

THE CLINICAL AND BIOCHEMICAL EFFECTS OF ATRIAL FIBRILLATION ON HEART FAILURE WITH REDUCED EJECTION FRACTION



WITS
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NONKANYISO MBOWENI

Student Number: 795139

A Thesis submitted to the Faculty of Health Sciences, University of the
Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree
of Doctor of Philosophy.

7 November 2025

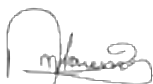
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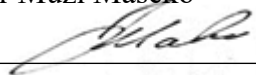
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DEDICATION

To God Almighty, Your divine guidance and Holy Spirit have been my anchor. You have sustained me .All glory and honour belong to You.

To my late paternal and maternal Grandparents, whose wisdom and love laid the foundation for my journey. Your unwavering belief in me, encouragement to dream beyond limits, and your gentle push toward excellence have been my source of inspiration. I dedicate this work to your memory.

To my parents, whose boundless love, unfathomable sacrifices, and never-ending support have carried me through every challenge and triumph of this journey. There could never be anyone like you both. I am indebted to you.

To my beloved husband, Bafo Kondlo, for your guidance, love, and unwavering support through this journey .

To my brother Velani Mboweni, for his constant love, presence and support. You have been a source of comfort and motivation throughout this journey.

To my supervisors Professor Nqoba Tsabedze and Professor Muzi Maseko, I would not have been able to complete this work without your guidance and support.

To Dr. Buhle Mokheseng for your invaluable mentorship and support. Your wisdom, guidance, and encouragement have been pivotal in shaping this journey. I am deeply grateful.

To Dr. Marketa Toman for your mentorship. Your guidance, generosity with your time, knowledge, and encouragement has made an immeasurable difference in my academic and personal growth.

With love and gratitude, this thesis is dedicated to you all.

PRESENTATIONS ARISING FROM THIS PROJECT

1. Echocardiographic and novel 2D-Speckle tracking in HFrEF patients with Atrial Fibrillation and sinus rhythm - **15th Pan African Society of Cardiology Congress, Kenya, 2021**
2. The Clinical and Biochemical Effects of Atrial Fibrillation on Heart Failure with Reduced Ejection Fraction: A Sub-Saharan Perspective – **European Society of Cardiology (ESC) Congress, Barcelona 2022**
3. Echocardiographic and Novel 2D-Speckle Tracking in HFrEF patients with Atrial Fibrillation and Sinus Rhythm – **Physiological Society of Southern Africa (PSSA) Congress, Johannesburg, 2021**
4. The Biochemical effects of Atrial Fibrillation on Heart Failure with Reduced Ejection; A South African Perspective. **South African Medical Research Council 16th Early Career Scientist Convention, Cape Town, 2022**

PUBLICATIONS ARISING FROM THIS PROJECT

1. Mboweni N, Maseko M, Tsabedze N. Heart failure with reduced ejection fraction and atrial fibrillation: a Sub-Saharan African perspective. ESC Heart Fail. 2023;10(3):1580-96. <https://doi.org/10.1002/ehf2.14332>

ACKNOWLEDGEMENTS

This project was made possible through the funding from the South African Medical Research Council (SAMRC) via the Clinician Researcher Scholarship. This funding funded all my research costs, tuition fees and accommodation for the duration of my PhD and MBBCh studies.

The Carnegie Corporation of New York and the South African Heart Association for additional travel and tuition funding.

This project would not have been possible without the support, staff and resources from the School of Physiology .

Acknowledgment is extended to the Division of Cardiology at Charlotte Maxeke Johannesburg Academic Hospital for permitting the recruitment of patients from their heart failure clinic and cardiac admission wards.

Dr Marketa Toman and the Department of Chemical Pathology for assisting me in conducting biomarker ELISAs and analysis.

Thank you to Dr Samantha Nel and Sihle Xulu for assistance with data collection and echocardiographic analysis.

Acknowledgement to Ms Mmoni Lehloo, Mrs Mokgadi Maseko and Peggy for their administrative support and assistance in recruiting patients.

Special thanks are extended to Ms. Brenda Kagodoro for expert guidance and support in statistical analysis.

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LIST OF ABBREVIATIONS

2D	Two dimensional
a	Peak late diastolic mitral inflow velocity
ADHF	Acutely decompensated heart failure
AF	Atrial fibrillation
ARG	Arginine
AOR	Adjusted odds ratio
β	Beta
BPM	Beats per minute
CAD	Coronary artery disease
CHF	Chronic heart failure
CI	Confidence Interval
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
CKD	Chronic kidney disease
CRP	C-reactive protein
cTnT	Cardiac Troponin T
DBP	Diastolic blood pressure
E	Peak early diastolic mitral inflow velocity
e'	Early diastolic mitral annular tissue velocity
ELISA	Enzyme-linked immunosorbent assay
ECG	Electrocardiogram
ED	End diastolic
eGFR	Estimated glomerular filtration rate
GAL-3	Galectin - 3
GDF-15	Growth differentiation factor – 15
GDMT	Guideline directed medical therapy
GLY	Glycine
GLS	Global longitudinal strain
HDL	High density lipoprotein
HF	Heart Failure (encompasses all heart failure phenotypes)
HFmrEF	Heart failure with mid-range ejection fraction

HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
HIC	High income country
HR	Heart rate
HWE	Hardy- Weinberg equilibrium
DCMO	Dilated cardiomyopathy
IHD	Ischemic heart disease
IL-6	Interleukin-6
IL-31	Interleukin-31
IQR	Interquartile range
JAK-STAT3	Janus kinase and signal transducer and activator of transcription 3
KCCQ	Kansas City Cardiomyopathy Questionnaire
LASr	Left atrial strain reservoir function
LAScd	Left atrial strain conduit function
LASp	Left atrial strain pump function
LDL	Low-density lipoprotein
LVEF	Left ventricular ejection fraction
LVIDd	Left ventricular internal diameter at end-diastole
LVIDs	Left ventricular internal diameter at end-systole
LVPWd	Left Ventricular Posterior Wall thickness in diastole
MAF	Minimum allele frequency
MALD - TOF	Matrix-Assisted Laser Desorption/Ionization-Time Of Flight
NT-proBNP	N-terminal pro-B-type natriuretic peptide
NYHA	New York Heart Association
OAC	Oral anticoagulant
OR	Odds ratio
PAP	Pulmonary artery pressure
PCR	Polymerase chain reaction
PPCMO	Post-partum cardiomyopathy
RAAS	Renin-Angiotensin-Aldosterone System
RV	Right ventricle
SBP	Systolic blood pressure

SNP	Single Nucleotide Polymorphism
SR	Sinus rhythm
SSA	Sub-Saharan Africa
ST2	Suppression of tumorigenicity 2
STE	Speckle tracking echocardiography
sST2	Soluble suppression of tumorigenicity 2
ST2L	Suppression of tumorigenicity 2 Ligand
T2DM	Type 2 Diabetes Mellitus
TAPSE	Tricuspid Annular Plane Systolic Excursion
OR	Odds ratio

ABSTRACT

Background: The increasing burden of non-communicable diseases in Sub-Saharan Africa has led to an increased prevalence of heart failure with reduced ejection fraction (HFrEF), often complicated by atrial fibrillation (AF). However, most evidence informing HF management is derived from high-income countries, overlooking the specific epidemiology, clinical and biomarker profiles, and genetic backgrounds of African populations.

Aim: This thesis aimed to characterise the clinical, echocardiographic, biochemical, and genetic characteristics of South African patients with HFrEF, both with and without concomitant AF, and to identify determinants of all-cause mortality in this population.

Methods: A prospective, single-centre observational cohort study was conducted among HFrEF adults attending a dedicated HF clinic as well as those admitted in cardiac admission wards at a tertiary hospital in Johannesburg. Patients with HFrEF were phenotyped using comprehensive clinical assessment, advanced echocardiography including 2D-speckle tracking, measurement of standard and novel biomarkers (NT-proBNP, sST2, IL-6, GDF-15, galectin-3, cathepsin-D, cTnT), and β 1-adrenergic receptor Arg389Gly single nucleotide polymorphism genotyping. The outcome of all-cause mortality was assessed after a follow-up period of 3 years. Predictors of all-cause mortality were identified through multivariable regression models. **Results:** Among 136 HFrEF patients, the prevalence of AF was 21%. HFrEF-AF patients were older, had more advanced structural heart disease, poorer functional status, and higher mortality compared to those in sinus rhythm. Notably, prescription rates and achievement of optimal guideline-directed medical therapy (GDMT) were suboptimal among HFrEF-AF patients, with fewer patients achieving target doses across drug classes. Similarly, oral anticoagulation (OAC) use was lower than guideline standards, despite elevated thromboembolic risk. Biomarker profiling revealed a distinct fibrotic and inflammatory signature in HFrEF-AF, with elevated sST2, GDF-15, and IL-6 correlating with clinical severity and quality of life. Echocardiographic analysis confirmed greater left atrial dysfunction and advanced right sided remodeling in HFrEF-AF. Independent predictors of all-cause mortality included worsening diastolic dysfunction, higher heart rate, prior stroke, poorer quality of life, and right ventricular dysfunction. Pharmacogenomic analysis demonstrated a predominance of the Arg389 allele, with the Gly389 loss-of-function variant notably absent in HFrEF-AF, diverging from other African populations. **Conclusions:** This thesis provides the first comprehensive multimodal characterisation of HFrEF-AF in South Africa. The findings

highlight that AF marks a more advanced and severe HFrEF phenotype, underpinned by inflammatory and fibrotic pathways and distinctive genetic profiles. Suboptimal GDMT and OAC implementation highlight significant care gaps. These data underscore the need for context-specific risk stratification and management strategies, and lay the groundwork for future precision medicine initiatives in this high-risk population

OVERVIEW OF THE THESIS

This thesis comprises of the following main chapters:

- a) CHAPTER 1: INTRODUCTION
- b) CHAPTER 2: LITERATURE REVIEW
- c) CHAPTER 3: SUBMISSIBLE MANUSCRIPT 1
- d) CHAPTER 4: SUBMISSIBLE MANUSCRIPT 2
- e) CHAPTER 5: SUBMISSIBLE MANUSCRIPT 3
- f) CHAPTER 6: CONCLUSION

CHAPTER 1 introduces the thesis by outlining the study background and key components of the overall study with a focus on heart failure with reduced ejection fraction (HFrEF) and concomitant atrial fibrillation (AF).

CHAPTER 2 is a contemporary narrative review of existing epidemiology on HFrEF-AF in SSA and knowledge gaps in managing this subpopulation.

CHAPTER 3 describes the baseline clinical characteristics of HFrEF patients in South Africa and the impact of AF on the clinical presentation mortality outcomes.

CHAPTER 4 describes the biomarker profile of standard and novel cardiac biomarkers in patients with HFrEF-AF and HFrEF-SR and evaluates their prognostic and clinical utility.

CHAPTER 5 describes the presence of an Arg389Gly single nucleotide polymorphism responsible for therapeutic response to B-blocker therapy, reporting the allelic and genotype frequencies in this population, comparing them with other populations.

CHAPTER 6 presents the overall conclusion of the thesis and recommendations for future studies.

CHAPTER 1: INTRODUCTION

1.1 Epidemiology of heart failure and atrial fibrillation

Over the last decade, heart failure (HF) has become a major public health concern. Owing to the effects of westernisation and urbanisation, an epidemiological transition from infectious to non-communicable diseases is evidenced by an increase in the prevalence of traditional risk factors for cardiovascular diseases i.e. obesity, diabetes mellitus and hypertension (1). Heart failure constitutes two main phenotypes, each with distinct pathophysiology, epidemiology, and management implications: heart failure with reduced ejection fraction (HFrEF), defined by a left ventricular ejection fraction (LVEF) $\leq 40\%$, and heart failure with preserved ejection fraction (HFpEF), defined by a LVEF $\geq 50\%$ with predominantly diastolic dysfunction. Heart failure with preserved ejection fraction is commonly associated with increasing age, hypertension, obesity, diabetes and sedentary lifestyle (2, 3). Heart failure with reduced ejection fraction is equally associated with longstanding hypertension and direct cardiomyocyte injury (3). In Sub-Saharan Africa (SSA), over 50% of the cases of HFrEF are attributable to cardiomyopathies and hypertensive heart disease (4, 5). On the contrary, ischaemic heart disease is the leading cause of HFrEF in high-income countries (HICs) (4). In SSA and South Africa, contemporary data indicates that HFrEF remains the most prevalent HF phenotype encountered in clinical practice, and is associated with increased morbidity and mortality (6, 7).). Although HFpEF is gaining recognition, it remains undercharacterised in SSA . The frequency of HF-related hospitalisations place a catastrophic financial and social burden on the South African economy (8, 9).

Atrial fibrillation (AF), a common comorbidity of both HF phenotypes, is characterised by rapid and haphazard electrical activity in the atria (10). The relative impact of AF and prevalence differs between HF phenotypes with a recent meta-analysis reporting a higher prevalence of AF (45%) amongst HFpEF patients and increases with worsening diastolic dysfunction (11). In HFrEF, the prevalence of AF is substantial but lower (32%) than that of HFpEF and increases with worsening New York Heart Association (NYHA) functional class (11). Atrial fibrillation is a major decompensating factor and determinant of hospitalisation, stroke risk and mortality amongst patients with both HFpEF and HFrEF (11, 12). Other effects of AF include a reduction in the quality of life as consequence of symptoms such as exercise intolerance and palpitations (13). The co-occurrence of HF and AF, in high-income countries, is reported in more than 40% of HF patients (14, 15). The prevalence of AF is predicted to double, in high-income countries, by 2060 (12, 14).

Due to an increase in the prevalence of risk factors such as hypertension, obesity, diabetes, HF, and a younger age of onset in SSA, a similar rise in the prevalence of AF is anticipated (4, 16).

1.2 Pathophysiology of HFrEF and AF

In HFrEF, the predilection to AF is said to increase with chronicity and severity (17). A bidirectional relationship between HFrEF and AF exists where adverse structural and electrical remodelling of the atria as a consequence of HFrEF predisposes to the onset of AF (17). Moreover, AF aggravates HFrEF eliciting an increase in HF symptoms, hospitalisations and mortality (13, 15). Left atrial enlargement and dysfunction have been implicated in the bidirectional relationship between HFrEF and AF. The maintenance of AF in the setting of HFrEF is, in part, a result of dysfunctional calcium handling in the atria secondary to atrial dilatation (10). An increase in left ventricular end diastolic pressure results in elevated left atrial pressures and an increase in atrial mechanical wall stress, oxidative stress and a subsequent disturbance in calcium handling within the atria (18). Atrial mechanical stress constitutes stretch and shear stress resulting from increased intra-atrial volume and pressure, respectively. Underlying these pathophysiological mechanisms of HFrEF are processes of fibrosis and inflammation. These pathophysiological mechanisms ultimately cause structural and electrophysiological remodelling of the atria which sets the milieu for spontaneous firing, and hence arrhythmogenicity (19). These changes in left atrial structure and function changes may have important prognostic implications in HFrEF independent of recognised parameters such as left ventricular ejection fraction (LVEF) (20).

1.3 Left atrial function

In recent years, two-dimensional speckle tracking echocardiography (2D-STE) has been shown to be a simple and non-invasive tool for assessing left atrial function in patients with heart disease. Atrial function is dependent on the haemodynamic conditions of LV filling, which conventional, transthoracic echocardiography does not encompass in its analysis of left atrial function (21). Two-dimensional speckle tracking echocardiography, however, does account for haemodynamic conditions which are: left atrial reservoir (during left ventricular systole), conduit (during diastasis) and contraction function (during ventricular systole). This property of 2D-STE enables early and accurate detection of atrial dysfunction (21). Thus, Left atrial dysfunction as determined by 2D-STE can provide useful prognostic information in addition to conventional echocardiographic

parameters. Left atrial 2D-STE could provide in phenotyping HFrEF-AF patients and potentially enhance prognostication in this high risk population.

1.3 Cardiac biomarkers

Early diagnosis and accurate risk stratification are essential for effective management of HFrEF and prevention of AF. Although substantial research has elucidated the biochemical mechanisms underlying HFrEF-AF in HICs, such data remain scarce in South Africa. A recent European study reported that patients with HFrEF-AF had a biomarker profile which correlated with their outcomes (22). However, this was not observed in patients with HFpEF and AF in the same study. This data suggests that AF has a distinct pathophysiological role in each HF phenotype. Biomarkers reflecting cardiac stress, inflammation, fibrosis, and maladaptive remodelling provide valuable insights into the pathophysiological mechanisms and prognostication of HFrEF-AF. However, their applicability and performance in SSA populations remain unexplored.

Biomarkers reflect distinct biological pathways, including neurohormonal activation, inflammation, oxidative stress, and myocardial injury, and thus facilitate a comprehensive understanding of disease progression (Figure 1.1). According to Ibrahim and Januzzi, an ideal biomarker should be specific to the pathophysiological process, easily measurable, cost-effective, predictive of clinical outcomes, and modifiable with targeted therapies (23, 24).

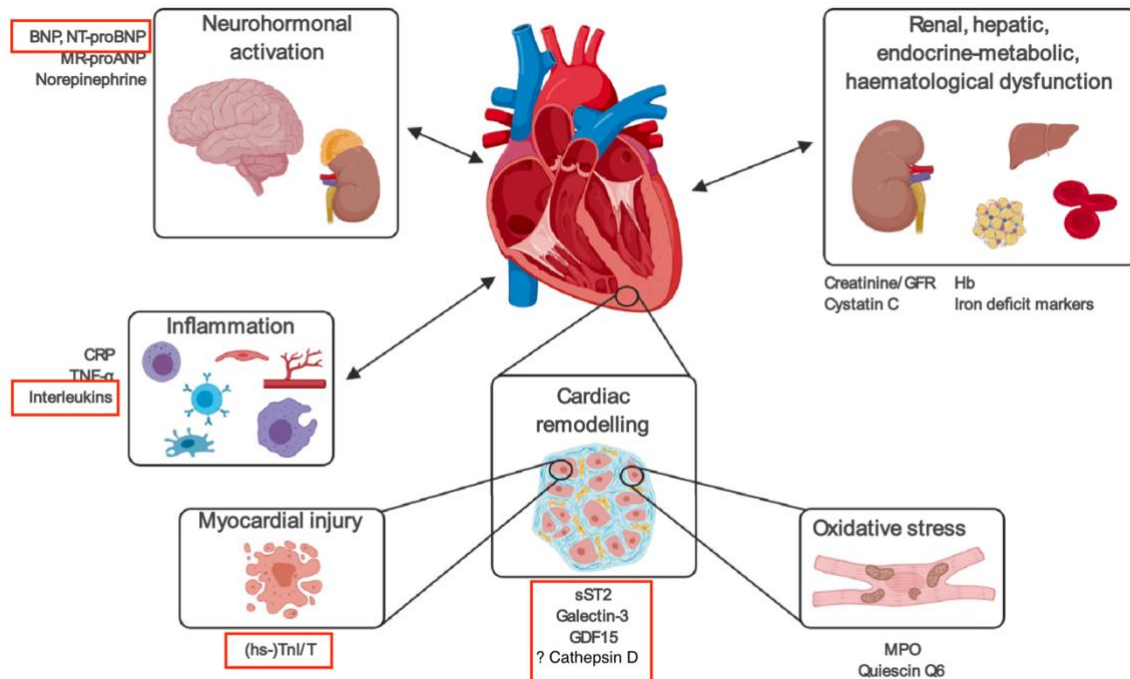


Figure 1.1 Schematic representation of the key pathophysiological mechanisms underlying HFrEF and their associated circulating biomarkers. N-terminal pro B type natriuretic peptide (NT-proBNP) reflects neurohormonal activation and is widely utilised for diagnostic and prognostic purposes. Inflammation and the imbalance between pro-inflammatory and anti-inflammatory cytokines are central to the pathophysiology of HFrEF. Interleukin-6 (IL-6), a pleiotropic cytokine involved in the Janus kinase and signal transducer and activator of transcription 3 (JAK-STAT3) signalling pathway and stimulates the release of c-reactive protein. Interleukin-6 is secreted by cardiomyocytes, fibroblasts, and adipose tissue in response to injury and inflammation and promotes hypertrophy, fibrosis, and ventricular dysfunction, and is increasingly recognised as a marker of poor prognosis in HFrEF. Cardiac troponins (e.g., hs-cTnT) indicate myocardial injury and are predictive of adverse outcomes. Soluble suppressor of tumorigenicity 2 (sST2) and Galectin-3 (GAL-3) are markers of fibrosis and cardiac remodelling, with sST2 offering incremental prognostic value. Growth differentiation factor – 15 (GDF-15) reflects oxidative stress, inflammation, and systemic disease burden, while Cathepsin-D is an emerging marker of cardiomyocyte stress and disease severity. Together, these biomarkers reflect the complex, multifactorial nature of HFrEF pathogenesis and support the rationale for a multi-marker risk stratification approach. Figure adapted from Castiglione et al., 2020 (14)

Recent evidence highlights the value of multi-marker strategies, which integrate complementary biological signals to enhance prognostic accuracy beyond that of individual biomarkers (25). In SSA, where healthcare resources are limited, such approaches could enable more precise, and affordable patient risk stratification.

1.3.2 Established Biomarkers

Brain natriuretic peptide (BNP) and **N-terminal pro B type natriuretic peptide (NT-proBNP)** (the inactive prohormone of BNP) are peptides secreted by ventricular cardiomyocytes in response to pressure or volume overload (26). Both peptides have been shown to correlate strongly with left ventricular dysfunction. Under normal physiological conditions, BNP and NT-proBNP levels remain low. However, in the context of HFrEF, they are released in an attempt to restore haemodynamic stability. Brain natriuretic peptide facilitates diuresis, natriuresis, arterial vasodilation, exhibits anti-fibrotic effects and counteracts an overactive sympathetic nervous system (SNS) and renin–angiotensin–aldosterone system (RAAS) in HF (26). NT-proBNP, with its longer half-life and renal clearance, is the preferred marker for both diagnosis and prognosis in HFrEF and retains its predictive value in the presence of AF (27, 28). However, its interpretation is confounded by renal impairment, age, and obesity. Racial differences in baseline levels of NT-proBNP have been documented but remain understudied in SSA populations (29).

Cardiac troponin T (cTnT), a marker of cardiomyocyte injury, reflects subclinical myocardial damage and oxidative stress (Figure 1.1) (20, 33). High-sensitivity cTnT (hs-cTnT) assays improve detection and predict worsening heart failure and mortality. When combined with NT-proBNP and growth differentiation factor-15 (GDF-15), hs-cTnT enhances risk stratification in HFrEF-AF (35, 36).

1.3.3 Emerging and Novel Biomarkers

Interleukin (IL)-6 is a pleiotropic cytokine produced by cardiomyocytes, fibroblasts and extracardiac sources such as adipose tissue, activates the Janus kinase and signal transducer and activator of transcription 3 (JAK-STAT3) pathway and stimulates acute phase responses including C-reactive protein (CRP) release (30). Elevated levels of IL-6 are increasingly recognised as mediators of adverse cardiac remodelling and a marker of poor prognosis in HF (31). Experimental evidence from pressure-overload and myocardial injury models show that IL-6 signalling amplifies inflammation, promotes hypertrophy and fibrosis, and impairs ventricular function (32). In human cohorts of HF (predominantly HFrEF, or mixed HF phenotypes), higher IL-6 levels have been associated with increased natriuretic peptide concentrations, greater symptom burden and reduced functional capacity (31). While much of the detailed mechanistic work has focused on HFpEF, theoretical and translational studies suggest that similar IL-6 driven pathways may contribute to the progression of HFrEF through exacerbation of neuro-humoral activation, endothelial dysfunction,

cardiomyocyte injury and extracellular matrix remodelling (33). Thus, IL-6 may serve both as a prognostic biomarker and a potential therapeutic target in HFrEF, particularly in patients exhibiting evidence of systemic inflammation or comorbidity burden.

Soluble suppression of tumorigenicity 2 (sST2), is a biomarker which belongs to the interleukin (IL)-1 receptor family with two main isoforms: a soluble form (sST2) and a membrane-bound receptor form, named suppression of tumorigenesis-2 ligand (ST2L) (35). Soluble ST2 is secreted by cardiomyocytes, cardiac fibroblasts, type 2 pneumocytes and various extracardiac tissues (34). Interleukin-33 (IL-33), a cytokine, released by stromal cells in both cardiac and extracardiac tissues, serves as a ligand for both sST2 and ST2L (35). The role of IL-33 as either a pro-inflammatory or anti-inflammatory cytokine is influenced by co-stimulatory factors (36). Acting as a decoy receptor for IL-33, sST2 inhibits cardioprotective anti-fibrotic pathways, thereby promoting myocardial remodelling and fibrosis (Figure 1.1) (37). Soluble ST2 is not cardiac specific and therefore cannot be used in the diagnosis of HFrEF. However, its prognostic utility as a measure of cardiac fibrosis and remodelling has become apparent in cardiovascular diseases (36). In chronic HFrEF, sST2 has been shown to provide additive prognostic value beyond NT-proBNP and cTnT, although optimal cut-offs in African populations are undefined (38, 39). A study by Santema et al, showed circulating levels of sST2 were significantly increased HFrEF-AF patients compared to those in sinus rhythm as well as a strong association with increased mortality (22, 28).

Galectin-3, a β -galactosidase-binding lectin which is ubiquitously expressed across different tissues, is involved in various biological processes such as apoptosis, regulation of cell-cell and cell-matrix interactions, proliferation, adhesion, and inflammation (40). Galectin-3 is implicated in the pathological remodelling and fibrosis in HFrEF through its induction of fibroblast proliferation and collagen deposition (Figure 1.1) (41). Galectin-3 but has shown prognostic utility in predicting re-hospitalisation and mortality HFrEF patients in ADHF and may identify HFrEF-AF patients who could benefit from catheter ablation (42-45). Similar to NT-proBNP, GAL-3 levels are influenced by renal function, limiting its specificity. The clinical utility and measured levels of GAL-3 in an African HFrEF-AF cohort remain undetermined. To the best of current knowledge, no studies have assessed this molecule in a HFrEF SSA population (25).

Growth Differentiation Factor-15 (GDF-15) is a cytokine which belongs to the transforming growth factor- β superfamily and is secreted from various cells including cardiomyocytes in response to strain, ischemia and oxidative stress (Figure 1.1) (46). Growth Differentiation

Factor-15 has been shown to improve mortality prediction in some HFrEF models but offers limited incremental value when combined with other clinical and biochemical predictors (47). Atrial fibrillation often complicates HFrEF by increasing the risk of thromboembolic events and worsening cardiac function, which might be reflected in higher GDF-15 levels due to increased stress and inflammation. In patients with AF alone, on oral anticoagulation, from the ARISTOTLE trial, levels of GDF-15 were independently associated with the risk of major bleeding, stroke and mortality (48).

Cathepsin D, a lysosomal protease released during oxidative stress, has been linked to myocardial injury and adverse outcomes in HF (49, 50). Although mechanistic evidence remains limited, circulating cathepsin D may reflect cardiomyocyte stress and disease severity, with potential as a novel biomarker in HFrEF.

Each biomarker provides unique insights into HFrEF progression (Figure 1.1), with potential implications for risk stratification and therapeutic targeting in HFrEF and potentially HFrEF-AF patients. A meta-analysis demonstrated a 20-30% reduction on mortality when a biomarker-guided management strategy was employed over standard care (51). Given the manifold and complex pathophysiological mechanisms involved in HFrEF, reliance on a single biomarker may be insufficient for comprehensive risk assessment. A multi-marker strategy integrates multiple pathological processes to improve and strengthen predictive accuracy, thereby enhancing individualised management approaches

1.4 Discrepancies in management of HFrEF-AF

Managing patients with AF and HFrEF is a growing point of uncertainty for many physicians as these patients do not respond to, pharmacological, mortality-benefitting therapy (6). Beta-blockers (β -blockers) are used for management of patients with AF alone (52). Similarly, β -blockers are used to manage stable patients with HFrEF (53). The use of β -blockers in individuals with HFrEF is founded on landmark randomised clinical trials demonstrating that β -blockers significantly reduce mortality in HFrEF patients (in sinus rhythm) with an ejection fraction below 50% (54). Studies show that patients with HFrEF-AF exhibit suboptimal response to β -blockers (55-57). Patients with HFrEF-AF carrying a homozygous Glycine (Gly)389 mutation in the β_1 -adrenergic receptor did not respond to β -blockers therapy, in contrast to those without the mutation, who demonstrated a significant reduction in adverse outcomes (58, 59). A recent sub-study analysis of the PARADIGM-

HF trial showed that patients with HFrEF-AF did not derive as much mortality benefit from the drug, sacubitril/valsartan, compared to patients without AF (60).

1.5 Pharmacogenomic role for the Arg389Gly single nucleotide polymorphism

The Arginine(Arg)389 on transmembrane seven (TM7), situated at the c-terminus of the β_1 -adrenergic receptor, plays a key role in the coupling of the stimulatory guanine nucleotide-binding protein (Gs protein) and hence signal transduction (61). The literature has proposed a role for the Arg389Gly polymorphism in rendering heart failure patients unresponsive to beta-blocker therapy (61). Patients with the loss-of-function homozygous Gly389 variant are proposed to be poor responders and patients with a homozygous Arg389 mutant, good responders (61). This effect has been demonstrated only in European, American and Asian patients (62). A randomised control trial comparing β -blockers in patients with HFrEF-AF and heart failure alone (sinus rhythm) showed that only patients with HFrEF-AF homozygous for the Arg389 allele showed little or no sustained response to carvedilol, a non-selective β -blocker commonly used in the South African public sector hospitals (55, 63). The cohort of patients in this study were of (South) Eastern European decent. Another study demonstrated that the frequency distribution of the Arg389Gly genetic variants differ significantly between European and Non-European populations (64).

Very little is known about the clinical relevance of this polymorphism, and its frequency distribution in South Africa. To date, only one study has looked for this polymorphism in a South African HF population and employed a very stringent selection criteria excluding patients on β -blocker therapy as well as patients with arrhythmias (65).

1.6 Study rationale

Heart failure with reduced ejection fraction and atrial AF represent a growing clinical burden in Sub-Saharan Africa (SSA), driven by the region's ongoing epidemiological shift from infectious to non-communicable diseases. The coexistence of these two conditions is associated with worse clinical outcomes. Despite the growing prevalence of HFrEF-AF in SSA, most existing data informing clinical practice are derived from HICs and may not be applicable to African populations due to differences in disease aetiology, underlying genetics, healthcare infrastructure, and age of onset.

There is an urgent unmet need to better characterise HFrEF-AF within SSA contexts to guide tailored clinical management. In this regard, phenotyping patients using advanced echocardiographic techniques and biomarker profiling may offer valuable insights. Two-dimensional speckle tracking echocardiography is a sensitive, non-invasive modality for assessing left atrial function, a key determinant in the pathophysiological interplay between HFrEF and AF. Unlike conventional echocardiography, 2D-STE can detect subclinical left atrial dysfunction that may precede structural changes and arrhythmia onset.

Simultaneously, cardiac biomarkers reflecting processes such as fibrosis, neurohormonal activation, inflammation, stress, myocardial injury, and maladaptive remodelling, including NT-proBNP, sST2, GDF-15, galectin-3, cathepsin-D, and IL-6, have shown promise in risk stratification and disease monitoring. However, their roles in South African patients with HFrEF, particularly those with AF, remain undefined.

Additionally, emerging evidence highlights a pharmacogenomic dimension to treatment responsiveness in HFrEF. The Arg389Gly SNP in the β 1-adrenergic receptor gene has been implicated in modulating the efficacy of β -blockers, a cornerstone therapy in HFrEF. While this genetic variant has been studied in European, Asian, and North American cohorts, there is a paucity of data on its frequency and clinical impact in SSA populations.

This study intends to bridge these critical knowledge gaps by systematically investigating the clinical, echocardiographic, biochemical, and (Arg389Gly) SNP frequencies of South African patients with HFrEF and AF. By comparing these findings with those from patients in sinus rhythm, this research seeks to advance understanding of the pathophysiological mechanisms underpinning HFrEF-AF in SSA, identify potential prognostic markers, and explore an opportunity for precision medicine approaches. The findings have the potential to inform context-specific management strategies and improve outcomes for this high-risk patient population.

1.7 Aim and objectives

The primary aim of this study was to investigate the clinical and biochemical effects of AF on patients with HFrEF at a dedicated HF clinic and cardiac admission wards at Charlotte Maxeke Academic Johannesburg Hospital (CMJAH).

The study objectives were:

- a) To determine the prevalence of HFrEF-AF within the division of Cardiology at CMJAH.
- b) To compare the demographic and clinical characteristics between patients with HFrEF-AF and HFrEF in sinus rhythm (HFrEF-SR).
- c) To compare left atrial function in patients with HFrEF-AF versus HFrEF-SR using 2D-speckle tracking echocardiography.
- d) To quantify the levels of standard and novel cardiac biomarkers, N-terminal BNP (NT-proBNP), sST2, cardiac troponin (cTnT), growth-differentiation factor-15 (GDF-15), cathepsin-D, galectin 3 (GAL-3), and IL-6, in HFrEF-AF patients
- e) To determine whether the abovementioned biomarkers are associated with HFrEF-AF and/or HFrEF-SR and to evaluate their potential to predict outcomes within a South African HFrEF cohort.
- f) To identify clinical characteristics and echocardiographic parameters associated with levels of standard and novel biomarkers in HFrEF patients using a multivariable linear regression model.
- g) To ascertain the presence of the Arginine389Glycine β_1 -adrenergic receptor mutation in HFrEF-AF and HFrEF-SR patients using Single Nucleotide Polymorphism genotyping using iPLEX® assays on a MassArray system.
- h) To determine the mortality in patients with HFrEF-AF versus HFrEF-SR, after 12 months.

1.8 REFERENCES

1. Keates AK, Mocumbi AO, Ntsekhe M, Sliwa K, Stewart S. Cardiovascular disease in Africa: Epidemiological profile and challenges. *Nature Reviews Cardiology*. 2017;14(5):273-93.
2. Savji N, Meijers WC, Bartz TM, Bhambhani V, Cushman M, Naylor M, et al. The Association of Obesity and Cardiometabolic Traits With Incident HFpEF and HFrEF. *JACC Heart Fail*. 2018;6(8):701-9.
3. Ho JE, Enserro D, Brouwers FP, Kizer JR, Shah SJ, Psaty BM, et al. Predicting Heart Failure With Preserved and Reduced Ejection Fraction: The International Collaboration on Heart Failure Subtypes. *Circ Heart Fail*. 2016;9(6).
4. Agbor VN, Essouma M, Ntusi NAB, Nyaga UF, Bigna JJ, Noubiap JJ. Heart failure in sub-Saharan Africa: A contemporaneous systematic review and meta-analysis. *Int J Cardiol*. 2018;257:207-15.
5. Nyaga UF, Bigna JJ, Agbor VN, Essouma M, Ntusi NAB, Noubiap JJ. Data on the epidemiology of heart failure in Sub-Saharan Africa. *Data in Brief*. 2018;17:1218-39.
6. Mboweni N, Maseko M, Tsabedze N. Heart failure with reduced ejection fraction and atrial fibrillation: a Sub-Saharan African perspective. *ESC Heart Fail*. 2023;10(3):1580-96.
7. Dzudie A, Hongieh Abanda M, Nkoke C, Barche B, Damasceno A, Edwards C, et al. Clinical characteristics and outcomes of black African heart failure patients with preserved, mid-range, and reduced ejection fraction: a post hoc analysis of the THESUS-HF registry. *ESC Heart Fail*. 2021;8(1):238-49.
8. Ogah OS, Stewart S, Onwujekwe OE, Falase AO, Adebayo SO, Olunuga T, et al. Economic burden of heart failure: investigating outpatient and inpatient costs in Abeokuta, Southwest Nigeria. *PLoS One*. 2014;9(11):e113032.
9. Adeniji FIP, Obembe TA. Cardiovascular Disease and Its Implication for Higher Catastrophic Health Expenditures Among Households in Sub-Saharan Africa. *J Health Econ Outcomes Res*. 2023;10(1):59-67.
10. Denham NC, Pearman CM, Caldwell JL, Madders GWP, Eisner DA, Trafford AW, et al. Calcium in the Pathophysiology of Atrial Fibrillation and Heart Failure. *Frontiers in Physiology*. 2018;9.
11. Mundisugih J, Franke KB, Tully PJ, Munawar DA, Kumar S, Mahajan R. Prevalence and Prognostic Implication of Atrial Fibrillation in Heart Failure Subtypes: Systematic Review and Meta-Analysis. *Heart Lung Circ*. 2023;32(6):666-77.
12. Chugh SS, Havmoeller R, Narayanan K, Singh D, Rienstra M, Benjamin EJ, et al. Worldwide epidemiology of atrial fibrillation: A global burden of disease 2010 study. *Circulation*. 2014;129(8):837-47.
13. Zafir B, Lund LH, Laroche C, Ruschitzka F, Crespo-Leiro MG, Coats AJS, et al. Prognostic implications of atrial fibrillation in heart failure with reduced, mid-range, and preserved ejection fraction: a report from 14 964 patients in the European Society of Cardiology Heart Failure Long-Term Registry. *Eur Heart J*. 2018;39(48):4277-84.

14. Krijthe BP, Kunst A, Benjamin EJ, Lip GY, Franco OH, Hofman A, et al. Projections on the number of individuals with atrial fibrillation in the European Union, from 2000 to 2060. *Eur Heart J*. 2013;34(35):2746-51.
15. Kotecha D, Chudasama R, Lane DA, Kirchhof P, Lip GY. Atrial fibrillation and heart failure due to reduced versus preserved ejection fraction: A systematic review and meta-analysis of death and adverse outcomes. *Int J Cardiol*. 2016;203:660-6.
16. Africa SS. Mortality and causes of death in South Africa: Findings from death notification 2018. *STATISTICAL RELEASE*. 2021:1-98.
17. Bergau L, Bengel P, Sciacca V, Fink T, Sohns C, Sommer P. Atrial Fibrillation and Heart Failure. *J Clin Med*. 2022;11(9).
18. Schönleitner P, Schotten U, Antoons G. Mechanosensitivity of microdomain calcium signalling in the heart. *Progress in Biophysics and Molecular Biology*. 2017;130:288-301.
19. Peter Libby ROB, Douglas L. Mann, Gordon F. Tomaselli, Deepak L. Bhatt, Scott D. Solomon, . Braunwald's Heart Disease, 2 Vol Set, 12th Edition. Philadelphia: Elsevier; 2011.
20. Gan GCH, Ferkh A, Boyd A, Thomas L. Left atrial function: evaluation by strain analysis. *Cardiovascular Diagnosis and Therapy*. 2018;8(1):29-46.
21. Yuda S, Muranaka A, Miura T. Clinical implications of left atrial function assessed by speckle tracking echocardiography. *J Echocardiogr*. 2016;14(3):104-12.
22. Santema BT, Kloosterman M, Van Gelder IC, Mordi I, Lang CC, Lam CSP, et al. Comparing biomarker profiles of patients with heart failure: atrial fibrillation vs. sinus rhythm and reduced vs. preserved ejection fraction. *Eur Heart J*. 2018;39(43):3867-75.
23. Ibrahim NE, Januzzi JL, Jr. Beyond Natriuretic Peptides for Diagnosis and Management of Heart Failure. *Clin Chem*. 2017;63(1):211-22.
24. Morrow DA, De Lemos JA. Benchmarks for the assessment of novel cardiovascular biomarkers. *Circulation*. 2007;115(8):949-52.
25. Badianyama M, Mpanya D, Adamu U, Sigauke F, Nel S, Tsabedze N. New Biomarkers and Their Potential Role in Heart Failure Treatment Optimisation-An African Perspective. *J Cardiovasc Dev Dis*. 2022;9(10).
26. Goetze JP, Bruneau BG, Ramos HR, Ogawa T, de Bold MK, de Bold AJ. Cardiac natriuretic peptides. *Nat Rev Cardiol*. 2020;17(11):698-717.
27. Hijazi Z, Lindback J, Alexander JH, Hanna M, Held C, Hylek EM, et al. The ABC (age, biomarkers, clinical history) stroke risk score: a biomarker-based risk score for predicting stroke in atrial fibrillation. *Eur Heart J*. 2016;37(20):1582-90.
28. Santema BT, Chan MMY, Tromp J, Dokter M, van der Wal HH, Emmens JE, et al. The influence of atrial fibrillation on the levels of NT-proBNP versus GDF-15 in patients with heart failure. *Clin Res Cardiol*. 2020;109(3):331-8.
29. Commodore-Mensah Y, Wang D, Jeon Y, Foti K, McEvoy JW, Coresh J, et al. Racial and ethnic differences in circulating N-terminal pro-brain-type natriuretic peptide (NT-proBNP) in US adults. *Am J Prev Cardiol*. 2023;15:100526.
30. Rose-John S. Interleukin-6 Family Cytokines. *Cold Spring Harb Perspect Biol*. 2018;10(2).

31. Roman-Pepine D, Serban AM, Capras RD, Cismaru CM, Filip AG. A Comprehensive Review: Unraveling the Role of Inflammation in the Etiology of Heart Failure. *Heart Fail Rev.* 2025;30(5):931-54.
32. Reina-Couto M, Pereira-Terra P, Quelhas-Santos J, Silva-Pereira C, Albino-Teixeira A, Sousa T. Inflammation in Human Heart Failure: Major Mediators and Therapeutic Targets. *Front Physiol.* 2021;12:746494.
33. Van Linthout S, Tschope C. Inflammation - Cause or Consequence of Heart Failure or Both? *Curr Heart Fail Rep.* 2017;14(4):251-65.
34. Pascual-Figal DA, Perez-Martinez MT, Asensio-Lopez MC, Sanchez-Mas J, Garcia-Garcia ME, Martinez CM, et al. Pulmonary Production of Soluble ST2 in Heart Failure. *Circ Heart Fail.* 2018;11(12):e005488.
35. Weinberg EO, Shimpo M, De Keulenaer GW, MacGillivray C, Tominaga S, Solomon SD, et al. Expression and regulation of ST2, an interleukin-1 receptor family member, in cardiomyocytes and myocardial infarction. *Circulation.* 2002;106(23):2961-6.
36. Riccardi M, Myhre PL, Zelniker TA, Metra M, Januzzi JL, Inciardi RM. Soluble ST2 in Heart Failure: A Clinical Role beyond B-Type Natriuretic Peptide. *J Cardiovasc Dev Dis.* 2023;10(11).
37. Pascual-Figal D, Lax A, Asensio Lopez MC. Circulating ST2, from biomarker to pathogenic mediator. *Rev Esp Cardiol (Engl Ed).* 2023;76(9):672-4.
38. Emdin M, Aimo A, Vergaro G, Bayes-Genis A, Lupon J, Latini R, et al. sST2 Predicts Outcome in Chronic Heart Failure Beyond NT-proBNP and High-Sensitivity Troponin T. *J Am Coll Cardiol.* 2018;72(19):2309-20.
39. Bayes-Genis A, de Antonio M, Vila J, Penafiel J, Galan A, Barallat J, et al. Head-to-head comparison of 2 myocardial fibrosis biomarkers for long-term heart failure risk stratification: ST2 versus galectin-3. *J Am Coll Cardiol.* 2014;63(2):158-66.
40. Blanda V, Bracale UM, Di Taranto MD, Fortunato G. Galectin-3 in Cardiovascular Diseases. *Int J Mol Sci.* 2020;21(23).
41. Zaborska B, Sikora-Frac M, Smarz K, Pilichowska-Paszkiel E, Budaj A, Sitkiewicz D, et al. The Role of Galectin-3 in Heart Failure-The Diagnostic, Prognostic and Therapeutic Potential-Where Do We Stand? *Int J Mol Sci.* 2023;24(17).
42. Meijers WC, Januzzi JL, deFilippi C, Adourian AS, Shah SJ, van Veldhuisen DJ, et al. Elevated plasma galectin-3 is associated with near-term rehospitalization in heart failure: a pooled analysis of 3 clinical trials. *Am Heart J.* 2014;167(6):853-60 e4.
43. Miro O, Gonzalez de la Presa B, Herrero-Puente P, Fernandez Bonifacio R, Mockel M, Mueller C, et al. The GALA study: relationship between galectin-3 serum levels and short- and long-term outcomes of patients with acute heart failure. *Biomarkers.* 2017;22(8):731-9.
44. Clementy N, Garcia B, André C, Bisson A, Benhenda N, Pierre B, et al. Galectin-3 level predicts response to ablation and outcomes in patients with persistent atrial fibrillation and systolic heart failure. *PLoS ONE.* 2018;13(8):1-12.
45. Marrouche NF, Brachmann J, Andresen D, Siebels J, Boersma L, Jordaens L, et al. Catheter Ablation for Atrial Fibrillation with Heart Failure. *New England Journal of Medicine.* 2018;378(5):417-27.

46. Assadi A, Zahabi A, Hart RA. GDF15, an update of the physiological and pathological roles it plays: a review. *Pflugers Arch*. 2020;472(11):1535-46.
47. Sharma A, Stevens SR, Lucas J, Fiuzat M, Adams KF, Whellan DJ, et al. Utility of Growth Differentiation Factor-15, A Marker of Oxidative Stress and Inflammation, in Chronic Heart Failure: Insights From the HF-ACTION Study. *JACC Heart Fail*. 2017;5(10):724-34.
48. Wallentin L, Hijazi Z, Andersson U, Alexander JH, De Caterina R, Hanna M, et al. Growth differentiation factor 15, a marker of oxidative stress and inflammation, for risk assessment in patients with atrial fibrillation: insights from the Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation (ARISTOTLE) trial. *Circulation*. 2014;130(21):1847-58.
49. Wu P, Yuan X, Li F, Zhang J, Zhu W, Wei M, et al. Myocardial Upregulation of Cathepsin D by Ischemic Heart Disease Promotes Autophagic Flux and Protects Against Cardiac Remodeling and Heart Failure. *Circ Heart Fail*. 2017;10(7).
50. Hoes MF, Tromp J, Ouwerkerk W, Bomer N, Oberdorf-Maass SU, Samani NJ, et al. The role of cathepsin D in the pathophysiology of heart failure and its potentially beneficial properties: a translational approach. *European Journal of Heart Failure*. 2020;22(11):2102-11.
51. Troughton RW, Frampton CM, Brunner-La Rocca HP, Pfisterer M, Eurlings LW, Erntell H, et al. Effect of B-type natriuretic peptide-guided treatment of chronic heart failure on total mortality and hospitalization: an individual patient meta-analysis. *Eur Heart J*. 2014;35(23):1559-67.
52. Van Gelder IC, Rienstra M, Bunting KV, Casado-Arroyo R, Caso V, Crijns H, et al. 2024 ESC Guidelines for the management of atrial fibrillation developed in collaboration with the European Association for Cardio-Thoracic Surgery (EACTS). *Eur Heart J*. 2024;45(36):3314-414.
53. McDonagh TA, Metra M, Adamo M, Gardner RS, Baumbach A, Bohm M, et al. 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. *Eur Heart J*. 2021;42(36):3599-726.
54. Rienstra M, Damman K, Mulder BA, Van Gelder IC, McMurray JJV, Van Veldhuisen DJ. Beta-Blockers and Outcome in Heart Failure and Atrial Fibrillation. *JACC: Heart Failure*. 2013;1(1):21-8.
55. Rau T, Düngen Hd, Edelmann F, Waagstein F, Lain M, Dimkovi S, et al. Impact of the β 1-Adrenoceptor Arg389Gly Polymorphism on Heart-Rate Responses to Bisoprolol and Carvedilol in Heart-Failure Patients. 2012;92(1).
56. Kotecha D, Holmes J, Krum H, Altman DG, Manzano L, Cleland JG, et al. Efficacy of beta blockers in patients with heart failure plus atrial fibrillation: an individual-patient data meta-analysis. *Lancet*. 2014;384(9961):2235-43.
57. Zhu M, Zhou X, Cai H, Wang Z, Xu H, Chen S, et al. Catheter ablation versus medical rate control for persistent atrial fibrillation in patients with heart failure: A PRISMA-compliant systematic review and meta-analysis of randomized controlled trials. *Medicine (Baltimore)*. 2016;95(30):e4377.
58. Kao DP, Davis G, Aleong R, Connor CMO, Fiuzat M, Carson PE, et al. Effect of bucindolol on heart failure outcomes and heart rate response in patients with reduced ejection fraction heart failure and atrial fibrillation. 2013;5:324-33.

59. Aleong RG, Sauer WH, Davis G, Murphy GA, Port JD, Anand IS, et al. Prevention of Atrial Fibrillation by Bucindolol Is Dependent on the Beta 1 389 Arg/Gly Adrenergic Receptor Polymorphism. *JACC: Heart Failure*. 2013;1(4):338-44.
60. Mogensen UM, Jhund PS, Abraham WT, Desai AS, Dickstein K, Packer M, et al. Type of Atrial Fibrillation and Outcomes in Patients With Heart Failure and Reduced Ejection Fraction. *Journal of the American College of Cardiology*. 2017;70(20):2490-500.
61. Brodde O-E. β 1 - and β 2 -Adrenoceptor polymorphisms and cardiovascular diseases. *Fundamental & Clinical Pharmacology*. 2008;22(2):107-25.
62. Liu W-N, Fu K-L, Gao H-Y, Shang Y-Y, Wang Z-H, Jiang G-H, et al. β 1 Adrenergic Receptor Polymorphisms and Heart Failure: A Meta-Analysis on Susceptibility, Response to β -Blocker Therapy and Prognosis. *PLoS ONE*. 2012;7(7):e37659-e.
63. Parikh KS, Fiuzat M, Davis G, Neely M, Blain-Nelson P, Whellan DJ, et al. Dose Response of β -Blockers in Adrenergic Receptor Polymorphism Genotypes. *Circulation: Genomic and Precision Medicine*. 2018;11(8):139-48.
64. Katsarou M-S, Karathanasopoulou A, Andrianopoulou A, Desiniotis V, Tzinis E, Dimitrakis E, et al. Beta 1, Beta 2 and Beta 3 Adrenergic Receptor Gene Polymorphisms in a Southeastern European Population. *Frontiers in Genetics*. 2018;9.
65. Woodiwiss AJ, Badenhorst D, Sliwa K, Brooksbank R, Essop R, Sareli P, et al. Beta1- and alpha2c-adrenoreceptor variants as predictors of clinical aspects of dilated cardiomyopathy in people of African ancestry. *Cardiovascular journal of Africa*. 19(4):188-93.

