CHARACTERISING HEART FAILURE WITH PRESERVED EJECTION FRACTION UTILISING CARDIOVASCULAR MAGNETIC RESONANCE IMAGING

Thesis submitted for the degree of Doctor of Philosophy at the University of Leicester

By

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January 2018

Abstract

Characterising heart failure with preserved ejection fraction utilising cardiovascular magnetic resonance imaging

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Background
Heart failure with preserved ejection fraction (HFpEF) represents a growing clinical entity that is incompletely understood.

Aims
We aimed to better phenotype HFpEF using cardiovascular magnetic resonance imaging (CMR) and assessed the relation of CMR parameters to clinical outcomes

Methods and Results
Recruitment was conducted as a single-centre, observational, cohort study. Subjects underwent transthoracic echocardiography, comprehensive stress-rest CMR, six-minute walk testing and Minnesota living with heart failure questionnaire evaluation. The composite endpoint was death and/or rehospitalisation with HF at minimum 6-month follow-up.

In suspected HFpEF (n=154), CMR detected new clinical diagnoses such as coronary artery disease, microvascular dysfunction, hypertrophic cardiomyopathy (HCM) and constrictive pericarditis in a significant proportion (27%) and those with a new diagnosis had adverse outcomes (hazard ratio (HR) 1.92; 95% confidence interval (CI) 1.07–3.45; p = 0.03).

Following exclusion of HCM and constrictive pericarditis, 140 age- and sex-matched 'purer' HFpEF patients were compared to controls (n=48) and HFrEF (n=46). Compared to controls, HFpEF was characterised by changes in the left ventricle (LV) e.g. reduced ejection fraction, increased mass and concentric remodeling, greater focal and diffuse fibrosis. Additionally, left atrial (LA) function was reduced and volumes increased with more prevalent right ventricular systolic dysfunction (RVD - 19%).

Compared to HFpEF, HFrEF patients had worse LV systolic and diastolic function, higher LV mass, more eccentric LV remodeling, more focal and diffuse fibrosis, worse LA function, higher LA volumes and worse RV function.

In HFpEF, indexed extra-cellular volume (iECV) - a novel marker of diffuse fibrosis (HR 2.157; CI 1.326–3.507; p = 0.002), LA ejection fraction (HR 0.703; CI 0.501–0.986; p = 0.041) and RVD were strongly associated with adverse outcomes (HR 2.439, CI 1.201–4.953; p = 0.014).

Conclusions
CMR evaluation highlights the marked clinical and pathophysiological heterogeneity of HFpEF, refines diagnosis and risk-stratifies patients.

This thesis is dedicated to my

Uncle and Auntie:

Dr Radha Krishna Murthy and

Mrs Suseela Devi

Acknowledgements

I would like to thank the following people who contributed to this thesis.

My supervisors Professors Gerry McCann, Iain Squire and Leong Ng have all been a constant source of guidance, provided close supervision and enhanced my critical thinking.

I would like to thank Dr Adrian Cheng and my fellow 'Fellows': Drs Asif Adnan, Jayanth Arnold, Amerjeet Banning, Jamal Khan, Sheraz Nazir and Anvesha Singh for their encouragement, humour and support throughout, especially during seemingly never ending analysis!

My gratitude and sincere thanks extends to Anna-Marie Marsh and John McAdam (physiologists), Mary Harrison and Sue Mackness (research nurses) and all the CMR radiographers for consistently putting up with my endless demands and last minute requests for study visits and CMR slots (and for always obliging).

The research study would not have been possible without the largely elderly cohort of patients and healthy volunteers who dedicated their valuable time and effort.

I would also like to thank my parents, my cousin Gopi and my mother- and father- in-law Ruth and Iain for their support.

My research period from inception to submission of my thesis has coincided with many life-changing events and great personal memories including marriage, the birth of our beautiful children, Anjali and Arjun and embarking upon a career as a Consultant Cardiologist. This entire journey has only been made possible because of the unwavering support of my beautiful wife Jen. She has made countless sacrifices, provided constant encouragement and has quite simply been 'my Rock'.

Academic outputs resulting from this thesis

Awards

Early Career Award (clinical) – Finalist. *Left atrial ejection fraction: a novel imaging biomarker for diagnosis and prognosis in heart failure with preserved ejection fraction.* The joint EuroCMR/ Society for Cardiovascular Magnetic Resonance (SCMR) Meeting – Barcelona, Spain 2018

Young Investigator Award Runner-up. *Diagnostic And Prognostic Utility Of Cardiovascular Magnetic Resonance Imaging In Heart Failure With Preserved Ejection Fraction.* Annual Meeting – British Society for Heart Failure, London, UK 2016

Publications

Kanagala P, Cheng ASH, Singh A, McAdam J, Marsh AM, Arnold JR, Squire IB, Ng LL, McCann GP. *Diagnostic and prognostic utility of cardiovascular magnetic resonance imaging in heart failure with preserved ejection fraction – implications for clinical trials*. J Cardiovasc Magn Reson. 2018 Jan 11;20(1):4. doi: 10.1186/s12968-017-0424-9.

Kanagala P, Squire IB, Ng LL, McCann GP. *Novel plasma and imaging biomarkers in heart failure with ejection fraction.* Int J Cardiol Heart Vasc 2015 Jul 30;9:55-62. doi: 10.1016/j.ijcha.2015.07.004

Kanagala P, Cheng ASH, Singh A, Khan JN, Gulsin GS, Patel P, Gupta P, Arnold JR, Squire IB, Ng LL, McCann GP. *Relation of focal and diffuse fibrosis assessed by cardiovascular magnetic resonance imaging to clinical outcome in heart failure with preserved ejection fraction.* (Accepted by JACC CVI)

Manuscripts written for journal submission

Kanagala P, Cheng ASH, Singh A, Khan JN, Patel P, Gupta P, Arnold JR, Squire IB, Ng LL, McCann GP. *Left atrial ejection fraction: a novel diagnostic and prognostic biomarker in heart failure with preserved ejection fraction.* (Submitted to JCMR)

Kanagala P, Cheng ASH, Singh A, Khan JN, Patel P, Gupta P, Arnold JR, Squire IB, Ng LL, McCann GP. *Prevalence of right ventricular dysfunction and prognostic significance in heart failure with preserved ejection fraction.*

Presentations and abstracts

International

Kanagala P, Cheng ASH, Singh A, Khan JN, Patel P, Gupta P, Arnold JR, Squire IB, Ng LL, McCann GP. *Left atrial ejection fraction: a novel imaging biomarker for diagnosis and prognosis in heart failure with preserved ejection fraction.* Oral presentation in the Early Career Award category (clinical) at the joint EuroCMR/ Society for Cardiovascular Magnetic Resonance (SCMR) Meeting – Barcelona, Spain 2018

Kanagala P, Cheng ASH, McAdam J, Marsh AM, Arnold JR, Patel P, Gupta P, Squire IB, Ng LL, McCann GP. *Relation of focal and diffuse fibrosis assessed by cardiovascular magnetic resonance imaging to clinical outcome in heart failure with preserved ejection fraction.* Poster presentation at the European Society of Cardiology (ESC) Congress, Barcelona, Spain 2017

Kanagala P, Cheng ASH, Singh A, McAdam J, Marsh AM, Arnold JR, Squire IB, Ng LL, McCann GP. *Left atrial ejection fraction: a novel diagnostic and prognostic biomarker in heart failure with preserved ejection fraction, irrespective of cardiac rhythm.* Poster presentation at the European Society of Cardiology (ESC) Congress, Barcelona, Spain 2017

Kanagala P, Cheng ASH, McAdam J, Marsh AM, Patel P, Gupta P, Arnold JR, Squire IB, Ng LL, McCann GP. *Prevalence and prognostic significance of right ventricular dysfunction in heart failure with preserved ejection fraction.* Poster presentation at the European Society of Cardiology (ESC) Congress, Barcelona, Spain 2017

Kanagala P, Cheng ASH, McAdam J, Marsh AM, Squire IB, Ng LL, McCann GP. *Diagnostic And Prognostic Utility Of Cardiovascular Magnetic Resonance Imaging In*

Heart Failure With Preserved Ejection Fraction. Oral presentation at the World Congress on Acute Heart Failure, Florence, Italy 2016

Kanagala P, Cheng ASH, Khan JN, Singh A, Nazir SA, Squire IB, Ng LL, McCann GP. *Unmasking the prevalence of silent myocardial infarction, ischaemia and microvascular dysfunction in HFPEF with CMR.* Moderated poster at the European Society of Cardiology (ESC) Congress, London, UK 2015

Kanagala P, Cheng ASH, Singh A, Khan JN, Nazir SA, Squire IB, Ng LL, McCann GP. *The many faces of HFPEF: insights from the CMR sub-study of DIAMOND-HFPEF.* Poster presentation at the Society for Cardiovascular Magnetic Resonance (SCMR) Congress, Nice, France 2015

National

Kanagala P, Cheng ASH, McAdam J, Marsh AM, Squire IB, Ng LL, McCann GP. *In heart failure with preserved ejection fraction (HFpEF), cardiovascular magnetic resonance imaging (CMR), detects new, alternative diagnoses which carry prognostic significance.* Moderated poster at the British Cardiovascular Society Congress, Manchester, UK 2016

Kanagala P, Cheng ASH, McAdam J, Marsh AM, Squire IB, Ng LL, McCann GP *Diagnostic And Prognostic Utility Of Cardiovascular Magnetic Resonance Imaging In Heart Failure With Preserved Ejection Fraction.* Oral presentation in the Young Investigator Award category at the British Society for Heart Failure Annual Meeting, London, UK 2016

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LIST OF ABBREVIATIONS

A wave = peak velocity of late atrial filling (mitral inflow)

ACEi = angiotensin converting enzyme inhibitor

AF = atrial fibrillation

AR velocity = peak atrial reversal velocity representing flow reversal in the pulmonary vein during atrial systole

ARB = angiotensin II receptor blocker

AS = aortic stenosis

ASE = American Society of Echocardiography

AUC = area under curve

AV = aortic valve

BMI = body mass index

BNP = B-type natriuretic peptide

BSA = body surface area

BSE = British Society of Echocardiography

CAD = coronary artery disease

CHQ = Chronic Heart Failure Questionnaire

CI = confidence interval

CK = creatine kinase

CMR = cardiovascular magnetic resonance imaging

COPD = chronic obstructive pulmonary disease

CPET = cardiopulmonary exercise test

CRF = case record form

CT = computed tomography

D wave = peak diastolic velocity representing flow into the left atrium during ventricular diastole (pulmonary venous flow)

DBP = diastolic blood pressure

DCM = dilated cardiomyopathy

DD = diastolic dysfunction

DHF = diastolic heart failure

DT = deceleration time of E velocity (mitral inflow)

E wave = peak velocity of early left ventricular filling (mitral inflow)

E/A ratio = ratio of E velocity and A velocity (mitral inflow)

ECG = Electrocardiogram

ECM = extra-cellular matrix

ECV = extra-cellular volume

EF = ejection fraction

eGFR = estimated glomerular filtration rate

EDPVR = end-diastolic pressure volume relationship

ESC = European Society of Cardiology

ESPVR = end-systolic pressure volume relationship

FEV1 = forced expiratory volume

FLASH = fast low angle shot

FOV = field of view

FVC = forced vital capacity

FWHM = full width half maximum technique

HCM = hypertrophic cardiomyopathy

HF = heart failure

HFmrEF = heart failure with mid-range ejection fraction

HFnEF = heart failure with normal ejection fraction

HFpEF = heart failure with preserved ejection fraction

HFrEF = heart failure with reduced ejection fraction

HR = hazard ratio

ICC = intra-class correlation coefficient

iECV = extra-cellular volume indexed to body surface area

IVRT = isovolumic relaxation time (mitral inflow)

IQR = interquartile range

KCCQ = Kansas City Cardiomyopathy Questionnaire

LA = left atrium

LAEF = left atrial ejection fraction

LAVmax = maximal left atrial volume

LAVmin = minimal left atrial volume

LAVI = left atrial volume indexed to body surface area

LAVImax = maximal left atrial volume indexed to body surface area

LAVImin = minimal left atrial volume indexed to body surface area

LCBRU = Leicester Cardiovascular Biomedical Research Unit

LGE = late gadolinium enhancement imaging

LV = left ventricle

LVEDP = left ventricular end-diastolic pressure

LVEDV = left ventricular end-diastolic volume

LVEDVI = left ventricular end-diastolic volume indexed to body surface area

LVEF = left ventricular ejection fraction

LVH = left ventricular hypertrophy

LVESV = left ventricular end-systolic volume

LVESVI = left ventricular end-systolic volume indexed to body surface area

LVMI = left ventricular mass indexed to body surface area

LVSV = left ventricular stroke volume

MI = myocardial infarction

MLHF = Minnesota living with heart failure questionnaire

MMPs = matrix metalloproteinases

MOLLI = Modified Inversion Recovery Look Locker

MV = mitral valve

MVD = microvascular dysfunction

MRS = magnetic resonance spectroscopy

NIHR = National Institute for Health Research

NPV = negative predictive value

NRI = net reclassification index

NRES = National Research Ethics Service

NT-proBNP = N-terminal pro-B-type natriuretic peptide

NYHA = New York Heart Association class

PASP = pulmonary artery systolic pressure

PCr:ATP = myocardial phosphocreatine: adenosine tri-phosphate ratio

PCWP = pulmonary capillary wedge pressure

PET = positron emission tomography

PPV = positive predictive value

PSIR = phase-sensitive inversion recovery

QOL = quality of life

ROC = receiver operator characteristic

RV = right ventricle

RVD = right ventricular dysfunction

RVEDV = right ventricular end-diastolic volume

RVEDVI = right ventricular end-diastolic volume indexed to body surface area

RVESV = right ventricular end-systolic volume

RVESVI = right ventricular end-systolic volume indexed to body surface area

RVSV = right ventricular stroke volume

RWMA = regional wall motion abnormalities

S wave = peak systolic velocity representing flow into the left atrium during ventricular systole (pulmonary venous flow)

SBP = systolic blood pressure

S/D ratio = ratio of S velocity and D velocity

SPECT = single photon emission computed tomography

TDI = tissue Doppler imaging

TI = inversion time

TIMPs tissue inhibitors of matrix metalloproteinases

TTE = transthoracic echocardiography

SD = standard deviation

6MWT = Six minute walk test

1 INTRODUCTION

Published (Review article):

Kanagala P, Squire IB, Ng LL, McCann GP. *Novel plasma and imaging biomarkers in heart failure with ejection fraction.* Int J Cardiol Heart Vasc 2015 Jul 30;9:55-62. doi: 10.1016/j.ijcha.2015.07.004

1.1 Heart failure classification

Heart failure (HF) is a clinical syndrome of typical symptoms (e.g. breathlessness, fatigue, oedema) and signs (e.g. elevated jugular venous pulse, pulmonary crepitations) that result as a consequence of abnormal cardiac structure or function. This clinical definition has been further refined to dichotomise HF patients on the basis of left ventricular ejection fraction (LVEF). An arbitrary cut-off for normal LVEF is 50%. The EF is derived by dividing stroke volume (end-diastolic volume minus end-systolic volume) by the end-diastolic volume[1,2].

