Myocardial Deformation Imaging and Prognostication in Acute Heart Failure

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Declaration of originality

I hereby declare that the work presented in this thesis is all my own, performed under the supervision of Professor Pankaj Sharma and Dr Aigul Baltabaeva. Work derived through collaboration and assistance has been acknowledged in the text, and a list of references is provided in the bibliography.

Copyright Declaration

Abstract

Heart failure (HF) is a pernicious medical syndrome which is growing in prevalence as the global population ages, while outcomes in acute heart failure (AHF) remain dire, with mortality approximating 10-30% at 1-year post hospital admission. Clearly delineating AHF subcategories may aid in the assessment and treatment of patients, allowing clinicians to better elucidate the underlying mechanisms, whilst better prognostication may help physicians to appropriately escalate therapies in AHF.

This thesis presents data from the Mitral Regurgitation in Acute Heart Failure (MRAHF) study. This thesis hypothesised that myocardial deformation imaging in standard clinical conditions was both feasible and would demonstrates substantial differences in global longitudinal strain and strain rate values between heart failure subcategories, and that a novel prognostic tool for AHF could be produced using only data available upon admission.

When compared to the EuroHeart Survey II (EHSII) cohort, a large prospective observational cohort of AHF, the MRAHF cohort is older (79.0 years ± 11.5 versus 69.9 years ± 12.5, $p<0.00001$), with reduced rates of hypertension (55.0% vs 62.5%, $p <0.005$), ischaemic heart disease (37.8% vs 53.6%, $p<0.00001$) and COPD (14.3% vs 19.3%, $p<0.05$), but with similar in-hospital mortality outcomes (4.9% vs 6.7%, $p<0.15$).

Peak systolic strain (PSS) varied significantly and substantially between the AHF subcategories - HFrEF, HFmrEF and HFpEF – in all cardiac regions assessed in the echocardiographic 4, 2 and 3 chamber views using standard clinical frame rates. The difference was most clearly seen when assessing global longitudinal peak systolic strain (GLS) where median GLS was - -6.62% (-4.41 – -8.83), -9.03% (-6.28 – -11.78) and -13.12% (-10.02 – -16.22) in HFrEF, HFmrEF and HFpEF respectively, $p<0.0001$ between all subgroups. The above was also seen for peak systolic strain rate (PSSR), where PSSR varied substantially and significantly between subcategories in all myocardial regions assessed. This difference was most marked for global longitudinal peak systolic strain rate (GLSR); -0.48 S^{-1} (-0.34 – -0.62), -0.66 S^{-1} (-0.49 – -0.83) and -0.87 S^{-1} (-0.70 – -1.04) respectively, $p<0.00001$ between all groups. These data demonstrate the feasibility and potential utility of stratifying AHF patients according to less load-dependent measures of systolic function such as GLS and GLSR.

Using demographic and biochemical data collected at the point of patient admission for AHF, a prognostic risk scoring system was created using binary logistic regression methods. Each variable was assigned one point in the score, and total scores were grouped together to produced low risk (0-2) medium risk (3-4) and high risk (≥5) groups. Significantly different 6-month mortality rates were seen between risk groups - 4.9%, 25.3% and 49.2% respectively, $p<0.0001$. The C-index for this risk score was 0.746 (0.695 – 0.798) indicating a test with fair predictive accuracy.

A further prognostic model was created which included echocardiographic variables. In this model age ≥80 was assigned two points due to an odds ratio approximately double that of the other variables. All other variables were assigned one point and the total score were grouped together to produce low risk (0-3), medium risk (4-5) and high risk (6-10). Significantly different 6-month mortality rates were seen between risk groups – 4.7%, 23.7% and 54.2% respectively, $p<0.0001$. The C-index for this risk score was 0.804 (0.758-0.849) indicating a test with good predictive accuracy. These data demonstrate the feasibility of producing and using such a risk scoring tool at the point of admission to hospital.

Acknowledgements

This work would not have been possible without the help and support of many individuals and organisations. From an academic point of view the input and aid of Professor Pankaj Sharma and his team in the Cardiovascular Disease Institute at Royal Holloway University of London has been invaluable. Thanks also to Dr Baltabaeva for her guidance and advice as my Clinical and Scientific Supervisor throughout the last two years. Large thanks must go to Dr David Crook for his invaluable assistance with complex statistical techniques included in the thesis.

The Research and Development department at Ashford & St Peters NHS trust has been an incredibly welcoming and supportive environment for someone new to research and I am incredibly grateful for the opportunities I have had there. I cannot overemphasise the importance of the support offered by Dr Wrigley who spent an inordinate amount of time helping me to develop my skills and support me through the introduction to research. In addition, Mrs Claire Atkinson provided much needed support in difficult times, thank you for pushing me on when I was slowing. I would like to thank the other members of the research team for their help with recruitment and data collection – specifically Dr Otar Lazariashvili who performed the echocardiography and extracted echocardiographic data, as well as Hugo Roque who stepped in to perform acquire images when Dr Lazariashvili was absent.

I would like to think Drs Nicola Raeside and my long-suffering partner Dr Katie Addy, both for their help and support during the research, their help with proof-reading the text and for putting up with me talking about heart disease for two years. I am also indebted to my non-medical family for putting up with my rantings and ravings concerning heart failure. To Ollie, I am grateful for your discount shirts in times of need, to Dad thank you for the occasional slap up meal or show to let off steam, and to Mum thank you for a place of refuge to get away and work when I needed some space.

There are many friends who have provided emotional support throughout the research period and particularly to Drs Olivia Szepietowski, David Thompson, Zayd Tippu, Michael Anderson and Mr Daniel Shutt I offer my unending thanks for their support.

Finally, many thanks to all the patients who kindly consented to take part in the study, without whose kindness and patience none of the research would have been possible.

Candidate Contribution

This section will outline the extent of the contribution made by myself towards the work presented in this thesis.

The Mitral Regurgitation in Acute Heart Failure (MRAHF) study was conceived of and planned prior to my employment as a research fellow within the NHS trust. All ethical approval had been acquired in the year prior to my involvement and patient recruitment began in the month prior to my arrival. Trial conception, planning, financing and organisational approval was orchestrated by Drs Aigul Baltabaeva – the principal investigator (PI), Isaac John and Martha Wrigley with help from the trust research team, specifically Claire Atkinson, Flabiola Mann and Jessica Law.

Subsequent to my arrival I took on the role of patient recruitment, which was then almost exclusively performed by myself, recruiting >95% of all patients recruited in the study. My role included identifying possible trial recruits, assessing their clinical history and examining them, obtaining consent for involvement in the trial and performing phlebotomy as per the inclusion/exclusion criteria. If not excluded from the study at this stage, I would pass the patient's details onto the senior research fellow who would perform bedside echocardiography. Where there were doubts over diagnosis these patients would be discussed with the other research fellow and PI, a consultant cardiologist.

After having obtained consent, I would then collect all of the required pre-admission and admission data, including demographic, biochemical, bedside investigation, clinical, comorbidity, pharmacotherapy and previous admission data. I then manually uploaded these to an online database whilst keeping an original copy for our records. I also helped to manage this database with assistance from an IT engineer from Metanoic Health Ltd.

All echocardiography was performed either by the senior research fellow or, in <5% cases where he was absent, by two of the senior echocardiographers within the trust. All initial echocardiographic data were assessed by the senior research fellow with advice from the PI. Once collected and recorded, I then uploaded these data to our collective database.

After echocardiography had been performed, I analysed left ventricular strain and strain rate profiles using the EchoPac software and input these data to our online database.

Subsequent to patient discharge I would then collect and upload mortality data from hospital records, bereavement records, GP records and the county registrar.

During recruitment I maintained the database by regularly screening for omissions and anomalies which were corrected with reference to the original data collection sheets. After completion of patient recruitment this was repeated to ensure completion and accuracy of the database, and then the data were downloaded to a joint SPSS file for the research group to use.

All subsequent data analysis and statistical work was performed by myself. Initial discussion regarding applicable statistical techniques was had with the statistical team at Royal Holloway University and the statistical methods were subsequently checked by Dr David Crook from Brighton & Sussex University.

The thesis was written in its entirety by myself. Additional support regarding form, layout of text and advice on writing style and content was provided by members of the trust cardiology group – specifically Drs Ian Beeton and Adam Jacques, as well as input from other colleagues involved in research projects and thesis production, most notably Drs Olivia Szepietowski and Martha Wrigley.

Original Findings

This section will outline the original findings of this thesis to demonstrate that the work is both substantial and original.

Chapter two outlines the methods and materials required in the development of, and completion of the MRAHF trial, a prospective observational cohort study. This has not previously been reported in publication.

Chapter four reports new findings in relation to global longitudinal strain (GLS) and global longitudinal strain rate (GLSR) in acute heart failure (AHF). It demonstrates that statistically discriminatory global longitudinal peak systolic strain (PSS) and peak systolic strain rate (PSSR) values can be determined in an AHF population in standard clinical settings with standard frame rates. It demonstrates that PSS and PSSR values in the three current HF subcategories of HF with reduced ejection fraction (HFrEF), HF with mid-range ejection fraction (HFmrEF) and HF with preserved ejection fraction (HFpEF) are substantially and significantly different. These data are the first reported regarding the discriminatory value of GLSR in standard clinical settings, and the first large dataset in AHF reporting GLS and GLSR variation between the above subgroups which also includes patients with AHF of an ischaemic aetiology.

In addition, observational data reported in this chapter show that GLS and GLSR values in patients recruited in < 2 days from their inpatient admission are statistically similar when compared to those recruited ≥ 2 days from their admission date. This is not seen with left ventricular ejection fraction (LVEF) which is statistically and quite substantially higher in those recruited and assessed in < 2 days compared with those recruited ≥ 2 days after admission. These data suggest that GLS and GLSR may be more stable markers of contractile function in the acute phase of an admission compared to LVEF. This finding is compatible with known data regarding the relative load-independency of GLS and particularly GLSR in comparison to LVEF but this has not previously been described in the AHF population.

Chapter five outlines the production and testing of a novel prognostic tool to be used to predict risk of 6-month mortality at admission for patients with AHF. This is produced using a binary logistic regression method standardly seen in other commonly used risk-scoring algorithms. It is the first risk-scoring tool in AHF that is applicable without recourse to either

a computer or online calculator that can offer prognostic information solely with data gathered upon admission, thus rendering it easier to use than current prognostic models available in AHF. The model is shown to have similar or greater predictive accuracy when compared to other commonly applied prognostic models in AHF.

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List of Abbreviations

Ɛ – Strain

ɛ́ – Strain rate

ACC – American College of Cardiology

ACEi – Angiotensin-converting enzyme inhibitor

AF – Atrial fibrillation

AHA – American Heart Association

AHF – Acute heart failure

ALARM-HF – Acute Heart Failure Global Survey of Standard Treatment

AMTS – Abbreviated mental test score

ANOVA – Analysis of variance

ANP – A-type natriuretic peptide

APACHE-HF - Acute Physiology and Chronic Health Evaluation in Heart Failure scoring system

APACHE II - Acute Physiology and Chronic Health Evaluation scoring system II

ARB – Angiotensin receptor blocker

ARDS – Acute respiratory distress syndrome

ARNI – Angiotensin-receptor neprolysin inhibitor

ASE – American Society of Echocardiography

AT2 – Angiotensin II

BB – Beta blocker

BMI – Body mass index

BNP – B-type natriuretic peptide

BP – Blood pressure

BSE – British Society of Echocardiography

CAD – Coronary artery disease

CART – Classification and regression tree

CCB – Calcium channel blocker

CHARM – Effects of Candesartan for the management of Patients with Chronic Heart Failure trial

CHF – Chronic heart failure

CI – Cardiac index

CKD – Chronic Kidney Disease

CNP – C-type natriuretic peptide

CO – Cardiac output

COPD – Chronic obstructive pulmonary disease

CVA – Cerebrovascular accident

CVD – Cardiovascular disease

CXR – Chest X-ray

DBP – Diastolic blood pressure

DM – Diabetes Mellitus

EASCVI – European Association of Cardiovascular Imaging

ECG – Electrocardiogram

EDTA - Ethylenediaminetetraacetic acid

EDV – End diastolic volume

EF – Ejection fraction

eGFR – Estimated glomerular filtration rate

EHS II – EuroHeart Failure Survey II

EFFECT - Enhanced Feedback for Effective Cardiac Treatment trial

ELAN-HF - European Collaboration on Acute Decompensated Heart Failure trial

EMPHASIS – Eplerenone in Mild Patients Hospitalisation and Survival Study

EPHESUS – Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study

ESC – European Society of Cardiology

ESC-HF-LT – European Society of Cardiology Heart Failure Long-Term study

ESCAPE – Evaluation Study of Congestive Heart Failure and Pulmonary Artery Catheterization Effectiveness trial

ESV – End systolic volume

GCP – Good clinical practice

GMP – Guanosine monophosphate

GLS – Global longitudinal strain

GLSR – Global longitudinal strain rate

GP – General practitioner

HES – Hospital episode statistic

HF – Heart failure

HFmrEF – Heart failure with mid-range ejection fraction

HFrEF – Heart failure with reduced ejection fraction

HFpEF – Heart failure with preserved ejection

HOPE – Heart Outcomes Prevention Evaluation study

HR – Heart rate

HTN – Hypertension

IBM – International Business Machines

IHD – Ischaemic heart disease

IPL – Inpatient lists

IQR – Interquartile range

IT – Information Technology

LV – Left ventricle

LVEDV – Left Ventricular End Diastolic Volume

LVEF – Left ventricular ejection fraction

LVESV – Left Ventricular End Systolic Volume

MAGGIC – Meta-analysis Global Group in Chronic Heart Failure study

MAP – Mean arterial pressure

MI – Myocardial infarction

MR – Mitral regurgitation

MR-FT – Magnetic resonance feature tracking

MR-T – Magnetic resonance tissue tagging

MRA – Mineralocorticoid receptor antagonist

MRAHF – Mitral regurgitation in Acute Heart Failure trial

MRI – Magnetic resonance imaging

NICE – National Institute for Health and Care Excellence

NP – Natriuretic peptide

NPV – Negative predictive value

NRI – Net reclassification index

NT pro-BNP – N terminal pro-b type natriuretic peptide

NYHA – New York Heart Association

OPTIMIZE-HF - Organized Program to Initiate Lifesaving Treatment in Hospitalized Patients with Heart Failure trial

OR – Odds ratio

PACS – Picture archiving and communication software

PARADIGM-HF – Prospective Comparison of ARNI with ACEi to Determine Impact on Global Mortality and Morbidity in Heart Failure trial

PARAGON-HF – Efficacy and Safety of LCZ696 Compared to Valsartan on Morbidity and Mortality in Heart Failure Patients with Preserved Ejection Fraction trial

PARAMOUNT – Prospective comparison of ARNI with ARB on management of Heart Failure with preserved ejection fraction trial

PCWP – Pulmonary capillary wedge pressure

POCT – Point of care test

PSS – Peak systolic strain

PSSR – Peak systolic strain rate

RAAS – Renal-angiotensin-aldosterone system

RALES – Randomised aldactone evaluation study

RCT – Randomised controlled trial

ROCAUC – Receiver operator characteristic curve area under the curve

ROI – Region of interest

RV - Right ventricle

RVFAC – Right ventricular fractional area change

SBP – Systolic blood pressure

SCD – Sudden cardiac death

SD – Standard deviation

SNS – Sympathetic nervous system

SOLVD – Studies of left ventricular dysfunction trial

SPAP – Systolic pulmonary artery pressure

SPSS - Statistical package for the Social Sciences

SR – Strain rate

ST2 – Suppression of tumorigenicity factor

SV – Stroke volume

TAPSE – Tricuspid annular plane systolic excursion

TOPCAT – Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist trial

TTE – Transthoracic echocardiography

UK – United Kingdom

US – United States of America

VBG – Venous blood gas

Chapter One: Introduction to the literature, current understanding and statement of hypotheses

1.1 Chapter introduction

This chapter reviews current literature regarding heart failure (HF) and is divided into three sections. The first describes the current understanding of the natural history of HF, the second describes the and reviews the literature regarding the main foci of this study, novel subclassifications of HF and prognostication in HF, while the last identifies the current deficits in knowledge and describes the purpose, proposed hypothesis and structure of the thesis.

1.2 Heart Failure

HF is a complex medical syndrome in which the heart fails to fill at low enough pressures to avoid pulmonary congestion and/or fails to provide adequate cardiac output to sustain the metabolic requirements of the body.

Clinically it is defined by the European Society of Cardiology (ESC) as a combination of signs and symptoms of HF (see Table 1.1), objective evidence of cardiac dysfunction and, in unclear cases, clinical response to standard HF therapy (Ponikowski, P., Voors et al. 2016).

This clinical syndrome can be caused by a range of underlying pathologies but is typically a result of disorders of the pericardium, myocardium, endocardium, heart valves, the great vessels or cellular metabolism (Yancy, Jessup et al. 2013).

Symptoms	Signs
Typical	More Specific
Breathlessness Orthopnoea Paroxysmal nocturnal dyspnoea Reduced exercise tolerance Fatigue Ankle swelling	Elevated jugular venous pressure Hepatojugular reflux Third heart sound (gallop rhythm) Laterally displaced apical impulse
Less Typical	Less Specific
Nocturnal cough Wheezing Bloated feeling Loss of appetite Confusion (particularly in the elderly) Depression Palpitations Dizziness Syncope Bendopnoea	Weight gain (>2 kg/week) Weight loss (in advanced HF) Cachexia Cardiac murmur Peripheral oedema (ankle, sacral, scrotal) Pulmonary crepitations Clinical signs of pleural effusion Tachycardia Irregular pulse Tachypnoea Cheyne-Stokes respiration Hepatomegaly Ascites Cold extremities Oliguria Aberrations of pulse pressure

Table 1.1 – **Symptoms and signs typical of heart failure** – adapted from 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure (Ponikowski, P., Voors et al. 2016).

1.2.1 Subclassification

HF can be subclassified in a variety of ways. With regards to time course, HF can be described either as chronic (CHF) or acute (AHF). The former is often considered synonymous with clinically stable disease, while the latter typically refers to episodes where there is an exacerbation of HF symptoms which requires either medical attention or hospital admission. Use of the term 'stable' is not without controversy as clinically stable HF is known to progress over time, but it is a term often used to delineate those patients typically treated in the community from those requiring acute inpatient or intensive management.

Episodes of AHF can occur due to either decompensations of known disease or a *de novo* expression of HF. Decompensations of known disease are slightly more common, but in the EuroHeart Failure Survey II *de novo* disease still represented approximately 40% of AHF cases (Nieminen, Brutsaert et al. 2006).

HF can be categorised according to metabolic requirements as either high or low output. High-output HF typically refers to conditions in which increased metabolic demand is unable to be met by increases in cardiac output, typically diseases such as thyrotoxicosis, sepsis and systemic arterio-venous fistulae (Mehta, Dubrey 2009). Therapy in this group is normally focussed on amelioration of the underlying pathology (Mehta, Dubrey 2009). In comparison, low-output HF occurs when the heart fails to provide adequate metabolic support due to sub-optimal cardiac output.

When considering symptomatic severity, the two most commonly used categorisation tools are the American College of Cardiology/American Heart Association (ACC/AHA) and New York Heart Association (NYHA) classifications. These are commonly used in clinical practice as symptomatic severity correlates with patient outcomes (Ammar, Jacobsen et al. 2007, Ahmed, Aronow et al. 2006, Gradman, Deedwania et al. 1989).

The NYHA classification of HF has been used since 1928, with multiple subsequent revisions and updates (New York Heart Association 1964). The NYHA classification ascribes a functional class depending upon the extent to which the patient's reported symptoms impact on functional status. Class I is attributed to patients with minimal symptomatic interference, while class IV refers to patients with maximal functional impairment – a more complete description can be seen in Table 1.2.

The ACC/AHA classification was introduced in 1994 and differs in three main ways from the NYHA functional classification. Firstly, it uses information regarding underlying cardiac structural abnormalities to discriminate between patients, secondly, it contains a category for patients at risk of developing HF, and thirdly patients cannot move backwards through its classes if functional status improves, unlike in the NYHA classification. A full description is given in Table 1.3.

NYHA Class	Functional impairment
I	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitations or dyspnoea.
II	Slight limitation of physical activity. Patients are comfortable at rest but ordinary levels of physical activity lead to fatigue, palpitations or dyspnoea.
III	Marked limitation of physical activity. Patients are comfortable at rest but less than ordinary levels of physical activity cause fatigue, palpitations or dyspnoea.
IV	Symptomatic at rest. Patients experience fatigue, palpitations or dyspnoea in the absence of activity. Patients are unable to perform any physical activity without discomfort. If activity is performed, discomfort increases.

Table 1.2 – New York Heart Association functional classification of heart failure – adapted from the NYHA Nomenclature and criteria for diagnosis of disease of the heart and blood vessels (New York Heart Association 1964).

ACC/AHA Class	Description
A	Patients at high risk of developing HF because of the presence of conditions that are strongly associated with the development of HF.
B	Patients who have developed structural heart disease that is strongly associated with the development of HF but who have never shown signs or symptoms of HF.
C	Patients who have current or prior symptoms of HF associated with underlying structural heart disease.
D	Patients with advanced structural heart disease and marked symptoms of HF at rest despite maximal medical therapy and who require specialized interventions.

Table 1.3 – ACC/AHA clinical classification of heart failure – adapted from 2013 ACCF/AHA guidelines for the management of heart failure (Yancy, Jessup et al. 2013).

HF can also be discussed in terms of right ventricular (RV) versus left ventricular (LV) failure, related to sub-optimal systolic or diastolic function of the affected side. LV systolic function is commonly discussed in terms of LV ejection fraction (LVEF), a parameter derived from either echocardiographic or magnetic resonance imaging while RV systolic function is difficult to measure in a similar manner given its non-geometric shape. Commonly used markers of RV systolic function include fractional area change (RVFAC) and tricuspid annular plane systolic excursion (TAPSE) while systolic pulmonary artery pressure (SPAP) is an important marker of RV afterload.

While the LV and RV are distinct in terms of their embryological origins (Anderson, Brown et al. 2018), there are similarities in terms of response to adverse loading and failure, with foetal gene reactivation seen bilaterally as an adaptive response (Lowes, Minobe et al. 1997, Schiano, Vietri et al. 2015). They also demonstrate similar molecular reorganisation in the setting of hypertrophy (Unverferth, Fetters et al. 1983), and are functionally interdependent; they share myocardial fibres which encircle both ventricles, share the pericardial space and alteration in contractility of either ventricle markedly affects the function of the other (Friedberg, Redington 2014). Given the interdependence, it is perhaps not pathophysiologically accurate to discuss failure in terms of unilaterality due to the extent to

which each ventricle affects contralateral function, and thus global output (Friedberg, Redington 2014).

They are also markedly different in terms of morphology, where there are differences in the macro and micro architectures of the left and right ventricles. The LV is substantially thicker with myocyte and myofibril orientation changing at different depths within the ventricular wall. In comparison, the right ventricle is much thinner and has a largely trabeculated apex, better adapted to deal with increases in volume than increases in pressure. The difference in muscle density is an adaptation to account for the different pressures against which the two ventricles must generate force, substantially lower in the pulmonary circulation compared to the systemic circulation (Scatteia, Baritussio et al. 2017).

The LV approximates a conical shape that is classically described as having three layers; the subendocardium, the mid-wall and subepicardium. The subepicardium consists of myocytes organised in a left-handed helix which ultimately allows for torsion of the cardiac apex relative to the base. The mid-wall region contains largely circumferential myocytes which allow for contraction in radial direction. The subendocardium contains fibres organised in a right-handed helix as well as longitudinal fibres. While these layers are described here as distinct, myocytes are largely similarly oriented to their neighbours and there is a gradual, rather than abrupt change in fibre direction between the three layers described above. These differently arranged fibres allow for deformation in longitudinal, circumferential and radial planes as well as causing torsion of the myocardium. Due to the larger radius of rotation of the subepicardium it provides greater torque than the subendocardium thus the rotation of the subepicardium is significantly expressed (Nakatani 2011).

Below the macro-architecture, the myocytes are arranged into sheetlets. These banks of sheetlets are arranged obliquely to the plane of the local wall and are able to change their angle during the cardiac cycle (Nielles-Vallespin, Khalique et al. 2017). This helps to significantly enhance localised contraction; ultimately the individual myocytes shorten by approximately 15% but the LV wall thickens radially by approximately 30-50%. It is thought that the substantial disparity demonstrated above is largely accounted for by myocyte sheetlet reorientation (Ghonim, Voges et al. 2017).

In comparison to the relatively simple shape of the LV, the crescenteric RV wraps around the LV. In comparison to the thicker LV, the RV free wall is relatively thin except at the

interventricular septum where there is marked ventricular interdependence as described above due to sharing of subendocardial fibres in the septum. The interventricular septum contains helical fibres but this is the only section of the RV to do so, with longitudinal fibres predominating throughout the rest of the RV. As a result, contraction of the RV occurs primarily due to long-axis shortening as opposed to the helical twisting of the LV (Buckberg, Hoffman 2014). In the RV free wall myocardial fibre orientation is longitudinal in the endocardial layer and circumferential in the epicardium. Due to its relatively thin wall the RV is not considered to have a middle layer unlike the LV except in the right ventricular outflow tract (Ozawa, Funabashi et al. 2016).

The terms 'Right' and 'Left' heart failure are often used to describe a particular pattern of symptoms which are typically ascribed to the dysfunction of each ventricle (see Table 1.4).

'Right-sided' Heart Failure	**'Left-sided' Heart Failure**
Symptoms Peripheral Oedema Abdominal Swelling Weight gain Fatigue	**Symptoms** Dyspnoea Cough Pink frothy sputum Orthopnoea Paroxysmal Nocturnal Dyspnoea
Signs Ascites Hepatomegaly Hepatic dysfunction – jaundice, coagulopathy, gynaecomastia Parasternal Heave Distended jugular vein	**Signs** Rales Laterally displaced apex beat Cyanosis

Table 1.4 – Signs & symptoms classically attributed to 'right sided' versus 'left sided' heart failure (Field, Kudenchuk et al. 2012).

Acute decompensations can be classified using the Forrester haemodynamic subsets (see Table 1.5), using intra-cardiac measurements to obtain pulmonary capillary wedge pressure (PCWP) and derive the Cardiac Index (CI) (Forrester, Diamond et al. 1976). These correlate

with typical clinical pictures as described in Table 1.5. These subclassifications are important given that 'cold and wet' patients have significantly raised mortality compared to other groups, and that these classifications improve the ability to prognosticate, even more than NYHA status (Nohria, Mielniczuk et al. 2005).

Forrester Haemodynamic Subsets	
Subset	**Description**
I - Warm and Dry (normal)	PCWP 15-18 mmHg and CI >2.2 L/min/m^2
II - Warm and Wet (congested)	PCWP >18 mmHg and CI >2.2 L/min/m^2
III - Cold and Dry (hypoperfused)	PCWP 15-18 mmHg and CI <2.2 L/min/m^2
IV - Cold and Wet (congested and hypoperfused)	PCWP >18 mmHg and CI <2.2 L/min/m^2

Table 1.5 – Forrester Haemodynamic Subsets of heart failure – adapted from Acute Decompensated Heart Failure (Galdo, Riggs et al. 2013) CI – Cardiac index, PCWP – Pulmonary capillary wedge pressure.

Current consensus guidelines largely subcategorise HF according to LV systolic function. Varied definitions of impaired systolic function exist, but the categories used are typically defined in terms of LVEF which describes the proportion of blood pumped from the LV in each cardiac cycle.

Lower limits for normal, or 'preserved' LVEF have been proposed at 35% (Heart Failure Society of America 2010), 40% (McMurray, John JV, Adamopoulos et al. 2012) and, more recently 50% (Ponikowski, P., Voors et al. 2016). The selection of these values as categories denoting distinct pathology is relatively arbitrary as they are derived from the selection criteria of major interventional studies rather than specific pathophysiological criteria (Merit-HF Study Group 1999, Poole-Wilson 1999, Packer, M., Fowler et al. 2002, Packer, Milton, Bristow et al. 1996, SOLVD Investigators* 1991). There are, however, consistent data available that patients with lower LVEF values fare worse than matched cohorts with higher LVEF in the context of a range of cardiovascular diseases including coronary arterial disease (Yeboah, Rodriguez et al. 2016), sudden death post myocardial infarction (Schulze Jr, Strauss et al. 1977, Ayesta, Martinez-Selles et al. 2018, Wellens, Schwartz et al. 2014) and HF

(Meta-analysis Global Group in Chronic Heart Failure (MAGGIC) 2011, Vasan, Larson et al. 1999),

Rather than define a specific disease, these LVEF values have historically helped label a syndrome that can be ameliorated with pharmacological interventions. The failure of these therapies to ameliorate the course of disease in HF with EF >40% has led to suspicion that HF with preserved ejection fraction (HFpEF) represents an alternate underlying pathology and phenotype, borne out by significant differences between the groups in terms of response to therapy, but also demographics, comorbidities and cardiac geometry (Butler, Fonarow et al. 2014).

Originally HF was only considered in patients with reductions in systolic function, though as early as1984 there was recognition of a HF syndrome with preserved markers of systolic function and evidence of diastolic abnormalities (Dougherty, Naccarelli et al. 1984). Until the mid-2010s patients have been commonly categorised as either HF with reduced ejection fraction (HFrEF) when LVEF is <50% or HFpEF when LVEF is ≥50%. Recently the ACC/AHA, and subsequently the ESC guidelines, introduced the novel intermediate category of HF with borderline ejection fraction and mid-range ejection fraction (HFmrEF) respectively, both describing a subgroup in whom LVEF is measured at 40-49% (Yancy, Jessup et al. 2013). To accommodate this extra group, the new criteria have reclassified HFrEF as LVEF <40% in both guidelines.

The nature and validity of this new subgroup remains unclear. As described above, HFrEF and HFpEF patients have such clearly different demographics, underlying aetiologies, comorbidity profiles, ventricular remodelling patterns and response to therapies that it is clear that these represent two separate pathways which lead to a similar clinical syndrome, however data regarding the nature of the HFmrEF have not been so unequivocal.

Re-examination of registry information and previous clinical trials has suggested that HFmrEF represents a substantial subgroup of patients, somewhere between 14-18% (Rickenbacher, Kaufmann, Maeder, Bernheim, Goetschalckx, Pfister, Pfisterer, Brunner-La Rocca et al. 2017). Patients with HFmrEF are more typically female and older which is more consistent with the HFpEF cohort (Toma, Ezekowitz et al. 2014, Steinberg, Zhao et al. 2012), while their underlying aetiology is more commonly ischaemic which is comparable to the HFrEF cohort (Solomon, SD 2005, Rickenbacher, Kaufmann, Maeder, Bernheim,

Goetschalckx, Pfister, Pfisterer, Brunner-La Rocca et al. 2017). In terms of ventricular remodelling, HFmrEF is commonly reported as demonstrating intermediate myocardial mass and phenotypically intermediate between the typical concentric remodelling seen in HFpEF and the eccentric hypertrophy seen in HFrEF (Rickenbacher, Kaufmann, Maeder, Bernheim, Goetschalckx, Pfister, Pfisterer, Brunner-La Rocca et al. 2017, Pascual-Figal, Ferrero-Gregori et al. 2017). As has been previously shown HFpEF has similarly deleterious but slightly improved mortality outcomes compared to HFrEF (Gottdiener, McClelland et al. 2002). HFmrEF has been shown to act correspondingly with similar rates of survival, rehospitalisation and HF rehospitalisation to HFpEF (Lam, Gamble et al. 2018). As yet there is little data regarding the effect of treatment options on HFmrEF as will be discussed later but retrospective analysis of the CHARM-Preserved and TOPCAT studies suggest HFmrEF may benefit from similar treatment pathways (Pitt, Pfeffer et al. 2014, Yusuf, Pfeffer et al. 2003), however these analyses are limited by the typical confounders of retrospective analysis and new RCTs focussing specifically on the HFmrEF subgroup are awaited.

Some groups have postulated that it is an overlap phase prior to transition to HFrEF or HFpEF, indeed in one study 35% patients with HFmrEF were shown to transition to HFmrEF and HFpEF in an almost 1:1 ratio over 3 years of follow-up (Webb, Draper et al. 2018). The fact that patients can transition both ways suggests notable heterogeneity within the group. Others have focussed on the similarities in terms of typical aetiology and ischaemic burden in both HFrEF and HFmrEF, hypothesising that HFmrEF is an early or transition stage in HFrEF (Lam, Solomon 2014).

The true nature of HFmrEF remains unclear. It does appear to be distinct in certain variables from HFrEF and HFpEF, acting differently enough from both to be clearly included in either. In fact, the paradigm of HF as defined by LVEF may be unhelpful in the long term, but what is clear is that further information regarding what is currently classified as HFmrEF will likely help us to understand and treat the underlying pathophysiology, and that further efforts to elucidate the underlying group characteristics and pathological mechanisms of the syndrome are both useful and necessary.

1.2.2 Pathophysiology

The symptomatic and pathological sequelae of heart failure arise either from a reduction in cardiac output leading to a failure to adequately provide metabolically active tissues with

required substrates for metabolism, or from congestion of the pulmonary vasculature as a result of impaired diastolic relaxation.

Cardiac output (CO) per minute is defined as:

Heart rate (HR) x stroke volume (SV)

Any factor negatively affecting either of the above will diminish CO, leading to reduction of tissue perfusion and thus metabolic support for active tissues. SV can also be affected by a decrease or persistent excess of preload, decrease in effective contractility or increase in afterload, all of which lead to a consequent fall in CO.

The body has several intrinsic compensatory mechanisms to counter reductions in cardiac output which include cardiac remodelling, the Frank-Starling mechanism and neurohormonal activation (Mann, Douglas L., Bristow 2005, LaCombe, Basit et al. 2019).

Cardiac remodelling occurs due to abnormalities in ventricular loading. In HF ventricular pressures are typically raised, either due to impaired ejection fraction and increased volume loading, or due to failure of myocardial relaxation and increased ventricular pressure loading. In the setting of persistent raised ventricular wall stress, the cardiac myocytes hypertrophy as part of their adaptive physiological response (Heusch, Libby et al. 2014). According to the Laplace-Young relationship, myocardial oxygen requirements are proportional to wall tension so when cardiac wall thickness increases due to hypertrophy and cavity radius is reduced, wall tension falls and myocardial oxygen demand is decreased.

Persistent supraphysiological preload leads to dilation of the ventricle, causing a transition from a small hyperkinetic ventricle to a large compliant ventricle capable of producing large stroke volumes (Gaasch, Meyer 2008). Pressure stimulates dissolution of extramyocardial structural collagen via fibroblast activation, which allows rearrangement of myofibrils and chamber enlargement to accommodate for a larger fluid volume. This causes myocytes to increase in length but allows sarcomere length to return to normal from their distended state (Gaasch, Meyer 2008). This normalises contractility and thus physiological contractile reserve. Systolic wall stress returns to baseline, with normal preload, afterload and LVEF while stroke volume increases due to the large end-diastolic volume. Eventually this process becomes maladaptive; fibroblast proliferation, oxidative stress and extracellular matrix

deposition leads to apoptosis and fibrosis within the myocardium (Mentz, O'connor 2016), but the manner and timing of this transition is still poorly understood.

The Frank-Starling Mechanism describes the process by which an increase in preload leads to increased myocardial contractility. Excess myocardial stretch leads to increased calcium sensitivity of myofibrils which increases actin-myosin cross bridge formation (Klabunde 2011). The increased stretch also distends the sarcomere. Maximal sarcomeric force is generated at a sarcomere length of 2.2 μm; sarcomeric length under this figure (or theoretically over) leads to diminution of potential contractile force (Sonnenblick, Spiro et al. 1963). This relationship is outlined in Figure 1.1 and is demonstrated by the theoretically parabolic nature of the length-force curves.

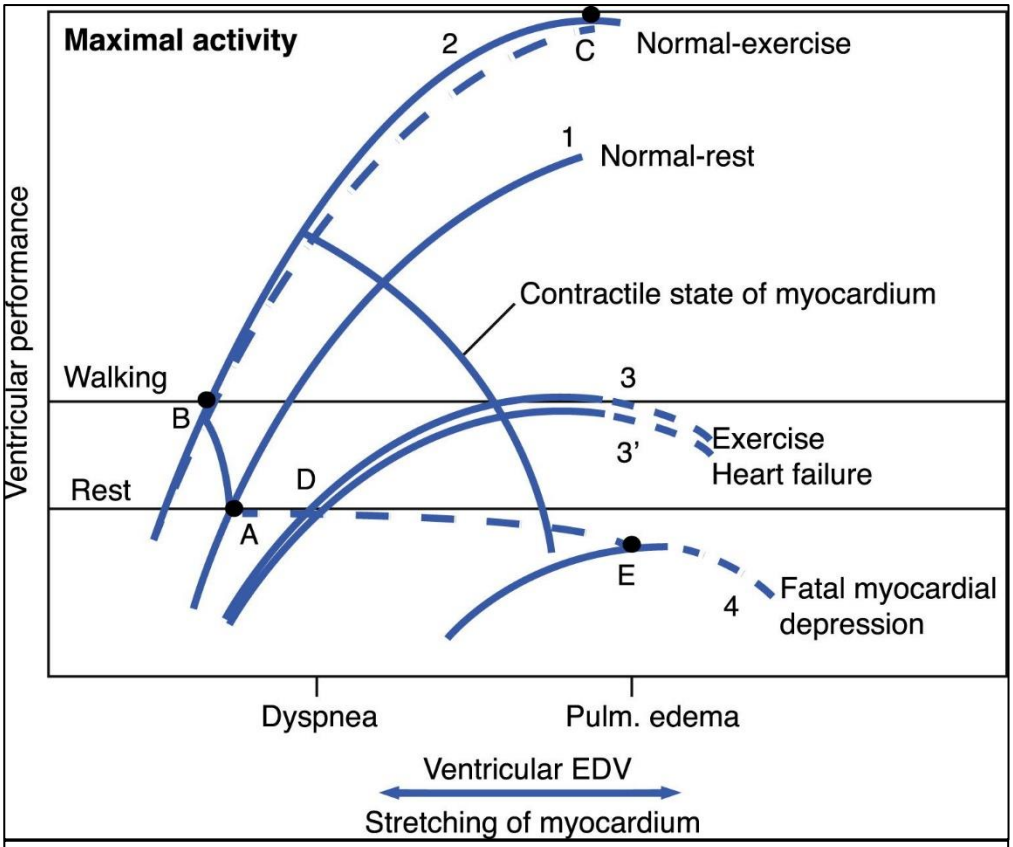

Figure 1.1 – The Frank-Starling relationship – Ventricular performance compared against ventricular end diastolic volume (EDV). 1 = Normal-rest; 2 = Normal-exercise; 3 = Heart failure-exercise; 3' = Heart failure; 4 = Fatal myocardial depression; A = normal at rest; B = normal walking; C = normal maximal exercise; D = heart failure at rest; E = heart failure while walking. The **dashed lines** are the descending limbs of the ventricular performance curves, which are rarely seen during life but show the level of ventricular performance if EDV could be elevated to very high levels. Taken from Harrison's Principles of Internal Medicine (Fauci 1998).

The sympathetic nervous system plays a vital role in control of cardiovascular output and helps to regulate cardiac rate, myocardial contractility and peripheral resistance while the parasympathetic system acts as a counterbalance through stimulation of the vagus nerve.

One of the most effective mechanisms to improve cardiac output is to increase heart rate, as is seen during exercise.

Reduction in cardiac output and subsequent reduction in aortic and carotid baroreceptor pressure leads to subsequent upregulation of the sympathetic response to improve systemic perfusion pressures. Peripheral beta-1 stimulation leads to activation of the renin-angiotensin-aldosterone system (RAAS) which causes vasoconstriction and sodium retention with

subsequent osmolar pressure to retain water, all leading to an increase in mean arterial pressure (MAP). This initially compensatory mechanism becomes maladaptive over time but in a manner and rate which is not well delineated.

Animal models have shown that when this response is prolonged it leads to apoptosis via the protein kinase A pathway (Communal, Singh et al. 1998) and induces isolated myocardial increases in levels of tumour necrosis factor alpha, interleukin 1-beta and interleukin-6 (Murray, Prabhu et al. 2000). It also leads to a reduction in beta-1 receptor density and uncouples the beta-2 receptors from their downstream effector molecules. This pathway normally increases calcium influx leading to an increase in myocardial contractility and when affected pathologically this response is attenuated (Houser, Margulies 2003). These pathways contribute to the pathogenesis of heart failure and to pathological remodelling (Triposkiadis, Karayannis et al. 2009).

The biochemical pathways described above are now routine targets for pharmacological agents used to preventing maladaptive remodelling; beta blockers (BB), angiotensin converting enzyme inhibitors (ACEi) and aldosterone inhibitors are now the mainstay of contemporary medical therapy in HF. These will be discussed in greater depth later in this chapter.

1.2.3 Diagnosis

The process by which HF is diagnosed has changed in accordance with the understanding of the disease. In with the absence of proven biomarkers and widespread access to echocardiography, the researchers from the Framingham study provided the first agreed clinical criteria for a HF diagnosis with minor and major criteria from clinical examination and history combined to form a diagnosis (McKee, Castelli et al. 1971)**.**

The Boston points-scoring criteria subsequently included chest radiography alongside the clinical assessment (Carlson, K. J., Lee et al. 1985), while later research studies included echocardiographic data as access to echocardiography became more widely available (Bangdiwala, Weiner et al. 1992).

Current ESC and AHA guidelines recommend a combination of clinical history, biochemistry, electrocardiographic data and echocardiographic imaging to confirm or refute a diagnosis (Ponikowski, P., Voors et al. 2016).

Common signs and symptoms that raise clinical suspicion of HF have already been described in Table 1.1, the confirmatory tools required for the diagnosis of HF will be outlined below.

1.2.3.1 Biochemistry

Biochemical assessment of HF was revolutionised with the discovery of circulating natriuretic peptides (NP) (Sudoh, Kangawa et al. 1988, De Bold, Borenstein et al. 1981)and subsequent research into their potential utility in HF diagnosis (de Lemos, McGuire et al. 2003). Circulating A-type (ANP), B-type (BNP) and C-type (CNP) natriuretic peptides are primarily produced by the heart in response to increased myocardial stretch due to either volume or pressure overloading. Physiologically they cause a variety of effects within the renal, adrenal, cardiac and vascular tissues including decreased sodium reabsorption (Kalra, Anker et al. 2001), increased natriuresis (Kalra, Anker et al. 2001), reduced aldosterone secretion (Yoshimura, Yasue et al. 2001), increased release of cyclic guanosine monophosphate (GMP) and thus vascular dilation (Yoshimura, Yasue et al. 2001), while absence of NPs leads to cardiac fibrosis in experimental models (Tamura, Ogawa et al. 2000). These effects combine to reduce cardiac stress and aid return to normal haemodynamics.

ANP and BNP levels have been well established as correlating with mortality and inversely correlating with LVEF (Harrison, Morrison et al. 2002, Koglin, Pehlivanli et al. 2001, Menezes Falcão, Pinto et al. 2004), as well correlating with clinical response to pharmacotherapy (Troughton, RW, Yandle et al. 2000, Richards, Doughty et al. 1999, Magnussen, Blankenberg 2018). Meta analyses have demonstrated the superiority of BNP versus ANP as a diagnostic tool (Doust, Glasziou et al. 2004), and BNP is now routinely used as a ‘rule out’ test, given its high negative predictive value (NPV). The ESC guidelines suggest a rule out diagnostic value of <100 pg/ml in the acute setting for which the NPV is 94-98% (Ponikowski, P., Voors et al. 2016), while ACC/AHA guidelines also recommend the use of BNP but do not suggest particular discriminatory values (Yancy, Jessup et al. 2013).

N terminal pro-BNP (NT pro-BNP) is the aminoterminal portion of pro-BNP and has a longer circulating half-life than BNP with similar diagnostic and prognostic value to BNP (Mueller, Gegenhuber et al. 2005, Linssen, Jaarsma et al. 2018). The structure and production of both BNP and NT-pro BNP are outlined in Figure 1.2.

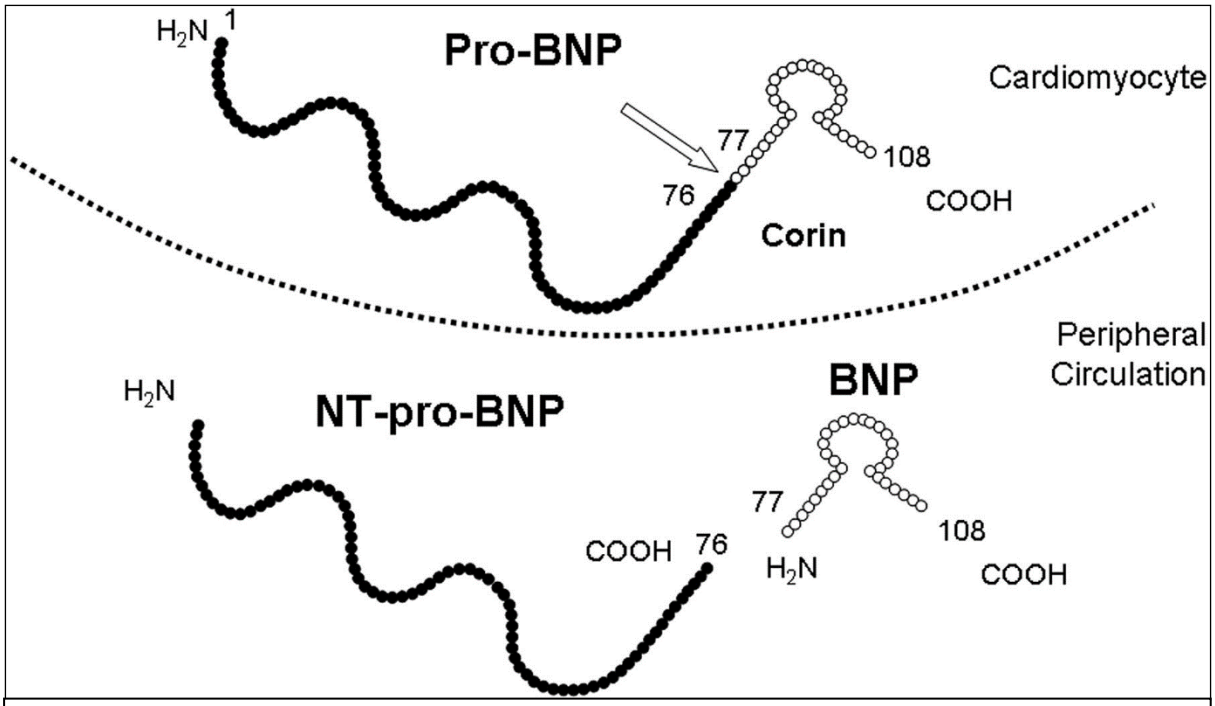

Figure 1.2 – Diagram illustrating the conversion of Pro-BNP to BNP and NT-pro-BNP via the action of the enzyme Corin. Taken from B-type natriuretic peptides and echocardiographic measures of cardiac structure and function (Troughton, Richard W., Richards 2009).

Both assays have been recommended as part of the screening process for patients with potential HF, however recent pharmacological developments may lead to increased use of NT-pro-BNP. Sacubitril-Valsartan is a novel angiotensin-receptor neprolysin inhibitor (ARNI) which has recently been shown to be superior to ACEi therapy (McMurray, John JV, Packer et al. 2014) and its use has been included in the 2016 ESC HF guidelines (Ponikowski, P., Voors et al. 2016). Its mechanism of action leads to decreased degradation of circulating BNP, ameliorating its physiological effect. This consequently has implications for the use of BNP as a diagnostic tool and for BNP-guided titration of therapy. The PARADIGM-HF trial demonstrated raised circulating BNP and cGMP levels in the Sacubitril-Valsartan treatment arm (McMurray, John JV, Packer et al. 2014), while NT-pro BNP levels were reduced, a finding mirrored in the PARAMOUNT trial (Solomon, Scott D., Zile et al. 2012). Use of BNP as a diagnostic or prognostic marker appears of diminished value in patients treated with ARNIs due to its pharmacodynamics, and a shift may be seen in either the current diagnostic screening levels or an increased use of NT-pro BNP given that it is not similarly affected.

Primary amongst other potential biochemical markers is suppression of tumorigenicity factor 2 (ST2) - a member of the interleukin-1 receptor family with soluble and ligand isoforms. These are upregulated when cardiac myocytes are strained, leading to transduction of the antifibrotic and antihypertrophic effects of interleukin-33. Several studies have demonstrated their potential use as a diagnostic aid, though it is often noted that NT-pro BNP levels are a better diagnostic discriminator than ST2 levels, primarily due to the lack of specificity (Januzzi, James L., Peacock et al. 2007, Dieplinger, Mueller 2015). Januzzi et al. demonstrated that ST2 was a stronger prognostic marker than NT-pro-BNP at 1 and 4 years (Januzzi, J. L.,Jr, Rehman et al. 2010), and is equally prognostic in both HFpEF and HFrEF (Manzano-Fernández, Mueller et al. 2011). Not only is it strongly prognostic, but Pascual-Figal et al. demonstrated that ST2 was a stronger classifier of mortality risk than other markers including BNP, NT-pro BNP and CRP (Pascual-Figal, Manzano-Fernández et al. 2011). Serial measurement of ST2 has been shown to identify decompensated HF patients at risk of mortality, transplantation and hospitalisation (Manzano-Fernandez, Januzzi et al. 2012) as well as act as a strong prognosticator in chronic HF (Anand, Rector et al. 2014). Much like BNP and NT-pro BNP, levels of ST2 have been shown to reduce with optimal medical therapy, while it has been suggested that raised ST2 levels may predict implantation of defibrillators and sudden death in HF (Pascual-Figal, Ordoñez-Llanos et al. 2009), as well as identifying patients most likely to benefit from aldosterone antagonists (Weir, Miller et al. 2010).

ST2 is now in recommended for use in risk stratification and prognostication by the ACC/AHA for both chronic and decompensated heart failure, and it appears likely that its use may become more widespread.

Markers of myocardial damage, structural remodelling and oxidative stress have also been investigated for utility in HF diagnosis and prognostication but are currently used primarily in research (Takeishi 2014). BNP and its precursor NT-pro-BNP remain the most common biochemical diagnostic tool in the field of HF, but as suggested there may be increased use of ST2 in the future.

1.2.3.2 Electrocardiography

The 2016 ESC guidelines reference the electrocardiogram (ECG) as one of its essential initial investigations in the diagnosis of HF. A normal ECG can rule out HFrEF with 89%

sensitivity (Mant, Doust et al. 2009) and in patients with suspected HF the ECG can be particularly useful when considering the underlying aetiology of HF, for example the typical changes ST segment and T wave changes seen in ischaemic heart disease, or the evidence of a cardiac arrhythmia.

Notably there is little to no data regarding the utility of ECGs in diagnosis of HFpEF or HFmrEF. Consensus statements suggest the use of ECG to aid diagnosis (Paulus, Tschope et al. 2007), while some data suggest more consistent appearance of ECG abnormalities such as left ventricular hypertrophy (McMurray, John JV, Carson et al. 2008), but there are little data which guide the clinician as to sensitivity or specificity in this patient group.

The ESC guidelines also note that generalised ECG abnormalities show understandably low specificity (Thomas, Kelly et al. 2002, Davie, Francis et al. 1996, Kelder, Cramer et al. 2011), but given its sensitivity and negative predictive value for HFrEF it remains a logical first-line test, and was a particularly useful discriminator for the necessity of an echocardiogram prior to the discovery of serum biomarkers.

1.2.3.3 Chest X-ray

In suspected HF patients, performing and assessing x-ray imaging of the chest is recognised as a useful test given that the largest proportion of HF patients presenting to hospital do so with signs and symptoms of systemic overload. In this setting a chest x-ray is particularly useful to rule out other causes of breathlessness (Ponikowski, Piotr, Jankowska 2015).

Classical features of HF on chest X-ray include cephalization (the rostral diversion of blood within the lungs), interstitial oedema, costophrenic angle blunting and cardiomegaly (Radiology Masterclass , Fonseca, Mota et al. 2004) – the latter of which is defined as a cardiothoracic ratio in excess of 0.5 on a posterior-anterior chest radiograph (Gaillard 2015). Interstitial oedema can also be seen in acute respiratory distress syndrome (ARDS) which can cause diagnostic uncertainty. Features which are known to aid the clinician in distinguishing underlying aetiology are shown below in Table 1.6 and typical features of heart failure on chest X-ray can be seen in Figure 1.3.

	Cardiac Cause	ARDS
Heart Size	Enlarged	Normal
Vascular Pedicle	Normal or enlarged	Normal or reduced
Pulmonary blood flow distribution	Inverted	Balanced
Peribronchial cuffs	Common	Not common
Regional distribution of lung oedema	Even	Peripheral/Patchy
Air bronchograms	Not common	Very common
Pleural effusions	Very common	Not common

Table 1.6 – Features aiding discrimination between Cardiac interstitial oedema and ARDS – Adapted from The radiologic distinction of cardiogenic and non-cardiogenic oedema (Milne, Pistolesi et al. 1985).

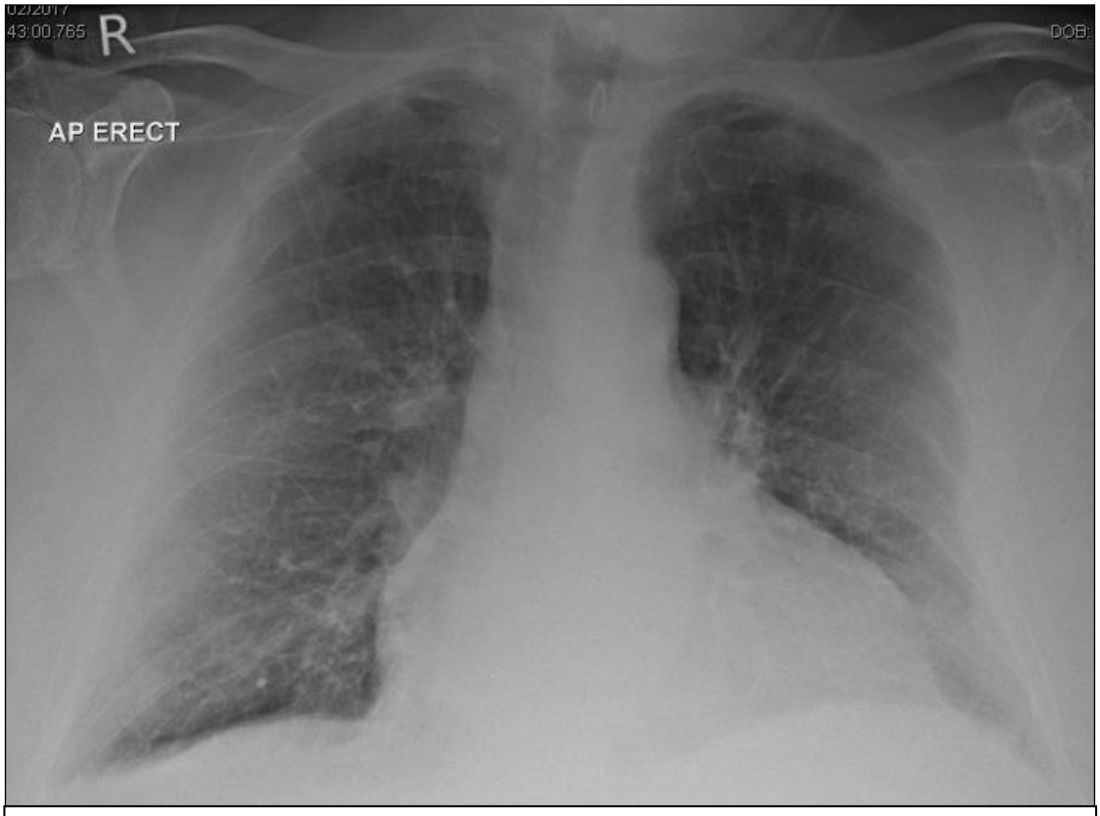

Figure 1.3 – Chest radiograph demonstrating typical features of cardiac pulmonary congestion. This includes an enlarged cardiac shadow, enlarged vascular pedicle, inverted pulmonary blood distribution, peribronchial cuffing and small bilateral pleural effusions.

