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PULSE WAVE VELOCITY, HYPERTENSION AND PLASMA ALDOSTERONE LEVELS

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INTRODUCTION

For a long time, European hypertension guidelines primarily focused on blood pressure (BP) values as the main factor determining the need and the type of treatment. In 1994, the European Society of Cardiology (ESC), European Society of Hypertension (ESH) and European Atherosclerosis Society (EAS) developed joint recommendations on prevention of coronary heart disease (CHD) in clinical practice, and emphasized that prevention of cardiovascular (CV) disease should be based on quantification individual global CV risk profile rather than on absolute levels of isolated CV risk factors, such as high blood pressure (BP), high cholesterol or glucose levels. In particular, clinical evaluation of all hypertensive outpatients should include comprehensive assessment of the so-called "hypertension-mediated organ damage" (HMOD), even in asymptomatic, otherwise healthy individuals.

The importance of HMOD has been demonstrated in numerous clinical studies. These consistently showed that, despite effective BP control achieved in treated hypertensive patients, the rate of major CV events remains higher than that reported in non-hypertensive subjects. This observation may be explained by other functional and structural alterations (stiffness) of cardiac and vascular system might be induced by hypertension, even at early stages of the disease.

Among different markers of vascular HMOD, pulse wave velocity (PWV) is a simple, non-invasive and reproducible tool for evaluate stiffness of large arteries, and thus, can be easily employed in the routine clinical practice. Data showed that PWV is an independent predictor of morbidity and mortality in patients with essential hypertension as well as in patients with diabetes and chronic kidney disease (CKD). Furthermore, several studies performed in various populations demonstrated a significant pro-fibrotic and pro-inflammatory effect of aldosterone on vascular endothelium leading to arterial stiffness. Assessment of carotid-femoral PWV, an index of aortic stiffness and underlying arteriosclerosis, is actually the 'gold standard' technique for measuring aortic stiffness (IIb B).

The aim of the research was to analyse PWV profile obtained with a validated and simple oscillometric method in a population of hypertensive patients and evaluate potential correlation with plasma aldosterone levels and CV risk factors.

CHAPTER 1

Pulse wave analysis in hypertensive patients

Endothelium is the largest organ of human body and contributes to maintain vascular homeostasis regulating blood pressure (BP), organ perfusion, platelet aggregation and selective wall permeability. Endothelial dysfunction of both small and large arteries may lead to an increase stiffness of the whole vascular system, causing alterations in arterial pressure and blood flow dynamic, thus impacting on heart work, coronary perfusion and multi-organ function.

Increased central arterial stiffness is the result of the natural ageing process of human vessels, but it is also induced by many other cardiovascular (CV) risk factors and diseases, including smoke, hypertension, metabolic syndrome, diabetes mellitus, renal impairment, and atherosclerosis. Whatever the case, it lead to high risk of developing major CV events (e.g. myocardial infarction, heart failure, stroke, chronic kidney disease), independently by age, gender and ethnicity.

The importance of arterial stiffness has been recognized for many years and a variety of techniques have been developed for assessing vascular stiffness, though the exact mechanisms underlying this condition are not completely understood. In experimental and clinical studies, both structural and functional changes have been observed within the medium layer. These may include deposition of collagen, calcium accumulation, and reduction in nitric oxide availability in smooth muscle cells. This process promotes the remodelling of small arteries (smooth muscle concentric remodelling, collagen and cellular infiltration), which further promotes increased peripheral vascular resistances and BP levels, and affects renal and brain function by increasing pulsatile load and heart function by increasing systolic load.

Although several conventional CV risk factors have been addressed to cause arterial ageing, there is a considerable inter-individual variability in developing arterial stiffness, suggesting that this process might be affected also by environmental and genetic influences. Moreover, ageing aggravates vascular stiffness, thus promoting the development of a “vicious cycle”.

Arterial stiffness is commonly considered as a marker of physiological arterial ageing. Young

individuals with long exposure to multiple CV risk factors could manifest “early vascular ageing”, whereas old subjects without traditional CV risk factors or genetic susceptibility may have supernormal vascular ageing.

Healthy large arteries physiologically transform pulsatile flow into continuous flow at arteriolar level without great energy dissipation through the vascular wall. The ability of arteries to adapt their lumen sectional area for a given change in BP defines for “arterial compliance”, which depends on the function and geometry of the vascular conduct. BP wave propagation and reflection in the arterial tree depends on arterial wall distensibility: the arterial pulse propagates in the arterial tree with a pulse wave velocity (PWV), a parameter for measuring arterial stiffness and vascular mechanical properties. Pulse wave analysis (PWA) is a simple and reproducible measure that provides the calculation of PWV, described by the Moens–Korteweg equation in the 1920s,

$$c_o = \sqrt{\frac{Eh}{2R\rho}}$$

In the formula: c_O is the wave speed, E is Young’s modulus in the circumferential direction, h is the wall thickness, R the vessel radius and ρ the fluid density.

PWV is obtained by measuring the time (in seconds) taken for a pulse wave to travel a distance (in meters). Distance is usually estimated using a site on the body surface, while time is recorded by measuring the interval between two points on a pressure or flow wave, using a proximal and distal transducer. Usually time is calculated through the ‘foot’ of a waveform, being this part least influenced by wave reflection, thus the stiffer the vessel, the faster the PWV.

Several errors may occur in measuring a given distance between pulse assessment points and time taken by the wave to travel between the two sites. For example, distance measure may be inaccurate in obese patients or in those with supraventricular arrhythmias. Currently available software and electronic tools are supplied with specially designed algorithms taking into account multi-parametric values, thus reducing errors.

In physiological condition, PWV varies from vessel to vessel. In a middle-aged subject, PWV in the ascending aorta would be of the order of 4 ms^{-1} compared with 5 ms^{-1} in carotid arteries and 7 ms^{-1} in the brachial artery. Since the aorta is responsible for most of the pathophysiological effects of arterial stiffness, the PWV recorded along the aortic level resulted to be the most clinically relevant. The hypothesis was supported by Paini et al. demonstrating that the aorta stiffened more than the carotid artery, according with age and other CV risk factors.

Another recognized parameter for evaluating arterial stiffness is the augmentation index (AIx), that quantify the wave reflection which can be derived using pressure waveform data. It can be defined as:

AIx = (*P*2–*P*1)/PP

In the formula: *P*1, the first systolic peak is ascribed to the forward pressure wave and results from the ejection of the blood from the heart; *P*2, the second systolic peak indicates the reflected pressure wave; *P*2–*P*1, the augmentation pressure and can thus either be negative or positive; PP, the systolic-diastolic pressure difference.

The AIx is a function of pulse wave reflection and there is a positive correlation with PWV since higher aortic PWV will result in faster transmission of pressure waves to the peripheral arteries and a faster return of reflected waves that determine AIx: the stiffer the arteries, the faster the pulse wave will travel in the vessel and the reflected wave (P2) arrives earlier in P1.

The AIx depends on the shape of the P1 wave, which in turn depends on left ventricular ejection and on the elasticity of the ascending aorta, as well as the timing of the reflected wave, in turn influenced by gender and height.

Higher AIx has been positively associated with left ventricular hypertrophy both in normotensive subjects and essential hypertensive patients. Nevertheless, the AIx can be affected by multiple factors (left ventricular ejection fraction, PWV, timing of reflection, arterial tone, structure at peripheral reflecting sites, BP, age, gender, height and heart rate). There are concern over the accuracy of central AIx derivation from pulse waveform analysis

and several question about its use remain unanswered. Therefore, the usefulness of AIx as a marker of CV events must be confirmed by further studies.

Available evidence demonstrated that aortic PWV and AIx are non-invasive measurements of large elastic artery stiffness and wave reflection, two independent predictors of future CV events and all-cause mortality. Interventions aimed at lowering aortic PWV and AIx may be effective in reducing risk of major CV outcomes. Antihypertensive treatments can reduce arterial stiffness beyond passive reductions of arterial BP; also, lipid-lowering, glucose-lowering and anti-inflammatory drugs may provide additional benefits on stiffness, independently from pressure reduction, although this hypothesis is not yet proven.

Another marker of endothelial function is the Compliance (C), reciprocal of elastance, calculated by the following equation:

$$C = V / P$$

Where V is the change in arterial blood volume (mL) and P is the change in arterial pressure (mmHg).

Several studies investigate the role in C changes in hypertensive patients, demonstrating that these C abnormalities can be detectable also in preclinical (asymptomatic) stages of the disease, thus representing a potentially sensitive marker for early detection of future CV events and a valuable tool for improving global CV risk stratification.

It should be noted, however, that the words “compliance”, “elasticity” and “stiffness” are often used interchangeably, although their physical definitions are rather different, as previously described.

