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Association of plasma renin and aldosterone concentration and its ratio with systolic and diastolic cardiac function and phenotypes of cardiac disorder

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Abbreviations

ACC: American College of Cardiology

ACE: angiotensin-converting enzyme

ACE-I: angiotensin converting enzyme inhibitors

ACTH: adrenocorticotropic hormone

AF: atrial fibrillation

AGT: angiotensinogen

AHA: American Heart Association

ARB: angiotensin receptor antagonists

ARR: aldosterone to renin ratio

ATC-code: anatomical therapeutic chemical classification

AT1R: type 1 Angiotensin II receptor

BMI: body mass index

BP: blood pressure

CAD: coronary artery disease

CAPI: computer assisted personal interview

CHF: chronic heart failure

CKD: chronic kidney disease

CLIA: automated chemiluminescence immunoassays

CMS: cardio-metabolic syndrome

CO: carbon dioxide

CRP: C-reactive protein

CV: cardiovasular

CVD: cardiovascular disease

CVRF: cardiovascular risk factors

DD: diastolic dysfunction

DDpEF: diastolic dysfunction with preserved ejection fraction

DDrEF: diastolic dysfunction with reduced ejection fraction

DecTime E': Deceleration time E' (DecTime E')

DHF: diastolic heart failure

DM: diabetes mellitus

DMC: diabetic mellitus cardiomyopathy

DT: deceleration time

DVT: deep venous thrombosis

ECG: electrocardiogram

eCRF: electronic Case Report File

EF: ejection fraction

ESV: end-systolic volume

ET: ejection time (ET)

ET-1: endothelin-1

EVD: end-diastolic volume of the left

FCD: functional cardiac disease

FEV1: forced expiratory volume in one second

FH: family history

FVC: forced vital capacity

GCP: Good Clinical Practice

GEP: Good Epidemiological Practice

GFR: glomerular filtration rate

GHS: Gutenberg Health Study

GHSID: Gutenberg Health Study Identity

GHDLID: Gutenberg Health Study Labor-Identity

HbA1c: glycated haemoglobin

HDL: high-density lipoprotein

HF: heart failure

HFpEF: heart failure with preserved ejection fraction

HFrEF: heart failure with reduced ejection fraction

HRT: hormonal replacement therapy

HTN: hypertension

IDOM: Online Erfassung von Medikamentendaten

IGF1R: insulin-like growth factor 1 receptor

IL-6: interleukin-6

IQR: interquartile range

IRS-1: insulin receptor substrate 1

ISVd: end- diastolic thickness of the intraventricular septum

IVCT: isovolumic contraction time

IVRT: isovolumic relaxation time

K: kalium

Lad: end-diastolic volume of the left atrium

LDL: low-density lipoprotein

LV: left ventricle

LVDD: left ventricular diastolic dysfunction

LVEF: left ventricular ejection fraction

LVIDd: end-diastolic diameter of the left ventricle

LVPWd: end- diastolic thickness of the free wand of the left ventricle

LVSD: left ventricular systolic dysfunction

MAP: mean arterial pressure

MAPK: mitogen-activated protein kinases

Ma-VA': maximal late-systolic velocity of the mitral ring

Ma-VE': maximal early-systolic velocity of the mitral ring

Ma-VS: maximal systolic velocity of the mitral annulus

MI: myocardial infarct

MR: mineralocorticoid receptor

MRA: mineralocorticoid receptor antagonists

mRNA: messenger ribonucleic acid

MV A-Duration: mitral valve duration of the passive filling

MV A-Vmax: flow velocity during the active filling of the ventricles due to the atrial contractility

MV DT: mitral valve deceleration time

MV E-Vmax: flow velocity during the passive filling of the ventricles

Na: natrium

NaCl: sodium chloride

NADPH: nicotinamide adenine dinucleotide phosphate

NO: nitric oxide

NT-proBNP: N-terminal of the prohormone brain natriuretic peptide

N: number

NYHA: New York Heart Association

OC: oral contraceptives

PAC: plasma aldosterone concentration

PAD: peripheral artery disease

PAH: pulmonary arterial hypertension

PDrEF: preserved diastolic dysfunction with reduced ejection fraction

PE: pulmonary embolism

PEF: peak expiratory flow

PH: pulmonary hypertension

PI3K: phosphoinositide 3-kinase

PKG: protein kinase G

PRC: plasma renin concentration

PW-Doppler: pulsed wave Doppler

Q: quartile

RAAS: renin-angiotensin-aldosterone system

Rad: end-diastolic volume of the right atrium

RAS: renin-angiotensin system

ROS: reactive oxygen species

RVIDd: End-diastolic diameter of the right ventricle

SD: systolic dysfunction

SHF: systolic heart failure

SOP: standard operating procedure

TDI: tissue Doppler imaging

TGF-b: tissue growth factor-b

TNF-a: tumor necrosis factor alpha

U.S. United States

U.S.A: united states of America

VCAM-1: vascular cell adhesion molecule 1

WHO: world health organization

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1. Introduction

Heart failure's worldwide high incidence and continuously rising prevalence emphasize the need of distinguishing different clinical phenotypes and understanding the multifactorial pathophysiological mechanisms involved in order to optimize the risk assessment, to improve and individualize the existing treating prospects as well as to develop new ones. Heart failure is a clinical entity, which includes a dominant functional cardiac disorder, diastolic or systolic, and the presence of its clinical manifestations, according to NYHA classification. As cardiac dysfunction is defined the situation of the asymptomatic diastolic or /and systolic cardiac dysfunction, determined by echocardiographic parameters (LVEF, E/E'). Under this concept five clinical phenotypes of cardiac disorder and heart failure were investigated in our study; the asymptomatic (NYHA I) isolated diastolic dysfunction (DD), the asymptomatic (NYHA I) isolated systolic dysfunction (SD), the asymptomatic (NYHA I) combined systolic and diastolic dysfunction, heart failure (NYHA>I) with preserved LVEF (HFpEF) and heart failure (NYHA>I) with reduced LVEF (HFrEF).

This data presents RAAS actions in cardiovascular system to have a potential double role with both repairing and deleterious effects: on the one side, it serves as a compensatory mechanism in case of myocardial or vascular injury to preserve left ventricular function and as a maladaptive mechanism when it promotes fibrosis or hypertrophy, inflammation or oxidative stress. Widely accepted is also the tremendous effect of RAAS system on heart tissue and its undoubted role in heart failure pathogenesis. Main purpose of this study is to investigate if a possible association of plasma renin level, serum aldosterone concentration as well as renin-to-aldosterone ratio (ARR) with these clinical phenotypes of heart failure (DD, SD, combined DD and SD, HFpEF, HFrEF) exists.

Surprisingly, HFpEF in females seems to appear almost twice as frequent as HFrEF (63% versus 38%). RAAS regulation seems to depend also upon other hormonal-to-hormonal interactions, such as the ovarian hormones. This knowledge implies that female heart is more sensitive to the aldosterone effects. Through echocardiographic examination of healthy population, it could be observed a correlation of aldosterone concentration and even HFpEF only in women. Mounting clinical data reveals that estrogens and hormonal replacement therapy in postmenopausal women antagonize the expression of RAAS system. These interesting

differences between two sexes urged us think that an investigation of the relation between RAAS and cardiac phenotypes under a sex-specific scope would elucidate more this issue.

Hypertension, diabetes mellitus and adiposity are undeniable risk factors of heart failure and RAAS involvement incontrovertible. In particular, RAAS is overexpressed in arterial hypertension, mediates the cardiovascular complications of diabetes and is produced in fat tissue. It seems that RAAS hormones are the linkage, which connects these three clinical situations, which consequently contribute to cardiac dysfunction. At this point, arouse the question to what degree these cardiovascular risk factors influence the association between renin, aldosterone and ARR and cardiac phenotypes, which we tried to answer with our analyses.

Furthermore, considering and evaluating deeper data obtained from large clinical meta-analyses trials testing thousands of patients, that RAAS blockade is not as beneficial in HFpEF as in HFrEF. It was proposed that, due to the observed decreased reduction in death rate in case of HFpEF maybe consequently HFpEF is characterized by a more limited RAAS activation. Since very early was expressed the idea that level of RAAS hormone concentration depends on the clinical severity of heart failure, which was supported by the SOLVD Investigators who suggested that low LVEF in absence of clinical symptoms is accompanied by low RAAS expression and by observations that patients with LVEF of 40-49% were more benefited that others with LVEF of 50% or more, indicating an increase of RAAS blockade effectiveness as LVEF declines. In order to confirm or reject this hypothesis and explain these observations we investigated the age- and sex- specific impact of standard drug medication for heart failure as well as of other drugs which can potentially influence RAAS hormones, on plasma renin level, serum aldosterone concentration and ARR.

2. Literature

Heart failure

Heart failure concerns 5.3 million people in America and is the number one discharge diagnosis (3, 4). It accounts for 1 in 7 deaths in the U.S. and someone in the U.S. dies from heart disease about once every 90 seconds (5). Statistic data demonstrate clearly the cardiovascular disease as the leading cause of death globally, accounting for 17.3 million deaths per year (5) with heart failure to consist almost the 7% of all cardiovascular deaths (6). Despite the medicine revolution with a consequent improvement of outcome, the absolute mortality rates for HF remain tremendous high, about 50%, within 5 years of diagnosis (7, 8).

NYHA classification and ACCF/AHA staging of heart failure two further classifications provide supplemental information; ACC/AHA stages of heart failure (9) and New York Heart Association (NYHA) (10) functional gauge, the first estimates the progression of heart failure and the latter the severity expressed by clinic symptoms of the disease, respectively.

‘’ NYHA Classification (10):

- Stage I: No limitation of physical activity. Ordinary physical activity does not cause symptoms of HF.
- Stage II: Slight limitation of physical activity. Comfortable at rest, but ordinary physical activity results in symptoms of HF.
- Stage III: Marked limitation of physical activity. Comfortable at rest, but less than ordinary activity causes symptoms of HF.
- Stage IV: Unable to carry on any physical activity without symptoms of HF, or symptoms of HF at rest.

A wide variety of damages which can cause diastolic or/and systolic impairment, are handled by the cardiovascular system in numerous ways (11), which progressively lead to reduced cardiac output or/and elevated filling pressures or/and the clinic express of the disease. Whereas hemodynamically, heart failure reflects the incapacity of heart to supply sufficient cardiac output when required (11), regarding the symptoms and signs, among others, dyspnea, fatigue and, occasionally angina, with subsequent reduction of exercise tolerance, as well as fluid retention, leading progressively to pulmonary and/or splanchnic congestion and/or peripheral edema (9) are the most typical clinical manifestations of HF. The extent and the severity of their expression can greatly diverse among patients with heart failure (6). On the other hand, the asymptomatic

LV dysfunction is not rare as it represents an incidence range of 6% (mild diastolic dysfunction is present) to 21% (moderate or severe diastolic dysfunction) among a community-based population. Surprisingly, this clinical entity is related with an increased mortality risk (12-14). However, it has been shown that asymptomatic presentation of diastolic dysfunction will progress in a symptomatic one almost 5 years after the first diagnosis (15, 16).

This complex clinical syndrome that constitutes the heart failure can derive from any structural (concentric or eccentric remodeling) or functional dysfunction (impaired ventricular filling or reduced ejection of blood), in accordance with the latest guidelines (6). In these terms, it should be emphasized that heart failure can result from a wide spectrum of systolic or diastolic abnormalities (SHF and DHF respectively), or both, and vary from clinic phenotypes with severe left ventricular remodeling and distinct reduction of EF to patients with normal LV mass, size and shape and preserved EF (6). Thinking heart failure more as a clinical syndrome with complex pathogenesis, than a disease with well-defined characteristics, cases where a particular impairment is prominent cannot be excluded, irrespectively of clinical symptoms. Therefore, it would be reasonable a taxonomic attempt of heart disorders (17). First of all, it must be clear that heart failure and heart dysfunction are two different concepts, which can be partially separated. Heart failure is a syndrome, regardless of the pathophysiological disorder, and is characterized by the clinical expression of symptoms, resulting typically from fluid retention and insufficient blood circulation. The dysfunction is determined by the exact abnormality involved, diastolic or systolic. In general, heart failure is an umbrella-term which includes the idea of dysfunction, as the latter is presumed in order for the first to occur. Accordingly differs also the diagnosis as heart failure, in terms of consisting a clinical syndrome, a big part is based on the clinical expression, whereas the heart dysfunction, under the conception of being a mechanical defect, is defined mostly by echocardiographic measurements (17) (e.g. LVEF or E/E'). It can be said that, observing heart failure as a part of the cardiovascular disease, there is a continuing progress beginning from the existence of cardiovascular risk factors, proceeding to asymptomatic alterations of the heart tissue and resulting to irreversible, in most of the cases, heart dysfunction accompanied by clinical symptoms (18). The asymptomatic stage seems to predict the final stage of heart failure (14). The importance of examining and treating separately asymptomatic cardiac dysfunction and symptomatic heart failure becomes obvious from the staging A to D as cardiac disorder progresses from mild to severe, regarding clinical symptoms. Particularly, people in stage B present systolic or diastolic abnormalities in cardiac structure, visible by echocardiography, but no symptoms are presented (19). On the other side Stage C characterizes patients who have developed the typical clinical manifestation or signs of heart failure (20). Surprisingly was the finding of Ammar et al. (19) regarding the frequency of each

stage in the community as it was reported that among randomly selected study population, people with an asymptomatic dysfunction appeared to be twice as more as these with symptomatic heart failure (34% vs.12%).

In respect of the main distinct heart disorders, taking into account the dependence on the clinical appearance and the expression of symptoms, types of heart failure, are the diastolic and the systolic. Collectively, diastolic dysfunction describes the weakness of the ventricle to fill with blood due to a mechanical impairment of its relaxation ability in the phase of diastole or the increase in wall stiffness, whereas the systolic function is normal (normal EF). Should this condition last long, it would come to a point when the increase in filling pressure or volume, responses in order to maintain an adequate amount of stroke volume, a condition which potentially cause the clinical expression of the disease (heart failure). On the other hand, a systolic dysfunction refers to the impaired contractility of ventricle during systole (possibly due to loss of cardiomyocytes, fibrotic myocardium alterations or ischemic vascular changes), which finally leads to decrease of stroke volume and EF and eventually to symptoms (systolic heart failure) (17).

LVEF (or simply EF) is used as a main criterion in the classification of heart failure, in order to enable the categorization of demographic characteristics, diagnostic methods, prognostic values and therapeutic means and responsiveness to medication among the total population and different subgroups of patients according to comorbidities (21).

Respecting this EF-based classification, the major categories represent two main groups of patients. Those with heart failure and reduced EF, defined as the clinical diagnosis of HF and EF less than or equal to 35% or 40%, according to different guidelines' definitions (9, 22) and those with heart failure and preserved EF, variably estimated from EF >40%, >45%, >50%, and 55%.There is also an intermediate subgroup of patients with an EF between 40% and 50% (6). A clearer classification based on the left ventricular ejection fraction was provided by Lang et al. (23) as follows:

LVEF:

- ≥55% Reference range
- 45-54% Mildly reduced
- 30-44% Moderately reduced
- <30% Severely reduced

The criteria of definition of HFpEF given by the Working Group for the European Society of Cardiology proposed that: “diagnosis of primary diastolic heart failure requires three obligatory conditions to be simultaneously satisfied: 1) presence of signs or symptoms of congestive heart failure (CHF); 2) presence of normal or only mildly abnormal left ventricular (LV) systolic function (with an EF >50% within 72 hours of the CHF event) 3) evidence of abnormal LV relaxation, filling, diastolic dispensability, or diastolic stiffness.” (24). Or of same meaning but differently expressed by Vasan et. al. and then adopted by the 2013 ACCF/AHA Guidelines for the Management of Heart Failure are: ‘’a) clinical signs or symptoms of HF; b) evidence of preserved or normal LVEF; and c) evidence of abnormal LV diastolic dysfunction that can be determined by Doppler echocardiography or cardiac catheterization’’ (25).

Definition of diastolic heart dysfunction by Bursi (1) and Kuwaki (2):

- Normal cardiac function: E/A $\geq$ 0.75 and DT>140msec and E/E’ <10
- Mild diastolic dysfunction: E/A< 0.75 and E/E’ <10
- Mild to moderate diastolic dysfunction: E/A$\leq$0.75, DT>140msec and E/E’$\geq$10 (2, 4)
- Moderate diastolic dysfunction: 0,75$\leq$E/A and DT>140msec and E/E‘ $\geq$10
- Severe diastolic dysfunction: E/A>2 and DT<140msec and E/E‘ $\geq$ 10

At this point it is worth mentioned that in the majority of patients signs of combined systolic and diastolic dysfunction coexist (26). Considering carefully the pathophysiology of the heart disorders which potentially can result to heart failure, it is worth to notice a common abnormality in pressure-volume relation characterizes both dysfunctions (in diastole or systole respectively). Nevertheless, there is a significant difference: diastolic dysfunction or diastolic heart failure can exist isolated (increased pressure to normal volume, normal EF) in contrary to the systolic (decreased EF) which progressively develops diastolic characteristic abnormalities (17). In a conceptual way of thinking we can conclude that diastolic heart failure can be considered as an independent clinical entity, likely to occur isolated (normal EF), while in contrast the systolic heart failure at late stages is almost always accompanied by diastolic-typical abnormalities (combined diastolic and systolic heart failure) (17).

HFpEF versus HFrEF

Victor et al. (27) tried to find which factor is capable of leading an asymptomatic diastolic disorder to a symptomatic heart failure. He explored the frequency that classical CVRF appear in both cases and observed no difference. The fatality by the incidence of diastolic heart failure aggregates 5-8%, while the death rate in systolic heart failure is slightly higher (10-15%). These distinct disorders present almost equal rates of re-hospitalization (approximately 50%) (27, 28).

No relevant difference is observed regarding the rate of 1-year re-hospitalization between diastolic and systolic heart failure (15, 29-35). Although numerous population and experimental studies show the explicit architectural and functional difference between SHF and DHF (36), still highly controversial remains the clarification of HFrEF and HFpEF. There has been evidence that illustrates an oxymoron: the idea of HFpEF and HFrEF being a part of the same disease spectrum (37) in terms of sharing similar clinical symptoms (1, 21, 38-42) and the contradictory conception, by multiple proven demonstrations, that clearly verify a wide heterogeneousness concerning the demographic characteristics, the risk factors and the comorbidities of insulted patients, the etiological causes, the diagnostic methods and parameters, the therapeutic measures and the prognosis.

HFpEF is a fascinating clinical entity of debatable area, regarding the arguable nomenclature to the multifaceted pathophysiological mechanisms involved in its pathogenesis, varying from abnormal diastolic relaxation and impaired systolic function but with preserved EF (irrespectively if the left or right ventricle is predominantly affected) (43), which, in spite of the existing enormous wealth of data, remain poorly and incompletely understood (44). In any case HFpEF depicts approximately half of the overall HF entity (45), represents an increasing prevalence among the hospitalized patient with heart failure (46) and, surprisingly, is proven to be as lethal as HFrEF (47) with the same, or even worse prognosis (48-50), comparing to HFrEF.

Over the past decades great effort has been put into understanding the pathophysiology of HFpEF and HFrEF and great strides have been made in this direction. Considering the new knowledge obtained from large studies about the myocardial remodeling, the cardiomyocyte and cardiomyoblast function and the biochemical and hormonal crosstalk pathway between them, an evolutionary paradigm is proposed which enlightens in a better way the pathogenetic mechanism of HFpEF (51).This supports the hypothesis that the development of HFpEF is based on proinflammatory signaling, systematic and cardiac, rather than volume overload. The main idea of this paradigm is that, this pro-inflammatory state, which can, potentially, be caused by many systematic comorbidities coexisting frequently with HFpEF, leads eventually to diastolic left ventricular dysfunction and, consequently to heart failure. The principal mediating points in this process are presumed to be the microvascular endothelial inflammation, which reduces nitric oxide bioavailability and protein kinase G (PKG) activity with the result of cardiomyocyte stiffness and interstitial fibrosis (51). As outlined above, the microvascular inflammation plays a key role to functional and structural alterations, through distinct myocardium stiffness and collagen fibrosis, respectively, which lead inevitably to the development of concentric remodeling and diastolic dysfunction (52), the main abnormality observed in patients with HFpEF (51, 53-55). The endothelial inflammatory state exposes the myocardium to increased collagen in two

ways: firstly, it causes the monocytes migration into the coronary microvasculature. The produced fibrotic factor TGF-β stimulates the conversion of fibroblasts into myofibroblasts (52, 56, 57). And secondly, it also retains the proliferation of fibroblasts and myofibroblasts, through reduction of NO bioavailability (52).

HFpEF and HFrEF represent two distinct disorders in the heart failure spectrum, and as such, should be studied separately. Though overlapped common characteristics (1, 21, 30, 38, 40, 58-60), population-based cohorts point fundamental differences between HFrEF and HFpEF, in terms of the patterns of ventricular remodeling (41) and pathophysiologic perturbations and mechanisms.

The paradigms of HFrEF and HFpEF appear to be significantly different. Toxic, infective and ischemic situations (51) are among the most common etiological factors of HFrEF, whereas the massive loss of cardiomyocytes (57) presents to be the main mechanism involved in the pathogenesis. The bridging factor that binds that process seems to be the oxidative stress (61-63). Apoptosis or necrosis result to an extended loss of cardiomyocytes and a reactive exaggerated fibroblast replacement. The latter occurs as the myocardium attempts to response to the subsequent extreme raised wall stress. The balance between collagen synthesis and degradation favors/ inclines toward the first with the result of excessive fibrosis in the extracellular matrix (64). These alterations are supported from observations of many studies whose compatible results confirm this pathophysiologic pattern of HFrEF. Electron microscopic imaging show lower cardiomyocyte density in HFrEF than in HFpEF (36) and biopsy samples demonstrate the presence of fibrotic tissue in HFrEF (65) comparing to HFpEF (36). These findings confirm the HFrEF paradigm, as a model based on the LV dilatation an eccentric remodeling (64). Although myocardial ischemia is on the one hand, a typical cause of HFrEF due to loss of cardiomyocytes, on the other hand calcium distortions can result in disturbance of ventricle relaxation and fibrotic process to increased stiffness, both of which can lead to diastolic dysfunction (66).

In other words, HFrEF is typically characterized by accelerated cardiomyocyte loss (9) and exaggerated fibrosis (64) with subsequent left ventricular dilatation (11) and reduction of the cardiac output. On the other hand, the paradigm of HFpEF represents an antipodal hypothesis. Increased wall thickness as well as ratio of wall thickness to chamber dimension and greater ventricular mass to volume (concentric remodeling) are observed in HFpEF, whereas chamber dimensions are at least nearly normal, shown by most of the studies (1, 36, 39, 55, 59, 67-71).

As emphasized above, HFrEF and HFpEF differ consistently, considering their pathogenesis. There is, though evidence that the pathological pathways of them cross each other, so that separating them completely would be at least naïve. It is a common truth that an advanced HFrEF is accompanied by systematic and coronary endothelial dysfunction and the inflammation represented by the expression of TNF-α as well as IL-6 (72, 73) is driven by the endothelial dysfunction itself, but not from other cardiac-independent comorbidities, like in HFpEF. The inflammatory reaction in this case is, yet, analogously and directly associated with the severity of heart failure (NYHA Stage and EF) (72, 73). The consequent depression of NO bioavailability due to the disturbed endothelial NO synthase results in diastolic dysfunction, the main disorder in HFpEF (74). Additionally, the concentric pattern of hypertrophy bases, among others, on the increased myocardial pressure overload. This triggers the typical structural and functional myocardial changes, such as cardiomyocyte hypertrophy (36) and interstitial fibrosis (36, 52, 65, 75) as well as stiffness (36, 65, 75, 76), alterations which induce disturbed relaxation and, finally, diastolic dysfunction. By maintenance of overload the hypertrophic cardiomyocytes are driven to apoptosis and loss with automatically progress of the initial concentric hypertrophy to eccentric.

Taking into account the different pathophysiological mechanisms or the different consequence of them, we can propose that HFrEF and HFpEF are two different sides of the same coin. As both parts of the heart failure entity, the conception of them being bound by a common ligand is not to wonder about. This thread could be presumed to be the endothelial dysfunction and the subsequent inflammation, both playing a key role in the pathogenesis of HFrEF and HFpEF.

In summary, the myocyte-loss/dysfunction in case of HFrEF, triggered from oxidative stress (61-63) in most of the cases, driven from acute myocardial infarction, myocarditis or toxic cardiomyopathy (9), occurs as a result of these ischemic, infective or toxic situations and leads to a fibrotic remodeling (eccentric hypertrophy). Whereas the myocyte-loss/dysfunction in HFrEF is the cause of remodeling, in HFpEF the hypertrophic remodeling (concentric hypertrophy) is the effect of the endothelial inflammation derived from other cardiac or commonly non-cardiac comorbidities, responsible for the occurrence of HFpEF.

Worth notice is that, although myocarditis in terms of being an infection can potential cause HFrEF, evidence from a recent study supports parvo-viral myocarditis to results in HFpEF (77). This could be explained by taking into account that the endothelium was insulted from the virus, but not the cardiomyocytes. Not surprisingly, therefore, the coronary inflammation and not the oxidative stress in the cardiomyocytes was the key pathogenic pathway which finally results in HFpEF, in accordance with the paradigm of its pathogenesis.

The observation that HFpEF is highly associated with non-cardiac comorbidities necessitates the deeper insight into the pathogenesis. Only in that way the diagnosis will stop being a diagnosis of exclusion, which is unsatisfactory (43), especially taking into account the large prevalence of HFpEF in total population. As outlined above, data from recent studies shifts the theory of the pathogenesis of HFpEF away from volume overload to the vascular and cardiac pro-inflammation (51). Under this perspective, could be explained the observed presence of systematic comorbidities in case of HFpEF, but not of HFrEF (78). It is recently reported that the inflammatory state produced by comorbidities can have an additional prognostic value as foretelling factors, able to prefigure the occurrence of HFpEF, but not in HFrEF (78). Obviously, the early detection and therapeutic handling of them are of utmost importance in preventing the onset of clinical heart failure (79-81). As mentioned above, the evolutionary novel paradigm of HFpEF indicates a pathophysiological model of HFpEF based on the inflammatory state (51). In principle, production of inflammatory cytokines can induce an oxidative stress state with, consequent, myocardium hypertrophy and stiffness driven by lowered microvasculary and myocardial NO bioavailability resulting in decreased cardiomyocyte PKG activity (51). In these terms it suggests a high prevalence of non-cardiac systematic comorbidities in HFpEF (82) which trigger a pro-inflammatory environment which gives birth to HFpEF.