1.2.3.4 Echocardiography

Transthoracic echocardiography (TTE) has developed from a tool that was able to indistinctly image the walls of the atria and ventricles as flat lines on an oscilloscope (Hertz, Edler 1954), to become the cornerstone of structural cardiology, able to create live three-dimensional images of the heart. It is the essential confirmatory tool in HF, being simultaneously portable, essentially devoid of risk to the patient and more widely available than other imaging modalities such as magnetic resonance imaging (MRI). TTE provides useful data regarding cardiac structure and function in almost all patients and is routinely preferred to transoesophageal echocardiography in part due to the risks inherent to the pharmacological sedation which is often required.

Diagnosis of HF is made by assessing left ventricular systolic and diastolic function, as well as right ventricular function and pulmonary artery pressures. TTE enables assessment of haemodynamics and consideration of underlying aetiology and is able to detect valvular abnormalities, regional wall motion abnormalities and evidence of other specific pathologies. It is markedly inferior to MRI in terms of tissue characterisation but is the reference standard for diagnosis and assessment of heart failure.

As noted earlier, one of the major criteria for diagnosing HF is based upon LVEF. This can be estimated visually but is typically prone to error in patients with abnormal ventricular geometry or extremes of ventricular size (Marwick 2015). To assess LVEF quantitatively, accurate volume measurements are required of the left ventricle in end systole and end diastole. Typically, these are measured using two separate planes, apical four chamber and apical two chamber views, from which volumes can be derived using different calculations, the most common of which is the biplane method of disks, frequently referred to as Simpson's rule (Otto 2013).

End diastolic volume (EDV) – end systolic volume (ESV) is equal to SV, which when divided by EDV is equal to the proportion of intracavity blood pumped out per cycle, termed the ejection fraction (EF). This can be mathematically represented thus: $EF = \frac{EDV - ESV}{EDV}$

Diagnosis of HFmrEF or HFpEF requires evidence of relevant structural disease or diastolic dysfunction. This can be readily seen on echocardiography, for example using measurement of myocardial wall thickness using echocardiographic M-mode, demonstration of valvular

pathology on Doppler tracing or pericardial abnormalities again detectable both on 2-dimensional imaging and changes to movements of the right ventricular wall. Evidence of increased atrial area can be demonstrated through increased atrial size on M-mode or via border tracing performed in apical 4 chamber view.

Diagnosis of diastolic dysfunction in the setting of normal LVEF can be difficult and is often complicated by the alteration to normal physiology which occurs with aging; slowing of LV relaxation is particularly common with age which must be considered when interpreting the data.

Multiple quantitative parameters can be used in assessing diastolic function, including, but not limited to, mitral inflow Doppler traces in early diastole (E) and in atrial contraction (A), pulmonary venous flow, mitral annular tissue Doppler velocities (e'), tricuspid regurgitation velocity and left atrial volume.

The detailed measurements of diastolic function are beyond the scope of this project but Figure 1.4 outlines the components of a standard quantitative assessment of diastolic function as required by the joint American Society of Echocardiography (ASE) and European Association of Cardiovascular Imaging (EASCVI) guidelines for diagnosis and quantification of severity of diastolic dysfunction.

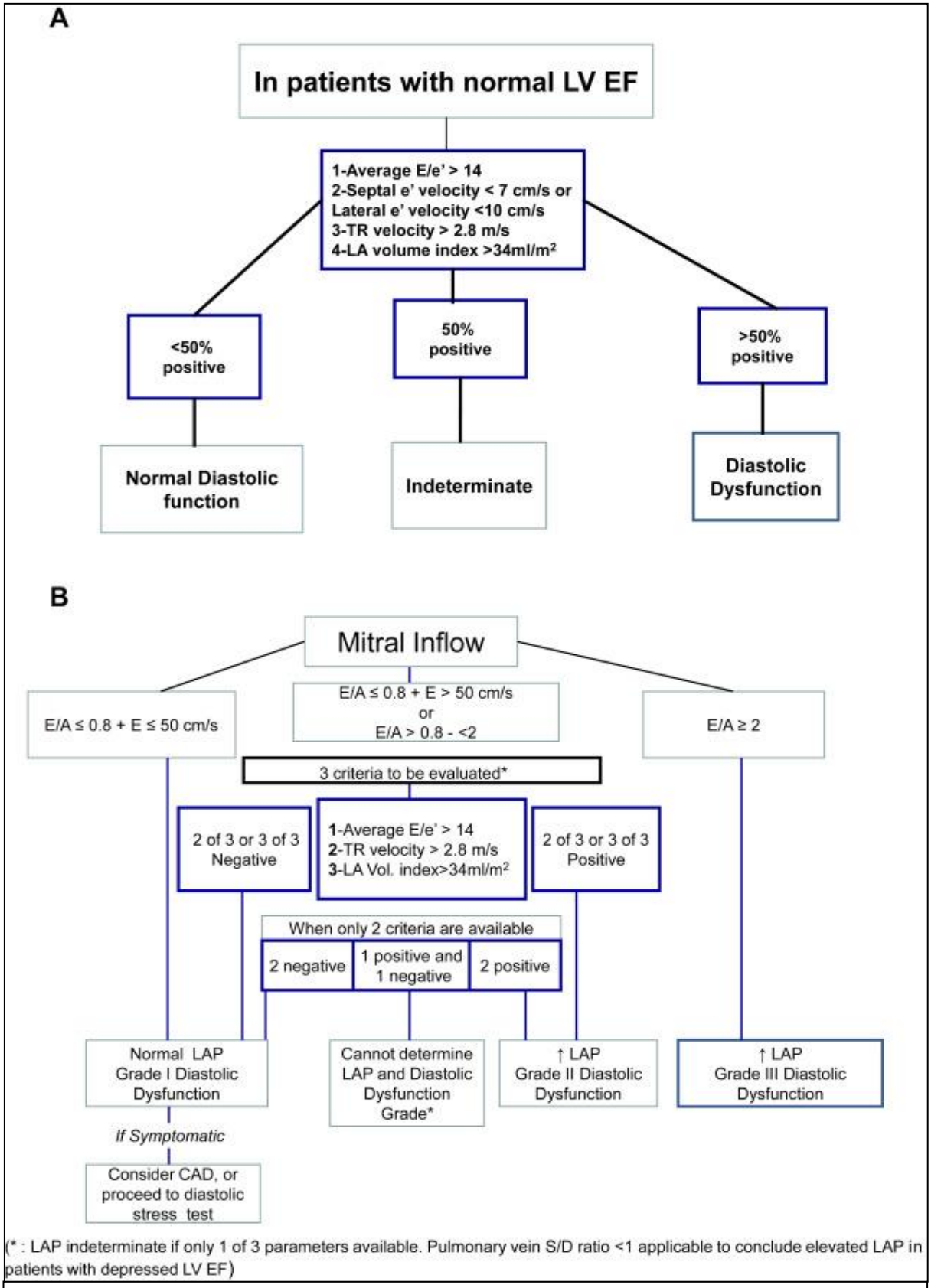

Figure 1.4 – Flow diagram for diagnosis and grading of diastolic dysfunction - taken from ASE/EASCVI recommendation for grading of severity of diastolic dysfunction(Nagueh, Smiseth et al. 2016). **(A)** Algorithm for diagnosis of LV diastolic dysfunction in subjects with normal LVEF. **(B)** Algorithm for estimation of LV filling pressures and grading LV diastolic function. in patients with depressed LVEFs and patients with myocardial disease and normal LVEF after consideration of clinical and

As outlined below in Table 1.7, typical signs and symptoms plus evidence of a reduced LVEF allow the physician to diagnose HFrEF, while the combination of clinical signs and symptoms, biochemical evidence and diastolic or structural abnormalities allows a physician to make the diagnosis of HFmrEF or HFpEF.

Type of HF		**HFrEF**	**HFmrEF/borderline**	**HFpEF**
Criteria	**1**	Symptoms ± Signs (as per Table 1.1)	Symptoms ± Signs (as per Table 1.1)	Symptoms ± Signs (as per Table 1.1)
	2	Left Ventricular Ejection Fraction <40%	Left Ventricular Ejection Fraction 40-49%	Left Ventricular Ejection Fraction ≥50%
	3		1. Elevated levels of natriuretic peptide 2. At least one of either relevant structural heart disease (left ventricular hypertrophy and/or left atrial enlargement) or diastolic dysfunction	1. Elevated levels of natriuretic peptide 2. At least one of either relevant structural heart disease (left ventricular hypertrophy and/or left atrial enlargement) or diastolic dysfunction

Table 1.7 – ESC guideline diagnostic criteria for heart failure – adapted from 2016 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure (Ponikowski, P., Voors et al. 2016).

When considering how intrinsically variable the measurement of LVEF is, categorising HF as described above becomes an immediate problem. 2-dimensional TTE is the most widely available diagnostic tool for calculating LVEF, but quantitative analysis is hugely dependent upon the acquisition of adequate acoustic windows and operator technique. Variations in these can lead to significant inter- and intra-observer variability of >10% (Jacobs, Salgo et al. 2005, Malm, Frigstad et al. 2004, Bellenger, Burgess et al. 2000, Pellikka, She et al. 2018). Given that 10% encompasses the entire range of the HFmrEF category, accurate categorisation becomes inherently difficult. In addition, due to acquisition technique and geometric assumptions there are significant discrepancies in measurement of ventricular volumes when compared to volumetric techniques employed in either MRI or 3-dimensional

echocardiography (Mistry, Halvorsen et al. 2010, Gouda, AbdelWahab et al. 2015, Foley, Mankad et al. 2012, Pellikka, She et al. 2018).

Even if imaging modalities were perfect and were affected by only minimal inter- or intra-observer variability, LVEF is a dynamic measurement; LVEF varies considerably according to contemporaneous haemodynamic conditions, be it due to alterations in preload or afterload (Silke, Verma et al. 1985, Berko, Gaasch et al. 1987, Gaasch, Meyer 2008) or alterations to inotropy (Horn, Teichholz et al. 1974, Lim, S. L., Lam et al. 2015). Small variations in the time from admission to echocardiography, experience of the sonographer or quality of the sonography equipment can lead to significant differences in LVEF measurements and thus patient subcategorisation. As a result, there has been much interest in markers of ventricular contractile function which are more in (Silke, Verma et al. 1985)dependent of haemodynamic state such as strain rate imaging. Indeed, studies using myocardial deformation imaging have suggested the existence of contractile abnormalities not previously detected when using LVEF as the sole quantitative marker (Kraigher-Krainer, Shah et al. 2014). Interestingly, there are little to no data that describe the differences between HF subcategories in terms of myocardial deformation imaging parameters.

1.2.3.4.1 Myocardial deformation imaging

Myocardial deformation imaging refers to strain and strain rate of the myocardium – the former describes 3-dimensional deformation of a cube of myocardium in relation to its original shape, while the latter describes the velocity at which this occurs.

Strain (ε) can be described thus:

$$\varepsilon = \frac{L - Lx}{Lx}$$

where L is the length of the object after deformation and Lx is the original length. This is a dimensionless quantity as it is defined relative to its original length and is often denoted in percent. By convention, negative strain standardly refers to shortening while positive strain denotes lengthening relative to original length (Weidemann, Kowalski et al. 2001).

Strain rate refers to the speed at which this deformation occurs and is denoted by the symbol έ and units are described as s^{-1}.

Both strain and strain rate have been demonstrated to correlate significantly with contractile function. Initially there was concern that myocardial deformation values were markedly influenced LV size, hypertrophy and haemodynamic loading conditions, however, experimental models have demonstrated that, while strain remains relatively load-dependent (Yingchoncharoen, Agarwal et al. 2013), strain rate is not (Ferferieva, Van den Bergh et al. 2011), and experimental studies have demonstrated a correlation between strain rate and intrinsic cardiac contractile function (Schlangen, Petko et al. 2014, Dahle, Stangeland et al. 2016, Abraham, Laskowski et al. 2003, Greenberg, Firstenberg et al. 2002). As such, assessing myocardial deformation parameters may allow us to potentially make more precise conclusions regarding overall systolic function as well as intrinsic cardiac contractility.

Myocardial deformation assessment was initially developed using colour-doppler echocardiography and validated as a tool using in-vitro cardiac phantoms and sonomicrometry; measuring the distance between surgically-implanted intracardiac piezoelectric crystals and the displacement and rate of displacement of these crystals allowed conclusions to be drawn regarding deformation of the myocardial tissues. Whilst a useful initial step, tissue doppler imaging was time-consuming to analyse, modestly robust and reproducible and has significant accuracy concerns regarding frame rate and angle dependency (Blessberger, Binder 2010).

Myocardial deformation assessment has potential benefits of assessing regional rather than global function in addition to the theoretical reduction in the load-dependence of the assessment when compared to volume-oriented assessment of ventricular function. It was subsequently developed using other modalities including MRI tissue tagging (MR-T) and has now developed to include feature tracking MRI (MR-FT) and speckle-tracking 2-d echocardiographic deformation assessments, each of which have their own strengths and weaknesses.

Specifically, echocardiography is widely available, relatively inexpensive and can deliver high temporal and spatial resolution, while MR-FT is easily reproducible and offers a better analysis of circumferential and radial strains. Regarding disadvantages, echocardiography is prone to foreshortening, can be difficult to reproduce in terms of image and plane acquisition, while there is inevitably some through-plane motion of the myocardium. MR-FT is based upon contour markers only, while MR-T is notably time consuming, and both offer lower

spatial and temporal resolution and are more resource intensive than echocardiography (Amzulescu, De Craene et al. 2019).

Both 2-dimensional echocardiography and MRI measurements of myocardial deformation have been shown to correlate well with in-vivo sonomicrometry models of myocardial deformtation, and similarly well when compared against each other (Yeon, Reichek et al. 2001, Amundsen, Helle-Valle et al. 2006, Amzulescu, Langet et al. 2017, Bansal, Cho et al. 2008, Korinek, Wang et al. 2005). Both appear to represent imaging modalities of substantial use for myocardial deformation assessment, with the selection of one modality over the other dependent upon the strengths and weaknesses as outlined above alongside considerations of patient and disease-specific factors.

In contemporary echocardiographic speckle-tracking deformation imaging, specialised software allows for tracking of individual interference patterns (speckles) within the myocardial tissue and this allows for production of a 2-dimensional displacement curve for each point in the myocardium. Speckles are typically defined as visual spots generated by the interaction between the ultrasound beam and myocardial fibres. For the purpose of tracking and analysis, speckles can be grouped into functional units known as kernels which have a unique ultrasonic appearance, an ultrasound fingerprint, and thus their movement can be traced throughout the cardiac cycle. This ultimately allows for the calculation of total displacement, rate of displacement, deformation and rate of deformation of the myocardial region (Mondillo, Galderisi et al. 2011, Perk, Tunick et al. 2007). This technique has been validated against sonomicrometry and tagged MRI demonstrating the feasibility and reproducibility of the technique (Amundsen, Helle-Valle et al. 2006).

In dividing the myocardium into a series of small cubes, localised deformation can be assessed along three orthogonal axes (typically longitudinal, circumferential and radial) across multiple areas of the myocardium (Weidemann, Kowalski et al. 2001). Strain and strain rate can thus be calculated for each distinct cube in each axis.

As described in paragraph 1.2.1, myofibril orientation varies throughout the myocardium allowing for the generation of force in different planes and ensuring efficiency of contraction. The gradual change in fibre orientation between myocardial layers means that these distinct layers have a greater and lesser role to play in developing myocardial deformation in each of the above axes. The subepicardium and subendocardium both have fibres that run

longitudinally, albeit at diametrically opposed oblique angles to aid development of rotational force, whilst the mid-wall fibres run circumferentially to aid the development of circumferential force, both combining to produce ‘passive’ radial thickening.

When considering myocardial deformation in each plane, the sub-layers of the myocardium will have a greater or lesser role to play; the subepicardium and particularly the subendocardium will contribute more substantially to longitudinal strain, while the mid-wall layer will contribute more to circumferential strain, and the combination of all fibre layers will be significant in the development of ‘passive’ radial deformation (Bijnens, Cikes et al. 2012, Kalam, Otahal et al. 2014).

In the methodology and results chapters of this thesis longitudinal deformation is the only plane assessed. Longitudinal strain has emerged as the most commonly measured strain tensor in part due to a number of studies validating its use as a marker of function and prognosis in a wide range of conditions including hypertrophic cardiomyopathy, HFpEF, amyloidosis and diabetes(Caselli, Montesanti et al. 2015, Imbalzano, Zito et al. 2011, Zhang, X., Wei et al. 2013, Lo, Haluska et al. 2016). Longitudinal strain and strain rate values can be determined for each myocardial segment as described above or averaged to produce a value known as global longitudinal strain (GLS). Reductions in peak systolic GLS have been seen in myocardial ischaemia, alongside characteristic patterns of systolic lengthening and post-systolic shortening (Kukulski, Jamal et al. 2003, Edvardsen, Aakhus et al. 2000). Similarly, GLS has been demonstrated to be an independent predictor of clinically-relevant coronary heart disease in patients with suspected stable angina (Biering-Sorensen, Hoffmann et al. 2014). GLS has also been demonstrated to be of use in subclinical cardiomyopathies, particularly in hypertrophic cardiomyopathies where LVEF values remain normal or supranormal. GLS is diminished in early cardiomyopathic disease but not in physiological hypertrophy (Afonso, Kondur et al. 2012), and thus may aid assessment and prognostication in this patient group, particularly with a view to demarcating physiological versus pathological hypertrophy.

GLS values are also able to delineate patients with HFpEF from both hypertensives without diastolic dysfunction and normal controls, demonstrating reduced strain in comparison (Kraigher-Krainer, Shah et al. 2014). This may be important as it is currently difficult to correctly identify many patients with HFpEF and HFmrEF given the complexity of the

current diagnostic criteria as described above. Strain and strain rate values have also been shown to fall as LVEF reduces, and it has been suggested that they could be used as a more accurate measure of declining systolic function than LVEF, particularly in patients with regional wall-motion abnormalities (Brown, Jenkins et al. 2009).

GLS has also been shown to predict outcome in those with HFrEF. In patients whose LVEF recovers due to therapeutic intervention, GLS is a predictive marker of further stability or deterioration (Adamo, Perry et al. 2017). In fact, several studies have shown superiority of GLS values to LVEF in predicting outcomes in patients with known HF (Stanton, Leano et al. 2009, Ersbøll, Valeur et al. 2013), as well as predicting the development of HF in patients with subclinical disease (Russo, Jin et al. 2014).

GLS is also the variable most widely assessed and reported due to the fact that it has, historically, technically been substantially easier to measure and thus there is more software developed for the assessment of these values. Studies have demonstrated significantly less inter-vendor concordance when assessing circumferential and particularly radial strain as opposed to longitudinal strain and thus, due to these practical implications, GLS has been more commonly adopted and assessed (Manovel, Dawson et al. 2010, Risum, Ali et al. 2012). As a result, there is an intrinsic selection bias in the number of studies demonstrating its worth for a range of cardiac pathologies. That being said, there are also data suggesting that depending upon in the underlying aetiology, longitudinal strain is a more sensitive marker of early pathology in comparison to circumferential and radial strains (Ilardi, Santoro et al. 2016). This is particularly seen in the myocardial ischaemia due to the greater effect of ischaemia on subendocardial fibres compared to subepicardial or midwall (Kalam, Otahal et al. 2014), and has also been shown to be an early marker of chemotherapeutic cardiotoxicity (Florescu, Magda et al. 2014) as well as being superior to circumferential and radial strains for prediction of outcome in aortic valve replacement surgery (Zhang, K., Sheu et al. 2019).

Despite the above, there are clearly roles for circumferential strain assessment; circumferential strain is useful in the assessment of ischaemic scar depth (Dohi, Sugiura et al. 2016) and has been demonstrated to predict adequate lead placement and subsequent reverse remodelling in cardiac resynchronisation therapy (Becker, Kramann et al. 2007). As noted above, much of the initial paucity in data regarding circumferential strain is due to difficulties and discrepancies between vendors in defining and assessing circumferential strain. As this is

resolved by newer imaging techniques a larger role for circumferential strain measurements is likely to emerge, particularly regarding conditions largely affecting the myocardial midwall such as non-ischaemic cardiomyopathies(Taylor, Umar et al. 2015), systemic vasculitides (Edwards, N., Ferro et al. 2007) and lipid-storage disorders such as Fabry's disease (Vijapurapu, Nordin et al. 2018).

'Normal' GLS values have been reported as being between -18 and -25% in healthy individuals, but there is substantial variation seen in diseased populations.

Limited information exists regarding GLS and global longitudinal strain rate (GLSR) in the AHF population, partially due to the typical requirements for echocardiographic frame rates of nearly 200 frames per second. These are typically only achievable in research conditions, and not in the typical clinical setting.

Further experimental information regarding strain and strain rate values may allow us to more clearly define the AHF population in the future, particularly in the context of HFpEF which remains a complex and difficult diagnosis to make in many patients.

As discussed above, a diagnosis of HF requires a combination of clinical signs and symptoms, bedside tests such as ECG and chest x-ray, biochemistry and echocardiographic data. This process continues to evolve and enhance the sensitivity and specificity of our diagnoses, with particular improvements and additions now seen in biochemical and echocardiographic parameters.

1.2.4 Epidemiology

Chronic HF is seen in approximately 1.2% of the UK population (Bhatnagar, Wickramasinghe et al. 2015), consistent with US data which put prevalence at approximately 1.7 % in 2005 (American Heart Association 2005)and 2.4% in 2012 (Roger, V. L., Go et al. 2012).

In the United Kingdom (UK) HF is rare in younger patients, particularly in those under 50, but rises with age to nearly 8% of those 75 years or older (Bhatnagar, Wickramasinghe et al. 2015). This approximates data from the United States of America (US) in which prevalence has been reported at 12% in those aged 80 years or older (Roger, V. L., Go et al. 2012).

When looking exclusively at the elderly population, HF is typically reported in approximately 6-13% of patients >65 years old across the western world (Mureddu, Agabiti et al. 2012, Sánchez, Leiro et al. 2008, Abhayaratna, Smith et al. 2006, Azevedo, Bettencourt et al. 2006, Ceia, F., Fonseca et al. 2005, Ceia, Fátima, Fonseca et al. 2002, Raymond, Pedersen et al. 2003, Di Bari, Pozzi et al. 2004, Davies, MK, Hobbs et al. 2001). Unfortunately, there is a relative paucity of data from non-western populations, though data from China do suggest similar population prevalence and age distribution (Jiang, Ge 2009).

Incidence of HF in the UK has been declining in the recent decade; currently it is reported as approximately 3.2 per 1000 patient years, falling from 3.6 per 1000 patient years in 2002 (Conrad, Judge et al. 2017). Data from Europe and the USA report similar incidence of 1-5 per 1000 patient years for the adult populations, with the trend remaining either stable or demonstrating declining incidence (Savarese, Lund 2017). As with prevalence, incidence is known to rise with age, with global estimates of >100/1000 patient years in those aged ≥65 years (McMurray, J. J., Stewart 2000).

Large prospective cohort studies such as the Framingham Heart Study (Levy, D., Kenchaiah et al. 2002) and Olmsted County group (Roger, Veronique L., Weston et al. 2004) have been invaluable in tracking trends within HF. As described above, both groups describe a steady or declining incidence of HF but interestingly show improved patient survival.

The trend of incidence and survival seen in both prospective and retrospective cohorts is leading to a significant increase in the healthcare burden of HF. Therapeutic options are improving and growing in number, but they are largely able to ameliorate rather than cure the underlying syndrome. This leads to improvements in long term survival but carries high costs for both the individual in terms of symptoms, therapeutic side-effects and hospitalisations, but also for the healthcare providers tasked with caring for the individual both in the community and in the hospital setting. As a result, the financial burden of HF is predicted to grow dramatically in line with increased HF survival (Savarese, Lund 2017).

Gender discrepancies are also seen in HF; prevalence of heart failure is higher in males than females at all age groups, with prevalence in males at least double that of females under the age of 75, after which the gap narrows (Bhatnagar, Wickramasinghe et al. 2015). Females

differentially express genes related to cardiac function (Mendelsohn, Karas 2005) and it is well understood that the clinical phenotype of HF in each gender is markedly different (see Table 1.8 below).

Clinical Phenotype		
Female		**Male**
Hypertensive/Valvular disease	**Aetiology**	Ischaemic
Preserved	**LV Function**	Reduced
Following decompensation	**Diagnosis**	Using BNP biochemistry
Better compliance	**Therapy**	Worse compliance
More easily affected	**Quality of life**	Fewer psychological problems
Better	**Outcome**	Worse

Table 1.8 – Phenotypic differences in the natural history of heart failure compared between the genders, each group in reference to the other. Adapted from Gender differences in heart failure: paving the way towards personalized medicine? (Schirmer, Hohl et al. 2010).

Ethnicity may also play a role in the predisposition to, and clinical course of, HF. Much of the data on racial differences within HF patients comes from US populations due to the diverse racial mix within the country. Aetiological and pathological differences have been described in these studies, with pathological increases in LV mass described in white and Hispanic populations when compared to African-American populations (Bahrami, Kronmal et al. 2008). Incidence has been shown to be highest in African-American populations, followed by Hispanic then age and sex-matched Caucasian groups (Bibbins-Domingo, Pletcher et al. 2009). This latter point is likely due to increased levels of reversible risk factors in terms of hypertension, diabetes and obesity in both the African-American and Hispanic populations. This may not represent a true genetic predisposition but may in fact reflect lower socio-economic status and higher atherosclerotic risk as was suggested by the authors of the MESA trial (Roger, V. L. 2013).

A similar trend is seen regarding hospital admissions; African-American groups have been demonstrated to have the most frequent hospitalisations for HF, followed by Hispanic, then

white then Asian populations, all at statistically different rates. Interestingly, in-hospital outcomes have been observed to be better in the African-American group compared to others (Sahni, Horwich et al. 2015). One interpretation of these data is that increased readmissions arise as a result of poor access to outpatient interventions but these disadvantaged patients may have a lower level of disease progression upon admission.

1.2.5 Aetiology

As outlined earlier, HF is a syndrome rather than a single disease process. As such, many conditions can give rise to the systolic and diastolic abnormalities which lead directly to the clinical symptoms described in Table 1.1.

These can be broadly grouped into diseases of the myocardium, abnormal loading conditions and arrhythmias, of which further examples can be seen in Table 1.9.

Pathophysiological abnormality	Example condition
Diseased myocardium	Ischaemic heart disease, toxic damage, metabolic derangements
Abnormal loading conditions	Hypertension, valvular defects, high output states (sepsis, hyperthyroidism)
Arrhythmias	Tachy/bradyarrhythmias

Table 1.9 – Common aetiologies of heart failure – adapted from 2016 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure (Ponikowski, P., Voors et al. 2016).

The Heart Failure the Global Burden of Disease Study estimated 17 primary aetiologies within HF but the majority of cases can be attributed to a handful of conditions, namely ischaemic heart disease, hypertension and rheumatic heart disease (Hawkins, Petrie et al. 2009).

Aetiology of HF also varies geographically. Coronary artery disease (CAD) and hypertension (HTN) are the commonest underlying cause seen in the western world but there is a substantial proportion of HF secondary to valvulopathies and nutritionally related cardiac disease seen in developing countries (Lip, G. Y., Gibbs et al. 2000).

The diseases above have been listed as separate entities but they are not mutually exclusive and some even have causal links or associations. Many patients suffer from multiple concomitant pathologies, all of which may lead to HF, therefore clearly delineating primary aetiology often remains a challenge.

The Framingham study offers useful information concerning aetiological trends. In the initial cohort HTN was reported as the most common cause but was superseded by CAD in the 1970s while HTN and valvular disease declined dramatically (Kannel 1990). This reflects trends in therapeutic interventions for HTN but may also represent an increased ability to detect CAD with the advent and increased availability of coronary angiography. Similarly, the overall burden of HF secondary to CAD has risen alongside the ability to intervene in the underlying disease and improve the mortality of acute coronary events. Reductions in short term mortality secondary to CAD has led to the advent of a large proportion of patients with underlying contractile dysfunction secondary to myocardial ischaemia and consequent HF. Recently this has shown signs of decreasing with rates of myocardial infarction declining in many western countries (Moran, Forouzanfar et al. 2014). This is likely due to increased uptake of primordial and primary prevention measures, particularly with regards to smoking cessation.

Rates of untreated HTN have also been diminishing, which may partly explain the gradual reduction in disease incidence described above (Unal, Critchley et al. 2004). In the USA treatment of hypertension is estimated to have led to a reduction in incidence of 50% (Levy, D., Kenchaiah et al. 2002). Nevertheless, for those who remain untreated, the lifetime risk of HF for those with blood pressure (BP) >160/90 is double that of those with BP <140/90 (Levy, D., Kenchaiah et al. 2002).

As mentioned, valvular heart disease remains a relatively common cause of HF in developing countries. While the incidence of rheumatic fever has declined in western populations to <0.01/1000 patient years, in Sudan the incidence has been reported to be 100 times greater (Essop, Nkomo 2005). Valvular disease does still play a role in the development of HF in Western countries, but typically in the context of degenerative valve diseases such as mitral regurgitation and aortic stenosis.

1.2.6 Morbidity

Morbidity in HF is substantial; over 80% of patients with chronic HF have been shown to suffer from physical symptoms including dyspnoea, fatigue, oedema and sleeping difficulties due to paroxysmal nocturnal dyspnoea, impacting upon all aspects of daily life (Schiff, Fung et al. 2003).

HF is also associated with poor subjective quality of life. The prevalence of clinically significant depression in this patient group is approximately 21% (Rutledge, Reis et al. 2006) which compares extremely poorly with a general population burden of 3.3% (Edwards, J. 2016). This can have disastrous consequences, as the presence of depression is known to lead to significantly poorer prognosis irrespective of comorbidity burden (Bush, Ziegelstein et al. 2001, Williams, Ghose et al. 2004), particularly with regards to its impact on cardiovascular mortality (Penninx, Beekman et al. 2001).

Furthermore, treatment comes with its own burdens. Typical pharmacological therapies have a range of known and relatively common side-effects, with adverse reactions reported in up to 10% of those taking bisoprolol (TRIAL 1999), 7-8% of those taking ramipril (Heart Outcomes Prevention Evaluation (HOPE) Study Investigators 2000) and up to 30% of those taking spironolactone (Aronson 2009).

HF is one of the most common causes of hospitalisation throughout the western world, with approximately 1-2% of all admissions secondary to HF as the primary diagnosis (Blecker, Paul et al. 2013). Readmissions rates are incredibly high for those hospitalised with AHF; typical all-cause readmission rates in HF have remained stable in the last 10-15 years at around 20% readmitted at 30 days (Ross, Chen et al. 2010), 25% at 3 months (Alla, Zannad et al. 2007) and rising to 40% by 6 months (Krumholz, Parent et al. 1997). HF is the most common single cause for readmission in this group (Vader, LaRue et al. 2016, Desai, Stevenson 2012).

As shown, HF has a profound symptomatic and psychological impact on patients' lives, but also leads to increased morbidity in terms of medication side effects, hospital admissions, incredibly high readmission rates and, as is discussed below, mortality.

1.2.7 Mortality

HF has long been understood to have a poor prognosis, both in the short and long term. In 2001 Stewart et al. described how 5-year mortality in HF was comparable or worse than that of many common types of cancer such as bowel, prostate, bladder, breast and ovarian (Stewart, MacIntyre et al. 2001).

Globally, inpatient mortality after admission due to AHF is approximately 5%, which rises to between 5 and 15% at 1-month post discharge, and 17-30% at 1 year (Ambrosy, Fonarow et al. 2014). Few of the global registries collect outcome data for longer than 1 year, but smaller prospective studies have suggested 5-year mortality between 45%.and 65% (Bleumink, Knetsch et al. 2004, Tribouilloy, Rusinaru et al. 2007).

As discussed earlier in reference to rising prevalence of HF, mortality has been falling in recent decades, but still remains worryingly high. A Danish HF registry describes a fall in mortality from 45% to 33% at 1 year and 59% to 43% at 5 years when comparing 1987 to 2012 (Schmidt, Ulrichsen et al. 2016).

Similar trends have also been described from the Olmsted county cohort, where a statistically significant improvement in 5-year mortality was noted from patients admitted in 1991 to those admitted in 2001; in multivariate analysis, year of admission conferred a hazard ratio of 0.99 (0.97-1.00) demonstrating improving mortality over time. This effect was only seen in the HFrEF and not the HFpEF subgroup (Owan, Hodge et al. 2006), and is likely related to the introduction of prognosis-modifying medications and interventional therapies with proven efficacy in HFrEF. This global trend appears to be driven by decreasing sudden cardiac death (SCD) leading a consequent proportional increase in the quantity of patients dying secondary to pump failure (Pereira-Barretto, Bacal et al. 2015).

Reviews of global mortality trends have reported that death from a cardiac cause typically accounts for around 50% of overall mortality in HF. They also describe quite substantial global variation with Indian, Chinese and middle eastern populations demonstrating a particularly large burden of cardiac death (Bleumink, Knetsch et al. 2004).

Regional studies report similar findings, with cardiovascular disease (CVD) mortality approximating at least 50% of all-cause mortality in HF patients (Ni, Xu 2015, Pons, Lupón et al. 2010), with some groups reporting up to 90% (Ørn, Dickstein 2002).

CVD mortality appears to be falling within the HFrEF group, likely due to the increased uptake of disease and outcome modifying drugs (Rush, Campbell et al. 2015). Data from the TOPCAT study suggest that CVD accounts for a similar proportion of mortality in HFrEF in comparison with HFmrEF and HFpEF (60-70%), but with a higher annualised incidence of death (Bajaj, Claggett et al. 2018).

HF has also been reported as the leading cause of CVD mortality amongst the general population, followed by sudden cardiac death (SCD) and acute myocardial infarction (MI) (Pons, Lupón et al. 2010).

As has been demonstrated above, the mortality of HF, irrespective of subcategorisation, remains high. Additional understanding of the underlying disease processes and ability to focus our therapeutic efforts on those whom they will best serve is imperative to reduce the mortality burden of HF worldwide.

1.2.8 Prognosis-altering pharmacological interventions

Despite the high rates of mortality, for the majority of the 20th century pharmacotherapy in HF consisted of digoxin, diuretics and little else despite these therapies being supported by no studies demonstrating prognostic benefit (Hurst 1974). Both digoxin and diuretics are still used in HF, though digoxin has fewer recommended applications and is typically used for patients with tachycardia related HF. It offers no prognostic benefit, though it may aid with symptom amelioration and readmission rates (Digitalis Investigation Group 1997). In contrast, diuretics are used in all forms of HF to reduce afterload and thus improve stroke volume (Wilson, Reichek et al. 1981) but again are used for symptomatic benefit only. Observational studies have demonstrated that increased diuretic use is an independent marker of increased mortality (Eshaghian, Horwich et al. 2006). There have been no large randomised controlled trials (RCTs) that demonstrate prognostic benefits of diuretics in any HF subset, though meta-analyses have suggested that loop and thiazide diuretics may reduce the risk of HF admission and death compared to placebo (Faris, Rajaa F., Flather et al. 2012, Faris, R., Flather et al. 2002).

Thankfully, due to progression in RCTs pharmaceutical companies have developed medications with demonstrable efficacy in improving cardiac geometry and function, as well as morbidity and mortality. The main foci of current prognosis-altering therapies are the

sympathetic nervous system (SNS), RAAS, vasopressin pathway and NPs which will be discussed in turn.

1.2.8.1 Sympathetic nervous system blockade

As discussed above, short term SNS activation is beneficial in increasing SV via heightened chronotropy and inotropy. Chronic SNS activation in response to decreased stroke volume leads to down-regulation and desensitisation of cardiac and baroreceptor catecholamine receptors. Excessive exposure to SNS stimulation eventually leads to maladaptive hypertrophy, apoptosis and necrosis of cardiac myocytes (Mann, D. L., Kent et al. 1992) while it is known that genetic polymorphisms which upregulate the response to catecholamines increase the likelihood of developing HF (Small, Wagoner et al. 2002).

Small trials conducted by two groups in the mid to late 1970s were the first to demonstrate that beta-adrenergic blockade improved cardiac geometry and survival in HFrEF patients (Swedberg, Hjalmarson et al. 1979, Waagstein, Hjalmarson et al. 1975). Further trials using carvedilol, bisoprolol and metoprolol indicated a similar effect for the class in general (Packer, M., Fowler et al. 2002, Merit-HF Study Group 1999, Poole-Wilson 1999).

There have been no convincing studies demonstrating a benefit in mortality conferred by BBs in HFpEF. Nebivolol has been shown to reduce admissions for patients with HFpEF in sinus rhythm (van Veldhuisen, Cohen-Solal et al. 2009), but meta-analysis of available observational data and RCTs does not support their general use to improve mortality or morbidity in HFpEF (Bavishi, Chatterjee et al. 2015).

1.2.8.2 Renin-angiotensin-aldosterone system blockade

Maladaptive SNS activation also contributes to excess RAAS activation, which can be separately stimulated by a fall either in pressure at the carotid baroreceptors or flow rate at the macula densa. The main downstream substrate of pathology is excess Angiotensin II (AT2) production, which contributes to the adverse cardiac remodelling and endothelial dysfunction seen in HF. Inhibition of AT2 production by ACEi or receptor blockade by ARBs leads to a combination of vasodilation, antagonism of the sympathetic nervous system and natriuresis, reducing intracardiac pressure and volume overload (Tham, Bernardo et al. 2015, Davies, M. K., Gibbs et al. 2000).

ACEis have been a mainstay of pharmacotherapy in HFrEF since the late 1980s; the CONSENSUS and SOLVD trials first demonstrated that patients with HFrEF given enalapril showed improvements in cardiac geometry, symptoms and significant mortality benefits when compared to placebo cohort (SOLVD Investigators* 1991, Consensus Trial Study Group* 1987). This has subsequently been confirmed by other trials using different ACEis suggesting a general class effect (Julian, Moss et al. 1993, Køber, Torp-Pedersen et al. 1995, Pfeffer, Braunwald et al. 1992).

Despite their action on a common physiological pathway, evidence of the prognostic benefit of angiotensin receptor blockers (ARBs) has been more equivocal. Meta-analysis of their effect has demonstrated that they do not appear to reduce mortality or admissions in HFrEF in comparison to ACEis (Heran, Musini et al. 2012), though they may be superior to placebo when ACEis cannot be tolerated (Jong, Demers et al. 2002).

Theoretically, inhibition of AT2-induced ventricular hypertrophy and fibrosis could attenuate pathological diastolic changes seen in HFpEF, however large RCTs have demonstrated no significant differences in hospitalisation or mortality in this cohort (Massie, Carson et al. 2008, Cleland, Tendera et al. 2006, Yusuf, Pfeffer et al. 2003).

Working on the terminal portion of the RAAS pathway, mineralocorticoid receptor antagonists (MRAs) are also widely used in HFrEF. The RALES, EPHESUS and EMPHASIS studies have variously delineated the clear benefits of MRAs on hospitalisation and survival when given in addition to guideline medical therapy (Pitt, Zannad et al. 1999a, Zannad, McMurray et al. 2011, Pitt, Remme et al. 2003). In the EMPHASIS trial, at median follow up of 21 months, the primary outcome of hospitalisation or mortality in was reduced from 25.9% in the placebo group to 18.3% of the Eplerenone group ($p<0.001$).

Considering the utility of MRAs in HFpEF, the recent TOPCAT study spironolactone did not demonstrate a beneficial effect on the composite outcome of CV death, aborted cardiac arrest or HF hospitalisation, though there was a reduction in HF hospitalisations (Solomon, Scott D., Claggett et al. 2015). Similar findings have been reported in other studies, though there is no clear evidence that MRAs improve mortality in the HFpEF group (Kurrelmeyer, Ashton et al. 2014, Edelmann, Wachter et al. 2013, Deswal, Richardson et al. 2011).

1.2.8.3 Natriuretic peptide pathway

Recently a new class of medications has been developed focused on neprilysin-mediated degradation of circulating NPs. NPs are released in response to excess pressure and volume overload of the ventricles and atria and cause natriuresis and vasodilation while counteracting maladaptive cardiac remodelling (Tamura, Ogawa et al. 2000, Yoshimura, Yasue et al. 2001, Kalra, Anker et al. 2001). Blocking the action of neprilysin leads to an increase in circulating NPs and thus increases their cardioprotective effects. The PARADIGM-HF trial demonstrated that angiotensin receptor-neprolysin inhibitors (ARNI) caused a reductions in symptoms, hospitalisations and mortality when compared to patients taking ACEi only (McMurray, John JV, Packer et al. 2014), and as such their use is now recommended in the HFrEF group (Ponikowski, P., Voors et al. 2016).

The PARAGON-HF study is currently assessing the impact of the ARNI sacubitril/valsartan on the HFpEF group. This may provide clinically relevant information about the response of the HFmrEF subgroup to these therapies, and thus new therapeutic options in these groups, so the data are eagerly anticipated.

Figure 1.5 summarises the pathological mechanisms and interactions of the above pharmacological agents.

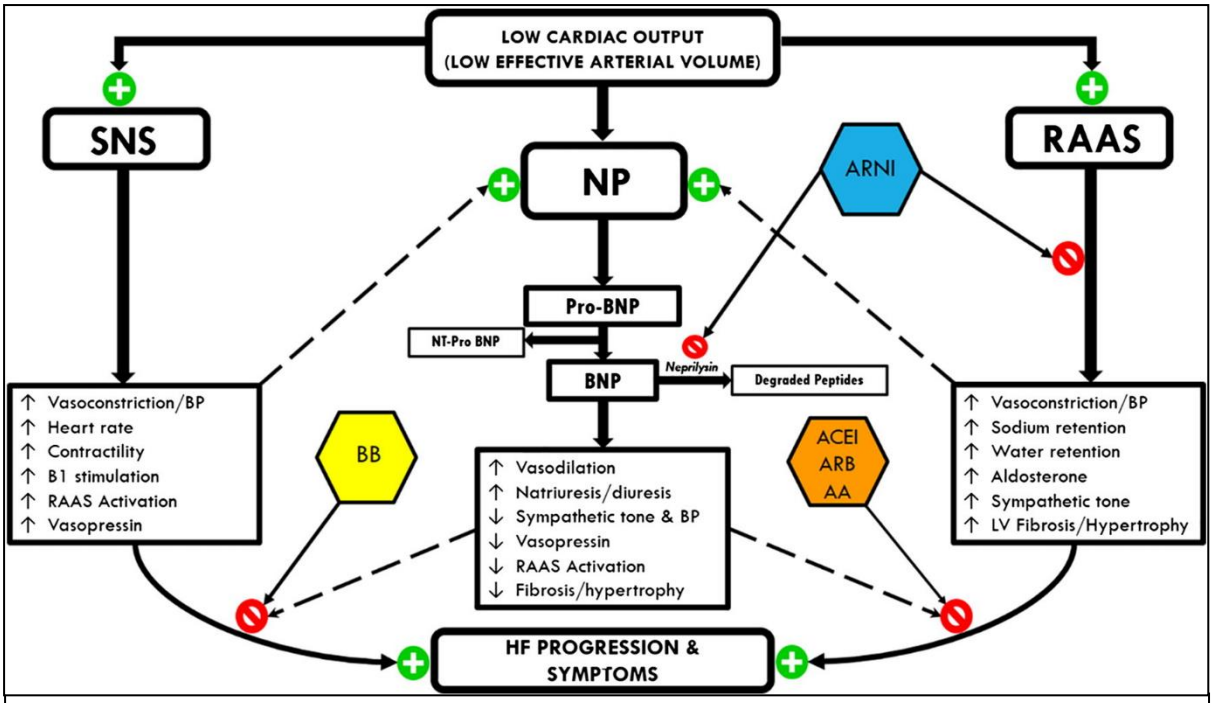

Figure 1.5 - Pathological mechanisms and the interactions of current pharmacological agents – Taken from Pharmacologic Therapy for Heart Failure With Reduced Ejection Fraction: Closing the Gap Between Clinical Guidelines and Practice(Biglane, Becnel et al. 2017).

ACEI – Angiotensin converting enzyme inhibitor, ARB – Angiotensin receptor blocker, ARNI – Angiotensin receptor neprolysin inhibitor, BB – Beta blocker, BNP – B natriuretic peptide, BP – Blood pressure, LV – Left ventricle, NP – Natriuretic peptide, NT-Pro BNP – N-terminal Pro BNP, RAAS – Renin angiotensin aldosterone system, SNS – Sympathetic nervous system.

Having reviewed the current research on HF and touched upon the discrepancies between HFrEF and the novel categorisations, the next section will look more in depth at the focus of this thesis: echocardiographic discriminators in novel subcategorisations of AHF and the ability to effectively prognosticate in AHF.

1.3 Thesis Foci – current literature and gaps in the evidence

This section will outline the main foci of the thesis, namely differences in myocardial deformation imaging between novel subclassifications of AHF, and the ability to prognosticate in HF. Each section will cover the current literature on the subject with critical review of the weaknesses of the available data, and the ways in which this study aims to add to the current understanding.

1.3.1 Novel categorisations of HF – current literature and gaps in the evidence

As discussed earlier, HF has typically been categorised according to LVEF. Until the 2013 AHA/ACC guidelines (and subsequent ESC guidelines) this led to two distinct categories: HFrEF which responded to pharmacotherapy, and HFpEF which did not.

HF with mid-range ejection fraction (HFmrEF) was introduced as a novel categorisation due to concerns regarding potential pathological and phenotypical differences that may exist in this subgroup (Yancy, Jessup et al. 2013) and was designed to stimulate research into the possible pathophysiological and therapeutic differences between the groups (Ponikowski, P., Voors et al. 2016). If differences do exist between HFmrEF and the traditional HFpEF group then the data heterogeneity induced by the inclusion of this group in previous clinical trials may have affected the results derived from these studies. As a result, much of what has just been discussed in terms of demographics, pathology and treatment may be inaccurate and it may have an impact on the ability to treat another subset of patients.

First the evidence from studies looking specifically at this novel subgroup will be discussed followed by a description of the gaps within the current understanding.

Retrospective evaluation of large CHF registries suggests that HFmrEF represents between 12% and 18% of patients (Coles, Tisminetzky et al. 2015, Tsuji, Sakata et al. 2017, Cheng, Cox et al. 2014, Kapoor, Kapoor et al. 2016). Demographically these registry studies show statistically intermediate characteristics of the HFmrEF group in terms of age and gender profiles (Kapoor, Kapoor et al. 2016, Tsuji, Sakata et al. 2017), as well as intermediate prevalence of comorbidities such as coronary artery disease (Kapoor, Kapoor et al. 2016), obesity (Cheng, Cox et al. 2014, Tsuji, Sakata et al. 2017), hypertension and atrial fibrillation (Tsuji, Sakata et al. 2017).

Estimated total prevalence of HFmrEF can be calculated as a function of the total HF population as described above. Registry studies have suggested that the typical HFmrEF patients are older and more commonly female than in than HFrEF, thus prevalence of HFmrEF is skewed towards these demographic groups (Kapoor, Kapoor et al. 2016). Incidence is harder to demonstrate as there is little to no data specifically regarding *de novo* HFmrEF. When looking at previous echocardiography in the HFmrEF subgroup, Rastogi et al. reported that 73% of their patients were improved HFrEF, 10% were static HFmrEF 17%

were deteriorated HFpEF. This may give an indication that while the HFmrEF subgroup represents a substantial proportion of HF patients, only a small share of these present initially as HFmrEF, rather than deteriorating or improving from their initial state. If so, incidence of HFmrEF is likely to be relatively low.

When looking at clinical status, HFmrEF patients appear to act as an intermediate haemodynamic phenotype in terms of systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate and cardiac geometry (Tsuji, Sakata et al. 2017).

Biochemically haemoglobin, creatinine, BNP and NT-pro-BNP levels in HFmrEF all appear intermediate between HFrEF and HFpEF (Tsuji, Sakata et al. 2017), while ST2 is significantly reduced in HFmrEF when compared to HFpEF (Moliner, Lupón et al. 2018). When looking at their ability to aid prognostication in the HFmrEF group, levels of commonly used markers of treatment response such as NT-pro-BNP, high sensitivity troponin T and ST2 appear to be of greater predictive value in this group than in either HFrEF or HFpEF (Moliner, Lupón et al. 2018).

When considering morbidity, the HFmrEF group has been described separately as experiencing intermediate length of stay and rates of all cause readmission (Cheng, Cox et al. 2014, Kapoor, Kapoor et al. 2016) but with higher cardiovascular (CV) readmission rates when compared to HFpEF, acting similarly to HFrEF (Cheng, Cox et al. 2014). In the chronic setting, HFmrEF patients appear to demonstrate an intermediate severity of clinical symptoms when categorised according to the NYHA scale with an intermediate prevalence of NYHA class III and IV symptoms (Tsuji, Sakata et al. 2017).

In terms of all-cause mortality and CV mortality HFmrEF patients have been shown to demonstrate either similar or statistically intermediate outcomes compared to HFrEF and HFpEF at up to 4 years post discharge (Tsuji, Sakata et al. 2017, Cheng, Cox et al. 2014). In an early study in this group, Bhatia et al. described unadjusted mortality rates in HFmrEF of 5.1% at 30 days and 21.3% at 1 year, compared to HFpEF (5.3% and 22.2% respectively) and HFrEF (7.1% and 25.5% respectively) (Hsu, Ziaeian et al. 2017, Bhatia, Tu et al. 2006).

There have been only limited studies directly assessing the response of the HFmrEF to therapeutic intervention. Meta-analysis of 11 trials using beta blockade has suggested that HFmrEF patients with sinus rhythm may also benefit, with a statistically similar response

seen in both the HFrEF and HFmrEF groups. In contrast, the data from the HFpEF cohort demonstrated no mortality benefit, as has been seen in previous trials, while patients with HFmrEF and atrial fibrillation also displayed no benefit (Cleland, Bunting et al. 2017). The CHARM study assessed the effect of ARBs against placebo on primary outcome of CVD or hospitalisation across a range of ejection fractions. They demonstrated a similar effect of RAAS inhibition on the primary outcome in the HFmrEF group to that seen in the HFrEF group, with hazard ratios of 0.82 and 0.76 respectively in the treatment arms. This effect was not replicated in the HFpEF group. The TOPCAT study also reassessed the effect of MRAs in this cohort. They demonstrated a reduction in HF hospitalisation for the whole cohort with LVEF >45%. This effect was most pronounced in the group with LVEF 45-50% and guidelines have been updated to suggest the use of MRAs in the >45% group to reduce hospitalisation. As discussed earlier, the addition of neprilysin inhibitors to the arsenal of pharmacotherapy in HFrEF offers new treatment options and the PARAGON-HF study will be able to delineate any clinical benefit in this HF subcategory (Solomon, Scott D., Rizkala et al. 2017). As yet, however, there is little to no evidence of their efficacy in HFmrEF.

Echocardiographic parameters appear to have been understudied in HFmrEF, particularly concerning novel parameters of ventricular systolic function. Studies in Chinese patients first demonstrated significant differences in ventricular geometry between groups when stratified according to LVEF groups which approximate the new categories. They showed significant differences between groups in both Doppler and geometrical parameters but were limited by the size of their study, recruiting only 284 patients of whom 38 were within the group LVEF 40-55% (He, Burkhoff et al. 2009). Cardiac geometry was studied in greater depth in CHF patients using data from the TIME-CHF study. These data demonstrated a progressive increase in LV dimensions with a falling LVEF, decreased wall thickness in HFrEF and suggested that concentric remodelling is seen preferentially in HFpEF compared to eccentric remodelling in HFrEF (Rickenbacher, Kaufmann, Maeder, Bernheim, Goetschalckx, Pfister, Pfisterer, and Rocca 2017).

In a study looking at exercise tolerance in stable CHF, myocardial deformation parameters have been shown to be statistically correlated with performance, but due to limited numbers the group were unable to demonstrate differences between LVEF subgroups (Tsougos, Angelidis et al. 2017).

The only notable study of AHF myocardial deformation parameters was undertaken by Park et al. Their study of 4,172 consecutive AHF patients suggested that strain imaging is more accurate than LVEF in prognostication for HF (Park, Park et al. 2018). This research is limited by their exclusion of HF patients with an ischaemic origin, an important omission given that this is a large proportion of the total HF burden cited as nearly a third of patients in the EuroHeart Survey II (Nieminen, Brutsaert et al. 2006). They also only collected all-cause mortality data, and thus did not assess the utility of GLS to predict cardiovascular mortality.

As described, there has been little assessment and characterisation of parameters of myocardial deformation in AHF and specifically between the new HF subcategories. This is, no doubt, in part due to the novelty of the recategorisation, also due to the lack of viable images from older registry studies. This is an important gap in contemporary knowledge; LVEF may not remain the definitive marker of HF categorisation due to its well-understood imprecision and the arbitrary nature of the values used to subcategorise patients, thus parameters of systolic function less dependent upon haemodynamic variation are likely to add to the ability to effectively define these groups. Specifically, given the large inter-observer variability of LVEF measurements and imprecision when assessing patients with regional wall motion abnormalities, the addition of an adjunctive parameter which is not similarly susceptible may offer an improved ability to clearly delineate and categorise these subgroups.

