BIOMARKERS OF HEART FAILURE WITH A PRESERVED EJECTION FRACTION IN PATIENTS WITH AORTIC DYSFUNCTION
by

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DISSERTATION

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(School of Pathology and Pre-Clinical Sciences)

at

SEFAKO MAKGATHO HEALTH SCIENCES UNIVERSITY

SUPERVISOR: Prof OHI Majane
CO-SUPERVISOR: Prof LH Böhmer

JANUARY 2019
DECLARATION OF ORIGINALITY

I, MARILET VAN HOOGLAND declare that BIOMARKERS OF HEART FAILURE WITH A PRESERVED EJECTION FRACTION IN PATIENTS WITH AORTIC DYSFUNCTION is my own work and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references. This work has not been submitted before for any other degree at any other institution.

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Marilet van Hoogland                     Date
Student Number: (201507150)

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Supervisor

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Co-supervisor
DECLARATION OF ETHICAL CLEARANCE

The study was approved by the Sefako Makgatho Health Sciences University Research Ethics Committee (SMUREC).

Protocol Number: SMUREC/M/08/2017:PG

(Clearance certificates attached – Appendix A)

____________________  ____________________
Marilet van Hoogland  Date
Student Number: (201507150)
DEDICATION

TO MY PARENTS AND GRANDPARENTS WHO HAVE ALWAYS GIVEN ME
UNCONDITIONAL LOVE, SUPPORT AND ENCOURAGEMENT.
ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my supervisors, Professors Harold Majane and Linde Böhmer for their guidance, mentorship, constant support and words of encouragement throughout the duration of my MSc. The invaluable skills and knowledge that you have imparted will never be forgotten. I would like to thank Professors Angela Woodiwiss and Gavin Norton for their invaluable skills and knowledge shared with me.

I am grateful for all the staff members in the Department of Cardiology at DGMAH for their assistance and support throughout the study. I would also like to thank the National Research Foundation/ Innovation Master’s Scholarship as well as the Department of Physiology, SMU, for financially supporting this project.

Lastly, the study would not have been possible without the voluntary collaboration of all the study participants, to whom I owe my sincere appreciation.
ABSTRACT

**Background:** Almost 50% of all heart failure cases have a preserved ejection fraction, which is often observed in the elderly (mean age of 75 years), more specifically in older females. Therapy targeting the known mechanisms of this disorder has not significantly improved the outcomes due to the multifactorial nature of the syndrome. Left ventricular diastolic dysfunction is a characteristic feature of heart failure with a preserved ejection fraction. It has been shown that an increased risk of heart failure is associated with increased aortic stiffness in the elderly which could potentially be a causative factor leading to the development of this heart failure phenotype. A better understanding of the mechanisms responsible for left ventricular diastolic dysfunction produced by common risk factors such as hypertension may lead to better therapeutic approaches in the prevention and treatment of this syndrome. Currently little is known about the onset, the population age to be targeted, the prevalence and the progression of heart failure with a preserved ejection fraction in sub-Saharan Africa. To date, no clinical study has been conducted on heart failure with a preserved ejection fraction in a South African population, more so in a middle-age black population.

**Aim:** To investigate two biochemical markers, N-terminal pro-brain natriuretic peptide and galectin-3, that predict heart failure with a preserved ejection fraction and specifically markers that predict or are associated with aortic dysfunction in heart failure with a preserved ejection fraction in a middle-age black population in South Africa.
**Methods:** The nature of the study was a case-control investigation. Sixty-six (66) participants with heart failure with a preserved ejection fraction and 213 participants without heart failure from African descent and older than 18 years of age were enrolled. All participants gave informed consent and completed a standardised questionnaire. Echocardiographic, anthropometric, central haemodynamic measurements and routine blood tests were conducted. Biomarker analysis was done using commercially available enzyme-linked immunosorbent assay kits.

**Results:** The mean age for the prevalence or onset of heart failure with a preserved ejection fraction in black South African patients was 54.88±13.51 years. Arterial stiffness as assessed by carotid to femoral pulse wave velocity was significantly increased in participants with heart failure with a preserved ejection fraction (9.97±2.78 m/s) when compared to participants without this pathology (6.11±2.18 m/s) with a p-value of p<0.0001, however there were no significant associations between central haemodynamic parameters, N-terminal pro brain natriuretic peptide (p = 0.9746) and galectin-3 (p = 0.2166). Lastly, N-terminal pro brain natriuretic peptide, but not galectin-3, was significantly associated with left ventricular hypertrophy (p = 0.0002), left atrial diameter in diastole (p = 0.0005) and left atrial to left ventricular ratio (p = 0.0448).

**Conclusion:** Heart failure with a preserved ejection fraction is more prevalent in a middle-aged black South African sample with aortic stiffness when compared to European and American populations as assessed by high pulse wave velocity
and longstanding hypertension. N-terminal pro b-type natriuretic peptide, but not galectin-3, is independently associated with left atrial diameter, left ventricular hypertrophy and hence could be used for the diagnosis of heart failure with a preserved ejection fraction in this community sample.
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LIST OF ABBREVIATIONS

A wave  Trans-Mitral Blood Flow Velocity in the Late Period of Left Ventricular Diastolic Filling
ACEi  Angiotensin Converting Enzyme Inhibitor
Ao  Diameter of the Aorta in Diastole
Alx  Augmentation Index
ARBs  Aldosterone Receptor Blockers
BMI  Body Mass Index
BNP  Brain Natriuretic Peptide
BP  Blood Pressure
CAD  Coronary Artery Disease
CKD  Chronic Kidney Disease
COPD  Chronic Obstructive Pulmonary Disease
DBP  Diastolic Blood Pressure
DBPc  Central Diastolic Blood Pressure
DD  Diastolic Dysfunction
DGMAH  Dr George Mukhari Academic Hospital
DM  Diabetes Mellitus
E/A  Ratio of E Wave to A Wave Velocity
E/e’  Index of Left Ventricular Filling Pressures
EDTA  Ethylenediaminetetraacetic Acid
EF  Ejection Fraction
ELISA  Enzyme-Linked Immunosorbent Assay
ESC  European Society of Cardiology
FS Fractional Shortening Measured in Percentage
E wave Trans-Mitral Blood Flow Velocity in the Early Period of Left Ventricular Diastolic Filling
HbA1c Haemoglobin A1c
HF Heart Failure
HFmrEF Heart Failure with Mid-Range Ejection Fraction
HFpEF Heart Failure with Preserved Ejection Fraction
HFrEF Heart Failure with Reduced Ejection Fraction
HR Heart Rate
HT Hypertension
IVSd Interventricular Septal Diameter in Diastole
LA Left Atrial
LA/Ao Left Atrial/Aorta Diameter Ratio
LAE Left Atrial Enlargement
LA/LV Left Atrial/Left Ventricular Diameter Ratio
LV Left Ventricular
LVEDd Left Ventricular End-Diastolic Diameter
LVEF Left Ventricular Ejection Fraction
LVESd Left Ventricular End-Systolic Diameter
LVH Left Ventricular Hypertrophy
LVPWd Left Ventricular Posterior Wall Diameter in Diastole
MAPc Central Mean Arterial Pressure
MI Myocardial Infarction
NT-proBNP N-Terminal Pro-Brain Natriuretic Peptide
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>OSA</td>
<td>Obstructive Sleep Apnea</td>
</tr>
<tr>
<td>PH</td>
<td>Pulmonary Hypertension</td>
</tr>
<tr>
<td>PP</td>
<td>Pulse Pressure</td>
</tr>
<tr>
<td>PPC</td>
<td>Central Pulse Pressure</td>
</tr>
<tr>
<td>PPP</td>
<td>Peripheral Pulse Pressure</td>
</tr>
<tr>
<td>PWV</td>
<td>Pulse Wave Velocity</td>
</tr>
<tr>
<td>RAAS</td>
<td>Renin Angiotensin Aldosterone System</td>
</tr>
<tr>
<td>RWT</td>
<td>Relative Wall Thickness</td>
</tr>
<tr>
<td>SBP</td>
<td>Systolic Blood Pressure</td>
</tr>
<tr>
<td>SBPc</td>
<td>Central Systolic Blood Pressure</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SMU</td>
<td>Sefako Makgatho Health Sciences University</td>
</tr>
<tr>
<td>SMUREC</td>
<td>Sefako Makgatho Health Sciences University Research Ethics Committee</td>
</tr>
<tr>
<td>SV</td>
<td>Stroke Volume</td>
</tr>
<tr>
<td>TCHOL/HDL</td>
<td>Total Cholesterol to High Density Lipoprotein Ratio</td>
</tr>
<tr>
<td>TMB</td>
<td>3,3',5,5'-Tetramethylbenzidine</td>
</tr>
<tr>
<td>TOPCAT</td>
<td>Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist Trial</td>
</tr>
<tr>
<td>WHR</td>
<td>Waist to Hip Ratio</td>
</tr>
</tbody>
</table>
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CHAPTER 1

INTRODUCTION AND BACKGROUND
Heart disease, including heart failure (HF) is currently one of the leading causes of death in the United States and the Western world (1,2). The prevalence of HF is accepted as being between 1% and 2% of the general population, where heart failure with preserved ejection fraction (HFpEF) is prevalent in almost 50% of all HF cases (3). With such an extensive range of factors (presence of hypertension (HT), diabetes mellitus (DM), valvular heart disease and myocardial infarction (MI)), it is clear that there is a relatively large population at risk, potentially in excess of 25% of the total adult population (4). However, as the population ages, the prevalence of HF will likely increase, exacerbated by continuing difficulties in the effective management of HT, the growing epidemic of DM Type II, and the improved survival of patients with HF following an acute MI. In contrast to higher-income countries, the burden of HT and HT-related diseases, such as HF, is currently increasing significantly in lower-income countries. This has been attributed to increasing urbanisation (5).

DEFINITION OF HEART FAILURE

The current 2016 European Society of Cardiology (ESC) guidelines defines HF as a clinical syndrome characterised by symptoms such as breathlessness, reduced exercise tolerance, increased recovery time after exercise, tiredness and fatigue (6). As HF progresses, it also has accompanying signs (elevated jugular venous pressure, pulmonary crackles and peripheral oedema), that are caused by a structural and/or functional cardiac abnormality that ultimately results in a reduced cardiac output and/or increased intracardiac pressures at rest or during
stress or exercise. Unfortunately, the current definition used for HF is limited to stages at which the above-mentioned clinical symptoms are already present (6).

Before clinical symptoms become apparent, patients can present with asymptomatic structural and/or functional cardiac abnormalities (diastolic or systolic left ventricular dysfunction) which are characterised as the precursors of HF. These precursors of HF are related to poor outcomes, and by diagnosing HF in the precursory stage, before the patient starts to experience any clinically significant symptoms of HF, the appropriate treatment in the early stage of HF may reduce morbidity and mortality in patients with asymptomatic left ventricular (LV) dysfunction. It is however vital to identify the underlying cause of the development of HF first as it will determine the patient’s treatment regimen.

HF includes a wide range of patients, from those with a normal ejection fraction (EF) to those with a reduced EF (Table 1.1). The main terminology used to describe HF is based on the measurement of the left ventricular ejection fraction (LVEF) (6,7,9), whereby heart failure with a reduced EF (HFrEF) is characterised by left ventricular systolic dysfunction (inadequate contraction) while HFpEF is characterised by LV diastolic dysfunction (DD) (inadequate relaxation). The differentiation of patients with HF based on the LVEF is vital as both of these phenotypes present with different underlying aetiologies, demographics, co-morbidities and responses to the treatment therapy currently available.
In previous guidelines and publications, it was acknowledged that a grey area exists between HFpEF and HFrEF (10, 11). The current ESC 2016 guidelines includes HF with mid-range ejection fraction (HFmrEF) as part of the definition of HF. Patients with HFmrEF would most probably have primarily systolic dysfunction that is mild, together with some features of DD (6). Patients with HFmrEF therefore do not meet the requirements for the diagnosis of HFpEF, but also do not present with all features necessary for the diagnosis of HFrEF.

Table 1.1 The Definition of HFpEF, HFmrEF and HFrEF (6)

<table>
<thead>
<tr>
<th>TYPE OF HF</th>
<th>HFrEF</th>
<th>HFmrEF</th>
<th>HFpEF</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRITERIA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Symptoms and/or signs(^a) of HF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>LVEF &lt;40%</td>
<td>LVEF 40-49%</td>
<td>LVEF ≥50%</td>
</tr>
<tr>
<td>3</td>
<td>1. Increased levels of natriuretic peptides(^b)</td>
<td>1. Increased levels of natriuretic peptides(^b)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2. At least one additional criterion:</td>
<td>2. At least one additional criterion:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a) Relevant structural heart disease (LVH and/or LAE)</td>
<td>a) Relevant structural heart disease (LVH and/or LAE)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>b) DD</td>
<td>b) DD</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)In the early stages of HF (particularly HFpEF patients or those receiving diuretic treatment) signs may not necessarily be present. \(^b\)Brain natriuretic peptide (BNP) and N-terminal pro-brain natriuretic peptide (NT-proBNP) levels; BNP >35 pg/mL and NT-proBNP >125 pg/mL.

LVH – Left Ventricular Hypertrophy, LAE – Left Atrial Enlargement

PREVALENCE OF HEART FAILURE

It is worth noting that in recent years HF has not only become a major public health concern of considerable socioeconomic burden, but also accounts for significant morbidity and premature mortality (12). The prevalence and incidence of HF has increased globally with countries such as the United States and Asia
at the forefront, compared to countries in Europe. The United States currently has 5.7 million people with diagnosed HF, however it is expected that the number of people diagnosed with HF will increase to more than 8 million by the year 2030. This increase of 2.3 million newly diagnosed HF patients in the United States will result in a HF prevalence increase of 46% (13). The prevalence of HF (Figure 1.1) in Asia seems to be higher than in European countries, ranging between 1.3% and 6.7% (14) compared to 2.2%, 1.4% and 1.7% in Sweden (15), Italy (16) and Germany (17) respectively. There is currently limited insight into the prevalence and incidence of HF, more specifically HFpEF in sub-Saharan Africa, moreover in South Africa, although clinical characteristics and aetiologies of HFrEF have been extensively studied previously (18,19). Further research is warranted to investigate the prevalence of HFpEF among South Africans.

