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PHD Thesis

CORRELATIONS BETWEEN PLASMA ALDOSTERONE LEVELS AND VENTRICULAR-ARTERIAL COUPLING IN ADULT OUTPATIENT WITH ESSENTIAL HYPERTENSION:

ROLE OF GENDER

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Index

Aldosterone: synthesis, regulation and action

Aldosterone is a mineralocorticoid, a steroid hormone mostly produced by the enzyme aldosterone synthase in the glomerulosa zone of the adrenal cortex and implicated in the regulation of blood pressure and the maintenance of water and electrolyte balance. Aldosterone activation of mineralocorticoid receptors (MRs) induces changes at the genomic level

In particular, in the late distal tubule and collecting duct of nephrons, aldosterone diffuses directly across the membrane and bind the inactive cytoplasmic form of the MR. This binding dissociates the MR from a multiprotein complex containing molecular chaperones, which in turn permits translocation of the MR in the nucleus, where the activated receptor modulates the expression of some proteins, including the serum and glucocorticoid inducible kinase 1 (sgk-1). Aldosterone- induced sgk-1 expression triggers a cascade of events that eventually increases the absorption of Na+ ions and water, indirectly increases K+ excretion and also contributes to acid-base balance acting on the epithelial sodium channel, the sodium-potassium exchange pumps, hydrogen ion ATPases, and bicarbonate-chloride antiporters. As a final effect of this cascade, intravascular volume expands, and blood pressure rises.

In the last decades studies have demonstrated that Aldosterone is synthesized not only in adrenal glomerulosa but also at other extra-adrenal sites, with localized paracrine role, and interacts with epithelial and nonepithelial tissues outside of the kidney, including heart and blood vessels. Moreover, Aldosterone could trigger cellular responses through non-genomic mechanism, altering activities of mitogen activated protein (MAP) kinase, Protein Kinase A (PKA), Protein Kinase C (PKC), ERK, and

Protein -Kinase D (PKD). These rapid effects may be coupled with the activation of MRs or of membrane bound receptors, for example the G-protein-coupled receptor-30 (GPR30), leading to alterations in second messengers, such as cAMP, diacylglycerol (DAG), Ca++, and Inositol 1,4,5-triphosphate. Regardless of the rapid onset, the induced effects may last up to several hours or even to several days. For example, the non-genomic effects of aldosterone on lowering the activity of the Na+/K+ pump of cardiomyocytes, which is solely mediated by epsilon PKC, can remain for up to 7 days.

Aldosterone secretion in stimulated by high plasma potassium concentration, adrenocorticotropic hormone, volume depletion or poor renal perfusion (dehydration or haemorrhage), low sodium blood levels and Angiotensin II.

Angiotensin II derives from Angiotensin I (cleaved by angiotensin-converting enzyme (ACE)) which in turn derives from the Angiotensinogen, that is synthesized by the liver, and it is cleaved by renin, which is secreted into the lumen of renal afferent arterioles by juxtaglomerular cells.

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: vascular and cardiac damage

It has been largely proved that Aldosterone plays a major role in the pathophysiology of cardiovascular disease

As mentioned before, MR have been detected in cardiac myocytes and fibroblasts and their activation can induce myocardial damage with mechanisms that are independent of blood pressure elevation, , since they regulate a variety of signal transduction mechanisms and cellular responses, which might result in tissue inflammation, hypertrophy, and fibrosis.

In 1991 Weber experiments in rats demonstrated that chronic administration of Aldosterone is associated with collagen accumulation in the myocardium, while Rocha et al demonstrated that aldosterone and salt treatment in uni-nephrectomized rats led to severe hypertension and the development of a vascular inflammatory phenotype in the heart; eplerenone administration attenuated proinflammatory molecule expression in the rat heart and subsequent vascular and myocardial damage. Another study from Mueller et al, reported the ability of Aldosterone to induce coronary vascular damage in double transgenic renin/angiotensinogen rat through mechanisms independent of blood pressure

It was therefore proposed that excessive aldosterone secretion is a causative factor of both myocardial fibrosis and diastolic dysfunction, although supporting evidence is still limited in humans, most likely because of the lack of models in which the effects of the RAS and aldosterone can be dissociated.

Studies in human, confirmed all data collected by animal experiments.

The most important study conducted to prove the harmful effects of aldosterone on the cardiovascular system is the Randomized Aldactone Evaluation Study (RALES), in 1999, which demonstrated that the addition of an aldosterone blocker in the treatment of patients with severe heart failure (improved mortality and morbidity by 30%-35%, respectively.

Later Muiesan et al demonstrated that the increase in aldosterone levels could contribute to the increase of LV mass exceeding the amount needed to compensate hemodynamic load.

Seong-Mi et al found that in young patients with never-treated HT, aldosterone significantly contributes to changes in LV geometry and functional impairment through its pro-hypertrophic and myocardial fibrosis effects beyond blood pressure, detecting signs of myocardial damage by using 2D speckle-tracking imaging, reduced e' velocity as an LV diastolic impairment.

So long term exposure to increased aldosterone levels contributes to the development of cardiac, vascular, and renal damage, also independent of blood pressure (BP) increase. This is demonstrated also by the fact that in experimental models of hypertension with excess aldosterone, fibrosis involved not only the left but also the right ventricle, i.e. a chamber exposed to the bloodstream but not to the pressure overload, and was prevented by administration of no antihypertensive dosages of the aldosterone-specific receptor antagonist spironolactone

There are three major mechanisms, involving the activation of MRs and glucocorticoid receptors (GRs), through genomic and nongenomic pathway, with whom aldosterone may determine LV remodelling and LV systolic and diastolic dysfunction: hypertrophy, chronic inflammation, dysregulation of extracellular matrix (ECM) metabolism, and finally

cardiac fibrosis.

1 HYPERTROPHTY: Left ventricular hypertrophy, represents a maladaptive response to the increased afterload, since it is an important independent predictor of cardiovascular complications and death. Brilla et al] In mice experiments Aldosterone excess has been found associated with increased mRNA levels of α- and β-myosin heavy chain in ventricular cardiomyocytes. Ithas been found also responsible of the increase of the level of cardiotrophin-1 (CT-1) a cytokine which can induce the hypertrophy of cardiomyocytes intensifying the expression of myosin light chain and skeletal α-actin and enhancing myosin light-chain phosphorylation in a dose-dependent manner.

2 Inflammation: Aldosterone can induce myocardial inflammation through the formation of reactive oxygen species (ROS) and increased expressions of pro- fibrotic and pro-inflammatory molecules, such as IL-6, transforming growth factor-β1, plasminogen activator inhibitor 1, endothelin 1, connective tissue growth factor, placental growth factor, osteopontin, and galectin-3. Aldosterone has also been found to increase oxidative stress, through the intensification of nicotinamide adenine dinucleotide phosphate oxidase activity and reduction of the expression of glucose-6-phosphate dehydrogenase (G6PD). In addition, Aldosterone can increase the expression of intercellular adhesion molecule on endothelial cells, which can then induce macrophage infiltration and facilitate the inflammatory process.

3 Dysregulation of extracellular matrix metabolism: Aldosterone seem to increase collagen types I and III synthesis and secretion from cardiomyocytes and fibroblasts, through the activation of MRs, and inhibit its degradation through the activation of GRa.

Aldosterone is also implicated in the development of arterial stiffness. It induces remodelling, oxidative stress and endothelial dysfunction, through the reduction of nitrite oxide bioavailability in vascular smooth muscle cells and reduction in endothelial glucose-6- phosphate dehydrogenase expression. Over more, it induces vascular inflammation through direct effects on MR, but it also enhances 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 remodeling characterized by an increase in the wall- to-lumen ratio in small arteries.