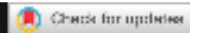
CHAPTER 2: NARRATIVE REVIEW: HEART FAILURE WITH REDUCED EJECTION FRACTION AND ATRIAL FIBRILLATION: A SUB-SAHARAN AFRICAN PERSPECTIVE

ESC HEART FAILURE

ESC Heart Failure 2023; 10: 1580–1596

Published online 19 March 2023 in Wiley Online Library (wileyonlinelibrary.com) DOI: 10.1002/ehf2.14332

REVIEW



Heart failure with reduced ejection fraction and atrial fibrillation: a Sub-Saharan African perspective

Nonkanyiso Mboweni^{1,2}, Muzi Maseko¹ and Nqoba Tsabedze^{2*}

¹School of Physiology, University of the Witwatersrand, Johannesburg, South Africa; and ²Department of Internal Medicine, Division of Cardiology, Charlotte Maseko Johannesburg Academic Hospital, University of the Witwatersrand, Gauteng/Johannesburg, South Africa

Abstract

Cardiovascular diseases are a well-established cause of death in high-income countries. In the last 20 years, Sub-Saharan Africa (SSA) has seen one of the sharpest increases in cardiovascular disease-related mortality, superseding that of infectious diseases, including HIV/AIDS, in South Africa. This increase is evidenced by a growing burden of heart failure and atrial fibrillation (AF) risk factors. AF is a common comorbidity of heart failure with reduced ejection fraction (HFrEF), which predisposes to an increased risk of stroke, rehospitalizations, and mortality compared with patients in sinus rhythm. AF had the largest relative increase in cardiovascular disease burden between 1990 and 2010 in SSA and the second highest (106.4%) increase in disability-adjusted life-years (DALY) between 1990 and 2017. Over the last decade, significant advancements in the management of both HFrEF and AF have emerged. However, managing HFrEF/AF remains a clinical challenge for physicians, compounded by the suboptimal efficacy of guideline-mandated pharmacotherapy in this group of patients. There may be an essential role for racial differences and genetic influence on therapeutic outcomes of HFrEF/AF patients, further complicating our overall understanding of the disease and its pathophysiology. In SSA, the lack of accurate and up-to-date epidemiological data on this subgroup of patients presents a challenge in our quest to prevent and reduce adverse outcomes. This narrative review provides a contemporary overview of the epidemiology of HFrEF/AF in SSA. We highlight important differences in the demographic and aetiological profile and the management of this subpopulation, emphasizing what is currently known and, more importantly, what is still unknown about HFrEF/AF in SSA.

Keywords Atrial fibrillation; Heart failure with reduced ejection fraction (HFrEF); Sub-Saharan Africa

Received: 23 June 2022; Revised: 6 January 2023; Accepted: 7 February 2023

*Correspondence to: Nqoba Tsabedze, Department of Internal Medicine, Division of Cardiology, Charlotte Maseko Johannesburg Academic Hospital, University of the Witwatersrand, Johannesburg, Gauteng, South Africa. Tel: +27 722722420. Email: nqoba.tsabedze@wits.ac.za

Introduction

Heart failure (HF) is a well-recognized public health concern in high-income countries (HICs). In the last two decades, HF has emerged as a major public health problem in Sub-Saharan Africa (SSA) owing to the effects of westernization and a subsequent epidemiological transition from infectious to non-communicable disease patterns.¹ This epidemiological transition is evidenced by an increasing prevalence of traditional risk factors for cardiovascular diseases (CVDs) such as obesity, sedentary lifestyle, diabetes mellitus, and hypertension.² HF as a clinical syndrome is associated with increased morbidity and mortality.³ The aetiological profile of HF in SSA differs greatly from that of HICs, with the majority

of causes of HF being non-ischaemic in nature.^{1,4–7} Many HF-related hospitalizations impose a major burden on the global economy, particularly those of SSA's low-middle-income countries (LMICs).⁸

Atrial fibrillation (AF) is a common comorbidity of HF and is characterized by rapid and haphazard electrical activity in the atria marked by an irregular heart beat and absent p-waves on an electrocardiogram (ECG).^{9,10} The aetiological spectrum of HF in SSA is broad. This has an impact on the relative AF prevalence across the HF spectrum in SSA. Rheumatic heart disease (RHD) is a common risk factor for AF in SSA, with a prevalence ranging from 10% to 46%.^{11–14} However, recent studies are beginning to demonstrate an increase in non-valvular AF in this setting with RHD not so prevalent

anymore in some regions.^{11,15–17} A few studies have demonstrated heart failure with preserved ejection fraction (HFpEF) and heart failure with mid-range ejection fraction (HFmrEF) to have a higher AF prevalence than heart failure with reduced ejection fraction (HFrEF).^{6,18} However, in patients with longstanding HF, the prevalence of HFrEF/AF becomes more apparent.¹⁶ Interesting insights from the multinational THESUS-HF registry reported a significantly higher prevalence of AF in HFmrEF (28.5%) compared with that of HFpEF (21.3%) and HFrEF (14.5%).¹⁹

Data from SSA suggest that AF is a major decompensating factor among HF patients in SSA.^{6,15,16,19–22} A Tanzanian study found a survival rate of 63% [vs. 96% ($P < 0.001$)] in HF/AF patients.²¹ Similarly, AF was found to be a significant predictor of death in one of the largest HF outcomes studies ($n = 1488$) undertaken in Ghana.⁶ However, AF was not significantly associated with all-cause death in HFmrEF or HFpEF patients, unlike HFrEF.⁶ Similarly, data from the THESUS-HF registry revealed that in the setting of HF, 6 month mortality was substantially greater in individuals with valvular AF compared with non-valvular AF.¹⁵

AF predisposes individuals to a five-fold greater stroke risk and an increased rehospitalization risk.^{26,27} Across the global population, 1 to 2 in 10 individuals with any cardiomyopathy, excluding post-partum cardiomyopathy, will develop AF.²⁸ Furthermore, HF/AF patients, in SSA, are at a 1.3- to 3.4-fold risk of death compared with HF patients without AF.^{21,29} This is particularly concerning given the significant morbidity and mortality associated with AF. AF is common in both HFpEF and HFrEF but portends a higher risk of mortality in patients with HFrEF.^{6,30,31} A recent study demonstrated that HFrEF/AF patients had a greater 1 year mortality rate (36.5%) than patients with HFmrEF/AF (27.7%) and HFpEF/AF (27.7%).^{7,31}

Over the last decade, significant advancements in the management of both HFrEF and AF have emerged. However, managing HFrEF with co-existing AF remains a clinical challenge for physicians, compounded by the suboptimal efficacy of guideline-mandated pharmacotherapy in this group of patients. Furthermore, there may be an important role for racial differences and genetic influence on therapeutic outcomes of HFrEF/AF patients, further complicating our overall understanding of the disease and its pathophysiology. In SSA, the lack of accurate and up-to-date epidemiological data on this subgroup of patients presents a challenge in our quest to prevent and reduce adverse outcomes.

This narrative review provides a contemporary overview of the epidemiology on HFrEF/AF in SSA. We further highlight important differences in this subpopulation's demographic and aetiological profile and, more importantly, what is still unknown about HFrEF/AF in SSA. With emerging data from HIC studies advocating for a timely aggressive rhythm control strategy in this cohort of patients, we were compelled to bring SSA to the conversation. We aim to raise awareness

about the growing burden of HFrEF/AF and provide a summary of new approaches to managing HFrEF/AF.

Methods

We searched PubMed/MEDLINE, EMBASE, and Scopus from inception to September 2022, without language restriction, to identify all published studies providing data on the prevalence, epidemiology, and outcomes of HFrEF and concomitant AF in Sub-Saharan African populations. Countries below the equator were considered SSA. Although this review aims to focus on the co-occurrence of HFrEF/AF, due to the lack of data, we independently screened AF and HF population studies to identify data on the relevant subgroup of interest (HFrEF/AF). These studies are summarized in *Table 1*.

The following keywords were used in the search strategy: 'heart failure', 'atrial fibrillation', and the name of all the SSA countries. We included all cross-sectional and cohort studies. The following definition of HF was employed: patients with signs and symptoms of HF as well as an echocardiographic diagnosis. We excluded studies where the study does not report on AF's impact on outcomes, AF populations where HF population is heterogeneous with HFpEF/HFmrEF predominance, and HF studies where HFrEF is a minority. A narrative review approach was adopted to enable a broad discussion on various aspects of HFrEF/AF.

PubMed search strategy

Search: heart failure atrial fibrillation Africa

('heart failure'[MeSH Terms] OR ('heart'[All Fields] AND 'failure'[All Fields]) OR 'heart failure'[All Fields]) AND ('atrial fibrillation'[MeSH Terms] OR ('atrial'[All Fields] AND 'fibrillation'[All Fields]) OR 'atrial fibrillation'[All Fields]) AND ('Africa'[MeSH Terms] OR 'Africa'[All Fields] OR 'Africa s'[All Fields] OR 'Africa's'[All Fields])

Translations

heart failure: 'heart failure'[MeSH Terms] OR ('heart'[All Fields] AND 'failure'[All Fields]) OR 'heart failure'[All Fields]
atrial fibrillation: 'atrial fibrillation'[MeSH Terms] OR ('atrial'[All Fields] AND 'fibrillation'[All Fields]) OR 'atrial fibrillation'[All Fields]
africa: 'Africa'[MeSH Terms] OR 'Africa'[All Fields] OR 'Africa's'[All Fields] OR 'Africa'[All Fields].

A PRISMA flow diagram is shown in *Appendix S1*.

Epidemiology

CVDs are a well-established cause of death in HICs. In the last 20 years, SSA has seen one of the sharpest increases in

Table 1 Studies reporting on the impact of atrial fibrillation on HF across Sub-Saharan Africa

Author	Sample size (n)	Country	Heart failure phenotype	Heart failure diagnosis	Study population	Mean age	Study design	Outcome	AF prevalence	Impact of AF on study outcome
Makubi (2014) ^{a,21}	427	Tanzania	ADHF LVEF = 41 (12)	Framingham criteria ²³	HF population	55	Prospective observational study	All-cause mortality	15.7%	The survival rate was significantly lower in patients with atrial fibrillation compared with those without 63% vs. 96% ($P < 0.001$).
Ogah (2014) ²⁴	262	Nigeria	ADHF LVEF = 39.7 (18)	Framingham criteria ²³	HF population	56.1	Prospective observational study	Hospital readmission	12.6%	The frequency of atrial fibrillation was higher in readmitted patients. This was not significant.
Malamba-Lez (2018) ^{a,25}	231	Democratic Republic of Congo	ADHF LVEF = 29 (15)	Framingham criteria ²³	HF population	56	Retrospective analysis	In-hospital mortality rate	20%	AF was significantly associated with in-hospital mortality rate ($P = 0.02$).
Mandi (2019) ^{a,16}	107	Burkina Faso	HF/AF (93.1%)	Framingham criteria ²³	AF population	66.6	Prospective observational study	All-cause mortality Rehospitalization Ischaemic stroke	HF/EF/AF: 59% HFmrEF/AF: 8.9% HFpEF/AF: 31.9%	Very high overall long-term mortality (40.59%). Patients with AF and idiopathic dilated cardiomyopathy were at higher risk of death than other patients (log-rank test = 11.88, $P < 0.001$).

Abbreviations: ADHF, acute decompensated heart failure; AF, atrial fibrillation; HFmrEF, heart failure with mid-range ejection fraction; HFpEF, heart failure with preserved ejection fraction; HFREF, heart failure with reduced ejection fraction; LVEF, left ventricular ejection fraction.

^aStudies where AF had a significant impact on study outcome.

CVD-related mortality, superseding that of infectious diseases and malignant neoplasms, combined in South Africa.^{29,49} This increase is evidenced by a growing burden of HF and AF risk factors. CVD-related deaths accounted for 17.7% of the total causes of death in South Africa, and among these were cerebrovascular diseases (5.1%), heart disease (5.1%), hypertensive heart disease (4.5%), and ischaemic heart disease (3.0%).⁴⁹ Furthermore, it is worth noting that in 2017, diabetes, an independent risk factor for HFREF and AF, was the second highest cause of death (5.9% of total deaths) in South Africa.⁴⁹ The co-occurrence of HF and AF, in HICs, is reported in more than 40% of HF patients.^{50,51} According to the few studies that have been conducted in SSA, the prevalence of AF in HF has been reported to range between 12.5% and 62.6%, with South Africa (56%) and Ivory Coast (62.6%) showing the highest prevalence^{11,21,22,24,52-54} (Table 1). This wide range reflects SSA's dearth of robust datasets. There are a number of possible explanations for this, including varying (outdated) definitions of HF and AF, and a paucity of research studies employing recent definitions and diagnostic criteria. Additionally, it is possible that estimated HFREF/AF incidence and prevalence underestimate the true burden in SSA due to under-reporting, insufficient AF screening, limited access to healthcare facilities, and geographical disparity in published data.

In SSA, the age and risk factor profile for AF differ compared with HIC, where most cases are recorded in the 8th decade of life.^{11,17,18,55} In SSA, AF presents throughout the lifespan, with most patients presenting before age 50.¹¹ On average, a South African cohort from the large Heart of Soweto Study was 8 years younger than AF patients in Cameroonian and Burkina Faso populations.^{16,17} Despite differences in the mean age of onset across SSA, it appears that the young age of onset is widespread.^{11,15,18,55-57}

It is worth noting that AF appears to be more common among females than in males.^{11,15,17,18,54,55} In the Heart of Soweto Study, women were more likely to be obese than their male counterparts (73% vs. 40%). The independent risk of developing AF with an increasing body mass index (BMI) is well documented in the literature. The study authors hypothesize that the preponderance of AF in women may be explained by the pattern of obesity in the cohort rather than gender per se. Other studies in SSA found a female predilection for AF, especially after excluding women with peripartum cardiomyopathy.^{15,54}

Significant sex-based differences persist against the backdrop of a heterogeneous aetiological HF spectrum. Women with non-valvular AF in South Africa are more likely to have hypertensive heart disease and less likely to have dilated cardiomyopathy. Men, on the other hand, frequently presented with left ventricular systolic dysfunction and larger cardiac dimensions.^{11,15}

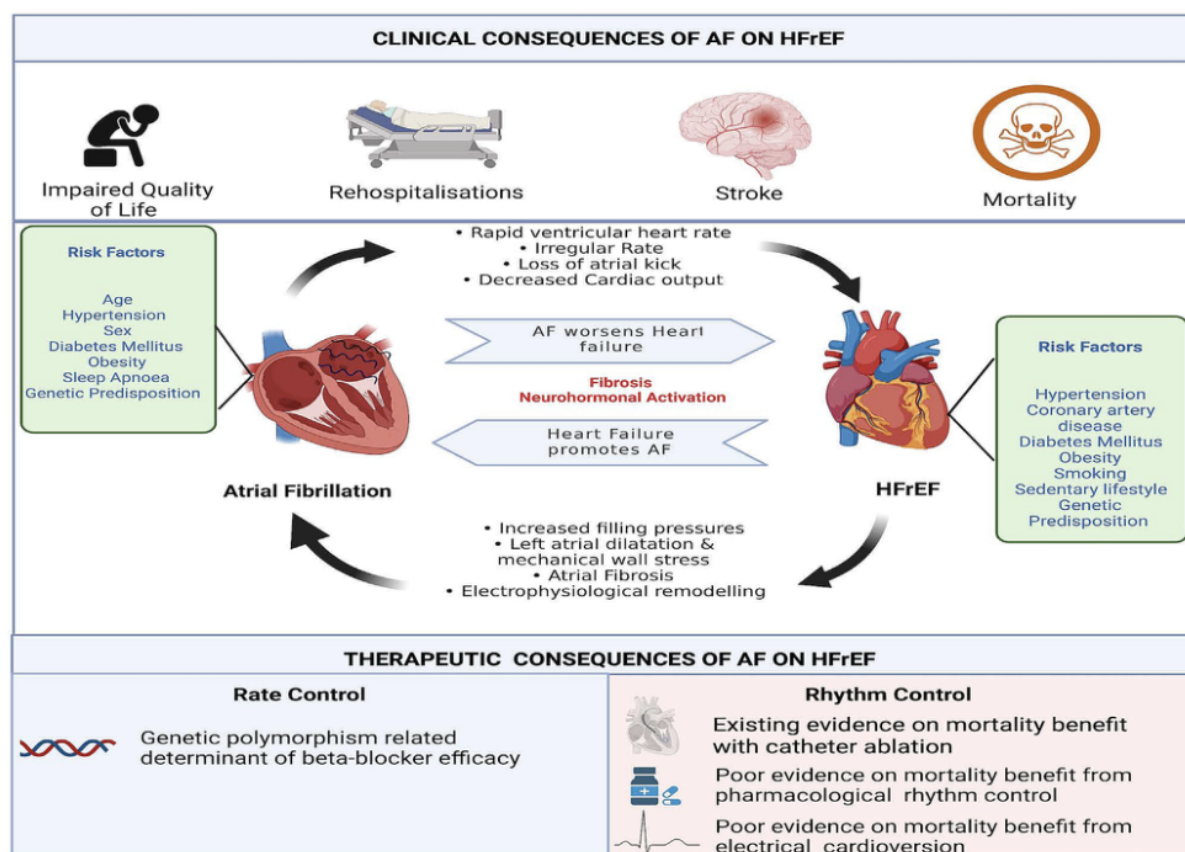
AF had the largest relative increase in CVD burden between 1990 and 2010 in SSA and the second highest

(106.4%) increase in disability-adjusted life-years (DALY) after peripheral vascular disease between 1990 and 2017.^{58,59} This increase was observed despite a decrease in RHD over the prescribed time period, suggesting that factors beyond RHD are likely to contribute to the rise in AF in SSA. Consistent with other systematic reviews, stroke emerged as the leading cause of CVD burden in SSA.⁵⁸ Given the established relationship between AF and stroke, it is worth speculating that the former could contribute to the latter's rise.

Independent risk factors for both AF and HFrEF are advanced age, hypertension, diabetes mellitus, and coronary artery disease (Figure 1). Hypertension, obstructive sleep apnoea (OSA), diabetes mellitus, and obesity are among some of the strongest predictors of AF in HIC.^{60,61} In SSA, hypertension is a common risk factor across all countries, whereas RHD varies by country. For example, RHD was one of the least significant risk factors for AF in Kenya, whereas hypertension, HF, and diabetes mellitus were the most prevalent predictors.⁵³ Coronary artery disease remains uncommon; however, emerging data and an increase in atherosclerosis

risk factors across SSA suggest that this may not remain at low prevalence rates indefinitely.²¹ Contemporary epidemiological studies hypothesize that the burden of AF in SSA will be double that of HIC by 2060.^{2,27,62} The rising burden of HFrEF, AF, and co-existing CVDs, many of which are risk factors for AF, is confirmed by the upward trend in HFrEF/AF-related hospitalizations in SSA.^{2,3,63} Characterizing the clinical profile of these patients in SSA is critical as it has therapeutic, socioeconomic, and policy implications. Such data may be used to advance strategies to mitigate modifiable risk factors. Reducing healthcare utilization by preventing AF recurrence and decreasing the AF burden has been shown to be extremely beneficial in HIC economies.^{64,65} A study conducted by Pathak *et al.* demonstrated that aggressive management of risk factors like OSA, weight, hypertension, and diabetes significantly reduced the AF burden after catheter ablation.^{64,65} This study highlights optimal modifiable risk factor management's potential benefits for upstream non-invasive therapy. Whether the same benefits are observed in the setting of HFrEF is unclear.

Figure 1 The effects of atrial fibrillation (AF) on heart failure with reduced ejection fraction (HFrEF). AF significantly impairs the quality of life and increases the risk of stroke, rehospitalization, and mortality in patients with HFrEF. AF is both a cause and a result of HFrEF with both entities sharing independent risk factors. Genetic polymorphisms in the beta-adrenergic receptor may modulate beta-blocker efficacy in patients with HFrEF/AF.



Pathophysiology

AF is caused by HFrEF, a process facilitated by adverse structural and electrical remodelling of the atria (*Figure 1*).^{10,66} Moreover, AF aggravates HF, eliciting an increase in HF symptoms, hospitalizations, and mortality (*Figure 1*).⁶⁷ AF is both a cause and a result of HFrEF, and in clinical practice, the index diagnosis of both conditions is not uncommon, making a causal link unclear.⁶⁸ A subgroup analysis of the temporal associations of AF and HF, from the Framingham Heart Study, found that AF occurring after HFrEF confers a worse prognosis than AF that occurs before HF.³⁰ Depending on whether AF or HF is the underlying condition, patient groups differ significantly in terms of outcomes and the required therapeutic approach. In SSA, there are currently no data on what proportion of HFrEF/AF patients are atrial tachycardia-induced HFrEF vs. AF secondary to HFrEF.

Most published literature on the pathophysiological mechanisms of HF/AF is based on HFrEF/AF in animal models. Left atrial enlargement and dysfunction have been implicated in the bidirectional relationship between HFrEF and AF (*Figure 1*). AF promotes HF through a persistently increased ventricular rate, sympathetic dysregulation, and loss of atrial contraction mediated by calcium 2^+ mishandling (*Figure 1*).⁶⁹ As a result, gross morphological and functional changes such as reduced systolic function, cardiac chamber dilatation, and greater wall tension result. In a matter of weeks, the full HF phenotype with reduced ejection fraction is established.⁶⁸ This is commonly referred to as tachyarrhythmia-induced HF.

In the setting of HFrEF, AF results from dysfunctional calcium handling in the atria secondary to atrial dilatation.¹⁰ The increase in left ventricular end-diastolic pressure results in elevated left atrial pressure. The elevated atrial pressure increases atrial mechanical wall stress, which subsequently alters calcium handling within the atria.⁷⁰ Atrial mechanical stress constitutes stretch and shear stress resulting from increased intra-atrial volume and pressure, respectively.⁷¹ Underlying these pathophysiological mechanisms of HFrEF are processes of inflammation and fibrosis (*Figure 1*). These pathophysiological mechanisms ultimately cause structural and electrophysiological remodelling of the atria, which sets the milieu for spontaneous depolarization, and hence arrhythmogenicity at the site of the pulmonary vein confluence with the left atria.⁷² These changes in left atrial structure and function have important prognostic implications in HFrEF, independent of recognized parameters such as left ventricular ejection fraction (LVEF).⁷³

In patients with ventricular dysfunction, left atrial contraction has an essential compensatory role to play in augmenting ejection force and stroke volume by increasing end-diastolic volume and pressure.⁷⁴ In the setting of AF, the atrial contraction component is lost and replaced by the ineffective quivering of the atria compounded by rapid ventricular activation and shortened diastolic filling time.⁷⁴ This

results in a 20–30% reduction in cardiac output.⁷⁴ This loss in cardiac output is particularly notable in the elderly population as they are highly dependent on the atrial pump function. Consequently, recurrent and worsened HF symptoms and syncopal episodes are observed.^{67,74} Therefore, restoration of atrial contraction can significantly stabilize the haemodynamics and symptoms in patients with AF and concomitant HFrEF.

Management of heart failure with reduced ejection fraction/atrial fibrillation

Irrespective of the temporal association of AF and HFrEF, managing both conditions concomitantly is a clinical challenge as these patients do not respond optimally to HFrEF pharmacological mortality-benefitting therapy. Optimal management of HFrEF constitutes a combination of neurohormonal antagonists such as angiotensin-converting enzyme inhibitors (ACE-I), angiotensin receptor blockers (ARBs), or a neprilysin inhibitor [angiotensin receptor neprilysin inhibitor (ARNI)], as well as mineralocorticoid receptor antagonists and beta-blockers.⁷⁵ These therapies have all been shown to provide significant mortality benefits and reduce rehospitalizations.^{76–78} With AF in the picture, this regimen includes a rate and rhythm control strategy and stroke prevention with oral anticoagulants (OAC).⁹ Following data from the PARADIGM-HF trial, the 2021 ESC Guidelines for diagnosing and treating acute and chronic HF now recommend using sacubitril/valsartan, an ARB and neprilysin inhibitor (ARNI), as first-line therapy in symptomatic HFrEF patients.⁷⁵ There is evidence that sacubitril/valsartan could lessen the AF burden in HFrEF/AF patients with non-permanent AF.⁷⁹ Human and animal model studies support this conclusion, implying that ARNIs may exert antiarrhythmic effects indirectly by preserving left atrial function and modulating atrial fibrosis in pressure-loaded subjects.⁸⁰ However, this antiarrhythmic effect was not observed in PARADIGM-HF and a recent meta-analysis. Rather, the incidence of de novo AF remained the same in the ARNI and enalapril arms.^{81,82} A sub-study analysis found that although HFrEF/AF patients do benefit from reduced mortality, the advantage is not as great as seen in sinus rhythm patients.⁸³ A prospective randomized control trial (RCT) is necessary to verify or refute these findings.

More recently, sodium-glucose cotransporter 2 (SGLT2) inhibitors, which are glucose-lowering agents, have evolved as HF therapy. They have recently been included as part of first-line HFrEF therapy for their significant cardiovascular death reduction, regardless of baseline HF medication use or diabetic status.⁸⁴ Data from the Dapagliflozin and Prevention of Adverse Outcomes in Heart Failure (DAPA-HF) revealed dapagliflozin demonstrated a significant reduction in

cardiovascular deaths and HF events in patients with symptomatic HFrEF.⁸⁴ The study comprised a small number of Black participants (5.1%) and did not include any patients from SSA. The reported prevalence of AF in this cohort was 38.6%.⁸⁴ Dapagliflozin may also reduce the incidence of AF, regardless of whether patients have a history of AF or pre-existing CVD or HF, according to new findings from a sub-analysis of the DECLARE-TIMI trial.⁸⁵

Most landmark HFrEF and AF RCTs enrolled <10% of Black patients and even lesser Africans. Therefore, caution should be exercised when generalizing findings from data in HICs. It is also worth noting that most African Americans are of West African ancestral descent and are not representative of the entire African population.⁸⁶ Even among Africans, there is a large degree of genetic and risk factor heterogeneity; thus, extrapolations from these large RCTs to Sub-Saharan Africans cannot be made definitively.

Rate control

Autonomic dysregulation is a hallmark of HF, independent of aetiology. The imbalance between the sympathetic nervous system (SNS) and parasympathetic nervous system (PNS) not only is reactive to HF, to try and maintain homeostasis acutely, but also contributes to the progression of chronic HFrEF.⁸⁷ These complex interactions' net effects are excessive SNS stimulation and a corresponding decrease in PNS tone.⁸⁷ Chronic SNS stimulation promotes myriad biological changes that lead to cardiac dysfunction. Among these changes are myocyte death and necrosis, desensitization of beta-adrenergic signalling, myocardial hypertrophy, and fibrosis. Beyond that, chronic SNS stimulation results in increased end-diastolic volume, left ventricular afterload, contractility, and heart rate.⁸⁸ Therefore, it seems implicit that the benefit of beta-blockade in HFrEF would translate into improved morbidity and mortality benefit. This mortality benefit was demonstrated in landmark trials for bisoprolol, carvedilol, and metoprolol; and was among the highest for these agents.^{89–91}

Beta-blockers and ACE-I/ARBs are used as first-line therapy to manage stable patients with HFrEF. Similarly, beta-blocker monotherapy is often the first-line rate-controlling agent used for the symptomatic management of patients with lone AF.^{9,92} Unlike sinus rhythm, slower heart rates are not associated with improved survival HFrEF/AF (Table 2).^{44,45,93} Carvedilol in the Carvedilol Prospective Randomized Cumulative Survival (COPERNICUS) trial, bisoprolol in Cardiac Insufficiency Bisoprolol Study (CIBIS)-II, and metoprolol succinate in the Metoprolol Controlled-Release Randomized Intervention Trial in Heart Failure (MERIT-HF) all showed a significant reduction in mortality, of 34–35%, when compared to placebo.^{89–91} On the other hand, Bucindolol in the Beta-Blocker Evaluation of Survival Trial (BEST) showed a

non-significant 13% risk reduction in mortality compared to placebo.⁹⁴ These findings suggest differences in outcomes by geographic region and race. Trials CIBIS-II, COPERNICUS, and MERIT-HF were conducted outside of the United States, and BEST recruited patients in the United States and Canada. Twenty-four per cent of patients in BEST were African American and demonstrated no mortality benefit from bucindolol [hazard ratio (HR) 1.17, $P = 0.27$], whereas non-Black patients did benefit [HR 0.82; confidence interval (CI): 0.70 to 0.96; $P = 0.01$].⁹⁴ Investigators of The Multicenter Oral Carvedilol in Heart Failure Assessment (MOCHA) trial reported a dose-dependent increase in LVEF and decreased mortality and rate of hospitalization in patients with HFrEF.⁹⁵ In another study, by McAlister *et al.*, a reduction in heart rate was significantly associated with mortality benefit, but this was not dose dependent.⁹⁶ The SHIFT trial evaluated the efficacy of ivabradine in HFrEF patients with a heart rate of ≥ 70 b.p.m. Ivabradine is a sinus node (I_f – Channel) inhibitor and hence does not work via the beta-adrenoreceptor. Ivabradine demonstrated a significant reduction in HF events but not in cardiovascular-related deaths.⁹⁷ These findings highlight the importance of heart rate reduction for improving clinical outcomes in HFrEF. There were no study participants from SSA in these trials.

A large meta-analysis done by Kotecha *et al.* concluded that beta-blockers do not provide any mortality benefit and reduction in hospitalizations in patients with HFrEF/AF.⁴⁵ The study analysed 10 landmark trials with a cumulative sample size of $n = 18\,254$ and an HFrEF/AF prevalence of 17% ($n = 3066$). The prevalence reported by Kotecha and colleagues is relatively low compared with what other studies have reported. This could be attributable to the fact that AF was documented only based on a single baseline ECG, predisposing potential misclassification errors in establishing a diagnosis of AF, especially for non-permanent AF.⁴⁵ All-cause mortality, cardiovascular death, and cardiovascular and HF-related hospitalizations were major outcomes. Patients with HFrEF/AF had no significant reduction in all primary and clinical composite outcomes compared to placebo.⁴⁵ However, this retrospective meta-analysis used relatively old trials and only 1 out of 10 trials differentiated between AF and atrial flutter. All other trials included in the analyses classified AF as both atrial flutter and fibrillation.⁴⁵ Suppose beta-blockers do not provide mortality benefits. In that case, it is unclear why the positive effects of beta-blockers, particularly on myocardial remodelling, do not correspond to prognostic benefits in patients with AF. This is an anomaly that requires further investigation.

On the contrary, more recent studies are at variance with the findings by Kotecha *et al.*, reporting a 25–29% reduction in mortality with the use of beta-blockers^{46–48} (Table 2). Cadrin-Tourigny and colleagues are commended for their thorough attempt to characterize AF in their cohort. In their study, AF was characterized according to baseline rhythm

Table 2 Studies on the outcomes of rate and rhythm control strategies in heart failure with reduced ejection fraction/atrial fibrillation

Intervention	Trial name/study type	Sample size	Study population	Effect in HFrEF/SR	Effect in HFrEF/AF	Reference
Antiarrhythmic drug therapy (dofetilide) vs. placebo	DIAMOND-CHF	N = 1518	- Patients with new or worsening HFrEF - NYHA class III or IV or paroxysmal nocturnal dyspnoea - Danish population	-	No effect on mortality.	Torp-Pedersen et al. (1999) ³²
Antiarrhythmic drug therapy vs. pharmacological rate control therapy	AF-CHF	N = 1376	- LVEF $\leq$ 35% - NYHA class II to IV - Canadian, North and South American, European, and Israeli population	-	HR 1.06 (0.86–1.30) in the rhythm control group. There was no difference with respect to primary (death from cardiovascular causes) and secondary outcomes.	Roy et al. (2008) ³³
Catheter ablation (PVI) vs. AV node ablation and with biventricular pacing (CRT)	PABA-CHF	N = 82	- Symptomatic, AAD-resistant AF - LVEF < 40% - NYHA class II to IV	-	Catheter ablation was superior with respect to LVEF, exercise capacity, and symptoms.	Khan et al. (2008) ³⁴
Catheter ablation vs. pharmacological rate control		N = 41	- Persistent AF - LVEF < 40% - NYHA class II to IV		No difference in MRI measured LVEF.	MacDonald et al. (2011) ³⁵
Catheter ablation vs. pharmacological rate control therapy	ARC-HF	N = 52	- Persistent AF - LVEF < 35% - NYHA class II to IV - British population	-	Catheter ablation was superior with respect to exercise capacity. Catheter ablation showed a non-significant trend towards LVEF improvement [mean difference, +5.6% (95% CI, -0.1 to +11.3); $P = 0.055$]. Catheter ablation was superior with respect to LVEF.	Jones et al. (2013) ³⁶
Catheter ablation vs. pharmacological rate control therapy	CAMTAF	N = 50	- Persistent AF - NYHA class II to IV - LVEF < 50%	-	Catheter ablation was superior with respect to LVEF. Catheter ablation was superior with respect to exercise capacity, BNP quality of life, and symptoms compared with rate control.	Hunter et al. (2014) ³⁷
Catheter ablation vs. amiodarone	AATAC	N = 203	- Persistent AF - LVEF $\leq$ 40% - NYHA class II or III - Dual-chamber ICD or CRT	-	Catheter ablation was superior with respect to freedom from AF.	Di Biase et al. (2016) ³⁸
Catheter ablation vs. rate control	CAMERA-MRI	N = 68	- Persistent AF - LVEF $\leq$ 45%		Catheter ablation was superior with respect to LVEF.	Prabhu et al. (2017) ³⁹
Catheter ablation vs. basic medical therapy	CASTLE-AF	N = 363	- Symptomatic, AAD-resistant AF - LVEF < 35%	-	Catheter ablation was superior with respect to composite primary endpoint	Marrouche et al. (2018) ⁴⁰

(Continues)

Table 2 (continued)

Intervention	Trial name/study type	Sample size	Study population	Effect in HFrEF/SR	Effect in HFrEF/AF	Reference
Catheter ablation vs. basic medical therapy	AMICA	N = 140	• NYHA class II or III • Dual-chamber ICD or CRT with home monitoring • Persistent AF • LVEF ≤ 35% • German, Hungarian, and Spanish participants	(death or hospitalization for worsening heart failure).	Catheter ablation was not superior to BMT with respect to LVEF.	Kuck <i>et al.</i> (2019) ⁴¹
AV node ablation + CRT vs. rate control	APAF-CRT	N = 102	• Permanent AF • N arrow QRS (≤110 ms) • ≥1 HF hospitalization in previous year		AV node ablation was superior with respect to composite outcome (death caused by HF, HF hospitalization, or worsening HF).	Brignole <i>et al.</i> (2018) ⁴²
Catheter ablation vs. medical rate or rhythm control	CABANA	N = 2204	• ≥2 paroxysmal or 1 persistent AF episodes in the last 6 months • Age ≥ 65 years • Age < 65 years + ≥1 risk factor for stroke		Catheter ablation did not reduce composite primary endpoint.	Packer <i>et al.</i> (2019) ⁴³
Rate control	Meta-analysis	N = 8680	• HFrEF/SR vs. HFrEF/AF	HR 0.63 (0.54–0.73)	HR 0.86 (0.66–1.13) (No mortality benefit)	Rienstra <i>et al.</i> (2013) ⁴⁴
Rate control	Meta-analysis	N = 18 254	• HFrEF/SR vs. HFrEF/AF	HR 0.73 (0.67–0.80)	HR 0.97 (0.83–1.14) (No mortality benefit)	Kotecha <i>et al.</i> (2014) ⁴⁵
Rate control	HF registry	N = 18 858	• HFrEF/SR vs. HFrEF/AF • Swedish participants	HR 0.77 (0.63–0.94)	HR 0.71 (0.61–0.84) (29% reduction in mortality)	Li <i>et al.</i> (2015) ⁴⁶
Rate control	AF registry	N = 39 741	• HFrEF/AF • Danish population	-	HR 0.75 (0.71–0.79) (25% reduction in mortality)	Nielsen <i>et al.</i> (2016) ⁴⁷
Rate control	AF-CHF sub-analysis, propensity score matching	N = 1376	• HFrEF/AF • North, South American and Israeli population	-	HR 0.72 (0.55–0.95) (28% reduction in mortality)	Cadrin-Tourigny <i>et al.</i> (2017) ⁴⁸

Abbreviations: AAD, antiarrhythmic drug; AF, atrial fibrillation; AV, atrioventricular; BMT, bone marrow transplantation; CI, confidence interval; CRT, cardiac resynchronization therapy; HFrEF, heart failure with reduced ejection fraction; HR, hazard ratio; ICD, implantable cardioverter defibrillator; LVEF, left ventricular ejection fraction; MRI, magnetic resonance imaging; NYHA, New York Heart Association; RCT, randomized control trial; SR, sinus rhythm.

and pattern, with an ECG taken in each participant every 4 months.⁴⁸ Furthermore, findings from a large-scale ($n = 18\,858$) prospective study conducted in Sweden demonstrated that a heart rate > 100 b.p.m. was associated with an increase in mortality.⁴⁶ These contrasting findings further highlight geographical and possible genetic and environmental factors that may influence the outcomes of beta-blocker therapy in HFrEF/AF.

Although there is no consensus on the therapeutic benefit of beta-blockers in patients with HFrEF/AF, their use did not predispose patients to death and hospital admissions, proving their use to be at least safe.⁴⁵ Using beta-blockers in HFrEF reduced the incidence of AF in sinus rhythm patients. This finding may be clinically relevant and an important component of the benefit seen in sinus rhythm.

Arg389Gly polymorphism

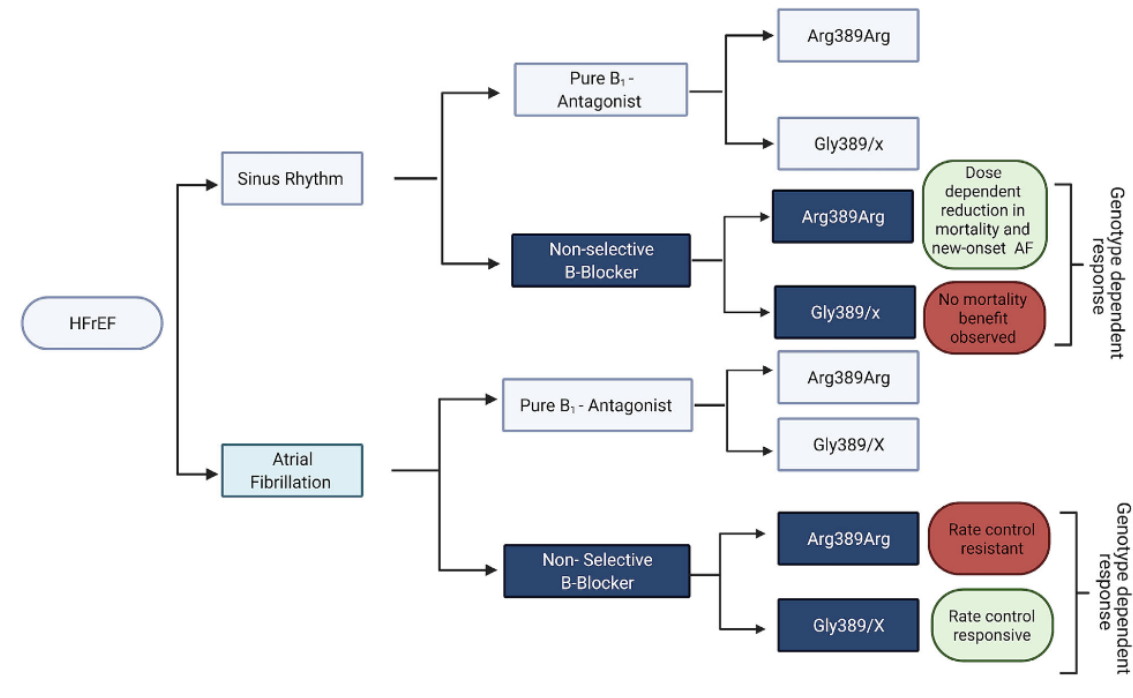
Given the heterogeneity of HF and the burden of idiopathic dilated cardiomyopathy in SSA, strides have been made to investigate HF's genetic undertones.^{4,98} Of note, the role of the β_1 -adrenoceptor (ADRB1) polymorphisms has been extensively

elucidated in hypertension and HF in the broader population context.^{4,99} Only recently, the response to rate control has been considered genotype dependent.¹⁰⁰ However, no studies have been done in our setting in this regard.

Data show that adverse drug responses, including failure to respond, may be attributable to polymorphisms in the ADRB1 gene. Identifying patients on this basis may be helpful in managing patients with HFrEF and HFrEF/AF. It has been hypothesized that the arginine389glycine (Arg389Gly) polymorphism modulates such differential responses to beta-blockers in HFrEF patients from HIC.^{101–103} These effects were also recently shown to be dose dependent in specific subgroups (Figure 2). Its significance in SSA HFrEF and HFrEF/AF patients remains undetermined but demonstrates the importance of precision-based medicine and its potential therapeutic utility in our setting.

The ADRB1 is a G-protein coupled receptor (GPCR) expressed on cell surfaces. It is activated by catecholamine binding, which leads to increased intracellular cyclic-adenosine monophosphate (cAMP) activity and a resultant increase in inotropic and chronotropic effects on the

Figure 2 Genotype-dependent efficacy of beta-blockers in heart failure with reduced ejection fraction/atrial fibrillation (HFrEF/AF). Grey: HFrEF pathways where the efficacy of beta-blockers are genotype independent. Navy blue: HFrEF pathways where the efficacy of beta-blockers are genotype dependent. In sinus rhythm patients, there is dose-dependent mortality benefit and reduction in new-onset AF in patients with the gain of function Arg389Arg variant. In patients with HFrEF/AF, the Arg389Arg variant was unresponsive to the rate-lowering effects of non-selective beta-blockers.



myocardium.^{104–106} Data from *in vitro* studies show that Gly389 is a 'loss of function variant' with less constitutive activity and a low affinity for norepinephrine. When bound to an agonist, the receptor elicits reduced cAMP production, thereby attenuating the cAMP-dependent beta-adrenergic signal transduction cascade.^{107,108} The gain of function, Arg389, mutant, on the other hand, has a higher affinity for norepinephrine and thus a higher potential for signal transduction.^{109,110} This receptor variant is also said to be more constitutively active (i.e. transducing signal/active in the absence of bound ligand).¹¹⁰

Heart failure with reduced ejection fraction and ADRB1 genotype

Available data from the BEST trial show a dose-dependent decrease in all-cause mortality and HF hospitalization in a select group of HFrEF patients treated with low-dose bucindolol, a non-selective beta-blocker, compared to placebo.¹⁰² Patients on low-dose bucindolol (0–25 mg daily) with the homozygous Arg389 variant had a significantly greater event rate, when corrected for common risk factors, compared to placebo ($P = 0.006$). When up-titrated to a high dose of bucindolol (>25 mg daily), Arg389 homozygotes had a 60% reduction in all-cause mortality ($P = 0.0002$).¹⁰³ This benefit was not observed in patients carrying the Gly389 allele.¹⁰² Another sub-study of the BEST trial showed that HFrEF patients with the Arg389Arg genotype had a 74% reduction in new-onset AF when treated with bucindolol [HR 0.26 (95% CI: 0.12–0.57)] and no effect observed in the Gly389 patients¹¹¹ (Figure 2). These findings support a pharmacogenetic role for the polymorphism in managing HFrEF patients, directly impacting clinical outcomes. The effects of high-dose bucindolol in HFrEF/AF are proposed to be a function of selective inverse agonism, which attenuates the receptor's constitutive activity, bucindolol's sympatholytic properties, which impact the gain of function mutant receptor, and/or its norepinephrine-lowering properties impacting the high-affinity ADRB1 Arg389 receptors.^{112,113}

Heart failure with reduced ejection fraction/atrial fibrillation and genotype

In Arg389 homozygous HFrEF/AF patients, bucindolol demonstrated a borderline significant reduction in all-cause mortality/HF hospitalization and cardiovascular mortality/cardiovascular hospitalization.¹⁰² A pharmacogenetic sub-study of the prospective, double-blind, randomized CIBIS-ELD (Cardiac Insufficiency Bisoprolol Study in Elderly study) trial ($n = 528$) demonstrated that the heart rate-lowering effects of carvedilol, a non-selective beta-blocker, in patients with HFrEF/AF were also dependent on the Arg389Gly polymorphism^{53,61} (Figure 2). The CIBIS-ELD trial assessed bisoprolol's and carvedilol's tolerability and feasibility in South-Eastern European patients with HFrEF/AF and HFrEF alone (sinus rhythm).¹¹⁴ Carvedilol appeared to have a smaller heart

rate-lowering effect in HFrEF/AF patients than in sinus rhythm.¹⁰⁰ Patients with HFrEF/AF who possessed the 'gain of function' homozygous Arg389 allele were resistant to rate control using carvedilol vs. patients with a Gly389 allele over a ~12 week period of dose up-titration (maximum daily dose = 50 mg)¹⁰⁰ (Figure 2). There was 12 b.p.m. difference between homozygous Arg389 HFrEF/AF patients compared with those with the 'loss of function' variant. This genotype-dependent effect was only observed during the chronic phase of treatment with carvedilol in HFrEF/AF patients.¹⁰⁰ Unlike the BEST trial, this study is limited by its lack of outcomes data, and therefore, findings cannot be used to infer prognosis.

It is unclear what the underlying molecular mechanisms explaining the interactions between the genotype, beta-blocker administered, dose, and heart rhythm are. However, some authors have attempted to hypothesize/attribute carvedilol's effect to its high binding affinity as an inverse agonist and suggest that it could modulate the phenotype of the atrioventricular (AV) node and/or atrial myocardium in Arg389 homozygotes.¹⁰⁰ Carvedilol's binding to the ADRB1 has negative intrinsic activity by way of decreasing cAMP-dependent signalling pathways and activating arrestin-dependent, cAMP-independent, pathways, for example, the activation of epidermal growth factor receptors. Epidermal growth factor has been shown to confer cardioprotection against the deleterious effects of catecholamines in murine models.¹¹⁵ The authors hypothesize that the cAMP-independent effects may be modulated by the Arg389Gly polymorphism.¹⁰⁰

The frequency distribution of the Arg389Gly genetic variants differs significantly between European and non-European populations.¹¹⁶ There is a paucity of data on this polymorphism in SSA. Despite compelling evidence to support differential therapeutic outcomes based on geographical region and genotype, little is known about the clinical relevance of this polymorphism and which genetic variant is dominant in our setting. To date, only one study has looked for this polymorphism in an African population and employed very stringent selection criteria excluding patients on beta-blocker therapy and patients with arrhythmias.⁹⁸

Rhythm control

Rhythm control has been and will remain a cornerstone of treatment in patients with AF. Rhythm control is accomplished by antiarrhythmic drugs (AADs), electrical cardioversion, or catheter ablation (cryoablation or radiofrequency). Depending on the approach used, the advantages of rhythm management include increased quality of life, decreased rehospitalizations, and, more recently, a reduction in mortality (Table 2).⁴⁰

Compared with pharmacological rate control, AADs had no clear advantage with regard to mortality, rehospitalization, and stroke reduction in patients with AF (Table 2). This is largely driven by the adverse effects of AADs, which marred the survival benefit.¹¹⁷ There has been a shift in the therapeutic paradigm of AF from a 'one size fits all' approach to a recent, AF-classification (temporal heterogeneity classification) and symptom-based approach. Despite their lack of superiority to rate control, the use of AADs to restore sinus rhythm is indicated in symptomatic AF patients.⁹ Patients with HFrEF/AF of SSA origin are non-existent in large clinical trials evaluating the efficacy and safety of AADs. Thus, findings from these data cannot be easily generalized to an SSA context. However, anecdotal evidence suggests similar trends to those observed in studies conducted in HICs.