Figure 1.1 The Prevalence and Incidence of HF Worldwide (20).
PATHOPHYSIOLOGY OF HEART FAILURE

The pathophysiology of HFrEF is reasonably well understood and forms the basis of the therapeutic approaches in the current treatment of HF. These approaches include the administration of several agents such as β-blockers that influence the sympathetic nervous system, angiotensin converting enzyme inhibitors (ACEi) that influence the renin angiotensin aldosterone system (RAAS) and aldosterone receptor blockers (ARBs) (21). Data from several large multicentre clinical trials specific for HFrEF have shown that these therapeutic treatment approaches can reduce the risk of hospitalisation or death in these patients (23,24,25). Hence, the survival of patients with HFrEF has improved considerably over the last few years.

However, the proportion of patients with HFpEF is increasing steadily as opposed to those suffering from HFrEF (25). The increase in the prevalence of HFpEF is possibly a combination of an aging population, an increase in the prevalence of obesity, and/or an increase in the recognition and diagnosis of HF by physicians. Generally, a poor understanding of the pathophysiology of HFpEF exists, and hence a fail in therapeutic approaches. Unfortunately there is currently not a standardised treatment protocol specific for HFpEF as most HFpEF clinical trials showed a neutral outcome (26). This approach has failed to address the mortality of patients with HFpEF, and it is therefore important to broaden the current understanding of the pathophysiology of HFpEF.
Origin of Heart Failure with a preserved Ejection Fraction and the Current Understanding of the Pathophysiology of Heart Failure with a preserved Ejection Fraction

LV DD with LV remodelling due to MI and LV DD in hypertrophied hearts were two research areas that developed an interest in the clinical presentation and the development of HFrEF (27). The first studies that indicated LV DD to notably contribute to hypertensive heart disease (28), increased aortic stiffness and LV DD, surfaced during the late seventies. These studies described HFrEF as the presence of normal LV systolic function, but with the presence of LV DD (29).

Soon after this influx of research contributions to the area of diastolic LV dysfunction, specifically in hypertrophied hearts, HFrEF was identified and addressed as a by-product of large clinical based studies that focused on the use of ACEi in patients with HFrEF (30), as well as in patients with post-MI remodelling of the LV. Patients with HFrEF from these large studies were however clearly different from other HF patients, as they consisted of patients at risk for unfavourable eccentric LV remodelling due to limited MI.

LV dysfunction was also previously explained as the presence of prolonged isovolumic LV relaxation accompanied with slow LV filling together with an increase in LV diastolic stiffness (31). With the development of Doppler echocardiography, DD could easily be seen in recordings of the mitral valve inflow velocity. Abnormal mitral valve inflow velocities, suggestive of LV DD, were
however not specific just for HFpEF, but also included patients with HFrEF, and was seen in the recordings of older patients (32).

This uncertain origin of HFpEF led to the confusion surrounding HFpEF as a distinct diagnosis and ultimately caused the neutral outcome of numerous large HFpEF clinical trials (33). Indeed, cardiac hypertrophy has little in common with limited MI, and in both of these conditions the mechanisms that drive LV remodelling are likely to be different and would therefore react differently to pharmacological treatment.

The uniform presence at rest of elevated diastolic LV stiffness and slow relaxation of the left ventricle was recently re-evaluated through invasive research. Zile et al., 2014, showed that the uniform presence also demonstrated elevated diastolic stiffness that results in limited cardiac performance during exercise (34). This re-evaluation was also evident in the latest European Guidelines for the diagnosis of diastolic LV dysfunction.

Even though LV DD plays an important role as a mechanism underlying HFpEF, it is not seen as the only contributor to the pathophysiology of HFpEF. Indeed, numerous other important mechanisms in the development of HFpEF have been identified. These mechanisms include the following: Resting and exercise-exacerbated systolic dysfunction (15,16), impaired ventricular-vascular coupling (17,18), abnormal exercise-induced and flow mediated vasodilation (15), chronotropic incompetence (18,19), pulmonary arterial HT (20,21) as well as a
novel paradigm for the development of HFPfEF through coronary microvascular endothelial inflammation proposed by Paulus and Tschöpe, 2013 (42).

LV DD results from increased myocardial stiffness in the absence of endocardial or pericardial disease. Two compartments within the myocardium regulate its diastolic stiffness. Extracellular matrix and cardiomyocytes determine myocardial stiffness and act via matricellular proteins (27). Stiffness of the extracellular matrix is firstly largely determined by collagen through the regulation of its total amount, secondly due to the relative abundance of collagen type I, and thirdly due to the degree of collagen cross-linking. All three of these mechanisms appear to be involved in the development of HFPfEF (43).

Of clinical relevance is the observation that pro-collagen type I carboxy-terminal pro-peptide is therefore a potential biomarker of HFPfEF, specifically in patients with arterial HT (44). This is due to downregulation of matrix metalloproteinases and the upregulation of tissue inhibitors of matrix metalloproteinases in hypertensive patients. Another clinically relevant observation is that in LV endomyocardial biopsies done on HFPfEF patients, normal collagen volume fractions were seen in about one third of those patients. Their LV end-diastolic volume, LV end-systolic wall stress and LV modulus were however comparable with patients showing an increase in collagen volume fraction. This finding therefore suggests that in addition to the deposition of collagen, intrinsic cardiomyocyte stiffness also contributes to LV DD in patients with HFPfEF (45).
Another characteristic feature of HFpEF is slow LV relaxation, which may cause a decrease in LV stroke volume (SV), especially at an increased heart rate (HR) (27). This finding is in contrast to the normal healthy heart, which causes an increase in LV relaxation at an elevated HR.

Although the LVEF is preserved in patients with HFpEF, the LVEF is more accurately regarded as a measure of ventricular-arterial coupling than contractility alone. Impaired exercise capacity in patients with HFpEF is thus strongly associated with a decrease in the distensibility of the aorta, due to ventricular and vascular stiffening (27). Ventricular and vascular stiffening increase with aging, HT, DM and are abnormally elevated in elderly patients with HFpEF (46).

Ageing, DM and more specifically HT (as indicated in Figure 1.2) are known factors that cause an abnormal increase in ventricular and vascular stiffening of HFpEF patients. Abnormal relaxation and increased stiffness of the LV due to pressure overload, ischaemia, hypertrophy caused by longstanding HT and MI, cause both diastolic abnormalities and DD that ultimately lead to the development of diastolic HF. Abnormal LV relaxation and increased stiffness abnormally affect early LV filling, elevated LV filling pressures and increased left atrial pressure and size (47). It is therefore important to evaluate all factors contributing to the development of HFpEF before a clinical diagnosis is made.
Numerous risk factors (gender, age, HT and obesity) for cardiovascular disease are associated with the incidence of LVH and remodelling of LV structure. Maintained systolic and diastolic function is present in the initial stages of diastolic abnormalities, after which systolic and diastolic (pump/filling) dysfunction occurs. Over a period of time, the presence of congestive symptoms known to HF may progress extensively and result in sudden cardiac death or end-stage HF.

In the most recent ESC Guidelines for the diagnosis and treatment of acute and chronic HF, strict criteria have been proposed for the diagnosis of HFpEF (6). The criteria for HFpEF does not only consist of signs and symptoms of fluid overload and an LVEF of above 50%, but also includes evidence that LV DD is present (6). Because of these specific guidelines, most HFpEF patients are presenting with a concentric remodelled LV due the presence of several co-morbid conditions such as longstanding arterial HT, obesity and DM Type I and II.
CO-MORBIDITIES AND RISK FACTORS THAT ACCOMPANY AND CAUSE HEART FAILURE WITH PRESERVED EJECTION FRACTION

Numerous studies encourage that a phenotype-orientated approach is required to improve the outcome of HFpEF patients. Such an approach would move the focus towards the conditions that led them to consult with a medical professional in the first place, thus identifying, treating and managing the patient’s co-morbidities. Samson et al., 2016, described four commonly encountered clinical phenotypes of HFpEF, namely aging, obesity, coronary artery disease (CAD) and pulmonary hypertension (PH) and their accompanying risk factors and comorbid conditions (48). Interestingly, the prevalence of CAD among HFpEF patients is low and has therefore recently been used as a guideline to accurately enrol patients into HFpEF trials (48).

Table 1.2 Commonly Encountered Phenotypes of HFpEF and the Accompanying Risk Factors and Co-morbidities (48)

<table>
<thead>
<tr>
<th>PHENOTYPE</th>
<th>AGING</th>
<th>OBESITY</th>
<th>CAD</th>
<th>PH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk factors</td>
<td>HT</td>
<td>HT</td>
<td>HT</td>
<td>Hypercholesterolaemia</td>
</tr>
<tr>
<td>Poor diet</td>
<td>Lack of exercise</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co-morbidities</td>
<td>Atrial fibrillation</td>
<td>OSA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anaemia</td>
<td>DM Type I/II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COPD Frailty</td>
<td>CKD</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

COPD – Chronic Obstructive Pulmonary Disease, OSA – Obstructive Sleep Apnea, CKD – Chronic Kidney Disease
ASSESSING LEFT VENTRICULAR FUNCTION

LV DD is thought to be the fundamental pathophysiological abnormality present in patients with HFpEF. The key determinants of diastolic function includes both extrinsic (ventricular activation, age, systolic function, incoordination and filling pressures) and intrinsic (passive ventricular stiffness) factors, and must therefore be taken into account when assessing HFpEF (49). The classification of diastolic function can be defined as the degree of leftward and upwards shifts of the diastolic pressure-volume relationship. The direct measurement of this relationship would however require invasive techniques. It was previously shown that increased left atrial size is a marker of LV DD, and that the left atrial to left ventricular diameter ratio (LA/LV) can identify the degree of upward and leftward shifts in the relationship of the LV diastolic pressure-volume (50). The assessment of LV DD therefore plays a significant role in the diagnosis of HFpEF. Although echocardiography is currently the preferred imaging technique for the diagnosis of DD, no single echocardiography variable is sufficiently accurate to be used in isolation to make a diagnosis of LV DD. A complete echocardiography examination incorporating all relevant two-dimensional and Doppler studies are therefore currently recommended (6).
NATRIURETIC PEPTIDES AS BIOMARKERS OF HEART FAILURE

BNP and NT-proBNP

Myocardial cells located in both atria and ventricles secrete BNP in response to mechanical stretch (51), yet physiologically endocrine function of the heart is largely located in the atria (52). In HF, BNP production increases in the atria together with atrial natriuretic peptide. BNP’s gene is located on the short arm of chromosome 1. BNP consists of 32 amino acids with a central ring of 17 amino acids formed by a disulphide bond between cysteine amino acids (Figure 1.3). The messenger ribonucleic acid of BNP is translated into a precursor molecule named proBNP that consists of 108 amino acids. ProBNP is then cleaved into BNP and NT-proBNP (31,32).
Since natriuretic peptides were first discovered in the early 1980’s (55), new knowledge has been accumulating over the years about their important role in cardiovascular homeostasis. Initially thought to be entirely of value in the management of HF, an expanded role in cardiovascular health has been established over the last decade. Links between natriuretic peptides, myocardial fibrosis, and systemic inflammation have been established, suggesting that the secretion of this peptide may be a prompt defence mechanism against early cardiovascular insult (56).

The likely explanation supporting the superiority of natriuretic peptides over other risk indicators in predicting the risk for HF or other cardiovascular events is that
increased levels of natriuretic peptides in the blood indicate the presence of emerging pathology, while other risk indicators such as DM or HT solely imply risk of developing pathology.

**Plasma BNP and NT-proBNP in Assessing HF: Selecting NT-proBNP over BNP**

Determining plasma NT-proBNP levels has significant advantages over routine BNP plasma measurements, even though the plasma levels of the two will be similar under ideal conditions. NT-proBNP has a half-life of 60-120 minutes compared to a half-life of 18-22 minutes of BNP. NT-proBNP is therefore considered more stable when compared to BNP. Once blood samples are collected, the levels of BNP are not stable *in vitro* for long periods of time, decreasing significantly over the first 24 hours. There is however very little variation in the level of NT-proBNP for at least 72 hours or even longer (57). NT-proBNP was found to be stable for at least one year when stored at -80°C in several different serum and plasma conditions despite undergoing a minimum of five freeze-thaw cycles (58). NT-proBNP can therefore be assayed from stored specimens with confidence that the biomarker has not been degraded. In addition, assaying NT-proBNP has been found to be easier than assaying BNP, the reason being a higher plasma concentration. NT-proBNP has also been found to be a good marker in identifying patients with HF.
GALECTIN-3 AS A NOVEL BIOMARKER USED TO ASSESS HEART FAILURE WITH PRESERVED EJECTION FRACTION

Galectins form part of a family of soluble β-galactoside binding lectins that perform many important regulatory roles, specifically in the process of inflammation (59). Galectin-3 can be classified as a chimaera-type galectin and is also the only member of the galectin family that has an extended N-terminal domain constituted of tandem repeats of short amino acid segments linked to a single C-terminal carbohydrate-recognition domain (60). Lectin activity is made possible by the C-terminal domain, whereas the full biological activity of galectin-3 is due to the N-terminal domain (61). Research done by Meijers et al., 2014 showed that frozen plasma samples of galectin-3 can be stored for up to ten years at -80 °C without the loss of stability and viability (62).