Among parameters defining arterial stiffness the measurement of Pulse Pressure (PP) is the simplest, as it is defined by the difference between systolic and diastolic BP and determined by cardiac stroke volume and arterial stiffness:

Pulse pressure (PP) = SBP − DBP

This simple parameter indicates the degree of impairment of function of larger arteries and is a valuable predictor of left ventricular mass, carotid–intima thickness and risk of future CV events. PP is physiologically affected by several factors and may be difficult to measure in patients with aortic valve disease or arteriovenous fistulae. Furthermore, PP not always indicates a change in aortic stiffness, since β-Adrenergic activation has been shown to increase PP, but not PWV.

Central PP has been shown to better relate to endothelial function if compared to brachial artery PP, while 24.hour PP has been shown to be a better predictor of mortality, than a single office PP measurement, by predicting the risk of stroke recurrence after an acute stroke. Furthermore, some studies evidenced PP to have an independent predictive value for CV risk in patients aged over 50 years, whilst diastolic BP better predicts CV risk in younger subjects. These data suggest that peripheral vascular resistance could be more important in the CV risk evaluation in younger individuals, whilst large artery stiffness seems to be more significant in older individuals.

A prospective cohort study published in 1999 demonstrated that PP is an independent predictor of chronic heart failure in a population of 1621 healthy elderly individuals, who were followed up for 3.8 years. PP resulted more predictive than systolic BP alone and was independent by diastolic BP. In hypertensive patients, the predictive role of PP showed to be affected by antihypertensive therapy. Data analysis derived from the First National Health and Nutrition Examination Survey Epidemiologic Follow Up Study has shown PP had significant predictive value for CV disease only in hypertensive treated subjects, but not in untreated individuals.

Non-invasive measurement of aortic stiffness, through carotid-femoral PWV and PP has been recommended (IIB b) by 2018 ESH/ESC Guidelines for the management of Hypertension, to determine asymptomatic hypertension-mediated organ damage (HMOD), a common and often undetected marker of pre-clinical cardiovascular disease (CVD), and personalize the intensity of blood pressure lowering treatment. HMOD increases CV risk independently of blood pressure level and includes structural and/or functional changes in different target organs (ie vasculature, retina, heart, kidney etc) caused by hypertension that have well-established adverse prognostic significance.

Since several HMOD categories are not included in the Systematic COronary Risk Evaluation (SCORE) charts, their identification may help to reclassify a patient's CV risk from low to moderate or from moderate to high and, especially if HMOD is pronounced, identify high-risk or very high-risk patients even in the absence of CV risk factors. Moreover, detecting HMOD in younger patients with grade 1 hypertension without previous CV events may provide a rationale to consider antihypertensive treatment and/or influence the choice of BP lowering agents. This has been established particularly in presence of left ventricular hypertrophy (LVH), albuminuria and arterial stiffening.

Of note, PWV is not only a marker and predictor of CV disease and HMOD, but several studies have shown an association between PWV reduction and regression of LVH. For this reason, PWV assessment should be adopted for evaluating BP treatment efficacy and better stratifying individual global CV risk profile during patients' follow up.

A recent review analysed data from 23 studies, including 2573 patients, reporting changes in arterial stiffness (estimated by means of PWV), systolic and diastolic BP and LVM index. Significant reductions in systolic BP, PWV and LVM index were observed in 16, 14 and 20 studies, respectively. Systolic BP reduction did not correlate with the proportion in reductions of the other two variables and a significant positive correlation (r=0.61; P= 0.003) has been found between arterial stiffness and reduction of LVM index (reduction in LVMI of 6.9 g/m2 per 1.0 m/s reduction in PWV).

Asymptomatic vascular HMOD related to arterial stiffening has been defined by current Guidelines as PP >=60 mmHg in older people and Carotid–femoral PWV >10 m/s. Vascular HMOD showed an independent predictive value for fatal and non-fatal CV events in hypertensive patients, even beyond major CV risk factors.

Different mechanisms may explain the association between increased arterial stiffness and risk of CV events. Stiffening causes earlier return of reflected waves in late systolic phase, thus increasing PWV, central PP and LV load, reducing stroke volume, and increasing myocardial oxygen demand. High systolic BP raises cardiac work, and lower diastolic BP, leading to reduced coronary perfusion and, potentially, in sub-endocardial ischemia. Furthermore, arterial stiffness promotes atherosclerosis, probably through oxidative stress, fibrosis, calcifications within the arterial wall; in addition, increased arterial stiffness can increase the risk of atherosclerotic plaque rupture.

There are multiple evidences that show how increased aortic PWV and PP are associated with poor CV outcome. A longitudinal study evaluated the association between PWV and coronary disease in 1045 hypertensive patients with essential hypertension and without previous CV events. Aortic stiffness was assessed by carotid-femoral PWV and CV risk assessment made by calculating the Framingham risk score with a mean follow-up of 5.7 years. In univariate analysis, the relative risk of CV event increased with level of PWV; for 1 SD, ie, 3.5 m/s, relative risks were 1.42 (95% confidence interval [CI], 1.10 to 1.82; P 0.01) and 1.41 (95% CI, 1.17 to 1.70; P 0.001), respectively. Framingham score significantly predicted the occurrence of coronary and all CV events (P 0.01 and P 0.0001, respectively). In multivariate analysis, PWV remained significantly associated with the coronary event even after adjustment either of Framingham score (for 3.5 m/s: relative risk, 1.34; 95% CI, 1.01 to 1.79; P 0.039) or classic CV risk factors (for 3.5 m/s: relative risk, 1.39; 95% CI, 1.08 to 1.79; P 0.01).

Detecting PWV changes can be useful in identifying patients with higher probability of developing renal disease. Ohya et al measured brachial-ankle PWV by using an automatic oscillometric method and estimated creatinine clearance by using the Cockcroft-Gault formula in 3,387 individuals. PWV increases with age, systolic BP, fasting glucose level, total cholesterol level, male gender, proteinuria and decreased creatinine clearance. All these factors were independently predicted by PWV in multiple regression analysis. Furthermore, a study showed a greater PWV with falling renal function from stages I to V; estimated GFR per 1.73 m2 and systolic BP resulted to be the major clinical determinants of vascular stiffness in patients with chronic kidney disease independently of traditional CV risk factors.

Although carotid–femoral PWV has been defined as the gold standard for quantify arterial stiffness during the last years, a large number of devices to measure arterial stiffness have been used, applying different avant-garde techniques available, for clinical and research purpose. Many studies have been carried out with the aim of validate different methods for measuring PWV, hence their conformity to current recommendations, underling their strengths and limitations.

Among various devices that had been developed to non-invasively measure of vascular stiffness transcutaneous tonometry is the most common one. Transit time to assess carotid-femoral PWV, defined as time delay between the two waves foot, is based on pulse wave acquisition at common carotid and femoral artery site through superficial measurements.

SphygmoCor is composed of a pen-like tonometric sensor for acquire pressure waves at the carotid and then at femoral site, with not simultaneous registration, since electrocardiogram is needed to synchronize the R peak with the two PW measures whilst the latest version, SphygmoCor Xcel (Xcel, Atcor Medical) allows femoral and carotid waves to be recorded simultaneously. SphygmoCor has been used in the phase V of The Caerphilly Prospective Study ruled out between 2002 and 2004 and aimed at identifying potential risk factors for arterial stiffening. It was also adopted in the Chronic Renal Insufficiency Cohort (CRIC) study, in order to determine potentially predictive factors for increased aortic PWV in CKD.

Complior (ALAM, Vincennes France) is a validated device for the measurement of PWV and central SBP composed by two non-invasive pressure sensors (piezoelectric mechano-transducers) for simultaneously record pulse waves in the carotid and femoral arteries.

Mobil-O-Graph (IEM, Stolberg, Germany) uses a cuff-based oscillometric method to evaluate carotid-femoral PWV from a single point pressure wave recording. After measuring both systolic and diastolic BP, the brachial cuff is inflated till the diastolic BP level and held for 10 seconds to record pulse waves. The device uses an ARCSolver application (Austrian Institute of Technology, Vienna, Austria) for process central pressure curves analyze multiple parameters (age central pressure and aortic impedance). Furthermore the Mobil-O-Graph 24h pulse wave analysis (PWA) monitor (IEM GmbH, Stolberg, Germany) is a validated monitor for 24-h blood pressure monitoring with the patented ARCSolver algorithm: PWA and aPWV are recorded over 24 h, allowing central hemodynamic measurements to be performed under ambulatory conditions.