From numerous population-based studies it has become quite evident that not only there is a relation between coexisting diseases, such as hypertension, diabetes mellitus, metabolic syndrome, and atherosclerotic disease (which also consist “important risk factors for HF” according to ACCF/AHA guidelines (6)), but also they provide the essential biological substrate for HFpEF to be created (11), through the induced structural (6) and functional perturbations, such as impaired cardiac reserve dysfunction (decreased of chronotropic and vasodilator reserves) (68, 83-85). The most important of them are age itself (11) and age-typical pathologies with great emphasis on hypertension which is the highest prevailing (86). Unlike to HFrEF, hypertensive, older women, with a lower incidence of an ischemic coronary event, is the most common clinical profile of patients with HFpEF, derived from observation from multiple studies (6, 11). Surprisingly, the prevalent rate of female sex is reported to appear in HFpEF is almost twice as high the rate of HFrEF (63% versus 38%) (87, 88). Hypertension is already correlated with inflammation in vasculatory and oxidative stress (89). Hypertension was initially presumed to contribute to the incidence of HFpEF through excessive volume afterload (90), which was considered to be the major pathophysiological mechanism of HFpEF. However it is shown that myocardial afterload is nothing more than an unbreakable hypertrophic stimulator, which can act unmolested due to inflammation, induced by hypertension (51). Hypertension, as a major comorbidity observed with high frequency in HFpEF, and typical remodeling changes in HFpEF

are so tightly connected with each other to such an extent that can be supposed that HFpEF is the progress and the clinical expression of asymptomatic hypertensive heart disease and forms a type of “accelerated hypertension” (59, 68, 70, 91).

Apart from age and hypertension, which consist the dominant coexisting comorbidities in HFpEF, overweight/obesity, diabetes mellitus (DM), chronic obstructive pulmonary disease, anemia, chronic kidney disease (51) as well as atrial fibrillation (AF), and in less degree CAD and hyperlipidemia are also commonly observed in HFpEF population according to many cohorts studies (58, 92). The thread that joins these, seemingly completely different diseases, is inflammation (51), upon which is pathophysiologically based the pathogenesis of HFpEF.

Prognosis of HFpEF is demonstrated to be worse than this of comorbidities (93). Moreover, data shows more severe structural and functional impairment in case of HFpEF comparing to hypertension alone (94). This obtained information indicates the hypothesis of an underlying specific HF-related mechanism such as neuroendocrine activation (95) involved in worsening of HFpEF’s outcome.

Taking into account the principal evidence of inflammation being the connecting pathophysiological pathway in the contribution of comorbidities to the pathogenesis of HFpEF, as well as the suspected neuroendocrine activation as part of the pathophysiological process of the development of HFpEF, we can reach the conclusion that a sequence of events, starting from inflammation, which enhances the formation proliferation of myofibroblasts (due to decreased NO bioavailability (96)), can freely lead to the potential pro-fibrotic effect of growth hormones such as RAAS-hormones (97). Renin is assumed to contribute to heart failure indirectly, through causing myocardial infarct, as reported in two epidemiological studies, which observed a correlation between high renin activity and increased incidence of MI (98, 99). Similar was the observation, regarding high renin levels and dilated cardiomyopathy (100).

RAAS System

The renin-angiotensin-aldosterone system (RAAS) is a major hormonal contributor to the hemodynamic and fluid homeostasis, through blood pressure regulation and fluid volume-sodium-potassium balance, respectively (101). Renin, firstly synthetized as a prohormone in the afferent arterioles of renal glomerulus, after undergoing proteolytic changes, it is then released into the plasma circulation, where metabolic mechanisms take place in order to cleave the inactive prohormone pro-renin to the active renin. Then, by acting upon angiotensinogen, (substrate of renin produced in liver), renin produces Angiotensin I. Angiotensin-converting enzyme, produced in lungs, catalyses the cleavage of Angiotensin I to generate the active

Angiotensin II the main hormone of RAAS, which effects by binding to the type 1 Angiotensin II receptor (AT1R) (102). Whereas Angiotensin II is a powerful hormone concerning the regulation of cardiovascular system in human body, through vasoconstriction, as well as pro-fibrotic and pro-hypertrophic actions (as it stimulates fibroblast proliferation and collagen synthesis) (103), aldosterone is a mineralocorticoid hormone which acts as an important mediator of the effects of RAAS system. Initially its production was found to take place in the adrenal cortex, after stimulated by angiotensin II, potassium and, less strong, by adrenocorticotropic hormone (ACTH) and sodium (104). In particular, the production of aldosterone, sensitive to high potassium or low sodium (105), acts on the epithelial sodium channel in nephron causing, direct, Na-absorption and, indirect, K-excretion, resulting to intravascular volume expansion and blood pressure raise (106).

Much effort and research work has been devoted to establish the dogmatic knowledge about the function of RAAS. Hence, at this point in order to conceive the progress of understanding the RAAS system, and particularly its actions on heart tissue, a short historical reference would be worth mentioned.

The first step was made almost twenty years ago when Swedberg et al. associated aldosterone direct with mortality in patients with heart failure (107) and Rossi et al. indirect with left ventricular mass (108), a strong predictor of mortality. These observations firstly pointed out the function of aldosterone, regarding its distinct effects in heart and its role in heart failure. Although too soon to be applied in clinical praxis, these data could allowed the reconsideration of cardiac fibrosis as important factor of its pathophysiology (109).

At this time, the knowledge that MR are significantly expressed also in the heart (110, 111) and blood vessels (112) was obtained, by immune-histochemical and biochemical evidence, which showed high affinity aldosterone binding sites in the rat heart. This could furnish elucidation to the demonstration that aldosterone is a hormone with impact in the heart tissue (109). The results of two more prominent studies confirm and enhance the crucial role of aldosterone in the development of heart failure, whose unpredictably positive outcomes made a huge step regarding the establishment of MRA as a therapeutic choice in heart failure. In particular, first to investigate the beneficial outcome or not from including spironolactone, the only approved MRA until this time, as part of the standard medical therapy of heart failure was the Randomized Aldactone Evaluation Study (RALES). A considerable sample 1163 patients with heart failure with clinical severity of NYHA III-IV classes (113) was examined under the administration of MRA. The results appeared to be so surprisingly positive, that the trial came prematurely to end, as the proposed primary end point (decrease in mortality) was at an early stage achieved.

Namely, not only the global all-cause mortality, but also the cardiovascular death and the hospitalizations due to heart failure demonstrated a remarkable reduction of more or equal to 30% in patients treated with spironolactone. Results of another trial, the Eplerenone Post-Acute Myocardial Infraction Heart Failure and Survival Study (EPHESUS) (114), comparing 6642 patients with heart failure treated with MRA, came in compliance with RALES (114). The manifested positive results of MRA, in terms of the risk of sudden death and death from cardiac and all causes, were also reported in a post hoc analysis reflecting an even more beneficial effect of this treatment regarding above mentions parameters up to 37%, 32%, 31%, respectively, 30 days after the induction of eplerenone therapy (115).

In the period when the relation of aldosterone and heart failure was revealed, evidence about the underlying mechanisms was brought to light. More than twenty years ago, Weber et al. found cardiac hypertrophy and fibrosis in uninephrectomized rats' hearts under simultaneous administration of aldosterone and NaCl (116, 117). Brilla et al. and Robert et al. witnessed the dependence of chronic aldosterone and NaCl effect on the cardiac fibrosis in both ventricles (118, 119). Both aldosterone and angiotensin II stimulate fibroblasts to synthetize collagen leading to cardiac fibrosis. Collagen formation induced in the presence of both hormones was increased, and the higher the level of hormone the more was the produced tissue collagen (120).

Having been extensively documented the detrimental functional and structural consequences of RAAS activation in heart; it has become of crucial importance the biological mechanisms that hide behind this association. Searching further the hypertrophic and fibrotic role of the major RAAS components, angiotensin II and aldosterone, has been shown, that these main hormones are closely bound to each other. Angiotensin II is among others an important stimulator of aldosterone production (via ''AT1R-up-regulation of cytochrome P450 oxidase B2 and increased level of hydrogen peroxide'' (121), whereas aldosterone consists an up-regulator of angiotensin II receptor, increasing the number of AT1R (122, 123) and mediating the angiotensin II cardiac hypertrophic effects (124). This evidence demonstrates a cross-talk between angiotensin II and aldosterone. Additional to their depending relation as far as the stimulation and regulation are concerned, it seems that angiotensin II and aldosterone exert their actions synergistically, at least respecting their vascular fibrotic effects. Examined rats presented an accelerated rhythmus of fibrosis under the concentration of both hormones, rather than of aldosterone alone (125).

Angiotensins II as well as aldosterone exert their action by binding with their receptors. Studies have shown the presence of these receptors in the heart and the vasculature. The biological event of hormone-receptor coupling appears to take place in the conduction system of heart

(sinoatrial and atrioventricular nodes) (126) in case of angiotensin II, while aldosterone receptors are sited in cardiac tissue (cardiomyocytes, endothelial cells and fibroblasts) (110). This data proves the crucial role of hormonal system in cardiac function and potentially dysfunction. Having obtained evidence concerning the localization of RAAS receptors intra-cardiac and the contribution of this hormonal system in the development of fibrotic process in heart, it would be interesting to combine this information by showing the expression of receptors in fibrotic tissue itself. Indeed, this demonstration came from Sun and Weber (123), who observed the anatomical existence of angiotensin II- and aldosterone receptors in rat cardiac sites, where fibrosis was developed after applying the hormones respectively.

The hypertrophic or apoptoticresponse of cardiomyocytes II and the consequent fibrotic replacement accompanies myocyte loss is considered to depend upon the intracellular produced angiotensin II and toxic environment caused by elevated angiotensin II concentration respectively (127). In 2002, Sun et al. (128) suggested that the perivascular fibrosis possesses a key role in the progress of cardiac fibrosis as one of the initial occurring events and emphasizing the importance of coronary fibrosis, which is related with intra-cardiac inflammation. Recently, in harmony with the already accepted correlation between fibrosis and inflammation in coronary microvascular vessels or in myocardium, Usher and colleagues (129) confirmed this theory by reporting the mediating role of macrophage polarization between MR activation and development of cardiac remodeling, reflecting a cause-to-result event sequence.

Tumor necrosis factor alpha (TNF) plays a central role in inflammation by modulating the whole cascade via promoting oxidative stress and controlling cross-talks between inflammatory cells participating in the process (130). Its hemodynamic impact concerns the vasoconstriction due to the induced NO vasoconstriction and hypertrophy of vascular neo-intima (131). C-reactive protein (CRP), typical for the acute-phase response, hinders the proper endothelial function and triggers the overexpression of AT1R (132, 133). Angiotensin II is shown to stimulate VCAM-1 through oxidative stress (134). The suppression of VCAM-1 after spironolactone administration (135) implicates that aldosterone might be involved too. Moreover IL-6 is a key cytokine in the balance of proliferation and apoptosis (102). Briefly the importance of inflammation regarding the pathophysiology of cardiac diseases is reviewed by Masiha et al. who demonstrated the role of inflammatory markers in left ventricle hypertrophy (LVH) and diastolic dysfunction (136). Results provided by Brilla et al. managed to support the idea of aldosterone affecting the vasculature, by showing that spironolactone administration in rats under aldosterone infusion inhibited the fibrotic process (117, 118). Vascular injury and in turn dysfunction seems to activate the infiltration of inflammatory cells endothelial, causing alteration of the vascular wall structure in a

cellular level, which inevitably leads to the incidence of cardiovascular disorders. Mainly through this process occurs atherosclerosis. It can be imposed that there is a vicious cycle concerning the involvement of Angiotensin II in the inflammatory cascade. In presence of vascular injury, angiotensin II stimulates leukocytes migration, which in turn upregulates more the production of angiotensin II preserving in that way the inflammatory environment in cardiac microcirculation (101). This mechanism, which causes the endothelium dysfunction, plays evidentially a pathogenic and bridging role between hypertension and atherosclerosis (137, 138) and consequently HFrEF.

Likewise to angiotensin II, similar are the effects of aldosterone concerning tissue fibrosis and vascular remodeling via inflammation and oxidative stress (139) interfering in the pathogenesis of atherosclerosis (140), as mentioned already above. More supporting data derives from S. J. Sherajee et al. (141), who reported an elimination of reactive oxygen species (ROS) after administration of a mineralocorticoid receptor antagonist, spironolactone, in rats ‘’fed’’ with aldosterone. State of chronic hyperaldosteronism via secondary hyperparathyroidism, resulting from electrolytic balance distortions (hypocalcemia and hypomagnesemia), increase the calcium level in cells. This overload triggers oxidative stress, loss of cardiomyocytes and fibrotic myocardial response (142, 143). It has been assumed that increased intra-cardiac calcium concentration is tight connected with inflammation and an important contributor in myocardial and vascular remodeling (144, 145)

Angiotensin II is a hormone with pleiotropic actions. It can induce oxidative stress by enhancing NADH/NADPH pathway, generating anions of superoxide and lowering NO expression (146, 147). Moreover, elevation of blood pressure such as inflammatory state originated from angiotensin II seem to be the major stimulating factors of the fibrotic process in myocardium (148) and angiotensin II promoted oxidative stress, among others, drives to cardiac hypertrophy (149). Similar is the role of aldosterone in architecture of tissue and vasculature. As a conclusion, the chief RAAS hormones and the dominant inflammatory cytokine, TNF, are the major regulators of multiple enzymatic and molecular cross-talks and biological pathways, which synergistically account for numerous cardiovascular disorders (102).

Brilla et al. and Sun et al. emphasized the fundamental NaCl contribution in the fibrotic effect of aldosterone (118, 150) and furthermore, Gekle and Grossmann (151) suggested that aldosterone although must essentially be a participant, a second trigger is also pre-required in order to exert its fibrotic actions, such as NaCl, oxidative stress, angiotensin II etc. On the other hand Garnier et al. and Ouvrard-Pascaud et al. presents an arguing finding, assuming that aldosterone alone locally expressed can stimulate the fibrotic process (152, 153).

Another fascinating outcome can be concluded, in respect of the indication and profit of applying MRA in heart failure irrespectively the aldosterone level. Taking into account the normal sodium and aldosterone expression in RALES and EPHESUS raises the question about the origin of MR stimulation as we can imply that MR stimulation is not only from aldosterone mediated (113, 114). In spite of this observation being encouraging, concerning the potentially beneficial usage of MRA as new therapeutic agents, the exact pathophysiological character of the MR is still not completely known and need therefore further research (109).

Summarizing, angiotensin II is a hallmark hormone with inflammatory, fibrotic and hypertrophic actions. Inflammation and oxidative stress both induced and maintained by angiotensin II appear to be the principal pathophysiological pathways involved in cardiovascular diseases. Particularly, is considered to being a marker of evaluating the cardiovascular risk (154) and IL-6 is involved in the pathogenesis of, cardiac or not, disorders, such as hypertension and diabetes (155) whereas recruitment and adhesion of inflammatory cells and molecules by angiotensin II can potential facilitate the endothelial disorganization and dysfunction with direct clinical consequences (138).

This data presents RAAS actions in cardiovascular system to have a potential double role with both repairing and deleterious effects: on the one side it serves as a compensatory mechanism in case of myocardial or vascular injury to preserve left ventricular function and as a maladaptive mechanism when it promotes fibrosis or hypertrophy without an underlying pathological etiology on the other (156). This place could be assumed to be the RAS system has a double role: neuroendocrine activation occurs as a response to pathological events undertaking a saving role or when prolonged overexpressed or inappropriate activated leads to harmful effects leading to more severe cardiac dysfunction, preserving in that way a vicious cycle.

According to principal knowledge, ACE is the major enzyme involved in angiotensin II generation. Further investigation has elucidated new aspects of RAAS system, which is produced locally in tissues and through alternative biological pathways, mediated by serin-proteinases such us chymase (157). Despite to what was, until then, concretely believed, regarding the significant role of ACE in Ang II formation, newly published results upset the whole concept. Evolutionary data support the theory of chymase, being the dominant contributor of angiotensin II generation in human. There is plenty of data provided by numerous studies, which even suggested that angiotensin II cardiac tissue generation is based principally on chymase-pathway instead of ACE Particularly, Urata et al. first represented quantitatively this evidence, claiming that 80% of the generated angiotenisn II in human ventricles is produced upon chymase, whereas only 10% of the locally synthetized hormone derives from ACE metabolic

enzyme (158-161).Persisting amount of active angiotensin II intra-cardiac as well as in circulation, despite sufficient doses of ACE-inhibitors, as medical treatment of heart failure, could be satisfyingly explained by the scenario, accounting for an alternative non-ACE dependent enzymatic pathway (162-164).

Concerning the formation of angiotensin II from angiotensin there are arguing statements about the mediated enzyme. Previous studies show ACE as the main contributor, whereas others, chymase instead (165-167). A middle ground solution to this conflicting issue came from Fleming, who suggested that chymase is the major enzyme in the tissue angiotensin II metabolism, while ACE is more responsible for the circulatory formation of angiotensin II from angiotensin I (168). In other words ACE is the metabolic ‘’chief’’ in circulation (169) and chymase in cardiac tissue (167, 170).

Furthermore, in order to form a firm statement about the significant role of chymase, local production of angiotensin II was searched deeper in a cellular level. Administering medication with ACE inhibitor, intracellular and extracellular measured concentrations of angiotensin II in the medium of vascular smooth muscle cells, resulted in very disconcerted results , which upset the oversimplified conception of predominant role of chymase; ACE inhibitor managed to suppress angiotensin II by 90% when glucose was normal, whereas in the presence of high levels of glucose only an Ang II formation decrease by 19% was shown (171). The observed divergences led us to assume that, unlike generation of plasma angiotensin II from renin and ACE, local production in cardiac tissue devotes other sources of enzymes-substrates for its formation sequence, according to the stimulating factor in each case (172).

Given the complexity of RAAS system, discrepant study outcomes are relative often. Due to the great variety of candidate components, involved biological and enzymatic mechanisms and target tissues, which can be affected by different stimuli and cause diverse cardiac disorders, concrete demonstrations should be handled with skepticism in order to avoid inaccuracies.

Renin is synthetized in the kidney, the liver is the main producer of angiotensinogen (AGT) and the pulmonary endothelium is rich in angiotensin-converting enzyme (ACE) (173). According to „classical” view, RAS system is a well coordinate circulating system with multiple effects on many tissue targets, whose production depends on different body organs and requires multiple active bio-enzymes. Under this aspect of this concrete knowledge about RAS system it has become widely acceptable that it constituted an endocrine hormonal system.

The main question, that has to be answered, is if all the components of RAS or only isolated hormones are expressed in heart tissue. In other words, if a local RAS system exists in heart. A

summary from the most important findings regarding the local production, or uptake of RAS hormones (pro-renin, renin, angiotensinogen, angiotensin-converting enzyme, angiotensin I and angiotensin II*)* from circulating plasma in cardiac cells as well as in hearts which have been isolated from animals, or even humans, would contribute to elucidate this mystery (174).

However, there are studies, whose data argues with this common belief, suggesting a locally produced, intra-cardiac RAS. Moreover, a recent study puts more light into the evolutionary knowledge or presumption of existing two major RAS systems, a circulating and a local one, suggesting a third category, the intra-cardiac intracellular RAS (173), meaning synthesis and activation of RAS completely in cardiac cells (175).

Given the already described intracellular RAS, the examination of presence or not and in first case, expression of which of the components of RAS consist a point of major significance. Regarding the ability of cardiomyocytes and endothelium of ''binding'' renin the supplementary site of its existence could be presumed to be the cardiac or endothelial cells' membrane (176, 177). Supporting this hypothesis appears to be the reported increase of angiotensin II in rat cardiac tissue (by genetic overexpression of human angiotensinogen) after the renin infusion had been interrupted and washed out (178).

According to data from several studies, the presence of angiotensin I and angiotensin II in heart is widely accepted (179-182). It is demonstrated by Müller D.N. et al. (178) that the local expression of angiotensin is mostly based upon ''tissue bound'' renin. Moreover, it is calculated that angiotensin II tissue concentrations (measured in grams) often exceed those in plasma circulation (measured in ml of plasma) up to 4-5 times (182). This evidence and in accordance to data suggesting minimal generation of angiotensin in the vascular tissue of coronaries (174, 182) as well as to the above mentioned almost exclusive expression of renin and angiotensinogen extracellular, indicates the surprising conclusion of angiotensin I local tissue synthesis of more than 90% and of angiotensin II over 75%, comparing to the amount of derived hormones from circulation. More interestingly, locally released angiotensin I, and not up-taken from circulation angiotensin I, consists also the main reservoir of the in-situ production of angiotensin II, as it is demonstrated (174, 182).

Angiotensin II seems to have a more global endogene endocardiac presence as it can be produced intacellurarly (173, 175), as described previously, or even to be able of endocytosis after being generated extracellular through binding with its AT1 receptor (183-185).

Data from exploring the angiotensin II tissue and intravascular levels in an isolated rat heart model and the effect of losartan, AT1 receptor antagonist, appears to amplify the idea of

intracellular angiotensin II production, hypothesis which extensively was described by Kumar et al. (173). Creating in that way the appropriate conditions in order to investigate the influence of losartan concerning the differently originated and located angiotensin II, the following interesting findings were observed: Whereas the extracellular angiotensin II and the locally generated angiotensin II during renin/angiotensinogen infusion were not affected by losartan, there was reported a major difference concerning the tissue angiotensin II following the angiotensin II perfusion. The tissue angiotensin II concentration, achieved after angiotensin II infusion appeared a distinct decrease, if not vanished almost completely, after the losartan administration. Summing up, it is obvious that losartan, as an AT1 receptor antagonist, can be applied where a procedure including the AT1 receptor takes place, implying that ‘’arterially delivered angiotensin II binds with its receptor at cardiac tissue’’ (186).

As mentioned previously, renin and angiotensinogen do not present in healthy hearts. However, a variety of several pathological situations can act as stimulators of intra-cardiac production of RAS (174). Two typical examples of such pathologies, that can potentially increase the local release of angiotensin II and overexpress ACE mRNA, are myocardial infraction and heart failure (187, 188).

In case of an acute myocardial infraction, two main pathophysiological reactions occur, serving two fundamental different purposes: on the one hand the preservation of the heart contractility and function after cardiomyocyte loss and on the other, the improvement of the rest myocardium’s behavior. In these terms the first one is the myocardial repair and concerns the insulted, from ischemia, area and the other one is the structural remodeling, through cardiac hypertrophy and fibrosis (189-191), of the non-infracted part of cardiac tissue (192).

A huge body of evidence has proved the existence of a local RAS intra-cardiac, whose release, or not, varies depending on the stimulating factor and the pathological or normal circumstances. While the vital role of local RAS in concern of the pathology of various heart diseases is established, there is still parameters of the same issue that remain puzzling, such as the possible interactions of circulating and endogenous RAS. According to Heller et al., investigating levels of renin intra-cardiac and in plasma in existing pressure-induced cardiac hypertrophy reported a clear positive correlation between them (100, 193). In spite of not existing concrete data relatively to angiotensinogen behavior, the low level of its concentration measured in heart failure could indicate its extensive consumption by increased renin (100). However examining RAS components levels after MI, early after its occurrence (<4 weeks), they appear to be high with rare contaminant increase in circulation (194), which could imply an independent relation of repairing RAS, locally produced, to plasma activation, at least in case of MI at early stage (192).

To sum up, angiotensin is proved to be mostly locally produced and plasma renin and angiotensinogen sequestered from circulation are the needed mediators for the endo-cardiac release of angiotensin (174, 180, 182). Regardless from the origin of the components of RAS we can presume that a local, functionally active, RAS system do exist in heart (175).

These new data proving the paracrine and autocrine role of RAS, apart from its endocrine action, (173) created the need of looking intensively into this still enigmatic, in many aspects, hormonal system. Taking into account the vital actions of RAS in cardiac function, after neuroendocrine response or dysfunction, when inappropriate activated, it is of crucial importance the complete understanding regarding the exact actions, the sites of its production, the stimulating and regulating factors and the degree of influence according to different circumstances. Under this aspect, much attention has been given to the circulating versus local RAS tissue, including a more particular, recently described, intracellular expression of RAS (173). Much effort has been put into clarifying debatable issues relatively the significant role of local compering to circulating RAS and the possible interactions between them without being completely understood so far.

Many studies have been conducted with the purpose of illuminate the issue. Nevertheless, despite the supporting results, as far the existence of a local active RAS system is concerned, still discrepancies are documented, leading to uncertainty of the validation of these findings. In order to find out the origin of RAS components (uptake from circulation versus local release in heart) measurements have to be done in cardiac tissue free from plasma affection. However, from practical view, culturing cells is possible only under serum-like biological environment. In these terms, RAS components can still be present even in cardiac cells cultured in ‘’plasma-free’’ medium, due to their being influenced from plasma previously. In other words, it is tricky to reassure the complete separation of plasma from tissue, so that it becomes very complicated for research outcomes to be exactly defined. This is the main reason explaining the difficulties regarding the interpretation of study results and evaluation of new obtained knowledge (174).

After first identifying and cloning RAAS genes, (genes of angiotensinogen, ACE and angiotensin receptors), many studies followed with the purpose to investigate the clinical relevance of RAAS polymorphisms in regard of cardiac disorders. Despite many studies having looked into an exciting new research area, two main major questions, concerning, firstly, the existence, or not, of a strong correlation between genetic variants and cardiac disorders and, secondly, their use as risk factors or predictors of cardiac dysfunctions, remain rhetorical (195).

Binding with its receptor, angiotensin becomes capable of applying its effects; therefore, antagonism of this binding is evident to be a major therapeutic target. Respecting the two types of receptors, AT1 and AT2, genetic studies have mostly focused on A1166C and G1675A polymorphism of them, respectively. These alleles have been recently associated with coronary disease, ischemic stroke as well as heart failure and left ventricular hypertrophy (196). Indeed, Wu CK et al. conducted a study with 666 enrolled patients, from whom 285 were echocardiographically diagnosed with DHF. He found that AT1R 1166 CC genotype was related to higher prevalence of DHF (197), supporting the results of other studies which suggest a link between A1166CC polymorphism and heart failure. This knowledge, in combination with the varying of the responsiveness to pharmacologic medication in presence of G1675 allele (196), could be used as useful tool in handling the treatment of different cardiac diseases.

Concerning angiotensinogen gene, previous studies have shown a link between angiotensinogen polymorphisms, and various cardiac diseases. For instance T174M genetic polymorphism of angiotensinogen gene is connected with clinical expression of heart failure (198). Moreover, SHF incidence was assumed to be genetic influenced by G-6A and M235T polymorphisms (199, 200). Supplementary to these results, Chang SN et al. examining different angiotensinogen gene polymorphisms in 509 patients , 123 from them with documented SHF (LVEF < 45%), could demonstrate that T174M CC and G-152A GG homozygotes were correlated with a higher risk of occurrence of SHF, so that T174M and G-152A were appeared as a clinically relevant prognostic factor (201). Presumably, RAAS system's activation when upregulated by specific polymorphisms in particular angiotensinogen gene sites (''coding or promoter regions'') may interfere to the development of SHF (201), hypothesis whose confirmation demands further investigation.