Recently, it has been shown that circulating aldosterone levels, even within the physiological range, are also related to an increased risk of cardiovascular mortality, fatal stroke, and sudden cardiac death. The results of a metanalysis conducted in 3838 patients with primary aldosteronism and 9284 patients with essential hypertension support the hypothesis that primary aldosteronism is associated with increased cardiovascular and cerebrovascular morbidity, including stroke, coronary artery disease, heart failure, and atrial fibrillation.

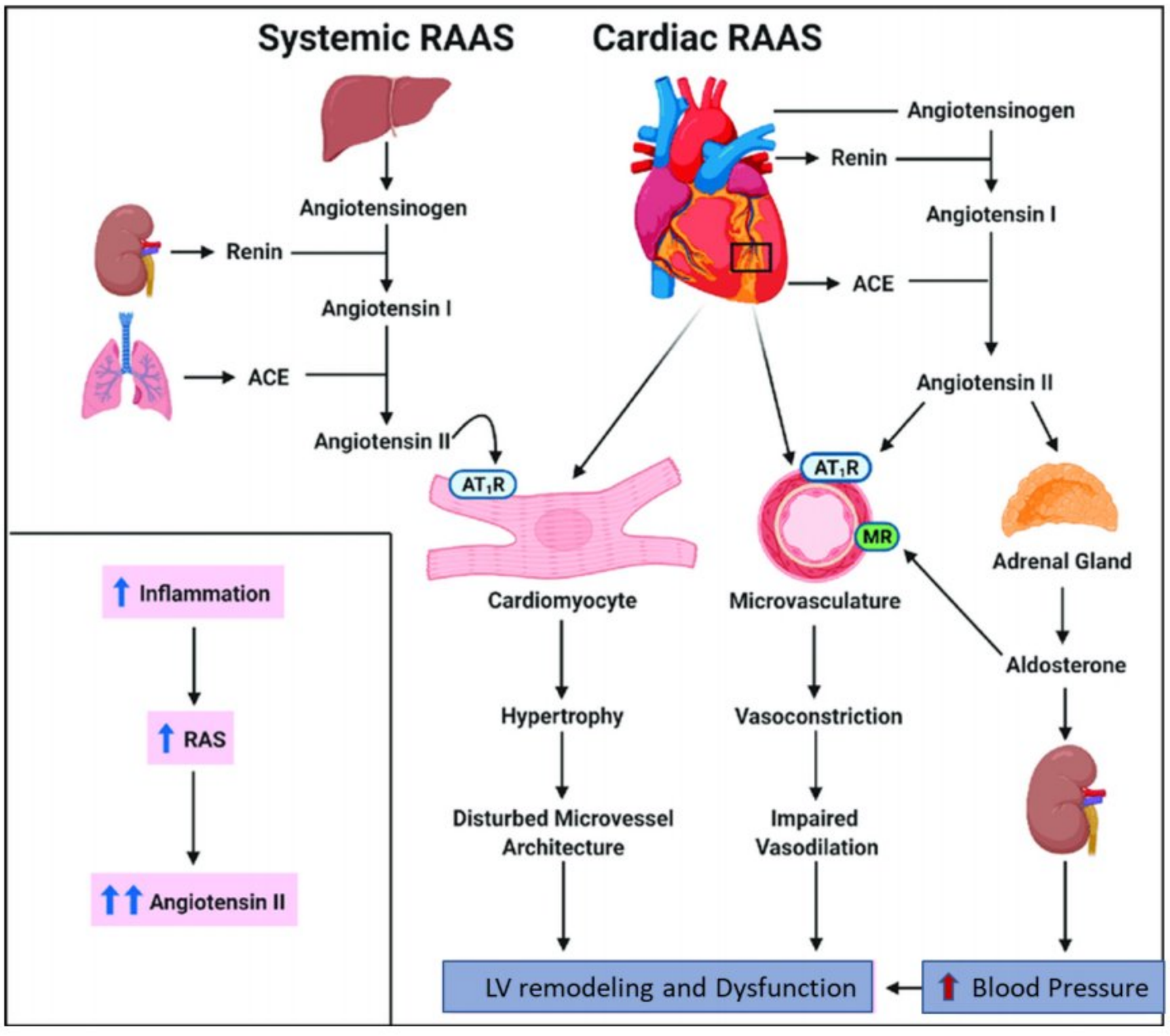
The process of LV and vascular remodelling and fibrosis influence diastolic and systolic function to different extents, causing subtle to overt cardiac failure.

Prolonged isovolumic LV relaxation, slower LV filling, and increased diastolic LV stiffness contribute to the elevation of LV end diastolic pressure (LVEDP), which are associated with the development of heart

failure with preserved, or as the changes in cardiac structure worsen, with reduced ejection fraction.

Arrhythmias are promoted by myocardial fibrosis and enlargement of left atrium, due to elevated LVEDP.

Cardiocerebrovascular complications, including coronary artery disease, nonfatal myocardial infarction, stroke, and transient ischemic attack are also related with the damage caused by excess of Aldosterone.



Ventricular-arterial coupling (VAC)

The cardiovascular system consists of the heart and the arterial system. The ventricle is a generator of hydraulic energy, which transfers the mechanical energy of the contraction to the blood, under the influence of the arterial system.

Heart pumps the blood into the vascular system in a pulsative way; Thus, the blood flow oscillates from the heart, generating a pulse wave that moves along the pulmonary and systemic circulation. The muscular arteries distribute the blood into the periphery and can modulate this pulse wave propagation through changes in vascular tone. Then the blood flow reaches the arterioles, which are the most important site of vascular resistance, and where smooth muscular cells regulate the capillary blood flow through relaxation and constriction as a response to local and nervous factors.

Then the blood reaches the capillaries, where the flow has by then became continuous and where tissue oxygenation, gas exchange and nutrition take place.