The EF is important since: a) it has prognostic implications (the lower the EF, the lesser the survival), b) it appears to describe differing epidemiological and aetiological profiles (see later) and c) the vast majority of HF patients have been recruited to clinical trials on the basis of the EF alone.

Typically, as systolic function worsens, the EF is lowered. Thus, the traditionally described 'systolic heart failure' is synonymous with EF ≤40% and now referred to as heart failure with reduced ejection fraction (HFrEF). It is only in this cohort of patients that effective therapies have been demonstrated to date. On the other hand, HF patients with EF ≥ 50% are now termed as heart failure with preserved ejection fraction (HFpEF). This terminology reflects the widely accepted beliefs that whilst EF maybe normal, subtle and more sensitive measures of systolic function may be abnormal. Patients with EF ranges between 40 to 49%, recently re-defined as heart failure with mid-range EF (HFmrEF), represent an intermediate group often described as a 'grey area' that most probably consists of mild systolic dysfunction and diastolic dysfunction[3].

1.2 Why is HFpEF important?

1.2.1 Prevalence

The true overall prevalence of HFpEF in the community has been estimated to be 1.14% – 5.5% of the general population[4]. Initial reports varied widely with reported prevalence ranging from 13% to 74% largely owing to selection biases (differing diagnostic criteria

and population profiles)[5]. However, data from more up-to-date and refined population based echocardiographic studies have shown the mean prevalence of HFpEF amongst HF patients to be 54% (range 40% – 70%)[4]. In addition, the prevalence of HFpEF relative to HFrEF is rising at a rate of ~ 1% year thereby ensuring that HFpEF will be the dominant epidemiological HF phenotype for the foreseeable future[6].

1.2.2 Mortality, morbidity and socio-economic costs

Compared to age and co-morbidity matched controls without HF, HFpEF portends poor prognosis[7]. Annual mortality rates with HFpEF range from 10% to 30%[8]. Mortality risk is comparable to HFrEF *(*141 deaths / 1000 patients years)[6,9] albeit slightly lower in those with HFpEF *(*121 deaths / 1000 patient years)[10].

In addition, readmission rates for those with HFpEF are very high: from acute decompensations (15% – 25% within 6 months, 1/3 within a year) or from any cause (45 – 60% at one year)[11]. Furthermore, since HFpEF predominates in the elderly, a greater burden of cardiovascular (obesity, diabetes, atrial fibrillation [AF]) and non-cardiovascular co-morbidities (renal dysfunction, chronic lung disease, anaemia, malignancy and hypothyroidism) co-exist[12]. In keeping with the above, a Charlson index (a weighted prognostic score of co-morbidity) of ≥ 3 has been found in 70 % of community HFpEF patients[13]. Given that HF admissions alone account for nearly 2% of total National Health Service expenditure and the demographic profile of the HFpEF is laden with significant co-morbidity in a predominantly elderly population, the implications for costing and service provisions are clear[14].

1.3 Defining HFpEF

In the past HFpEF was originally referred to as diastolic heart failure (DHF) since diastolic dysfunction was thought to be the main pathophysiological driver behind this syndrome[15-18]. However, it soon became apparent that diastolic dysfunction is not unique to DHF and is also commonly found in HFrEF[13,19] (and HFmrEF)[3], as well as in patients without HF[7]. Hence, the initial shift in terminology to heart failure with *normal* ejection fraction (HFnEF*)* and most recently to HFpEF[1,20,21].

1.3.1 Overview of HFpEF guidelines

To date, five sets of guidelines (see Table 1.1) have been published to diagnose HFpEF[3,15-17,20].

All guidelines share the following criteria:

- Signs and/or symptoms of HF
- Evidence of preserved EF
- Evidence of diastolic dysfunction

All guidelines recognise elevated filling pressures (left ventricular end-diastolic pressure [LVEDP], pulmonary capillary wedge pressure [PCWP]) measured invasively as stand-alone evidence for diastolic dysfunction. The original proposal by the European Society of Cardiology (ESC) used a lower EF cut-off (>45%)[15]. Subsequent guidance[16] mandated EF measurement within 72 hours of presentation as well as invasive assessment of diastolic function. However, this was shown to lack sensitivity and impractical in a real world setting. Additionally, the emphasis on timely gathering of EF data was subsequently removed from future guidelines since acute measurements of EF during decompensation were found to be similar when re-measured after 72 hours[22].

The third set of guidance[17] introduced structural LV abnormalities (left ventricular hypertrophy [LVH], left atrial [LA] enlargement) as surrogates of diastolic dysfunction. Finally, the latter two reports from the ESC[3,20] have incorporated the use of tissue Doppler imaging (TDI) by echocardiography and plasma natriuretic peptides. In the latest guidelines[3] (see Figure 1.1), the diagnostic thresholds for TDI E/E'[23] and natriuretic peptides[24] have been lowered to reflect recent evidence on normal ranges. An exercise component has also been proposed to identify subjects with normal E/E' at rest but in whom E/E' rises with stress testing[25,26].

Table 1.1 Summary of HFpEF guidelines

	Guidelines				
	ESC[15] 1998	**NHLBI[16] 2000**	**LAHEY[17] 2005**	**ESC[20] 2007**	**ESC[3] 2016**
HF signs & symptoms	Present	Present	Present	Present	Present
Normal LV systolic function	LVEF > 45% LVEDVI < 102 ml/m^2	LVEF > 50% within 72 hours of HF episode	LVEF > 50% LVEDVI < 97 ml/m^2	LVEF > 50% LVEDVI < 97 ml/m^2	LVEF ≥ 50%
LV diastolic dysfunction (DD)	Invasively measured high filling pressures **Or** Echo measures of DD - transmitral flow	Invasively measured high filling pressures	Invasively measured high filling pressures **Or** Echo measures of DD - including previous *plus* - Left atrial enlargement *or* - left ventricular hypertrophy	Invasively measured high filling pressures **Or** Echo measures of DD - including previous *plus* - Tissue Doppler E/E' > 15 as stand alone *or* - E/E' > 8 plus elevated plasma natriuretic peptides BNP > 200pg/ml or NT-pro BNP > 220 pg/ml	Elevated plasma natriuretic peptides: BNP > 35pg/ml or NT-pro BNP > 125 pg/ml ***And*** At least one additional criterion: 1. Relevant structural heart disease i.e. Left atrial enlargement *or* left ventricular hypertrophy ***Or*** 2. Measures of DD a) Echo measures Resting or exercise induced E/E'> 13 ***Or*** b) Invasively measured high filling pressures

Current diagnostic criteria for HFpEF

A schematic outlining the latest guidelines for diagnosing HFpEF is illustrated in Figure 1.1 below.

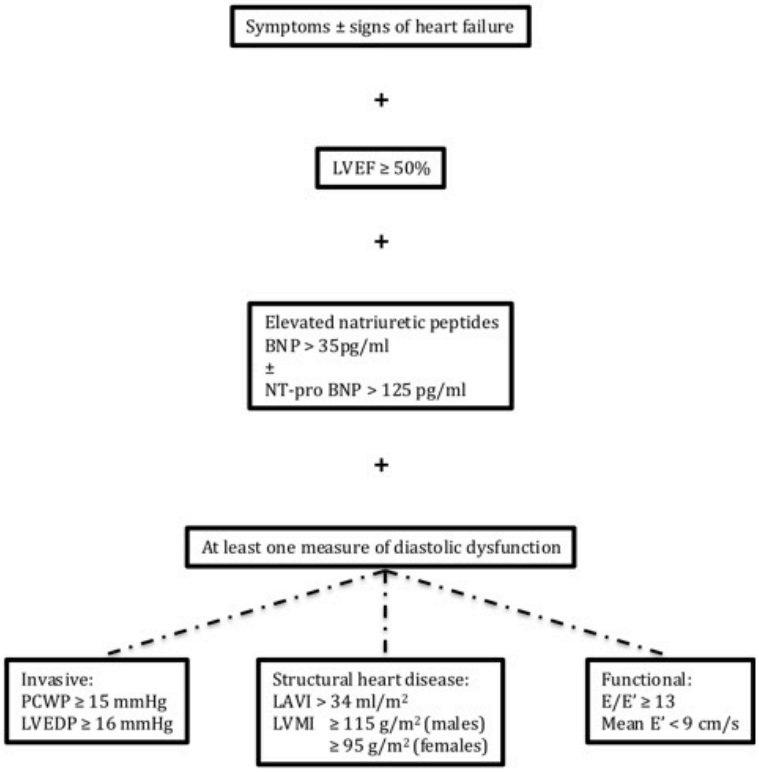


Figure 1.1 Flow diagram illustrating latest diagnostic guidelines for HFpEF

BNP = B-type natriuretic peptide; LAVI = left atrial volume indexed to body surface area; LVEDP = left ventricular end-diastolic pressure; LVEF = left ventricular ejection fraction; LVMI = left ventricular mass indexed to body surface area; NT-proBNP = N-terminal pro-B-type natriuretic peptide; PCWP = pulmonary capillary wedge pressure

1.3.2 Signs and symptoms

Both HFpEF and HFrEF patients have similar presentations[27]. Using clinical acumen alone (signs and symptoms), misdiagnosis of HF may result in 50% of cases[28]. In general, clinical features tend be over-sensitive and non-specific for the diagnosis of HF (see Table1.2)[27]. In addition, the typical phenotypes of HFpEF (e.g. elderly, obese, AF) make diagnosis more challenging as clinical signs are often more difficult to elicit[29-31]. Besides, symptoms and signs may be multi-factorial in origin given the greater prevalence of co-morbid conditions (e.g. lung disease, renal impairment, anaemia, hypothyroidism)[12]. Symptoms disproportionate to the degree of cardiac pathology in HFpEF patients have also been reported[32].

Table 1.2 Prevalence of clinical features in HFpEF versus HFrEF

	HFpEF (%)	**HFrEF (%)**
Symptoms		
Dyspnoea on exertion	85	96
Paroxysmal nocturnal dyspnoea	55	50
Orthopnoea	60	73
Signs		
Elevated jugular venous pulse	35	46
Pulmonary crepitations	72	70
Displaced apex	50	60
Third heart sound	45	65
Fourth heart sound	45	66
Hepatomegaly	15	16
Oedema	30	40
Chest radiography		
Cardiomegaly	90	96
Pulmonary venous congestion	75	80
Adapted from Zile[27]		

1.3.3 Evidence of preserved EF

Controversy still exists as to what constitutes a 'normal' EF[33-36]. Firstly, the LVEF in HF patients has been shown to demonstrate a 'unimodal' pattern of distribution (see Figure 1.2)[37-40]. Secondly, the distinction between 'normal' and 'abnormal' EFs has varied markedly in previous guidelines, clinical trials and registry data ('normal' EFs ranging from 40% to >50%)[20,41-47]. Besides, a 'normal' EF does not equate to normal systolic function[48].

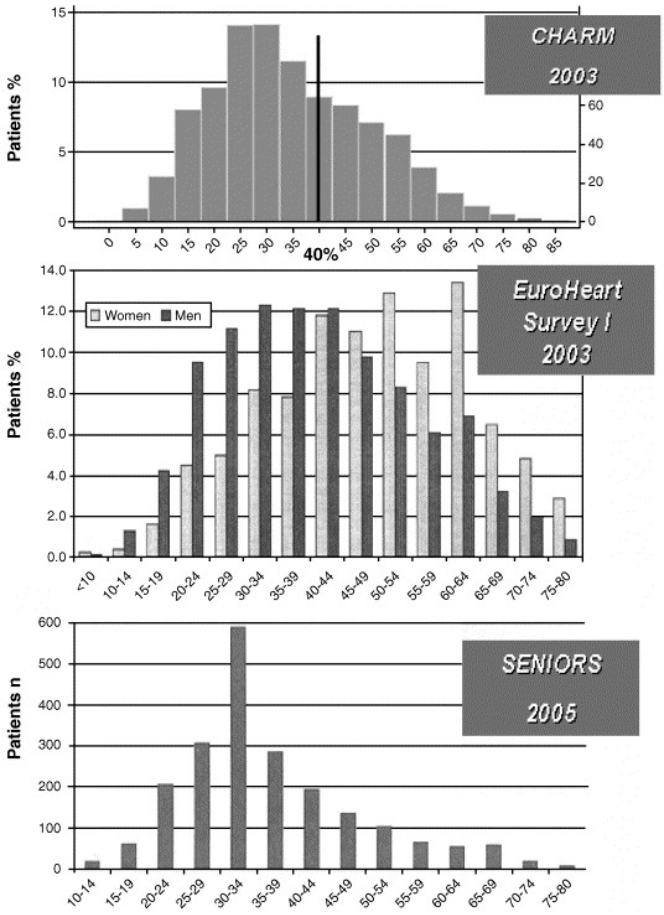


Figure 1.2 Unimodal distribution of left ventricular ejection fraction from clinical trials

Reproduced with permission from Brutsaert[37]. Data were obtained from 3 recent studies: CHARM[40], EuroHeart Survey[49] and SENIORS[39].

The EF as a surrogate measure of systolic function has been incorporated into the guidelines for many reasons: it is non-invasive, reproducible, provides a crude measure of overall pump function, is readily available in routine clinical practice and is useful as a prognostic indicator[50]. The EF however, merely reflects the change in ventricular volumes between cardiac cycles and thus is an imprecise measure of systolic function and has intrinsic methodological limitations.