Excessively investigated is the ACE gene. The ACE trait is already demonstrated to influence the ACE expression especially the I/D polymorphic site (195). Genetic work by Rigat B.et al. (202) described the first recognised polymorphism as insertion (I) or deletion (D) of a 287 DNA-pair sequence base, where the existence of I- and D-allele was based on. Trying to conceive the possible clinical significance of its high prevalence, given the fact that the degree of ACE expression and tissue activation may be strongly positive related to ACE polymorphisms many studies have investigated an association of different genotypes of ACE gene and occurrence of clinical disorders. Taking as sample the population who suffer from ischemic heart disease, Cambien et al. (203) was the first investigator, who researched the ACE polymorphisms and cardiac infraction relation, assuming even an existing correlation between ACE genotype and risk estimation. Opposite to these results too, de-emphasizing a possible genetic relation to

cardiac infraction, Ruiz et al. examining patients with diabetes mellitus and D-genotype, reported an important incidence of atherosclerosis when D-allele coexisted, suggesting that genetically-specific individuals are disposed to other cardiovascular risk factors in a way, which could affects tremendous the disease presentation and development (204).

Given that ACE genotypes interfere with ischemic heart disease remained only a hypothesis, a possible affection of ischemic cardiomyopathy by ACE gene could answer this challenging question. In these terms Raynolds et al. (205) was the first to conduct a relative investigation, which resulted to an interesting observation: DD genotype appearance was increased not only in subjects with ischemic cardiomyopathy (39,2%), but also in patients with idiopathic dilated cardiomyopathy group (35,7%).

Contradictory results were reported by Andersson & Sylven (206), suggesting no association between prevalence of DD genotype and idiopathic congestive heart failure. Surprising, nevertheless, were the, significantly, increased 5-year rates of death in patients presenting the DD genotype comparing to individuals with ID/II genotypes (49 versus 72%). This finding, by itself, denotes clinical impact of homozygote D-allele on the expression of cardiac disorders and prediction of mortality (odds ratio 1.69).

Analyzing further the clinical impact of genetically determined ACE expression on cardiac disorders, an association with hypertension was also investigated. The results noted by Zee et al. (207) and the Framingham Study, demonstrating a positive correlation between ACE gene I/D polymorphism and hypertension (especially in males, according to the Framingham Study) (208).

Evident is the knowledge that hypertension, among others, consists a main factor of left ventricular hypertrophy. With this regard, extending the research, a relation between ACE gene I/D polymorphism and ventricular hypertrophy was investigated. Decisively contributed first with his study Schunkert et al. (209), who defined ventricular hypertrophy with electrocardiographic criteria. Hypertrophy was demonstrated to be positively correlated with D-allele presence. Besides, evidence of D-allele being a predictor factor of hypertrophy was addressed only in male population, and surprisingly, even in normotensive subjects. The most exciting finding was that the more normal were the blood pressure, the more robust appeared to be the association with D-allele (209) .

Additionally, Schunkert's et al. findings concerning the relation of D-allele with ventricular hypertrophy were compatible with those of the Framingham Study regarding D-allele and hypertension. This could imply that hypertension cause primarily ventricular hypertrophy in the

presence of D-allele. However, conflicting to this theory arises to be the observation of D-allele being strongly related with normotensive individuals, not with the hypertensive ones. No concrete clarification, sufficient to elucidate these results, was given. As mentioned above, DD-genotype may enhance the ACE expression and consequently the angiotensin II generation. Angiotensin II, acting as a potential hypertrophic factor in the myocardium, could lead to ventricular hypertrophy. This could interpret the link between D- allele and left ventricular hypertrophy (210, 211). This hypothesis seems to be supported by data showing that degree of hypertension is not always linearly related to the severity of hypertrophy assuming the contributory pathophysiological role of ‘’non-hemodynamic factors’’ and genetic background mechanisms (212). Indeed, overexpressed ACE gene mRNA is documented in pressure overloaded cardiac tissue (213).

The great genetic variability of RAS components complicates more the, already, multifactorial pathophysiology of cardiac disorders. The obtained knowledge of the genetic variants that could potentially be involved in the clinical outburst of cardiac dysfunctions and the observed range of benefit regarding the medical treatment remains insufficiently understood. Despite genetic polymorphisms are much investigated, the impact of genetic variable expression of RAAS genes to an increased incidence of cardiac events remains confusing The fact that despite much research work has devoted to genetics of RAS, the results have barely contributed to development of new therapeutic aspects of heart diseases. This unfortunate truth leads to the need in further molecular, individualized, investigation.

Although HF is a clinical syndrome which enjoys wide recognition due to its growing prevalence globally, this heart disease in female population remains incomplete explainable and needs further elucidation. An enigma is the fact that despite women present the clinical symptoms of heart failure, just like men, the mortality rates favor them more than men. (214).The presumption that sex-specific characteristics can determine RAAS hormones, and consequently the differences in the heart disease prevalence between the two sexes, is supported by investigations of the male mRen2.Lewis rat. It has been observed that angiotensin II, angiotensinogen, renin and ACE levels were much more increased in female rats (215). Nevertheless, after menopause the difference degrades as women have similar levels of ACE (216-218). On the other side, hormonal replacement therapy with estrogens, usually administrated postmenopausal, seems to lower ACE in these women (219, 220).

Another interesting finding derived from Framingham Heart Study, a community-based study. Vasan et al. (221) examined via echocardiography, healthy population, regarding ischemic cardiovascular events and heart failure and managed to relate positively aldosterone serum level

with structural alteration of left ventricle (concentric hypertrophy) and even HFpEF only in female subjects. Moreover mounting clinical data reveals the crucial contribution of RAAS in diastolic disorder, presented by the absence of estrogens (222). In these terms, synthesis of all RAAS components is controlled by estrogens (223-226). This implication of female heart being more sensitive to the aldosterone effects is supported from the knowledge that progesterone, estrogen and aldosterone receptors are all identified to be located in the arterial wall (227, 228), coexisted estrogens and aldosterone receptors in fibroblasts and cardiomyocytes (229) and the reported intolerance of spironolactone, a mineralocorticoid receptor, as a result of gynecomastia and abnormalities of menstrual cycle women in pre-menopause ("androgenic and progesteronergic properties") (230).

According to these observations, although it is widely evidenced, that disturbance of sodium and hemodynamic balance with decrease of blood pressure, are the main stimuli of RAAS activation, RAAS regulation seems to depend also upon other hormonal-to-hormonal interactions, such as the ovarian hormones. Ovarian hormones, estrogen and progesterone fluctuate during the menstrual cycle, which could explain variability between female population and between two sexes depending on the menstrual phase of woman (231). In this regard, pregnancy with increase of estrogen, oral contraceptive pills as well as hormonal replacement therapy can certainly influence the hormonal environment having also a contributory role in affecting interactions of ovarian hormones with other endogenous hormonal systems.

Since decades has been investigated the possible interaction of female hormones in the modulation of RAAS system.. An explanation for this "phenomenon" might be the way of contraceptives intake; in oral administration of contraceptive medication follows the hepatic metabolism of estradiol. Liver is also the source of plasma angiotensinogen and therefore is believed that only oral contraceptives could result in rise of angiotensin II and aldosterone (232).

Generally estrogens antagonize the RAAS system by suppressing its formation and activation (233). ACE levels, while higher in men in young population (234), are similar in among postmenopausal women and men (216-218). Under hormonal replacement therapy with estrogens, the ACE action was lowered (216, 219), angiotensin II formation was weakened via blocking the chymase pathway blocking by estrogens (233) and aldosterone levels were reduced (235).. Concomitant to this data and under the scope of estrogen influencing RAAS was put forth the hypothesis that estrogen hinder the ACE/ angiotensin II/AT1 or AT2R, which is the responsible RAAS "arm" for inducting hypertension and diastolic dysfunction (236) and enhance the cardio-protective "arm" of ACE2/ angiotensin (1,7)/Mas receptor (237).Antithetically to

estrogens, it is imposed that high progesterone is the initial stimulator for the subsequent aldosterone expression (238, 239).

To put it in a nutshell, although estrogens and progesterone are principally reproductive hormones, they also seem to exert other actions interacting with different hormonal systems affecting among others the cardiovascular system. Examining deeper the already established knowledge that menopause with the tremendous depression of estrogen consists a major cardiovascular risk factor for women, as postmenopausal increased insulin resistance (240, 241)

Summing up the pathophysiological mechanisms involved in the regulation of RAAS by ovarian hormones we can conclude to the following crosstalk signaling: in case of estrogens, they increase angiotensinogen production but the consequent rise of angiotensin II formation acts as part of a negative feedback loop discouraging its further production resulting to a global inhibition of RAAS.

Cardiovascular risk factors

Diabetes mellitus

Diabetes mellitus is a chronic disease with prevalence with epidemic propensity considering the enormous number of 346 million affected individuals globally, according to the World Health Organization (WHO) (242). The fast rate of increasing prevalence is reflected by the astonishing evaluation of the International Diabetes Federation's global that 52 million of 2nd to 7th decade of age suffer from diabetes in 2011 and more than 64 million people will present diabetes by the year 2030 in Europe (243).

Findings from epidemiological studies resulted to the observation that, in the presence of diabetes, the risk of HF, as well as of cardiovascular diseases in general, comparing to healthy population (244). Diabetics are probably exposed independently to an elevated hazard of HF, as reported by the Framingham study, United Kingdom Prospective Diabetic Study and Euro Heart Failure Survey. A nationwide case-control study supported this presumption demonstrating that from a population with idiopathic dilated cardiomyopathy without any identified etiology coexisted with diabetes in a population up to 75% (245). Furthermore, 4 out of 10 diabetic, normotensive people present diastolic dysfunction (246) and the possibility of occurrence of HF is 2 to 5 times higher than in euglycemic people (247). One good explanation could be given from the RAAS involvement (248) in the mechanism which results, under circumstances regulated by individual

factors, in insulin resistance (249). This is implied from clinical studies whose evidence demonstrated that RAAS blockade prohibits insulin resistance (250, 251).

It seems that cardiovascular diseases bears the brunt of diabetic complications as they consist the main cause of death in a percent greater than 50%, whereas other DM-induced complications do not exceed the rate of 15% (252). Analyzing and interpreting 29 clinical studies with more than 1000 enrolled subjects, a meta-analyses pointed CVD and proteinuria as two parameters with dangerous potential in diabetic population (253).

The assumption that diabetes is related with the pathogenesis of heart dysfunction was expressed early and this association was described under the name of diabetic cardiomyopathy in 1972 (254). Large epidemiological studies such us Framingham study (247) and provided later data which confirmed and fortified the idea of the interference of DM in the pathogenesis of HF. Established is the knowledge of existing a vicious cycle between DM and HF: each of them consist the cause and concomitantly the result of one another (255-258). After published evidence of the distinct correlation between diabetes and several-fold increased mortality rates due to CVD (253, 259), it can easily be conceived the meaning of the statement that '' heart failure and diabetes is a deadly intersection'' commented in the European Association for the Study of Diabetes (EASD) symposium 2013. As it has been demonstrated repeatedly that DM and HF are inextricably tied with each other, the next step would be to define exactly this association and study it deeper at a pathophysiological level. The initial definition of this incompletely understood clinical entity was shifted from the remodeling of coronary microvasculature (254, 260) and promoted fibrotic process in cardiac tissue (260) towards an updated concept of DCM being'' a clinical condition diagnosed when ventricular dysfunction occurs in the absence of coronary atherosclerosis and hypertension'' according to the most updated ACCF/AHA and the ESC/EASD guidelines (261).

The clinical state of diabetes mellitus cardiomyopathy constitutes a very confusing clinical state concerning the vagueness of diagnosis and poor awareness of pathology. The challenge of the unknown nature of DMC and the detrimental impact of DM on the HF development has risen the scientific interest, which is depicted by the numerous clinical studies (262). DMC is presented as heart failure syndrome with an extended clinical spectrum (263). Despite being initially considered as an almost identical type of HFrEF, it has been shown that HFrEF is nothing else but the late stage of the disease following the diastolic dysfunction, on which HFpEF is pathophysiologically based (264-266). In this respect, a sequence of events starts from alteration of diabetic cardiac tissue with cardiomyocyte hypertrophy and interstitial fibrosis (245)

contributing to disturbance of diastolic relaxation and finally resulting to systolic dysfunction with contractile disorders (245, 267).

Hollenberg et al. indicated contrasting results, namely that in diabetics aldosterone level is increased (268). Plasma renin level seemed to be higher in presence of hyperglycemia, whereas serum aldosterone concentration remained unchanged comparing to normoglycemia (269).

Arterial hypertension

About 20-55% (more than 1 out of 4) suffers from hypertension, after age and sex- assessment, in a global scale, according to large trials conducted in Europe and Canada (270, 271). A worldwide data analysis reveals the gigantic number of hypertension prevalence in 2000: 972 million people or 26 % (272) whereas 8 years later the percentage of diagnosis of high blood pressure reaches the unconceivable 40% of adult population, which is interpreted into 1 billion people (273).
Cardiovascular diseases are the main death cause worldwide, responsible for more than 17.5 million deaths in 2012 (274), while hypertension or its complications are involved in almost the half of them (275). The importance of blood pressure control in reducing CVD risk becomes obvious by looking the recommendation of ‘’The European Guidelines on cardiovascular disease prevention in clinical practice 2012 (ESC 2012)’’, according to which, giving up smoking, following a healthy diet, maintaining physical active, restriction of alcohol consumption, losing body weight, controlling blood pressure (BP) and lipids level would be an effective change of lifestyle (276).

Considering the occurrence of hypertension among women and men under and over the 45 year of age, it is observed a clear difference. Among the first group, the prevalence of hypertension is sharply higher in men, whereas among more aged people (over 55 years old) this difference tends to flatten (277). It is when women enter menopause, a situation of progressive permanent loss of estrogens that they simultaneously begin to lose their cardiovascular protection and undergo the same hazard comparing to men of same age. In particular, 10 years after menopause hypertension appears with prevalence twice as frequent among women (278) and 3 women out of 4 women suffer hypertension post-menopausal (277). Among others hypertension is the major comorbidity predisposing for diastolic dysfunction and among women in post-menopause attributes the most in the occurrence of diastolic heart failure (279, 280) through LV hypertrophy which, in turn, disturbs the myocardial relaxation.

Since HF affects about 2,5 million women in the United States (281) and consists the cause of death in almost the one third of them (282) deserves a separate discussion in order to understand the sex-specific pathophysiological mechanism participating in its pathogenesis in order to explore new predictors, evaluate risk factors, discover reliable screening tests, develop efficient therapeutic strategies. As mentioned previously, epidemiologically data concerning the prevalence of hypertension, the main cause for the development of diastolic dysfunction, reveals the role of estrogen loss, which could offer an explanation to the same or even higher rates of hypertension occurrence in postmenopausal women comparing to this in men.

The possibility of developing heart failure is higher in people with high systolic or diastolic blood pressure on the basis of hypertension being an undeniable risk factor of heart failure. Indeed, approximately in 74% of patients with congestive heart failure pre-existed elevated blood pressure. 13% of death cases happen due to hypertension or hypertension-mediated diseases and under this aspect of view, it is easily explainable the statement of World Health Organization (WHO) that hypertension is the primary and leading mortality cause globally (283).

It is nowadays widely known that, among others, arterial stiffness, older age, and female gender are the link of hypertension with HFpEF as the most usually coexistent comorbidity (86) and that it is proven to be the number one cause of occurrence of cardiovascular diseases globally (284, 285). The dominant trinity of the first stages of hypertensive heart disease is: left ventricular hypertrophy, myocardial fibrosis, and disorder of diastolic function whereas the systolic one remains unaffected (90).

RAAS activation leads to diastolic dysfunction through the mechanism of hypertension (286, 287) but also the overload of hypertension acts as trigger for the activation of RAAS system, which then exerts its effects in heart tissue through collagen formation and fibrosis, causing consequently structural and functional heart abnormalities (288, 289). As discussed before, RAAS is a hormonal system with both hemodynamic and metabolic role preserving in that way the ‘’delicate’’ homeostatic balance in body. In this respect, supportive to this idea is the fact that principal aim of hypertension therapeutic management is the inhibition of RAAS (272).

Initially, as an effort to enlighten the underlying mechanism of hypertension and investigate the impact of RAAS in its pathogenesis, a review based on clinical experiments on adult dogs was published (90). It was reported that structural myocardial alterations, typical for hypertension, were present when angiotensin II was not. This result led then the investigators to support the idea of the load being the pivotal contributor to the hypertensive-related perturbations. Nevertheless, a recent work searching further the clinical entity of HFpEF came to upset the till

then established knowledge about the predominant role of afterload in hypertension pathogenesis, demonstrating controversial arguments to this hypothesis (51). It proposed a new concept of microvascular inflammation and not excessive afterload being the major trigger of hypertension (51). The new HFpEF suggests that pathophysiologic hypertension bases on the unobstructed hypertrophy resultant due to increased myocardial afterload and on eliminated NO bioavailability, both derived from the key of microvascular inflammation.

The hypertrophic, fibrotic as well as inflammatory role of aldosterone seems to correlate aldosterone with the prevalence of hypertension processing a crucial role in its development (290, 291). Testing aldosterone level in subjects with normal blood pressure revealed high serum concentration of the hormone, result which implies that aldosterone interferes in hypertension's mechanism and increase blood pressure (290).

Interestingly, regarding RAAS system, hypertension is categorized into low-renin hypertension (292) and high/normal renin level (293). Patients from the first category present a borderline aldosterone concentration although plasma renin is low, implying an inappropriately high production of aldosterone triggered by angiotensin II (294, 295). On the contrary, in people with normal/high renin, "non-modulators", aldosterone generation does not depend on sodium intake. This division of hypertension seem to have an important clinical impact as hypertensive people with low renin are more sodium-sensitive and therefore, diuretics are more effective in this group (292), whereas the "non-modulators" benefit more from RAAS blockade (296).

Obesity

Obesity is a detrimental ''mute'' health problem, which concerns approximately the one third of the population in eastern world (297) whereas one out of two individuals is obese in western societies (298) and proven as one from the most significant factors leading to HFpEF (33, 299, 300). Adipose tissue, despite previously thought to be just energy storage, is actually much more active than expected. In particularly, it undertakes an endocrine role. In this regard, it is capable of generating and releasing the RAAS hormones (301). It has been shown that the components of RAAS are located in adipose tissue (301). Considering the observation that adiposity-related RAAS interfere in dysregulation of blood pressure (302), one could claim that obesity is the connecting link between diabetes and hypertension (303) through RAAS, two clinical entities that are intimately related with each other.

Obesity is related with local RAAS activation leading to insulin resistance due to chronic low-level pro-inflammation (304, 305). Confirmation of the conceptual idea that aldosterone production and obesity are pathophysiologically correlated, demonstrates the Primary Aldosteronism Prevalence in Hypertension (PAPY) prospective study examining a considerable number of patients (1125) with recent diagnosis of hypertension observing that increase in aldosterone level in plasma was correspondent to an increase of BMI units, exhibiting an association between aldosterone concentration and visceral adiposity (306).

Metabolic syndrome

Shortly, the metabolic, from many described as cardio-metabolic syndrome (CMS) , is a clinical entity which is characterized by the combination and interaction of not only metabolic but also hemodynamic major factors: central or visceral obesity, hypertension (HTN), dyslipidemia, insulin resistance/hyperinsulinemia, and microalbuminuria and endothelial dysfunction (307, 308).

In terms of the major underlying pathophysiological mechanism and causal origin which bind these different components are assumed to be insulin resistance and endothelial dysfunction respectively (309). Having become unignorable the strong association of CMS, microalbuminuria, CKD and CVD with consequently higher rates of mortality, it is of utmost importance to elucidate the pathophysiological mechanisms hiding behind. It is assumed that microalbuminuria reflects an inflammatory state as well as oxidative stress condition, both of which can potential result to endothelial dysfunction, augmented or even caused by RAAS activation (310).

Undeniably, hypertension, diabetes and obesity concern separately each of them a great number of patients. Taking into account the tremendous prevalence of these diseases globally, interesting would be to investigate and discover in what extend these distinct disorders, which are pathophysiological closely connected, coexist. Epidemiological data provided from Sharabi et al. (311) predicts a tremendous concurrence of 200 million individuals all over the world which could also explain the term of this evil triad ‘’diabesotension’’, which is suggested to be a defined clinical condition in terms of complex perturbations involving hormonal and hemodynamic pathological alterations (311). As each one of these diseases is undoubtedly proven to be relevant risk factors for cardiovascular death, due to the dysfunctional abnormalities in microvascular as well as macrovascular level, is expected that their cardiovascular complications contribute synergistically in an additive way to the increase of risk (311). High

blood pressure and diabetes in presence of body mass index (BMI) over 25 kg/m² are claimed to increase twofold the cardiovascular mortality risk, according to reports of a meta-analysis of clinical studies (312). Recently, it has become clear that hypertension and diabetes are related with each other and even consist a vicious cycle; the presence of the one fuels the occurrence of the other (311). Particularly, individuals with insulin resistance and hyperinsulinemia are more possible to develop hypertension (313) and the opposite, hypertension contributes to the appearance of diabetes mellitus (314, 315). The clinical praxis, as suggested by the ESC/EASD guidelines of 2013, reveals the association between DM and hypertension as RAAS inhibitors administrated as antihypertensive medication are highly indicated in diabetic patients with microalbuminuria (class I, level A) (316). Furthermore the blood pressure target should be adjusted with the ‘’target of <140/85 mmHg’’ in presence of diabetes (316).

Studying deeper this evil triangle of diabetes, hypertension and obesity, conceiving numbers reflect their relation. In particular, it seems that BMI and hypertension depend on one another (313) as each increase of BMI by one single unit is accompanied by 16% rise of the hypertension odds ratio, whereas each additional year of age increase it by only 6% (317). Likewise, obesity plays a crucial role in diabetes multifactorial pathogenesis creating insulin resistance and being a cause of diabetes mellitus (318). Aldosterone seems to bridge the pathogenesis of all of them as it is associated to adiposity and glucose metabolism and, in fact, independently (319).

Insulin resistance is the main determinant involved in the multifactorial pathogenesis of ‘’diabesotension’’, possessing a principal position in the genesis of this clinical entity (311, 320). It is widely acceptable that obesity and diabetes are typical examples of insulin-resistant states, and hypertension, whose role was underestimated in the beginning, too, as suggested by Ferrannini et al. (320). Beyond the biochemical and metabolic alterations depending on the condition of cardiovascular status, insulin can affect also hemodynamically the cardiovascular system through modulation of different biological processes. In particular PI3K and MAPK, whose activation is responsible for the cardiac hypertrophy (321), are the major mediating signaling pathways with protective and harmful role for the vasculature respectively. It is simply conceivable that any hyperinsulinemia-like disturbance would shift this elegant balance towards vasoconstriction resulting to hypertension and would benefit the development of atherosclerosis leading to CVD, as it happens in insulin resistance and consequent hyperinsulinemia states like diabetes and obesity (322) and therefore, potentially to HFrEF.

Apart from insulin resistance, inappropriate over-activation of RAAS, can also be considered as a common pathophysiological thread between these clinical entities as it is inextricably tied with the pathogenesis of each of them (323, 324).Interesting is the crosstalk between RAAS and insulin signaling; RAAS is believed to be often derived from insulin resistance itself through upregulation of AT1 receptors in vascular smooth cells and overproduction of angiotensinogen intra-arterial by insulin (325, 326), while RAAS (aldosterone with MR receptor as well as angiotensin II with AT1R) can block on the other hand the insulin signaling (327, 328) causing therefore insulin resistance.

Clinical pharmaceutical studies- RAAS-blockade in heart failure

In short, the main RAAS blockade agents aim the conversion of angiotensin I to angiotensin II by inhibiting ACE enzyme (ACE inhibitors), the binding of the formed angiotensin II to its receptors (ARBs) and the blocking of aldosterone receptor in order to prevent aldosterone to exert its actions (MRA) (329). The blockage of RAAS in different stages of this hormonal cascade could explain some of the clinical differences in regard of, among others, their benefits (329). Although taking into consideration their similar indications, such as heart failure (330), one could assume, firstly, that they are brother agents with fast identical actions and effectiveness (329) and, secondly, that most patients with heart failure (HFrEF or FHpEF) would respond the same way to the ACE inhibitor, ARB or MR antagonism medication (331). However considering and evaluating deeper data from large clinical meta-analyses trials testing thousands of patients, one realizes that this generalized hypothesis is at least obsolete as it has been clearly demonstrated that ACE inhibitors possess a much more effective potential in CV risk and mortality reduction, in particular in the presence of hypertension (4-6) and that RAAS blockade is not as beneficial in HFpEF as in HFrEF (40).

As mentioned previously, given that our conception of HFrEF and HFpEF is updated, considering them as different clinical entities and two distinct phenotypes (11) as well as the undeniable role of inappropriate expression of RAAS hormonal system in heart failure pathogenesis (332, 333), is of utmost importance to decide if and in what degree should therapy of heart failure be based on RAAS blockade, namely if therapeutic response depends on RAAS activation in each case of reduced or preserved EF (43). Whereas it is clearly shown the effectiveness of RAAS blockers in HFrEF, challenging enigma remains the role of RAAS blockade in the treatment and management of HFpEF. A clinical cohort was designed with the aim to test and possibly correlate the RAAS inhibition medication with the improvement of mortality rates in patients with HFpEF and HFrEF. Indeed, it has been shown that the 1- year as

well as the 5-year survival was higher when RAAS antagonists were administrated (47). Despite the relative overall benefit of RAAS antagonism suggested by Lund et al. (47), mounting data discourage RAAS blockade as effective therapeutic choice for HFpEF. The large OPTIMIZE-HF trial (21) taking as samples ‘’ 20,118 patients with left ventricular systolic dysfunction (LVSD) and 21,149 patients with preserved systolic function (left ventricular ejection fraction [EF] ≥40%)’’ did not succeed to demonstrate an advantage neither of ACE-I nor of ARBs on mortality improvement in HFpEF, in contrary to HFrEF.

Additionally, observing quantified evidence obtained from many clinical HFPEF and HFrEF cohorts presented that patients with LVEF of 40-49% were more benefited that others with LVEF of 50% or more, indicating an increase of RAAS blockade effectiveness as LVEF declines (47, 334). Indeed, investigators have also suggested that the reduced LVEF acts as stimulator for the RAAS system to express (335). Paulus et al. summarizing large clinical trials emphasized once again the non-positive outcome of HFpEF when treated with RAAS inhibitors (336).

However, long therapy with aldosterone receptor blockade with spironolactone is showed to be beneficial as far the diastolic function is concerned (337). The idea that MRA contribute to the improvement of the diastolic function of heart (337) through reducing fibrosis and oxidative stress, regardless sex-specific characteristics and blood pressure control is supported by Habibi et al. who tested the effectiveness of mineralocorticoid receptor blockade when RAAS is overexpressed (338). Furthermore, Orea-Tejeda et al. (339) have demonstrated encouraging results of spironolactone in reducing the LV wall thickness in patients with HFpEF in a preliminary level, results that should be, although, further investigated.