The use of AADs in patients with HF and concomitant AF can be especially challenging because of their suboptimal efficacy and dangerous side effects profile.¹¹⁸ Amiodarone and dofetilide are the only two antiarrhythmics that have demonstrated safety in patients with HFrEF but are not without side effects.^{32,33} The Atrial Fibrillation and Congestive Heart Failure (AF-CHF) trial and the Danish Investigators of Arrhythmia and Mortality on Dofetilide in Congestive Heart Failure (DIAMOND-CHF) trial cast scepticism on the mortality benefit of sinus-rhythm maintenance vs. rate control in patients with co-existing HFrEF and AF.^{32,33} The AAD of choice was predominantly amiodarone and exclusively dofetilide in the AF-CHF and DIAMOND-CHF trials, respectively. These trials concluded that neither drug was associated with lower mortality in patients with co-existing AF and HF despite lowering the AF burden. Dofetilide had a greater rate of reduction in AF burden (81%); however, it is not available in SSA. Amiodarone has been documented to facilitate successful electro-cardioversion in patients with persistent AF regardless of their ejection fraction and is currently the most widely used AAD in SSA.^{119,120}

Results from the South African (SAFIR-RSA) survey demonstrated that the majority (74%) of patients were on rate control therapy, in the form of beta-blockers, and the remainder rhythm control therapy primarily with class III agents. In the SAFIR-RSA survey, hospitalizations for HF occurred more frequently in patients on AADs.¹²⁰ The study participants were not stratified according to HF modality. Thus, it is impossible to extrapolate what proportion of these HF admissions were attributable to HFrEF/AF patients. It is also unclear whether these HF-related hospitalizations result from the AADs or the AF itself. Furthermore, this study was done in the South African private-health sector, not representative of the overall SSA population.

It is widely known that AF in the setting of HFrEF is a marker of poorer outcomes.⁸³ Furthermore, data from the GENETIC-AF study have shown that longer durations of AF and HF attenuate treatment efficacy.¹²¹ Similarly, rhythm control improved outcomes in HFrEF/AF when initiated

within 1 year of AF diagnosis.¹²² Hence, there is a lot of debate around early and aggressive rhythm control of these patients in order to improve outcomes. In recognition of this, recent National Heart Foundation of Australia and the Cardiac Society of Australia and New Zealand's Clinical Guidelines for the Diagnosis and Management of Atrial Fibrillation (2018) and the ESC 2020 guidelines recommend catheter ablation for HFrEF/AF as first-line therapy.^{75,123,124} This is in contrast to previous guidelines that recommended catheter ablation for AF patients refractory to AADs.

The potential benefit of catheter ablation in patients with HFrEF/AF has not been fully elucidated until recently.⁴⁰ Several observational studies have illustrated that catheter ablation improves the quality of life, functional class, rehospitalizations, and LVEF by 8.5%.^{125–128} However, this improvement in LVEF initially did not translate into any mortality benefits.¹²⁷ Results from the CASTLE-AF trial and a meta-analysis by Alturki *et al.* (2019) have shown that catheter ablation, as an aggressive rhythm control strategy, does indeed offer mortality benefit in AF patients with co-existing HFrEF compared to the standard pharmacotherapy (rate control or rhythm control) (Table 2).^{40,129} A significant reduction in cardiovascular-related deaths drove this mortality benefit. The investigators of CASTLE-AF did not mandate a specific strategy for the reason that previous studies show neither rate control nor rhythm control are superior to one another.⁴⁰

The benefit of catheter ablation in this cohort of interest may not be homogenous and there may be underlying genetic and racial determinants of procedural success. A small study demonstrated that the PITX2 single nucleotide polymorphism, known to predispose to AF, predicts AF recurrence in European patients undergoing catheter ablation.^{130,131} However, the same association was not observed in Korean patients.¹³² These findings warrant large cross-population studies investigating the genetic determinants of AF and the response to therapeutic interventions across different populations, inclusive of SSA.

Four in five AF patients in SSA are managed via a rate control strategy and/or via pharmacological rhythm control/AADs.^{28,133} This demonstrates that evidenced-based AF rhythm control strategies with catheter ablation are not widely available in SSA. It is unlikely that catheter ablation will soon become routine practice in SSA, attributable to a lack of infrastructure and various barriers to access. South Africa is one of the very few countries in SSA performing catheter ablation with a slowly growing repository of data with a preponderance to the private sector. Awareness and understanding of AF's prognostic effect on HFrEF in SSA is critical. We do not want SSA to remain behind in the diaspora, as successful management of HFrEF/AF in our young cohort will be time dependent. The aforementioned warrants more inter-Africa initiatives/collaborations to conduct large-scale studies across SSA.

Conclusions

The future risk of CVDs, predominantly driven by the rising prevalence of cardiovascular risk factors, is a growing public health concern in SSA. The incidence of previously rare forms of CVD, such as coronary artery disease, will increase, in concert with historically prevalent forms of disease, such as RHD, that are yet to be optimally eradicated. In SSA, where infrastructure and resources remain a major barrier to definitive treatment of both HFrEF and AF, the success of strategies designed to curtail the evolving and increasing burden of HFrEF/AF will be dependent upon accurate and up-to-date epidemiological data and well-defined RCTs evaluating the efficiency and efficacy of pharmacological and non-pharmacological interventions.

There are evident huge gaps in the SSA diaspora as it pertains to HFrEF/AF. There seems to be a slowly growing knowledge base in specific geographical locations in SSA like South Africa, Botswana, Ghana, and Nigeria. However, these data do not reflect SSA's overall status quo and demographics. There also exists wide heterogeneity across studies conducted in SSA with regard to the diagnosis of HF, and few studies report on the modalities of HF. To date, there is not a single systematic review published on HFrEF/AF in SSA, and most extrapolations about this subpopulation are made from HF or AF studies. Contrary to extensive data available in HIC on the types of AF prevalent among HFrEF patients, very little is known in our setting. Prioritized research areas to address knowledge gaps should encompass the following: pathophysiology, risk factor profiles and clinical phenotypes of HFrEF/AF, genetic predictors of disease onset, response to therapy, and factors influencing therapeutic outcomes in SSA.

References

1. Damasceno A, Mayosi BM, Sani M, Ogah OS, Mondo C, Ojji D, Dzudie A, Kouam CK, Suliman A, Schrueder N, Yonga G, Ba SA, Maru F, Alemayehu B, Edwards C, Davison BA, Cotter G, Sliwa K. The causes, treatment, and outcome of acute heart failure in 1006 Africans from 9 countries. *Arch Intern Med*. 2012; **172**: 1386–1394.
2. Keates AK, Mocumbi AO, Ntsekhe M, Sliwa K, Stewart S. Cardiovascular disease in Africa: epidemiological profile and challenges. *Nat Rev Cardiol*. 2017; **14**: 273–293.
3. Stewart S, Wilkinson D, Hansen C, Vaghela V, Mvungi R, McMurray J, Sliwa K. Predominance of heart failure in the Heart of Soweto Study cohort. *Circulation*. 2008; **118**: 2360–2367.
4. Ajayi AA, Sofowora GG, Ladipo GO. Explaining heart failure hypermortality in Sub Saharan Africa: global genomic and environmental contribution review. *J Natl Med Assoc*. 2020; **112**: 141–157.
5. Sliwa K, Mayosi BM. Recent advances in the epidemiology, pathogenesis and prognosis of acute heart failure and cardiomyopathy in Africa. *Heart*. 2013; **99**: 1317–1322.
6. Bonsu KO, Owusu IK, Buabeng KO, Reidpath DD, Kadirvelu A. Clinical characteristics and prognosis of patients admitted for heart failure: a 5-year retrospective study of African patients. *Int J Cardiol*. 2017; **238**: 128–135.
7. Mandi DG, Bamouni J, Yameogo RA, Naibe DT, Kabore E, Kambire Y, et al. Spectrum of heart failure in sub-Saharan Africa: data from a tertiary hospital-based registry in the eastern center of Burkina Faso. *Pan Afr Med J*. 2020; **36**: 30.
8. Agbor VN, Essouma M, Ntusi NAB, Flore U, Joel J, Jacques J. Heart failure in sub-Saharan Africa: a contemporaneous systematic review and meta-analysis. *Int J Cardiol*. 2018; **257**: 207–215.
9. Hindricks G, Potpara T, Dagres N, Arbelo E, Bax JJ, Blomström-Lundqvist C, Boriani G, Castella M, Dan G-A, Dilaveris PE, Fauchier L, Filippatos G, Kalman JM, La Meir M, Lane DA, Lebeau J-P, Lettino M, Lip GYH, Pinto FJ, Thomas GN, Valgimigli M, Van Gelder IC, Van Putte BP, Watkins CL. ESC scientific document group, 2020 ESC Guidelines for the diagnosis and management of atrial fibrillation developed in collaboration with the European Association for Cardio-Thoracic Surgery (EACTS): the task force for the diagnosis and management of atrial fibrillation of the

Acknowledgements

The authors thank Dr Dineo Mpanya for her expertise and assistance in writing the manuscript. Mpanya provided technical editing, language editing, and help in reviewing the manuscript for grammar and syntax.

Conflict of interest

None declared.

Funding

The work reported herein was made possible through funding by the South African Medical Research Council (SAMRC) through its Division of Research Capacity Development under the SAMRC Institutional Clinician Researcher Programme. The content hereof is the sole responsibility of the authors and does not necessarily represent the official views of the SAMRC.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Supporting Information.

- European Society of Cardiology (ESC) Developed with the special contribution of the European Heart Rhythm Association (EHRA) of the ESC. *Eur Heart J*. 2021; **42**: 373–498.
10. Denham NC, Pearman CM, Caldwell JL, Madders GWP, Eisner DA, Trafford AW, Dibb KM. Calcium in the pathophysiology of atrial fibrillation and heart failure. *Front Physiol*. 2018; **9**: 1380.
 11. Sliwa K, Carrington MJ, Klug E, Opie L, Lee G, Ball J, Stewart S. Predisposing factors and incidence of newly diagnosed atrial fibrillation in an urban African community: insights from the Heart of Soweto Study. *Heart*. 2010; **96**: 1878–1882.
 12. Okello E, Wanzhu Z, Musoke C, Twalib A, Kakande B, Lwabi P, Wilson NB, Mondo CK, Odoi-Adome R, Freers J. Cardiovascular complications in newly diagnosed rheumatic heart disease patients at Mulago Hospital, Uganda: cardiovascular topics. *Cardiovasc J Afr*. 2013; **24**: 76–79.
 13. Zühlke L, Engel ME, Karthikeyan G, Rangarajan S, Mackie P, Cupido B, Mauff K, Islam S, Joachim A, Daniels R, Francis V, Ogendo S, Gitura B, Mondo C, Okello E, Lwabi P, al-Kebsi MM, Hugo-Hamman C, Sheta SS, Haileamlak A, Daniel W, Goshu DY, Abdissa SG, Desta AG, Shasho BA, Begna DM, ElSayed A, Ibrahim AS, Musuku J, Bode-Thomas F, Okeahialam BN, Ige O, Sutton C, Misra R, Abul Fadl A, Kennedy N, Damasceno A, Sani M, Ogah OS, Olunuga T, Elhassan HHM, Mocumbi AO, Adeoye AM, Mntla P, Ojji D, Mucumbitsi J, Teo K, Yusuf S, Mayosi BM. Characteristics, complications, and gaps in evidence-based interventions in rheumatic heart disease: the Global Rheumatic Heart Disease Registry (the REMEDY study). *Eur Heart J*. 2015; **36**: 1115–1122.
 14. Yadeta D, Semeredin N, Mekonnen GE. Prevalence and predictors of atrial fibrillation and its embolic complications in patients with rheumatic heart disease at Tikur Anbessa specialized hospital, Addis Ababa, Ethiopia. *Ethiop J Health Dev*. 2019; **33**: 12–16.
 15. Sani MU, Davison BA, Cotter G, Mayosi BM, Edwards C, Ogah OS, Damasceno A, Ojji DB, Dzudie A, Mondo C, Kouam CK, Suliman A, Yonga G, Abdou Ba S, Maru F, Alemayehu B, Sliwa K. Prevalence, clinical characteristics and outcomes of valvular atrial fibrillation in a cohort of African patients with acute heart failure: insights from the THESUS-HF registry. *Cardiovasc J Afr*. 2018; **29**: 139–145.
 16. Mandi DG, Bamouni J, Naïbé DT, Yaméogo RA, Kaboré E, Kambiré Y, Kologo KJ, Millogo GRC, Yaméogo NV, Thiam Tall A, Zabsonré P. Epidemiology and long-term prognosis of atrial fibrillation in rural African patients. *Egypt Heart J*. 2019; **71**: 6.
 17. Ntep-Gweth M, Zimmermann M, Meilitz A, Kingue S, Ndobu P, Urban P, Bloch A. Atrial fibrillation in Africa: clinical characteristics, prognosis, and adherence to guidelines in Cameroon. *Europace*. 2010; **12**: 482–487.
 18. Mwita JC, Ocampo C, Molefe-Baikai OJ, Goepamang M, Botsile E, Tshikuka JG. Characteristics and 12-month outcome of patients with atrial fibrillation at a tertiary hospital in Botswana. *Cardiovasc J Afr*. 2019; **30**: 168–173.
 19. Dzudie A, Hongieh Abanda M, Nkoke C, Barche B, Damasceno A, Edwards C, Davison B, Cotter G, Sliwa K, THEUS-HF investigators, Damasceno A, Sani M, Ojji D, Suliman A, Yonga G, Ogah OS, Mondo C, Dzudie A, Charles KK, Abdou Ba S, Maru F, Alemayehu B, Edwards C, Davison BA, Cotter G, Sliwa, Sliwa K. Clinical characteristics and outcomes of black African heart failure patients with preserved, mid-range, and reduced ejection fraction: a post hoc analysis of the THESUS-HF registry. *ESC Heart Fail*. 2021; **8**: 238–249.
 20. Chen Y, Alphonse E, Mujuni E, Kisigo GAGA, Kingery JRJR, Makubi A, et al. Atrial fibrillation and mortality in outpatients with heart failure in Tanzania: a prospective cohort study. *BMJ Open*. 2022; **12**: 1–9.
 21. Makubi A, Hage C, Lwakatare J, Kisenge P, Makani J, Ryden L, et al. Contemporary aetiology, clinical characteristics and prognosis of adults with heart failure observed in a tertiary hospital in Tanzania: the prospective Tanzania Heart Failure (TaHeF) study. *Heart*. 2014; **100**: 1235–1241.
 22. Familoni OB, Olunuga TO, Olufemi BW. A clinical study of pattern and factors affecting outcome in Nigerian patients with advanced heart failure. *Cardiovasc J Afr*. 2007; **18**: 308–311.
 23. McKee PA, Castelli WP, McNamara PM, Kannel WB. The natural history of congestive heart failure: the Framingham study. *N Engl J Med*. 1971; **285**: 1441–1446.
 24. Ogah OS, Stewart S, Falase AO, Akinyemi JO, Adegbite GD, Alabi AA, Ajani AA, Adesina JO, Durodola A, Sliwa K. Predictors of rehospitalization in patients admitted with heart failure in Abeokuta, Nigeria: data from the Abeokuta heart failure registry. *J Card Fail*. 2014; **20**: 833–840.
 25. Malamba-Lez D, Ngoy-Nkulu D, Steels P, Tshala-Katumbay D, Mullens W. Heart failure etiologies and challenges to care in the developing world: an observational study in the Democratic Republic of Congo. *J Card Fail*. 2018; **24**: 854–859.
 26. Dries DL, Exner DV, Gersh BJ, Hil DP, Domanski MJ, Waclawiw MA, et al. Atrial fibrillation is associated with an increased risk for mortality and heart failure progression in patients with asymptomatic and symptomatic left ventricular systolic dysfunction: a retrospective analysis of the SOLVD trials. *Int J Cardiol*. 1998; **32**: 695–703.
 27. Chugh SS, Havmoeller R, Narayanan K, Singh D, Rienstra M, Benjamin EJ, Gillum RF, Kim YH, McAnulty JH Jr, Zheng ZJ, Forouzanfar MH, Naghavi M, Mensah GA, Ezzati M, Murray CJL. Worldwide epidemiology of atrial fibrillation: a global burden of disease 2010 study. *Circulation*. 2014; **129**: 837–847.
 28. Noubiap JJ, Bigna JJ, Agbor VN, Mbanga C, Ndoadougue AL, Nkeck JR, Kamguia A, Nyaga UF, Ntusi NAB. Meta-analysis of atrial fibrillation in patients with various cardiomyopathies. *Am J Cardiol*. 2019; **124**: 262–269.
 29. Nyaga UF, Bigna JJ, Agbor VN, Essouma M, Ntusi NAB, Noubiap JJ. Data on the epidemiology of heart failure in Sub-Saharan Africa. *Data Brief*. 2018; **17**: 1218–1239.
 30. Santhanakrishnan R, Wang N, Larson MG, Magnani JW, McManus DD, Lubitz SA, et al. Atrial fibrillation begets heart failure and vice versa. *Circulation*. 2016; **133**: 484–492.
 31. Horodinschi RN, Diaconu CC. Comorbidities associated with one-year mortality in patients with atrial fibrillation and heart failure. *Healthcare*. 2021; **9**: 830.
 32. Torp-Pedersen C, Møller M, Bloch-Thomsen PE, Køber L, Sandøe E, Egstrup K, Agner E, Carlsen J, Videbæk J, Marchant B, Camm AJ. Dofetilide in patients with congestive heart failure and left ventricular dysfunction. *N Engl J Med*. 1999; **341**: 857–865.
 33. Roy D, Talajic M, Nattel S, Wyse DG, Dorian P, Lee KL, Bourassa MG, Arnold JMO, Buxton AE, Camm AJ, Connolly SJ, Dubuc M, Ducharme A, Guerra PG, Hohnloser SH, Lambert J, le Heuzey JY, O'Hara G, Pedersen OD, Rouleau JL, Singh BN, Stevenson LW, Stevenson WG, Thibault B, Waldo AL. Rhythm control versus rate control for atrial fibrillation and heart failure. *N Engl J Med*. 2008; **358**: 2667–2677.
 34. Khan MN, Jais P, Cummings J, Di Biase L, Sanders P, Martin DO, et al. Pulmonary-vein isolation for atrial fibrillation in patients with heart failure. *N Engl J Med*. 2008; **359**: 1778–1785.
 35. MacDonald MR, Connolly DT, Hawkins NM, Steedman T, Payne J, Shaw M, et al. Radiofrequency ablation for persistent atrial fibrillation in patients with advanced heart failure and severe left ventricular systolic dysfunction: a randomised controlled trial. *Heart*. 2011; **97**: 740–747.
 36. Jones DG, Haldar SK, Hussain W, Sharma R, Francis DP, Rahman-Haley SL, McDonagh TA, Underwood SR, Markides V, Wong T. A randomized trial to assess catheter ablation versus rate control in the management of persistent atrial fibrillation in heart failure. *J Am Coll Cardiol*. 2013; **61**: 1894–1903.

37. Hunter RJ, Berriman TJ, Diab MI, Kamdar MR. A randomized controlled trial of catheter ablation versus medical treatment of atrial fibrillation in heart failure (the CAMTAF trial). *Circ Arrhythm Electrophysiol.* 2014; 7: 31–38.
38. Di Biase L, Mohanty P, Mohanty S, Santangeli P, Trivedi C, Lakkireddy D, et al. Ablation versus amiodarone for treatment of persistent atrial fibrillation in patients with congestive heart failure and an implanted device: results from the AATAC multicenter randomized trial. *Circulation.* 2016; 133: 1637–1644.
39. Prabhu S, Taylor AJ, Costello BT, Kaye DM, McLellan AJA, Voskoboinik A, et al. Catheter ablation versus medical rate control in atrial fibrillation and systolic dysfunction: the CAMERA-MRI study. *J Am Coll Cardiol.* 2017; 70: 1949–1961.
40. Marrouche NF, Brachmann J, Andresen D, Siebels J, Boersma L, Jordaens L, Merkely B, Pokushalov E, Sanders P, Proff J, Schunkert H, Christ H, Vogt J, Bänsch D. Catheter ablation for atrial fibrillation with heart failure. *N Engl J Med.* 2018; 378: 417–427.
41. Kuck KH, Merkely B, Zahn R, Arentz T, Seidl K, Schlüter M, Tilz RR, Piorkowski C, Gellér L, Kleemann T, Hindricks G. Catheter ablation versus best medical therapy in patients with persistent atrial fibrillation and congestive heart failure: the randomized AMICA trial. *Circ Arrhythm Electrophysiol.* 2019; 12: 1–12.
42. Brignole M, Pokushalov E, Pentimalli F, Palmisano P, Chieffo E, Occhetta E, Quartieri F, Calò L, Ungar A, Mont L, APAF-CRT Investigators, Menozzi C, Alboni P, Bertero G, Klersy C, Noventa F, Brignole M, Oddone D, Donato O, Maggi R, Croci F, Solano A, Pentimalli F, Palmisano P, Landolina M, Chieffo E, Taravelli E, Occhetta E, Quartieri F, Bottoni N, Iori M, Calò L, Sgueglia M, Pieragnoli, Giorni A, Nesti M, Giannini I, Ungar A, Padeletti L, Pokushalov E, Romanov A, Peregodov I, Vidorreda S, Nunez R, Mont L, Corbucci G, Valsecchi S, Lovecchio M. A randomized controlled trial of atrioventricular junction ablation and cardiac resynchronization therapy in patients with permanent atrial fibrillation and narrow QRS. *Eur Heart J.* 2018; 39: 3999–4008.
43. Packer DL, Mark DB, Robb RA, Monahan KH, Bahnson TD, Poole JE, Noseworthy PA, Rosenberg YD, Jeffries N, Mitchell LB, Flaker GC, Pokushalov E, Romanov A, Bunch TJ, Noelker G, Ardashev A, Revishvili A, Wilber DJ, Cappato R, Kuck KH, Hindricks G, Davies DW, Kowey PR, Naccarelli GV, Reiffel JA, Piccini JP, Silverstein AP, al-Khalidi HR, Lee KL, for the CABANA Investigators. Effect of catheter ablation vs antiarrhythmic drug therapy on mortality, stroke, bleeding, and cardiac arrest among patients with atrial fibrillation. *JAMA.* 2019; 321: 1261.
44. Rienstra M, Damman K, Mulder BA, Van Gelder IC, McMurray JJV, Van Veldhuisen DJ. Beta-blockers and outcome in heart failure and atrial fibrillation. *JACC: Heart Failure.* 2013; 1: 21–28.
45. Kotecha D, Holmes J, Krum H, Altman DG, Manzano L, Cleland JG, Lip GY, Coats AJ, Andersson B, Kirchhof P, von Lueder T, Wedel H, Rosano G, Shibata MC, Rigby A, Flather MD, Beta-Blockers in Heart Failure Collaborative Group. Efficacy of beta blockers in patients with heart failure plus atrial fibrillation: an individual-patient data meta-analysis. *Lancet.* 2014; 384: 2235–2243.
46. Li SJ, Sartipy U, Lund LH, Dahlstrom U, Adiels M, Petzold M, et al. Prognostic significance of resting heart rate and use of β -blockers in atrial fibrillation and sinus rhythm in patients with heart failure and reduced ejection fraction: findings from the Swedish Heart Failure Registry. *Circ Heart Fail.* 2015; 8: 871–879.
47. Nielsen PB, Larsen TB, Gorst-Rasmussen A, Skjøth F, Lip GYH. β -Blockers in atrial fibrillation patients with or without heart failure: association with mortality in a nationwide cohort study. *Circ Heart Fail.* 2016; 9: 1–9.
48. Cadrin-Tourigny J, Shohoudi A, Roy D, Talajic M, Tadros R, Mondésert B, Dyrda K, Rivard L, Andrade JG, Macle L, Guerra PG, Thibault B, Dubuc M, Khairy P. Decreased mortality with beta-blockers in patients with heart failure and coexisting atrial fibrillation: an AF-CHF substudy. *JACC Heart Fail.* 2017; 5: 99–106.
49. Africa SS. Mortality and causes of death in South Africa: findings from death notification. *Statistical Release.* 2018; 2021: 1–98.
50. Maisel WH, Stevenson LW. Atrial fibrillation in heart failure: epidemiology, pathophysiology, and rationale for therapy. *Am J Cardiol.* 2003; 91: 2–8.
51. Krijthe BP, Kunst A, Benjamin EJ, Lip GYH, Franco OH, Hofman A, Witteman JCM, Stricker BH, Heeringa J. Projections on the number of individuals with atrial fibrillation in the European Union, from 2000 to 2060. *Eur Heart J.* 2013; 34: 2746–2751.
52. Coulibaly I, Anzouan-Kacou JB, Konin KC, Kouadio SC, Abouo-N'Dori R. Atrial fibrillation: epidemiological data from the Cardiology Institute in Abidjan, Côte d'Ivoire. *Med Trop: revue du Corps de sante coloniale.* 2010; 70: 371–374.
53. Shavadia J, Yonga G, Mwanzi S, Jinah A, Moriasi A, Otieno H. Clinical characteristics and outcomes of atrial fibrillation and flutter at the Aga Khan University Hospital. *Nairobi Cardiovasc J Africa.* 2013; 24: 6–9.
54. Ogah OS, Davison BA, Sliwa K, Mayosi BM, Damasceno A, Sani MU, Mondo C, Dzudie A, Ojji DB, Kouam C, Suliman A, Schrueder N, Yonga G, Ba SA, Maru F, Alemayehu B, Edwards C, Cotter G. Gender differences in clinical characteristics and outcome of acute heart failure in sub-Saharan Africa: results of the THESUS-HF study. *Clin Res Cardiol.* 2015; 104: 481–490.
55. Oldgren J, Healey JS, Ezekowitz M, Commerford P, Avezum A, Pais P, Zhu J, Jansky P, Sigamani A, Morillo CA, Liu L, Damasceno A, Grinvalds A, Nakamya J, Reilly PA, Keltai K, van Gelder I, Yusufali AH, Watanabe E, Wallentin L, Connolly SJ, Yusuf S, RE-LY Atrial Fibrillation Registry Investigators. Variations in cause and management of atrial fibrillation in a prospective registry of 15 400 emergency department patients in 46 countries. *Circulation.* 2014; 129: 1568–1576.
56. Stewart S, Hart CL, Hole DJ, McMurray JJV. A population-based study of the long-term risks associated with atrial fibrillation: 20-year follow-up of the Renfrew/Paisley study. *Am J Med.* 2002; 113: 359–364.
57. Lugero C, Kibirige D, Kayima J, Mondo CK, Freers J. Atrial fibrillation among the black population in a Ugandan tertiary hospital. *Int J Gen Med.* 2016; 9: 191–198.
58. Moran A, Forouzanfar M, Sampson U, Chugh S, Feigin V, Mensah G. The epidemiology of cardiovascular diseases in sub-Saharan Africa: the global burden of diseases, injuries and risk factors 2010 study. *Prog Cardiovasc Dis.* 2013; 56: 234–239.
59. Gouda HN, Charlson F, Sorsdahl K, Ahmadzade S, Ferrari AJ, Erskine H, Leung J, Santamauro D, Lund C, Amini LN, Mayosi BM, Kengne AP, Harris M, Achoki T, Wiysonge CS, Stein DJ, Whiteford H. Burden of non-communicable diseases in sub-Saharan Africa, 1990–2017: results from the Global Burden of Disease Study 2017. *Lancet Glob Health.* 2019; 7: e1375–e1387.
60. Schnabel RB, Sullivan LM, Levy D, Pencina MJ, Massaro JM, D'Agostino RB, et al. Development of a risk score for atrial fibrillation (Framingham Heart Study): a community-based cohort study. *The Lancet.* 2009; 373: 739–745.
61. Cadby G, McArdle N, Briffa T, Hillman DR, Simpson L, Knudman M, Hung J. Severity of OSA is an independent predictor of incident atrial fibrillation hospitalization in a large sleep-clinic cohort. *Chest.* 2015; 148: 945–952.
62. Stambler BS, Nguna LM. Atrial fibrillation in sub-Saharan Africa: epidemiology, unmet needs, and treatment options. *Int J Gen Med.* 2015; 8: 231–242.
63. Agbor VN, Tianyi FL, Amini LN, Mbanga CM, Petnga SJN, Simo LP, Dzudie A, Chobuo MD, Noubiap JJ. Burden of atrial fibrillation among

- adults with heart failure in sub-Saharan Africa: a systematic review and meta-analysis. *BMJ Open*. 2022; **12**: e061618.
64. Pathak RK, Middeldorp ME, Lau DH, Mehta AB, Mahajan R, Twomey D, Alasady M, Hanley L, Antic NA, McEvoy RD, Kalman JM, Abhayaratna WP, Sanders P. Aggressive risk factor reduction study for atrial fibrillation and implications for the outcome of ablation. *J Am Coll Cardiol*. 2014; **64**: 2222–2231.
 65. Pathak RK, Elliott A, Middeldorp ME, Meredith M, Mehta AB, Mahajan R, Hendriks JML, Twomey D, Kalman JM, Abhayaratna WP, Lau DH, Sanders P. Impact of CARDIOrespiratory FITNESS on arrhythmia recurrence in obese individuals with atrial fibrillation. *J Am Coll Cardiol*. 2015; **66**: 985–996.
 66. Nattel S, Harada M. Atrial remodeling and atrial fibrillation. *J Am Coll Cardiol*. 2014; **63**: 2335–2345.
 67. Zafir B, Lund LH, Laroche C, Ruschitzka F, Crespo-Leiro MG, Coats AJS, Anker SD, Filippatos G, Seferovic PM, Maggioni AP, de Mora Martin M, Polonski L, Silva-Cardoso J, Amir O, ESC-HFA HF Long-Term Registry Investigators. Prognostic implications of atrial fibrillation in heart failure with reduced, mid-range, and preserved ejection fraction: a report from 14 964 patients in the European Society of Cardiology Heart Failure Long-Term Registry. *Eur Heart J*. 2018; **39**: 4277–4284.
 68. Sossalla S, Vollmann D. Arrhythmia-induced cardiomyopathy. *Deutsches Arzteblatt Online*. 2018; **115**: 2018.
 69. Huizar JF, Ellenbogen KA, Tan AY, Kaszala K. Arrhythmia-induced cardiomyopathy. *J Am Coll Cardiol*. 2019; **73**: 2328–2344.
 70. Schönleitner P, Schotten U, Antoons G. Mechanosensitivity of microdomain calcium signalling in the heart. *Prog Biophys Mol Biol*. 2017; **130**: 288–301.
 71. Eckstein J, Verheule S, de Groot N, Allesie M, Schotten U. Mechanisms of perpetuation of atrial fibrillation in chronically dilated atria. *Prog Biophys Mol Biol*. 2008; **97**: 435–451.
 72. Santangeli P, Zado ES, Hutchinson MD, Riley MP, Lin D, Frankel DS, Supple GE, Garcia FC, Dixit S, Callans DJ, Marchlinski FE. Prevalence and distribution of focal triggers in persistent and long-standing persistent atrial fibrillation. *Heart Rhythm*. 2016; **13**: 374–382.
 73. Gan GCH, Ferhik A, Boyd A, Thomas L. Left atrial function: evaluation by strain analysis. *Cardiovasc Diagnosis Therapy*. 2018; **8**: 29–46.
 74. Mehrzad R, Rajab M, Spodick D. The three integrated phases of left atrial macrophysiology and their interactions. *Int J Mol Sci*. 2014; **15**: 15146–15160.
 75. McDonagh TA, Metra M, Adamo M, Gardner RS, Baumbach A, Böhm M, et al. 2021 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure. *Eur Heart J*. 2021; **42**: 3599–3726.
 76. SOLVD Investigators, Yusuf S, Pitt B, Davis CE, Hood WB Jr, Cohn JN. Effect of enalapril on mortality and the development of heart failure in asymptomatic patients with reduced left ventricular ejection fractions. *N Engl J Med*. 1992; **327**: 685–691.
 77. Pitt B, Zannad F, Remme WJ, Cody R, Castaigne A, Perez A, Palensky J, Wittes J. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. *N Engl J Med*. 1999; **341**: 709–717.
 78. Granger CB, McMurray JJV, Yusuf S, Held P, Michelson EL, Olofsson B, Ostergren J, Pfeffer MA, Swedberg K, CHARM Investigators and Committees. Effects of candesartan in patients with chronic heart failure and reduced left-ventricular systolic function intolerant to angiotensin-converting-enzyme inhibitors: the CHARM-Alternative trial. *The Lancet*. 2003; **362**: 772–776.
 79. Guerra F, Pimpini L, Flori M, Contadini D, Stronati G, Gioacchini F, et al. Sacubitril/valsartan reduces atrial fibrillation and supraventricular arrhythmias in patients with HFrEF and remote monitoring: preliminary data from the SAVE THE RHYTHM. *Eur Heart J*. 2020; **41**.
 80. Suo Y, Yuan M, Li H, Zhang Y, Li Y, Fu H, Han F, Ma C, Wang Y, Bao Q, Li G. Sacubitril/valsartan improves left atrial and left atrial appendage function in patients with atrial fibrillation and in pressure overload-induced mice. *Front Pharmacol*. 2019; **10**: 1285.
 81. McMurray JJV, Packer M, Desai AS, Gong J, Lefkowitz MP, Rizkala AR, Rouleau JL, Shi VC, Solomon SD, Swedberg K, Zile MR, PARADIGM-HF Investigators and Committees. Angiotensin-neprilysin inhibition versus enalapril in heart failure. *N Engl J Med*. 2014; **371**: 993–1004.
 82. Liu X, Liu H, Wang L, Zhang L, Xu Q. Role of sacubitril-valsartan in the prevention of atrial fibrillation occurrence in patients with heart failure: a systematic review and meta-analysis of randomized controlled trials. *PLoS ONE*. 2022; **17**: e0263131.
 83. Mogensen UM, Jhund PS, Abraham WT, Desai AS, Dickstein K, Packer M, Rouleau JL, Solomon SD, Swedberg K, Zile MR, Køber L, McMurray JJV. Type of atrial fibrillation and outcomes in patients with heart failure and reduced ejection fraction. *J Am Coll Cardiol*. 2017; **70**: 2490–2500.
 84. McMurray JJV, Solomon SD, Inzucchi SE, Køber L, Kosiborod MN, Martinez FA, et al. Dapagliflozin in patients with heart failure and reduced ejection fraction. *N Engl J Med*. 2019; **381**: 1995–2008.
 85. Zelniker TA, Bonaca MP, Furtado RHM, Mosenzon O, Kuder JF, Murphy SA, Bhatt DL, Leiter LA, McGuire DK, Wilding JPH, Budaj A, Kiss RG, Padilla F, Gause-Nilsson I, Langkilde AM, Raz I, Sabatine MS, Wiviott SD. Effect of dapagliflozin on atrial fibrillation in patients with type 2 diabetes mellitus: insights from the DECLARE-TIMI 58 trial. *Circulation*. 2020; **141**: 1227–1234.
 86. Zakharia F, Basu A, Absher D, Assimes TL, Go AS, Hlatky MA, et al. Characterizing the admixed African ancestry of African Americans. *Genome Biol*. 2009; **10**: R141–R.
 87. Florea VG, Cohn JN. The autonomic nervous system and heart failure. *Circ Res*. 2014; **114**: 1815–1826.
 88. Hartupée J, Mann DL. Neurohormonal activation in heart failure with reduced ejection fraction. *Nat Rev Cardiol*. 2017; **14**: 30–38.
 89. Investigators C-I, Committees. The Cardiac Insufficiency Bisoprolol Study II (CIBIS-II): a randomised trial. *Lancet (London, England)*. 1999; **353**: 9–13.
 90. Hjalmarson Å, Goldstein S, Fagerberg B, Wedel H, Waagstein F, Kjekshtus J, Wikstrand J, el Allaf D, Vitovec J, Aldershvile J, Halinen M, Dietz R, Neuhaus KL, Jánosi A, Thorgeirsson G, Dunselman PHJM, Gullestad L, Kuch J, Herlitz J, Rickenbacher P, Ball S, Gottlieb S, Deedwania P, MERIT-HF Study Group. Effects of controlled-release metoprolol on total mortality, hospitalizations, and well-being in patients with heart failure. *JAMA*. 2000; **283**: 1295.
 91. Packer M, Fowler MB, Roecker EB, Coats AJS, Katus HA, Krum H, Mohacsi P, Rouleau JL, Tendera M, Staiger C, Holcslaw TL, Amann-Zalan I, DeMets DL. Effect of carvedilol on the morbidity of patients with severe chronic heart failure. *Circulation*. 2002; **106**: 2194–2199.
 92. Gelder ICV, Rienstra M, Crijns HJGM, Olshansky B. Atrial fibrillation 2 Rate control in atrial fibrillation. *The Lancet*. 2016; **388**: 818–828.
 93. Cullington D, Goode KM, Ng BE, Zhang J, Cleland JGF, Clark AL. Is heart rate important for patients with heart failure in atrial fibrillation? *JACC Heart Fail*. 2014; **2**: 213–220.
 94. Beta-Blocker Evaluation of Survival Trial I, Eichhorn EJ, Domanski MJ, Krause-Steinrauf H, Bristow MR, Lavori PW. A trial of the beta-blocker bucindolol in patients with advanced chronic heart failure. *N Engl J Med*. 2001; **344**: 1659–1667.
 95. Bristow MR, Gilbert EM, Abraham WT, Adams KF, Fowler MB, Hersherberger RE, Kubo SH, Narahara KA, Ingersoll H, Krueger S, Young S, Shusterman N. Carvedilol produces dose-related improvements in left ventricular function and survival in subjects with chronic heart failure. *Circulation*. 1996; **94**: 2807–2816.

96. McAlister FA, Wiebe N, Ezekowitz JA, Leung AA, Armstrong PW. Meta-analysis: β -blocker dose, heart rate reduction, and death in patients with heart failure. *Ann Intern Med.* 2009; **150**: 784.
97. Swedberg K, Komajda M, Böhm M, Borer JS, Ford I, Dubost-Brama A, Lerebours G, Tavazzi L, SHIFT Investigators. Ivabradine and outcomes in chronic heart failure (SHIFT): a randomised placebo-controlled study. *The Lancet.* 2010; **376**: 875–885.
98. Woodiwiss AJ, Badenhorst D, Sliwa K, Brooksbank R, Essop R, Sareli P, et al. Beta1- and alpha2c-adrenoceptor variants as predictors of clinical aspects of dilated cardiomyopathy in people of African ancestry. *Cardiovasc J Afr.* **19**: 188–193.
99. Taylor MR, Sun AY, Davis G, Fiuzat M, Liggett SB, Bristow MR. Race, common genetic variation, and therapeutic response disparities in heart failure. *JACC: Heart Failure.* 2014; **2**: 561–572.
100. Rau T, Dungen H, Edelmann F, Waagstein F, Lain M, Dimkovi S, et al. Impact of the β 1-adrenoceptor Arg389Gly polymorphism on heart-rate responses to Bisoprolol and carvedilol in heart-failure patients. *Clin Pharmacol Ther.* 2012; **92**: 21–28.
101. Parvez B, Chopra N, Rowan S, Vaglio JC, Muhammad R, Roden DM, Darbar D. A common β 1-adrenergic receptor polymorphism predicts favorable response to rate-control therapy in atrial fibrillation. *J Am Coll Cardiol.* 2012; **59**: 49–56.
102. Kao DP, Davis G, Aleong R, Connor CMO, Fiuzat M, Carson PE, et al. Effect of bucindolol on heart failure outcomes and heart rate response in patients with reduced ejection fraction heart failure and atrial fibrillation. *Eur J Heart Fail.* 2013; **5**: 324–333.
103. Parikh KS, Fiuzat M, Davis G, Neely M, Blain-Nelson P, Whellan DJ, et al. Dose response of β -blockers in adrenergic receptor polymorphism genotypes. *Circ Genom Precis Med.* 2018; **11**: 139–148.
104. Joseph SS, Lynham JA, Grace AA, Colledge WH, Kaumann AJ. Markedly reduced effects of (–)-isoprenaline but not of (–)-CGP12177 and unchanged affinity of β -blockers at Gly389- β 1-adrenoceptors compared to Arg389- β 1-adrenoceptors. *Br J Pharmacol.* 2004; **142**: 51–56.
105. Madamanchi A. β -Adrenergic receptor signaling in cardiac function and heart failure. *McGill J Med.* 2007; **10**: 99–104.
106. Mason RP, Giles TD, Sowers JR. Evolving mechanisms of action of beta blockers: focus on nebivolol. *J Cardiovasc Pharmacol.* 2009; **54**: 123–128.
107. Sandilands AJ, O'Shaughnessy KM, Brown MJ. Greater inotropic and cyclic AMP responses evoked by noradrenaline through Arg389 β 1-adrenoceptors versus Gly389 β 1-adrenoceptors in isolated human atrial myocardium. *Br J Pharmacol.* 2003; **138**: 386–392.
108. Amanfu RK, Saucerman JJ. Modeling the effects of β 1-adrenergic receptor blockers and polymorphisms on cardiac myocyte Ca^{2+} handling. *Mol Pharmacol.* 2014; **86**: 222–230.
109. Mason DA, Moore JD, Green SA, Liggett SB. A gain-of-function polymorphism in a G-protein coupling domain of the human β 1-adrenergic receptor. *J Biol Chem.* 1999; **274**: 12670–12674.
110. Liggett SB. Pharmacogenomics of β 1-adrenergic receptor polymorphisms in heart failure. *Heart Fail Clin.* 2010; **6**: 27–33.
111. Aleong RG, Sauer WH, Davis G, Murphy GA, Port JD, Anand IS, Fiuzat M, O'Connor CM, Abraham WT, Liggett SB, Bristow MR. Prevention of atrial fibrillation by bucindolol is dependent on the beta1389 Arg/Gly adrenergic receptor polymorphism. *JACC Heart Fail.* 2013; **1**: 338–344.
112. Liggett SB, Miale-Perez J, Thaneemit-Chen S, Weber SA, Greene SM, Hodne D, Nelson B, Morrison J, Domanski MJ, Wagoner LE, Abraham WT, Anderson JL, Carlquist JF, Krause-Steinrauf HJ, Lazzaroni LC, Port JD, Lavori PW, Bristow MR. A polymorphism within a conserved beta1-adrenergic receptor motif alters cardiac function and beta-blocker response in human heart failure. *Proc Natl Acad Sci.* 2006; **103**: 11288–11293.
113. O'Connor CM, Fiuzat M, Carson PE, Anand IS, Plehn JF, Gottlieb SS, et al. Combinatorial pharmacogenetic interactions of bucindolol and β 1, α 2C adrenergic receptor polymorphisms. *PLoS ONE.* 2012; **7**: e44324-e.
114. Inkrot S, Tahirovic E, Apostolovic S, Du H-D, Mehrhof F, Prettin C, et al. Titration to target dose of bisoprolol vs. carvedilol in elderly patients with heart failure: the CIBIS-ELD trial. *Eur J Heart Fail.* 2011; **13**: 670–680.
115. Noma T, Lemaire A, Naga Prasad SV, Barki-Harrington L, Tilley DG, Chen J, le Corvoisier P, Violin JD, Wei H, Lefkowitz RJ, Rockman HA. β -Arrestin-mediated β 1-adrenergic receptor transactivation of the EGFR confers cardioprotection. *J Clin Invest.* 2007; **117**: 2445–2458.
116. Katsarou M-S, Karathanasopoulou A, Andrianopoulou A, Desiniotis V, Tzinis E, Dimitrakis E, Lagiou M, Charmandari E, Aschner M, Tsatsakis AM, Chrousos GP, Drakoulis N. Beta 1, Beta 2 and Beta 3 adrenergic receptor gene polymorphisms in a Southeastern European population. *Front Genet.* 2018; **9**: 560.
117. Wyse DG, Waldo AL, DiMarco JP, Domanski MJ, Rosenberg Y, Schron EB, Kellen JC, Greene HL, Mickel MC, Dalquist JE, Corley SD, Atrial Fibrillation Follow-up Investigation of Rhythm Management (AFFIRM) Investigators. A comparison of rate control and rhythm control in patients with atrial fibrillation. *N Engl J Med.* 2002; **347**: 1825–1833.
118. Zimetbaum P. Antiarrhythmic drug therapy for atrial fibrillation. *Circulation.* 2012; **125**: 381–389.
119. Singh SN, Tang XC, Reda D, Singh BN. Systematic electrocardioversion for atrial fibrillation and role of antiarrhythmic drugs: a substudy of the SAFE-T trial. *Heart Rhythm.* 2009; **6**: 152–155.
120. Jardine RM, Fine J, Obel IWP. A survey on the treatment of atrial fibrillation in South Africa. *S Afr Med J.* 2014; **104**: 623.
121. Piccini JP, Abraham WT, Dufton C, Carroll IA, Healey JS, van Veldhuisen DJ, et al. Bucindolol for the maintenance of sinus rhythm in a genotype-defined HF population. *JACC: Heart Failure.* 2019; **7**: 586–598.
122. Rillig A, Magnussen C, Ozga A-K, Suling A, Brandes A, Breithardt G, Camm AJ, Crijns HJGM, Eckardt L, Elvan A, Goette A, Gulizia M, Haegeli L, Heidebuchel H, Kuck KH, Ng A, Szumowski L, van Gelder I, Wegscheider K, Kirchhof P. Early rhythm control therapy in patients with atrial fibrillation and heart failure. *Circulation.* 2021; **144**: 845–858.
123. Brieger D, Amerena J, Attia J, Bajorek B, Chan KH, Connell C, et al. National Heart Foundation of Australia and the Cardiac Society of Australia and New Zealand: Australian clinical guidelines for the diagnosis and management of atrial fibrillation 2018. *Heart Lung Circ.* 2018; **27**: 1209–1266.
124. Calkins H, Hindricks G, Cappato R, Kim YH, Saad EB, Aguinaga L, Akar JG, Badhwar V, Brugada J, Camm J, Chen PS, Chen SA, Chung MK, Cosedis Nielsen J, Curtis AB, Davies DW, Day JD, d'Avila A, (Natasja) de Groot NMS, di Biase L, Duytschaever M, Edgerton JR, Ellenbogen KA, Ellinor PT, Ernst S, Fenelon G, Gerstenfeld EP, Haines DE, Haissaguerre M, Helm RH, Hylek E, Jackman WM, Jalife J, Kalman JM, Kautzner J, Kottkamp H, Kuck KH, Kumagai K, Lee R, Lewalter T, Lindsay BD, Macle L, Mansour M, Marchlinski FE, Michaud GF, Nakagawa H, Natale A, Nattel S, Okumura K, Packer D, Pokushalov E, Reynolds MR, Sanders P, Scanavacca M, Schilling R, Tondo C, Tsao HM, Verma A, Wilber DJ, Yamane T, Document Reviewers, Blomström-Lundqvist C, de Paola AAV, Kistler PM, Lip GYH, Peters NS, Pisani CF, Raviele A, Saad EB, Satomi K, Stiles MK, Willems S. 2017 HRS/EHRA/ECAS/APHSR/SOLAECE expert consensus statement on catheter and surgical ablation of atrial fibrillation. *Europace.* 2018; **20**: e1–e160.
125. Chen MS, Marrouche NF, Khaykin Y, Gillinov AM, Wazni O, Martin DO, Rossillo A, Verma A, Cummings J,

- Erciyes D, Saad E, Bhargava M, Bash D, Schweikert R, Burkhardt D, Williams-Andrews M, Perez-Lugones A, Abdul-Karim A, Saliba W, Natale A. Pulmonary vein isolation for the treatment of atrial fibrillation in patients with impaired systolic function. *J Am Coll Cardiol*. 2004; **43**: 1004–1009.
126. Anselmino M, Matta M, D'Ascenzo F, Bunch TJ, Schilling RJ, Hunter RJ, et al. Catheter ablation of atrial fibrillation in patients with left ventricular systolic dysfunction. *Circ Arrhythm Electrophysiol*. 2014; **7**: 1011–1018.
127. Halabi SA, Qintar M, Hussein A, Alraies MC, Jones DG, Wong T, et al. Catheter ablation for atrial fibrillation in heart failure patients: a meta-analysis of randomized controlled trials. *JACC Clin Electrophysiol*. 2015; **1**: 200–209.
128. Ullah W, Ling L-H, Prabhu S, Lee G, Kistler P, Finlay MC, Earley MJ, Sporton S, Bashir Y, Betts TR, Rajappan K, Thomas G, Duncan E, Staniforth A, Mann I, Chow A, Lambiase P, Schilling RJ, Hunter RJ. Catheter ablation of atrial fibrillation in patients with heart failure: impact of maintaining sinus rhythm on heart failure status and long-term rates of stroke and death. *Europace*. 2016; **18**: 679–686.
129. AlTurki A, Proietti R, Dawas A, Alturki H, Huynh T, Essebag V. Catheter ablation for atrial fibrillation in heart failure with reduced ejection fraction: a systematic review and meta-analysis of randomized controlled trials. *BMC Cardiovasc Disord*. 2019; **19**: 18.
130. Shoemaker MB, Bollmann A, Lubitz SA, Ueberham L, Saini H, Montgomery J, Edwards T, Yoneda Z, Sinner MF, Arya A, Sommer P, Delaney J, Goyal SK, Saavedra P, Kanagasundram A, Whalen SP, Roden DM, Hindricks G, Ellis CR, Ellinor PT, Darbar D, Husser D. Common genetic variants and response to atrial fibrillation ablation. *Circ Arrhythm Electrophysiol*. 2015; **8**: 296–302.
131. Zhang M, Hill MC, Kadow ZA, Suh JH, Tucker NR, Hall AW, Tran TT, Swinton PS, Leach JP, Margulies KB, Ellinor PT, Li N, Martin JF. Long-range Pitx2c enhancer-promoter interactions prevent predisposition to atrial fibrillation. *Proc Natl Acad Sci*. 2019; **116**: 22692–22698.
132. Choi EK, Park JH, Lee JY, Nam CM, Hwang MK, Uhm JS, Joung B, Ko YG, Lee MH, Lubitz SA, Ellinor PT, Pak HN. Korean atrial fibrillation (AF) network: genetic variants for AF do not predict ablation success. *J Am Heart Assoc*. 2015; **4**: e002046.
133. Yuyun MF, Bonny A, Ng GA, Sliwa K, Kengne AP, Chin A, Mocumbi AO, Ngantcha M, Ajijola OA, Bukhman G. A systematic review of the spectrum of cardiac arrhythmias in sub-Saharan Africa. *Glob Heart*. 2020; **15**.