Many of the studies looking at this patient subgroup have been, understandably, retrospective registry-based cohort studies. As a result, they can be prone to selection biases due to a heavily reliance on accurate recordkeeping of the involved parties. Unfortunately studies of hospital episode statistic (HES) data have demonstrated that accurate documentation of primary diagnosis has been as low as 76% (Burns, Rigby et al. 2011)while appropriate documentation of HF diagnosis has been shown to be worryingly inaccurate (Goff, Pandey et al. 2000) and assessment of large registers has demonstrated significant underreporting of HF diagnoses(Kümler, Gislason et al. 2008). These factors, when combined, make it more difficult for large scale retrospective registries to include the relevant population in their datasets. Despite this, registry studies still represent very large datasets from which useful, statistically powerful conclusions can be derived, however large single-centre trials offer benefits in terms of homogeneity as discussed above and can be used to validate registry data.

The majority of previous studies into this subgroup are derived from large multi-centre registries. While this is immensely beneficial in the accrual of greater patient numbers, this markedly reduces the homogeneity of the data collected due to intrinsic variations in local practice.

Aside from the variation inherent in multi-centre trials, these trials also introduce variation in terms of sonographical practice. As discussed above, echocardiography suffers from significant inter-observer variation, dependent upon the skill, experience and equipment of the sonographer. Local echocardiography protocols also vary leading to variations in data collection and reporting practices.

Given the growing numerical and financial inpatient burden of AHF it is important to focus on underlying pathological differences in HF subcategories in an effort to improve the therapeutic options.

1.3.2 Prognostication in AHF – current literature and gaps in the evidence

The ability to effectively estimate prognosis in terms of readmission rates and mortality in any disease group is essential. Good prognostic information helps patients to develop realistic expectations of their disease course, aids in self-management and future planning as well as helping them to understand why medical professionals intervene with therapeutic options. For the physician it is similarly vital, allowing for evidence-based planning with regards to therapeutic interventions, intensity of treatment, as well as allowing for the appropriate organisation of health and social resources at a local or state level.

Tools to aid with prognostication in CHF have been developed over the last 20 years with multiple validated tools in existence for use in ambulatory patients.

Initially, small studies such as that conducted by Aaronson et al. demonstrated the possibility of risk stratifying and prognosticating in the ambulatory HF group using invasive parameters such as peak oxygen consumption and mean pulmonary capillary wedge pressure and non-invasive parameters such as aetiology, clinical observations, LVEF and biochemistry (Aaronson, Schwartz et al. 1997). These data were derived from a small cohort of only 268 patients, solely selected from a group of patients with advanced HF being considered for heart transplant, but it demonstrated the possibility and value of prognostication.

Lucas et al. demonstrated the utility of clinical findings in prediction of outcome, showing that freedom from clinical congestion conferred a beneficial prognosis upon patients, but did not use this in any formal prediction model. Theirs was again a small patient cohort of 146 taken exclusively from an outpatient setting (Lucas, Johnson et al. 2000).

Pocock et al. advanced the concept, constructing a prognostic model in chronic heart failure in both HFrEF and HFpEF. This was created using data from the CHARM programme with 7599 patients, a sizeable CHF cohort, and produced a multivariable model for prediction of 2-year mortality or HF readmission. This model used a large variety of clinical and comorbid criteria, including over 20 variables, while also using a complex algorithm to approximate mortality and readmission risks (Pocock, Wang et al. 2005).

This model was improved upon by Levy et al. who created the Seattle model of risk prediction. They developed an online tool based on a prognostic model derived from 1125 CHF patients followed up to 3 years post clinical evaluation. They produced an online model which allowed for the automatic calculation of risk profile by the clinician, markedly simplifying the process of risk stratification. They used a similar mix of clinical variables to produce their model, using a multi-variate Cox regression method as had been used in previous studies (Levy, W. C., Mozaffarian et al. 2006).

Lupón et al. created a similar model with a large population but using additional novel biomarkers which had come to prominence in the mid-2000s (Lupón, de Antonio et al. 2013). This, amongst a range of other clinical and biochemical parameters allowed them to produce a comprehensive mortality and readmission scoring scale for use in outpatient heart failure. It does require a large quantity of data to use and can be time consuming for the physician, but again this group produced an online calculator for ease of use, and their data advanced the ability to prognosticate in the CHF population.

These studies and their models have provided useful long-term mortality risk prediction models for patients with CHF. Practical models for use in AHF have remained elusive, with different gaps existing in both knowledge and application.

In a similar fashion to those described above, Lee et al. used a cohort of 2624 from the EFFECT study to produce a novel model for prognostication of AHF patients at 30 days and 1-year post admission, using variables easily acquired by the hospital physician. They

selected 11 variables which are added to produce an overall score corresponding to overall risk. Similar to models described above, their scoring scale requires relatively complex mathematical calculation and reference to risk score tables to assess risk for the individual patient but it is a useful initial tool in the AHF population (Lee, Austin et al. 2003).

Similar efforts have been produced by Fonarow et al. using a large AHF inpatient cohort of 33,046 patients (Fonarow, Adams et al. 2005). This group used their data to instead perform a classification and regression tree (CART) analysis to describe inpatient mortality only. CART is perhaps slightly better suited to clinical decision making but requires direct access to the tool for use as it again would otherwise require large amounts of complex data to be remembered by the acute physician at the patient's bedside.

Komajda et al. created a risk stratifying tool using readily available bedside parameters but have described risk stratification up to 3-year mortality in HFpEF only (Komajda, Carson et al. 2011). While this is a useful tool, it is, by definition, restricted to its target population, a population which is now obsolete in view of new guideline definitions regarding HFmrEF.

Okazaki et al assessed a more broadly applicable risk scoring tool, using bedside parameters only in the APACHE-HF, APACHE II and Modified APACHE II scoring systems (Okazaki, Shirakabe et al. 2014). In their single-centre study they collected data from 824 patients admitted to the intensive care unit in their centre and derived a scoring system for risk stratification of in-hospital mortality. The most substantial disadvantage of the APACHE II and Modified APACHE II risk-prediction tools are their complexity, both in memorising and in application of the scoring systems. The APACHE II score uses 17 variables rendering it complex to use in the clinical setting, Modified APACHE II uses only 7 variables but each variable can confer up to 6 points to the cumulative risk score rendering it complex to calculate in the acute clinical setting and the much simpler APACHE score uses non-standard variables values which are thus much more difficult to retain and transfer into use (Brod, Werkle-Bergner et al. 2013). In addition, the C-statistic of both the APACHE II and Modified APACHE II scores are <0.600 which would lead to both being standardly classified as Poor or Worthless tests (Obuchowski 2003).

Data from the OPTIMIZE-HF trial were used to produce a more simplified model predicting mortality at 60-90 days post discharge. The model produced uses only 8 variables, all easily acquired in the acute setting, though allocation of points to each variable is complex as each

variable is associated with a range of possible scores dependent on the value of the variable. As with previous scoring systems this renders it difficult to calculate manually in the acute setting without recourse to the scoring tool itself, and interpretation of the sum of these scores requires access to a nomogram to determine risk. The nomogram is actually very useful, allowing the clinician to give more accurate predictions about risk to each individual patient rather than allocate them a broad risk of low, medium or high as is done in many other studies. It does, however, preclude the use of this tool in a setting in which you do not have access to a visual or tabulated version of the nomogram (O'connor, Abraham et al. 2008).

Similarly, the ELAN-HF score provides a relatively simple risk score with only 8 variables, utilisation of NT-pro BNP levels and a C-statistic of 0.78, but they use certain variables only available at discharge. This does not preclude the use of the tool but restricts its application to the post-clinical stabilisation phase (Salah, Kok et al. 2014).

The ESCAPE discharge score has the same disadvantage of using variables only available at discharge (though predicting risk at discharge is acknowledged as the explicit intent of the study), while it also includes less-commonly performed tests such as the 6-minute walk test and requires a discharge BNP level which is not always performed (O'Connor, Hasselblad et al. 2010).

As can be seen from the review of current risk-prediction tools, there are notable problems or disadvantages in each. Firstly, the majority of these tools are derived from large retrospective registry studies. As described above in relation to the HFmrEF recategorisation, there are difficulties intrinsic to this study design. Specifically, they often do not characterise the whole burden of HF due to an inability to detect all HF patients. This can particularly stem from failures of misdiagnosis or detection. When predicting outcome of HF patients, if models are created from an incomplete patient cohort it is unlikely to be fully representative of true HF patients.

Secondly, the majority of current tools are simply too cumbersome to use for the acute physician at the bedside. Tools such as CURB65 and CHA_2DS_2-VaSc are routinely used in part due to their ease of use (Blatchford, Murray et al. 2000, Lim, W. S., van der Eerden et al. 2003). In his seminal work on educational theory Miller posited that information is best learnt and retained in units of 7 +/- 2 individual ‘chunks’ (Miller 1956) and is a theory which has subsequently been validated by experimental models (Shiffrin, Nosofsky 1994). This

correlates with contemporaneous predictive risk stratifying tools in pneumonia, atrial fibrillation and gastrointestinal bleeds. The tools produced and described above for both CHF and AHF all require retention of large volumes of information, substantially in excess of 9 variables, and, as such, are understandably difficult to use at the bedside without access to visual reminders of the tool, or access to computer-based calculation tools. The authors of the Seattle study comment that 'The calculation of estimated survival included 14 continuous variables and 10 categorical values, making it impractical for computation by hand' (Levy, W. C., Mozaffarian et al. 2006), and this remains a common problem for the majority of risk scoring models in AHF.

Those that do require fewer variables use a composite endpoint of mortality or rehospitalisation. While this is useful information for the clinician, they are very different endpoints from the point of view of the patient. The use of this composite endpoint in the study by Xanthopoulos et al appears driven by the relatively limited number of patients within their model derivation cohort, and thus the requirement to increase the number of potential 'events' to be able to satisfactorily derive and validate their model (Xanthopoulos, Giamouzis et al. 2017).

Some of the models which are simpler in their application, such as those derived from the ELAN and ESCAPE trials, use data only available at discharge which preclude the use of the tool at admission, where it could otherwise be used to make useful predictions regarding need for admission and intensity of inpatient therapy due to post-discharge mortality risk.

Lastly, none of the current models in AHF use echocardiographic information to improve their models. Considering that both European and American guidelines recommend prompt echocardiography in all patients admitted with AHF it is likely that this information would be available for a large proportion of patients early within the course of their admission. It may be that echocardiographic parameters offer significant additional predictive power and this is something that should at least be assessed.

In short, the production of a novel predictive tool - one derived from a real life AHF patient cohort, which predicts mortality and readmission rates, and which is easily memorisable and usable by the acute physician - would be of considerable benefit both to those at the hospital front door and those further along the path of the patient journey.

1.4 Study synopsis & Hypotheses

This study uses data from a large, consecutively recruited real-life patient cohort capturing the entire range of acute inpatient HF patients, both those with and without a formal diagnosis of AHF. In particular, efforts have been made to ensure homogeneity of recruitment and echocardiographic practice and interpretation.

This study will look specifically at whether strain and strain rate values derived from the LV align with LVEF categories.

This study hypothesises that left ventricular strain and strain rate values will vary according to subcategory, as defined by LVEF.

This study will also assess whether a predictive risk-scoring tool can be produced to improve the ability to prognosticate in a truly representative AHF cohort in terms of short to mid-term mortality.

This study hypothesises that a simple, valid, tool to stratify 6-month mortality risk in AHF can be produced using only data available to the clinician in the acute phase of admission for AHF.

1.5 Chapter summary & Thesis structure

This chapter has discussed the current understanding of HF as a clinical syndrome and outlined the current approach to assessment, diagnosis and management. It has also described the current morbidity and mortality profile associated with a diagnosis of HF. It has then discussed current gaps in contemporary understanding of two subsections of the above, namely current HF categorisations and prognostication in AHF.

To summarise, further information regarding echocardiographic parameters of novel HF subcategories is likely to offer further information to help clarify underlying pathologies.

Furthermore, there are gaps in ability to prognosticate in the AHF population that can be clarified, specifically for the ability to produce a genuinely usable prognostic tool during the acute phase of the hospital admission.

The first results chapter of the thesis will outline the baseline characteristics of the MRAHF study cohort and compare this to an existing large AHF registry cohort, the second will assess

the differences in myocardial deformation imaging values between heart failure subcategories and the third will assess prediction of mortality in AHF using variables easily acquired in the acute phase of a hospital admission.

To do so, data have been collected from consecutive AHF patients admitted to a single-centre hospital site. Data collected includes a variety of demographic, clinical, biochemical, interventional and echocardiographic information. Echocardiographic data will be compared against current HF categorisation subgroups. Post-hoc data collection has been performed to assess outcomes at 6-months post recruitment. This will allow for assessment of outcome and for production of predictive models.

The following chapter will describe the materials and methods used in the design and implementation of the study, as well as the statistical analyses performed to demonstrate statistical significance of the acquired data.

Chapter Two: Study Design, Methods and Materials

2.1 Chapter introduction

This chapter describes the study design and methodology. The chapter is divided into five sections: The first describes the parent study from which this thesis is derived, the second clarifies the overriding aims of the study as outlined in the introductory chapter, the third describes the methods used to achieve the study aims, the fourth describes the materials required to achieve the study aims and the fifth summarises what has been described within the chapter.

2.2 The MRAHF Cohort

The participants included in this study were recruited as part of a trial entitled 'Mitral Regurgitation in Acute Heart Failure' (MRAHF), identifier NCT02728739. This is a single-centre prospective cohort study of consecutive acute heart failure (AHF) patients with a specific focus on the effect of mitral regurgitation in AHF in terms of economic burden and prognostic impact.

This thesis is derived from the data acquired as part of the MRAHF trial and the methods and materials described in this chapter focus on the recruitment, data collection and analysis required to meet the aims of this thesis. Outcome data are still being collected as part of the MRAHF trial, but 6-month follow-up data are available for all patients recruited within the trial and are included in this thesis.

2.3 Thesis Aim and Hypothesis

This thesis aims to study the AHF population of a typical district general hospital in the United Kingdom. In doing so, the main foci are twofold:

Firstly, with reference to the recent European Society of Cardiology guidelines call for data, the study aims to characterise and elucidate the differences present within and between the new categories of heart failure (HF), namely heart failure with preserved ejection fraction (HFpEF), heart failure with mid-range ejection fraction (HFmrEF) and heart failure with reduced ejection fraction (HFrEF). Specifically, it aims to look at distinguishing echocardiographic features between these groups in terms of myocardial deformation

imaging. This study hypothesises that left ventricular strain and strain rate values will vary according to subcategory, as defined by LVEF.

Secondly, the study aims to elucidate clinical parameters, available to the typical medical practitioner within the first day of patient admission, that can be used to estimate risk of mortality at 6 months post discharge. In doing so it aims to provide information regarding factors which can better inform the practitioner and patient about medium term prognosis. This can help to guide intensification or relaxation of medical or interventional management. This study hypothesises that a simple, valid, tool to stratify 6-month mortality risk in AHF can be produced using only data available to the clinician in the acute phase of admission for AHF.

2.4 Methods

The following section describes the methods used to achieve the study aims. It will cover the process of study design, and then the practical logistics of the screening and recruitment processes as well as data capture and data management.

2.4.1 Study design

This study was completed using data from the MRAHF study. The MRAHF study was designed as a prospective cohort study of consecutive patients admitted to a single hospital centre with AHF as the presenting complaint over a period of one year. As outlined in chapter one, prospective consecutive studies reduce study selection bias and are recognised as the optimum study design for assessing and producing clinical risk scores, specifically as it allows the trial team to ensure complete data collection at the time of enrolment (Han, Song et al. 2016). It also helps to produce a study cohort that is as true to real-life AHF populations as possible. This enables us to more confidently use the data to describe differences within that cohort as applicable to real-world scenarios, or to make predictions about future scenarios with AHF patients.

While multi-centre studies are able to provide greater numbers of patients, and therefore often statistically more powerful results, a single centre offers the ability to reduce study variability. By reducing variables such as echocardiography technique, recruitment intensity and data collection, inherent variability in the study can be substantially reduced.

In the four calendar years preceding the study, the number of HF patients admitted to the single hospital site selected numbered 357, 370, 412 and 460 respectively. These data were sourced from HES coding data, which are extracted from discharge summaries. Reflecting the gradual growth of HF admissions by approximately 30-40 patients per year, a final recruitment target of 500 patients was set.

Ethical approval was received from the NHS Research Ethics Committee (REC) North of Scotland 2 with a letter of Favourable Opinion received on 28/04/2016. REC reference for this is 16/NS/0047. IRAS project ID is 194815 and Protocol number is MHL-2016-001. Ethical approval from the HRA was not applicable for the study as it was to be performed at a single hospital site with an existing research contract in place.

Future project applications wishing to access the study data will be assessed and will have to follow strict Medical Research Council ethical guidelines (MRC working group 2012).

2.4.2 Study population and sampling

Patients were screened and recruited at the sole participating clinical site, a large district general hospital in the south of England. The study population includes patients from the surrounding catchment area which encompasses a population of over 410,000 people.

Sampling of a specific subset of the population in question is typically performed to ensure a representative or reproducible cohort. This can be performed via a multitude of means, including, but not limited to, random, systematic, stratified and cluster sampling. These methods attempt to sample a statistical population in order to avoid the necessity of recruiting the entire population of interest. In order to ensure true population representation this study has opted for systematic sampling. While this is time-consuming, it guarantees that the population data collected is truly representative as it is exhaustive. Consecutive recruitment was ensured by screening daily throughout the recruitment period including weekends, bank holidays and holiday seasons.

2.4.3 Inclusion and exclusion

Inclusion criteria for the study:

1) Clinical signs and symptoms consistent with AHF as the primary cause for admission
2) Inpatient admission of <7 days by time of consent

3) The ability to give informed consent

Exclusion criteria for the study:

1) Point-of-care test (POCT) B natriuretic peptide (BNP) level <100 pg/ml
2) Echocardiography inconsistent with a diagnosis of HF

Clinical criteria for diagnosis are based on guidance from the European Society of Cardiology (ESC) (Ponikowski, P., Voors et al. 2016) and signs and symptoms consistent with AHF is a clinical judgement as described in the first chapter (see Table 1.1). The study aimed to identify all consecutive patients admitted with AHF as their primary complaint. For the purposes of the study AHF was defined as rapid onset or worsening of symptoms of HF requiring non-elective admission to hospital for investigation or treatment as per the ESC HF guidelines (Ponikowski, P., Voors et al. 2016) .

Any patients admitted with AHF as a concomitant issue but not the primary cause for admission were not considered for recruitment. CHF is relatively common in the elderly population, a population who account for the majority of hospital inpatients. As such many patients will be admitted with a concomitant diagnosis of HF which is unrelated to their current admissions and thus does not reflect decompensated AHF.

To reduce heterogeneity of the echocardiographic dataset, patients were required to be recruited within a standardised timeframe, preferably as close to admission as possible and excluded if time since admission exceeded 6 days. POCT BNP assay was used rather than laboratory based biochemical assay to improve speed of assessment. While formal laboratory assays of BNP levels are returned to the requesting physician in approximately one to two hours, POCT for BNP can be performed by the recruiting physician in less than a quarter of the time. This increases the efficiency of the recruitment process with minimal loss of diagnostic accuracy; when tested against laboratory analysers, linear regression analysis demonstrated that the i-STAT POCT BNP analyser has a correlation coefficient of 0.997 compared to the laboratory values (Shah, Terracciano et al. 2010). BNP level of <100 pg/ml was selected as the exclusion level as data from the manufacturer of the POCT analyser notes that in their disease-free sample population, physiological levels were <100 pg/ml in 90% of patients (see Appendix 1.1). This also reflects ESC guidance which recommends a using BNP levels of <100 pg/ml as a rule-out screening tool for diagnosis of AHF (Ponikowski, P., Voors et al. 2016). In comparison with other POCT devices, the Abbott i-STAT has been

found to offer significant improvements in accuracy and speed of data availability, while it correlates with laboratory values. It is more costly than other POCT devices and laboratory methods, but this was not considered a barrier in the administration of this study (Shah, Terracciano et al. 2010).

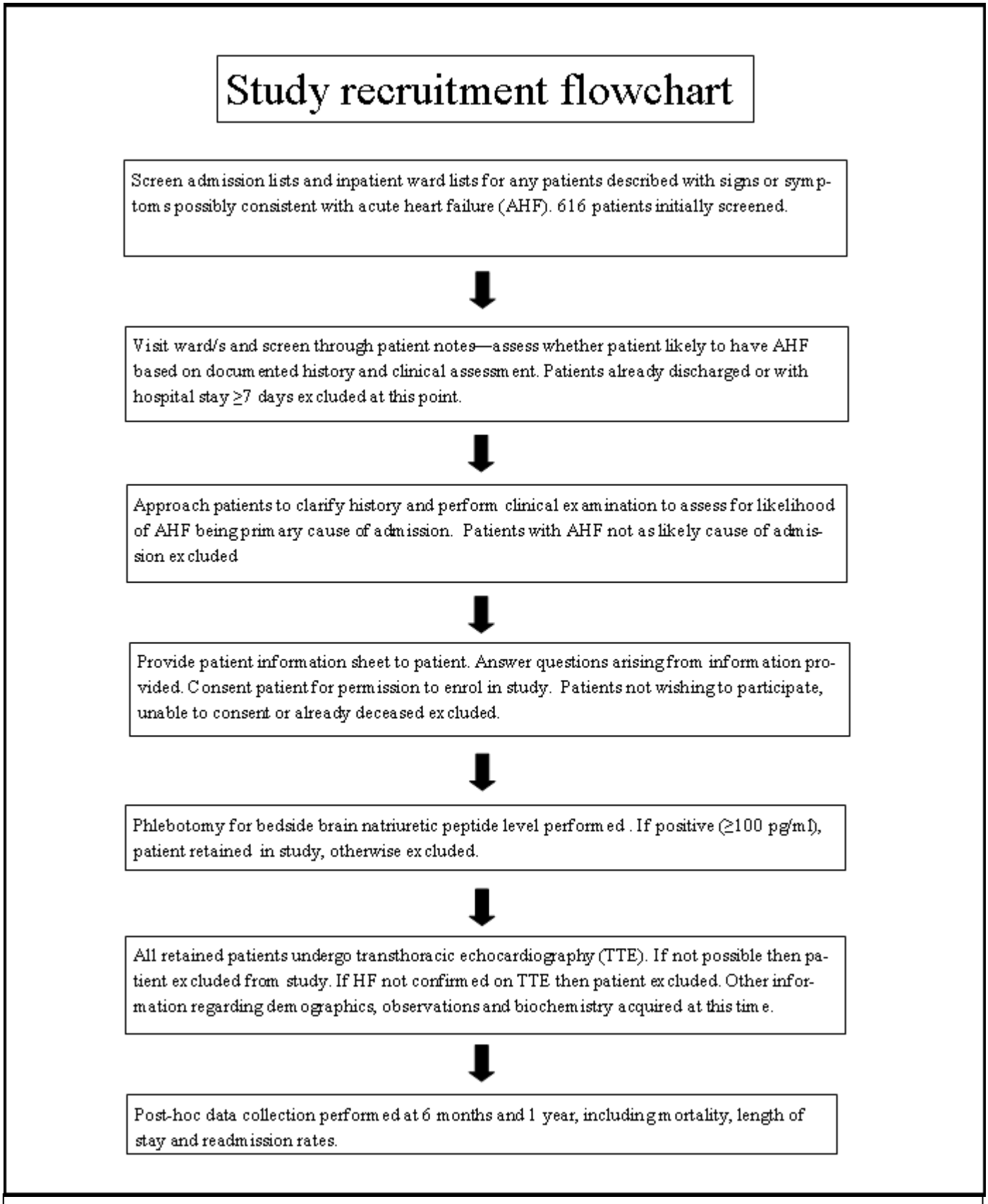

Figure 2.1 – Study Flowchart demonstrating the process of screening and recruitment to the MRAHF trial.

2.4.4 Ethical considerations

Informed written consent was obtained from all patients recruited to the study prior to any investigations being performed or data collected. If the patient was unable to give consent due to lack of capacity or writing difficulties, then next of kin were consulted for written consent. If this was not possible then patients were excluded from recruitment.

Necessarily, patients were often approached for recruitment very shortly after admission. To facilitate true informed consent patients were given as much time as they wished with the patient information sheet and all questions that arose were answered where possible. Relatives were encouraged to be part of the discussion. In doing so the patient was offered as much information as possible to make an informed decision on their involvement. Where requested, patients were given time overnight to await family members to discuss the decision and alleviate any specific concerns. This allowed for greater family engagement and patients were thus typically keener to engage with the study.

Where language barriers were an issue, formal translators were requested, and where not possible, other members of hospital clinical staff were asked for their assistance in conveying the required information. Use of family members was avoided where possible. This was done to aid patient understanding and informed consent, while non-clinical staff were not used in order to avoid errors of clinical mistranslation and thus misleading the patient.

Each patient enrolled in the trial was allocated a study number for use in data analysis. This enabled anonymization of all data and ensured confidentiality of patient details. A reference file was maintained on two secure hard-drives obtained from Western Digital as described below in the materials section. This reference file allows matching of patient identifiable details to their study number in case this is required at a later date. As the trial intended to look at outcomes in all AHF patients under contemporary conditions, biochemical results and information obtained via echocardiography performed as part of the study were not revealed to either patient or the clinical team unless either test suggested life-endangering or life-altering pathology. This was done to ensure that clinical practice remained as close to normal as possible and that the study itself did not influence outcomes.

If there were concerns regarding pathology identified on research echocardiography that was not already known by clinicians, these cases were discussed with the academic and clinical supervisors to advise on when and how to reveal this data to the clinical team.

2.4.5 Screening Process

Screening began in July 2016 and study recruitment ended in September 2017. During this period all patients admitted to the hospital were screened for potential recruitment to the study. Screening occurred daily, including weekends and holidays, throughout this period to ensure complete capture of the dataset.

Screening was performed by using a range of computer-based tools. Two key pieces of hospital software were accessed each morning to search for patients likely to meet the inclusion criteria. The first, Inpatient Lists (IPL), is a bespoke piece of software that allows tracking of any patient admitted to the hospital. Within this software is a subsection which allows for viewing of all patients admitted to the hospital through the medical team in a 24-hour period, including their presenting complaint. With this tool patients could be identified and tracked within the hospital for further screening (see Figure 2.2). The second, RealTime, is a bespoke piece of software which displays current bed occupancy throughout the hospital with accompanying clinical details. Any patient whose digitally-recorded clinical information on RealTime described signs or symptoms consistent with heart failure were noted and considered for further screening (see Figure 2.3).

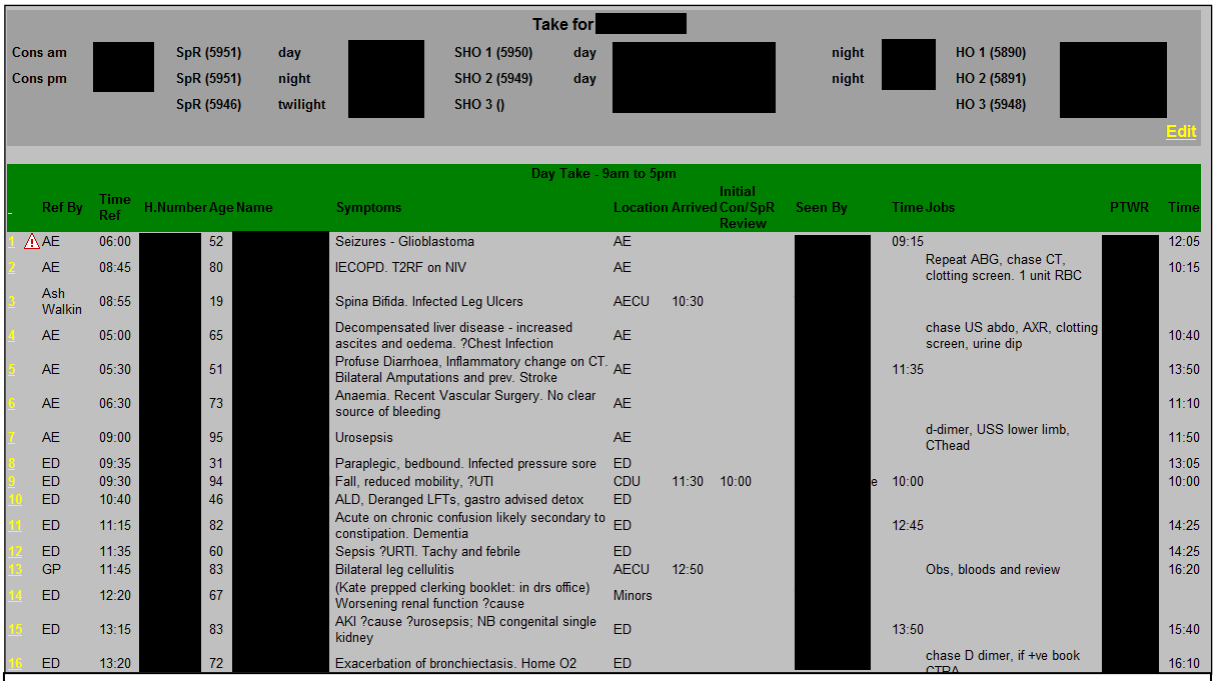

Figure 2.2 – Screenshot from Inpatient lists software, medical admissions screen. From this screen one can look at the details of patients admitted to hospital and screen for signs and symptoms related to AHF.

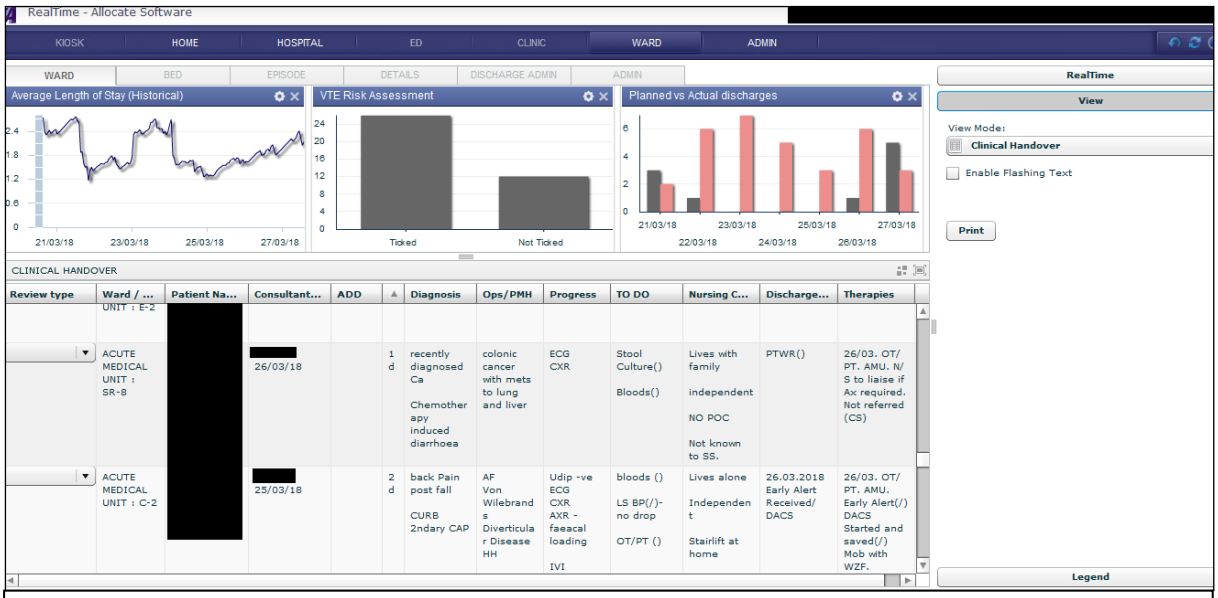

Figure 2.3 – Screenshot taken from RealTime clinical software. This demonstrates the ability to locate patients and screen clinical symptoms and provisional diagnosis.

Documented clinical notes for each identified patient were examined. If signs and symptoms documented within suggested a primary diagnosis of AHF then patient was approached to clarify signs and symptoms via clinical history and examination.

Patients with HF clearly as a concomitant problem rather than primary cause of admission were excluded from the study. An example of this from the study would include a patient admitted due to fracture of the femur who, post-surgery, developed clinical signs and symptoms of HF. As this was not the primary admitting pathology they were not considered for recruitment.

Those patients who had been discharged prior to assessment or recruitment were documented in the screening list. This typically occurred if patients were admitted and discharged within a 24-hour period. The total numbers of patients who were suitable for screening but who declined assessment or enrolment were noted for completeness and no further data were recorded.

Patients with signs and symptoms consistent with AHF as the cause of their admission were provided with patient information sheet version 1.1 25th April 2016 for their consideration (see Appendix 1.2). After reading this and answering any questions that had arisen from the information provided, they were then then asked for their consent to recruitment into the study. If they agreed then written consent was obtained formally using patient consent form 1.1 25th April 2016 (see Appendix 1.3). Recruited patients then underwent phlebotomy for a POCT BNP level. All patients underwent POCT BNP only, with no formal lab BNP used throughout the study in order to maintain homogeneity.

POCT BNP tests were used to increase the speed of screening and efficiency of echocardiography; formal laboratory values for BNP are typically returned between 1 and 2 hours after sampling at the clinical site. If this had been used then the maximum number of patients screened per day would have been approximately 5, limiting the number of patients possibly recruited each day and negatively impacting upon the feasibility of truly recruiting all consecutive patients.

POCT BNP and formal laboratory BNP assay values correlate well (Shah, Terracciano et al. 2010). As such, formal inpatient biochemistry values were not reviewed prior to assessment of the patient and all documented BNP values were taken from POCT BNP assays.

Bedside BNP was measured using the i-STAT BNP monitor (Abbott POCT ltd). Regular calibration of this device was performed as advised by the user guide (see Appendix 1.4). To perform a POCT BNP assay, a sample of peripheral venous blood is taken via phlebotomy,

transferred directly onto the i-STAT BNP cartridge and inserted into the i-STAT BNP analyser. The test runs for 10 minutes and provides the user with a point-of-care BNP level. The BNP range possible using the device is 15-5000 ng/L. Outside of this range it either provides the result '<15 pg/mL' or '>5000 pg/mL'.

Patients with a POCT BNP level <100 pg/ml underwent no further investigations and were informed of their exclusion from the study.

2.4.6 Admission data collection

Patients enrolled in the study with a BNP level ≥100 pg/ml, underwent clinical assessment. Clinical characteristics data were collected using a standard data collection form. This was subsequently entered to a password-protected online database. Data variables collected are outlined in Table 2.1 below:

Demographic data	Biochemistry data	Previous admissions data	Initial investigations
Date of Birth (dd/mm/yyyy) Sex Ethnicity Date of admission (dd/mm/yyyy)	Haemoglobin level (g/L) Estimated glomerular filtration rate (ml/min) Creatinine level (µmol/L) Sodium level (mmol/L) Potassium level (mmol/L) White cell count level (10^9/L) Urea level (mmol/L) C-reactive Protein level (mg/L)	Admissions of any cause at 1,2 and 3 years Admissions due to heart failure at 1,2 and 3 years prior to index admission Length of previous hospital stay Length of stay by consent for study	ECG – rate (bpm), rhythm, QRS duration (ms) CXR – Orientation, cardiac width (cm), thoracic width (cm), radiologist report

Clinical data	Comorbidity data	Community pharmacotherapeutic use on admission (true/false)
Symptoms leading to admission Aetiology of HF Height (m) and Weight (kg) on admission Non-invasive systolic and diastolic blood pressure on admission (mmHg) Heart rate on admission (bpm) Non-invasive blood oxygen saturation at admission (%) Presence or absence of cardiac murmur on admission assessed by the research team Presence or absence of cardiac murmur as documented by the admitting team AMTS on admission Oxygen device requirements on admission Venous blood gas pH and lactate on admission	Heart Failure Mitral Regurgitation Hypertension Diabetes Mellitus Chronic Kidney Disease Current smoking status Chronic obstructive pulmonary disease Cerebrovascular accident Dementia	Compliance with medications ACEi ARB ARNI BB MRA Nitrate-based vasodilators CCB Digoxin Diuretic Selective sinus node inhibitors

Table 2.1 – Data parameters collected from patient admission records

ACEi – Angiotensin Converting Enzyme inhibitor, AMTS – Abbreviated mental test score, ARB – Angiotensin Receptor Blocker, ARNI – Angiotensin Receptor Blocker and Neprolysin Inhibitor, BB – Beta Blocker, CCB – Calcium Channel Blocker, CXR – Chest x-ray, ECG – Electrocardiogram, MRA – Mineralocorticoid Receptor Antagonist.

All demographic data were recorded as they had been documented in hospital patient records and clarified with the patient.

All clinical data were recorded as documented by clinical staff. Presence of a cardiac murmur was assessed by the research team prior to recording documented auscultation by the clinical team. This avoided their interpretation biasing the research assessment.

Aetiology of HF was defined using the CHAMP aetiology headings as outlined by ESC guidelines (Ponikowski, P., Voors et al. 2016) - acute coronary syndrome (C), hypertensive crises (H), arrhythmia (A), mechanical (M), or pulmonary disease (P). Two additional categories were used, namely Medication Withdrawal (MW) and Unknown Aetiology (U). Decisions regarding aetiology were taken by the recruiting clinicians' assessment using biochemical, radiological and echocardiographic information. Where there was doubt or concern over aetiology this was discussed with the other research fellow and PI, a consultant cardiologist, to determine likely aetiology according to clinical, biochemical and radiological evidence available both upon and prior to admission to hospital. Where no evidence of a specific underlying cause was evident then patients were categorised as Unknown Aetiology.

Biochemical data were taken from the earliest available biochemical results at admission. eGFR was calculated using the abbreviated modified diet in renal disease equation.

Comorbidity profile was recorded according to either patient self-reporting, documentation of comorbidity in their medical notes or in previous discharge summaries. Documentation of chronic kidney disease (CKD) is often unclear so this was recorded as positive as per NICE guidelines (National Institute for Health and Care Excellence 2014).

Admissions data and length of stay were sourced from electronic discharge summaries recorded on the IPL software.

Pharmacological use on admission and compliance data were both recorded as reported by patients and/or medicines reconciliation information routinely collected and documented by pharmacy staff.

Electrocardiographic (ECG) data were extracted from ECG studies routinely performed by qualified hospital staff. Separate analysis of the ECG was performed, and results documented. CXR report from the reporting radiologist was printed and retained, either at the time of recruitment or retrospectively if not available. Orientation of X-ray image was documented. Cardiac shadow width, thoracic width and cardiothoracic ratio were measured and calculated as described by Chana et al (Chana, Martin et al. 2015).

Bedside transthoracic echocardiography (TTE) was then performed within 2 hours of recruitment by one of 3 ultrasonographers accredited by the British Society of Echocardiography (BSE). This was performed according to a standardised image acquisition protocol (see Appendix 1.5). The primary sonographer underwent re-accreditation during the study period to demonstrate ongoing proficiency at a standard recognised by the BSE.

2.4.7 Offline and post-discharge data collection

Further data were collected after recruitment was completed and all enrolled patients had been discharged from the hospital. Data variables collected are outlined in Table 2.2 below:

Outcome data	Admission data	Echocardiographic data
Date of discharge (dd/mm/yyyy) Length of stay (days) Number of readmissions at 1, 3 and 6 months: Total, Cardiovascular disease and HF Mortality at index admission, 1, 3 and 6 months Cause of mortality Time from discharge to death (days) Medication prescription on discharge – ACEi, ARB, ARNI, BB, MRA, nitrate-based vasodilators, CCB, Digoxin or diuretic	Diagnosis of a cardiovascular disease Diagnosis of acute heart failure on admission Cardiology review on index admission Echocardiography performed on index admission	LV geometry – LV end diastolic diameter (cm), LV end systolic diameter (cm), LV end diastolic volume (ml), LV end systolic volume (ml), LVEF (%) Transmitral flow – LV dp/dt, E (cm/s), A (cm/s), E/A ratio Tissue Doppler – E' peak velocity (cm/s), S' peak velocity (cm/s) RV geometry – RV diastolic area (cm^2), RV systolic area (cm^2), RVFAC Mitral and tricuspid valve geometry – RV basal diameter (cm), tricuspid annular diameter (cm), mitral valve annular diameter (cm), mitral valve commissural annular diameter (cm) Severity of mitral regurgitation Severity of tricuspid regurgitation Systolic pulmonary artery pressure (mmHg) Left ventricular strain (%) and strain rate (s^{-1})

Table 2.2 – List of variables collected post-discharge and via offline analysis of echocardiography. ACEi – Angiotensin Converting Enzyme inhibitor, ARB – Angiotensin Receptor Blocker, ARNI – Angiotensin Receptor Blocker and Neprolysin Inhibitor, BB – Beta Blocker, CCB – Calcium Channel Blocker, LV – Left ventricle, LVEF – Left ventricular ejection fraction, MRA – Mineralocorticoid Receptor Antagonist, RV – Right ventricle, RVFAC – Right ventricular fractional area change.

Length of stay data were obtained from the IPL software and calculated including both the day of admission and discharge.

Readmission data were taken from the IPL lists. For the purposes of this study, CVD is defined as diseases including coronary heart disease (CHD), cerebrovascular accident (CVA), peripheral arterial disease, rheumatic heart disease, congenital heart disease, deep vein thrombosis, valvular heart disease, inflammatory heart disease and cardiac arrhythmias.

Mortality data were acquired via summary care records online system. Cause of death 1a, 1b and 2 were taken from death certificates. These were accessed either through the hospital bereavement service for inpatient deaths, general practice records where available or local council records.

Diagnosis data were taken from admission clerking notes as documented by either registrar or consultant, whichever most senior doctor saw the patient on admission. Cardiology review is defined as documented evidence of review by cardiology registrar or a more senior cardiologist. Evidence of echocardiography during admission was taken from records of echocardiography documented on the IntelliSpace® picture archiving and communication software (PACS) enterprise software. Discharge medication lists were taken from digital discharge summary accessed via IPL.

All echocardiographic parameters were measured according to BSE recommendations.

Myocardial deformation measurements (strain and strain rate) were made using echocardiography derived tissue speckle tracking analysis. As noted earlier a typical echo protocol was used with a specific focus on the mitral valve (see appendix 1.4).

2.4.8 Myocardial deformation analysis

Myocardial deformation analysis of the left ventricle was performed using EchoPac software. Each patient had deformation imaging assessed in the 4, 2 and 3 chamber views.

Mechanical systole was timed according to aortic valve opening and closure, assessed using pulse wave Doppler.

Analysis was considered feasible if the patient had more than 2 segments available for analysis. Endocardial border tracing was performed, and the software automatically tracks speckles within the echo image to derive strain and strain rate values for 6 segments in each view.

Deformation analysis was performed in each view and global longitudinal strain and strain rate was derived by the software.

2.4.9 Data management

The data recorded from both the data record sheet and the echocardiography were uploaded to a secure online database maintained by an independent outsourced company, Metanoic Health Limited, UK. Copies of the ECG from admission as well as the report of chest radiography on admission were digitally scanned and uploaded to the online database.

Data were exported each month to Microsoft Excel spreadsheet software to screen for accidental omissions in data. Any omissions in the data were completed by recourse to the original data collection proforma, or to the appropriate source material where data had not been clearly collected in the data proforma. When all omissions had been replaced the data were transferred to the software package Statistical Package for the Social Sciences (SPSS) version 24, produced by IBM. Histograms were performed on all continuous data to screen for statistical outliers, defined according to visual assessment of the histogram (see Figure 2.4). Any outlying data were then rechecked via recourse to the original data collection proforma or alternate source material to screen for input errors or errors of measurement. Errors were corrected and updated on the initial data proforma as well as the online database where necessary.

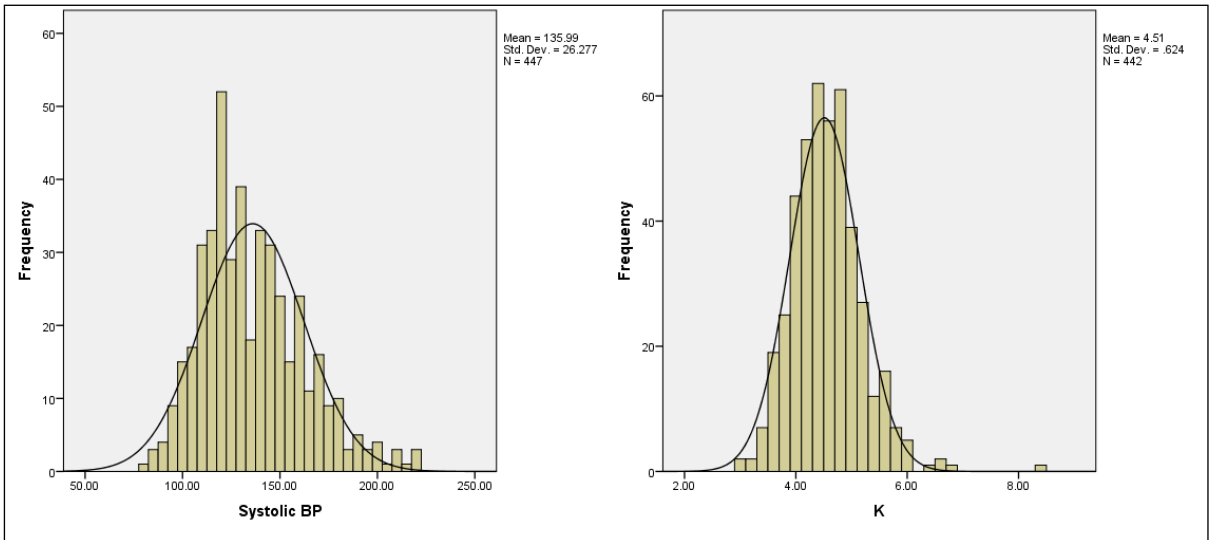

Figure 2.4 – Screenshots of histograms produced to assess for data anomalies. In the left histogram (SBP) the data fit the normal distribution whereas in the right (K) there is a statistical outlier. These data were checked for accuracy and for data entry errors.

The echocardiography data were retained on two separate hard-drives in two separate locations to allow for off-site analysis and to reduce the risk of data loss in accordance with Good Clinical Practice (GCP) research standards.

2.5 Materials

The following section describes the materials required to conduct the study using the above methods. These materials are required to ensure accuracy and quality of the data collected, and thus the validity of the study itself.

2.5.1 Data proforma

The data collection proforma was developed by the cardiology research team for the MRAHF study, forming part of the research protocol and received favourable opinion. Blank proformas were stored digitally in a secure research drive, with password protected access and were accessed and printed when required for use in recruitment. No specific training was required for their use. Once completed these were input onto the online database and the hard copies with copies of the ECGs and reports from the chest x-ray radiographs were maintained in a folder in a secure research office which has a combination lock for entry.

2.5.2 Abbott BNP analyser

The Abbott i-SITE1 BNP machine was obtained from Abbott Laboratories (Abbott) who funded the study. Training was provided on use of the device upon initial receipt of the device from Abbott, as well as written documentation regarding its use.

2.5.3 Abbott cartridges

i-STAT BNP cartridges were obtained from Abbott. They were refrigerated as per instruction from Abbott at between 2 and 8^{o}C, using a Labcold Pharmacy Refrigerator.

Quality control was routinely performed on the i-STAT cartridges as per Abbott's instruction (see Appendix 1.6). For each new batch of received cartridges or control materials the temperature of transfer was verified using four-window temperature indicator strips included in the shipping container. This was followed by testing of a sample cartridge using i-STAT

Control levels. These control vials were samples of human serum with pretested levels of BNP against which the i-STAT BNP analyser could be tested. Three control levels were provided for testing. To test the cartridges a control sample vial was mixed then opened, a drop of fluid was transferred to the i-STAT cartridge using the dropper tip then the control level was returned to storage. The cartridge was sealed and inserted into the i-STAT BNP machine. After 10 minutes this produced a sample value which was compared against expected values as per Value Assignment Sheets available from www.abbottppointofcare.com. There were no discrepancies noted throughout the study period.

When used for patient screening individual cartridges were left to stand for 5 minutes at room temperature prior to use as per manufacturer's instructions. Whole blood samples were collected via peripheral venous phlebotomy. Phlebotomy was performed using a butterfly-needle method attached to an Ethylenediaminetetraacetic acid (EDTA) collecting tube, which was filled to capacity as per instructions from Abbott.

The i-STAT BNP analyser was then turned on, the operator identification number was entered, the patient identification number was entered, and the barcode of the i-STAT BNP cartridge packet was scanned to the device. The cartridge packet was opened, and the cartridge removed for use.

The blood sample in the EDTA tube was then transferred to a syringe, from which the first 1ml of blood were discarded. Remaining blood was then added to the i-STAT BNP cartridge which was subsequently closed and inserted into the handheld i-STAT device for analysis. After 10 minutes the device provided a result. If <15 pg/ml the device would read '<15 pg/mL', if between 15 and 5000 pg/ml then the number would be produced, and >5000 pg/ml the device would read '>5000 pg/mL'. This data was noted in the specialised data proforma.

Training was provided on use of the device upon initial receipt of the device from Abbott, as well as written documentation and regarding its use.

2.5.4 Refrigeration

As noted above, cartridge storage required refrigeration. Once cartridges had been delivered and checked as above regarding temperature window assessment, they were transferred to the Research Refrigerator. The refrigerator used was sourced from Labcold and was maintained at approximately 6-7^{o}C. If temperatures deviated outside of 2-8 ^{o}C an attached alarm logs the aberration. It was monitored daily to ensure that no alarm had occurred, and no alarms were encountered throughout the study period. The refrigerator was stored in the research clinic room which requires a combination lock for entry. No specific training was required for its use.

2.5.5 Materials used for phlebotomy

Tourniquets used were standard single-use disposable V-Grip tourniquets manufactured by Richardson Healthcare. Blood collection safety needles (23-gauge, Reference number 450086) were used for phlebotomy and were supplied by Vacuette. These were stored on all medical wards and the accident & emergency department within the hospital with no special requirements for storage. Training in phlebotomy was received during training at medical school with subsequent revalidation during training years as a Foundation Year 1 and Foundation Year 2 doctor.

EDTA collection tubes (4ml, reference number 454032) were sourced from the hospital supply and were manufactured by Vacutainer. These were stored on all medical wards and the accident & emergency department within the hospital with no special requirements for storage.

2.5.6 Auscultation

Auscultation within the research team was performed using a 3M Littmann Master Cardiology Stethoscope. This was supplied personally. Required training was provided during medical school with yearly assessment of clinical skills assessment and no further training was required nor performed. This was stored within the research office which requires a combination lock code for entry.

2.5.7 Chest radiography

Chest x-ray radiography was performed by trained radiographers employed by the on-site radiography department. The Philips Digital Diagnost HP was used for patients admitted to the hospital wards and the Carestream DRX-1 Evolution was used in the Accident & Emergency department.

2.5.8 Echocardiography

Echocardiography was performed by three individuals. The primary sonographer was a research fellow in cardiology. When he was absent due to sickness or annual leave this was performed by two other BSE accredited echocardiographers employed by the NHS Trust. To reduce inter-observer variability of interpretation, all images were analysed and underwent routine data extraction by the primary sonographer. All echocardiograms were performed using the G.E Vivid S70 Cardiovascular Ultrasound system. This was sourced from GE Healthcare Ltd. All staff practising echocardiography had up-to-date accreditation from the British Society of Echocardiography (BSE) to perform Transthoracic Echocardiography to a standard expected by the society.

Images obtained via echocardiography were stored dually on IntelliSpace PACS Enterprise software and on EchoPac Software Version 201 (see Figure 2.5) with analysis performed using the latter software.

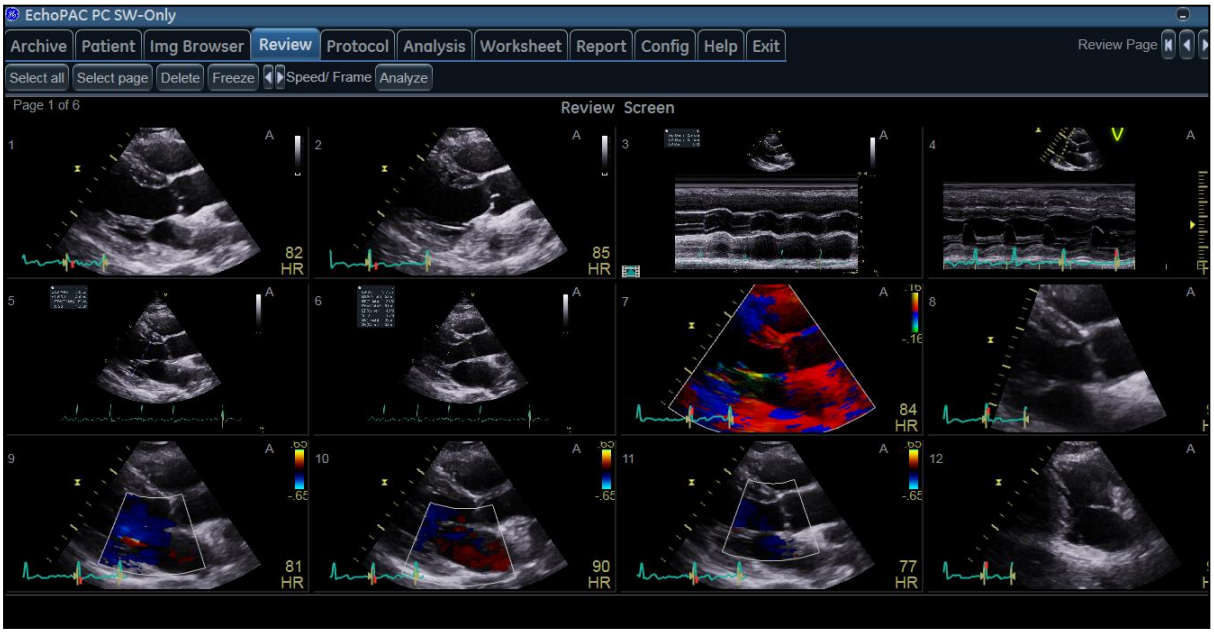

Figure 2.5 – Screenshot demonstrating the review function of Echopac software. Echopac is used to store, view and analyse echocardiographic images.

2.5.9 Chest radiography storage and Echocardiography image storage

Chest radiograph images with attached interpretation were stored electronically using the Philips IntelliSpace® PACS Enterprise Version 4.4.516.21 (PACS) as used within the NHS Trust. This was maintained by the Information Technology (IT) department at the NHS Trust and required no specific training to use. Access to this software was granted using an individual username and password.

2.5.10 Electrocardiography

All ECGs were performed on admission by nursing and healthcare assistant staff, trained to perform electrocardiography. The machine used was the GE Healthcare MAC 5500 HD with standard calibration. Values such as rate and QRS duration are calculated automatically by the machine. Where not calculated, these calculations were performed manually. Assessment of rhythm was also performed manually. Where unclear, ECG data was reviewed with the primary investigator, a consultant cardiologist.

2.5.11 Data manipulation and statistical analysis software

Data were exported initially to Microsoft Excel 2010, used by the local NHS Trust as part of the Microsoft Office 2010 software bundle.