The principal role of aldosterone in HF pathogenesis is elucidated indirectly by evidence which clearly showed an improvement of mortality (25%) by adding MR antagonists on the basis HF therapy with ACE inhibitors (340), compared with ARBs addition which had a neutral outcome (341), and strongly indicated a reduction of hypertrophy and fibrosis in heart tissue when mineralocorticoid receptor was blocked (342). Supportive data, namely reduced hospitalization rates due to heart failure, was demonstrated by the clinical randomized trial TOPCAT investigating patients with HFpEF, proposing that aldosterone antagonism has much to offer to the improvement of the clinical outcome of the disease (343).

Interestingly, controversial appear to be the results of the meta-analysis of these three studies (CHARM preserved, PEP-CHF, and I-PRESERVE) revealing an effective action of RAAS blockade (344), which may explains the assumption that individual trials expose underestimated data (345). It was proposed that, despite to an observed decreased reduction in death rate in

case of HFpEF, which imposes consequently a more limited RAAS activation (332), the HF form may play not such a significant role in the response to RAAS blockade (47).

Nevertheless, investigating the possible benefit of RAAS blockade dependently on co-existent elevated or lowered NT-proBNP blood levels the PEP-CHF study demonstrated a positive correlation between NT-proBNP concentration and RAAS antagonism effectiveness (346) whereas the I-PRESERVE trial suggested the exact opposite, namely the lower the NT-proBNP, the greater the benefit from RAAS inhibition (347).

Benedict et al. suggested that the association between RAAS activation and decreasing LVEF cannot be affected by the clinical status or influenced by the prescribed medication (348). However, a relation is implied to exist between benefit of RAAS antagonism and clinical status. Since very early was expressed the idea that level of RAAS hormone concentration depends on the clinical severity of heart failure which was supported by the SOLVD Investigators who suggested that low LVEF in absence of clinical symptoms is accompanied by low RAAS expression and therefore enalapril is not indicated in that case (349). Regarding aldosterone antagonism, a recent investigation revealed benefit mortality and morbidity in systolic HFrEF with mild symptoms (NYHA II), where eplerenone was tested in 2737 patients with clinically mild systolic heart failure with LVEF<35% (350).

In case of prescription of RAAS blockers as part of antihypertensive therapy however, a very recent large meta-analysis of ‘’18 prospective, randomized, controlled morbidity-mortality trials (68 343 RAAS inhibitor; 84 543 control), included only modern hypertension trials’’ examining both classes, ACE inhibitors and ARBs, with more than 158,998 recruited patients with hypertension, favors the first towards ARBs as major determinant responsible for the 10% decrease of all-cause mortality and 12% of CV mortality (283). However, an interesting previous large meta-analysis with collective data from 26 clinical trials testing the influence of RAAS blockade on cardiovascular outcome have demonstrated no benefit independent from hypertension for neither ACE inhibitors nor ARBs (351). Given the reliability provided by the tremendous number of these meta –analysis, one could assume that the admitted positive effect of RAAS blockers, in terms of tissue damage and outcome improvement, are based mainly on their antihypertensive action rather than reduction of local angiotensin II levels, and imply that these agents do not have complete access in tissue-sites where RAAS hormones are locally produced (352).

The large ALLHAT Trial, after recruiting a more than sufficient sample of 42,418 hypertensive patients in high-risk, evaluating lisinopril in comparison with other drug categories used as therapeutic agents in HFpEF failed to favor RAAS blockade as more effective (353). Generally

clinical studies of heart failure and hypertension failed to support an undeniable and strong benefit of RAAS inhibition, despite the enormous amount of clinical evidence indicating a favorable effect of these pharmacologic agents (354).

Examining separately ACE inhibitors and ARBs compared with placebo, large studies support discouraging or neutral results. In particular, The CHARM-Preserved study (n=3023) (50) and the I-PRESERVE trial (n=4128) (48) investigated the influence of candesartan and irbesartan, respectively, on the decrease of mortality and hospitalizations due to CV events and found no relevant reduction. No beneficial effect seem to have ACE inhibitors in the hospitalization and mortality rates according to the PEP-CHF trial (n=850) which compared perindopril with placebo (49).

Challengingly interesting are evidence from two trials, ELITE (355) and RESOLVD (356), which aimed on comparing the two RAAS blocker categories, losartan to captopril in patients with LVEF<40% and candesartan to enalapril in symptomatic left-ventricular dysfunction, respectively. Unexpectedly, these clinical trials demonstrated controversial results, namely, the ELITE showed that losartan is favored towards captopril concerning the mortality rates, whereas the pilot RESOLVD reported a tendency of fewer clinical events under enalapril, which implies an improvement of clinical outcome.

Turnbull et al. analyzing results from 31 trials and 190,606 recruited patients not only confirmed the ALLHAT trial's reports that RAAS blockers do not appear superior to other antihypertensive drugs but also suggested ''any difference between the effects of the drug classes on major cardiovascular events."

''Escape'' phenomenon

After the report of these discrepant data in regard of their different effect on mortality, it became essential to clarify this divergence. Several explanations appear to be reasonable, such as the ''escape phenomenon'', negative feedback mechanisms and effects of RAAS blockade agents on bradykinin. ACE inhibitors prevent the formation of angiotensin II from angiotensin I by blocking the ACE enzyme. Nevertheless, ACE enzyme is not the only one substrate for the generation of angiotensin II as chymase is an alternative pathway. Especially when negative feedback loops triggered by long ACE-inhibition allow the rise of renin and angiotensin I (357). The hormonal cascade can be continued ''escaping'' the medical inhibition of ACE leading to unobstructed generation of angiotensin II and aldosterone, despite the long-therapy with ACE inhibitors, leading to high levels of these hormones (358). Respectively, similarly could be

explained the statement of Strauss et al. “ARB MI paradox” due to elevated levels of angiotensin II after infraction because of the blockage of binding with the receptor under ARB inhibition (359). At first look, one could claim that ACE-inhibitors are not that effective after long administration but thinking deeper, another supplementary action of ACE inhibitors on bradykinin, peptide with antagonistic effects on angiotensin II (360), is what may preserve their therapeutic value (329).

Although ‘’escape’’ events are able to provide a reason for incomplete RAAS blockage, do not consist a clear explanation for imperfections of RAAS inhibition therapy, as implied by several studies investigating outcomes under the combination of different pharmaceutical agents such as ONTARGET (ramipril was combined with telmisartan) (361, 362), the Altitude trial (aliskiren with valsartan) (363, 364) as well as a meta-analysis of studies of individuals presenting a symptomatic left ventricular dysfunction (365).

RAAS blockade in new onset of diabetes mellitus

As discussed elaborately before, RAAS inappropriate over-activation leads to DM type 2 through increased insulin resistance and glycose intolerance as well as to hypertension and consequent CV diseases. Having become clear the pathogenic role of RAAS hormonal system in hypertension and diabetes, the expectation of being an efficient therapeutic choice was expectedly arisen. Numerous clinical animal and human studies and large trials are conducted in order to investigate in what degree RAAS inhibitors agents would deserve a place in clinical praxis. Enough evidence on a clinical or experimental level has been I selected and evaluated from a tremendous number of trials and meta-analyses respectively demonstrated the advantages of RAAS blockade under the aspect of metabolic syndrome and particularly in regard of insulin and glucose homeostatic balance.

With aim to eliminate oxidative stress with subsequent goal to normalize the endothelial function, RAAS blockade is proposed to be the target of therapy according as suggested by many clinical studies. The VALUE trial after enrolling and following 15,245 patients with hypertension, presetting age and risk assessment and predetermining the end points, examined the CV outcome under valsartan (ARB) and amlodipine (calcium channel blocker). Identical results for both groups derived as far as all-cause mortality was concerned but the possibility of developing DM in these patients was 23% lower in valsartan group (366).

At the point that clinical trials based on hypertension coincidently revealed observational findings with the concept that RAAS blockade influences the occurrence of diabetes, much interest was focused on that issue. This led to the conduction of further studies whose meta-analyses acknowledged this idea, namely that ACE inhibitors and ARBs can lower the appearance rates of diabetes when administrated in non-diabetics (367, 368). In these terms the ‘’prospective, double-blind, international, randomized clinical trial’’ NAVIGATOR (369) (Nateglinide and Valsartan in Impaired Glucose Tolerance Out-comes Research) was designed and recruited 9,306 individuals with glucose intolerance with purpose to test the ARB valsartan or oral hypoglycemic nateglinide in comparison with placebo regarding the new-onset of diabetes and CV events. Two more studies, ALLHAT and ALPINA, reported a relevant difference of 14% in lowering the progression to DM (113, 250) and in decreasing the risk of diabetes new-onset, favoring valsartan towards nateglinide. Similarly, The DREAM study (Diabetes Reduction Assessment with Ramipril and Rosiglitazone Medications) (370) highlights ramipril as an agent which despite not affecting the prevalence of diabetes, it helps to return to a normoglucemic condition which points the positive effect of RAAS blockade in glycose metabolism. Additionally, mounting evidence of numerous studies (HOPE (371), LIFE (372), ALLHAT (373), ALPINE (374), SOLVD (375), CHARM (376), CAPPP (377), VALUE (366), LIFE (378)) support this hypothesis that RAAS inhibition helps in the reduction of new onset of diabetes or the progression to it in presence or absence of hypertension, as RAAS hormones are involved in glucose metabolism and interfere to insulin signaling.

As discussed previously, given the proven crucial role of inflammation, mainly caused by RAAS activation, as key factor binding the metabolic syndrome, whose part are among others diabetes and hypertension, and CV disease, two possible reasons could be assumed to explain the difference of RAAS inhibition benefiting in comparison with other agents. On the one hand, anti-diabetic drugs do not seem to exert anti-inflammatory actions (379) whereas on the other hand ACE inhibitors and ARBs possess the potential of increasing insulin sensitivity contributing likely to the prevention of DM incidence (380). Bangalore et al. (381) searched and analyzed the results of 13 selected large clinical trials with a sample of 92,408 individuals with hypertension but without diabetes mellitus. Half of them received ACE inhibitors or ARBs whereas the other half were treated with other medicine classes such as calcium channel blockers, thiazide diuretics, or β-blockers or placebo. Nine of these studies demonstrated a significant decrease of new-onset diabetes (27%), while four of them reported an even greater reduction (33%) in patients presenting vascular disorders or impairment of left ventricular function (381).

As demonstrated by various clinical studies, RAAS blockade agents as treatment of hypertension reduce the rates of new-onset diabetes. It seems that the opposite is also possible, namely RAAS inhibitors (ACE inhibitors and ARBs) when administrated in presence of diabetics type II benefit, through blood pressure reduction, the CHF progress and outcomes on a cardiovascular level in general (382).

Role of RAAS blockade in arterial hypertension

Established data has revealed that although drug categories for hypertension treatment influence to comparable degree the blood pressure, among antihypertensive medication classes, RAAS blockade seems to be significantly more effective in amelioration of the coexistent diabetic nephropathy (383-387). This evidential knowledge resulted to the officially and widely accepted perception that ACE inhibitors or ARBs should be considered as first-line therapy of hypertension in patient manifesting albuminuria as renal dysfunction marker (384).

In particular, interesting are the results demonstrated by three large clinical studies, the Irbesartan in Patients With Type 2 Diabetes and Microalbuminuria (IRMA-2) study, the Reduction of End Points in Non-Insulin Dependent Diabetes Mellitus With the Angiotensin II Antagonist Losartan (RENAAL) study, and the Irbesartan Type 2 Diabetic Nephropathy Trial (IDNT) (385-387) and supported by the Telmisartan Randomized Assessment Study in ACE Intolerant Subjects With Cardiovascular Disease (TRAN-SCEND) which evaluated the telmisartan administrated due to intolerance towards ACE inhibitor of the examined subjects (388). It was suggested that ARBs interfere in the development of diabetic nephropathy independently of the renal dysfunction stage.

The Ongoing Telmisartan Alone and in Combination With Ramipril Global Endpoint Trial (ONTARGET) investigated then the possible additional profit from the combined medicine with both an ACE inhibitor (ramipril) and ARB (telmisartan) and showed an increased benefit compared with alone therapy concerning the progression albuminuria in view of diabetic nephropathy (389, 390). Besides this study showed similar outcome results for ACE inhibitors and ARBs in patients with high cardiovascular risk (362). The equality of these agents concerning diabetic nephropathy was demonstrated also by the DETAIL trial (391).

An interesting and worth mention clinical observation is the relation of these agents' therapeutic effectiveness with the type of diabetes mellitus; ARBs seem to influence positive the renal disorder in diabetes type II (392), whereas ACE inhibitors exert their beneficial actions in presence of diabetes type I (393). Indeed the Diabetes Control and Complications Trial (DCCT) elucidated the role of ramipril (ACE inhibitor) in renal disease in patients with DM type I (384).

Divided are the opinions if RAAS inhibition pharmacologic agents are superior to other classes of antihypertensive medicine (311). Most of the studies can prove no benefit of RAAS blockers towards other antihypertensive drug categories (ABCD, STOP-DM, INSIGHT-DM, ALLHAT-DM, ASCOT-DM). Nevertheless two of them favor RAAS inhibitors in hypertensive patients who suffer from diabetes too (CAPP-DM, LIFE-DM). For instance, Zappe et al. compared the competence of valsartan (ARB) and hydrochlorothiazide in patients with metabolic syndrome, hypertension and obesity and found that both effect similarly in blood pressure control but the first presented as more valuable concerning the level of fasting glucose (394).

Despite that the beneficial role of MR antagonism, regarding CV outcomes, is confirmed by two large clinical studies, Clinical trial data from the Randomized Aldactone Evaluation Study (RALES) and Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS) (113, 395), it remains unclear or incompletely evidence based the effects of MR antagonists in diabetes as part of metabolic syndrome (303). However, encouraging for the role of MR antagonists as part of hypertension management, concerning CHF and CKD (396) appears to be experimental data, testing in what degree reduction of oxidative stress as well as enhanced glucose tissue uptake due to unstrengthen insulin resistance influence the modulatory potential of MR antagonism in terms of blood pressure and insulin sensitivity (396-398).

In conclusion, taking into account that although RAAS hormonal system is widely accepted to play a principal role in both types of HF pathogenesis (HFpEF and HFrEF), controversial results derive from clinical trials and their meta-analysis, it becomes clear that gaps in our complete understanding still remain. Despite the huge bunch of evidential data in literature, this topic, if RAAS blockade should be considered a major participant in the therapeutic strategy of all forms of HF consist a matter of debate universally. In order to elucidate discrepancies concerning the effectiveness of RAAS blockade drug categories, to investigate deep the pathophysiological background of their pharmacological action in heart failure and its subtypes (regarding LVEF and clinical symptoms), to clarify if other comorbidities influence the effect of these agents and in what degree and to explain substantial differences between ACE-inhibitors, ARBs and MR-antagonists, more clinical research is needed. In pursuit of this purpose larger longer, randomized, population -based studies with wisely chosen sample of sufficient number of patients and control groups are necessitated in order multi-targeted approaches to insight into this multi-factorial theme of heart failure as well as into the clinical aspects and therapeutic strategies.

3. Methods and materials

3.1 Gutenberg health study (GHS)

In order to fulfill the aims of our study, data from the Gutenberg Health Study (GHS) was used.

3.1.1 Study-design

The Gutenberg health study is an age-, sex-, and residence stratified, population-based, single-center, prospective cohort study, which examines the cardiovascular system, tumor and eye diseases, metabolic disorders as well as abnormalities of the immune system and psychological condition of the population.

The study as well as the sampling design, was designed according to the tenets of the revised Helsinki protocol, after being approved by the local ethics committee and by the local and federal data safety commissioners. Every participant underwent a comprehensive, standardized 5-hour clinical investigation. All individuals went through a clinical assessment recording the current drug medication intake such as any medical records and history, when possible. After clinical examination and laboratory measurements the cardiovascular risk factors (CVRFs) of each individual were recorded. Furthermore, the conducted biochemical and genetic analysis were based on a large biobank, which was established for this purpose.

3.1.2 Study's goal

The study's primary purpose was the individualization cardiovascular risk assessment and stratification and consequently the prevention of the occurrence of cardiovascular diseases in population. Furthermore, it was explored the influence of subclinical parameters such as the endothelial dysfunction, different biochemical markers and genetic variables on defining or even improve the predictive accuracy of the risk score concerning the possibility of the onset of a cardiovascular disease was expplored. This required evidence and deep knowledge of the pathophysiology and etiology hidden behind as well as the suspect risk factors, which could potentially predispose these disorders. This vital information was obtained through a combination of biological and clinical data, namely biomaterial for genetic and molecular analysis and interpretation of clinical data and information provided from the examined population. In particular, factors concerning the influence of social and environmental factors, as well as the

lifestyle on an individual level, and the impact of genetic and biologic variants on the development and progress of a disease were explored, in order a possible correlation to be found which could probably allow the prediction of the clinical outcome and therefore, the evaluation of new diagnostic tools.

3.1.3 Inclusion criteria and study population

The population sample of this epidemiological study concerned participants from the Rhine-Main region in Rhineland-Pfalz in West Germany and in these terms included residents in Mainz and Bingen (196.425 residents in Mainz and 201.371 residents in the area Mainz-Bingen in 2007) (resource: statistic state office of Rhineland-Palatinate). Between 2007 and 2012, a total of 15,010 participants aged 35 to 74 years were enrolled to the GHS and the individuals were drawn randomly from governmental local registry offices in the City of Mainz and the district of Mainz-Bingen.

Women and men of age between 35-74 years old (210.867 residents in 2007) defined the target group whereas the random selection was orientated in that way, so that a 1:1 stratification was achieved in regard of sex, age and region characteristics. A random sample of 35.000 participants, with the same distribution concerning sex-, age-characteristics and place of residence (City of Mainz or the district Mainz-Bingen), was chosen based on the findings of similar population-studies and recruited following the same process so that the desired number of 15.000 was achieved.

3.1.4 Exclusion criteria

Criteria for exclusion were the insufficient knowledge of German language which brings communication difficulties and mental or physical disabilities which unable the proper conduction of the examinations.

3.1.5 Recruitment process

Regarding the contact of people, a multi-optional approach was designed and algorithms were developed with standard process and precise deadlines. As first step, among the registered residents of Stadt Mainz oder Landkreis Mainz-Bingenthe recruitment office contacted the chosen persons by sending them information about the study. Supplementary was sent a free

reply card, whose filling was an express of interest, or not, in participating in the study. As next step an appointment was made with the willing candidates. On the contrary, if no written notice was sent back, it was then tried to contact telephonically the potential participants according to an algorithm or by no success by sending up to three information letters.

3.1.6 Acceptance of participation

Participation was voluntary and written informed consent was obtained from each subject upon entry into the study. Before starting study investigations, a voluntary approval was required, officially by signing an acceptance declaration. This consisted a detailed and informative brochure, regarding conduct, goal and purpose of the study which provided also the necessary protection of data privacy on the one side and the official permission of the participant for taking biomaterial, for further clinical, biochemical and genetic analysis, for obtaining relevant for the study information from other doctors reassuring the medical confidentiality. Provided that interest was expressed, potential candidates were invited to participate in the study and undergo the audit program.

Just after the presentation of the participant in the study center and before the start of the standard procedure, followed a conversation with informative and explaining character about the above mentioned issues by an experienced study-assistant. Only under the condition of free agreement of the ‘’Informed Consent’’ participant could the study begin.

No laboratory analyses, clinical examinations, sampling of biomaterial were performed without informed written consent by every individual prior to participating in the study.

3.1.7 Ethic aspects and protection of medical data

For the conception of the study and the guarantied protection of privacy data, the Good Clinical Practice (GCP) and the Good Epidemiological Practice (GEP) as well as the ethical principles of the Declaration of Helsinki were taken into account and the proper performance of the study was defined by those limitations. Furthermore, the obligations of the Federal Data Protection Act were respected and the Ethics Commitee of the State Medical Association Rhineland-Palatinate and the Data Summary Protection Officer of the University Medical Center of the Johannes Gutenberg University Mainz gave a positive vote on the documentation of the study. The drawing of the sample over the registration offices has been approved by State Data Protection Commissioners of Rhineland-Pfalz.

All medical results and possible clinical diagnoses were used with pseudonymization. Besides, all the study participants were given an identification number (GHSID: Gutenberg Health Study Identity) instead of using their name whereas for the laboratory values had an additional ID-Number (GHSLID: Gutenberg Health Study Labor-identity). The working personal of the study were obliged to keep the medical confidentiality and examine the participants obeying the Article 42 EGStGB.

3.2 Study procedure and conduction of examinations

The Gutenberg Health Study took place in a specially set up center in the area of the Johannes University Clinic Mainz. The clinical examinations were operated by a well-educated and certificated assistant personnel in a technologically developed environment under standard operating procedures (SOPs) and protocols with an investigation- and research- orientated thinking and extreme precision and respect of the study regulation and limitations. After the signing the acceptance form, the investigated individuals could then start with the examinations, which were conducted with a certain sequence keeping the defined timetable. After the competition of the program a summary of medical results was given them for eventual further discussion with their personal doctor. Before discharged, the participants were provided with a study identity and a notebook where possible future diseases or disorders which last longer than 3 weeks should be documented

Preparation

In order to ensure as high reliability of the results as possible, a proper preparation is essential. Primary aims were the high standardization of the procedure and the low variability of the data. In this regard a specific list concerning the information of the people planned to participate in the study was sent per post. This list concerned indications about activities that should be avoided before the operation day of the study or any other restrictions. Some examples were fasting 8 hours before the begin of the examinations and stop smoking at least 2 hours before and no sport activity the last 8 hours. In case that the chosen people had a regular or daily medication the further administration was allowed and were kindly requested to bring the packages and medical history in order to facilitate the process of the interview.

3.3 Examinations in the study center of Gutenberg Health Study

a. Social- demographic data

As part of the GHS investigation program, the following data were collected in a computer-assisted interview (CAPI) using standardized items:

- Benefit from medical treatment
- Cancer prevention
- Gender issues
- Medical history - Health and diseases
- Traditional risk factors
- Disease-specific symptoms and pathology
- Family history
- Children
- Health Behavior
- Hobbies and leisure activities
- Smoking, passive smoking, alcohol consumption
- Occupational history
- Particulate matter and noise pollution
- Satisfaction with life and environmental factors
- Home environment

b. Medical technical investigations

Furthermore documentation of medical history, assessment of clinical data and evaluation of data obtained from different clinical examinations were an important part of the study.

- Detection of medication
- Spirometry
- Measurement of carbon monoxide in the alveolar
- Resting blood pressure and resting heart rate
- Periodontal pocket swabs
- Simultaneous determination of flussmedierter vasodilation and arterial stiffness by measuring the reactivity, the brachial artery using ultrasound, Volume-plethysmography the digital artery using Endo-PAT and digital photo-plethysmographic pulse curve analysis
- Neuro-cardiac regulation

- Closure pressure measurement with the legs determine the Ankle Brachial Index
- Acquisition of current weather data
- Anthropometry
- Body temperature
- Electrocardiogram
- Venous Blood with determination routine laboratory chemical parameters
- Ultrasonography of the carotid arteries
- Two and three dimensional echocardiography
- Ophthalmological examination with determination of visual acuity and refraction, perimetry (FDT), fundus photography, pachymetry, tonometry and slit lamp examination

c. Survey questionnaires

Survey questionnaires were also used in order to gain further information about the participants,

- Personality, mental illness and emotional distress
- Everyday loads
- Social Integration
- Psychosocial stress at work
- Life Events
- Visual quality of life
- Nutrition
- Short Questionnaire to Assess Health-Enhancing (SQUASH) Physical Activity

d. Sampling of biomaterials for bio-banking

For the purpose of the study essential was the laboratory examination. Of utmost importance for the study was the measurement of biomarkers in blood sample and, in particular, of plasma renin and aldosterone concentration as well as HDL, LDL, HbAc1 and GFR.

In summary the following examinations were carried out:

- Blood EDTA- and citrate-plasma
- Blood serum
- DNA (isolated)
- RNA (isolated)
- Washed erythrocytes

- Urine
- Periodontal pocket swabs

3.4 Measurement of clinic and medical history

3.4.1 Medication record

The Version 10/2000 of the PC-Software ‘’Instrument zur Datenbankgestützten Online-Erfassung von Medikamentendaten’’ (IDOM, KORA, Neuherberg/München) was used for the correct recording of the medication. Scanning the barcode of drug packages and asking people about the exact dose or the duration of the therapy, the medical therapy could be completely documented in most of the cases. If this was not possible, the medication, obtained from the doctor’s report or telephonically, was recorded manually by the study personnel or sent per post by the participant.

3.4.2 Measure of blood pressure and heart rate at rest

After the participant having relaxed, taken a comfortable position, uncovered his arm, the blood pressure as well as the pulse was measured with the automatic device Omron 705CP-II (Omron, Japan) under the temperature of 22° C. Very important is the right choice of the arm machete depending on the thickness of the arm; the small machete has a diameter of 17-22cm, the medium of 22-32cm and the big one of 32-42cm. In any case the machete was applied approximately 3cm over the elbow. The participants were requested not to move or speak during the measurement and to remain relaxed. To the latter contributed also the fact that the device is place on the table in such a way so that the examined person could not see the result during the measure. The first phase included evaluation of blood pressure and heart rate in both sides (right and left arm) at the same time after a rest period of 5 minutes. Then, after an interval of 3 minutes, followed the second and third measurement exactly in the same way as the first one, but only on the left arm. Should a difference of >20mmHg systolic or >15mmHg diastolic was noticed, the second and third measure should have been in both sides just like the first one and should unrealistic values occur, the measures should be repeated. Each measurement of blood pressure and pulse was in electronic Case Report File (eCRF) documented and the accuracy of the device is tested once annually.

3.4.3 Venous blood analysis

The participant lied on an examination bed. For a sufficient blood analysis, circa 120-140ml blood was taken using butterflies Wero-Medical (Safety-Multifly-Set, 20G, 200mm) and monovettes Sarstedt which were filled following a certain sequence. From this amount of blood almost 97,3ml is used for the EDTA-Plasma, Citrat-Plasma, Platelets Poor Plasma und Serum examination, 8ml from the first 9 participants for the RNA isolation of PBMC and the rest 2,5ml for the isolation of the total-RNA from blood. The most preferable was an elbow vena, but alternatively venous blood can be also taken from other arm or foot veins. Right after the blood collection, the monovettes were sent direct and without any delay to the GHS-laboratory and bio-bank and central laboratory of the university clinic Mainz for a routine examination of the sample.

3.4.4 Echocardiography

The transthoracic echocardiography is a widely applied cardiologic diagnostic method which allows the general assessment of heart tissue in a simple, accurate and non-invasive way and therefore without risks. It is a sonogram of heart, consists one of the most useful examinations of heart for diagnosis and follow up evaluation of structural and functional disorders.

Additionally, the Doppler echocardiography, which is differentiated in pulsed or continuous wave Doppler ultrasound, includes specifically the tissue Doppler and color Doppler technique. The first is used for the recognition of the motion of the heart and the second allows the evaluation of the flow of blood through the different cardiac departments, useful for the estimation of the valvar function.

In the Gutenberg Health Study for the operation of this examination the echocardiographic-system iE33 (Philips, Royal Philips Electronics, Netherlands) and the sector-array ultrasonic head X5-1 Royal Philips Electronics, Netherlands were used. The service of the device by the firma Phillips was planned regularly.