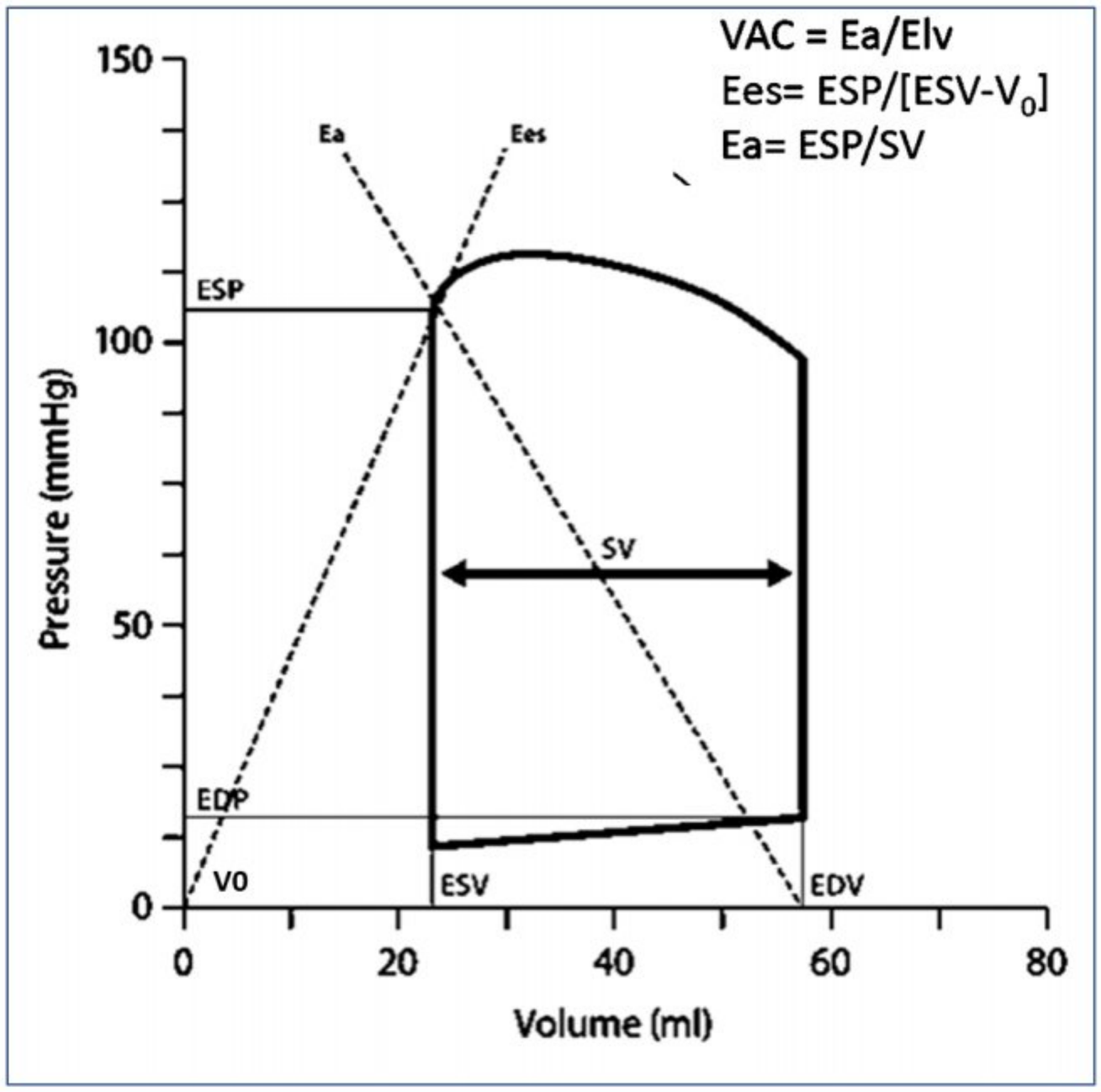
Considered the strict anatomical and functional relation among those structures, it is evident that the cardiovascular efficency depends on a complex interaction between heart performance and vascular system, and valuable method for assessing cardiovascular performance should relate both cardiac and arterial functions.

The evaluation of the interaction between the heart and the arterial system, which is called ventricular-arterial coupling, requires first that both ventricular systolic function and systemic arterial properties must be expressed in the same units.

Suga and Sagawa, by obtaining pressure-volume curves under different load conditions in experimental models, defined the characteristic of the ventricular-arterial system through the concept of end-systolic ventricular elastance (Ees) and arterial elastance (Ea), both terms express as the change in pressure for a change in volume.

Left ventricular end-systolic elastance represents heart stiffness and is an important index of myocardial contractility. It corresponds, on a pressure-volume loop (PVL), to the slope of the straight line deriving from the union of end-systolic points of different cardiac cycles obtained in the same heart at different loading conditions [Fig 1]. The value of ventricular elastance, given by the angular coefficient of the line ($\Delta P/\Delta V$), was observed to be an index of the contractility of the left ventricle, independent of load conditions. it can be expressed as ESP divided by end-systolic volume (ESV) subtracted V0, which is the theoretical volume associated with zero ventricular pressure

Arterial elastance expresses the afterload that opposes to left ventricular ejection independently of EF. According to recent studies it is s reliable estimate of peripheral resistance, total vascular compliance, characteristic impedance, and systolic and diastolic time intervals, and considers the pulsatile characteristics of blood flow in arteries. It is represented by the slope of a line intersecting with end-diastolic volume on the volume axis and the end-systolic pressure–volume point on the PVL. It can be expressed as the ratio between end-systolic pressure and stroke volume.



The relationship between the two systems, ventricular and arterial elastance, is called **ventricular-arterial coupling (VAC),** and provides information on the performance and the efficiency of the system, including the capability to provide the right stroke volume and arterial pressures for adequate organ perfusion. From the analysis of the pressure-volume curves, it is possible to calculate the cardiac work (or stroke work), assuming that the curve of the cardiac cycle has a rectangular shape, multiplying the base (EDV-ESV) and height (ESP). Cardiac performance is the ratio between the work done and the energy supplied to the system during the energy-conversion process. The performance – or efficiency – is therefore obtained from the ratio between the systolic work and the potential energy is represented by the

area subtended by the end-systolic elastance line relation, spent to perform it.

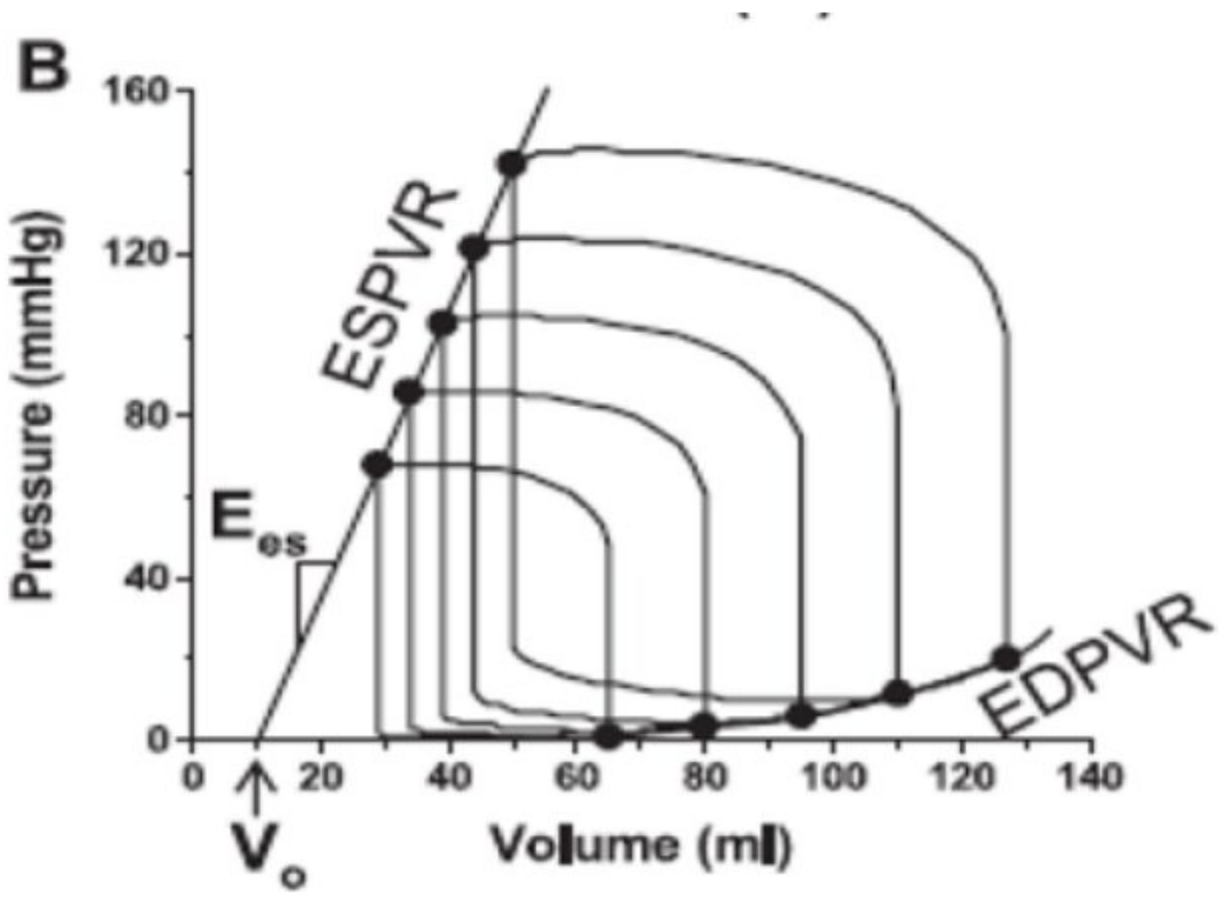
The greatest efficiency is achieved when elastances are matched. Some studies have shown that for maximal cardiac work, power, and efficiency, a normal VAC is 0.5 to 1.3. In failing hearts, VAC increases as cardiac function reduces and arterial load rises to maintain systolic pressure. At very high VAC, the transfer of blood from the ventricle to the aorta is significantly inefficient, leading to hemodynamic derangement and ineffective contraction.