Subtle but significant systolic abnormalities are readily missed using this approach[19]. This technique fails to provide useful information about contractile function nor accounts for any compensatory mechanisms at play. It reflects radial function better than longitudinal function. EF values are further affected by loading conditions and the degree of LV mass (especially LVH which is a common finding in both HFpEF and the elderly)[51]. In addition, mathematical models have demonstrated that LVEF can be spuriously increased in the setting of LVH and normal LV volumes despite significant reductions in both stroke volume and longitudinal function[52].

1.3.4 Assessment of diastolic function

Traditionally, diastole describes the time frame from aortic valve (AV) closure i.e. end-systole to mitral valve (MV) closure i.e. end-diastole. This period (see Figure 1.3) is divided into four phases as described below.

- **Phase 1** (isovolumic relaxation): Describes the onset of LV relaxation whereby LV pressure falls rapidly following AV closure. Eventually, when LV pressure drops below LA pressure, the MV opens. LV relaxation is both an active (energy utilised by the myocardium) and passive (elastic myocardial recoil) process. This phase is influenced by alterations in myocardial loading and inactivation as well as dyssynchrony.

- **Phase 2** (the early, rapid diastolic filling phase): Following MV opening, a trans-mitral pressure gradient is created ‘suctioning’ blood rapidly from the LA into the

LV. This period contributes approximately 80% of normal LV filling and is affected by active LV relaxation properties and compliance.

- **Phase 3** (diastasis): Occurs when there is equalisation of pressures between the LV and LA resulting in slow (or minimal) blood flow between the compartments. This is predominantly influenced by compliance.

- **Phase 4** (the late, diastolic filling period due to atrial contraction): With LA contraction, LA pressure again exceeds LV pressure resulting in a second pulse of blood flow between the LA and LV, contributing approximately 20 % of normal LV filling. This process is dependent on LVEDP and LA function.

Globally, LV filling is determined by the complex interplay between LV filling pressures and filling properties (described with *stiffness* and *compliance*). These factors in turn are governed by extrinsic (e.g. pericardial restraint, ventricular interaction) and intrinsic factors such as chamber *stiffness* (cardiomyocytes and extra-cellular matrix [ECM]), myocardial tone, chamber geometry and wall thickness. Increased LV filling pressures are the primary pathophysiological consequence of diastolic dysfunction. They are considered elevated when the mean PCWP is > 12 mmHg or when the LVEDP is > 16 mmHg[53].

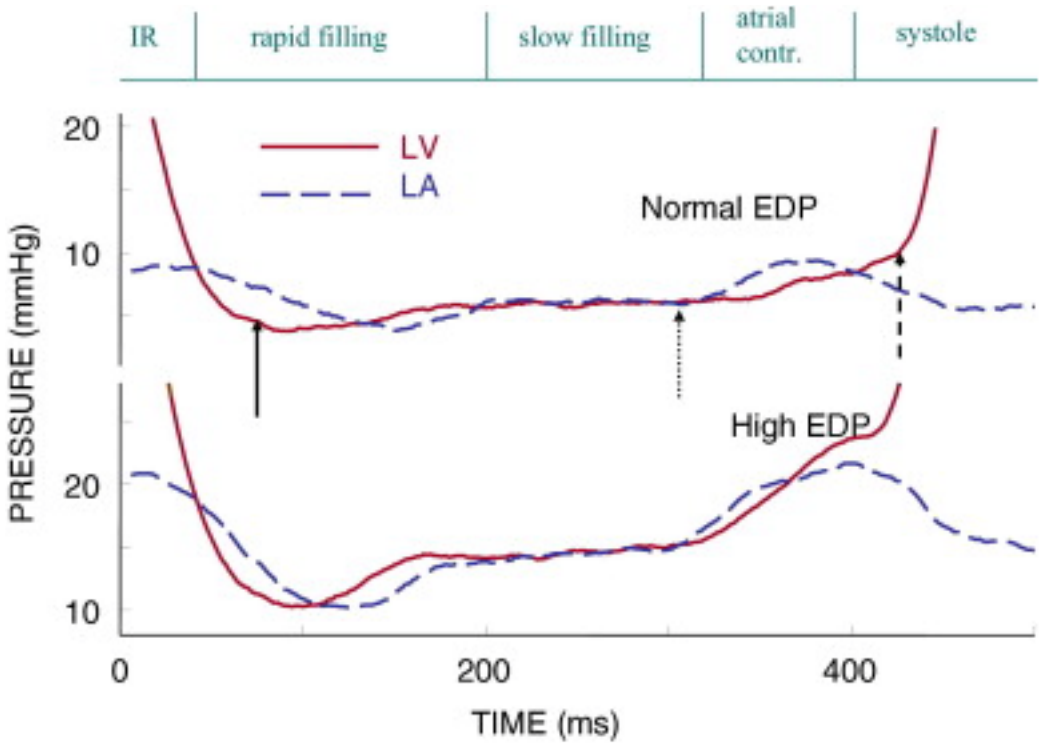


Figure 1.3 The four phases of diastole in relation to pressure recordings from the left atrium and left ventricle

Reproduced with permission from Nagueh[53].The first pressure crossover corresponds to the end of isovolumic relaxation and mitral valve opening. In the first phase, left atrial pressure exceeds left ventricular pressure, accelerating mitral flow. Peak mitral E roughly corresponds to the second crossover. Thereafter, left ventricular pressure exceeds left atrial pressure, decelerating mitral flow. These two phases correspond to rapid filling. This is followed by slow filling, with almost no pressure differences. During atrial contraction, left atrial pressure again exceeds left ventricular pressure. The solid arrow points to left ventricular minimal pressure, the dotted arrow to left ventricular pre-A pressure, and the dashed arrow to left ventricular end-diastolic pressure. The upper panel was recorded at a normal end-diastolic pressure of 8 mm Hg. The lower panel was recorded after volume loading and an end-diastolic pressure of 24 mm Hg. Note the larger pressure differences in both tracings of the lower panel, reflecting decreased operating compliance of the left atrium and left ventricle. Atrial contraction provokes a sharp rise in left ventricular pressure, and left atrial pressure hardly exceeds this elevated left ventricular pressure.

1.3.5 Evidence of diastolic dysfunction

1.3.5.1 Invasive assessment

Pressure-volume loops (see Figure 1.4) derived from measurements at the time of cardiac catheterisation provide unique insights into LV diastolic (and systolic) function. The end-systolic pressure volume relationship (ESPVR) reflects LV chamber *stiffness* (the ratio of change in *pressure* to change in *volume*) and the slope of ESPVR is a measure of end-systolic *elastance* (Ees). Essentially, changes in ESPVR are indicative of systolic pump function. On the other hand, the end-diastolic pressure volume relationship (EDPVR) reflects passive mechanical properties of the LV. Therefore, changes in the EDPVR are indicative of abnormalities of ventricular *elastance* and *compliance*. *Compliance* represents the ratio of change in *volume* to change in *pressure*. Therefore, it is represented by the slope of the EDPVR and varies accordingly with LV filling pressures.

In patients with classical HFrEF (i.e. dilated and remodeled LV with impaired pump performance), the EDPVR is shifted to downward and to the right. This reflects impaired LV ejection capacity whilst maintaining the end-diastolic pressure. In HFpEF, the EDPVR is shifted upwards and to the left. This reflects impaired LV filling capacity and elevated end-diastolic pressures[54,55].

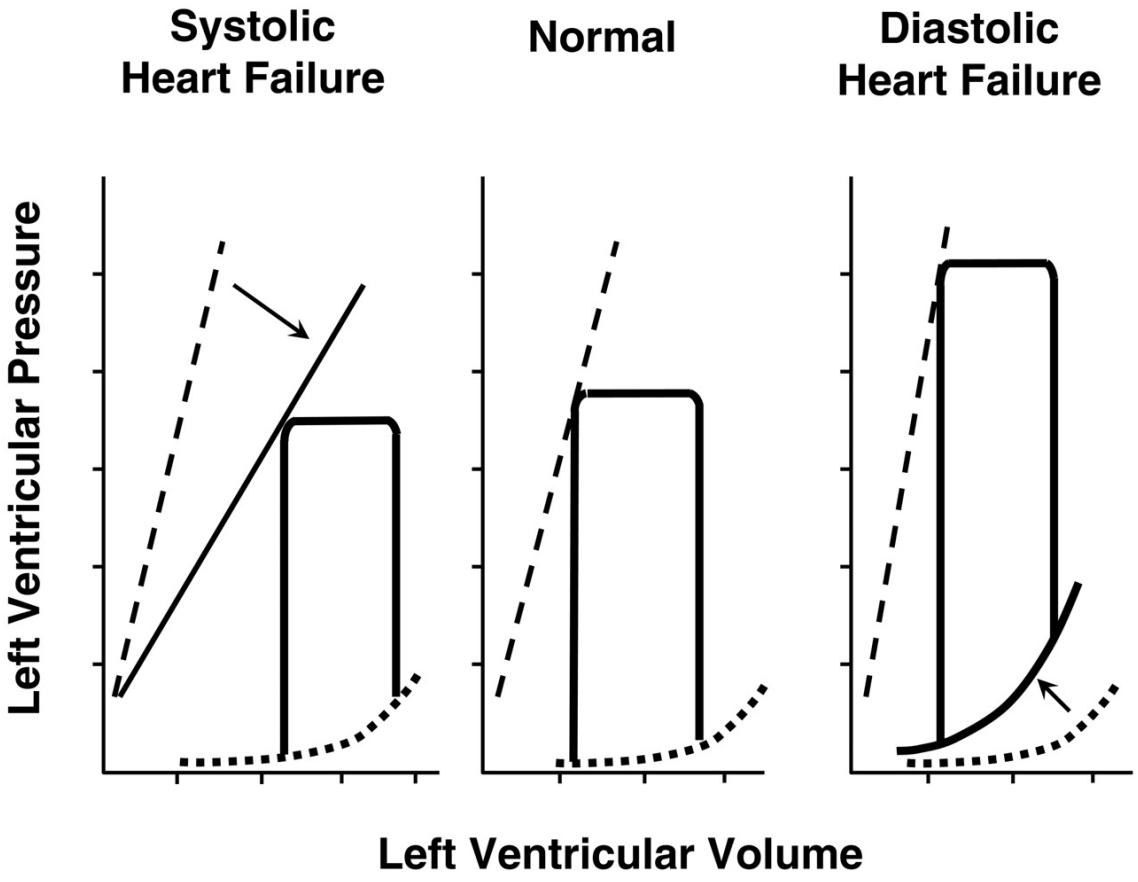


Figure 1.4 Pressure-volume relationships in HFrEF, normal and HFpEF subjects

Reproduced with permission from Aurigemma[54]. Schematic LV pressure-volume relationship through 1 cardiac cycle in systolic heart failure (left), a normal control (center), and diastolic heart failure (right). The dominant functional abnormality in systolic heart failure is a decrease in LV contractility, as evidenced by a decrease in the slope of the end-systolic pressure-volume relationship (systolic elastance). By contrast, the predominant functional abnormality in diastolic heart failure is an increase in diastolic stiffness, as evidenced by an upward and leftward shift of the diastolic pressure-volume relationship.

In a landmark study, it was demonstrated that patients labeled with 'DHF' had uniform diastolic abnormalities in both active relaxation (measured by prolonged time constant of isovolumic pressure decline – τ) and LV *stiffness* (as measured by an increased passive *stiffness* constant – β)[18]. Symptoms of HF are thought to result from this association of *stiffness* with elevated LVEDP (and PCWP) even after very small changes in LV end-

diastolic volumes[28]. Exertional symptoms in HFpEF are further thought to be a consequence of failure to sufficiently raise cardiac output during exercise due to abnormal LV filling and an inability to utilise the Frank-Starling mechanism[28,54].

Interpretation of pressure volume curves is also loading dependent. Conceptually, *compliance* is the inverse of *stiffness*. With time, a normal ventricle when exposed to non-cardiac causes of increased volumes such as with excessive fluid administration or renal failure may render the LV less compliant. Therefore, the EDPVR is not truly a measure of *stiffness*, rather the extent to which *stiffness* depends on volume. Hence, the measurements of EDPVR and such catheter derived data whilst useful, cannot be considered as definitive indices for diastolic dysfunction and may also carry peri-procedural risk[56]. In addition, data from HFpEF subjects reveals marked heterogeneity in EDPVR curves which may exhibit shifts towards all ranges of ventricular volumes (lower, higher and normal). This data variability (see Figure 1.5 and also Table 1.5) further suggests differing pathophysiological mechanisms are implicated in HFpEF [55,57,58].

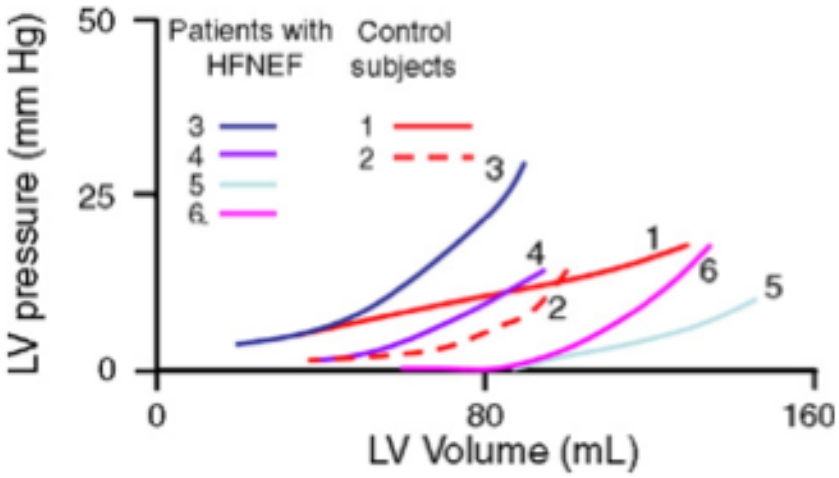


Figure 1.5 Heterogeneity of end-diastolic pressure volume relationships in HFpEF

Reproduced with permission from Maurer[55]. End-diastolic pressure-volume (PV) relations re-plotted from the work of Kawaguchi et al[57] and from Liu et al[58].End-diastolic PV relations of the patients with HFNEF may be shifted to the left (curve 3), shifted to the right (curves 5 and 6), or may not be significantly different (curve 4) from those of control subjects (curves 1 and 2).

1.3.5.2 Echocardiographic measures

1.3.5.2.1 Doppler Mitral inflow

Based on Doppler mitral inflow, the following measurements can be derived: peak of early filling (E velocity), peak of late atrial filling (A velocity), E/A ratio, deceleration time (DT) of E velocity and isovolumic relaxation time (IVRT).