The standardized preparation of the examined person included the undress of the upper part of his body and lying position on the left side with the left arm under the head.

Through two-dimensional and three-dimensional ultrasound, information could be gained about the structure of the heart, whereas through Doppler ultrasound pictures could be taken for the evaluation of the blood flow.

According to the standard operation procedures for the production of images were used the following echocardiographic standard views of:

- ❖ 2D-Echocardiography:

a. Parasternal views:

- Parasternal long axis view
- Parasternal short axis view
- M-Mode parasternal

b. Apical views:

- Apical 2-chamber view
- Apical 3-chamber view
- Apical 4-chamber view

- ❖ Doppler-Echocardiography:

- PW-Doppler in the mitral valve
- PW-Doppler in the mitral annulus in tissue Doppler
- PW-Doppler in mitral annulus in tissue Doppler in stress

Nevertheless, in terms of the study, for the measure of defined parameters for the estimation the condition of the heart structure and function in our study, the apical two-, three- and four chamber view and left parasternal log axis view were used. Regarding the 2D-echocardiography, the measured parameters for the further evaluation of the heart that were used for further analysis were the following:

a. <u>M-Mode:</u>

- End-diastolic diameter of the right ventricle (RVIDd)
- End- diastolic thickness of the intraventricular septum (ISVd)
- End-diastolic diameter of the left ventricle (LVIDd)
- End- diastolic thickness of the free wand of the left ventricle (LVDWd)
- End-systolic diameter of the left ventricle (LVIDs)

b. 2D-echocardiography/Apical 4 chamber view:

- End-diastolic volume of the left ventricle (EDV)
- End-systolic volume (ESV)
- Ejection fraction (EF)
- End-diastolic volume of the left atrium (LAd)
- End-diastolic volume of the right atrium (RAd)

c. PW-Doppler Mitral valve:

- Flow velocity during the passive filling of the ventricles (MV E-Vmax)
- Flow velocity during the active filling of the ventricles due to the atrial contractility (MV A-Vmax)
- Duration of the passive filling (MV A-Duration)
- Deceleration time (MV DT)
- E/A ratio
- E/E' ratio

d. Tissue Doppler (TDI):

- Maximal systolic velocity of the mitral annulus (Ma-VS)
- Maximal early-systolic velocity of the mitral ring (Ma-VE')
- Maximal late-systolic velocity of the mitral ring (Ma-VA')
- Deceleration time (DecTime E')
- Isovolumic relaxation time (IVRT)
- Ejection time (ET)

e. Stress tissue Doppler (s-TDI):

- Maximal systolic velocity of the mitral annulus under stress: Ma-VS stress
- Maximal early-systolic velocity of the mitral ring under stress: Ma-VE' stress
- Maximal late-systolic velocity of the mitral ring under stress: Ma-VA' stress
- Deceleration time under stress: DecTime E' stress
- Isovolumic relaxation time under stress: IVRT stress
- Ejection time under stress: ET stress
- Isovolumic contraction time under stress: IVCT stress

Supplementary to the above mentioned measurements belonged also the same PW-Doppler and tissue Doppler measures under stress. Therefore the examined person was requested to do 10 sit-ups.

There was a monitor where the saved pictures can be displayed and recorded in a digital form which enables a retrospective analysis. Notes relative to the difficulties of the ultrasound, measure, editing or saving of the images as well as possible difficulties or problems that could occur before, during or after the examination were documented in the eCRF system.

3.5 Quality control and data management

All measurements of the echocardiographic parameters were based on the following guidelines of: the American Society of Echocardiography's Nomenclature and Standards Committee, the American Heart Association, the European Society of Cardiology, the European Association of Echocardiography and the Task Force on Chamber Quantification (23).

The records of the images created from the echocardiographic examination were digitally saved and then stored in a program with multiple features of editing the pictures and a great potential of analyzing them. This system was the Xcelera, Royal Philips Electronics, Netherlands. This obtained data was then controlled by an experienced science cooperator as far as the quality, sufficiency and completeness of the images and the rightness, plausibility and accuracy of the measurements. Additionally, a specific data management unit with competent supervisory authority was responsible for the further controls based on certain algorithms in order to ensure the best possible quality of the results.

Regarding the calculation and estimation of the left ventricular ejection fraction derives from the use of the modified Simpson method. According to guidelines, the percent of the ejection fraction defines the severity of cardiac function as follows (23):

LVEF:

- ≥55% normal EF
- 45-54% mild reduced
- 30-44% moderately reduced
- <30% severely reduced

In order to evaluate the diastolic function of the heart three main parameters were principally used, namely the flow velocity during the passive and active filling of the left ventricle, measuring the E and A wave in the early and late phase of the diastole, respectively. The left ventricular end-diastolic pressure was estimated by the calculation of the value of E/E' which reflects the relation between the flow velocity during the passive filling of the ventricles through the mitral valve (E) and the ring of the mitral valve (E'). These parameters were measured by depicturing the heart through PW-Doppler in the mitral valve for the E wave and Tissue-Doppler in the apical four chamber view for the E', in particular focusing in the lateral side of the ring of the mitral valve.

The disturbance of the filling of left ventricle during diastolic phase depends fundamentally on these two parameters, the wave E and, consequently, on the E/E' ratio. The above is limited only in patients with EF more than 30% without ventricular dyssynchrony and with comparable E waves on the lateral and septal side of the annulus of the mitral valve, as the evaluation E/E' is not of a meaningful significance (399).

Supplementary the deceleration time of the E wave was also calculated allowing us to more accurate estimation of the diastolic function.

According the criteria of Bursi (1) and Kuwaki (2) based primarily on the above determinant parameters, the diastolic function was defined in summary as followed:

Definition of diastolic heart dysfunction by Bursi (1) and Kuwaki (2):

- Normal cardiac function: E/A > 0.75 and DT>140msec and E/E' <10
- Mild diastolic dysfunction: E/A< 0.75 and E/E' <10
- Mild to moderate diastolic dysfunction: E/A<0.75, DT>140msec and E/E'>10 (2)
- Moderate diastolic dysfunction: 0,75<E/A and DT>140msec and E/E' >10
- Severe diastolic dysfunction: E/A>2 and DT<140msec and E/E' > 10

Further parameters should be also estimated in order to estimate the diastolic function such as the size of the left atrium (400).

Given the diagnostic value derived from the measure of these echocardiographic parameters, the heart disorder can be differentiated into two main categories, the diastolic and the systolic dysfunction. Each of them can be characterized as symptomatic or asymptomatic. The latter includes situations of proven dysfunction without clinical presentation of symptoms which, therefore, do not demand a therapeutic treatment. These two discreet clinic entities of the

asymptomatic diastolic and systolic disorder can be further classified as isolated or combined. The case that a diastolic dysfunction is present but without reduction of the cardiac output is known as isolated diastolic dysfunction (DD). On the contrary, the normal diastolic function and, however, the ejection fraction is low, despite the absence of symptoms, represents an isolated systolic dysfunction with reduced cardiac output (SD). The combined asymptomatic diastolic and systolic disorder concerns the situation where the diastolic function of the heart is affected as well as the systolic function reflected by the reduced ejection fraction (combined DD and SD).

Regarding the symptomatic heart disorder or heart failure, it consists the concomitant presence of echocardiographic signs of diastolic or systolic dysfunction, based on the criteria of Bursi and the EF classification as mentioned previously, and clinic symptoms, subject to the NYHA classification, which at least once demanded medical treatment. In particular, the respective classification concerns the diastolic or systolic heart failure with reduced (HFrEF) or preserved (HFpEF) ejection fraction.

3.6 Definition and determination of traditional cardiovascular risk factors and cardiovascular disease

For the definition and determination of the classic cardiovascular risk factors were derived from the certain, below mentioned, factors, which were obtained from results and measurements in terms of the clinical examinations as well as the anamnestic information provided from the participants by the computer assisted personal interview (CAPI).

Data of cardiovascular disease were collected in a computer-assisted personal interview. Patients were classified as having a cardiovascular event based on the medical history or diagnosis of myocardial infarction (MI), stroke, arterial fibrillation (AF), peripheral artery disease (PAD), cardiac arterial disease (CAD), or congestive heart failure (CHF). In general, all examinations were performed according to standard operating procedures by certified personal.

a. Smoking

For the definition of smoking as one of the risk factors people were categorized into non-smokers, ex-smokers, occasional-smokers and smokers.

In the non-smoker category belong people who have never smoked in their live or have they but regularly for a period of time under 6 months. On the contrary, as ex-smokers were defined people who have smoked on a regular basis of more than 6 months in their live but at the time of the participation in the study do not smoke. People who smoked less than one cigarette at the

time of the study were the occasional smokers whereas smokers were people who smoked more than one cigarette at the time of the study.

b. Arterial hypertension

In terms of the study the hypertension was measured. Systolic values more than or equal to 140mmHg and diastolic more than or equal to 90mmHg when measured two or three times in a sitting position always following a relaxed and recovered phase of 8 and 11 minutes respectively as well as the medical anamneses regarding the prescription and administration of antihypertensive drugs the last two months before the day of the study allowed the definition of hypertension and determination of its presence or absence.

c. Diabetes mellitus

Besides previous medical diagnosis of diabetes or antidiabetic drugs were taken (Anatomical Therapeutic Chemical code or ATC-code A10), two more criteria were used in order to characterize a participant as diabetic or not. Diabetes mellitus was defined by a HbA1c ≥ 6,5%.

d. Dyslipidemia

Similarly to diabetes mellitus, a medical diagnosis of dyslipidemia or a medication with ATC-code C10 including subcodes or disorder of lipid metabolism or a LDL/HDL ratio more than 3.5 derived from the blood analysis of the study or triglycerides > 150 mg/dL were the criteria of the determination of dyslipidemia as cardiovascular risk factor.

e. Obesity

Obese individuals were considered to be the ones who had BMI of more than or equal to 30kg/m². The estimation of the BMI was enabled from the anthropometric examination.

f. Family history of myocardial infarction or stroke”

A myocardial infarction in the family constituted a risk factor for the participant when the affected member was female under the age of 65 including the sister or of male under the age of 60 including the brother. In both cases this concerned only first-degree relatives and characterized the medical family history as positive and consequently as an additional cardiovascular risk factor.

3.7 Clinical Assessment and Biosampling

Venous blood sampling was performed in supine position after an overnight fasting period (i.e. overnight fast, if subject was examined before 12 a.m. and 5 hour fast, if subject was examined

after 12 a.m.). All routine laboratory parameters were determined by standard methods in the central laboratory of the Mainz University Medical Center. All samples were immediately stored at -80°C within 2 hours after blood draw until further analysis. Serum sodium, potassium, and creatinine levels were measured within 90 minutes after withdrawal in the central laboratory of the University of Mainz Medical Center by routine methods. All biosamples were directly processed after withdrawal for storage and centrifuged for 10 min with 2,000 g. EDTA plasma for measurement of PRC and PAC was aliquoted in portion of 300 µl in 2D-barcoded polypropylene tubes (Micronic Europe B.V., Lelystad, The Netherlands).

3.8 Laboratory Analyses

Direct renin and aldosterone concentrations were measured by using automated chemiluminescence immunoassays CLIA using fully-automated LIAISON® platform (DiaSorin, Dietzenbach, Germany). To avoid a cryoactivation of prorenin, all frozen samples were thawed at 37°C (water bath) for 10 min, carefully turned 10 times, and immediately processed for the analysis and subsequent measurement. Both renin and aldosterone measurements were performed from one single EDTA tube. According to the manufacturer's instructions the analytical range for renin was 1.96-500 µU/mL and for aldosterone 1.91-100 ng/dL. Samples containing a PRC above 500µIU/mL were diluted in a diluent supplied by Diasorin (code-Endo 319133) and recalculated by the dilution factor. With regard to intra-assay and inter-assay coefficients of variation high and low controls provided by the manufacturer and aliquots from an `in-house human` plasma pool (prepared analog to the population samples, -80”C) was used to check reproducibility and long-term stability of the assay. Intra-assay variation of PAC was less than 6.3% over the range of 11.1 to 29ng/dL and inter-assay variation was less than 13.2% over the range of 7.1 to 30ng/dL aldosterone. For renin intra-assay variation was less than 5.3% (range 15.4-94.3µIU/mL) and inter-assay variation less than 11.0% (range 14.9-101µIU/mL).

3.9 Statistical analysis

First of all descriptive and explorative analyses were used in order to illustrate the distribution of echocardiographic parameters (LVEF, E/E’) in the study population, sex-specifically among men and women and in different age groups. Moreover renin and aldosterone concentrations such as ARR were tested in different stages of systolic and diastolic dysfunction, defined by LVEF and E/E’ and their mean values were measured in both male and female populations. We showed

also the distribution of the different clinic cardiac phenotypes in our total population and we tried to detect sex-specific differences.

The present statistical analysis was based on data collected from the Gutenberg Healthy Study (GHS) and conducted sex-specifically. The clinical and personal data collected from the examinations underwent statistical analysis in order to evaluate their significance regarding the pre-set endpoints.

Statistically, female and male sex divided our total sample into two more populations therefore the results were sex-specifically investigated and separately for female and male individuals presented. Continuous variables were reported by means with standard deviation or median values with 25th/75th percentiles (interquartile range, IQR), where appropriate. In concern of the calculation of dichotomous variables, absolute as well as relative frequencies were used. Regarding the distribution of continuous variables with the purpose of descriptive analysis, the average value with the analogue deviation was taken as standard. In case of an asymmetry of more than 1 or under -1, concerning characteristics or different examined features, the median was estimated with the help of the interquartile range.

Multivariable linear regression analysis in adjusted models was carried out to investigate the correlation of baseline renin, aldosterone concentrations and ARR with the systolic and diastolic heart dysfunction defined by the echocardiographic parameters LVEF in % and E/E' ratio respectively as well as the different clinical phenotypes of cardiac disorders. Furthermore logistic analysis was applied in order to study the relation of direct plasma renin and aldosterone concentration and ARR to the presence of asymptomatic functional cardiac disorder and heart failure.

Results were reported as relative risk (RR) with 95% confidence intervals (CIs). Due to the exploratory nature of this study, no threshold for statistical significance was set for the p-value. The p-values were considered a continuous measure of the evidence for an association. A p-value <0.05 was seen as relevant association.

Due to the explorative nature of the study, a threshold of statistical significance was not defined and p-values should therefore be interpreted as a continuous measure of statistical evidence, with lower values reflecting stronger association.

With linear regression analysis, plasma renin and aldosterone concentration as well as ARR were correlated with LVEF, E/E' and the different heart failure phenotypes sex-specifically, even

after adjusting for factors which potential can influence this association. In our first model (model 1) no adjustment was made (crude model). The second model (model 2) was adjusted for age, cardiovascular risk factors and GFR. In females, data were additionally adjusted for sex-specific confounders (i.e. information on menopausal status, intake of oral contraceptives (OC) and hormone replacement therapy (HRT) in model 2. The third model (model 3) was adjusted for drug intake that can affect the concentrations of plasma renin, aldosterone and ARR (37) (drugs for acid related disorders, antihypertensives, diuretics, beta-blockers, calcium channel blockers, agents acting on the hormonal level of plasma renin and aldosterone concentration, corticosteroids, antigout preparations). Finally, when aldosterone and renin were tested, a fourth model (model 4) was analyzed with additional adjustment for renin when aldosterone was controlled and vice versa.

All statistical analyses were performed using R version 3.14.2 software (http://www.r-project.org).

4. Results

4.1 Demographic characteristics of Gutenberg study sample

The Gutenberg Health Study included 15.010 individuals; 50.5% of them were men (n=7,584) and 49.5% were women (n=7,426). The table 1 shows main demographic and clinical characteristics of the study sample. In 14,740 was measured the renin and aldosterone plasma concentration.

4.1.1 Demographic and clinical characteristics of the study sample, according to sex

In the following table are presented the demographic, clinical characteristics, laboratory parameters, CVRF and comorbidities of the study sample, sex-specifically.

Table 1 Sex- specific demographic and clinical characteristics of the study sample (n=14,740)

	Men	**Women**
n	7,474	7,266
Sex, %	50.7	49.3
Age, years	55.3±11.1	54.8±11.1
Clinical characteristics		
BMI , kg/m²	27.9±4.3	26.9±5.6
SBP, mmHg	134±16	129±18
DBP, mmHg	83.9±9.5	81.1±9.4
Laboratory parameters		
LDL, mg/dl	139±35	139±36
HDL, mg/dl	50.4±12.4	64.5±15.4

Triglycerides, mg/dl	117.0 (85.4/164.0)	96.0 (72.0/130.0)
HbA1c, %	5.50 (5.20/5.80)	5.50(5.20/5.80)
eGFR (ml/min/1.73m²)	91.2 (81.4/100.0)	88.1 (78.2/97.3)
CVRF		
Obesity, %	26.3	24.1
Diabetes mellitus, %	11.4	7.2
Smoking, %	20.8	18.0
Arterial hypertension, %	54.6	44.8
Dyslipidaemia, %	54.7	33.7
FH of MI/Stroke, %	20.2	24.1
Comorbidities†		
History of MI, %	4.5	1.4
History of Stroke, %,	2. 4	1.3
History of AF, %	3.7	1.8
History of PAD, %	3.7	3.1
History of CAD, %	6.5	2.1
History of CHF, %	1.3	1.3
History of DVT, %	2.9	4.9
History of PE, %	0.1	0.2

Data of are presented as a relative and absolute values such as medians (Q1/Q3) with their interquartile ranges (HbA1c and GFR) and in %. † Medical records and CAPI. BMI stands for body mass index; SBP for systolic blood pressure; DBP for diastolic blood pressure; LDL for low density lipoproteins; HbA1c for glycosylated hemoglobin; eGFR for estimated glomerular filtration rate; FH for family history; MI for myocardial infarction; AF for atrial fibrillation; PAD; peripheral artery disease; CAD

for cardiac artery disease; CHF for congestive heart failure; DVT for deep venous thrombosis; PE for pulmonary embolism.

4.1.2 Sex-specific distribution of echocardiographic parameters of systolic and diastolic cardiac and cardiac phenotypes in the study sample.

The mean age (±SD) of the total sample was 55.0 ± 11.1 years (women 55.3 ±11.1 and men 54.8 ±11.1 years old. Regarding the distribution of traditional cardiovascular risk factors in the total sample, a higher prevalence of almost most of them was observed among men, compared to women, with only exception for family history of MI/Stroke. Exploring the prevalence of comorbidities among the male population CAD, MI and stroke appeared three-fold as frequent in males as in women, as expected, whereas, in females the prevalence of DVT and PE was slightly higher. The medical history of CHF did not differ between two sexes. The distribution of echocardiographic parameters, reflecting cardiac function and clinical phenotypes among male and female study participants is demonstrated in table 2.

Table 2 Sex-specific distribution of echocardiographic parameters of cardiac function in the study sample

		Men (n=7,584)	**Women (n=7,426)**
		Median (Q1/Q3)	**Median (Q1/Q3)**
Echocardiographic parameters of left ventricle	LVEF, %	62.8 (59.0/66.5)	64.1 (61.0/67.5)
	E/E' ratio	7.00 (5.7/8.8)	7.39 (6.1/9.2)
Clinical phenotypes	No FCD, %	50.2	49.8
	DD, %	49.6	50.4
	Combined	69.9	30.1

dysfunction, %		
SD, %	65.2	34.8
HFpEF, %	35.5	64.5
HFrEF, %	62.7	37.3

Data are presented as a relative values and median with their interquartile range (Q1/Q3). † Echocardiographic parameters of left ventricle. LVEF stands for left ventricular ejection fraction. † Clinical phenotypes. No FCD stands for no functional cardiac disorder; DD for isolated diastolic dysfunction SD for isolated systolic dysfunction; HFpEF for heart failure with preserved ejection fraction; HFrEF heart failure with reduced ejection fraction.

Within the present study sample, a median LVEF was found to be similar between men and women being 62.8% and 64.1 % respectively. Also, measured E/E' was higher in women than in men. Regarding the prevalence the symptomatic and asymptomatic clinical phenotypes of cardiac disorders among male and female individuals, it has been shown that distortion of systolic function (SD, combined DD and SD, as well as HFrEF) affects much more the men than the women (69.9%, 65.2% and 62.7% versus 30.1%, 34.8% and 37.3% respectively), whereas diastolic dysfunction (DD and HFpEF) occur more frequently in women than in men (50.4% and 64.5% versus 49.6% and 35.5% respectively).

4.1.3 Distribution of plasma renin, aldosterone and ARR, sex-specifically

Furthermore the levels of plasma renin and aldosterone, as well as ARR, were investigated in our total population and sex-specifically. The founding of these sample characteristics regarding the hormonal level is presented in table 3.

Table 3 Distribution of renin, aldosterone and ARR, sex-specifically

	Men (n=7,584)	**Women (n=7,426)**
	Median (Q1/Q3)	**Median (Q1/Q3)**
Renin (μU/mL)	14.40 (7.9/26.0)	9.10 (4.6/17.0)
Aldosterone (ng/dL)	7.45 (5.6/10.0)	7.55 (5.7/10.0)
ARR (μU/mL)/ (ng/dL)	5.26 (2.3/9.4)	8.38 (4.6/16.0)

Data are presented as medians (Q1/Q3) with their interquartile ranges. ARR for aldosterone to renin ratio

Interestingly, renin level was distinctly higher among males, whereas aldosterone concentration does not show a relevant difference between the two sexes, although seems to be slightly higher in females. Subsequently, ARR appeared also higher in women.

4.1.4 Sex-specific prevalence of cardiovascular risk factors and comorbidities in the different phenotypes of cardiac disorder

A further look was taken in the distribution of demographic and clinical variables in accordance to different echocardiographic phenotypes of cardiac disorder, which are presented in the Table 4.

Table 4 Sex-specific prevalence of cardiovascular risk factors and comorbidities in the different phenotypes of cardiac disorder

Men	**No FCD**	**DD**	**Combined dysfuncytion**	**SD**	**HFpEF**	**HFrEF**
n	5,386	1,090	116	351	205	104
Clinical characteristics						
BMI, kg/m²	27.4±4.1	28.9±4.3	29.1±4.0	27.8±4.0	31.4±5.7	30.2±4.9
SBP, mmHg	132±15	142+/-17	143+/-20	132+/-15	138+/-18	138+/-20
DBP, mmHg	83.6±9.0	85.7±10.2	85.3±12.6	84.2±9.2	82.6±10.9	83.0±13.0
Laboratory parameters						
LDL, mg/dl	139±34	139±36	135±38	139±36	128±37	128±35
HDL, mg/dl	50.7±12.2	49.7±12.4	51.3±11.9	50.9±12.5	48.9±13.9	47.7±14.6
Triglycerides, mg/dl	113.0	131.0	119.2	111.0	131. 0	157.5

	(83.0/185.0)	(96.0/182.3)	(90.4/159.6)	(82.5/156.8)	(99.3/181.3)	(103.6/186.7)
HbA1c, %	5.50 (5.20/5.80)	5.70 (5.40/6.10)	5.75 (5.30/6.20)	5.50 (5.20/5.70)	5.90 (5.50/6.30)	5.80 (5.44/6.20)
eGFR (ml/min/1.73m²)	93.1 (84.0/102.0)	86.3 (75.5/93.0)	84.8 (75.0/93.0)	92.7 (83.5/101.0)	83.8 (72.0/91.0)	84.3 (74.0/94.5)
CVRF						
Diabetes mellitus, %	8.2	19.4	23.3	9.4	30.2	24.0
Obesity, %	22.6	34.2	35.3	25.6	56.1	51.0
Smoking, %	22.1	13.9	19.0	22.8	19.5	30.8
Arterial hypertension, %	47.7	78.3	78.4	49.6	80.5	77.9
Dyslipidaemia, %	50.8	65.0	62.1	52.1	75.1	76.0
FH of MI/Stroke, %	19.9	18.6	17.2	24.8	28.8	31.7
Comorbidities						
History of MI, %	2.8	4.0	21.9	7.2	15.7	29.8

History of Stroke, %	1.6	3.9	3.5	2.0	7.9	6.7
History of AF, %	2.4	2.2	5.3	2.0	9.2	12.2
History of PAD, %	2.5	5.8	7.0	4.3	12.8	8.9
History of CAD, %	4.6	7.7	16.4	7.5	21.4	34.7
History of CHF, %	0.5	0	0	0	12.8	8.9
History of DVT, %	2.7	2.9	0.9	3.7	5.9	4.9
History of PE, %	0.1	0	0	0.3	1.0	0
Women	**No FCD**	**DD**	**Combined dysfunction**	**SD**	**HFpEF**	**HFrEF**
n	5,348	1,108	50	187	372	62
Clinical characteristics						
BMI, kg/m²	26.2±5.4	27.9±5.0	29.3±6.6	25.4±5.2	31.9±6,7	30.8±6.6
SBP, mmHg	126±17	139±18	140±17	127±18	137±18	134±17
DBP, mmHg	80.3±9.1	83.8±9.7	86.8±10.4	81.7±9.8	82.1±9.4	81.6±11.3
Laboratory parameters						

LDL, mg/dl	137±36	150±37	154±33	133±33	145±36	135±36
HDL, mg/dl	57.8±15.7	56.9±15.5	55.2±13.6	56.2±15.2	55.5±15.5	52.6±15.8
Triglycerides, mg/dl	101.0 (75.0/141.3)	118.4 (88.0/163.0)	117.5 (91.9/159.1)	102.0 (76.0/143.0)	124.0 (96.7/164.3)	133.5 (93.2/7173.1)
HbA1c, %	5.40 (5.10/5.70)	5.70 (5.40/6.00)	5.60 (5.30/5.90)	5.30 (5.00/5.60)	5.80 (5.50/6.20)	5.70 (5.40/6.00)
eGFR (ml/min/1.73m²)	89.8 (80.0/99.0)	82.5 (73.0/90.0)	79.9 (73.1/92.0)	90.8 (80.0/89.0)	79.9 (72.0/89.0)	86.0 (73.0/92.0)
CVRF						
Diabetes, %	5.2	10.4	12.0	1.6	24.1	16.4
Obesity, %	20.4	29.4	42.0	16.6	56.2	45.2
Smoking, %	18.9	14.6	18.0	23.0	11.4	21.3
Arterial hypertension, %	36.6	70.4	76.0	36.6	78.8	66.1
Dyslipidaemia, %	29.1	46.2	46.0	25.1	58.9	50.0

FH of MI/Stroke, %	22.8	29.0	24.0	21.9	27.2	35.5
Comorbidities						
History of MI, %	1.0	1.6	4.0	0.5	3.5	9.8
History of Stroke, %	0.9	1.7	4.0	1.1	3.2	4.8
History of AF, %	0.9	2.0	4.0	0.5	5.2	10.2
History of PAD, %	2.5	3.3	2.1	2.2	7.7	9.8
History of CAD, %	1.5	2.4	2.0	0	8.6	15.3
History of CHF, %	0.7	0	0	0	9.4	21.0
History of DVT, %	4.2	5.5	12.5	3.2	10.6	8.2
History of PE, %	0.2	0.1	0	0	0.8	0

Data are presented as relative and absolute values such as medians (Q1/Q3) with their interquartile ranges (Hb1Ac and GFR). † Medical records. BMI stands for body mass index; SBP for systolic blood pressure; DBP for diastolic blood pressure; LDL for low density lipoproteins; HbA1c for glycosylated hemoglobin; eGFR for estimated glomerular filtration rate; FH for family history; MI for myocardial infarction; AF for atrial fibrillation; PAD; peripheral artery disease; CAD for cardiac artery disease; CHF for congestive heart failure; DVT for deep venous thrombosis; PE for pulmonary embolism.