Among validated cuff-based oscillometric devices there are also Arteriograph (TensioMed, Budapest, Hungary) and Vicorder (Skidmore Medical, Bristol, UK). An increased aPWV (≥10 m/s; log-rank $P < 0.05$) obtained with Mobil-O-Graph independently predicted all-cause mortality in 135 patients affected by CKD stage II-IV. Another study, using ambulatory PWV validated ARCSolver algorithms, provides evidence for U-shaped association between peripheral ambulatory SBP or PP and all-cause mortality in in hemodialysis patients.

Benas et Al showed acceptable agreement between Mobil-O-Graph, Complior and Arteriograph regarding pulse wave analysis markers and Luzardo et al reported a similar measurement between carotid-femoral PWV evaluated by tonometry and PWV estimated using the oscillometric method, irrespective of measurement conditions. Oscillometric and

tonometric PWV showed a good and satisfactory agreement within the general population, even in healthy subjects, and although oscillometric PWV values showed to underestimate arterial stiffness in younger and to overestimate in older subject, the differences in PWV estimation between tonometric and oscillometric methods appears to do not to be significant in routine clinical practice. Furthermore, PWV analyses obtained using Mobil-O-Graph showed acceptable accuracy compared with intra-aortic readings as demonstrated by a study on 120 patients (mean age 61.8±10.8 years) undergoing elective cardiac catheterization for suspected coronary artery disease. Estimated values of PWV analyses derived from brachial cuff readings were compared with those obtained using invasive measurement. The mean difference between Mobil-O-Graph measurement and intra-aortic values was 0.43±1.24 m/s. Comparison of aPWV measured by the two methods showed a significant linear correlation (Pearson's R = 0.81, P<0.0001). The mean difference for repeated oscillometric measurements of aPWV was 0.05 m/s, with 95% confidence interval limits from – 0.47 to 0.57 m/s. More recently, additional methods for measuring arterial stiffness and PWV at both central and peripheral evidence have demonstrated to be useful tools in the setting of clinical practice for helping physicians in early identifying high risk individuals who have susceptibility to develop acute CV complications.

All these findings aroused scientific interest on pathophysiological and clinical aspects of treated uncontrolled hypertension, which still represents the major unresolved clinical issue for healthcare systems, worldwide. Despite the increasing evidence that non-invasive arterial function and PWV may be better predictors of CV events than brachial blood pressure alone these measurements are still not in common use in routine clinical practice.

CHAPTER 2

Aldosterone effects on cardiac and vascular system

Renin-angiotensin-aldosterone system (RAAS) regulates aldosterone production, as schematically illustrated in Figure 1.

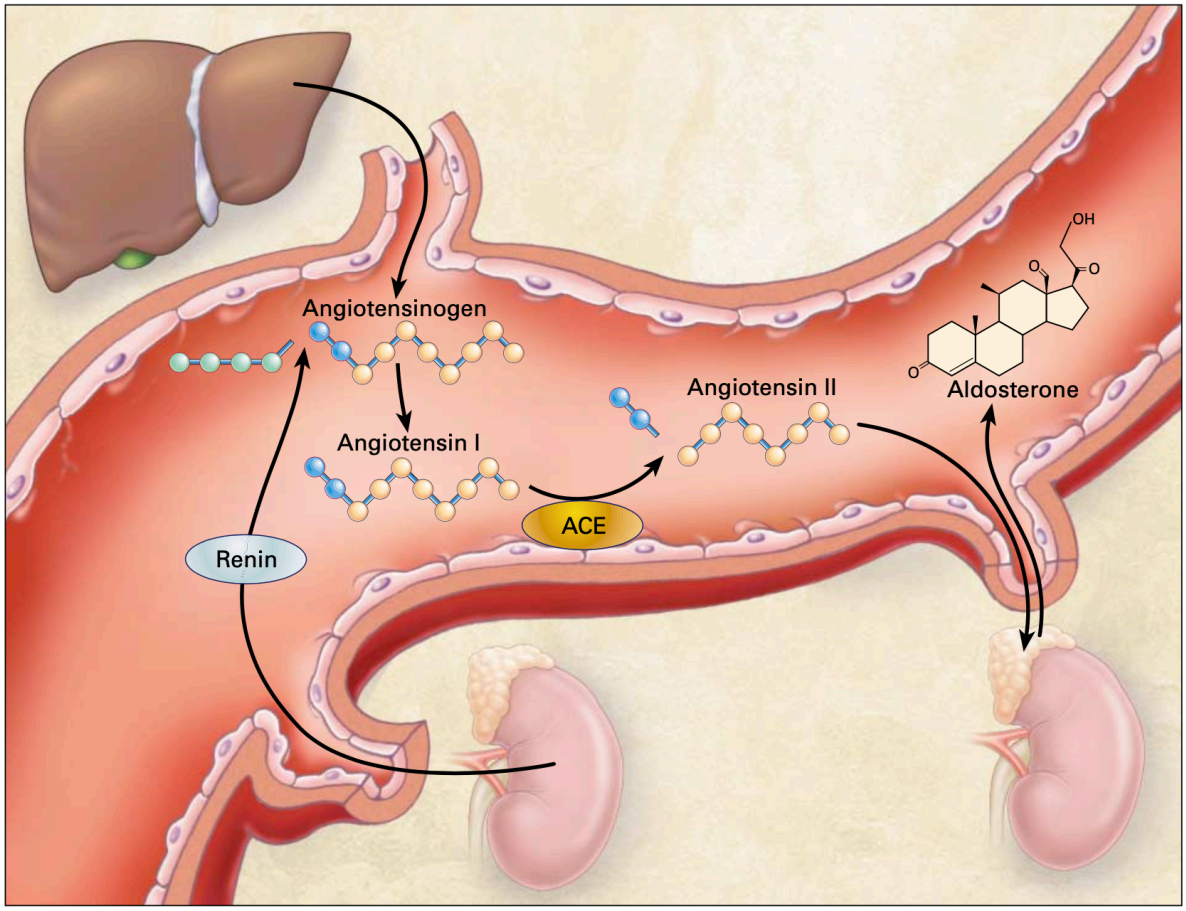


Fig 1 Angiotensinogen, is synthesized by the liver and it is cleaved by renin, which is secreted into the lumen of renal afferent arterioles by juxtaglomerular cells. Renin cleaves angiotensinogen forming angiotensin I. In turn, angiotensin I is cleaved by angiotensin-converting enzyme (ACE), to form angiotensin II. In the zona glomerulosa of the adrenal cortex, angiotensin II stimulates aldosterone production that is also stimulated by potassium, corticotropin, catecholamines (e.g., norepinephrine), and endothelins. From Weber KT. New Engl J Med 2001;345(23):1689-1697.

Juxtaglomerular cells in kidneys convert the blood circulating precursor pro-renin into renin secreting it into circulation, which cleaves angiotensinogen, released by the liver, to angiotensin I. Subsequently angiotensin-converting-enzyme (ACE) located on the surface of vascular endothelial cells (mainly on lungs and in smaller quantities on kidneys) converts the decapeptide angiotensin I into the octapeptide angiotensin II, a potent vasoconstrictive peptide that binds two different G-protein coupled receptors, AT1 (that mediated most of RAAS functions) and AT2.

Angiotensin II acts as autocrine/paracrine and endocrine hormone by stimulating the Gq protein of vascular smooth muscle cells rising intracellular calcium levels causing vasoconstriction. Furthermore, angiotensin II acts at the Na+/H+ exchanger in the proximal tubules of kidneys leading to sodium and bicarbonate reabsorption and H+ excretion. In addition, angiotensin II stimulates the production of aldosterone synthase and the secretion of this hormone from the zona glomerulosa of the adrenal cortex, increases the release of noradrenaline from sympathetic nervous system and acts on hypothalamus stimulating anti-diuretic hormone production. High plasma potassium concentration, adrenocorticotropic hormone, volume depletion or poor renal perfusion (dehydration or haemorrhage) and low sodium blood levels stimulates aldosterone secretion. RAAS pathway is not only regulated by the mechanisms that stimulate renin release, but it is also inhibit by atrial natriuretic peptides released by stretched atria.

Aldosterone is a mineralocorticoid, a steroid hormone that binds the mineralocorticoid receptor (MR) translocating into the cellular nucleus and acting as transcription factors of target genes. MR is widely expressed on epithelial cells of distal convoluted tubule and cortical collecting duct of kidneys, colon, cardiomyocytes, adipose tissue, vascular cells, neurons and macrophages. The secretion of aldosterone is also stimulated by adrenocorticotropic hormone.

In the kidney aldosterone acts on the collecting ducts of the nephron increasing the expression of epithelial sodium channels (ENaC) and consequently promotes water and sodium reabsorption in the distal renal tubule and collecting duct and urinary secretion of both hydrogen ions and potassium to maintain electrolyte balance. Sodium retention is also a response of aldosterone receptor stimulation sited in the distal colon and sweat glands, as schematically illustrated in Figure 2.