Typical patterns based on the E/A ratio and DT allow diastolic function to be graded as follows (see Figure 1.6):

- normal (E/A ratio 1-2)
- mild (impaired relaxation)
- moderate (‘pseudo-normal’ LV filling)
- severe (restrictive filling)

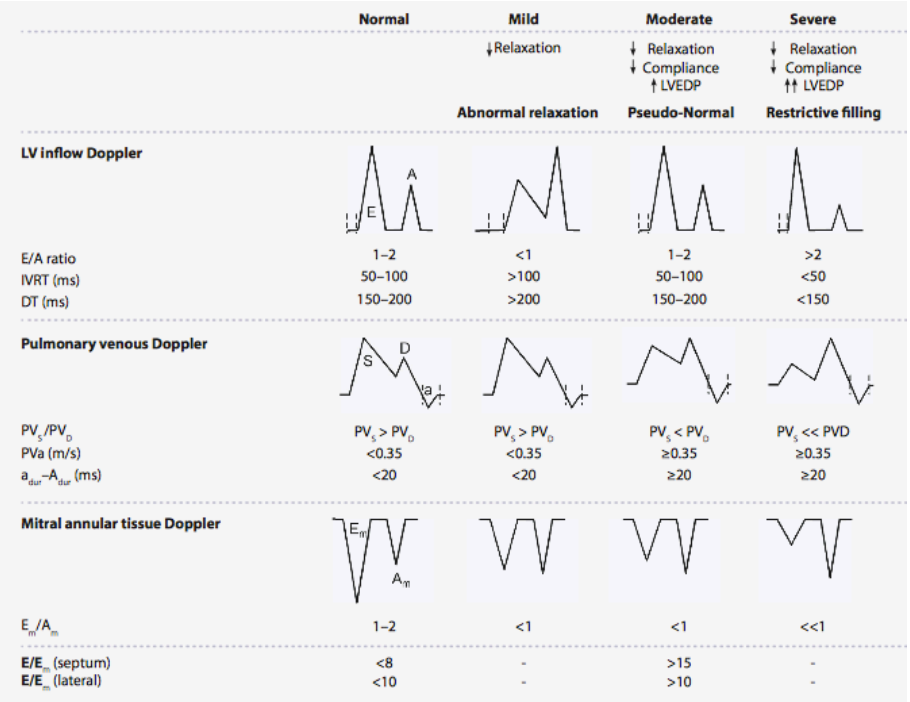


Figure 1.6 Echocardiographic grading of diastolic function

Reproduced from the British Society of Echocardiography: Guidelines for Chamber Quantification (https://www.bsecho.org/media/40506/chamber-final-2011_2_.pdf)

Mild diastolic dysfunction is associated with impaired relaxation prolonging IVRT, causing a slower rate of decline in LV pressure and thus a smaller LA to LV pressure gradient, which in turn reduces the E velocity. Compensatory mechanisms promote a greater contribution from late filling and hence a higher A velocity. The E/A ratio is thus lowered (<1).

With disease progression to moderate diastolic dysfunction, LA pressure rises to increase early LV filling. Hence, the E velocity is increased and a ‘pseudo-normal’ pattern is established with a normal E/A ratio (1-2) again.

When diastolic dysfunction approaches the severe spectrum, even higher LA pressures become established and greater E velocities are found (classically E/A ratios > 2 are seen).

Mitral inflow indices are subject to many variables including age, pre-load, heart rate, PR interval, arrhythmia, mitral valve disease and LA function. In addition, the filling patterns demonstrate a 'U' shaped relation with diastolic function such that similar values may be seen in both healthy subjects and in patients with cardiac dysfunction, reducing diagnostic clarity[15,59-62]. Used alone, these filling patterns have shown variable predictive values to detect HFpEF and therefore do not provide standalone evidence of HFpEF[20,63].

1.3.5.2.2 Doppler Pulmonary venous flow

Based on pulmonary venous flow sampling, the following measurements can be derived: peak systolic velocity (S) representing flow into the LA during ventricular systole, peak diastolic velocity (D) representing flow into the LA during ventricular diastole, the S/D ratio, peak atrial reversal (AR) velocity representing flow reversal in the pulmonary vein during atrial systole (unless in AF), the time duration of AR velocity (Ard), the time difference between AR duration and mitral inflow A velocity duration (AR – A duration)[53].

In general, pulmonary venous flow patterns are heavily influenced by age, mitral valve disease and the presence AF. Routine use to detect diastolic dysfunction is further limited by technical challenges of data acquisition (~ 80% of ambulant patients) and interpretation in the presence of artefacts. However, the AR – A duration (> 30 milliseconds) when obtained is useful as it correlates highly with elevated LVEDP[53,64]. When combined with mitral inflow measures, 93% of suspected HFpEF patients had evidence of diastolic dysfunction[20,65].

1.3.5.2.3 TDI for mitral annular velocities

TDI measures tissue velocity with high temporal and spatial resolution. The high feasibility, reproducibility, relative ease of operator use in clinical practice and the absence of a 'pseudonormal' pattern of LV filling make it the first-choice echocardiographic technique to assess diastolic function. Given that the LV apex is relatively fixed, TDI measurement of the movement of the mitral valve annulus is analogous to assessment of LV longitudinal function.

Based on TDI mitral annulus sampling, the following measurements can be derived: early diastolic velocity (E') measured at the septal and lateral insertion sites (and also expressed in the literature as Ea, Em, e'), late (atrial) diastolic velocity (A'), E'/A' ratio, systolic velocity (S').

It is recommended that measurements should be taken from both sides of the annulus (see Figure 1.7) and averaged (lateral E' values are typically higher than septal E'). Similar to other indices of diastolic function, TDI values are influenced by age. With advancing age, E values drop whereas A and E/E' ratios increase.[53]

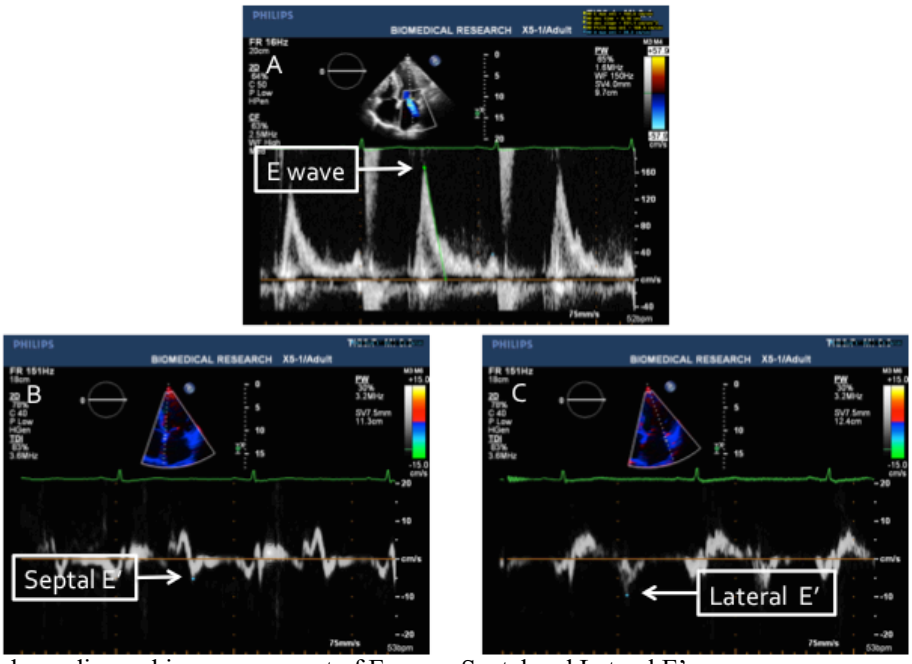


Figure 1.7 Echocardiographic measurement of E wave, Septal and Lateral E'

Although all the parameters described above provide insights into diastolic function, the ratio of mitral inflow E divided by E' (E/E') has been shown to be the best surrogate marker of LV filling pressure and correlates with catheter derived PCWP[66]. As discussed previously, whilst the E' is synonymous with the amount of blood flow from LV to LA, the

mitral inflow E velocity represents the pressure gradient required to make this blood shift. In simpler terms, a high E/E' equates to high LV to LA pressure gradient for a low shift in volume. Typically, E/E' ratios < 8 reflect normal LVEDP whilst E/E' ratios > 15 correlate with high LVEDP and provide definitive evidence of diastolic dysfunction. E/E' values ranging from 8 to 15 require additional echocardiographic or plasma biomarker evidence to confirm diastolic dysfunction [20,67].

1.3.5.2.4 Left atrial volume

A dilated LA is a marker of chronically elevated mean LA pressure i.e. LVEDP. Observational studies of patients without prior history of AF nor significant valvular dysfunction have shown that left atrial volume index (LAVI) ≥ 34 ml/m^2 is an independent predictor of mortality, HF, future AF development and ischaemic stroke[68]. In another population based study, indexed LA volumes (see Figure 1.8 for echocardiographic method illustration) closely correlated with the degree of diastolic dysfunction [69]. In suspected HFpEF, LAVI > 26ml/m^2 has shown to be a strong independent predictor of diastolic dysfunction as revealed by natriuretic peptides[70]. In a retrospective analysis of 1229 echocardiograms to test ESC guidelines[20] for HFpEF, LAVI > 40 ml/m^2 showed a high sensitivity & specificity for the diagnosis of HFpEF[71].

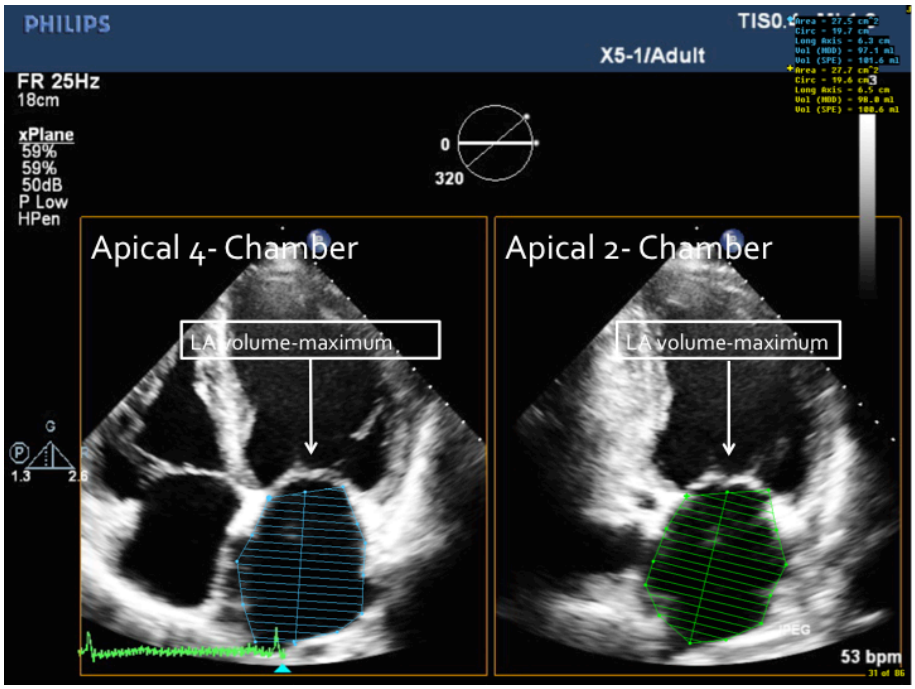


Figure 1.8 Echocardiographic calculation of left atrial volumes using Simpson's method

1.3.5.2.5 Left ventricular hypertrophy

Increased LV mass defines the presence of LVH. Calculation of the relative wall thickness (RWT) by the formula: *RWT = 2 x posterior LV wall thickness in end-diastole divided by the LV internal end-diastolic diameter* categorises LVH into either concentric or eccentric patterns. Essentially, RWT describes the relationship of LV wall thickness to LV cavity size i.e. concentricity[72]. In HFpEF, LVH has been proposed as an expression of advanced hypertensive heart disease[73], since preceding hypertension has been noted in up to 90% of subjects from epidemiological data[12,73]. Across both epidemiological studies[74,75] and registry data[76], LV cavity size appears to be predominantly normal with varying degrees of wall thickness. Concentric hypertrophy (LVH with increased RWT) has been the most commonly observed phenotype in this setting[74-76]. Hence, HFpEF guidelines[3,15-17,20] have stipulated the presence of a non-dilated LV with accompanying LVH for diagnosis.

Current diagnostic thresholds for LVH which have been incorporated into HFpEF guidelines are based upon sex-specific normal reference ranges published by the American Society of Echocardiography (ASE)[77]. The prolate ellipse model is the ASE recommended method for LV mass calculation (see Chapter 2 for formula) and an illustration of the echocardiographic measurements to be undertaken are shown in Figure 1.9.

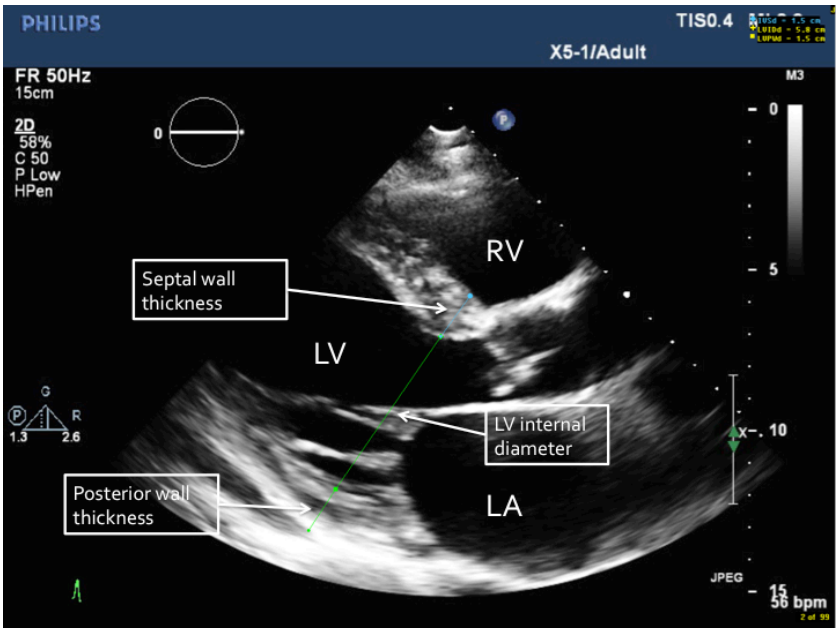


Figure 1.9 Echocardiographic measurements for calculation of LV mass and relative wall thickness

1.3.5.3 Plasma Natriuretic peptides

The natriuretic peptide pro-BNP is secreted by ventricular myocardium in direct response to LV (and LA) wall stress and LVEDP in both HFrEF & HFpEF[78] (see Figure 1.10) and adversely affects prognosis[78,79]. In plasma, pro-BNP is then cleaved to release B-type natriuretic peptide (BNP) and N-terminal pro-B-type natriuretic peptide (NT-pro BNP). The use of both BNP[80] and NT-pro BNP[81] for diagnosis in HF patients is now well established. Furthermore, natriuretic peptides correlate with echocardiographic indices of diastolic dysfunction and with worsening grades[82,83]. Additional correlation with invasive measures of diastolic dysfunction have also been shown[84,85]. For HFpEF exclusion, levels of BNP[80] (< 100) and NT-pro BNP[84] (< 120) exhibit high negative predictive values (96% and 93%) respectively.