A wide range of tissues produce galectin-3 and like other galectins (63), galectin-3 lacks a secretion signal peptide for classical vesicle-mediated exocytosis (64). Galectin-3 is found primarily in the cytoplasm and rarely in the nucleus and mitochondria (65). When galectin-3 is secreted into the extracellular space (via a non-classical secretory pathway that circumvents the endoplasmic reticulum and Golgi complex), it can interact with receptors on the cell surface as well as on glycoproteins to initiate transmembrane signalling pathways for different cellular functions (66). In human hearts, the expression of galectin-3 is generally low, however as HF progresses, rapid and significantly upregulation of galectin-3 takes place (67).
Cardiac stressors such as the release of angiotensin II and pressure overload within the heart causes upregulation galectin-3 in myocardial cells (65). The increase of galectin-3 production leads to cardiac remodelling and ultimately the development of HF (Figure 1.4). The expression level of galectin-3 mirrors the progression and severity of HF and therefore, galectin-3 is being used as a novel biomarker for HF. Galectin-3 is currently not used as a diagnostic biomarker for HF in clinical settings, and therefore still considered as a novel biomarker with the potential to assist in the diagnosis and prognosis of patients with HFpEF. Reference values for galectin-3 in HFpEF patients have not yet been established and an upper limit cut-off of 17.7 pg/mL has been proposed by Lok et al., 2010 (68). However, as galectin-3 is causally involved in pathological myocardial fibrosis, it has been suggested that galectin-3 also actively contributes to HF development (69).

Figure 1.4 The Structure and Effects of Galectin-3 (69).
The above figure shows the effects of galectin-3 on the kidneys, cardiac system and remodelling of the vascular system that could result in hypertrophy and fibrosis.
The majority of published articles on galectin-3 described patients with HFrEF and not HFpEF (70). Thus, more studies are needed to investigate the potential role of galectin-3 and the importance thereof as a possible diagnostic and prognostic biomarker in populations with HFpEF.

Galectin-3, which can be detected in plasma, is associated with several processes that are thought to play an important role in the disease process of HFpEF including myofibroblast proliferation, inflammation and ventricular remodelling (70). Future research is needed to establish whether galectin-3 in combination with other biomarkers has the potential to serve as a diagnostic tool for different disease states of HFpEF, and whether its prognostic and risk stratification abilities shown in acutely decompensated HFpEF patients is also apparent in compensated and clinically stable patients (71).

**PULSE WAVE VELOCITY AS A MEASURE OF AORTIC STIFFNESS**

PWV is emerging as the gold standard for evaluation of aortic stiffness (72). Bearing in mind that HFpEF occurs due to aortic dysfunction and that both age and HT increase arterial stiffness, assessment of aortic function is critical in such patients. Blood pressure (BP) measurements together with applanation tonometry at the radial, carotid and femoral arteries allow for reproducible assessments of several indexes of arterial stiffness. Indexes of arterial stiffness include the following: peripheral pulse pressure (PPp), central pulse pressure (PPc), peripheral augmentation index (Alp), central augmentation index (Alx) and
PWV. PWV, which is related to the stiffness of large arteries and inversely related to compliance (73), is possibly the best and most widely used non-invasive technique to determine the distensibility and stiffness of the aorta and proximal vessels. A number of studies support the notion that PWV is a simple and reliable index of arterial stiffness that is a strong independent predictor of cardiovascular outcomes (74). In this regard, arterial stiffness and wave reflections could add additional information with regard to the diagnostic process of patients diagnosed with HFPpEF.

PROBLEM STATEMENT AND RATIONALE

A prospective investigation of a large community-based sample of middle-aged to elderly individuals, done by Connie et al., 2015, revealed that an increased risk for HF is associated with increased aortic stiffness (75). Furthermore Chow et al., 2015 suggested that arterial stiffness of the LV could be a potential causative factor leading to the development of HFPpEF (76). Therefore, there is a necessity to investigate the prevalence of aortic stiffness among patients with HFPpEF and how it relates to the progression thereof.

Previous studies exploring HFPpEF (diastolic HF) have been conducted in the elderly (mean ages: 73.9±13.2 years), moreover on patients who were already receiving treatment (77,78,79,80). Although there are no sound population-based studies describing the prevalence of acute HF in sub-Saharan Africa, there is ample evidence to indicate important and unique characteristics when compared to developed countries and other parts of the world. Generally, the mean age of
HF presentation is considerably lower (40-55 years) in sub-Saharan Africa (81) when the majority of patients are of working age (82). This mean age suggests that HF affects people of sub-Saharan Africa in the prime of their life (working age) with the majority presenting with HT and CAD. HFpEF is therefore unlikely to be due to aortic dysfunction nor related to aortic stiffness as assessed by PWV. The risk factors associated with HFpEF might also be different in a middle-age population group when compared to the elderly, where a mean age of ≥75 years in most studies conducted on this phenotype in developed countries is observed. Similarly, according to hospital audits at Dr George Mukhari Academic hospital (DGMAH), the majority of patients admitted with HF are middle-aged. In this regard, it can be hypothesised that HFpEF is unlikely to be associated with aortic dysfunction and/or aortic stiffness in African patients living in South Africa.

I therefore aim to investigate biochemical markers that predict HF in these patients and specifically markers that predict or are associated with aortic dysfunction in middle-aged HFpEF participants.
CHAPTER 2

AIMS, HYPOTHESIS AND OBJECTIVES
2.1 Background and Research Aim

The aim of this study is to measure two different biochemical markers, NT-proBNP and galectin-3, of HFpEF in middle-aged patients with aortic dysfunction at the DGMAH.

2.2 Hypothesis

HFpEF as associated with increased levels of NT-proBNP and galectin-3 levels is likely to be more prevalent in a middle-aged population, however unlikely to be associated with aortic dysfunction and/or aortic stiffness.

Galectin-3 levels in a middle-aged African population is likely to be associated with LAE and LVH and would therefore contribute to the diagnosis HFpEF.

2.3 Objectives

2.3.1 To determine the prevalence of HFpEF with aortic dysfunction among middle-aged patients diagnosed with HFpEF at the DGMAH.

2.3.2 To determine whether there is an association between central haemodynamic parameters and the different biomarkers of HFpEF.

2.3.3 To determine whether there is an association between the different biomarkers (NT-proBNP and galectin-3) and the degree of HFpEF by comparing the concentration of biomarkers in the blood with the patient's LA and LV hypertrophy.
2.4 Research Questions

2.4.1 What is the mean age of patients diagnosed with HFpEF?

2.4.2 What is the prevalence of HFpEF with aortic dysfunction based on central haemodynamics among diagnosed HFpEF patients?

2.4.3 Is there an association between LA/Ao and LA/LV ratio and biomarker concentration?

2.4.4 Is there an association between central haemodynamics and biomarker concentration?
CHAPTER 3

METHODS
3.1 Study Group

The nature of the study was a case-control investigation. This study was conducted in the Department of Cardiology at the DGMAH, Odi District Hospital, Brits District Hospital, Shoshanguve BB clinic and at the Department of Physiology, Sefako Makgatho Health Sciences University (SMU), Ga-Rankuwa, South Africa. This is part of an ongoing study titled; Aortic function in HFrEF. The research proposal was approved by the SMU Research Ethics Committee (SMUREC) (SMUREC/M/08/2017:PG) and is in compliance with the declaration of Helsinki. Potential study participants were screened based on a definite diagnosis of HFrEF by the Cardiology division of DGMAH and the Department of Physiology as described in the 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic HF (6). Figure 3.1 shows the diagnostic algorithm for a diagnosis of HF of non-acute onset. All participants gave informed consent before participating in the study. Potential study participants were referred by both general and family physicians in primary and secondary health care settings. Sixty-six (66) participants presenting with HFrEF signs and symptoms were enrolled between July 2017 and September 2018. Inclusion criteria: Participants who were from African descent, 18 years and older and diagnosed with HFrEF. A maximum age was not capped in this study for comparison purposes, however the young-to-middle age was considered to be 18-44 years old and the middle-age 45-64 years as per Statistics South Africa guidelines (83). Potential participants who were younger than 18 years, not from African descent or those that warranted immediate emergency care, were not included in the study.
Figure 3.1 Diagnosis of HF According to the Recommendations of the ESC (6).

**Patient with Suspected HF**
(Non-acute onset)

**Assessment of HF Probability**

1. **Clinical History:**
   - History of CAD (MI, revascularisation)
   - History of arterial HT
   - Exposition to cardiotoxic drugs or radiation
   - Use of diuretics
   - Orthopnoea or paroxysmal nocturnal dyspnœa

2. **Physical Examination:**
   - Rales
   - Bilateral ankle oedema
   - Heart murmur
   - Jugular venous dilation
   - Laterally displaced or broadened apical beat

3. **ECG:**
   - Any abnormality

**Natriuretic Peptides**

- NT-pro BNP ≥ 125 pg/mL
- BNP ≥ 35 pg/mL

**Echocardiography**

If HF is Confirmed (based on all available data):
- Determine aetiology
- Start appropriate treatment

**HF Unlikely**
(Consider other diagnosis)
Participants enrolled in the study underwent echocardiographic and central haemodynamic measurements on the same day that blood samples were collected for routine blood and biomarker analysis. Each participant had random glucose, HbA1c (haemoglobin A1c), lipid profile, urea, creatinine, thyroid function and full blood count assessed. Routine blood analysis was performed at a central certified laboratory. Blood samples for HF biomarkers were processed in the Department of Physiology. Aliquoted plasma samples were stored at -80°C until assayed for galectin-3 and NT-proBNP.

3.2 Questionnaire

All participants completed a standardised English version of the African Project on Genes in Hypertension (APOGH) questionnaire (Appendix D), that was adjusted to comply with the requirements for this study. The questionnaire has shown to be reliable and feasible. Setswana is one of the major local languages in South Africa and one of the main languages spoken in the areas around the various hospitals and clinics where the study was conducted. The majority of the patients were reasonably proficient in the English language. Nevertheless, where there was need for interpretation, both study researchers and medical staff of the DGMAH Cardiology Department assisted. A patient information sheet written in both English and Setswana was also available to study participants. The questionnaire requested specific answers to date of birth, sex, background on diagnosis and treatment of HT and DM Type I and II. This questionnaire enabled the study to gather information with regard to the patient’s lifestyle, previous illness and family history.
3.3 Transthoracic Echocardiography

Before participating in the study, participants were diagnosed with HFpEF by a trained echocardiographer using transthoracic echocardiography. Echocardiography measurements were performed using a commercially available ultrasound system (GE Vivid 9). Participants were examined in the left lateral decubitus position using standard parasternal, short axis and apical views. Echocardiograph studies of participants were performed by an experienced senior echocardiographer and diagnosed according to the recommendations of the 2016 ESC guidelines for the diagnosis and treatment of acute and chronic HF (Refer to Table 1.1 in Chapter 1).

LVEF was measured using the modified biplane Simpson’s rule. Left ventricular end-diastolic diameter (LVEDd) and left ventricular end-systolic diameter (LVESd) were obtained from m-mode using parasternal short axis and parasternal long axis views. LVH and/or LAE were used as markers of structural heart disease.

3.4 Clinical Data Collection

Clinical data was obtained from all participants through detailed history taking, clinical examination that included a transthoracic echocardiograph and accessing available previous hospital records. Demographic data was recorded for each patient and anthropometric measurements, including weight, height, waist and hip circumference were measured on first consultation (visit one), one month
follow-up (visit two) and again two to four months later during follow-up (visit three) according to the physician’s recommendation.

3.5 Anthropometric Measurements

Anthropometric measurements included the participant’s height, body weight, waist and hip circumference. Readings were done in triplicate and the average, to the nearest first decimal, was recorded as the final measurement. A standard digital weighing scale was used to measure weight and a stadiometer with a fixed vertical backboard and adjustable head piece for height (Seca 763 Digital Column Scale). Weight and height were measured with the patient standing, wearing indoor clothes with no shoes. The body mass index (BMI) of a participant was calculated as weight per square metre (kg/m$^2$). Participants were identified as being overweight if their BMI was $\geq 25$ kg/m$^2$ and obese if their BMI was $\geq 30$ kg/m$^2$ according to the European guidelines for obesity management in adults (84). Waist circumference was measured to the nearest 0.1 cm by locating the upper hip bone and the top of the right iliac crest, and placed horizontally around the abdomen at the level of the iliac crest. Measurements were taken at the end of a normal respiration, using a non-elastic measuring tape. Hip circumference was measured to the nearest 0.1 cm by locating the greater trochanter, and placed horizontally at the widest lateral extension of the hips. The waist-to-hip ratio (WHR) was also calculated.
3.6 Conventional Blood Pressure Measurements

BP measurements were obtained, according to standard guidelines, with a manual sphygmomanometer and a stethoscope. BP was measured five consecutive times after the participant rested in a supine position for five minutes. The BP cuff was deflated whereby Korotkov phases I and V were employed to identify systolic and diastolic BP respectively. Standard cuffs were used with an inflatable bladder with a length of 22 cm and a width of 12 cm except when arm circumference exceeded 31 cm, when larger cuffs with a 31 x 15 cm bladder were used. The five consecutive BP readings were averaged to obtain a single systolic and diastolic BP reading for each participant. PPp was calculated as the difference between systolic blood pressure (SBP) and diastolic blood pressure (DBP), while mean arterial pressure (MAP) was calculated as a third of DBP plus PPp.

HT was defined as the use of antihypertensive treatment or a BP ≥ 140/90 mmHg; high normal levels from 130-139 mmHg systolic and 85-89 mmHg diastolic; optimal BP <130/85 mmHg. A mean conventional systolic <120 and diastolic <80 mmHg was considered as normal (85) as shown in Table 3.1.

<table>
<thead>
<tr>
<th>CATEGORY</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>120-129</td>
<td>80-84</td>
</tr>
<tr>
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<td>130-139</td>
<td>85-89</td>
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<tr>
<td>Grade 1 HT</td>
<td>140-159</td>
<td>90-99</td>
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<tr>
<td>Grade 2 HT</td>
<td>160-179</td>
<td>100-109</td>
</tr>
<tr>
<td>Grade 3 HT</td>
<td>≥180</td>
<td>≥110</td>
</tr>
<tr>
<td>Isolated Systolic HT</td>
<td>≥140</td>
<td>&lt;90</td>
</tr>
</tbody>
</table>


3.7 Aortic Stiffness and Central Haemodynamic Parameters

PWV was determined using applanation tonometry and PulsePen software. After participants had rested for 15 minutes in the supine position, arterial waveforms were determined at the carotid, radial and femoral pulse by applanation tonometry using a tonometer (PulsePen) interfaced with a computer employing PulsePen, version 6.21 software (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia). Recordings where the systolic or diastolic variability of consecutive waveforms exceeded 5%, or where the amplitude of the pulse wave signal was less than 80 mV, were discarded. The pulse wave was calibrated by manual BP measurements (auscultation) taken immediately before the recordings. From a validated inbuilt transfer function, an aortic waveform was generated from which central SBP (SBPc), central DBP (DBPc) and central MAP (MAPc) were derived. PPC was calculated as the difference between SBPc and DBPc and MAPc calculated as DBPc + 1/3(PPc).