Data were then transferred to SPSS software package. All data analysis was performed using this software. Training in the use of SPSS was provided by Royal Holloway University of London as part of the postgraduate research course.

2.5.12 Data storage

After echocardiography had been performed, the image data were transferred to two separate hard drives, stored separately so as to avoid the possibility of data loss. The hard drives used were 1 Terabyte Western Digital My Passport Ultra External Hard drives. They required no specific training prior to use.

2.5.13 Online database

Data were input to a bespoke online database created and maintained by an independent outsourced company; Metanoic Health limited. This database contained data input sections for each of the above data streams. It is password protected, with access granted only to the administrator and the two research fellows working with the data. Data from the database could be downloaded as a Microsoft Excel file during the study to allow for data screening and correction. Upon completion of the study and subsequent to error correction this database was locked, with the option to download a read-only excel file to ensure accuracy and consistency of the data used within the research group.

2.5.14 Data analysis

All data were analysed using SPSS software version 24 as described above.

Continuous variables were tested for normality using the Shapiro-Wilk tests, and evidence of skew was inspected visually using histograms, Q-Q plots and box plots.

Parametric continuous data are displayed as mean +/- standard deviation (SD). Non-parametric continuous data are presented as median with interquartile range (IQR). Categorical variables are presented as a percentage proportion of the population.

Continuous parametric variables were analysed using Student's T test when categorised as two groups or using one-way analysis of variance (ANOVA) when more than 2 groups existed, as for AHF subcategories.

Categorical data were compared statistically using Pearson's Chi-squared test.

Statistical differences in non-parametric continuous variables were assessed using the Mann-Whitney U test or Kruskal-Wallis test when more than 2 groups were present.

Binomial logistic regression analysis was performed to assess contribution of variables within models assessing mortality and binary presence of readmission at 6 months post discharge. Receiver operator characteristic curve area under the curve (ROCAUC) analysis was performed on the models created and Net reclassification index analysis was performed on the two models to assess for evidence of statistical improvement.

An alpha value of 0.05 was set as the threshold for statistical significance and familywise multiple testing correction was performed where appropriate.

2.6 Chapter Summary

This chapter has described the various methods used in the design of this study and has subsequently described the materials required to execute it. The following chapters will describe results obtained from analysis of the dataset obtained.

Firstly, the dataset will be examined according to HF subcategory as defined by LVEF and compared to a large prospective registry study, the EuroHeart Survey II. Secondly, the difference between myocardial deformation parameters within novel heart failure subcategories with be assessed, and finally this study will assess the ability of using data collected from this patient cohort to produce risk scoring models to predict all-cause mortality at 6 months post discharge. This first will use only clinical, demographic and

biochemical data, while the second will include echocardiographic data. The two will be compared to assess the statistical and predictive benefit of the additional data. Both will look at variables which are feasibly acquired during the acute admission period to allow for early prognostication and assessment of the likely patient journey.

Chapter Three: Baseline characteristics of the MRAHF cohort and comparison against the EuroHeart Failure II survey

3.1 Chapter introduction

This chapter describes the baseline characteristics of the acute heart failure (AHF) cohort included in this thesis and compares the patients according to the novel HF subcategories outlined in the 2016 European society of cardiology (ESC) guidelines (Ponikowski, P., Voors et al. 2016). This comparison demonstrates the extent to which the mitral regurgitation in acute heart failure (MRAHF) cohort is consistent with previous literature on inter-category differences. It then compares the MRAHF cohort to that of an international registry study, the EuroHeart Failure Survey II (EHS II) (Nieminen, Brutsaert et al. 2006) to demonstrate the advantages and disadvantages of each study, as well as the inter-cohort differences which may affect generalisability of the results.

This chapter first summarises the current literature regarding the novel HF subclassifications. It then describes the recruitment criteria for the MRAHF study and outlines the baseline characteristics of the MRAHF cohort stratified by left ventricular ejection fraction (LVEF) subcategory. Finally, it outlines the recruitment criteria for the EHS II study and contrasts the characteristics and advantages and disadvantages inherent to the study design of the two cohorts.

3.1.1 Current literature and aims

The concept of HF as a consequence of reduced systolic function has existed for as long as the concept of the disease and is now formally recognised as HF with reduced ejection fraction (HFrEF), while HF with preserved ejection fraction (HFpEF) was recognised in the 1980s (Dougherty, Naccarelli et al. 1984).

Since 2016, both European and American guidelines have included three HF subcategories, defined according to LVEF (Ponikowski, P., Voors et al. 2016, Yancy, Jessup et al. 2013). The subcategory of HF with borderline or mid-range ejection fraction (HFmrEF) was introduced due to concerns that patients with this intermediate LVEF value may represent an alternate HF phenotype with different pathology when compared to either HFrEF or HFpEF.

By better identifying HFmrEF patients and delineating the characteristics of this group, the underlying pathological mechanisms provoking their disease may be identified. Ultimately, this may aid the development and provision of prognosis-altering medications. This is important as currently only patients in the HFrEF group have evidence-based pharmacotherapeutics, despite HFrEF only accounting for approximately 50% of the total burden of HF (Chioncel, Mebazaa et al. 2017).

Since the introduction of the novel category, HFmrEF has been well characterised in terms of demographic, clinical and biochemical parameters by re-evaluating large registry studies. Typically, parameters from the HFmrEF group have been found to act as an intermediate of those found in HFrEF and HFpEF (Tsuji, Sakata et al. 2017, Cheng, Cox et al. 2014). Data derived from registry studies are often hindered by the study design, and frequently fail to recruit all eligible patients as they are largely non-consecutive studies.

The MRAHF study recruited a consecutive cohort of AHF patients, representative of typical AHF patients within a district general hospital in the United Kingdom. In addition, the aim was to focus on recruiting every admitted AHF patients, specifically attempting to recruit those who may have been initially misdiagnosed as having a non-cardiological admission, to ensure representation of the entire possible AHF cohort. This therefore includes the full range of patients in the HFrEF, HFmrEF and HFpEF subcategories.

The EHS II study was a multi-centre prospective study, designed to assess characteristics of patients with AHF throughout Europe, with a specific focus on aetiology, treatment and outcome. It was designed to represent typical AHF patients and ran from 2004 to 2005 (Nieminen, Brutsaert et al. 2006).

It is the most recent large AHF registry with adequate data for quantitative comparison; other more recent studies such as the European Society of Cardiology Heart Failure Long-Term study (ESC-HF-LT) do not provide data in terms of standard deviations to allow comparative quantitative analysis (Chioncel, Mebazaa et al. 2017).

In the following chapter the baseline characteristics of the MRAHF cohort used in this study will be outlined, with data stratified according to HF subcategories, and then the data from the MRAHF cohort will be compared to existing registry data from the EHS II study to demonstrate similarities and difference from an existing cohort of AHF patients. In addition,

the advantages and disadvantages inherent to both studies will be discussed. This will help to assess the generalisability of the results and the conclusions drawn from them.

3.2 Methods

Chapter two describes the materials and methods required for recruitment of the MRAHF cohort in detail. A summary of this is outlined below.

3.2.1 The MRAHF study cohort

The patient population used in this thesis is derived from the MRAHF study of AHF patients. Patients were recruited into the study cohort as described in detail in the previous chapter, but major inclusion and exclusion criteria are included below in Table 3.1**.** Patients within this study were recruited from throughout the hospital, in all medical, surgical, high dependency and intensive care wards, and included patients seen in the Accident and Emergency department.

Inclusion criteria	**Exclusion criteria**
Clinical Signs or symptoms consistent with AHF as the primary cause for admission	Point-of-care BNP level <100 pg/ml
Inpatient for <7 days by time of consent	Echocardiography inconsistent with a diagnosis of AHF
Ability to give informed consent	

Table 3.1 – Inclusion and exclusion criteria for recruitment to the MRAHF study.

The entire patient cohort recruited in the MRAHF study was used for analysis in this study. 616 patients were screened in a period of 13 months from July 2016 to September 2017. 500 patients gave informed consent to take part in the study and were recruited. 447 patients remained after subsequent exclusions based upon Point-of-care test B natriuretic peptide (POCT BNP) level and Echocardiography. A flow chart demonstrating patient recruitment numbers is included below in Figure 3.1.

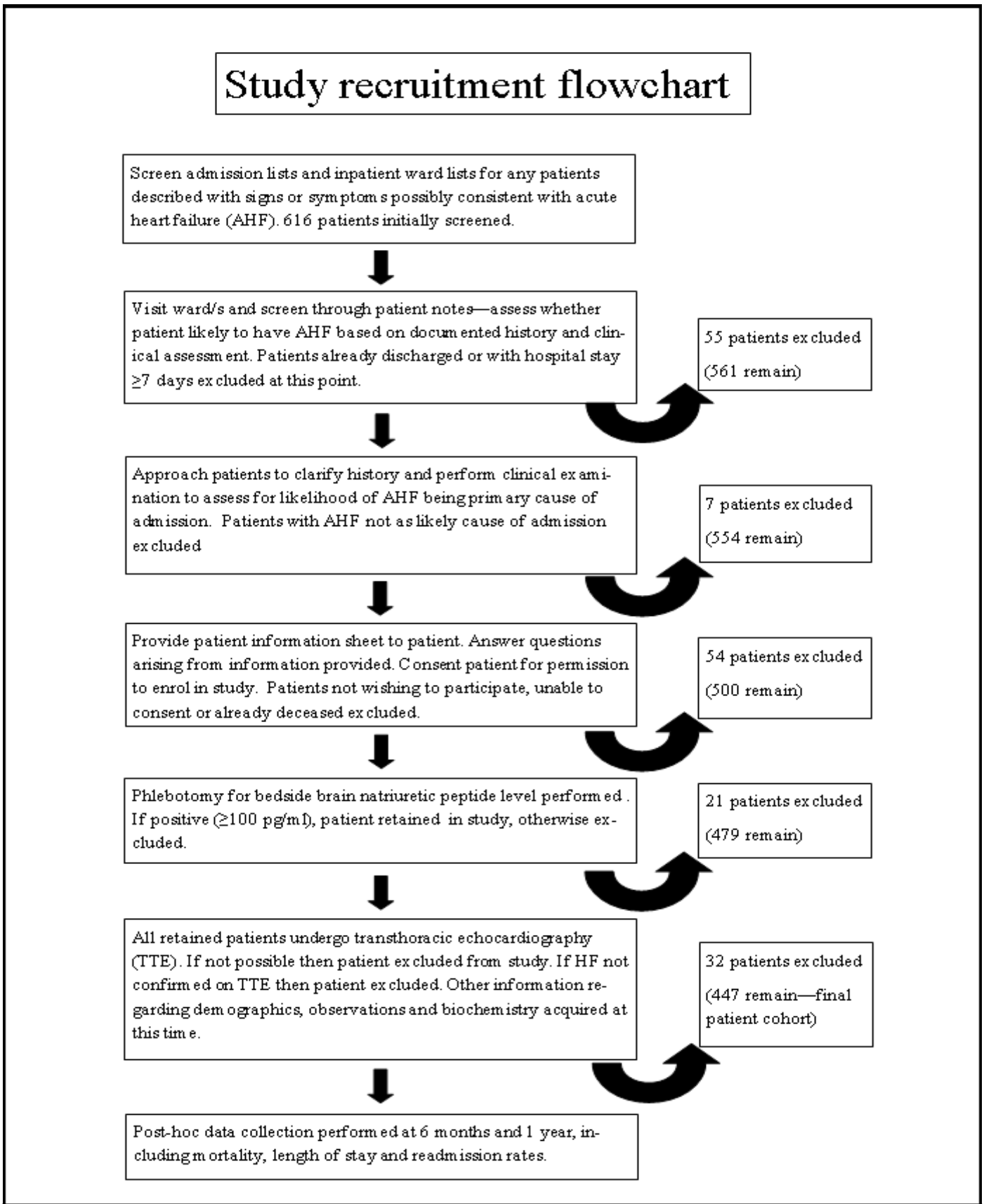

Figure 3.1 – Study flowchart demonstrating the screening and exclusions process. Included to the right are final numbers of patients recruited and excluded at each stage.

3.2.2 Categorisation of the MRAHF cohort

Patients were subdivided into categories according to LVEF as per Table 3.2 and compared to assess for statistical differences between demographic, observational, biochemical and radiographical parameters.

HFrEF	HFmrEF	HFpEF
LVEF <40%	LVEF 40-49%	LVEF ≥50%

Table 3.2 – AHF cohort subcategories according to LVEF values.

3.2.3 Statistical analysis

All statistical tests were performed using the Statistical Package for the Social Sciences (SPSS) version 24 developed by International Business Machines (IBM).

Continuous variables were tested for normality using the Shapiro-Wilk tests, and evidence of skew was inspected visually using histograms, Q-Q plots and box plots.

Continuous parametric variables were analysed using two-tailed Student's T test when categorised as two groups or using one-way analysis of variance (ANOVA) when more than 2 groups existed, as for AHF subcategories. Tukey's post-hoc test was used to determine statistical significance of specific inter-group variations.

Statistical differences in non-parametric continuous variables were assessed using the Mann-Whitney U test, or Kruskal-Wallis test when more than 2 groups were present. Dunn's post-hoc test was used to delineate the statistical significance of specific inter-group variations.

Pearson's Chi-squared test was used to assess for statistical differences between categorical variables.

Baseline characteristics of the MRAHF cohort are reported stratified by HF subcategory as described in Table 3.2.

When comparing data from the MRAHF and EHS II cohorts, outlying data from the MRAHF cohort were excluded from analysis to allow for parametric testing. Student's T-test was then

performed comparing the remaining data to the available means and standard deviations from the EHS II study.

Parametric continuous data are presented as a mean ± standard deviation. Non-parametric continuous data are presented as a median with interquartile range. Categorical variables are presented as a proportion within the population.

An alpha value of 0.05 was set as the threshold for statistical significance.

3.3 Results

447 patients were recruited into the study. LVEF was measurable in 444 patients with 3 patients having images in which assessment of LVEF was not feasible due to the image quality. Of the remaining 444 patients, 180 were categorised as HFrEF, 103 as HFmrEF and 161 as HFpEF.

	Whole cohort	HFrEF	HFmrEF	HFpEF	Significance (p)
N	444 (100%)	180 (40.5%)	103 (23.2%)	161 (36.3%)	-
Demographics					
Median age, years (IQR)	**82** (76-88)	**82** (75–89)	**85** (80–85)	**81** (76–86)	0.06
Gender, % female	**47.7**	**39.4**	**45.6**	**57.8**	<0.01 [a,c]
European Caucasian, %	**91.7**	**92.8**	**87.7**	**93.2**	0.20
Median BMI, kg/m^2 (IQR)	**26.5** (22.5-31.0)	**24.7** (21.5-27.9)	**27.0** (21.0–33.0)	**28.8** (23.7–33.9)	<0.01 *
Hospitalisation in last year, %	**41.2**	**43.3**	**35.0**	**42.9**	0.34
Median length of stay by recruitment, days (IQR)	**1** (0-2)	**1** (0-2)	**1** (0-2)	**1** (1-1)	0.06
Median length of stay on index admission, days (IQR)	**7** (3-11)	**7** (4-10)	**7** (3-11)	**6** (1-11)	0.59
Alternate primary diagnosis on admission, %	**3.7**	**4.0**	**2.0**	**4.4**	0.58
HF Aetiology, n (%)					<0.05
Ischaemic	**69 (**15.4)	**37** (20.6)	**11** (10.7)	**21** (13.0)	
Arrhythmogenic	**117** (26.2)	**48** (26.7)	**23** (22.3)	**45** (28.0)	
Hypertensive	**6** (1.3)	**3** (1.7)	**2** (1.9)	**1** (0.6)	
Pulmonary Disease	**9** (2.0)	**0** (0.0)	**2** (1.9)	**7** (4.3)	
Mechanical	**63** (14.1)	**15** (8.3)	**23** (22.3)	**25** (15.5)	
Medication withdrawal	**26** (5.8)	**12** (6.7)	**4** (3.9)	**10** (6.2)	
Unclear	**157** (35.1)	**65** (36.1)	**38** (36.9)	**52** (32.3)	

Comorbidities, %					
HF	**58.2**	**57.2**	**64.1**	**55.3**	0.35
IHD	**37.8**	**47.2**	**31.1**	**31.7**	<0.001 [a]
HTN	**55.0**	**56.1**	**47.6**	**58.4**	0.21
DM	**31.5**	**30.6**	**28.2**	**33.5**	0.64
CKD	**46.5**	**48.3**	**48.5**	**42.9**	0.52
COPD	**14.3**	**11.1**	**13.6**	**18.0**	0.18
CVA	**15.4**	**16.7**	**10.7**	**16.8**	0.33
Admission medication, %	**Whole Cohort**	**HFrEF**	**HFmrEF**	**HFpEF**	**Significance (p)**
ACEi	**28.4**	**33.7**	**20.4**	**28.1**	0.06
ARB	**15.1**	**12.9**	**17.5**	**15.0**	0.58
BB	**57.9**	**56.2**	**51.5**	**64.4**	0.09
MRA	**12.6**	**12.4**	**7.8**	**15.6**	0.17
Diuretic	**52.5**	**52.2**	**48.5**	**54.4**	0.65
CCB	**18.2**	**15.2**	**14.6**	**23.8**	0.07
Digoxin	**12.4**	**14.6**	**8.7**	**12.5**	0.36
ARNI	**0.0**	**0.0**	**0.0**	**0.0**	-

Biochemistry, Median (IQR)					
Haemoglobin, g/L	**118** (104 – 132)	**118** (105-131)	**112** (100-124)	**119** (104-134)	<0.05 ¥
White cell count, 10*9/L (IQR)	**8.2** (6.4 – 10.0)	**8.5** (7.2-9.8)	**6.6** (4.9-7.5)	**7.9** (6.0-9.8)	0.23
Sodium, mmol/L	**140** (137 – 143)	**139** (136-142)	**140** (138-142)	**140** (137-143)	0.52
Potassium, mmol/L	**4.4** (4.0 – 4.8)	**4.4** (4.0-4.8)	**4.3** (4.0-4.6)	**4.4** (4.0-4.8)	0.65
Urea, mmol/L	**9.0** (5.6 – 12.4)	**9.0** (5.4-12.6)	**9.5** (6.0-13.0)	**8.7** (5.6-11.8)	0.08
Creatinine, µmol/L	**96** (65-127)	**97** (71-123)	**101** (67-135)	**91** (59-123)	0.108
POCT BNP tertile, %:					
100-999 pg/ml	**52.8**	**35.6**	**50.5**	**72.6**	<0.005 [a,c]
1000-2999 pg/ml	**35.3**	**43.3**	**42.7**	**22.4**	<0.00001 [a,c]
≥3000 pg/ml	**11.9**	**21.1**	**6.8**	**5.0**	<0.001 [a,c]
Clinical Observations, Median (IQR)	**Whole cohort**	**HFrEF**	**HFmrEF**	**HFpEF**	**Significance (p)**
SBP, mmHg (IQR)	**135** (118 – 152)	**128** (113-143)	**141** (124-158)	**140** (124-156)	<0.05 *¥
DBP, mmHg (IQR)	**73** (61 – 85)	**73** (61-85)	**80** (70-90)	**71** (59-83)	0.09
AMTS, n/10 (IQR)	**10** (10-10)	**10** (10-10)	**10**(10-10)	**10** (10-10)	0.31
NYHA class, %:					0.78
II	**8.7**	**10.0**	**6.8**	**8.1**	
III	**38.5**	**36.1**	**42.7**	**39.1**	
IV	**52.8**	**53.9**	**50.5**	**52.8**	

ECG					
Mean heart rate, bpm (SD)	**93** (± 29)	**97** (± 27)	**92** (± 29)	**88** (± 31)	<0.005 *
Rhythm, %:					0.17
Sinus	**40.5**	**41.9**	**40.6**	**39.0**	
Atrial Fibrillation	**45.9**	**41.9**	**48.5**	**49.1**	
Paced	**9.3**	**11.7**	**8.9**	**6.2**	
Other	**4.3**	**4.5**	**2.0**	**5.7**	
Median QRS duration, ms (IQR)	**98** (73 – 123)	**114** (91-137)	**90** (63-117)	**90** (70-110)	<0.005 *¥
Echocardiography, Median (IQR)	**Whole Cohort**	**HFrEF**	**HFmrEF**	**HFpEF**	**Significance (p)**
LVEDV, ml	**110** (76-144)	**127** (92-165)	**109** (81-137)	**75** (53-97)	<0.005 *¥ Ω
LVESV, ml	**64** (35-93)	**91** (64-118)	**60** (44-76)	**31** (22-40)	<0.00001 *¥ Ω
LVEF, %	**44** (33-55)	**30** (24-36)	**44** (42-46)	**58** (54-62)	<0.00001 *¥ Ω
E/A, ratio	**1.35** (0.57-2.13)	**1.60** (0.90-2.30)	**1.00** (0.50-1.50)	**1.30** (0.65-1.95)	0.05
RVFAC, %	**40** (30-50)	**35** (25-45)	**35** (28-42)	**40** (32-48)	<0.00001 *
SPAP, mmHg	**51** (38-64)	**50** (37-63)	**52** (39-65)	**53** (40-66)	<0.05 *
LAs, cm^2	**26** (21-31)	**29** (25-34)	**30** (26-34)	**27** (22-32)	0.74

6-month outcomes, %	Whole Cohort	HFrEF	HFmrEF	HFpEF	Significance (p)
All-cause readmission	**59.1**	**62.8**	**57.8**	**55.0**	0.34
HF readmission	**23.7**	**24.0**	**23.5**	**23.2**	0.99
All-cause mortality	**28.1**	**31.7**	**18.6**	**30.0**	0.05
CVD mortality	**13.8**	**16.8**	**9.1**	**13.1**	0.20

Table 3.3 – Baseline characteristics of the MRAHF study stratified by LVEF subcategory

* = HFrEF versus HFpEF, ¥ = HFrEF versus HFmrEF, Ω= HFmrEF versus HFpEF a – HFrEF, b – HFmrEF, c – HFpEF

ACEi – Angiotensin Converting Enzyme Inhibitor, ARB – Angiotensin Receptor Blocker, ARNI – Angiotensin Receptor Blocker and Neprolysin Inhibitor, AMTS – Abbreviated Mental Test Score, BB – Beta Blocker, CKD – Chronic Kidney Disease, COPD – Chronic Obstructive Pulmonary Disease, CVA – Cerebrovascular Accident, CVD – Cardiovascular disease, DBP – Diastolic Blood Pressure, DM – Diabetes Mellitus, E/A – E:A Ratio, ECG – Electrocardiogram, HF – Heart failure, HTN – Hypertension, IHD – Ischaemic Heart Disease, IQR – Interquartile range LAs – Left Atrial Area in systole, LVEDV – Left Ventricular End Diastolic Volume, LVEF – Left Ventricular Ejection Fraction, LVESV – Left Ventricular End Systolic Volume, MRA – Mineralocorticoid Receptor Antagonist, NYHA – New York Heart Association, POCT BNP – Point of care test B Natriuretic Peptide, RVFAC – Right Ventricular Fractional Area Change, SaO2 – Oxygen Saturations, SBP – Systolic Blood pressure, SPAP – Systolic Pulmonary Artery Pressure.

3.3.1.1 Demographics

The median age of the cohort is 82 years (IQR 76-88) and 91.2% are of Caucasian European descent. These were statistically comparable between subcategories. 39.4% of patients with HFrEF were female, compared to 47.7% in HFmrEF and 57.8% in HFpEF ($p<0.01$). Median BMI increased from HFrEF to HFmrEF then HFpEF, BMI was significantly lower in HFrEF than HFpEF (24.7 kg/m^2 (IQR 21.5-27.9) versus 28.8 kg/m^2 (IQR 23.7–33.9), $p<0.01$). A diagnosis of a cause other than cardiovascular disease was made by the admitting physician in 3.7% of cases, a proportion that was seen statistically comparably throughout all HF subcategories.

3.3.1.2 Aetiology

Aetiology varies between HF subgroup – Ischaemic aetiology is more common in HFrEF at 20.6% compared to 10.7% and 13.0% for HFmrEF and HFpEF respectively, while Mechanical abnormalities are more common in the HFmrEF and HFpEF subgroups compared to HFrEF (22.3% and 15.5% compared to 8.3% respectively). Due to the nature of the testing performed none of these are significant when the Bonferroni correction is applied but these demonstrate similar signal regarding underlying aetiology seen in each subgroup as has been previously described in literature.

3.3.1.3 Comorbidities

Comorbidity burden was similar across all groups except in IHD which was significantly more prevalent in HFrEF versus the whole cohort (47.2% versus 37.8%, $p<0.001$).

3.3.1.4 Admission medications

Pre-admission medication use was similar across all subcategories with no statistically significant differences evident.

3.3.1.5 Biochemistry

Median haemoglobin levels were increased in HFrEF compared to HFmrEF (118 g/L (IQR 105-131) versus 112 g/L (IQR 100-124), $p<0.05$). POCT BNP levels were relatively higher in the HFrEF group and lower in HFpEF; 35.6% of HFrEF patients had BNP levels of 100-999 pg/ml compared to 52.8% of the total cohort, in contrast to 72.7% of HFpEF patients

compared to 52.8% of the total cohort (both p<0.005). 21.1% of HFrEF patients had BNP levels in the ≥3000 pg/ml group compared to 11.9% of the total cohort, in contrast 5.0% of HFpEF patients compared to 11.9% of the total cohort (both p<0.005). This is demonstrated in graphical form below in Figure 3.2:

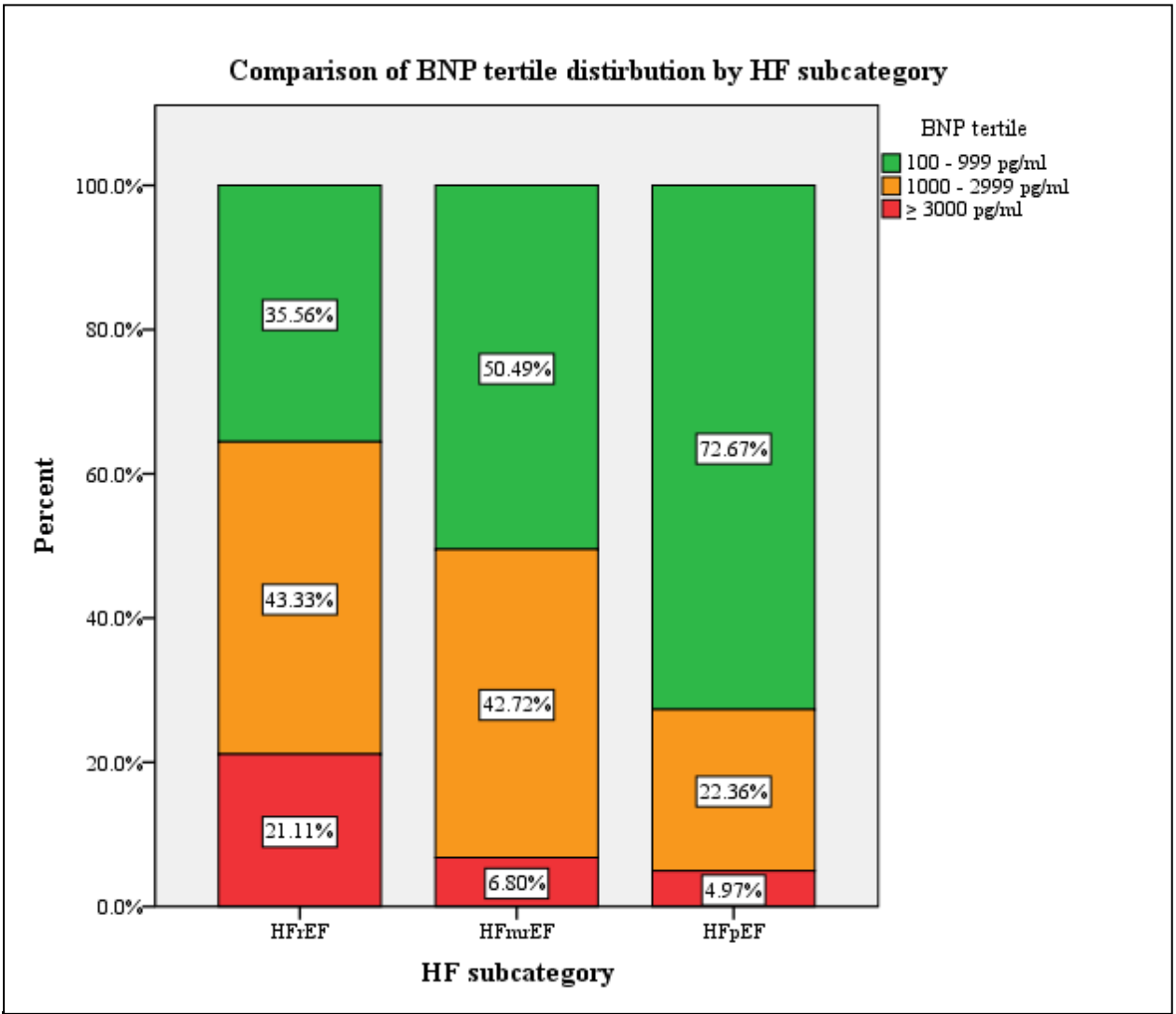

Figure 3.2 – Comparison of BNP tertile distribution by HF subcategory. The proportion of patients with BNP levels in the first tertile (100-999 pg/ml) increases from 35.56% in HFrEF to 72.67% in HFpEF. A corollary diminution in the proportion of patients with BNP levels in the third tertile (≥3000 pg/ml) is seen, from 21.11% in HFrEF to 4.97% in HFpEF. In both cases HFmrEF has intermediate characteristics.

3.3.1.6 Observations

Median systolic blood pressure was lower in the HFrEF subcategory upon admission when compared to both HFmrEF and HFpEF (128 mmHg (IQR 113-143) versus 141 mmHg (IQR

124-158) versus 140 mmHg (IQR 124-156), p<0.05). Other observations and NYHA functional class were similar across all groups.

3.3.1.7 ECG

Mean ECG rate was higher in HFrEF compared to HFpEF (93 bpm ± 29 versus 88 bpm ± 31, p<0.005) and median QRS duration was increased in HFrEF compared to both HFmrEF and HFpEF (114 ms (IQR 91-137) versus 90 ms (IQR 63-117) versus 90 ms (IQR 70-110), p<0.005). Underlying cardiac rhythms were similarly distributed in all groups.

3.3.1.8 Echocardiography

Left ventricular size was statistically distinct across all three groups, with ventricular size inversely correlated with ejection fraction. Median LVESV was 91 ml (IQR 64-118) in HFrEF, 60 ml (IQR 44-76) in HFmrEF and 31 ml (IQR 22-40) in HFpEF (p <.00001).

3.3.1.9 Outcomes

Outcomes were similarly poor across all groups. Whole cohort readmission rate at 6 months was 59.1% of which 23.7% was accounted for by readmission due to HF.

Whole cohort 6-month all-cause mortality rate was 28.1% of which 13.8% was accounted for by mortality due to cardiovascular disease.

3.3.2 The EuroHeart Survey II cohort

The EHS II is a large prospective registry cohort derived from 133 centres across 30 European countries, all of which were specifically invited to participate by the ESC EuroHeart network. Each site was expected to contribute at least 20 patients to the study.

Patients were considered appropriate for inclusion to the EHS II according to symptoms, signs and radiological findings as described below in Table 3.4. Patients in the EHS II cohort were recruited only from the emergency department, internal medicine and cardiology wards or intensive care units.

Inclusion criteria	Exclusion criteria
Symptomatic dyspnoea	No specific exclusion criteria
Signs of heart failure including rales, hypotension, hypoperfusion or right ventricular heart failure	
Lung congestion evident on chest X-ray	

Table 3.4 – Inclusion and exclusion criteria from the EHS II study.

The EHS II cohort comprises 3580 patients recruited throughout Europe, of whom 3.5% were from Northern Europe, 20.4% from Western Europe, 34.4% Central Europe and 42.4% from Mediterranean Europe.

3.3.3 Comparison of the MRAHF and EHS II cohorts

The baseline characteristics of the two cohorts will now be described and compared. These will be described sequentially in terms of patient demographics, comorbidity profile, haemodynamic profile, echocardiographic parameters and patient outcomes.

Statistical outliers in the continuous variables from the MRAHF dataset have been omitted where necessary to compare the two datasets using parametric statistical tests. Parametric continuous data are presented below as mean ± standard deviations and non-parametric data are presented as median value with interquartile range.

	MRAHF	EHS II	Significance (p)
Number	447	3580	-
Demographics			
Mean age, years (SD)	**79.0** (±11.5)	**69.9** (±12.5)	<0.00001
Female, %	**47.7**	**38.7**	<0.0005
Mean BMI, kg/m^2 (SD)	**26.7** (±8.1)	**26.8** (SD not reported)	-
De Novo AHF, %	**41.8**	**37.1**	0.051
Hospitalisation for HF within last 12 months, %	**28.0**	**44.5**	<0.00001
Comorbidities, %			
HTN	**55.0**	**62.5**	<0.005
IHD	**37.8**	**53.6**	<0.00001
DM	**31.5**	**32.8**	0.59
AF	**45.2**	**38.7**	<0.01
CVA	**15.4**	**13.3**	0.21
CKD	**46.5**	**16.8**	<0.00001
COPD	**14.3**	**19.3**	<0.05

Haemodynamics, median (IQR)	**MRAHF**	**EHS II**	**Significance (p)**
SBP, mmHg (IQR)	**135** (100-170)	**135** (110-160)	-
HR, bpm (IQR)	**89** (65-123)	**95** (77-114)	-
Admission Medications, %			
ACEi	**28.4**	**55.0**	<0.00001
ARB	**15.1**	**9.3**	<0.0005
BB	**57.9**	**43.2**	<0.00001
MRA	**12.6**	**28.1**	<0.00001
Echocardiography			
Echo availability	447 **(100%)**	3062 **(85%)**	-
Median LVEDD, mm (IQR)	**50** (46-56)	**58** (51-65)	-
Mean LVEF, % (SD)	**43** (±15)	**38** (±15)	<0.00001
Outcome			
Median length of stay, days (IQR)	**8** (2-14)	**9** (6-14)	-
In-hospital mortality, n (%)	22/447 (**4.9%)**	239/3580 (**6.7%)**	0.15

Table 3.5 – Comparison of the MRAHF and EHS II cohorts. ACEi – Angiotensin converting enzyme inhibitors, AF – Atrial fibrillation, ARB – Angiotensin receptor blockers, BB – Beta blockers, BMI – Body mass index, CKD – Chronic kidney disease, COPD – Chronic obstructive pulmonary disease, CVA – Cerebrovascular accident, DM – Diabetes mellitus, HR – Heart rate, HTN – Hypertension, IHD – Ischaemic heart disease, IQR – Interquartile range, LVEDD – Left ventricular end diastolic diameter, LVEF – Left ventricular ejection fraction, MRA – Mineralocorticoid receptor antagonist, SBP – Systolic blood pressure.

The data included in the table below will now be discussed in greater depth.

3.3.3.1 Demographics

Mean age of patients in the MRAHF cohort was higher (79.0 years ± 11.5 versus 69.9 years ± 12.5, $p<0.00001$), the cohort was proportionally more female (47.7% versus 38.7%, $p<0.0005$) and had substantially lower rates of hospitalisation in the 12 months prior to index admission than patients from the EHS II cohort (28.0% versus 44.5%, $p<0.00001$).

3.3.3.2 Comorbidities

Comorbidity burden was significantly higher in the EHS II cohort except in the cases of atrial fibrillation (38.7% versus 45.2%, $p<0.01$) and chronic kidney disease (16.8% versus 46.5% $p<0.00001$) which were more prevalent in the MRAHF cohort.

3.3.3.3 Haemodynamics

Haemodynamic parameters appear similar between cohorts, but inadequate data are available from the EHS II to compare statistically.

3.3.3.4 Admission medications

The use of both ACE inhibitors and mineralocorticoid receptor antagonist was lower in MRAHF compared to the EHS II cohort (28.4% versus 55.0%, $p<0.00001$ and 12.6% versus 28.1%, $p<0.00001$ respectively), while beta blockade and ARB use were higher (57.9% versus 43.2%, $p<0.00001$ and 15.1% versus 9.3%, $p<0.0005$ respectively).

3.3.3.5 Echocardiography

Echocardiography was performed in 85% of subjects in the EHS II survey compared to 100% in the MRAHF study. Mean LVEF was higher in the MRAHF cohort compared to EHS II (43% ± 15 versus 38% ± 15, $p<0.00001$).

3.3.3.6 Outcomes

Length of stay was comparable between cohorts though data were unavailable to make a full comparison. In hospital mortality was comparable at 4.9% in the MRAHF cohort and 6.7% in the EHS II cohort.

3.4 Discussion

This section will, in turn, discuss the differences within the MRAHF cohort according to LVEF subcategorisation, and then the differences between the MRAHF and EHS II cohorts.

3.4.1 MRAHF cohort – HFrEF, HFmrEF and HFpEF subcategories

Demographically, the subcategories are relatively homogeneous in terms of age and ethnicity but the prevalence of females within each subcategory increases in each category from HFrEF to HFpEF. A similar trend is seen for BMI. There is also a trend to increasing age in HFmrEF and HFpEF when compared to HFrEF which is typically seen in other studies, but this is not statistically significant in the MRAHF cohort. The data from the MRAHF cohort appear consistent with existing literature in terms of demographic breakdown. A small but important proportion of patients across all subcategories were diagnosed with an alternative condition upon admission. When not actively searching for these patients they are likely to be missed in larger registry studies which can negatively impact on the completeness of the data collected.

Comorbidity profile is relatively uniform across the groups except in the case of IHD. IHD is routinely reported as higher in the HFrEF subcategory (Kapoor, Kapoor et al. 2016)and that is again something that has been demonstrated here. History of ischaemic heart disease typically causes insufficient metabolic supply to myocardial regions and has long been understood to depress systolic function, hence an overrepresentation of IHD in the HFrEF subcategory is seen (Braunwald, Kloner 1982). The burden of IHD is often reported as statistically similar in the HFrEF and HFmrEF groups but this is not seen in the MRAHF cohort (Chioncel, Mebazaa et al. 2017). These data are borne out in this study by the aetiology of HF. Whilst not achieving significance due to the Bonferroni correction, there is signal that IHD is the substantially and significantly more common in HFrEF when compared to the other groups, whilst mechanical disease is more prevalent in HFmrEF and HFpEF, again without achieving statistical significance.

Preadmission medication prescription is similar across groups which is somewhat surprising. Intuitively, prognosis-altering medications should be more prevalent in the HFrEF group in which their use is proven, however this is not the case. The relative dearth of ACE inhibitor and MRA use could be explained by the high prevalence of chronic kidney disease

throughout the cohort and especially in the HFrEF subgroup. This is often considered a relative contraindication to ACEi and MRA use in primary care settings and may preclude their use. Beta blockade may be high in the HFpEF group due to the high prevalence of comorbid atrial fibrillation seen in this group as is commonly described in the literature (Chioncel, Mebazaa et al. 2017).

Biochemically the subgroups appear largely similar however POCT BNP levels vary across subgroups. A markedly increased proportion of the HFrEF subgroup has BNP levels in the highest tertile compared to the other groups, while HFmrEF appear intermediate between HFrEF and HFpEF. This may be explained by the significant differences in ventricular dimensions also demonstrated. Increased intraventricular pressure and wall stress is the stimulus for both BNP release and ventricular remodelling, thus increases in ventricular volumes are consistent with elevated BNP levels (Karakilic, Kepez et al. 2010).

Clinical observations are largely consistent across the subgroups except for a reduction of SBP seen in HFrEF. Arterial pressure is a function of cardiac output and systemic vascular resistance. A reduction in cardiac output due to falling contractile function seen in HFrEF will reduce SBP and is likely to account for this difference. This is perhaps supported by the fact that heart rate is elevated in the HFrEF group which, as described in chapter one, is a physiological response to attempt to maintain cardiac output, a function of heart rate and stroke volume.

Also, within ECG parameters, the HFrEF subgroup has a comparatively increased QRS duration. This is likely secondary to the increase in ventricular volumes previously described and concomitant increase in myocardial fibrosis that occurs with ventricular remodelling. This leads to a prolongation of electrical passage through the myocardium and elongation of the QRS complex.

Outcomes are similar across the groups with similar prevalence of readmission and mortality at 6 months. This is important as it demonstrates the poor outcomes still seen in over 50% of the MRAHF patient cohort for whom there are no prognosis-improving medication. This is consistent with other groups who report similar mortality across subcategories (Hsu, Ziaeian et al. 2017, Bhatia, Tu et al. 2006).

3.4.2 MRAHF cohort versus EHS II cohort

This section compares the MRAHF and EHS II cohorts. Firstly, it must be noted that the EHS II cohort is larger and more heterogeneous, recruited from the entirety of Europe, but with only a small proportion from northern Europe (3.5%). Secondly, the recruitment criteria for EHS II are dependent exclusively upon clinical and chest x-ray parameters which may lead to inclusion of non-HF patients. Specifically, echocardiography is available in only 85% of the EHS II cohort which leaves the study open to type 1 error in terms of patient selection. This is a recognised deficiency of the EHS II cohort.

This deficiency aside, there remain many differences between patients recruited in each study. The MRAHF cohort is typically older, with a larger proportion of female patients and reduced requirement for hospital admissions in the preceding 12 months.

The reduced comorbidity burden seen in the MRAHF cohort and indeed in Britain compared to particularly southern eastern Europe likely accounts for the increased age of patients in the MRAHF cohort, as these patients remain healthy for longer (Carlson, P. 2004) and is perhaps a reflection of the difference in life-expectancies. The variance in comorbidities and mortality has been variously linked to diet, exercise and smoking (Meslé 2004), the latter of which is likely higher in the EHS II cohort as suggested by comparatively raised rates of chronic obstructive pulmonary disease (COPD).

The notable exceptions are AF and CKD. The prevalence of AF is known to increase with age which may explain the higher prevalence in the MRAHF cohort which is significantly older than the population described in EHS II (Heeringa, van der Kuip, Deirdre AM et al. 2006). The increased prevalence of CKD appears to represent a classification artefact. The MRAHF study defined and recorded CKD status according to NICE guidelines on Chronic Kidney Disease (National Institute for Health and Care Excellence 2014), while in the EHS II survey it is defined as present in patients with creatinine >177umol/L or awaiting/undergoing dialysis. This is equivalent to NICE guideline CKD stage 4/5, hence the significantly lower prevalence of CKD in EHS II due to the omission of patients with CKD stages 1, 2 or 3 in their comorbidity statistics.

Haemodynamic variables upon admission appear qualitatively similar, but inadequate data are provided to quantitatively compare the two cohorts.

Community prognostic medication prescription is reduced in the MRAHF cohort in terms of ACEi and MRA, almost half that of the EHS cohort. This is disappointingly low given the known benefits of these medications but may be somewhat explained by the higher mean LVEF in the MRAHF cohort, thus fewer patients are likely to be eligible for prognosis-altering medications. This is, however, unlikely to account for the entire difference between cohorts. Beta blockade use is higher in the MRAHF cohort, likely secondary to the increased prevalence of AF within this cohort, given the relative dearth of other prognosis-altering medications.

Some of the variation noted between the two cohorts is likely to exist due to the methodology of patient capture. As noted, no echocardiographic criteria were included in the EHS II study, relying solely on clinical selection, while the MRAHF study had strict biochemical and echocardiographic criteria. It also represented consecutive patients, recruited from all areas within the hospital. Large registry studies can suffer from under-representation of cases of HF misdiagnosed as respiratory or dermatological complaints such as community acquired pneumonia or bilateral peripheral cellulitis. If, and how, this is likely to skew the data is unclear, however it must be acknowledged that this is likely to play a role in inter-cohort variation.

Despite the variations described above, inpatient mortality is consistent across both groups. While patients in the MRAHF cohort may live healthier for longer with fewer comorbidities, once they become inpatients due to AHF, prognosis appears similar to other AHF patients.

3.4.3 Limitations

The MRAHF and ESH cohorts differ in important ways. There are differences in patient demographics, comorbidity profiles, pre-hospital treatments and cardiac geometry which prevent results derived from the MRAHF cohort from being generalisable to a pan-European cohort.

Despite this the MRAHF cohort is a robustly collected consecutively acquired AHF dataset, representative of the local patient group. While results may not be immediately generalisable, conclusions drawn from the dataset may be used to suggest further tests in larger more heterogeneous populations to better establish the hypotheses tested.

In fact, it could be argued that the MRAHF cohort is more representative of an AHF cohort than EHS II given that it had stringent exclusion criteria to avoid recruiting non-AHF patients and had echocardiographic data for all patients retained within the study.

The EHS II study is recognised to have limitations intrinsic to its study design. As a multi-centre registry study, ensuring homogeneity of practice is always difficult, but this is rendered particularly relevant by the flexibility of the inclusion criteria; inclusion into the EHS II study is essentially a clinical diagnosis only, with no confirmatory biochemistry or echocardiographic testing. As such the reliability of the data is highly reliant upon uniformity of clinical acumen across clinical sites and the authors indeed note that neither diagnosis nor echocardiography was assessed or confirmed centrally, limiting confidence in the accuracy of the original diagnosis (Nieminen, Brutsaert et al. 2006). As demonstrated above, incorrect alternate diagnoses are a small but relevant occurrence in hospital admissions and can lead to type II errors when considering recruitment or inclusion in a trial.

Certain data points are not provided by the EHS II study in enough detail to perform comparative statistical analysis between the MRAHF and EHS II cohorts, particularly in the cases of BMI, haemodynamic variables and left ventricular dimensions. This is unfortunate as it detracts from the ability to fully compare the two datasets. A qualitative analysis of these data is still feasible, but conclusions cannot be drawn about the statistical significance of any similarity or difference noted. In addition, certain data points had to be excluded from the MRAHF dataset to render it parametric and permit inter-cohort analysis. This potentially reduces the validity of conclusions drawn regarding differences and similarities between the two cohorts.

The EHS II cohort is not the largest, nor the most recent AHF registry, with others such as the ALARM-HF (Follath, Yilmaz et al. 2011) and ESC HF LT studies (Chioncel, Mebazaa et al. 2017) enrolling a greater number of patients in a more recent timeframe. Unfortunately, the requisite data are not available from these studies to make a statistical comparison (standard deviations are not provided) and thus only a qualitative analysis is feasible. This may be qualitatively useful, but it is difficult to draw definite numerical conclusions from such data. As such, I have compared the MRAHF cohort to the most recent large European registry study of AHF patents that reports adequate data for quantitative comparisons of the two datasets.

3.5 Chapter summary

This chapter has described the baseline characteristics of the MRAHF cohort used in this study, with specific comparison of the three HF subgroups demonstrating consistency with what is already known in the literature regarding these subclassifications.

In addition, it has compared the MRAHF cohort to the EHS II cohort and described the underlying differences between the two cohorts so as to appreciate the context in which this data has been collected. In general, the MRAHF cohort was older, with fewer comorbidities and was less heavily medicated upon admission, but similar inpatient outcomes were seen in both cohorts.

Chapter Four: Myocardial deformation imaging parameters in novel heart failure subcategories

4.1 Chapter Introduction

This chapter describes myocardial deformation imaging values in the Mitral Regurgitation in Acute Heart Failure (MRAHF) cohort and contrasts them according to left ventricular ejection fraction (LVEF) based subcategories.

The chapter briefly reviews the current literature regarding heart failure (HF) subcategorisation, the gaps in understanding and what this study can add to the literature. It then outlines the materials and methods used to obtain the dataset. Myocardial deformation parameters are then shown to vary significantly and substantially between HF subcategories, with deformation values of patients with HF with mid-range ejection fraction (HFmrEF) intermediate between HF with reduced ejection fraction (HFrEF) and HF with preserved ejection fraction (HFpEF). Further results demonstrate the likely stability of myocardial deformation values compared to LVEF in the acute phase, and finally an interpretation of these results is offered with discussion of the limitations of the study and suggestions for further work.

4.2 Current literature – evidence and omissions

HF has typically been subclassified according to LVEF, with both European and American guidelines recognising separate clinical syndromes of HFrEF and HFpEF.

The 2013 ACC/AHA and 2016 ESC guidelines recognised the novel concept of HF with borderline ejection fraction and mid-range ejection fraction respectively, defined as HF in patients with LVEF of 40-49%. This was introduced due to concerns that patients with LVEF in this range may represent an alternate HF phenotype (Ponikowski, P., Voors et al. 2016, Yancy, Jessup et al. 2013).

While many studies have examined the relationships between HFrEF, HFmrEF and HFpEF concerning demographic, biochemical, and outcome discrepancies, minimal data exists regarding the differences in myocardial deformation parameters of the AHF cohort (Cheng, Cox et al. 2014, Kapoor, Kapoor et al. 2016, Tsuji, Sakata et al. 2017).

Myocardial strain and strain rate parameters are of interest due to their ability to more accurately demonstrate regional and global deformation and contractile function than other commonly used markers of systolic function such as LVEF. Particularly in the case of strain rate values, the speed of deformation has been shown to be much more closely correlated to intrinsic systolic function, independent of contemporaneous loading conditions which are known to significantly affect the representative accuracy of LVEF (Ferferieva, Van den Bergh et al. 2011). In addition, deformation imaging parameters more accurately represent contractile function in patients with regional wall abnormalities than measurement of LVEF (Brown, Jenkins et al. 2009).

When investigated in other cardiological conditions strain and strain rate parameters have been shown to identify clinically relevant coronary disease and systolic dysfunction in both hypertrophic cardiomyopathies and chronic HF (Kraigher-Krainer, Shah et al. 2014, Kukulski, Jamal et al. 2003, Afonso, Kondur et al. 2012).

Limited data have been reported regarding strain and strain rate in AHF. Park et al did suggest that strain and strain rate parameters could be of value to aid prognostication in AHF, above and beyond LVEF but their study excluded patients with AHF of an ischaemic aetiology. Ischaemic AHF accounts for approximately 20-30% of AHF admissions and thus it could be argued that their study is not representative of the typical AHF population (Park, Park et al. 2018).

In this study differences have been assessed between regional and global strain and strain rate parameters in HF subcategories, using consecutively acquired AHF patients in a large district general hospital.

4.3 Study hypotheses

This chapter will assess the following hypotheses:

a) Left ventricular strain values differ between patients labelled as HFrEF, HFmrEF and HFpEF in the MRAHF cohort and this difference is statistically significant.
b) Left ventricular strain rate values differ between patients labelled as HFrEF, HFmrEF and HFpEF in the MRAHF cohort and this difference is statistically significant.

4.4 Methods

Chapter two of this thesis described the main methods and materials used for study design and patient recruitment. Below is a summary of these with emphasis on echocardiographic data capture and offline strain and strain rate analysis.

4.4.1 Study design and population

The MRAHF study was designed as a prospective cohort study of all consecutive patients admitted to a single hospital site with signs and symptoms consistent with AHF as the primary driver of admission.

The hospital receives patients from a catchment area containing approximately 410,000 people and has typically seen between 300-400 patients admitted secondary to AHF in each of the previous four years. This study uses the entire dataset captured from the MRAHF study cohort.

4.4.2 Inclusion and exclusion criteria

Inclusion criteria for the study:

1) Clinical signs and symptoms consistent with AHF as the primary cause for admission
2) Inpatient admission of <7 days by time of consent
3) The ability to give informed consent

Exclusion criteria for the study:

1) Point-of-care test (POCT) B natriuretic peptide (BNP) level <100 pg/ml
2) Echocardiography inconsistent with a diagnosis of HF

4.4.3 Patient recruitment and data capture

Patient recruitment began in July 2016 and concluded in September 2017. 616 patients were approached for recruitment, 500 gave informed consent to join the study of whom 447 remained post exclusions. Data capture was carried out at the time of recruitment and included baseline demographic, biochemical, observational and radiological data as outlined in chapter two.

4.4.4 Echocardiography

Echocardiography was performed on all patients within 48 hours of recruitment. Offline analysis was subsequently performed to assess cardiac geometric, haemodynamic and myocardial deformation parameters. Cardiac geometry and haemodynamic status were assessed according to a standardised echocardiography protocol (see Appendix 1.4) and quantified as per standard British Society of Echocardiography (BSE) guidelines (Wharton, Steeds et al. 2015)**.** Patients were excluded from the study if echocardiography did not support a diagnosis of HF. For those patients retained within the study, they were categorised as either HFrEF, HFmrEF or HFpEF according to their measured LVEF - <40%, 40-49% and ≥50% respectively.

Reproducibility is a key factor in the utility of any investigative modality. Typically, this can be discussed in terms of intra-observer and inter-observer variability, whilst in the case of strain and strain rate, there is also the factor of inter-software variability to consider.

Many developers produced software to resolve myocardial deformation values from 2-dimensional, and more latterly 3-dimensional, echocardiography. Due to different in-house decisions regarding reference points and definitions of cardiac borders there was initially quite considerable variation in terms of strain and strain rate values from one software vendor to another within subjects. As recently as 2012, studies demonstrated the non-interchangeability of vendors and software due to substantial and significant differences in strain measurements in longitudinal, radial and circumferential axes (Takigiku, Takeuchi et al. 2012). Concerted international efforts by the EASCVI-ASE-industry task force regarding consensus on definitions and measurements have led to a reduction in inter-vendor variability (Voigt, Pedrizzetti et al. 2014, Farsalinos, Daraban et al. 2015) though they do still caution single segment strains demonstrate significant variability and GLS has the greatest reproducibility and (Mirea, Oana, Pagourelias et al. 2018).

Inter-observer and intra-observer variability in strain measurements has always been relatively low, at least comparable if not better than other common echocardiographic measurements such as EF (Stanton, Leano et al. 2009). This has been a longstanding concern for LVEF which is commonly considered to be +/- 7% of the stated value (Himelman, Cassidy et al. 1988), whilst GLS values have been demonstrated to have a variance coefficient up to half that of LVEF depending on the vendor(Farsalinos, Daraban et al. 2015).

Studies have demonstrated that expert level competency with intra-observer correlation coefficients of >0.9 are achievable for GLS within 50 patients assessed and monitored (Chan, Shiino et al. 2017). To achieve this and thus enhance intra-observer reproducibility in this study, both research fellows attended an off-site training event in Berlin with industry and academic experts where >100 cases were examined, discussed and assessed.

In this study inter-observer variability for strain measurements was eliminated by the use of one sole data analyser for all LV strain measurements. In order to reduce inter-observer variability of basic cardiac measurements including LVEF, of 447 patients retained within the study, 426 (95.3%) were scanned by the principle ultrasonographer, 12 (2.6%) by the second sonographer and 9 (2.1%) by the third. All off-line assessment of cardiac geometry and basic functional parameters was performed by the principle ultrasonographer, again to ensure homogeneity of practice and eliminate inter-observer variability.