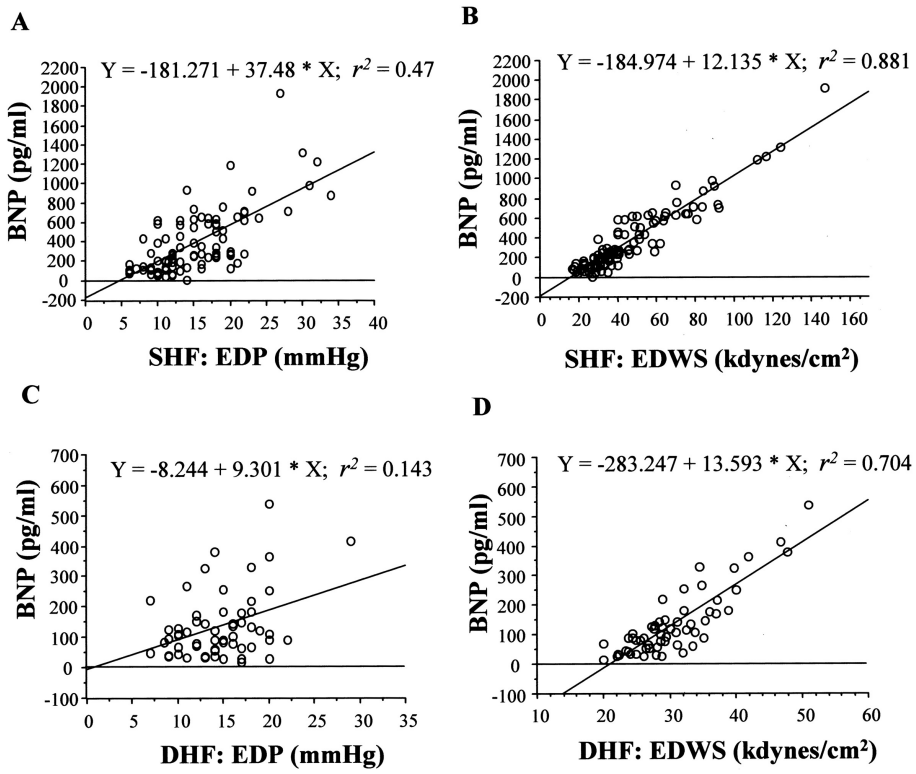


Figure 1.10 The relation of BNP with left ventricular wall stress and end-diastolic pressure

Reproduced with permission from Iwanaga[78]. Correlation between B-type natriuretic peptide (BNP) and left ventricular functional parameters in 98 patients with systolic heart failure (SHF) (A and B) and in 62 patients with diastolic heart failure (DHF) (C and D); (A and C) end-diastolic pressure (EDP) (mm Hg) and (B and D) end-diastolic wall stress (EDWS) (kdynes/cm2).

Conversely, levels can independently increase with age, in females and in co-morbid conditions such as sepsis, renal impairment, arrhythmia and chronic lung disease[24]. These findings are again frequently encountered in HFpEF populations. Hence, the latest guidance[3] suggests that elevated BNP and NT-pro BNP values do not provide standalone evidence of HFpEF and must be supplemented with other surrogate markers of diastolic dysfunction[20].

Are HFpEF and HFrEF part of the same syndrome or two separate entities?

It is now widely recognised that HFpEF as a syndrome does exist[2,20]. HFpEF patients account for approximately half the HF population in epidemiological data[6]. Significantly, the classical haemodynamic changes (e.g. elevated LVEDP and impaired LV relaxation)[18] and neurohormonal mechanisms typical of HF subjects have also been noted in HFpEF[86]. Conceptually, much debate still remains however as to whether HFpEF and HFrEF reflect different ends of the same HF spectrum or indeed whether HFpEF is a separate syndrome in its own right[87,88]. These two divergent hypotheses will have an obvious impact when trying to develop suitable biomarkers for diagnosis[89].

1.3.6 Evidence for the same syndrome hypothesis

The single syndrome hypothesis arose as a result of similar clinical features being present in both HFpEF and HFrEF[9,27,28]. This concept is further reinforced by epidemiological and earlier clinical trial data displaying a 'unimodal' distribution of LVEF (see earlier Figure 1.2)[37-40,49]. It is postulated that HFpEF represents a precursor that transitions across the HF spectrum in a continuum and eventually becomes HFrEF. Furthermore, it is the degree of remodeling that ultimately dictates this rate of temporal progression[19].

Such progression to eccentric remodeling and then HFrEF, has been highlighted in longitudinal studies of hypertensive heart disease[90-93] and in patients with hypertrophic cardiomyopathy (HCM)[94]. Importantly, many of these studies did not report interval rates of myocardial infarction (MI), which may alternatively have contributed to worsening LV dysfunction. However, in a study that did report such a finding, the rates were not significantly higher[92]. In a small echocardiographic study of HFpEF (n = 38), 21% of patients developed significant worsening of LV systolic function at 3-month follow-up[95].

Despite global measures of systolic function i.e. EF appearing to be *normal* in HFpEF, several studies have highlighted subtle yet definitive systolic abnormalities which progressively worsen over time[48,96-100]. Crucially, in support of the same syndrome

hypothesis, diastolic dysfunction the marker from which the 'DHF' label originated is present in both HFpEF and HFrEF[101-104].

1.3.7 Evidence for the separate syndrome hypothesis

To the contrary, subsequent registry and larger clinical trial data have now confirmed a 'bimodal' distribution (see Figure 1.11) of LVEF[47,88,105,106]. This provides a strong counter-argument supporting HFpEF and HFrEF as two separate disease entities. Furthermore, at the structural level, two morphologically distinct phenotypes have now been described: concentric hypertrophy/remodeling in HFpEF and eccentric hypertrophy/remodeling in HFrEF. Classically, concentric remodeling depicts a high LV wall mass: volume ratio in contrast to the (dilated LV) low wall mass: volume ratio seen in eccentric remodeling[74,86,107,108]. The haemodynamic consequences of such alterations in structure can be appreciated from invasive pressure/volume curves as described earlier (see Figure 1.5) which demonstrate divergent shifts of EDPVR[55]. Likewise, the end-systolic *elastance* (i.e. the slope of the ESPVR) is elevated in HFpEF but depressed in HFrEF.

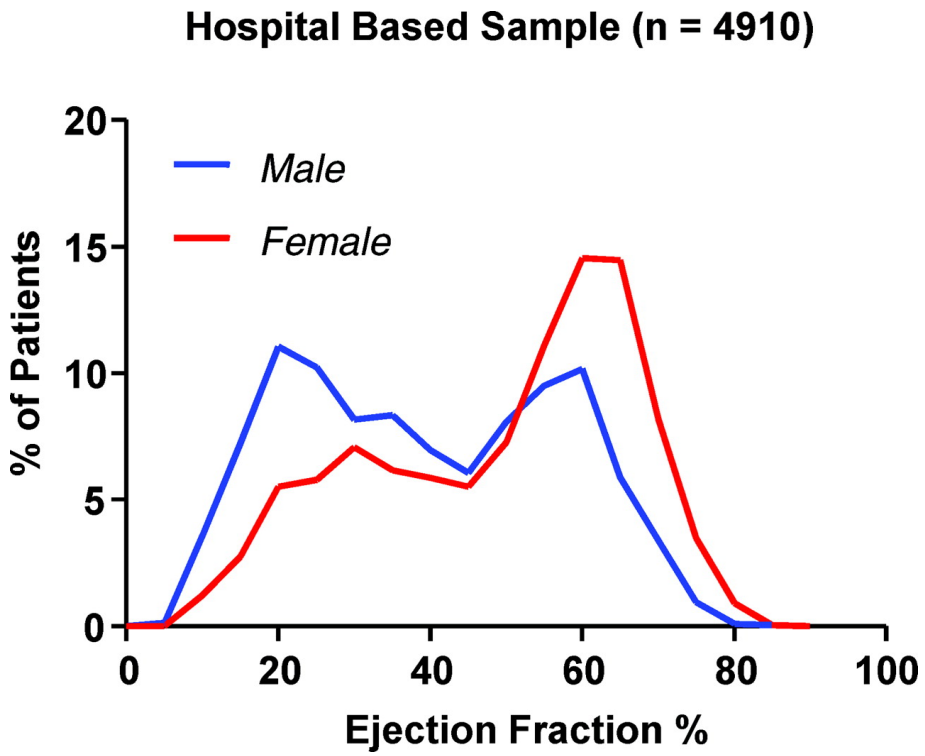


Figure 1.11 Bimodal distribution of left ventricular ejection fraction in hospitalised heart failure patients

Reproduced with permission from Borlaug et al[88]

Marked differences also exist at the cellular, sub-cellular levels and extend into the interstitium. HFpEF patients have larger diameter cardiomyocytes (hypertrophy) and increased resting tension compared to HFrEF. In addition, the stiffer isoform of the protein Titin predominates in HFpEF and is thought to contribute further to this resting tension. On the other hand, HFrEF exhibits narrower and more elongated cardiomyocytes with reduced myofilamentary density[107,109].

In the extra-cellular matrix (ECM), changes in collagen turnover and handling are altered such that matrix degradation appears less in HFpEF. This is thought to be secondary to down-regulation of matrix metalloproteinases (MMPs) and up-regulation of tissue

inhibitors of matrix metalloproteinases (TIMPs) which may serve as potential biomarkers for the future[110,111].

Finally and perhaps of greatest relevance, is the paucity of positive outcome data from clinical HF treatment trials in HFpEF (see Table 1.3). Unlike the compelling evidence base for HFrEF groups with demonstrable improvements in prognosis, HFpEF treatment response has been neutral at best despite the use of similar pharmacotherapy[35,39,41,43-45,112-114].

Table 1.3 Summary of major (neutral) clinical trials in HFpEF

Trial	Year	Intervention	n =	HFpEF Inclusion criteria	F/U (mths)	Primary end-points	Outcomes
PEP-CHF[41]	2006	Perindopril 2–4 mg PO/day	850	Age ≥ 70; LVEF >40%; Receiving diuretics for diagnosis of HF secondary to LV diastolic dysfunction; CV hospitalisation within 6 months	26.2	All cause mortality; HF hospitalisation	Neutral
CHARM-preserved[45]	2003	Candesartan 4-32 mg PO/day	3025	Age ≥ 18; LVEF > 40%; Hospital admission for CV reason	36.6 (median)	CV death; HF hospitalisation	Neutral
I-PRESERVE[44]	2008	Irbesartan 75–300 mg PO/day	4128	Age ≥ 60; LVEF ≥ 45%; Symptomatic HF; Hospitalised for HF within previous 6 months and NYHA class II–IV symptoms or No recent hospitalisation and NYHA class III–IV symptoms	49.5	All cause mortality; CV hospitalisation	Neutral
SENIORS[39]	2005	Nebivolol 1.25 – 10 mg PO/day	2128	Age ≥ 70; Hospitalisation with HF in previous 12 months; LVEF ≥ 35%	20.4	All cause mortality; CV hospitalisation	Neutral
Aldo-DHF[43]	2013	Spironolactone 25 mg PO/day	422	Age ≥ 50; LVEF ≥ 50%; TTE evidence of diastolic dysfunction (grade ≥ 1) or AF; Peak VO_2 ≤ 25 ml/kg/min	11.6	Peak VO_2; Diastolic function	Neutral
TOPCAT[114]	2014	Spironolactone 15–45 mg PO/day	3445	Age ≥ 50; LVEF ≥ 45%; At least one sign and symptom of HF; Controlled SBP; Serum potassium <5 mmol/L; HF hospitalisation within the previous 12 months or elevated BNP/NT-proBNP within the previous 60 days	39.6	Cardiovascular death; Cardiac arrest; HF hospitalisation	Neutral
DIG-ancillary trial[112]	2006	Digoxin 0.125–0.5 mg PO/day	988	Age ≥ 21; LVEF > 45%; Sinus rhythm; Clinical HF	37.2	HF mortality; HF hospitalisation	Neutral

Pathophysiology of HFpEF

Classically, HFpEF has been attributed to diastolic dysfunction in conjunction with concentric remodeling. Progression of diastolic dysfunction has been shown to be the primary mechanism distinguishing HFpEF from age, sex and body mass indexed (BMI) matched controls and hypertensive patients without HF[74]. Both invasive[18,115] and non-invasive[74,116] measures of diastolic dysfunction have confirmed abnormalities in LV relaxation and stiffness when compared to healthy or hypertensive subjects without HF.

In the absence of pericardial disease, diastolic dysfunction is primarily governed by myocardial *stiffness*, which in turn is regulated at the tissue level by alterations in cardiomyocytes and the ECM. Significant changes in intra- & extra-cellular calcium loading and handling results in greater myocardial calcium deposition[110]. Whilst predominant interstitial fibrosis is seen in HFpEF, both replacement and interstitial fibrosis are noted in HFrEF (dilated cardiomyopathy [DCM] patients)[107]. In nearly two-thirds of endo-myocardial biopsies from patients with HFpEF, the collagen volume fraction was found to be increased[109]. Furthermore, the presence of fibrosis was associated with higher LVEDP and stiffness. It is thought that these abnormalities ultimately predispose to pulmonary venous congestion and dyspnoea, especially on exercise[117].