Practically speaking, VA uncoupling occurs when Ea exceeds the value of Ees (Ea/Ees >1), which results in a compromised myocardial SW and efficiency. If the primary mechanism leading to this condition is an Ea increase, then VA coupling will eventually depend on the basal myocardial contractility and its ability to compensate for the afterload mismatch. If the ventricular pump performance can cope with the increased afterload, VA coupling will be maintained at expenses of an impaired mechanical efficiency. This scenario characterizes the compensatory increase in Ees in response to the augmented Ea associated with normal aging and arterial hypertension. Changes in the structural and biochemical ventricular properties allow to increase Ees and preserve VA coupling despite a progressive increase in Ea, but at expenses of limited exercise capacity.

On the other hand, if the first event if a decreased Ees, as in systolic heart failure or cardiogenic shock, the reduced myocardial performance will make the failing heart very sensitive to changes in Ea. Any increase in Ea will significantly impair VA coupling and, any therapy aimed at reduced Ea, such as the use of vasodilators, will substantially improve VA

coupling and ventricular efficiency.

The calculation of VA coupling requires invasive measures of ventricular pressure and volume. The gold standard for the estimation of ventricular elastance, described by Kono et al, is obtained invasively through cardiac catheterization, with the direct measurement of different intracardiac pressure and volume at different beats (multiple beats), by modifying the ventricular preload.



Pressure-volume curve under different loading conditions with construction of ESPVR and EDPVR

A non-invasive method, based on a single-beat model, have been developed for estimation of ventricular elastance, by Chen et al. using the information from non-invasive hemodynamic monitoring tools, such as standard echocardiography and peripheral blood pressure

measurements.

The calculation of Ea requires obtaining SV and Pes measurements. While SV can be easily estimated at the bedside using standard hemodynamic monitors or echocardiography, the P es still requires cardiac catheterization. However the radial systolic pressure has been extensively used as a surrogate for Pes. However, considering how the arterial wave reflections may influence on peripheral measurements and the constancy of mean arterial pressure (MAP) across the arterial system, the Ea estimate based on MAP/SV seems to offer a better surrogate over different hemodynamic conditions and interchangeably when measured in any peripheral arterial site.

METHODS

Aim of the Study

The primary aim of this study is to evaluate the potential correlations between plasma aldosterone (PA) levels and ventricular-arterial coupling (VAC), a parameter of myocardial efficiency, in adult outpatients with essential hypertension, and to estimate potential impact of gender differences.

Study population

Consecutive adult outpatients 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 full BP assessment, including home, clinic and 24-hour ambulatory blood pressure monitoring (ABPM), global CV risk estimation, including assessment of cardiac, renal and vascular hypertension-mediated organ damage (HMOD), and a screening for secondary forms of HT, between October 2019 and October 2022, were considered for our investigation.

To be included in the study protocol, participants must be adult individuals aged more than 18 years and have signed informed consent for study participation. In addition, the following exclusion criteria were considered: history of cardiovascular disease, including coronary artery disease, atrial fibrillation, stroke, congestive heart failure, cardiomyopathy, severe valve disease or peripheral artery disease; secondary hypertension ;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, chronic autoimmune or inflammatory diseases under steroids treatment.

From an overall population of 200 potentially eligible patients, 18 patients have been excluded because of a diagnosis of secondary hypertension (primary hyperaldosteronism). The remaining 182 patients were divided into two gender groups.

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

All BP measurements were performed according to recommendations by European guidelines.

In particular, clinic BP measurements were performed in the hypertension clinic during the morning section (8:00 am to 10:00 am). Sequential BP measurements were performed after 10 minutes of rest on the same arm with the participant in the sitting position in a quiet room using an automated oscillometric device (Omron 705 IT). The average of 3 consecutive BP measurements and heart rate was considered as clinic systolic/diastolic BP levels. ABPM was performed using an oscillometric device (Spacelabs 90207) in the hypertension unit after completion of the clinic BP measurements and started at approximately 10:00 am. Automatic BP readings were obtained every 15 minutes during the daytime period (6:00 am to 22:00 pm) and every 30 minutes during the night-time period (from 22:00 pm to 6:00 am) over

the 24 hours (25). Each patient was instructed not to alter her/his usual schedule during the monitoring period, to avoid unusual physical activities, and to maintain stillness of the arm during the BP measurements. Average values for the 24-hour, daytime and night-time systolic and diastolic BP levels, and heart rate were reported

Plasma aldosterone evaluation

According to current guidelines, the basal evaluation was performed after discontinuation of any antihypertensive treatment, able to alter the aldosterone-renin ratio (ARR), such as diuretics, ACE inhibitors and angiotensin II receptor blockers, mineralocorticoid receptor antagonists and beta-blockers or commonly used drugs able to affect aldosterone and/or plasma renin levels (e.g. dopaminergic and antihistaminergic medications, selective serotonin reuptake inhibitor antidepressants, liquorice) for at least 4-6 weeks before sample drawn. If needed, the antihypertensive drugs were 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. . Patients with hypokalaemia were given adequate oral potassium supplements before the hormonal evaluations. Blood samples were obtained standing and 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. Plasma renin has been assessed as activity (PRA, ng/ml/h) by measuring angiotensin I

generated over time, and plasma aldosterone concentration (PAC, pg/ml) has been measured with RIA

Echocardiogram

All participants underwent Doppler echocardiographic examination, performed according to the ASE guidelines, by Philips Epic 7 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, as recommended by current guidelines. LV ejection fraction was calculated according to the Simpson method.

Ventricular long axis myocardial velocities were also studied using Doppler tissue imaging technique with the sample volume placed at the basal segment of LV lateral and septal segments as well as RV free wall. Systolic (s'), as well as early and late (e' and a') diastolic myocardial velocities were measured. A mean value of the lateral and septal e' velocity was calculated. Left atrial diameter was measured from aortic root recordings with the M-mode cursor positioned at the level of the aortic valve leaflets.

Spectral transvalvular flow velocities were obtained using pulsed and continuous wave Doppler techniques as proposed by the American Society of Echocardiography. Peak LV early (E wave), and late (A wave) diastolic velocities were measured, and E/A ratio was 20 calculated. Systolic interval times (including IVRT and ICRT) has been assessed by using ECG traces and TDI.

In addition, SV, EF, pre-ejection, and total systolic interval times,

measured using echocardiography, and systolic and diastolic blood pressure cuff measurement, were introduced in a specifically implemented calculator (iElastance© - Apple iOS App) designed for determining non-invasive single-beat end-systolic Ees according to the Chen method as previously described. Ea was calculated as median arterial pressure (MAP x 0,9) and VAC has been then calculated as Ea/Ees.