CHAPTER 3 : THE CLINICAL CHARACTERISTICS AND MORTALITY OUTCOMES OF ATRIAL FIBRILLATION COMPLICATING HEART FAILURE WITH REDUCED EJECTION FRACTION: A PROSPECTIVE STUDY FROM SOUTH AFRICA

3.1 ABSTRACT

Background: A growing burden of hypertension, diabetes, and ischemic heart disease has increased cardiovascular disease-related mortality in Sub-Saharan Africa (SSA). This trend has resulted in increased prevalence of heart failure with reduced ejection fraction (HFrEF), which, due to synergistic pathophysiological mechanisms, complicates with atrial fibrillation (AF). To our knowledge, no prospective study has investigated AF's clinical impact on HFrEF patients in SSA.

Aim: To determine the prevalence of AF in patients with HFrEF, describe the clinical characteristics of HFrEF-AF patients, and determine the impact of AF on mortality.

Methods: This single-center prospective observational study was conducted at a tertiary hospital in Johannesburg. We enrolled 136 HFrEF patients and categorised them into HFrEF-SR (sinus rhythm) and HFrEF-AF. Baseline clinical characteristics were recorded, and cardiac biomarkers analysed. Comprehensive echocardiography and novel left atrial strain analysis using 2D-speckle tracking were performed. Patients were followed up for a median of 30.6 months.

Results: Twenty-eight (21 %) patients had AF. The mean age was 58.7 ± 14.9 years (males, 52.9%), and differed significantly between the two groups ($p < 0.001$). Hypertensive heart disease was the leading cause of HFrEF (36%). Patients with HFrEF-AF had significantly lower Kansas City Cardiomyopathy Questionnaire scores (KCCQ) (27 (16-43)) compared to those in SR (45(32;60), $p < 0.001$). Left atrial strain reservoir function (%) was significantly lower in HFrEF-AF patients (26.2 ± 11.3 , $p < 0.001$). Pharmacological therapy was suboptimal in the AF group: although anticoagulation use was higher in HFrEF-AF patients (60% vs. 9.5%, $p < 0.001$), the overall was inadequate. Additionally, HFrEF- AF patients received significantly lower doses of carvedilol (median 15.6 mg vs. 25 mg, $p = 0.002$), enalapril (median 10 mg vs. 20 mg, $p = 0.004$), and fewer HFrEF-AF patients received spironolactone (50% vs. 75.3%, $p = 0.013$). Patients with HFrEF-AF had a significantly lower overall survival rate (0.73 (0.61-0.82) vs 0.41 (0.22-0.61), $p < 0.001$). Previous stroke, a low TAPSE and KCCQ, a high E/e' and heart rate were independently associated with all-cause mortality.

Conclusion: Our study has demonstrated that AF is common in patients with HFrEF but comparatively low relative to high income countries and is associated with a more severe clinical presentation and a high mortality rate in comparison to their SR counterparts.

3.2 INTRODUCTION

As the global life expectancy increases, the incidence of heart failure (HF) and atrial fibrillation (AF) has increased and is projected to rise further (1). Heart failure is estimated to account for approximately 25.6-30% of hospital admissions cardiology units in Africa (2). Owing to common cardiometabolic risk factors such as hypertension, diabetes and coronary artery disease as well as electrophysiological and neurohormonal changes, HF with reduced ejection fraction (HFrEF) and AF frequently coexist and the presence of one precipitates the severity of the other. In the setting of HFrEF, AF confers a significant additional risk of morbidity and mortality compared to patients in sinus rhythm (SR) (3).

Despite a well-established synergistic link between HFrEF and AF and associated adverse outcomes, there is a paucity of original research focusing specifically on HFrEF-AF in Sub-Saharan Africa (SSA) and even fewer comparative studies comparing HFrEF-AF, and HFrEF-SR. Amongst available studies conducted in SSA, the majority focus on heterogeneous HF populations. Studies done in high-income countries (HIC) and SSA have identified disparities between HF and AF in the West and SSA (4-7). Therefore, this study aimed to estimate the prevalence of HFrEF-AF, amongst patients with HFrEF, and evaluate the clinical impact of AF on HFrEF, and mortality associated with this patient population in Johannesburg, South Africa.

3.3 METHODS

3.3.1 Study design and participants

A prospective observational study was conducted at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), South Africa. Two hundred and forty-eight (248) patients were consecutively screened from a specialised out-patient HF clinic and in-patient cardiac admission wards between June 2019 and December 2021. The study recruitment phase was affected by COVID-19 lockdown regulations, consequently impacting the overall sample size.

3.3.2 Inclusion & Exclusion criteria

Patients known with HF, chronically stable and/or acutely decompensated as well as patients with new-onset (“de novo”) acute HF were enrolled. The diagnosis was based on the 2016 European Society of Cardiology Heart Failure Guidelines (8). Individuals with a left ventricular ejection fraction (LVEF) of less than or equal to 45% on echocardiography were enrolled into the study. An LVEF of $\leq 45\%$ was chosen due to the subjectivity associated with LVEF measurements and the absence of evidence-based therapies for managing patients with mildly reduced ejection fraction (HFmrEF) (patients with an LVEF between 41% and 49%) at the study's inception. At study enrolment, patients were screened for AF with a standard 12-lead resting electrocardiogram (ECG), which was corroborated with clinical findings (9). Patients were divided into two groups based on their rhythm status: those with HFrEF in sinus rhythm (HFrEF-SR) and those with HFrEF and concomitant atrial fibrillation (HFrEF-AF). Patients with an LVEF greater than 45%, significant valvular heart disease, pacemaker rhythm, or any other rhythm were excluded from the study.

3.3.3 Data Collection

A comprehensive history and clinical examination was carried out on all patients. Resting blood pressure was measured using a calibrated, automated digital sphygmomanometer. Participants were seated comfortably in a chair with their back supported and feet flat on the floor for at least five minutes prior to measurement. The measurement was taken on the right arm, supported at heart level, using an appropriately sized cuff. Two readings were taken at one-minute intervals, and the average of the two was recorded as the participant's blood pressure. If the two readings differed by more than 10 mmHg, a third measurement was obtained and the average of the two closest values was used. Systolic and diastolic blood pressure values were recorded in millimetres of mercury (mmHg).

Weight was measured to the nearest 0.1 kilograms using a calibrated digital scale with participants wearing no shoes. Height was measured to the nearest 0.1 centimetres using a wall-mounted stadiometer, with participants standing upright, barefoot, with heels together and eyes facing forward. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2).

Relevant clinical information, such as the age, race, estimated duration of HF, aetiology, and comorbid conditions, was obtained from patient medical records and corroborated through

documented history and clinical findings. A focused history and physical examination were specifically performed to identify clinical signs (peripheral edema, raised jugular venous pressure, pulmonary crackles on auscultation, third heart sound) and symptoms (dyspnea, orthopnea, paroxysmal nocturnal dyspnea, reduced exercise tolerance, fatigue) indicative of acute decompensated heart failure (ADHF).

Heart failure symptom severity was assessed using the New York Heart Association (NYHA) functional class and the Kansas City Cardiomyopathy Questionnaire (KCCQ) -12 was administered to evaluate the quality of life within the preceding 2 weeks of presentation (10). The CHA₂DS₂-VASc (Congestive heart failure, Hypertension, Age ≥ 75 years, Diabetes mellitus, Stroke, Vascular disease, Age . 65-74 years, sex category (female) score) was determined to assess stroke risk in patients with AF (11). A score of 1 in females and 0 in males identified patients who were low risk for developing a thromboembolic event. Clinical investigations on all patients recruited into the study included a 12-lead ECG in which heart rate and rhythm were determined.

3.3.4 Etiological diagnosis

Determination of HF aetiology was at the discretion of the treating physicians and confirmed by a consultant cardiologist, using all available clinical, laboratory and echocardiographic data. A diagnosis of hypertensive heart disease was based on the presence of echocardiogram abnormalities such as left ventricular hypertrophy (concentric or eccentric), increased left ventricular mass index, enlarged left ventricular or left atrial (LA) size and increased volumes and diastolic or systolic left ventricular dysfunction in patients with hypertension (12). The diagnosis of ischemic heart disease was made on the basis of a confirmed diagnosis of coronary artery disease (CAD), which was confirmed on history or diagnostic coronary angiogram. As part of a routine etiological work-up, all patients with HFrEF at CMJAH undergo diagnostic coronary angiograms. A diagnosis of alcohol induced cardiomyopathy was considered where an excessive intake (80 to 90 standard units per day) for a minimum of 5 years was documented (13, 14). Dilated cardiomyopathy (IDCMO) was considered in the presence of left ventricular dilatation and left ventricular systolic dysfunction in the absence hypertension, valvular heart disease, (abnormal loading conditions), coronary artery disease or any obvious clinical condition sufficient to cause global systolic impairment (15). In this cohort, we were unable to determine if there was familial dilated cardiomyopathy (DCMO).

3.3.5 Blood sample collection and preparation

Two venous blood samples (3 mL each) were collected from each patient into EDTA-containing tubes (purple-top vacutainers; Becton Dickinson (BD) Diagnostics; New Jersey, USA).

Immediately after collection, samples were placed in an insulated ice box and transported promptly to the laboratory. Samples were centrifuged at 3000 rpm for 10 minutes at 4°C to separate plasma. Plasma aliquots were transferred into cryogenic vials and stored at -80°C until subsequent biomarker analysis.

3.3.6 Biomarker analysis and baseline biochemistry

Plasma concentrations of the N-terminal pro B type natriuretic peptide (NT-proBNP) and cardiac troponin T (cTnT) were analysed using commercial sandwich enzyme-linked immunosorbent assay (ELISA) kits with a colorimetric detection, obtained from the Elabscience (Wuhan, Hubei, China), following the manufacturer's instructions. Baseline biochemical parameters (haemoglobin, white cell count, creatinine, potassium, sodium and lipid lipogram) were obtained from National Health Laboratory Services records within the last week of presentation in the event these records were available. The Modification of Diet in Renal Disease ($175 \times \text{plasma creatinine} - 1.154 \times \text{age} - 0.203$ [$\times 0.742$ if female; $\times 1.21$ if black]) calculation was used to calculate the estimated glomerular filtration rate (eGFR) (16).

3.3.7 Echocardiography

A detailed two dimensional transthoracic echocardiographic assessment was performed using a Philips CX50 ultrasound system (Eindhoven, Netherlands). Left ventricular systolic and diastolic function, right ventricular function and valvular structure and function were evaluated in accordance with the American Society of Echocardiography guidelines (17). Left ventricular ejection fraction was assessed using the modified biplane Simpson's method using the apical two-chamber and four-chamber views.

3.3.8 Left atrial strain

Left atrial strain was measured using two-dimensional speckle tracking echocardiography (2D-STE) (18). Left atrial speckle tracking necessitated a minimum of three consecutive heart cycles using a frame rate of 50-80 frames per second. The endocardial border of the LA was traced manually, in the apical 4-chamber and 2-chamber views. The starting point for LA strain analysis was set at the

onset of the QRS-complex. Reservoir strain was defined as the peak positive strain value, contraction strain was defined as the peak value at the onset of the p-wave from the ECG trace, and conduit strain was calculated as the difference between reservoir and contraction strain. In patients with AF, the conduit and contraction strain were not measured due to the absence of atrial pump function (19). An experienced echocardiographer carried out all the measurements. Images were acquired, stored and analysed offline using a commercially available Philips QLAB 13 (Eindhoven, Netherlands) software.

3.3.9 Follow-up and clinical outcomes

Participants were followed up telephonically, on a three-monthly basis until June 2024. The primary outcome of interest was all-cause mortality.

3.3.10 Statistical analysis

Demographic, clinical and biochemical characteristics are presented in frequency tables and stratified according to the presence and absence of AF. Shapiro-Wilk test was used to determine the distribution among continuous variables. Normally distributed data were reported as mean $\pm$ standard deviation, median (interquartile range) for non-parametric data and number (percentages) for categorical data. Categorical data were compared using Pearson's chi-square test or Fisher's exact test. For normally distributed continuous variables, a Student's unpaired t-test was performed and a Wilcoxon rank sum test was employed for non-parametric data. For the primary outcome of interest, all-cause mortality, a univariate logistic regression model was used to calculate the unadjusted odds ratio for each variable of interest, and a p-value of <0.05 qualified as significant to use these variables to build a multivariate logistic regression model. Multicollinearity diagnostics were conducted and variables with a variance inflation factor (VIF) greater than five were removed from the model (20). Backwards stepwise logistic regression was employed to compute the final model. Overall survival was measured from the date of enrolment to the date of death or the last follow-up date in June 2024. Times for patients without the event of interest were censored at the last date the patient was known to be alive. Survival curves were plotted by the Kaplan-Meier method. A log-rank test was used to compare survival probability between AF and SR patients. A statistical significance threshold of 5% and a confidence interval of 95% was used. Statistical analyses were performed using STATA (version 17, College Station, Texas).

3.3.11 Ethics declaration

Approval to conduct the study was granted by the University of the Witwatersrand Human Research Ethics Committee (HREC; Medical Ethics Clearance certificate number: M190349). The study complied with the Declaration of Helsinki, and written informed consent was obtained from all study patients.

3.4 RESULTS

3.4.1 Demographic and clinical characteristics

A total of 248 patients were screened in the current study. One hundred and eleven patients were excluded, leaving a total of 136 patients included in the final analysis (Figure 3.1). Patients lost to follow-up were excluded from the survival analysis.

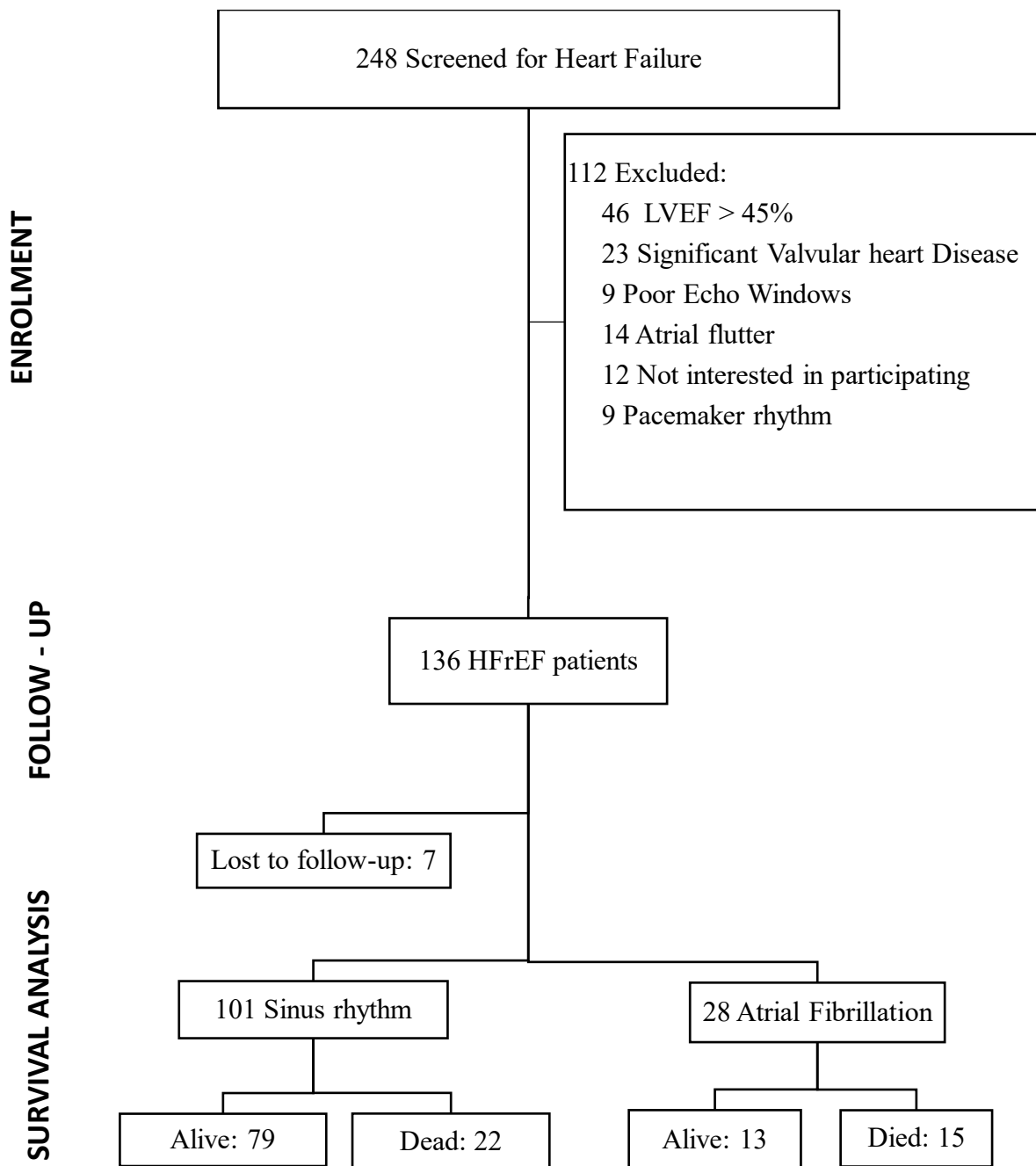


Figure 3.1 Flow diagram illustrating patient enrolment, exclusion criteria, rhythm classification, and survival status in the study cohort. A total of 248 patients were screened for heart failure. Of these, 112 were excluded due to a left ventricular ejection fraction (LVEF) > 45%, significant valvular heart disease, poor echocardiographic windows, atrial flutter, pacemaker rhythm, or lack of consent. The final cohort included 136 patients with heart failure with reduced ejection fraction (HFrEF). During follow-up, 7 patients were lost. Of the remaining 129 patients, 101 were in sinus rhythm (SR) and 28 had atrial fibrillation (AF). Survival analysis showed that 22 patients in SR and 15 in AF had died by the end of the study period.

The baseline demographic and clinical characteristics are summarised in Tables 3.1a and 3.1b. Males represented a majority (52.9%) of participants and the mean age of participants was 58.7 ± 14.9 years. One hundred (74%) of patients were of black African ethnicity ($p < 0.001$) and a greater percentage of Caucasians (35%) were present in the HFrEF-AF group compared to the HFrEF-SR group (8.3%) (Table 3.1a). Hypertensive heart disease was the leading cause of HFrEF (33.8%) followed by idiopathic dilated cardiomyopathy (19.1%) and ischemic heart disease (19.1%). The median duration of HFrEF was 3.6 [1.1-7.1] years.

The prevalence of AF in the current study was 21% (Table 3.1a). Fourteen (50%) patients with AF were black ($p = 0.001$). Patients with AF were significantly older (69.5 ± 12.3) years than those in SR (55.4 ± 14.3 years; $p < 0.001$). Patients with HFrEF-AF had significantly lower KCCQ (27 [16-43]) compared to those in SR (45 [32-60]) ($p < 0.001$) as well as worsening functional class (NYHA III-IV; 60.7% vs 21.5%, $p < 0.001$).

Table 3.1a. Baseline characteristics of patients with HFrEF categorised according to the presence and absence (SR) of atrial fibrillation.

Variable	Rhythm			p - value
	Total (n= 136)	HFrEF-SR (n=108)	HFrEF-AF (n=28)	
Gender, n (%)				
Male	72 (52.9)	56 (51.9)	16 (57.1)	0.617
Age (years)	58.7 ± 14.9	55.4 ± 14.3	69.5 ± 12.3	<0.001
BMI (Kg/m ²)	28.9 (25.7; 34)	29.4 (25.9; 33.9)	28.7 (24.2; 33.6)	0.549
Race (%)				<0.001
Black	101 (74.3)	87 (81.0)	14 (50.0)	
Caucasian	19 (13.9)	9 (8.3)	10 (35.7)	
Indian	11 (8.1)	7 (6.5)	4 (14.3)	
Coloured	5 (3.7)	5 (4.6)	0	
ADHF, n (%)	38 (27.9)	21 (19.4)	17 (60.7)	<0.001
HF Duration (years)	3.6 (1.1; 7.1)	3.5 (1.3; 6.8)	5.2 (0.5; 8.2)	0.666
Heart rate (bpm)	72 (63; 85)	72.8 ± 14.3	81 (70; 104)	0.003
SBP (mmHg)	124 (109; 138)	125 (114; 138)	117 (101; 138)	0.177
DBP (mmHg)	78.5 (69; 86)	79 (71; 86)	77 (66; 86.3)	0.376
CHA ₂ DS ₂ -VASc	4 (3; 5)	-	4 (3; 5)	-
KCCQ	42 (28; 59)	45 (32; 60)	27 (16; 43)	<0.001
NYHA Class				<0.001
I	50 (37.3)	47 (43.9)	3 (11.1)	
II	44 (32.9)	37 (34.6)	8 (28.6)	
III	30 (22.4)	17 (15.9)	13 (46.4)	
IV	10 (7.5)	6 (5.6)	4 (14.3)	

Data presented as mean ± standard deviation, median (interquartile range) or number (percentage); HFrEF (Heart failure with reduced ejection fraction), SR (Sinus rhythm), AF (Atrial fibrillation), BMI (Body Mass Index), SBP (Systolic blood pressure), ADHF (Acutely decompensated heart failure), DBP (Diastolic blood pressure), bpm (Beats per minute), KCCQ (Kansas City cardiomyopathy questionnaire), CHA₂DS₂-VASc (congestive heart failure, hypertension, age ≥ 75 years, diabetes mellitus, stroke, vascular disease, age 65-74 years, sex category), NYHA (New York Heart Association).

Amongst patients with AF, a greater proportion had coronary artery disease (14.3 % vs 3.7%, $p = 0.034$) and a history of previous stroke compared to patients in SR (Table 3.1b). Amongst patients with HIV-related cardiomyopathies, none had AF (2.9% vs 0%, $p = 0.301$).

Table 3.1b. Baseline characteristics of patients with HFrEF categorised according to the presence and absence (SR) of atrial fibrillation: Comorbidities and Etiologies

Variable	Rhythm			p - value
	Total	HFrEF-SR	HFrEF-AF	
	(n= 136)	(n=108)	(n=28)	
Comorbidities, n (%)				
Diabetes	34 (25)	26 (24.1)	8 (28.6)	0.624
Dyslipidaemia	39 (28.7)	29 (26.9)	10 (35.7)	0.355
Previous myocardial infarction	19 (13.9)	12 (11.1)	7 (25.0)	0.059
CKD	25 (18.4)	17 (15.7)	8 (28.6)	0.118
CAD	8 (5.9)	4 (3.7)	4 (14.3)	0.034
Previous stroke	10 (7.4)	5 (4.6)	5 (17.9)	0.017
Hypertension	86 (63.2)	66 (61.1)	20 (71.4)	0.313
HIV	20 (14.7)	20 (18.5)	0	0.014
Aetiology, n (%)				
Hypertensive heart disease	46 (33.8)	35 (32.4)	11 (39.3)	0.493
Ischemic heart disease	26 (19.1)	18 (16.7)	8 (28.6)	0.153
IDCMO	26 (19.1)	21 (19.4)	5 (17.9)	0.849
PPCMO	12 (8.8)	11 (10.2)	1 (3.6)	0.272
Chemo-induced	7 (5.2)	7 (6.5)	0	0.167
Alcohol-related	5 (3.7)	5 (4.6)	0	0.246
Diabetic cardiomyopathy	4 (2.9)	4 (3.7)	0	0.301
HIV-related	4 (2.9)	4 (3.7)	0	0.301
Tachy-induced	4 (2.9)	2 (1.9)	2 (7.1)	0.140
Thyrocardiac	2 (1.5)	1 (0.9)	1 (3.6)	0.300
Viral myocarditis	2 (1.5)	2 (1.9)	0	0.468
Non rheumatic VHD	1 (0.7)	1 (0.9)	0	0.609

Data presented as number (Percentage); HFrEF (Heart failure with reduced ejection fraction), SR (Sinus rhythm), AF (Atrial fibrillation), CKD (Chronic kidney disease, CAD (Coronary arterial

disease), IDCMO (Idiopathic dilated cardiomyopathy), PPCMO (Post-partum cardiomyopathy), VHD (Valvular heart disease)

3.4.2 Pharmacotherapy profile of the enrolled patients with HFrEF

Table 3.2 presents the pharmacotherapy profile of enrolled patients stratified by cardiac rhythm status: HFrEF with sinus rhythm (SR) and HFrEF with atrial fibrillation (AF). Beta-blockers were the most prescribed class of drugs, with 111 out of 125 patients (88.8%) receiving therapy. The prescription rate was significantly higher in patients with SR compared to those with AF (91.9% vs. 76.9%, $p = 0.031$). Among β -blocker users, carvedilol was the predominant agent, used in 97.3% of patients, with universal use in the AF group (100%) and 96.7% in the SR group. Median carvedilol dose was significantly lower in the AF group compared to the SR group (15.6 mg [IQR 12.5–25] vs. 25 mg [IQR 12.5–50]; $p = 0.002$).

Furosemide was prescribed to 80.2% of patients, with comparable usage between rhythm groups (SR = 81.3% vs. AF = 76%; $p = 0.578$). However, although not statistically significant, the median furosemide dose tended to be higher in the AF group (80 mg [IQR 40–80]) than in the SR group (40 mg [IQR 40–80]; $p = 0.077$).

Spironolactone use differed significantly between rhythm groups, with higher usage in the SR group (75.3% vs. 50%; $p = 0.013$). The median dose remained consistent across groups (25 mg [IQR 25–25]).

Anticoagulation was significantly more common in AF patients, with 60% receiving anticoagulation versus only 9.5% in the SR group ($p < 0.001$), reflecting appropriate management.

Enalapril was prescribed to 69.7% of patients, with significantly higher usage among SR patients (75%) compared to AF patients (50%; $p = 0.033$). Median enalapril dose was also higher in the SR group (20 mg [IQR 10–20] vs. 10 mg [IQR 5–10]; $p = 0.004$).

Table 3.2 Pharmacotherapy profile of enrolled patients with HFrEF stratified by heart rhythm: Sinus rhythm (SR) vs atrial fibrillation (AF).

Variable	RHYTHM			P - value
	Total (n= 136)	HFrEF-SR (n=108)	HFrEF-AF (n=28)	
Medication				
Enalapril, n (%) (n=122)	85 (69.7)	72 (75)	13 (50)	0.033
Enalapril dose (mg)	10 (10; 20)	20 (10; 20)	10 (5; 10)	0.004
Beta-blocker, n (%) (n=125)	111 (88.8)	91 (91.9)	20 (76.9)	0.031
Carvedilol, n (%)	107 (97.3)	87 (96.7)	20 (100)	
Atenolol, n (%)	2 (1.82)	2 (2.22)	0	
Bisoprolol, n (%)	1 (0.91)	1 (1.11)	0	
Carvedilol dose (mg)	25 (12.5; 50)	25 (12.5; 50)	15.6 (12.5; 25)	0.002
Losartan, n (%) (n=124)	18 (14.5)	15 (15.3)	3 (11.5)	0.628
Furosemide, n (%) (n=121)	97 (80.2)	78 (81.3)	19 (76)	0.578
Furosemide dose	40 (40; 80)	40 (40; 80)	80 (40; 80)	0.077
Spironolactone, n (%)	86 (69.9)	73 (75.3)	13 (50)	0.013
Spironolactone dose	25 (25;25)	25 (25; 25)	25 (25; 25)	
Anticoagulation, n (%) (n=119)	24 (20.2)	9 (9.5)	15 (60)	<0.001

Data presented as mean $\pm$ standard deviation, median (interquartile range) or number (percentage); HFrEF (Heart failure with reduced ejection fraction), SR (Sinus rhythm), AF (Atrial fibrillation)

3.4.3 Biochemistry

Estimated glomerular filtration rate (mL/min) was significantly lower in HFrEF-AF patients (52 [37-70.9]) compared with SR counterparts (71.9 [50-98.1]) (p=0.046). Baseline NT-proBNP, cTnT and creatinine levels were numerically higher in patients with HFrEF-AF but not significantly different. Baseline urea was significantly higher in patients with AF compared to those in SR. The

international normalized ratio (INR) was significantly higher amongst HFrEF-AF patients compared to the HFrEF-SR group ($p=0.040$) (Table 3.3).

Table 3.3. Baseline biochemical parameters of patients with HFrEF categorised according to the presence and absence (SR) of AF.

Variable	Rhythm			p - value
	Total (N=136)	HFrEF-SR (n=93)	HFrEF-AF (n=23)	
Haemoglobin (g/dl) ^a	13 ± 2.3	13 ± 2.2	13 ± 2.5	0.960
Platelet count ^b (x10 ⁹ /L)	245 (195; 315)	251 (201; 306)	241 (193.5; 327.5)	0.933
Creatinine umol/l ^c	99.5 (79; 137)	97 (76; 131)	116.5 (97; 146)	0.060
Urea ^c (mg/dL)	6.6 (4.6; 12.1)	6 (4; 11.8)	10.5 (6.1; 13.7)	0.020
eGFR, mL/min ^c	65.6 (48; 95.9)	71.9 (50; 98.1)	52 (37; 70.9)	0.046
Total cholesterol (mmol/L) ^d	3.5 ± 1.1	3.8 ± 1.1	3.1 ± 1.0	0.058
LDL (mmol/L) ^e	2.3 ± 0.9	2.4 ± 0.9	2.1 ± 0.8	0.235
HDL (mmol/L) ^e	0.96 (0.8; 1.2)	0.96 (0.82; 1.3)	0.9 ± 0.2	0.834
HBA1C (%) ^f	6.9 (6.1; 7.4)	6.5 (5.8; 7.4)	6.9 (6.5; 7.5)	0.447
INR (mmol/L) ^g	1.27 (1.1; 1.5)	1.16 (1.0; 1.4)	1.4 (1.2; 2.1)	0.040
NT-proBNP (pg/mL)	1030 (678; 1841)	993.5 (582; 1779)	1243 (847; 2040)	0.164
cTnT (pg/mL)	26.5 (19.1; 36)	26.7 (19.3; 35.3)	28.4 (17.9; 42.9)	0.644

Data presented as mean ± standard deviation, median (interquartile range); HFrEF (Heart failure with reduced ejection fraction), SR (Sinus rhythm), AF (Atrial fibrillation), eGFR (Estimated glomerular filtration rate), LDL (Low-density lipoprotein), HDL (High-density lipoprotein), HBA1C (Glycated haemoglobin), INR (International normalized ratio), NT-ProBNP (N-terminal pro brain natriuretic peptide), cTnT (Cardiac troponin T)

^a n = 91; ^b n = 85; ^c n = 96; ^d n = 47; ^e n = 45; ^f n = 29; ^g n = 38

3.4.4 Echocardiography

Patients with HFrEF-AF had a significantly greater LA volume (ml) (83.9 [64.5-96.9]) than those with HFrEF-SR (58.9 ± 25.1) ($p < 0.001$) (Table 3.4a).

Table 3.4a. Left ventricular and left atrial echocardiographic parameters categorised according to the presence (SR) and absence of AF.

Variable	Rhythm			
	Total (N= 119)	HFrEF-SR (n=93)	HFrEF-AF (n=23)	p- value
LVEF (%)	32 (22; 39.5)	32.5 (22; 40)	30.3 (20.6; 38)	0.463
IVSD (mm)	10.8 ± 2.6	10.8 ± 2.6	10.3 ± 2.5	0.454
LVIDd (mm)	58.0 ± 9.5	58.4 ± 9.7	55.5 (50.5; 61.5)	0.318
LVIDs (mm)	51.3 ± 11.3	51 (43.5; 61.0)	49.8 (42.0; 57.8)	0.401
LVPWD (mm)	10.3 (9; 12)	10.7 (9; 12)	9.4 (8; 12)	0.674
LV mass index (g/m ²)	139.7 ± 45.2	142.6 (105.7; 171.6)	123.7(96.5; 145.9)	0.072
LA area (cm ²)	20.8 (16.8; 26.3)	19.2 (16.4; 24.5)	28.5 ± 12.1	<0.001
LA volume (mL)	60.6 (41.9; 83.9)	58.9 ± 25.1	83.9 (64.5; 96.9)	<0.001
LA index (mL/m ²)	23.7 (21.1; 26.5)	23.2 (20.7; 26.3)	25.3 (23.5; 29.3)	0.019
E/E' ratio	12.8 (8.6; 16.9)	12.8 (8.9; 16.4)	13.4 (7.9; 19.2)	0.529
E/A ratio	1.3 (0.9; 2.2)	1.5 ± 0.9	-	
Normal MV morphology (%)	107 (90.0)	89 (92.7)	18 (78.3)	0.039
Functional MR	91 (93.8)	70 (94.6)	20 (90.9)	0.531
MR severity				0.228
Nil	23 (19.3)	22 (22.9)	1 (4.4)	
Trivial	12 (10.1)	9 (9.4)	3 (13.0)	
Mild	41 (34.5)	31 (32.3)	10 (43.5)	
Moderate	32 (26.8)	24 (25.0)	8 (34.8)	
Severe	11 (9.2)	10 (10.4)	1 (4.4)	

Data presented as mean ± standard deviation, median (Interquartile range) or number (Percentage); HFrEF (Heart failure with reduced ejection fraction), SR (Sinus rhythm), AF (Atrial fibrillation), LVEF (Left ventricular ejection fraction), IVSD (Interventricular septal diameter), LVIDd (Left ventricular internal diameter in diastole), LVIDs (Left ventricular internal diameter in systole), LVPWD (Left ventricular posterior wall dimension at end-diastole) LV (Left ventricle), LA (Left atrium), E (Peak early diastolic mitral inflow velocity), e' (Early diastolic mitral annular tissue velocity), a (Peak late diastolic mitral inflow velocity), MV (Mitral valve), MR (Mitral regurgitation).

Left atrial strain reservoir function (%) was significantly lower in patients with HFrEF-AF (29.00 ± 13.60) compared to those with HFrEF-SR (41.56 ± 15.46) (p < 0.001) (Table 3.4b).

Table 3.4b 2D speckle tracking strain measurements categorised according to heart rhythm.

Variable	Rhythm			p-value
	Total (N= 119)	HFrEF-SR (n=93)	HFrEF-AF (n=23)	
Global longitudinal strain (%)	-7.3 (-9.3; -4.6)	-7.3 ± 3.8	-	
LA reservoir function (LASR) [#]	39.5 (26.7; 50.9)	42.5 (30.1; 54.4)	26.2 ± 11.3	<0.001
LA conduit function	18.6 (11.4; 27.1)	18.8 (12.5; 27.6)	-	-
LA pump function (LASP)	26.9 ± 13.0	27.2 ± 13.3	-	-

Data presented as mean ± standard deviation, median (interquartile range); HFrEF (Heart failure with reduced ejection fraction), SR (Sinus rhythm), AF (Atrial fibrillation);

[#] Measurement conducted in both SR and AF patients

HFrEF-AF patients had significant right ventricular (RV) remodelling (basal RV dimension: 46.7 ± 7.2; mid RV dimension: 34.7 [29; 38]) compared to those without AF ((basal RV dimension: 41.5 ± 9.2; mid RV dimension: 29.5 [26; 34])). Patients in the HFrEF- AF group had significantly greater right atrial volumes compared to the SR group (75.1 (64.4; 122.5) vs 42.1 (29.4; 65), $p < 0.001$). The systolic pulmonary artery pressures did not differ significantly between the two groups (Table 3.4c).

Table 3.4c Right ventricular, right atrial and pulmonary echocardiographic parameters categorised according to the presence (SR) and absence of atrial fibrillation.

Variable	Rhythm			p-value
	Total (N= 119)	HFrEF-SR (n=93)	HFrEF-AF (n=23)	
Basal RV Dimension (mm)	42.5 ± 9.0	41.5 ± 9.2	46.7 ± 7.2	0.009
Mid RV Dimension (mm)	30.5 (26; 36)	29.5 (26; 34)	34.7 (29; 38)	0.024
RV wall thickness (mm)	7 (6; 9)	8 (6; 9)	7 (5.5; 8)	0.217
Pulmonary artery Diameter (mm)	22 (20; 24)	22 (20; 24)	22.3 ± 4.5	0.788
Normal tricuspid valve	115 (98.3)	93 (98.9)	22 (95.7)	0.276
RA Volume	46.3 (32; 75.1)	42.1 (29.4; 65)	75.1 (64.4; 122.5)	<0.001
TAPSE (mm)	16.0 ± 4.2	16.3 ± 4.3	14.9 ± 3.5	0.175
Tricuspid Regurgitation				<0.001
Nil	34 (28.6)	32 (33.3)	2 (8.7)	
Trivial	17 (14.3)	16 (16.7)	1 (4.4)	
Mild	31 (26.1)	26 (27.1)	5 (21.7)	
Moderate	23 (19.3)	10 (10.4)	13 (56.5)	
Severe	14 (11.7)	12 (12.5)	2 (8.7)	
TR Vmax (m/sec)	2.6 (2.4; 3.1)	2.6 (2.4; 2.9)	3.0 (2.5; 3.2)	0.109
Systolic Pulmonary artery pressure (mmHg)	38.8 ± 14.2	37.6 ± 14.5	41.6 ± 13.6	0.242

Data presented as mean ± standard deviation, median (Interquartile range) or number (Percentage); HFrEF (Heart failure with reduced ejection fraction), SR (Sinus rhythm), AF (Atrial fibrillation), RV (Right ventricle), RA (Right atrium), TAPSE (Tricuspid annular plane systolic excursion), TR Vmax (Tricuspid regurgitation peak velocity at tricuspid valve).

3.4.5 Outcomes

The overall study time at risk was 42 months, and the median was 30.63 months. A total of 37 patients died during the study period. Of patients who died, 40% had concomitant AF (p = 0.001) (Supplementary Table 1a.) The overall probability of survival at the end of the study was 0.66 (0.56-0.75). The probability of survival at 42 months in SR and AF was 0.73 (0.61-0.82) and 0.41 (0.22-0.61) (p < 0.000), log-rank) respectively. The median survival time for patients in AF was

27.1 months. The majority of patients in SR survived beyond the median survival time at risk (Figures 3.2 & 3.3).

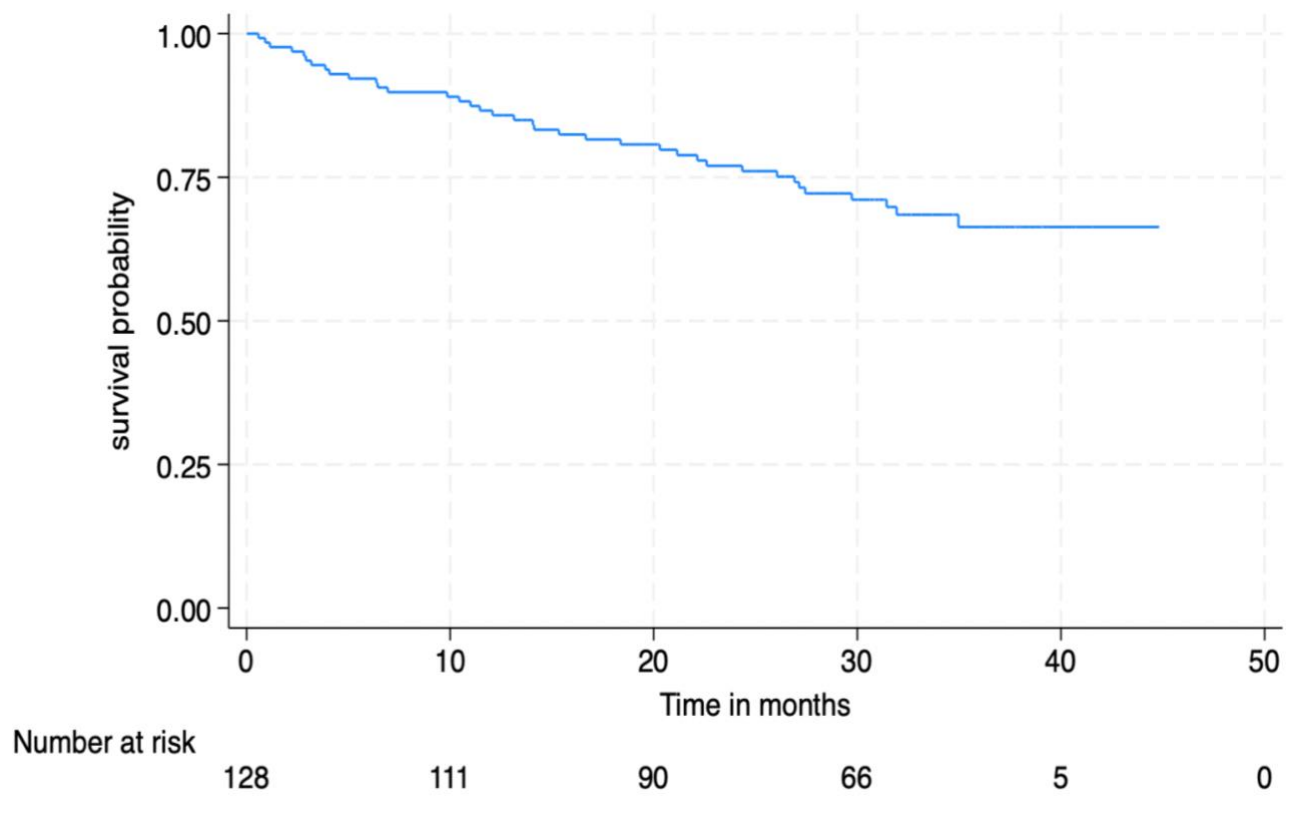


Figure 3.2 A Kaplan Meier plot illustrating the overall survival rate of patients.

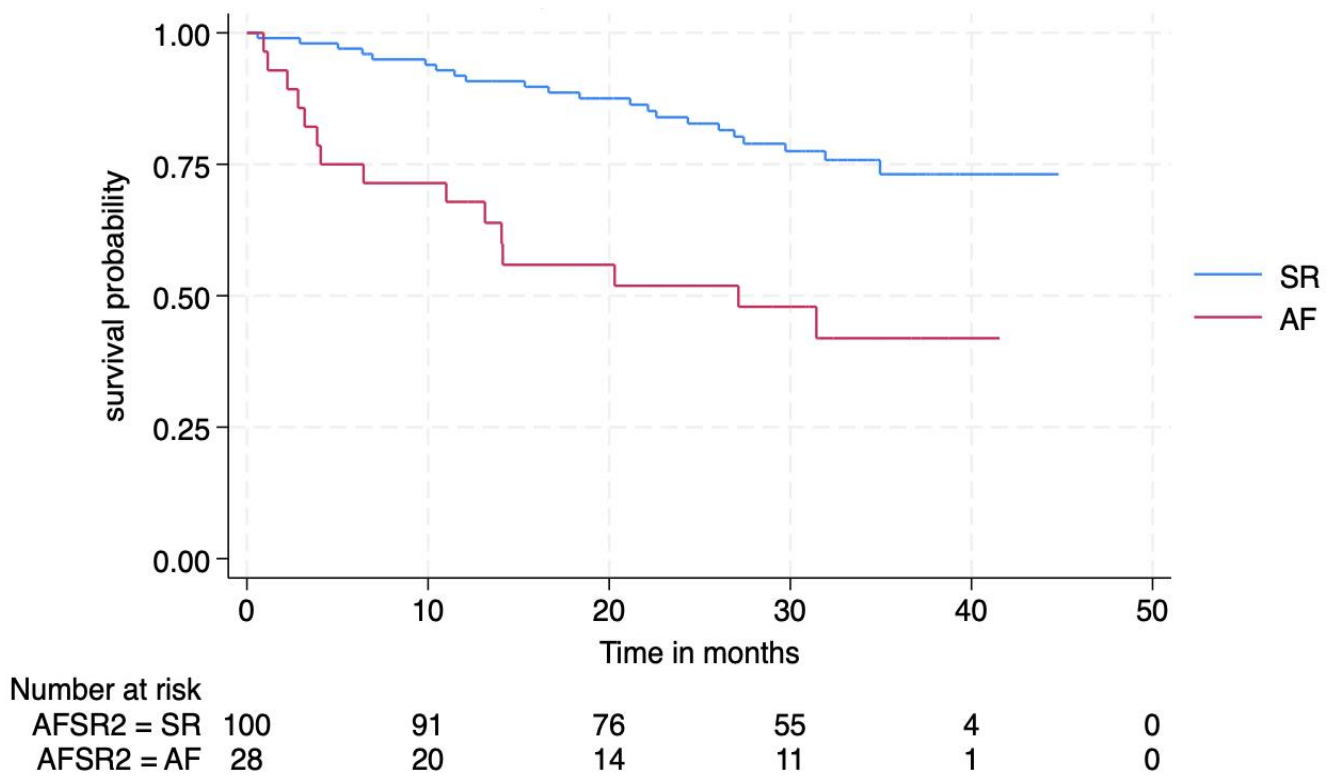


Figure 3.3 A Kaplan Meier plot illustrating the survival rate of patients with atrial fibrillation (red) compared to those in sinus rhythm (blue) ($p < 0.001$, log rank).

The survival rate of patients with HFrEF-SR at 12 months was 0.92 (CI: 0.84-0.96) and that of HFrEF-AF patients was 0.68 (CI:0.47-0.82) (Table 3.5).

Table 3.5 Survival rate of patients over 3-month intervals within the first year of survival time at risk.

Survival time at Risk (Months)	<i>Survival Rate (95%CI)</i>
HFrEF-SR	
3	0.98 (0.92-0.99)
6	0.97 (0.91-0.99)
9	0.95 (0.88-0.98)
12	0.92 (0.84-0.96)
15	0.91(0.83-0.95)
HFrEF-AF	
3	0.86 (0.66-0.94)
6	0.75 (0.55-0.87)
9	0.71 (0.51-0.85)
12	0.68 (0.47-0.82)
15	0.57 (0.37-0.73)

HFrEF (Heart failure with reduced ejection fraction), SR (Sinus rhythm), AF (Atrial fibrillation), CI (Confidence interval)

In a multivariate logistic regression model ($R^2=0.43$, $p < 0.001$) tricuspid annular plane systolic excursion (TAPSE), heart rate, early diastolic mitral inflow velocity/ early diastolic mitral annular tissue velocity ratio (E/e') and prior stroke were independently associated with all-cause mortality (Table 3.6). A higher heart rate (Odds ratio (OR) =1.05 [1.00- 1.10], $p = 0.028$) and E/e' (1.24 [1.05- 1.47], $p = 0.012$), a measure of diastolic dysfunction, were associated with increased odds of death. A prior history of stroke was a strong predictor of death (OR= 129.49 [2.44-1503], $p = 0.014$). Conversely, elevated TAPSE (OR = 0.77 [0.59-0.99], $p = 0.039$), a surrogate marker for right ventricular function, was protective.

Table 3.6 Univariate and multivariable logistic regression analysis for predictors of all- cause mortality

Variable	Univariate		*Multivariate	
	Odds Ratio (95% CI)	p- value	Odds Ratio (95% CI)	p- value
Age	1.03(1.004-1.06)	0.022		
Heart rate	1.04 (1.01-1.07)	0.002	1.05 (1.00- 1.10)	0.028
KCCQ	0.94 (0.92-0.97)	<0.001		
ADHF	4.5 (1.94-10.4)	<0.001		
Diabetes	2.1 (0.89-4.8)	0.088		
Previous stroke	4.3 (1.1-16.1)	0.033	129.5 (2.44-1503)	0.014
NT-PROBNP	1.00 (1.00-1.00)	0.031		
eGFR	0.97 (0.96-0.99)	0.004		
Creatinine	1.01 (1.00-1.02)	0.015		
Chronic kidney disease	3.2 (1.3-8.0)	0.013		
LVEF	0.93 (0.89-0.98)	0.002		
LVIDS	1.04 (1.01-1.09)	0.025		
E/e'	1.1 (1.02-1.2)	0.018	1.24 (1.05- 1.47)	0.012
Basal RV dimension	1.1 (1.0-1.1)	0.003		
TAPSE	0.79 (0.7-0.9)	0.001	0.77 (0.59- 99)	0.039
RA volume	1.01 (1.00-1.02)	0.006		
Systolic pulmonary artery pressure	1.03 (0.99-1.1)	0.127		
LA reservoir strain	0.93 (0.91-0.97)	0.001		

BMI (Body mass index), KCCQ (Kansas City Cardiomyopathy Questionnaire), MR (Mitral regurgitation), ADHF (Acute decompensated heart failure), NT-proBNP (N-terminal pro natriuretic peptide), eGFR (Estimated glomerular filtration rate), LVEF (Left ventricular ejection fraction), LVIDs (Left ventricular internal diameter in systole), LA (Left atrium), E (Early diastolic mitral inflow velocity), e' (Early diastolic mitral annular tissue velocity), RV (Right ventricle), TAPSE (Tricuspid annular plane systolic excursion), RA (Right atrium), GLS (Global longitudinal strain).

*Backwards elimination using a p-value = 0.15

In an age-adjusted regression model, KCCQ score, heart rate, E/e', and prior stroke were significantly associated with all-cause mortality ($R^2 = 0.40$, $p < 0.001$). A higher quality of life in the two weeks preceding presentation (as indicated by a lower KCCQ score) was protective (OR =

0.94 [0.90–0.99], $p = 0.026$). TAPSE showed a trend toward being protective but did not reach statistical significance ($p = 0.070$) (Table 3.7)

Table 3.7 Age-adjusted multivariate logistic regression model of variables associated with all-cause mortality.