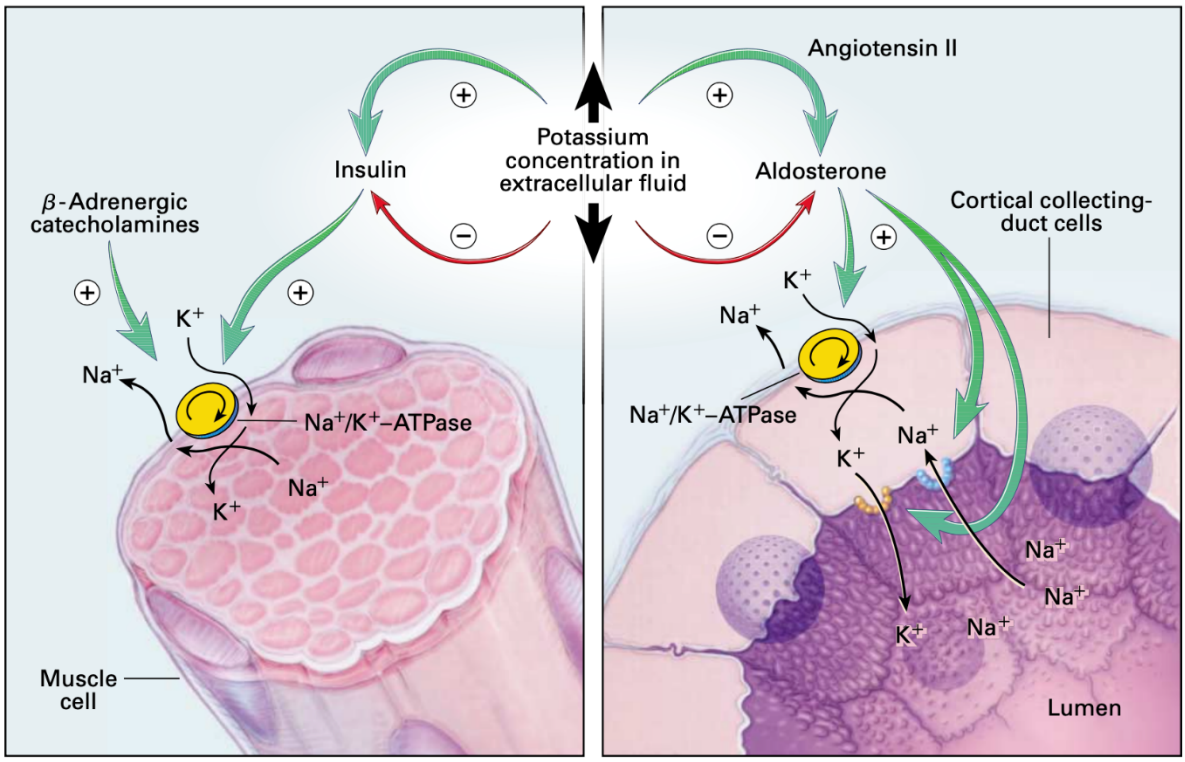


Fig 2 Principal hormones involved in potassium homeostasis. Insulin and b-adrenergic catecholamines promote the entry of potassium into muscle cells by stimulating NA+/K+–ATPase. Aldosterone induces potassium excretion through the activation of NA+/K+–ATPase and epithelial sodium and potassium channels sited in collecting-duct cells. An increase in the blood potassium concentration stimulates the secretion of each of these hormones, and a decrease inhibits their secretion. Angiotensin II has a synergistic effect on the stimulation of aldosterone production induced by hyperkalaemia. Derived from Gennari FJ. New Engl J Med 1998;339(7):451-458.

A chronic activation of RAAS promotes and perpetuates congestive heart failure, chronic kidney disease and arterial hypertension. The association between aldosterone and hypertension was established by Conn in secondary hypertension syndrome but is also present in primary hypertension, due to the evidences of mild elevations of aldosterone levels in patients affected by essential hypertension.

Oxidative stress and inflammation play a central role in vascular remodelling and arterial stiffness. Beside their physiological effect an excess of angiotensin II and aldosterone levels have a pro-fibrotic and pro-inflammatory consequences that induces remodelling and endothelial dysfunction, promoting arterial stiffness. Aldosterone also induces endothelial

dysfunction and oxidative stress through a mechanism involving reduction of nitrite oxide bioavailability in vascular smooth muscle cells and reduction in endothelial glucose-6-phosphate dehydrogenase expression. Aldosterone induces vascular inflammation through direct effects on MR but it also enhance the profibrotic effects of Angiotensin II, since it increases vascular ACE expression and upregulates vascular AT1 receptor expression; in turn, Angiotensin II activates MR response in smooth muscle cells. Furthermore, aldosterone may act on endothelial progenitor cells reducing vascular migration, differentiation, and proliferation and promote hypertrophic remodelling characterized by an increase in the wall-to-lumen ratio in small arteries.

There are significant associations between arterial stiffness and RAAS. In particular, it has been demonstrated that Angiotensin II infusion increases PWV, AIx and aortic PP, and that these effects are only partially explained in a BP-dependent manner. Long-term aldosterone exposure leads to increased collagen content and fibrosis, both in the heart ventricle and in the arterial wall. Experimentally, aldosterone increases arterial stiffness and PP in salt-fed rats modifying elastin and collagen quantity, an effect that is significantly reduced by MR antagonists involving predominantly a reduction in collagen content.

Blacher et al have shown a strong relationship between high plasma aldosterone levels and decreased systemic arterial compliance in patients with long-standing hypertension, but not in normotensive subjects, even after adjustment for age and BP. A study published in 2004 demonstrated a statistically significant inverse correlation between plasma renin activity and both large and small artery compliance in normotensive subject. Another study found a significant positive correlation between the aldosterone-renin ratio (ARR) and aortic systolic BP, aortic PP and AIx, but not PWV in untreated hypertensive patients. Lim et Al demonstrated that exercise systolic BP and systolic BP during exercise were independently and significantly related to ARR, a relationship caused by a reduced vascular compliance which impairs the systolic response to exercise.

Primary aldosteronism is associated with an impaired baroreflex function related in part to a reduced arterial compliance and despite a reduction of BP values and aldosterone levels, treatment did not significantly change compliance as demonstrated by Veglio et Al. Further studies are necessary to evaluate if these alterations may play a remarkable prognostic and therapeutic role but all these evidences underling an important role played by aldosterone in promoting arterial stiffening.

CHAPTER 3

Methods

3.1 Study objectives

The primary aim of our analysis was to evaluate the potential correlations between plasma aldosterone levels and different parameters of vascular stiffness in adult outpatients with essential hypertension, who were referred to our Hypertension Unit, Division of Cardiology, Department of Clinical and Molecular Medicine, University of Rome Sapienza, Sant'Andrea Hospital, Rome (IT), for BP assessment, including home, clinic and 24-hour ambulatory blood pressure monitoring (ABPM), and global CV risk estimation, including cardiac, renal and vascular HMOD assessment, between January 2017 and October 2021.

3.2 Study population

For the purposes of the present analysis, we extracted data from our medical database, which included clinical records derived from adult individuals, who were consecutively evaluated at the outpatient service of our Hypertension Unit at Sant'Andrea Hospital in Rome, Italy.

To be included in the study protocol, participants have to present the following inclusion criteria: adult individuals aged more than 18 years, signature of informed consent for study participation. In addition, the following exclusion criteria were considered: recent (<6 months) history of acute CV diseases, including at least one of the following: coronary artery disease, stroke, congestive heart failure, severe valve disease or peripheral artery disease; any neurological or psychiatric disease that may at least, in part, affect the BP assessment or the signature of the informed consent, drug abuse, chronic kidney disease with eGFR <30 mg/ml/min or dialysis, patients included in clinical trials or affected by chronic autoimmune or inflammatory diseases under steroids treatment.

The study conformed to the Declaration of Helsinki and its subsequent modifications. The confidentiality of the data of each patient included in the current study was carefully and strictly protected. Informed consent was obtained in all individuals included in the current study, which was approved by the local Ethical Committee.

Blood pressure measurements and PW analysis

All BP measurements were performed according to recommendations by European guidelines and in line with the study protocol adopted at our site.

