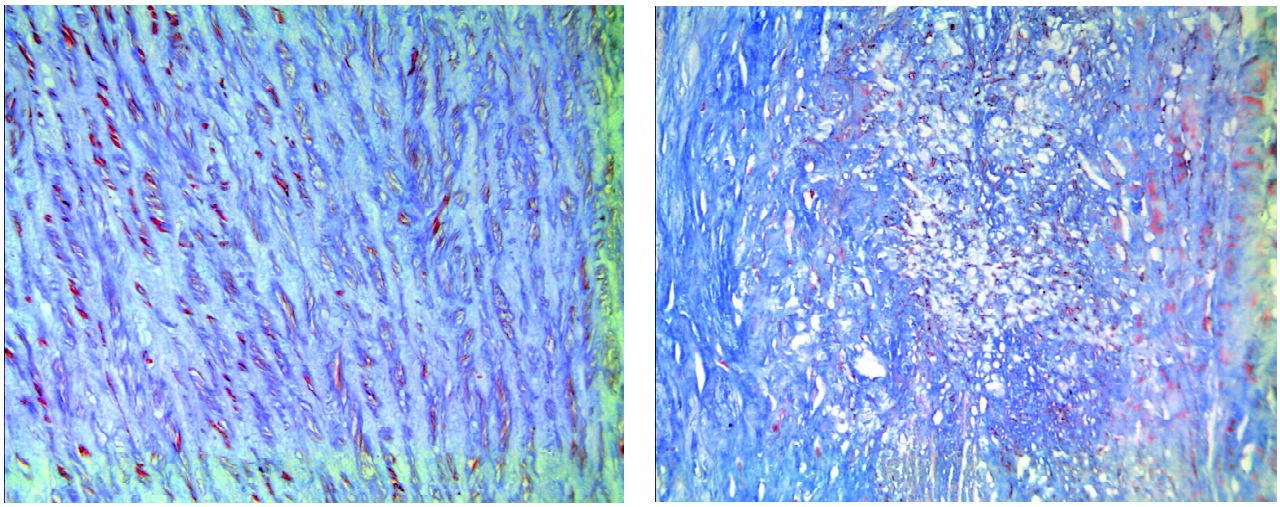


Figure 19. Graphical representation of the Windkessel model. Compliance and peripheral resistances, combined with the aortic valve, allow a constant peripheral flow [9]

Evaluating age-related changes in the biomechanics of the human thoracic aorta by both CT [45] and MRI [46], it was demonstrated that elasticity and distensibility progressively decreased. Aging in fact determines a progressive degeneration of the elastic fibers inside the aortic wall and a progressive imbalance of the structural histological architecture with an increase in collagen at the expense of the elastic components [47].



A B

Figure 20. A) Histological appearance of the aortic media and intima within the normal structures of a healthy subject. The normal aorta had very few collagens;**B) Cystic medial necrosis** of ascending aortic aneurysm of a 47-year-old male patient diagnosed of ascending aortic aneurysm, showing much collagen deposition in the intima and smooth muscle cell fragmentation with few collagen and cystic-like lesions in the media[48]. *Masson×200*

Aortic strain could represent a marker of early vascular aging, potentially useful to better describe patient cardiovascular risk.

At the moment, cfPWV is considered the gold standard non-invasive technique to evaluate aortic stiffness. However, it provides information on the whole descending aorta, not considering ascending aorta and including stiffer tracts of the arterial tree (carotid artery, iliac artery, and femoral artery), leading to a potential overestimation of aortic stiffness [13]. Furthermore, the real-life availability of PWV measuring instruments is limited by their cost and complexity.

Similarly, AIx assessment is based on applanation tonometry and therefore has the same disadvantages and is even less accurate than PWV in identifying increased aortic stiffness [49].

Another ultrasound method that may play a role in the local aA elastic properties evaluation is distensibility evaluated in M-mode during echocardiography [Figure 15, [50]]. However, M-mode measurement of the aA diameter can be inaccurate due to longitudinal movement of the vessel during the cardiac cycle, which leads to sampling different sections of the aorta during systole and diastole.

Second-level imaging methods can provide aA functional assessment. However, they also showed some drawbacks, such as exposure to ionizing radiation for CT, or high costs and limited availability in daily clinical practice for cardiac MRI. Instead, echocardiography is the first-line imaging tool in assessing cardiac organ damage, widely available and relatively low-cost.

Speckle tracking can measure the deformation of any anatomical structure by capturing a sequence of digital images on a cardiac cycle, with the advantage of being angle-independent and showing

good reproducibility. Previously, both Kim et al.[51] and Teixeira et al. [52] tried to apply softwares designed for the left ventricle to a cross section of the aorta (in the abdominal and ascending tracts, respectively). This required manual tracing of the internal contour of the short axis vessel and fictitious identification within this cross-sectional area of the six standard segments into which the ventricle is traditionally subdivided.

Instead, as previously explained, PaAS is the peak of transverse deformation during a cardiac cycle. It requires the identification of three pairs of spekles within the anterior and posterior wall of the aA, technically faster and easier to perform than tracing the internal vessel area. It can provide non-invasive, accurate and reproducible data on the transverse deformation of the ascending aorta and its stiffness. Furthermore, this technology is low cost and widely available in echocardiography laboratories, where it is already in use for left ventricular evaluation. The functional assessment by strain analysis that we propose can be routinely performed in patients with arterial hypertension and aA dilation.

We focused on ascending aorta due to its intrinsic characteristics like parallel vessel walls and good reproducibility of axial movements. Indeed, the overall PaAS feasibility rate of 71% in our population was related to very strict image quality criteria: a high-quality parasternal view, with a clear visualization of an aA segment of adequate length, was considered fundamental to follow the speckles during the entire cardiac cycle.

The deformation curves obtained reflect aortic walls stretch in response to the movement of blood column traveling from left ventricle to periphery, inducing an increase in the aA transverse diameter, visualized as a global positive deformation. The presence of a positive mean PaAS during the cardiac cycle was the expected physiological description of the normal aortic deformation. The significant association of PaAS with heart rate and systolic output underlined a close physiological connection between aortic deformation and blood flow. Mean values of PaAS were comparable, in terms of scale, to those previously reported [52], corroborating the strength of our simplified method.

In patients with normal aortic size and without significant cardiovascular organ damage, aortic wall biomechanical characteristics could be considered preserved. PaAS and β2 SI were strongly related to age and cfPWV, confirming previous results [53], supporting that PaAS was a good descriptor of arterial stiffness.

With aging, aortic capability to change its diameter to accommodate systolic blood volume is reduced and aortic stiffness is increased. Consequently, in our study, PaAS progressively decreased with increasing age and cfPWV, while β2 SI was progressively higher.

There is a supposed link between arterial stiffening and hypertension [54], but we did not observe a significant correlation between PaAS, β2 SI and hemodynamic parameters (SBP, DBP, MBP). It can be reasonably explained by the relative narrow range of blood pressure values that characterized our population. All study subjects were normotensive at the time of evaluation, limiting our possibility to explore the correlation of PaAS and β2 SI with patients' hemodynamics.

Hypertensive patients with greater local aortic stiffness showed greater antihypertensive therapy in terms of number of drugs, in which vasodilators and beta-blockers were more present than patients with normal β2 SI, hypothesizing that these categories of drugs may affect central pressures and therefore local arterial stiffness as a possible confounder. Further analyzes showed that only 28% of patients taking fewer than three drugs were taking vasodilators, while 83% of those taking three or more antihypertensive drugs were also taking vasodilators ($P < 0.001$). Furthermore, nonvasodilating beta-blockers can even stiffen large arteries through a direct "pro-fibrotic" effect [55,56]. However, this study was not focused on studying the effect of drugs on central hemodynamics of these complex patients, belonging to a third level center for the study and treatment of hypertension. These findings can presumably be explained by the fact that they could be patients with more difficult hypertension control. Further randomized prospective studies are needed to demonstrate an association between different drug classes and arterial stiffness indices.

Local aortic stiffness and ascending aorta size

Interestingly, PaAS and β2 SI were also strongly correlated with aortic diameters, even considering only patients with aA size in a normal range. If indeed PaAS is a good marker of aortic elastic properties, it would be reasonable to assume that an increased aortic size would be correlated with lower tension or reduced capacity to provide further expansion. The first phase of our study focused on individuals with no cardiac organ damage and normal aortic size. Therefore, it was obviously not adapted to explore this hypothesis in detail. We therefore sought to understand the link between biomechanical properties of the aortic walls and aortic remodeling, which was object of the second phase of our study.

In this phase of the study, we performed for the first time a functional assessment of the ascending aorta in hypertensive patients with known dilated ascending aorta. Our data showed that, in hypertensive patients, aA dilatation is associated with an increased local aortic stiffness, as measured by transverse aortic deformation by speckle tracking analysis. Abnormal β2 SI was observed much more frequently in patients with dilated ascending aorta than in those with normal ascending aortic diameter, suggesting that a larger ascending aorta diameter corresponds to a more rigid vessel.

Dividing our study population into three groups based on aA diameter, prevalence of a β2 SI above the reference values increased with increasing size.

aA local stiffness and cardiovascular organ damage

The role of mechanical characteristics of the aortic walls in cardiovascular pathophysiology is unequivocal. Greater aortic stiffness is linked to an increase in left ventricle afterload, with a consequent increase in cardiovascular work. In some predisposed subjects, increased arterial stiffness seems to be linked to unexpected proximal aorta remodeling, a condition previously defined early vascular aging [3,12]. Ascending aorta dilatation, if associated with a reduced elastic function, could represent a marker of increased afterload and of impaired ventricular-arterial coupling, which progressively worsens with larger diameters. In line with this, we demonstrated that patients with ascending aortic dilatation showed reduced local elastic function and that this condition is related to LVMi and PWV, known indices of cardiovascular hypertension mediated damage. Indeed, patients with increased β2 SI showed a prevalence of LVH and PWV > 10 m/s double than that observed in patients with normal β2 SI. However, we also observed that some patients with only mild aA ectasia, or even normal aA diameter, had abnormal β2 SI. These subjects, lacking inherent "damping capabilities" (the possibility to reduce pressure during cardiac systole) could be predisposed to a faster aortic dilatation aortic and possibly a higher incidence of cardiovascular events, but more longitudinal studies are needed to demonstrate it.