3.3 Statistical analysis

All data were entered into Microsoft Excel for Windows (Microsoft Office; Microsoft Corp., Redmond, Washington, USA) and then analysed using SPSS, version 27.0 (SPSS Inc., USA). Continuous data are expressed as the mean ± s.d. and categorical variables as a percentage of the group. Normal distribution of data was assessed using histograms and Kolmogorov – Smirnov test.

Differences between continuous variables were assessed using Student's t-test, while distribution of categorical variables between groups was evaluated by chi-square test. Relationships between variables were assessed by the calculation of Pearson correlation.

All tests were two-sided, and a P value of less than 0.05 was considered statistically significant.

RESULTS

From an overall sample of 200 outpatients who were consecutively evaluated at our Hypertension Unit at Sant'Andrea Hospital in Rome, Italy from October 2019 to October 2021, we excluded 18 (9%) records due to diagnosis of secondary hypertension. The study population was then stratified into two groups, according to gender (34.6% female; 65% male).

General characteristics, distribution of CV risk factors and clinical parameters of the overall study sample, male and female outpatients are reported in **Table 1**.

Table1.

Parameters	Overall sample	Female	Male	P value
Outpatients (%)	182	63 (34,6)	119 (65)	-
Age (years)	40.4±14.6	40.7±15.8	40.2±14.0	0,202
BMI (kg/m2)	26.6±4.5	24.7±4.7	27.6±4.1	0.186
Smoking (%)	47 (27.3)	12 (20.7)	35(30.7)	0.206
Obesity (%)	105 (57.7)	25 (39.7)	80 (67.2)	<0.001
Dyslipidaemia (%)	30 (16.6)	12 (19.4)	18 (15.1)	0.529
Diabetes (%)	9 (5)	4 (6.5)	5 (4.2)	0.495
BUN (mg/dl)	22.8±12.9	19.7±4.6	23.3±13.8	0.304
Creatinine (mg/dl)	0.92±0.3	0.8±0.2	0.9±0.3	0.453
Uric acid (mg/dl)	5.3±1.2	3.4±1.1	5.8±1.0	0.869
Sodium (mmol/lt)	140.0±2.6	139.3±3.2	140.4±2.2	0.508
Potassium(mmol/lt)	4.7±0.5	4.2±0.4	5.0±0.3	0,242
Glucose (mg/dl)	95.5±14.95	91.9±19.7	97.4±20.6	0,776
Total Chol. (mg/dl)	192±40.4	194.5±38.2	191.0±41.3	0,549
HDL Chol. (mg/dl)	58.4±69.0	69.6±80.0	52.2±62.0	0.684
LDL Chol. (mg/dl)	126±85.6	117.7±34.3	131.2±101.8	0.300
Triglycerides(mg/dl)	113.3±61.2	97.2±50.2	121.7±65.0	0.042
PAC (pg/ml)	124.9±86.2	128.9±104.9	122.8±75.5	0.168

PRA (ng/ml/h)	3.3±5.3	3.1±4.1	3.4±4.8	0,329
ARR	8.6±7.0	7.9±6.1	8.9±7.3	0.055

As illustrated in the table, study population included predominantly middle-aged male individuals, with high prevalence of obesity (57,7%) and relatively low prevalence of other CV risk factors (smoking habit, diabetes, and dyslipidaemia, respectively 25,8%, 16,5% and 4,9%).

Average values of PA levels, plasma renin activity (PRA) and aldosterone-renin ratio (ARR) are also reported in **Table 1**. No significant differences between the two gender groups were observed for these parameters.

As shown in **Table 2**, there were no significant differences Clinic, 24-h and central systolic and diastolic BP levels and parameters of vascular HMOD between the two groups. In the overall sample, 108 (71,1%) received BP lowering drugs.

Table 2

Parameters	**Overall**	**Female**	**Male**	**P value**
Clinic SBP (mmHg)	142±18.6	140,3±18.9	143.5±18.3	0,958
Clinic DBP (mmHg)	89.7±11.6	88,0±11.45	90.7±11.7	0,676
Clinic Heart Rate (bpm)	71.5±11.7	73,3±13.2	70.4±10.6	0,083
24-hour SBP (mmHg)	130.3±14.1	124,9±13.9	133.7±13.2	0,414
24-hour DBP (mmHg)	81,74±10.3	77,8±10.1	84.2±9.8	0,452
24-hour Heart Rate(bpm)	73,33±9.2	74,5±10.3	72.6±8.5	0,207
Central SBP (mmHg)	135,2±16.2	133,4±18.3	136.2±15.1	0,170
Central DBP (mmHg)	90,2±12.6	86,7±13.8	91.9±11.7	0,160
Peripheral Resistances (dyn*s/cm5)	20,0±10.9	1736,7±259.8	1738.3±245.8	0,475
AI@75 [95% CI] (%)	1737,8±284.9	23,8±10.1	18.1±10.9	0,529
PWV (m/sec)	7,2±1.7	6,9±1.9	7.3±1.5	0,734

Echocardiographic parameters in the overall population and in the

subgroups are shown in **Table 3**. LV mass was higher in man than in women, but those differences become not significant when the LV mass is indexed by body surface area and by height^2.7. Deceleration time was higher in woman compared to man, while other echocardiographic parameters was similar between the two groups

Table 3

Parameters	Overall	Female	Male	P value
LV mass	176,9±58,5	141.8±35.4	195.5±60.0	0.010
LV mass BSA	91,8±26.6	83.173±18.9	96.4±28.9	0.069
LV mass H^2.7	40,7±13.2	37.95±10.3	42.2±14.3	0.181
LV stroke volume (ml)	82,7±18.6	72.9±17.5	87.9±17.1	0.992
LV EF (%)	71,4±9.5	62.3±10.1	60.8±9.1	0.230
LV FS (%)	41,5±8.1	42.36±9.11	41.1±7.5	0.054
Cardiac Output	5,8±1.7	5.2±1.7	6.1±1.5	0.346
Cardiac Index	2,8±0.9	2.9±1.0	2.8±0.8	0.068
Pre-ejection time	68,0±14.3	68.2±13.1	67.9±14.9	0.518
Total ejection time	349,8±46.3	356.8±42.8	346.1±47.8	0,744
E wave	67,0±17.9	71.2±18.3	64.7±17.3	0.887
E/A ratio	1,1±0.6	1.11±0.7	1.05±0.5	0.700
DT	175,3±93.8	194.97±130.7	165.5±65.2	0.003
E' wave	0,2±0.1	0.18±0.11	0.16±0.1	0.161
E/E' ratio	4,5±1.8	4.58±2.1	4.40±1.49	0.325
IVCT	68,0±14.3	68.2±13.1	67.9±14.9	0.518
IVRT	76,9±20.6	73.3±18.7	78.8±21.4	0.181
Ea	1,6±0.5	1.8±0,4	1.5±0.4	0.186
Ev	2,2±0.8	2.4±0.7	2.0±0.8	0,172
VAC	0,8±0.2	0,757±0.16	0.793±0.19	0.276

Correlations between PAC and arterial elastance (**Figure 1**), ventricular elastance (**Figure 2**) and VAC (**Figure 3**) in the overall population are

shown in the following figures. Plasma aldosterone levels resulted significantly and positively correlated with VAC (Pearson r: 0.236; P=0.001), whilst no significance correlations were found between its single components, even if a trend towards augmentation (Pearson r: 0.107; P=0.145) and reduction (Pearson r: -0.63; P=0.391) was found, respectively for arterial and ventricular elastance

Figure 1.