In all symptomatic cardiac disorders (HFpEF, HFrEF) there was a higher prevalence of traditional risk factors and comorbidities than in asymptomatic cardiac disorders. Interestingly, smoking was the only CVRF with higher prevalence only in HFrEF but not in HFpEF comparing to the population with normal heart function in both sexes. Although considered as a traditional risk factor for HFrEF, dyslipidemia demonstrated a higher prevalence in female HFpEF subjects. Arterial hypertension was present more often in HFpEF in both sexes, but in women the difference of hypertension occurrence between HFpEF and HFrEF was markedly higher. History of MI and CAD was more prevalent in men than in women and particularly in the phenotypes of heart failure (HFpEF and HFrEF) as well as in phenotypes of cardiac disorder with systolic dysfunction (SD) and combined cardiac dysfunction.

With regard to the asymptomatic phenotypes, there was a lower prevalence of diabetes mellitus, obesity and arterial hypertension among the population with isolated systolic dysfunction (SD), compared to clinical phenotypes with impaired diastolic function (DD and combined DD and SD) in both male and female subjects.

4.1.5 Sex-specific distribution of plasma renin, aldosterone and ARR according to the severity of cardiac dysfunction

Within the next step it has been shown interest in how direct plasma renin concentration, aldosterone level as well as aldosterone-to-renin ratio were distributed according to the severity of cardiac dysfunction, defined by the echocardiographic parameters LVEF in % and E/E' ratio (Table 5).

Table 5 Sex-specific distribution of plasma renin, aldosterone and ARR according to the severity of systolic and diastolic cardiac dysfunction

		Men (n=7,584)	Women (n=7,426)
		Median (Q1/Q3)	**Median (Q1/Q3)**
Renin (µU/mL)		14.40 (7.9/26.0)	9.10 (4.6/17.0)
LVEF, %	- ≥55%	14.1 (7.8/25.0)	9.10 (4.7/17.0)
	- 45-55%	16.3 (8.9/33.0)	10.2 (4.6/20.0)
	- 30-44%	21.4 (8.6/64.0)	5.90 (1.2/12.0)
	- <30%	21.8 (16.0/47.0)	6.60 (1.2/12.0)
E/E‘	- ≥ 10	14.5 (7.0/35.0)	9.00 (4.2/19.0)
	- <10	14.3 (8.1/25.0)	7.56 (4.7/16.0)
Aldosterone (ng/dL)		7.45 (5.6/10.0)	7.55 (5.7/10.0)
LVEF, %	- ≥55%	7.42 (5.6/10.0)	7.54 (5.7/10.0)
	- 45-55%	7.66 (5.5/11.0)	7.72 (5.7/11.0)
	- 30-44%	8.52 (5.1/11.0)	8.59 (5.8/12.0)
	- <30%	10.8 (5.6/14.0)	14.1 (12.0/25.0)

	- ≥ 10	7.70 (5.6/10.5)	7.50 (5.6/10.0)
E/E'	- <10	7.41 (5.6/10.0)	7.56 (5.8/10.0)
ARR (μU/mL)/ (ng/dL)		5.26 (2.3/9.4)	8.38 (4.6/16.0)
LVEF, %	- ≥55%	5.36 (2.9/9.5)	8.42 (4.7/16.0)
	- 45-55%	4.47 (2.4/8.7)	7.89 (4.3/15.0)
	- 30-44%	3.87 (1.3/8.1)	15.5 (7.6/33.0)
	- <30%	2.43 (0.2/8.5)	22.3 (8.1/38.0)
E/E'	- ≥ 10	5.23 (2.0/11.0)	8.32 (3.8/18.0)
	- <10	5.28 (2.3/9.2)	8.40 (4.8/16.0)

Data are presented as medians (Q1/Q3) with their interquartile ranges. † echocardiographic parameters. LVEF stands for left ventricular ejection fraction; ARR for aldosterone to renin ratio.

Within male subjects, renin and aldosterone concentration increased with decreasing LVEF. Interestingly, the renin concentration in men with LVEF <30%, was substantially higher than in women with the same grade of systolic dysfunction (21.8 vs. 6.60 (μU/mL)). Aldosterone concentrations in severe reduced systolic function were also higher as in normal cardiac function in both sexes. Pronounced differences have been further found for ARR between men and women, especially in those with LVEF <30%. Although ARR was gradually decreasing with decreased LVEF in males, exactly the opposite direction was observed among females: female subjects with severe systolic dysfunction had the highest ARR-values.

With regard to the diastolic function, all studied biomarkers showed no significant difference according to the severity of diastolic function in men. Oppositely, a greater difference in the renin concentration was observed in women. Particularly, a higher concentration of renin was shown

as E/E', the major component for classifying the diastolic dysfunction into normal or mild (E/E'<10) and moderate or severe (E/E' >10), was higher.

4.1.6 Sex-specific distribution of plasma renin, aldosterone and ARR in the different phenotypes of cardiac disorders.

Interest was paid in the distribution of renin, aldosterone and ARR in these with normal heart function and in accordance to five clinical phenotypes of cardiac dysfunction, DD, SD, combined dysfunction, HFpEF, HFrEF sex-specifically. The results are presented in the Table 6.

Table 6 Sex-specific distribution of plasma renin, aldosterone and ARR in the different phenotypes of cardiac disorders

	Men (n=7,584)			Women (n=7,426)		
	Median (Q1/Q3)			Median (Q1/Q3)		
	Renin (μU/mL)	**Aldosterone (ng/dL)**	**ARR (μU/mL)/ (ng/dL)**	**Renin (μU/mL)**	**Aldosterone (ng/dL)**	**ARR (μU/mL)/(ng/dL)**
Clinical phenotypes						
No FCD, %	14.00	7.31	5.36	9.00	7.55	8.51
	(8.0/24.0)	(5.5/9.8)	(3.0/9.3)	(4.7/16.0)	(5.7/10.0)	(4.9/16.0)
DD, %	14.10	7.70	5.46	9.00	7.46	8.29
	(7.0/33.0)	(5.6/10.2)	(2.2/11.0)	(4.6/18.0)	(5.7/9.9)	(4.3/17)
Combined dysfunction, %	17.25	7.70	4.41	8.55	8.02	8.07
	(7.0/49.0)	(5.3/11.0)	(1.8/11.0)	(3.5/21.0)	(5.8/10.0)	(4.0/18.0)
SD, %	16.40	7.38	4.46	9.30	7.71	8.14
	(9.40/28.0)	(5.3/10.0)	(2.4/8.0)	(4.3/17.0)	(5.8/11.0)	(4.8/17.0)

HFpEF, %	19.30	8.55	4.46	10.80	7.65	7.19
	(8.5/68.0)	(6.2/12.0)	(1.5/9.6)	(4.1/37.0)	(5.6/11.0)	(2.4/19.0)
HFrEF, %	22.10	8.88	4.11	13.60	7.99	7.41
	(10.0/49.0)	(6.2/12.0)	(1.3/9.0)	(5,8/26.0)	(5.1/11.0)	(2.7/12.0)

Data are presented as medians (Q1/Q3) with their interquartile ranges. † Clinical phenotypes. No FCD stands for no functional cardiac disorder; DD for isolated diastolic dysfunction; SD for preserved diastolic function with reduced ejection fraction; HFpEF for heart failure with preserved ejection fraction; HFrEF heart failure with reduced ejection fraction; ARR for renin aldosterone ratio.

In general, the heart failure phenotypes, namely HFrEF and HFpEF, were associated with higher level of renin and aldosterone concentrations and lower ARR compared to asymptomatic cardiac dysfunction in men. In females, a similar trend has been seen for direct renin concentration and ARR, whereas for aldosterone, slightly higher values were observed in cardiac dysfunction, associated with reduced LVEF, than in subjects in whom LVEF was preserved.

In regard with the asymptomatic disorders, plasma renin concentration in men with combined diastolic and systolic disorder was higher than in those with isolated diastolic or systolic dysfunction (DD and SD). In women, aldosterone concentration revealed a similar pattern of association like it was seen for renin in men. Females with asymptomatic combined diastolic and systolic disorder had a higher level of aldosterone concentration in serum than isolated diastolic or systolic dysfunction (DD and SD).

4.2. Results of the regression analysis

4.2.1 Association between plasma renin, aldosterone and ARR with the systolic and diastolic cardiac dysfunction

To assess the association between aldosterone, direct renin and their ratio with echocardiographic measures of systolic (i.e. LVEF) and diastolic (i.e. E/E') function a multivariable linear regression analysis was conducted sex specifically. Several levels of adjustment have been performed to eliminate a possible impact of confounding factors on the above-mentioned association. Within the first model (model 1) no adjustment has been done (so called crude model). In the second model (model 2), it was adjusted for age, traditional cardiovascular risk factors (diabetes mellitus, hypertension, hyperlipidemia, smoking, obesity and positive family history of MI/stroke) and eGFR. In females, an additional adjustment for sex-specific variables as well as information on menopausal status, intake of oral contraceptives (OC) and hormone replacement therapy (HRT) was done. In the next model (model 3) drugs, which affect the concentration of the studied biomarkers within the same population (previous results from Hermann et al (REF)) such as drugs for acid related disorders, diuretics, beta-blockers, calcium channel blockers, agents acting on the RAAS, corticosteroids, antigout preparations were accounted. Finally, an additional fourth model, it was calculated only for aldosterone and renin, where an additional adjustment for renin in case of aldosterone was done and vice versa (please see Table 7).

Table 7 Relation between plasma renin, aldosterone and ARR with the systolic and diastolic cardiac function, as determined by the echocardiographic parameters LVEF and E/E'

	LVEF, %		E/E'	
	B-estimate 95% (CI)	**p-value**	**B-estimate 95% (CI)**	**p-value**
Men (n=7,294)				
Direct renin concentration (µU/mL)				
Crude (Model 1)	-0.00150 (-0.003/-0.001)	**0.0025**	0.000126 (0.0001/0.0002)	**<0.0001**
Model 2	-0.00114 (-0.002/-0.0002)	**0.024**	-0.0000297 (-0-0001/0.00002)	0.20
Model 3	-0.000739 (-0.002/0.0003)	0.16	-0.000053 (-0.0001/-0.0001)	**0.029**
Model 4	-0.000514 (-0.002/0.001)	0.33	-0.0000523 (-0.0001/-0.000004)	**0.033**

Aldosterone concentration (ng/dL)				
Crude (Model 1)	-0.0944 (-0.1/-0.1)	**<0.0001**	0.00397 (0.002/0.006)	**<0.0001**
Model 2	-0.0799 (-0.1/-0.05)	**<0.0001**	0.000566 (-0.001/0.002)	0.43
Model 3	-0.0603 (-0.1/-0.029)	**0.00016**	-0.000638 (-0.002/0.001)	0.62
Model 4	-0.0585 (-0.090/-0.03)	**0.00028**	-0.000183 (-0.002/0.001)	0.81
ARR(µU/mL)/ (ng/dL)				
Crude (Model 1)	0.000496 (-0.003/0.004)	0.76	0.000363 (0.0002/0.001)	<0.0001
Model 2	0.000652 (-0.003/0.004)	0.68	0.000121 (-0.00002/0.0003)	0.10

Model 3	0.000886	0.58	0.0000845	0.25
	(-0.002/0.004)		(-0.0001/0.0002)	
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Women (n=7,092)				
Direct renin concentration (µU/mL)				
Crude (Model 1)	-0.000537	0.40	0.000183	<0.0001
	(-0.002/0.001)		(0.0001/0.0003)	
Model 2	-0.000447	0.49	-0.0000203	0.55
	(-0.002/0.001)		(-0.0001/0.0001)	
Model 3	-0.000172	0.80	-0.0000474	0.18
	(-0.002-0.001)		(-0.0001/0.00002)	
Model 4	0.0000119	0.99	-0.0000444	0.21
	(-0.001/0.001)		(-0.0001/0.00002)	

Aldosterone concentration (ng/dL)				
Crude (Model 1)	-0.0612 (-0.09/-0.04)	**<0.0001**	-0.00258 (-0.004/-0.001)	**0.0011**
Model 2	-0.0600 (-0.09/-0.03)	**<0.0001**	-0.000735 (-0.002/0.001)	0.30
Model 3	-0.0542 (-0.08/-0.03)	**<0.0001**	-0.000971 (-0.002/0.0004)	0.18
Model 4	-0.0542 (-0.08/-0.03)	**<0.0001**	-0.000909 (-0.002/0.001)	0.21
ARR(μU/mL)/ (ng/dL)				
Crude (Model 1)	-0.00237 (-0.005/0.0001)	0.059	0.000404 (0.0003/0.001)	**<0.0001**
Model 2	-0.00262 (-0.01/-0.001)	**0.038**	0.000125 (-0.000004/0.0003)	0.058

Model 3	-0.00273	**0.034**	0.000105	0.12
	(-0.01/-0.0002)		(-0.00003/0.0002)	
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Data are presented as medians (Q1/Q3) with their interquartile ranges. CI, confidence interval; OR stands for odds ratio. Model 1: crude model. Model 2: adjustment for age, CVRF (diabetes mellitus, hypertension, hyperlipidemia, smoking, obesity and positive family history of MI/stroke), GFR and menopausal status and OC/HRT intake (in females only). Model 3: additionally adjusted for drug medication that influence the hormonal level (drugs for acid related disorders, antihypertensives, diuretics, beta-blockers, calcium channel blockers, agents acting on plasma renin and serum aldosterone, corticosteroids, antigout preparations) Model 4: additionally adjusted for renin if aldosterone used as independent variable and vice versa. LVEF stands for left ventricular ejection fraction.

With regard to the association between the RAAS biomarkers with systolic function, assessed by LVEF, it has been found that in men increasing renin concentration was related to decreased systolic function independently from age, traditional cardiovascular risk factors and eGRF. However, when medication, affecting direct renin concentration was taken into account, the initially observed association disappeared. In females no association was found neither in crude nor in the fully adjusted model.

In contrast, strong results have been revealed for aldosterone concentration. Indeed, it could be demonstrated that increasing aldosterone concentration was associated with worsening of systolic function and neither age nor traditional risk factors, renal function, medication or renin concentration affected this association in significant way. Each increase in aldosterone concentration (per unit increase) resulted in the reduction of left ventricular systolic function by 0.06 % in men and 0.05% in women in a fully adjusted model.

With regard to ARR, a significant sex differences were observed with regard to it association with systolic function of LV. Although in men ARR was not related to LVEF neither in crude not in multivariable adjusted analyses, in females increase in ARR was associated with significant reduction of LVEF in a fully adjusted model.

Regarding the relation of renin concentration to diastolic function, defined by the E/E' ratio, in men, it has been presented that increasing renin concentration level was associated with an increase of E/E' and worsening of diastolic cardiac function, even after adjustment for age, traditional CVRF, GFR, drug agents that affect direct renin concentration in plasma and aldosterone. Nevertheless, if only adjusted for age, CVRF and GFR and not for medication with impact on renin, this significant association was faded. Each increase in renin concentration (per unit increase) resulted in the increase of E/E' by 0.00005 (worsening of diastolic function) in men in a fully adjusted model. In woman, no significance between renin and diastolic function was shown at all.

Studying the association of aldosterone with diastolic dysfunction in men, a statistical strong relation was observed only in the crude model, whereas adjusting for age, traditional CVRF, GFR, medication that could affect aldosterone, and renin, the initially found association disappeared. Exactly the same result was observed examining the relation of aldosterone concentration and diastolic dysfunction in women. A statistical important association was seen only in the crude model, while after confounder factors were taken into account, it was not to be noticed anymore.

Referring to ARR and its relation to E/E', a similar interesting result was shown, comparing to that of ARR and LVEF. Once again significant sex differences were observed with regard to it association with diastolic cardiac function. Although in men ARR was not related to E/E' in

any model, in females increase in ARR was associated with significant increase of E/E', though just in the crude model.

4.2.2 Association between plasma renin, aldosterone and ARR with the clinical phenotypes of cardiac function

Finally, the relationship of renin, aldosterone and ARR to the different clinical phenotypes of cardiac dysfunction was investigated. For this purpose, a logistic linear regression analysis with similar level of adjustment (i.e. four similar models, which has been used for linear regression analysis) was performed. Phenotypes of asymptomatic cardiac disorder (DD, SD and combined systolic and diastolic dysfunction), as well as of heart failure (HFpEF and HFrEF) were compared to the group of subjects in whom no FCD could be diagnosed. The results of this analysis are reported in the table 8.

Table 8 Association of plasma renin, aldosterone and ARR to the clinical phenotypes of cardiac disorders

	DD vs. No FCD		Combined dysfunction vs. No FCD		SD vs. No FCD		HFpEF vs. No FCD		HFrEF vs. No FCD	
	OR 95% (CI)	**p-value**	**OR 95% (CI)**	**p-value**	**OR 95% (CI)**	**p-value**	**OR 95% (CI)**	**p-value**	**OR 95% (CI)**	**p-value**
Men	**(n= 1,078)**		**(n= 115)**		**(n= 344)**		**(n= 202)**		**(n= 103)**	
Direct renin concentration (µU/mL)										
Crude (Model 1)	1.001 (1.000/1.001)	0.0017	1.000 (1.000/1.001)	0.51	1.000 (1.00/1.00)	0.97	1.001 (1.001/1.002)	**<0.0001**	1.001 (1.001/1.002)	**<0.0001**
Model 2	1.000 (1.00/1.00)	0.78	1.000 (1.000/1.001)	0.61	1.000 (1.00/1.00)	0.68	1.000 (1.00/1.00)	0.17	1.001 (1.000/1.001)	**0.012**
Model 3	1.000 (1.00-1.00)	0.48	0.999 (0.997/1.000)	0.38	1.000 (1.00/1.00)	0.38	1.000 (1.00/1.00)	0.50	1.000 (1.00/1.00)	0.29
Model 4	1.000	0.47	0.999	0.35	1.000	0.41	1.000	0.64	1.000	0.59

	(1.00/1.00)		(0.997/1.000)		(1.00/1.00)		(1.00/1.00)		(1.00-1.00)	
Aldosterone concentration (ng/dL)										
Crude (Model 1)	1.016 (1.002/1.030)	**0.023**	1.037 (1.01/1.06)	**0.0041**	0.996 (0.97/1.02)	0.76	1.051 (1.03/1.07)	**<0.0001**	1.055 (1.04/1.09)	**<0.0001**
Model 2	1.002 (0.99/1.02)	0.84	1.019 (1.00/1.05)	0.19	0.988 (0.96/1.02)	0.42	1.029 (1.001/1.05)	**0.0051**	1.049 (1.03/1.07)	**<0.0001**
Model 3	1.002 (0.99/1.02)	0.76	1.015 (1.00/1.04)	0.34	0.986 (0.96/1.01)	0.34	1.021 (1.00/1.04)	0.064	1.030 (1.00/1.06)	**0.033**
Model 4	1.003 (0.99/1.02)	0.73	1.016 (1.00/1.05)	0.29	0.987 (0.96-1.01)	0.37	1.021 (1.00/1.04)	0.075	1.028 (1.00/1.06)	0.056
ARR(μU/mL)/ (ng/dL)										
Crude (Model 1)	1.002 (1.000/1.003)	0.015	1.000 (0.99/1.00)	0.84	0.995 (0.99/1.00)	0.16	1.002 (1.00/1.004)	0.013	1.001 (1.000/1.004)	0.42
Model 2	1.001	0.42	0.999	0.78	0.995	0.15	1.001	0.18	1.001	0.60

	(1.000/1.002)		(0.99/1.01)		(0.99/1.00)		(1.00/1.004)		(1.000/1.004)	
Model 3	1.001	0.27	0.999	0.65	0.996	0.22	1.001	0.25	1.001	0.74
	(1.000/1.002)		(0.99/1.01)		(0.99/1.00)		(1.00/1.003)		(1.000/1.004)	
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Women	**(n= 1,097)**		**(n= 48)**		**(n= 181)**		**(n= 368)**		**(n= 60)**	
Direct renin concentration (µU/mL)										
Crude (Model 1)	1.000	0.81	1.001	0.52	0.995	0.097	1.001	**<0.0001**	1.001	0.26
	(1.00/1.00)		(1.00/1.002)		(0.99/1.00)		(1.00/1.002)		(1.00/1.002)	
Model 2	0.999	**0.017**	1.000	0.77	0.995	0.13	1.000	0.35	1.000	0.87
	(0.998/1.00)		(1.00/1.00)		(0.99/1.00)		1.00/1.00)		(1.00/1.00)	
Model 3	0.999	**0.028**	1.000	0.99	0.996	0.22	1.000	0.87	0.999	0.60
	(0.998/1.00)		(1.00/1.00)		(0.99/1.00)		(1.00/1.00)		(0.996/1.00)	
Model 4	0.999	**0.029**	1.000	0.95	0.996	0.21	1.000	0.94	0.999	0.57
	(0.998/1.00)		(1.00/1.00)		(0.99/1.00)		(1.00/1.00)		(0.996/1.001)	

Aldosterone concentration (ng/dL)										
Crude (Model 1)	0.984 (0.97/1.00)	**0.030**	1.006 (0.95/1,05)	0.82	1.011 (0.98/1.04)	0.42	0.018 (1.00/1.04)	**0.049**	1.016 (1.00/1.05)	0.45
Model 2	0.999 (0.98/1.02)	0.89	1.020 (0.96/1.07)	0.46	1.001 (0.97/1.03)	0.93	1.017 (1.00/1.04)	0.13	1.020 (1.00/1.06)	0.37
Model 3	0.998 (0.98/1.02)	0.85	1.045 (0.98/1.09)	0.10	1.002 (0.97/1.03)	0.92	1.018 (0.99/1.04)	0.13	1.020 (1.00/1.06)	0.41
Model 4	1.000 (0.98/1.02)	0.98	1.045 (0.98/1.09)	0.10	1.005 (0.97/1.03)	0.77	1.017 (0.99/1.04)	0.14	1.021 (1.00/1.06)	0.38
ARR(μU/mL)/ (ng/dL)										
Crude (Model 1)	1.001 (1.00/1.002)	0.38	1.003 (1.00/1.005)	**0.018**	1.000 (1.00/1.002)	0.99	1.002 (1.00/1.003)	**0.023**	0.999 (0.990/1.003)	0.82
Model 2	0.999	0.061	1.002	0.15	1.000	0.99	1.001	0.47	0.998	0.55

	(0.997/1.00)		(1.00/1.004)		(1.00/1.002)		(1.00/1.002)		(0.900/1.003)	
Model 3	0.998	**0.042**	1.002	0.13	1.000	0.95	1.001	0.28	0.998	0.58
	(0.997/1.00)		(1.00/1.004)		(1.00/1.002)		(1.00/1.003)		(0.990/1.003)	
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Data are presented as medians (Q1/Q3) with their interquartile ranges. CI, confidence interval; OR stands for odds ratio. Model 1: crude model. Model 2: adjustment for age, CVRF (diabetes mellitus, hypertension, hyperlipidaemia, smoking, obesity and positive family history of MI/stroke), GFR and menopausal status and OC/HRT intake (in females only). Model 3: additionally, adjusted for drug medication that influence the hormonal level (drugs for acid related disorders, antihypertensives, diuretics, beta-blockers, calcium channel blockers, agents acting on plasma renin and serum aldosterone, corticosteroids, antigout preparations) Model 4: additionally adjusted for renin if aldosterone used as independent variable and vice versa. † Clinical phenotypes. No FCD stands for no functional cardiac disorder; DD for diastolic dysfunction with preserved ejection fraction; SD for isolated systolic dysfunction; HFpEF for heart failure with preserved ejection fraction; HFrEF heart failure with reduced ejection fraction; ARR for renin aldosterone ratio.

With regard to the association between various RAS biomarkers (aldosterone and renin) as well as their ratio (ARR) with different clinical phenotypes of cardiac dysfunction, it has been found that in men increasing renin concentration was related to DD in the crude model, while after adjusting for age, traditional cardiovascular risk factors and eGRF as well as medication that affect RAAS-hormones, and aldosterone concentration, showed no significance. Oppositely, in women, although no important relation of renin with DD was presented in the crude model, when after adjusting for all the above mentioned confounder factors, a strong association has been shown. Each increase in renin concentration (per unit increase) resulted in thincrease of possibility to occur DD in women in a fully adjusted model.

Aldosterone was strongly associated to DD in men and women only in the crude model. However, if age, traditional CVRF, GFR, medication, affecting direct renin concentration have been taken into account, an initially observed association disappeared completely and became statistically insignificant.

Investigating the ARR in concern of DD, an interesting sex-specific difference has been shown. In men the relation of ARR to DD was presented only in the crude model, whereas in women in every other adjusted model except from the crude.

Regarding renin and combined dysfunction in men and women, an association at all was found, neither in crude nor in a fully adjusted model. Comparing to women, in whom aldosterone and combined dysfunction did not prove to be associated, in men aldosterone has been shown to be statistically significant associated with combined dysfunction in the crude model. However, taking into account the confounder factors that can potentially affect aldosterone, this initially strong association shown, was lost.

ARR was found to be associated to combined dysfunction only in women, if no adjustment was applied. In men no relation of ARR to combined dysfunction has been shown.

Examining the possible relationship of renin, aldosterone and ARR to SD sex-specifically, no association of any hormone or their ratio has been demonstrated in any model of our analysis, neither in women nor in men.

Analyzing further the relation of renin with HFpEF in men, significance has been found in crude model. However, if age, CVRF, GFR, medication, affecting direct renin concentration and aldosterone concentration have been adjusted, this initially observed strong association disappeared completely and became statistically not significant. Exactly the same result has been demonstrated in women.

Although aldosterone was related in a statistical important way to HFpEF in the crude model in women, in men significance has also been shown, not only in the crude model, but also if age, CVRF and GFR were taken into account.

Regarding ARR and HFpEF only in women and only in the crude model has been shown a significant association. In males, no association was found at all; neither in crude nor in a fully adjusted model.

Concerning the association of renin to HFrEF in men, a significant relation has been presented when studying the crude model as well as after adjusting for age, CVRF, and GFR. Aldosterone was also significantly associated to HFrEF in men, after additional adjustment for medication which could affect the levels of renin concentration in plasma. In women a strong association of either renin or aldosterone to HFrEF has been failed to be proven. Similary, ARR has been related to HFrEF neither in men nor in women.