4.5 LIMITS

It is necessary to underline some limitations of the present study. Data came from a relatively small population, recruited in a single specialized center, introducing an obvious risk of selection bias. However, the included patients were consecutively enrolled from a larger population assessed in our Center, and underwent TTE and cfPWV evaluation for clinical reasons, regardless of the purpose of this study.

We focused our strain assessment only on the proximal ascending aorta, a standard aortic segment routinely evaluated, in order to get the best clinical applicability. Also sinus of Valsalva evaluation was considered a potential target for the same reason. However, its complex geometry, non-linear morphology, and its significant compliance reduction compared to aA would have complicated both sampling method and interpretation of results. In this regard, aA is in fact the most elastic segment and the most sensitive to degenerative phenomena, with important consequences on arterial-ventricular coupling. The aforementioned elastic action on the pulse wave generated by the left

ventricular contraction is in fact mainly carried out by the ascending aorta and therefore the aortic stiffness measured at this level would seem to be more effective in predicting the morphological and structural changes of the left ventricle, compared to other aortic segments [57].

In our main analysis, we used the absolute aA diameter > 40 mm to define aA dilatation. Although patients with dilated aA had significantly greater body size and higher male prevalence than those with normal aA diameter, these results were confirmed by adopting a definition of ascending aortic dilation based on the expected diameter for age, gender and BSA. We also arbitrarily defined different degrees of dilation of the ascending aorta. This was done according to the clinical practice carried out in our center, in which patients with an ascending aorta ≥45 mm receive a more intensive follow-up [[58] figure 21].

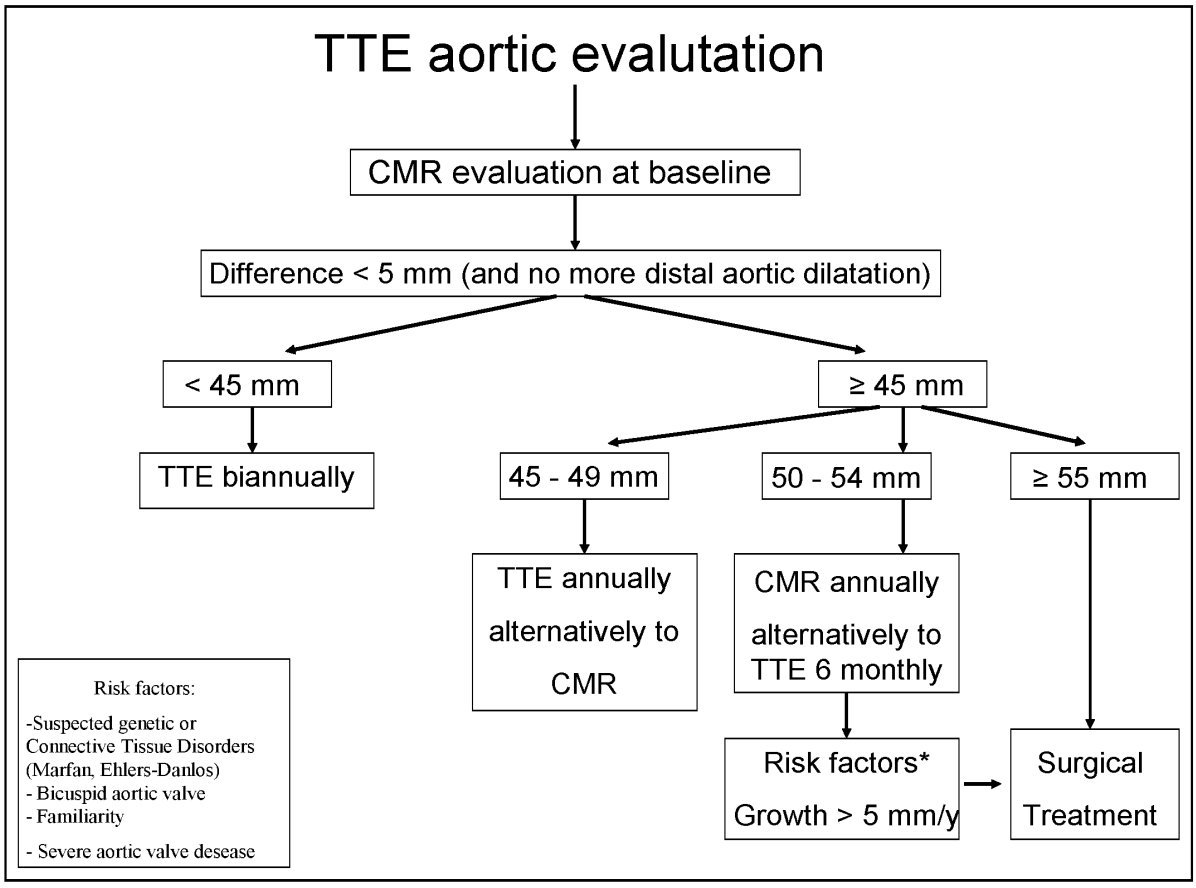


Figure 21. Proposed algorithm for the surveillance and surgical indication work-up of proximal thoracic aorta dilation.

In this regard, with this study we were trying to identify those subjects with clinical or echocardiographic characteristics that could predict a possible unfavorable evolution of the aortic pathology and therefore to require greater attention over time.

5. Evolution of the proximal aorta diameter in hypertensive patients with known mild-moderate dilation of the proximal aorta: results of a 5-year follow-up

5.1 Summary

242 hypertensive outpatients with known mild to moderate aortic dilatation (37-53 mm) were followed for at least 5 years. Mean growth rate was 0.08 ± 0.35 mm/year, similar to the observed in general population. No clinical or anthropometric parameters were significantly different in patients with and without aortic diameter increase. Aortic diameter at first visit, demographic and echocardiographic variables were the main determinants of the aortic diameter at the second visit, accounting for approximately 90% of its total variability [58].

5.2 Background

Proximal aorta dilatation is relatively frequent in hypertensive patients, with an estimated prevalence up to 16.9%, and is associated with cardiac organ damage. Furthermore, it represents an important risk factor for acute aortic disease (dissection, wall hematoma and penetrating ulcer) and is associated with an increased risk of cardiovascular events [6]. We observed that proximal aortic diameter is related to anthropometric [such as age, sex and body surface area (BSA)] and cardiovascular factors (LVM, LVH and PWV), but less is known about factors capable of predicting its evolution over time. Some pathological conditions are known to affect aortic expansion. For example, bicuspid aortic valve (BAV) is known to be related to a higher prevalence of aortic dilation, while its impact on the risk of aortic dissection is unclear. Current international guidelines propose follow-up with computed tomography (CT) or cardiac magnetic resonance imaging annually for mildy dilated (35–44 mm) cases or twice a year for moderately dilated (45–54 mm) cases. However, there is little evidence supporting this timing and the role of clinical and echocardiographic features in guiding physicians in the management of aortic dilation is unknown [1,12,59].

5.3 MATERIALS AND METHODS

We prospectively analyzed a cohort of essential hypertensive outpatients evaluated at the Hypertension Unit from 2003. Patients underwent serial complete anamnestic assessment, clinical evaluation and imaging exams for the study of hypertension-related cardiovascular organ damage. Proximal aortic dilatation was at first defined by absolute, sex-specific aortic diameter criteria (> 40mm in men and > 38mm in women) as suggested in previous studies [60]. Patients with aortic dimensions exceeding the threshold value were enrolled in a standardized follow-up program

from 2003 to 2016 a first echocardiographic check was performed at 6 months. If aortic diameter remained stable, subsequent control was postponed to 3 years. If SOV diameter increased at a rate greater than 2mm/year or reached greater than 45mm, cardiac MRI or CTscan as second level examination were performed. If agreement between diameters measured by transthoracic echocardiographic (TTE) and CTscan or MRI were similar, TTE was used in clinical practice for serial imaging follow-up of the dilated proximal aorta, as previously reported [61]. We included in present analysis only patients assessed with at least two TTE in a follow-up period of at least 5 years. The study has been approved by our local ethic committee (Comitato Etico Interaziendale A.O.U. Citta` della Salute e della Scienza di Torino – A.O. Ordine Mauriziano – A.S.L. TO1 – CEI/330) and all patients provided written informed consent to participate in this study. Exclusion criteria were documented connective tissue disorders (Marfan, Loeys- Dietz, and Ehlers-Danlos syndromes), inflammatory aortic diseases, BAV, or a history of thoracic aortic surgery. Patients scheduled to receive proximal aortic surgery or with more than moderate valve disease were also excluded.

In every visit, SBP and DBP (mmHg) were measured immediately before the TTE examination, in the supine position after 5 min of rest, according to the European Society of Hypertension/Euro- pean Society of Cardiology (ESH / ESC) recommendations (1). Blood pressure (BP) levels were optimized if they were above reference levels. Mean arterial pressure (MAP, mmHg) was defined as 1/ 3 (SBP) þ 2/3 (DBP), and pulse pressure (PP, mmHg) was the difference between SBP and DBP. We defined four categories of hypertension based on measured ambulatory pressure levels:

1. Category 1: patients with normal blood pressure values according to the guidelines (SBP 120 - 129 mmHg and DBP 80 - 84 mmHg);
2. Category 2: patients with normal-elevated blood pressure values according to the guidelines (SBP 130 - 139 mmHg and DBP 85 - 89 mmHg);
3. Category 3: patients with 1st degree hypertension according to the guidelines (SBP 140–159 mmHg and DBP 90–99 mmHg);
4. Category 4: patients with hypertension according to the guidelines (SBP> 160 mmHg and DBP> 100 mmHg).

Furthermore, in order to evaluate treatment efficacy, we defined “controlled BP” the improvement in BP category compared with the previous evaluation (e.g. conversion from grade III to normal values), and “uncontrolled BP” the worsening in BP category compared with the previous evaluation (e.g. conversion from normotensive to grade III hypertension). The presence of the same BP category at subsequent visit was classified as “controlled BP” only for category 1 and category 2; contrariwise the persistence of category 3 or category 4 was classified as “uncontrolled BP”.