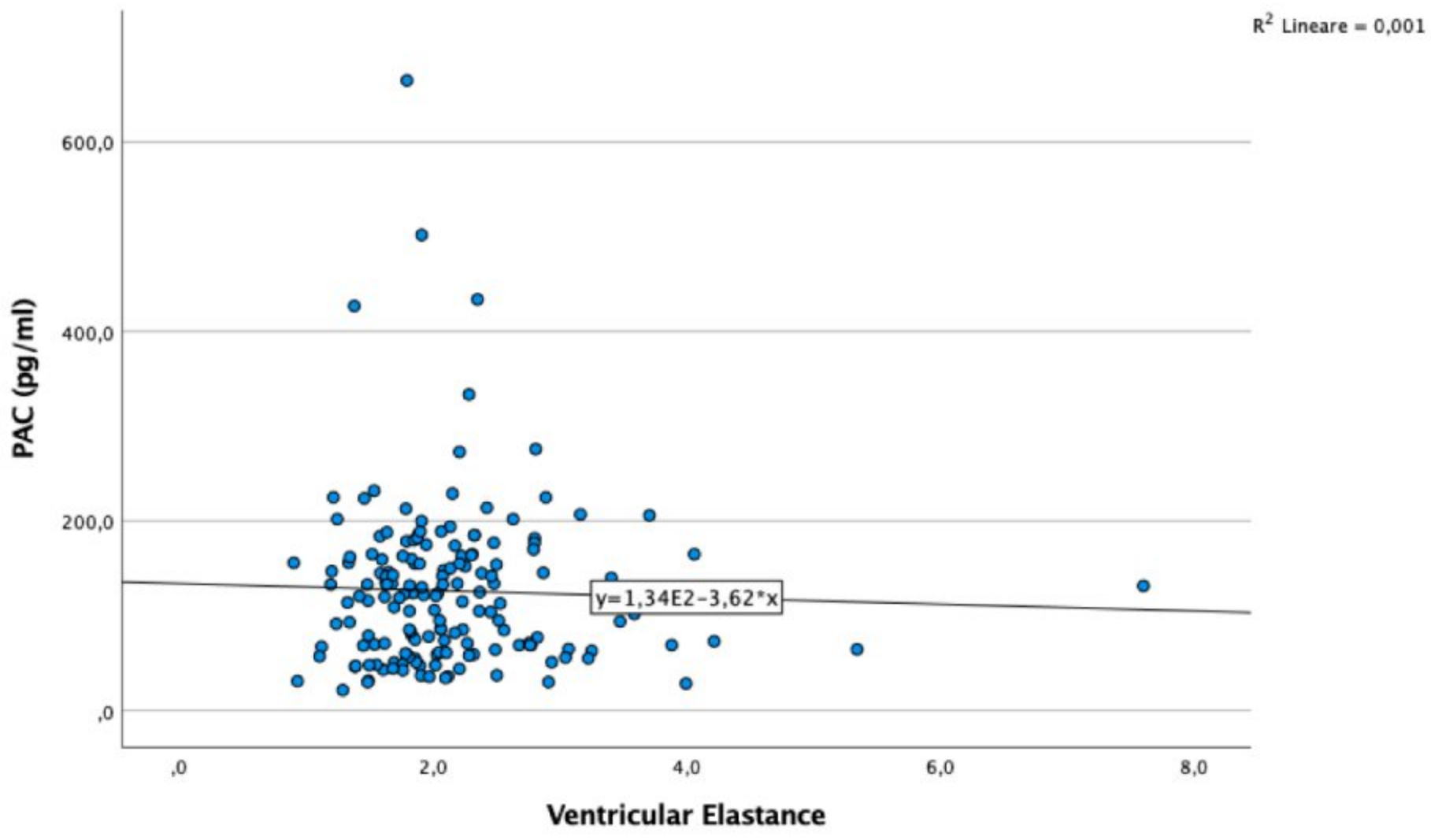


Figure 2.