Clinic BP measurements were performed in the Hypertension Clinic during the morning section (from 08:00 to 10:00 h). Sequential BP measurements and PWV analyses were performed in a quiet room, after 10 min of rest, on the same arm and with the participant in the sitting position, by using an automated, oscillometric device Mobil-O-Graph PWA Monitor (I.E.M. GmbH, Stolberg, Germany) using validated ARCSolver algorithms (Austrian Institute of Technology GmbH, Vienna, Austria). The Mobil-O-Graph's brachial BP measurement unit was validated according to standard protocols and the arm circumferences were measured to allow the correct choice of cuff size. On the basis of individual arm circumference, different cuff sizes (small 6–11 cm, small/medium 10–19 cm, medium 18– cm, large 22–32 cm and extra-large 33–47 cm) were applied, in order to have proper BP measurement, as recommended by current guidelines.

After estimation of peripheral systolic and diastolic BP, the cuff instantly reinflates and recordings for central BP are carried out at diastolic pressure levels for 10 s. Using this method, the central aortic pressure is automatically calculated from the brachial BP using a transfer function and PWV is estimated from the time difference between the derived forward and reflected waves. During the estimation of vascular stiffness parameters, the device was used according to the instructions of their manufacturers as well as in accordance with the ESC consensus. All clinic BP measurements were attended.

ABPM and 24-hour PWA were performed by the oscillometric device Mobil-O-Graph 24h PWA Monitor. The device was set in the Hypertension Unit after completion of the clinic BP measurements and the monitoring was started at about 10:00 AM. Automatic BP readings were obtained every 15 min during the day-time period (from 6:00 AM to 22:00 PM) and every 30min during the night-time period (from 22:00 PM to 6:00 AM) over the 24 h. Each patient was instructed not to alter her/his usual schedule during the monitoring period, asked to avoid unusual physical activities, and to maintain the arm still during BP measurements. Average values for the 24h, day-time, and night-time systolic and diastolic BP levels, heart rate and PW analysis were extracted. In addition, standard deviation from average values, as well as

number of BP measurements above the normal BP thresholds were reported for each time period (24h, day-time and night-time) in each participant.

Plasmatic aldosterone evaluation

According to current guidelines, commonly used drugs able to affect aldosterone and/or plasma renin levels (e.g. dopaminergic and antihistaminergic medications, selective serotonin reuptake inhibitor antidepressants, licorice) were withdrawn at least 4-6 weeks before sample drawn. Likewise, all antihypertensive drugs able to alter the aldosterone-renin ratio (ARR), such as diuretics, ACE inhibitors and angiotensin II receptor blockers, mineralocorticoid receptor antagonists and beta-blockers, were stopped at least 4-6 weeks before sample collection and, if needed, replaced by long-acting dihydropyridines calcium channel blockers (amlodipine 5-10 mg OD) and/or alpha-blocker (doxazosin 2-4 mg OD) for ensuring BP control during wash-out period.

Blood samples were obtained the after 60 min of quiet supine or sitting rest (the conditions that provided optimal results in the PAPY Study). In premenopausal women blood sample were taken avoiding exams during the luteal phase of menstrual cycle.

All blood was collected slowly from 8.00 to 9.00 am and samples kept at room temperature during transportation to the laboratory. Marked hypokalemia if detect, has been corrected before blood test. Plasma renin has been assessed as activity (PRA, ng/ml/h) by measuring angiotensin I generated over time, and plasma aldosterone concentration (PAC, ng/dL) has been measured with RIA.

Definition of cardiovascular risk factors and comorbidities

Diagnosis of hypertension was defined in the presence of systolic BP levels ≥140 mmHg and/or diastolic BP levels ≥ 90 mmHg in untreated subjects or in the presence of stable (≥6 months) antihypertensive drug treatment.

Hypercholesterolemia was defined for total cholesterol levels ≥ 190 mg/dl or low-density lipoprotein (LDL) cholesterol levels ≥ 130 mg/dl, while hypertriglyceridemia for triglyceride levels ≥ 150 mg/dl or stable lipid-lowering drug treatment in both conditions.

Diabetes was defined in the presence of plasma glucose levels ≥ 126 mg/dl or in the presence

of glucose-lowering therapy.

Based on anthropometric data, calculation of BMI was made and it was expressed as body weight in kilograms divided by the square of height in meters (kg/m^2).

Coronary artery disease (CAD), including not only non- fatal myocardial infarction (MI), but also other recurrent angina and coronary revascularizations, was diagnosed based on the presence of at least two of the following three criteria: symptoms (e.g., chest pain) lasting longer than 15 min, transient increase in serum enzyme concentrations (more than twice the upper limit of normal) and electrocardiographic changes (new persistent ST-segment elevation or pathological Q waves in two contiguous leads) indicating cardiac damage.

Echocardiogram

Doppler echocardiographic examination was available in 458 (51.35%) outpatients, and was performed by Philips Epic 7 C with a multi-frequency transducer (2.5–4 MHz). Images were implemented using standardized acquisition methods. LV dimensions were measured at end-diastole and end-systole, just below the mitral leaflets, through the standard left parasternal window. LV mass was calculated and then normalized by body surface area (BSA) and/or by height elevated to 2.7, as recommended by current guidelines. LV hypertrophy was defined in the presence of LV mass ^2.7 more than 47 g/m^2.7 in women and 50 g/ m^2.7 in men.

3.3 Statistical analysis

All data were entered into Microsoft Access for Windows (Microsoft Office; Microsoft Corp., Redmond, Washington, USA). Baseline characteristics of patients are presented as number and percentage for dichotomous variables and mean SD of the mean for continuous variables. Normal distribution of data was assessed using histograms and Kolmogorov – Smirnov test. Differences between continuous variables were assessed using Student's t-test. Odds ratio (OR) and 95% confidence interval (CI) were derived from logistic regression analysis. A multivariable model was fitted with baseline covariates which showed differences at the less than 0.05 significance level. All tests were two-sided, and a P value of less than 0.05 was considered statistically significant. All calculations were generated using SPSS, version 20.0 (SPSS Inc., Chicago, Illinois, USA).

CHAPTER 4

RESULTS

From an overall sample of 9,570 outpatients who were consecutively evaluated at the outpatient service of our Hypertension Unit at Sant'Andrea Hospital in Rome, Italy until from January 2005 to October 2021, we excluded 800 (8.3%) records due to poor or insufficient quality of the BP measurements, 43 records (0.4 %) due to gestational hypertension, 500 (5.2 %) records due to partial or missing data, 238 (2.5%) records without valid clinic BP, 115 (1.2%) patients aged less than 18 years, 6982 (72.9%) patients without available PWA data, thus resulting in an overall sample of 892 adult outpatients with valid BP data (women 38.7%, age 61.4 ± 15.2 years, 9.4% smokers, 16.4% diabetic). General characteristics, distribution of CV risk factors and clinical parameters are reported in Table 1.

Table 1

Parameters	
Outpatients	892 (100.0)
Female (%)	345 (38.7)
Age (years)	61.4±15.2
BMI (kg/m2)	27.3±6.6
Elderly (%)	400 (44.8)
Smoking (%)	84 (9.4)
Obesity (%)	565 (63.3)
Dyslipidaemia (%)	444 (49.8)
Diabetes (%)	146 (16.4)
CAD (%)	129 (14.5)
Stroke (%)	69 (7.7)
PAD (%)	33 (3.7)
CKD(%)	30 (3.4)

Aa illustrated, study population included predominantly middle aged male individuals, with high prevalence of obesity (64%) and dyslipidaemia (50%) and relatively low prevalence of smoking habit (9%), diabetes (16%), and comorbidities (24%). In the overall sample, 81.6%

patients received BP lowering drugs, 41.1% lipid lowering drugs, and 14.6% glucose lowering drugs.

Clinic systolic and diastolic BP levels and parameters of vascular HMOD in the overall population sample and in treated/untreated hypertensive outpatients are reported in Table 2.

Table 2.

Parameters	Overall	Untreated	Treated	P value
Clinic brachial SBP (mmHg)	141.4±18.2	139.5±17.9	141.8±18.3	0.151
Clinic brachial DBP (mmHg)	85.9±12.2	88.2±13.1	85.3±11.9	0.007
Clinic brachial PP (mmHg)	50.0±13.4	47.2±12.9	50.7±13.9	0.004
Clinic central SBP (mmHg)	125.1±15.4	126.1±14.9	124.9±15.4	0.356
Clinic central DBP (mmHg)	86.7±12.5	89.5±13.3	86.1±12.3	0.002
Clinic central PP (mmHg)	38.2±11.2	36.7±10.9	38.5±11.3	0.069
Clinic PWV (m/sec)	9.4±4.2	8.02±2.08	9.76±4.54	<0.001
Clinic AIx	24.0±14.1	21.3±13.6	24.5±14.1	0.009
Clinic Vascular Age (years)	65.4±16.1	55.9±16.2	67.5±15.3	<0.001

No significant differences were found with regard to systolic BP levels between treated and untreated individuals at both brachial and central BP assessment, whereas diastolic BP levels resulted significantly higher in untreated than in treated patients. Clinic PWV and vascular age resulted significantly higher in treated outpatients than in untreated individuals, whereas Aix resulted significantly lower in the former than in the latter group.