To determine aortic PWV, distances from the suprasternal notch to the carotid sampling site (distance A) and from the suprasternal notch to the femoral artery (distance B) were measured. PWV distance was calculated as distance B minus distance A. Pulse transit time, calculated as the mean time difference between sites A and B were determined from the average of 12 consecutive beats. Aortic PWV was calculated as the ratio of the distance in metres to the transit time in seconds. To determine central BP, the radial waveform was recorded at the left arm over an eight second period and the pulse wave calibrated by auscultatory measurement of the brachial BP immediately before the recordings. From an
inbuilt transfer function an aortic waveform was generated from which SBPc, DBPc and MAPc were derived. The magnitude of the forward and reflected waves was determined with wave separation analysis using the triangular flow wave method. The ratio between the reflected and forward waves was employed as an index of arterial stiffness.

3.8 Biomarker Blood Sample Collection and Storage

Apart from routine clinical blood samples taken, approximately 10 mL blood was collected from each participant by a registered nurse to be used for NT-proBNP and galectin-3 analysis. Samples were collected on the first consultation (visit one), one-month follow-up (visit two) and again two to four months later during follow-up (visit three) according to the physician’s recommendation. Blood samples were collected in ethylenediaminetetraacetic acid (EDTA) vacutainer tubes and centrifuged within 30 minutes at 2000 x g (Hermle Z326k) for 20 minutes, plasma aspirated, aliquoted and then stored at -80°C in a biofreezer (Thermo Scientific Forma 900 Series) until further analysis.

3.9 Biomarker Plasma Sample Measurements

3.9.1. Biomarker Assays

NT-proBNP and galectin-3 levels were measured in the stored plasma samples with commercially available enzyme-linked immunosorbent (ELISA) assays. The
measurement of NT-proBNP and galectin-3 concentrations were determined blind to the clinical details of the participants enrolled into the study. The assays were done according to manufacturer’s instructions within two months to one year of sample collection.

Furthermore, using the NT-proBNP and galectin-3 assays for this study, NT-proBNP and galectin-3 in frozen plasma samples were proven to have long-term stability when stored at -80°C for up to one year (58) and ten years (62) respectively.

3.9.2. Plasma NT-proBNP Assay Procedure

The sandwich ELISA kit used for the quantification of NT-proBNP was acquired from Cloud Clone Corp, USA (Catalogue number: SEA485Hu). The following reagents and materials were provided in the kit: a 96-well strip plate pre-coated with a capture antibody specific to NT-proBNP, wash buffer concentrate, human NT-proBNP standard with a standard diluent, assay diluent, detection reagents containing horseradish peroxidase conjugated antibody, 3,3',5,5'-tetramethylbenzidine (TMB) substrate and a stop solution.

In brief, 100 µL of each target antigen (dilutions of standard, blank and samples) was added to each microwell and covered with a plate sealer. The microplate was then placed in an oven (Labcon 5016U) set at 37°C for one hour to incubate. After aspirating the liquid in the microplate wells, 100 µL of detection antibody
(reagent A working solution) was added to each well followed by another one-hour incubation period in the oven set at 37°C. Subsequently the plate was washed three times with 350 µL of wash solution using a plate washer (Biotek ELx50). The wash solution was left for one to two minutes to incubate at room temperature during each cycle. Thereafter, 100 µL of horseradish peroxidase conjugated antibody (detection reagent B) was added and allowed to bind to the antigen for a period of 30 minutes at 37°C. Unbound reagents were removed by washing (as described above) for a total of five times. Finally, 90 µL of TMB substrate solution was added followed by a 20-minute incubation period at 37°C. The enzyme-substrate reaction was terminated by adding 50 µL of stop solution to each well. A spectrophotometer was used to measure the optical density immediately at 450 nm using a microplate reader (Biotek ELx800). All determinations were done in triplicate.

3.9.3. Plasma Galectin-3 Assay Procedure

The sandwich ELISA kit for human galectin-3 quantification was bought from R&D Systems, Minneapolis, USA (Catalogue number: DGAL 30). The following reagents and materials were provided in the kit: a 96-well microplate pre-coated with a monoclonal antibody specific for human galectin-3, wash buffer concentrate, human galectin-3 standard, polyclonal antibody specific for galectin-3 and linked to horseradish peroxidase, assay and calibrator diluent, colour reagent A (stabilised hydrogen peroxide), colour reagent B (stabilised TMB) and a 2 N sulphuric acid stop solution.
In brief, 100 µL assay diluent was added to each microwell before 50 µL of target antigen (standards, controls and samples) was dispensed and incubated for two hours at room temperature (23°C). After aspirating and washing four times with 400 µL of wash buffer using a plate washer (Biotek ELx50), 200 µL of detection antibody was added to each well followed by another two-hour incubation period at room temperature. After a second washing step (as described above), 200 µL substrate solution containing colour reagent A and B was added. A third incubation period of 30 minutes at room temperature followed. Finally, 50 µL 2 N sulphuric acid stop solution was added to each well and the optical density was determined within 30 minutes at 450 nm using a microplate reader (Biotek ELx800). All determinations were done in triplicate.

3.9.4. Calculation of Plasma NT-proBNP and Galectin-3 Concentrations

Triplicate absorbance readings for each standard, control and sample were averaged and the average zero standard optical density was subtracted. A standard curve was generated by plotting the mean absorbance for each standard on the y-axis against the concentration of each standard on the x-axis. A best fit linear trend line, with a set intercept of 0, using Microsoft Excel ® software was drawn through the points on a scatter plot. The linear equation of the trend line was generated by the software and used to determine the participant’s NT-proBNP and galectin-3 concentrations in plasma.
3.10 Statistical Analysis

Statistical analysis was done using SAS software package version 9.4 (SAS Institute Inc., Cary, NC). Data were expressed as mean ± the standard deviation (SD). Unadjusted means and proportions were compared by the large-sample z-test and the $\chi^2$-test. An independent t-test was performed to compare those with and those without HFpEF. A Wilcoxon signed rank test was performed in those instances where variables did not follow a normal distribution.

The dependent t-test was performed to compare HFpEF patient visits and the Wilcoxon rank sum test was performed where variables failed the test of normality. Multivariate unadjusted and adjusted regression analysis was also performed to determine independent relationships. Confounders that were included in the regression analysis were identified from univariate analysis.
CHAPTER 4

RESULTS
4.1 Demographic and Anthropometric Characteristics

Table 4.1 gives the demographic and anthropometric characteristics of the study participants. More participants from middle age (45-64 years) (83) and more females (56%), of whom 61% were postmenopausal, compared to males (44%) presented with HFpEF. Specifically (not shown in Table 4.1), the prevalence of HFpEF was 32% in young patients (18-44 years), 44% in middle age and 24% in the elderly (≥65 years). In addition, a high proportion of participants were overweight or obese (BMI >25kg/m²) with obesity statistically significant pronounced in HFpEF participants (86%). No significant differences between the two groups were observed in other indexes of adiposity (hip circumference, waist circumference and WHR).

Generally, a lower percentage of participants presented with DM Type I/II in participants without HFpEF (14%) when compared to those with HFpEF (20%). On average a low proportion of participants in both groups reported either regular smoking or regular consumption of alcohol. However, the percentage alcohol consumption was statistically significantly greater in those with HFpEF compared to those without HFpEF. A greater proportion of HFpEF participants were hypertensive compared to the control group (69,23% vs 55,39%). The percentage of participants with HFpEF receiving antihypertensive treatment was also significantly higher than the participants without HFpEF (83% vs 35%). Normal blood creatinine values were measured in HFpEF patients (95,56±91,64 µmol/L) excluding renal disease as a co-morbidity.
<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>PARTICIPANTS WITHOUT HFpEF</th>
<th>PARTICIPANTS WITH HFpEF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Number (n)</td>
<td>213</td>
<td>66</td>
</tr>
<tr>
<td>% Male</td>
<td>42.25</td>
<td>43.94</td>
</tr>
<tr>
<td>% Female</td>
<td>57.75</td>
<td>56.06</td>
</tr>
<tr>
<td>% Postmenopausal Females</td>
<td>70.27</td>
<td>60.66</td>
</tr>
<tr>
<td>Age (years)</td>
<td>52.08±16.69</td>
<td>54.88±13.51</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.61±0.09</td>
<td>1.61±0.13</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77.05±19.43</td>
<td>84.26±20.67*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.85±7.68</td>
<td>33.16±9.56**</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>93.55±17.14</td>
<td>97.48±16.76</td>
</tr>
<tr>
<td>Hip Circumference (cm)</td>
<td>107.12±15.24</td>
<td>112.16±16.49</td>
</tr>
<tr>
<td>WHR</td>
<td>0.87±0.12</td>
<td>0.87±0.12</td>
</tr>
<tr>
<td>% Overweight/Obese</td>
<td>69.48</td>
<td>85.71**</td>
</tr>
<tr>
<td>% Smokers</td>
<td>17.84</td>
<td>13.85</td>
</tr>
<tr>
<td>% Alcohol</td>
<td>16.90</td>
<td>33.85**</td>
</tr>
<tr>
<td>% With HT</td>
<td>55.39</td>
<td>69.23</td>
</tr>
<tr>
<td>% Treated for HT</td>
<td>34.74</td>
<td>83.08***</td>
</tr>
<tr>
<td>% With DM I/II</td>
<td>13.62</td>
<td>19.70</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>-</td>
<td>95.56±91.64</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, ***p<0.001 Participants without HFpEF vs participants with HFpEF. Normal reference range for creatinine is 64 – 104 µmol/L.
4.2 Clinical, Haemodynamic and Laboratory Characteristics

The clinical, haemodynamic and laboratory characteristics are shown in Table 4.2. The PPp was significantly higher in HFpEF participants (p<0.0001) when compared to participants without HFpEF. However, the PPp for both groups was above the normal range (40 mmHg) (86). In the participants with HFpEF with a mean age of 54.88±13.51 years, a high carotid to femoral PWV of 9.97±2.78 m/s was observed when compared to those without HFpEF (6.11±2.18 m/s). With regard to biomarkers of HF, a significantly higher galectin-3 level (p = 0.0026) in the HFpEF group was noted compared to the control group. Galectin-3 concentrations in both groups were still below the upper limit cut-off point of 17,7 pg/mL (68). The NT-proBNP level was not measured in the participants without HFpEF as those participants were not presenting with signs and symptoms of HF. Importantly, the level of NT-proBNP of participants with HFpEF was lower than the 125 pg/mL cut-off given by the ESC guidelines for the diagnosis and management of HFpEF.

In addition, statistically significant differences between participants without and with HF were observed in peripheral haemodynamic measures (SBP, PP and HR) with p-values <0.001, <0.0001 and 0.008 respectively. Carotid to femoral PWV (p<.0001) and PPC (p = 0.0002) showed statistically significant differences between the two groups. There were no statistically significant differences between peripheral DBP, SBPc and DBPc.
Table 4.2 Clinical and Laboratory Characteristics of Study Participants

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>PARTICIPANTS WITHOUT HFpEF</th>
<th>PARTICIPANTS WITH HFpEF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peripheral Haemodynamic Measurements</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP/DBP (mmHg)</td>
<td>130.43±22.62/84.22±11.77</td>
<td>138.11±17.45/84.23±11.25</td>
</tr>
<tr>
<td>PP (mmHg)</td>
<td>46.33±18.97</td>
<td>53.88±12.49***</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>65.47±11.62</td>
<td>69.62±12.60**</td>
</tr>
<tr>
<td><strong>Central Haemodynamic Measurements</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carotid-Radial PWV (m/s)</td>
<td>-</td>
<td>8.75±1.93</td>
</tr>
<tr>
<td>SBP/DBP (mmHg)</td>
<td>-</td>
<td>129.16±20.00/83.89±11.53</td>
</tr>
<tr>
<td>PP (mmHg)</td>
<td>-</td>
<td>45.11±16.23</td>
</tr>
<tr>
<td>Carotid-Femoral PWV (m/s)</td>
<td>6.11±2.18</td>
<td>9.97±2.78***</td>
</tr>
<tr>
<td>SBP/DBP (mmHg)</td>
<td>131.40±21.64/84.92±12.43</td>
<td>129.57±20.53/84.34±11.91</td>
</tr>
<tr>
<td>PP (mmHg)</td>
<td>37.18±17.25</td>
<td>44.88±16.68***</td>
</tr>
<tr>
<td><strong>Biomarker Concentrations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NT-proBNP (pg/mL)</td>
<td>-</td>
<td>120.86±258.79</td>
</tr>
<tr>
<td>Galectin-3 (pg/mL)</td>
<td>8.32±2.92</td>
<td>9.41±2.24**</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, ***p<0.001 Participants without HFpEF vs participants with HFpEF.
4.3 Echocardiography Data

Patients generally presented with symptoms of breathlessness, tiredness, fatigue and signs of peripheral oedema such as swelling of the ankles. The following common co-morbid conditions associated with HFpEF were observed in South African HFpEF participants: HT, DM Type I/II, obesity, OSA, CKD, COPD and PH. None of the participants with HFpEF reported CAD.

The echocardiographic data of participants with and without HFpEF is presented in Table 4.3. A LVEF of above 50% was noted in both groups. Surprisingly the HFpEF group presented with a significantly higher EF. An increased fractional shortening percentage (FS) and LVEDd was observed in participants without HFpEF, with a higher interventricular septum thickness diameter in diastole (IVSd) and left ventricular posterior wall diameter at end diastole (LVPWd) in the HFpEF group. A large difference between the two groups in the LV mass, relative wall thickness (RWT) and LV mass index was noted, with an LV mass of 22.38±70.33 g in those with HFpEF compared to 142.91±54.38 g in those without HFpEF. The diameters of the left atria as well as the diameter of the aorta in diastole (Ao), were higher in HFpEF participants compared to the control group. Furthermore, the ratios of the LA diameter to the diameter of the aorta in diastole (LA/Ao) and LA/LV ratio measurements were increased in HFpEF participants compared to the participants without HFpEF.