However, the concept that diastolic dysfunction is the sole contributor to HFpEF has been challenged such that the latest guidance accepts that diastolic dysfunction alone is not sufficient for a definitive diagnosis[3]. As described earlier, diastolic dysfunction is highly prevalent in both HFpEF and HFrEF[13], and in elderly patients without HF[7]. Of equal importance, diastolic function is reportedly normal in approximately one third of HFpEF patients enrolled in clinical trials[118,119]. The phenotypic variability, presence of systolic abnormalities, non-uniform responses seen in EDPVR curves, poor clinical trial outcomes and recent mechanistic studies further reinforce the marked heterogeneity of pathophysiology in HFpEF. These additional mechanisms (summarized in Table 1.4) include: deranged ventricular-arterial coupling[57,120], increased arterial stiffness[121], attenuated systemic vasorelaxation[120,122], pulmonary hypertension[123], chronotropic incompetence[124,125], endothelial dysfunction[122,126], LA dysfunction[127], enhanced sensitivity

to volume overloading[128] and subtle abnormalities in systolic parameters despite a 'normal' EF[48,96-100].

Table 1.4 Heterogeneity of HFpEF reflected by differing pathophysiology and clinical phenotypes

Pathophysiological mechanisms	Clinical phenotypes
LV diastolic dysfunction	'Pure' diastolic heart failure
Systolic LV-arterial stiffening	'Common' HFpEF associated with hypertension, diabetes, obesity
Abnormal LV-arterial coupling	
Myocardial contractile dysfunction	Coronary artery disease associated
Impaired exercise reserve	Early HFpEF with exercise induced diastolic dysfunction
Chronotropic incompetence	
Left atrial dysfunction	Atrial fibrillation predominant
Pulmonary hypertension	Pulmonary hypertension ± right heart failure
Volume overload	Non-cardiac cause – related volume overload (such as chronic kidney disease or anaemia)
Endothelial dysfunction	
Amended from Komajda[35]	

Limitations, challenges and the need to develop imaging biomarkers in HFpEF

A biomarker has been defined as a "characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention"[129]. The medical condition of interest should: be sufficiently common, significantly impact upon morbidity & mortality, be well defined and with effective treatments available. Likewise, for the biomarker being developed, it should ideally: be a stable product, discriminate between pathology and normal (and between pathologies), enhance clinical care, be acceptable to patients, exhibit a

linear relation with change in pathology as well as being reproducible and replicated across multiple studies [89].

Adopting this approach to HFpEF reveals a series of disease- and biomarker-specific factors (see Table 1.5.) that make biomarker development challenging[20,35,111,113,130,131]. The primary limiting factor is the marked heterogeneity that characterises HFpEF populations. To date, various diagnostic criteria (including differing EF thresholds) have been employed to define HFpEF. Phenotypic diversity (e.g. obesity, diabetes, AF, right heart failure) coupled with a high prevalence of co-morbidities makes patient identification difficult. Imaging phenocopies such as hypertrophic cardiomyopathy and amyloid are additional confounders. Alternate explanations for pathophysiological mechanisms add to the uncertainty. Furthermore, the discriminatory capabilities of biomarkers (to distinguish HFpEF from HFrEF) are hindered by supportive evidence to suggest the existence of both entities in continuum as part of a single syndrome. Whilst invasive pressure assessments best illustrate the haemodynamic consequences of diastolic dysfunction, they are limited by inherent procedural risks. On the other hand, non-invasive measures of diastolic dysfunction are within normal range in up to a third of subjects. These factors highlighted above therefore ensure that existing and newer markers described in this Chapter do not wholly fulfill the aforementioned biomarker criteria[52,53,132-136].

Table 1.5 Summary of challenges and limitations of existing biomarkers in HFpEF

Disease specific factors	Biomarker specific factors
Population not well defined[113] Variable diagnostic criteria in guidelines and clinical trials ***Confounders of diagnosis***[35,130] Phenotypic variability High prevalence of co-morbidities may alternatively explain clinical features Imaging phenocopies (e.g. hypertrophic cardiomyopathy, amyloid) Atrial fibrillation (challenging clinical and imaging assessment) ***No clear and effective therapies available***[113] ***Evidence for* HFpEF *as a continuum with* HFrEF**[20,35,111] Similar clinical signs and symptoms Unimodal distribution of EF in clinical trials Co-existence of systolic abnormalities and progression over time Eccentric remodeling over time seen in hypertensives ***Heterogeneity of pathophysiology***[35,111,131] Diastolic dysfunction – in HFpEF & HFrEF, in normal subjects, absent in ≈ 1/3 of HFPEF Various pathophysiological mechanisms proposed	***Invasive approach (assessment of diastolic dysfunction or biopsy quantification of fibrosis)***[55,57,58,137] Procedural risk Sampling error Non-uniform responses in end-diastolic pressure volume relationship curves ***Traditional echocardiographic measures for diagnosis***[52,53,132-135,138] Not the recognised gold standard for EF, LV & LA volumes, LV mass Limitations of methodology and feasibility, less reproducible compared to CMR Markers of diastolic dysfunction: loading dependent Haemodynamic disturbances may not be apparent at rest ***Plasma natriuretic peptides***[3,24,27,139] Lower values in HFpEF versus HFrEF Lower values in obesity Higher levels in non-HFpEF conditions but commonly encountered in HFrEF

Rationale for CMR evaluation of HFpEF and possible biomarker substrates

1.3.8 Distinguishing from other differential diagnoses

CMR is currently well placed for the evaluation of potential HFpEF subjects. A wide range of pathologies such as silent MI & ischaemia due to coronary artery disease (CAD)[19,28], HCM[28,130] and constrictive pericarditis[28,130,140] may masquerade as HFpEF. These differential diagnoses or imaging 'phenocopies' (see Figure 1.12) may share many features of the HFpEF phenotype i.e. signs & symptoms, preserved EF, LVH, diastolic dysfunction, atrial dilatation and elevated natriuretic peptides. The superior diagnostic capabilities of CMR above standard echocardiography for the detection of such pathologies have already been reported[130,135,140-144]. The ability of CMR to interrogate any imaging plane and

perform in vivo tissue characterisation makes it the reference standard for detection of such diagnoses[130,135].

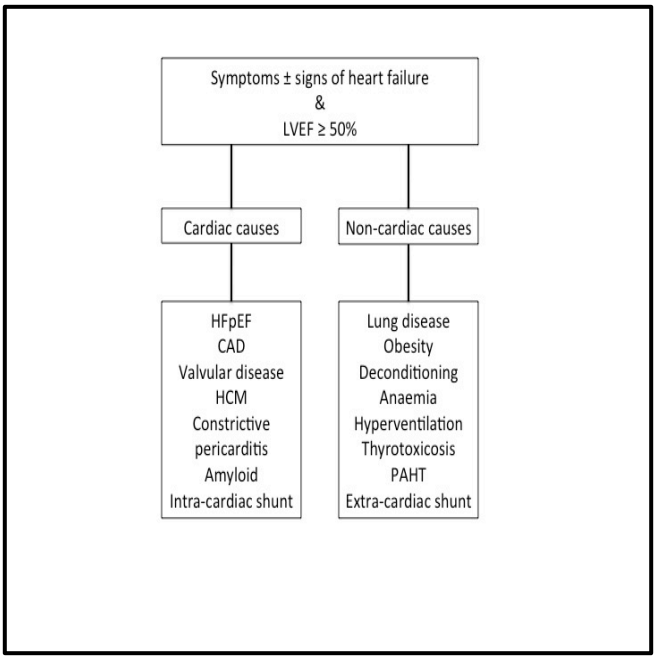


Figure 1.12 Differential diagnoses of HFpEF

Amended from Maeder[28]. PAHT = pulmonary arterial hypertension

1.3.9 More accurate assessment of existing parameters in diagnostic guidelines

CMR is the recognised imaging gold standard for assessing the majority of parameters that comprise latest HFpEF guidelines[3]. However, it is not part of the existing framework for routine use and CMR diagnostic thresholds in HFpEF have yet to be established. Compared to echocardiography, CMR affords superior spatial resolution and has excellent reproducibility for measuring LVEF (and volumes)[132,145], LA volume[146] and LV mass[132,147].

1.3.10 Providing alternative non-invasive metrics for assessing diastolic dysfunction (ECM quantification)

Without the need for invasive measurements, evolving CMR techniques (e.g. late gadolinium enhancement imaging [148] and T1 mapping including ECV) to assess fibrosis may enable accurate quantification of derangements in myocardial architecture

(cardiomyocytes and ECM), which directly influence diastolic function as reported earlier. These techniques have been extensively validated against histology and with excellent reproducibility[149-153].

Non-invasively, focal myocardial fibrosis (ischaemic or non-ischaemic) is best detected with CMR[154,155]. LGE imaging was initially developed upon an understanding that infarcted (scarred) myocardium is associated with regional increases in collagen content, extra-cellular volume (ECV) expansion and a slower washout of extra-cellular contrast agents (e.g. gadolinium) from such areas. Due to the accumulation of gadolinium based contrast agents in these areas, T1 times (relaxation properties of tissue) are reduced such that fibrotic regions appear as areas of high signal intensity compared to 'nulled' (black) normal myocardium using inversion recovery CMR sequences[135]. Due to its excellent spatial resolution and high contrast-to-noise ratio, LGE is able to detect even very small infarcts with high accuracy[156].

The identification and quantification of fibrosis has been shown to reduce survival across a range of clinical conditions including HFrEF, HCM and amyloid and most recently in a small cohort of HFpEF, albeit the quantification technique used was a significant limitation[135,157]. The pattern of LGE (see Figure 1.13) potentially allows discrimination between aetiologies (e.g. ischaemic versus non-ischaemic and HFpEF 'phenocopies' such as HCM, amyloid or pericardial constriction), provides prognostic information and identifies vulnerable myocardium amenable to targeted therapies[130,135,142].

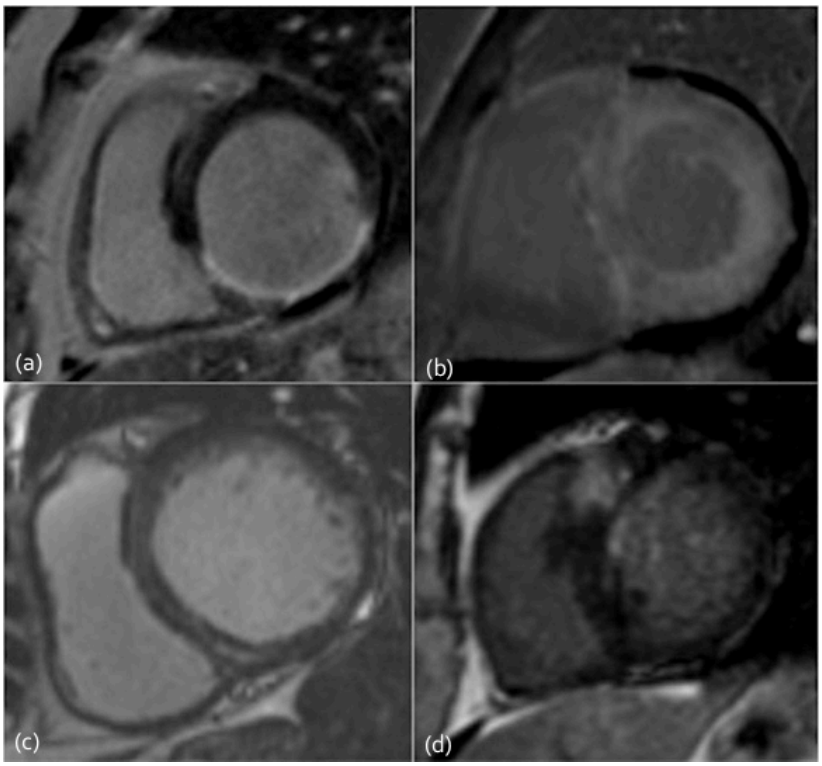


Figure 1.13 LGE patterns of focal fibrosis in differing aetiologies of heart failure

Image reproduced from Kanagala et al[158].(a) Sub-endocardial pattern in myocardial infarction; (b) global sub-endocardial pattern with mid-myocardial extension in amyloidosis; (c) mid-wall pattern typical of non-ischaemic dilated cardiomyopathy; (d) marked focal "scar" in the region of maximal left ventricular hypertrophy and the superior right ventricular insertion point seen in hypertrophic cardiomyopathy.

In HFpEF however, the pattern of fibrosis, at least in the early stages, is typically diffuse[111] and the signal differences between diseased and normal myocardium is less distinct, rendering the LGE technique insensitive. T1 mapping and ECV quantification techniques are promising recent developments in CMR addressing this issue (see Figure 1.14). Native T1 values (non-contrast) are a reflection of myocardial tissue properties (such as fat and water content) and may be altered in diseased states. Estimates of T1 values encoded within pixel intensity of images enable both focal and diffuse myocardium to be studied. T1 values can discriminate pathology from normal (e.g. high T1 in diffuse fibrosis and amyloid, low T1 in iron overload) and may detect pre-clinical disease. ECV quantification (reliant on

measurement of hematocrit, contrast administration and pre- and post-contrast T1 values) permits the myocardium to be further dichotomized into both intra- and extra-cellular compartments. Differing ECV techniques have been validated against collagen volume fraction measured at histology [159] and also tested across a range of pathologies (HFrEF, aortic stenosis [AS], HCM, amyloid) whereby derived values discriminated between healthy controls and disease[160]. Recently in small studies, post-contrast T1 times (n = 61) have shown association with adverse outcomes (hospitalisation or death)[161] and ECV values (n = 62) appear to correlate with CMR measures of diastolic dysfunction in HFpEF[162].

Before the aforementioned T1 mapping techniques enter routine clinical practice however, significant limitations need to be addressed including: a lack of consensus on scanning parameters and ECV techniques, the absence of normative reference ranges across sex and age, potential confounders of T1 values such as heart rate, respiratory motion, magnet strength and the lack of large scale multi-centre studies[163]. The reproducibility of T1 mapping ECV is excellent but there is a large overlap between ECV measurements in most disease states and age-matched controls which is likely to render this technique unsuitable for guiding diagnosis or therapy in an individual patient [164,165].