5.4 RESULTS

We analysed a total of 242 patients for the present study (median follow-up 5.47 ± 1.22 years). The great majority of our patients were men (85%), overweight, with a fair control of blood pressure (54 (22.3%) patients at baseline with BP < 130/80, compared to 81 (33.5%) patients after 5 years follow up, p = 0.280). At baseline, our population showed a mild-to-moderate (37–53mm) aortic ectasia at SOV level. Mean aortic diameter was significantly lower compared with final assessment at the end of follow-up (39.3 ± 4.0 vs. 39.8 ± 4.1 mm, P<0.05) and, as expected, more patients were nonactively treated at their first visit, with significantly higher blood pressure (MAP 104.9 ± 12.4 vs. 100.6 ± 12.6 mmHg, P<0.05). Likewise, LVM and diastolic function parameters were significantly different at baseline and after 5 years. Clinical and echocardiographic features are listed in Tables 14 and 15, respectively.

Variable	Baseline (n=242)	After 5 years (n=242)	p value
Age (years)	54.8 ± 11	60.9 ± 11.0	< 0.01
Gender (male, %)	206 (85.1)		
Height (cm)	174.1 ± 7.7	172.6 ± 8.4	< 0.01
Weight (Kg)	81.8 ± 13.4	83.7 ± 14.3	< 0.01
BMI (Kg/m2)	26.9 ± 3.8	28.1 ± 4.4	< 0.01
SBP (mmHg)	141.1 ± 17.3	138.9 ± 19.4	0.316
DBP (mmHg)	86.8 ± 11.3	81.5 ± 11.7	< 0.01
MAP (mmHg)	104.9 ± 12.4	100.6 ± 12.6	< 0.01
PP (mmHg)	54.3 ± 11.9	57.4 ± 16.0	0.02
HR (bpm)	70.4 ± 11.2	69.7 ± 12.0	0.79
BSA (m2)	1.96 ± 0.18	1.97 ± 0.18	0.06
Never treated (%)	14.2	5.3	< 0.01
BP < 130/80 (%)	54 (22.3)	81 (33.5)	0.280

Table 14 - Clinical characteristics at baseline vs 5-year follow-up. BMI Body Mass Index (BMI); Body Surface Area (BSA); Systolic blood pressure (SBP); diastolic blood pressure (DBP); Mean arteril pressure (MAP); Pulse pressure (PP); Heart rate (HR);

Variable	Baseline (n=242)	After 5 years (n=242)	p value
Aorta			
SoV (mm)	39.3 ± 4.0	39.8 ± 4.1	< 0.01
LV morphology			
LVH (%)	9.9	14.5	< 0.01
RWT	0.42 ± 0.08	0.44 ± 0.09	< 0.01
LVM (g)	180.4 ± 47.8	183.0 ± 48.1	< 0.01
LVMi (g/m^2)	91.9 ± 22.8	93.7 ± 25.5	< 0.01
Systolic function			
EF (%)	58.1 ± 3.9	60.5 ± 5.4	< 0.01
Diastolic function			
E/A	1.02 ± 0.36	0.92 ± 0.34	< 0.01
E' (cm/s)	8.9 ± 2.7	7.7 ± 2.5	< 0.01
E/E'	7.1 ± 2.4	8.2 ± 2.6	< 0.01

Table 15 - Echocardiographic parameters at baseline and after 5 years of follow up aortic diametere at sinus of valsalva level (SoV); LVH: Left Ventricular Hypertrophy; RWT: Relative Wall Thickness; LVM: Left Ventricular mass; E wave on Transmitralic Doppler (E); A wave on Transmitralic Doppler (A), mean Tissutal Doppler E wave (Etdi mean); E/e' ratio (E/Etdi mean)

Mean aortic growth rate at SOV level was 0.08 ± 0.35 mm/year. Growth rate was significantly related to height, weight, BSA, PP and ejection fraction at baseline, but not to heart rate or change in LVM or LVMi. No significant difference was found in SOV growth rate between patients with controlled or uncontrolled blood pressure (mean SOV growth rate for stable/decreasing vs increasing in BP grading 0.055 ± 0.37 vs. 0.062 ± 0.29mm/year, respectively, P = 0.89, figure 22).

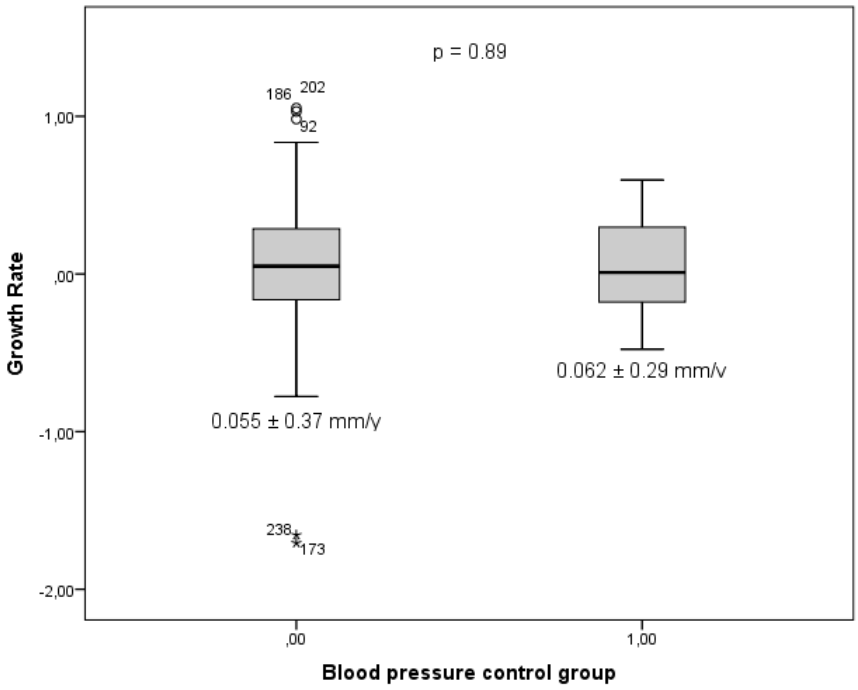


Figure 22. Aortic diameter growth rate in relation to blood pressure control (BP). Blood pressure control: Group 0: stable or decreased hypertension class; Group 1: increased hypertension class, p 0.89

In our selected population, the prevalence of aortic dilatation (according to the new criteria based on z score definition) was of 47.5% (115 patients). Compared with patients with normal aortic z score, patients with dilated aorta showed significantly higher LVM, LVMi and were more likely to be off drug treatment for hypertension. Clinical and echocardiographic features of patients with and without aortic dilatation based on z score are listed in Table 16.

Variable	Not dilated at baseline	After 5 years	p value	Dilated at baseline	After 5 years	p value	P test N vs D at baseline
N (%)	127 (52.5%)			115 (47.5%)			
Age (years)	54.8 ± 10.6	61.9 ± 10.9	< 0.01	54.9 ± 11.4	60.1 ± 11.5	< 0.01	0.96
Gender (Male, %)	103 (81.1)			104 (90.4)			0.06
Height (cm)	173.8 ± 7.8	172.3 ± 8.6	< 0.01	174.4 ± 7.5	172.8 ± 8.3	< 0.01	0.58
Weight (Kg)	79.9 ± 13.0	83.5 ± 14.9	0.012	83.9 ± 13.6	87.0 ± 15.0	< 0.01	0.08
BMI (Kg/m2)	26.4 ± 3.6	27.2 ± 4.0	< 0.01	27.5 ± 3.8	29.1 ± 4.6	< 0.01	0.43
SBP (mmHg)	141.9 ± 19.5	139.7 ± 18.0	0.35	140.7 ± 15.0	138.9 ± 17.8	0.60	0.75
DBP (mmHg)	87.5 ± 12.4	80.8 ± 12.5	< 0.01	85.7 ± 10.3	82.8 ± 10.8	< 0.01	0.65
MAP (mmHg)	105.6± 13.8	100.1 ± 14.8	< 0.01	104.0 ± 10.9	100.0 ± 11.5	0.02	0.69
PP (mmHg)	54.4 ± 13.2	57.1 ± 19.7	0.25	55.0 ± 11.1	58.4 ± 13.9	0.06	0.93
HR (bpm)	70.3 ± 11.5	69.0 ± 11.7	0.70	70.1 ± 10.7	69.8 ± 13.3	0.95	0.44
BSA (m2)	1.94 ± 0.18	1.94 ± 0.18	0.78	1.99 ± 0.18	2.00 ± 0.18	0.04	0.32
Never treated (%)	18.3	4.4	< 0.01	10.1	6.3	< 0.01	< 0.01
Aorta							
SoV (mm)	36.7 ± 3.1	37.3± 3.7	0.04	42.3 ± 2.7	42.4 ± 2.8	0.23	< 0.01
LV morphology							
LVH (%)	7	15	< 0.01	13	14.2	0.63	< 0.01
RWT	0.42 ± 0.7	0.44 ± 0.8	< 0.01	0.43 ± 0.9	0.44 ± 0.1	0.16	0.61
LVM (g)	169.7 ± 43.4	181.4 ± 46.9	0.35	192.1 ± 49.9	200.9 ± 48.5	0.80	< 0.01
LVM/BSA (g/m^2)	87.1 ± 19.5	93.7 ± 22.7	0.27	97.3 ± 25.0	100.5 ± 23.3	0.62	< 0.01
Systolic function							
EF (%)	58.4 ± 3.8	60.5 ± 5.4	< 0.01	57.5 ± 4.0	60.6 ± 5.4	< 0.01	0.82
Diastolic function							
E/A	1.03 ± 0.36	0.97 ± 0.36	0.03	0.99 ± 0.35	0.87 ± 0.32	< 0.01	0.17
E' (cm/s)	8.8 ± 2.6	7.9 ± 2.3	< 0.01	8.7 ± 2.5	7.5 ± 2.7	< 0.01	0.36
E/E'	7.4 ± 2.6	8.1 ± 2.6	< 0.01	7.1 ± 2.3	8.2 ± 2.6	< 0.01	0.45

TABLE 16. Clinical and echocardiographic features of normal aorta versus dilated aorta based on z score BMI Body Mass Index (BMI); Body Surface Area (BSA); Systolic blood pressure (SBP); diastolic blood pressure (Diastolic blood pressure); mean arterial pressure (MAP); Pulse pressure (PP); heart rate (HR); aortic diameter at sinus of Valsalva level (SoV); LVH: Left Ventricular Hypertrophy; RWT: Relative Wall Thickness; LVM:Left Ventricular mass; E wave on Transmitralic Doppler (E); A: A wave on Transmitralic Doppler (A), mean Tissue Doppler E wave (Etdi mean); E/e ratio (E/Etdi mean)

A regression analysis was performed to pinpoint parameters that might be useful to predict aortic diameter evolution. Demographic (sex, age, BSA, height), hemodynamic (SBP, DBP, MPB, PP) and echocardiographic variables (ejection fraction, indexes of diastolic function) alternately analyzed in the regression model did not reach statistical power to reliably predict aortic dimensions (R^2 = 0.3-0.4). Only adding the aortic diameter at first visit to the selected regressors, we improved quality of our model. The model taking in consideration weight, PP, LVM, ejection fraction, time between visits and aortic diameter at baseline (Table 17) was able to describe 85% of the total variability in aortic dimensions (Figure 23, $R^2 = 0.85$, $P < 0.05$).