24-hour systolic and diastolic BP levels and parameters of vascular HMOD in the overall population sample and in treated/untreated hypertensive outpatients are reported in Table 3.

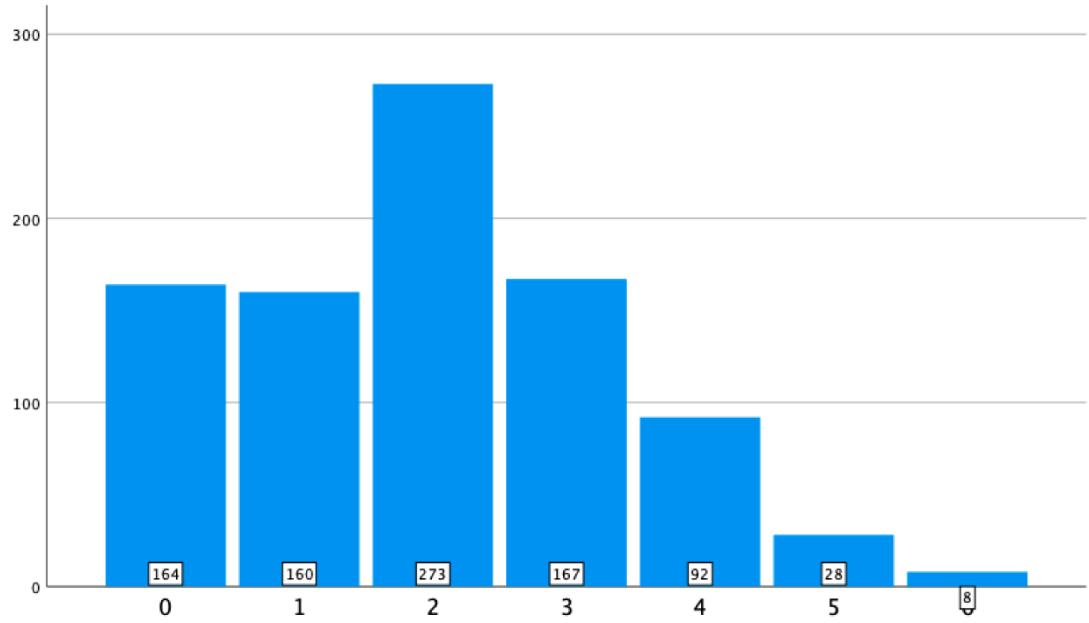
Table 3.

Parameters	Overall	Untreated	Treated	P value
24-h brachial SBP (mmHg)	131.9±14.9	131.1±15.3	132.2±14.8	0.514
24-h brachial DBP (mmHg)	82.1±11.9	85.2±12.5	81.4±11.7	0.004
24-hour brachial PP (mmHg)	46.7±8.5	45.9±8.7	50.8±7.8	<0.001
24-hour central SBP (mmHg)	123.6±20.2	120.5±20.8	125.4±19.7	0.129
24-hour central DBP (mmHg)	89.1±16.2	87.5±16.9	89.9±15.9	0.357
24-hour central PP (mmHg)	46.6±8.5	32.9±19.1	35.4±15.2	0.353
24-hour PWV (m/sec)	7.3±1.6	6.8±1.5	7.6±1.6	0.001
24-hour AIx	22.7±7.9	22.0±8.1	23.1±7.8	0.362
24-hour Vascular Age (years)	51.0±13.7	47.4±12.5	52.9±14.0	0.014

No significant differences were found with regard to 24-hour systolic and diastolic, brachial and central BP levels between treated and untreated individuals, with the only exception of 24-hour brachial diastolic BP, which resulted significantly lower in treated than in untreated individuals. Of note, 24-hour brachial PP, PWV and vascular age were all significantly higher in treated outpatients than in untreated individuals, whereas no significant differences were observed for 24 hour central PP and Aix between the two groups.

Distribution of antihypertensive drugs is illustrated in Figure 1.

Figure 1.



Number of antihypertensive drugs

About 20% did not received any antihypertensive drug classes, about 20% of hypertensive patients received monotherapy, 30% dual combination therapy, while the remaining proportion (about 35%) received at least three antihypertensive drugs classes. Among treated hypertensive outpatients (n=728; 81.6%), 289 (39.7%) resulted normotensive (controlled), 47 (6.5%) had uncontrolled diastolic hypertension, 190 (26.1%) uncontrolled systolic hypertension and 202 (27.7%) had uncontrolled systolic/diastolic hypertension.

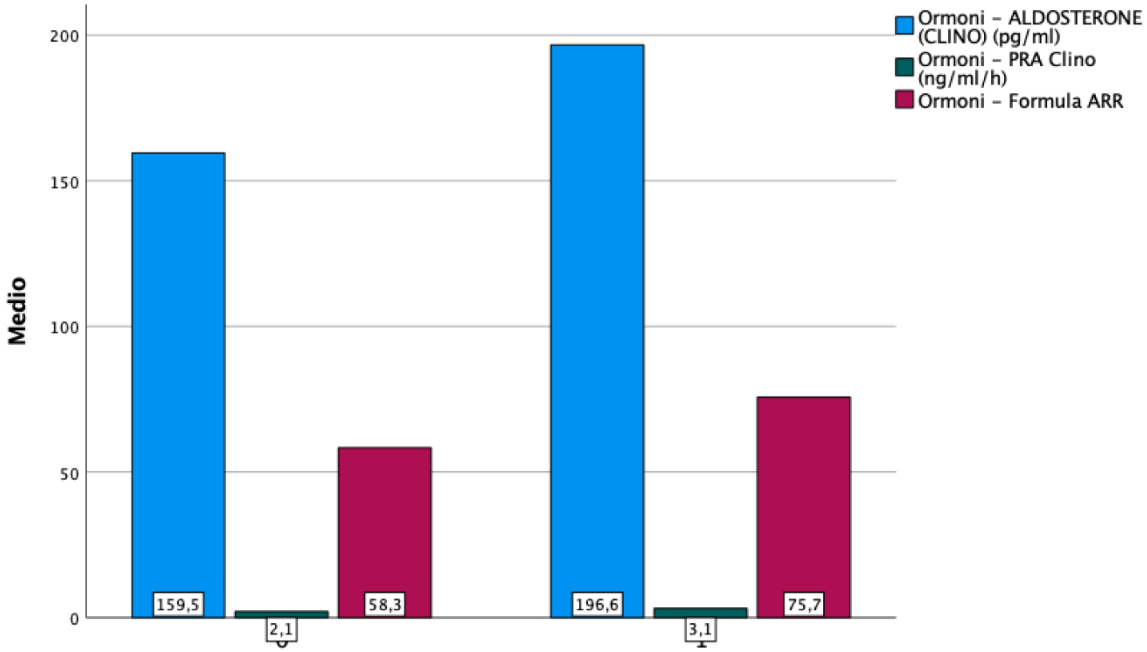
Determination of plasma aldosterone levels (PA), plasma renin activity (PRA) and aldosterone-renin ratio (ARR) was available in 147 outpatients with valid PWA and suspected secondary hypertension, who underwent screening for primary hyperaldosteronism. Average values of these parameters are reported in Table 4.

Table 4.

Parameters	Overall	Untreated	Treated	P value
PA	180.4±147.8	156.0±136.0	193.4±152.8	0.145

PRA	2.9±5.1	2.2±3.5	3.2±5.8	0.276
ARR	66.7±15.3	54.1±14.9	73.3±15.8	0.477

No significant differences between the two groups were observed for these parameters, as illustrated in Figure 2.



Correlations between PA (table 5), PRA (table 6) or ARR (table 7) and brachial or central systolic/diastolic BP, brachial or central PP, and clinic or 24 hour AIx, PWV and vascular age are reported in the following tables.

Table 5.