Large AHF studies use multiple echocardiography-trained sonographers, but by using one sonographer for >95% of images in this study, inter-observer variability of technique is reduced. This is intended to reduce the inter-observer variability found in acquisition of acoustic windows. In addition, all studies were assessed and reported by the principal ultrasonographer, reducing inter-observer interpretation variability. Echocardiographic studies were performed within 48 hours of admission. This limit was intended to reduce the variability of haemodynamic status caused by therapeutic interventions provided during inpatient admission. The above measures were instigated to ensure that measurement of LVEF was consistent with minimal confounders. This aimed to ensure that patients were consistently attributed to the same subcategory and are genuinely representative of these subcategories.

Unless echocardiographic findings indicated life or management-altering pathology, the results of the scans were not passed to the clinical team. In cases where such pathology was detected, these were discussed with the Principal Investigator of the MRAHF study when not time-sensitive or with the on-call consultant when immediate action was required.

Subsequent to image acquisition, offline analysis of myocardial deformation imaging parameters was then performed as described below.

4.4.5 Myocardial deformation imaging assessment

All images were exported to EchoPac for statistical analysis.

The timing of the cardiac cycle was assessed by timing aortic valve opening and closure for each patient using Pulse wave Doppler signal through the aortic valve (see Figure 4.1 for illustration).

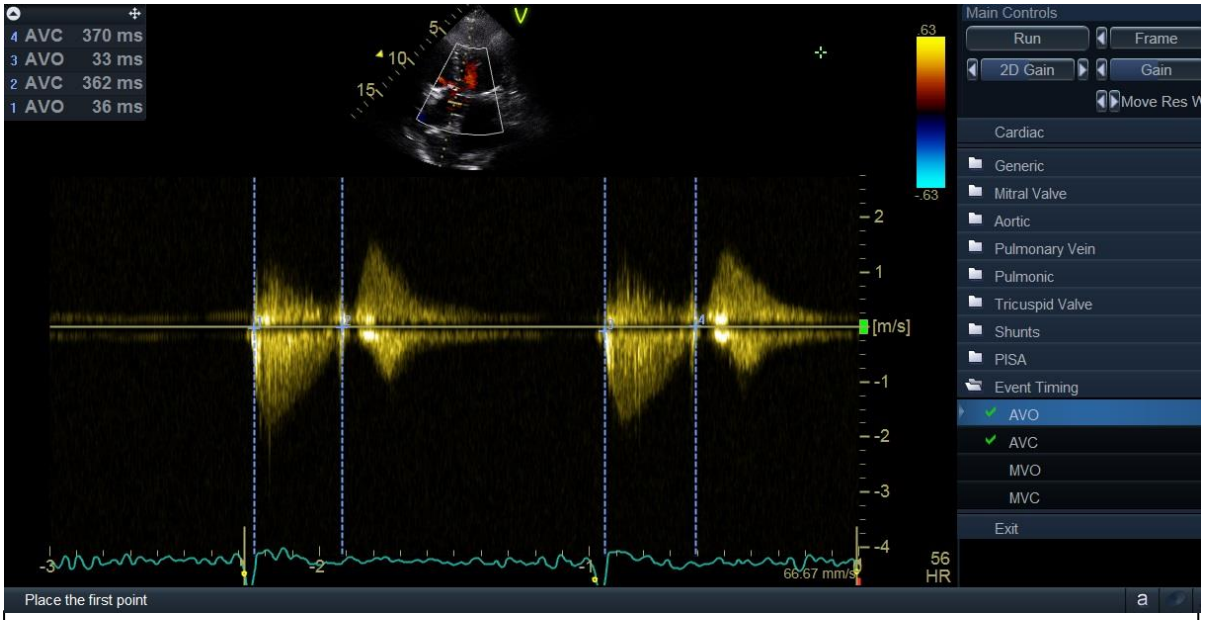

Figure 4.1 – Aortic valve opening and closure assessment using pulse wave Doppler images. Valve opening and closure are timed with commencement and cessation of flow through the aortic valve as demonstrated above.

Myocardial deformation assessment was then performed in each of the apical 4, 2, and 3 chamber views of the left ventricle (LV) in order to obtain comprehensive strain and strain rate measurements for the majority of the LV. The following approach was used:

Once the appropriate image of the LV had been selected, the Strain function on the GE EchoPac software was selected and the frame rate of the selected image was recorded. The endocardial border was then traced in the end systolic frame from one end of the mitral annulus to the other. The software automatically generates a 'region of interest' (ROI) which includes the depth of the myocardium. This was adjusted when necessary to reflect myocardial wall thickness. When satisfied with accurate border tracing and ROI depth, the software was run.

The software automatically reports the ability to perform speckle tracking in all segments of the cardiac wall in this view. If all segments were not trackable using the ROI, attempts were

made to retrace the endocardial border until the software was able to track the individual segments. If this remained non-feasible for all segments then this image was considered non-feasible and this was noted in the dataset. If tracking was feasible in at least 2 segments, then the data were considered usable and the frame rate of the used image was noted - see Figure 4.2 below.

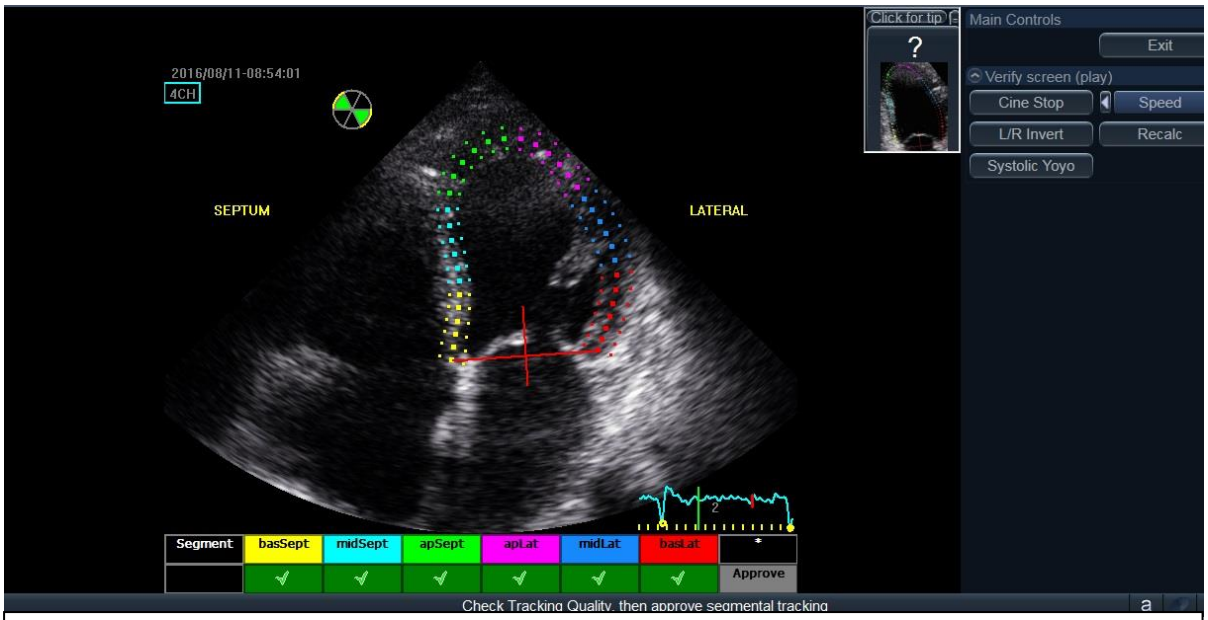

Figure 4.2 – Left ventricular border tracing in EchoPac software.

Once an acceptable trace had been performed and image tracking was feasible, the strain analysis would be performed automatically by the software. This would provide 6 strain values for 6 individual myocardial segments in each view. In addition, strain and strain rate values for all segments are recorded and averaged to produce GLS and GLSR. Segments acquired in each view can be seen in Table 4.1.

Acoustic View	Segment assessed
4 Chamber View	Basal Inferior septum Mid inferior septum Apical Inferior septum Basal anterior lateral wall Mid anterior lateral wall Apical anterior lateral wall
2 Chamber View	Basal inferior wall Mid inferior wall Apical inferior wall Basal anterior wall Mid anterior wall Apical anterior wall
3 Chamber View	Basal inferior lateral wall Mid inferior lateral wall Apical inferior lateral wall Basal anterior septum Mid anterior septum Apical anterior septum

Table 4.1 – Myocardial segments assessed for myocardial deformation parameters in each acoustic view.

Timing of the strain calculation was automatically calculated but adjusted to ensure that analysis begins at end diastole, as defined by the peak of the R wave, and ends at the same point of the next cardiac cycle. End systole was defined according to aortic valve closure as measured earlier.

Visual assessment of the strain curve was made to assess for obvious abnormalities. If these were distinctly abnormal the above process was repeated to try and obtain a normal appearing strain curve. If it remained abnormal in morphology these values were considered likely representative of ventricular strain patterns.

Peak systolic strain (PSS) was then noted, defined as the peak value achieved between the end of diastole and the closure of the aortic valves as ascertained earlier through Doppler traces. These values were noted for all segments measurable in each image with an additional averaged GLS value.

To ascertain strain rate (SR) values, the SR function was then selected. The software would then provide SR values for each of the available 6 myocardial segments in each image and an averaged GLSR value. Peak SR values were noted in the first half of systole as maximal

physiological systolic SR is generated in the first half of systole (Weidemann, Jamal et al. 2002). This is defined as half the duration between end diastole and aortic valve closure.

By measuring only physiological strain and strain rate prior to aortic valve closure the measurement of myocardial deformation which does not contribute to systolic force generation is avoided. Pathological deformation curves with post-systolic strain peaks are commonly seen in myocardial ischaemia but the deformation measured subsequent to closure of the aortic valve is not an indication of true contractile function of the myocardium, but rather is an artefactual representation of the relaxation of other local segments and subsequent rebound contraction of the ischaemic region(Sutherland, Hatle et al. 2006). As such, values measured after the aortic valve closure do not represent true underlying myocardial contractile function, the variable of interest, hence why it is typical throughout the literature to measure peak systolic strain values prior to aortic valve closure.

Different areas of the myocardium produce peak physiological strain at marginally but different times and amplitudes. While this is important in the understanding of the underlying mechanism of force generation, physiological maximums still occur prior to aortic valve closure thus measurement at this time point will still capture maximal physiological force in all regions assessed(Sutherland, Hatle et al. 2006).

4.4.6 Statistical analysis

All statistical tests were performed using the Statistical Package for the Social Sciences (SPSS) version 24 developed by IBM.

Continuous variables were tested for normality using the Shapiro-Wilk tests, and evidence of skew was inspected visually using histograms, Q-Q plots and box plots.

All myocardial deformation variables demonstrated statistically significant skew in the Shapiro-Wilk test, rejecting the null hypothesis. Visual inspection of histograms, Q-Q and box plots confirmed positive skew throughout the dataset. This is likely due to the typical lower value of systolic strain and strain rate values of 0, with no upper limit defined.

Mean strain and strain rate values were compared across AHF subcategories, defined according the LVEF as described above. Due to the non-parametric nature of the data, a Kruskal-Wallis test was used to compare groups and demonstrate statistically significant

differences between the three subcategories. Dunn's pairwise post-hoc test was used to assess for specific inter-group variations.

An analysis is then made of differences seen in markers of systolic function categorised according to time of recruitment into the study.

One-way analysis of covariance (ANCOVA) was performed to assess the effect of confounding variables on mortality differences between groups.

Parametric continuous data are presented as a mean ± standard deviation. Non-parametric continuous data are presented as a median with interquartile range. Categorical variables are presented as a proportion within the population. An alpha value of 0.05 was set as the threshold for statistical significance.

4.5 Results

In this section the baseline characteristics of the study population will be presented and compared according to LVEF subcategory, then the differences in strain and strain rate values between the subgroups will compared and the effect of time to recruitment on LVEF, strain and strain rate values will be demonstrated.

4.5.1 Baseline characteristics of the AHF subcategories

Baseline characteristics of the MRAHF cohort have been described according to HF subcategories in chapter three. A summary of these is presented below.

Of 447 patients, LVEF was measurable in 444 patients. Of these, left ventricular strain analysis was possible in 442 patients as two patients did not have images adequate for deformation analysis.

40.5% patients were categorised as HFrEF, 23.2% as HFmrEF and 36.3% as HFpEF.

The median age of the total cohort was 82 (IQR 76-88), 47.7% were female, median BMI was 26.5 kg/m^2 (IQR 22.5-31.0) and the median LVEF was 44% (IQR 33-55).

Ischaemic heart disease was more prevalent in the HFrEF subcategory compared to HFmrEF and HFpEF (47.2% versus 31.1% and 31.7%, $p<0.005$). The HFrEF subcategory also demonstrated lower median systolic blood pressures (128 mmHg (IQR 113-143) versus 141

mmHg (IQR 124-158) and 140 mmHg (IQR 124-156), $p<0.05$) and faster mean heart rates (97 bpm ± 27 versus 92 bpm ± 29 and 88 bpm ± 31, $p<0.005$).

POCT BNP values were higher in the HFrEF group compared to HFmrEF and HFpEF (proportion of patients with BNP ≥3000 pg/ml - 21.1% versus 6.8% and 5.0%, $p<0.0001$).

Left ventricular geometry varied significantly between the groups; median left ventricular end systolic volume was significantly different in all groups, HFrEF, HFmrEF and HFpEF respectively (91 ml (IQR 64-118) versus 60 ml (IQR 44-76) versus 31 ml (IQR 22-40), $p<0.05$)

4.5.2 Left ventricular myocardial deformation imaging – Peak systolic strain values

Frame rate values were comparable between groups. LV PSS values were consistently statistically distinct between HF subcategories in almost all myocardial regions studied, with intermediate LV PSS values in HFmrEF when compared to HFrEF and HFpEF. GLS values were significantly and substantially different in each subcategory; median GLS increased by nearly half from HFrEF to HFmrEF and nearly double from HFrEF to HFpEF (-6.62% (IQR -4.41 – -8.83) versus -9.03% (IQR -6.28 – -11.78) versus -13.12% (IQR -10.02 – -16.22), $p<0.0001$).

	HFrEF n = 180 (40.5%)	**HFmrEF** n = 103 (23.2%)	**HFpEF** n = 161 (36.3%)	**Significance (p)**
Mean frame rate, fps (SD)	**48** (±6)	**49** (±6)	**49** (±7)	0.07
Median peak systolic strain, % (IQR)				
4 Chamber View				
Basal inferior septum	**-5.08** (-2.63 – -7.53)	**-7.05** (-4.53 – -9.67)	**-10.25** (-6.65 – -13.85)	<0.0001 *¥Ω
Mid inferior septum	**-7.20** (-4.04 – -10.36)	**-10.32** (-7.27 – -13.37)	**-15.00** (-11.22 – -18.78)	<0.0001 *¥Ω
Apical inferior septum	**-7.11** (-1.11 – -13.11)	**-13.20** (-7.50 – -18.90)	**-19.47** (-13.39 – -25.55)	<0.0001 *¥Ω
Basal anterior lateral wall	**-6.64** (-3.32 – -9.96)	**-7.91** (-4.13 – -11.69)	**-9.50** (-4.36 – -14.64)	<0.01 *
Mid anterior lateral wall	**-6.88** (-3.48 – -10.28)	**-8.10** (-4.42 – -11.78)	**-10.12** (-4.96 – -15.28)	<0.05 *Ω
Apical anterior lateral wall	**-6.64** (-1.84 – -11.44)	**-10.00** (-4.67 – 15.33)	**-15.00** (-8.89 – -21.11)	<0.05 *¥Ω
2 Chamber View				
Basal inferior wall	**-7.27** (-3.39 – -11.15)	**-8.83** (-4.65 – -13.01)	**-12.34** (-8.18 – -16.50)	<0.01 *Ω
Mid inferior wall	**-7.42** (-3.53 – -11.31)	**-11.46** (-7.83 – -15.09)	**-14.69** (-10.80 – -18.58)	<0.005 *¥Ω
Apical inferior wall	**-7.66** (-2.68 – -12.64)	**-15.13** (-10.17 – -20.09)	**-19.38** (-12.92 – -25.84)	<0.001 *¥Ω
Basal anterior wall	**-4.84** (-1.75 – -7.93)	**-6.88** (-3.01 – -10.75)	**-8.59** (-3.85 – -13.43)	<0.05 *
Mid anterior wall	**-4.80** (-1.64 – -7.96)	**-7.19** (-3.21 – -11.17)	**-9.63** (-4.00 – -15.26)	<0.05 *¥
Apical anterior wall	**-4.49** (0.00 – -8.98)	**-10.56** (-5.48 – -15.64)	**-13.59** (-6.62 – -20.56)	<0.05 *¥Ω
3 Chamber View				
Basal inferior lateral wall	**-5.55** (-0.56 – -10.54)	**-7.83** (-3.94 – 11.72)	**-9.25** (-4.80 – -13.70)	<0.05 *
Mid inferior lateral wall	**-6.17** (-3.00 – -9.34)	**-9.53** (-4.55 – -14.51)	**-11.72** (-7.72 – -15.72)	<0.05 *¥Ω
Apical inferior lateral wall	**-7.19** (-2.99 – -11.39)	**-12.25** (-6.22 – -18.28)	**-16.56** (-11.45 – -21.67)	<0.005 *¥Ω
Basal anterior septum	**-5.31** (-2.26 – -8.36)	**-7.81** (-4.55 – -11.07)	**-10.78** (-6.97 – -14.59)	<0.001 *¥Ω
Mid anterior septum	**-6.25** (-2.46 – -10.04)	**-9.06** (-4.45 – -13.67)	**-13.13** (-8.30 – -17.96)	<0.005 *¥Ω
Apical anterior septum	**-6.41** (-1.56 – -11.26	**-10.47** (-4.57 – -16.37)	**-15.78** (-9.45 – -22.11)	<0.005 *¥Ω
Global Longitudinal Strain	**-6.62** (-4.41 – -8.83)	**-9.03** (-6.28 – -11.78)	**-13.12** (-10.02 – -16.22)	<0.0001 *¥Ω

Table 4.2 – Left ventricular peak systolic strain versus AHF subcategory * = HFrEF versus HFpEF, ¥ = HFrEF versus HFmrEF, Ω= HFmrEF versus HFpEF.

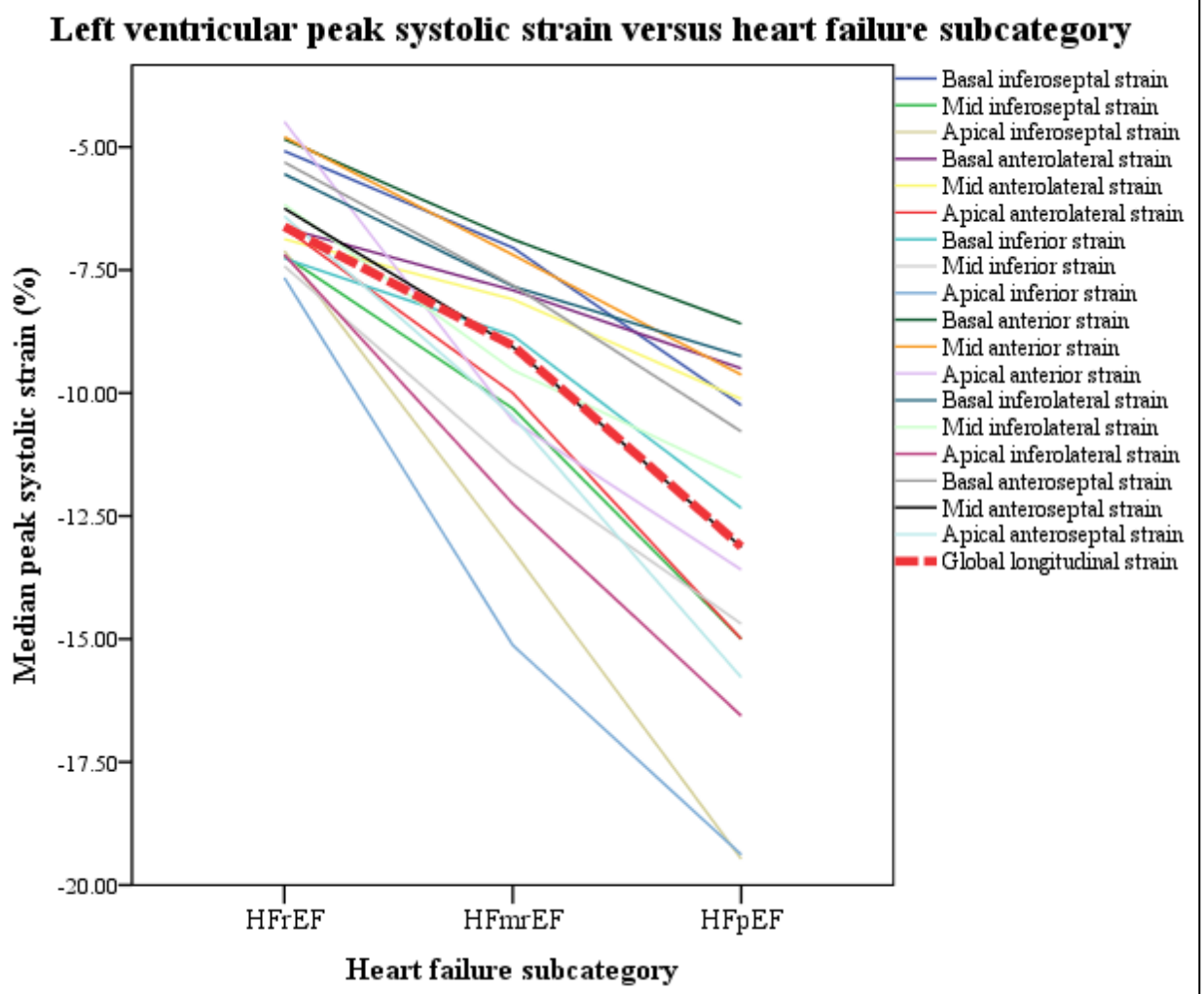

Figure 4.3 – Left ventricular peak systolic strain versus heart failure subcategory. Median peak systolic strain values increase (become more negative) from HFrEF to HFmrEF and then HFpEF in all myocardial areas assessed. Global longitudinal strain shows the same pattern.

4.5.3 Left ventricular myocardial deformation imaging – strain rate values

Frame rate values were comparable between groups. Median LV SR values were consistently statistically distinct between HF subcategories in almost all myocardial regions studied, with intermediate LV SR values in HFmrEF when compared to HFrEF and HFpEF. GLSR values were statistically and substantially different between groups; Median GLSR increased by nearly half from HFrEF to HFmrEF and nearly double from HFrEF to HFpEF (-0.48 (IQR -0.34 – -0.62) versus -0.66 (IQR -0.49 – -0.83) versus -0.87 (IQR -0.70 – -1.04), $p<0.0001$).

	HFrEF n = 180 (40.5%)	**HFmrEF** n = 103 (23.2%)	**HFpEF** n = 161 (36.3%)	**Significance (p)**
Mean frame rate, fps (SD)	**48** (±6)	**49** (±6)	**49** (±7)	0.07
Median strain rate, S^{-1} (IQR)				
4 Chamber View				
Basal inferior septum	**-0.44** (-0.31 – -0.57)	**-0.50** (-0.37 – -0.63)	**-0.72** (-0.50 – -0.94)	<0.05 *¥Ω
Mid inferior septum	**-0.52** (-0.67 – -0.67)	**-0.65** (-0.48 – -0.82)	**-0.85** (-0.63 – -1.07)	<0.0001 *¥Ω
Apical inferior septum	**-0.56** (-0.27 – -0.85)	**-0.86** (-0.56 – -1.16)	**-1.22** (-0.83 – -1.61)	<0.0001 *¥Ω
Basal anterior lateral wall	**-0.41** (-0.18 – -0.64)	**-0.56** (-0.39 – -0.73)	**-0.75** (-0.48 – -1.02)	<0.01 *Ω
Mid anterior lateral wall	**-0.41** (-0.26 – -0.58)	**-0.49** (-0.29 – -0.69)	**-0.70** (-0.45 – -0.95)	<0.0001 *Ω
Apical anterior lateral wall	**-0.47** (-0.21 – -0.73)	**-0.64** (-0.40 – -0.88)	**-0.93** (-0.56 – 1.30)	<0.01 *¥Ω
2 Chamber View				
Basal inferior wall	**-0.52** (-0.30 – -0.74)	**-0.65** (-0.42 – -0.88)	**-0.88** (-0.56 – -1.20)	<0.05 *¥Ω
Mid inferior wall	**-0.53** (-0.34 – -0.72)	**-0.70** (-0.49 – -0.91)	**-0.98** (-0.73 – -1.21)	<0.005 *¥Ω
Apical inferior wall	**-0.57** (-0.27 – -0.87)	**-0.86** (-0.55 – -1.17)	**-1.22** (-0.90 – -1.54)	<0.005 *¥Ω
Basal anterior wall	**-0.38** (-0.21 – -0.55)	**-0.54** (-0.32 – -0.76)	**-0.65** (-0.35 – -0.95)	<0.01 *¥
Mid anterior wall	**-0.35** (-0.16 – -0.54)	**-0.51** (-0.27 – -0.75)	**-0.62** (-0.33 – -0.91)	<0.01 *¥Ω
Apical anterior wall	**-0.41** (-0.14 – -0.68)	**-0.72** (-0.45 – -0.99)	**-0.91** (-0.56 – -1.26)	<0.05 *¥Ω
3 Chamber View				
Basal inferior lateral wall	**-0.44** (-0.25 – -0.63)	**-0.61** (-0.39 – -0.83)	**-0.74** (-0.45 – -1.03)	<0.01 *¥Ω
Mid inferior lateral wall	**-0.39** (-0.20 – -0.58)	**-0.58** (-0.35 – -0.81)	**-0.82** (-0.57 – -1.07)	<0.0001 *¥Ω
Apical inferior lateral wall	**-0.53** (-0.27 – -0.79)	**-0.78** (-0.47 – -1.09)	**-1.03** (-0.71 – -1.35)	<0.01 *¥Ω
Basal anterior septum	**-0.38** (-0.24 – -0.52)	**-0.48** (-0.19 – -0.67)	**-0.66** (-0.39 – -0.83)	<0.005 *¥Ω
Mid anterior septum	**-0.44** (-0.22 – -0.66)	**-0.61** (-0.36 – -0.86)	**-0.81** (-0.49 – -1.13)	<0.005 *¥Ω
Apical anterior septum	**-0.48** (-0.21 – -0.75)	**-0.74** (-0.40 – -1.08)	**-1.08** (-0.67 – -1.49)	<0.005 *¥Ω
Global longitudinal strain rate	**-0.48** (-0.34 – -0.62)	**-0.66** (-0.49 – -0.83)	**-0.87** (-0.70 – -1.04)	<0.00001 *¥Ω

Table 4.3 – Left ventricular systolic strain rate versus heart failure subcategory. * = HFrEF versus HFpEF, ¥ = HFrEF versus HFmrEF, Ω= HFmrEF versus HFpEF.

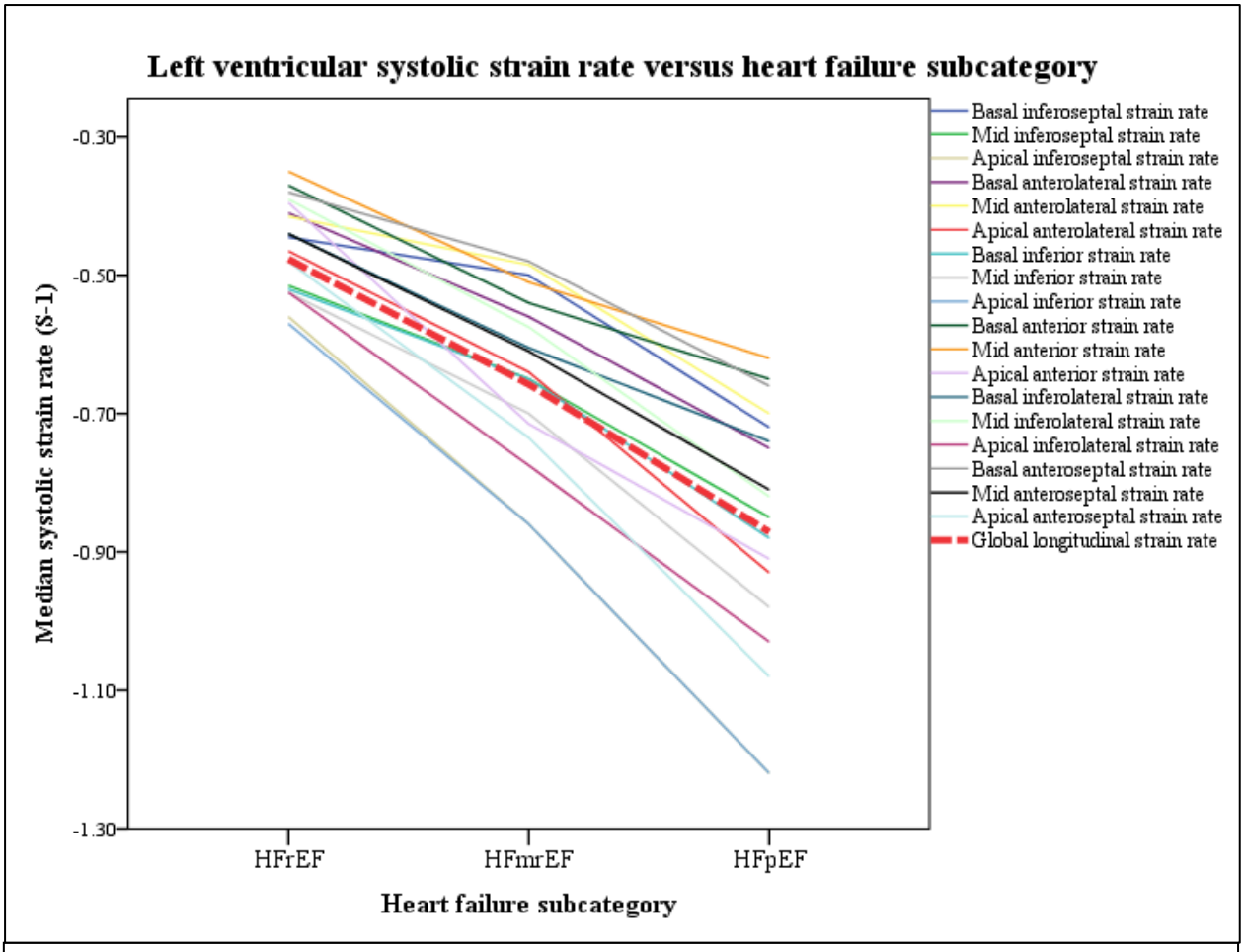

Figure 4.4 – Left ventricular strain rate versus heart failure subcategory. Median strain rate values increase (become more negative) from HFrEF to HFmrEF and then HFpEF in all myocardial areas assessed. Global longitudinal strain rate shows the same pattern.

4.5.4 Global Longitudinal Strain vs LVEF

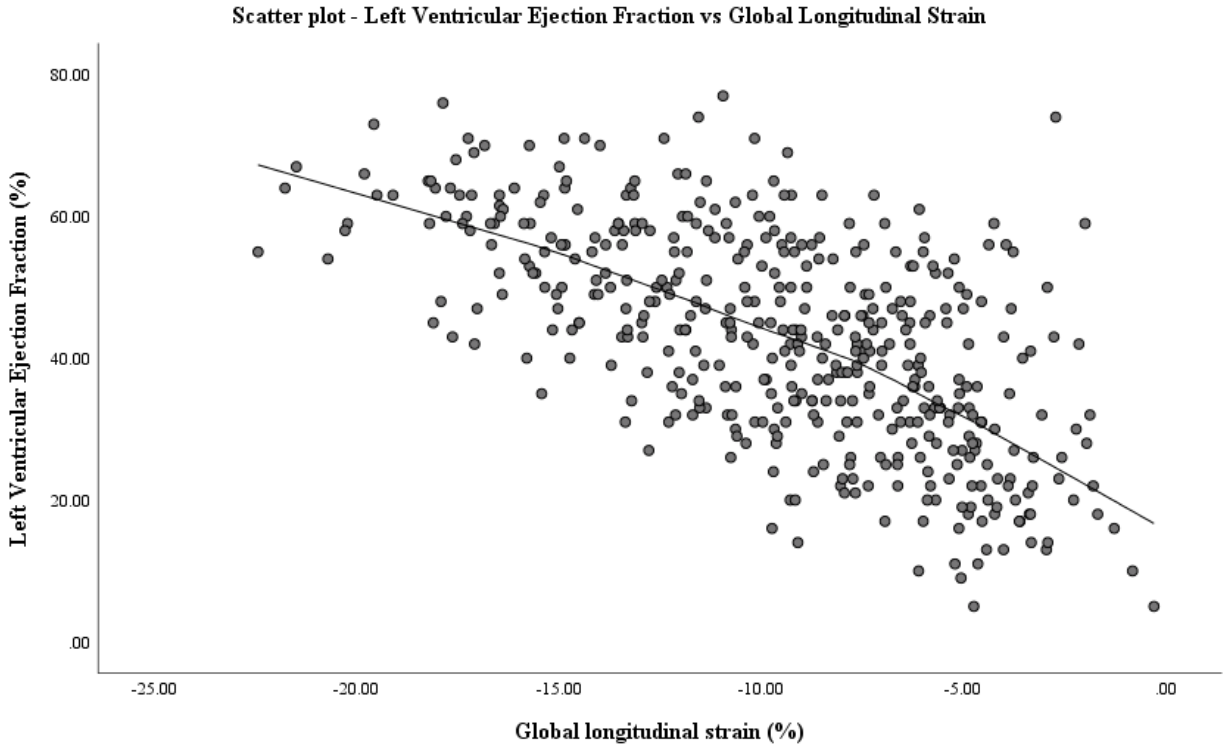

Figure 4.5 – Scatter plot – Left ventricular Ejection Fraction vs Global Longitudinal Strain. Left ventricular ejection fraction compared against global longitudinal strain. A Loess line of best fit demonstrates a curvilinear relationship at higher ejection fractions.

When LVEF is plotted against Global Longitudinal Strain there is a curvilinear relationship seen, with a gradual flattening of the Loess line of best fit curve at higher levels of LVEF.

4.5.5 Global Longitudinal Strain Rate vs LVEF

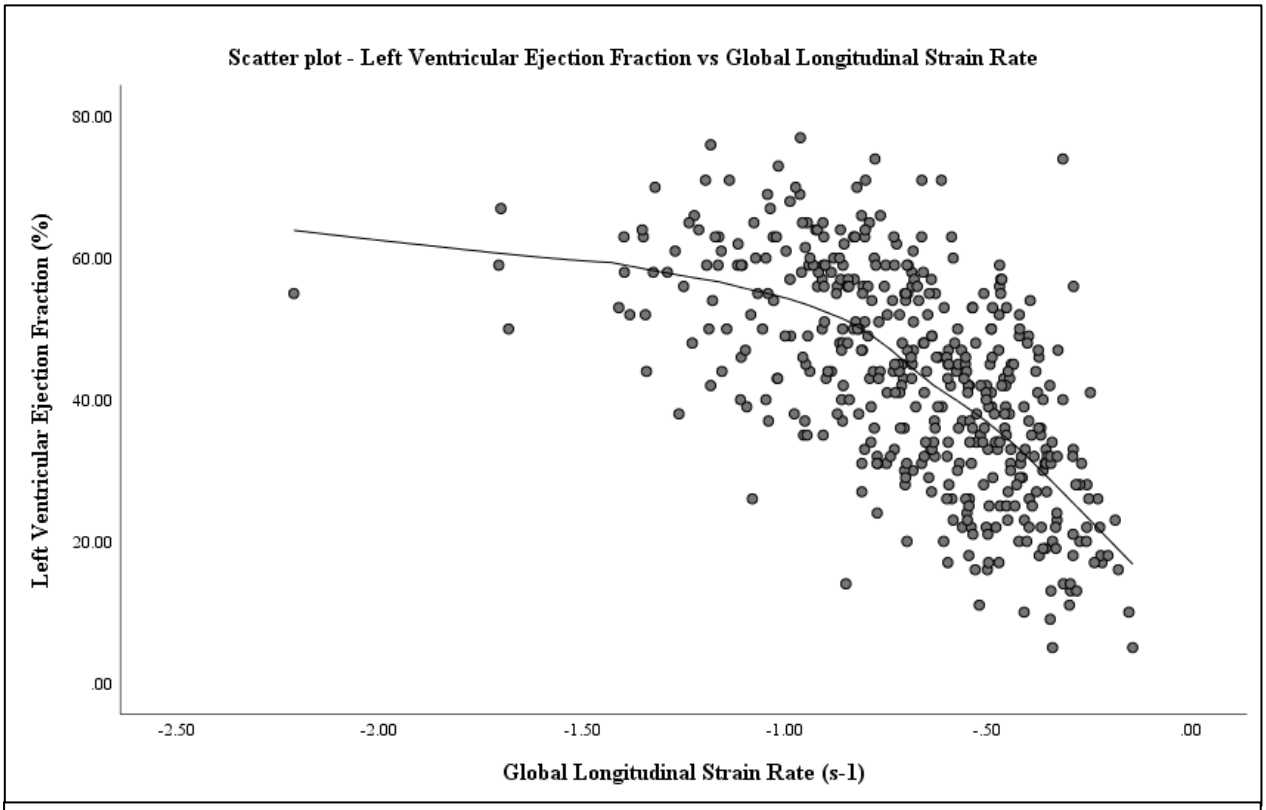

Figure 4.6 – Scatter plot – Left ventricular Ejection Fraction vs Global Longitudinal Strain rate. Left ventricular ejection fraction compared against global longitudinal strain rate. A Loess line of best fit demonstrates a curvilinear relationship at higher ejection fractions.

When LVEF is plotted against Global Longitudinal Strain Rate there is a curvilinear relationship seen, with a gradual flattening of the Loess line of best fit curve at higher levels of LVEF.

4.5.6 Time to recruitment and markers of left ventricular contractile function

Median LVEF values were significantly lower in patients recruited ≥2 days after admission (44% (IQR 33-55) versus 39% (IQR 24-54), $p<0.05$). This was not seen in terms of median GLS or GLSR values (both $p>0.05$).

Patients recruited to the study ≥2 days post admission were demographically similar with statistically comparable age and gender profiles. They were significantly more likely to have been admitted to hospital during a weekend (68.2% versus 21.9%, $p<0.0001$).

They had a similar burden of comorbidities and biochemical profiles were comparable between groups. They had significantly higher rate of mortality at 6 months post discharge (36.4% versus 24.6%, $p<0.05$).

A one-way ANCOVA was conducted to determine a statistically significant difference between patients recruited <2 days and ≥2 days post admission in terms of mortality controlling for variables including demographic profile, comorbidity burden, biochemical parameters and weekend admission. The statistically significant difference in mortality between the two groups is eliminated after performing an ANCOVA controlling for weekend admission, $F(1,441) = 3.279$, $p = 0.07$. This indicates that statistical differences in mortality are no longer significant when adjusting for the effect of weekend admission.

	Time to recruitment <2 days n = 315	**Time to recruitment ≥2 days** n = 132	**Significance (p)**
Demographics			
Median age, years (IQR)	**82** (76-88)	**81** (73-89)	0.62
Gender, % female	**48.9**	**44.7**	0.40
Weekend admission, %	**21.9**	**68.2**	<0.0001
Comorbidities, %			
HF	**58.4**	**57.6**	0.87
IHD	**38.4**	**36.4**	0.68
HTN	**56.5**	**51.5**	0.33
CKD	**44.1**	**52.3**	0.12
Biochemistry, median (IQR)			
Haemoglobin, g/L	**122** (107 – 137)	**119** (102 – 136)	0.30
Creatinine, µmol/L	**99** (70-128)	**115** (86 – 144)	0.14
Sodium, mmol/L	**139** (136 – 142)	**140** (136 – 144)	0.21
Potassium, mmol/L	**4.4** (4.0 – 4.8)	**4.5** (4.1 – 4.9)	0.08
Urea, mmol/L	**9.0** (6.1 – 11.9)	**9.9** (6.2 – 13.8)	0.07
POCT BNP Tertile:			
100-999 pg/ml, %	**54.9**	**47.7**	0.32
1000-2999 pg/ml, %	**34.3**	**37.9**	
≥3000 pg/ml, %	**10.8**	**14.4**	
Outcomes			
6-month readmission, %	**61.7**	**53.0**	0.09
6-month mortality, %	**24.6**	**36.4**	<0.05
Echocardiographic parameter, median (IQR)			
LVEF, %	**45** (34-56)	**41** (30-52)	<0.05
GLS, %	**-9.15** (-5.97 – -12.33)	**-9.10** (-5.64 – -12.56)	0.44
GLSR, S^{-1}	**-0.65** (-0.45 – -0.85)	**-0.66** (-0.46 – -0.86)	0.59

Table 4.4 – A comparison of markers of left ventricular contractile function according to time recruited post admission to hospital.
CKD – Chronic kidney disease, GLS – Global longitudinal strain, GLSR – Global longitudinal strain rate, HF – Known heart failure, HTN – Hypertension, IHD – Ischaemic heart disease, IQR – Interquartile range, LVEF – Left ventricular ejection fraction, POCT BNP – Point of care test B natriuretic peptide.

4.6 Discussion

This chapter has demonstrated differences found in myocardial deformation parameters between HF subcategories in the MRAHF cohort. It has also demonstrated the feasibility and applicability of myocardial deformation imaging in the AHF setting.

Myocardial deformation imaging has traditionally been constrained by difficulties achieving adequate frame rates. In a research setting it has typically been performed with frame rates approximating 200 frames per second due to concerns about the introduction of statistical noise in strain rate values when frame rate falls below 100 frames per second. It has been demonstrated that strain values can be robustly acquired at lower frame rates of 50-60 Hz (Rösner, Barbosa et al. 2015), but concerns have been raised concerning estimating strain rate values at this speed (Mirea, O., Duchenne et al. 2016).

In this study of AHF patients, in the acute setting with a standard echocardiography protocol, frame rates of 30-50 frames per second were typical. Even with these frame rates values, useful strain and strain rate values can be derived from the dataset; the results from the study demonstrate the ability of strain and strain rate values to discriminate between patients with varying gross contractile function as assessed by LVEF and this may suggest a further use for myocardial deformation values outside of the carefully constrained research settings.

As has been described for many other clinical, demographic, biochemical and echocardiographic factors, in the MRAHF cohort HFmrEF appears to be an intermediate group, with both strain and strain rate values located between those of HFrEF and HFpEF and statistically distinct from both groups. Both left ventricular strain and strain rate values appear to vary significantly according to LVEF-based subcategorization of HF. Throughout all measured segments of the LV, there is either a statistically significant difference between each group or demonstration of a trend from lowest deformation values in HFrEF to highest in HFpEF.

Both strain and strain values demonstrate a trend, with the least negative values seen in HFrEF, most negative in HFpEF, and HFmrEF acting as a statistically distinct intermediate class. The differences between median values of each subcategory are large, with both strain and strain rate values increased by between 50-100% in HFpEF compared to HFrEF and HFmrEF values intermediate between these values.

The trends demonstrated in both strain and strain rate values are consistent throughout the different segments of the myocardium in each of the assessed views, and this is represented by the trend seen in GLS and GLSR values.

These trends are more frequently statistically significant for strain rate values than strain values. This may be an artefact and could resolve with a greater quantity of measurements but may reflect the fact that strain rate is less dependent upon contemporaneous haemodynamic status. As such, strain rate values may be more likely to demonstrate statistically significant intrinsic contractile differences present between each subcategory. This is consistent with the known data on the relative load dependence of strain versus strain rate values (Ferferieva, Van den Bergh et al. 2011, Greenberg, Firstenberg et al. 2002, Yingchoncharoen, Agarwal et al. 2013), but also with data demonstrating that strain and strain rate values correlate well with accurately and consistently measured LVEF (Adamo, Perry et al. 2017).

When plotting GLS vs LVEF and GLSR vs LVEF in Figures 4.5 and 4.6 respectively, in both cases we see that the Loess line of best fit demonstrates a curvilinear relationship with flattening of the curve at higher ejection fractions. This is consistent with previous data from other research groups who demonstrate a curvilinear relationship between GLS/GLSR and LVEF (Pedrizzetti, Mangual et al. 2014, Yip, Zhang et al. 2011, Dalen, Thorstensen et al. 2009). This has particularly been demonstrated to be of use in patients with pathological vs physiological hypertrophy and patients with HFpEF compared to those without; in both cases, those with pathology demonstrate depressed GLS compared to their control counterparts (Wasfy, Weiner 2015, Ersbøll, Valeur et al. 2013).

Patients recruited ≥2 days post admission were demonstrably similar in terms of their demographic, comorbidity and biochemical profiles to those recruited <2 days, but did have a higher rate of mortality. Due to the gross similarities it is unlikely that this patient group is phenotypically or pathologically distinct, but the discrepancy in mortality may be explained by the fact that the majority of them were admitted to hospital on a weekend, a recognised risk factor for higher mortality (Kostis, Demissie et al. 2007, Sharp, Choi et al. 2013). Rather than representing a group with different anatomical profile and higher mortality, this may be a function of the effect of variance in weekend healthcare provision. In the MRAHF cohort, when adjusting for the effect of weekend admission, these differences in mortality were no longer statistically significant.

Despite the fact that patients from both groups shared similar demographic, comorbidity and biochemical profiles, there is a statistically significant difference between their LVEF values which is not seen in terms of GLS and GLSR values. LVEF values are known to be heavily affected by preload (Konstam, Abboud 2017) and acute management of AHF is typically centred around diuresis which leads to a fall in cardiac preload. As a result, LVEF typically falls in accordance with the Frank-Starling mechanism and this may be the cause of the fall in LVEF seen in patients recruited later in their hospital stay.

If this reduction in median LVEF is purely due to diuresis, this change is not only statistically significant but the effect size is clinically relevant, as the median patient is almost recategorised from HFmrEF to HFrEF which would entirely change their prospective treatment pathway. The ESC guidelines recommend that patients with AHF undergo echocardiography within 48 hours (Ponikowski, P., Voors et al. 2016); if LVEF is variable in the acute phase and likely to fall after this period, prognostic management guided solely by the LVEF measured within 48 hours may lead to the inappropriate omission of prognostic medications for patients whose LVEF subsequently falls post diuresis.

In contrast with LVEF, GLS and GLSR values are not significantly different in patients recruited ≥2 days post admission. This is consistent with previous literature in which both values, but particularly GLSR, have been demonstrated to be less load dependent (Ferferieva, Van den Bergh et al. 2011). As a result, these parameters are more consistent across time and may better represent intrinsic contractile function of the patient independent of acute haemodynamic profile. One should note that these findings are not proof of a causal relationship but serve as an interesting marker and guide to further work and assessment of these findings.

The differences in deformation parameters between the subgroups are interesting for their own sake and for the understanding of the underlying pathologies but may also be useful in terms of aiding diagnosis, subcategorisation and discriminating between HF categories. Strain and strain rate parameters are discriminators between subgroups and are consistent across time periods unlike LVEF. This is valuable given that there are currently only evidence-based pharmacotherapeutic interventions for the HFrEF subgroup. Inadvertently commencing therapies in patients unlikely to benefit from them will likely only increase the frequency of well-recognised side effects of prognostic medications such as bradycardia and

hyperkalaemia with no morbidity or mortality benefit. The corollary of that is that the failure to start therapies in patients who would benefit will inevitably worsen their prognosis.

Stratifying HF patients using deformation imaging values in addition to standard LVEF assessment could add additional diagnostic weight and accuracy given that the supplementary information is derived from a preload-independent source. For instance, a patient with an LVEF of 42% but poor strain and strain rate values may be better placed in the HFrEF group rather than HFmrEF due to the evidence of underlying intrinsic contractile dysfunction. Furthermore, the combination of LVEF and deformation imaging may allow us to better subcategorise patients with AHF according to loss of intrinsic myocardial contractile function, rather than due to the gross change in cavity size, with the inherent flaws as described earlier. Averaged GLS and GLSR values demonstrate less statistical variance than individual segment data when comparing between the various vendors of strain and strain rate software (Mirea, Oana, Pagourelias et al. 2017), and this may prove a more fruitful avenue to pursue.

This has already been suggested in the context of the intensive care setting or to aid novice echocardiographers (Benyounes, Lang et al. 2015), whilst the ESC guidelines had previously recognised that ‘deformation imaging is more sensitive than ejection fraction in detecting minor changes in LV systolic function’ (McMurray, John JV, Adamopoulos et al. 2012). Given that small differences in LVEF can have an important impact on management and outcome this is not something that should be overlooked. As limitations in its use such as inter-vendor or software variability and consensus on normal values improve, opinion is shifting to suggest an increased use for deformation imaging (Duncan 2015). Since 2015, guidelines have recommended the use of GLS and GLSR values in many cardiovascular conditions, including, but not limited to, assessment of patients with mitral valve pathology (Ponikowski, P., Voors et al. 2016), hypertension (Marwick, Gillebert et al. 2015), restrictive cardiomyopathies (Habib, Bucciarelli-Ducci et al. 2017) and prior to cardiotoxic chemotherapy in patients with cancer (Ponikowski, P., Voors et al. 2016), so extension of the assessment to patients with AHF is a realistic possibility.

Due to the variety of software programmes which can now automatically derive strain and strain rate measurements, the training required to produce these results at an expert level via offline data analysis has been suggested at 50 cases (Chan, Shiino et al. 2017)**,** and would not

represent an onerous addition to the routine training cardiac physiologists or clinicians. As such, adding myocardial deformation imaging values to the standard assessment would be feasible and may add additional discriminatory power for assessment of borderline cases. This would help to suggest or refute intrinsic contractile dysfunction, and thus enable us to appropriately classify the severity of systolic dysfunction in AHF patients.

4.6.1 Limitations

There are limitations intrinsic to the study. The mean frame rates achieved in the study are noticeably lower than those typically used in strain and strain rate studies. This is largely due to the use of a standard echocardiographic protocol in a standard acute clinical setting. As frame rates fall, strain, and particularly strain rate values become complicated by an increasing amount of statistical noise, and strain rate curves become less smooth in appearance due to loss of data points. While this is certainly true, the results above suggest that strain and strain rate values can still be analysed in the acute population to derive meaningful values, irrespective of lower frame rate speeds. It cannot be claimed that these values are an absolute representation of the underlying contractile function, but certainly as a discriminatory tool in AHF patients they appear to be consistent, with significantly different and widely variable values seen between subcategories.

There remains some caution in the interpretation of strain and strain rate values, particularly with reference to inter-vendor software variations. This has certainly been a concern historically but newer studies suggest this variation may be improving (Yang, Marwick et al. 2015). Indeed, inter-vendor and inter-operator differences between strain values are now significantly less than differences between LVEF measurements and other measurements such as E and E/A (Farsalinos, Daraban et al. 2015). There are, however, still difficulties in agreeing standardised norms for strain and strain rate values due to the small but statistically significant variation encountered when assessing similar patient cohorts using different machines and software (Farsalinos, Daraban et al. 2015). Deformation values have even been shown to vary between intra-vendor software upgrades (Nagata, Takeuchi et al. 2015). As such, the absolute values contained within this study may not be transferrable to other centres using different materials, however the trends described are likely still applicable and informative. Nevertheless, a consensus on strain and strain rate algorithms and their application would be beneficial in order to expand the use of deformation imaging. This was

recognised by the ESC and led to the first consensus document on speckle tracking imaging to attempt to improve uniformity of reporting (Voigt, Pedrizzetti et al. 2014).

While the above results refer to specific PSS and SR values, it is understood that specific patterns of strain and strain rate curves are of diagnostic and qualitative value in a variety of cardiac conditions with post-systolic shortening in ischaemic the best described (Smiseth, Torp et al. 2015). The results above focus very specifically on the utility of absolute PSS and SR values as this is perhaps easier to standardise and compare, but the qualitative value of strain and strain rate curve patterns should not be forgotten in clinical practice.

The MRAHF patient cohort is significantly older than the typical European patient as described in chapter three, and overwhelmingly white and Caucasian. This introduces difficulties in generalising the findings outlined here to a generalised European population. This must be acknowledged, but the demographic breakdown of the MRAHF study population is qualitatively similar to that described in the British National Heart Failure Audit in terms of age, gender and comorbidity burden (Donkor, Cleland et al. 2015), and thus is likely to be representative of the British AHF population.

4.6.2 Further Work

This study has demonstrated both the feasibility and utility of myocardial deformation in an AHF but would benefit from being repeated in other AHF populations with different demographic characteristics to assess the reproducibility and generalisability of the results reported here.

Verification of these findings with larger datasets, representative of different geographical populations, will be beneficial in assessing the extent to which these findings can be generalised throughout the AHF population, though there is no clear theoretical reason why other populations should act in a different manner.

Normal values for strain and strain rate are still not clear, particularly given the inter-vendor and inter-software variability. Consensus regarding algorithms and values would be of immense value if strain and strain rate are to be more widely used in assessment and management of the failing heart.

LVEF is lower in patients recruited after ≥2 days of admission while GLS and GLSR remain statistically similar between patients recruited at <2 days of admission. To clarify this finding, one could perform a study assessing LVEF, GLS and GLSR on all AHF patients at the time of admission and 2 days subsequent to admission. This would help clarify if LVEF does indeed fall in these patients while GLS and GLSR remain static. If this is the case, they may represent a more consistent marker of LV function, particularly in the acute phase.

If this is the case then the utility of using GLS/GLSR with LVEF versus LVEF in isolation could be assessed as a guide to prognostic management. If this was of additive benefit this could offer a more accurate and useful diagnostic or prognostic test which may improve the ability to accurately delineate and subcategorise patients with AHF, and thus focus therapeutic efforts upon patients more likely to benefit from their use. It would also help to shift the focus from a crude marker of systolic function to incorporate more precise and representative correlates of systolic function.

Ultimately, classifying and treating patients according to a preload-independent marker such as strain or strain rate values may be more accurate and more useful than LVEF. In order for this to be tested, one could envisage a randomised controlled trial in which patients were allocated to current guideline therapies according to strain or strain rate values, while the other arm is treated according to LVEF values. Changes in cardiac geometry, function and patient outcome could be compared between the two groups to assess the utility of categorising patients in this manner. The data may not yet exist to support such a study but this could represent a highly interesting avenue of research.

4.7 Chapter summary

This chapter has outlined the current literature regarding myocardial deformation parameters in HF and the gaps within the evidence. It has then recovered the materials and methods used in the MRAHF study to acquire and analyse the dataset.

There are large, statistically significant differences in myocardial deformation imaging values between HF subcategories. These findings add to the understanding of different HF phenotypes, particularly with a view to accurately distinguishing between the groups which can be challenging using standardly available and used diagnostic tools. In addition, LVEF values are significantly lower in patients recruited later in their hospital admission but

myocardial deformation values are not. This may suggest a use for either repeated measurement of LVEF or an enhanced role for deformation imaging in the acute phase.