Variable	t	P value
Intercept (β0)	9,157	0.0097
Weight (Kg)	0,021	0.14
PP (mmHg)	-0,031	0.04
LVM (g)	0,002	0.62
FE %	-0,059	0.18
Time (days)	$4{,}606*10^{-4}$	0,26
SoV_1 (mm)	0,820	<< 0.01

Table 17: coefficients for the aortic model PP: pulse pressure; EF: ejection fraction; LVM: left ventricualr mass; SoV_1: aortic diameter at SoV level at first visit

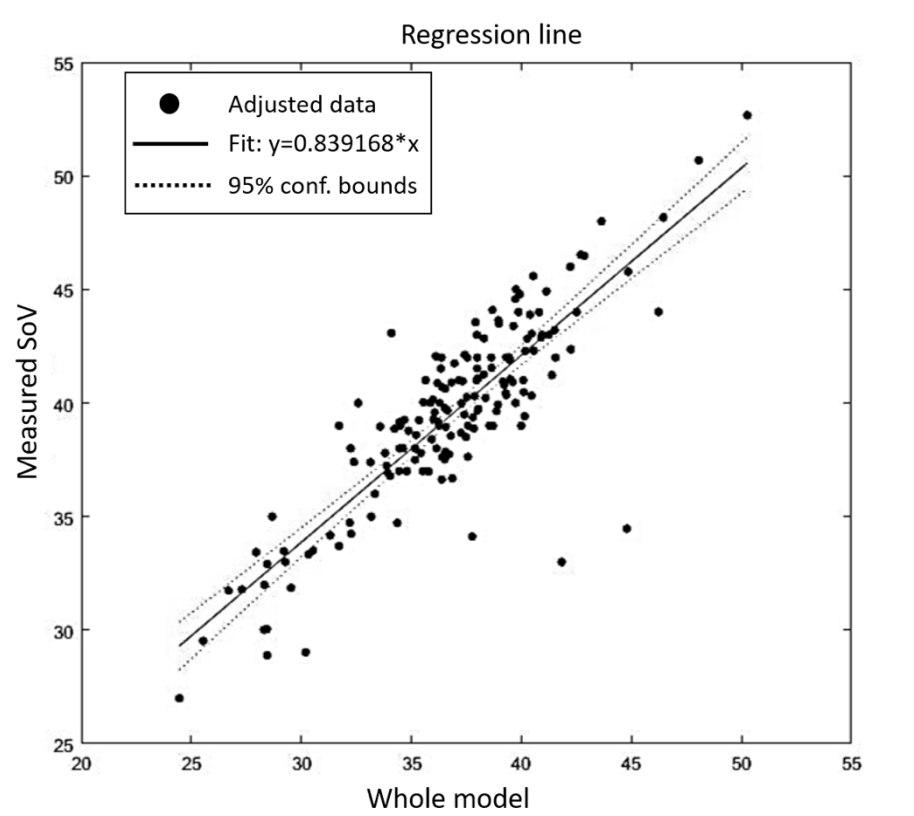
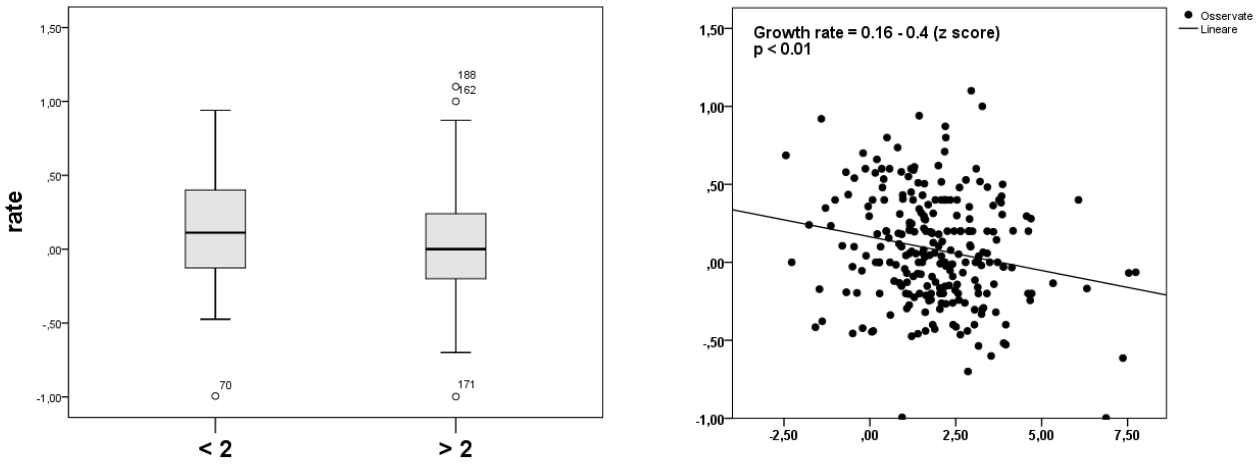


FIGURE 23. Linear regression model aortic diameter predicted by whole model compared to aortic diameter measured at the sinus of Valsalvae (SOV) taking into account weight, PP, LVM, FE, time between visits and aortic diameter at baseline, R^2=0,85, P <0,05). EF, ejection fraction; LVM, left ventricular mass; PP pulse pressure; SOV, sinus of Valsalva

We observed an inverse association between aortic z score at baseline and SOV growth rate (R^2 = 0.04, P<0.05). Observed aortic growth rates were indeed higher for normal vs. dilated patients (0.13 ± 0.35 and 0.03 ± 0.36 mm/year respectively, P<0.05, Figure 24). A minority of our patients (n = 53, 21.9 %) showed significant increase (> 2 mm in 5 years follow up) in SOV dimensions. Baseline aortic diameter was the only echocardiographic parameter showing significant differences between patients with and without such increase. Focusing on them, dividing our population into dilated and not dilated patients at baseline on the base of z score value, we observed a trend toward a greater percentage of patients not dilated at baseline with a significant growth [33 (26% of not dilated at baseline) vs 20 (17.4% of dilated at baseline), p = 0.102)] but a growth rate not significantly different (0.52 ± 0.23 versus 0.53 ± 0.19 mm/year).



A **B**

FIGURE 24. A) Aortic diameter growth rate for patients with normal aorta (<2 z score) vs. dilated aorta (> 2 z score) at baseline (P <0.05). z score: standard deviations from the mean value of the aortic diameter considered in the general population. **B) Growth rate dependent on baseline z score, linear regression.**

5.5 DISCUSSION

A recent review [62] reported mean growth rates for ascending aortic aneurysm ranging from 0.2 to 2.8 mm/year, a distinctively wide range. Although large aortic size, distal aneurysm locations, presence of Marfan's syndrome, and BAV have been consistently associated with accelerated growth, association between hypertension and aneurysm expansion are not clearly established [14].

In our study, we tried for the first time to predict aortic diameter at subsequent visits using baseline clinical and echocardiographic data.

The anthropometric variables that have been proved to be major determinants of vessel diameter are sex, age and BSA[10]. Hence, we used z score as suggested in recent international guidelines [27] to overcome biases induced by a definition of dilatation based on the sole absolute value of aortic diameter. The prevalence of aortic dilatation (defined as z score >2) was 47.5% of our population.

We report a slow but significant progression of aortic root dilatation at the SOV level in a population of hypertensive patients with baseline mild-to-moderate aortic dilatation followed for an average of 5.47 ± 1.22 years. Interestingly such progression occurred at a rate of 0.1 mm/year, similarly to the rate observed in the general population [63]. Only a small percentage of patients (n = 53, 21.9 %) presented a significant increase (i.e. > 2mm) in aortic size during a follow-up of at least 5 years.

No clinical or anthropometric parameters were significantly associated with the enlargement of aortic diameter over time in the regression analysis, whereas the major role of the vessel size clearly emerged as baseline aortic SOV diameter was the only variable significantly different between patients with and without further aortic growth.

Laplace's law states that tangential stress is directly

proportional to applied pressure and vessel radius and inversely proportional to wall thickness. A chronic exposition to greater shear stress might accelerate aortic wall degeneration. The typical histological finding in thoracic aorta aneurysms is cystic medial degeneration, seen as smooth muscle cell and elastic fiber degeneration [figure 20, [64]]. This condition can be secondary to acquired factors (such as aging or atherosclerosis), connective tissue disorders or familial diseases with an inherited pattern. Our population of hypertensive patients presented early vascular ageing with a greater prevalence of aortic enlargement than general population. Growth rate was significantly related to anatomical (height, weight, BSA) and hemodynamic characteristics (PP and ejection fraction at baseline). In particular, the negative association between PP and growth rate could be a marker of increased impedance at the level of proximal aorta in this population. However, we did not observe any statistically significant association between blood pressure levels or blood pressure control and aortic expansion rate, in accordance with previous studies [12,65]. Instead, we observed that people with baseline aortic dilatation showed a slower dilatation over time than people with normal diameter. This finding could be related to aortic wall characteristics specific for our population. Patients with mild-to-moderate dilatation could present some kind of compensatory biological mechanism, such as histological differences in the composition of the aortic wall, eventually affecting its mechanical properties, as hypothesized in previous in-vitro studies [66]. These compensatory changes in aortic wall might reduce aortic growth rate at lesser degrees of dilatation, but not in the presence of greater increases in aortic diameter. Otherwise, we could suppose that patients with dilated aorta, being more likely treated with antihypertensive drugs (89.9 vs. 81.7% of patients with normal aortic diameter at baseline, $P<0.01$ - Table 14), showed a reduced aortic growth rate related to the pharmacological blood pressure control. In order to avoid regression to the mean phenomenon, at the time of the study design, the aortic dimensions were measured several times and the average of at least three values was considered: this proved to reduce the random variability of the values with respect to the average. Furthermore, we used a cut-off value to define aortic dilatation with a z score > 2. However, analyzing the few patients with significant growth (> 2 mm over 5 years), a higher percentage of patients with significant growth in the subgroup of non-dilated patients (with z score <2) was observed. We could think that it was not reached a statistical significance due to the low sample size. However, further studies are needed to test these assumptions.