5. Discussion

The main purpose of the present analysis was to investigate the association between aldosterone, renin and concentration as well as their ARR to the systolic and diastolic cardiac function, as defined by LVEF and E/E' respectively. In addition, the relationship of above mentioned biomarkers and different clinical phenotypes of cardiac dysfunction, namely the asymptomatic (NYHA I) isolated diastolic dysfunction (DD) the asymptomatic (NYHA I) isolated systolic dysfunction (SD) the asymptomatic (NYHA I) combined systolic and diastolic dysfunction heart failure (NYHA>I) with preserved LVEF (HFpEF) and heart failure (NYHA>I) with reduced LVEF (HFrEF) has been also studied.

Our analyses were performed sex- specifically in order to detect any sex-related differences of mechanisms involved in the pathogenesis of clinical phenotypes of heart failure.

As supported ''DHF and SHF as successive stages or distinct phenotypes of the HF syndrome'' (401). In this regard, it has been suggested that the risk factors and comorbidities HFpEF and HFrEF patients differ between men and women.

General, ,clinically manifested HF, on the contrary to asymptomatic cardiac dysfunction, reflects the hemodynamic failure to supply efficient blood, regardless of rest or exertional state and independently of increased filling pressure and is a clinical entity characterized by symptoms (principally dyspnea) and signs (predominately edema) accompanying a heart dysfunction (9). Although the clinical symptoms of both HF forms is similar, the pathophysiological underlying mechanisms and risk factors differ significantly among these two forms, resulting in various prevalence, the rates of mortality and survival (92), the prognosis as well as the discrepancies in medical treatment.

Numerous studies have already showed that the pathophysiological model of HFpEF might be linked to pro inflammatory state. Additionally, it is observed that HFpEF is highly associated with non-cardiac comorbidities, which trigger a pro-inflammatory environment, which gives birth to HFpEF. This data suggests a high prevalence of non-cardiac systematic comorbidities, such as diabetes mellitus and obesity and CVRF in HFpEF. Hypertension is also a major comorbidity observed with high frequency in HFpEF. Furthermore, overweight/obesity, diabetes mellitus (DM), chronic kidney disease are also commonly observed in HFpEF population. The thread that joins these, seemingly completely different diseases is inflammation, which consist the pathophysiological base of HFpEF.

In line with these observations are the results from our study,that have been shown that obesity, hypertension and diabetes were more frequent in the HFpEF than in HFrEF in both sexes. The same difference in the prevalence of these CVRF was observed regarding the

asymptomatic diastolic dysfunction, isolated or combined with reduction of the systolic function (DD, combined dysfunction) comparing to isolated systolic dysfunction with normal diastolic function (SD). That is an interesting observation, as the idea of the existence of a common link between these diseases and diastolic dysfunction is supported, independently from the presence of clinical symptoms or sex-differences.

The paradigms of HFrEF and HFpEF development appear to be significantly different. Whereas HFpEF is, at least in particular, caused by inflammation, HFrEF is rather the result of massive loss of cardiomyocytes. As the main pathogenetic mechanism differs, a difference in the prevalence of CVRF and comorbidities is also expected. Indeed, comorbidities such as CAD and MI such as the CVRF that cause theses cardiovascular diseases such as smoking and dyslipidemia, through endothelial injury and atheromatosis, are more often present in people with systolic dysfunction in compare to these with normal systolic function.

As already described in the results' section, it seems that men suffering from MI and CAD are three-fold as many as women, whereas women suffering from DVT and PE are approximately twice as many as men. A possible assumption to explain these observations might be the higher frequency of CVRF such as smoking and dyslipidemia, in men on the one side and the consumption of anti-contraceptive pills by premenopausal women and hormone replacement therapy by the postmenopausal ones, drugs which increase the risk of thrombosis and consequently of DVT and PE, on the other. The different prevalence of CVRF and comorbidities between men and women may support the idea of existence of sex-specific different underlying mechanisms.

An interesting observation regarding the arterial hypertension, is that its prevalence, presented mainly in patients with diastolic dysfunction, is almost as high as in patients with asymptomatic isolated diastolic dysfunction or combined dysfunction (DD or combined dysfunction) and symptomatic diastolic heart failure (HFpEF) in both sexes. The prevalence of remaining CVRF and CV comorbidities, though, was higher in symptomatic cardiac disorders in compare to asymptomatic.

Exploring the prevalence of hypertension in the five clinical phenotypes of cardiac dysfunction, it was observed that, although the prevalence of arterial hypertension is very similar in both HFpEF and HFrEF among men (difference of 3% favoring HFpEF), it is clearly higher among women with HFpEF than in female patients with HFrEF (difference of 12% favoring HFpEF). This is consistent to evidence from the literature that suggest that women present more frequent a concentric hypertrophy pattern, whereas men eccentric remodeling. On the other side it can be assumed that not only hypertension is involved in the pathogenesis of HFpEF, as already proved by numerous studies, but also that there should

be another component in this association between hypertension and HFpEF, particularly in women.

Another interesting observation from our study was the prevalence of dyslipidemia and hypertension in male and female patients with HFpEF and HFrEF. Whereas the prevalence of both risk factors was found to be very similar between mela subjects with HFpEF and HFrEF, in women an important difference was revealed. The prevalence of arterial hypertension and dyslipidemia in female patients with HFpEF was distinctly higher than in those with HFrEF. A possible explanation would be that women with HFrEF are more frequent medical treated comparing to those suffering from HFpEF, possible because the diagnosis of HFpEF is oft more complicated. As CAD and MI are more frequent comorbidities among men, it is easily to understand that dyslipidemia, one of the main risk factors, is in most of the cases diagnosed and appropriately treated, before a reduction of systolic dysfunction due to loss of cardiomyocytes occurs.

Men have dyslipidemia twice as frequently as women, which is a causal factor of MI, which in turn can result in HFrEF. Indeed, we observed that males with HFrEF were more than women. Women have higher rates of obesity, people with HFpEF are more frequent obese and women with HFpEF are twice as more as these with HFrEF. Moreover, unlike to HFrEF, hypertensive, older women, with a lower prevalence of an ischemic coronary event, is the most common clinical profile of patients with HFpEF, as observed by multiple studies. This simple observation of our study supports the established knowledge that the occurrence of the clinical phenotypes of cardiac disorders differ between men and women.

Looking into observational data from literature, it becomes clear that, although heart failure is more frequent among males comparing to females (2.5% vs. 1.8%), cardiovascular mortality of heart failure seems to be higher among female population (58.2% vs. 41.8%) (402). Surprisingly, one out of three women in the US die from HF (282) and almost 2.5 million women suffer from this disease (282).

As mentioned above, the NYHA classification is generally used in order to divide the clinical phenotypes of heart failure into asymptomatic (NYHA I) or symptomatic ones (NYHA>I). From out total population, 87.8% (n=12,919) were asymptomatic (NYHA I), whereas 1,250 study participants were classified as symptomatic (n=) with NYHA II (9.6%), NYHA III (1.9%), NYHA IV (0.8%).

Looking further into distribution of cardiac phenotypes among present study sample, regardless of sex, some interesting observations can be done. The largest part of the randomly chosen, examined population, namely 74.7% (n=10,734) had no functional cardiac dysfunction, while only almost 25.3% (n=4,006) had an abnormal diastolic, systolic or

combined asymptomatic or symptomatic disorders. With regard to the asymptomatic dysfunctions, 15.3 % (n=2,198) of entire study population had DD, the 3.7% (n=538) had SD and the 1.2% (n=166) had combined dysfunction. From the symptomatic ones, 4.0% (n=577) presented with HFpEF and only 1.2% (n=166) with HFrEF.

Our results seem to be in line with those of Ammar et al. (19) , who reported that more subjects from the general population-based study have an asymptomatic disorder, comparing to symptomatic (34% versus 12%, respectively). Our results showed that that asymptomatic phenotypes of cardiac disorders (DD, SD and combined dysfunction) represented in 20.2% study population, and they are four-fold as frequent as the symptomatic ones (HFpEF and HFrEF) which were only the 5.2%. Such an observation deriving from our study gains more statistical strength as our sample was 15,010 people comparing that of Ammar et al. which was just 2,029. Nevertheless, our findings are in agreement with the statement by Zile et al. (55) that whereas diastolic dysfunction or heart failure can coexist isolated, systolic dysfunction or heart failure is in most of the cases followed by diastolic abnormalities. Indeed, as observed from our study, DD and HFpEF consisted 19.3% in the total population and the 18.8% of people with abnormal heart function while SD and HFrEF with isolated systolic dysfunction presented in only 4.9% of total population.

Moreover, although combined diastolic and systolic dysfunction usually appear together, we found that this combined dysfunction existed only in almost 1.2% comparing to each of isolated diastolic and systolic dysfunction (DD and SD, respectively) which is presented in 15.3% and 3.7% of GHS subjects respectively. Moreover, diastolic heart failure with preserved EF (HFpEF), which affect predominantly a diastolic dysfunction, appeared in the 4.0% of the population comparing to systolic heart failure with or without diastolic abnormalities (HFrEF) which affected only the 1.2% of our total sample. Owan et al. (92) suggested that, epidemiologically, HFpEF portrays half of the cases of HF. However, we observed that this clinical phenotype is not only very often among people with HF, but also it concerns the 76.9% of the overall HF entity.

Worth mentioned is that although the dominant abnormality in HFpEF is the diastolic dysfunction and in HFrEF the systolic, both types of HF present a degree of diastolic disorder as defined by restricted relaxation, higher degree of stiffness and increased filling pressures (41, 55) and at least limited, comparing to HFrEF, systolic dysfunction can also occur in HFpEF (41, 68, 91). Under the conception that HFpEF and HFrEF are two links of the same chain, it is also implied that HFpEF may be a precursor of HFrEF, transformation which is not, though, seem to take place when the major predisposing, ischemic, coronary event for HFrEF has not happened before (403). Decrease of LVEF is a main characteristic of HFrEF. It does not define the ventricular contractility (91), it is just a measurable criterion for its

evaluation and a result of the increased LV mass, basically in eccentric hypertrophy (remodelling in HFrEF) and not in concentric alteration of LV (remodelling in HFpEF) (404).

Diastolic dysfunction or diastolic heart failure can exist isolated (increased pressure to normal volume, normal EF), in contrary to the systolic (decreased EF) which progressively develops diastolic characteristic abnormalities as the loss of myocardium is replaced from myofibroblasts causing fibrosis and stiffness. In these terms, it can be concluded, that diastolic heart failure can be considered as an independent clinical entity, likely to occur isolated (normal EF), while in contrast the systolic heart failure at late stages is almost always accompanied by diastolic-typical abnormalities (combined diastolic and systolic heart failure).

Data from several studies has already showed that the prevalent rate of female sex in HFpEF is almost twice as high as in HFrEF. Indeed, the same result derived from the epidemiological study of our population, as the clinical phenotypes with reduced systolic function, isolated (SD) or in combination to diastolic dysfunction, as well as HFrEFwere much more frequent in male population, whereas the prevalence of diastolic disorders (DD and HFpEF) where presented mainly in females.

It is well known, the fact that sex difference exists for renin concentration that also has been confirmed by the present analysis. In men, plasma direct renin concentration levels seem to be distinctly higher than in women (14.40 vs. 9.10 µU/ ml). That raises the question on underlying pathophysiologic mechanisms and especially on clinical impact of renin on the cardiac function. On the other side, a minimally higher level of aldosterone concentration was observed in women (7.55 vs. 7.45ng/dL). Despite of the evidence by Lang (23) which suggests that estrogens are able to antagonize RAAS system, in the literature is also reported that oral anti-contraceptives or HRT with estradiol for example, which are followed by hepatic metabolism, stimulates angiotensinogen production and aldosterone generation, which could also explain the slightly higher concentration of aldosterone in women.

The present results show a relation of renin level to the worsening of systolic and diastolic cardiac function, defined by LVEF and E/E'. The worse the heart function, the higher was the plasma renin in men. In particularly, a linear increase was shown. Especially a higher renin concentration was to observe as the LVEF under 30% fell in men. Interestingly, no relevant increase of renin was shown in women, in which, surprisingly, a lower level of plasma renin concentration was presented in the severe systolic dysfunction. Oppositely to renin levels in women in the different stages of systolic dysfunction, which fail to show a linear increase, aldosterone concentration was found to be clearly higher in the severe systolic dysfunction in women. Similarly to renin levels, aldosterone was increased in the late stages of systolic dysfunction in men.

Aldosterone concentration presented to rise as the diastolic function of heart worsened in both men and women.

As far as the diastolic dysfunction defined by one major echocardiographic parameter E/E', was presented, some interesting results were to be observed. Plasma renin concentration presented no increase of its level worsening the diastolic dysfunction in men, but not in women. On the contrary, aldosterone level was higher in the severe diastolic dysfunction in men, comparing to the normal diastolic function of the mild dysfunction. In women, no difference was shown regarding the plasma aldosterone level in different stages of diastolic dysfunction.

RAAS regulation seems to depend also upon other hormonal-to-hormonal interactions, such as the ovarian hormones. Ovarian hormones, estrogen and progesterone fluctuate during the menstrual cycle, which could explain variability between female population and between two sexes depending on the menstrual phase of woman (231). Summing up, the pathophysiological mechanisms involved in the regulation of RAAS by ovarian hormones is the following crosstalk signaling: estrogens, increase the angiotensinogen production, but the consequent rise of angiotensin II formation acts as part of a negative feedback loop discouraging the production of renin, such us to increased production of aldosterone, due to the increased activation of angiotensin II. That could possibly explain the different levels of plasma renin and aldosterone concentration among men and women.

Regarding ARR, a linear decrease was presented worsening the LVEF in men. The opposite was shown in women. A higher ARR ratio was found in Individuals with normal diastolic function or mild dysfunction, independently from sex.

Numerous experimental and clinical studies indicate the role of RAAS in heart function and cardiac remodeling. Additionally, many large pharmacological trials have tested the effectiveness of anti-RASS medication in different phenotypes of heart failure and compared the benefit of this medication, in symptomatic and asymptomatic disorders, Mainly HFpEF and HFrEF, two clinical phenotypes which represent a dominant a diastolic or a systolic dysfunction respectively, were examined, in order to understand the pathology as well as the role and the degree of RAAS activation in each case.

In particular, very interesting came from the large OPTIMIZE-HF trial (96), showing that there was no clear benefit in HFpEF, comparing to HFrEF, regarding the rates of mortality, thereby indicating a lower degree of RAAS. Furthermore Bishu et al. (95) and Zannad et al. (97) implied an increase of RAAS blockade effectiveness as LVEF declines and the SOLVD investigators assumed supplementary that low LVEF with no clinical symptoms is accompanied by lower RAAS level (349).

Some of the clinical differences in respect of their benefits (329) could be explained by the blockage of RAAS in different stages of this hormonal cascade. Taking into consideration their common indications in HF treatment(330) one could assume a similar responsiveness of patients with HF (HFrEF or HFpEF), independently from the drug category of anti-RAAS agent (ACE inhibitor, ARB or MR antagonist) (331). However, considering and evaluating deeper data from large clinical meta-analyses trials testing thousands of patients, one realizes that this generalized hypothesis is at least obsolete, as it has been clearly demonstrated by many studies, RAAS blockade not being as beneficial in HFpEF as in HFrEF (40). Whereas the benefit of RAAS blockers in HFrEF has been clearly shown, an enigma remains the role of RAAS blockade in the treatment and management of HFpEF. A clinical cohort has been shown that the 1- year as well as the 5-year survival was higher when RAAS antagonists were administrated (47). However, mounting data discourage RAAS blockade as effective therapeutic choice for HFpEF, such as the large OPTIMIZE-HF trial (21) who enrolled as sample population of 20,118 patients with left ventricular systolic dysfunction (LVSD) and 21,149 patients with preserved systolic function (PSF) (left ventricular ejection fraction [EF] ≥ 40%)'. Dissapointing were the data demonstrating no effect of either ACE-I or ARBs on reduction of the cardiovascular mortality in HFpEF, in contrary to HFrEF. Paulus et al. summarizing the results from large clinical trials emphasized once again the non-positive outcome of HFpEF, when treated with RAAS inhibitors (336).

Investigators have also suggested that the reduced LVEF acts as stimulator for the RAAS system to express (335). Indeed, this idea was supported by observing quantified evidence obtained from many clinical HFpEF and HFrEF cohorts presented that patients with LVEF of 40-49% were more benefited that others with LVEF of 50% or more, indicating an increase of RAAS blockade effectiveness as LVEF declines (47, 334).

Furthermore, evidence which strongly indicated a reduction of hypertrophy and fibrosis in heart tissue when mineralocorticoid receptor was blocked (342), elucidated once again practically the principal role of aldosterone in HF pathogenesis. Long therapy with aldosterone receptor blockade with spironolactone is showed to be beneficial as far the diastolic function is concerned (337). Supportive data, namely reduced hospitalization rates due to heart failure, was demonstrated by the clinical randomized trial TOPCAT investigating patients with HFpEF, proposing that aldosterone antagonism has much to offer to the improvement of the clinical outcome of the disease (343).

In the literature was proposed that, despite to an observed decreased reduction in death rate in case of HFpEF which imposes consequently a more limited RAAS activation (332), the HF form may play not such a significant role in the response to RAAS blockade (47).

Benedict et al. suggested that the association between RAAS activation and decreasing LVEF cannot be greatly influenced by the clinical status or the prescribed medication (348). However, benefit of RAAS antagonism and clinical status are assumed to be related. The idea that level of RAAS hormone concentration depends on the clinical severity of heart failure (405, 406), supported also by the SOLVD investigators who suggested that low LVEF in absence of clinical symptoms is accompanied by low RAAS expression (349).

Paulus et al. (51) described a new conception of the HF spectrum and in his novel paradigm expressed the idea of HFpEF being a pro- inflammatory state, in the creation of which many coexisted cardiac or non-cardiac comorbidities are involved. The main idea behind was the crucial importance of inflammation, which inevitably enhances functional and structural alterations, through distinct myocardium stiffness and collagen fibrosis, respectively. These abnormalities lead, in turn, to the development of concentric remodeling and diastolic dysfunction (41), the main characteristic of patients with HFpEF (51, 53-55).

Older studies claimed that RAAS activity as well as the responsiveness of anti RAAS-medication to this system is limited among the elderly and that aldosterone generation becomes lower with ageing (407), as reduced plasma renin and aldosterone hormonal concentrations were measured (408). However experimental data from a recent animal study addressed, that among others, a decrease of degradation of RAAS results in a higher level of aldosterone which is partially responsible for age-specific alterations such as cardiorenal dysfunction due to fibrosis (409). Furthermore, there is evidence that demonstrates that age-related abnormalities in the heart tissue structure, such as LVH, or function, such as clinical heart failure, happen due to increased expression of RAAS system (410, 411) through NADPH oxidase-derived ROS (412, 413). In that way RAAS seems to be involved in cardiac remodeling (414).

In order clarify the statistical importance of this observational data a multivariable linear regression analysis was conducted, in order to investigate if a relation between plasma renin and aldosterone levels and LVEF or E/E' exists.

Furthermore, the associaton between renin, aldosterone and ARR and major echocardiographic parameters, which determine the diastolic and systolic dysfunction, namely LVEF and E/E', was analysed. In order to explore the possible involvement of the hormonal level of renin and aldosterone in the pathogenesis of cardiac disorders after adjusting for confounder factors that eventually could influence the relation between renin and aldosterone such as ARR and phenotypes of cardiac dysfunction.

Firstly, the relation of plasma renin and aldosterone concentration to the systolic dysfunction, defined by the echocardiographic parameter LVEF, was tested. A significant association was

observed in men in the crude model and after adjusting for age (p-value: 0.0025 and under 0.024 respectively), whereas no significant association was shown in females at all. This founding agrees with the information that renin levels are higher in men comparing to women. However, after adjusting for CVRF, GFR, medication and aldosterone, this strong statistical relation lost its power, indicating a possible co-interference and probably stronger influence of these factors in the deterioration of the LVEF, rather than the plasma renin level. Indeed from other studies, in which the impact of the standard drug therapy of heart failure on plasma renin secretion was tested, derived also the result that diuretics and beta-blocker can significantly influence the plasma renin level. It is demonstrated by several studies since a long time ago, that diuretic therapy can stimulate renin secretion (415) through reduction of the pressure in the renal arterioles by depleting plasma volume and sodium (416, 417). From the literature is known that beta-blockers block the secretion of renin from juxta-glomerular cells of kidney (418), reduce the renin release by inhibiting the process of pro-renin to renin and weaken also the plasma renin activity (419). As LVEF decreases, presentation of heart failure symptoms becomes more possible and a medical treatment is consequently required. Among other drug agents for heart failure, beta-blockers and diuretics belong in the first line therapy. Diuretics are effective in the symptom control in these patients by reducing the filling pressures in heart through lowering the intravascular volume and thereby the pre-load leading to an increase of cardiac output. That could explain the loss of significance of the association of renin to LVEF after adjusted for drug medication, as patients with reduced LVEF present more frequent symptoms and are earlier treated with medicine, that eventually influence the plasma renin level.

Studying the relationship of plasma renin to the E/E', as definer of the diastolic function, a strong association has been shown. On the contrary to the result derived from testing the relation of renin to LVEF, in case of E/E' not only a statistical significance was found, but also it remained even after adjustment for drug medication and aldosterone. This finding could imply, that the influence of renin on the E/E' is stronger and independent from factors that affect its level in plasma, comparing to its influence on the LVEF. Considering just the founding of the analysis of renin, it could be assumed that renin plays a more important role in the diastolic function than in the systolic.

In women, on the other side, a significant relation of renin and LVEF or E/E' failed to be shown, in every of the studied models, indicating clearly a less important involvement of renin in the systolic and diastolic dysfunction, at least as defined by echocardiographic parameters, in the female population. This indicates that other confounders other than plasma renin concentration, which could also cause an increase of renin level, may be the cause of the diastolic dysfunction, through a pathogenic mechanism independent from renin, at least in the early stages. Renin showed no strong association with LVEF in women and this could also be

explained by the protective effect of estrogens, as numerous studies have proved. That could be also a possible explanation of the observed lower degree of increase of renin comparing to men and no existence of linearity between renin level and systolic dysfunction in our descriptive analysis when LVEF was severe reduced.

Furthermore, it was investigated the association of aldosterone concentration to LVEF and E/E'. As expected, a significant relation was found between aldosterone level and LVEF in all statistical models independently from age, CVRF, medication, hormonal status in case of women and plasma renin levels, in both sexes. This emphasizes the importance of aldosterone in the systolic function, regardless other factors which may also play a role in its pathogenesis. This founding is also supported from the observational data of our study, which presented a linear increase of plasma aldosterone concentration according to the deterioration of systolic dysfunction and decrease of the LVEF in both sexes.

A large number of studies demonstrated that aldosterone is a hormone with impact in the heart tissue (109). Aldosterone stimulates fibroblasts to synthetize collagen leading to cardiac fibrosis. The effects of aldosterone concerning tissue fibrosis and vascular remodeling via inflammation and oxidative stress (139) interfering in the pathogenesis of atherosclerosis (140) could be a possible explanation of the significant role of aldosterone levels in the systolic dysfunction of heart in both men and women and even after adjustment for other possible confounder factors.

In conclusion, investigating the association of plasma renin and aldosterone on the echocardiographic parameters, by which the systolic and diastolic dysfunction are defined, some important observations have to be made. Whereas renin is significantly associated with LVEF and E/E' in men, it does not seem to play an important role in the increase of E/E' or decrease of LVEF in women.

On the contrary, aldosterone is proved to be crucial as affecting factor of LVEF and E/E' in both men and women. Parallel, the significance of the association of hormonal level to cardiac function presents a relative variability in dependence from the adjustment made. Surprisingly, although aldosterone level appeared to be higher when E/E'$\geq$ 10 only in men, a significant statistical association between aldosterone with E/E' has been shown in both men and women, though, only in the unadjusted model. These results indicate a possible and maybe more important role of other confounding factors in the pathogenesis of each cardiac disorder, on the one hand, and a different response of females to the influence of renin and aldosterone on LVEF and E/E' , on the other.

Studying the association of ARR to LVEF and E/E', no association was found between ARR and LVEF of E/E' in men. In women, ARR was associated to LVEF in models 2 and 3,

indicating an important impact on LVEF after adjusting for age, CVRF and drug therapy. However, ARR was statistically related to E/E' in women only in the crude model, whereas after adjustment did not.

Having demonstrated the associations between the plasma renin and aldosterone and ARR and the major echocardiographic parameters LVEF and E/E', which defines the systolic and diastolic dysfunction respectively, can be now reported the findings of deeper and further analyses concerning the main purpose of this study, namely the possible associations between plasma renin and serum aldosterone, as well as ARR and the five clinical phenotypes of cardiac disorders.

Regarding the influence of renin on the different clinic phenotypes of cardiac disorder, a multivariable linear regression analysis was carried out. A significant association of plasma renin was shown only in the symptomatic disorders, namely HFpEF (p-value <0.0001) and HFrEF (p-value < 0.0001) in men in the unadjusted statistical model (crude). This result of our study agrees with the evidence from the literature that the RAAS-system is more activated in the symptomatic cardiac disorders.

Renin remained also significantly associated to HFrEF in men when confounders such as age, CVRF and GFR were adjusted, whereas to HFpEF not. According to evidence from the literature, renin is assumed to contribute to heart failure indirectly, through causing myocardial infarct. It was observed a correlation between high renin activity and increased incidence of MI. From this observation it could be also claimed, that renin plays an important role in the occurrence of heart failure with dominant systolic dysfunction, independently from comorbidities accompanying the increase of age (such as CAD or MI, which are undeniably causes of HFrEF as clearly emphasized before), CVRF and renal dysfunction, which can cause deviation of the level of plasma renin. The growing the age, the more frequent are the coexisting comorbidities in the population. In addition, these comorbidities, such as MI and CAD, which consist the main causes of systolic impairment and systolic heart failure, appear almost twice as oft in the age group 64-75 comparing to 55-64 in men (MI 10.3% vs. 4,9% respectively and CAD 15,1% vs. 7,5%). Moreover, from our descriptive analysis observations agree that the rates of some risk factors are more common in men than in women. For example, as mentioned before, myocardial ischemia is more frequent among men with HFrEF, comparing to women, suggesting a possible important role of these factors in the pathogenetic mechanismus of HFrEF in men.

Oppositely, in case of HFpEF, where the diastolic dysfunction is the dominant disorder, and the systolic dysfunction secondary or even absent, increase of plasma renin level could not be proved as an adequate causal associating factor of HFpEF after adjustment for the above mentioned confounders, as the initially proven significant relation lost its significance. From

numerous population-based studies it has become quite evident, that not only there is a relation between coexisting diseases, such as hypertension, diabetes mellitus, metabolic syndrome, and atherosclerotic disease (which also consist “important risk factors for HF” according to ACCF/AHA guidelines (6)), but also they provide the essential biological substrate for HFpEF to be created (11). The most important of them are age itself (11) and age-typical pathologies with great emphasis on hypertension which is the highest prevailing (86).