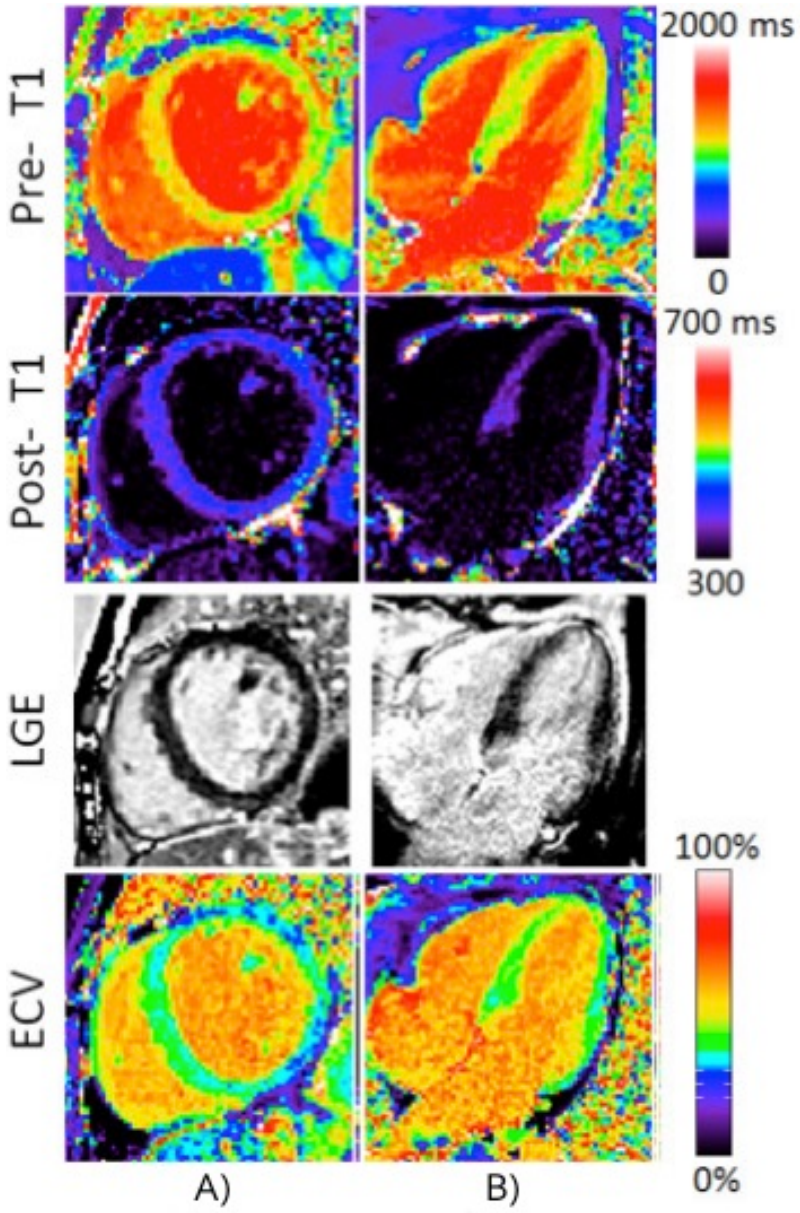


Figure 1.13 Diffuse fibrosis in the presence of 'normal' appearing myocardium with LGE

Adapted from Kellman[166]. CMR examples of "normal" appearing late gadolinium enhancement but with diffuse abnormalities in myocardial extra-cellular volume. Pre-contrast (top row) and post-contrast (2nd row) T1 maps, late gadolinium enhancement (3rd row) and extra-cellular volume maps (bottom row) in (a) non-ischaemic dilated cardiomyopathy (DCM); (b) amyloidosis.

Accurate phenotyping and evaluation of other pathophysiological substrates (? biomarkers)

Other pathophysiological mechanisms implicated in HFpEF and their prevalence may be studied, allowing more comprehensive phenotyping and characterisation than has been possible to date. Such undertakings include: testing for myocardial ischaemia /CAD evaluation, LA dysfunction and right ventricular dysfunction.

1.3.10.1 Evaluating CAD and ischaemia

At present, the role of CAD and ischaemia in the natural history of HFpEF is incompletely defined. Not only is CAD associated with an increased risk of developing HFpEF but worsens prognosis in this setting[167]. Epidemiological studies have reported lower prevalence of CAD in HFpEF compared to HFrEF. However, pooled analysis of prospective studies suggests that CAD is present in nearly half of all HFpEF cases[168]. Unfortunately, the majority of these studies failed to systematically look for CAD and were further hindered by the lack of a universal definition and incomplete documentation in many.

Ischaemia in HFpEF may result from macrovascular (CAD) or microvascular disease (MVD). Ischaemia reduces LV chamber compliance, increases LVEDP, causes diastolic dysfunction and accentuates adverse ECM remodeling. In conjunction with pressure overload typical of HFpEF, LV wall stress is further increased, blunting sub-endocardial perfusion and coronary reserve[131,167]. Non-invasive imaging can identify haemodynamically significant CAD with good sensitivity (84%) and specificity (86%) utilising stress perfusion CMR[169]. CMR best detects infarction (which may be silent) and alternatively may explain symptoms (angina equivalent), provide prognostic information and enables effective primary and secondary prevention therapies[142,170].

Invasive (angiography) or non-invasive (CMR) detection of diminished coronary flow reserve (CFR) and MVD confer adverse prognosis in the presence or absence of CAD[171]. Furthermore, these imaging biomarkers appear to be overrepresented in populations typical of HFpEF: increasing age, female, obese, diabetic, hypertensive and in similar pressure

overloaded conditions e.g. AS, HCM[171]. Indeed MVD, ECM remodeling and microvascular endothelial inflammation appear intimately linked and have recently been proposed as a novel paradigm for HFpEF[172]. In HCM patients with preserved EF at baseline, MVD predicted transition to HFrEF and development of symptoms[173]. Diminished myocardial perfusion reserve (MPR) as measured by CMR may further detect pre-clinical disease. In a recent study of severe AS patients[174], MPR independently predicted exercise capacity and was determined by the degree of fibrosis and LV mass (remodeling).

1.3.10.2 Evaluating LA dysfunction

The left atrium displays important mechanical functions throughout the cardiac cycle (see Figure 1.15). Initially during ventricular systole, it acts as a reservoir, collecting blood from the pulmonary veins. During early diastole, it acts as a conduit allowing passive emptying of blood into the LV driven by a high transient LA-LV pressure gradient. Finally, in sinus rhythm, active emptying occurs during end-diastole as a result of LA contraction[175].

With chronic exposure to volume- and pressure over-loading, disturbances during any of these phases may result in LA remodeling typically characterised by LA dilation, AF and diminished contractility. An enlarged LA is a predictor of diastolic dysfunction in HFpEF[68,70] correlates with worsening grades of diastolic dysfunction [69] and is a useful prognosticator, independently predicting incident HFPEF[68,176,177]. Hence, LA dilatation currently provides supportive evidence for HFpEF diagnosis[3]. In HFpEF, where AF is highly prevalent, the loss of atrial contraction can reduce cardiac ouput by one-fifth[178], and may explain the higher symptom burden, poorer quality of life and diminished exercise capacity seen in such patients[179].

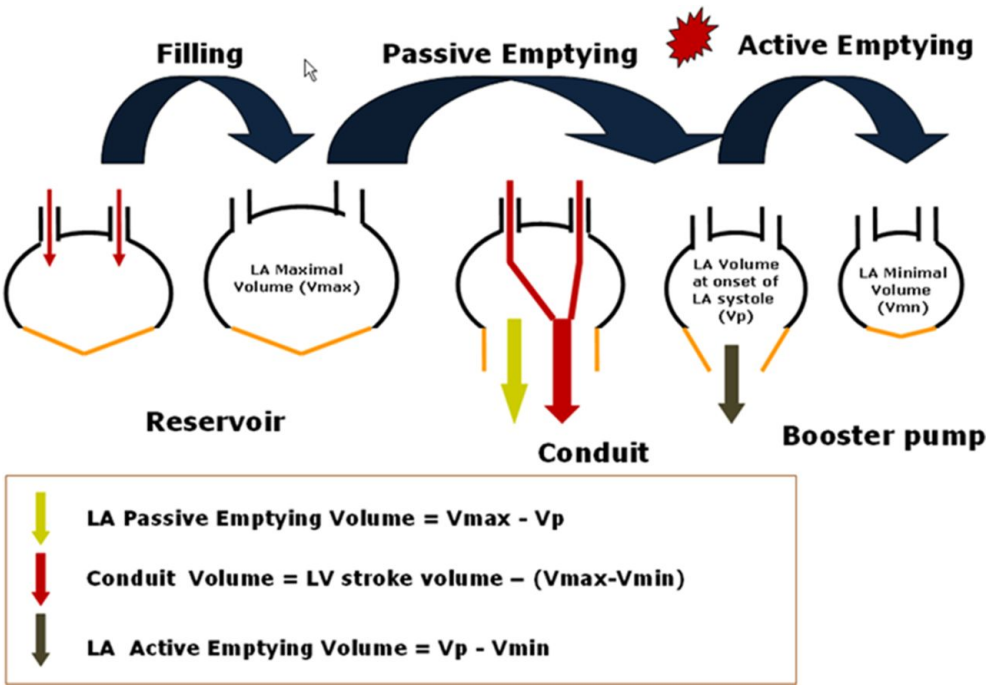


Figure 1.14 Phases of left atrial function

Reproduced from Rossi[175]. LA indicates left atrial; LV, left ventricle; Vp, left atrial volume before atrial contraction; Vmax, maximal volume (as defined at left ventricular end-systolic phase); and Vmin, left atrial minimal volume (as defined at left ventricular end-diastolic phase).

Further highlighting the role of the LA in HF, increased LA size and decreased LA emptying were associated with future development of HF: either HFrEF or HFpEF[180]. As a marker of diastolic dysfunction in HF, speckle tracking echocardiography performed better than E/E' (AUC = 0.93 versus 0.69) and correlated strongly with LV filling pressures[181].

Echocardiographic strain measures of LA dysfunction in HFpEF have previously shown that LA functional impairment may precede LA remodeling[180], reduced systolic strain distinguishes asymptomatic subjects with diastolic dysfunction from HFpEF[182], resting LA function is independently associated with exercise capacity[183] and impaired LA function relates to symptom onset[184]. Furthermore, abnormal measures of LA strain have also been noted in the HFpEF antecedent conditions of hypertension and diabetes despite normal LA dimensions, highlighting the potential for early disease profiling[185]. Although limited data

exists on prognostic implications in HFpEF, LA dysfunction does appear to be related to adverse outcomes[186,187].

As described above, the evidence base for LA evaluation in HFpEF is primarily TTE based and reliant upon adequate LA endocardial border definition for both volumetric and strain assessments[188]. CMR however, affords superior spatial resolution, has excellent reproducibility, and is the current gold standard for LA volumetric[146] and functional assessment in sinus rhythm[189] or AF[190]. Recently, CMR measures of LA function identified subjects from the general population at heightened cardiovascular risk[191] as well as those who developed incident HF.[192] For prognostic evaluation, CMR measured left atrial ejection fraction (LAEF) in sinus rhythm was also associated with adverse outcomes in HFrEF[193].

1.3.10.3 Evaluating RV dysfunction

RV disturbance in HFrEF has been extensively studied and well established[194], with clear relation to worse functional status[195] and mortality[196]. Recently however, there has been growing interest in the role of the RV in the setting of HFpEF. Pathophysiological mechanisms implicated include intrinsic myocardial processes, load dependent (pulmonary hypertension) and load independent conditions such as CAD, AF and obesity[197]. Right ventricular systolic dysfunction (RVD) is reportedly less prevalent in HFpEF than HFrEF[198], although prevalence varies widely ranging from 4%[199] to 33%[200], dependent upon differing echocardiographic criteria.

Furthermore, either surrogate markers of RVD such as pulmonary hypertension (elevated pulmonary artery systolic pressure [PASP])[201], RV hypertrophy[202], tricuspid annular plane systolic excursion (TAPSE)[203] or both semi-quantitative[203] and quantitative (fractional area change [FAC])[200] measures of RV contractile performance have been associated with worse prognosis in HFpEF. In the latter study, RVD was associated with an incremental risk of mortality beyond that conferred by pulmonary hypertension alone. A FAC < 35% was associated with more than double the risk compared to subjects without RVD, independent of PASP[200].

In contrast to the ellipsoid LV, the RV is a complex crescent shaped structure. In addition, the typically thin free wall, hyper-trabeculation and the presence of a moderator band make RV endocardial border definition more complicated, limiting functional assessment and quantitation, compared to the LV[204]. Concomitant lung disease and obesity, typically associated with HFpEF, adds to these difficulties. Whilst, there appears to be a strong signal for RVD from the aforementioned echocardiography studies, CMR might be better placed for more robust RV assessment in HFpEF, where only 2 such outcome studies have been undertaken. In both studies, RVD assessed by CMR was associated with adverse outcomes[205,206]. The inter-study reproducibility for CMR assessment of the RV compared to the LV is lower. However, overall reproducibility for the RV is good[204] and CMR is the current accepted imaging gold standard[3].

1.3.10.4 Evaluating altered myocardial mechanics

Remodeling of the ECM compartment alters myocardial tissue mechanics resulting in abnormalities of both diastole and systole in HFpEF[99]. Longitudinal LV function is typically depressed in HFpEF and can be measured with echocardiography (tissue Doppler) and CMR (velocity-encoded or tissue phase mapping)[99,154]. A more detailed assessment of LV performance can now be made using strain (or deformation) analysis. Simplistically, strain imaging assesses myocardial tissue lengthening, shortening or thickening in orthogonal planes.

Significant correlations between early diastolic strain rates, regional stiffness and the extent of myocardial fibrosis were initially described in animal studies[207]. Subsequently, regional strain disturbances have demonstrated a strong relation with LV catheter derived relaxation abnormalities and LVEDP in HCM[208]. Furthermore, the ratio of mitral E wave velocity: global strain rate correctly predicted LVEDP and was more accurate than E/E′ ratios in patients with preserved EF and regional dysfunction[209]. In an exercise echocardiographic study of 56 patients with HFPEF, both resting and exertional reductions in longitudinal & radial strain as well as apical rotation were observed[103]. As prognostic biomarkers, strain parameters (global longitudinal peak strain and longitudinal early diastolic strain) are important predictors of adverse outcomes (one-year follow up) in HFpEF[210].