We also used z score evaluation to estimate real prevalence of aortic dilatation in our population and found that conventional parameters (age, sex and BSA) used in calculating z score are weak predictors of patient-specific aortic size, implying that additional parameters, such as innate aortic properties, should be considered in future research endeavors.

We observed that demographic, hemodynamic and echocardiographic variables commonly measured in out- patient visits were inadequate in the prediction of the evolution of SOV diameter in the middle term. Therefore, we used a multiparadigm numerical computing environment to implement the regression analysis and proposed an innovative model that, for the first time, provides an estimate of future growth of aortic diameter with a good accuracy, based on easily available parameters (weight, PP, LVM, ejection fraction, time between visits and aortic diameter at baseline). We expect that our model will improve the clinical management of hypertensive patients with mild-to-moderate aortic dilatation.

Aortic dilatation is common in hypertensive patients with cardiac organ damage, such as left ventricular hypertrophy (LVH) and arterial stiffness (PWV increase), validated prognostic markers. We observed a strong association of aortic diameter with left ventricular mass, suggesting that patients with dilated aorta may have a higher cardiovascular risk. Longitudinal studies are needed to confirm its possible prognostic role.

6. The "RECALL" project

6.1 Summary

Then we evaluated the long-term impact of ascending aortic remodeling (AAD) in hypertensive patients and its possible prognostic value as a predictor of cardiovascular (CV) events.
423 hypertensive patients were included and underwent transthoracic echocardiography, cfPWV, and clinical evaluation. During a median follow-up of 7.4 years, 52 CV events were observed. AAD was associated with an increased risk of cardiovascular events ($p < 0.010$), even after correcting for major confounding factors in multivariate analysis ($p < 0.010$). Furthermore, we observed that ascending aortic assessment improves risk stratification compared to PWV alone and that in AAD absence, sinus of Valsalva Dilatation (SVD) lost any prognostic value [67]

6.2 BACKGROUND

Ascending aorta (ASC) is the aortic segment between the sinotubular junction and the aortic arch. Ascending Aorta Dilatation (AAD) has been associated with hypertension-mediated cardiovascular organ damage: hypertensive individuals with AAD have greater arterial stiffness (as measured by PWV) and a three-fold greater prevalence of hypertrophy left ventricle (LVH) than subjects without this condition. The dilatation of the first tract of the proximal aorta, sinuses of Valsalva diameter (SOV), has so far received more attention: it has recently been observed that the dilatation of SOV (SVD) has prognostic value both in patients with hypertension [6] in patients with LVH [68].
Conversely, the predictive value of AAD in terms of cardiovascular events has not yet been assessed. Therefore, in this study we aimed to evaluate the prognostic role of AAD in predicting the risk of cardiovascular events in a population of hypertensive patients.

6.3 METHODS

Patients evaluated for cardiovascular damage at the tertiary Hypertension Unit of the University of Turin between December 2007 and November 2013 were prospectively enrolled; and the assessment of cardiovascular damage was carried out through standard clinical evaluation, transthoracic echocardiography (TTE) and arterial stiffness assessment with carotid–femoral PWV.
AAD was defined following three different methods:
- an ASC diameter more than 36mm for female and 41mm for male subjects (AADa) [69];
- an ASC diameter indexed to height$^{0.5}$ (ASCh$^{0.5}$) exceeding the 75th percentile of the population distribution;

- an ASC diameter indexed to $BSA^{0.4}$ ($ASCi^{0.5}$) exceeding the 75th percentile of the population distribution.

In the last two methods the 75^{th} percentile has been arbitrarily chosen as a threshold.

Predicted SOV was obtained from age, gender and height as previously reported[10]. SOV diameter z-score (SOVz) was generated by the difference between observed SOV and predicted SOV, divided by sex specific SD of observed SOV. SOVz exceeding the 75^{th} percentile of the population distribution was considered as SVD. The primary outcome of this study was a composite of first cardiovascular event, defined as: nonfatal MI, nonfatal stroke, sudden cardiac death, heart failure requiring hospitalization, transient ischemic attack (TIA), coronary revascularization, unstable angina (meant as typical symptoms and ECG changes without significant troponin movement, which underwent coronary angiography and possibly revascularization), need for surgery involving aorta or its major branches, implanted cardioverter defibrillator (ICD) implantation and arrhythmias (atrial fibrillation and major ventricular arrhythmias). The event identification occurred on November 2018 and was based on chart review from clinical documentation present on the electronic medical record within our hospital system (AOU Città della Salute e della Scienza di Torino, TrackCare, InterSystems Corporation, Milano, Italy), the Hypertension Centre of the University of Torino (HyperMacondo) and direct telephone contact with all the patients.

Statistical analysis

Statistical analysis was performed by using a dedicated software (R: A Language and Environment for Statistical Computing, v4.0.0 for Mac OSX, R Core Team., Vienna, Austria). The normal distribution of variables was verified by graphical evaluation (histogram and Q–Q graph) and Shapiro–Wilk test. Data were presented as "mean ± SD" or "median (inter-quartile range)" and as "observations (percentage frequency)" as appropriate. Continuous variables were compared by t test or Mann–Whitney test, while categoric ones by the $\chi 2$ test. Allometric indexes to scale ASC diameter have been computed as follow: logarithmic transformation of the formula $Y=aX^b$ was performed and scaling exponents obtained by linear regression. The equality of scaling exponents between genders was assumed. The homoschedasticity and normality of residual variance were tested using the Breusch–Pagan/Cook–Weisberg tests and the Shapiro–Wilk tests, respectively. Univariate Cox regression analysis was performed for all clinical variables and penalized model has been performed for selecting variables to be included in the multivariate Cox model. Different AAD definitions have also been tested by correcting for age, sex and BSA. A P value less than 0.05 for two-tail tests was considered significant in all statistical analysis.

6.4 RESULTS

Overall population

Out of 627 hypertensive patients evaluated, 493 (78.6%) had available ASC diameter and met inclusion/exclusion criteria. The drop out was 8.5% and follow-up data were available for 423 patients. PWV data were available in only 360 subjects due to the exclusion of patients with low quality acquisition or noncompliance to the exam. Demo- graphic features of the study population divided by presence of AADa is summarized in Table 18.

		AADa		
Variable	**Population (n=423)**	**No (n=355)**	**Yes (n=68)**	**p value**
Age (years)	52.9±12.9	51.2±12.5	62.2±10.9	**<0.001**
Gender male (n,%)	330 (78.0%)	283 (79.7%)	47 (69.1%)	0.076
BMI (kg/m^2)	26.7±3.26	26.6±3.32	27.3±2.85	0.054
BMI >25 (n,%)	283 (66.9%)	232 (65.4%)	51 (75.0%)	0.159
BSA (m^2)	1.93±0.19	1.93±0.19	1.95±0.21	0.508
SBPp (mmHg)	138.3±15.2	138.2±14.8	139.0±17.0	0.725
DBPp (mmHg)	82.0±10.2	81.8±9.86	83.3±11.8	0.321
PPp (mmHg)	56.3±11.8	56.4±11.5	55.6±13.4	0.620
SBPc (mmHg)	126.25±14.8	125.7±14.5	129.0±16.1	0.093
DBPc (mmHg)	83.34±10.2	83.1±9.9	84.8±11.5	0.210
PPc (mmHg)	42.91±10.96	42.66±10.75	44.26±12.04	0.274
Augmentation Index (%)	23.9±11.4	23.0±11.5	28.5±10.0	**<0.001**
HR (bpm)	71.0±11.1	71.5±10.8	67.9±12.1	**0.034**
Smoke (n, %)	122 (29.0)	107 (30.1)	15 (22.7)	0.223
Diabetes (n, %)	16 (3.8%)	12 (4.3%)	4 (5.9%)	0.303
Hypertension period (years)	6.50 [2.00-14.0]	6.00 [2.00-12.5]	11.0 [5.00-19.0]	**<0.001**
Hematochemicals:				
Total Cholesterol (mg/dl)	212±41.6	213±42.2	209±38.1	0.561
HDL Cholesterol (mg/dl)	53.4±15.5	53.1±15.3	54.9±16.4	0.497

LDL Cholesterol (mg/dl)	133.2±35.9	134.1±36.5	128.1±32.5	0.283
Triglycerides (mg/dl)	106 [75.0-163]	105 [74.8-165]	109 [76.0-152]	0.913
eGFR (ml/min)	101.3±30.0	103.4±29.6	90.6±26.8	**0.004**
Pharmacotherapy				
WO (n, %)	102 (24.1%)	97 (27.3%)	5 (7.4%)	**0.001**
Group A (n, %)	251 (59.3%)	202 (56.9%)	48 (70.5%)	0.058
Group B (n, %)	70 (16.5%)	55 (15.5)	15 (22.1%)	**0.026**
SCORE risk (%)	1.40 [0.50-3.40]	1.20 [0.45-2.70]	3.25 [1.17-6.03]	**<0.001**
Framingham (%)	17.4 [9.10-30.0]	16.4 [8.60-27.9]	26.7 [15.0-30.0]	**<0.001**
Events (n(%))	52 (12.3%)	33 (9.3%)	19 (27.9%)	**<0.001**

Table 18 - Demographic characteristics at baseline with division based on AADa presence A significant difference between patients with and without AADa was shown in **bold**. Legend: AADa (ascending aorta dilatation according to ARGO-SIIA project); BMI: body mass index; BSA: body surface area; SBPp: peripheral Systolic blood pressure; DBPp: peripheral diastolic blood pressure; PPp: peripheral pulse pressure; SBPc: cedntral systolic blood pressure: DBPc: central diastolic blood pressure; PPc: central pulse pressure; HR: heart rate; eGFR: estimated glomerular filtration rate; WO: wash out; Group A: up to 3 drugs none of which are diuretic or up to 2 drugs including a diuretic; Group B: 3 drugs (including a diuretic) or ≥ 4 drugs

AADa was found in 16.1% of study population. Patients with AADa were older [62.2 ± 10.9 vs. 51.2 ± 12.5 years] than the ones without AADa and presented a longer history of hypertension [11.0 (5.00–19.0) vs 6.00 (2.00–12.5) years, P<0.001], a worse eGFR [90.6 ± 26.8 ml/min vs. 103.4 ± 29.6 ml/min, P = 0.004], and higher cardiovascular risk, obtained by both Systemic Coronary Risk Evaluation [SCORE[70]] and Framingham [71] score. No differences were found between AADa groups with regard to peripheral or central BP, but patients with AADa showed higher aortic augmentation index (23.0 ± 11.5 vs. 28.5 ± 10.0 %, P<0.0.001).