No significance was to be seen between renin and asymptomatic disorders with reduced LVEF such as SD or combined dysfunction, observation that maybe implies that there is possibly another factor which causes systolic dysfunction in the asymptomatic disorder in men rather than renin or factors with stronger influence on the myocardium. As already known from the literature smoking is a main CVRF causing heart failure. In particular, results from the PREVEND study demonstrated that tobacco consumption is highly related with the heart failure with reduced ejection fraction. From our baseline characteristics, we recognized that smoking was incidentally more frequent in people presenting a combined dysfunction (19%) or isolated SD (22.8%) comparing to isolated DD (13.9%), supporting the data from PREVEND study that smoking is clearly associated to a systolic dysfunction more than to an isolated diastolic one, so that it could be claimed that smoking causes decrease of LVEF through mechanisms independent from plasma renin. Similar not significant were the results presented, when plasma renin and DD was studied. The fact that in male population renin failed to be strongly associated to the asymptomatic disorders (DD, SD or combined dysfunction) support the evidence, derived from studies that tested anti-RAAS agents and their effectiveness in symptomatic and asymptomatic population with cardiac disorders, as mentioned above, that RAAS-system is less activated in asymptomatic disorders.

Investigating the same relation of renin to the phenotypes of cardiac disorder in the female population of our study, there is a remarkable result to be noted. Although renin remained significantly associated to HFpEF, even only in the unadjusted model, no association at all was to be presented in case of HFrEF. Additionally, oppositely to men, where an important role of renin in the presence of asymptomatic cardiac dysfunctions was failed to be proven, in case of women, there is a statistically strong association to DDpEF. Interestingly, significance was shown in all adjusted models, but not in the crude model. In other words, renin is related to DDpEF in women when confounder factors are excluded, whereas in their presence, it did not, observation, which implies a stronger relation of confounder factors (such as hypertension, renal function, drug medication or serum aldosterone) than renin. The observation that plasma renin is significantly associated to DDpEF, without age to be a meaningful factor, encourage more the hypothesis that renin may be an adequate factor

causing diastolic dysfunction among females, even in the early stages before symptoms occur.

Female heart may also be more vulnerable to the loss of the protective role of estrogens. Moreover, the important role of hypertension in the pathogenesis of diastolic dysfunction has been already underlined. Accepting this evidence derived from many studies, it becomes clear and even totally explainable the loss of the significance importance after adjusting for age and consequently menopausal status, CVRF such as hypertension, OC or HRT, as these factors are proven to be main influencer of the diastolic function or dysfunction.

Furthermore, as anticipated, due to the fact, that plasma renin has no great impact to LVEF; renin demonstrated no significant correlation to the appearance of systolic disorders expressed by reduced LVEF such as isolated SD, combined dysfunction or HFrEF among females. This could indicate that there must be another factor causing relevant systolic impairment in female heart tissue than renin, such as CAD or MI.

To summarize, plasma renin is strongly associated only to the symptomatic disorders in men, independently from the dominant dysfunction. There was presented no statistical importance between renin and asymptomatic cardiac disorders, despite the results, that renin is associated significantly to the deterioration of the systolic (decrease of LVEF) and diastolic function (increase of E/E'), implying a crucial role of renin or an increased RAAS activation in severe disorders (systolic and diastolic), and therefore symptomatic in men. Oppositely, renin seem to be significantly associated to the cardiac disorders with diastolic dysfunction in women, regardless the severity and presentation of symptoms, even though no relation between renin and E/E' was shown in female population.

Investigating further the role of plasma aldosterone concentration to the presence of the clinical phenotypes of cardiac disorder there are some similarities but also a main noticeable difference comparing the results in regard of plasma renin. Once again, aldosterone was presented to be significantly associated with HFpEF and HFrEF in men, whereas no relation was shown regarding the HFrEF in women. This supports the hypothesis, already expressed, that females are ''protected'' from the RAAS activation because of their hormonal status due to estrogen. In this part of population there must be some other main mechanism participating in the pathogenesis of the systolic dysfunction, such as loss of cardiomyocytes after a MI. Nevertheless, this assumption does not seem to be true in case of the study of diastolic dysfunction, as aldosterone was significantly associated to DD and HFpEF in women. The knowledge that progesterone, estrogen and aldosterone receptors are all identified to be located in the arterial wall (227, 228) and that estrogens and aldosterone receptors coexist in fibroblasts and cardiomyocytes (229) may indicate that the female heart could be more sensitive to the aldosterone effects. Growing the female population and

because of the hormonal change with reduction of estrogen, women loose the protective role of estrogens, which they enjoy premenopausal. This leads and explains probably the remarkable increase of hypertension in women in the menopause, in age groups 55-64 and 64-75 years, from 34.7% to 72.6%, reaching the levels of hypertension among the male population (74.5%) and presenting a double incidence in the female population as it is growing older. Under this perception it becomes easier to understand the results of our study, pointing out that the pathogenesis of diastolic dysfunction could, to a reasonable extend, though hypertension explained. This could be a possible explanation of the significance of aldosterone on DD and HFpEF in women. After adjusting for age and CVRF the significant association of aldosterone to DDpEF and HFpEF in women faded, implying once again the important impact of CVRF such as hypertension such as hormonal changes growing the age on the diastolic function in women.

Another remarkable difference derives from the study of aldosterone and clinical phenotypes of cardiac disorders in male population. In particular, aldosterone was significantly associated with DD in men. Contrarily to plasma renin, when the influence of aldosterone on the asymptomatic combined dysfunction was investigated, a significant statistical result was found. This implies that aldosterone is closely related to the appearance of asymptomatic systolic dysfunction in men, whereas renin is not. This could be interpreted by the knowledge from the literature that aldosterone, among other mechanisms, promote fibrosis and consequently diastolic cardiac impairment, whereas renin does not have a direct effect on the heart tissue, but a trigger-role in order to activate the RAAS-system.

Another interesting finding derived from Framingham Heart Study, a community-based study. Vasan et al. (221) examined via echocardiography, healthy population, regarding ischemic cardiovascular events and heart failure and managed to relate positively aldosterone level with structural alteration of left ventricle (concentric hypertrophy) and even HFpEF only in female subjects.

Interesting is, though, the fact that this observation was only in women to be made, whereas in men was not. There is also a significant association of aldosterone to DD, just like in case of renin, an expected result considering the strong relation of aldosterone to E/E', as a parameter of diastolic dysfunction, in both sexes. Confirming mounting clinical data from several studies reveals the crucial contribution of RAAS in diastolic disorder, presented by the absence of estrogens (222), indicating clearly that RAAS regulation seems to depend also upon other hormonal-to-hormonal interactions, such as the ovarian hormones.

As mentioned before, the same mechanisms which adapt to the volume overload in case of pregnancy become maladaptive as growing the age resulting in hypertension and cardiac alterations in the elderly (420). In concert with findings from the large Framingham study

which reported an increase of hypertension rate every ten years in women more than three-times the increase of that in men (69% vs. 15%) (421). In our study, hypertension rates at age under 65 was higher in men than in women, in age group 65-74 appeared a double rate of increase of hypertension in females with the noticeable result of almost similar rates between men and women over the age of 65 years (74.5% in men versus 72.6% in women). These observational findings indicate an interfering role of female hormones, or their decrease, in pathogenesis of hypertension. This observation, although the prevalence of hypertension in women was not higher than that of men as suggested by Kannel et al. (278), agrees with Roger et al. (7), who reported a discreet increase of hypertension rates in female objects of age over 55 years with the result that more than almost 75% postmenopausal women present hypertension. Hypertension, consists a pressure overload situation, which can consequently trigger the cardiac remodeling of the left ventricle (422) and therefore result to diastolic disorder and even diastolic heart failure (279, 280), sequence which occurs principally postmenopausal when estrogen are detrimentally reduced and women are no longer under their protecting effect (92, 423). Indeed, investigations of the possible association of menopausal status of the female population to the symptomatic or asymptomatic diastolic disorders, independently from the systolic cardiac function, it was clearly confirmed the significant role of the estrogen loss in the concentric cardiac remodeling. The crucial role of hypertension, among other factors, in the diastolic dysfunction, in a way other than RAAS-activation, is also supported from our results. Particularly, our analysis presented that the initial significant association of renin and aldosterone to the HFpEF in women in the unadjusted model lost its statistical strength when adjusted for age and CVRF (model 2).

Notably, aldosterone does not prove to be a crucial causal factor, regarding the HFrEF in women. Probably heart failure with reduced ejection fraction in women is caused, mainly, through other mechanisms than activation of RAAS-system, despite the higher degree of its activation in HFrEF, as also being a severe heart disorder. This data implying and support the knowledge that women are protected from the harmful effects of RAAS-hormones, in terms of causing heart damage and in last stages failure by decreasing the aldosterone production in females premenopausal or under hormonal replacement therapy with estrogens.

However, it has to be remarked that aldosterone was not proved to be significantly related to the isolated systolic dysfunction without diastolic dysfunction (PDrEF). A possible pathophysiological explanation would be the consideration of aldosterone of being a hormone, which leads to sodium retention, water retention causing parallel to fibrosis, increase of blood pressure and therefore diastolic dysfunction. Indeed, from our study derives the conclusion that aldosterone is related to all clinical phenotypes of cardiac

disorder with diastolic dysfunction, isolated or not, symptomatic or not, in men, whereas in women only to the cardiac disorders with dominant or at least coexisted diastolic dysfunction.

Investigating further the association of plasma renin and aldosterone concentration to cardiac dysfunction in women there are some interesting supporting demographical data from the sample characteristics of our study population. First of all the measurable level of plasma renin in women is relevantly lower than that in men, as already noticed. In addition, epidemiological data from our study showed a double prevalence of cardiac disorder with preserved LVEF (DDpEF and HFpEF) comparing to the heart failure phenotypes with reduced LV-function (PDrEF, DDrEF and HFrEF) in women. The systolic dysfunction in women, though, does not necessarily contradicts this assumption of estrogen's protective role, as systolic dysfunction may be caused by comorbidities such as CAD or MI rather than RAAS-activation.

The ARR demonstrated a significant association to the asymptomatic isolated diastolic disorder (DD) and heart failure with preserved EF (HFpEF) as well as to asymptomatic combined dysfunction, whereas no was presented in male population association at all. These results are concordant to the results deriving from the study of ARR in regard to LVEF and E/E', where similarly no relation was shown among men comparing to women.

5.1 Perspectives and conclusions

Heart failure accounts for 1 in 7 deaths in the U.S. and consists almost the 7% of all cardiovascular deaths (6). The absolute mortality rates for HF remain tremendous high, about 50%, within 5 years of diagnosis. This complex clinical syndrome that constitutes the heart failure can derive from any structural (concentric or eccentric remodeling) or functional dysfunction (impaired ventricular filling or reduced ejection of blood). heart failure can result from a wide spectrum of systolic or diastolic abnormalities (SHF and DHF respectively), or both, and vary from clinic phenotypes with severe left ventricular remodeling and distinct reduction of EF to patients with normal LV mass, size and shape and preserved EF. Observing heart failure as a part of the cardiovascular disease, there is a continuing progress beginning from the existence of cardiovascular risk factors, proceeding to asymptomatic alterations of the heart tissue and resulting to irreversible, in most of the cases, heart dysfunction accompanied by clinical symptoms. Diastolic dysfunction describes the weakness of the ventricle to fill with blood due to a mechanical impairment of its relaxation ability in the phase of diastole or the increase in wall stiffness. On the other hand, a systolic dysfunction refers to the impaired contractility of ventricle during systole, which finally leads to decrease of stroke volume. There has been evidence that illustrates an oxymoron: the

idea of HFpEF and HFrEF being a part of the same disease spectrum (37)in terms of sharing similar clinical symptoms) and the contradictory conception, by multiple proven demonstrations, that clearly verify a wide heterogeneousness concerning the demographic characteristics, the risk factors and the comorbidities of insulted patients, the etiological causes, the diagnostic methods and parameters, the therapeutic measures and the prognosis. The development of HFpEF is based on proinflammatory signaling, systematic and cardiac, which can, potentially, be caused by many systematic comorbidities coexisting frequently with HFpEF, especially hypertension, diabetes mellitus and obesity, leads eventually to diastolic left ventricular dysfunction, cardiomyocyte stiffness and interstitial fibrosis through activation of the RAAS-System. Toxic, infective and ischemic situations are among the most common etiological factors of HFrEF, whereas the massive loss of cardiomyocytes presents to be the main mechanism involved in the pathogenesis. The thread joining them could be presumed to be the endothelial dysfunction and the subsequent inflammation, both playing a key role in the pathogenesis of HFrEF and HFpEF. Having been extensively documented the detrimental functional and structural consequences of RAAS activation in heart; it has become of crucial importance the biological mechanisms that hide behind this association. Searching further the hypertrophic and fibrotic role of the major RAAS components, including aldosterone, the undeniable role of inappropriate expression of RAAS hormonal system in heart failure pathogenesis becomes clear.

From numerous studies that investigated the role of pharmacological agents for RAAS-blockade, it is implied that the level of RAAS hormone concentration depends on the clinical severity of heart failure suggesting that low LVEF in absence of clinical symptoms is accompanied by low RAAS expression. Furthermore a large amount of evidence appear to doubt the effectiveness of anti-RAAS medication in case of HFpEF comparing to HFrEF, suggesting that the degree of RAAS-activation in this heart failure entity may be debatable.

Data from several studies has already presented that the prevalent rate of female sex in HFpEF is almost twice as high the rate of HFrEF. Indeed, the same result derived from our epidemiological study of our population, as the clinical phenotypes with reduced systolic function, isolated or in combination to diastolic dysfunction, (DDrEF, PDrEF and HFrEF) were much more frequent in male population, whereas the prevalence of asymptomatic isolated diastolic disorder (DD) and HFpEF where presented mainly in females. Many studies have been conducted with the purpose of illuminate the issue. Mounting clinical data reveals the crucial contribution of RAAS in diastolic disorder, presented by the absence of estrogens. In these terms, the synthesis of all RAAS components is controlled by estrogens. The pathophysiological mechanisms involved in the regulation of RAAS are based on crosstalk signaling between RAAS and ovarian hormones. Generally, estrogens antagonize the RAAS system by suppressing its formation and activation.

Many studies have been conducted with the purpose of illuminate the impact of RAAS-activation in the pathogenesis of cardiac disorders. In conclusion, some highlight findings deriving from our study are worth mentioned. In our analysis it has been demonstrated that renin and aldosterone are both significantly associated with both symptomatic disorders (HFrEF and HFpEF) in male population, whereas in women only with HFpEF, indicating possibly a different degree of activation of RAAS or a different pathophysiological impact in women. Furthermore, it has been observed that aldosterone was associated with asymptomatic disorders with diastolic dysfunction in men, whereas renin was related only to the phenotypes of symptomatic heart failure. Interestingly, although renin has been significantly associated to diastolic cardiac disorders (DD and HFpEF) in women, this relation has not been demonstrated, as the association of renin with E/E', one of the main echocardiographic determinators of diastolic function, was examined in women. Oppositely, despite the significant association of aldosterone with LVEF, in women no strong relation has been shown between aldosterone and heart failure phenotypes with systolic dysfunctions. Last but not least, ARR was stongly associated with LVEF, E/E' as well as with some of the cardiac phenotypes only in women, while in men no association has been proven at all.

This diverse results and discrepancies, to some point, referring the results of the investigation of the association of renin, aldosterone and ARR to the echocardiographic parameter which define the diastolic and systolic dysfunction as well as the clinical phenotypes of cardiac disorders emphasize the necessity to obtain new knowledge. Much effort has been already put into clarifying the role of RAAS regarding the cardiac function. Though, due to the complexity of the biochemical and hormonal interactions, the excessive degree of pathogenetic systematic functions of RAAS, the multivariable genetic components involved in both expression of RAAS and presence of cardiomypathy on the one side, as well as the heterogenity of the pathology and prevalence of numerous of CVRF and comorbidities pre-indicating cardiac dysfunctions on the other, a complete understanding of the impact of RAAS and, particularly, sex-specifically, remains to be illuminated.

6. Limitations and strengths of the GHS (Gutenberg Health Study)

Like all population-based studies, the Gutenberg Health Study was also exposed to possible bias by non response bias. The study population was based on a self-selection of persons of the sample. For voluntary surveys these are unavoidable possible mistakes. The Gutenberg health study showed comparable recruitment rates with similar studies.

The participants of the GHS (Gutenberg Health Study) were predominantly caucasian and of European origin, so that the results presented were limited to this specific ethnic group of population. In addition, people with insufficient knowledge of the german language as well as with physical or mental limitations that make it impossible for them to visit the study center and take part in the examinations of the study were also excluded from the participation. In this excluded population belong probably people with severe, symptomatic or with acute onset of a disease, such as heart failure, whose symptomes were restricted factors and unable them to take part in the study. However, the number of persons excluded was very small and the study population very high. However, the primary objective of this analysis was the investigation of the association of renin, aldosterone and ARR with cardiac function. For this purpose, the large study population was more than sufficient in ordert to obtain statistical important results (15,010).

The greatest limitation of the available data is certainly the chracteristics of the conduction of the ultrasound examination and function principals of ultrasound. The sound is reflected off structures inside the body, and the ultrasound machine analyzes the information from the sound waves. The ultrasound beam may produce different properties in different directs. It should be perpendicular to the structure of the beam in order to bounce back for the image to be created successfully. Ultrasound leads to an increased depth which affects the frequency required for optimal imaging. This leads to a lower resolution of the image produced. That is the reason of the limited penetration in obese patients. Poor penetration is also observed through bone, which explains the difficulties of gaining high quality of images in very thin persons. These are the disadvantages in U/S-imaging domain, which result to different image quality and consequently to different degree of evaluation the information gained. All ultrasound examinations were conducted with the same equipment (ultrasound gel) and machine providing a high degree of homogeneity of image resolution. Nevertheless, there were several ultrasound operators throughout the study duration. A main disadvantage of the ultrasound examination is the subjectivity of the examination in regard of the fact that the quality of results and the use of equipment depend on skills of the operator. In these terms, in our study this consisted an inevitable limitation. Furthermore, ultrasound examination was conducted by medical assistants with no deep medical knowledge, though adequately

trained in carrying out an ultrasound examination according to the SOPs (Standard Operation Procedures). Although the conduction of ultrasounds was carried out by medical assistants, the interpretation and the final evaluation of the images were controlled by the study doctor. However all cases of poor image quality may resulted in questionable and sometimes in falsified interpretation.

Regarding the assessment of the medical history, the documentation of medical treatment and the registration of comorbidities of the study participants, an additional possible limitation should be noted. This data was obtained by means of a questionnaire forms. These forms are an important and good tool for recording information, can be, though, sometime relative unreliable, when not based on official medical report and partially filled out by the participating people themselves.

Last but not least, labor investigation was an important part of our study, as renin and aldosterone were measured in plasma. Almost every participant was gone through a blood examination. For this purpose, the blood examinations were made in the laboratory of the Gutenberg University Clinic in Mainz. The difference in the transport duration of the blood samples to the laboratory and the waiting time in the laboratory until the process of the examination of blood may could have affected slightly the blood results. Similar laboratory deviations may have been caused by the eating condition of the participants at the time of the blood examination, affecting for example the level of glucose, and the previous take of medication that potentialy can affect the level of renin and aldosterone in plasma.

7. Summary

Heart failure and cardiac dysfunction can be two different clinical entities as well as different stages of the same disease. There are five clinical phenotypes of cardiac dysfunction according to the presentation of clinical symptoms, symptomatic heart failure (HF) or asymptomatic cardiac disorder (CD), and the pathogenetic mechanism, diastolic, systolic or combination of both dysfunctions. These are DD, SD, combined dysfunction, HFpEF and HFrEF. Sex, age, cardiovascular factors, comorbidities, hormonal status in case of women and drug medication has been shown to play an important role in the prevalence and pathophysiology of heart failure. Structural remodeling due to myocyte's loss or hypertrophy (eccentric and concentric respectively), as well as functional alterations of the heart tissue due to inflammation partially caused from overexpression of hormones result in the occurence of the clinical phenotypes of asymptomatic cardiac dysfunction or heart failure. Undeniable is the crucial role oft he RAAS-activation, as already been shown by numerous studies and tremendous ammount of data.

The aim of this work was to investigate the association of plasma renin, plasma aldosterone and their ratio (ARR) with the five clinical phenotypes of cardiac dysfunction sex-specifically. Furthermore, the influence of possible confounder factors, which can potentially can affect this association, such as age, cardiovascular factors, GFR, menopausal status and hormonal replacement therapy or anticontraceptive therapy, and medication with impact on the RAAS, were tested and adjusted in different statistical models.

For this purpose clinical data from 14,740 participants of the Gutenberg Health Study (GHS), an age-, sex-, and residence stratified, large population-based, prospective, single-center, prospective cohort study, was used. In 14,740 was measured plasma renin and aldosterone concentration. The age was registered, the cardiovascular risk factors were assessed on the basis of clinical and laboratory chemical parameters and the comorbidities on the medical history of the study participants. The assignment to the subgroups of the cardiac function was based on echocardiographic criteria (LVEF and E/E').

Our results showed some interesting differences between men and women. Whereas plasma renin and aldosterone concentration was significantly associated with both phenotypes of heart failure (HFpEF and HFrEF) in men, in women they were associated only with HFpEF. This sex-specific difference observed in our results emphasizes the knowledge that sex-hormones, such as estrogens, can interactively und significantely influence the production and the activation of RAAS- hormones.

Data from several studies has already presented that the prevalent rate of female sex in HFpEF is almost twice as high the rate of HFrEF, data in accordance to our observational data.

Regarding the asymptomatic disorders, in men the hormonal level of concentration of aldosterone in plasma, but not of renin, was statistically related to the phenotypes of asymptomatic isolated diastolic disorder (DD) and combined dysfunction. Oppositely, both renin and aldosterone were associated only with asymptomatic isolated DD, but not with asymtomatic combined dysfunction, in women. It could be impied that in men aldosterone can cause asymptomatic isolated diastolic (DD) or combined cardiac dysfunction, whereas in women both hormones where statistically important in the isolated asymptomatic diastolic isolated dysfunction (DD).

In summary, our results confirm partially the conception that RAAS activation is generally more in both clinical phenotypes of heart failure (HFpEF and HFrEF), as severe (symptomatic) stages of cardiac dysfunction in men. In women renin and aldosterone do not seem to play a significant role in the phenotypes of systolic dysfunction or heart faulure, indicating a hormonal interaction and involvement of estrogens in the expression of RAAS and the occurence of cardiac disorders in female population.

The exact role of RAAS-hormones as pathogenetic factors in the asympromatic cardiac dysfunction sex-specifically, as well as the precise influence of each hormone in the systolic and diastolic function should still be investigated.

Zusammenfassung

Herzinsuffizienz und kardiale Dysfunktion sind verschiedene klinische Entitäten und Stadien desselben komplexen Krankheitsbildes. In Abhängigkeit vom Vorhandensein klinischer Symptome und der pathogenetischen Mechanismen unterscheidet man die symptomatische Herzinsuffizienz (HF) und die asymptomatischen kardialen Dysfunktionen (CD), sowie die diastolisch, systolisch und kombinierte diastolische-systolische Dysfunktion. Demnach gibt es fünf klinische Phänotypen: die isolierte diastolische Dysfunktion (DD), die isolierte systolische Dysfunktion (SD) die kombinierte diastolische und systolische Dysfunktion sowie die Herzinsuffizienz mit erhaltener EF (HFpEF) und mit reduzierter EF (HFrEF). Es konnte gezeigt werden, dass Geschlecht, Alter, kardiovaskuläre Risikofaktoren, Komorbiditäten und weibliche Hormone einen Einfluss auf die Prävalenz und Pathophysiologie der Herzinsuffizienz haben. Strukturelles Remodelling durch Verlust von Myozyten, exzentrische bzw. konzentrische Hypertrophie des Myokards sowie funktionelle Veränderungen des Herzgewebes aufgrund von Inflammation führen zum Auftreten der oben genannten fünf klinischen Phänotypen der asymptomatischen Herzfunktionsstörung und Herzinsuffizienz. Zudem wurde in zahlreichen Studien belegt, dass die Aktivierung des RAAS-Systems eine wesentliche Rolle in der Entstehung von Störungen der Herzleistung spielt.

Ziel dieser Arbeit war geschlechtsspezifische Unterscheide von Plasma-Renin, Plasma-Aldosteron und deren Verhältnis (ARR) in den fünf klinischen Phänotypen der kardialen Dysfunktion zu untersuchen. Hierfür wurden die klinischen Daten von 14.740 Teilnehmer der Gutenberg-Gesundheitsstudie (GHS), einer monozentrischen prospektiven Kohortenstudie, eingeschlossen. Alter, Geschlecht, kardiovaskuläre Risiken, Komorbiditäten, die glomeruläre Filtrationsrate (GFR), der Menopausenstatus von Frauen sowie Hormonersatztherapie bzw. Antikontrazeptiva und Medikamente, die in das RAAS eingreifen, wurden aus den Krankenakten erhoben um deren Einfluss auf die Herzleistung zu untersuchen. Die Zuordnung zu den Untergruppen der Herzfunktion erfolgte anhand der echokardiographischen Messungen LVEF und E/E'.

Die Studie ergab mehrere geschlechtsspezifische Unterschiede. Bei Männern war die Renin- und Aldosteronkonzentration im Blut signifikant mit den Phänotypen HFpEF und HFrEF assoziiert, bei Frauen jedoch nur mit HFpEF. Die Ergebnisse unterstreichen die Erkenntnis zahlreicher anderer Arbeiten, die gezeigt haben, dass Sexualhormone die Aktivierung des RAAS beeinflussen. Zudem haben mehrere Studien in Übereinstimmung mit unserer Beobachtung gezeigt, dass bei Frauen die Prävalenz von HFpEF fast doppelt so hoch ist als die Prävalenz von HFrEF.

Bei Männern zeigte sich eine Assoziation zwischen der Konzentration von Aldosteron im Plasma mit den isolierten und kombinierten asymptomatischen kardialen Dysfunktionen. Die Höhe des Plasmaspiegels von Renin scheint keinen besonderen Einfluss auf diese Phänotypen zu haben. Im Gegensatz dazu war bei Frauen die Höhe der Plasmakonzentration von Renin und Aldosteron mit DD assoziiert, nicht jedoch mit der kombinierten Dysfunktion. Möglicherweise beeinflusst bei Männern Aldosteron die Entstehung der asymptomatischen DD und der kombinierten kardialen Dysfunktion. Bei Frauen scheinen beide Hormone nur bei der isolierten asymptomatischen diastolischen Dysfunktion (DD) eine Rolle zu spielen.

Zusammenfassend konnte gezeigt werden, dass bei Männern die RAAS-Aktivierung für die symptomatischen Stadien der kardialen Dysfunktion HFpEF und HFrEF eine wichtige Rolle einnimmt. Bei Frauen scheinen Renin und Aldosteron für die Phänotypen der systolischen Dysfunktion und der Herzinsuffizienz von untergeordneter Rolle zu sein, möglicherweise durch Interaktionen von Östrogenen mit RAAS. Weitere Studien sind notwendig zur genaueren Untersuchung des Einflusses der Geschlechtshormone auf die kardialen Dysfunktionen bei Frauen und Männern.

8. References

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