Chapter Five: Prediction of 6-month mortality in an acute heart failure cohort using variables available upon admission to hospital

5.1 Chapter Introduction

This chapter describes the production of novel risk scoring tools, designed to help stratify 6-month mortality risk in patients with acute heart failure (AHF), early within their inpatient hospital admission.

This chapter first reviews the current literature and gaps in current understanding then outline what this study has aimed to address. It then outlines the materials and methods required to produce the risk scoring tools and describes the production of two separate scoring tools, one which includes echocardiographic data and one which does not. Both tools are demonstrated to have good calibration, predictive power and discriminatory ability. A comparison is then made of the improvement to the scoring models with the inclusion of biochemical data from Point of Care test B type natriuretic peptide levels. Finally, there is a discussion of the conclusions that can be drawn from the data and the limitations intrinsic to the study, and lastly suggestions for further work.

5.2 Current literature – evidence and omissions

The ability to predict risk of adverse outcomes in a given disease is essential in modern medicine. Therapeutic interventions are predicated on the concept that they reduce the risk of adverse outcomes, be that symptom burden, admission to hospital, or ultimately death. Similarly, clinical management decisions regarding intensification or relaxation of treatment are made based upon recognition of factors which confer prognostic risks to the individual patient. In order to know this, it is important to first understand the original mortality and morbidity risk of the underlying disease and the variables which contribute negatively to both.

The risk or probability of a particular outcome, be that hospital admission, disease manifestation or mortality, can be predicted using a number of statistical and arithmetic means. These include correlations, linear and logistic regressions to produce hazard and odds ratios. The conversion of these into statistical risk prediction models and simple clinical risk scores has become commonplace in many medical specialties.

Well-known risk stratification models include CURB-65 which predicts mortality in community acquired pneumonia (Lim, W. S., van der Eerden et al. 2003), the Blatchford score which predicts requirement for invasive intervention, transfusion and mortality in gastrointestinal bleeding (Blatchford, Murray et al. 2000) and the CHA_2D_2S VaSc score (Lip, Gregory YH, Nieuwlaat et al. 2010) which is used in patients with atrial fibrillation to predict risk of cerebrovascular accident and requirement for anticoagulative therapy. All three models use simple, easy-to-remember variables which are applicable by the emergency or acute medical physicians to aid risk stratification and subsequent patient management. In each study the authors emphasise the benefits of simplicity of their risk scoring formulae, noting how this translates into adoption of the risk scoring model.

Similar, if more complicated, scores are available for the chronic heart failure (CHF) population; online tools such as the Seattle risk score and Barcelona Bio-Heart Risk calculator use the input of more than 12 variables, both continuous and categorical, to produce readmission and mortality risk probabilities (Lupón, de Antonio et al. 2013, Levy, W. C., Mozaffarian et al. 2006).

As yet, there is no simple tool that can be used in the AHF population for prediction of mortality risk using data available upon admission. Many tools exist, but limitations exist in their application due to a variety of factors including, but not limited to, the inclusion of variables not available at admission (O'connor, Abraham et al. 2008b, Salah, Kok et al. 2014), the use of a very large number of predictive variables (Okazaki, Shirakabe et al. 2014), complex scoring algorithms (Lee, Austin et al. 2003), non-routine tests (O'Connor, Hasselblad et al. 2010) and the omission of echocardiographic data.

These factors can render it difficult to make early, evidence-based, management decisions regarding patient admission, treatment and follow-up care using the currently available risk-stratification tools.

This study describes the creation and application of two novel risk scoring tools for use upon acute admission, able to offer useful information about 6-month mortality risk with data easily obtainable at an early stage in the patient journey.

In doing so it aims to demonstrate the feasibility of such a task and suggest avenues for expansion of such a model with validation in a larger patient cohort.

5.3 Study hypotheses

This chapter will assess the following hypotheses:

a) Using data from the Mitral Regurgitation in Acute Heart Failure (MRAHF) cohort it is possible to produce a risk-scoring tool, able to give prognostic information to healthcare professionals regarding likely 6-month mortality risk in the AHF patient cohort.
b) It is possible to produce the above risk-scoring tool using information readily available to the acute physician directly upon admission to allow for early patient prognostication without necessitating recourse to specialist or complex data.

5.4 Methods

Chapter two describes the methods and materials used for study design and patient recruitment. Below is a brief summary of these with a specific emphasis on the statistical methods employed to produce a risk-stratifying and scoring tool.

5.4.1 Study design and population

This study uses data from the entire cohort of the MRAHF study. This is a prospective, cohort study of consecutive patients admitted to a single hospital site with signs and symptoms consistent with AHF as the primary driver of admission.

5.4.2 Inclusion and exclusion criteria

Inclusion criteria for the study:

1) Clinical signs and symptoms consistent with AHF as the primary cause for admission
2) Inpatient admission of <7 days by time of consent
3) The ability to give informed consent

Exclusion criteria for the study:

1) Point-of-care test (POCT) B natriuretic peptide (BNP) level <100 pg/ml
2) Echocardiography inconsistent with a diagnosis of HF

5.4.3 Patient recruitment and data capture

Patient recruitment began in July 2016 and ceased in September 2017. 616 patients were approached for recruitment, 500 patients gave informed consent to be recruited into the trial of whom 447 remained subsequent to exclusions. Data capture was carried out at the time of recruitment and included baseline demographic, biochemical, observational and radiological data including echocardiography as outlined in chapter two.

Mortality data, including date of death, for patients within the cohort was obtained from the online NHS Summary Care Record at 6-months post discharge. Cause of death for each patient was obtained from the patient's death certificate via either general practitioner (GP) records or, where unavailable, the local County Registrar's office.

5.4.5 Statistical analysis

5.4.5.1 Baseline characteristics

All statistical analysis was performed using SPSS version 24 as accessed at Royal Holloway University, with all statistical analysis researched, planned and performed by myself with subsequent checking of methods and suggestion for further analysis provided by Dr David Crook.

Continuous variables were tested for normality using the Shapiro-Wilk tests, and evidence of skew was inspected visually using histograms, Q-Q plots and box plots.

Continuous parametric variables were analysed using Student's T test when categorised as two groups. One-way analysis of variance (ANOVA) was used to compare data when more than 2 groups existed, as for AHF subcategories. Tukey's post-hoc test was used to ascertain specific inter-group variations within the ANOVA.

Statistical differences in non-parametric continuous variables were assessed using the Mann-Whitney U test or Kruskal-Wallis test when more than 2 groups were present with Dunn's post-hoc test used to assess for specific inter-group variations.

Pearson's Chi-squared test was used to assess for statistical differences between categorical variables.

Parametric continuous data are presented as a mean ± standard deviation. Non-parametric continuous data are presented as a median with interquartile range. Categorical variables are presented as a proportion within the population.

An alpha value of 0.05 was set as the threshold for statistical significance.

5.4.5.2 Risk prediction modelling

Logistic regression analysis cannot be performed using repeated measurements or matched data therefore the data from any repeat admission within the study period was excluded from analysis (Dayton 1992).

Logistic regression requires minimal assumptions regarding linearity, normality or homoscedasticity of the included variable. Assessment of multicollinearity is not mandatory in prediction models as one is not attempting to demonstrate causality, but rather produce a risk probability model.

Variables were selected according to clinical availability within 48 hours of patient admission to hospital and remaining variables were selected according to previously reported predictive value.

These variables were used in forward conditional binary logistic regression modelling to select the model that accounted for the greatest variance of mortality according to Nagelkerke R^2 values.

Each continuous independent variable included in the model was assessed for linearity with the logit transformation of 6-month mortality. This was performed using the Box-Tidwell test. All continuous variables demonstrated linearity and thus results of the Box-Tidwell test were not statistically significant.

Continuous variables which were selected for the model were then converted into categorical variables using values which provided maximum statistical discriminatory power. Where possible, these values were assessed at commonly used discriminatory or reference values. Binary logistic regression analysis was then repeated using only these derived categorical variables to assess for significance. Using the derived model, predictive scoring systems were produced using the odds ratios (OR) of each categorical variable to allocate points relative to

the other variables. In the context of a prospective cohort study, B(exp) is synonymous with the OR for each variable. Variables for whom the exponentiation of the coefficient (B(exp)) values produced were <1.000 were inverted to simplify comparison of B(exp) values.

Having assessed model association statistically, tests of model performance were then performed.

The total score for each patient was then calculated using the model. The predictive utility of the scores were assessed using receiver operator characteristic curve area under the curve (ROCAUC) analysis. This compared the patients' risk score, and thus their predicted mortality risk to actual 6-month mortality. The ROCAUC is equal to the concordance statistic or concordance index (C-Index) for binary outcomes as assessed in this study. The C-index is the standard measure of a predictive model's goodness of fit in a binary logistic regression and can be defined as the estimated probability that the predictive model will predict higher risk of event in a 'case' (6-month mortality) than in a 'control' (alive at 6-months). A C-index of 0.500 would indicate that the model is no better than chance at predicting the increased probability of case vs control, whereas a C-index of 1.000 would demonstrate that in 100% of pairings the predictive model would indicate a higher risk of an event for a 'case' than a 'control' (Uno, Cai et al. 2011).

The accuracy of the predictive score was assessed according to values as described in Table 5.1, as has been standardly reported in clinical literature (Obuchowski 2003, Metz 1978).

C-Index	Predictive accuracy
0.900-1.000	Excellent
0.800-0.899	Good
0.700-0.799	Fair
0.600-0.699	Poor
0.500-0.599	Worthless test

Table 5.1 C-Index ranges and their associated predictive accuracy.

Actual patient mortality was then assessed for each individual risk score. Risk scores were then grouped into three categories in which there were different levels of 6-month mortality

risk, namely <10%, 10-29% and ≥30%. These point score ranges were then denoted as low, medium and high-risk for 6-month mortality.

The discriminatory value of these three points' categories was demonstrated using Kaplan-Meier survival curves and statistical independence was determined using Pearson's Chi-squared test.

The risk modelling process was performed first using only clinical data, and then subsequently repeated including echocardiographic variables to assess the predictive value of echocardiographic data.

Net reclassification index (NRI) analysis was performed to assess the additional discriminatory power of the model which included echocardiographic variables.

5.5 Results

5.5.1 Baseline characteristics

52 cases were excluded due to repeat recruitment into the study. Baseline characteristics of the study cohort post these exclusions are included below in Table 5.2.

Excluded cases were typically of a higher median age (83 years (IQR 79-87) versus 81 years (IQR 75-87), $p<0.05$), were more likely to have known HF (96.2% versus 53.2%, $p<0.0001$) and were more likely to have been admitted in the previous year for any cause (80.8% versus 35.9%, $p<0.0001$). Otherwise they shared similar demographic, comorbidity and outcome profiles.

After exclusions, the median age of cases remaining for inclusion in risk modelling was 81 years (IQR 75-87), 47.1% of the cohort were female.

Median length of stay on index admission was 6 days (IQR 3-9). At 6 months 58.5% of patients had been readmitted, 22.6% due to decompensation of HF and 27.7% of patients had died, 13.0% due to cardiovascular causes.

	Whole cohort	HFrEF	HFmrEF	HFpEF	Significance (p)
N	392 (100%)	156 (40.5%)	91 (23.2%)	145 (36.3%)	-
Demographics					
Median age, years (IQR)	81 (75-87)	80 (72-88)	83 (77-89)	81 (74-88)	0.07
Gender, % female	46.9	38.5	46.2	56.6	<0.01 [a,c]
European Caucasian, %	92.1	93.6	89.0	92.4	0.43
Median BMI, kg/m^2 (IQR)	27.3 (22.8-31.8)	26.5 (22.5-30.5)	26.7 (22.7-30.7)	28.6 (24.0-33.2)	0.10
Hospitalisation in last year, %	36.0	36.5	30.8	38.6	0.47
Median length of stay on index admission, days (IQR)	6 (3-9)	7 (3-11)	5 (2-8)	6 (3-9)	0.18
Comorbidities, %					
HF	52.8	51.9	59.3	50.3	0.36
IHD	36.2	44.9	29.7	31.0	<0.05 [a]
HTN	55.6	57.1	49.5	57.9	0.39
DM	30.4	29.5	26.4	33.8	0.46
CKD	45.2	48.1	49.5	40.0	0.28
COPD	14.0	10.3	13.2	17.9	0.21
CVA	15..1	14.7	12.1	17.9	0.43
6-month outcomes, %					
All-cause readmission	58.5	62.2	56.7	54.9	0.35
HF readmission	22.5	22.4	22.2	22.7	0.99
All-cause mortality	27.7	30.8	20.0	29.2	0.17
CVD mortality	12.8	15.9	9.2	11.7	0.29

Table 5.2 – Baseline characteristics of the AHF cohort used for model production post exclusions of data from patients' repeat admissions.
a – HFrEF, b – HFmrEF, c – HFpEF
BMI – Body mass index, CKD – Chronic kidney disease, COPD – Chronic obstructive pulmonary disease, CVA – Cerebrovascular accident, CVD – Cardiovascular disease, DM – Diabetes mellitus, HF – Heart failure, HTN – Hypertension, IHD – Ischaemic heart disease, IQR – Interquartile range.

5.5.2 Variable selection

All 316 collected variables in the dataset were initially considered for inclusion. 251 variables which would not be available to the clinician within 48 hours of admission were excluded. One variable with <50% data available was excluded (Abbreviated Mental Test Score (AMTS)). POCT BNP level was excluded from the main models as it is typically replaced by laboratory BNP values in clinical practice and laboratory BNP values were not recorded in this study.

A literature search was performed to search for evidence of the predictive value of the remaining 63 variables. Variables with no evidence within the literature of increasing mortality risk in HF were excluded. 33 variables remained of which 7 were collected from echocardiography. The variables included in model assessment and production and can be seen in Table 5.3. All variables were collected and are defined according to the methods described in chapter two.

Demographic	Medications on admission	Clinical Observations	Common investigations
Age, years [1]	Mineralocorticoid receptor antagonist [5]	NYHA functional class, I-IV [2]	ECG Rate, bpm [1]
Sex [2]	ACE inhibitor [6]	SBP, mmHg [1]	ECG Rhythm [1]
BMI, Kg/m^2 [3]	Angiotensin receptor blocker [7]	DBP, mmHg [6]	ECG QRS duration, ms [8]
Weekend admission [4]	Beta blocker [6]		

Clinical History	Biochemistry	Echocardiogram
Previous admissions – all cause, and HF [9]	Creatinine, µmol/L [6]	LVEF, % [6]
Cause of acute decompensation [2]	Haemoglobin, g/L [1]	RVFAC, % [11]
Known HF [6]	Sodium, mmol/L [1]	TAPSE, cm [12]
Known IHD [1]	Potassium, mmol/L [1]	SPAP, mmHg [13]
Known CKD [6]	Urea, mmol/L [1]	Severity of mitral regurgitation, I-IV [14]
	VBG pH [10]	Severity of tricuspid regurgitation, I-IV [14]
	VBG lactate, mmol/L [10]	Left atrial area in systole, cm^2 [15]

Table 5.3 – Retained variables used in binary logistic regression to produce a scoring model. Numeric suffixes denote the literature evidence supporting their use in HF prognostication.

ACE – Angiotensin converting enzyme, BMI – Body mass index, CKD – Chronic kidney disease, DBP – diastolic blood pressure, ECG – electrocardiogram, HF – Heart failure, IHD – Ischaemic heart disease, LVEF – Left ventricular ejection fraction, NYHA – New York Heart Association, RVFAC – Right ventricular fractional area change, SaO2 – Blood oxygen saturation, SBP – Systolic blood pressure, SPAP – systolic pulmonary artery pressure, TAPSE – Tricuspid annular plane systolic excursion, VBG – Venous blood gas.

1 – (Lee, Austin et al. 2003), 2 - (Levy, W. C., Mozaffarian et al. 2006), 3 - (Oreopoulos, Padwal et al. 2008), 4 - (Horwich, Hernandez et al. 2009), 5 - ((Pitt, Zannad et al. 1999), 6 - (Siirilä-Waris, Lassus et al. 2006), 7 - (Pocock, Wang et al. 2005), 8 - (Harjola, Follath et al. 2010), 9 - (Gheorghiade, Vaduganathan et al. 2013), 10 - (Park, Choi et al. 2015), 11 - (Melenovsky, Hwang et al. 2014), 12 - (Burke, Katz et al. 2014), 13 - (Komajda, Jais et al. 1990), 14 - (Grayburn, Appleton et al. 2005), 15 - (Quiñones, Greenberg et al. 2000).

Two models were then assessed – one which omits echocardiographic variables (Clinical model) and one including all variables (Clinical & Echo model).

5.5.3 Clinical model

5.5.3.1 Model production

Forward conditional binary logistic regression was performed for prediction of 6-month mortality using the variables in Table 5.3 omitting echocardiography. The model produced is included below in Table 5.4.

Variable	Significance (p)	Exp(B)	95% C.I for Exp(B)	
			Lower	Upper
Age	<0.0001	1.087	1.049	1.127
BMI	<0.0001	0.940	0.910	0.970
Sodium	<0.05	0.939	0.888	0.993
Urea	<0.0001	1.101	1.046	1.159
SBP	<0.005	0.977	0.964	0.990

Table 5.4 – Predictive model produced using continuous variables.
Exp(B) – Exponentiation of the coefficient, SBP – Systolic blood pressure.

All potential continuous variables were converted to binary categorical variables and the forward conditional regression was repeated to determine a model. This model can be seen in Table 5.5. Of the 7 categorised causes of decompensation, only arrhythmogenic/non-arrhythmogenic decompensation was found to contribute significantly to the prognostic model with non-arrhythmogenic HF conferring an OR of 2.537 compared to arrhythmogenic HF.

Variable	Significance (p)	Exp(B)	95% C.I for Exp(B)		Nagelkerke R^2	Variance accounted for by each variable, %
			Lower	Upper		
Age ≥80 years	<0.005	2.717	1.541	4.794	0.094	9.4
Admission in previous year	<0.01	2.026	1.189	3.449	0.157	6.3
Non-arrhythmogenic decompensation	<0.01	2.537	1.257	5.122	0.199	4.2
BMI <26 kg/m^2	<0.05	1.847	1.078	3.165	0.242	4.3
Known CKD	<0.05	1.749	1.003	3.051	0.261	1.9
Sodium <135 mmol/L	<0.05	2.122	1.166	3.859	0.278	1.7
Urea ≥12 mmol/L	<0.0001	2.642	1.489	4.689	0.295	1.7
SBP <130 mmHg	<0.005	2.364	1.408	3.967	0.308	1.3

Table 5.5 - Predictive model produced after conversion of continuous variables into categorical variables.
BMI – Body mass index, CKD – Chronic kidney disease, Exp(B) – Exponentiation of the coefficient, SBP – Systolic blood pressure.

5.5.3.2 Regression Coefficient

The regression coefficient of the model produced is described below:

Regression coefficient = -4.248 + 0.971*Non-Arrhythmogenic + 0.588* Known CKD + 0.721* Admission in last year +0.779* Na<135 mmol/L + 1.034*Age ≥80 + 0.622* BMI <26 Kg/M^2+ 1.008*Urea ≥12 mmol/L + 0.877* SBP<130 mmHg

Boundary score for group membership, i.e. prediction of alive or dead, is 0.50.

5.5.3.3 Model Calibration

Prior to modelling the null model predicts that all patients will remain alive as seen in table 5.6.

		Predicted mortality		**Percentage Correct**
		Alive	**Dead**	
Observed 6-month mortality	**Alive**	283	0	100.0
	Dead	108	0	0.0
Accuracy				72.4
Table 5.6 – Clinical model calibration plot – prior to addition of predictive variables.				

Table 5.7 demonstrates the changes to the predicted vs observed outcomes after application of the clinical model:

		Predicted mortality		**Percentage Correct**
		Alive	**Dead**	
Observed 6-month mortality	**Alive**	259	24	91.5
	Dead	67	41	38.0
Accuracy				76.7
Table 5.7 -Clinical model calibration plot – post addition of predictive variables.				

Table 5.8 compares the diagnostic test statistics pre and post application of the clinical model:

Test statistic	**Pre-model value**	**Post-model value**
Sensitivity	0.0%	38.0%
Specificity	100.0%	91.5%%
Positive Predictive Value	NA	63.1%
Negative Predictive Value	72.4%	79.5%%
Accuracy	72.4%	76.7%%
Table 5.8 – Comparison of diagnostic test statistics pre and post application of the clinical model		

Table 5.8 demonstrates substantial improvements in test sensitivity, positive predictive value, negative predictive value and test accuracy, while specificity falls from 100% to 91.5%.

The Hosmer and Lemeshow test was not statistically significant for the model, suggesting good statistical calibration (N = 391, X^2 = 9.975, p = 0.267).

The cumulative Nagelkerke R^2 of the model produced can be seen in Figure 5.1.

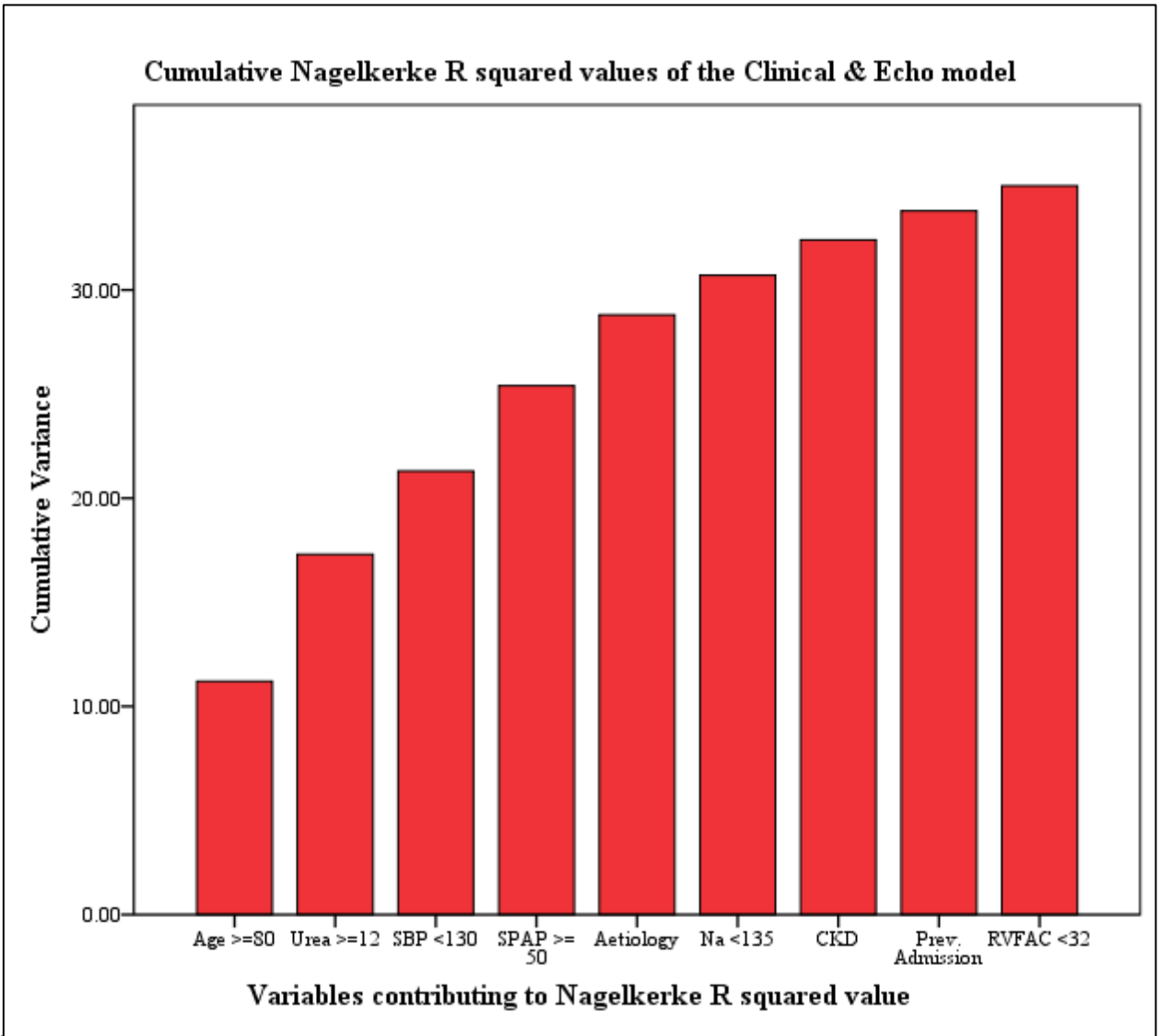

Figure 5.1 – Cumulative Nagelkerke R^2 values of the variables included in the clinical model
BMI – body mass index, CKD – chronic kidney disease, Na – sodium, SBP – Systolic blood pressure.

5.5.3.4 Risk score point allocation

Each variable was allocated a point value according to the ratio of exponentiation of the coefficients (Exp (B)) which are equivalent to the OR. One point was allocated per variable

as all OR were approximately on a 1:1 ratio. The minimum score possible per patient is 0 and maximum is 8.

5.5.3.5 Discriminatory ability - Receiver operator curve assessment

Risk score was calculated for each patient in the dataset. Predictive power for mortality of the risk scores were assessed using a receiver operator curve (ROC). Area under the curve statistics are presented in Table 5.9 and the ROC is shown in Figure 5.2.

			Asymptotic 95% confidence interval	
Area under the curve	**Standard error**	**Asymptotic significance (p)**	**Lower bound**	**Upper bound**
0.746	0.026	<0.0001	0.695	0.798

Table 5.9 – Area under the curve statistics for the Clinical model.

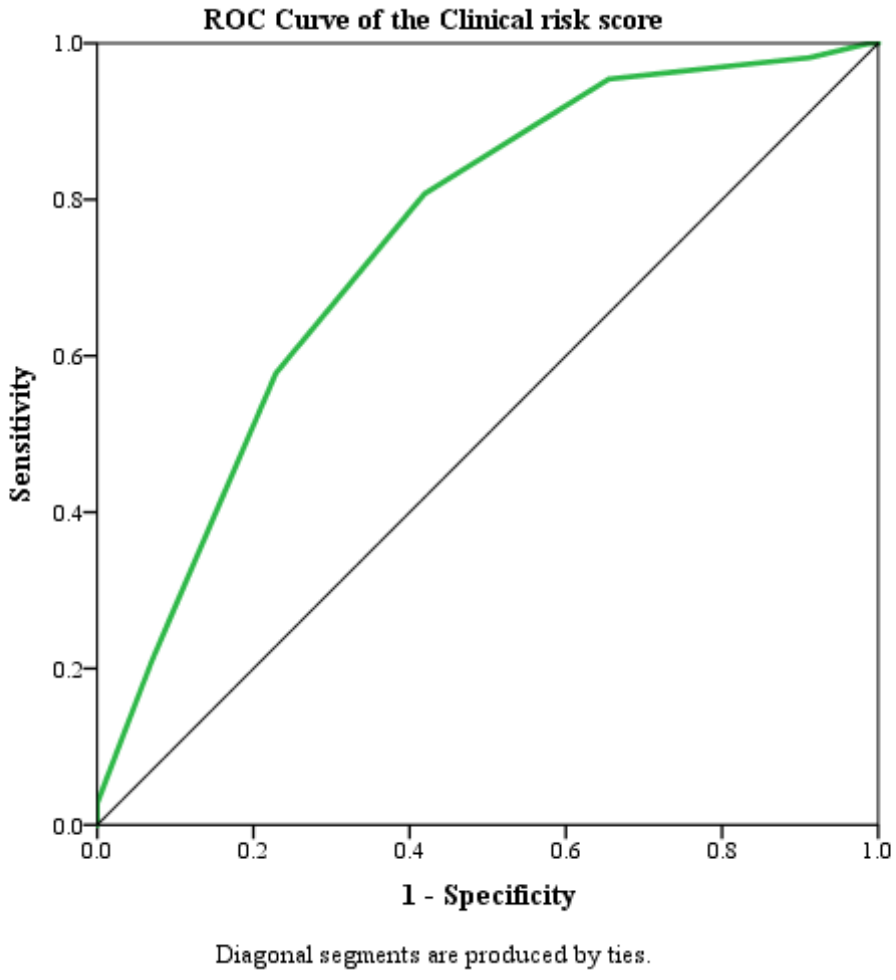

Figure 5.2 – Receiver operator curve for the Clinical model. The black diagonal line is the 50% reference.

5.5.3.6 Score stratification

Frequency of mortality was assessed for each point score from 0-8 and can be seen below in Table 5.10.

Clinical risk score	N	6-month mortality, %
0	3	0.0
1	24	8.3
2	76	3.9
3	83	19.3
4	79	31.6
5	85	47.1
6	36	50.0
7	4	50.0
8	3	100.0

Table 5.10 – Percentage 6-month mortality of each Clinical risk score.

Clinical risk score values were then grouped together and stratified into low, medium and high risk. Low risk was defined as 6-month mortality <10%, medium risk as 6-month mortality of 10-29% and high risk as 6-month mortality ≥30%. This corresponded with Clinical risk scores of 0-2, 3-4 and ≥5 respectively.

Chi squared testing demonstrated that these scores allowed for statistically significant discrimination of mortality risk between groups; mortality in the low risk group was 0%, 25.6% in the medium risk group and 65.6% in the high-risk group ($p<0.0001$).

Risk Group	N	Mortality	Significance (p)
Low	103	4.9%	<0.0001
Medium	162	25.3%	
High	128	49.2%	

Table 5.11 – Risk stratification table for the Clinical risk score demonstrating mortality risk in each group.

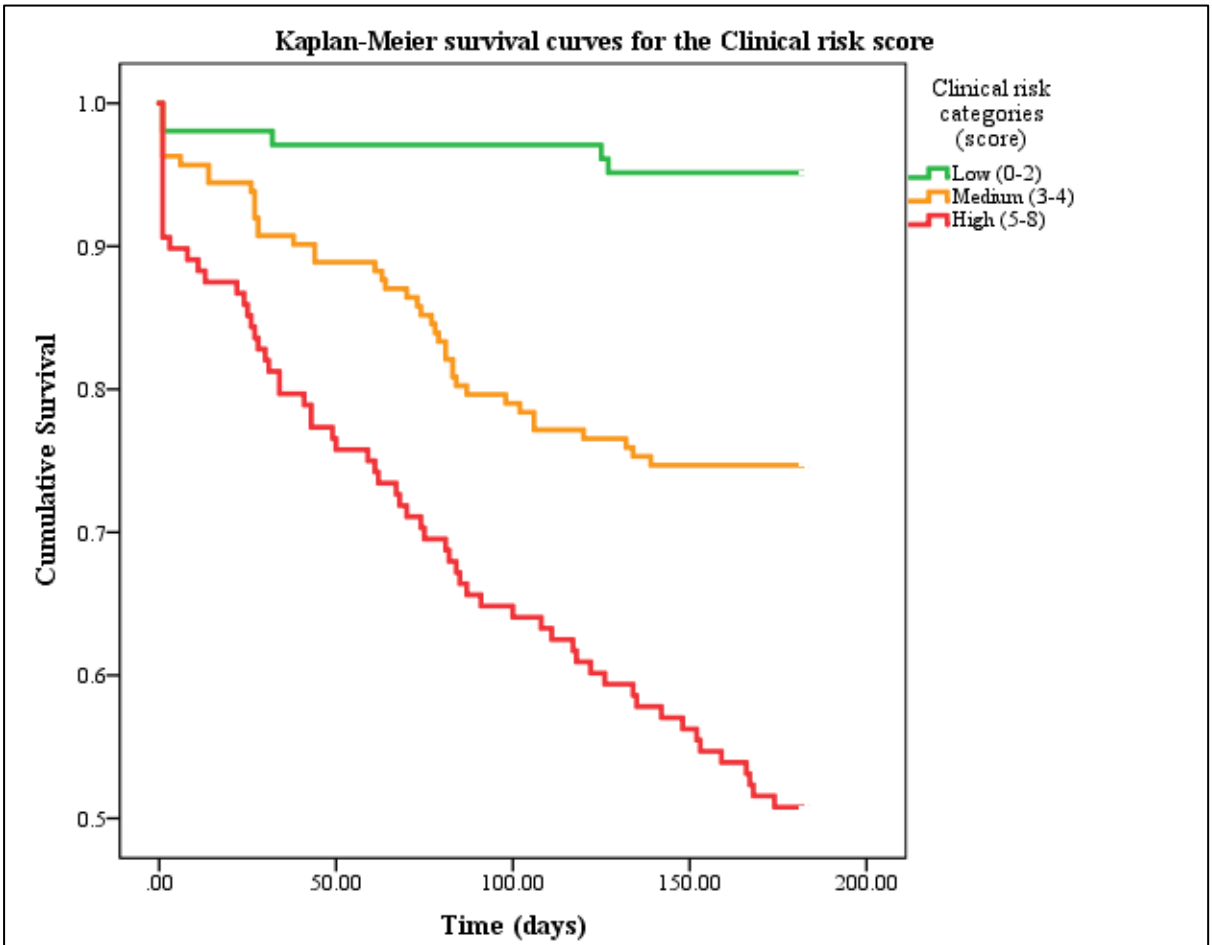

Figure 5.3 – Kaplan-Meier curve demonstrating survival versus Clinical risk score category.

5.5.3.7 Clinical risk score summary

The Clinical risk score can be summarised thus:

Risk Variable		**Point score**
Age ≥80 years		1
Hospital admission in previous year		1
Non-arrhythmogenic aetiology of HF		1
BMI <26 kg/m^2		1
Known CKD		1
Sodium <135 mmol/L		1
Urea ≥12 mmol/L		1
Systolic blood pressure <130 mmHg		1
Points scored	**Risk category**	**6-month mortality risk, %**
0-2	Low	4.9
3-4	Medium	25.3
5-8	High	49.2

Table 5.12 – Summary table of the Clinical risk score including variables, allocated point score per variable and risk category stratification table for the Clinical model.
BMI – Body mass index, CKD – Chronic kidney disease.

5.5.4 Clinical & Echo Model

5.5.4.1 Model production

Echocardiographic parameters were added to the list of variables used in the clinical model and forward conditional binary logistic regression for prediction of 6-month mortality was repeated with the new variables.

The model produced is included below in Table 5.13.

Variable	Significance (p)	Exp(B)	95% C.I for Exp(B)	
			Lower	Upper
Age ≥80 years	<0.0001	3.415	1.843	6.328
Admission in previous year	<0.05	2.040	1.171	3.553
Non-arrhythmogenic aetiology	<0.05	2.567	1.241	5.310
BMI <26 kg/m^2	<0.05	1.767	1.011	3.087
Known CKD	<0.05	2.567	1.241	3.318
Sodium <135 mmol/L	<0.01	2.402	1.299	4.441
Urea ≥12 mmol/L	<0.005	2.523	1.396	4.559
SBP <130 mmHg	<0.005	2.235	1.301	3.842
RVFAC	<0.05	0.975	0.953	0.998
SPAP	<0.05	1.015	1.000	1.030

Table 5.13 – Variables included in logistic regression in the Clinical & Echo model
BMI – Body mass index, Exp(B) – Exponentiation of the coefficient, RVFAC – Right ventricular fractional area change, SBP – Systolic blood pressure, SPAP – Systolic pulmonary artery pressure.

Continuous variables were converted to categorical variables and the regression was repeated to assess for validity of the model. Body mass index <26 kg/m^2 was eliminated from the model by the regression algorithm at this stage.

Variable	Significance (p)	Exp(B)	95% C.I for Exp(B)		Nagelkerke R^2	Variance accounted for by each variable, %
			Lower	Upper		
Age ≥80	<0.0001	3.963	2.164	7.259	0.112	11.2
Urea ≥12 mmol/L	<0.01	2.252	1.263	4.016	0.173	6.1
SBP<130 mmHg	<0.01	2.293	1.334	3.943	0.213	4.0
SPAP ≥50 mmHg	<0.01	2.166	1.240	3.786	0.254	4.1
Non-arrhythmogenic	<0.01	2.696	1.311	5.545	0.288	3.4
Na <135 mmol/L	<0.01	2.422	1.306	4.491	0.307	1.9
Known CKD	<0.05	1.907	1.070	3.401	0.324	1.7
Admission in previous year	<0.05	1.818	1.059	3.123	0.338	1.4
RVFAC <32%	<0.05	1.815	1.027	3.207	0.350	1.2

Table 5.14 – Variables included in the model after conversion of continuous variables to categorical variables.
CKD – Chronic kidney disease, Exp(B) – Exponentiation of the coefficient, Na – Sodium, RVFAC – Right ventricular fractional area change, SBP – Systolic blood pressure, SPAP – Systolic pulmonary artery pressure.

5.5.4.2 Regression Coefficient

The regression coefficient of the model produced is described below:

Regression coefficient = -4.811 + 0.992*Non-arrhythmogenic + 0.646*Known CKD + 0.598*Admission in last year + 0.885* Na <135 mmol/L + 1.377*Age ≥80 + 0.812*Urea ≥12 mmol/L + 0.830*SBP <130 mmHg + 0.596* RVFAC<32% + 0.773*SPAP ≥50 mmHg

Boundary score for group membership, i.e. prediction of alive or dead, is 0.50.

5.5.4.3 Model Calibration

Prior to modelling the null model predicts that all patients are alive as seen in table 5.15.

		Predicted mortality		**Percentage Correct**
		Alive	**Dead**	
Observed 6-month mortality	**Alive**	272	0	100.0
	Dead	105	0	0.0
Accuracy				72.1
Table 5.15 – Clinical & Echo model calibration plot – prior to addition of variables.				

Table 5.16 demonstrates the changes to the predicted vs observed outcomes after application of the clinical & echo model:

		Predicted mortality		**Percentage Correct**
		Alive	**Dead**	
Observed 6-month mortality	**Alive**	247	25	90.8%
	Dead	63	42	40.0%
Accuracy				76.7%
Table 5.16 – Clinical & Echo model calibration plot – post addition of variables.				

Table 5.17 compares the diagnostic test statistics pre and post application of the clinical & echo model:

Test statistic	**Pre-model value**	**Post-model value**
Sensitivity	0.0%	40.0%
Specificity	100.0%	90.8%
Positive Predictive Value	NA	62.7%
Negative Predictive Value	72.1%	80.0%
Accuracy	72.1%	76.7%
Table 5.17 - Comparison of diagnostic test statistics pre and post application of the clinical & echo model		

Table 5.17 demonstrates substantial improvements in test sensitivity, positive predictive value, negative predictive value and test accuracy, while specificity falls from 100% to 90.8%.

The Hosmer and Lemeshow test was not statistically significant for the model, suggesting good statistical calibration (N = 375, X^2 = 5.302, p = 0.725).

The cumulative Nagelkerke R^2 of the model produced can be seen in Figure 5.4.

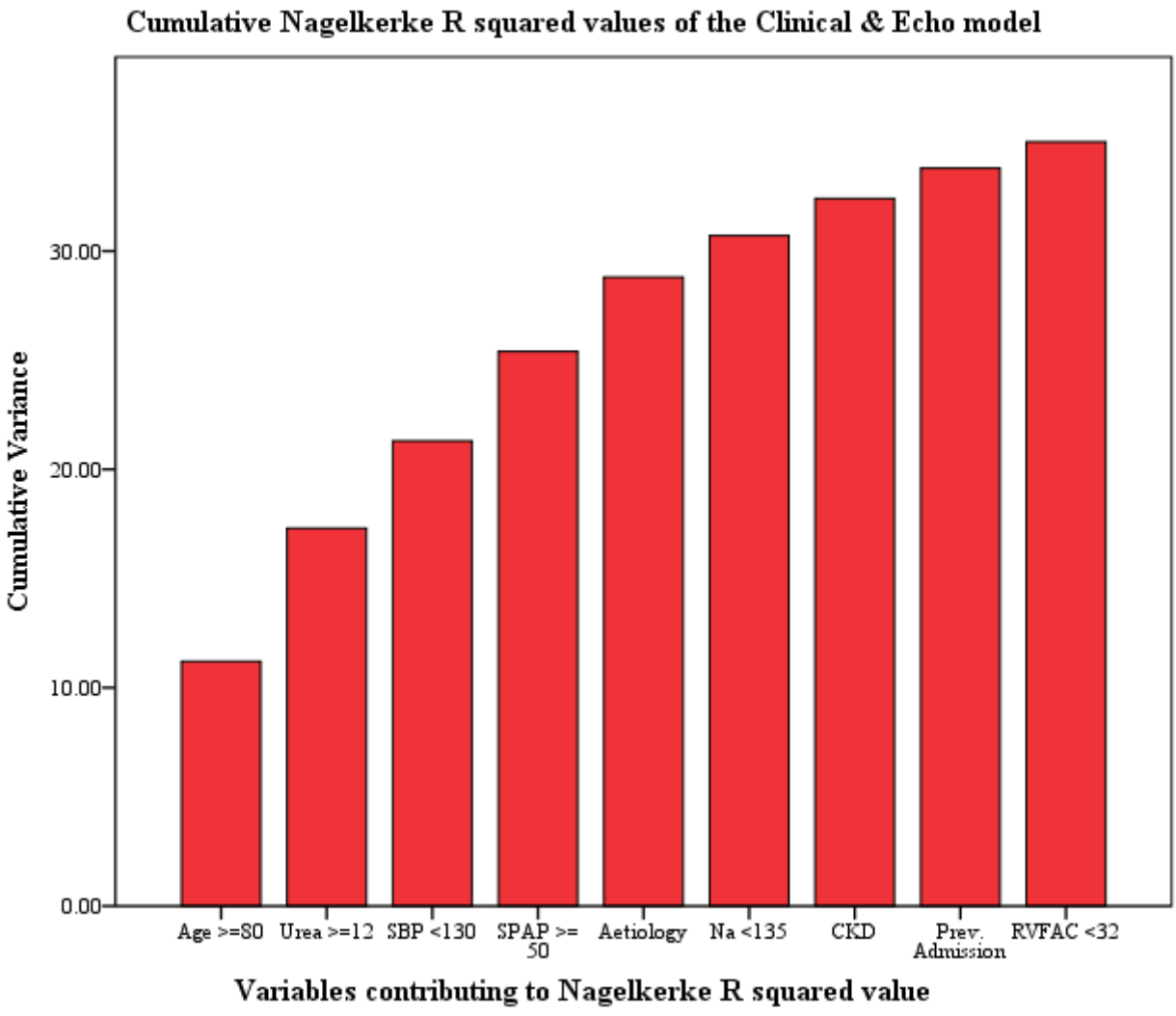

Figure 5.4 - Cumulative Nagelkerke R^2 values of the variables included in the Clinical & Echo model
CKD – chronic kidney disease, Na – Sodium, RVFAC – Right ventricular fractional area change, SPAP – Systolic pulmonary artery pressure.

5.5.4.4 Risk score point allocation

Each variable was allocated a point value according to the ratio of exponentiation of the coefficients (Exp (B)) which are equivalent to the OR. The median OR was approximately 2. The Exp(B) for Age ≥80 was almost double the median so a positive answer in this category was allocated a score of two points, all other variables were allocated one point. The minimum score possible is 0 and maximum is 10.

5.5.4.5 Discriminatory ability - Receiver operator curve assessment

Risk score was calculated for each patient in the dataset. Predictive power for mortality of the risk scores were assessed using a receiver operator curve (ROC). Area under the curve statistics are presented in Table 5.18 and the ROC is shown in Figure 5.5.

			Asymptotic 95% confidence interval	
Area under the curve	**Standard error**	**Asymptotic significance (p)**	**Lower bound**	**Upper bound**
0.804	0.023	<0.0001	0.758	0.849

Table 5.18 – Area under the curve statistics for the Clinical & Echo risk score.

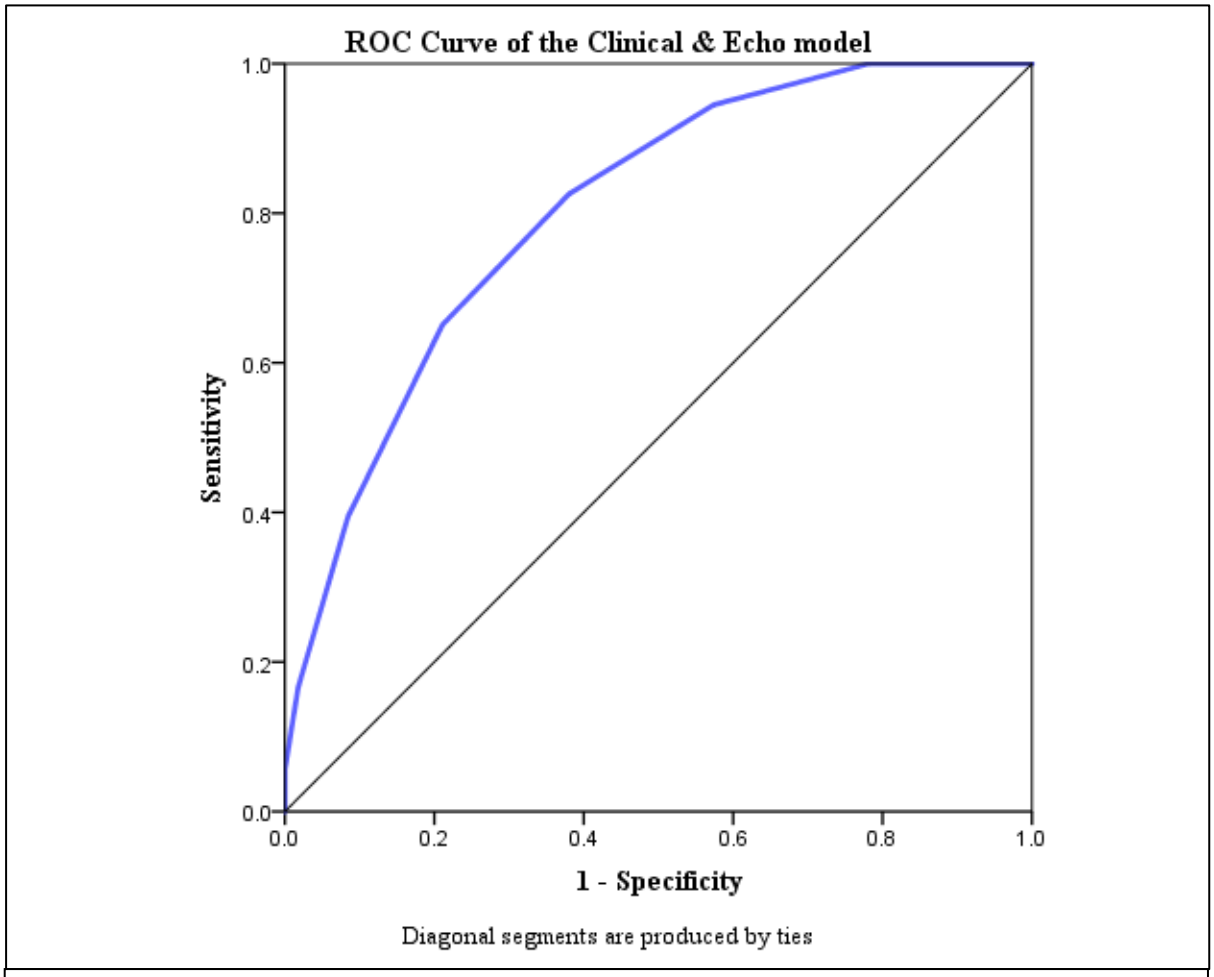

Figure 5.5 – Receiver operator curve for the Clinical & Echo model. The black diagonal line is the 50% reference.

5.5.4.6 Score stratification

Frequency of mortality was assessed for each point score from 0-10 and can be seen below in Table 5.19.

Clinical & Echo risk score	N	6-month mortality, %
0	5	0.0
1	33	0.0
2	24	0.0
3	65	9.2
4	68	19.1
5	67	28.4
6	64	43.8
7	44	56.8
8	17	70.6
9	6	100.0
10	0	-

Table 5.19 – Percentage 6-month mortality of each Clinical & Echo risk score.

Clinical & Echo risk score values were then stratified into low, medium and high risk. Low risk was defined as 6-month mortality <10%, medium risk as 6-month mortality of 10-29% and high risk as 6-month mortality ≥30%. This corresponded with Clinical & Echo risk scores of 0-3, 4-5 and ≥6 respectively.

Chi squared testing demonstrated that these scores allowed for statistically significant discrimination of mortality risk between groups; mortality in the low risk group was 4.7%, 23.7% in the medium risk group and 54.2% in the high-risk group ($p<0.0001$).

Risk Group	N	Mortality	Significance (p)
Low	135	4.7%	<0.0001
Medium	150	23.7%	
High	160	54.2%	

Table 5.20 – Risk stratification table for the Clinical & Echo risk score demonstrating mortality risk in each group.

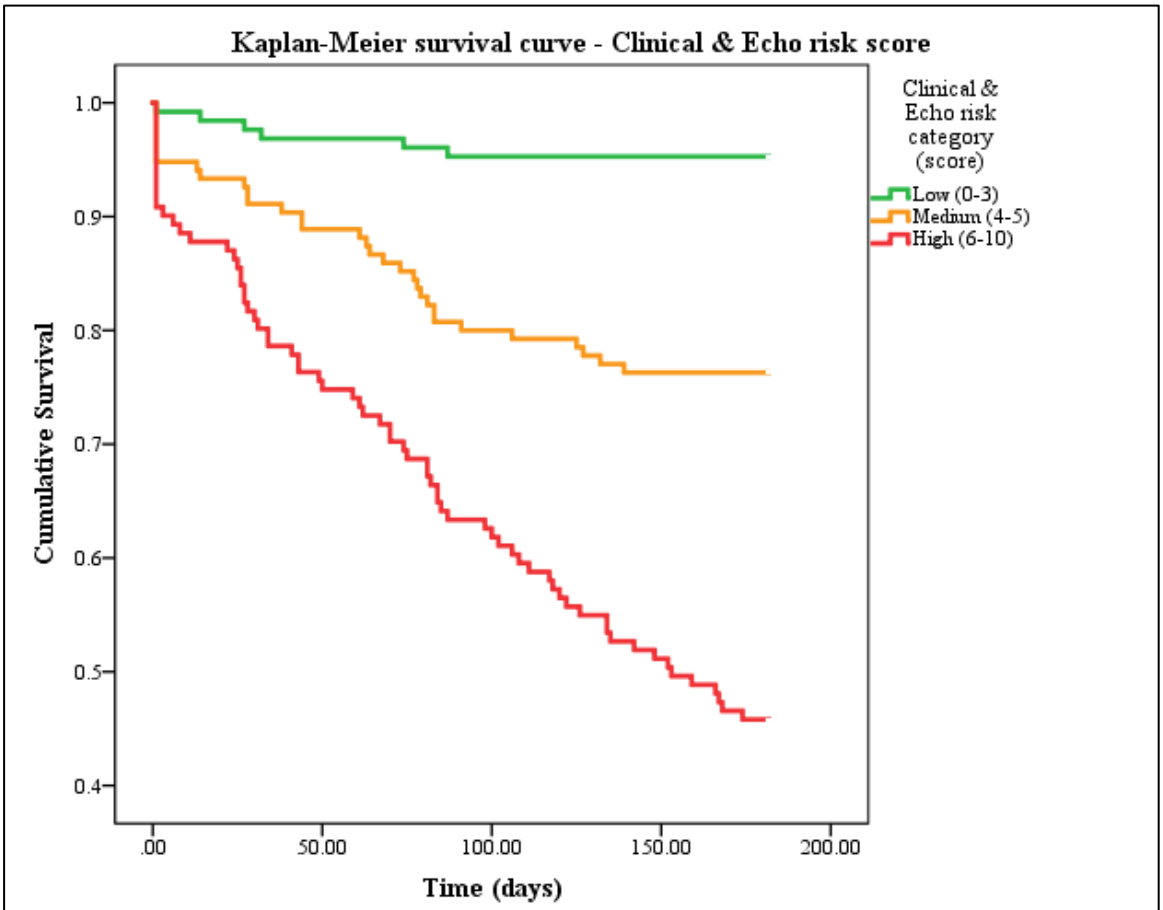

Figure 5.6 – Kaplan-Meier curve demonstrating survival versus Clinical & Echo risk score category.

5.5.4.7 Clinical & Echo risk score summary

The Clinical & Echo risk score can be summarised thus:

Risk Variable		**Point score**
Age ≥80		2
Urea ≥12 mmol/L		1
Systolic blood pressure <130 mmHg		1
SPAP ≥50 mmHg		1
Non-arrhythmogenic aetiology		1
Sodium <135 mmol/L		1
Known CKD		1
Admission in previous year		1
RVFAC <32%		1
Points scored	**Risk category**	**6-month mortality risk, %**
0-3	Low	4.7%
4-5	Medium	23.7%
6-10	High	54.2%

Table 5.21 – Summary table of the Clinical & Echo risk score including variables, allocated point score per variable and risk category stratification table for the Clinical & Echo model.
CKD – Chronic kidney disease, RVFAC – Right ventricular fractional area change, SPAP – Systolic pulmonary artery pressure.

5.5.5 Model comparison

The ROC curves of the two models are compared visually in Figure 5.7.