All echocardiographic parameters showed a statistically significant difference between participants without HFpEF and participants with HFpEF with the
exception of LVEd, LVPWs and Ao. The IVSd, LVPWd, LV mass, RWT, LV mass index, LA, LA/Ao and LA/LV parameters were significantly increased (\(p<0.0001\)) in participants with HFpEF compared to the control group.

### Table 4.3 Echocardiography Data of Study Participants

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>PARTICIPANTS WITHOUT HFpEF</th>
<th>PARTICIPANTS WITH HFpEF</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEF (%)</td>
<td>66.19±10.03</td>
<td>68.75±5.77*</td>
</tr>
<tr>
<td>FS (%)</td>
<td>39.46±8.66</td>
<td>29.27±16.89**</td>
</tr>
<tr>
<td>LVEDd (cm)</td>
<td>4.73±0.65</td>
<td>4.62±0.64</td>
</tr>
<tr>
<td>IVSd (cm)</td>
<td>0.87±0.17</td>
<td>1.28±0.18***</td>
</tr>
<tr>
<td>LVPWd (cm)</td>
<td>0.87±0.15</td>
<td>1.23±0.17***</td>
</tr>
<tr>
<td>LVPWs (cm)</td>
<td>1.27±0.25</td>
<td>1.37±0.26</td>
</tr>
<tr>
<td>LV Mass (g)</td>
<td>142.91±54.38</td>
<td>224.38±70.33***</td>
</tr>
<tr>
<td>RWT</td>
<td>0.38±0.07</td>
<td>0.54±0.11***</td>
</tr>
<tr>
<td>LV Mass Index (g/m²)</td>
<td>39.77±15.31</td>
<td>68.68±34.68***</td>
</tr>
<tr>
<td>LA Diameter (cm)</td>
<td>2.66±0.48</td>
<td>3.74±0.54***</td>
</tr>
<tr>
<td>Ao (cm)</td>
<td>2.52±0.44</td>
<td>2.62±0.46</td>
</tr>
<tr>
<td>LA/Ao</td>
<td>1.07±0.20</td>
<td>1.40±0.27***</td>
</tr>
<tr>
<td>LA/LV</td>
<td>0.56±0.10</td>
<td>0.82±0.12***</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, ***p<0.001 Participants without HFpEF vs participants with HFpEF. LV Mass Index indexed to height (2.7).
4.4 Demographic Anthropometric Characteristics of Heart Failure with a Preserved Ejection Fraction Study Participants (Visit 1, 2 and 3)

Table 4.4 indicates that more females compared to males were seen for follow-up. Anthropometric measurements did not differ significantly throughout the participant follow-up period. No significant differences between the visits were observed in lifestyle habits (smoking and alcohol consumption) and comorbidities such as HT and DM III.
<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>VISIT 1</th>
<th>VISIT 2</th>
<th>VISIT 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Number (n)</td>
<td>66</td>
<td>43</td>
<td>20</td>
</tr>
<tr>
<td>% Male</td>
<td>43.94</td>
<td>40.91</td>
<td>41.67</td>
</tr>
<tr>
<td>% Female</td>
<td>56.06</td>
<td>59.09</td>
<td>58.33</td>
</tr>
<tr>
<td>% Postmenopausal Females</td>
<td>39.39</td>
<td>40.91</td>
<td>45.83</td>
</tr>
<tr>
<td>Age (years)</td>
<td>54.88±13.51</td>
<td>56.52±13.26</td>
<td>59.79±14.14</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.61±0.13</td>
<td>1.61±0.10</td>
<td>1.59±0.13</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>84.26±20.67</td>
<td>85.66±18.04</td>
<td>88.28±20.85</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>33.16±9.56</td>
<td>33.56±9.85</td>
<td>35.72±12.19</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>97.48±16.76</td>
<td>98.95±13.82</td>
<td>101.14±14.33</td>
</tr>
<tr>
<td>Hip Circumference (cm)</td>
<td>112.16±16.49</td>
<td>111.40±14.96</td>
<td>112.16±15.34</td>
</tr>
<tr>
<td>WHR</td>
<td>0.87±0.12</td>
<td>0.89±0.11</td>
<td>0.90±0.08</td>
</tr>
<tr>
<td>% Overweight/Obese</td>
<td>48.48</td>
<td>56.82</td>
<td>58.33</td>
</tr>
<tr>
<td>% Smokers</td>
<td>13.64</td>
<td>11.36</td>
<td>8.33</td>
</tr>
<tr>
<td>% Alcohol</td>
<td>33.33</td>
<td>36.36</td>
<td>37.5</td>
</tr>
<tr>
<td>% With HT</td>
<td>68.18</td>
<td>72.73</td>
<td>75</td>
</tr>
<tr>
<td>% Treated for HT</td>
<td>68.89</td>
<td>56.82</td>
<td>58.33</td>
</tr>
<tr>
<td>% With DM I/II</td>
<td>19.70</td>
<td>13.64</td>
<td>8.33</td>
</tr>
</tbody>
</table>
4.5 Clinical, Haemodynamic and Laboratory Characteristics of Heart Failure with a Preserved Ejection Fraction Study Participants (Visit 1, 2 and 3)

Statistically significant differences between visit one and visit two were observed in PPp ($p = 0.0402$), SBPc carotid to radial ($p = 0.0346$) and SBPc carotid to femoral ($p = 0.0289$). As shown in Table 4.5, PPc decreased between visits one and two and one and three, particularly from visit one to two, but was however not statistically significant. When visit two and three were compared, carotid to femoral PWV showed a significant decrease with $p = 0.0150$. A downward trend was observed in PWV from visit one to three; however, not statistically significant. Similarly, a downward trend was observed in biomarkers of HF (NT-proBNP and galectin-3) between visits one and three. All the other parameters measured remained stable during the period from the initial visit to visit three.
<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>VISIT 1</th>
<th>VISIT 2</th>
<th>VISIT 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peripheral Hemodynamic Measurements</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP/DBP (mmHg)</td>
<td>138.11±17.45/84.23±11.25</td>
<td>132.40±14.91/82.96±10.76</td>
<td>132.57±17.7/80.93±10.16</td>
</tr>
<tr>
<td>PP (mmHg)</td>
<td>53.88±12.49</td>
<td>48.32±16.18*</td>
<td>51.64±14.43</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>69.62±12.60</td>
<td>70.31±12.04</td>
<td>71.53±15.25</td>
</tr>
<tr>
<td><strong>Central Hemodynamic Measurements</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carotid-Radial PWV (m/s)</td>
<td>8.75±1.93</td>
<td>8.30±2.78</td>
<td>8.75±3.03</td>
</tr>
<tr>
<td>SBP/DBP (mmHg)</td>
<td>129.16±20.00/83.89±11.53</td>
<td>121.45±11.58*/82.73±10.97</td>
<td>123.05±14.51/82.10±9.27</td>
</tr>
<tr>
<td>PP (mmHg)</td>
<td>45.11±16.23</td>
<td>40.23±13.78</td>
<td>40.95±12.77</td>
</tr>
<tr>
<td>Carotid-Femoral PWV (m/s)</td>
<td>9.97±2.78</td>
<td>9.60±2.04</td>
<td>9.2±2.14*</td>
</tr>
<tr>
<td>SBP/DBP (mmHg)</td>
<td>129.57±20.53/84.34±11.91</td>
<td>123±14.80*/83.15±10.46</td>
<td>120.42±13.04/80.05±9.25</td>
</tr>
<tr>
<td>PP (mmHg)</td>
<td>44.88±16.68</td>
<td>39.85±12.85</td>
<td>40.37±14.49</td>
</tr>
<tr>
<td><strong>Biomarker Concentrations</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NT-proBNP (pg/mL)</td>
<td>120.86±258.79</td>
<td>168.26±230.18</td>
<td>102.16±189.29</td>
</tr>
<tr>
<td>Galectin-3 (pg/mL)</td>
<td>9.41±2.24</td>
<td>9.53±2.29</td>
<td>9.33±2.24</td>
</tr>
</tbody>
</table>

*p<0.05 visit 1 vs visit 2, ‡p<0.05 visit 2 vs visit 3.
4.6 Echocardiography Data of Heart Failure with a Preserved Ejection Fraction Study Participants (Visit 1, 2 and 3)

No statistically significant differences were noted in cardiac structure and function between visits of the HF participants, except for IVSd that showed a significant difference ($p = 0.0381$) between visit two and visit three as indicated in Table 4.6.
<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>VISIT 1</th>
<th>VISIT 2</th>
<th>VISIT 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEF (%)</td>
<td>68.75±5.77</td>
<td>68.33±10.82</td>
<td>72.40±8.38</td>
</tr>
<tr>
<td>FS (%)</td>
<td>29.27±16.89</td>
<td>38.92±8.05</td>
<td>42.00±8.00</td>
</tr>
<tr>
<td>LVEDd (cm)</td>
<td>4.62±0.64</td>
<td>4.90±0.82</td>
<td>4.71±0.37</td>
</tr>
<tr>
<td>IVSd (cm)</td>
<td>1.28±0.18</td>
<td>1.40±0.35</td>
<td>1.72±0.20†</td>
</tr>
<tr>
<td>LVPWd (cm)</td>
<td>1.23±0.17</td>
<td>1.67±0.23</td>
<td>1.51±0.44</td>
</tr>
<tr>
<td>LVPWs (cm)</td>
<td>1.37±0.26</td>
<td>1.67±0.48</td>
<td>1.67±0.32</td>
</tr>
<tr>
<td>LV mass (g)</td>
<td>224.38±70.33</td>
<td>265.67±108.28</td>
<td>297.74±92.07</td>
</tr>
<tr>
<td>RWT</td>
<td>0.54±0.11</td>
<td>0.48±0.13</td>
<td>0.61±0.25</td>
</tr>
<tr>
<td>LV mass index (g/m²)</td>
<td>68.68±34.68</td>
<td>79.11±39.37</td>
<td>108.26±59.85</td>
</tr>
<tr>
<td>LA Diameter (cm)</td>
<td>3.74±0.54</td>
<td>3.82±0.59</td>
<td>3.75±0.49</td>
</tr>
<tr>
<td>Ao (cm)</td>
<td>2.62±0.46</td>
<td>2.86±0.52</td>
<td>2.84±0.32</td>
</tr>
<tr>
<td>LA/Ao</td>
<td>1.40±0.27</td>
<td>1.36±0.25</td>
<td>1.30±0.26</td>
</tr>
<tr>
<td>LA/LV</td>
<td>0.62±0.12</td>
<td>0.82±0.59</td>
<td>0.80±0.10</td>
</tr>
</tbody>
</table>

‡p<0.05 visit 2 vs visit 3. LV Mass Index indexed to height (2.7).
4.7 Association between Central Haemodynamic Measurements, Biomarker Concentrations and Echocardiography Parameters

The strongest relationships were shown between PPp and PPC, NT-proBNP and LA diameter, LA diameter and LA/LV and galectin-3 and NT-proBNP ($p<0.001$) as indicated in Table 4.7. Statistically significant relationships were observed between PWV and PPp, NT-proBNP and PPp (Figure 4.4), LA diameter and PWV (Figure 4.7), NT-proBNP and PWV, LV mas index and PWV, LV mass index and LA diameter, LA/Ao and LA diameter, NT-proBNP and LA/LV and galectin-3 and NT-proBNP. Furthermore, only a trend effect was noted between LA/Ao and NT-proBNP ($p = 0.065$).

Table 4.8 shows the linear regression analysis for NT-proBNP and echocardiography parameters as well as PWV and echocardiography parameters. A strong relationship was observed between NT-proBNP and LA diameter ($p = 0.0005$). A statistically significant regression relationship was seen between biomarkers of HF (NT-proBNP and galectin-3) and LA diameter above 3.5 cm in diastole (Figure 4.2).

NT-proBNP was independently associated with both LV mass and LA diameter after adjustments for physiological and cardiovascular risk factors (Figure 4.5 and 4.6).
Table 4.7 Correlation Matrix between Central Haemodynamic Measurements, Biomarker Concentrations and Echocardiography Parameters

<table>
<thead>
<tr>
<th></th>
<th>PPp</th>
<th>PPC</th>
<th>PWV</th>
<th>LA Diameter</th>
<th>LV Mass Index</th>
<th>LA/LV</th>
<th>LA/Ao</th>
<th>NT-proBNP</th>
<th>Galectin-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPp (mmHg)</td>
<td>-</td>
<td>0.908***</td>
<td>0.284*</td>
<td>-0.129</td>
<td>0.122</td>
<td>-0.108</td>
<td>0.106</td>
<td>0.273*</td>
<td>0.267</td>
</tr>
<tr>
<td>PPC (mmHg)</td>
<td>0.908***</td>
<td>-</td>
<td>0.215</td>
<td>-0.056</td>
<td>0.341</td>
<td>-0.110</td>
<td>0.186</td>
<td>0.005</td>
<td>0.195</td>
</tr>
<tr>
<td>PWV (m/s)</td>
<td>0.284*</td>
<td>0.215</td>
<td>-</td>
<td>0.381*</td>
<td>0.633**</td>
<td>0.209</td>
<td>-0.195</td>
<td>0.311*</td>
<td>0.207</td>
</tr>
<tr>
<td>LA Diameter (cm)</td>
<td>-0.129</td>
<td>-0.056</td>
<td>0.381*</td>
<td>-</td>
<td>0.505**</td>
<td>0.575***</td>
<td>0.508</td>
<td>0.573***</td>
<td>0.157</td>
</tr>
<tr>
<td>LV Mass Index (g/m²)</td>
<td>0.122</td>
<td>0.341</td>
<td>0.633**</td>
<td>0.505**</td>
<td>-</td>
<td>0.026</td>
<td>-0.140</td>
<td>0.259</td>
<td>0.250</td>
</tr>
<tr>
<td>LA/LV</td>
<td>-0.108</td>
<td>-0.110</td>
<td>0.209</td>
<td>0.575***</td>
<td>0.026</td>
<td>-</td>
<td>0.438</td>
<td>0.363*</td>
<td>0.119</td>
</tr>
<tr>
<td>LA/Ao</td>
<td>0.106</td>
<td>0.186</td>
<td>-0.045</td>
<td>0.508**</td>
<td>-0.140</td>
<td>0.438*</td>
<td>-</td>
<td>0.353</td>
<td>-0.004</td>
</tr>
<tr>
<td>NT-proBNP (pg/mL)</td>
<td>0.273*</td>
<td>0.005</td>
<td>0.311*</td>
<td>0.573***</td>
<td>0.259</td>
<td>0.363*</td>
<td>0.353</td>
<td>-</td>
<td>0.433**</td>
</tr>
<tr>
<td>Galectin-3 (pg/mL)</td>
<td>0.267</td>
<td>0.195</td>
<td>0.207</td>
<td>0.157</td>
<td>0.250</td>
<td>0.119</td>
<td>-0.004</td>
<td>0.433**</td>
<td>-</td>
</tr>
</tbody>
</table>

Numbers are correlation coefficients (r) and *p<0.05, **p<0.01, ***p<0.001 for the relationships. LV Mass Index was indexed to height (2.7).