Table 19 displays echocardiographic features in different AADa groups. Subjects with AADa presented a greater prevalence of LVH, particularly with concentric geometry, LAe, SVD, PWV more than 10 m/s and higher values of mean E/tdiE (P<0.050 for all). No differences in ejection fraction were observed.

		AADa		
Variable	**General Population (n = 423)**	**No (n=355)**	**Yes (n=68)**	**p value**
LVMI (g/m^2)	89.9±22.7	87.9±20.9	100.0±28.7	**0.001**
LVH (n, %)	70 (16.6%)	47 (13.3%)	23 (33.8%)	**<0.001**
Normal (n, %)	263 (62.2%)	230 (64.8%)	33 (48.5%)	**0.017**
Concentric remodeling (n, %)	90 (21.2%)	78 (21.9%)	12 (17.6%)	0.557
Eccentric hypertrophy (n, %)	41 (9.69%)	31 (8.73%)	10 (14.7%)	0.193
Concentric hypertrophy (n, %)	29 (6.86%)	16 (4.5%)	13 (19.1%)	**<0.001**
EF (%)	60.2±4.4	60.3±4.3	58.4±4.6	0.157
LAVi (ml/m^2)	30.1±9.09	29.8±9.15	32.0±8.62	**0.093**
LAe (n(%))	103 (28.4%)	80 (25.9%)	23 (42.6%)	**0.019**
SOV (mm)	36.5±4.84	35.9±4.51	40.0±5.08	**<0.001**
SVD (n(%))	106 (25.1%)	72 (20.3%)	34 (50.0%)	**<0.001**
ASC (mm)	35.2±5.31	33.7±4.26	42.9±3.22	**<0.001**
E/A	1.04±0.35	1.08±0.35	0.86±0.26	**<0.001**
E/e'	7.21±2.33	7.07±2.10	7.94±3.26	**0.042**
PWV (m/s)	7.40 [6.50;8.80]	7.20 [6.40;8.50]	8.80 [7.20;9.90]	**<0.001**
PWV > 10m/s (n(%))	45 (12.5%)	31 (10.2%)	14 (25.5%)	**0.003**

Table 19 - Ultrasound and hemodynamic characteristics at baseline, divided by presence of "AADa" A significant difference between patients with and without AADa was shown in **bold**. Legend: AADa: ascending aorta dilatation according to ARGO-SIIA project; LVH: Left ventricular hypertrophy; LVMI : left ventricular mass index; LVH : Left ventricular hypertrophy; LAVi: left atrial volume index; LAe: left atrial enlargement; SOV: sinuses of Valsalva; SVD: sinuses of Valsalva dilatation according to z-score; ASC: ascending aorta; PWV: pulse wave velocity; E: E wave at transmitral Doppler; A: A wave at transmitral Doppler; e': mean wave E at tissue transmitral Doppler

Survival analysis

During a median follow-up of 7.4 years (5.6–9.1 years) 52 events occurred: 19 among patients with AADa and 33 among patients without AADa (27.9 vs. 9.3%, P<0.001). In particular we observed 16 atrial fibrillations (30.8%), 13 myocardial infarction (25.0%), 6 coronary revascularizations (11.5%), 6 surgeries involving aorta or its major branches (11.5%), 4 ICD implants (7.6%), 3 strokes (5.7%), 3 TIA (5.7%), 1 unstable angina (1.9%, Table 20).

Variable	Events n = 52	AADa	
		No (n=33)	Yes (n=19)
Myocardial infarction	13 (25.0)	10 (30.3)	3 (15.8)
Stroke	3 (5.7)	3 (9.1)	3 (15.8)
Sudden cardiac death	0 (0.0)	0 (0.0)	0 (0.0)
Heart failure	0 (0.0)	0 (0.0)	0 (0.0)
TIA	3 (5.7)	2 (6.0)	1 (1.5)
Unstable angina	1 (1.9)	0 (0.0)	1 (1.5)
Cardiac resynchronization (without event)	6 (11.5)	3 (9.1)	3 (15.8)
Ao/SAV surgery	6 (11.5)	2 (6.0)	4 (21.0)
ICD	4 (7.6)	2 (6.0)	2 (10.5)
Arrhythmias			
AF	16 (30.8)	12 (36.4)	4 (21.0)
VF	0 (0.0)	0 (0.0)	0 (0.0)

Table 20. Cardiovascular events observed, divided by the presence or absence of aortic dilatation. **Legend**: AADa: ascending aorta dilatation according to ARGO-SIIA project; AF: atrial fibrillation; VF: ventricular fibrillation; Ao: aortic surgery; SAV: sovra-aortic surgery; TIA: Transient ischemic attack; ICD: Implanted Cardioverter Defibrillator

In univariate Cox regression and after adjusting for age, gender and BSA, the increasing of ASC diameter, as well ASCh0.5 and ascending aorta diameter/BSA0.4 (ASCi0.4), resulted related to a greater risk of event (P<0.001 for all). Similar outcomes were obtained for the presence of AAD defined according to different criteria (ARGO-SIIA project, $height^{0.5}$ e $BSA^{0.4}$, P <0.05) (Table 21).

	Univariate regression			Adjusted for age and sex		
	beta	**HR (95% CI for HR)**	***p value***	**beta**	**HR (95% CI for HR)**	***p value***
ASC	0.12	1.12 (1.07-1.18)	**<0.001**	0.12	1.13 (1.06-1.20)	**<0.001**
ASCi$^{0.4}$	0.18	1.2 (1.12-1.29)	**<0.001**	0.18	1.20 (1.10-1.30)	**<0.001**
ASCh$^{0.5}$	0.18	1.19 (1.11-1.28)	**<0.001**	0.17	1.18 (1.10-1.29)	**<0.001**

Table 21a. Uni and multivariate regression with the different definitions of ascending aorta. *Event risk after correction for age, gender and BSA for ASC, ASCi$^{0.4}$ e ASCh$^{0.5}$* Significant p results are shown in **bold**. Legend: ASC: absolute ascending aorta diameter; ASCh$^{0.5}$: indexed to height$^{0.5}$ ascending aorta diameter; ASCi$^{0.4}$: indexed to BSA$^{0.4}$ ascending aorta diameter

		Univariate Regression			Adjusted for age and sex		
	Obs.	**beta**	**HR (95% CI for HR)**	***p value***	**beta**	**HR (95% CI for HR)**	***p value***
AADa	423	0.85	2.34 (1.57-3.49)	**<0.001**	0.65	1.92 (1.25-2.95)	**0.003**
AADi$^{0.4}$	423	1.1	3.10 (1.80-5.34)	**<0.001**	0.92	2.52 (1.36-4.65)	**0.003**
AADh$^{0.5}$	423	1.10	3.12 (1.81-5.37)	**<0.001**	1.02	2.77 (1.49-5.16)	**0.001**

Table 21b. Uni and multivariate regression with the different definitions of ascending aorta dilatation. *Event risk after correction for age, gender and BSA for ASC, ASCi$^{0.4}$ e ASCh$^{0.5}$* Significant p results are shown in **bold**. Legend: AAD: ascending aorta dilatation based on absolute value; AADh$^{0.5}$: AAD indexed to height$^{0.5}$; AADi$^{0.4}$: AAD indexed to BSA$^{0.4}$

Univariate Cox regression was performed for all demographic and echocardiographic variables investigated: variables which showed to be associated with event (age, hypertension duration, wash-out from antihypertensive drugs, pharmacotherapy group A, eGFR, LAVi, PWV, ASC diameter, Framingham score risk) were included in a penalized regression to perform variables selection. As shown in Table 22, the selected variables were: duration of hypertension, eGFR and PWV in the case of ASC [hazard ratio = 1.13 (1.04 - 1.23), P = 0.005] and duration of hypertension, eGFR and PWV> 10 m / s in the case of AADa [hazard ratio = 2.58 (1.47 - 4.54), P = 0.001].

*	beta	HR (95% CI for HR)	*p value*	*	beta	HR (95% CI for HR)	*p value*
Hypertension duration	0.02	1.02 (0.98-1.05)	0.391	**Hypertension duration**	0.02	1.03 (0.98-1.06)	0.170
eGFR	-0.02	0.98 (0.98-0.99)	**0.036**	**eGFR**	-0.02	0.98 (0.97-1.01)	0.060
ASC	0.12	1.12 (1.04-1.22)	**0.005**	**AADa**	0.94	2.58 (1.47-4.54)	**0.001**
PWV	0.70	1.07 (0.88-1.31)	0.494	**PWV>10m/s**	0.37	1.45 (0.75-2.82)	0.270

Table 4: multivariate regression. Event risk after correction for main confounders for ASC (column A) and AADa (column B). Significant p results are shown in **bold**. Legend: eGFR: estimated glomerular filtration rate; PWV: pulse wave velocity. * 243 events, number of events = 26.

Both ASC diameter and AADa (Fig.25) were associated with an increased risk of events after correction for these variables.