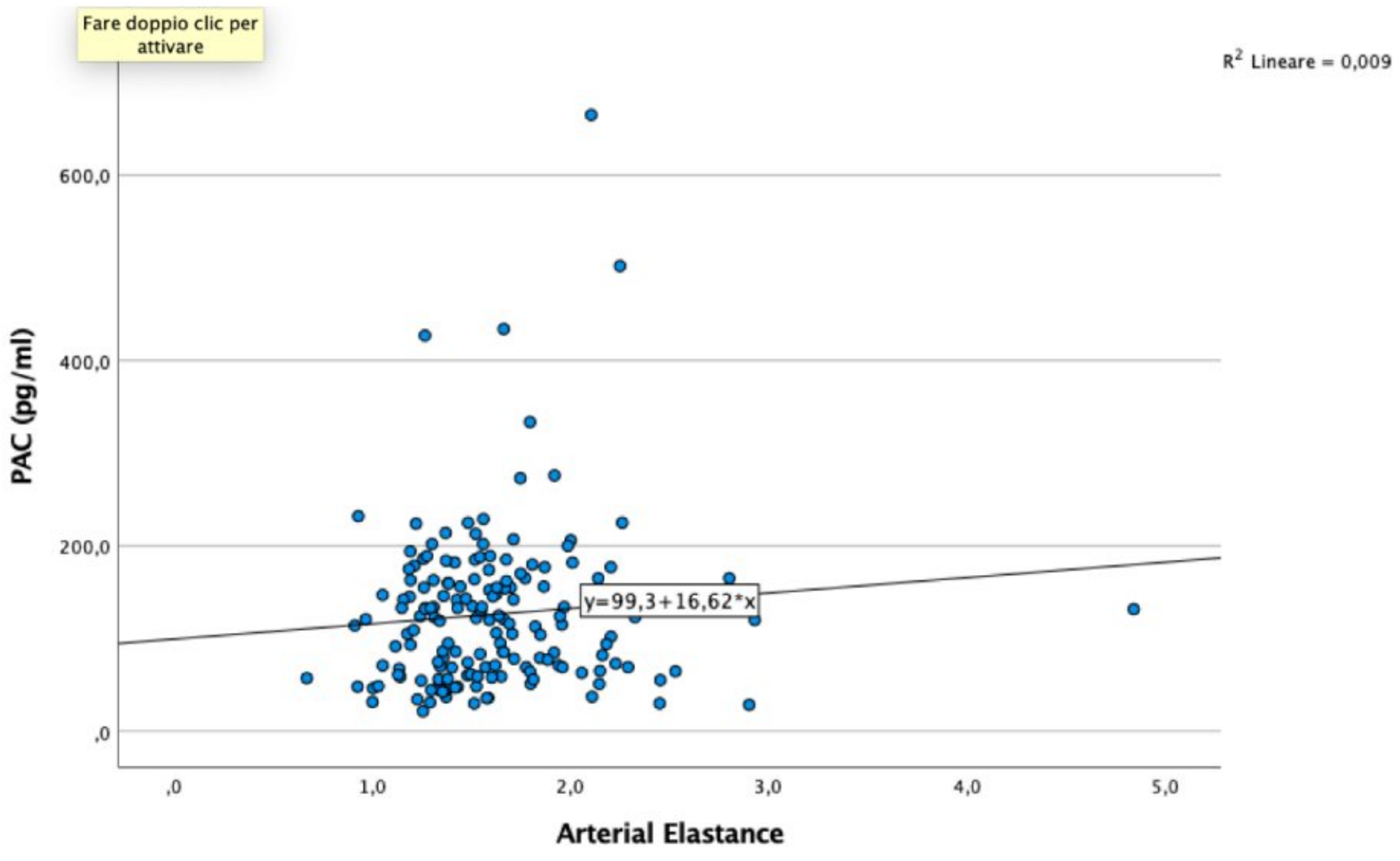
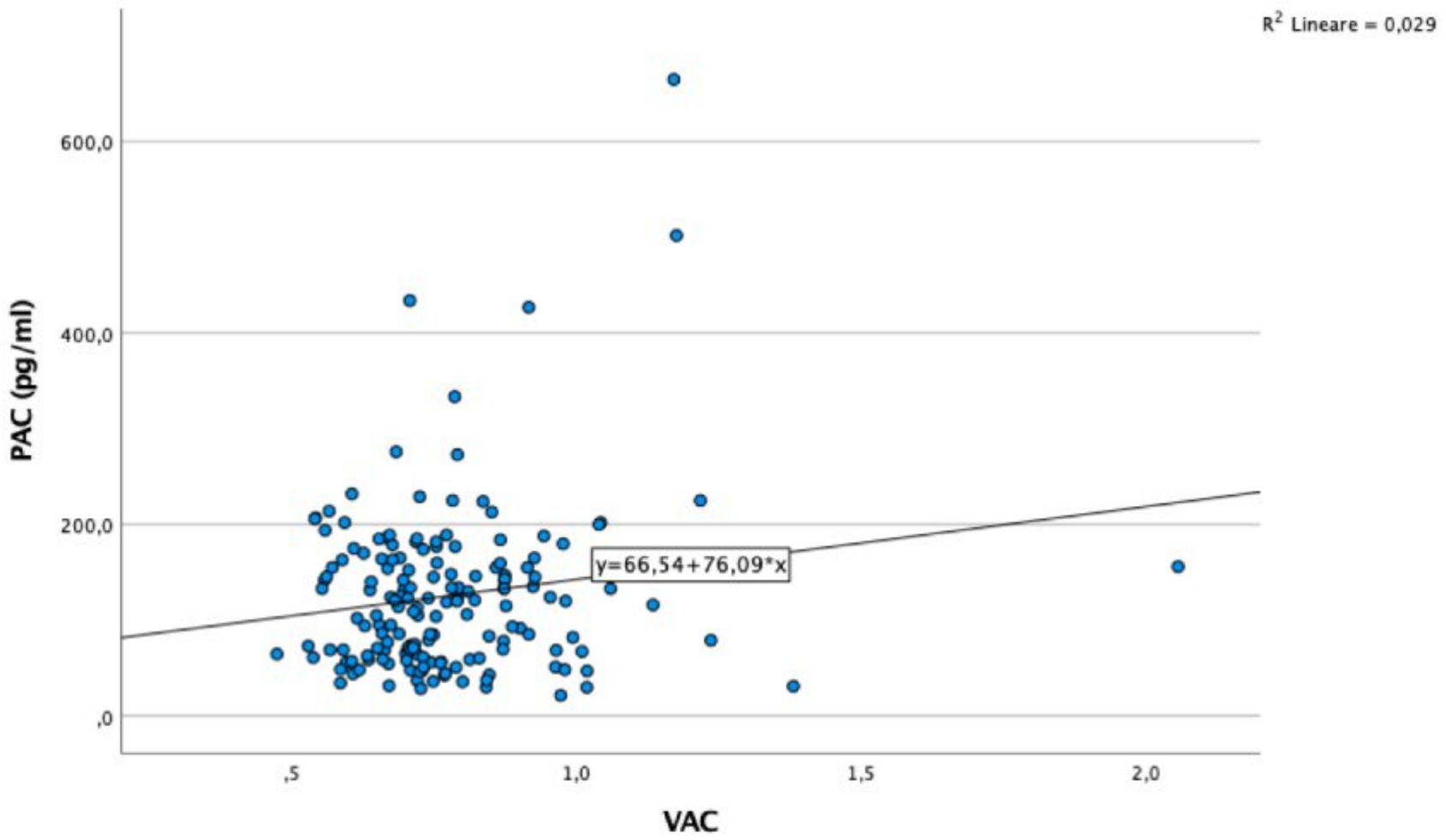


Figure 3.



Correlations between PAC and ventricular elastance, arterial elastance and VAC

By analysing the correlation among PAC and BP level, echocardiographic parameters of LV structure and LV diastolic and systolic function some differences have been observed in women and men (**table 4**)

PA levels correlate with BP levels in men, but not in women, as well as with LV mass and VAC.

Late-wave ratio (E/A) and the early mitral annular velocity (E wave) shown a significant negative correlation with PAC in Men, but not in women.

On the contrary E wave deceleration time was significantly correlated with PAC only in woman. IVCT remained significantly correlated with PAC in both groups.

Table 4

Overall sample				
	Pearson's correlation	Two-side Significance	CI 95%	
			Inferior	Superior
VAC	0,169	0,029*	0,017	0,312
Arterial Elastance	0,095	0,222	-0,058	0,243
Ventricular Elastance	-0,029	0,714	-0,180	0,124
Systolic BP	0,180	0,020*	0,028	0,322
Diastolic BP	0,072	0,352	-0,081	0,222
SBP-24h	0,168	0,064	-0,011	0,335
DBP-24h	0,217	0,016*	0,040	0,380
LV mass	0,094	0,225	-0,059	0,242
LV mass BSA	0,133	0,087	-0,020	0,279
LV mass H^2.7	0,125	0,106	-0,027	0,272
E wave	-0,112	0,151	-0,260	0,041
E/A	-0,105	0,178	-0,254	0,049
DT	0,337	<0,001*	0,192	0,466
E'	-0,083	0,297	-0,236	0,074
E/E'	-0,030	0,703	-0,185	0,126
IVCT	0,225	0,003*	0,075	0,364
IVRT	0,073	0,351	-0,080	0,222

Table 5

Women				
	Pearson's correlation	Two-side Significance	CI 95%	
			Inferior	Superior
VAC	0,166	0,223	-0,103	0,409
Arterial Elastance	0,025	0,853	-0,239	0,286
Ventricular Elastance	-0,076	0,575	-0,332	0,191
Systolic BP	-0,090	0,510	-0,344	0,178
Diastolic BP	-0,230	0,088	-0,463	0,037
SBP-24h	0,130	0,391	-0,168	0,403
DBP-24h	0,157	0,298	-0,142	0,426
LV mass	-0,141	0,300	-0,388	0,128
LV mass BSA	-0,160	0,238	-0,405	0,109
LV mass H^2.7	-0,135	0,320	-0,383	0,134
E wave	0,025	0,857	-0,240	0,286
E/A	-0,028	0,837	-0,289	0,237

DT	0,491	<0,001*	0,252	0,668
E'	-0,054	0,699	-0,317	0,218
E/E'	0,013	0,926	-0,256	0,280
IVCT	0,315	0,018*	0,054	0,532
IVRT	0,009	0,947	-0,255	0,271

Table 6.

Men				
	Pearson's correlation	**Two-side Significance**	**CI 95%**	
			Inferior	**Superior**
VAC	0,186	0,05*	-0,002	0,359
Arterial Elastance	0,136	0,156	-0,053	0,314
Ventricular Elastance	-0,015	0,878	-0,200	0,172
Systolic BP	0,397	<0,001*	0,225	0,542
Diastolic BP	0,317	<0,001*	0,137	0,474
SBP-24h	0,186	0,108	-0,043	0,394
DBP-24h	0,252	0,028*	0,026	0,450
LV mass	0,235	0,013*	0,050	0,403
LV mass BSA	0,286	0,002*	0,104	0,448
LV mass H^2.7	0,270	0,004*	0,087	0,434
E wave	-0,226	0,018*	-0,396	-0,039
E/A	-0,189	0,049*	-0,363	0,000
DT	0,159	0,099	-0,031	0,336
E'	-0,159	0,106	-0,339	0,035
E/E'	-0,061	0,540	-0,249	0,133
IVCT	0,179	0,050*	-0,008	0,353
IVRT	0,122	0,201	-0,066	0,301

Correlation among PAC and BP level, echocardioghrphic parameters of LV structure and LV diastolic and systolic function in overall population, in women and in men.