Table 4.8 Linear Regression Analyses for Haemodynamic Parameters, NT-proBNP and Echocardiography Parameters

<table>
<thead>
<tr>
<th>REGRESSION</th>
<th>DEPENDENT VARIABLE</th>
<th>PWV (m/s)</th>
<th>NT-proBNP (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>INDEPENDENT VARIABLE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPp (mmHg)</td>
<td>-</td>
<td>0.0745*</td>
<td></td>
</tr>
<tr>
<td>NT-proBNP (pg/mL)</td>
<td>0.0969*</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>LA Diameter (cm)</td>
<td>0.1450*</td>
<td>0.3281***</td>
<td></td>
</tr>
<tr>
<td>LA/LV</td>
<td>-</td>
<td>0.1317*</td>
<td></td>
</tr>
</tbody>
</table>

Numbers are coefficients of determination (r²) and *p<0.05, **p<0.01, ***p<0.001.
Figure 4.1 Linear Regression Relationships between Haemodynamic, Echocardiography and Biomarker Parameters in HFpEF patients (n=66). *p<0.05, **p<0.01, ***p<0.001

Figure 4.2 Linear Regression Relationships between Biomarkers of HF and LA diameter (≥3.5 cm). *p<0.05, **p<0.01, ***p<0.001
Figure 4.3 Linear Regression Relationship between PWV Carotid to Femoral and LA Diameter in Participants with HF. *p<0.05, **p<0.01, ***p<0.001
**Figure 4.4** Partial Correlation Coefficients (r) and 95% Confidence Intervals for the Relationship between NT-proBNP and PPp with Adjustments for Physiological and Cardiovascular Risk Factors. *P*-values are for significant independent relationships.

<table>
<thead>
<tr>
<th>NT-proBNP</th>
<th>Partial r</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPp</td>
<td>0.273</td>
<td>(0.011;0.496)</td>
<td>0.0396</td>
</tr>
<tr>
<td>Age</td>
<td>0.177</td>
<td>(-0.092;0.419)</td>
<td>0.1927</td>
</tr>
<tr>
<td>Sex</td>
<td>0.274</td>
<td>(0.010;0.499)</td>
<td>0.0404</td>
</tr>
<tr>
<td>BMI</td>
<td>0.343</td>
<td>(0.059;0.571)</td>
<td>0.0176</td>
</tr>
<tr>
<td>HT</td>
<td>0.200</td>
<td>(-0.070;0.441)</td>
<td>0.1431</td>
</tr>
<tr>
<td>Treatment</td>
<td>0.247</td>
<td>(-0.022;0.479)</td>
<td>0.0693</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.272</td>
<td>(0.005;0.499)</td>
<td>0.0444</td>
</tr>
<tr>
<td>Alcohol</td>
<td>0.231</td>
<td>(-0.039;0.466)</td>
<td>0.0905</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.369</td>
<td>(0.056;0.610)</td>
<td>0.0200</td>
</tr>
<tr>
<td>TCHOL/HDL</td>
<td>0.468</td>
<td>(0.135;0.698)</td>
<td>0.0063</td>
</tr>
<tr>
<td>LA Diameter</td>
<td>0.293</td>
<td>(-0.066;0.579)</td>
<td>0.1037</td>
</tr>
</tbody>
</table>

**Figure 4.5** Partial Correlation Coefficients (r) and 95% Confidence Intervals for the Relationship between NT-proBNP and LV Mass with Adjustments for Physiological and Cardiovascular Risk Factors. *P*-values are for significant independent relationships.

<table>
<thead>
<tr>
<th>NT-proBNP</th>
<th>Partial r</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV Mass</td>
<td>0.604</td>
<td>(0.315;0.783)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Age</td>
<td>0.577</td>
<td>(0.271;0.769)</td>
<td>0.0005</td>
</tr>
<tr>
<td>Sex</td>
<td>0.572</td>
<td>(0.264;0.766)</td>
<td>0.0006</td>
</tr>
<tr>
<td>BMI</td>
<td>0.742</td>
<td>(0.493;0.872)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>HT</td>
<td>0.571</td>
<td>(0.262;0.765)</td>
<td>0.0006</td>
</tr>
<tr>
<td>Treatment</td>
<td>0.608</td>
<td>(0.314;0.788)</td>
<td>0.0693</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.618</td>
<td>(0.329;0.794)</td>
<td>0.0444</td>
</tr>
<tr>
<td>Alcohol</td>
<td>0.648</td>
<td>(0.372;0.811)</td>
<td>0.0905</td>
</tr>
<tr>
<td>TCHOL/HDL</td>
<td>0.655</td>
<td>(0.283;0.846)</td>
<td>0.0200</td>
</tr>
<tr>
<td>PPp</td>
<td>0.538</td>
<td>(0.219;0.746)</td>
<td>0.0015</td>
</tr>
<tr>
<td>EF</td>
<td>0.584</td>
<td>(0.259;0.782)</td>
<td>0.0008</td>
</tr>
</tbody>
</table>
Figure 4.6 Partial Correlation Coefficients (r) and 95% Confidence Intervals for the Relationship between NT-proBNP and Echocardiography Parameters with Adjustments for Physiological and Cardiovascular Risk Factors. P-values are for significant independent relationships.
Figure 4.7 Partial Correlation Coefficients (r) and 95% Confidence Intervals for the Relationship between PWV and LA diameter with Adjustments for Physiological and Cardiovascular Risk Factors. P-values are for significant independent relationships.
5.1 Discussion

The main findings of this study are as follows: Firstly, the mean age for the prevalence or onset of HFpEF in black South African patients in DGMAH and hospitals and clinics that feed into this health institution is 54.88±13.51 years. With regard to the age prevalence, 76% of HFpEF participants in this study were young to middle-aged and 24% were older participants (>65 years). Secondly, arterial stiffness as assessed by PWV was significantly increased in participants with HFpEF when compared to participants without this pathology. However, there were no significant associations between central haemodynamic parameters, NT-proBNP and galectin-3. Lastly, NT-proBNP, but not galectin-3, was significantly associated with LVH, LA diameter and LA/LV, in both univariate and multivariate analysis, suggesting the favourable use of NT-proBNP in the diagnosis of HFpEF, particularly in patients with both structural heart disease as marked by LVH and LAE, and those with LV DD.

This study is the first to show that in a community with a high prevalence of HT and obesity, HFpEF occurs more frequently in the middle-aged as opposed to the elderly. Furthermore, the study shows a high PWV in HFpEF patients (9.97±2.78 m/s) at a young age which is not common in developed countries (6.5±1.6 m/s) (87). The study further confirms the presentation of concentric remodelled left ventricle in this age group which has only been reported previously in the elderly (88), probably due to longstanding HT and obesity. Evidence of abnormal LV relaxation and increased LV stiffness was confirmed by increased FS which effects early ventricular filling.
The mean age for HFpEF in black South Africans in this study is 54.88±13.51 years. Generally, the mean age of HF presentation is considerably lower (40-55 years) in sub-Saharan Africa (81) where the majority of patients are of working age (82). This mean age suggests that HF affects people of sub-Saharan Africa mainly at the prime of their life (working age) with the majority presenting with HT and CAD. This is in agreement with studies done in Nigeria and Ghana (90,91). However, it is in contrast with the mean age of 75 years in studies conducted in American and European countries where HFpEF is more common in the elderly population (78,92,93). This is also in accordance with previous studies which showed that the complications of HT tend to occur more often, and at a much earlier age, specifically in black populations when compared to Caucasians in European countries (94,95,96,97). The differences in the prevalence and onset of HFpEF observed in black South Africans can be attributed to the high prevalence and early onset of HT. This was evident in the current study population, where the percentage of HT was 69% in the HFpEF group and 55% in the group without HFpEF compared to a prevalence of 35% observed in the United States (97). Studies conducted in a similar study population in South Africa also showed a similar prevalence of HT for participants without HF (99,100).

Hypertensive HFpEF presenting in a black middle-aged population in South Africa is a further reflection of the effects or complications of HT presenting at an early stage. Long distances to health care facilities, a high rate of unemployment, and lack of education are important aspects of late presentation to health care providers (101,102), and therefore result in late diagnosis and/or ineffective
treatment of common co-morbidities. The presentation of HFpEF at a relatively young to middle age (working age) has the potential to undermine national productivity, as a consequence of the number of active life years lost by the most active workforce of the population. It also has detrimental consequences to the entire family as these patients are more often the breadwinners of the household. More saddening is the fact that it is not common practise to target this phenotype in a clinical setting due to its multifactorial causes, for example DM, obesity, CAD etc. Treatment is therefore challenging. In addition, patients wait for 6-12 months to undergo echocardiographic assessment, hence it minimises the early detection of this specific phenotype. Accordingly, only 24% of participants were above 65 years (elderly), and the question that therefore remains is: does this imply that more of the potential elderly participants already progressed to the HFrEF phenotype?

In relation to the high prevalence of HT in this study population, SBPp and PPp were significantly higher in HFpEF participants when compared to those without HFpEF. However, both were above normal limits (120-129 mmHg (SBPp) (85) and 40 mmHg (PPp)) in both groups. The above normal values of PPp could be due to isolated systolic HT seen in both groups. However, the statistically different PPp in participants with HF compared to those without HF could possibly be explained by the presentation of signs and symptoms of HF in this particular group. Indeed, the onset of HFpEF in HF patients remain difficult to identify as signs and symptoms only become apparent during later stages of the syndrome.
The Coronary Artery Risk Development in Young Adults (CARDIA) Study recently questioned the classic view that DBP more often identifies cardiovascular disease events than SBP in all individuals younger than 50 years. The result being that SBP in young black individuals and DBP in young Caucasian individuals were identified as the most robust indicators of future cardiovascular disease. Nevertheless, in middle-age, SBP in both races were identified as a risk of incident cardiovascular disease (102). PPp was elevated in HFpEF participants in this study. In HF, PPp may reflect on vascular stiffness and ventricular function. PPp increases as HF progresses and is therefore associated with worse outcomes. Indeed, higher PPp values were independently associated with mortality by trend in HFmrEF and HFpEF (103).

Accordingly, BP and more specifically PPp is one of the most important contributing factors to PWV, a classic measure of arterial stiffness (105,106). The significant difference observed in carotid to femoral PWV between those without HFpEF compared to those with HFpEF was 6.11±2.18 and 9.97±2.78 m/s respectively. This is in keeping with studies conducted in Brazil (military police officers from African and European descent) (106), London (population based study on Caucasians and African Caribbeans) (107) and in Germany (HFpEF study sample) (108). As expected PWV was positively correlated with LV mass index indicating effects of high afterload on the left ventricle due to peripheral vasoconstriction. It is noteworthy that arterial stiffness (PWV) is further correlated with LA diameter signifying the progression of disease in these patients.
Importantly, PWV did decrease within the HFpEF study population from visit one to three which could be explained by vasodilatory treatment given to participants for HT and HF. This shows that a possible improvement in some HFpEF participants was evident in a follow-up period of two to four months, suggesting that if identified on time, HFpEF, whether HT induced or due to other co-morbidities such as DM, PH and obesity (48) could be managed with therapy.

Obesity around the world has reached epidemic proportions. In both groups, participants were predominantly overweight or obese, with BMI averages of 29.85±7.68 kg/m² in participants without HFpEF and 33.16±9.56 kg/m² in participants with HFpEF. However, those with HFpEF had a significantly higher BMI. Higher BMI values were also observed in several other HFpEF studies (110,111), including the TOPCAT (Treatment of Preserved Cardiac Function Heart Failure With an Aldosterone Antagonist) trial (111). Previous studies have suggested an “obesity paradox,” whereby HF patients with a high BMI had a better rate of survival than those with a low BMI (contrary to observations in the general population). Recent evidence suggests this effect may possibly be limited to patients with HF, more specifically HFpEF (113,114,115). The possible causes of the “obesity paradox” are debatable, yet there is no doubt that BMI decreases as HF becomes more severe. Some believe that the decline in BMI values simply reflects a more advanced stage in progression of HF. Those that adhere to the above mentioned view, suggest that lifestyle interventions to reduce an above normal BMI will benefit patients not only symptomatically, but also perhaps prognostically, by reducing pro-inflammatory visceral adiposity and could
possibly have a favourable effect on haemodynamic stress (115). Others believe that a higher BMI may provide a metabolic reserve as HF progresses or even be intrinsically beneficial (117,118), in which case efforts should be made to increase rather than reduce BMI. Indeed, one of the frequently used HF treatments, beta-blockers, increases BMI (118). Thus, high BMI values in the participants without HF might possibly predict the development of HF, whereas higher BMI levels in the participants with HFpEF are linked to more favourable outcomes. Further investigation into a black middle-aged South African group in this regard is needed. Thus, both an elevated BMI and HT (often linked to obesity) in middle-age are associated with an increased risk for HF in later life (119).