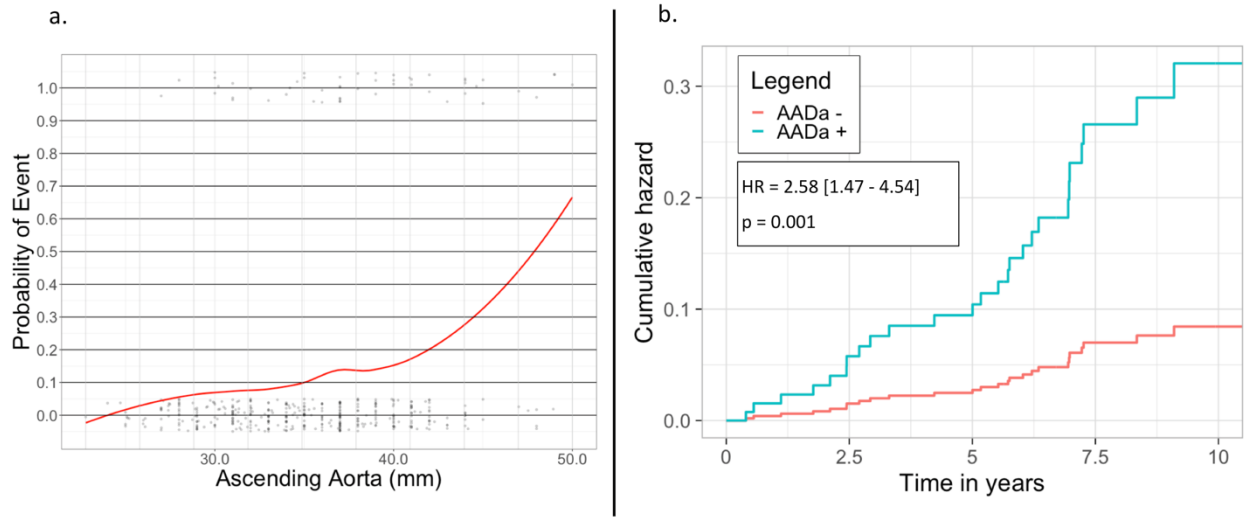
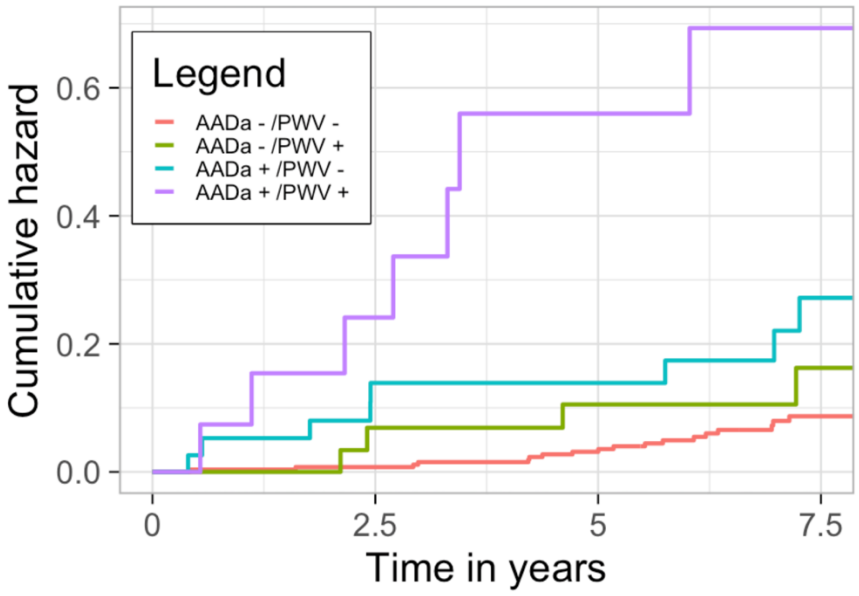


FIGURE 25. Event risk curves in relation to ascending aorta diameter. Probability of incurring an event with increasing diameter of the ascending aorta (a) and risk curve of events with or without dilation of the ascending aorta (according to the definition of the ARGO-SIIA project).Legend: AAD, ascending aorta dilatation; ASC, ascending aorta diameter; HR, Hazard Ratio.

Similar results were obtained for all the other indexations of the diameter and for all the definitions of AAD considered.

Adding LVMi and mean E/tdiE to confounders didn't affect ASC significance in pre- dicting cardiovascular events (data not shown).

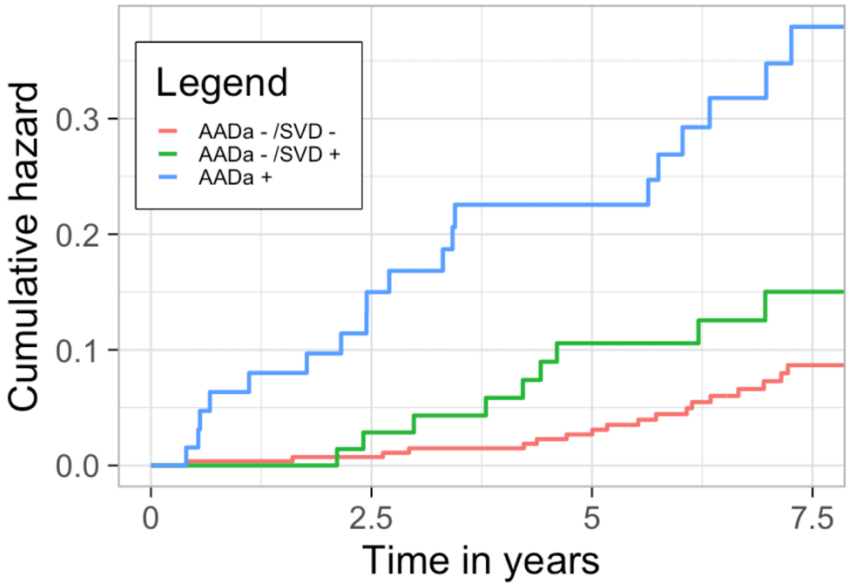
In Figure 26 survival curves of a first model including AADa and PWV > 10 m/s are displayed. In Kaplan–Meier analysis presenting AADa alone (AADa+/PWV-) (P = 0.024) conferred a higher risk of event compared with patients with no AADa nor PWVmore than 10 m/s (AADa-/ PWV-). Furthermore, the presence of both conditions (AADa+/PWV+) was associated with a greater risk of event than both AADa and PWV more than 10 m/s alone (P = 0.036 and 0.006, respectively).



	Combined AAD and PWV>10m/s	AADa - / PWV -		AADa + / PWV +	
		Chi-squared	*p value*	Chi-squared	*p value*
Log-Rank	AADa - / PWV -			29.163	<0.001
	AADa - / PWV +	0.532	0.466	7.707	0.006
	AADa + /PWV -	5.068	0.024	4.353	0.036
	AADa + / PWV +	29.163	<0.001		

FIGURE 26. Kaplan - Meier curve of cardiovascular risk based on the presence of dilation of the ascending aorta and PWV> 10 m / s. Cumulative cardiovascular risk curve in relation to ascending aorta dilation presence according to the ARGO-SIIA project definition and PWV> 10 m / s. AADa, ascending aorta dilation according to the ARGO-SIIA project; PWV, pulse wave velocity

Survival curves of a second model including AADa and SVD are shown in Figure 27. Presence of AADa (with or without SVD) resulted in greater risk of events than t than no dilatation on either levels (AAD- / SVD-) (P <0.001) and SVD only (AAD- / SVD +) (P = 0.026).



	Combined AAD and SVDz	AADa - / SVDz -		AADa +	
		Chi-squared	*p value*	Chi-squared	*p value*
Log-Rank	AADa - / SVDz -			20.625	<0.001
	AADa - / SVDz +	1.260	0.262	4.963	0.026
	AADa +	20.625	<0.001		

FIGURE 27. Kaplan-Meier curves of cardiovascular risk based on the presence of dilation of the aorta at the ascending and / or sinus of Valsalva. Cumulative cardiovascular risk curves in relation to the presence of ascending aorta dilation (defined according to the ARGO-SIIA project) and sinus of Valsalva dilation (based on the z-score). AADa, ascending aorta dilatation according to the ARGO-SIIA project; SVD, aortic dilatation at the level of the sinus of Valsalva according to z-score.

6.5 DISCUSSION

The current study is to the best of our knowledge the first one to evaluate the prognostic value of AAD in hypertensive patients. Our main findings can be summarized as follows: first, in our population of hypertensive patients, increasing ASC diameter and AAD according to ARGO-SIIA definition resulted associated with a greater risk of cardio-vascular events; second, AADa seemed to be able to stratify cardiovascular risk in patients without PWV more than 10 m/s; third, the prognostic value of proximal aorta dilatation could depend on the presence of AAD, since in its absence, SVD seemed to not be able to predict patients' risk of cardiovascular events in our population. Ageing has been proven to cause an alteration in physiological elastic property of aortic vessel, which may occur in the form of aortic remodeling and dilatation. In agreement with this evidence, in our study patients who presented AADa were on average 10 years older and with a longer arterial hypertension history than patients without aortic enlargement.

Our work seems to confirm the marginal role, among hypertensive patients, of both peripheral and central BP on aortic remodeling, as previously reported in literature [59]. On the other hands surrogates of arterial stiffness are reported to be significant determinant of aortic remodeling, and our results seem to support this hypothesis.

Patients with AADa showed a higher rate of established cardiovascular risk factors, such as LVH, LAe and PWV > 10 m/s, supporting previous evidence of a role of AAD in cardiovascular risk stratification. In particular LVMi, arterial stiffness (in terms of PWV) and ASC dimension could be considered as three different anatomical expression of the same adaptive phenomenon. Hypertension, such as ageing, are associated with an increase in arterial stiffness [in terms of PWV [3,72]] , affecting the summation between incident and reflexed pulse pressure waves at aortic root level [26]. This complex interplay leads to a mismatch between cardiac output and vascular impedance with consequent LVH and AAD.

Nevertheless, the prognostic value of the AAD has never been specifically studied. In our population ASC dimension showed a significant prognostic value, even when corrected for main confounders we identified. This was true not only for ASC diameter itself (Table 21a), but even for $ASCi^{0.4}$ and $ASCh^{0.5}$ (Table 21bSupplementary material), providing a strong evidence that ASC dimensions have a significant role in cardiovascular risk stratification. In this perspective, AAD definition pro- posed by ARGO-SIIA project resulted clinically effective in identifying at-risk patients, and the same was true for dilatation of the ASC as defined based on other criteria ($ASCi^{0.4}$ and $ASCh^{0.5}$).