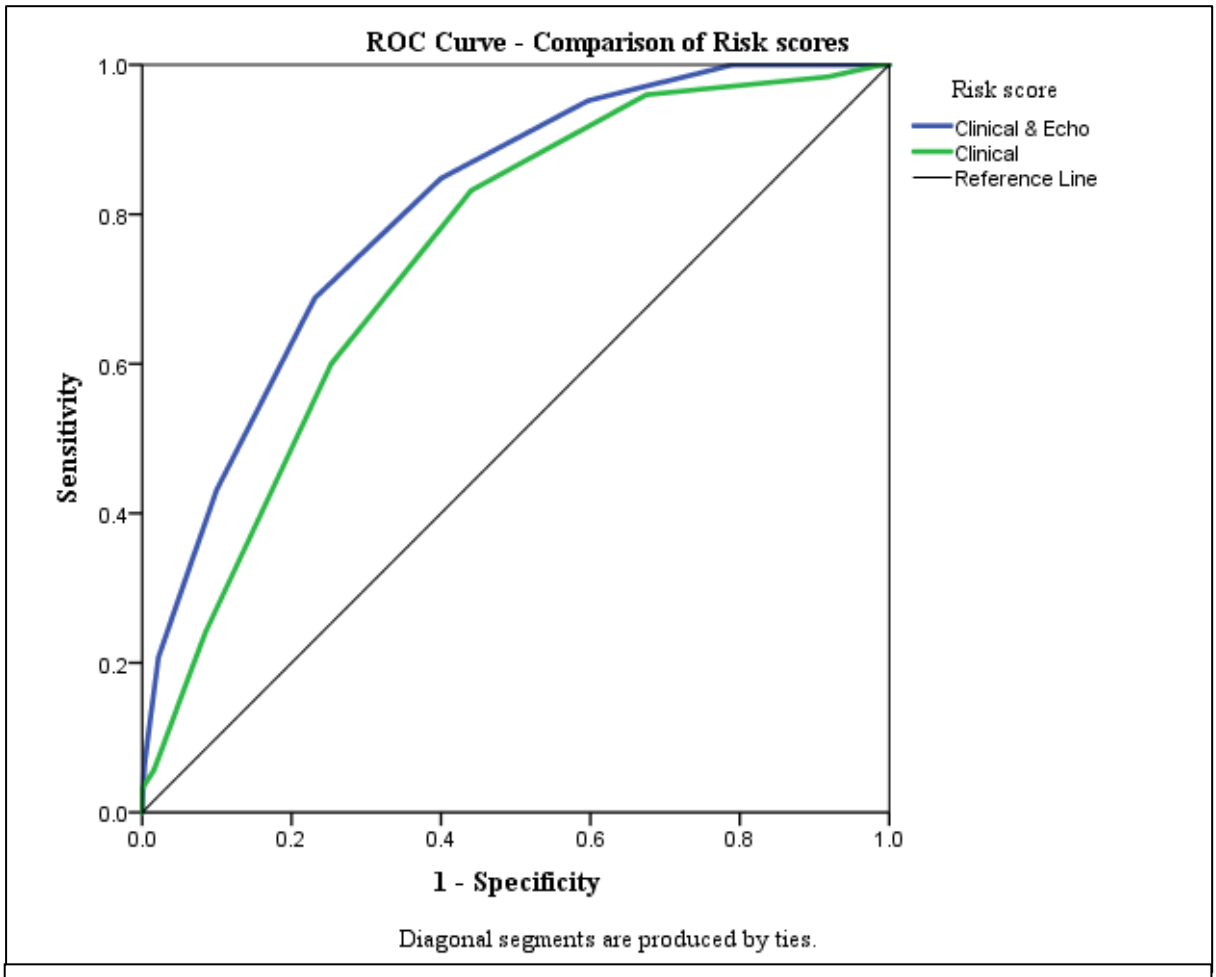

Figure 5.7 – Visual comparison of the two risk scoring methods using receiver operator characteristic. The black diagonal line is the 50% reference.

The two models produced were then compared statistically using NRI analysis.

28 events moved up category, 9 events moved down category, 7 non-events moved up category and 3 non-events moved down category.

NRI value for the Clinical & Echo model versus Clinical model =0.164 (S.E 0.05, $p<0.005$). This is equivalent to a 16.4% improvement in within-patient performance of the Clinical & Echo model compared to the Clinical model. This improvement is statistically significant.

5.5.6 Addition of POCT BNP as a variable to both models

POCT BNP was omitted from both models due to the lack of its availability in many hospitals. When the variable 'BNP ≥ 1000 pg/ml' was added to both models, statistically significant additional discriminatory power was seen.

Model calibration of the Clinical Model improved from 76.7% to 78.0%, whilst the Clinical & Echo model improved from 77.1% to 78.7% and in both cases the regression modelling demonstrated that this additional variable added discriminatory value at $p<0.05$.

Total Nagelkerke R^2 value of the Clinical model rose from 30.8% to 32.1%, while the Clinical & Echo model rose from 35.0% to 37.2%.

In both models the OR of BNP ≥ 1000 pg/ml was approximate to other variables (excepting age ≥80) so would be allocated 1 point for a positive result.

C-statistic of the Clinical model improved from 0.746 (0.695 – 0.798) to 0.769 (0.723 – 0.815) while the Clinical & Echo model improved from 0.804 (0.758 – 0.849) to 0.817 (0.776 – 0.859). These are demonstrated using ROC curves in Figures 5.8 and 5.9 below:

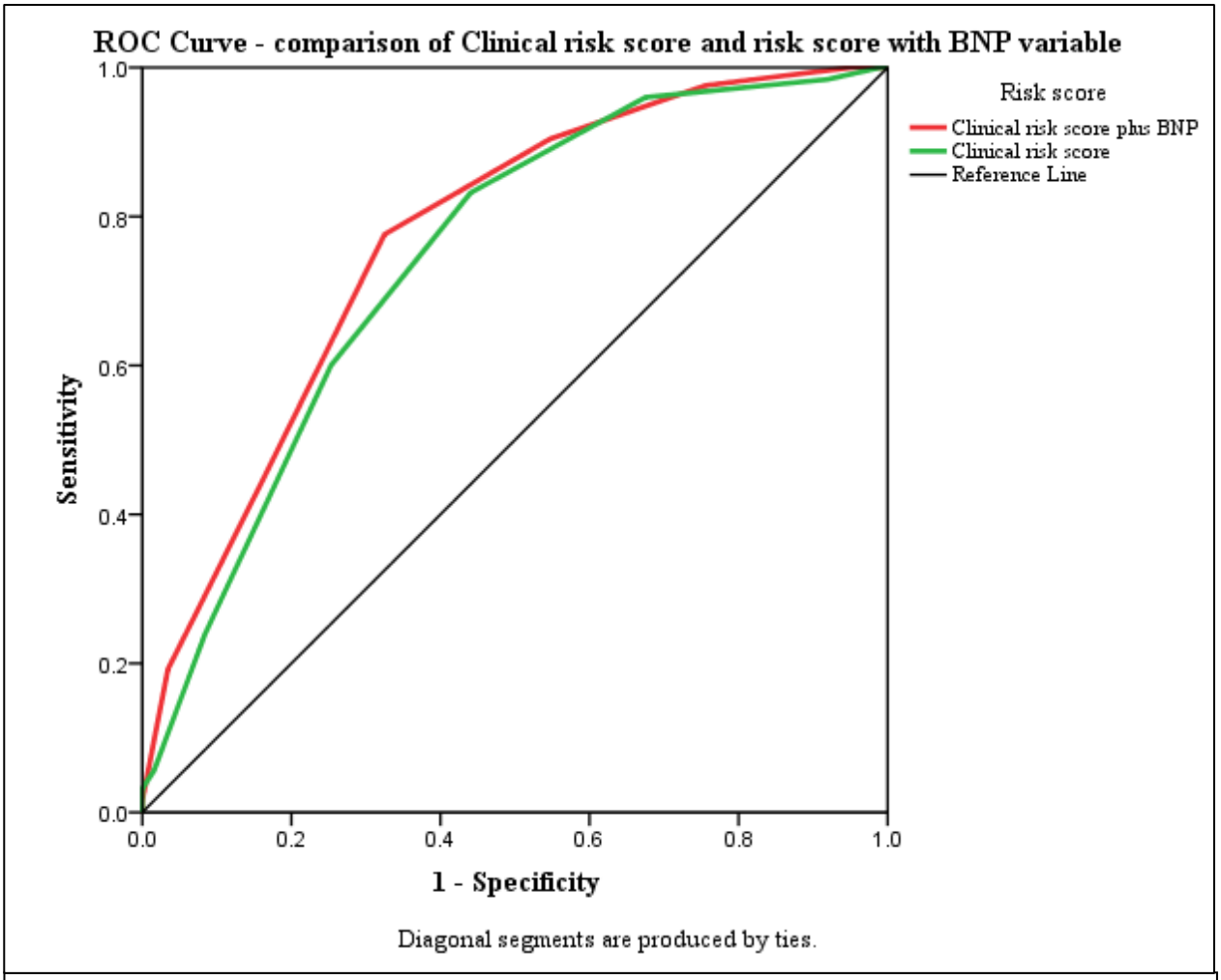

Figure 5.8 – Visual comparison of the Clinical risk score with and without POCT BNP variable using receiver operator characteristic curves. The black diagonal line is the 50% reference.

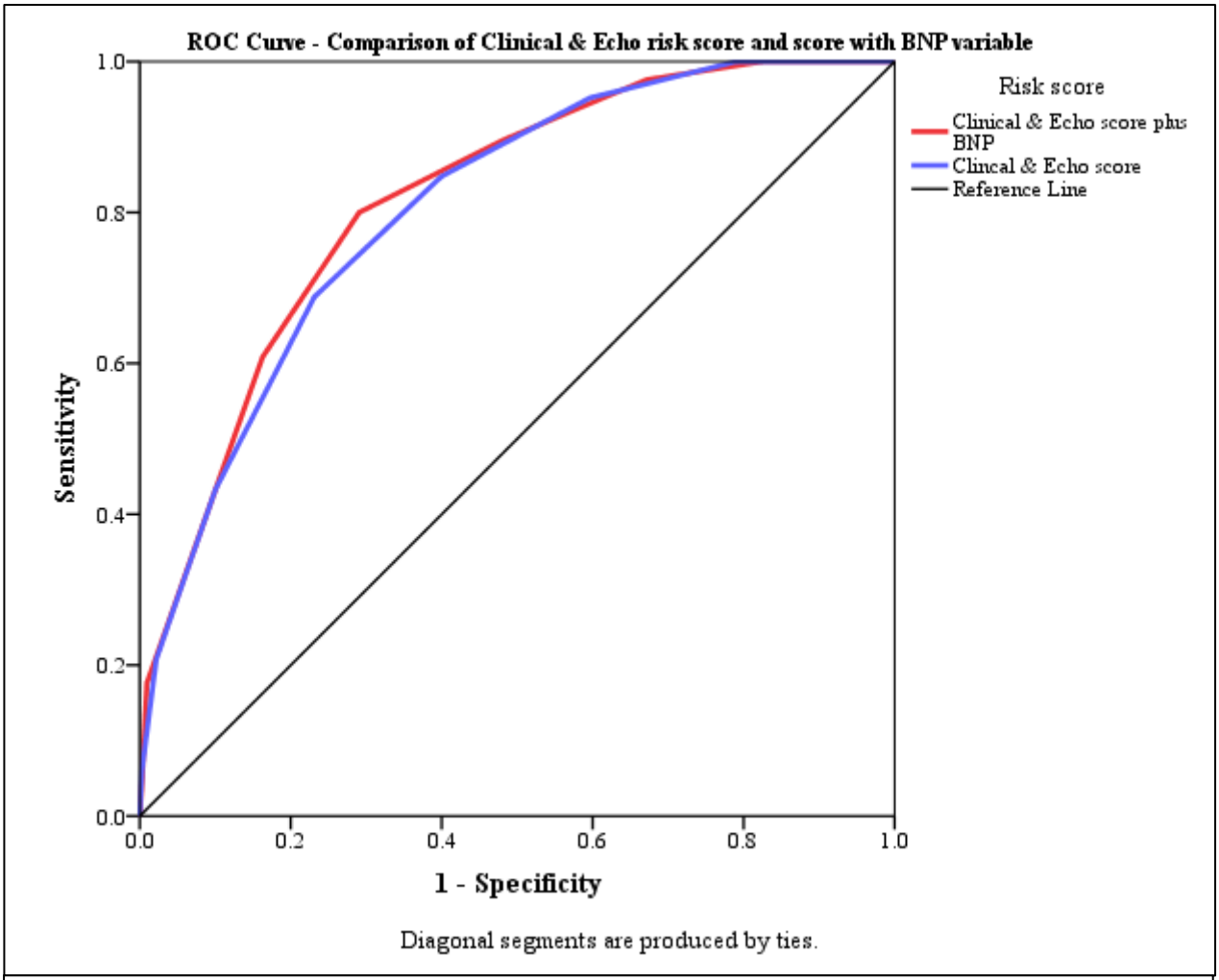

Figure 5.9 – Visual comparison of the Clinical & Echo risk score with and without POCT BNP variable using receiver operator characteristic curves. The black diagonal line is the 50% reference.

The two models produced were then compared statistically using NRI analysis.

When adding BNP to the Clinical model, 6 events moved up category, 7 events moved down category, 3 non-events moved up category and 2 non-events moved down category.

NRI value for the addition of BNP to the Clinical model =-0.013, p=0.54. This is equivalent to a 1.3% net reduction in within-patient performance of the model. This is not statistically significant.

When adding BNP to the Clinical & Echo model, 6 events moved up category, 5 events moved down category, 7 non-events moved up category and 1 non-event moved down category.

NRI value for the addition of BNP to the Clinical model =-0.012, p=0.69. This is equivalent to a 1.2% net reduction in within-patient performance of the model. This effect is not statistically significant.

5.6 Discussion

This study was performed to assess the ability to produce and use a novel risk scoring tool for prognostication in AHF. It was intended to assess the ability to use only those variables and data available to the clinician within the acute phase of a patient's hospital admission, and whether with these data alone useful predictions can be made about likely mortality outcomes at 6 months post discharge.

The strength of the study lies in the study design and patient cohort, with prospective enrolment, systematic data collection and statistical tests focussed on variables already demonstrated to show prognostic value, helping to avoid the common pitfalls of overfitting and specious associations. It also uses the data from consecutive patients of all ages and aetiologies.

From the data and results presented above, one can see that not only is it feasible to produce such a scoring tool, but that the model produced using these variables is Fair to Good in its predictive accuracy, or at least Good when including echocardiographic variables in addition to demographic, clinical and biochemical data. The C-statistic of the Clinical model is 0.746 (0.695-0.798), and the C-statistic of the Clinical & Echo model is 0.804 (0.758-0.849). These are both consistent with the reported C-statistics of other similarly produced models in both AHF and CHF. It is favourable when compared to the C-statistic of other widely adopted predictive models such as the $CHADS_2$ and subsequent CHA_2DS_2 VaSc scores which reported a C-statistic of 0.606 (Lip, Gregory YH, Nieuwlaat et al. 2010). Within the field of HF, it compares favourably to standards in CHF mortality prediction such as the Seattle risk score in which C-statistics variably ranged from 0.682 to 0.810 depending upon population (Levy, W. C., Mozaffarian et al. 2006), and compared to other AHF models, C-statistic values have been reported from 0.72 to 0.84 (Scrutinio, Ammirati et al. 2013, O'Connor, Hasselblad et al. 2010b, Felker, Leimberger et al. 2004, O'connor, Abraham et al. 2008).

When initially producing the model, the data from 52 cases were omitted due to the assumptions necessary to perform logistic regression. This reduces the size of the dataset but

is required to correctly produce logistic regression models of outcome. The patients omitted from the data were older, were more likely to have known heart failure and had increased levels of previous admissions in the past year. HF is known to progress with age and progression of disease leads to increased rates of hospital admission, therefore those with repeated admissions in the recruitment period are more likely to be older and have had recent admissions.

From the remaining dataset, forward conditional regression modelling selected 7 variables in the Clinical model and 9 in the Clinical & Echo model. Both models used a combination of demographic, biochemical and observational data, as well as echocardiographic data in the Clinical & Echo model. The selection of variables was not externally controlled as the automated regression model inherently selects all of the most statistically significant variables which add to the predictive power of the model. The fact that fewer than 10 variables were selected is very helpful for the clinician, the ultimate beneficiary of such a tool. A risk scoring tool is only as good as it is practical, and so producing a risk scoring tool with a relatively small number of variables required for its calculation adds to the practical utility of the score. This is in marked contrast with some of the other scores previously discussed which require access to a computer-based calculator (Levy, W. C., Mozaffarian et al. 2006), regression tree diagram (Fonarow, Adams et al. 2005) or complex mathematical calculation (Lee, Austin et al. 2003).

Calibration of both models is good; both tests add to the predictive ability when compared to chance, increasing accurate prediction by more than 5% in each case. Given the intended purpose of the test to predict risk of mortality, the pre-model sensitivity of 0% is useless thus an increase to 38% in the clinical model and 40% in the clinical & echo model demonstrates a substantial and imperative improvement.

In a similar vein, the pre-model calibration plot demonstrated a non-calculable positive predictive value which becomes 63.1% and 62.7% respectively while negative predictive value increases from 72.4% to 79.5% and 72.1% and 80.0%.

Prior to use of a model, the guess of the calibration plot, whilst relatively accurate, is useless in terms of predicting mortality as it predicts that all patients will not die in 6-months post admission.

The models both deliver a substantial improvement in sensitivity, positive and negative predictive values, with only a small loss of specificity. This demonstrates the purpose of producing such a model, allowing a physician to make better predictions regarding the outcome of interest, and the statistics presented above demonstrate the utility of both models.

Hosmer and Lemeshow tests were not statistically significant which suggests a good statistical fit. The Hosmer and Lemeshow test must be interpreted with caution as it can be over-reassuring in tests using small sample sizes so typically sample sizes of 400 or greater is recommended. In this study the sample size is approximately 400 and the probability estimates are not close to being significant therefore the risk of type II error is small.

The variables included within the model are, necessarily, consistent with variables selected by other groups due to the manner in which they were selected. The ORs are also illustrative; several groups have previously described age as an important predictive factor, and in both models it is the variable that accounts for the greatest proportion of variance in mortality.

Arrhythmias have been described as detrimental in the CHF and AHF population, contributing greatly to the mortality burden (Nattel, Maguy et al. 2007, Ehrlich, Nattel et al. 2002). In both predictive models arrhythmogenic HF is found to be prognostically protective when compared to other forms of decompensation. This is unlikely to suggest that arrhythmogenic heart failure is benign, but that decompensation by other cause is simply even more damaging. It may also reflect a proportion of patients admitted with transient arrhythmias as the sole driver of their presentation and minimal structural or vascular abnormalities, so that if/when the arrhythmia is appropriately controlled their risk profile is substantially reduced.

When adding echocardiographic variables, the calibration of the model improved as demonstrated by improvements in C-statistic and Nagelkerke R^2 values. In addition, NRI analysis suggested a large, statistically significant improvement of the Clinical & Echo model in comparison to the Clinical model when comparing the models within individual patients.

One surprise omission from the Clinical & Echo model is left ventricular ejection fraction. Many other groups have created models which included LVEF as a prognostic indicator in their risk scores (Lupón, de Antonio et al. 2013, Xanthopoulos, Giamouzis et al. 2017, Levy, W. C., Mozaffarian et al. 2006), but LVEF was not included in the Clinical & Echo model

described above. Falling LVEF is widely recognised as a poor marker of prognosis but was not found to add significant prognostic value in combination with the variables included in the model. This may simply be a function of the logistic regression model, but may reflect the fact that in the acute decompensated phase of HF LVEF may be enhanced by volume overload and consequent elevation of preload. This may give a falsely high value which does not reflect the true severity of systolic dysfunction which in term may not be of additional prognostic value in comparison to other clinical, biochemical and echocardiographic features. Alternatively, the MRAHF cohort had a relatively high mean and median LVEF. Lower LVEF is known to confer poorer prognosis in comparison to higher LVEF throughout the entire range but the effect size may be smaller at higher LVEF which could render LVEF less statistically relevant than other variables in this cohort.

In contrast, RVFAC and SPAP were selected when including echocardiographic data. Both are correlates of right ventricular function, and, as such, it is not surprising that impaired right ventricular function should confer a prognostic disadvantage. It is, perhaps, unexpected that in this modelling the effect was larger than that of LVEF and remained statistically significant. The reasoning behind this is not entirely clear, but it may be that in the acute phase markers of right ventricular function act as more useful prognostic variables. Other common echocardiographic parameters were omitted from the model. As with LVEF, this does not suggest that parameters such as severity of valvular disease or TAPSE are devoid of prognostic use. As discussed above, all of these have been demonstrated to be of use when considering prognosis, however regression modelling selects only those variables which add the greatest discriminatory power in the derivation cohort. Within this cohort, markers of right ventricular function were found to be particularly predictive of prognosis, and perhaps require more attention than is currently being afforded. The discovery of medications to ameliorate LV morphology and clinical outcome has led to a substantial focus on the LV in the previous decades, but there is an increasing focus on parameters of right ventricular function and the benefit RV specific therapy which may prove of great importance given the ventricular interdependence discussed in the introduction (Harjola, Mebazaa et al. 2016).

Recategorisation of risk scores for both models was performed to achieve good discrimination between patients at low, medium and high risk of mortality at 6-months post discharge. In addition, it is easier for clinicians to both remember the information and convey it to their patients. The Kaplan-Meier curve for mortality in each risk group demonstrates that

actual mortality correlates well with risk group assignment. The 6-month mortality differences between risk categories in each model are statistically significant and the Kaplan-Meier survival curves demonstrate the trend well, showing the large differences in mortality between each group. This is useful for the clinician as it demonstrates good discriminatory power to the terms Low, Medium and High risk used to further stratify AHF patients.

BNP level has been demonstrated by many contemporary studies to be a useful guide to clinical status, response to therapy and mortality risk and has been included in risk prediction models by other groups (Lupón, de Antonio et al. 2013). Due to the fact that point of care testing B natriuretic peptide (POCT BNP) levels were measured in this study rather than laboratory serum BNP levels, this was omitted from the original models; the majority of hospitals will not have access to similar equipment and thus its inclusion is potentially counterproductive. Despite this, an assessment of its impact on the predictive models was made for intellectual interest. When POCT BNP ≥1000 pg/ml was added as a variable to both models it has significantly and substantially increased the discriminatory power of both models. Model calibration, the total Nagelkerke R^2 value and C-statistic all improved for both models, suggesting benefit of its use in such a model but NRI analysis did not suggest a significant change to either model when applied in this cohort. POCT BNP levels are known to correlate well with formal laboratory values (Shah, Terracciano et al. 2010), and certainly may play a role, but there is substantial variation in values derived from different vendors of POCT. As a result, absolute values from the POCT BNP device used in this study are unlikely to be generalisable to other POCT devices. If POCT BNP devices become more widely available and a reduction in variability of BNP results between devices is seen then it is likely that their use in prognostic risk scores would be beneficial. Alternatively, the use of formal laboratory BNP or NT-pro-BNP levels is likely to be of additional value, in line with the improvements to the models seen secondary to the inclusion of POCT BNP.

NRI analysis should be interpreted with caution. NRI is not a measure of model calibration but rather compares the old and new risk values within individual patients (Kerr, Wang et al. 2014). In the first instance NRI demonstrates substantial and significant improvement from the Clinical to the Clinical & Echo model which is in line with the improvement in indices of model performance such as C-statistic and Nagelkerke R^2 value. When used later to compare the effect of adding the POCT BNP variable to both models it shows non-significant net worsening of the model despite C-statistic and Nagelkerke R^2 both demonstrating

improvements. Whilst a useful guide to model comparison, its results should be interpreted in context (Kerr, Wang et al. 2014). NRI analysis is also particularly sensitive to an increasing number of risk categories and tends to increase in line with this number. Its use in a study with only two categories – death or non-death – is less constrained by this but this inherent flaw should be noted (Pickering, Endre 2012).

The results presented above demonstrate the feasibility of producing risk scoring models using data available to the clinician during the early stages of hospital admission. Valid risk scoring models can be produced using clinical data, and potentially improved by the addition of echocardiographic variables where these are available.

The models produced here are derived from a real-life AHF cohort. Other larger AHF and CHF risk prediction models use data acquired from randomised case-control studies with selection criteria that inevitably restrict patient involvement. This introduces selection bias, whereas the prospective consecutive cohort study design used here is recognised as the best model for studying prognosis due to the ability to control and measure predictors and outcomes (Han, Song et al. 2016). It also restricts the numbers lost to follow up, and particularly reduces the quantity of information missing which is a common problem in larger retrospective registry studies.

Both models also use only those variables that are available to the clinician within hours of presentation to hospital, enabling calculation and decision making at an early juncture within the patient journey. Use of this information to more accurately risk-stratify patients may have important beneficial consequences for patient management, admission, discharge and follow-up.

5.6.1 Limitations

As has been discussed in the general limitations of previous results chapters, this study is limited by the nature of its population. Due to the demographic makeup of the local area in which the study was conducted, it has an older, predominantly Caucasian population and these demographics are not generalizable to either the nationwide or global AHF population. Patients of different ages, genders and ethnicities have different outcomes in AHF thus it is difficult to generalise these findings to a larger more diverse population without extensive external validation in alternate populations with varying demographic makeup.

In addition, when comparing sample size to other studies of a similar nature, one can see that typical cohort sizes range from 500-5000, so the MRAHF patient cohort is notably smaller which may reduce reliability and generalisability of the findings.

Though POCT BNP has been discussed as a useful additional discriminatory variable in this cohort, the gradual increase in the use of angiotensin-receptor neprolysin inhibitors (ARNIs) may influence the interpretation of BNP levels, both POCT and formal laboratory values. As discussed in the introduction, BNP is degraded by neprolysin, the action of which is directly targeted by ARNIs for therapeutic effect. The use of ARNIs leads to a rise in circulating BNP levels (McMurray, John JV, Packer et al. 2014, Solomon, Scott D., Zile et al. 2012), thus their use as a marker of worsening overload or indeed as part of a prognostic risk score appears hampered by this iatrogenic rise. As discussed earlier, further investigation as to the utility of NT-pro BNP in this setting may be of greater benefit.

In terms of the statistical analysis itself, when conducting binary logistic regression there is a risk of a statistical error termed overfitting. If the complexity of the statistical model is too great for the quantity of data collected, the variables included in the model can represent statistical noise rather than statistically significant relationships between the variable and the outcome (Peduzzi, Concato et al. 1996). Typically, a rule of 10 or 15 is applied to logistic regression analyses in which 10 or 15 events must occur per variable included in the model. After the exclusions required for logistic regression, 109 patients had died at 6 months, with 283 alive. In this case 109 events had occurred so using the rule of 10, 10 variables could be included into the model without risking overfitting but in this case up to 33 variables were included in the Clinical & Echo model. The risk of overfitting the data can be mollified by selection of variables with a known link to the outcome and this study has aimed to reduce the risk of statistical overfitting by selecting only those clinical and echocardiographic variables with evidence of prognostic value provided by previous studies. Nevertheless, it is still possible that, though the results are interesting, due to the relatively small sample size these results may not replicate well with a larger dataset. This adds weight to the need for these results to be assessed in a larger cohort.

Including variables in linear regression models that are correlated, such as creatinine and history of CKD, may lead to multicollinearity. This can reduce clarity regarding the precise role of each individual variable. In risk prediction modelling, however, this is not necessarily

a negative consequence as the model is not concerned with the exact role of each variable, but rather which markers can accurately predict the desired outcome. Neither model selected clinically correlated variables, but their co-selection in future models should be accompanied by consideration of the purpose of the model.

5.6.2 Further Work

Due to the relatively small sample size, demographic homogeneity and concern regarding overfitting, these models would benefit from external validation using a large external cohort with a heterogeneous population.

Widely adopted risk stratification models such as the CURB-65 and CHA_2DS_2-VaSc were validated using large heterogeneous populations acquired by performing large multi-centre prospective cohort studies (Lim, W. S., van der Eerden et al. 2003, Lip, Gregory YH, Nieuwlaat et al. 2010). Designing a follow-up large multi-centre study to validate the above findings would help reduce concerns regarding the validity of the results acquired above when applied to larger more heterogeneous populations.

An alternative option would be to perform a similar study in which the patient population as divided into a derivation cohort and a validation cohort, allowing for internal validation as has been performed by other groups (Xanthopoulos, Giamouzis et al. 2017). This allows for validation of the model and methods but is perhaps of less use when attempting to demonstrate further generalisability of the model to general AHF cohorts with different demographic constituents. Studies have demonstrated that the effect of individual risk factors on AHF can vary widely across discrete geographical patient populations and therefore large, multi-regional validation is often of greater benefit (Wessler, Ruthazer et al. 2017).

In addition, data regarding cardiac biomarkers such as BNP, NT pro-BNP and ST2 could be usefully included in a risk prediction model. Further studies could collect formal laboratory biomarker values in addition to the use of POCT BNP values as a rapid screening tool, in order to assess the utility of formal laboratory values in a risk stratification model.

Further work is required to produce similar risk stratifying scores in terms of cardiovascular mortality rather than simple all-cause mortality as has been described in this study. All-cause mortality is perhaps the most pertinent outcome for the patient, but it would further the understanding of the disease and perhaps improve clinical practice if the specific factors that

are predictive of cardiovascular mortality in AHF patients could be identified. There are inherent difficulties in this task. Cause-of-death in this study has been categorised according to data retrieved from death certificates. Death certificates are produced largely without access to post-mortem information, and therefore the primary aetiology is often based on clinical judgement alone. This is perhaps the best marker available, aside from universal post-mortems, but is inherently flawed by the fact that often it is based on insufficient or incomplete evidence.

There still remains a dearth of information regarding prediction of readmission which is of great importance for both the patient and healthcare provider. For the individual, each hospital admission causes incredible upheaval in their life, destabilising personal and family life with consequent physical and mental health burdens (Vaccarino, Kasl et al. 2001). For the healthcare provider, each hospital admission for AHF is known to worsen prognosis and incur substantial additional costs (National Institute for Health and Care Excellence 2014b, Gheorghiade, Vaduganathan et al. 2013). If these risks are better predicted, and thus mollified, it is likely to serve in the best interests of both parties.

5.7 Chapter summary

This chapter has reviewed the existing literature on the subject of risk prediction scores in both CHF and AHF. It has then described the methods and materials used to produce two risk stratification scores, both in terms of data collection and statistical analyses.

These scoring systems have then been described and analysed for their predictive value, both individually and then compared.

These predictive tools demonstrate the feasibility of risk stratifying AHF patients using information routinely acquired both immediately upon admission and with the addition of echocardiographic data, to produce informative risk stratification tools. In addition, both tools require <10 variables and risk scores are easily calculated, improving their practicality compared to many existing models which are limited by the quantity of variables and/or calculations required to accurately predict risk.

Chapter 6: Summary and Conclusions

6.1 Chapter introduction

This chapter will summarise the important literature regarding heart failure (HF) as discussed in the introduction and then restate the hypotheses set within this thesis, with a summary of the methods and strategies used to investigate these hypotheses.

It will then summarise the results of each subsequent chapter, discuss the novel observations derived from the results, possible interpretations of these observations and the broader implications of these with reference to the practical consequences and suggestions for further work.

6.2 Literature

Since its recognition as a medical syndrome, HF has been of great interest to the medical community due to the large burden of morbidity and mortality, as well as a gradual but persistent increase in prevalence. Despite this, until the 1980s very few treatments existed with proven efficacy to ameliorate prognosis. Since this time, progress in treating HF patients has been mixed, even with the advent of sympathetic nervous system antagonists such as beta blockers (Packer, M., Fowler et al. 2002), and inhibitors of the renin-angiotensin-aldosterone system such as angiotensin converting enzyme inhibitors (SOLVD Investigators* 1991) and mineralocorticoid receptor antagonists (Pitt, Zannad et al. 1999). These medications have proven efficacious in patients with HF with reduced ejection fraction (HFrEF) but are of unproven value in patients outside of this subcategory who account for up to 50% of all HF patients (Bavishi, Chatterjee et al. 2015).

Due to concerns that the two classic subcategories of HFrEF and HF with preserved ejection fraction (HFpEF) were too broad and arbitrary, a third, intermediate subcategory of HF with mid-range ejection fraction (HFmrEF) was introduced to stimulate research into phenotypic and pathological differences between patients with intermediate left ventricular ejection fractions (LVEF), defined as 40-49% (Ponikowski, P., Voors et al. 2016).

Subsequent to guidelines recognising this additional subcategory, evidence has emerged that HFmrEF acts as an intermediate category in many respects, certainly in terms of demographic profile, haemodynamic status and comorbidity burden (Tsuji, Sakata et al. 2017). Perhaps due to the difficulty in obtaining sufficient data suitable for analysis, data regarding potential

differences in myocardial deformation parameters between these three subcategories have not been published in any depth. Myocardial deformation imaging is an emerging component within echocardiography which is able to detect subtler systolic dysfunction and may offer an easily accessible preload-independent marker of systolic function. This could be useful in more accurately delineating between subcategories and may have broader implications for identification of systolic dysfunction and therapeutic intervention.

Aside from looking at gaps in the evidence regarding novel subcategorisations, prediction of mortality risk in acute HF(AHF) remains hindered by issues of complexity and calculation. Risk stratification is important in medicine as it allows us to effectively concentrate therapeutic effort on those most in need, and those most likely to benefit. Widely used, well-validated scoring systems exist for risk stratification of patients presenting acutely with many medical conditions; CURB 65 is used in patients presenting acutely with community acquired pneumonia and the Blatchford score is used in upper gastrointestinal bleeds to name but two of the most commonly used (Blatchford, Murray et al. 2000, Lim, W. S., van der Eerden et al. 2003). Many risk scores exist for the prediction of risk in chronic heart failure (CHF), and online tools are available which can use a combination of demographic, clinical, biochemical and echocardiographic data to provide useful prognostic information (Lupón, de Antonio et al. 2013, Levy, W. C., Mozaffarian et al. 2006). There is a lack of simple, easily applicable tools in AHF to effectively risk-stratify patients upon admission to hospital which is a critical period in the patient journey. Of the tools that do currently exist in the context of AHF, some are complicated to use (Okazaki, Shirakabe et al. 2014, O'connor, Abraham et al. 2008) while others require information not readily available to the acute physician (Salah, Kok et al. 2014, O'Connor, Hasselblad et al. 2010) and thus they become either too cumbersome or infeasible to use at the point of admission.

6.3 Hypotheses

The main hypotheses of this thesis were stated in relation to two key areas, myocardial deformation parameters in HF subcategories and prognostication in AHF.

These were stated thus:

Firstly, left ventricular strain and strain rate values will vary according to HF subcategory, as defined by LVEF, in a statistically significant fashion.

Secondly, using only data available to the clinician in the acute phase of admission, a simple, valid risk stratification tool can be produced to predict risk of 6-month mortality in patients admitted with AHF.

6.4 Strategy

The data required for the above tasks were acquired as part of the mitral regurgitation in acute heart failure (MRAHF) study. The MRAHF study is a prospective cohort study of consecutive AHF patients admitted to a single district general hospital.

It was designed to recruit all patients admitted to the study site with AHF and assess the burden of mitral regurgitation (MR) in this population, with a specific focus on the financial, morbidity and mortality burden of the disease, as well as tracking the journey of patients with AHF and MR to tertiary centres for mitral valve intervention.

Patients were approached for recruitment into the study if they had:

1) Clinical signs and symptoms consistent with AHF as the primary cause for admission
2) Been an inpatient for <7 days by the time of recruitment to the study
3) The ability to give informed consent

After consenting for phlebotomy and echocardiography, patients were subsequently excluded from the study if they had:

1) Point-of-care test (POCT) B natriuretic peptide (BNP) level <100 pg/ml
2) Echocardiography inconsistent with a diagnosis of HF

The target for patient recruitment was 500, selected as the likely burden of AHF patients in the hospital in a year. Patients were recruited from July 2016 until September 2017.

616 patients were screened from the hospital admissions of which 500 patients were recruited to the study. 53 patients were subsequently excluded due to B natriuretic peptide (BNP) level or echocardiography not diagnostic of heart failure. The final patient cohort numbered 447.

Baseline demographic, clinical, biochemical and radiographical data were collected at the time of recruitment and echocardiography was performed within 48 hours.

Offline analysis of echocardiographic data was subsequently performed including cardiac geometry, function and, latterly, myocardial deformation parameters.

Patients outcomes were monitored in terms of readmission or mortality at 6 months post discharge, and cause of both admission and mortality were recorded within the dataset.

The data from all patients recruited into the MRAHF study were used for the production of this thesis.

6.5 Chapter Three – Baseline characteristics and comparison of the MRAHF & EHS II cohorts

The baseline characteristics of the MRAHF cohort were first stratified according to LVEF subcategory. These subcategories were compared to assess for consistency or discrepancy with the growing literature on characteristic differences between HFrEF, the novel category of HFmrEF and HFpEF.

Patients categorised as HFrEF were more likely to be male, more likely to have a history of ischaemic heart disease and had significantly higher BNP levels upon admission. Left ventricular (LV) volumes were larger in these patients and QRS duration was also increased compared to the other subcategories.

Patients categorised as HFpEF were more likely to be female, had a significantly higher BMI than patients with HFrEF and BNP values were significantly lower. LV volumes were lowest in this group.

Patients categorised as HFmrEF had intermediate values in terms of gender breakdown, BNP values, ECG rate and QRS duration. Left ventricular volumes were intermediate between HFrEF and HFpEF, as were systolic pulmonary artery pressures. History of ischaemic heart disease (IHD) was statistically similar to that of the HFpEF patients, not HFrEF.

These results are in keeping with much of the current research regarding the characteristics of HFmrEF patients. In most parameters measured, HFmrEF acts as an intermediate category (Tsuji, Sakata et al. 2017). The only substantial difference to reported literature was the statistical similarity of HFmrEF to HFpEF in terms of the prevalence of IHD as a comorbidity. Patients with HFmrEF are commonly described as having a similar IHD burden to patients with HFrEF (Tsuji, Sakata et al. 2017), but this is not the case in the cohort of patients recruited for the MRAHF study.

The MRAHF study was subsequently compared to the EuroHeart survey II (EHS II), a large multi-centre prospective registry study of AHF patients conducted in Europe.

Compared to the EHS II cohort, the MRAHF cohort was older, had a higher proportion of female patients, had a lower rate of hospitalisation in the previous 12 months and a lesser comorbidity burden. Community prescription of prognostic medications was more common in the EHS II cohort with the exception of beta blocker usage, likely related to the higher burden of atrial fibrillation seen in the MRAHF cohort. LVEF was statistically higher in the MRAHF group and LV volumes were comparably higher. Despite the demographic differences, in-patient mortality burden was similar between the two groups.

The MRAHF and EHS II cohorts are notably different in many respects. This may be due to the larger geographic area from which their patients were recruited, but also likely due to the less stringent selection criteria used in EHS II and lack of formal exclusion criteria. As a result, they are likely to have included many patients in whom a HF was either a concomitant diagnosis or a diagnosis of dubious accuracy. Drawing conclusions as to the generalisability of findings derived from the MRAHF cohort is rendered somewhat difficult by these facts but, given the selection criteria of the MRAHF study and consecutive cohort study design, it is likely that the MRAHF cohort represents the typical AHF cohort in the local area.

The purpose of this comparison is to establish the baseline characteristics of the MRAHF cohort, aid assessment of the generalisability of the results and to demonstrate the benefits of the study design in comparison to previous projects. The differences shown between the two cohorts may in part be due to the recruitment criteria of the EHS II, but also serve as a reminder that results derived from a single population in a single geographical location require validation from larger datasets. Ideally, external validation would occur using patients from a variety of geographical areas and demographic backgrounds to ensure generalisability of the conclusions drawn.

6.6 Chapter Four – Myocardial deformation parameters in heart failure subcategories

In this chapter, an assessment was made of differences in myocardial deformation parameters, stratifying the MRAHF cohort by LVEF subcategory.

Both longitudinal strain and longitudinal strain rate values were found to be highest (most negative) in the HFpEF group and lowest (least negative) in the HFrEF group. Patients in the HFmrEF group represented an intermediate category. These findings were statistically significant, both in analysis of individual myocardial segments, and when considering the averaged values, global longitudinal strain (GLS) and global longitudinal strain rate (GLSR).

There were large differences seen when comparing the medians of each subcategory, all of which were statistically significant. In both strain and strain rate analyses, the deformation values of HFpEF were approximately double those in HFrEF.

In a separate analysis, LVEF was found to be lower in patients recruited ≥2 days after admission while GLS and GLSR were statistically comparable in patients recruited both < and ≥2 days after admission. Patients recruited ≥2 days after admission were comparable in terms of demographic data, comorbidity burden, admission biochemistry and readmission rates, but did have a slightly higher mortality rate.

This study first demonstrates the feasibility of echocardiographic myocardial deformation imaging in the acute clinical setting, with clinically and statistically relevant data collected in this cohort.

The results from this cohort summarised above indicate that the difference in median strain and strain rate values between HF subcategories is found to be large and statistically significant. Myocardial deformation parameters in HFmrEF represent an intermediate phenotype, with intermediate levels of contractile function as shown by the strain and strain rate values.

In addition, in those patients recruited ≥2 days since admission, LVEF was substantially and significantly lower than those recruited <2 days subsequent to admission. This is in contrast to their myocardial deformation values which remained statistically similar across both groups. This is unlikely to be a function of an intrinsic phenotypical or pathological difference between the groups as they are otherwise statistically comparable in terms of demographic characteristics, comorbidity burden, biochemical parameters and readmission rate. It is more probable that this finding further demonstrates the relative load-independence of myocardial deformation parameters in AHF patients.

Strain and strain rate values appear to offer useful information to help delineate and subcategorise patients. Given the significant variance in LVEF values seen between ultrasonographers, machine types and software, additional information on alternate markers contractile function with less inter-user and inter-machine variance may benefit the accurate subcategorisation of patients with AHF. This is important as their allocation to a HF subcategory can significantly alter their management in terms of pharmacotherapy or device-based therapies. In this study, inter-observer variability was effectively eliminated as >95% of echocardiograms and all basic offline analysis was performed by the same highly experienced ultrasonographer. As such, subcategorisation of patients by LVEF value is as consistent as possible using standard echocardiographic techniques.

Deformation parameters are also known to be relatively load-independent (Ferferieva, Van den Bergh et al. 2011). Other measures of intrinsic cardiac contractility such as pressure-volume loops can be impractical to perform, such as pressure-volume loops. As such, information from myocardial deformation parameters, particularly GLSR, may offer the clinician the ability to better make inferences about intrinsic contractile function of the AHF patient's myocardium.

The data presented in chapter four indicate that GLS and GLSR values may vary less than LVEF in the acute phase. LVEF is known to be preload dependent (Gaasch, Meyer 2008, Silke, Verma et al. 1985) and thus will fall in response to therapeutic offloading of the heart with diuretics. It is possible that this accounts for the statistical reduction in LVEF values seen in patients recruited ≥2 days after admission. In contrast to median LVEF values, median GLS and GLSR values remained statistically similar.

6.6.1 Further Work

Having already discussed the disadvantages of a single-centre study and the concerns regarding generalisability, the results regarding significant variation in deformation parameters between HF subcategories would benefit from repetition in alternate sites with different patient demographics. Nevertheless, it would seem likely that myocardial deformation parameters in HFmrEF are intermediate to HFrEF and HFpEF as this is in keeping with current literature and understanding of the biomechanics of HF.

A follow up study to ascertain the veracity of the finding regarding variance of LVEF, GLS and GLSR values after admission would also be beneficial. Determining whether LVEF is in fact a reliably stable marker in the setting of AHF is of great importance given the degree upon which it is relied. If deformation parameters offer a more stable indication of systolic function in the acute phase, this could greatly add to current practice and patient assessment.

This could feasibly be assessed by performing a similar study in which AHF patients undergo echocardiography at admission and 2 days hence to assess whether the factor likely contributing to the apparent change in parameter is in fact time and diuresis.

The study and use of myocardial deformation parameters have grown hugely in the preceding two decades and the enthusiasm with which they are being trialled and implemented in a wide range of conditions affecting cardiac function appears unabated. Currently the widespread use of deformation imaging for clinical decision making appears relatively constrained by a lack of consensus over normal values, particularly between vendors and software manufacturers. Nevertheless, this state of affairs appears to be improving, and the use of deformation parameters appears destined for greater use in the future (Yang, Marwick et al. 2015). This may enable more accurate assessment of contractile function which could ultimately revolutionise the way that HF is discussed and subcategorised.

6.7 Chapter Five – Prediction of 6-month mortality risk in acute heart failure

This chapter describes the production and assessment of a risk score for predicting mortality in AHF patients at 6 months post discharge.

From the 316 variables collected in the MRAHF study, 33 variables were selected for testing within a risk prediction model. Variables were considered for inclusion if they were available to the clinician within 48 hours of admission and there was literature which indicated their utility as variables predictive of mortality in AHF. Seven of these variables were derived from echocardiography. Two models were produced, the first (Clinical model) using demographic, clinical, biochemical and common investigations only, and the other (Clinical & Echo model) also including echocardiographic data.

The Clinical and Clinical & Echo models used 7 and 9 variables respectively to predict 6-month mortality outcomes and were able to predict mortality with accuracy of 76.7% and 77.1%.

C-statistic for the models were 0.746 and 0.804, so the models would standardly be assessed as ‘Fair’ and ‘Good’ respectively. This compares favourably with other widely-used medical prediction models such as CURB-65, and in the context of HF compares favourably with the commonly-used Seattle risk score.

Scores derived from both models were stratified into tertiles to simplify the process for the clinician, and these allocated risk groups delineated well between patients at low, medium and high risk of mortality in both cases.

The inclusion of echocardiographic variables appeared to add diagnostic accuracy to the models but this difference did not appear statistically significant.

This study has demonstrated the feasibility of producing a risk score for prediction of 6-month mortality in AHF using only those variables available to the acute physician. Both risk scores produced are of comparably high predictive accuracy and use a relatively limited number of variables. Producing two separate scores using similar variables allows for risk prediction either in the absence or presence of echocardiographic information.

These risk scores use a small number of variables enabling both scores to be easily remembered and used by clinicians in the acute period of hospital admission. This could aid the process of patient risk stratification in the acute phase, and guide decisions regarding admission, escalation and intensity of community or outpatient follow-up.

6.7.1 Further Work

Evidently this study and the proposed models require external validation. Multi-centre data collection from prospective AHF cohorts would be ideal for external validation and would allow for confident conclusions to be made about the generalisability of the risk score to the general AHF population.

Further information could be acquired from this trial regarding need for escalated medical care and involvement of intensive care unit support. This additional data would help to

support decision making regarding escalation of individual care, as has been successfully implemented with the CURB-65 scoring model.

In summary, the risk stratification models produced offer novel, easily usable risk stratification tools for the acute physician in the early stages of patient admission. They appear to work well in the derivation population but for more widespread use they require validation with an external cohort. This will either confirm or refute the feasibility of usage outside of the derivation population.

6.8 Thesis summary

The work described in this thesis contributes new evidence to the field of AHF. Specifically, this thesis has demonstrated the feasibility, utility and potential future applications of myocardial deformation imaging, and established the potential for novel risk scoring methods to enhance early risk stratification of patients with AHF.

Amelioration of the mortality and morbidity burden is required in HF, which is rapidly becoming a global epidemic as populations live longer in the presence of multiple cardiac risk factors. Improving the accurate assessment of AHF patients, both in terms of their systolic function and prognostic risk, will enable us to better identify patients requiring therapeutic intervention and improve patient outcomes.

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Publications, abstracts and presentations

Included below is a list of pertinent publications, abstracts and podium presentations undertaken during the research period.

Publications

Stewart, J., Manmathan, G. and Wilkinson, P., 2017. Primary prevention of cardiovascular disease: A review of contemporary guidance and literature. *JRSM cardiovascular disease,* **6**, pp. 2048004016687211.

Abstracts & Poster Presentation

J. Stewart, O. Lazariashvili, A. Baltabaeva. Right ventricular function is a strong predictor of outcome in acute heart failure. Poster presented at Heart Failure 2018, European Society of Cardiology; Vienna; Sunday 27th May 2018; Poster number 1136.

J. Stewart, O. Lazariashvili, A. Baltabaeva. Mitral Regurgitation worsens prognosis in acute heart failure. Poster presented at Heart Failure 2018, European Society of Cardiology; Vienna; Monday 28th May 2018; Poster number 1834.

J. Stewart, O. Lazariashvili, A. Baltabaeva. Precipitant of hospitalization predicts mortality in acute heart failure. Poster presented at Heart Failure 2018, European Society of Cardiology; Vienna; Monday 28th May 2018; Poster number 1835.

J. Stewart, O. Lazariashvili, A. Baltabaeva, I. Beeton, D. Fluck. Significant mitral regurgitation in acute heart failure is associated with adverse ventricular geometry, function and prognosis. Poster accepted for presentation at EuroEcho-Imaging 2018, European Association of Cardiovascular Imaging; Milan; Thursday 6th December 2018; Poster Number 303.

Podium Presentations

Optimisation of medical therapy in community heart failure patients – Lessons learned from the MRAHF study. Oral presentation made at the North West Surrey Clinical Commissioning Group and Surrey Community Educational Provider Meeting, St Peter's Hospital, Chertsey, April 2018

Appendices

Appendix 1.1 POCT BNP Assay and Instrument Calibration Data

510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT COMBINATION TEMPLATE

A. 510(k) Number:
k053597

B. Purpose for Submission:
New device

C. Measurand:
B-type natriuretic peptide

D. Type of Test:
Quantitative

E. Applicant:
i-STAT Corporation

F. Proprietary and Established Names:
i-STAT BNP test
i-STAT Control Level 1
i-STAT Control Level 2
i-STAT Control Level 3
i-STAT BNP Calibration Verification Control Set

G. Regulatory Information:
1. Regulation section:
862.1117, B-type natriuretic peptide test system
862.1660, Single (specified) analyte controls (assayed and unassayed)
2. Classification:
Class II, Class I
3. Product code:
NBC, JJX
4. Panel:
75 Chemistry

H. Intended Use:
1. Intended use(s):
The i-STAT BNP test is an in vitro diagnostic test for the quantitative measurement of Btype natriuretic peptide (BNP) in whole blood or plasma samples using EDTA as the anticoagulant. BNP measurements can be used as an aid in the diagnosis and assessment

of severity of congestive heart failure.
The i-STAT Controls are assayed liquid plasma used to verify the integrity of newly received i-STAT BNP cartridges.
The i-STAT BNP Calibration Verification Controls are assayed liquid plasma used to verify the calibration of i-STAT BNP cartridges throughout the reportable range.
2. Indication(s) for use:
See Intended use(s) above.
3. Special conditions for use statement(s):
Prescription use only
4. Special instrument requirements:
i-STAT 1 Analyzer

I. Device Description:
Each i-STAT BNP cartridge provides a sample inlet, sensors to detect the BNP, and all the necessary reagents needed to perform the test. The cartridge contains a buffer and preservatives. A list of reactive ingredients is indicated below:

Reactive Ingredient	Biological Source
Antibody/Alkaline Phosphatase Conjugate	Murine IgG:Bovine Intestine
IgG	Caprine IgG: Murine IgG
Sodium Aminophenyl Phosphate	N/A
Heparin	Porcine Intestine

The i-STAT BNP Controls are supplied as assayed frozen liquid plasma at 3 levels, Control Level 1, Control Level 2 and Control Level 3. The human sera used in the preparation of this product has been tested by FDA approved test methods and found negative/non-reactive for HIV-1, HIV-2, HBsAg, HCV, HTLV-1 and HTLV-2.

The i-STAT Verification Control Set is supplied as 3 levels of assayed frozen liquid plasma at 3 levels, Level 1, Level 2 and Level 3. The human sera used in the preparation of this product has been tested by FDA approved test methods and found negative/nonreactive
for HIV-1, HIV-2, HBsAg, HCV, HTLV-1 and HTLV-2.

J. Substantial Equivalence Information:
1. Predicate device name(s):
Biosite Triage BNP Test
2. Predicate 510(k) number(s):
k021317
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Assay methodology	Two-site ELISA	Two-site ELISA
Capture site	Heterogeneous	Heterogeneous
Capture antibodies	Monoclonal	Monoclonal
Enzyme label antibody	Monoclonal	Monoclonal
Sample type	Whole blood or plasma	Whole blood or plasma
Acceptable samples	EDTA anti-coagulated blood or plasma	EDTA anti-coagulated blood or plasma

Differences		
Item	Device	Predicate
Enzyme label	Fluorescent dye	Alkaline phosphatase
Enzyme detection	Fluorescent	Electrochemical
Sample volume	250 µL	20 µL
Reportable range	15-5000 pg/mL	5-5000 pg/mL

K. Standard/Guidance Document Referenced (if applicable):
CLSI Guideline EP7-A; CLSI Guideline EP9-A2; CLSI Guideline C-28-A2

L. Test Principle:
The i-STAT BNP test cartridge uses a two-site enzyme-linked immunosorbant assay (ELISA) method. Antibodies specific for BNP are located on an electrochemical sensor fabricated on a silicon chip. Also deposited in another location on the sensor silicon chip is an antibody/alkaline phosphatase enzyme conjugate specific to a separate portion of the BNP molecule. The whole blood or plasma sample is brought into contact with the sensors allowing the enzyme conjugate to dissolve into the sample. The BNP within the sample becomes labeled with alkaline phosphatase and is captured onto the surface of the electrochemical sensor during an incubation period of approximately seven minutes. The sample is washed off the sensors, as well as excess enzyme conjugate. Within the wash fluid is a substrate for the alkaline phosphatase enzyme. The enzyme bound to the antibody/antigen/antibody sandwich cleaves the substrate releasing an electrochemically detectable product. The electrochemical (amperometric) sensor measures this enzyme product which is proportional to the concentration of BNP within the sample.

M. Performance Characteristics (if/when applicable):
1. Analytical performance:
a. Precision/Reproducibility:
Precision data were collected as follows: duplicates of each control were tested daily for a period of 20 days for each of 3 lots of cartridges, resulting in a total of 434 replicates. The average statistics are presented below.

Aqueous Control	Mean	% CV (within-run)	% CV (total)
Level 1	126	9.0	11.1
Level 2	1551	6.6	8.1
Level 3	3337	8.0	9.8

Whole blood imprecision data were collected as follows: whole blood samples from 5 healthy donors were spiked to low, intermediate and high BNP concentrations affording 15 samples, each of which was measured in 10 i-STAT BNP cartridges from a single cartridge lot; three lots of cartridges were employed. The mean withinsample BNP concentration ranged from 84 – 3925 pg/mL and the within-sample imprecision (%CV) ranged from 3.4 to 9.4%; the average BNP concentration and imprecision were 1464 pg/mL and 6.5% respectively. The individual results are presented in the table below:

Donor	Mean BNP in pg/mL	% CV
1	99	6.3
1	765	3.4

Donor	Mean BNP in pg/mL	% CV
1	3803	4.1
2	107	7.8
2	1049	7.8
2	2638	5.7
3	108	9.2
3	1036	6.5
3	3805	5.4
4	84	9.1
4	783	9.4
4	3925	7.6
5	95	7.0
5	763	3.9
5	2900	4.8

b. Linearity/assay reportable range:

The dilution linearity of the i-STAT BNP test was studied using EDTA whole blood and plasma samples derived from 3 separate donors. For each donor, the original BNP negative sample and a BNP spiked sample were prepared. This process yielded three BNP positive whole blood samples that were then assayed in duplicate for each of 3 separate i-STAT BNP cartridge lots. These whole blood samples were then diluted using an equal mass of the original unspiked whole blood and assayed in duplicate. From this whole blood data, the BNP recovery was calculated.

Whole blood	Concentration	Diluted concentration	% recovery
A	590	312	106%
B	2765	1429	103%
C	5123	2803	109%

The plasma derived from these three donors was combined in all pair-wise combinations in equal volumes. These combinations were then assayed in duplicate

for each of 3 separate i-STAT BNP cartridge lots. The BNP recovery for each pair was calculated using the average of the 6 results.