Variables	Univariate		Multivariate	
	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value
Age	1.03 (1.0-1.06)	0.022	0.98 (0.92- 1.04)	0.965
TAPSE	0.79 (0.7-0.9)	0.001	0.80 (0.64- 1.02)	0.070
Heart rate	1.04 (1.0-1.1)	0.002	1.04 (1.00- 1.09)	0.047
E/e'	1.1 (1.02-1.2)	0.018	1.28 (1.05- 1.54)	0.014
Previous stroke	4.3 (1.1-16.1)	0.033	443 (3.07- 641)	0.016
KCCQ	0.94 (0.92-0.97)	<0.001	0.94 (0.89- 0.99)	0.026

CI (Confidence interval), TAPSE (Tricuspid annular plane systolic excursion), E (Early diastolic mitral inflow velocity), e' (Early diastolic mitral annular tissue velocity), KCCQ (Kansas City Cardiomyopathy Questionnaire).

3.5 DISCUSSION

This study compared the clinical characteristics of South African patients with HFrEF-SR and HFrEF-AF as well as the factors that were associated with their mortality over a 30 month period. Our findings demonstrate that (1) Patients with HFrEF-AF were significantly older than patients in SR; (2) The estimated prevalence of HFrEF-AF was comparatively low (21%) in this cohort; (3) Patients with HFrEF-AF had advanced structural heart disease compared to those in SR, (4) Patients with HFrEF-AF, in South Africa, receive less intensive pharmacotherapy and is administered at lower doses compared to their SR counterparts.. (5) Patients with HFrEF-AF had higher morbidity and mortality compared to their SR counterparts.

The mean age of HFrEF patients was 58.7 ± 14.9 years, which is comparable to findings in other SSA countries (21, 22). Patients with HFrEF-AF were significantly older than those in SR by over a decade, consistent with global trends (23). Our cohort of HFrEF-AF patients was significantly younger than that of their North American and European counterparts, despite a similar trend towards advanced age (23-25). We postulate that this is largely attributable to the high prevalence of a more malignant form of long-standing hypertension in Black South Africans (26). Hypertension is

an established etiology of HFrEF. Given the well-documented genetic predisposition to salt sensitivity in individuals of black African ancestry as well as increased dietary salt intake, hypertension manifests at a younger age and presents with greater severity (27, 28). Furthermore, patients in this demographic are more likely to present with hypertension-mediated organ damage compared to other racial groups (26, 29). An overactive Renin-Angiotensin-Aldosterone System (RAAS) and sympathetic nervous system are pathophysiological hallmarks of salt sensitivity as well as HFrEF, potentially exacerbating disease progression (30, 31). This is further compounded by poor blood pressure control and limited access to healthcare in our setting (7, 32, 33). Approximately 15.4% of individuals achieve adequate blood pressure control in South Africa, a figure significantly lower compared to other regions globally (7, 32, 33). A recent study demonstrated that the index presentation of hypertension-mediated organ damage in SSA is approximately 15 years younger than in patients in HICs (5, 22). Thus, HFrEF is often a manifestation of severe, long-standing, poorly controlled hypertension.

The prevalence of HFrEF-AF within this cohort of HFrEF patients in the Department of Cardiology at CMJAH was comparable to other low- to middle-income countries (12-20%) but markedly lower than that of HICs (~49%) (21, 23). Our findings suggest that the burden of HFrEF-AF, in South Africa, is a function of advanced, severe HF rather than advancing age. The prevalence of HFrEF-AF, in HICs, is largely driven by advanced age (34). This is supported by a longer average life expectancy of approximately 80 years in HICs compared to approximately 60 years in South Africa, as well as a larger geriatric population compared to SSA (35). In addition to this, we postulate that a large proportion of our patients may succumb to mortality before reaching a tertiary institution or, although known to our facility, may be critically ill and demise outside of the institution, resulting in loss to follow-up. The significantly lower KCCQ score and low survival rate within the first 12 months observed in patients with HFrEF-AF in this study lend support to this hypothesis. The ARIC study showed that African Americans had a 41% lower age-adjusted and gender-adjusted risk of AF compared with Caucasians, despite a greater risk factor burden amongst African Americans (36). A similar racial variation was observed in the United Kingdom (37). This may also be a contributing factor to the low AF prevalence observed in our cohort. These observations, highlight the potential complexity of the underlying pathophysiological mechanisms of AF in the setting of HFrEF. Exploring these potential pathophysiological undertones may provide valuable insights for managing this unique demographic.

The presence of AF in HFrEF was associated with a more advanced form of HFrEF as evidenced by significantly lower KCCQ scores, higher functional class (NYHA), worse echocardiographic indices of disease severity such as LA volume, strain and extensive right ventricular and atrial remodelling as well as numerically higher levels of NT-proBNP in comparison to those in SR. Our findings suggest that these patients with HFrEF-AF have extensive structural heart disease, severe symptomatology and are more likely to deteriorate rapidly. A similar impact of AF in HFrEF with respect to quality of life and mortality is observed globally (34, 38, 39). The onset of AF in HFrEF is facilitated by adverse structural and electrophysiological remodelling of the atria. Furthermore, AF exacerbates these pathophysiological processes, via increased ventricular heart rate, loss of atrial systolic function and subsequent decrease in cardiac output eliciting an increase in HF symptoms, worsening cardiac function, morbidity and mortality (34, 40).

Despite representing a subgroup with greater morbidity and severity, patients with HFrEF-AF received significantly less guideline-directed medical therapy (GDMT) compared to their counterparts in SR. Beta-blockers were the most frequently prescribed agents overall but their use was significantly lower in HFrEF-AF patients compared to those in SR, and the median carvedilol dose substantially lower in the AF group. A similar trend was observed for angiotensin converting enzyme inhibitors (ACE-I) and spironolactone. This is clinically important, as GDMT including β -blockers, has been shown to reduce mortality and improve functional status in HFrEF, regardless of rhythm status (8). Suboptimal GDMT titration in HFrEF-AF may reflect clinical inertia in complex multimorbid patients, hesitancy to initiate or up-titrate GDMT in the context of acutely decompensated patients due to hemodynamic instability, renal dysfunction or fear of low tolerance amongst AF patients (41). Furthermore, the significantly higher use of oral anticoagulation (OAC) in HFrEF-AF patients reflects appropriate stroke prevention measures. However, the finding that only 60% of HFrEF-AF patients were anticoagulated, despite clear indication, suggests barriers to implementation of OAC or possible contraindications. Factors may include concerns regarding bleeding risk, poor INR control with warfarin (the only available OAC in the public sector at the time of enrolment), adherence challenges, limited access to regular monitoring, and healthcare provider caution (42). These pharmacological disparities, may partially explain the excess morbidity and mortality observed in the HFrEF-AF patient group and highlight potential opportunities to strengthen inpatient and early outpatient optimisation of GDMT.

The observation that RA volume was significantly larger in the HFrEF-AF cohort compared to the SR group despite no significant difference in pulmonary artery pressures (PAP) or HF duration,

merits discussion. The Pulmonary artery systolic pressure (PASP) estimated via echocardiography is a surrogate measure which does not equate to the invasive haemodynamic gold-standard measurement obtained via right heart catheterisation (43, 44). In cases of advanced RV dysfunction, the RV loses its ability to generate high systolic pressures despite progressive dilation and remodelling; as a result, PAPs may appear relatively “normal” or inadequately elevated despite severe underlying disease (45). In this context, significant RA enlargement may reflect longstanding volume and pressure load, atrial remodelling and congestion, even though the RV can no longer mount a full pressure response. The “duration” of HF measured in this study was based on the time from the index presentation with acute decompensated heart failure to the study enrolment. This approach likely underestimates the true chronicity of disease, particularly in patients with AF, who may have had prolonged subclinical or untreated disease. Often, the onset of AF serves as the “precipitant” that propels the patient into symptomatic ADHF. Consequently, these patients may present later in the disease trajectory with advanced atrial and right-side remodelling, yet still demonstrate modest PAP estimates on echocardiography. Collectively, the discordance between RA enlargement and “normal” PAPs in this cohort may thus reflect advanced right-sided remodelling and methodological limitations of non-invasive pressure estimation, rather than a less severe physiology in the HFrEF-AF group.

Individuals with HFrEF-AF had a significantly reduced survival rate compared to patients in SR. This finding supports the hypothesis that patients with HFrEF-AF present with more severe clinical manifestations. In this cohort, a large percentage of patients with HFrEF-AF presented in acute decompensated HF, and succumbed to the outcome of all-cause mortality within the first year. The evidence regarding whether AF itself is an independent predictor of all-cause mortality in HFrEF is inconsistent. A large European multinational long-term registry reported that AF was not independently associated with all-cause mortality (34). It is worth noting however, that given our study design, we could not determine this.

In light of the extent of structural heart damage as well as clinical severity in patients with HFrEF-AF, other variables better performed in predicting the outcome of all-cause mortality. Worsening diastolic dysfunction, increasing heart rate, prior stroke and lower quality of life two weeks prior to presentation were independently associated with an increased risk of all-cause mortality, while better right ventricular function was protective. These findings support the view that in the setting of HFrEF, AF serves as a marker of disease severity rather than as an independent predictor of mortality. To the best of our knowledge, this is the first study of its kind in SSA to focus

on this specific subset of HF patients. There are few studies in SSA that have been able to establish AF as an independent predictor of all-cause mortality (41, 42). While these studies had a much larger sample size, their populations were predominantly heterogeneous HF (Malamba et al) and AF populations (Mandi et al.) (41, 42). Furthermore, there is now recent evidence to suggest a much more significant burden of AF in heart failure with preserved ejection fraction (34, 43).

Clinical Perspective and Future directions

To the best of our knowledge, this study is the first prospective observational study to report on the clinical impact of AF on HFrEF in SSA patients. The study highlights the complex interplay between AF and HFrEF within a South African cohort. Our findings suggest that AF in the setting of HFrEF reflects a more advanced stage of cardiac dysfunction, characterised by structural remodelling, poorer functional status, and higher mortality. These observations have important clinical and public health implications, particularly in resource-limited settings where hypertension and late presentation remain major contributors to the burden of HFrEF.

Additional large-scale, racially diverse multi-centre prospective studies are required to more accurately define the actual clinical impact of AF in HFrEF within this high-risk population and to determine whether the lower observed prevalence of HFrEF-AF is due to underdiagnosis, differences in access to healthcare, or population-specific characteristics. The pharmacotherapy gaps, particularly in high-risk patients, underscore the need for early rhythm-specific treatment optimisation during hospitalisation and structured follow-up in outpatient settings. Further investigation into the impact of guideline-directed medical therapy, catheter ablation and anticoagulation strategies in this population could provide insights into improving clinical outcomes. Should these findings be replicated in subsequent studies, they may support the necessity for earlier and more aggressive interventions in this demographic of patients.

Limitations

Our study had several limitations. The main limitation of the study was a small sample size. The recruitment of patients for the current study was halted due to the COVID-19 pandemic lockdown regulations. Moreover, the pandemic itself may have influenced the study's mortality outcomes and completeness of follow-up data. Individuals with HFrEF were at an increased risk of severe COVID-19 infection and related complications, potentially contributing to higher mortality rates

within this cohort. This introduces potential bias and limits the generalisability of our findings to a pre-pandemic HFrEF population. In the detection of AF, we utilized standard 12-lead ECG and did not conduct prolonged cardiac rhythm monitoring using holter monitors or loop recorders, which may have led to underdiagnosis of paroxysmal AF. The predominance of permanent AF in our cohort may have further influenced mortality. The study was a single-centre study done at a tertiary institution, and hence, our findings may not be generalisable to the general population.

Conclusion

Our study has demonstrated that AF is common in patients with HFrEF but comparatively lower than reported in HICs. When present, AF was associated with a more severe clinical presentation, greater structural heart disease, and higher mortality in comparison to patients in SR. These findings highlight the importance of recognising AF as a marker of disease severity in patients with HFrEF rather than as an independent predictor of mortality.

3.6 REFERENCES

- Chugh SS, Havmoeller R, Narayanan K, Singh D, Rienstra M, Benjamin EJ, et al. Worldwide epidemiology of atrial fibrillation: A global burden of disease 2010 study. *Circulation*. 2014;129(8):837-47.
2. Agbor VN, Essouma M, Ntusi NAB, Nyaga UF, Bigna JJ, Noubiap JJ. Heart failure in sub-Saharan Africa: A contemporaneous systematic review and meta-analysis. *Int J Cardiol*. 2018;257:207-15.
 3. Mogensen UM, Jhund PS, Abraham WT, Desai AS, Dickstein K, Packer M, et al. Type of Atrial Fibrillation and Outcomes in Patients With Heart Failure and Reduced Ejection Fraction. *Journal of the American College of Cardiology*. 2017;70(20):2490-500.
 4. Bahrami H, Kronmal R, Bluemke DA, Olson J, Shea S, Liu K, et al. Differences in the incidence of congestive heart failure by ethnicity: the multi-ethnic study of atherosclerosis. *Arch Intern Med*. 2008;168(19):2138-45.
 5. Bibbins-Domingo K, Pletcher MJ, Lin F, Vittinghoff E, Gardin JM, Arynchyn A, et al. Racial differences in incident heart failure among young adults. *N Engl J Med*. 2009;360(12):1179-90.
 6. Mou L, Norby FL, Chen LY, O'Neal WT, Lewis TT, Loehr LR, et al. Lifetime Risk of Atrial Fibrillation by Race and Socioeconomic Status: ARIC Study (Atherosclerosis Risk in Communities). *Circ Arrhythm Electrophysiol*. 2018;11(7):e006350.
 7. Yuyun MF, Sliwa K, Kengne AP, Mocumbi AO, Bukhman G. Cardiovascular Diseases in Sub-Saharan Africa Compared to High-Income Countries: An Epidemiological Perspective. *Glob Heart*. 2020;15(1):15.
 8. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. *European Heart Journal*. 2016;37(27):2129-200.
 9. Van Gelder IC, Rienstra M, Bunting KV, Casado-Arroyo R, Caso V, Crijns H, et al. 2024 ESC Guidelines for the management of atrial fibrillation developed in collaboration with the European Association for Cardio-Thoracic Surgery (EACTS). *Eur Heart J*. 2024;45(36):3314-414.
 10. Faller H, Steinbuechel T, Schowalter M, Spertus JA, Stork S, Angermann CE. [The Kansas City Cardiomyopathy Questionnaire (KCCQ) -- a new disease-specific quality of life measure for patients with chronic heart failure]. *Psychother Psychosom Med Psychol*. 2005;55(3-4):200-8.
 11. Olesen JB, Torp-Pedersen C, Hansen ML, Lip GY. The value of the CHA2DS2-VASc score for refining stroke risk stratification in patients with atrial fibrillation with a CHADS2 score 0-1: a nationwide cohort study. *Thromb Haemost*. 2012;107(6):1172-9.
 12. Marwick TH, Gillebert TC, Aurigemma G, Chirinos J, Derumeaux G, Galderisi M, et al. Recommendations on the Use of Echocardiography in Adult Hypertension: A Report from the European Association of Cardiovascular Imaging (EACVI) and the American Society of Echocardiography (ASE). *J Am Soc Echocardiogr*. 2015;28(7):727-54.
 13. Guzzo-Merello G, Cobo-Marcos M, Gallego-Delgado M, Garcia-Pavia P. Alcoholic cardiomyopathy. *World J Cardiol*. 2014;6(8):771-81.

14. Piano MR. Alcoholic cardiomyopathy: incidence, clinical characteristics, and pathophysiology. *Chest*. 2002;121(5):1638-50.
15. Arbelo E, Protonotarios A, Gimeno JR, Arbustini E, Barriales-Villa R, Basso C, et al. 2023 ESC Guidelines for the management of cardiomyopathies. *Eur Heart J*. 2023;44(37):3503-626.
16. Levey AS, Coresh J, Greene T, Stevens LA, Zhang YL, Hendriksen S, et al. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Intern Med*. 2006;145(4):247-54.
17. Camm AJ, Kirchhof P, Lip GYH, Schotten U, Savelieva I, Ernst S, et al. Guidelines for the management of atrial fibrillation. *European Heart Journal*. 2010;31(19):2369-429.
18. Badano LP, Kolas TJ, Muraru D, Abraham TP, Aurigemma G, Edvardsen T, et al. Standardization of left atrial, right ventricular, and right atrial deformation imaging using two-dimensional speckle tracking echocardiography: a consensus document of the EACVI/ASE/Industry Task Force to standardize deformation imaging. *Eur Heart J Cardiovasc Imaging*. 2018;19(6):591-600.
19. Donal E, Lip GY, Galderisi M, Goette A, Shah D, Marwan M, et al. EACVI/EHRA Expert Consensus Document on the role of multi-modality imaging for the evaluation of patients with atrial fibrillation. *Eur Heart J Cardiovasc Imaging*. 2016;17(4):355-83.
20. Kim JH. Multicollinearity and misleading statistical results. *Korean J Anesthesiol*. 2019;72(6):558-69.
21. Mboweni N, Maseko M, Tsabedze N. Heart failure with reduced ejection fraction and atrial fibrillation: a Sub-Saharan African perspective. *ESC Heart Fail*. 2023;10(3):1580-96.
22. Damasceno A, Mayosi BM, Sani M, Ogah OS, Mondo C, Ojji D, et al. The causes, treatment, and outcome of acute heart failure in 1006 Africans from 9 countries: Results of the sub-Saharan Africa survey of heart failure. *Archives of Internal Medicine*. 2012;172(18):1386-94.
23. Kotecha D, Chudasama R, Lane DA, Kirchhof P, Lip GY. Atrial fibrillation and heart failure due to reduced versus preserved ejection fraction: A systematic review and meta-analysis of death and adverse outcomes. *Int J Cardiol*. 2016;203:660-6.
24. Veenis JF, Brunner-La Rocca HP, Linssen GCM, Smeele FJJ, Wouters N, Westendorp PHM, et al. Atrial fibrillation in chronic heart failure patients with reduced ejection fraction: The CHECK-HF registry. *Int J Cardiol*. 2020;308:60-6.
25. Justin D, Mark RAC, Carlos E. Alfonso, Alexander Llanos, Elias Collado, Joshua M. Larned, German Giese, Michael D. Dyal, Michael G. Nanna, Abdulla A. Damluji. The Impact of Frailty on Patients With AF and HFrEF Undergoing Catheter Ablation: A Nationwide Population Study. *JACC: Advances*. 2024;3(11).
26. Rayner B. Hypertension: detection and management in South Africa. *Nephron Clin Pract*. 2010;116(4):c269-73.
27. Ware LJ, Charlton K, Schutte AE, Cockeran M, Naidoo N, Kowal P. Associations between dietary salt, potassium and blood pressure in South African adults: WHO SAGE Wave 2 Salt & Tobacco. *Nutr Metab Cardiovasc Dis*. 2017;27(9):784-91.

28. Schutte AE, Botha S, Fourie CMT, Gafane-Matemane LF, Kruger R, Lammertyn L, et al. Recent advances in understanding hypertension development in sub-Saharan Africa. *J Hum Hypertens*. 2017;31(8):491-500.
29. Gafane-Matemane LF, Craig A, Kruger R, Alaofin OS, Ware LJ, Jones ESW, et al. Hypertension in sub-Saharan Africa: the current profile, recent advances, gaps, and priorities. *J Hum Hypertens*. 2024.
30. Maaliki D, Itani MM, Itani HA. Pathophysiology and genetics of salt-sensitive hypertension. *Front Physiol*. 2022;13:1001434.
31. Schwinger RHG. Pathophysiology of heart failure. *Cardiovasc Diagn Ther*. 2021;11(1):263-76.
32. Yoruk A, Boulous PK, Bisognano JD. The State of Hypertension in Sub-Saharan Africa: Review and Commentary. *Am J Hypertens*. 2018;31(4):387-8.
33. Kandala NB, Nnanatu CC, Dukhi N, Sewpaul R, Davids A, Reddy SP. Mapping the Burden of Hypertension in South Africa: A Comparative Analysis of the National 2012 SANHANES and the 2016 Demographic and Health Survey. *Int J Environ Res Public Health*. 2021;18(10).
34. Zafir B, Lund LH, Laroche C, Ruschitzka F, Crespo-Leiro MG, Coats AJS, et al. Prognostic implications of atrial fibrillation in heart failure with reduced, mid-range, and preserved ejection fraction: a report from 14 964 patients in the European Society of Cardiology Heart Failure Long-Term Registry. *Eur Heart J*. 2018;39(48):4277-84.
35. Victor Gaigbe-Togbe. Lina Bassarsky DG, Thomas Spoorenberg, Lubov Zeifman, Patrick Gerland, Sara Hertog, Mark C. Wheldon, Vladimira Kantorova, Giulia Gonnella, Ivan Williams, Guiomar Bay, Helena Cruz Castanheira, Yumiko Kamiya, . World Population Prospects 2022: Summary of results. United Nations Department of Economic and Social Affairs PD; 2022. Contract No.: UN DESA/POP/2022/TR/NO. 3.
36. Go AS, Hylek EM, Phillips KA, Chang Y, Henault LE, Selby JV, et al. Prevalence of diagnosed atrial fibrillation in adults: national implications for rhythm management and stroke prevention: the AnTicoagulation and Risk Factors in Atrial Fibrillation (ATRIA) Study. *JAMA*. 2001;285(18):2370-5.
37. Shen AY, Contreras R, Sobnosky S, Shah AI, Ichijui AM, Jorgensen MB, et al. Racial/ethnic differences in the prevalence of atrial fibrillation among older adults--a cross-sectional study. *J Natl Med Assoc*. 2010;102(10):906-13.
38. Santhanakrishnan R, Wang N, Larson MG, Magnani JW, McManus DD, Lubitz SA, et al. Atrial Fibrillation Begets Heart Failure and Vice Versa. *Circulation*. 2016;133(5):484-92.
39. Horodinschi RN, Diaconu CC. Comorbidities Associated with One-Year Mortality in Patients with Atrial Fibrillation and Heart Failure. *Healthcare*. 2021;9(7):830-.
40. Nattel S, Harada M. Atrial remodeling and atrial fibrillation: recent advances and translational perspectives. *J Am Coll Cardiol*. 2014;63(22):2335-45.
41. Mbanze I, Spracklen TF, Jessen N, Damasceno A, Sliwa K. Heart failure in low-income and middle-income countries. *Heart*. 2025;111(8):341-51.
42. Ebrahim I, Bryer A, Cohen K, Mouton JP, Msemburi W, Blockman M. Poor anticoagulation control in patients taking warfarin at a tertiary and district-level prothrombin clinic in Cape Town, South Africa. *S Afr Med J*. 2018;108(6):490-4.

43. Seyyedi SR, Mozafari M, Sharif-Kashani B, Sadr M, Emami H, Mehrzmay A. Correlation of Echocardiographic and Right Heart Catheterization Estimations of Pulmonary Artery Systolic Pressure. *Tanaffos*. 2022;21(1):78-84.
44. Herrmann EJ, Herrmann E, Tello K, Mantzsch K, Tekeste M, Fichtlscherer S, et al. A Word of Caution-Potential Limitations of Pulmonary Artery Pressure Monitoring in Detecting Congestion Caused by Right-Sided Heart Failure. *Biomedicines*. 2025;13(6).
45. Bosch L, Lam CSP, Gong L, Chan SP, Sim D, Yeo D, et al. Right ventricular dysfunction in left-sided heart failure with preserved versus reduced ejection fraction. *Eur J Heart Fail*. 2017;19(12):1664-71.
46. Malamba-Lez D, Ngoy-Nkulu D, Steels P, Tshala-Katumbay D, Mullens W. Heart Failure Etiologies and Challenges to Care in the Developing World: An Observational Study in the Democratic Republic of Congo. *J Card Fail*. 2018;24(12):854-9.
47. Germain Mandi D, Bamouni J, Aristide Yaméogo R, Temoua Naïbé D, Kaboré E, Kambiré Y, et al. Spectrum of heart failure in sub-Saharan Africa: data from a tertiary hospital-based registry in the eastern center of Burkina Faso. *Pan African Medical Journal*. 2020;36:1-11.
48. Son MK, Park JJ, Lim NK, Kim WH, Choi DJ. Impact of atrial fibrillation in patients with heart failure and reduced, mid-range or preserved ejection fraction. *Heart*. 2020;106(15):1160-8.

CHAPTER 4: BIOMARKER SIGNATURES OF FIBROSIS AND INFLAMMATION IN SOUTH AFRICAN PATIENTS WITH HFREF AND THEIR CLINICAL CORRELATES

4.1 ABSTRACT

Background: Heart failure (HF) with reduced ejection fraction (HFrEF) and atrial fibrillation (AF) frequently coexist, leading to worse outcomes. The use of cardiac biomarkers to phenotype these patients may provide valuable insights into the underlying pathophysiology and aid in better risk stratification of this high risk population for early therapeutic intervention. There is a paucity of data on the levels of standard and cardiac biomarkers in Sub-Saharan Africa (SSA) as well as their clinical and prognostic utility in HFrEF-AF patients. The current study aimed to determine the quantities of standard and novel cardiac biomarkers in South African HFrEF patients with AF and assess their association with all-cause mortality and clinical characteristics.

Methods: In this prospective, single-centre study, 136 HFrEF patients were enrolled. Patients were categorised into HFrEF with AF (HFrEF-AF) and those without AF, sinus rhythm (HFrEF-SR). One hundred and twenty-five plasma samples were analysed for concentrations of N-terminal pro B type natriuretic peptide (NT-proBNP), cardiac troponin T (cTnT), soluble suppression of tumorigenicity 2 (sST2), Galectin-3 (GAL-3), growth differentiation factor 15 (GDF-15), interleukin-6 (IL-6) and cathepsin-D using ELISA. Regression models were employed to determine biomarkers associated with the primary outcome of all-cause mortality and to identify clinical and echocardiographic determinants of each biomarker.

Results: Patients with HFrEF-AF had significantly higher levels of GDF-15, cathepsin-D, and sST2 compared to those with HFrEF-SR. Biomarker levels were also significantly elevated in patients with acute decompensated heart failure (ADHF). Right ventricular dysfunction was consistently associated with elevated levels of sST2, GDF-15, Cathepsin-D, and IL-6. Poorer patient-reported quality of life was linked to higher levels of IL-6, GDF-15, and sST2.

Conclusion: The present study has demonstrated that South African HFrEF-AF patients have a predominantly fibrotic and inflammatory biomarker profile. Clinical and echocardiographic measures of disease severity and poor quality of life closely correlate with systemic inflammatory and fibrotic biomarkers.

4.2 INTROCUCTION

Heart failure (HF) is a complex clinical syndrome characterized by the heart's inability to fill or pump blood efficiently to meet the body's metabolic demands (1, 2). This syndrome, resulting from underlying functional or structural impairment, is classified on the basis of left ventricular ejection fraction (LVEF). Heart failure with reduced ejection fraction (HFrEF) is defined by an LVEF of less than 40% and is predominantly characterized by systolic dysfunction (3). HFrEF, on its own, is associated with significant morbidity and mortality.

The pathophysiological mechanisms in HFrEF are complex, encompassing an index event that leads to direct damage of the cardiac muscle. Injury to the myocyte leads to the heart's inability to pump effectively (2). This initial decline in pump function triggers an upregulation of neurohormonal mechanisms which constitute increased sympathetic nervous system activity, increased renin-angiotensin-aldosterone system (RAAS) activity and activation of cytokine pathways (2). Sustained neurohormonal upregulation and hemodynamic (volume) overload leads to maladaptive remodelling of the LV. This cardiac remodelling is underpinned by wall stress and stretch which leads to fibrosis, myocyte hypertrophy, necrosis and apoptosis and resultant LV dilatation and concomitant LV dysfunction (2).

Atrial fibrillation (AF) frequently coexists with HFrEF, exacerbating these pre-existing pathophysiological processes (4). In HFrEF, elevated LV end diastolic pressures, due to pump dysfunction, lead to elevated left atrial pressures and hence atrial mechanical wall stretch and stress (4). This, similar to the LV, induces maladaptive remodelling in the atria, altering electrophysiological handling and hence sets the milieu for AF (4, 5). Once AF is present, there is loss of atrial contraction, an increase in HR, haemodynamic instability and neurohormonal activation is further upregulated (4, 5). The result of this is a superimposed worsening of atrial and overall structural heart remodelling, chronic inflammation and worsening cardiac function (6). Biomarkers reflecting these pathophysiological processes provide insights into disease severity, response to therapeutic interventions as well as prognosis.

Patients with HFrEF and AF have poorer outcomes and compared to those with sinus rhythm (SR), including more frequent hospitalisations, worsening structural heart disease, and increased mortality (7, 8). Moreover, they do not respond optimally to mortality benefitting medical therapy

(9). In SSA, we have previously demonstrated that HFrEF-AF patients experience rapid deterioration, underscoring the urgent need for improved risk stratification and early intervention.

4.2.1 The role of biomarkers in HFrEF and HFrEF-AF

Biomarkers have emerged as an essential tool in HFrEF management, offering prognostic, diagnostic, and therapeutic insights. With the advent of catheter ablation, biomarkers may aid in identifying HFrEF-AF patients likely to benefit from this intervention (10). The identification of high-risk patients through biomarkers could improve clinical decision-making in SSA, where resources remain scarce.

According to Ibrahim and Januzzi, an ideal biomarker for HF should be 1) Specific to the pathophysiological process; 2) Easily measurable with standardized assays; 3) Cost-effective and readily available; 4) Predictive of clinical outcomes; 5) Modifiable with targeted therapies (11, 12).

4.2.2 NT- proBNP (N-terminal pro-B-type natriuretic peptide)

Brain natriuretic peptide (BNP) and NT-proBNP are natriuretic peptides secreted by ventricular cardiomyocytes in response to pressure or volume overload (13). These peptides are synthesized from a pre-hormone encoded by the NPPB gene (13). Following enzymatic cleavage and processing by signal peptidase and furin or corin, respectively, BNP₁₋₃₂, the biologically active peptide, and NT-proBNP, the inactive N-terminal fragment, are produced (14). Under normal physiological conditions, both BNP and NT-proBNP levels remain low. However, in the context of HFrEF, they are released in an attempt to restore haemodynamic stability. Brain natriuretic peptide facilitates diuresis, natriuresis, arterial vasodilation, exhibits anti-fibrotic effects and counteracts an overactive sympathetic nervous system (SNS) and renin–angiotensin–aldosterone system (RAAS) in HF (13). N-terminal pro-B-type natriuretic peptide is passively excreted via the kidneys, whereas only 25% of BNP is excreted by the kidneys, with the remainder undergoing enzymatic degradation by neprilysin (15). Due to their different clearance mechanisms, NT-proBNP has a longer half-life and higher plasma concentration compared to BNP, thus making it an easier molecule to measure. N-terminal pro-B-type natriuretic peptide has emerged as a successful diagnostic and prognostic cardiac biomarker and has recently proven to be useful in monitoring response to HF therapies (3, 16). While NT-proBNP is considered the closest to an ideal biomarker (according to the Ibrahim and Januzzi's criteria), it is not without confounders. Levels of NT-proBNP increase in acute

decompensated heart failure (ADHF), advancing age and in cases of kidney dysfunction. Conversely, its levels decrease in obese patients (17). Studies done in the United States of America have also demonstrated racial differences in NT-proBNP levels in both normal and HFrEF patients, which remain unexplored (18). In the context of HFrEF-AF, the biomarker retains its negative prognostic value regardless of AF status (19)

4.2.3 Cardiac Troponin T (cTnT)

Cardiac Troponin T (cTnT) is a protein released during cardiomyocyte injury or death traditionally used to diagnose acute myocardial infarction (14). Proposed mechanisms include imbalances between oxygen supply and demand at the level of the sub-endocardium, as well as direct myocardial injury resulting from neurohormonal activation, oxidative stress and inflammation (14). Elevated levels of both standard and high-sensitivity cTnT (hs-cTnT) are predictive of worsening HF and all-cause mortality in patients with chronic and acute HFrEF, with hs-cTnT demonstrating greater sensitivity (20-22). In patients with HFrEF-AF, hs-cTnT identified those at risk of clinical deterioration when used in combined multi-marker panel with growth differentiation factor (GDF-15) and NT-proBNP (23, 24). Cardiac Troponin T's role as a prognostic marker in HFrEF is still understudied, necessitating further investigation (25).

4.2.4 Soluble Suppression of Tumorigenicity 2 (sST2)

Suppression of Tumorigenicity 2 (ST2) is a biomarker which belongs to the interleukin (IL)-1 receptor family with two main isoforms: a soluble form (sST2) and a membrane-bound receptor form, named Suppression of Tumorigenesis-2 ligand (ST2L) (26). Soluble ST2 is secreted by cardiomyocytes, cardiac fibroblasts, type 2 pneumocytes and various extracardiac tissues (27). Interleukin-33 (IL-33), a cytokine, released by stromal cells in both cardiac and extracardiac tissues, serves as a ligand for both sST2 and ST2L (28). The role of IL-33 as either a pro-inflammatory or anti-inflammatory cytokine is influenced by co-stimulatory factors (26). At a cardiac level, the binding of IL-33 to ST2L is cardioprotective, initiating a complex biochemical cascade that results in anti-hypertrophic, anti-apoptotic and anti-fibrotic effects (29). However, when the heart is subjected to injury or mechanical stress, sST2 is secreted from cardiomyocytes and cardiac fibroblasts and competes with ST2L for the IL-33 binding site, coining the term “decoy receptor” (29). This competition antagonises the cardioprotective effects of the IL-33/ST2L pathway, thereby contributing to myocardial fibrosis and remodelling (29). Soluble ST2 is not cardiac specific and

therefore cannot be used in the diagnosis of HFrEF. However, its prognostic utility as a measure of cardiac fibrosis and remodelling has become apparent in cardiovascular diseases (26). In the setting of chronic HFrEF, its prognostic utility is independent of cTnT, NT-proBNP and age suggesting potential additive value in a multi-marker model with other standard cardiac biomarkers (30). Increasing concentrations of sST2 (e.g >35ng/mL) have been strongly independently associated with adverse outcomes in patients with chronic HFrEF however there is no consensus on optimal prognostic cut-offs (30). In multivariable models, it has demonstrated additive prognostic value to NT-proBNP and superiority to GAL-3 in chronic HFrEF patients (30, 31). Population specific cut-offs and its utility in a multi-marker approach is undetermined in SSA.

4.2.5 Galectin- 3 (GAL-3)

Galectin-3 is a β -galactosidase-binding lectin. It is ubiquitously expressed across different tissues and is involved in various biological processes such as apoptosis, regulation of cell-cell and cell-matrix interactions, proliferation, adhesion, and inflammation (32). Galectin-3 is implicated in the pathological remodelling and fibrosis in HFrEF through its induction of fibroblast proliferation and collagen deposition (33). Unlike NT-proBNP, GAL-3 is not useful in the diagnosis of HFrEF but has shown prognostic utility in predicting re-hospitalisation and mortality in ADHF (34, 35). However, there is lack of strong evidence supporting its prognostic utility in HFrEF alone, as its performance is often surpassed by other biomarkers like NT-proBNP and ST2. In patients with HFrEF-AF, GAL-3 has been shown to predict which individuals may benefit from catheter ablation, a novel rhythm management strategy that offers superior mortality benefits compared to medical rate and rhythm control (36, 37). Patients with GAL-3 concentration less than 26 ng/mL were shown to benefit from catheter ablation (36). Similar to NT-proBNP, GAL-3 levels are elevated in cases of kidney dysfunction. The clinical utility and measured levels of GAL-3 in an African HFrEF-AF cohort remain undetermined. To the best of current knowledge, no studies have assessed this molecule in a HFrEF SSA population (38).

4.2.6 Growth differentiation factor-15 (GDF-15)

Growth Differentiation Factor-15 is a cytokine which belongs to the transforming growth factor- β superfamily and is secreted from various cells including cardiomyocytes in response to strain, ischemia and oxidative stress (39). It is therefore recognized as a biomarker in HF due to its association with oxidative stress, inflammation, and fibrosis. Numerous studies in HICs, where the

aetiology is predominantly ischemic, have demonstrated GDF's prognostic utility in HFrEF (40-42). While GDF-15 improves discrimination for mortality in some models, its incremental value is less pronounced when added to robust models that include other biomarkers and clinical variables (42). Studies on GDF-15 in HFrEF-AF are scarce. Atrial fibrillation often complicates HFrEF by increasing the risk of thromboembolic events and worsening cardiac function, which might be reflected in higher GDF-15 levels due to increased stress and inflammation. In patients with AF alone, on oral anticoagulation, from the ARISTOTLE trial, levels of GDF-15 were independently associated with the risk of major bleeding, stroke and mortality (43).

4.2.7 Cathepsin-D

Cathepsin-D is a lysosomal aspartic protease that is ubiquitously expressed and plays a role in protein degradation as well as the proteolytic activation of growth factors and hormones. It is released following oxidative stress (44). Although its role in cardiovascular disease remains largely unclear, it has been illustrated in a rat model of myocardial infarction (44). This study demonstrated that the upregulation of cathepsin-D was cardioprotective and prevented the subsequent development of HF. More recently, circulating levels of cathepsin-D have been associated with advanced disease and high mortality rates in a heterogeneous population of patients with HF (45). The same study demonstrated intracellular cathepsin-D release from human stem cell-derived cardiomyocytes under mechanical stress in concert with cTnT release. The silencing of cathepsin-D in this study further increased the release of cTnT under induced stress (45). These findings suggest intracellular cathepsin-D may be important for the survival of cardiomyocytes under mechanical stress and circulating levels may serve as a potential surrogate for disease severity (45). No association between AF and cathepsin-D has been reported. There is limited evidence for cathepsin-D in HFrEF and its clinical utility.

Each biomarker provides unique insights into HF progression, with potential implications for risk stratification and therapeutic targeting in HFrEF and potentially HFrEF-AF patients. A meta-analysis demonstrated a 20-30% reduction on mortality when a biomarker-guided management strategy was employed over standard care (46). Given the manifold and complex pathophysiological mechanisms involved in HFrEF, reliance on a single biomarker may be insufficient for comprehensive risk assessment. A multi-marker strategy integrates multiple pathological processes to improve and strengthen predictive accuracy, thereby enhancing individualised management approaches.

4.2.8 Knowledge gap in SSA

Heart failure with reduced ejection fraction presents a major health challenge in SSA, where it affects a younger demographic compared to high-income regions (9). The etiological spectrum of HFrEF in SSA is unique, often driven by non-ischemic causes such as hypertensive heart disease, idiopathic dilated cardiomyopathy and rheumatic heart disease.

Despite the well-recognised role of cardiac biomarkers in improving outcomes in HFrEF, data on their clinical utility in SSA populations is limited. To the best of our knowledge studies done in SSA have investigated established biomarkers such as NT-proBNP and cTnT however, none have looked at sST2, GAL-3, GDF-15 or Cathepsin-D (38). There is a pressing need to evaluate the clinical utility of both standard and novel biomarkers in this unique patient population.

This study aimed to quantify the levels of standard (NT-proBNP, cTnT) and novel cardiac biomarkers (sST2, GAL-3, GDF-15, cathepsin-D) in South African HFrEF-AF patients. Additionally, it aimed to determine their association with all-cause mortality and to identify clinical characteristics and echocardiographic parameters associated with levels of novel biomarkers in this population.

4.3 METHODS

4.3.1 Study design and Population

This was an observational, prospective, single-centre study. A total of 136 patients with HFrEF were consecutively enrolled from a specialised outpatient HF clinic and cardiac admission wards at Charlotte Maxeke Johannesburg Academic Hospital between June 2019 and December 2021.

A comprehensive clinical history and physical examination were performed on patients, including the measurement of height, weight, body mass index (BMI), systolic and diastolic blood pressures. Relevant clinical information, such as the age, race, estimated duration of HF, aetiology, and comorbid conditions, was obtained from patient medical records and corroborated through documented history and clinical findings. A focused history and physical examination were specifically performed to identify clinical signs (peripheral oedema, raised jugular venous pressure, pulmonary crackles on auscultation, third heart sound) and symptoms (dyspnoea, orthopnoea, paroxysmal nocturnal dyspnoea, reduced exercise tolerance, fatigue) indicative of acute

decompensated heart failure (ADHF). Additionally, all patients underwent a standard 12-lead electrocardiogram (ECG) to determine heart rate and rhythm.

4.3.2 Blood Sample collection and preparation

Two venous blood samples (3 mL each) were collected from each patient into EDTA-containing tubes (purple-top BD vacutainers; Becton Dickinson Diagnostics; New Jersey, USA). Immediately after collection, samples were placed in an insulated ice box and transported promptly to the laboratory. Samples were centrifuged at 3000 rpm for 10 minutes at 4°C to separate plasma. Plasma aliquots were transferred into cryogenic vials and stored at -80°C until subsequent biomarker analysis.

Patients were divided into two groups according to rhythm status; those with HFrEF in sinus rhythm (HFrEF-SR) and those with HFrEF and concomitant atrial fibrillation (HFrEF-AF). The numbers of patients with available biomarker levels were as follows: NT-proBNP = 125; sST2 = 124; GAL-3 = 123; cTnT = 122; GDF-15 = 125; cathepsin-D = 125; and IL-6 = 122.

4.3.3 Biomarker analysis

The plasma concentrations of the investigated biomarkers were analysed using commercial sandwich enzyme-linked immunosorbent assay (ELISA) kits with a colorimetric detection, obtained from the Elabscience (Wuhan, Hubei, China) and following the manufacturer's instructions. These assays included: sST2 (E-EL-H6082; assay range: 0.31-20 ng/mL, analytical sensitivity 0.19 ng/mL, precision CV (intra-assay ≤ 6.1 %, inter-assay ≤ 5.4%)), (GDF-15 (E-EL-H0080; assay range 23.44 – 1500pg/mL, anal. sens. 14.06 pg/mL, precision CV (intra-assay ≤ 5.9 %, inter-assay ≤ 6.2%)), NT-proBNP (E-EL-H6126; assay range 0.16 - 10 ng/mL, anal. sens. 0.09 ng/mL, precision CV (intra-assay ≤ 6.5 %, inter-assay ≤ 5.1 %)), cTnT (E-EL-H0646; assay range 15.63 – 1000pg/mL, anal. sens. 9.38 pg/mL, precision CV (intra-assay ≤ 6.1 %, inter-assay ≤ 5.3%)), IL-6 (E-EL-H0102; assay range 7.81 – 500 pg/mL, anal. sens. 4.69pg/mL, precision CV (intra-assay ≤ 6.7 %, inter-assay ≤ 6.6 %)), and cathepsin-D (E-EL-H0669; assay range 46.88 – 3000 pg/mL, anal. sens. 28.13 pg/mL, precision CV (intra-assay ≤ 5.5 %, inter-assay ≤ 6.6 %)). Further, the kit for GAL-3 (SEA303Hu; obtained from Cloud-Clone Corp., CCC-Texas, USA; assay range 0.156-10 ng/mL, analytical sensitivity 0.054 ng/mL, and precision CV (intra-assay <10 %; inter-assay <12 %)) was also sandwich ELISA on a similar principle as the Elabscience kits. Samples were

applied directly, results exceeding the assay range were repeated in appropriate dilutions. Biotin-avidin binding was applied in two subsequent incubation steps; biotinylated detection antibody was added first into the wells to create capture antibody-analyte-biotinylated antibody complex. Avidin-enzyme conjugate was applied next. Unbound fractions were removed by washing after each incubation step. A substrate for the enzyme was added into the reaction mixture to facilitate a conversion of the substrate into a coloured product. The reaction was terminated by a stopping reagent and absorbance in the wells was determined on a plate reader (Synergy-HT; BioTek, Vermont, USA) at 450 nm. The concentration of unknown samples was derived from the standard curve.

4.3.4 Statistical analysis

Demographic and clinical characteristics are presented in tables, stratified according to the presence or absence of AF as well as patient outcome (deceased or alive) at the end of the study. Continuous variable with normal distribution were reported as mean $\pm$ standard deviation, while non-parametric data are presented as median (interquartile range). Categorical variables are reported as number (percentages).

Comparisons of categorical data between groups were compared using Pearson's chi-square test. For normally distributed, continuous variables, Student's t-test was utilised. For non-parametric continuous data, including biomarker measurements between two groups (e.g HFrEF-SR vs HFrEF-AF; ADHF vs CHF; and deceased vs alive), the Wilcoxon rank-sum test was employed.

Clinical and echocardiographic data were used to assess the relationship between traditional and novel cardiac biomarkers. Baseline biochemical data obtained from the NHLS were not included in this analysis given the large amount of missing data. A multivariable linear regression model was employed to evaluate the clinical and echocardiographic determinants of sST2, cathepsin-D, GDF-15, IL-6 and NT-proBNP (Supplementary Figures 1 & 2). For the purposes of accurate model interpretation, biomarker outliers (values below the 1st percentile and beyond the 99th percentile) were removed. A Spearman's correlation was performed to select continuous variables for the model and a linear regression model for categorical variables. All clinical correlates with a $p < 0.2$ were selected. Multicollinearity statistics were done and variables with a variance inflation factor > 5 were removed. Clinical variables with an $n < 80$ were excluded from the analysis to optimise model

generalisability. A final model was built for each biomarker employing a backwards stepwise regression method ($p < 0.2$).

For the primary outcome of interest, all-cause mortality, a univariate logistic regression model was used to calculate the unadjusted odds ratio for each variable. A final multivariate logistic regression model was built employing priori clinical interest and statistical significance ($p < 0.1$) for variable selection (47, 48). Goodness-of-fit statistics were employed to compare different models with alternating variable combinations, given the small number of events, for selection of the final model. Statistical analyses were performed using STATA (version 18, College Station, Texas).

4.4 RESULTS

4.4.1 Baseline demographics

The baseline clinical demographics are reported in Table 4.1. A total of 136 patients were enrolled in the study. Males represented a majority (52.9%) of participants and the mean age of participants was 58.7 ± 14.9 years. One hundred (74%) of patients were of black African ethnicity ($p < 0.001$) (Table 1). Twenty eight patients (20 %) had AF and majority (60.7%) of them were in ADHF. On the contrary, majority of HFrEF-SR patients were chronic and clinically stable HFrEF patients (ADHF = 19.4%, $p < 0.001$). The median duration of HFrEF was 3.6 (1.1; 1.7) years with no significant difference between the two groups (Table 4.1). Patients with HFrEF-AF displayed a trend towards longer duration of HFrEF, however this was not significantly different. Hypertensive heart disease was the leading cause of HFrEF (33.8%) followed by idiopathic dilated cardiomyopathy (19.1%) and ischemic heart disease (19.1%). The median duration of HFrEF was 3.6 [1.1-7.1] years.

Table 4.1. Baseline characteristics of patients with HFrEF categorised according to the presence and absence (SR) of atrial fibrillation.

Variable	Rhythm			p -value
	Total (n= 136)	HFrEF-SR (n=108)	HFrEF-AF (n=28)	
Gender, n (%)				
Male	72 (52.9)	56 (51.9)	16 (57.1)	0.617
Age (years)	58.7 ± 14.9	55.4 ± 14.3	69.5 ± 12.3	<0.001
BMI (Kg/m ²)	28.9 (25.7; 34)	29.4 (25.9; 33.9)	28.7 (24.2; 33.6)	0.549
Race, n (%)				<0.001
Black	101 (74.3)	87 (81.0)	14 (50.0)	
Caucasian	19 (13.9)	9 (8.3)	10 (35.7)	
Indian	11 (8.1)	7 (6.5)	4 (14.3)	
Coloured	5 (3.7)	5 (4.6)	0	
ADHF, n (%)	38 (27.9)	21 (19.4)	17 (60.7)	<0.001
HF duration (years)	3.6 (1.1; 7.1)	3.5 (1.3; 6.8)	5.2 (0.5; 8.2)	0.666
Heart rate (bpm)	72 (63; 85)	72.8 ± 14.3	81 (70; 104)	0.003
NYHA class				<0.001
I	50 (37.3)	47 (43.9)	3 (11.1)	
II	44 (32.9)	37 (34.6)	8 (28.6)	
III	30 (22.4)	17 (15.9)	13 (46.4)	
IV	10 (7.5)	6 (5.6)	4 (14.3)	

Data presented as mean ± standard deviation, median (interquartile range) or number (percentage); HFrEF (Heart failure with reduced ejection fraction), SR (Sinus rhythm), AF (Atrial fibrillation), BMI (Body Mass Index), SBP (Systolic blood pressure), ADHF (Acutely decompensated heart failure), DBP (Diastolic blood pressure), BPM (beats per minute), NYHA (New York Heart Association)

4.4.2 Measured levels of biomarkers between HFrEF-SR and HFrEF-AF patients

Standard and novel cardiac biomarkers were analysed on 125 plasma samples (Table 4.2). Measured levels of GDF-15 (696.3 [375.6;973.4] vs 452.1 [263.4; 763.8] ng/mL, p= 0.030) and sST2 (40.5 [15; 79.7] vs 20.8 [7.5; 39.9] ng/mL, p= 0.030) were significantly higher amongst patients with HFrEF-AF when compared to HFrEF-SR patients (Table 4.2). Blood concentrations of NT-proBNP, cTnT, cathepsin D and IL-6 were numerically higher among HFrEF-AF patients but not significantly different (Table 4.2).

Table 4.2. Measured levels of standard and novel cardiac biomarkers in patients with HFrEF categorised according to the presence and absence (SR) of atrial fibrillation.