		ALDOSTERONE (CLINO) (pg/ml)
ALDOSTERONE (CLINO) (pg/ml)	Pearson Correlation	1
	Two-side Significance	
	N	147
Clinic PAS (mmHg) M	Pearson Correlation	,057
	Two-side Significance	,495
	N	146
Clinic PAD (mmHg) M	Pearson Correlation	-,033
	Two-side Significance	,690
	N	146

cPP clinic	Pearson Correlation	,108
	Two-side Significance	,192
	N	146
PWA - cSis (mmHg)	Pearson Correlation	,030
	Two-side Significance	,720
	N	147
PWA - cDia (mmHg)	Pearson Correlation	-,011
	Two-side Significance	,891
	N	147
PWA - cPP (mmHg)	Pearson Correlation	,057
	Two-side Significance	,495
	N	147
PWA - Augmentation Index	Pearson Correlation	-,002
	Two-side Significance	,983
	N	146
PWA - PWV (m/s)	Pearson Correlation	,241**
	Two-side Significance	**,003**
	N	147
PWA - VA	Pearson Correlation	,240**
	Two-side Significance	**,007**
	N	126
24 h PAS (mmHg) M	Pearson Correlation	,233**
	Two-side Significance	**,007**
	N	132
24 h PAD 24 ore (mmHg) M	Pearson Correlation	,183*
	Two-side Significance	**,036**
	N	132
PP24 h	Pearson Correlation	,169
	Two-side Significance	**,052**
	N	132
PWA 24 h - cSis Media	Pearson Correlation	,261**
	Two-side Significance	**,008**
	N	101
PWA 24 h - cDia Media	Pearson Correlation	,273**
	Two-side Significance	**,006**
	N	101
cPP 24 h	Pearson Correlation	,062
	Two-side Significance	,537
	N	101
PWA 24 h - Alx@75 Media	Pearson Correlation	,070
	Two-side Significance	,489
	N	101
PWA 24 h – PWV Media	Pearson Correlation	,260
	Two-side Significance	,009
	N	101
PWA 24 h - VA	Pearson Correlation	,198
	Two-side Significance	,057
	N	93

Plasma aldosterone levels resulted significantly and positively correlated with PWV at both clinic (Pearson r: 0.241; P=0.003) and 24-hour (Pearson r: 0.260; P=0.009) BP assessments, clinic Vascular Age, clinic and 24-hour systolic/diastolic BP levels, 24-hour central systolic and diastolic BP levels.

Table 6.

		PRA Clino (ng/ml/h)
PRA (ng/ml/h)	Pearson Correlation	1
	Two-side Significance	
	N	136
Clinic PAS (mmHg) M	Pearson Correlation	,107
	Two-side Significance	,217
	N	135
Clinic PAD (mmHg) M	Pearson Correlation	,075
	Two-side Significance	,387
	N	135
Clinic cPP	Pearson Correlation	,071
	Two-side Significance	,412
	N	135
PWA - cSis (mmHg)	Pearson Correlation	,011
	Two-side Significance	,895
	N	136
PWA - cDia (mmHg)	Pearson Correlation	-,020
	Two-side Significance	,813
	N	136
PWA - cPP (mmHg)	Pearson Correlation	,034
	Two-side Significance	,695
	N	136
PWA - Augmentation Index	Pearson Correlation	-,133
	Two-side Significance	,123
	N	135
PWA - PWV (m/s)	Pearson Correlation	-,154
	Two-side Significance	,073
	N	136
PWA - VA	Pearson Correlation	-,175
	Two-side Significance	,060
	N	116
24 h PAS (mmHg) M	Pearson Correlation	,259**

	Two-side Significance	,004
	N	121
24 h PAD (mmHg) M	Pearson Correlation	,184*
	Two-side Significance	,043
	N	121
24 h PP	Pearson Correlation	,222*
	Two-side Significance	,014
	N	121
24 h PWA - cSis Media	Pearson Correlation	,249*
	Two-side Significance	,016
	N	93
24 h PWA - cDia Media	Pearson Correlation	,171
	Two-side Significance	,101
	N	93
24 h cPP	Pearson Correlation	,291**
	Two-side Significance	,005
	N	93
24 h PWA - Alx@75 Media	Pearson Correlation	,069
	Two-side Significance	,513
	N	93
24 h PWA - PWV Media	Pearson Correlation	-,006
	Two-side Significance	,956
	N	93
24 h PWA - VA	Pearson Correlation	-,092
	Two-side Significance	,400
	N	85

PRA resulted significantly positively associated with systolic clinic and 24-hour BP levels and clinic and 24-hour PP levels at both brachial and central BP assessments, whereas no significant correlations were found with other parameters of vascular HMOD.

Table 7.

		ARR
ARR	Pearson Correlation	1
	Two-side Significance	
	N	146
Clinic PAS (mmHg) M	Pearson Correlation	,114
	Two-side Significance	,173
	N	145

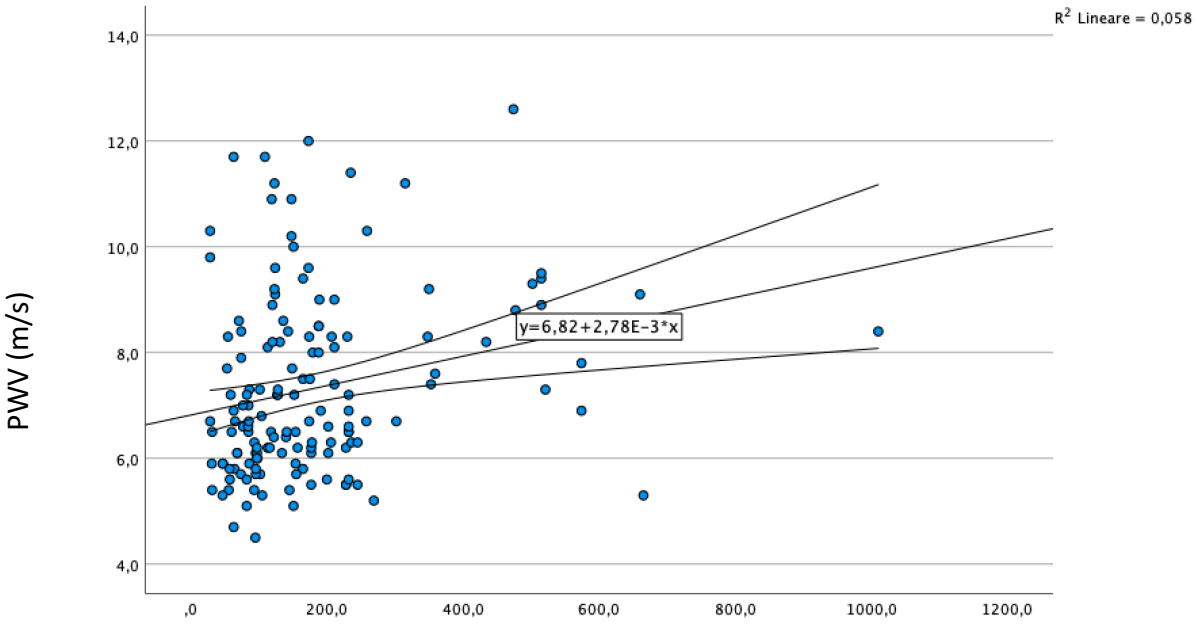
Clinic PAD (mmHg) M	Pearson Correlation	,077
	Two-side Significance	,357
	N	145
Clinic cPP	Pearson Correlation	,082
	Two-side Significance	,327
	N	145
PWA - cSis (mmHg)	Pearson Correlation	,042
	Two-side Significance	,616
	N	146
PWA - cDia (mmHg)	Pearson Correlation	,008
	Two-side Significance	,925
	N	146
PWA - cPP (mmHg)	Pearson Correlation	,052
	Two-side Significance	,536
	N	146
PWA - Augmentation Index	Pearson Correlation	,009
	Two-side Significance	,916
	N	145
PWA - PWV (m/s)	Pearson Correlation	,169*
	Two-side Significance	**,042**
	N	146
PWA - VA	Pearson Correlation	,173
	Two-side Significance	,056
	N	123
24 h PAS (mmHg) M	Pearson Correlation	,288**
	Two-side Significance	**,001**
	N	132
24 h PAD (mmHg) M	Pearson Correlation	,251**
	Two-side Significance	**,004**
	N	132
24 h PP	Pearson Correlation	,177*
	Two-side Significance	**,042**
	N	132
24 h PWA - cSis Media	Pearson Correlation	,307**
	Two-side Significance	**,002**
	N	101
24 h PWA - cDia Media	Pearson Correlation	,306**
	Two-side Significance	**,002**
	N	101
24 h cPP	Pearson Correlation	,132
	Two-side Significance	,190

	N	101
24 h PWA - Alx@75 Media	Pearson Correlation	,126
	Two-side Significance	,208
	N	101
24 h PWA - PWV Media	Pearson Correlation	,246*
	Two-side Significance	**,013**
	N	101
24 h PWA - VA	Pearson Correlation	,243*
	Two-side Significance	**,019**
	N	92

Similarly, ARR resulted significantly and positively associated with systolic clinic and 24-hour BP levels and clinic and 24-hour PP levels at both brachial and central BP assessments. Of note, among other parameters of vascular HMOD, ARR resulted significantly and positively associated with clinic and 24-hour PWV, and showed a significant correlation with 24-hour vascular age and borderline significant correlation with clinic vascular age.