DISCUSSION

The results of the current study confirm and further expand previous findings indicating that Aldosterone determine several preclinical alterations in LV structure and function. Our study had the purpose to evaluate if augmented level of Aldosterone, even within the physiological range, could be associated with preclinical alterations in LV structure and function in a population of hypertensive patients without hyperaldosteronism. The data collected provide some interesting insights, considering that the population examined is composed by relatively young patients with mild hypertension, and that they are therefore at an early stage of the disease, that is supposed not to be yet resulted in overt organ damage.

The study population included predominantly middle-aged male individuals, with high prevalence of obesity. This can be explained by the fact that obesity is a major cause of hypertension in young middle-aged patients, once excluded causes of secondary hypertension.

In our study we observed that Ees alone was not significantly associated with aldosterone levels, but the tendency to its decrease in patients with a tendency to increase of Ea, as observed in our simple of patients, determined a significant augmentation of VAC.

The direct correlation between plasma aldosterone levels and **VA**C suggest that aldosterone may favour the development of subclinical LV systolic dysfunction with impair of mechanical energetic efficiency and a reduction in contractility reserve, acting both on the ventricular and on the arterial side.

It is well known that the direct effect of aldosterone on cardiomyocytes and cardiac fibroblasts determines an increased deposition of interstitial collagen.

Previous studies have demonstrated the presence of receptors for mineralocorticoid hormones in human cardiomyocytes and cardiac fibroblasts, and their stimulation may favour the development of myocardial structural alterations, also independent of blood pressure (BP) increase, and, consequently, progressive disfunction with mechanisms that involve chronic inflammation, dysregulation of extracellular matrix (ECM) metabolism, and deposition of myocardial collagen,

This is demonstrated also by the fact that in experimental models of hypertension with excess aldosterone, fibrosis involved not only the left but also the right ventricle, a chamber exposed to the bloodstream but not to the pressure overload, and was prevented by administration of non-antihypertensive dosages of the aldosterone-specific receptor antagonist spironolactone.

On the other hand, aldosterone increases arterial stiffness modifying elastin and collagen quantity, so decreasing systemic arterial compliance and contributing to deteriorate arterial-ventricular coupling

These finding suggests a direct and early effect of circulating aldosterone levels on myocardial efficiency.

A recent study from Salvetti et al demonstrated that in patients with primary aldosteronism myocardial the myocardial mechano-energetic efficiency index (MEEi) is lower as compared with essential hypertensive patient

In our study PA levels resultedsimilar in males and females, even if it has been found in literature that usually are lower in premenopausal women than in men. Also, BP levels and echocardiographic parameters didn't differ significantly between the two groups.

Nevertheless, we found dome gender-related differences in the correlation between aldosterone level and BP levels and echocardiographic parameters.

We found that BP levels correlates positively with aldosterone level in men but not in women, as well as with LV mass. This data differs from previous finding, in particular in the Framingham Heart Study, elevated plasma levels of aldosterone correlated with cardiac wall thickness in women but not men.

IVCT remained significantly correlated with PAC in both groups. It has been demonstrated that cardiac time intervals change during disease progression, and that prolonged IVCT is associated with greater risk of congestive heart failure and cardiovascular mortality.

Some indices of diastolic function - late-wave ratio (E/A) and the early mitral annular velocity (E wave) - shown a significant negative correlation with PAC in men, but not in women. Thisseems to suggest a greater impairment of indices of LV diastolic function in men compared to woman according to aldosterone level.

Previous studies have shown that indices of impaired diastolic function derived by TDI are strictly correlated with circulating markers of myocardial fibrosis. Over more, diastolic dysfunction, which is characterized by the inability of the heart chambers to dilate in order to accommodate volumes of blood due to the increased rigidity of the ventricular walls, may be the mirror of the initial decrease in left

ventricular elastance and, therefore, of VAC.

Actually, analysing the correlations in gender subgroups, we also found that aldosterone level correlates with VAC in men but not in women.

All these discrepancies may be due to a crosstalk between androgens and the cardiovascular effects of aldosterone.

Some studies conducted on animals demonstrated that the hormonal environment is thus important and influences the RAA system effects.

It is well established that oestrogen have an anti-fibrotic and anti-inflammatory activity, and it has been also already studied in animal models that oestrogens may decrease aldosterone concentrations.

It has been found that in ovariectomized premenopausal female dogs, oestrogen decreases aldosterone concentrations. A study conducted in ovariectomized rats, demonstrated that estrogen decreases aldosterone content in the adrenal glands and aldosterone secretion induced by angiotensin II.

On the other hand, in ovariectomized female Wistar rats, aldosterone with high salt induces hypertension, cardiac hypertrophy, vascular fibrosis, and increased osteopontin expression. Activation of either oestrogen receptor α or β protects against these pathologic changes. Together, these findings suggest that oestrogens might attenuates aldosterone-induced tissue injury, even if there are not evidences in human studies.

The Randomized Aldactone Evaluation Study for Congestive Heart Failure (RALES) showed that spironolactone improves the prognosis of patients with heart failure, and the Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS) showed that eplerenone improved the prognosis of patients with acute myocardial

infarction complicated by heart failure. However, risk reduction was higher in spironolactone than eplerenone although patient characteristics are different in two studies. Spironolactone, a nonselective MR antagonist, also blocks androgen receptors and stimulates progesterone receptors, whereas eplerenone is a selective MR antagonist. The beneficial effects of spironolactone might occur, in part, via the inhibition or activation of sex hormone receptors. However, in the sub analysis of RALES, the beneficial effects of spironolactone were identical in both men and women.

POTENTIAL LIMITATIONS

We acknowledge that our study has several limitations. First of all, this study analysed data that were retrospectively extracted from our medical database, which were not prophetically collected during clinical consultations.

Our study shows an association between aldosterone and other clinical and instrumental parameters but cannot provide proof of the causality of this association. Moreover, the small sample size might have led to a low-powered analysis to exclude possible relationships.

Considering the parameter used to evaluate ventricular efficiency, even if the use of single beat method to calculate the VAC is fairly well validated, extensive use of formulas with mathematical assumptions may lead to incorrect estimations, together with the possible error derived by the fact that measurements are affected by the quality of the echocardiographic images.

CONCLUSIONS

Our study demonstrated that PA levels, even within the normal range, may affect at early-stage LV efficiency, which was non-invasively evaluated thorughout the echocardiographic measurement of VAC.

Although the use of VAC as a prognostic parameter in the ICU setting is well established, its use in the outpatient setting has not yet been extensively studied and evaluated. In this context, the results obtained from our study may suggest that VAC and its components (namely, ventricular elastance and arterial elastance) may represent useful parameters to detect subclinical LV systolic dysfunction and impairment of cardiovascular efficiency, and should therefore be used as an additional parameter in routinely practice

Another interesting and innovative aspect of our study is represented by the pathogenic role of aldosterone in CVD, may be influenced by hormonal environment, leading to a different susceptibility of the ventricle to aldosterone in women and men.

Understanding the interplay between Aldosterone and sex hormone may be very interesting because may change the pharmacological approach individualizing treatment according to gender to improve patient outcomes.

Further prospective studies are needed to confirm the results.

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