Biomarker	Rhythm			p-value
	Total (N=125)	HFrEF-SR (n=98)	HFrEF-AF (n=27)	
NT-proBNP (ng/L)	1030 (678; 1841)	993.5 (582; 1779)	1243 (847; 2040)	0.164
cTnT (ng/L) ^a	26.5 (19.1; 36.0)	26.5 (19.2; 35.1)	28.4 (17.9; 42.9)	0.584
sST2 (ng/mL) ^b	23.9 (8.3; 48.0)	20.8 (7.5; 39.9)	40.5 (15.0; 79.7)	0.030
Cathepsin-D (ng/L)	4056 (1608; 8413)	3616 (1566; 8007)	6474.5 (3190; 10454)	0.053
GDF-15 (ng/mL)	485 (300.4; 828.1)	452.1 (263.4; 764)	696.3 (375.6; 973.4)	0.030
GAL-3 (ng/mL) ^c	1.0 (0.4; 1.5)	1.1 (0.4; 1.6)	0.6 (0.5; 1.4)	0.327
Il-6 (ng/L) ^a	9.4 (3.9; 27.0)	8.9 (3.3; 25.3)	16.6 (4.9; 37.8)	0.204

Data presented as mean $\pm$ standard deviation or median (interquartile range); NT-ProBNP (N-terminal pro natriuretic brain peptide), cTnT (Cardiac troponin T), sST2 (Soluble suppressor of tumorigenicity 2); GDF-15 (Growth differentiation factor – 15), GAL-3 (Galectin-3), IL-6 (Interleukin-6), ^a n = 122; ^b n =124; ^c n= 123

4.4.3 Measured levels of biomarkers between ADHF and chronic (stable) HF patients

Measured levels of NT-proBNP (1779 [927; 2610] ng/L vs 941.5 [539.5; 1535] ng/L, $p < 0.001$), sST2 (54.6 [14.4; 88.6] ng/mL vs 19.2 [7.5; 38.1] ng/mL, $p < 0.001$), cathepsin-D (8511.5 [2815.5; 12933] ng/L vs 3609 [1566; 6893] ng/L, $p < 0.001$), GDF-15 (848.3 [549.8; 1287.8] ng/mL vs 375.6 [259.2; 636.6] ng/mL, $p < 0.001$) and IL-6 (24.9 [13.2; 57.7] ng/L vs 7.3 [3.0; 18.2] ng/L, $p < 0.001$) were significantly higher amongst patients in ADHF compared to patients with chronic HF (CHF) (Table 4.3).

Table 4.3. Measured levels of both novel and standard cardiac biomarkers, classified by acute and chronic heart failure with reduced ejection fraction (HFrEF)

Biomarker	Total (N=136)	ADHF (n=45)	CHF (n=91)	p-value
NT-proBNP (ng/L)	1030 (678; 1841)	1777 (838; 2728)	950 (540; 1476)	0.002
cTnT (ng/L) ^a	26.5 (19.1; 36.0)	26.8 (17.0; 42.8)	26.5 (19.2; 33.5)	0.583
sST2 (ng/mL) ^b	23.9 (8.3; 48.0)	49.2 (13.7; 85.3)	18.9 (7.1; 36.2)	<0.001
Cathepsin-D (ng/L)	4056 (1608; 8413)	8007 (2618; 11758)	3572.5 (1534; 6893)	<0.001
Gdf-15 (ng/mL)	485 (300; 821)	764 (475; 1179)	386 (259; 637)	<0.001
GAL-3 (ng/mL) ^c	1.0 (0.4; 1.5)	0.8 (0.5; 1.4)	1.1 (0.4; 1.6)	0.775
Il-6 (ng/L) ^a	9.4 (3.9; 27.0)	22.7 (7.3; 47.8)	7.3 (3.0; 18.4)	<0.001

Data presented as mean $\pm$ standard deviation or median (interquartile range); ADHF (Acute decompensated heart failure); CHF (Chronic heart failure), SR (Sinus rhythm), AF (Atrial fibrillation), NT-ProBNP (N- terminal pro natriuretic brain peptide), cTnT (Cardiac troponin T), sST2 (Soluble suppressor of tumorigenicity 2), GDF-15 (Growth differentiation factor – 15), GAL-3 (Galectin-3), IL-6 (Interleukin-6); ^a n = 122; ^b n =124; ^c n= 123 ;

4.4.4 Measured levels of biomarkers by all-cause mortality and association with all-cause mortality

Levels of GDF-15 differed significantly between deceased (708.9 [481.3; 1056.5] ng/mL) and surviving patients (380.5 [250; 666.9] ng/mL, $p < 0.001$) (Table 4.4). N-terminal pro-B-type natriuretic peptide, cTnT, ST2, Cathepsin-D, GAL-3, and IL-6 did not reach statistical significance.

Table 4.4. Measured levels of standard and novel cardiac biomarkers by outcome

Biomarker	(N= 129)	Deceased (n=37)	Alive (n=92)	p-value
NT-ProBNP (ng/L)	1030 (674; 1841)	1216 (691; 2610)	995 (674; 1686)	0.155
cTnT (ng/L) ^a	25.9 (18.9; 36.0)	27.9 (21.1; 44.0)	24.9 (17.9; 35.1)	0.213
ST2 (ng/mL) ^b	24.9 (8.4; 49.2)	32.7 (12.3; 66.5)	20.8 (8.2; 38.7)	0.124
Cathepsin-D (ng/L)	4083 (1608; 8413)	4720 (2744.5; 11168)	4061.5 (1597; 7734)	0.111
GDF-15 (ng/mL)	470.4 (285.3; 810.5)	708.9 (481.3; 1057)	380.5 (250; 667)	< 0.001
GAL-3 (ng/mL) ^c	1.0 (0.5; 1.5)	0.9 (0.5; 1.7)	1.1 (0.4; 1.5)	0.645
IL-6 (ng/L) ^a	9.0 (3.4; 24.9)	11.9 (5.9; 33.9)	8.5 (3.1; 24.3)	0.078

Data presented as mean $\pm$ standard deviation or median (Interquartile range); NT-ProBNP (N-terminal pro-B-type natriuretic peptide); cTnT (Cardiac troponin T); sST2 (Soluble suppressor of tumorigenicity 2); GDF-15 (Growth differentiation factor – 15); IL-6 (Interleukin-6); ^a n = 122; ^b n = 124; ^c n = 123

Univariate logistic regression analysis showed that elevated levels of GDF-15 were significantly associated with all-cause mortality. Elevated levels of sST2 showed a borderline association with all-cause mortality in a univariate logistic regression. In a multivariate logistic regression model, good right ventricular function (higher TAPSE) was protective and a higher quality of life (lower KCCQ) was significantly associated with a lower odds of death.

Table 4.5. Univariate and multivariate logistic regression models for biomarkers and clinical characteristics associated with all-cause mortality.

Variables	Univariate		Multivariate *	
	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value
NT-ProBNP (ng/L)	1.00 (1.00-1.00)	0.073		
sST2 (ng/mL)	1.01 (1.00- 1.02)	0.069		
Age (Years)	1.02 (0.99- 1.05)	0.024	1.05 (1.00-1.11)	0.037
KCCQ	0.94 (0.92-0.97)	0.047	0.95 (0.91- 0.98)	0.010
GDF-15 (ng/mL)	1.001 (1.00-1.00)	0.001		
IL-6 (ng/L)	1.01 (0.99- 1.02)	0.322		
TAPSE (mm)	0.79 (0.7-0.9)	0.001	0.74 (0.61- 0.92)	0.005
Heart rate (bpm)	1.04 (1.01-1.07)	0.002		

CI (Confidence interval), NT-ProBNP (N-terminal pro-B-type natriuretic peptide), sST2 (Soluble suppressor of tumorigenicity 2), KCCQ (Kansas City Cardiomyopathy Questionnaire), GDF-15 (Growth differentiation factor – 15); IL-6 (Interleukin-6), TAPSE (Tricuspid Annular Plane Systolic Excursion); *Priori clinical interest, statistical significance ($p < 0.1$) as well as goodness-of-fit statistics were employed to select the final model

4.4.5. Clinical and echocardiographic determinants of sST2

In a univariable analysis evaluating the clinical correlates of sST2, the following variables were selected after accounting for multicollinearity and missing data: HF duration, heart rate, TAPSE, right ventricle (RV) basal dimensions, left atrial area, left atrial strain, sex, ADHF, dilated cardiomyopathy (DCMO), post-partum cardiomyopathy (PPCMO) and white race. The variables were included in a backwards, stepwise multivariable linear regression model ($R^2 = 0.36$, $p < 0.0001$) and the results are presented in Table 4.7. This model shows that a larger left atrial area ($\beta = 0.03$, $p = 0.034$) is associated with higher levels of sST2. Female patients with PPCMO ($\beta = -1.06$, $p = 0.035$) are associated with lower levels of sST2 and worse right ventricular function is (TAPSE; $\beta = -0.09$, $p = 0.005$) associated with higher levels of sST2.

Table 4.7. Multivariable linear regression model for clinical and echocardiographic determinants of sST2

Variable	Univariate			*Multivariable linear regression		
	β -coefficient	Standard error	P - value	β -coefficient	Standard error	P - value
ADHF	0.89	0.24	<0.001	0.49	0.29	0.098
Heart rate	0.03	0.01	<0.001	0.01	0.00	0.115
TAPSE	- 0.12	0.03	<0.001	- 0.09	0.02	0.005
LA area	0.04	0.01	0.005	0.03	0.01	0.034
PPCMO	- 1.04	0.44	0.020	- 1.06	0.49	0.035
Caucasian race	0.22	0.08	0.009	0.56	0.36	0.122
DCMO	0.29	0.33	0.363			
RV basal dimensions	0.05	0.13	0.001			
Sex	-0.45	0.24	0.064			
LA strain	-0.02	0.01	0.005			

ADHF (Acute decompensated heart failure), TAPSE (Tricuspid annular plane systolic excursion), LA (Left atrial), PPCMO (Post-partum cardiomyopathy), DCMO (Dilated cardiomyopathy), RV (Right Ventricle); *Backwards stepwise regression method was employed ($p < 0.2$).

4.4.6 Clinical and echocardiographic determinants of cathepsin-D

Following a univariable analysis for potential determinants of cathepsin-D, the following variables were selected for the multivariate model: KCCQ, heart rate, idiopathic dilated cardiomyopathy, left ventricular internal diameter in diastole (LVIDd), sex and left ventricular mass index, ADHF, RV basal dimensions and TAPSE. In a multivariable linear regression model ($R^2 = 0.29$, $p < 0.0001$), worse right ventricular function (TAPSE; $\beta = - 0.12$, $p = 0.001$) was strongly associated with higher levels of cathepsin-D (Table 4.8).

Table 4.8. Multivariable linear regression model for clinical and echocardiographic determinants of cathepsin-D

Variable	Univariate			*Multivariable linear regression		
	β -coefficient	Standard error	P - value	β -coefficient	Standard error	P- value
ADHF	0.80	0.24	0.001	0.52	0.33	0.119
BASAL RV DIMENSIONS (MM)	0.05	0.01	0.001	0.03	0.02	0.072
TAPSE	- 0.12	0.03	<0.001	- 0.12	0.03	0.001
LVMI	0.01	0.00	0.058			
DCMO	0.29	0.32	0.358			
Sex	-0.40	0.24	0.096			
LVIDd	0.02	0.01	0.111			
Heart rate	0.02	0.01	0.001			
KCCQ	-0.02	0.01	0.002			

ADHF (Acute decompensated heart failure), RV (Right ventricle), TAPSE (Tricuspid annular plane systolic excursion), LVMI (Left ventricle mass index), DCMO (Dilated cardiomyopathy), LVIDd (Left ventricular internal diameter at the end of diastole), KCCQ (Kansas City cardiomyopathy Questionnaire;

*Backwards stepwise regression method was employed ($p < 0.2$).

4.4.7 Clinical and echocardiographic determinants of GDF-15

The following variables were included in a multivariable model: KCCQ, left atrial area, heart rate, HF duration, left atrial reservoir strain, LVIDd, Left Ventricular Posterior Wall thickness in diastole (LVPWd), heart rate, ADHF, LVEF, RV basal dimensions, PPCMO and chronic kidney disease (Table 4.10). The KCCQ, LVEF, RV basal dimensions and PPCMO were independent associated with GDF-15 in multivariable linear regression model ($R^2 = 0.49$, $p < 0.001$). Higher GDF-15 levels were significantly associated with lower LVEF ($\beta = -0.02$, $p < 0.001$), larger RV size ($\beta = 0.02$, $p = 0.002$), worse quality of life (higher KCCQ score; $\beta = -0.01$, $p = 0.012$), and absence of PPCMO ($\beta = -0.43$, $p = 0.036$). Chronic kidney disease was a borderline predictor of elevated GDF-15 ($\beta = 0.29$, $p = 0.053$) (Table 4.9).

Table 4.9. Multivariable linear regression model for clinical and echocardiographic determinants of GDF-15

Variable	Univariate			*Multivariable linear regression		
	β -coefficient	Standard error	P - value	β -coefficient	Standard error	P - value
LVEF	-0.03	0.01	<0.001	- 0.02	0.01	< 0.001
Basal RV dimensions	0.04	0.01	<0.001	0.02	0.01	0.002
KCCQ	- 0.02	0.00	<0.001	- 0.01	0.00	0.012
PPCMO	-0.79	0.22	<0.001	- 0.43	0.19	0.036
CKD	0.42	0.16	0.008			
ADHF	0.63	0.12	<0.001			
LVIDd	0.02	0.01	0.000			
Heart rate	0.01	0.00	0.002			
HF duration	-0.01	0.01	0.346			
LA strain						
LVPWd	-0.07	0.03	0.010			

LVEF (Left ventricular ejection fraction), RV (Right ventricle), KCCQ (Kansas City cardiomyopathy Questionnaire), PPCMO (Post-partum cardiomyopathy), CKD (Chronic kidney disease), ADHF (Acutely decompensated heart failure), LVIDd (Left ventricular internal diameter at the end of diastole), HF (Heart failure), LA (Left atrial), LVPWd (Left Ventricular Posterior Wall thickness in diastole) ; *Backwards stepwise regression method was employed ($p < 0.2$).

4.4.8 Clinical and echocardiographic determinants of IL-6

Univariable analysis identified the following variables as potential determinants of IL-6: KCCQ, heart rate, HF duration, sex, ADHF, left atrial area, left atrial reservoir strain, TAPSE and type 2 diabetes mellitus. In a multivariable linear regression model ($R^2 = 0.20$, $p < 0.001$), lower TAPSE ($\beta = - 0.09$, $p = 0.023$) and a poor quality of life (higher KCCQ; $\beta = - 0.03$, $p = 0.001$) were strongly associated with higher levels of IL-6 (Table 4.10).

Table 4.10. Multivariable linear regression model for clinical and echocardiographic determinants of IL-6

Variable	Univariate			*Multivariable linear regression		
	β -coefficient	Standard error	P - value	β -coefficient	Standard error	P - value
KCCQ	-0.03	0.01	<0.001	- 0.03	0.00	0.001
TAPSE	0.04	0.01	<0.001	- 0.09	0.03	0.023
Heart rate	0.02	0.01	0.026			
T2DM	0.63	0.32	0.051			
LA strain	- 0.02	0.01	0.074			
ADHF	1.07	0.28	<0.001			
Sex	- 0.19	0.28	0.500			
LA area	0.03	0.02	0.135			
HF duration	- 0.03	0.03	0.248			

KCCQ (Kansas City Cardiomyopathy Questionnaire), PPCMO (post-partum cardiomyopathy), TAPSE (Tricuspid annular plane systolic excursion), T2DM (Type 2 diabetes mellitus), LA (Left atrial), ADHF (Acutely decompensated heart failure), HF (Heart failure); *Backwards stepwise regression method was employed ($p < 0.2$).

4.4.9 Clinical and echocardiographic determinants of NT-proBNP

The following clinical and echocardiographic clinical correlates, from the univariate analyses, were included: KCCQ, heart rate, sex, ADHF, PPCMO, chronic kidney disease, type 2 diabetes mellitus. In a linear regression model ($R^2 = 0.23$, $p < 0.001$) patients with Type 2 diabetes mellitus are independently associated with higher NT-proBNP levels ($\beta = 0.35$, $p = 0.044$). Heart rate is a strong independent predictor of elevated NT-proBNP ($\beta = 0.01$, $p = 0.003$) (Table 4.11).

Table 4.11. Multivariable linear regression model for clinical and echocardiographic determinants of NT-proBNP

Variable	Univariate			*Multivariable linear regression		
	β -coefficient	Standard error	P - value	β -coefficient	Standard error	P - value
T2DM	0.42	0.16	0.009	0.35	0.17	0.044
HEART RATE	0.01	0.00	<0.001	0.01	0.00	0.003
PPCMO	-0.66	0.24	0.008	-0.37	0.28	0.184
RA	0.00	0.00	0.011	0.00	0.00	0.127
VOLUME						
KCCQ	- 0.01	0.00	0.001			
ADHF	0.51	0.14	<0.001			
Sex	- 0.28	0.14	0.044			
CKD	0.35	1.67	0.040			

T2DM (Type 2 diabetes mellitus), PPCMO (post-partum cardiomyopathy), RA (Right atrial), KCCQ (Kansas City Cardiomyopathy Questionnaire), PPCMO (post-partum cardiomyopathy), ADHF (Acutely decompensated heart failure), CKD (Chronic kidney disease) *Backwards stepwise regression method was employed ($p < 0.2$).

4.5. DISCUSSION

In this study circulating levels of both standard and novel biomarkers were measured in South African patients with HFrEF- AF. We assessed their prognostic utility and evaluated their association with clinical characteristics and echocardiographic parameters. The study revealed the following findings : 1) Soluble ST2 and GDF-15 were significantly elevated in HFrEF-AF patients when compared to their SR counterparts, 2) Right ventricular dysfunction was consistently associated with elevated levels of sST2, GDF-15, cathepsin-D and IL-6, 3) A poorer patient-reported quality of life over the preceding 2 weeks was linked to higher levels of IL-6 and GDF-15.

4.5.1 Elevated levels of GDF-15 in HFrEF-AF patients

Growth differentiation factor- 15, is documented to have increased expression in HFrEF (15). The hypothesis of elevated levels of GDF-15 was based on the pathophysiological basis of extensive cardiac remodelling and inflammation in HFrEF-AF and the oxidative stress, inflammatory and fibrotic pathways GDF-15 is involved in (42). Interestingly, previous studies have not reported an association between GDF-15 and HFrEF-AF, instead citing no association with AF in HFrEF after adjusting for clinical confounders (19). Growth differentiation factor-15 is released in 60-fold higher concentrations in the liver, kidney, adipocytes and endothelial tissue compared to the heart (47). Under normal physiological conditions, levels of GDF-15 are low (39). In the context of HFrEF, elevated plasma levels of GDF-15 levels are predominantly produced from extracardiac tissues (47). Elevated GDF-15 levels therefore, may be a reflective of the overall (comorbid) condition of HFrEF patients. Indeed, literature has shown an association between GDF-15 with various comorbidities such as CKD and anemia in HF (40, 48) . Levels of GDF-15 were significantly elevated in ADHF in our study, consistent with previous studies (41, 49, 50). Majority of HFrEF-AF patients in this cohort were in ADHF potentially confounding what appeared to be elevated levels of GDF-15 in HFrEF-AF patients. Growth differentiation factor-15 reflects the severity and overall comorbid condition of the HFrEF-AF patients rather than worsening cardiac dysfunction, inflammation and remodelling attributable solely to the presence of AF. The observation in our study that GDF-15 was independently associated with poor RV function (RV basal dimensions: $\beta = 0.02$, $p = 0.002$), quality of life (KCCQ: $\beta = -0.01$, $p = 0.012$) and CKD ($\beta = 0.29$, $p = 0.053$) is consistent with this hypothesis.

4.5.2 Elevated levels of sST2 in HFrEF-AF patients

In the present study, circulating levels of sST2 were twice as high in patients with HFrEF-AF compared to those with HFrEF-SR. Evidence for increasing sST2 levels based on AF presence in HFrEF has been documented (51). Increased production of cardiac sST2 in response to mechanical stress results in sST2 binding to IL-31 and thus antagonises the anti-fibrotic, anti-hypertrophic and anti-apoptotic IL-31/STL dependent pathway (29). The result of this is unopposed cardiac remodelling at the level of the myocardium. In HFrEF-AF, where there is a large degree of LV and LA mechanical stress, this sST2/IL-31 maladaptive remodelling pathway is upregulated and may explain the significantly higher levels of sST2 observed in HFrEF-AF patients compared to their SR

counterparts (52). The additional finding in this study that an increase in LA area was independently associated with increasing sST2 levels lends credence to these findings. Atrial structural remodelling, as previously described, is the basis for AF incidence and its maintenance, hence the adage “AF begets AF” (2). The greater the AF burden, the greater the degree of atrial structural remodelling, and consequently, a greater AF burden. The finding of significantly elevated LA areas in HFrEF-AF (Chapter 3) confirms the presence of extensive atrial remodelling in this cohort. This as well as the independent association between sST2 levels in LA area positions sST2 as a surrogate for atrial remodelling in this sub-population. Yan and colleagues, as well as other authors have demonstrated similar findings (52, 53). Moreover, supporting its role as a surrogate for atrial remodelling, sST2 levels have previously been shown to predict AF recurrence in patients undergoing catheter ablation (2).

Soluble ST2 levels are also reported to be elevated in cases of ADHF, a finding observed in this cohort. Extracardiac release of soluble ST2 is observed in type II pneumocytes in response to mechanical strain as well as in endothelial vascular cells (27, 54). In the context of pulmonary congestion, sST2 is produced by type II pneumocytes in response to alveolar strain (27). This production of sST2 has been shown to positively correlate with the degree of pulmonary congestion in ADHF (27). In HFrEF, grade 2 pulmonary hypertension is significantly more common amongst patients with AF compared to those in SR, which may to an extent account for the significantly elevated sST2 levels observed amongst HFrEF-AF patients in our study (55). Interestingly, ADHF did not emerged as an independent predictor of sST2 levels. Moreover, echocardiographic measures of pulmonary hypertension such as the tricuspid regurgitation peak velocity and pulmonary artery systolic pressures did not differ significantly between HFrEF-AF and HFrEF-SR patients in our study (Chapter 3).

4.5.3 Clinical and echocardiographic determinants of biomarkers

Inflammation plays a significant role in pathological remodelling following acute insults and in chronic HF (CHF) (2). In patients with CHF, an inflammatory phenotype is evident, characterised by elevated circulating levels of pro-inflammatory cytokines (IL-6, tumour necrosis factor- α etc.) and correlate with disease severity and comorbid status (56). The association between poor quality of life and advanced HF, as evidenced by RV dysfunction and elevated IL-6 levels aligns with previous studies (57, 58). Plenz et al. demonstrated significantly increased IL-6 expression in the RV tissues of patients with advanced dilated cardiomyopathy undergoing transplantation (58). The

results of our study suggest that IL-6 serves as a biomarker of disease severity and morbidity in this cohort. However, adequately powered studies are necessary to clearly elucidate IL-6's prognostic utility and role in HFrEF.

Poor RV function as measured by TAPSE was strongly associated with increasing levels of cathepsin-D ($\beta = -0.12$, $p = 0.001$) and sST2 ($\beta = -0.09$, $p = 0.005$). To the best of our knowledge, no studies have demonstrated an association between cathepsin-D and RV dysfunction in a HFrEF population. Soluble ST2, on the other hand has been reported to be associated with RV dysfunction, aligning with its role in maladaptive cardiac remodelling (59). There is minimal literature on cathepsin-D's pathophysiological role and clinical utility in HFrEF with one study citing a strong association with a more advanced form of HFrEF, consistent with the abovementioned observations (45). In the present study, a strong correlation between sST2 and cathepsin-D ($\rho = 0.97$, $p < 0.001$; Supplementary Figure.1) was observed supporting synergistic or shared pathophysiological pathways in this subpopulation. While the exact pathophysiological pathway of cathepsin-D in HFrEF is unclear, Hoes and colleagues demonstrated that it is secreted from cardiac myocytes following hypoxia and mechanical stress and essential for myocyte survival under stress suggesting a cardioprotective effect.

While PPCMO is beyond the scope of the study, its strong association with lowering levels of novel markers GDF-15 ($\beta = -0.43$, $p = 0.036$) and sST2 ($\beta = -1.06$, $p = 0.035$) in this population is worth mention. These findings highlight different pathophysiological mechanisms within the different sub-populations of HFrEF patients and justify the role of biomarkers in phenotyping HFrEF patients to risk-stratify and develop targeted interventions. The exact reasons that result in circulatory GDF-15 and sST2 in PPCM are unknown. Furthermore, there is limited evidence establishing the link between circulating GDF-15 and sST2 levels and PPCMO.

4.5.4 Clinical Perspective and Future direction

To the best of our knowledge, this study is the first in SSA to profile HFrEF and HFrEF-AF patients using both standard and novel cardiac biomarkers. The finding of elevated sST2 and GDF-15 in HFrEF-AF patients validates our hypothesis that South African HFrEF-AF exhibit greater disease severity clinically and biochemically compared to their SR counterparts, characterised by a structurally more fibrotic heart and increased morbidity. The association between clinical characteristics and echocardiographic parameters supports the integration of multi-marker

biomarker profiling with clinical, biochemical and echocardiographic parameters tailored to this population. In particular, RV dysfunction and patient-reported quality of life appear to correlate closely with systemic inflammatory and fibrotic biomarkers, which may aid in risk stratification or monitoring response to therapy in this high risk population. Our findings are merely hypothesis generating and require large, multi-centre studies in SSA to validate the clinical utility of these markers individually as well as in a multi-marker panel. Further studies should also be directed towards determining the exact prognostic cut-off levels in South Africa.

4.5.5 Limitations

Our study had several limitations. The main limitation of the study was a small sample size. The recruitment of patients for the current study was halted due to the COVID-19 pandemic lockdown regulations. Loss of plasma samples due to haemolysis further decreased our sample size. The small sample size and number of events limited our ability to build robust prediction models that are generalisable. Biochemical data collected from NHLS records was not available for all participants resulting in a large amount of missing data and thus we could not fully explore their effect of common comorbidities in HF such as anaemia, dyslipidaemia etc. on all-cause mortality as well as the biomarkers. The use of highly sensitive cTnT, instead of standard assays would have enabled better detection of cardiac troponin levels. The study is a single-centre study done at a tertiary institution, and hence, our findings may not be generalisable to the general population.

4.5.6 Conclusion

The present study has demonstrated that South African HFrEF-AF patients have a predominantly fibrotic and inflammatory biomarker profile. Clinical and echocardiographic measures of disease severity and poor quality of life closely correlate with systemic inflammatory and fibrotic biomarkers.

4.6 REFERENCES:

1. Heidenreich PA, Bozkurt B, Aguilar D, Allen LA, Byun JJ, Colvin MM, et al. 2022 AHA/ACC/HFSA Guideline for the Management of Heart Failure: A Report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. *Circulation*. 2022;145(18):e895-e1032.
2. Peter Libby ROB, Douglas L. Mann, Gordon F. Tomaselli, Deepak L. Bhatt, Scott D. Solomon, . Braunwald's Heart Disease, 2 Vol Set, 12th Edition. Philadelphia: Elsevier; 2011.
3. McDonagh TA, Metra M, Adamo M, Gardner RS, Baumbach A, Bohm M, et al. 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. *Eur Heart J*. 2021;42(36):3599-726.
4. Carlisle MA, Fudim M, DeVore AD, Piccini JP. Heart Failure and Atrial Fibrillation, Like Fire and Fury. *JACC Heart Fail*. 2019;7(6):447-56.
5. Denham NC, Pearman CM, Caldwell JL, Madders GWP, Eisner DA, Trafford AW, et al. Calcium in the Pathophysiology of Atrial Fibrillation and Heart Failure. *Frontiers in Physiology*. 2018;9.
6. Nattel S, Harada M. Atrial remodeling and atrial fibrillation: recent advances and translational perspectives. *J Am Coll Cardiol*. 2014;63(22):2335-45.
7. Kotecha D, Chudasama R, Lane DA, Kirchhof P, Lip GY. Atrial fibrillation and heart failure due to reduced versus preserved ejection fraction: A systematic review and meta-analysis of death and adverse outcomes. *Int J Cardiol*. 2016;203:660-6.
8. Mamas MA, Caldwell JC, Chacko S, Garratt CJ, Fath-Ordoubadi F, Neyses L. A meta-analysis of the prognostic significance of atrial fibrillation in chronic heart failure. *European Journal of Heart Failure*. 2009;11(7):676-83.
9. Mboweni N, Maseko M, Tsabedze N. Heart failure with reduced ejection fraction and atrial fibrillation: a Sub-Saharan African perspective. *ESC Heart Fail*. 2023;10(3):1580-96.
10. Serban T, Hennings E, Strebel I, Knecht S, du Fay de Lavallaz J, Krisai P, et al. Biomarkers to predict improvement of left ventricular ejection fraction after atrial fibrillation ablation. *Heart Rhythm*. 2024;21(9):1581-8.
11. Ibrahim NE, Januzzi JL, Jr. Beyond Natriuretic Peptides for Diagnosis and Management of Heart Failure. *Clin Chem*. 2017;63(1):211-22.
12. Morrow DA, De Lemos JA. Benchmarks for the assessment of novel cardiovascular biomarkers. *Circulation*. 2007;115(8):949-52.
13. Goetze JP, Bruneau BG, Ramos HR, Ogawa T, de Bold MK, de Bold AJ. Cardiac natriuretic peptides. *Nat Rev Cardiol*. 2020;17(11):698-717.
14. Castiglione V, Aimo A, Vergaro G, Saccaro L, Passino C, Emdin M. Biomarkers for the diagnosis and management of heart failure. *Heart Fail Rev*. 2022;27(2):625-43.
15. Koniari I, Artopoulou E, Velissaris D, Ainslie M, Mplani V, Karavasili G, et al. Biomarkers in the clinical management of patients with atrial fibrillation and heart failure. *J Geriatr Cardiol*. 2021;18(11):908-51.
16. Tsutsui H, Albert NM, Coats AJS, Anker SD, Bayes-Genis A, Butler J, et al. Natriuretic peptides: role in the diagnosis and management of heart failure: a scientific

statement from the Heart Failure Association of the European Society of Cardiology, Heart Failure Society of America and Japanese Heart Failure Society. *Eur J Heart Fail.* 2023;25(5):616-31.

17. Bayes-Genis A, Lloyd-Jones DM, van Kimmenade RR, Lainchbury JG, Richards AM, Ordonez-Llanos J, et al. Effect of body mass index on diagnostic and prognostic usefulness of amino-terminal pro-brain natriuretic peptide in patients with acute dyspnea. *Arch Intern Med.* 2007;167(4):400-7.

18. Commodore-Mensah Y, Wang D, Jeon Y, Foti K, McEvoy JW, Coresh J, et al. Racial and ethnic differences in circulating N-terminal pro-brain-type natriuretic peptide (NT-proBNP) in US adults. *Am J Prev Cardiol.* 2023;15:100526.

19. Santema BT, Chan MMY, Tromp J, Dokter M, van der Wal HH, Emmens JE, et al. The influence of atrial fibrillation on the levels of NT-proBNP versus GDF-15 in patients with heart failure. *Clin Res Cardiol.* 2020;109(3):331-8.

20. Latini R, Masson S, Anand IS, Missov E, Carlson M, Vago T, et al. Prognostic value of very low plasma concentrations of troponin T in patients with stable chronic heart failure. *Circulation.* 2007;116(11):1242-9.

21. Aimo A, Januzzi JL, Jr., Mueller C, Miro O, Pascual Figal DA, Jacob J, et al. Admission high-sensitivity troponin T and NT-proBNP for outcome prediction in acute heart failure. *Int J Cardiol.* 2019;293:137-42.

22. Berg DD, Docherty KF, Sattar N, Jarolim P, Welsh P, Jhund PS, et al. Serial Assessment of High-Sensitivity Cardiac Troponin and the Effect of Dapagliflozin in Patients With Heart Failure With Reduced Ejection Fraction: An Analysis of the DAPA-HF Trial. *Circulation.* 2022;145(3):158-69.

23. D'Amato A, Severino P, Prosperi S, Mariani MV, Germano R, De Prisco A, et al. The Role of High-Sensitivity Troponin T Regarding Prognosis and Cardiovascular Outcome across Heart Failure Spectrum. *J Clin Med.* 2024;13(12).

24. McEvoy JW, Chen Y, Ndumele CE, Solomon SD, Nambi V, Ballantyne CM, et al. Six-Year Change in High-Sensitivity Cardiac Troponin T and Risk of Subsequent Coronary Heart Disease, Heart Failure, and Death. *JAMA Cardiol.* 2016;1(5):519-28.

25. Lazar DR, Lazar FL, Homorodean C, Cainap C, Focsan M, Cainap S, et al. High-Sensitivity Troponin: A Review on Characteristics, Assessment, and Clinical Implications. *Dis Markers.* 2022;2022:9713326.

26. Riccardi M, Myhre PL, Zelniker TA, Metra M, Januzzi JL, Inciardi RM. Soluble ST2 in Heart Failure: A Clinical Role beyond B-Type Natriuretic Peptide. *J Cardiovasc Dev Dis.* 2023;10(11).

27. Pascual-Figal DA, Perez-Martinez MT, Asensio-Lopez MC, Sanchez-Mas J, Garcia-Garcia ME, Martinez CM, et al. Pulmonary Production of Soluble ST2 in Heart Failure. *Circ Heart Fail.* 2018;11(12):e005488.

28. Weinberg EO, Shimp M, De Keulenaer GW, MacGillivray C, Tominaga S, Solomon SD, et al. Expression and regulation of ST2, an interleukin-1 receptor family member, in cardiomyocytes and myocardial infarction. *Circulation.* 2002;106(23):2961-6.

29. Pascual-Figal D, Lax A, Asensio Lopez MC. Circulating ST2, from biomarker to pathogenic mediator. *Rev Esp Cardiol (Engl Ed).* 2023;76(9):672-4.

30. Emdin M, Aimo A, Vergaro G, Bayes-Genis A, Lupon J, Latini R, et al. sST2 Predicts Outcome in Chronic Heart Failure Beyond NT-proBNP and High-Sensitivity Troponin T. *J Am Coll Cardiol*. 2018;72(19):2309-20.
31. Bayes-Genis A, de Antonio M, Vila J, Penafiel J, Galan A, Barallat J, et al. Head-to-head comparison of 2 myocardial fibrosis biomarkers for long-term heart failure risk stratification: ST2 versus galectin-3. *J Am Coll Cardiol*. 2014;63(2):158-66.
32. Blanda V, Bracale UM, Di Taranto MD, Fortunato G. Galectin-3 in Cardiovascular Diseases. *Int J Mol Sci*. 2020;21(23).
33. Zaborska B, Sikora-Frac M, Smarz K, Pilichowska-Paszkiel E, Budaj A, Sitkiewicz D, et al. The Role of Galectin-3 in Heart Failure-The Diagnostic, Prognostic and Therapeutic Potential-Where Do We Stand? *Int J Mol Sci*. 2023;24(17).
34. Meijers WC, Januzzi JL, deFilippi C, Adourian AS, Shah SJ, van Veldhuisen DJ, et al. Elevated plasma galectin-3 is associated with near-term rehospitalization in heart failure: a pooled analysis of 3 clinical trials. *Am Heart J*. 2014;167(6):853-60 e4.
35. Miro O, Gonzalez de la Presa B, Herrero-Puente P, Fernandez Bonifacio R, Mockel M, Mueller C, et al. The GALA study: relationship between galectin-3 serum levels and short- and long-term outcomes of patients with acute heart failure. *Biomarkers*. 2017;22(8):731-9.
36. Clementy N, Garcia B, André C, Bisson A, Benhenda N, Pierre B, et al. Galectin-3 level predicts response to ablation and outcomes in patients with persistent atrial fibrillation and systolic heart failure. *PLoS ONE*. 2018;13(8):1-12.
37. Marrouche NF, Brachmann J, Andresen D, Siebels J, Boersma L, Jordaens L, et al. Catheter Ablation for Atrial Fibrillation with Heart Failure. *New England Journal of Medicine*. 2018;378(5):417-27.
38. Badianyama M, Mpanya D, Adamu U, Sigauke F, Nel S, Tsabedze N. New Biomarkers and Their Potential Role in Heart Failure Treatment Optimisation-An African Perspective. *J Cardiovasc Dev Dis*. 2022;9(10).
39. Assadi A, Zahabi A, Hart RA. GDF15, an update of the physiological and pathological roles it plays: a review. *Pflugers Arch*. 2020;472(11):1535-46.
40. Kempf T, von Haehling S, Peter T, Allhoff T, Cicoira M, Doehner W, et al. Prognostic utility of growth differentiation factor-15 in patients with chronic heart failure. *J Am Coll Cardiol*. 2007;50(11):1054-60.
41. Anand IS, Kempf T, Rector TS, Tapken H, Allhoff T, Jantzen F, et al. Serial measurement of growth-differentiation factor-15 in heart failure: relation to disease severity and prognosis in the Valsartan Heart Failure Trial. *Circulation*. 2010;122(14):1387-95.
42. Sharma A, Stevens SR, Lucas J, Fiuzat M, Adams KF, Whellan DJ, et al. Utility of Growth Differentiation Factor-15, A Marker of Oxidative Stress and Inflammation, in Chronic Heart Failure: Insights From the HF-ACTION Study. *JACC Heart Fail*. 2017;5(10):724-34.
43. Wallentin L, Hijazi Z, Andersson U, Alexander JH, De Caterina R, Hanna M, et al. Growth differentiation factor 15, a marker of oxidative stress and inflammation, for risk assessment in patients with atrial fibrillation: insights from the Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation (ARISTOTLE) trial. *Circulation*. 2014;130(21):1847-58.

44. Wu P, Yuan X, Li F, Zhang J, Zhu W, Wei M, et al. Myocardial Upregulation of Cathepsin-D by Ischemic Heart Disease Promotes Autophagic Flux and Protects Against Cardiac Remodeling and Heart Failure. *Circ Heart Fail*. 2017;10(7).
45. Hoes MF, Tromp J, Ouwerkerk W, Bomer N, Oberdorf-Maass SU, Samani NJ, et al. The role of cathepsin-D in the pathophysiology of heart failure and its potentially beneficial properties: a translational approach. *European Journal of Heart Failure*. 2020;22(11):2102-11.
46. Troughton RW, Frampton CM, Brunner-La Rocca HP, Pfisterer M, Eurlings LW, Erntell H, et al. Effect of B-type natriuretic peptide-guided treatment of chronic heart failure on total mortality and hospitalization: an individual patient meta-analysis. *Eur Heart J*. 2014;35(23):1559-67.
47. Zabor EC, Reddy CA, Tendulkar RD, Patil S. Logistic Regression in Clinical Studies. *Int J Radiat Oncol Biol Phys*. 2022;112(2):271-7.
48. Collins GS, Reitsma JB, Altman DG, Moons KG. Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): the TRIPOD Statement. *BMC Med*. 2015;13:1.
49. Du W, Piek A, Schouten EM, van de Kolk CWA, Mueller C, Mebazaa A, et al. Plasma levels of heart failure biomarkers are primarily a reflection of extracardiac production. *Theranostics*. 2018;8(15):4155-69.
50. Ueland T, Gullestad L, Kou L, Young JB, Pfeffer MA, van Veldhuisen DJ, et al. Growth differentiation factor 15 predicts poor prognosis in patients with heart failure and reduced ejection fraction and anemia: results from RED-HF. *Clin Res Cardiol*. 2022;111(4):440-50.
51. Bouabdallaoui N, Claggett B, Zile MR, McMurray JJV, O'Meara E, Packer M, et al. Growth differentiation factor-15 is not modified by sacubitril/valsartan and is an independent marker of risk in patients with heart failure and reduced ejection fraction: the PARADIGM-HF trial. *Eur J Heart Fail*. 2018;20(12):1701-9.
52. Monzo L, Jarolim P, Borlaug BA, Benes J, Jurcova I, Jenca D, et al. Growth Differentiation Factor-15 Is Associated With Congestion-Related Anorexia and Weight Loss in Advanced Heart Failure. *JACC Heart Fail*. 2025;13(2):315-29.
53. Santema BT, Kloosterman M, Van Gelder IC, Mordi I, Lang CC, Lam CSP, et al. Comparing biomarker profiles of patients with heart failure: atrial fibrillation vs. sinus rhythm and reduced vs. preserved ejection fraction. *Eur Heart J*. 2018;39(43):3867-75.
54. Yan X, Guo Y, Li L, Wang Z, Li Z. The sST2 level is an independent influencing factor associated with atrial fibrillation in heart failure patients: a case-control study. *J Thorac Dis*. 2022;14(5):1578-87.
55. Tan R, Yu H, Han X, Liu Y, Yang X, Xia YL, et al. Circulating Soluble Suppression of Tumorigenicity 2 Predicts Recurrence After Radiofrequency Ablation of Persistent Atrial Fibrillation. *Front Cardiovasc Med*. 2021;8:653312.
56. Demyanets S, Kaun C, Pentz R, Krychtiuk KA, Rauscher S, Pfaffenberger S, et al. Components of the interleukin-33/ST2 system are differentially expressed and regulated in human cardiac cells and in cells of the cardiac vasculature. *J Mol Cell Cardiol*. 2013;60:16-26.

57. Rottlaender D, Motloch LJ, Schmidt D, Reda S, Larbig R, Wolny M, et al. Clinical impact of atrial fibrillation in patients with pulmonary hypertension. *PLoS One*. 2012;7(3):e33902.
58. Li H, Chen C, Wang DW. Inflammatory Cytokines, Immune Cells, and Organ Interactions in Heart Failure. *Front Physiol*. 2021;12:695047.
59. Markousis-Mavrogenis G, Tromp J, Ouwerkerk W, Devalaraja M, Anker SD, Cleland JG, et al. The clinical significance of interleukin-6 in heart failure: results from the BIOSTAT-CHF study. *European Journal of Heart Failure*. 2019;21(8):965-73.
60. Plenz G, Song ZF, Tjan TD, Koenig C, Baba HA, Erren M, et al. Activation of the cardiac interleukin-6 system in advanced heart failure. *Eur J Heart Fail*. 2001;3(4):415-21.
61. deFilippi C, Daniels LB, Bayes-Genis A. Structural heart disease and ST2: cross-sectional and longitudinal associations with echocardiography. *Am J Cardiol*. 2015;115(7 Suppl):59B-63B

CHAPTER 5: B1-ADRENERGIC RECEPTOR ARG389GLY POLYMORPHISM AND ATRIAL FIBRILLATION IN HFrEF: GENOTYPIC DISPARITIES IN A SOUTH AFRICAN COHORT

5.1 ABSTRACT

Background: The presence of atrial fibrillation (AF) in patients with heart failure with reduced ejection fraction (HFrEF) presents a clinical challenge, as these patients do not respond optimally to pharmacological therapy, including beta-blockers (β -blockers). The Arginine389Glycine (Arg389Gly) polymorphism in the β 1-adrenergic receptor has been implicated in altering the response to β -blocker therapy in European, American, and Asian populations. However, little is known about the clinical relevance of this polymorphism in African populations.

Aim: This study aimed to identify the presence of the Arg389Gly or rs1801253 (1165 G > C) single nucleotide polymorphism (SNP) and to analyse the distribution of allelic frequencies in a South African cohort of patients with HFrEF-AF and HFrEF-SR. The study also aimed to compare minimum allele frequencies (MAF) among other populations.

Methods: One hundred and eighteen DNA samples belonging to patients with HFrEF were genotyped for the Arg389Gly SNP using the iPLEX MassArray platform. The genotype and allele frequencies were compared between HFrEF patients with and without AF. The minor allele frequency (MAF) was compared to other global population from the Ensembl genome databank.

Results: The heterozygous Arg/Gly genotype predominated in the overall cohort (47%) and the HFrEF-AF group (69%). Genotype frequencies differed significantly between HFrEF patients with and without AF ($p=0.030$), with the homozygous Gly389Gly variant absent in the HFrEF-AF group. The Gly389 MAF (32%) was lower than West African and African American populations but comparable to European populations.

Conclusion: These findings suggest a potential role of the Arg389Gly polymorphism in AF susceptibility among HFrEF patients in South Africa.

5.2 INTRODUCTION

The presence of atrial fibrillation (AF) presents a challenge in the management of heart failure with reduced ejection fraction (HFrEF). Managing patients with AF and HFrEF is a growing point of uncertainty for many physicians as these patients do not respond optimally to pharmacological, mortality-benefitting therapy (1). Beta-blockers (β -blockers) constitute one of the main pillars of mortality benefitting therapy in patients with HFrEF (2, 3). Similarly, β -blockers are the mainstay of rate control management in patients with AF (4). Given their affordability and accessibility, non-selective β -blockers are preferred in the South African public healthcare system.

The use of β -blockers in patients with HFrEF and concomitant AF (HFrEF-AF) presents a clinical challenge, with patients not deriving optimal rate control or mortality benefit (1, 5, 6). Moreover, African Americans in the Bucindolol in the Beta-Blocker Evaluation of Survival Trial (BEST) received no mortality benefit from β -blockers compared to non-black patients (7). Studies have highlighted the potential role of a mutant β_1 -adrenergic receptor in modulating HFrEF patients' response to β -blocker therapy in European, American and Asian populations (8, 9). This differential response is further modulated by the presence of AF (5). An Arginine at position 389 (Arg389) on transmembrane 7, situated at the c-terminus of the β_1 -adrenergic receptor, plays a key role in Gs coupling and hence signal transduction (10). To this effect, the Arginine389Glycine (Arg389Gly) polymorphism has been implicated in altering a dose-dependent response to β -blocker therapy (10).

Heart failure with reduced ejection fraction patients with the loss-of-function homozygous Gly389 variant are proposed to be poor responders and do not derive mortality benefit. Patients with a homozygous Arg389 mutant, are proposed to be good responders (10-12). The inverse is observed for rate control in HFrEF-AF patients homozygous for the Arg389 allele, showing little or no sustained response to carvedilol, a non-selective β -blocker therapy commonly used HFrEF management (2, 5). These effects have been demonstrated only in European, American, and Asian patients (8). Moreover, the frequency distribution of the Arg389Gly genetic variants differ significantly between European and Non-European populations (13)

Despite compelling evidence to support differential therapeutic outcomes based on geographical region and genotype, little is known about the clinical relevance of this polymorphism, and which genetic variant is dominant in our setting. To the best of our knowledge, a single study has looked

for this polymorphism in a Sub-Saharan African HFrEF population (14). The study by Woodiwiss et al sought to assess the relationship between the Arg389Gly and Del322-325 variants and the presence, severity, and progression of “idiopathic” dilated cardiomyopathy in black South African patients. This study employed a stringent selection criteria excluding patients on β -blocker therapy as well as patients with HFrEF-AF (14). Their findings were negative, finding no association between the Arg389Gly SNP and the risk for IDC nor its severity.

This study aimed to identify the presence of the Arg389Gly or rs1801253 (1165 G > C) single nucleotide polymorphism (SNP) and to analyse the distribution of allelic frequencies in a South African cohort of patients with HFrEF and to compare them with other populations.

5.3 METHODS

5.3.1 Ethical clearance

The study was performed according to the principle of the declaration of Helsinki. Ethical approval for this study was granted by the University of Witwatersrand, Faculty of Health Sciences Human Research Ethics Committee (HREC; medical; M190349). Participants were issued an information sheet detailing the purpose and process of the study. Written consent was obtained from all participants.

5.3.2 Participants and sample selection

One hundred thirty six patients with HFrEF were consecutively enrolled from a specialised outpatient heart failure clinic and inpatient cardiac admission wards at Charlotte Maxeke Johannesburg Academic Hospital between June 2019 and December 2021.

A comprehensive history and clinical examination was conducted on all patients. We extracted further information from patients’ medical records. The age, race and presence of any comorbidities was documented. Information on prescribed drugs for each patient was retrieved from their medical records and the patient. A focused history and a physical examination to identify HF signs and symptoms was conducted. Clinical investigations on all patients recruited into the study included a 12-lead ECG in which the heart rate and rhythm were determined.

Two venous blood samples were drawn from each patient into 3ml purple top EDTA-BD vacutainer blood collection tubes. Thereafter, samples were placed in an ice box and transported to the laboratory. Samples were centrifuged and buffy coat extracted and stored at -80 degree Celsius with DNA shield until the time of extraction.

Patients were divided into two groups according to rhythm status; those with HFrEF in sinus rhythm (HFrEF-SR) and those with HFrEF and concomitant atrial fibrillation (HFrEF-AF

5.3.3 DNA Extraction

DNA was extracted from buffy coat samples using DNA Miniprep Plus kit (Zymo Research Corp., Freiburg, Germany) according to the manufacturer's instructions.

5.3.4 Primer design

The Agena Assay Designer software was employed to construct target-loci polymerase chain reaction (PCR) and extension primers that targeted the Arg389Gly or rs1801253 (1165 G > C) SNP (Table 5.1). These primers were synthesised unmodified under standard purification conditions. Primers were synthesised by Inqaba Biotec Laboratories (Pretoria, SA).

Table 5.1. Primers designed for amplification PCR of target loci and extension primer.