Plasma Blood Sample	Concentration pg/mL)	Diluted Concentration (pg/mL)	% Recovery
A	590	—	—
B	2764	—	—
C	5123	—	—
A+B	—	1570	94%
B+C	—	3992	101%
A+C	—	2734	96%

A plasma sample was spiked with BNP to a value of approximately 5000 pg/mL. This sample was subjected to a series of dilutions with fresh, un-spiked plasma in order to prepare a range of concentrations. The concentration of each sample/dilution was calculated based on the measured concentration of the initial solution and the dilutions performed. The diluted samples were then measured in i-STAT BNP test cartridges (N = 6-10). The procedure was repeated with a whole blood sample. The results of these experiments are summarized in the following table:

Sample	Dilution	Calculated [BNP] (pg/mL)	Measured [BNP] (pg/mL)	%Recovery
Plasma	1	52	57	110%
Plasma	2	104	114	110%
Plasma	3	259	265	103%
Plasma	4	518	560	108%
Plasma	5	1036	1002	97%
Plasma	6	2072	2277	110%
Plasma	7	3107	3384	109%
Plasma	8	4143	4222	102%
Whole Blood	1	44	41	93%
Whole Blood	2	88	88	100%
Whole Blood	3	269	287	107%
Whole Blood	4	537	554	103%
Whole Blood	5	725	720	99%
Whole Blood	6	1450	1367	94%
Whole Blood	7	3042	2826	93%
Whole Blood	8	4056	3856	95%

c. Traceability, Stability, Expected values (controls, calibrators, or methods):
The i-STAT BNP calibrators are traceable to an internal reference standard that has been prepared gravimetrically with synthetic BNP. The internal reference standard underwent a one-time value assignment to align with the ARCHITECT BNP assay with a decision threshold of 100 pg/mL. Manufacturers working calibrators are prepared by gravimetric manipulation of the standard and incorporate a one-time value assignment for alignment of methods. The i–STAT, AxSYM and ARCHITECT assays have been designed, by virtue of their calibration, to report comparable values. The i-STAT vs. ARCHITECT method comparison data exhibits a correlation slope of 0.97 (see method comparison section). Similar data for the ARCHITECT vs. AxSYM exhibited a slope of 1.03.

Stability studies were performed to evaluate the intended storage (open and closed vial) for the i-STAT controls and calibration verification materials. The real-time

frozen stability of BNP control/calibration verification materials was established for 3 lots of material, each comprised of 3 levels. Stability was judged to be acceptable provided that the mean BNP concentration measured at each test event be within ± 20 % of the original mean concentration. The stability was acceptable over 5 months frozen storage. The stability studies are ongoing.

The labeling for the i-STAT controls and calibration verification materials states that, after thawing, the opened or unopened vial is stable for 4 hours when capped and stored at 2 – 8 º. Stability studies performed support the 4 hour time limit.

d. Detection limit:
The limit of the blank for the BNP method is 15 pg/mL, which is the lowest BNP level that can be distinguished from zero. The value was estimated using a control material with < 5 pg/mL BNP during a 20 day precision study in which 3 separate lots of BNP test cartridges were tested in duplicate using a pool of 6 i-STAT 1 analyzers for a total of 147 test results.

e. Analytical specificity:
The following muscle proteins were tested at both 1000 pg/mL and 20,000 pg/mL concentrations and found to have no detectable cross-reactivity for BNP: ANP, CNP, and N-terminal pro-BNP.

The i-STAT BNP assay employs electrochemical rather than optical detection. An electrogenic substrate is cleaved by an enzyme label giving rise to an electroactive product that can be oxidized at a sensor electrode generating a signal comprised of electrical current, therefore optical interferents, including hemoglobin, bilirubin, and chylomicrons, do not interfere with this mode of detection.

The following substances were found to have no significant effect (less than 10%) on the BNP method, when added to a plasma pool containing approximately 1000 pg/mL of B-type natriuretic peptide at the concentrations indicated:

Compound	Test Level (µmol/L unless otherwise indicated)
Acetaminophen	1660
Allopurinol	294
Ampicillin	152
Ascorbic Acid	227
Acetyl Salicylic Acid	3333
Atenolol	37.6
Caffeine	308
Captopril	23
Chloramphenicol	155
Diclofenac	169
Digoxin	6.15
Dopamine	5.87
Enalaprilat	0.86
Erythromycin	81.6
Furosemide	181
Sodium Heparin	90 U/mL
Ibuprofen	2425
Isosorbide dinitrate	636
Methyldopa	71
Nicotine	6.2
Nifedipine	1.156
Phenytoin	198
Propanolol	7.71
Salicylic Acid	4.34
Theophylline	222
Verapamil	4.4
Warfarin	64.9

f. Assay cut-off:
BNP results less than or equal to 100 pg/mL are representative of normal values in patients without CHF. See Clinical cut-off section below.

2. Comparison studies:

a. Method comparison with predicate device:
Method comparison data were collected using CLSI guideline EP9-A2. Venous blood samples were collected in EDTA evacuated tubes and analyzed in duplicate on the i-STAT System. A portion of the specimen was centrifuged and the separated plasma was analyzed in duplicate on the i-STAT 1 System and on the comparative method, the Abbott ARCHITECT BNP assay, within 1 hour of collection. Deming regression analysis was performed on the first replicate of each sample. In the method comparison table, n is the number of specimens in the first data set, Sxx and Syy refer to estimates of imprecision based on the duplicates of the comparative and the i-STAT methods respectively. Sy.x is the standard error of the estimate, and r is the correlation coefficient. The samples had BNP values ranging from 5-5000 mg/dL.

Method Comparison

	Abbott ARCHITECT
N	433
Mean (pg/mL)	482.1
Sxx (pg/mL)	38.1
Syy (pg/mL)	97.6
Slope	0.971
Intercept	-14.4
Sy.x	198.0
Xmin	5
Xmax	4797.7
Correlation, r	0.961

b. Matrix comparison:
EDTA plasma is the only sample type indicated. The labeling states that performance characteristics have not been established for samples taken from capillary tubes and direct skin punctures (e.g. fingersticks) so these sample types should not be used with the BNP cartridge.

3. Clinical studies:

Clinical studies performed with the Abbott AxSYM BNP assay are included in the labeling for the i-STAT BNP assay. The applicant provided the following to support the transfer of reference ranges:

• The AxSYM, ARCHITECT and i-STAT BNP assays employ an identical antibody set. The average imprecision is similar for the 3 assays as follows: AxSYM average %CV = 7.9 %; ARCHITECT average %CV = 5.2 %; i-STAT average %CV = 9.7 %.
• The i–STAT, AxSYM and ARCHITECT assays have been designed, by virtue of their calibration, to report comparable values.
• The CLSI document C28-A2, How to Define and Determine Reference Intervals in the Clinical Laboratory, provides guidance concerning the transferability of reference ranges from one measurement system to another. The i-STAT vs. ARCHITECT method comparison data exhibits a correlation slope of 0.97 (see method comparison section above). Also, similar data for the ARCHITECT vs. AxSYM exhibited a slope of 1.03 (see k060964).

a. Clinical Sensitivity:
In studies performed with the AxSYM BNP Assay, age-matched analysis of the heart failure and non-heart failure populations was performed based on the data published by the American Heart Association in the 2000 Heart and Stroke Statistical Update and according to the age structure of the United States population. The age distributions in the intended use population are approximately as follows: individuals less than 45 years old comprise 9%, individuals 45-54 years old comprise 11%, individuals 55-64 years old comprise 22%, individuals 65-74 years old comprise

26%, and individuals 75 years and older comprise 32%. The resulting combined AUC is 0.87 (0.85 to 0.90, 95%CI). The clinical sensitivity and specificity using a decision threshold of 100 pg/mL is presented in the table below.

	Males (Age Group)					
	All	<45 Years	45-54 Years	55-64 Years	65-74 Years	75+ Years
Sensitivity	71.0% (328/462)	47.1% (8/17)	57.1% (24/42)	57.3% (51/89)	70.6% (115/163)	86.1% (130/151)
95% Confidence Interval	66.6 to 75.1%	23.0 to 72.2%	41.0 to 72.3%	46.4 to 67.7%	62.9 to 77.4%	79.5 to 91.2%
Specificity	94.8% (403/425)	97.2% (104/107)	100.0% (71/71)	97.9% (92/94)	88.7% (102/115)	89.5% (34/38)
95% Confidence Interval	92.3 to 96.7%	92.0 to 99.4%	94.9 to 100.0%	92.5 to 99.7%	81.5 to 93.8%	75.2 to 97.1%

	Females (Age Group)					
	All	<45 Years	45-54 Years	55-64 Years	65-74 Years	75+ Years
Sensitivity	80.5% (186/231)	44.4% (4/9)	73.3% (11/15)	50.0% (13/26)	80.6% (58/72)	91.7% (100/109)
95% Confidence Interval	74.8 to 85.4%	13.7 to 78.8%	44.9 to 92.2%	29.9 to 70.1%	69.5 to 88.9%	84.9 to 96.2%
Specificity	88.4% (411/465)	95.9% (94/98)	90.7% (68/75)	89.6% (69/77)	85.7% (114/133)	80.5% (66/82)
95% Confidence Interval	85.1 to 91.2%	89.9 to 98.9%	81.7 to 96.2%	80.6 to 95.4%	78.6 to 91.2%	70.3 to 88.4%

b. Clinical specificity:
See Clinical Sensitivity section above.

c. Other clinical supportive data (when a. and b. are not applicable):

4. Clinical cut-off**:**
Data from the clinical studies performed with the AxSYM BNP assay were used to generate The Receiver Operating Characteristic (ROC) curve of BNP decision thresholds versus clinical sensitivity and clinical specificity. At a decision threshold of 100 pg/mL, the BNP assay demonstrated a clinical sensitivity and specificity of 74.2% and 91.5% respectively. The area under the curve is 0.90 (0.86 to 0.92, 95% CI).

5. Expected values/Reference range:
Plasma samples from 890 individuals (465 females, 425 males) who had not been diagnosed with heart failure were tested with the AxSYM BNP assay. This population included non-hospitalized patients with renal disease (not on dialysis), diabetes, hypertension and chronic obstructive pulmonary disease. BNP levels for these patients were not statistically different from the population of apparently healthy individuals. The data are summarized below.

Non-Heart Failure Population - All (Age Group)

	All	<45 Years	45-54 Years	55-64 Years	65-74 Years	75+ Years
Sample Size (N=)	890	205	146	171	248	120
Median (pg/mL)	21	17	9	24	23	31
Mean (pg/mL)	39	28	21	37	47	63
SD (pg/mL)	66	36	30	48	80	109
95th Percentile	135	85	87	119	160	254
Percentage < 100 pg/mL	91.5%	96.6%	95.2%	94.2%	87.1%	83.3%
Minimum (pg/mL)	0	0	0	0	0	0
Maximum (pg/mL)	907	263	142	380	907	837

Non-Heart Failure Population - Males (Age Group)

	All	<45 Years	45-54 Years	55-64 Years	65-74 Years	75+ Years
Sample Size (N=)	425	107	71	94	115	38
Median (pg/mL)	14	12	1	17	21	37
Mean (pg/mL)	30	23	9	26	47	49
SD (pg/mL)	61	34	14	45	96	51
95th Percentile	104	73	40	80	150	121
Percentage < 100 pg/mL	94.8%	97.2%	100.0%	97.9%	88.7%	89.5%
Minimum (pg/mL)	0	0	0	0	0	0
Maximum (pg/mL)	907	200	57	380	907	254

Non-Heart Failure Population - Females (Age Group)

	All	<45 Years	45-54 Years	55-64 Years	65-74 Years	75+ Years
Sample Size (N=)	465	98	75	77	133	82
Median (pg/mL)	26	23	23	37	23	25
Mean (pg/mL)	46	34	34	51	46	69
SD (pg/mL)	70	37	36	48	63	126
95th Percentile	150	89	111	155	159	266
Percentage < 100 pg/mL	88.4%	95.9%	90.7%	89.6%	85.7%	80.5%
Minimum (pg/mL)	0	0	0	0	0	0
Maximum (pg/mL)	837	263	142	230	374	837

Plasma samples from 693 patients with diagnosed heart failure (231 females, 462 males) were tested with the AxSYM BNP assay. All patients in this population were categorized according to the functional classification system published by the New York Heart Association (NYHA). This system divides heart failure patients into one of four categories of increasing disease progression (classes I to IV) based upon a subjective assessment of the patient's clinical signs and symptoms. The data from this study are summarized below.

Heart Failure Population – All

		NYHA Functional Class			
	All	I	II	III	IV
Sample Size (N=)	693	124	319	190	60
Median (pg/mL)	298	133	266	335	1531
Mean (pg/mL)	578	320	432	656	1635
SD (pg/mL)	771	388	574	841	1097
5th Percentile	14	9	15	12	188
95th Percentile	2154	1257	1534	2516	>4000
Percentage ≥ 100 pg/mL	74.2%	58.1%	73.0%	79.0%	98.3%
Minimum (pg/mL)	0	3	0	0	14
Maximum (pg/mL)	>4000	1651	>4000	>4000	>4000

Heart Failure Population – Males

	NYHA Functional Class				
	All	I	II	III	IV
Sample Size (N=)	462	94	215	121	32
Median (pg/mL)	268	122	258	293	1645
Mean (pg/mL)	524	314	409	597	1646
SD (pg/mL)	719	390	539	821	1032
5th Percentile	12	9	14	22	265
95th Percentile	1976	1281	1356	2288	3654
Percentage ≥ 100 pg/mL	71.0%	56.4%	70.7%	76.0%	96.9%
Minimum (pg/mL)	0	3	0	0	14
Maximum (pg/mL)	>4000	1408	3782	>4000	>4000

Heart Failure Population - Females

	NYHA Functional Class				
	All	I	II	III	IV
Sample Size (N=)	231	30	104	69	28
Median (pg/mL)	385	174	298	466	1408
Mean (pg/mL)	685	341	481	760	1623
SD (pg/mL)	858	388	641	870	1186
5th Percentile	16	14	21	12	244
95th Percentile	2593	1022	2031	2718	>4000
Percentage ≥ 100 pg/mL	80.5%	63.3%	77.9%	84.1%	100.0%
Minimum (pg/mL)	0	10	0	0	173
Maximum (pg/mL)	>4000	1651	>4000	>4000	>4000

N. Instrument Name:
i-STAT 1 Analyzer

O. System Descriptions:
1. Modes of Operation:

Single use cartridge

2. Software:
FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:
Yes ___X_____ or No _____

3. Specimen Identification:
Bar code reader is incorporated into the system

4. Specimen Sampling and Handling:
Whole blood samples are applied directly into the sample well of the cartridge

5. Calibration:
Factory set

6. Quality Control:
The reliability of the results is maintained through a combination of user testing and instrument self-checks. The self checks occur with every cartridge run and verify performance of the analyzer and cartridge sub-systems. This includes checks on the individual sensor's performance, the integrity of the calibrant fluid, the response of the pressure and thermal transducers, and the flow of calibrant and sample within the cartridge. Any values that are statistically deviant from the factory established expectation values would cause the test results to be suppressed. Daily monitoring is through the use of internal and external electronic simulators. Liquid controls are provided for the verification of cartridge lot performance for all newly received cartridge lots.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Q. Proposed Labeling:
The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:
The submitted information in this premarket notification is complete and supports a substantial equivalence decision.

Appendix 1.2 Patient Information Sheet 1.1 25th April 2016 for MRAHF study

Study Title: ***Incidence of significant mitral regurgitation in patients presenting with acute heart failure. Journey to Tertiary Centre (MRAHF).***

Patient Information Sheet

Dear Patient,

We would like to ask you to take part in our clinical investigation study. Before you decide whether you would like to take part it is important that you understand why this research is being done and what it will involve. One of our team will go through this information sheet with you and answer any questions or concerns you may have. Please ask us if there is anything that is not clear or if you would like more information and talk to others if you wish. You will have to decide on the first day of your admission to the hospital whether you would like to take part in this study. This information sheet will explain the purpose of the study and what will happen to you if you take part.

Thank you for taking the time to read this.

Part 1

What is the purpose of the study?

The purpose of this study is to assess the prevalence of moderate-to-severe **Mitral Regurgitation (MR)**, also known as leaky valves in patients presenting to hospital in acute **Heart Failure (HF)**. Patients requiring hospital admission.

Why have I been invited?

You have been asked to participate in this study because you have been admitted to hospital with symptoms of heart failure.

Do I have to take part?

Your participation in this study is completely voluntary. You do not have to take part. Please take the time to read this information sheet carefully and discuss it with relatives, friends.

It is up to you to decide whether or not to take part in this clinical study. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. You will be free

to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

If you have any questions or concerns about this study, or if you do not fully understand any part of it, please ask your research doctor.

What will happen to me if I take part?

If you agree to take part in this clinical study you will be asked to have a recording of heart sounds with a special stethoscope, have a heart scan and a bedside **B-type Natriuretic Peptide Blood Test (BNP)**. The level of BNP will be checked using a small device (i-STAT BNP) at the bedside. The BNP test results will determine your eligibility for inclusion into this study.

If your test results indicate elevated BNP level of (≥100 pg/ml) you will undergo special procedure called **Transthoracic Echocardiography (TTE)** for grading of MR severity within 2 days of your hospitalisation.

A transthoracic echocardiogram (TTE) is the most common type of echocardiogram, which is a still or moving image of the internal parts of the heart using ultrasound. In this case, the probe (or ultrasonic transducer) is placed on the chest or abdomen of the patient to get various views of the heart.

Will expenses be paid?

We are not anticipating expenses to incur as all the investigations will be completed within current hospital admission.

What do I have to do?

Your participation in the study will last for the time you are in hospital.

You will not be eligible to participate in the study if you have other causes of breathlessness or palpitations.

What are the possible disadvantages and risks of taking part?

Participation involves having a heart scan whilst in hospital and additional skin prick to take blood for BNP test.

What are the possible benefits of taking part?

Your participation will be important as it will help us establish whether hospital admissions with heart failure are caused with leaky valves. We will also be in a position to find out if assessment of heart

sounds on auscultation is good enough to detect valvular problems as well as value of bedside assessment of BNP. These tests are not routinely available in current clinical settings.

What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed.

What will happen if I don't want to continue in the study?

You are free to withdraw your participation at any time with no prejudice to your standard of care. We will need to use the data collected on you up until the time of your withdrawal.

What if there is a problem?

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do this by contacting **Patient Advice and Liaison Service (PALS)** within the hospital:

Telephone: 01932 723553

Email: pals@asph.nhs.uk

Will my taking part in the study be kept confidential?

Yes. If you consent to take part in the clinical study, any of your medical records may be inspected by the company sponsoring the research for purposes of analysing the results. They may also be looked at by people from the company and from regulatory authorities to check that the study is being carried out correctly. Your name, however, will not be disclosed outside the hospital. The Trust Information Governance Policy and Data Protection Act 1998 will be strictly followed.

We will follow ethical and legal practice and all information which is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the hospital/surgery will have your name and address removed so that you cannot be recognised from it.

What will happen to the results of the research study?

After the end of this clinical study the results will be analyzed and published in medical scientific journals. As all information that is available from you is collected anonymously you will of course not be identified in any report or publication.

The study outcome will be posted on the Trust research and development website, which is located at: http://www.ashfordstpeters.nhs.uk/quality/research . Participants will be provided with the web link to access the information.

Who is organising and funding the study?

This study has been funded by Abbott Vascular Company, and sponsored by Metanoic Health Ltd.

Who has reviewed the study?

The formal review by R&D committee took place on 15.10.2015. Similar detailed review took place at ABBOTT Laboratories Abbott Vascular. Both panels have come to a conclusion that this is an innovative and interesting research project. This study will also be reviewed and approved by Research Ethics Committee (REC) **[REC Name: North of Scotland 2]**.

Contact for Further Information

Please feel free to ask any question you have about this study. If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions.

Contact Details:

Name: Dr A. Baltabaeva

Email: Aigul.Baltabaeva@asph.nhs.uk

Tel No.: 01932723534

Appendix 1.3 Abbott POCT BNP user guide

B-TYPE NATRIURETIC PEPTIDE/ (BNP)

Intended Use

The i-STAT® BNP test is an *in vitro* diagnostic test for the quantitative measurement of B-type natriuretic peptide (BNP) in whole blood or plasma samples using EDTA as the anticoagulant. BNP measurements can be used as an aid in the diagnosis and assessment of the severity of congestive heart failure.

Method Explanation

The i-STAT BNP test cartridge uses a two-site enzyme-linked immunosorbant assay (ELISA) method. Antibodies specific for BNP are located on an electrochemical sensor fabricated on a silicon chip. Also deposited in another location on the sensor silicon chip is an antibody/alkaline phosphatase enzyme conjugate specific to a separate portion of the BNP molecule. The whole blood or plasma sample is brought into contact with the sensors allowing the enzyme conjugate to dissolve into the sample. The BNP within the sample becomes labeled with alkaline phosphatase and is captured onto the surface of the electrochemical sensor during an incubation period of approximately seven minutes. The sample is washed off the sensors, as well as excess enzyme conjugate. Within the wash fluid is a substrate for the alkaline phosphatase enzyme. The enzyme bound to the antibody/antigen/antibody sandwich cleaves the substrate releasing an electrochemically detectable product. The electrochemical (amperometric) sensor measures this enzyme product which is proportional to the concentration of BNP within the sample.

Contents

Each i-STAT BNP cartridge provides a sample inlet, sensors to detect the BNP as described above, and all the necessary reagents needed to perform the test. The cartridge contains a buffer and preservatives. A list of reactive ingredients is indicated below:

Reactive Ingredient	Biological Source	Minimum Quantity
Antibody/Alkaline Phosphatase Conjugate	Murine IgG : Bovine Intestine	0.009 µg
IgG	Caprine IgG : Murine IgG	8.5 µg : 8 µg
Sodium Aminophenyl Phosphate	N/A	0.9 mg
Heparin	Porcine Intestine	0.45 IU
IgM	Murine IgM	0.3 µg

Metrological Traceability
The i-STAT System test for B-type natriuretic peptide (BNP) measures BNP amount-of-substance concentration in plasma or the plasma fraction of EDTA anticoagulated whole blood (units of measure: pg/mL or ng/L) for *in vitro* diagnostic use. BNP values assigned to i-STAT's controls and calibration verification materials are traceable to i-STAT's working calibrator prepared from synthetic BNP (Peptide International, Louisville, KY, Cat# 4212v). i-STAT System controls and calibration verification materials are validated for use only with the i-STAT System and assigned values may not be commutable with other methods. Further information regarding metrological traceability is available from Abbott Point of Care Inc.

Reportable Range
The i-STAT BNP test will report 15 to 5000 pg/mL (ng/L). Samples below the reportable range will yield " <15 pg/mL" on the analyzer display screen. Samples above the reportable range will yield ">5000 pg/mL".

Reference Range
Whole blood and plasma samples from 165 apparently healthy donors were assayed. The upper 95% reference range was determined to be 50 pg/mL (ng/L).

Clinical Significance
Congestive heart failure (CHF) is a complex clinical syndrome resulting in decreased cardiac output that is insufficient to meet the body's metabolic needs.1 It may result from dysfunction of either ventricle in systole (contraction), diastole (relaxation) or both.2 The most common underlying cause of CHF is coronary artery disease. Other causes include: hypertension, myocarditis, valvular heart disease and idiopathic (unknown).3

Common symptoms include: paroxysmal nocturnal dyspnea (PND), orthopnea, dyspnea on exertion (DOE), nocturnal cough and peripheral edema.2 Clinical signs include elevated jugular venous pressure, rales on lung auscultation, the presence of a third heart sound and peripheral edema.2 Unfortunately, these signs and symptoms are variable, and when present, non-specific as other clinical entities such as chronic obstructive pulmonary disease can produce a similar clinical picture.4

B-type natriuretic peptide (BNP) is one of a family of structurally similar peptide neurohormones that also includes atrial natriuretic peptide (ANP) and C-type natriuretic peptide (CNP) whose function is to regulate blood pressure, electrolyte balances, and fluid volume. ANP is stored in granules within the atria and released rapidly in response to atrial stretch. In contrast, BNP is synthesized, stored, and released primarily by the ventricular myocardium in response to volume expansion and pressure overload.1 Pre-pro-BNP (134 amino acids) is synthesized in the cardiac myocytes and is processed to a pro-BNP (108 amino acids) precursor molecule. The pro-BNP is then subsequently cleaved into the physiologically active BNP (32 amino acids) and an N-terminal fragment referred to as N-Terminal pro-BNP (76 amino acids).3

Numerous clinical trials suggest the potential clinical usefulness of plasma BNP in:

1. the diagnosis of dyspnea and CHF4,5
2. the detection of left ventricular systolic and diastolic dysfunction6,7
3. the prognosis of patients with CHF and acute coronary syndromes8,9 and
4. therapy monitoring for CHF patients10,11

Multiple studies establish the value of BNP for facilitating the diagnosis of CHF in patients presenting with dyspnea.12 Davis et al. measured levels of ANP and BNP in 52 patients presenting with acute

dyspnea.12,13 They found that admission plasma BNP concentrations more accurately reflected the final diagnosis than did ejection fraction (EF) levels or ANP plasma concentrations. Morrison et al. also showed that rapid testing of BNP could help differentiate pulmonary from cardiac etiologies of dyspnea.4 Furthermore, the Task Force of the European Society of Cardiology for the Diagnosis and Treatment of Chronic HF has included the use of natriuretic peptide (e.g., BNP) testing along with electrocardiography and chest x-rays in their guidelines for the diagnosis or rule out of HF.14

The Breathing Not Properly study, a 1586 patient multinational prospective study, validated the clinical utility of rapid measurement of BNP, used in conjunction with other clinical information, for the diagnosis or exclusion of CHF in the emergency department.15 BNP levels were much higher in patients with subsequent CHF than in those with non-cardiac dyspnea (675 pg/mL vs 110 pg/mL). A BNP cutoff value of 110 pg/mL had a sensitivity of 90% and a specificity of 76% for differentiating CHF from other causes of dyspnea, and a cutoff value of 50 pg/mL had a negative predicative value of 96%. There was a 43% indecision rate among physicians in the ED trying to make a diagnosis in patients with dyspnea. Had BNP levels been available to those physicians, the indecision rate would have been reduced to 11%. In multivariate analysis, BNP levels always contributed to the diagnosis, even after consideration of the history and physical exam.

BNP levels are also raised in patients with left ventricular dysfunction, and the values can be used to assess the severity of CHF, as they correlate with both New York Heart Association (NYHA) functional class and patient prognosis.16

Steg et al. indicated in 2005 that BNP measurement is consistently superior to a single echocardiographic determination of left ventricular EF in identifying patients with CHF, regardless of the threshold value.16 Two-dimensional echocardiography was less sensitive than a single determination of BNP in diagnosing CHF. However, the two variables have marked additive diagnostic value and when combined have a much improved accuracy compared to either method alone. This strongly suggests that, where applicable, they should be used together.16

Studies also indicate that BNP also has a burgeoning role in the prognostic assessment of patients with heart failure.17 BNP is a powerful prognostic indicator for patients with CHF at all stages of the disease and seems to be a better predictor of survival than many traditional prognostic indicators, such as New York Heart Association class, serum creatinine values, and possibly left ventricular ejection fraction.18 The relative risk of death increases by about 35% for each 100 pg/mL increase in BNP in patients with CHF.18 Raised BNP values also predict the survival in patients not known to have CHF, with the risk doubled in patients with a BNP value >20 pg/mL.18

BNP has also been shown to predict morbidity and mortality in other cardiovascular conditions, such as acute coronary syndromes and acute myocardial infarction.19 ACS patients with increased BNP levels have a higher rate of cardiac complications and higher mortality post myocardical infarction.

When a panel of neurohormones (including BNP and catecholemines) was measured one to four days after acute infarction, BNP was the only independent predictor of late ejection fraction (EF <40%) and was the most powerful predictor of death within four months after infarction.[20] In 2,525 AMI patients, the magnitude of BNP elevation correlated with mortality, heart failure, and recurrent infarction at both 30 days and 10 months.[8] A strategy of combining EF and BNP improved risk stratification beyond using either alone.[21]

EXPECTED VALUES

Non-heart Failure Population

Plasma samples from 890 individuals (465 females, 425 males) who had not been diagnosed with heart failure were tested with the AxSYM® BNP assay. This population included non-hospitalized patients

with renal disease (not on dialysis), diabetes, hypertension and chronic obstructive pulmonary disease. BNP levels for the patients with renal disease, diabetes, hypertension and chronic obstructive pulmonary disease were not statistically different from the population of apparently healthy individuals. The data from this study are summarized in the following table.*

Non-Heart Failure Population - All (Age Group)						
	All	<45 Years	45-54 Years	55-64 Years	65-74 Years	75+ Years
Sample Size (N=)	890	205	146	171	248	120
Median (pg/mL)	21	17	9	24	23	31
Mean (pg/mL)	39	28	21	37	47	63
SD (pg/mL)	66	36	30	48	80	109
95th Percentile	135	85	87	119	160	254
Percentage < 100 pg/mL	91.5%	96.6%	95.2%	94.2%	87.1%	83.3%
Minimum (pg/mL)	0	0	0	0	0	0
Maximum (pg/mL)	907	263	142	380	907	837

Non-Heart Failure Population - Males (Age Group)						
	All	<45 Years	45-54 Years	55-64 Years	65-74 Years	75+ Years
Sample Size (N=)	425	107	71	94	115	38
Median (pg/mL)	14	12	1	17	21	37
Mean (pg/mL)	30	23	9	26	47	49
SD (pg/mL)	61	34	14	45	96	51
95th Percentile	104	73	40	80	150	121
Percentage < 100 pg/mL	94.8%	97.2%	100.0%	97.9%	88.7%	89.5%
Minimum (pg/mL)	0	0	0	0	0	0
Maximum (pg/mL)	907	200	57	380	907	254

Non-Heart Failure Population - Females (Age Group)						
	All	<45 Years	45-54 Years	55-64 Years	65-74 Years	75+ Years
Sample Size (N=)	465	98	75	77	133	82
Median (pg/mL)	26	23	23	37	23	25
Mean (pg/mL)	46	34	34	51	46	69
SD (pg/mL)	70	37	36	48	63	126
95th Percentile	150	89	111	155	159	266
Percentage < 100 pg/mL	88.4%	95.9%	90.7%	89.6%	85.7%	80.5%
Minimum (pg/mL)	0	0	0	0	0	0
Maximum (pg/mL)	837	263	142	230	374	837

Heart Failure Population

Plasma samples from 693 patients with diagnosed heart failure (231 females, 462 males) were tested with the AxSYM BNP assay. All patients in this population were categorized according to the functional classification system published by the New York Heart Association (NYHA).[22] This system divides heart failure patients into one of four categories of increasing disease progression (classes I to IV) based upon a subjective assessment of the patient's clinical signs and symptoms. The data from this study are summarized in the following table.*

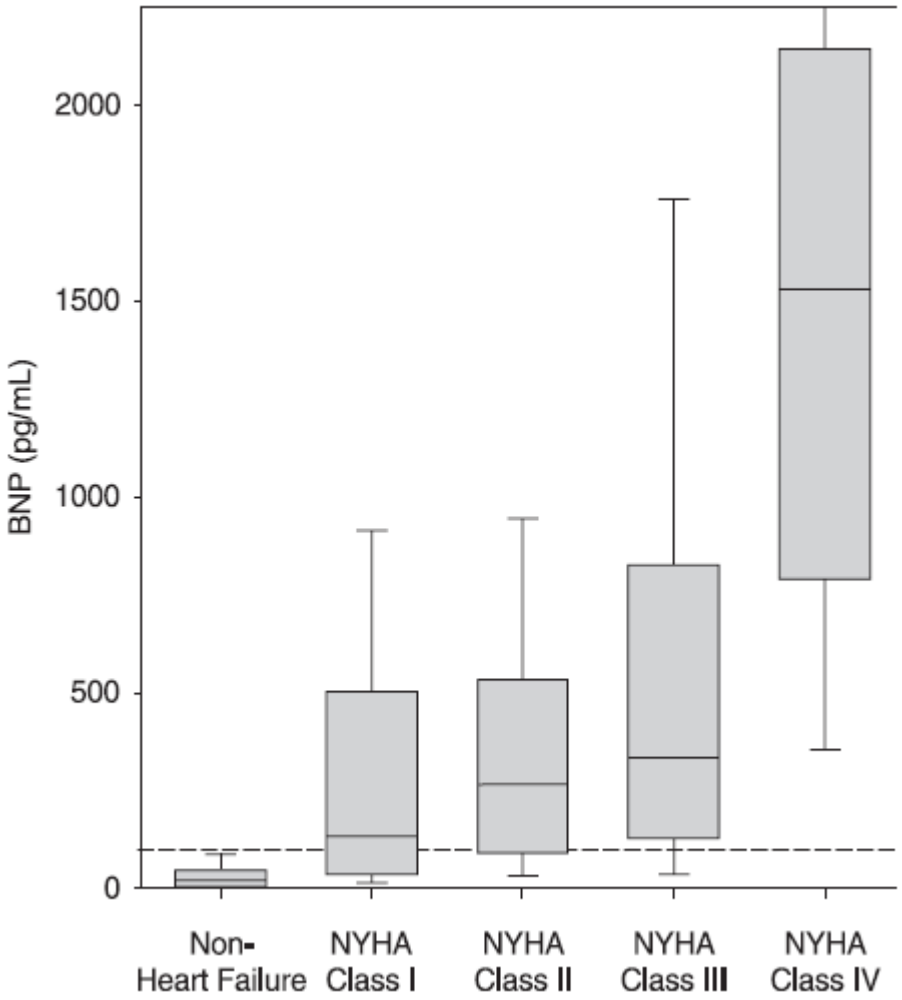
Heart Failure Population - All					
		NYHA Functional Class			
	All	I	II	III	IV
Sample Size (N=)	693	124	319	190	60
Median (pg/mL)	298	133	266	335	1531
Mean (pg/mL)	578	320	432	656	1635
SD (pg/mL)	771	388	574	841	1097
5th Percentile	14	9	15	12	188
95th Percentile	2154	1257	1534	2516	>4000
Percentage ≥ 100 pg/mL	74.2%	58.1%	73.0%	79.0%	98.3%
Minimum (pg/mL)	0	3	0	0	14
Maximum (pg/mL)	>4000	1651	>4000	>4000	>4000

Heart Failure Population - Males					
		NYHA Functional Class			
	All	**I**	**II**	**III**	**IV**
Sample Size (N=)	462	94	215	121	32
Median (pg/mL)	268	122	258	293	1645
Mean (pg/mL)	524	314	409	597	1646
SD (pg/mL)	719	390	539	821	1032
5th Percentile	12	9	14	22	265
95th Percentile	1976	1281	1356	2288	3654
Percentage ≥ 100 pg/mL	71.0%	56.4%	70.7%	76.0%	96.9%
Minimum (pg/mL)	0	3	0	0	14
Maximum (pg/mL)	>4000	1408	3782	>4000	>4000

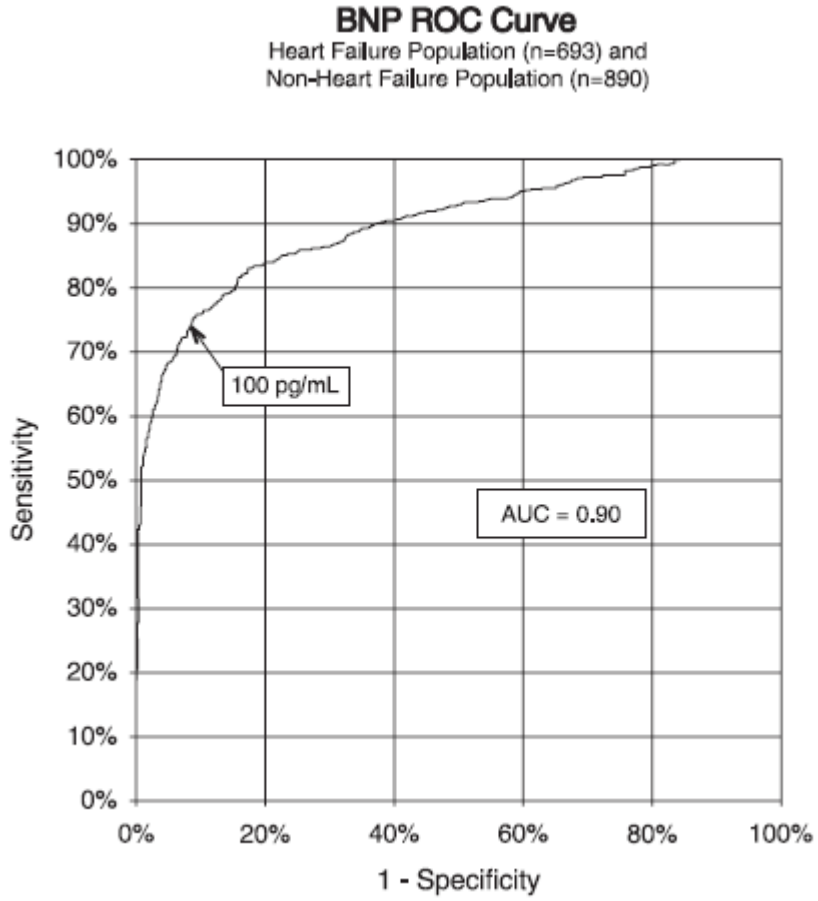
Heart Failure Population - Females					
		NYHA Functional Class			
	All	**I**	**II**	**III**	**IV**
Sample Size (N=)	231	30	104	69	28
Median (pg/mL)	385	174	298	466	1408
Mean (pg/mL)	685	341	481	760	1623
SD (pg/mL)	858	388	641	870	1186
5th Percentile	16	14	21	12	244
95th Percentile	2593	1022	2031	2718	>4000
Percentage ≥ 100 pg/mL	80.5%	63.3%	77.9%	84.1%	100.0%
Minimum (pg/mL)	0	10	0	0	173
Maximum (pg/mL)	>4000	1651	>4000	>4000	>4000

A box and whiskers plot of the clinical study population, broken down by NYHA classification, is presented in the following graph. The dashed line represents 100 pg/mL, the suggested decision threshold for the AxSYM BNP assay. In support of previous literature reports,23 these data show a progressive increase in BNP concentrations with increases in NYHA classifications. This analysis

indicates that BNP measurements provide objective information for use in the assessment of the severity of heart failure.



Data from the above clinical study were used to generate the Receiver Operating Characteristic (ROC) curve of BNP decision thresholds versus clinical sensitivity and clinical specificity as shown in the following graph. At a decision threshold of 100 pg/mL, the BNP assay demonstrated a clinical sensitivity and specificity of 74.2% and 91.5%, respectively, in this study. The area under the curve (AUC) is 0.90 (0.86 to 0.92, 95% CI).



The i-STAT BNP Calibrators are traceable to an internal reference standard that has been prepared gravimetrically with synthetic BNP. The internal reference standard underwent a one-time value assignment to align with the ARCHITECT BNP assay with a decision threshold of 100 pg/mL.

An age-matched analysis of the heart failure and non-heart failure populations was performed based on the data published by the American Heart Association in the 2000 Heart and Stroke Statistical Update24 and according to the age structure of the United States population.25 The age distributions in the intended use population are approximately as follows: individuals less than 45 years old comprise 9%, individuals 45-54 years old comprise 11%, individuals 55-64 years old comprise 22%, individuals 65-74 years old comprise 26%, and individuals 75 years and older comprise 32%. The resulting combined AUC is 0.87 (0.85 to 0.90, 95% CI).

The clinical sensitivity and specificity using a decision threshold of 100 pg/mL is presented in the following table.

Males (Age Group)						
	All	<45 Years	45-54 Years	55-64 Years	65-74 Years	75+ Years
Sensitivity	71.0% (328/462)	47.1% (8/17)	57.1% (24/42)	57.3% (51/89)	70.6% (115/163)	86.1% (130/151)
95% Confidence Interval	66.6 to 75.1%	23.0 to 72.2%	41.0 to 72.3%	46.4 to 67.7%	62.9 to 77.4%	79.5 to 91.2%
Specificity	94.8% (403/425)	97.2% (104/107)	100.0% (71/71)	97.9% (92/94)	88.7% (102/115)	89.5% (34/38)
95% Confidence Interval	92.3 to 96.7%	92.0 to 99.4%	94.9 to 100.0%	92.5 to 99.7%	81.5 to 93.8%	75.2 to 97.1%

Females (Age Group)						
	All	<45 Years	45-54 Years	55-64 Years	65-74 Years	75+ Years
Sensitivity	80.5% (186/231)	44.4% (4/9)	73.3% (11/15)	50.0% (13/26)	80.6% (58/72)	91.7% (100/109)
95% Confidence Interval	74.8 to 85.4%	13.7 to 78.8%	44.9 to 92.2%	29.9 to 70.1%	69.5 to 88.9%	84.9 to 96.2%
Specificity	88.4% (411/465)	95.9% (94/98)	90.7% (68/75)	89.6% (69.77)	85.7% (114/133)	80.5% (66/82)
95% Confidence Interval	85.1 to 91.2%	89.9 to 98.9%	81.7 to 96.2%	80.6 to 95.4%	78.6 to 91.2%	70.3 to 88.4%

Performance Characteristics

Precision data were collected as follows: Duplicates of each control were tested daily for a period of 20 days for each of 3 lots of cartridges, resulting in a total of 434 replicates. The average statistics are presented below.

Whole blood imprecision data were collected as follows: whole blood samples from 5 healthy donors were spiked to low, intermediate and high BNP concentrations affording 15 samples, each of which was measured in 10 i-STAT BNP cartridges from a single cartridge lot; three lots of cartridges were employed. The mean within-sample BNP concentration ranged from 84 – 3925 pg/mL and the within-sample imprecision (%CV) ranged from 3.4 to 9.4%; the average BNP concentration and imprecision were 1464 pg/mL and 6.5% respectively.

Method comparison data were collected using CLSI guideline EP9-A2.26 Venous blood samples were collected in EDTA evacuated tubes and analyzed in duplicate on the i-STAT System. A portion of the specimen was centrifuged and the separated plasma was analyzed in duplicate on the i-STAT System and on the comparative method within 1 hour of collection. Deming regression analysis27 was performed on the first replicate of each sample. In the method comparison table, n is the number of specimens in the first data set, Sxx and Syy refer to estimates of imprecision based on the duplicates of the comparative and the i-STAT methods respectively. Sy.x is the standard error of the estimate, and r is the correlation coefficient.* Method comparisons may vary from site to site due to differences in sample handling, comparative method calibration and other site specific variables.

The i-STAT BNP assay is designed for quantitation of BNP in whole blood or plasma samples. A series of samples for comparison of whole blood and plasma results was prepared from blood drawn from 25 nominally healthy donors. For each donor, whole blood (unspiked) and plasma obtained via centrifugation were first run simultaneously in duplicate i-STAT BNP test cartridges. A whole blood

sample was then spiked with BNP and, following a short equilibration period, a plasma sample was prepared by centrifugation and the whole blood and plasma samples were run simultaneously in duplicate. Three lots of i-STAT BNP test cartridges were employed with a single lot being used for each donor. The results of Deming regression of whole blood vs plasma (x-axis) correlation data are summarized below for all samples ([BNP] < 5000 pg/mL) and separately for samples with [BNP] < 1000 pg/mL.

*The usual warning relating to the use of regression analysis is summarized here as a reminder. For any analyte, "if the data is a narrow range, the estimate of the regression parameters are relatively imprecise and may be biased. Therefore, predictions made from estimates may be invalid".[26] The correlation coefficient, r, can be used as a guide to assess the adequacy of the comparative method range in overcoming the problem. As a guide, the range of data can be considered adequate if r>0.975.

Precision Data (pg/mL)

Aqueous Control	Mean	%CV (within-run)	%CV (total)
Level 1	126	9.0	11.1
Level 2	1551	6.6	8.1
Level 3	3337	8.0	9.8

Method Comparison

	Abbott ARCHITECT
N	433
Mean (pg/mL)	482.1
Sxx (pg/mL)	38.1
Syy (pg/mL)	97.6
Slope	0.971
Intercept	-14.4
Sy.x	198.0
Xmin	5
Xmax	4797.7
Correlation, r	0.972

Equivalence of Whole Blood and Plasma (x-axis)

	Plasma([BNP]<5000pg/mL)	Plasma([BNP]<1000pg/mL)
N	49	36
Mean (pg/mL)	776	146
Sxx (pg/mL)	122.0	18.5
Syy (pg/mL)	98.1	16.5
Slope	0.946	1.01
Intercept	50.2	-0.2
Sy.x	107.3	28.3
Xmin	0	0
Xmax	4173	922
Correlation,r	0.997	0.996

Analytical Sensitivity

The limit of blank (commonly termed analytical sensitivity) was estimated at 14 pg/mL by calculating two times the total imprecision determined using a BNP-depleted plasma material (measured to be <5 pg/mL BNP) over a 20-day imprecision study using three separate lots of BNP cartridges and six i-STAT 1 analyzers.

Analytical Specificity

The BNP method is specific for the B-type natriuretic peptide. The following muscle proteins were tested at both 1000 pg/mL and 20000 pg/mL concentrations and found to have no detectable crossreactivity for BNP: ANP, CNP, and N-terminal pro-BNP.

Recovery

The dilution linearity of the i-STAT BNP test was investigated using EDTA whole blood and plasma samples derived from three separate donors. For each donor, the original BNP negative sample and a BNP spiked sample were prepared. This process yielded three BNP positive whole blood samples that were then assayed in duplicate for each of three separate i-STAT BNP cartridge lots. These whole blood samples were then diluted using an equal mass of the original unspiked whole blood and assayed in duplicate. From this whole blood data, the BNP recovery was calculated.

Whole Blood Sample	Concentration (pg/mL)	Diluted Concentration (pg/mL)	% Recovery
A	590	312	106%
B	2765	1429	103%
C	5123	2803	109%

The plasma derived from these three donors was combined in all pairwise combinations in equal volumes. These combinations were then assayed in duplicate for each of three separate i-STAT BNP cartridge lots. The BNP recovery for each pair was calculated using the average of the six results.

Plasma Blood Sample	Concentration pg/mL)	Diluted Concentration (pg/mL)	% Recovery
A	590	—	—
B	2764	—	—
C	5123	—	—
A+B	—	1570	94%
B+C	—	3992	101%
A+C	—	2734	96%

A plasma sample was spiked with BNP to a value of approximately 5000 pg/mL and the concentration was determined by duplicate measurements with i-STAT BNP test cartridges; the result was found to be within 200 pg/mL of the intended target. This sample was subjected to a series of dilutions with fresh, unspiked plasma in order to prepare a range of concentrations. The concentration of each sample/dilution was calculated based on the measured concentration of the initial solution and the dilutions performed. The diluted samples were then measured in i-STAT BNP test cartridges (N = 6-10). The procedure was repeated with a whole blood sample. The results of these experiments are summarized in the following table.

Sample	Dilution	Calculated [BNP] (pg/mL)	Measured [BNP] (pg/mL)	%Recovery
Plasma	1	52	57	110%
Plasma	2	104	114	110%
Plasma	3	259	265	103%
Plasma	4	518	560	108%
Plasma	5	1036	1002	97%
Plasma	6	2072	2277	110%
Plasma	7	3107	3384	109%
Plasma	8	4143	4222	102%
Whole Blood	1	44	41	93%
Whole Blood	2	88	88	100%
Whole Blood	3	269	287	107%
Whole Blood	4	537	554	103%
Whole Blood	5	725	720	99%
Whole Blood	6	1450	1367	94%
Whole Blood	7	3042	2826	93%
Whole Blood	8	4056	3856	95%

Test Limitations

The frequency of suppressed results is affected by atmospheric pressure. Suppressed result rates may increase with higher elevations (decreased barometric pressure) and may become persistent if testing is performed at more than 7500 feet above sea level. Where unavailability of results is unacceptable, i-STAT recommends having an alternate test method available.

Samples from patients who have been exposed to animals or who have received therapeutic or diagnostic procedures employing immunoglobulins or reagents derived from immunoglobulins may

contain antibodies, e.g., HAMA or other heterophile antibodies, which may interfere with immunoassays and produce erroneous results.[28-34] The generation of potentially interfering antibodies in response to bacterial infections has been reported.[28] While this product contains reagents that minimize the effect of these interferents and QC algorithms designed to detect their effects, the possibility of interference causing erroneous results should be evaluated carefully in cases where there are inconsistencies in the clinical information.

Partially clotted samples can result in elevated BNP readings above the reference range, as well as quality check codes. To prevent this from occurring, upon drawing the whole blood sample into an EDTA collection tube, the sample should be inverted gently at least 10 times to ensure even dissolution of the anticoagulant.

Grossly hemolyzed samples can cause a decreased alkaline phosphatase activity, resulting in decreased detection of BNP, increased assay backgrounds, and/or quality check codes.

Hematocrits in the range of 0-60% PCV have been demonstrated not to affect results. Samples with hematocrit levels above this range have demonstrated increases in the test imprecision and quality check codes.

The analyzer must remain on a flat surface with the display facing up during testing. Motion of the analyzer during testing can increase the frequency of suppressed results or quality check codes. A level surface includes running the handheld in the downloader/recharger.

Measurements of BNP should occur prior to nesiritide (Natrecor) recombinant BNP treatment, or 2 hours post-treatment.[35]

Interference Testing

The following substances were found to have no significant effect (less than 10%) on the BNP method, when added to a plasma pool containing approximately 1000 pg/mL of B-type natriuretic peptide at the concentrations indicated:

Compound	Test Level (µmol/L unless otherwise indicated)
Acetaminophen	1660
Allopurinol	294
Ampicillin	152
Ascorbic Acid	227
Acetyl Salicylic Acid	3330
Atenolol	37.6
Caffeine	308
Captopril	23
Chloramphenicol	155
Diclofenac	169
Digoxin	6.15
Dopamine	5.87
Enalaprilat	0.86
Erythromycin	81.6
Furosemide	181
Sodium Heparin	90 U/mL
Ibuprofen	2425
Isosorbide dinitrate	636
Methyldopa	71
Nicotine	6.2
Nifedipine	1156
Phenytoin	198
Propranolol	7.71
Salicylic Acid	4340
Theophylline	222
Verapamil	4.4
Warfarin	64.9

Interference studies were based on CLSI guideline EP7-A.[36]

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Appendix 1.4 Transthoracic Echocardiography Image Acquisition Protocol

Echo image acquisition protocol: Parasternal long axis (PLAX) view:

- 2d loop at 50-70/second frame rate.
- 2d loop with colour Doppler.
- 2d loop with colour tissue Doppler at >120/sec frame rate.
- M mode of aortic root/left atrium.
- M mode of left ventricle.
- 2d loop of aortic root for left ventricular outflow tract dimension.
- 2d loop RV inflow view.
- 2d loop RV inflow view with continuous wave Doppler.

Parasternal short axis (PSAX) view:

- 2d loop at aortic valve level.
- Pulsed wave of right ventricular outflow tract.
- 2d loop of mitral valve.
- 2d loop with colour Doppler at mitral valve level >120/second frame rate.
- 2d loop at papillary muscle level at 50-70/second frame rate.
- 2d loop at mid left ventricular level.
- 2d loop at apical left ventricular level.

Apical four chamber (A4C) view:

- 2d loop at 50-70/second frame rate focusing on all 4 chambers.
- 2d loop with colour flow Doppler on all 4 chambers.
- 2d loop zoom at 50-70/second frame rate focusing on left and right ventricles.
- 2d loop zoom with colour tissue Doppler at >120/sec frame rate focusing on left and right ventricles.
- Pulsed tissue Doppler of the lateral mitral annulus.
- Pulsed tissue Doppler of the septal mitral annulus.
- Pulsed tissue Doppler of the right ventricular free wall annulus.
- 2d with colour flow Doppler focusing on mitral regurgitation – assess proximal isovelocity surface area, vena contracta and colour jet in left atrium.
- Zoomed 2d loop with colour flow Doppler focusing on mitral regurgitation - assess proximal isovelocity surface area, vena contracta and colour jet in left atrium.
- 2d loop with colour flow Doppler focusing on tricuspid regurgitation.
- 2d loop at >120/sec frame rate including both atria and pulmonary veins.
- Pulsed Doppler of the mitral inflow.
- Pulsed wave Doppler of the right upper pulmonary vein.
- Continuous wave Doppler of the mitral regurgitation jet.
- Pulsed Doppler of the left ventricular outflow.
- Continuous wave Doppler of the aortic outflow.
- Pulsed Doppler of the tricuspid inflow.
- Continuous wave Doppler of the tricuspid regurgitation jet.

Apical two chamber (A2C) view:

- 2d loop at 50-70/second frame rate looking at left atrium and left ventricle.
- 2d loop with colour flow Doppler.
- 2d loop at 50-70/second frame rate focusing on left ventricle.
- 2d loop with colour tissue Doppler at high frame rate focusing on left ventricle.
- 2d loop zoom at high frame rate on left atrium and pulmonary veins.
- 2d loop with colour flow Doppler focusing on mitral regurgitation – focus on proximal isovelocity surface area, vena contracta and colour jet in left atrium.
- Zoomed 2d loop with colour flow Doppler focusing on mitral regurgitation – focus on proximal isovelocity surface area, vena contracta and colour jet in left atrium.

Apical long axis (ALAX) (or three chamber) view:

- 2d loop at 50-70/second frame rate.
- 2d loop at 50-70/second rate focusing on left ventricle.
- 2d loop with colour tissue Doppler.
- 2d loop with colour flow Doppler focusing on mitral regurgitation - focus on proximal isovelocity surface area, vena contracta and colour jet in left atrium.
- Zoomed 2d loop with colour flow Doppler focusing on mitral regurgitation - focus on proximal isovelocity surface area, vena contracta and colour jet in the left atrium.

Subcostal (SC) view:

- IVC dimension and sniff test with M-mode.
- 2d loop of subcostal long axis view.
- 2d loop of subcostal short axis view at papillary muscle level.
- 2d loop of subcostal short axis view at aortic valve level.

Qualitative assessment of primary vs secondary aetiology of mitral regurgitation if present.

Appendix 1.5 Cardiac Marker Quality Control Information

Cardiac Marker & Endocrinology

Quality Controls

cTnl Control Level 1 06P1709
cTnl Control Level 2 06P1710
cTnl Control Level 3 06P1711
BNP Control Level 1 06P1705
BNP Control Level 2 06P1706
BNP Control Level 3 06P1707
BhCG Control Level 1 05P5901
BhCG Control Level 2 05P5902
BhCG Control Level 3 05P5903

Storage: Fridge 2-8C or Room Temperature 4 Hours unopened/recapped.
Equibriation before Use: 15 Minutes at room temperature.

1. Set up the Analyser – Turn the Analyser on, press the Menu key for the Administration menu, then select number 3 for Quality Tests, then 1 for Control, then scan or enter your Operator ID (you can just enter through this), scan the bar code on the vial of the QC for the control lot number, the scan the bar code on the cartridge pouch for the cartridge lot number.

2. Thoroughly mix by gently swirling the bottle, avoid foaming the sample.

3. Dispense a drop of the sample directly from the vial into the cartridge

4. Insert cartridge into the i-STAT.

5. Check values against Value Assignment Sheet for Control Lot Number.

Value Assignment Sheets located:
https://www.abbottpointofcare.com/support/value-assignment-sheets