The average level of NT-proBNP in the HFpEF participant group was below the suggested cut-off point of 125 pg/mL given by the ESC (6). This finding concurs with several other studies that reported low levels of circulating NT-proBNP in obese HF patients despite having increased LV filling pressures (121,122,123,124). In a disease setting, such as in HF, increased levels of NT-proBNP reflect the attempt of the body to restore homeostasis in a response to increased LV filling pressures (57). BNP and NT-proBNP are nonetheless depressed in obesity despite higher LV end-diastolic pressures (122). The increased circulating levels of NT-proBNP is therefore secondary to LV dysfunction and also independent of adipose tissue functioning. Hence, NT-proBNP is involved in very different and independent physiological processes that include both the cardiovascular system and the function of adipose tissue. Increased NT-proBNP clearance by adipose tissue in HFpEF patients and
decreased NT-proBNP production due to ‘cardiac cachexia’ have also been proposed as possible mechanisms (123), but the reduced production or secretion of NT-proBNP in the atrial or ventricular myocardium in HFpEF remains to be explored (124). This was also observed in a study done on 30 stable, mostly obese middle-aged HFpEF patients (120). In contrast, analysis from the TOPCAT trial showed that among obese HFpEF patients with elevated natriuretic peptides levels, such as NT-proBNP, identify a higher risk phenotype with a significantly increased incidence of both mortality and HF hospitalisation (26).

The Heart Dallas Study does not support the above-mentioned hypothesis that decreased levels of BNP and NT-proBNP seen in obese patients are due to increased clearance of BNP and NT-proBNP by adipose tissue. Researchers suggests that it could rather be due to a substance secreted by lean muscle mass or the possibility that lean mass contributes directly to renal clearance of BNP and NT-proBNP (125). The clearance of BNP and NT-proBNP is equally dependent on renal function and a moderate inverse, but significant correlation between glomerular filtration rate and BNP or NT-proBNP concentration has been shown by multiple studies (126). Nevertheless, increased BNP or NT-proBNP concentrations are mainly related to and a result of the presence and extent of cardiac pathology rather than impaired renal clearance (127).

These studies therefore recommend that excluding the diagnosis of HFpEF based solely on NT-proBNP levels should be discouraged and that natriuretic peptide levels in obese patients should be interpreted with caution (128).
Furthermore, a positive association between both biomarkers for HF (NT-proBNP and galectin-3) and an LA diameter above 3.5 cm was observed. Bearing in mind that galectin-3 is a marker of fibrosis and inflammation associated with long-term cardiovascular outcomes in patients with HF (129). This suggests that in an African middle-aged HF population, moderate increases in LA diameter due to above normal elevated galectin-3 levels can already be seen in participants with early onset HF. This association could be as a result of atrial remodelling. Galectin-3 in the HFpEF group was only modestly elevated, and though increased when compared to those without HFpEF, not statistically significant. LA diameter measurements were significantly increased in HFpEF participants compared to those without HFpEF. Indeed, LAE is a sign of atrial remodelling, however only observed in about half of the patients with stable chronic HF (52). Large variations between studies are observed and thus most likely reflecting disease aetiologies and stages of HF that are heterogeneous (52). It has previously been shown that galectin-3, as a marker of atrial remodelling (130), correlates directly with the extent of fibrosis in the left atria (77, 129, 130). HFpEF participants from our study showed only modestly elevated galectin-3 and LA diameter measurements, suggestive of relatively early stages of LA remodelling in stable HFpEF patients. This finding is similar to that of Edelmann et al., 2014, who found galectin-3 levels to be modestly elevated in patients with stable HFpEF and that galectin-3 relates to functional performance and quality of life in these patients. Increasing galectin-3 was therefore associated with worse outcomes, independent of treatment for HT or NT-proBNP levels (130).
Contractility (FS) of the left ventricle in HFpEF participants was greatly reduced (p<0.01) when compared to those without HFpEF, but still above the normal value of 28%. This suggests that myocardial contractility of the left ventricle possibly increases to match arterial load in hypertensive participants without HFpEF, but that the progression to HFpEF could be mediated by mechanisms that impair myocardial LV contractility and increase passive myocardial LV stiffness simultaneously. It has been reported previously that reduced contractility may occur together with hypertensive HFpEF patients with concentric remodelling (133). Endocardial motion is therefore allowed to stay preserved even though shortening of individual myofibres are reduced. This allows the EF to still remain within a normal range (134).

Measures of LVH (LWPWd, LV mass, RWT and LV mass index) showed significantly higher values when compared to participants without HFpEF, although LVEDd was not significantly different in both groups. Increased LV size is associated with advanced LV remodelling and is known to result in a higher incidence of adverse clinical outcome in patients with HFrEF, but it is still unclear whether this also applies to those with HFpEF. A statistically significant increase in IVSd was observed in participants with HFpEF when comparing visit two to three. However, the observed increase in IVSd should be interpreted carefully as these results are based on only those participants that followed up for a third time.

LA/Ao and LA/LV were significantly increased in participants with HFpEF compared to those without HFpEF. Increased LA/LV (a marker of LV filling
pressures) was also previously observed in not only patients with HF but also subjects with HT, DM and LVH. An increased LA/LV also predicted worse exercise capacity in the above-mentioned groups and was associated with more frequent loop diuretic use. This is also consistent with our findings, as diuretic treatment was given to all HFpEF patients also diagnosed with HT. These data are therefore consistent with the hypothesis that LA/LV is a non-invasive marker of the LV diastolic pressure-volume relationship as discussed in chapter one (assessing LV function) (50). It remains unclear whether treatment and the duration of treatment given for HFpEF assists in the improvement of LV structure and thus function.

5.2 Limitations of the Study

The study has a number of limitations that require comment. Firstly, the HFpEF study has incomplete data, particularly at the third visit participant mark (due to a relatively high mortality rate, transport to and from hospitals or clinics and financial difficulties), that resulted in weakening of statistical power. Due to the above-mentioned difficulties experienced, not all participants had data collected from them in equal monthly intervals as mentioned in chapter three (3.4 clinical data collection).

Secondly, this study was performed in a population sample of African ancestry with a high prevalence of HT (55% in participants without HFpEF and 69% of participants with HFpEF) and a high proportion of participants who were
overweight or obese (69% in participants without HFpEF and 86% of participants with HFpEF). Therefore, deductions from the results obtained may not necessarily be applicable to other population groups with a different prevalence of both HT and overweight or obesity.

Thirdly, more women compared to men participated in the study, and sex differences may exist in the pathophysiology of HFpEF. In this regard, it was not statistically powered to evaluate measures of aortic stiffness and biomarkers of HF in sex-specific groups. Hence, the present results may reflect a dominant effect in females when compared to males.
CHAPTER 6

CONCLUSION
6.1 Conclusion

HFpEF is more prevalent in a middle-aged black South African sample with aortic stiffness when compared to European and American populations as assessed by high PWV and longstanding HT. NT-proBNP, but not galectin-3, is independently associated with LA diameter, LVH and hence could be used for the diagnosis of HFpEF in this community sample.
6.2 Recommendations

Plasma NT-proBNP concentration levels could offer a good means of initial screening for LVH in asymptomatic patients with HT. Lim et al., 2017 showed that while NT-proBNP analysis is the most cost-effective method in detecting any causes of HF, portable echocardiography used to assess patients with suspected HF in the community is the most cost-effective strategy in identifying HF (135). This recommendation could pose a problem in a South African setting as government hospitals are understaffed and performing and interpreting echocardiography involves considerable skill and training. In this regard NT-proBNP analysis would be the recommended screening method for suspected HF before echocardiography is used. This would allow the identification of those who would benefit from echocardiography in screening for early signs of DD that could potentially lead to the development of HFP EF. This would also provide a better evaluation of cardiovascular risks associated with not only HT, but also HFP EF.

Larger studies of patients with LVH due to HT, but without clinically apparent HFP EF, are needed to clarify the role of NT-proBNP in the diagnosis of LVH in the routine follow-up of patients with HT, and in the monitoring of the regression of LVH with treatment for HT. Markers for LV filling pressures, such as the ratio of trans-mitral blood flow velocity in the early (E wave) and late (A wave) period of LV diastolic filling (E/A wave ratio) and an index of LV filling pressure ratio (E/e’) should be included in future studies when looking at the effects of increased LV filling pressures in DD on biomarkers of HF such as NT-proBNP and galectin-
3 in young to middle-aged HFpEF patients of African descent. A combination of established biomarkers elevated during HF (such as NT-proBNP and galectin-3) and associated with atrial remodelling might contribute to the development of risk scores. Both of these HF biomarkers have previously been shown to be markers of atrial remodelling and to directly correlate with the extent of LA fibrosis, but not in a South African population with middle-aged HFpEF patients.
APPENDIX A

SMUREC: Ethical Clearance Certificates
02 February 2017

Ms M van Hoogland
Department of Human Physiology
P.O. Box 130
Medunsa, 0204

MEETING: 01/02/2017

SMUREC Ethics Reference Number: SMUREC/08/2017: PG

The New Application received on 17 January 2017, was reviewed by members of Sefako Makgatho University Research Ethics Committee on 02 February 2017 and was approved on 02 February 2017.

Title: Biomarkers of heart failure with preserved ejection fraction in patients with aortic dysfunction

Researcher:  
Supervisor: Prof L Böhmer
Co-supervisor: Prof H Majere
Hospital Superintendent: Dr MC Holm (GAPAHT)
Other Involved HOD: Prof PS Mintu (Cardiology)
Department: Human Physiology
School: Medicine
Degree: MSc (Medicine) Physiology

Please note the following information about your approved research protocol:

Protocol Approval Period: 02 February 2017 – 02 February 2018

Please remember to use your protocol number (SMUREC/08/2017: PG) on any documents or correspondence with the REC concerning your research protocol.

Please note that the REC has the prerogative and authority to ask further questions, seek additional information, require further modification, or monitor the conduct of your research and the consent process.

After Ethical Review: Please note a template of the progress report is obtainable in the Research Office and should be submitted to the Committee before the year has expired. The Committee will then consider the continuation of the project for a further year if necessary. Annually a number of projects may be selected randomly for an external audit. Translation of the consent document in the language applicable to the study participants should be submitted.


Sincerely

PROF SA OMODANA
CHAIRPERSON SMUREC
APPROVAL NOTICE – CONTINUATION OF STUDY

01 March 2018

Ms M van Hoogland
Department of Physiology
P.O. Box 105
Medunsa, 0204

MEETING: 01/2017
01/2018

SMUREC Ethics Reference Number: SMURECM/18/2017: PG

The New Application received on 17 January 2017, was reviewed by members of Sefako Makgatho University Research Ethics Committee on 02 February 2017 and was approved on 02 February 2017.

On the 01 February 2018 SMUREC approved continuation of this study

Title: Biomarkers of heart failure with preserved ejection fraction in patients with aortic dysfunction

Researcher: Ms M van Hoogland
Supervisor: Prof L Botmer
Co-supervisor: Prof T Manne
Hospital Superintendent: Dr MC Ham (DGMA/H)
Department: Physiology
School: Medicine
Degree: MSc (Medicine) Physiology

Please note the following information about your approved research protocol:

Approval Period: 02 February 2017 – 31 January 2022

After Ethical Review: Kindly remember to use your protocol number (SMURECM/18/2017: PG) on any documents or correspondence concerning your research protocol with the REC. The REC has the prerogative and authority to ask further questions, seek additional information, require further modification or monitor the conduct of your research and the consent process. A template of the progress report is obtainable from the Research Office and is due on an annual basis for your study irrespective of the approval period. Please note that a number of projects may be selected randomly for an external audit every year. Translation of the consent document in the language applicable to the study participants should be submitted if required.


Sincerely,

PROF C BAKER
DEPUTY CHAIRPERSON SMUREC

Date: 01/03/2018
APPENDIX B

PATIENT INFORMATION SHEET
PATIENT INFORMATION SHEET

Biomarkers of Heart Failure with a Preserved Ejection Fraction in Patients with Aortic Dysfunction

Hello! My name is Marilet van Hoogland, and along with, Professors Olebogeng Harold I. Majane (principal researcher), Pindile Mntla (head of Department of Cardiology), Leon Hay (head of Department of Physiology), co-researcher Dr Leon Scott and postgraduate students Mrs Odette Heyneke, Ms Mangaka Dibetso and Mr Tshepo Lechaba, we are interested in studying aortic function in heart failure patients with preserved ejection fraction. That is where systolic chamber function or contraction of the heart is still within normal limits. We would therefore like to invite you to consider participating in our research study.

Before agreeing to participate, it is important that you read the aims of the study, and understand the procedures to be done, discomforts, as well as your right to withdraw from the study at any time. Withdrawal will not have a negative impact on you in any way. The regular care that you receive from your regular doctor for your condition will not be influenced.
AIMS OF THE STUDY:

1) To determine the prevalence of heart failure with a preserved ejection fraction with aortic dysfunction among middle-aged patients diagnosed with heart failure with a preserved ejection fraction at the Dr George Mukhari Academic Hospital.

2) To determine whether there is an association between central haemodynamic parameters and the different biomarkers of heart failure with a preserved ejection fraction.

3) To determine whether there is an association between the different biomarkers (NT-proBNP and galectin-3) and the degree of heart failure with a preserved ejection fraction by comparing the concentration of biomarkers in the blood with the patient’s left atrial diameter and left ventricular hypertrophy.

In order to answer our research questions, we need to compare aortic function in heart failure with preserved ejection fraction participants to healthy participants. Since you have recently had heart failure and however still have relatively normal pump function, you are invited to participate in our study.

PROCEDURES:

If you agree to take part in this study, you will first be asked questions and be examined to see if you qualify to participate in the study.

For your safety, you would not be eligible to participate in this study if you have any of the following:
• Heart failure with reduced pump function

• Having suffered a heart failure, you may have, or may be in the process of completing multiple routine heart failure investigations, which are done on all heart failure patients.