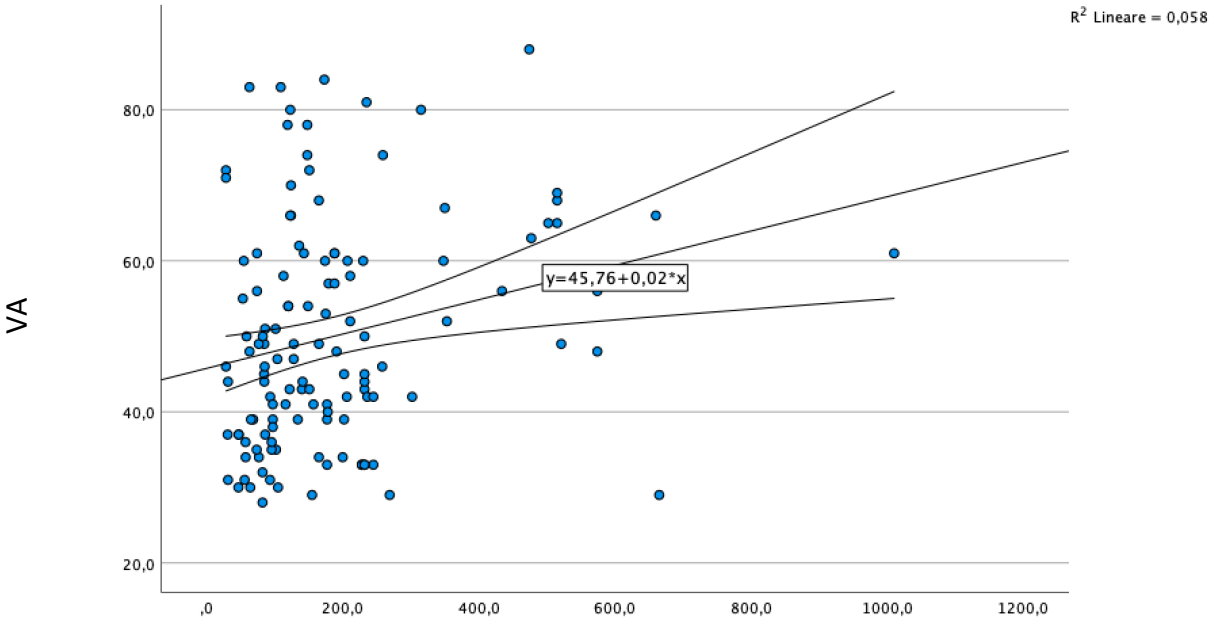
As discussed before, plasma aldosterone levels resulted significantly and positively associated with clinic PWV (Figure 3), clinic vascular age (figure 4), as well as with 24-hour PWV and vascular age (data not shown).

Figure 3.



PAC (pg/ml)

Figure 4



Similar findings were observed even after corrections for confounding factors, including age, gender, BMI and presence of antihypertensive therapy

CHAPTER 5

DISCUSSION

During the last years epidemiologic evidences showed that arterial stiffness, thought to be only part of natural aging process, is instead a marker of cardiovascular risk. Increased PWV values are an important tool for identify subjects with target vessels HMOD and can be used to reclassify CV risk especially in low or very low risk subjects. Several evidences show how PWV may add a prognostic value beyond the parameters included in the SCORE risk charts such as the systolic blood pressure thus its measurement should be integrated in the hypertensive patient evaluation for better manage treatment and reducing future CV events. Furthermore, PWV and AIx values may be used to assess drug effect during follow up and better understand the pathophysiology of hypertension with particular regard to hyperaldosteronism.

In our study PAC resulted significantly and positively correlated with PWV at both clinic (Pearson r: 0.241; P=0.003) and 24-hour (Pearson r: 0.260; P=0.009) BP assessments, clinic Vascular Age, clinic and 24-hour systolic/diastolic BP levels, 24-hour central systolic and diastolic BP levels. Our findings were consistent with those reported by Park et al in a study which included 438 hypertensive patients. The analysis demonstrated that serum aldosterone is significantly associated with central aortic PWV in hypertensive patients, thus supporting the role for aldosterone in developing central aortic stiffness and increased PWV in hypertensive patients.

A recent study performed in 36 patients with confirmed PA, 28 patients with primary hypertension and 20 normotensive subjects which demonstrated significantly higher PWV in PA patients if compared to hypertensive and control group, although the research used Sphygmocor tonometer, it underlies the clinical impact of the deleterious effects of aldosterone excess on arterial fibrosis and remodeling.

The most used devices for evaluating stiffness are those based on transcutaneous tonometry but studies confirmed the reliability of the oscillometric based analysis. In the current study, the Mobil-O-Graph was used for both clinic and 24-h monitoring PWA, the device has been previously compared and validated to other invasive and noninvasive methods.

Although the importance of detecting PWV and AIx has been extensive demonstrated in clinical and observational studies, they are not been applied in daily clinical practice yet. In our study, the technique used to assess PW analyses has different advantages. Firstly, the Mobil-O-Graph is simple to use since trough a common cuff provides several information on vascular function. Secondly derived measures are operator independent therefore to execute correct evaluation correctly, the operator doesn't need extensive knowledge, thus can be performed also by a technician. Thirdly although the device perform an indirect estimation of stiffness, which could influence the reliability of the measurements, the ARCSolver algorithm used by the Mobil-O-Graph is feasible and provides reproducible values as extensively demonstrated in literature. Noteworthy the single pressure waves are verified in order to recognize artifacts; hence, an aortic PW is generated by a transfer function. After the analysis of the various vascular and anthropometric parameter the device calculate vascular age defined as the age that an individual would have if he had the same absolute risk but with controlled CV risk factors. Furthermore Mobil-O-Graph has been test and compared to invasive cardiac catheterization not only in adult population but showing a certain degree of measurements accuracy also in children and adolescents, but further data are needed to understand the potential implication and validate cut off in this in this category of patients.

Current Guidelines report carotid-femoral PW analysis as a gold standard for diagnosis of vascular HMOD but our data support the implementation in future studies investigating the role of oscillometric methods since the comfortable and non-invasive devices and relatively limited costs for the examination in a setting of clinical practice.

The results of our study, if confirmed, may have several implications in the everyday clinical practice of hypertension. Firstly, they further emphasize the central role of arterial stiffness in the evaluation of CV risk of hypertensive patients.

Secondly, they underline the effect of aldosterone levels leading to alteration of elastic arteries since it increases arterial stiffness by pro-fibrotic effects with subsequent potential implication also in diagnosis and prognosis of patients with hyperaldosteronism and hypertension. Currently there are limited data available on how mineralocorticoid affect stiffness markers. According to our results a recent cross-sectional study reported ARR to be directly and independently associated with large arterial stiffness in individuals without clinical suspicion of PA.

Aldosterone-to-renin ratio (ARR) is a screening tool for primary aldosteronism (PA) but evaluation of PW analysis may add useful information for refer patients to screening for secondary hypertension. The relationship found between high ARR and PWV may have future clinical implication since they suggest a potential role of PW analysis in detecting primary aldosteronism even when the PA criteria for identify patients with suspicious secondary hypertension are not met.

Finally, our data encourage the assessment of PW analysis in all adults with hypertension, aimed at early identifying and promptly treating those individuals at higher risk or even at apparently low risk without symptomatic or manifest HMOD.

These findings may have potential clinical implications, in view of the persistently high burden of hypertension-related CV diseases.

Potential limitations

Some potential limitations should be acknowledged. First of all, this study analysed data that

were retrospectively extracted from our medical database, which were not prospectically collected during clinical consultations. In addition, we do not have data on sleeping quality nor on prevalence of sleep disorders, which have been reported to affect 24h and, mostly night-time BP levels during 24h ambulatory BP monitoring. In this latter regard, information on work activities as well as circadian pattern of involved patients may also have had potential impact on the observed results. Finally, data on non-CV comorbidities have not been addressed, being out of the aim of the present analysis and no data about antihypertensive treatment and how it can affect PW analyses parameters has been considered. Further prospective studies are needed to confirm the results.

CONCLUSION

Our analysis demonstrated the correlations between PAC and different parameters of vascular stiffness in adult outpatients with essential hypertension even after corrections for confounding factors, including age, gender, BMI and presence of antihypertensive therapy

Despite the increasing evidence that non-invasive arterial function and PWV may be better predictors of CV events than brachial blood pressure alone these measurements are still not in common use in routine clinical practice.

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Conflicts of interest There are no conflicts of interest.

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