SEQUENCE ID	SEQUENCE	LENGTH (NUCLEOTIDES)
S1801253_W1_FORWARD	CGTTGGATGATCATCTACTGCCGCAGCC	29
S1801253_W1_REVERSE	CGTTGGATGGGTCTCCGTGGGTCGCGTG	29
S1801253_W1_EXTENSION	CCGCAAGGCCTTCCAG	17

5.3.5 Genotyping

Samples were sent to Inqaba Biotec Laboratories (Pretoria, SA) for genotyping using the iPLEX MassArray platform. Genotyping was successfully done on 118 samples. This method of genotyping method involves an initial PCR reaction specific to the locus followed by single base extension using mass-modified primers containing terminal deoxyribonucleotides that binds immediately upstream of the polymorphism site (15). Matrix-Assisted Laser Desorption/Ionization-

Time Of Flight (MALDI-TOF) Mass Spectrometry (MS) was used to measure specific mass of the amplified primer which, identifies the SNP allele. This procedure involves the following steps: Pre-PCR for DNA (5 to 50 ng / μ l) and oligonucleotides preparation, PCR for target locus amplification using the designed primers, post-PCR for purification of the reaction with SAP (Shrimp alkaline phosphatase), single base amplification with iPLEX system, cleaning products with resin, deployment of amplified products on SpectroCHiPs, and products tracking with MS with appropriate MALDI-TOF matrix (15).

5.3.6 Minor allele frequency comparison across other populations

The minor allele frequency observed for the Arg389Gly SNP was compared to world populations on Ensembl (16). In this study, the minor allele corresponded to the Glycine (G) variant, while the major allele was Arginine (C). Minor allele frequencies from Kenyan, Nigerian, Gambian, African American, American, European, and South Asian populations were selected for comparison. The American population excluded African Americans and comprised individuals of Colombian descent, Americans of Mexican ancestry residing in Los Angeles, as well as Puerto Rican and Peruvian Americans.

5.3.7 Statistical Analysis

Demographic and clinical characteristics are presented in frequency tables and stratified according to the presence and absence of AF. Normally distributed data were reported as mean $\pm$ standard deviation and median, interquartile range for non-parametric data and percentages for categorical data. Categorical data were compared using Pearson's chi-square test. For normally distributed, continuous variables, a student t-test was performed and a Wilcoxon rank sum test was employed for non- parametric data.

Genotype frequency of each SNP was compared between HFrEF-SR and HFrEF-AF groups using Pearson's chi square test. Due to the absence of Gly389Gly homozygotes in the HFrEF-AF group, genotype categories were collapsed into Arg389 homozygotes vs. all other genotypes. Deviation of allelic and genotype frequencies from the Hardy-Weinberg equilibrium (HWE) was determined using a Pearson's Chi square test. A Kruskal–Wallis test was used to compare heart rate across different genotypes. A statistical significance threshold of 5% and a confidence interval of 95% was used. Statistical analyses were performed using STATA (version 17, College Station, Texas).

5.4 RESULTS

5.4.1 Baseline characteristics

A total of 118 patients' had their DNA successfully genotyped. The baseline demographic and clinical characteristics are summarised in Table 5.2. Sixty-one (52%) patients were males and the mean age of patients was 59.9 ± 14.8 years. Twenty three (20 %) patients had AF. Majority of patients were of black African ethnicity. Ninety five (89%) patients were on β -blockers and rate controlled (heart rate= 70 [61-83] beats per min).

Table 5.2. Baseline Characteristics of patients

Variable	N=118, %
Male	61 (52)
Age (Years)	59.9 ± 14.8
BMI (m ² /kg)	29.5 (25.8; 33.8)
Atrial fibrillation	23 (20)
Sinus rhythm	95 (80)
Race (%)	
Black	87 (73.7)
Caucasian	18 (15.3)
Indian	9 (7.6)
Coloured	4 (3.4)
ADHF, n (%)	32 (27.1)
Heart failure duration (years)	3.4 (0.9;7.1)
Heart rate (BPM)	70 (61; 83)
B-blocker (yes)	95 (89)
Dosage (mg)	25 (12.5; 50)
ACE-I (yes)	73 (70)
Dosage (mg)	20 (10; 20)

Data presented as mean ± standard deviation, median (interquartile range) or numbers (percentage); BMI (Body Mass Index), BPM (Beats per minute), Acute decompensated heart failure (ADHF), ACE-I (Angiotensin-converting enzyme inhibitor)

5.4.2 Genotype frequency Distribution

The heterozygous Arg/Gly genotype was predominant in the entire cohort as well as amongst patients with HFrEF-AF (Table 5.3). There was a significant difference ($\chi^2 = 6.98$; $p = 0.030$) between the genotype frequencies of HFrEF-SR (Arg/Arg = 48%, Arg/Gly = 41%, and Gly/Gly = 11%; HWE = 1: $p = 0.69$, $q = 0.31$) and HFrEF-AF patients (Arg/Gly = 69%, Arg/Arg = 30% and Gly/Gly = 0%; HWE = 1: $p = 0.65$, $q = 0.35$).

Table 5.3. Genotype frequency distribution for the overall cohort, categorised by heart rhythm

Genotype	Total	HFrEF-SR	HFrEF-AF
Arg/Arg, n (%)	53 (45)	46 (48)	7 (30)
Arg/Gly, n (%)	55 (47)	39 (41)	16 (69)
Gly/Gly, n (%)	10 (8)	10 (11)	0

Data presented numbers (percentage); HFrEF (Heart Failure with Reduced Ejection Fraction), SR (Sinus Rhythm), AF (Atrial Fibrillation), Arg (Arginine), Gly (Glycine); Arg389Arg was significantly more common in the HFrEF-AF group ($p = 0.030$)

5.4.3 Allelic Frequency Distribution

There were no differences in allelic distribution of the major and minor alleles between the two groups ($p > 0.05$) (Table 5.4). The allelic distribution amongst patients with HFrEF-AF significantly deviates from HWE ($\chi^2 = 6.44$; $p = 0.011$). The allelic distribution amongst patients with HFrEF-SR did not deviate from HWE ($\chi^2 = 0.16$; $p = 0.922$).

Table 5.4. Allelic frequency distribution in the overall cohort and categorised by heart rhythm

Allele	Total	HFrEF-SR	HFrEF-AF
C	161 (0.68)	131 (0.69)	30 (0.65)
G	75 (0.32)	59 (0.31)	16 (0.35)

Data presented numbers (percentage); HFrEF (Heart Failure with Reduced Ejection Fraction), SR (Sinus Rhythm), AF (Atrial Fibrillation).

5.4.4 Minimum allele frequency comparison across other populations

The minor allele frequency (MAF) for the selected SNP in our cohort was compared to global populations retrieved from Ensembl, including the Yoruba of Nigeria, Luhya of Kenya, African American, American, European, and South Asian populations. The MAF in our South African cohort was lower compared to Nigerian, Gambian, and African American populations, higher than American populations, and comparable to European and Kenyan population (Table 5.5).

Table 5.5 Minor allele frequency distribution across different population groups

	South Africa	Kenya	Nigeria	Gambia	African American	American	Europe	South Asian
MAF (%)	32	31	48	46	45	19	32	27

MAF (Minor allele frequency)

5.5 DISCUSSION

Our study provides novel insights into the genetic landscape of the rs1801253 (1165 G > C) SNP among South African adult patients with HFrEF-SR and HFrEF-AF. We reported the allelic and genotype frequency distribution, and compared them with other populations. Our study revealed that the Arg389, gain of function, allele was predominant in our cohort while the loss-of-function allele Gly389 was minimal and the homozygous variant completely absent in patients with HFrEF-AF and did not conform to HWE. The minimum allele frequency observed in our cohort differed substantially when compared to American and West African populations.

5.5.1 Population genetics and allelic differences

Upon comparing MAF across populations, our data show that both Arg and Gly alleles are common, but their frequencies vary by ancestry. Our South African cohort showed a 32% frequency of the Gly389 allele, comparable to Europeans. In contrast, Nigerian, Gambian and African American populations have a 45-48% Gly389 allele frequency, suggesting that the Gly389 allele is more frequent in Africans than in Europeans (~31%) and Americans (~19%) (13, 15). Africa is recognised as the cradle of human evolution and the origin of our species' global migration. Thus, Africa is more genetically diverse with approximately one million more genetic variants than the average non-African genome (16). Consequently, the genetic diversity within African populations is more extensive than that between Africans and Eurasians, lending support to the "Out of Africa" model of human evolution (17).

5.5.2 Arg389Gly variants and AF susceptibility

In our cohort, the Arg389 allele predominated – an expected finding given the above frequencies. However, a striking observation was the complete absence of the loss-of-function Gly389Gly genotype among HFrEF- AF patients whereas, HFrEF patients without AF showed the usual

distribution according to HWE principles. In other words, where one would anticipate ~10% of the Gly389 allele in a group of South African ancestry, we observed none in the HFrEF-AF group. The absence of the Gly389Gly variant in HFrEF-AF patients must be interpreted in light of both sample size and potential biological selection.

In light of the overall small sample size and even smaller number of HFrEF-AF patients (n=20), it is a possibility that the complete absence of Gly389Gly individuals in this group of patients is a size artefact and just by chance.

The more intriguing possibility is that patients with the Arg389 allele are predisposed to developing AF in HFrEF, whereas the Gly389Gly variant is relatively protective. The Arg389 variant's constitutively active state could facilitate AF onset in the setting of a stressed myocardium (18). Studies show that HFrEF patients homozygous for the Arg389 variant have an elevated resting heart rate and a greater ease of inducing tachyarrhythmias (18). Hyper-adrenergic activity leads to higher resting heart rates, greater chronotropic and ionotropic responses to catecholamines and potentially more ectopic activity – all factors that can promote AF (19). An invasive electrophysiology study found Arg389Arg patients were far more prone to trigger supraventricular arrhythmia, whereas those carrying a Gly389 allele had difficulty inducing the arrhythmia under the same conditions (20). In HFrEF, a Gly389Gly patient, by contrast, has diminished receptor function (20). Consequently, their cardiac response to adrenergic surges may be less pronounced, potentially mitigating the risk of AF (21). Thus, the absence of Gly389Gly among HFrEF-AF patients likely reflects a form of “clinical selective pressure,” rendering them more likely to remain in SR.

5.5.3 The Gly389Gly variant as a potential functional disadvantage in HFrEF

It is worth considering that the Gly389Gly variant may confer a disadvantage in HFrEF progression, which could indirectly affect AF occurrence. One might hypothesize, that if Gly389Gly patients had significantly worse systolic function or inability to mount a compensatory adrenergic response, they might succumb to HF early in the disease, thus “removing” them from the HFrEF-AF pool. However, current evidence does not support the hypothesis that Gly389Gly patients fare worse in HF. Instead, literature suggests that patients with the Arg389Arg genotype have more maladaptive remodelling and outcomes. Notably, in a cohort of African-American heart failure patients, Arg389Arg homozygotes had worse event-free survival than Gly allele carriers (22). This

is consistent with the previous hypothesis and seems unlikely that Gly389Gly patients are underrepresented in our cohort due to negative survival bias.

It is a well-established fact that the polymorphism has been implicated in response to β -blockers in HFrEF patients from Europe, America and Asia (8). However, this has never been elucidated in a Sub-Saharan African population. We did not investigate the effect of the polymorphism on patients' response to β -blockers in our cohort however, we did observe that the heart rate did not differ significantly across the genotypes. Majority of patients were on a β -blocker.

5.5.4 Limitations

The small sample size limits our ability to draw significant inferences from these data and moreover, we cannot rule out a sample size artifact in our findings. A much larger sample size is required. The inclusion of healthy South African controls could have improved the interpretation of these findings.

5.5.5 What our study adds and future studies

Our findings contribute to the expanding body of evidence that highlights the importance of genetic polymorphisms in cardiovascular diseases. Understanding the distribution and functional role of SNPs, such as rs1801253, across diverse populations is essential for personalized therapeutic strategies and improving clinical outcomes in HFrEF-AF management. These findings augment the limited data available on this SNP in SSA. Our findings are primarily descriptive and hypothesis generating. Future research should aim to build on these findings in larger, multi-ethnic cohorts and to elucidate the functional role of the Arg389Gly SNP in SSA HFrEF-AF populations.

5.5.6 Conclusion

The Gly389 allele is less prevalent among South Africans and Europeans in comparison to West African populations. The Gly389Gly, a loss-of-function variant, was absent in South African patients with HFrEF-AF. The Arg389 gain of function allele was predominant in a South African HFrEF and HFrEF-AF cohort.

5.6 REFERENCES

1. Kotecha D, Holmes J, Krum H, Altman DG, Manzano L, Cleland JGF, et al. Efficacy of β blockers in patients with heart failure plus atrial fibrillation : an individual-patient data meta-analysis. *The Lancet*. 384(9961):2235-43.
2. McDonagh TA, Metra M, Adamo M, Gardner RS, Baumbach A, Bohm M, et al. 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. *Eur Heart J*. 2021;42(36):3599-726.
3. Rienstra M, Damman K, Mulder BA, Van Gelder IC, McMurray JJV, Van Veldhuisen DJ. Beta-Blockers and Outcome in Heart Failure and Atrial Fibrillation. *JACC: Heart Failure*. 2013;1(1):21-8.
4. Van Gelder IC, Rienstra M, Bunting KV, Casado-Arroyo R, Caso V, Crijns H, et al. 2024 ESC Guidelines for the management of atrial fibrillation developed in collaboration with the European Association for Cardio-Thoracic Surgery (EACTS). *Eur Heart J*. 2024;45(36):3314-414.
5. Rau T, Düngen Hd, Edelmann F, Waagstein F, Lain M, Dimkovi S, et al. Impact of the β 1-Adrenoceptor Arg389Gly Polymorphism on Heart-Rate Responses to Bisoprolol and Carvedilol in Heart-Failure Patients. 2012;92(1).
6. Zhu M, Zhou X, Cai H, Wang Z, Xu H, Chen S, et al. Catheter ablation versus medical rate control for persistent atrial fibrillation in patients with heart failure: A PRISMA-compliant systematic review and meta-analysis of randomized controlled trials. *Medicine (Baltimore)*. 2016;95(30):e4377.
7. Beta-Blocker Evaluation of Survival Trial I, Eichhorn EJ, Domanski MJ, Krause-Steinrauf H, Bristow MR, Lavori PW. A trial of the beta-blocker bucindolol in patients with advanced chronic heart failure. *N Engl J Med*. 2001;344(22):1659-67.
8. Liu W-N, Fu K-L, Gao H-Y, Shang Y-Y, Wang Z-H, Jiang G-H, et al. β 1 Adrenergic Receptor Polymorphisms and Heart Failure: A Meta-Analysis on Susceptibility, Response to β -Blocker Therapy and Prognosis. *PLoS ONE*. 2012;7(7):e37659-e.
9. Huntgeburth M, La Rosee K, ten Freyhaus H, Bohm M, Schnabel P, Hellmich M, et al. The Arg389Gly beta1-adrenoceptor gene polymorphism influences the acute effects of beta-adrenoceptor blockade on contractility in the human heart. *Clin Res Cardiol*. 2011;100(8):641-7.
10. Brodde O-E. β 1 - and β 2 -Adrenoceptor polymorphisms and cardiovascular diseases. *Fundamental & Clinical Pharmacology*. 2008;22(2):107-25.
11. Kao DP, Davis G, Aleong R, Connor CMO, Fiuzat M, Carson PE, et al. Effect of bucindolol on heart failure outcomes and heart rate response in patients with reduced ejection fraction heart failure and atrial fibrillation. 2013;5:324-33.
12. Parikh KS, Fiuzat M, Davis G, Neely M, Blain-Nelson P, Whellan DJ, et al. Dose Response of β -Blockers in Adrenergic Receptor Polymorphism Genotypes. *Circulation: Genomic and Precision Medicine*. 2018;11(8):139-48.
13. Katsarou M-S, Karathanasopoulou A, Andrianopoulou A, Desiniotis V, Tzinis E, Dimitrakis E, et al. Beta 1, Beta 2 and Beta 3 Adrenergic Receptor Gene Polymorphisms in a Southeastern European Population. *Frontiers in Genetics*. 2018;9.

14. Woodiwiss AJ, Badenhorst D, Sliwa K, Brooksbank R, Essop R, Sareli P, et al. Beta1- and alpha2c-adrenoreceptor variants as predictors of clinical aspects of dilated cardiomyopathy in people of African ancestry. *Cardiovascular journal of Africa*. 19(4):188-93.
15. Gabriel S, Ziaugra L, Tabbaa D. SNP genotyping using the Sequenom MassARRAY iPLEX platform. *Curr Protoc Hum Genet*. 2009;Chapter 2:Unit 2 12.
16. Dyer SC, Austine-Orimoloye O, Azov AG, Barba M, Barnes I, Barrera-Enriquez VP, et al. Ensembl 2025. *Nucleic Acids Res*. 2025;53(D1):D948-D57.
17. Sibomana O. Genetic Diversity Landscape in African Population: A Review of Implications for Personalized and Precision Medicine. *Pharmgenomics Pers Med*. 2024;17:487-96.
18. Yu N, Chen FC, Ota S, Jorde LB, Pamilo P, Patthy L, et al. Larger genetic differences within africans than between Africans and Eurasians. *Genetics*. 2002;161(1):269-74.
19. Nascimento BC, Pereira SB, Ribeiro GS, Mesquita ET. Beta1-adrenergic receptor polymorphisms associated with atrial fibrillation in systolic heart failure. *Arq Bras Cardiol*. 2012;98(5):384-9.
20. La Rosee K, Huntgeburth M, Rosenkranz S, Bohm M, Schnabel P. The Arg389Gly beta1-adrenoceptor gene polymorphism determines contractile response to catecholamines. *Pharmacogenetics*. 2004;14(11):711-6.
21. Boris DJ, Luz Leiria TL, Chemello D, Saffi MAL, de Lima GG. Polymorphism of the Beta-1 Gly389Arg receptor in patients with dual atrioventricular nodal physiology. *Indian Pacing Electrophysiol J*. 2020;20(3):112-6.
22. Liggett SB, Mialet-Perez J, Thaneemit-Chen S, Weber SA, Greene SM, Hodne D, et al. A polymorphism within a conserved beta1-adrenergic receptor motif alters cardiac function and beta-blocker response in human heart failure. *Proceedings of the National Academy of Sciences*. 2006;103(30):11288-93.
23. Johnson AE, Hanley-Yanez K, Yancy CW, Taylor AL, Feldman AM, McNamara DM. Adrenergic Polymorphisms and Survival in African Americans With Heart Failure: Results From A-HeFT. *J Card Fail*. 2019;25(7):553-60.

CHAPTER 6: CONCLUSION & FUTURE DIRECTIONS

In this thesis, a narrative review employing a systematic search was conducted. A key finding emanating from this review is the paucity of robust datasets on HFrEF-AF in SSA and hence its undetermined prevalence, impact and incidence. Only 4 articles from SSA, with none originating from South Africa, were identified to provide insight into the true burden of HFrEF-AF in SSA. Notably, none of these studies were dedicated HFrEF populations with the aim to determine the burden and impact of AF; rather the AF burden and impact on HFrEF were incidental findings. The age of onset for both HFrEF and AF is considerably younger in SSA compared to HICs, and the aetiology of HFrEF is predominantly non-ischemic, with hypertensive heart disease being the leading cause of HFrEF (Figure 6.1). Although a broad range of mortality-benefitting therapy is available for HFrEF patients, this benefit is not observed in HFrEF-AF patients globally. Sub-Saharan Africans represent less than 5% of the population in most HFrEF and AF landmark randomised controlled trials. The differential response to β -blocker therapy, contingent upon the Arg389Gly polymorphism, is elucidated by the observation that HFrEF-AF patients possessing the Arg389Arg genotype exhibit minimal to no therapeutic response.

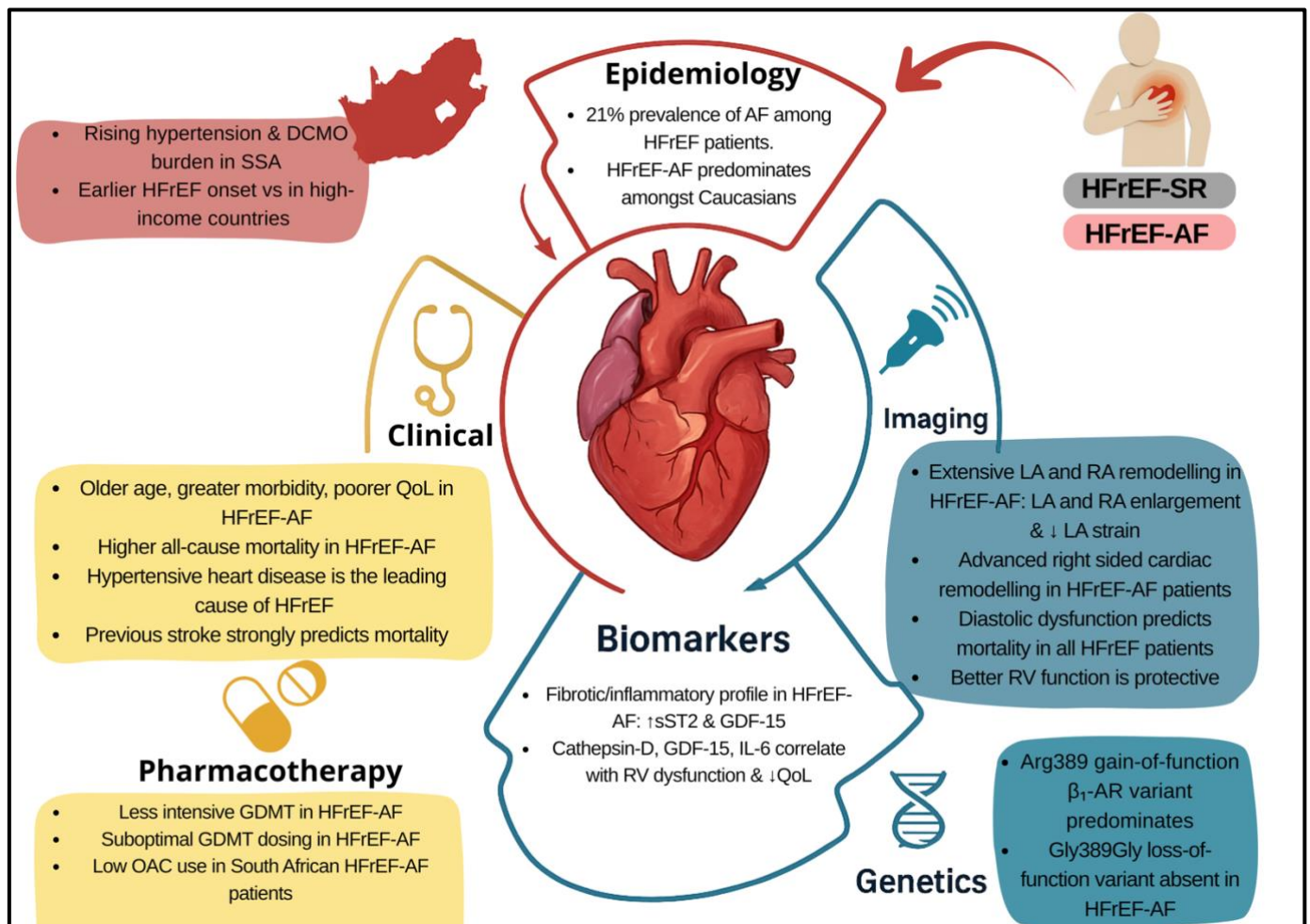


Figure 6.1 illustrates the multimodal characterisation of patients with heart failure with reduced ejection fraction and concomitant atrial fibrillation (HFrEF-AF) in South Africa. One hundred and thirty-six patients with HFrEF from the Division of Cardiology at Charlotte Maxeke Academic Hospital in Johannesburg, South Africa were enrolled in the current study. The prevalence of HFrEF-AF was 21%, with an earlier age of onset compared to that observed in high-income countries. Amongst patients with AF in this HFrEF cohort, majority were Caucasian. Clinically, patients with HFrEF-AF were worse off than their sinus rhythm (SR) counterparts, presenting at an advanced age, with greater morbidity and poor patient reported quality of life (QoL). Hypertensive heart disease was identified as the leading cause of HFrEF in this South African population. The mortality rate was higher in patients with HFrEF-AF compared to the SR group, with a history of previous stroke serving as a strong predictor of all-cause mortality. Echocardiographic assessment revealed extensive left atrial (LA) and right atrial (RA) remodelling and in the HFrEF-AF group. Additionally, these patients exhibited extensive right ventricular (RV) remodelling. Diastolic dysfunction and RV dysfunction were independently associated with all-cause mortality across all HFrEF patients. Biomarker analyses revealed a predominantly fibrotic and inflammatory profile in HFrEF-AF, with elevated levels of sST2 and GDF-15. Additional inflammatory markers—cathepsin D, GDF-15, and IL-6—were associated with RV dysfunction and reduced QoL. Upon exploration of a single nucleotide polymorphism of the β_1 -adrenergic receptor, proposed to be implicated in the pharmacogenomics of HFrEF, patients with HFrEF-AF predominantly exhibited the Arg389 gain-of-function polymorphism, with a notable absence of the Gly389 loss-of-function allele, suggesting a pharmacogenomic basis for differential treatment responses.

The baseline demographics, clinical and echocardiographic characteristics of 136 HFrEF patients, either in a state of chronic HFrEF or acute decompensated HF were described. The prevalence and the clinical and prognostic impact of AF on these patients were assessed. The mean age of the cohort was 58.7 ± 14.9 years, with HFrEF-AF patients older than those in SR, yet generally younger than patients in HICs (Figure 6.1). The overall AF prevalence was 20%, comparatively lower to HICs (Figure 6.1). The primary aetiology of HFrEF was hypertensive heart disease, followed by DCMO. These findings, coupled with an overall younger age of onset, suggest a potential underlying genetic predisposition in this predominantly black SSA cohort. Atrial fibrillation in the context of HFrEF was associated with a more severe clinical presentation, poor patient reported quality of life as well as more advanced structural remodelling on standard echocardiography and strain analysis (Figure 6.1). This translated into a poorer outcome among patients with HFrEF-AF, underpinned by rapid deterioration. We postulate that the large burden of a more severe and malignant form of hypertension, underpinned by salt-sensitivity and unique to black South Africans, may partially account for the younger age of onset and severity. Limited early access to healthcare and poor health seeking behaviour may contribute as well. However, more research is needed to further look into the underlying pathophysiological mechanisms in this sub-population of HFrEF-AF patients to better define the problem and its impact. Right ventricular dysfunction, indicative of advanced structural remodelling in HFrEF, previous stroke and poor quality of life in the preceding 2 weeks to presentation emerged as independent predictors of mortality.

Our findings demonstrate that although the proportion of HFrEF-AF patients receiving oral anticoagulation (OAC) was higher than that of their SR counterparts, the overall rate remained suboptimal. This may reflect ongoing hesitancy among healthcare professionals to initiate anticoagulation in this population, particularly in a public sector setting where warfarin is often the only available OAC. In such contexts, challenges including poor therapeutic international normalized ratio (INR) control, monitoring difficulties (such as inability to frequently attend prothrombin clinics), and concerns regarding adherence likely contribute to under-prescription. The low rate of anticoagulation may partly account for the observed higher prevalence of stroke and mortality in this HFrEF-AF cohort. Additionally, we observed a reduced uptake of guideline-directed medical therapy (GDMT) for rate control and anti-remodelling drugs—specifically β -blockers and angiotensin-converting enzyme (ACE) inhibitors—in patients with HFrEF-AF compared to those in SR. This discrepancy may be attributable to a higher proportion of HFrEF-AF patients being acutely decompensated at the time of enrolment, thereby limiting the opportunity for initiation or up-titration of these agents. Nonetheless, this finding warrants further investigation as it

reflects missed opportunities for early in-patient optimisation in this high-risk subgroup. Addressing these treatment gaps could potentially improve outcomes and reduce morbidity in patients with coexistent HFrEF and AF.

One hundred and twenty five plasma samples belonging to patients enrolled in the study were subjected to biomarker profiling using ELISAs. Seven biomarkers markers were analysed, namely : NT- proBNP, cTnT, sST2, GDF-15, cathepsin-D, GAL-3 and IL-6. Patients with HFrEF-AF exhibited a predominantly fibrotic and inflammatory biomarker profile, consistent with clinical and echocardiographic findings in Chapter 3. Moreover clinical and echocardiographic measures of disease severity closely correlate with these systemic inflammatory (IL-6) and fibrotic (sST2 and GDF-15) biomarkers. Less well -studied biomarker cathepsin-D was elevated amongst HFrEF-AF patients and strongly correlated with poor right ventricular dysfunction and sST2, suggesting a pathophysiological pathway in cardiac remodelling. This finding requires further investigation in an adequately powered cohort. We draw to the conclusion that in this cohort of HFrEF, AF is a marker of a more severe form of structural and clinical HFrEF.

The incidental finding of lower levels of GDF-15 and sST2 with PPCMO simply demonstrates the importance of using biomarkers to better phenotype specific sub-groups of HFrEF and hence risk stratify patients for timeous and targeted intervention. While we did not evaluate the impact of therapeutic intervention on levels of biomarkers, GDF-15 and sST2 have been shown to predict which HFrEF-AF patients will benefit from successful catheter ablation (1).

Lastly, the intriguing finding of complete absence of the Gly389Gly genotype among patients with HFrEF-AF (contrasted with their presence in HFrEF-SR) suggests a potential meaningful biological pattern. Arg389 allele carriers (Arg/Arg or Arg/Gly) are more prone to AF when facing the hemodynamic and neurohormonal upregulation in HFrEF. Patients with the Gly389Gly appear less susceptible to developing tachyarrhythmias given their attenuated adrenergic response to catecholamines. Our findings are supportive of a potential protective effect of the Gly389 variant in preventing AF in the context of HFrEF. However, the study is underpowered to make substantial inferences based on these findings.

Limitations

Our study had several limitations. The main limitation of the study was a small sample size. The recruitment of patients was halted due to the COVID-19 pandemic lockdown regulations. The pandemic itself may have influenced the study's mortality outcomes and completeness of follow-up data. Individuals with HFrEF were at an increased risk of severe COVID-19 infection and related complications, potentially contributing to higher mortality rates within our study population. This introduces potential bias and limits the generalisability of our findings to a pre-pandemic HFrEF population. In the detection of AF, we utilized standard 12-lead ECG and did not conduct prolonged cardiac rhythm monitoring using holter monitors or loop recorders, which may have led to underdiagnosis of paroxysmal AF. The predominance of permanent AF in our cohort may have further influenced mortality. The utilisation of highly sensitive cTnT, rather than standard assays, may have enabled better detection of cTnT levels. Access to a South African control dataset containing the Arg389Gly SNP for comparison of observed genotype and allelic frequencies would have enhanced interpretation of our findings. The study was a single-centre study conducted at CMJAH, and hence, our findings may not be generalisable to the general population.

Future Directions

Findings from chapter 3 of the current study underscores the need for early diagnosis, aggressive risk factor management, and the implementation of population-based preventive strategies to mitigate the burden of advanced HF in South Africa and the broader SSA region. Targeted management of hypertension, community screening for AF and HF symptoms, timely initiation and up titration of anti-remodeling therapy, appropriate anticoagulation and improved access to cardiac care could contribute to reducing the burden and progression to advanced disease. Health departments may leverage these findings through public awareness campaigns that promote early presentation to healthcare facilities, routine blood pressure monitoring, and adherence to antihypertensive therapy.

With the advent of catheter ablation, an aggressive rhythm strategy shown to improve outcomes in HFrEF-AF, precise and cost-effective tools for risk stratification can enable early and successful intervention in this young and high risk population. Future research should focus on employing a multi-omics approach to phenotype, risk stratify and explore pathophysiological mechanisms in these patients. Soluble ST2 and NT-proBNP are well-established biomarkers that are increasingly

recognised for their role in the diagnosis and prognostication of HF, as recently acknowledged in the 2024 European Society of Cardiology HF guidelines. However, specific cut-off values for South African patients remains undetermined. Although the current study is under-powered to propose routine clinical use of the studied biomarkers, it provides a foundation for future studies to investigate their clinical utility. It is imperative to ascertain appropriate prognostic cut-offs for biomarkers and to conduct both internal and external validation of predictive biomarker models specific to patients with HFrEF-AF in South Africa. This necessitates larger, adequately powered multi-center studies.

The findings from Chapter 5 are purely descriptive and hypothesis-generating. Larger and adequately powered studies will be essential to build on these preliminary findings and further investigate the pharmacogenomic relevance of the Arg389Gly SNP in our local setting. Although this study did not assess treatment response, our findings provide early support for the promise of precision medicine approaches in South African HFrEF cohorts. The increasing availability and affordability of genetic testing in the country—using MALDI-TOF, a technique employed in this study—paves the way for wider implementation of SNP analysis in routine clinical research and future clinical care. The large genetic diversity among Africans alone necessitates the establishment of a South African reference genome bank. Such a resource would facilitate the identification of population-specific pathogenic variants and those modulating drug response, ultimately contributing to more equitable and personalised approaches to HF management.

SUPPLEMENTARY MATERIAL

Supplementary Table 1a. Baseline characteristics of patients with HFrEF categorised according to the outcome of all-cause mortality (deceased) and alive.

Variable	Rhythm			p - value
	Total (n= 129)	Deceased (n=108)	Alive (n=92)	
Atrial Fibrillation	28 (21.7)	15 (40.5)	13 (14.1)	0.001
Gender, n (%)				
Male	61 (47.3)	17 (45.9)	51 (55.4)	0.329
Age (yrs)	58.7 ± 15.1	62.9 ± 15.7	56.2 ± 14.4	0.019
BMI (Kg/m ²)	29.4 (25.2; 34)	27.2 (24.3; 32.3)	30.9 (26.2; 34.9)	0.068
Race (%)				0.491
Black	94 (72.9)	26 (70.3)	68 (73.9)	
Caucasian	19 (14.7)	4 (10.8)	15 (16.3)	
Indian	11 (8.5)	5 (13.5)	6 (6.5)	
Coloured	5 (3.9)	2 (5.4)	3 (3.3)	
ADHF, n (%)	34 (26.4)	18 (48.7)	16 (17.4)	<0.001
HF Duration (years)	3.6 (1.3; 7.4)	3.3 (0.7; 6.7)	3.6 (1.7; 7.4)	0.464
Heart rate (bpm)	72 (62; 85)	85.5 ± 15.4	68.5 (60; 81)	<0.001
SBP	125 (109; 138)	119.8 (108; 130)	125.5 (113.5; 141.5)	0.098
DBP	61 (47.3)	17 (45.9)	51 (55.4)	0.329
KCCQ	42 (28; 59)	28 (19.5; 39.5)	47 (34; 60)	<0.001
NYHA Class				<0.001
I	48 (37.2)	2 (5.4)	46 (50.0)	
II	45 (34.9)	17 (45.9)	28 (30.4)	
III	27 (20.9)	17 (32.4)	15 (16.3)	
IV	9 (6.9)	6 (16.2)	3 (3.3)	

Data presented as mean ± standard deviation, median (interquartile range) or number (percentage); HFrEF (Heart failure with reduced ejection fraction), SR (Sinus rhythm), AF (Atrial fibrillation), BMI (Body Mass Index), SBP (Systolic blood pressure), ADHF (Acutely decompensated heart failure), DBP (Diastolic blood pressure), bpm (beats per minute), KCCQ (Kansas city cardiomyopathy questionnaire), NYHA (New York Heart Association)

Supplementary Table 1b. Baseline characteristics of patients with HFrEF categorised according to the outcome of all-cause mortality (deceased) and alive: Comorbidities and Etiologies

Variable	Rhythm			p - value
	Total (n= 129)	Deceased (n=37)	Alive (n=92)	
Comorbidities, n (%)				
Diabetes	32 (24.8)	13 (35.1)	19 (20.7)	0.085
Dyslipidaemia	38 (29.5)	12 (32.4)	26 (28.3)	0.672
Previous myocardial infarction	18 (13.9)	8 (21.6)	10 (10.9)	0.111
CKD	24 (18.6)	12 (32.4)	12 (13.0)	0.010
CAD	2 (1.6)	1 (2.7)	1 (1.1)	0.502
Previous stroke	1 (0.8)	1 (2.7)	0	0.113
Hypertension	10 (7.8)	6 (16.2)	4 (4.4)	0.023
HIV	1 (0.8)	1 (2.7)	0	0.113
Aetiology, n (%)	80 (62.0)	22 (59.5)	58 (63.0)	0.704
Hypertensive heart disease	20 (15.5)	3 (8.1)	17 (18.5)	0.141
Ischemic heart disease				
DCMO	43 (33.3)	15 (40.5)	28 (30.4)	0.271
PPCMO	25 (19.4)	9 (24.3)	16 (17.4)	0.368
Chemo-induced	25 (19.4)	6 (16.2)	19 (20.7)	0.564
Alcohol-related	12 (9.3)	2 (5.4)	10 (10.9)	0.334
Diabetic cardiomyopathy	7 (5.4)	6 (2.7)	6 (6.5)	0.386
HIV-related	5 (3.8)	2 (5.4)	3 (3.3)	0.568
Tachy-induced	3 (2.3)	2 (5.4)	1 (1.1)	0.141
Thyrocardiac	4 (3.10)	1 (2.7)	3 (3.26)	0.869
Viral myocarditis	3 (2.3)	0 (0.0)	3 (3.3)	0.266
Non rheumatic VHD	2 (1.5)	0 (0.0)	2 (2.2)	0.366

Data presented as number (percentage); HFrEF (Heart failure with reduced ejection fraction), SR (Sinus rhythm), AF (Atrial fibrillation), CKD (Chronic kidney disease, CAD (coronary arterial disease), DCMO (Idiopathic dilated cardiomyopathy), PPCMO (Post-partum cardiomyopathy), VHD (Valvular heart disease)

Supplementary Table 2a. Left ventricular and left atrial echocardiographic parameters categorised according to the outcome of all-cause mortality (deceased) and alive.

Variable	Rhythm			p - value
	Total (n= 129)	Deceased (n=108)	Alive (n=92)	
LVEF (%)	32 (22; 39.5)	24 (19; 30.5)	36 (24.5; 41)	<0.001
IVSD (mm)	10.7± 2.5	10.3 ± 2.5	10.8 ± 2.3	0.294
LVIDd (mm)	57.8 ± 9.4	68 (45.2; 68)	56 (51; 64)	0.060
LVIDs (mm)	51 ± 11.1	57 (50; 62)	49.8 ± 10.9	0.018
LVPWD (mm)	10.2 ± 2.1	9.9 ± 2.2	10.4 ± 2.2	0.294
LA area (cm ²)	22.5±8.4	24.0 ± 4.9	18.5 (16.4; 23.8)	<0.001
LA reservoir function	39.6 [26.7-50.9]	28.3 [22.6-34.2]	43.6 [30.1-54.6]	<0.000
E/E' ratio	12.9±5.3	14.9 ± 5.0	10.9 (7.9; 15.1)	0.006
MR severity				0.008
Nil	22 (19.3)	2/33 (6.1)	20/81 (24.7)	
Trivial	12 (10.5)	0	12/81 (14.8)	
Mild	40 (35.1)	14/33 (42.4)	26/81 (32.1)	
Moderate	30 (26.3)	12/33 (36.4)	18/81 (22.2)	
Severe	10 (8.8)	5/33 (15.2)	5/81 (6.2)	

Data presented as mean ± standard deviation, median (interquartile range) or number (percentage); HFrEF (Heart failure with reduced ejection fraction), SR (Sinus rhythm), AF (Atrial fibrillation), LVEF (left ventricular ejection fraction), IVSD (interventricular septal diameter), LVIDd (left ventricular internal diameter in diastole), LVIDs (left ventricular internal diameter in systole), LVPWD (left ventricular posterior wall dimension at end-diastole) LV (left ventricle), LA (left atrium), E (Peak early diastolic mitral inflow velocity), e' (Early diastolic mitral annular tissue velocity), a (Peak late diastolic mitral inflow velocity), MV (mitral valve).

Supplementary Table 2b. Right ventricular, right atrial and pulmonary echocardiographic parameters categorised according to the outcome of all-cause mortality (deceased) and alive.

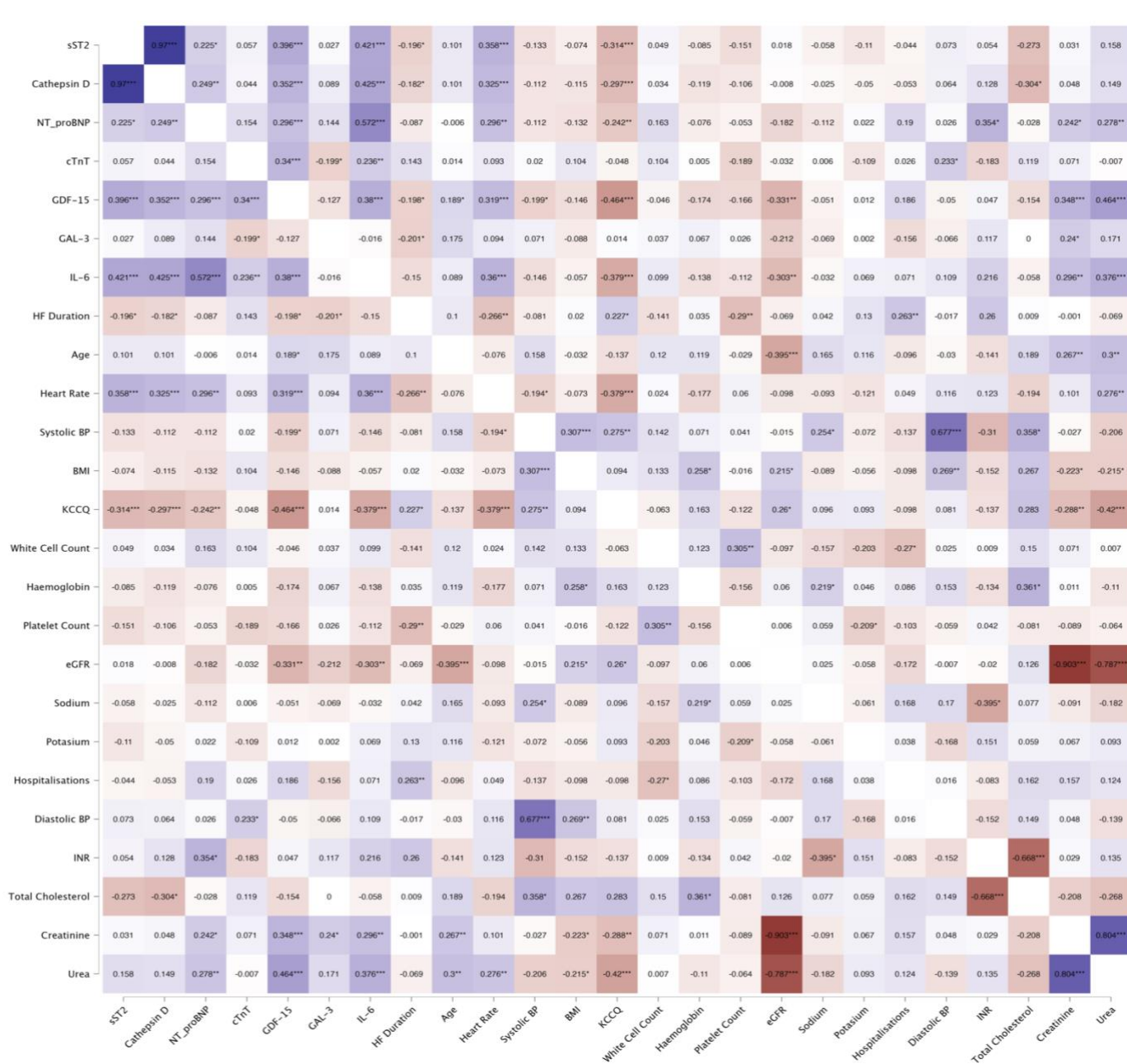
Variable	Rhythm			p - value
	Total (n= 129)	Deceased (n=108)	Alive (n=92)	
Basal RV Dimension (mm)	42.3 ± 9.0	46.5 ± 8.8	40.5 ± 8.6	<0.001
Mid RV Dimension (mm)	31.1 ± 8.2	33.5 (27.6; 38.5)	29 (26; 34)	0.059
Pulmonary artery Diameter (mm)	22.3 ± 3.7	22.7 ± 3.5	21 (20; 24)	0.348
Normal tricuspid valve	115 (98.3)	93 (98.9)	22 (95.7)	0.276
RA Volume	45.5 (32; 73.1)	61 (55; 66)	41.1 (30; 65)	0.003
TAPSE (mm)	16.1 ± 4.1	14.1 (11; 19)	17.0 ± 3.9	<0.001
TR Vmax (m/sec)	2.6 (2.4; 3.1)	2.9 (2.5; 3.3)	2.6 (2.4; 2.9)	0.093
Systolic Pulmonary artery pressure (mmhg)	38.9 ± 14.5	41.4 (33; 50)	33 (28; 40)	0.032

Data presented as mean ± standard deviation, median (interquartile range) or number (percentage); RV (right ventricle), RA (right atrium), TAPSE (tricuspid annular plane systolic excursion), TR Vmax (tricuspid regurgitation peak velocity at tricuspid valve).

Supplementary Table 3. Univariable logistic regression analysis for predictors of all- cause mortality

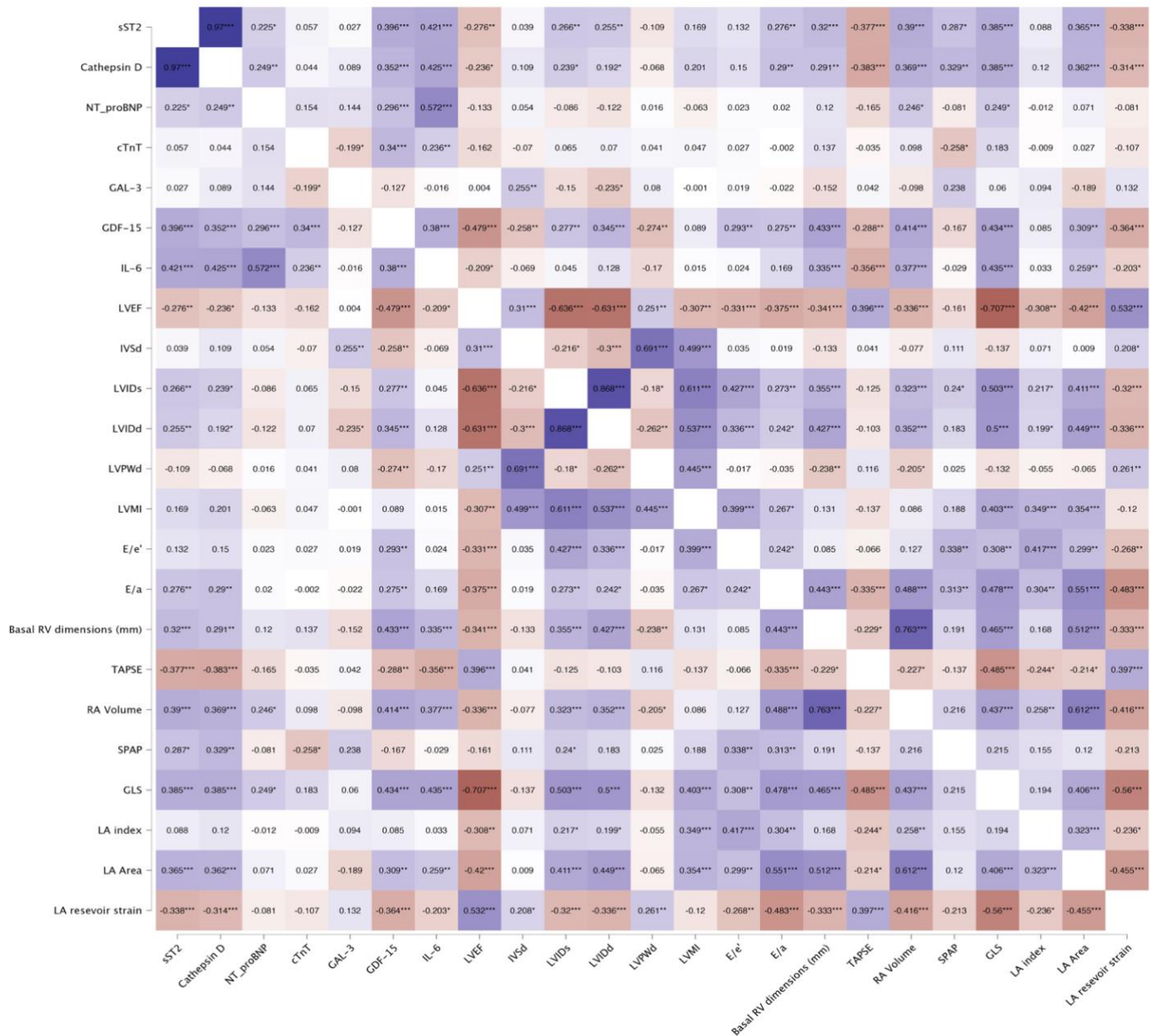
Predictor variable	Univariate OR (95%CI)	p-value
Age (years)	1.03 (1.00-1.06)	0.022
BMI	0.98 (0.94-1.03)	0.504
Heart rate	1.04 (1.01-1.07)	0.002
KCCQ	0.94 (0.92-0.97)	<0.001
MR severity	1.04 (0.75-1.46)	0.800
ADHF	4.5 (1.94-10.4)	<0.001
Diabetes	2.1 (0.89-4.8)	0.088
Previous stroke	4.3 (1.1-16.1)	0.033
NT-proBNP	1.00 (1.00-1.00)	0.031
eGFR	0.97 (0.96-0.99)	0.004
Creatinine	1.01 (1.00-1.02)	0.015
Chronic kidney disease	3.2 (1.3-8.0)	0.013
LVEF	0.93 (0.89-0.98)	0.002
LVIDs	1.04 (1.01-1.09)	0.025
LA AREA	1.03 (0.9-1.1)	0.226
E/e'	1.10 (1.02-1.2)	0.018
Basal RV dimension	1.10 (1.0-1.1)	0.003
Mid RV dimension (mm)	1.04 (0.99-1.1)	0.158
TAPSE	0.79 (0.7-0.9)	0.001
RA volume	1.01 (1.00-1.02)	0.006
SPAP	1.03 (0.99-1.1)	0.127
LA reservoir function	0.93 (0.91-0.97)	0.001

CI (Confidence Interval), BMI (body mass index), KCCQ (Kansas city cardiomyopathy questionnaire), MR (mitral regurgitation), ADHF (acutely decompensated heart failure), eGFR (estimated glomerular filtration rate), LVEF (left ventricular ejection fraction), LVIDs (left ventricular internal diameter at end-systole), LA (left atrium), E (Early diastolic mitral inflow velocity), e' (Early diastolic mitral annular tissue velocity), RV (right ventricle), RA (right atrium), TAPSE (tricuspid annular plane systolic excursion), SPAP (systolic pulmonary artery pressure).