These would routinely include chest x-ray, electrocardiogram, and echocardiogram which is an ultrasound (sonar, similar to that used in pregnant women) of your heart will be performed in order to assess the size and function of your heart), as well as numerous blood tests.

We request your permission to use this information in our study. Furthermore, we would like to conduct a few more tests. These are explained below:

1. Completion of Questionnaire
   a. You will be asked a series of questions regarding possible risk factors for heart failure. Your answers will be recorded for you by the interviewer, who will be a doctor or study co-ordinator. This will take approximately 10 minutes.

2. Physical Examination
   a. Conducted by a qualified doctor (cardiologist)
   b. Non-invasive examination, particularly of your cardiovascular (heart).

3. Blood Measurement
   a. 20 ml (about 4 teaspoons) of blood from your arm vein will be collected by a qualified doctor or registered nurse. Blood will be collected on visit 1, visit 2 and on visit 3 and will be used for biomarkers of heart failure.
4. Blood Pressure (Brachial & Central) and Pulse Wave Velocity Measurement

a. Brachial blood pressure measurements will be obtained in a seated/supine position after 10 minutes of rest using a standard mercury sphygmomanometer in order to calibrate the central blood pressure measurement.

b. Central blood pressure and pulse wave velocity: After you have rested for 15 minutes lying on a bed, pressure pulse waves will be recorded non-invasively using a small blunt probe (the size of a ballpoint pen). The pressure pulse waves will be recorded from the surface of your left arm (placed on your wrist), the surface of your neck on the left side, and from the surface of your upper leg/thigh. There is no pain or discomfort in this procedure however you are required to lie very still for a few seconds.

c. The central blood pressure and pulse wave velocity help us to assess the blood pressure at your heart and the stiffness of your arteries.

How long will it take?

It will take approximately one hour to complete all of the above tests. We will try to do them all on the same day (admission day), before discharge and on follow-up. Approximately four hundred and fifty patients who suffered a heart failure will participate in this study and 800 previous and current participants from APOGH study will serve as control group.
**Where will the tests be performed?**

All the tests will be performed in the Dr George Mukhari Academic Hospital, Division of Cardiology or at the cardiology ward, while you are in hospital and on follow-up.

**What will it cost me?**

All costs will be paid by the investigators and the hospital. There will be no cost to you.

**RIGHT TO WITHDRAW FROM THE STUDY:**

You are completely free to choose whether or not you wish to volunteer for this study. You are also free to withdraw from the study at any time, should you wish to. Should you decide either not to volunteer, or withdraw your participation, this decision will not impact negatively on you and your continued medical treatment in any way.

**GENERAL INFORMATION:**

If any further clinical abnormalities are detected we will arrange for further medical follow-up as appropriate.
PLEASE NOTE THAT ALL OF YOUR INFORMATION AND RESULTS WILL BE KEPT STRICTLY CONFIDENTIAL

Should you have any questions regarding this study, please contact us at:

Marilet van Hoogland 072 327 8367
Prof Harold Majane 079 549 7341
Prof Pindile Mntla 012 521 4627
Prof Leon Hay 012 521 4535
Dr Leon Scott 012 521 3935
SMUREC Chairperson 012 521 4321
APPENDIX C
WRITTEN CONSENT FORM
WRITTEN CONSENT FORM

TITLE OF THE STUDY: BIOMARKERS OF HEART FAILURE WITH A PRESERVED EJECTION FRACTION IN PATIENTS WITH AORTIC DYSFUNCTION

Name of Participant: ______________________________________

Participant Study Number: ______________________________________

The aims and procedures of this study have been explained to me by the doctor/study co-ordinator.

I have read and understood the information sheet provided. I have had the opportunity to ask questions and to consider the answers given to me.

I understand that participation in this study is voluntary, that I may withdraw my consent at any time and that if I choose not to consent my decision will have not impacted negatively on me in any way. The study will have no influence on the regular treatment that holds for my condition neither will it influence the care that I receive from my regular doctor.

I hereby freely give my informed consent to taking part in this study.

Name of Participant: ______________________________________

Date: ______________________________________

Signature of Participant: ______________________________________
(Or thumb print if participant cannot sign)

Signature of Guardian: ______________________________________
(where applicable)
STATEMENT BY THE RESEARCHER

I confirm that I have fully explained the nature of the study and the procedure to be performed to the above-named participant. I will adhere to the approved protocol and answer any future questions concerning the study as best as I am able.

Name of Researcher: ________________________________
Date: ________________________________
Place: ________________________________
Signature of Researcher: ________________________________

RESEARCHERS:
Prof. Olebogeng Harold I. Majane (Principal researcher), Professor Pindile Mntla (Head of department of Cardiology)
Professor Leon Hay (Head of department of Physiology) and Dr Leon Scott (Co-researcher).

POSTGRADUATE STUDENTS:
Mrs Odette Heyneke, Ms Marilet van Hoogland, MS Mangaka Dibetso and Mr Tshepo Lechaba
WRITTEN CONSENT FORM
LEINA LA PATLISISO: BIOMARKERS OF HEART FAILURE WITH A PRESERVED EJECTION FRACTION IN PATIENTS WITH AORTIC DYSFUNCTION

Leina lame: ________________________________

Nomoro ya sephira ya patlisiso: ________________________________

Ke begetswe le go thalosetswa maikaelelo kgotsa maithomo le tsamaiso ya patlisiso. Ke neetswe sebaka / tshono ya go botsa dipotso ke batsamaise / ba batlisisi (ngaka), ka bona dikarabo tse di maleba mme ke thalosetsa tshedimosetso e ke e neetsweng.

Ke thalosetsa gore go tsaya karolo mo patlisisong e ke boithaopo le gore nka ikgogela morago mo go yone nako ngwe le ngwe ntle le go neela mabaka. Se ga se kitla se nna le seabe sepe mo kalafong ya me, mo bolwetseng le mo kalafing kgotsa tlhokomelo e ke e amogelang mo ngakeng yame ya go le gale.

Fano ke neela tumelelo ya go tsaya karolo mo patlisisong e:

Leina la molwetse: ________________________________

Letlha: ________________________________

Tshaeno ya molwetse:
(O ka dirisa monwana o mogolo f aka gongwe molwetse a retelelwa ke go saena)

Seteitemente ka mmatlisi: ________________________________
Ke tlametse tshedimosetso ka molomo / e e kwadilweng malebana le patlisiso e.
Ke dumela go araba dipotso dingwe le dingwe mo nakong e e tlang tse di
amanang le patlisisoe ka moo nka kgonang ka teng. Ke tla tshegetsa lenaneo /
thulaganyo/ "protokolo" e e rebotsweng

Leina: 

Letlha: 

Lefelo: 

Tshaeno: 

BA BATLISISI:
Prof. Olebogeng Harold I. Majane (Principal researcher), Professor Pindile Mntla
(Head of department of Cardiology)
Professor Leon Hay (Head of department of Physiology), Dr Leon Scott (Co-
researcher).

BA ITHUTI BA THUTO E KGOLWANE:
Mrs Odette Heyneke, Ms Marilet van Hoogland, Ms Mangaka Dibetso and Mr
Tshepo Lechaba
APPENDIX D

QUESTIONNAIRE AND DATA COLLECTION SHEET
QUESTIONNAIRE

IDENTIFICATION OF THE PARTICIPANT
Surname: ________________________________
Name(s): ________________________________
Sex: ________________________________
Date of Birth: ________________________________
Hospital Number: ________________________________

CONTACT DETAILS
Telephone Number: ________________________________
Alternative Telephone Number: ________________________________
Residential Address: ________________________________

NEXT OF KIN
Surname: ________________________________
Name(s): ________________________________
Relationship to Patient: ________________________________
Telephone Number: ________________________________

DATE OF NEXT VISIT
__________________________________________
HOW TO COMPLETE THE QUESTIONNAIRE

You may complete the questionnaire yourself, or you may request the help of one of the researchers or your next of kin.

*Researcher to store this section separate from the remainder of the questionnaire.*
**Past Medical History**

Heart Disease ☐  Dyslipidaemia ☐  Diabetes I ☐

TB ☐  Previous Coronary Artery Disease ☐  Diabetes II ☐

Autoimmune ☐  Renal Disease (e.g. kidney stones) ☐

Other: __________________________________________________________

Details (specify disease type, starting date – when symptoms occurred, date of cure etc.)

_________________________________________________________________

_________________________________________________________________

_________________________________________________________________

Hypertension ☐

Previously diagnosed but **not** receiving treatment?  Y ☐  N ☐

If yes, then reason/ explanation:

_________________________________________________________________

_________________________________________________________________

Previously diagnosed but **stopped** treatment?  Y ☐  N ☐

If yes, then reason/ explanation:

_________________________________________________________________

_________________________________________________________________

Previously diagnosed but **inadequate** treatment?  Y ☐  N ☐

If yes, then reason/ explanation:

_________________________________________________________________

_________________________________________________________________
Medication History (Present – within the past 6 months)

<table>
<thead>
<tr>
<th>Medication</th>
<th>Dose</th>
<th>Duration</th>
<th>Indication (past/present)</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>

- Diabetic patients to indicate if they are being treated with pills or insulin injection
- Researcher to interrogate treatment for hypertension
- Researcher to check patient's file to complete or verify this section
Habits

Do you currently smoke?  Y □  N □

If yes, age at which started: _________________________________

- Number of cigarettes per day: _______________________________
- Number of years: _________________________________
- Other: __________________________________________________

Past smoking history  Y □  N □

- Number of cigarettes per day: _______________________________

Do you consume alcoholic beverages?  Y □  N □

- Number of glasses of beer per day: _______________________________
- Number of bottles of wine per week: _______________________________
- Number of bottles of liquor per week: _______________________________

Family History

Hypertension  Y □  N □  Unknown □

Stroke  Y □  N □  Unknown □

Diabetes I/II  Y □  N □  Unknown □

Heart Disease  Y □  N □  Unknown □

Females Only

Post-menopausal  Y □  N □  Age at Menopause: _______________________________

Oral Contraceptive  Y □  N □

If yes,

- Names & Doses: _____________________________________________
- Duration: __________________________________________________

Are you pregnant at present?  Y □  N □
**Physical Activity**

Do you walk regularly?  Y☐   N☐

If yes, age at which started: ____________________________
• Number of hours per day: ____________________________
• Number of kilometres per day: _______________________

Do you practice any sport activities at regular basis?  Y☐   N☐

• Number of hours per week spend on practicing sport: ______________

**Questionnaire Filled out by:**

Name: ____________________________
Signature: ____________________________
Date: ____________________________
(As Answered by the Patient)
DATA COLLECTION SHEET

IDENTIFICATION OF THE PARTICIPANT

Surname: ____________________________________________
Name(s): ____________________________________________
Sex: _______________________________________________
Date of Birth: _______________________________________
Hospital Number: ____________________________________
Conventional Blood Pressure (BP)

- Researcher to Ensure that Patient has Rested before Measurements and that the Cuff Size is Appropriate.

<table>
<thead>
<tr>
<th>Time of BP Collection:</th>
<th></th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Left Arm:</th>
<th>Right Arm:</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP</td>
<td>SBP</td>
</tr>
<tr>
<td>DBP</td>
<td>DBP</td>
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<td></td>
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</table>

<table>
<thead>
<tr>
<th>Average SBP</th>
<th>Average DBP</th>
<th>Average SBP</th>
<th>Average DBP</th>
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</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Heart Rate:</th>
<th></th>
</tr>
</thead>
</table>
# Echocardiography

**M-mode Echocardiographic Images: Parasternal Long-Axis (PLAX)**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Value 1</th>
<th>Value 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEd</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVEs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FS%</td>
<td></td>
<td></td>
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<td>EF%</td>
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<tr>
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<td>IVSs</td>
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<tr>
<td>LVPWs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ao</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E Wave</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A Wave</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ao/LA Ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E/A Wave Ratio</td>
<td></td>
<td></td>
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<tr>
<td>E/e'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ao Root</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asc Ao</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ao Annulus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ao</td>
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</tr>
</tbody>
</table>

**Est Right Sided Pressures:**

<table>
<thead>
<tr>
<th>Pressure</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary Artery</td>
<td></td>
</tr>
<tr>
<td>Pressures:</td>
<td></td>
</tr>
</tbody>
</table>

**Notes:**

____________________________________________________________________

____________________________________________________________________

____________________________________________________________________

____________________________________________________________________
<table>
<thead>
<tr>
<th>Anthropometric Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suprailliac (cm):</td>
</tr>
<tr>
<td>BW (kg):</td>
</tr>
<tr>
<td>BH (cm):</td>
</tr>
</tbody>
</table>

*Body Weight (BW); Body Height (BH); Waist Circumference (Waist C); Hip Circumference (Hip C); Body Mass Index (BMI)*
Central Blood Pressure Measurements & Pulse Wave Velocity

Date of Collection: _____________________________
Time of Collection: _____________________________
Average Conventional SBP (mmHg): _____________________________
Average Conventional DBP (mmHg): _____________________________
SSn-Fem: _____________________________
SSn-Car: _____________________________
Car-Fem: _____________________________
SSn-Rad: _____________________________
Car-Rad: _____________________________

*Supra-Sternal Notch (SSn); Femoral (Fem); Carotid (Car); Radial (Rad)

** (PRINT EVALUATION AND PWV REPORTS: PulsePen)
# Blood Collection and Results

Collected: [ ] Yes [ ] No

<table>
<thead>
<tr>
<th>BLOOD COLLECTION FOR BNP/GALECTIN-3 ANALYSIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>On Visit 1/ Visit 2/ Visit 3</td>
</tr>
<tr>
<td>--------------------------------</td>
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<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>LABORATORY POST ELISA RESULTS FOR NT-proBNP/GALECTIN-3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Visit 1</td>
</tr>
<tr>
<td>Visit 2</td>
</tr>
<tr>
<td>Visit 3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HOSPITAL BLOOD RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>RECEIVED: [ ] YES [ ] NO</td>
</tr>
</tbody>
</table>
REFERENCES


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