Although aortic dimensions and PWV, as previously discussed, can be thought of two different ways to describe the same phenomenon, the information they provide is not necessarily equivalent, as proven by the fact that the presence of both AADa and PWV more than 10m/s is a more powerful predictor of cardiovascular events compared with the presence of either one alone. Moreover, since AADa alone resulted able to stratify cardiovascular risk in patients with both presence or absence of PWV more than 10 m/s, combined with greater availability of TTE technology, relatively short time needed for ASC diameters acquisitions and measurements compared with the dedicated instrument for PWV assessment, suggests that ascending aortic dimensions should be considered as marker for vascular organ damage in routine evaluation of cardiovascular risk.

The prognostic role of the proximal aorta dilatation has already been proven in several studies at the level of SOV. SVD has been shown to be an additional risk factor in patients with LVH [68] and an independent cardiovascular risk factor in patients without LVH [6] and > 65 years old [73]. In our population SVD resulted effective in cardiovascular risk stratification only when associated with AADa as well (Figure 27), suggesting ASC enlargement to be the main determinant in prognostic

value of aortic root dilatation. The pathophysiological basis of these findings is not yet completely understood, although differences in microscopic structure and mechanical characteristics between ASC and SOV has been proven. Biomechanical experiments proved that ASC has a greater compliance, with more gradual variation after mechanical stretching compared with SOV [74]. Also morphometric and hemodynamic determinants exert a different role in determining the diameters of these different sectors of the aorta: for example, males tend to have larger SOV than females, whereas the BSA-indexed diameter of the ASC is not influenced by sex and in some studies was found to be even larger in female patients [12]. On the other hand, age seems to influence the ASC more than SOV [75]. It is therefore evident that these two segments of the aorta are two distinct units able to influence and be influenced in a different way by the hemodynamic of the subject.

So, our results suggest that during the risk stratification process of hypertensive patients, ASC diameter may be evaluated, as well as other HMODs such as LAVi and LVMi. Indeed, AADa showed to be able to stratify the cardiovascular risk of hypertensive patients independently from the other HMODs.

7. Future perspectives

The ATHOS project

Validation of the ATHOS device with invasive methods or with non-invasive methods that determine an accurate measurement of the aortic length, such as magnetic resonance imaging, will be necessary. Furthermore, validation of the instrument will have to be confirmed in subjects with cardiovascular diseases, and patients with a broader range of PWV should be assessed. Its simplicity of use could help in increasing clinical application of PWV assessment and improving patients' cardiovascular risk stratification.

At the present time, our collaboration with *Politecnico di Torino* continues. We are investigating the application of new sensors based on the innovative graphene technology. They have a high sensitivity even for small pressure variations. This allowed its use in wearable and therefore operator-independent mode, a property currently not guaranteed by any of the new technologies on the market. Once the signal acquisition of the new sensors would be optimized, it will be necessary to compare the performance of the new graphene sensors with those obtained by Sphygmocor, but at the moment it was not possible due to COVID-19 pandemic health restrictions.

Aortic strain analysis

Our aim was to test a new technique with the purpose of providing a new potentially useful descriptor of cardiovascular pathophysiology, and we hope that our data will guide further investigations in this context. To our knowledge, it has not yet been validated in direct comparison to current gold standard, represented by magnetic resonance or invasive investigations.

It will be essential to identify the reference values in normotensive subjects and verify its sensitivity and specificity in determining the subgroup of patients at higher cardiovascular risk. A functional assessment of the ascending aorta could play an important role in early identification of subjects with normal aortic dimensions but compromised elastic properties. Previous studies showed that β2 SI is related to cerebrovascular events and the development of hypertension in non-hypertensive patients [76]. The results obtained so far showed that it could be possible to obtain more specific information on cardiovascular health status, identifying patients with a higher cardiovascular risk and with a greater risk of aortic dilation or more rapid progression, providing adequate therapeutic management and setting the long-term follow-up. Further longitudinal studies are needed to confirm this hypothesis and give a prognostic value to this new technique and to the data that can be derived from its application.

A future step would be to make the instrument automatic and therefore operator independent. In this regard, we are collaborating for the development of a new software that automatically measures the longitudinal strain of the ascending aorta on DCOM images, with consequent savings of time and standardization of the measurement process.

Follow up of hypertensive patients and second phase of the "RECALL" project

Future analysis of follow-up data will attempt to evaluate the predictors of aortic diameter progression in patients followed in our center. We will try to overcome current work limitations. First of all, we were not able to establish a direct causal link between aortic dilation and cardiovascular events because it was an observational study, in a highly selected population. The limited number of events did not allow for a more accurate analysis of the impact of AAD on individual cardiovascular endpoints. Therefore, it will be necessary to expand study population, designing the study prospectively for a longer follow-up time, in order to increase the number of observed events as well. The purpose of these analyzes will be to deepen the timing of follow up and to identify patients at greater risk of dilation. In this regard, we are evaluating the prognostic role of ascending aortic remodeling (AAD) in a multicenter study phase.

8. ABSTRACT

Background

Arterial hypertension is the main cardiovascular risk factor in western population. Less is known about the role of proximal aortic dilatation as an expression of hypertension-related organ damage. Proximal aorta dilatation at Sinus of Valsalva level is an independent predictor of CV events regardless of LVH and other common confounders. It is relatively frequent in hypertensive patients, with an estimated prevalence up to 16.9 %. Focusing on ascending aorta (AA) diameter, it is significantly related to changes in cardiac morphology, central hemodynamics and arterial stiffness. Currently there are nomograms that allow to estimate the expected aortic diameter according to age, gender and body size, but it is unknown which anthropometric, hemodynamic and clinical factors have the most important influence over aortic diameters and rate of aortic dilatation.

Recently, we observed a mean growth rate of proximal aorta in hypertensive patients with known aortic dilatation of about 0.1 mm/year, similarly to the rate observed in the general population. It is then fairly clear that aortic size alone is an inadequate predictor of the development of acute aortic pathology, so we concentrated on aortic elastic properties to predict aortic diameter evolution.

At the moment the gold standard for the non-invasive evaluation of regional aortic stiffness is magnetic resonance imaging (MRI), with the known limits of high costs and reduced availability. Assessment of large artery stiffness by carotid femoral Pulse Wave Velocity (cfPWV) represents a strategic parameter in the evaluation of hypertension mediated organ damage, commonly used for cardiovascular risk stratification.

Aims

Firstly, we aimed to develope a new easy to use, reproducible, low-cost and non-invasive tool for the assessment of the cfPWV (The ATHOS project).

Then, we tried to evaluate the association between arterial stiffness and aortic remodeling, focusing on local aortic mechanics by echocardiographic aortic strain analysis (Aortic strain analysis).

Eventually, we evaluated the prognostic value of AA dilatation as predictor of cardiovascular (CV) events in essential hypertensive patients (The “RECALL” project).

The ATHOS project

Currently, the reference device for non-invasive cfPWV is SphygmoCor, but its cost and technically challenging use limit its diffusion in clinical practice. For this reason, we are completing the patenting of a new device for non-invasive assessment of cfPWV, ATHOS (Arterial sTiffness faitHful tOol aSsessment, patent protocol number P3640IT00, 2020-025, dated 11/20/2020), designed in collaboration with the Politecnico di Torino and compared to the reference SphygmoCor. 90 healthy subjects were recruited. In each subject, we assessed cfPWV, using SphygmoCor (PWV_SphygmoCor) and ATHOS (PWV_ATHOS) devices in an alternate fashion, following the ARTERY Society guidelines. Mean PWV_ATHOS and mean PWV_SphygmoCor were 7.88 ± 1.96 m/s and 7.72 ± 1.95 m/s, respectively. Mean difference between devices

was 0.15 ± 0.56 m/s, with a high correlation between measurements (r = 0.959, p < 0.001). Considering only PWV values ≥ 8 m/s (n= 30), mean difference was 0.1 ± 0.63 m/s. The interclass correlation coefficient (ICC) was 97.7 % with ATHOS. Its simplicity and manageability could help PWV assessment spreading, to get a better cardiovascular risk stratification.

Aortic strain analysis

In order to verify the association between local aortic mechanics and aortic remodeling, we tested the feasibility and usefulness of aortic strain analysis in estimating ascending aortic elastic properties, using a simplified transthoracic echocardiography (TTE) speckle-tracking (ST) based method in terms of β2-Stiffness index (Beta-SI).

In a second moment, we tested its behavior in 100 hypertensive outpatients with increasing dilation of proximal thoracic aorta. Beta-SI resulted to rise exponentially with AA dimensions (p<0.001). A progressively impaired Beta-SI in groups identified by progressively greater AA was observed. Beta-SI was also related to cardiovascular organ damage in terms of left ventricular mass (LVMi, p=0.030) and PWV (p=0.028). Patients with high Beta-SI showed grater LVMi (94±24 vs. 117±47 g/m2; p=0.010) and PWV (8.63±1.88 vs. 10.20±2.99 m/s; p=0.013).

The "RECALL" project

Eventually, we evaluated the long-term impact of ascending aorta remodeling (AAD) in hypertensive patients and its possible prognostic value as predictor of cardiovascular (CV) events.

423 hypertensive patients were included and underwent transthoracic echocardiography, cfPWV and clinical evaluation. During a median follow-up of 7.4 years (interquartile range 5.6-9.1 years) a total of 52 events were observed. After adjusting for age, sex and BSA, AAD resulted associated with a greater risk of cardiovascular event (p<0.010), even after adjusting for major confounders in multivariate analysis (p<0.010). Moreover, we observed that the assessment of ascending aorta improves risk stratification compared to PWV alone, and that in absence of AAD, Sinus of Valsalva Dilatation (SVD) lost any prognostic value (p=0.262, Figure 1). In conclusion, AAD is associated with a greater risk of CV events and its evaluation may optimize risk stratification in hypertensive patients.

Conclusions:

ATHOS simplicity and manageability could help PWV assessment spreading, to get a better cardiovascular risk stratification.

With aortic strain analysis we were trying to identify those subjects with clinical or echocardiographic characteristics that could predict a possible unfavorable evolution of the aortic pathology and therefore to require greater attention over time, so we started a follow up program in our Hypertension Center. In these years, we observed that AAD is associated with a greater risk of CV events and its evaluation may optimize risk stratification in hypertensive patients.

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