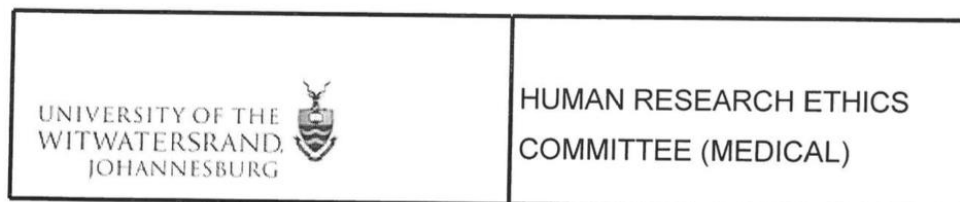
Supplementary Figure. 1 Spearman's rho heatmap illustrating the correlation between continuous clinical Characteristics and standard and novel cardiac biomarkers. The intensity of the colour indicates the strength of the correlation; sST2 (soluble suppression tumorigenicity 2), GDF-15 (growth differentiation factor – 15), NT-proBNP (N-terminal pro b-type natriuretic peptide), cTnT (cardiac troponin T), GAL-3 (galectin-3), IL-6 (interleukin-6), HF (heart failure), BP (blood pressure), BMI (body mass index), KCCQ (Kansas city cardiomyopathy questionnaire), eGFR (estimated glomerular filtration rate), INR (international normalised ratio)

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$



Supplementary Figure 2. Spearman's rho heatmap illustrating the correlation between echocardiographic parameters and standard and novel cardiac biomarkers. The intensity of the colour indicates the strength of the correlation. sST2 (soluble suppression tumorigenicity 2), GDF-15 (growth differentiation factor – 15), NT-proBNP (N-terminal pro b-type natriuretic peptide), cTnT (cardiac troponin T), GAL-3 (galectin-3), IL-6 (interleukin-6), LVEF (left ventricular ejection fraction), IVSd (interventricular septum thickness at end-diastole), LVIDs (left ventricular internal diameter at end-systole), LVIDd (left ventricular internal diameter at end-diastole), LVPWd (left ventricular posterior wall thickness in diastole), LVMI (Left ventricular mass index), E (Early diastolic mitral inflow velocity), e' (Early diastolic mitral annular tissue velocity), RV (Right ventricle), TAPSE (Tricuspid annular plane systolic excursion), RA (Right atrium), SPAP (systolic pulmonary artery pressure), GLS (Global longitudinal strain), LA (Left atrium). *p < 0.05, ** p < 0.01, *** p < 0.001

APPENDIX A:



Office of the Deputy Vice-Chancellor (Research & Post Graduate Affairs)

TO: Ms N Mboweni
School of Physiology
Medical School
University

E-mail: nene.mboweni@gmail.com

CC: Supervisor: Dr N Tsabedze and Professor M Maseko
<Muzi.Maseko@wits.ac.za>
and <HREC-Medical.ResearchOffice@wits.ac.za>

FROM: Iain Burns
Human Research Ethics Committee (Medical)
Tel: 011 717 1252

E-mail: Iain.Burns@wits.ac.za

DATE: 2019/06/07

REF: R14/49

PROTOCOL NO: M190349 *(This is your ethics application study reference number. Please quote this reference number in all correspondence relating to this study)*

PROJECT TITLE: *The clinical and biochemical effects of atrial fibrillation on heart failure with reduced ejection fraction*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to the Government funding of the University.



MSWorks2000/Iain0007/Clearscan.wps



R14/49 Ms N Mboweni

**HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M190349**

NAME: Ms N Mboweni
(Principal Investigator)
DEPARTMENT: School of Physiology
Medical School
University

PROJECT TITLE: The clinical and biochemical effects of atrial fibrillation on heart failure with reduced ejection fraction

DATE CONSIDERED: 29/03/2019

DECISION: Approved Unconditionally

CONDITIONS:

SUPERVISOR: Dr N Tsabedze and Professor M Maseko

APPROVED BY: 
Dr CB Penny, Chairperson, HREC (Medical)

DATE OF APPROVAL: 2019/06/07

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the 3rd Floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to submit details to the Committee. I **agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **March** and will therefore reports and re-certification will be due early in the month of **March** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

Date

PLEASE QUOTE THE CLEARANCE CERTIFICATE NUMBER IN ALL ENQUIRIES

APPENDIX B:

Study Protocol

Project Title: The Clinical and Biochemical Effects of Atrial Fibrillation on
Heart Failure with
Reduced Ejection Fraction

Principle Investigator: Nonkanyiso Mboweni

Degree: Doctor of Philosophy (PhD)

Supervisors: Prof. Muzi Maseko and Dr. Nqoba Tsabedze

Institution: University of Witwatersrand , Faculty of Health Sciences

1. Background

Over the last decade, heart failure (HF) has become a major public health concern. Owing to the effects of westernization and urbanisation, an epidemiological transition from infectious to non-communicable diseases (NCDs) is evidenced by an increase in the prevalence of traditional risk factors for cardiovascular diseases i.e. obesity, diabetes mellitus and hypertension (Keates *et al.*, 2017). In Sub-Saharan Africa, over 50% of the cases of HF are attributable to cardiomyopathies and hypertensive heart disease (Agbor *et al.*, 2018; Nyaga *et al.*, 2018). On the contrary, ischaemic heart disease is the main cause of HF in high-income countries (Agbor *et al.*, 2018). Early literature (Stewart *et al.*, 2008; Ntusi & Mayosi 2009) on the epidemiology of HF in South Africa, reported that people living with HF frequently present with heart failure with reduced ejection fraction (HFrEF) (dilated cardiomyopathy and hypertensive heart disease being the underlying causes). HF is associated with increased morbidity and mortality (Agbor *et al.*, 2018). The frequency of heart failure-related hospitalisations places a huge burden on our economy (Agbor *et al.*, 2019).

Atrial fibrillation, a common comorbidity in HF, is characterised by a rapid and haphazard electrical activity in the atria (Denham *et al.*, 2018). Atrial fibrillation is a major decompensating factor and determinant of stroke risk and mortality amongst patients with HFrEF (Chugh *et al.*, 2014). Other effects of AF include a reduction in the quality of life as consequence of symptoms such as exercise intolerance and palpitations (Thrall *et al.*, 2006; Zafir *et al.*, 2018). The co-occurrence of HF and AF, in high-income countries, is reported in more than 50% of HF patients (Maisel & Stevenson, 2003; Krijthe *et al.*, 2013). The prevalence of AF is predicted to double, in high-income countries, by 2060 (Chugh *et al.*, 2014). Due to an increase in the prevalence of risk factors such as hypertension, obesity, diabetes, heart failure, and a younger age of onset in Sub-Saharan Africa, a similar rise in the prevalence of

AF is anticipated (Stewart *et al.*, 2008; Agbor *et al.*, 2019). The prevalence of AF is reported to increase with HF chronicity and severity (Wongcharoen & Chen, 2012). There is a bidirectional relationship between HF and AF, where adverse structural and electrical remodeling of the atria as a consequence of heart failure predisposes to the onset of AF (Nattel & Harada, 2014; Denham *et al.*, 2018). Moreover, AF aggravates heart failure, eliciting an increase in heart failure symptoms, hospitalisations and mortality (Zafir *et al.*, 2018).

The bulk of published literature on the pathophysiological mechanisms of HF/AF is predominantly based on that of HFrEF/AF. Left atrial enlargement and dysfunction have been implicated in the bidirectional relationship between HFrEF and AF. The maintenance of AF in the setting of HFrEF is, in part, a result of dysfunctional calcium handling in the atria secondary to atrial dilatation (Denham *et al.*, 2018). In HFrEF, an increase in left ventricular end diastolic pressure results in elevated left atrial pressure. The elevated atrial pressure leads to an increase in atrial mechanical wall stress, which subsequently alters calcium handling within the atria (Schönleitner *et al.*, 2017). Atrial mechanical stress constitutes stretch and shear stress resulting from increased intra-atrial volume and pressure, respectively (Eckstein *et al.*, 2008). Underlying these pathophysiological mechanisms of HFrEF are processes of fibrosis and inflammation. These pathophysiological mechanisms ultimately cause structural and electrophysiological remodeling of the atria which sets the milieu for spontaneous firing, and hence arrhythmogenicity, at the pulmonary veins (Lin *et al.*, 2016). These changes in left atrial structure and function changes have important prognostic implications in HFrEF (Gan *et al.*, 2018), independent of recognised parameters such as left ventricular ejection fraction.

In recent years, two-dimensional speckle tracking echocardiography (2D-STE) has been shown to be a simple and noninvasive tool for assessing left atrial function in patients with heart

disease. Atrial function is dependent on the haemodynamic conditions of left ventricular (LV) filling, which conventional, transthoracic echocardiography does not encompass in its analysis of left atrial function (Yuda *et al.*, 2016). Two-dimensional speckle tracking echocardiography, however, does account for haemodynamic conditions which are: left atrial reservoir (during left ventricular systole), conduit (during diastasis) and contraction function (during ventricular systole). This property of 2D-STE enables early and accurate detection of atrial dysfunction (Yuda *et al.*, 2016). Thus, Left atrial dysfunction as determined by 2D-STE can provide useful prognostic information in addition to conventional echocardiographic parameters. Thus, assessing left atrial 2D-STE in patients with HFrEF/AF against patients with HFrEF/SR will help clinicians to better prognosticate patient outcomes.

The diagnosis and management of HFrEF in its early stages is critical and could aid the prevention of AF. In recent years, clinical studies have revealed some insight into the clinical characteristics and prognostic outcomes of heart failure with concomitant AF. However, the majority of research is targeted to high-income countries. Despite a growing number of HFrEF patients with independent risk factors for AF such as obesity and hypertension, there is a paucity of data on the demographics, clinical and biochemical profile of HFrEF/AF in South Africa. A recent European study (Santema *et al.*, 2018) reported that patients with HFrEF/AF had a biomarker profile which correlated with their outcomes. However, this was not observed in patients with heart failure with preserved ejection fraction and AF where their biomarker profile did not correlate with patient outcomes (Santema *et al.*, 2018). This data not only suggests that AF plays a different pathophysiological role in each HF modality but that biomarkers may have the potential to aide our understanding of the underlying pathophysiology of HFrEF/AF.

Brain natriuretic peptide (BNP) and N-terminal-pro brain natriuretic peptide (NT-proBNP) (the inactive prohormone of BNP) are peptides released from the ventricles in response to increased ventricular volume and pressures (Bonow *et al.*, 2018). Both peptides have been shown to correlate strongly with left ventricular dysfunction (Gluba *et al.*, 2012). Given its long half-life, NT-proBNP is the preferred natriuretic peptide for clinical use (Bonow *et al.*, 2018). Recent literature showed that NT-proBNP predicts mortality (Santema *et al.*, 2018) and stroke (Hijazi *et al.*, 2016) in patients with HFrEF/AF. In the study conducted by Santema *et al.* (2018), soluble suppressor of tumorigenicity 2 (ST2), a marker of maladaptive cardiac remodeling, matrix metalloproteinase-2 (MMP2) and spondin 1 (SPON 1) were significantly increased in patients with AF compared to those in sinus rhythm (Santema *et al.*, 2018). A sub-study analysis of the ‘Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation (ARISTOTLE)’ trial (Horowitz *et al.*, 2018) reported that increased levels of asymmetric dimethylarginine (ADMA) were weakly associated with thromboembolic events in anticoagulated patients with AF. Similarly, elevated levels of symmetric dimethylarginine (SDMA) were weakly associated with bleeding events. Both markers showed a strong association with increased mortality. These data encourage additional cross-population studies to further interrogate the underlying mechanisms and clinical relevance of such findings in attempts to identify new therapeutic targets for the treatment of HFrEF/AF.

The presence of atrial fibrillation presents a challenge in the diagnosis and treatment of HF. Managing patients with AF and HFrEF is a growing point of uncertainty for many physicians as these patients do not respond to mortality-benefitting therapy (Kotecha *et al.*, 2014). Beta-blockers are used for management of patients with AF alone (Kirchhof *et al.*, 2016; Van Gelder *et al.*, 2016). Similarly, beta-blockers are used to manage all stable patients with HFrEF. This use of beta-blockers in HFrEF patients is based on findings from clinical trials that showed

beta-blockers significantly reduce outcomes in HFrEF patients (in sinus rhythm) with an ejection fraction below 50% (Rienstra *et al.*, 2013; Van Gelder *et al.*, 2016). However, recent data has shown that patients with HFrEF/AF do not respond to beta-blockers (Rau *et al.*, 2012; Kotecha *et al.*, 2014; Zhu *et al.*, 2016). It was reported that HFrEF/AF patients with a homozygous Glycine389 mutation in the beta₁-adrenergic receptor did not respond to beta-blocker therapy compared to those without the mutation, who showed significant reduction in adverse outcomes (Kao *et al.*, 2013; Aleong *et al.*, 2014).

The **Arginine389** on transmembrane seven is situated at the c-terminus of the beta₁-adrenergic receptor where it plays a key role in Gs coupling and hence signal transduction (Brodde, 2008). The literature has proposed a role for the Arginine389Glycine polymorphism in rendering heart failure patients unresponsive to beta-blocker therapy (Brodde, 2008). Patients with the loss-of-function homozygous Glycine389 variant are proposed to be poor responders and patients with a homozygous Arginine389 mutant, good responders (Brodde, 2008). This effect has been demonstrated only in European, American and Asian patients (Liu *et al.*, 2012). A randomised control trial (Rau *et al.*, 2012; Parikh *et al.*, 2018), comparing beta-blockers in patients with HFrEF/AF and heart failure alone (sinus rhythm) showed that only patients with HFrEF/AF homozygous for the Arginine389 allele show little or no sustained response to carvedilol, a non-selective beta-blocker commonly used in the South African public sector hospitals. The cohort of patients in this study were of (South) Eastern European decent. Moreover, a recent study (Katsarou *et al.*, 2018) focused particularly on a South-Eastern European population, demonstrated that the frequency distribution of the Arginine389Glycine genetic variants differ significantly between European and Non-European populations. Despite the growing numbers of beta-blocker unresponsive HFrEF/AF patients in our setting and Sub-Saharan Africa at large, very little is known about the clinical relevance of this polymorphism, and which genetic

variant is dominant in our setting. To date, only one study has looked for this polymorphism in an African population and employed a very stringent selection criteria excluding patients on beta-blocker therapy as well as patients with arrhythmias (Woodiwiss *et al.*, 2008).

These data and the lack of definitive evidence for the pharmacological management of patients with HFrEF/AF, necessitates the development of novel therapies aimed at the pathophysiological pathways of HFrEF/AF. Furthermore, thorough interrogation of whether or not adopting an aggressive rhythm strategy to treat patients with HFrEF/AF is superior to the current guideline therapy is warranted.

Given the differences in aetiology, genetic make-up, and age of onset of HFrEF/AF, we know that data obtained from studies conducted in high-income countries cannot be easily appropriated to low-to-middle income regions such as sub-Saharan Africa. The global health burden of HFrEF/AF and the paucity of epidemiological and clinical characterization of these patients in a South African context warrants further, targeted investigation.

Aim

The aim of the present study is to investigate the clinical and biochemical effects of atrial fibrillation on patients with heart failure with reduced ejection fraction at a dedicated HFrEF clinic in a South African tertiary hospital.

2. Study Objectives

- To determine the prevalence of HFrEF/AF at the Charlotte Maxeke Academic hospital (CMJAH) HFrEF clinic,
- To compare the epidemiology and clinical characteristics between patients with HFrEF/AF and HFrEF/SR at the CMAJH HFrEF clinic,

- To ascertain the presence of the Arginine389Glycine beta₁-adrenergic receptor mutation in HFrEF/AF and HFrEF/SR patients using Single Nucleotide Polymorphism genotyping using iPLEX® assays on a MassArray system.
- To determine the significance of clinically available biomarkers and determine the levels of novel biomarkers; N-terminal BNP (NT-proBNP), suppression of tumorigenicity (ST2), tumour necrosis factor-alpha (TNF-α), Fas/Apo1, Transforming growth factor beta (TGF-β) matrix metalloproteinase 2 (MMP2), interleukin-4 (IL-4) and 6 (IL-6), interferon gamma (INF-γ), midregional-proadrenomedullin (MR-proADM), asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) in HFrEF/AF patients and assess if these markers are associated with the condition and potentially predict prognosis,
- To determine whether an association exists between clinical characteristics, observed levels of standard, and novel cardiac biomarkers in patients with HFrEF/AF and those with HFrEF/SR using a multivariate linear regression model,
- To compare left atrial function in patients with HFrEF/AF versus HFrEF/SR using 2D-speckle tracking echocardiography,
- To determine the mortality and hospitalisations in patients with HFrEF/AF versus HFrEF/SR, after 12 months.

3. Methods

3.1 Study Design

The present study is an observational, prospective study conducted at Charlotte Maxeke Academic Hospital in Johannesburg. Study participants will be recruited from the HFrEF clinic within the division of Cardiology.

3.2 Study population and sample

Adult (≥ 18 years) patients will be recruited from a dedicated HFrEF clinic, at Charlotte Maxeke Academic Hospital. All patients attending the HFrEF clinic will be recruited consecutively (enumerative sampling) to participate in the study. The HFrEF clinic sees 500 patients within a period of six months. The sample size for the current study is $n = 500$. The prevalence of atrial fibrillation in patients with HF is 20- 30% (Chugh *et al.*, 2014) therefore at 95% CI, the minimum recommended sample size of HFrEF/AF patients is 109 for the present study.

3.2.1 Inclusion and exclusion criteria

Patients 18 years of age and above, who have given verbal and written consent, with an ejection fraction $< 40\%$; diagnosed with heart failure with reduced ejection fraction (HFrEF) confirmed by echocardiography will be enrolled. Within the enrolled group, patients with atrial fibrillation will be identified. The diagnosis of atrial fibrillation (AF) will be defined according to a documented history of AF or a finding of AF by 12-lead electrocardiogram upon consultation. Patients with valvular heart disease will be excluded from the study. Similarly, patients with a pacemaker rhythm, unknown or any other rhythm will be excluded.

3.2.1 Study groups

Patients will be desegregated into two arms namely, patients diagnosed with heart failure with reduced ejection fraction in sinus rhythm (HFrEF/SR) and patients with heart failure with reduced ejection fraction and concomitant atrial fibrillation (HFrEF/AF).

3.3 Data Collection

Data will be collected using REDCap electronic data capture tools hosted at the University of Witwatersrand. Data captured will consist of patient data obtained from patient medical records and upon patient consultation. Past medical records will be used to ascertain a number of

variables such as HF aetiology, risk factor profile, co-morbidities as well as concomitant pharmacological therapy.

3.4 Variables

3.4.1 Clinical characteristics

a) Demographic and anthropometric measurements

Detailed socio-demographic and clinical data will be attained recorded. Age, race and sex will be captured. Standard anthropometric measures such as height and weight will be measured. Patients will be classified as overweight if their body mass index (BMI) is $\geq 25 \text{ kg/m}^2$ and obese if their BMI is $\geq 30 \text{ kg/m}^2$.

b) Clinical characteristics

Signs and symptoms of HF (dyspnea, heart murmurs, rales, elevated jugular venous pressure, peripheral edema orthopnea and paroxysmal nocturnal dyspnea) will be assessed during consultation. Similarly, functional class as indexed by the New York Heart Association and vital signs (temperature, respiratory rate, heart rate and blood pressure) will be documented. Nurse-derived blood pressure and heart rate will be recorded using a mercury sphygmomanometer. The Kansas City Cardiomyopathy Questionnaire (KCCQ) (Faller *et al.*, 2005) will be used to obtain information on patient quality of life. CHADS-VASc score (Olesen *et al.*, 2012) will be documented to assess stroke risk.

c) Co-morbidities and risk factor profile

Patients will be screened for Hypertension, dilated cardiomyopathy, diabetes

mellitus, chronic kidney disease (defined as an eGFR <60 mL/min per 1.73 m²), coronary artery disease, anaemia, dyslipidemia, family history of heart failure, prior stroke, smoking history and alcohol intake. Hypertension will be defined as the use of antihypertensive medication or if the mean blood pressure measurements are >140/90 mm Hg in patients not taking medication.

d) Concomitant pharmacological therapy

Concomitant pharmacological treatment will be documented. The following medications will be considered: angiotensin converting enzyme (ACE) inhibitors, Beta blockers, angiotensin II receptor blockers (ARBs), diuretics, mineralocorticoid receptor blockers, ivabridine, digoxin, potent vasodilators, anti-arrhythmics, statins, calcium channel blockers, anticoagulant and antiplatelet therapy.

e) Echocardiography

Upon enrollment, all patients will undergo echocardiographic assessment of ventricular function as indexed by left ventricular function, valvular integrity and function, and regional wall abnormalities. Left atrial strain parameters will be obtained using two-dimensional speckle tracking echocardiography (2D-STE) to assess left atrial function.

f) Electrocardiography

Baseline ECG parameters (RR interval, P wave, PR interval, QRS complex, ST segment, T wave, ST interval, bundle branch morphology, and axis) will be analysed.

3.4.2 Blood biochemistry

Venous blood will be drawn by a trained phlebotomist. Blood samples will be centrifuged and stored at -80 degrees celcius until analysis. The following standard and novel prognostic and diagnostic cardiac biomarkers will be measured: N-terminal BNP (NT-proBNP), creatine kinase-muscle/brain (CKMB), cathepsin-D, high sensitivity cardiac troponin T, suppression of tumorigenicity (ST2), Fas/Apo1, matrix metalloproteinase 2 (MMP2), tumour necrosis factor-alpha (TNF-a), Transforming growth factor beta (TGF-B), interleukin- 6 (IL-6) and 4(IL-4), interferon gamma (INF-y), midregional-proadrenomedullin (MR-proADM), asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA).

3.4.3 Arginine389Glycine Beta₁-adrenergic Single Nucleotide Polymorphism (SNP) Detection

Primer Design

Primers will be designed and synthesized for the Arg389Gly or rs1801253 (1165 G > C) single nucleotide polymorphism (SNP) by iNqaba Biotec (Pretoria, South Africa), using Assay Design Suite (ADS) software.

DNA Extraction and SNP Detection

DNA will be extracted from the buffy coat layer in blood samples as using standard protocols**. DNA extraction will be done in the Department of Internal Medicine Laboratory at the University of Witwatersrand.

Purified DNA samples will be sent to iNqaba Biotec (Pretoria, South Africa) where they will be subjected to single nucleotide polymorphism genotyping using an iPLEX® Assay and MassARRAY® System. DNA will be subjected to PCR and subsequent primer extension using the iPLEX® assay. Analytes will be transferred onto a SpectroCHIP® Array and loaded into

the MassARRAY® where they will be analysed using highly sensitive matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. INqaba Biotech will generate a genotyping report which will be used for data analysis.

3.4.3. Patient Follow up and Outcome measures

Telephonic patient follow-up will be conducted one year following enrollment to ascertain mortality and HF-related hospitalisations.

3.5 Statistical analysis

Normally distributed data will be reported as mean (SD), median (interquartile range) for non-parametric data and percentages for categorical data. Categorical data will be compared using chi-square test or Fisher's exact. For normally distributed, continuous variables a student t-test will be performed and a Wilcoxon rank sum test or Kruskal Wallis test will be employed for non-parametric data. Multivariate linear regression model will be used to determine whether associations exist between measured levels of standard and novel biomarkers, and clinical characteristics and imaging indices. Cox proportion regression models will be used to determine outcomes. Demographic, clinical and biochemical characteristics will be presented in frequency tables and stratified according to the presence of AF and the absence of AF (sinus rhythm). Kurtosis and skewness normality tests will be performed on continuous variables. A significance threshold of 5% and confidence interval of 95% will be used. Statistical analyses will be performed using STATA (version 15, College Station, Texas).

3.6 Ethical consideration

Ethical approval will be obtained from the Human Research Ethics Committee (HREC) of the University of the Witwatersrand, Johannesburg, South Africa. Verbal and written informed consent will be obtained from patients prior to their participation. Participant identities will be

anonymised upon data capturing. REDCap software will automatically generate random study participant ID numbers.

3.7 Ethics and Hospital approval

Ethics and hospital approval.

An ethics application has been made to the University of Witwatersrand Human Research Ethics Committee (HREC) for the current study. Approval from the CEO of Charlotte Maxeke Academic Hospital to conduct the current study has been obtained.

4. Timelines

4.1 Gantt chart of the study running from January 2019 to February of the year 2021

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb
Protocol Preparation (2019)														
Protocol Submission (2019)														
Ethics application (2019)														
Systematic review preparation (2019)														
Submit review for publication (2019)														
Data collection (2019)														
Data analysis (2019)														
Write up first paper (2019-2020)														
Write up second paper (2020)														
Data collection (Patient follow-up) (2020)														
Data analysis (2020)														
Write up third paper (2020-2021)														
Write up thesis (2021)														

5. Budget/Funding

PROJECT: CLINICAL AND BIOCHEMICAL EFFECTS OF AF IN HFREF (3 YEAR BUDGET)				
CATEGORY	ITEM	COST PER ITEM	NUMBER NEEDED	TOTAL COST
PARTICIPANT REIMBURSEMENT	Transport and time reimbursement (1 Visit)	250	500	125 000
Research assistant/nurse		20 000 pm	24	240 000
Phlebotomist		1000 pm	8	8000
ECHOCARDIOGRAPHY	RESEARCH ECHOCARDIOGRAPHY FORMAL RECORDING			
	1 DVD disks	10	500	5 000
	2 ECHO TECH ASSISTANCE	500	500	250 000
	3 Linen savers (200 per box)	400	3	12000
	4 Ultrasound gel (5000ml)	122	2	244
CARDIAC BIOMARKER ANALYSIS				
CARDIACMYOCYTE DYSFUNCTION				
	PROGNOSTIC AND DIAGNOSTIC			
	NT-proBNP (ELISA 1x96 wells)	8 508	6	51 048
	DIAGNOSTIC			
	BNP			
	CKMB			
	Cathepsin D			
	PROGNOSTIC			
	hs-cTNT			
	hs-cTNI			
CARDIAC REMODELLING				
	PROGNOSTIC AND DIAGNOSTIC			
	Fas/Apo1			
	INF-y			
	DIAGNOSTIC			
	ST2			
	MMP2			
	TGF-B			
	TNF-a			
	INF-y			
	IL-6			
	IL-4			
ENDOTHELIAL DYSFUNCTION				
	DIAGNOSTIC			
	MR-proADM			
	PROGNOSTIC			
	ADMA (Atrial Fibrillation)			
	SDMA (Atrial Fibrillation)			
WHOLE BLOOD	Thermofischer ChargeSwitch™ gDNA Serum Kit (50 purifications)	2 170	12	26 040
LAB CONSUMABLES				
	1 Set of pipettes (4 adjustable pipettes)	13 031	1	13 031
	2 Pipette tips	5 000	1	5 000
	3 1.5ml microcentrifuge tubes (500)	250	4	1 000
	4 Cryovials (2ml) pack of 1000	5 626	1	5 626
	6 Nitrile Gloves	280	10	2 800
BLOOD COLLECTION				
	1 Purple top tubes (EDTA) (pack of 100)	921	15	13 815
	2 5ml Syringes	7	580	4 060
	3 Needles (x50)	350	12	4 200
	4 Nitrile Gloves	280	13	3 640
	5 Medi-tape (2 rolls)	35	10	350
	6 cotton (100g)	24	20	480
	7 webcols (200pcs)	24	3	72
	8 D-germ Alcohol & Chlorhexidine Handrub (500ml)	50	10	500
ECG				
	2 Disposable pregelled ECG electrodes (50/pkt)	59	100	5900
SNP Genotyping of Arg389Gly		130.53	500	75 574
INCIDENTALS	Printing (PAPER, FILLING, STATIONARY etc)	10 000	1	10 000
RESEARCH WORKSHOPS AND CONFERENCES				
	RESEARCH WORKSHOPS	350	5	1 750
	BIOETHICS: CONDUCTING RESEARCH RESPONSIBLY	7000	1	7 000
	Good clinical practice training	3500	1	3 500
	REDCAP Training	750	2	1 500
	LOCAL CONFERENCE REGISTRATION (SA HEART CONGRESS 2019)	6000	1	6 000
				860849
Student exchange /visit to Glasgow				
	Visa	Pending		
	Air travel	Pending		
	Accommodation	Pending		

The present study will be supported by the Division of Cardiology will support the present study. In the meantime, funding will be solicited from the National Research Fund, South African Medical Research Fund and other research funding bodies.

References

1. Agbor, V., Essouma, M., Ntusi, N., Nyaga, U., Bigna, J. and Noubiap, J. (2018). Heart failure in sub-Saharan Africa: A contemporaneous systematic review and meta-analysis. *International Journal of Cardiology*, 257, pp.207-215.
2. Agbor, V., Aminde, L., Tianyi, F., Mbanga, C., Petnga, S., Ditah, C. and Noubiap, J. (2019). Atrial fibrillation among adults with heart failure in sub-Saharan Africa — prevalence, incidence and all-cause mortality: a systematic review and meta-analysis protocol. *BMJ Open*, 9(2)
3. Aleong, R., Sauer, W., Davis, G., Murphy, G., Port, J., Anand, I., Fiuzat, M., O'Connor, C., Abraham, W., Liggett, S. and Bristow, M. (2013). Prevention of Atrial Fibrillation by Bucindolol Is Dependent on the Beta 1 389 Arg/Gly Adrenergic Receptor Polymorphism. *JACC: Heart Failure*, 1(4), pp.338-344.
4. Baher, A. and Marrouche, N. (2018). Treatment of Atrial Fibrillation in Patients with Co-existing Heart Failure and Reduced Ejection Fraction: Time to Revisit the Management Guidelines. *Arrhythmia & Electrophysiology Review*, 7(2), p.91.
5. Cheng, M., Lu, X., Huang, J., Zhang, J., Zhang, S. and Gu, D. (2014). The prognostic significance of atrial fibrillation in heart failure with a preserved and reduced left ventricular function: insights from a meta-analysis. *European Journal of Heart Failure*, 16(12), pp.1317-1322.
6. Chugh, S., Roth, G., Gillum, R. and Mensah, G. (2014). Global Burden of Atrial Fibrillation in Developed and Developing Nations. *Global Heart*, 9(1), pp.113-119.
7. Eckstein, J., Verheule, S., de Groot, N., Allessie, M. and Schotten, U. (2008). Mechanisms of perpetuation of atrial fibrillation in chronically dilated atria. *Progress in Biophysics and Molecular Biology*, 97(2-3), pp.435-451.
8. Faller, H., Steinbüchel, T., Schowalter, M., Spertus, J., Störk, S. and Angermann, C. (2005). Der Kansas City Cardiomyopathy Questionnaire (KCCQ). *Psychotherapie · Psychosomatik · Medizinische Psychologie*, 55(3/4), pp.200-208.
9. Gluba, A., Bielecka, A., Mikhailidis, D., Wong, N., Franklin, S., Rysz, J. and Banach, M. (2012). An update on biomarkers of heart failure in hypertensive patients. *Journal of Hypertension*, 30(9), pp.1681-1689.
10. Hoit, B. (2014). Left Atrial Size and Function. *Journal of the American College of Cardiology*, 63(6), pp.493-505.

11. Horowitz JD., De Caterina R., Heresztyn T., Alexander JH., Andersson U., Lopes RD., Steg PG., Hylek EM., Mohan P., Hanna M, Jansky P., Granger CB., Wallentin L; ARISTOTLE Investigators. (2018). Asymmetric and Symmetric Dimethylarginine Predict Outcomes in Patients With Atrial Fibrillation: An ARISTOTLE Substudy. *Journal of the American College of Cardiology*, 72(7), pp.721-733.
12. Kao, D., Davis, G., Aleong, R., O'Connor, C., Fiuzat, M., Carson, P., Anand, I., Plehn, J., Gottlieb, S., Silver, M., Lindenfeld, J., Miller, A., White, M., Murphy, G., Sauer, W. and Bristow, M. (2013). Effect of bucindolol on heart failure outcomes and heart rate response in patients with reduced ejection fraction heart failure and atrial fibrillation. *European Journal of Heart Failure*, 15(3), pp.324-333.
13. Keates, A., Mocumbi, A., Ntsekhe, M., Sliwa, K. and Stewart, S. (2017). Cardiovascular disease in Africa: epidemiological profile and challenges. *Nature Reviews Cardiology*, 14(5), pp.273-293.
14. Kirchhof, P., Benussi, S., Kotecha, D., Ahlsson, A., Atar, D., Casadei, B., Castella, M., Diener, H., Heidbuchel, H., Hendriks, J., Hindricks, G., Manolis, A., Oldgren, J., Popescu, B., Schotten, U., Van Putte, B., Vardas, P., Agewall, S., Camm, J., Baron Esquivias, G., Budts, W., Carerj, S., Casselman, F., Coca, A., De Caterina, R., Deftereos, S., Dobrev, D., Ferro, J., Filippatos, G., Fitzsimons, D., Gorenek, B., Guenoun, M., Hohnloser, S., Kolh, P., Lip, G., Manolis, A., McMurray, J., Ponikowski, P., Rosenhek, R., Ruschitzka, F., Savelieva, I., Sharma, S., Suwalski, P., Tamargo, J., Taylor, C., Van Gelder, I., Voors, A., Windecker, S., Zamorano, J. and Zeppenfeld, K. (2016). 2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS. *European Heart Journal*, 37(38), pp.2893-2962.
15. Kotecha, D., Holmes, J., Krum, H., Altman, D., Manzano, L., Cleland, J., Lip, G., Coats, A., Andersson, B., Kirchhof, P., von Lueder, T., Wedel, H., Rosano, G., Shibata, M., Rigby, A. and Flather, M. (2014). Efficacy of β blockers in patients with heart failure plus atrial fibrillation: an individual-patient data meta-analysis. *The Lancet*, 384(9961), pp.2235-2243.
16. Kotecha, D., Chudasama, R., Lane, D., Kirchhof, P. and Lip, G. (2016). Atrial fibrillation and heart failure due to reduced versus preserved ejection fraction: A systematic review and meta-analysis of death and adverse outcomes. *International Journal of Cardiology*, 203, pp.660-666.

17. Krijthe, B., Kunst, A., Benjamin, E., Lip, G., Franco, O., Hofman, A., Witteman, J., Stricker, B. and Heeringa, J. (2013). Projections on the number of individuals with atrial fibrillation in the European Union, from 2000 to 2060. *European Heart Journal*, 34(35), pp.2746-2751.
18. Liu, W., Fu, K., Gao, H., Shang, Y., Wang, Z., Jiang, G., Zhang, Y., Zhang, W. and Zhong, M. (2012). β 1 Adrenergic Receptor Polymorphisms and Heart Failure: A Meta-Analysis on Susceptibility, Response to β -Blocker Therapy and Prognosis. *PLoS ONE*, 7(7), p.e37659.
19. Maisel, W. and Stevenson, L. (2003). Atrial fibrillation in heart failure: epidemiology, pathophysiology, and rationale for therapy. *The American Journal of Cardiology*, 91(6), pp.2-8.
20. Nishimura, M. and Maisel, A. (2018). Biomarkers: a window into the pathophysiology of atrial fibrillation in heart failure with reduced vs. preserved ejection fraction. *European Heart Journal*, 39(43), pp.3876-3878.
21. Nyaga, U., Bigna, J., Agbor, V., Essouma, M., Ntusi, N. and Noubiap, J. (2018). Data on the epidemiology of heart failure in Sub-Saharan Africa. *Data in Brief*, 17, pp.1218-1239.
22. Schönleitner, P., Schotten, U. and Antoons, G. (2017). Mechanosensitivity of microdomain calcium signalling in the heart. *Progress in Biophysics and Molecular Biology*, 130, pp.288-301.
23. Olesen, J., Torp-Pedersen, C., Hansen, M. and Lip, G. (2012). The value of the CHA2DS2-VASc score for refining stroke risk stratification in patients with atrial fibrillation with a CHADS2 score 0–1: A nationwide cohort study. *Thrombosis and Haemostasis*, 107(06), pp.1172-1179.
- 24.
25. Parikh, K., Fiuzat, M., Davis, G., Neely, M., Blain-Nelson, P., Whellan, D., Abraham, W., Adams, K., Felker, G., Liggett, S., O'Connor, C. and Bristow, M. (2018). Dose Response of β -Blockers in Adrenergic Receptor Polymorphism Genotypes. *Circulation: Genomic and Precision Medicine*, 11(8).
26. Stewart, S., Wilkinson, D., Hansen, C., Vaghela, V., Mvungi, R., McMurray, J. and Sliwa, K. (2008). Predominance of Heart Failure in the Heart of Soweto Study Cohort. *Circulation*, 118(23), pp.2360-2367.
27. Thrall, G., Lane, D., Carroll, D. and Lip, G. (2006). Quality of Life in Patients with Atrial Fibrillation: A Systematic Review. *The American Journal of Medicine*, 119(5), pp.448.e1-448.e19.

28. Van Gelder, I., Rienstra, M., Crijns, H. and Olshansky, B. (2016). Rate control in atrial fibrillation. *The Lancet*, 388(10046), pp.818-828.
29. Verma, A., Kalman, J. and Callans, D. (2017). Treatment of Patients With Atrial Fibrillation and Heart Failure With Reduced Ejection Fraction. *Circulation*, 135(16), pp.1547-1563.
30. Yasuda, R., Murata, M., Roberts, R., Tokuda, H., Minakata, Y., Suzuki, K., Tsuruta, H., Kimura, T., Nishiyama, N., Fukumoto, K., Aizawa, Y., Tanimoto, K., Takatsuki, S., Abe, T. and Fukuda, K. (2015). Left atrial strain is a powerful predictor of atrial fibrillation recurrence after catheter ablation: study of a heterogeneous population with sinus rhythm or atrial fibrillation. *European Heart Journal - Cardiovascular Imaging*.
31. Yuda, S., Muranaka, A. and Miura, T. (2016). Clinical implications of left atrial function assessed by speckle tracking echocardiography. *Journal of Echocardiography*, 14(3), pp.104-112.
32. Zafir, B., Lund, L., Laroche, C., Ruschitzka, F., Crespo-Leiro, M., Coats, A., Anker, S., Filippatos, G., Seferovic, P., Maggioni, A., De Mora Martin, M., Polonski, L., Silva-Cardoso, J. and Amir, O. (2018). Prognostic implications of atrial fibrillation in heart failure with reduced, mid-range, and preserved ejection fraction: a report from 14 964 patients in the European Society of Cardiology Heart Failure Long-Term Registry. *European Heart Journal*
33. Zhu, M., Zhou, X., Cai, H., Wang, Z., Xu, H., Chen, S., Chen, J., Xu, X., Xu, H., ... Mao, W. (2016). Catheter ablation versus medical rate control for persistent atrial fibrillation in patients with heart failure: A PRISMA-compliant systematic review and meta-analysis of randomized controlled trials. *Medicine*, 95(30)

INFORMATION AND INFORMED CONSENT FORM

Study Title: The clinical and biochemical effects of atrial fibrillation in heart failure with reduced ejection fraction

Site: Charlotte Maxeke Johannesburg Academic Hospital Cardiology Unit

Investigator: Nene Mboweni

Cell Phone No: 0738125878

Email: nene.mboweni@gmail.com

This Informed Consent Form has two parts:

- **Information Sheet (to share information about the research with you)**
- **Certificate of Consent (for signatures if you agree to take part)**

You will be given a copy of the full Informed Consent Form

PART I: Information Sheet

Good day, my name is Nene Mboweni; I am a PhD student at the University of the Witwatersrand doing my research in the division of cardiology. I am going to give you information and invite you to be a part of this research. You have been selected because you are a patient attending this clinic dedicated to heart failure. You do not have to decide today whether or not you will participate in the research. Before you decide, you can talk to anyone you feel comfortable with about the research.

There may be some words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain. If you have questions later, you can ask me, the study doctor or staff.

What is the study about?

Heart failure has recently become one of the most common diseases in South Africa. Heart failure occurs when your heart can no longer pump enough blood to the rest of your body. As heart failure gets worse, it may cause the upper regions of your heart (the atria) to beat in a fast and uncontrolled fashion (fibrillation). This is called atrial fibrillation. In this study, we want to learn about the effects of atrial fibrillation when it occurs together with heart failure. By doing so, we can better understand its mechanisms and find ways to prevent, identify and treat the condition early. We would like to also find out whether patients who do not respond to medication have a different gene compared to those who do respond to medication.

Why is the study being done ?

A growing number of patients in South Africa have heart failure and later develop atrial fibrillation. It has been noted that patients who have heart failure together with atrial fibrillation do not respond to medication. These patients are also at a greater risk for getting strokes and re-hospitalisation compared to those without atrial fibrillation. If we can learn more about its effects on the heart, by thoroughly screening patients who have heart failure with and without atrial fibrillation. In this way, we can prevent atrial fibrillation in patients with heart failure and find ways to treat those with atrial fibrillation already.

The type of research assessments and procedures

The research will involve me asking you a few questions. The questions are to assess how your poor heart function has affected you, and what difficulties you have because of your heart's poor function. I will then examine you, measure your blood pressure and the electrical activity of your heart to determine whether you have atrial fibrillation with your heart failure. I will do this using an electrocardiogram (also called an ECG). This is a test that records the electrical activity of your heart through small electrode patches that I will attach to the skin of your chest, arms, and

legs. This will be quick, safe, and painless. I will then record how well your heart works using a machine called an Echocardiograph, with help from my colleague, Samantha. This test takes "moving pictures" of your heart with sound waves. You don't have to stay in the hospital for it. You'll lie on a bed on your left side or back and Samantha will put special jelly on a probe and move it over your chest area. The sound waves will pick up images of your heart and valves and produce a videotape and images of your heart. You can sometimes watch during the test. It usually takes one hour. It's painless and has no side effects. I will use your past medical records to obtain additional information such as the following: Other illnesses you have, medications you are currently on and your most recent baseline blood results. Finally, either myself or a trained nurse will take blood from your arm using a syringe and a needle. This may cause you some physical discomfort for a short moment. We will take about 4 tablespoons of blood. In total, we will take your bloods once for the duration of the study. The blood samples will be frozen and stored at the genetics and biochemistry laboratory in the department of Internal Medicine until we are ready to begin analysis. We are going to screen your bloods for various standard heart 'markers' that tell us about your heart's function as well as new heart markers that could potentially provide us with more information about your condition. We will extract your DNA to search for a specific gene involved with your ability to respond to the medication. Only the approved investigations related to this research will be done on your blood and your blood will be destroyed once the research is complete.

Do you know why we are asking you to take part in this study? Do you know what the study is about?

Voluntary Participation

Your participation in this research is entirely voluntary. You may choose to participate or not. If you decide not to participate your treatment in the hospital will not be affected in any way. At any moment or point in the study you may decide to withdraw participation without any consequences even if you agreed earlier. Your participation in this research will however be appreciated.

If you decide not to take part in this research study, do you know what your options are? Do you know that you do not have to take part in this research study, if you do not wish to? Do you have any questions?

Duration of the study?

The research takes place over 7 months (enrollment). During this time, it will be necessary for you to come to the heart failure clinic once, aside from your routine visit. We will then call you 12 months later to do a telephonic follow-up.

Risks of being involved in the study

There are no physical risks involved in participating in the study except the needle prick during withdrawal of blood which may cause some physical discomfort.

Benefits of being involved in the study

There may not be immediate benefit for you but your participation is likely to help us find the answer to the research question which will benefit generations ahead of you.

Reimbursement

We will give you R250.00 to reimburse you for your time and travel to the hospital. You will not be given any other money or gifts to take part in this research.

Can you tell me if you have understood correctly the benefits that you will have if you take part in the study? Do you know if the study will pay for your travel costs? Do you know how much you will be re-imbursed? Do you have any other questions?

Confidentiality

The information that we collect from this research project will be kept confidential. Information about you that will be collected during the research will be put away and no-one but the researchers will be able to see it. Any information about you will have a number on it instead of your name. Only the researchers will know what your number is and we will lock that information up with a code. It will not be shared with or given to anyone except our research colleagues. With this research, something out of the ordinary is being done in the hospital. It is possible that if others in the community are aware that you are participating, they may ask you questions. We will not be sharing the identity of those participating in the research.

Did you understand the procedures that we will be using to make sure that any information that we as researchers collect about you will remain confidential? Do you have any questions about them?

Sharing the Results

The knowledge that we get from doing this research will be shared with you directly through myself, my supervisors or the dedicated research nurse before it is made widely available to the public. Confidential information will not be shared. You will be told in a timely manner of our findings. After you have been informed, we will publish the results in order that other interested doctors may learn from our research.

Right to Refuse or Withdraw

You do not have to take part in this research if you do not wish to do so and refusing to participate will not affect your treatment at the heart failure clinic in any way. You will still have all the benefits that you would otherwise have at this clinic.

Who to Contact

If you have any questions you may ask them now or later, even after the study has started. If you wish to ask questions later, you may contact me directly on the details provided above. If you wish you may also contact my supervisor, Dr Nqoba Tsabedze. His contact details are as follows:

Email address: Nqoba.tsabedze@wits.ac.za

Telephone No: (011) 488 3611/6452

This proposal has been reviewed and approved by the Human Research Ethics Committee (medical) of the University of the Witwatersrand, Johannesburg; which is a committee whose task it is to make sure that researchers and participants are protected from harm. If you wish to find out more about the HREC their contact details are:

Ms Zanele Ndlovu and Mr Rhulani Mukansi, Medical School, Parktown, Phillip Tobias Building, 2nd Floor, Cnr York Road and Princess of Wales Terrace, Mon-Fri 08h00-17h00 Tel: 011-717-1234/1252/ 2700 or Room SH1005, 10th Floor Senate House,

Emails: zanele.ndlovu@wits.ac.za or rhulani.mukansi@wits.ac.za

You can ask me any more questions about any part of the research study, if you wish to. Do you have any questions?

If you feel you have not been treated according to the descriptions in this form, or your rights as a participant in this research have been violated during the course of this project, you may contact the chair of the University of the Witwatersrand's Ethics Committee, Professor Clement Penny. His contact details are as follows:

Email address: Clement.penny@wits.ac.za

Telephone no: [\(011\) 717 2301](tel:(011)7172301)

Thank you for your consideration. Your participation is greatly appreciated.

Your signature below indicates that you have read and understand the above information, are at least 18 years of age and agree to participate in the study titled "The clinical and biochemical effects of atrial fibrillation on heart failure with reduced ejection fraction"

PART II: Certificate of Consent

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research.

Print Name of Participant:

Signature of Participant:

Date

Day/month/year

If illiterate

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness _____ **AND Thumb print of participant**

Signature of witness _____

Date _____

Day/month/year



Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands that the following will be done:

- 1. Access to my medical records clinical examination.**
- 2. Clinical Examination**
- 3. Blood Sampling**
- 4. Echocardiographic analysis**
- 5. ECG readings**

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this ICF has been provided to the participant.

Print Name of Researcher/person taking the consent _____

Signature of Researcher /person taking the consent _____

Date _____

Day/month/year



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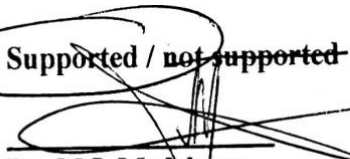
Enquiries:
Ms. N. Mzila
Office of the Clinical Director
Email:
Nolwazi.Mzila@gauteng.gov.za
Tell: 011) 488
06 March 2019

Dear Ms. N Mboweni

STUDY TITLE: The Clinical and Biomedical effects of Atrial Fibrillation on Heart Failure with Reduced Ejection Fraction.

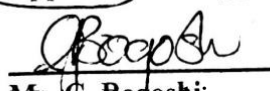
Permission to conduct the above mentioned study is provisional approved. Your study can only commence once ethics approval is obtained. Please forward a copy of your ethics clearance certificate as soon as the study is approved by the ethics committee for the CEO's office to give you the final approval to conduct the study.

~~Supported / not supported~~


Dr. M.I. Mofokeng
Clinical Director

DATE: 7/03/2019

~~Approved / not approved~~


Ms. B. Boshui

Chief Executive Officer
DATE: 08/03/2019

APPENDIX C:

THESIS CHAPTER 1-6 incl refs.docx

by Nonkanyiso Mboweni

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