Whilst CMR tagging is well established as a method for strain and strain rate assessment, CMR feature tracking has recently emerged as a promising alternative[154,211]. Compared to tagging, feature tracking does not necessitate prolonged breath-holding for image acquisition, has been recently studied in HFpEF[211] with good feasibility, has shorter analysis times and shows good reproducibility at both 1.5- and 3-Tesla magnet strengths[212].

1.3.10.5 Assessing Metabolic function

Existing nuclear and MRI techniques permit the detection of metabolic derangements of energetic status and substrate utilisation (e.g. free fatty acids) implicated in HF[213]. Irrespective of HF aetiology, reductions in energy levels (by measuring phosphocreatine) of approximately 70% have already been shown in human and animal studies[214]. Using magnetic resonance spectroscopy (MRS), the association between reduced myocardial phosphocreatine: adenosine tri-phosphate ratio (PCr:ATP) (phosphocreatine: adenosine tri-phosphate ratio) and diastolic dysfunction has been shown in hypertensive patients[215] and in HFpEF during exercise[216]. Furthermore, diminished ATP flux through creatine kinase (CK) may distinguish those patients with LVH who transition to HF [217]. Recently, CMR hyperpolarized imaging (artificially increasing molecular alignment within a magnetic field) has emerged as an exciting new methodology allowing cardiac metabolism to be studied with dramatic increases in signal-to-noise and early studies in HF are keenly awaited[218].

Reduced substrate uptake and oxidation may also limit cardiac performance. Published literature provides conflicting data from studies of cardiac metabolism: both fatty acid and glucose utilization appear enhanced in early stages but diminishes with advancing HF[213]. Several PET radionuclide tracers that reflect utilisation and oxidative metabolism (e.g. analogues of fatty acid and glucose) may be of potential benefit in HFpEF[219]. Increasing evidence implicates the role of excessive myocardial triglyceride accumulation (steatosis) in conditions highly prevalent in HFpEF: obesity, diabetes and pressure overload[220]. Steatosis, as quantified by MRS is independently associated with echocardiographic measures of diastolic dysfunction[221], strain parameters derived from CMR tagging and correlates with histology[220].

1.3.10.6 Assessing Molecular function

Molecular targeting of the key markers implicated in ECM turnover has recently shown good capabilities, albeit almost exclusively in animal models. Potential targets studied include MMPs, ECM proteins, the renin-angiotensin axis and myofibroblasts. Post-infarct studies have already demonstrated the feasibility of assessing collagen deposition[222] and increased probe activity closely approximates with histological findings[223]. Although most studies have employed nuclear techniques (limited signal from poor tissue penetration), hybrid imaging with PET, SPECT, CT or CMR may further improve spatial resolution, which is the current major limitation. Cost and limited radiotracer availability are additional factors[224].

Overall, many potential targets and pathophysiological substrates exist in HFpEF which lend themselves to evaluation by existing imaging modalities. The respective strengths and their relative weaknesses compared to CMR are summarized in Table 1.6.

Table 1.6 Summary of strengths and potential applicability of imaging biomarkers in HFpEF

	LVEF	Contractile function (LV/LA)	Chamber quantification	ECM quantification (fibrosis)	Myocardial mechanics	CAD/ischaemia/flow reserve	Molecular imaging	Metabolic imaging
TTE	++	++	++	+	++	+	NA	NA
CMR	+++	+++	+++	+++	+++	+++	+	++
PET	+	+	+	++	NA	+++	++	++
SPECT	+	+	+	+	NA	++	++	++
CT	+	+	+++	+	+	+	+	NA

Adapted from Paterson[225] and Jellis[154]. Abbreviations: LVEF = left ventricular ejection fraction; LV = left ventricle; LA = left atrium; ECM = extra-cellular matrix; CAD = coronary artery disease; TTE = trans-thoracic echocardiography; CMR = cardiac magnetic resonance; PET = positron emission tomography; SPECT = single-photon emission computed tomography; CT = computed tomography; NA = not applicable or not assessed; + = limited evidence but potential future role; ++ = supportive evidence from either at least one large study or registry data; +++ = accepted reference standard or strongly supportive evidence base including meta-analyses or randomized controlled trials.

Gaps in the current knowledge and study aims

The previous sections have highlighted the potential role of CMR in HFpEF. To date, CMR studies in this setting are sparse. In particular, significant gaps in our knowledge about HFpEF persist regarding:

- the proportion of alternative diagnoses in patients with suspected HFpEF
- the proportion of underlying CAD/myocardial ischaemia
- the presence of fibrosis
- the role of LA dysfunction
- the role of RV dysfunction
- the structural and functional differences in comparison with healthy controls and HFrEF using gold standard imaging
- the association of some of the above measures in relation to clinical outcomes

Ultimately, the aims of this thesis are, utilising CMR to:

- better phenotype and characterise HFpEF (also in comparison with HFrEF and age- and sex- matched healthy controls)
- provide mechanistic insights into HFpEF pathophysiology
- describe potential biomarkers and their relation to relevant clinical outcomes (exercise capacity, HF quality of life and prognosis)

Original hypotheses

The following hypotheses will be tested:

1. H_1: In patients with suspected HFpEF, CMR identifies alternative pathologies in a significant proportion compared to standard evaluation and may impact upon clinical outcomes
   H_0: In patients with suspected HFpEF, CMR does not identify alternative pathologies in a significant proportion compared to standard evaluation and these will not impact upon clinical outcomes

2. H_1: CMR quantified LV fibrosis will be more prevalent in HFpEF compared to healthy controls and may impact upon clinical outcomes
   H_0: CMR quantified LV fibrosis will be more prevalent in HFpEF compared to healthy controls and will not impact upon clinical outcomes

3. H_1: CMR measures of LA dysfunction will discriminate between HFpEF and healthy controls and may impact upon clinical outcomes
   H_0: CMR measures of LA dysfunction will not discriminate between HFpEF and healthy controls and will not impact upon clinical outcomes

4. H_1: CMR measured RV dysfunction will be more prevalent in HFpEF compared to healthy controls and impact upon clinical outcomes
   H_0: CMR measured RV dysfunction will be not be more prevalent in HFpEF compared to healthy controls and will not impact upon clinical outcomes

5. H_1: CMR will identify structural and functional differences between HFpEF, healthy controls and HFrEF
   H_0: CMR will not identify structural and functional differences between HFpEF, healthy controls and HFrEF

2 METHODS

Study design, funding and rationale

The study design was observational, prospective, cohort and conducted at a single tertiary cardiac centre (Glenfield Hospital). The CMR sub-study, the main focus of this thesis was part of an overall research project funded by the Leicester Cardiovascular Biomedical Research Unit (LCBRU). The umbrella study was conceived with the aims of developing both plasma and imaging biomarkers in HFpEF. The study was funded by the LCBRU via a project grant from the National Institute for Health Research.

Study registration

The trial was initially entitled the 'Diastolic Heart Failure study'. The title was subsequently changed to 'Developing Imaging And plasMa biOmarkers iN Describing Heart Failure with preserved Ejection Fraction' (DIAMOND-HFpEF) and registered retrospectively on February 06, 2017 with ClinicalTrials.gov. The study identifier code was NCT03050593.

Subject screening and recruitment

The initial aim was to recruit a total of 300 subjects: HFpEF (n = 200), HFrEF (n= 50) and healthy controls (n = 50). Patients were screened retrospectively from an existing clinical Hospital database comprising subjects with a coded label of HF as the primary reason for hospitalisation in the preceding 2 years. Screening was also undertaken prospectively in the out-patients department and the hospital wards. The results of latest clinical echocardiography were reviewed from electronic discharge summaries or the in-house Hospital imaging databases.

2.1.1.1.1 Study personnel

Individuals involved in the study conduct and analysis that forms part of this thesis are referred to throughout by the following initials:

- *AMM* = Anna-Marie Marsh (Physiologist / Echocardiographer)

- *AS* = Anvesha Singh (Clinical Research Fellow)
- *ASHC* = Adrian Cheng (Consultant Cardiologist)
- *GPM* = Gerry McCann (Consultant Cardiologist)
- *JM* = John McAdam (Physiologist / Echocardiographer)
- *JRA* = Jayanth Arnold (Clinical Research Fellow)
- *MH* = Mary Harrison (Research Nurse)
- *PK* = Prathap Kanagala (Clinical Research Fellow)
- *SM* = Susan Mackness (Research Nurse)

2.1.2 Inclusion criteria

The study was comprised of three cohorts: HFpEF, HFrEF and healthy controls.
HFpEF was defined as:

- clinical or radiographic evidence of HF
- **and**
- LVEF > 50% on transthoracic echocardiography (TTE)

Our definition of HFpEF was not in accordance with latest ESC guidelines[20] at the time of recruitment. However, we took a pragmatic approach to reflect a real world setting. In particular, the presence of diastolic dysfunction was not a pre-requisite for study entry since recent contemporary clinical trials have highlighted normal diastolic function (assessed by echocardiography) at rest in approximately a third of such patients[114]. Furthermore, the reported prevalence of diastolic dysfunction is wide ranging with marked inter-study heterogeneity owing to variable definitions in use[226].

HFrEF was defined as:

- clinical or radiographic evidence of HF
- **and**
- LVEF < 40% on TTE

2.1.3 Exclusion criteria

The exclusion criteria were:

- age < 18 years
- known MI in the preceding 6 months
- suspected or confirmed cardiomyopathy
- suspected or confirmed constrictive pericarditis
- non-cardiovascular life expectancy < 6 months
- severe native valve disease
- severe chronic obstructive pulmonary disease (or forced expiratory volume [FEV_1] < 30% predicted or forced vital capacity [FVC] <50% predicted)
- estimated glomerular filtration rate (eGFR) < 30 ml/min/m^2
- patient inability to provide informed consent e.g. dementia

2.1.4 Healthy Controls

For comparison with HF, asymptomatic controls (age and sex-matched) without known heart disease were also recruited. Subjects with hypertension were included in this group so as to detect the effects of heart failure alone rather than combined hypertension. Controls were recruited through advertising (see Appendix 9.1.1) and none had been referred for a clinical CMR scan. Fourteen volunteers had also served as healthy controls in another study[227] at our centre.

Study protocol

Potentially eligible subjects from screening were either posted (with an attached reply slip) or personally given an information sheet (see Appendix 9.1.2) detailing the study. For those interested in participating, an appropriate date and time for the study visit was arranged.

2.1.5 Ethics and Consent

The study was approved by the National Research Ethics Service (NRES) on the 24th of July 2012. (see Appendix 9.1.3) and conducted according to the Declaration of Helsinki. Written informed consent was obtained from all subjects prior to participation.

2.1.6 Study visit and investigations

Consent was obtained prior to any investigations (see Appendix 9.1.4 and 9.1.5). All study investigations were performed during a single visit, on the same day if possible. Rarely, CMR was deferred to a later date dependent upon scanner availability. Typically, study visits were 4 hours in duration, allowing for recovery time in between investigations (bearing in mind the typically elderly cohort of HF patients). A summary of the investigations is detailed below in Table 2.1.

Table 2.1 Summary of study visit and investigations

Order	Investigation
1	Clinical assessment, history taking & examination
2	Venepuncture & blood sample processing
3	Spirometry
4	Electrocardiogram
5	Transthoracic Echocardiography
6	6 Minute Walk Test
7	Quality of Life Questionnaires
8	CMR

2.1.7 Clinical assessment, history taking and examination

A paper case report form (CRF) was used to document relevant findings from the history & examination and review of the medical records (see Appendix 9.1.6). Particular attention was paid to the presence or absence of the following:

1. **Clinical features of HF as per ESC guidelines**

Typical HF symptoms: breathlessness, orthopnoea, paroxysmal nocturnal dysponoea, reduced exercise capacity, fatigue/tiredness, increased time to recover following exercise, ankle swelling

Less typical HF symptoms: nocturnal cough, wheeze, weight gain > 2kg/week, weight loss (in advanced HF), bloated feeling, loss of appetite, confusion, depression, palpitations, syncope

More specific HF signs: Raised jugular venous pulse, hepatojugular reflux, gallop rhythm, laterally displaced apex, cardiac murmur
Less specific HF signs: peripheral oedema (ankle/sacral/scrotal), pulmonary crepitations, reduced air entry at lung bases/effusion, tachycardia, irregular pulse, tachypnoea (>16 breaths per minute), hepatomegaly, ascites, cachexia (tissue wasting)

2. **Radiology reports of prior chest X-rays**

Prior chest radiographic reports were sourced from the electronic Hospital Radiology reporting systems. The presence of a raised cardiothoracic ratio, pulmonary congestion or pleural effusion were documented.

3. **Medical History**

- Prior hospitalisation with HF (and dates)
- Assessment of coronary artery disease (Angina/ previous MI / coronary angiography/ revasularisation / stress testing and dates)
- Diabetes
- Hypertension

- Hypercholesterolaemia
- Smoking history
- Cerebrovascular disease
- Lung Disease
- AF

4. **Medications**

5. **New York Heart Association (NYHA) status**

I	No symptoms; no limitation in ordinary physical activity
II	Mild symptoms; slight limitation during ordinary activity
III	Marked limitation; symptoms even with less than ordinary activity
IV	Severe limitation; symptoms at rest

2.1.7.1 Anthropometric and other data

The height and weight were measured using the same scale in the Biomedical Research Unit. The BMI was calculated using the formula: BMI = weight in kilograms ÷ (height in metres)^2. Using the dominant arm, heart rate and blood pressure were recorded (average of 3 measurements) with the patient being seated and rested for a period of 10 minutes.

2.1.8 Venepuncture and blood sample processing

Following 15 minutes of rest (patient supine on a couch), up to 25 mls of blood was collected by venepuncture and placed in tubes with EDTA anticoagulant. Approximately 10 mls of blood was promptly transported to the Hospital laboratory for analysis of BNP (immunoassay, Siemens, Erlangen, Germany), haematocrit, haemoglobin and renal function. eGFR was calculated from the simplified Modification of Diet in Renal Disease formula[228].

The remaining blood samples were centrifuged and the resulting supernatant plasma was stored at -80^{0}C in cryotubes (labeled with a study identification number) until future processing (for potential plasma biomarkers) at a later stage.

2.1.9 Spirometry

The primary objective of hand-held spirometry was to assess study eligibility and to ensure that subjects did not have severe lung disease. The best of 3 recordings was used for analysis. Testing was performed in accordance with national guidance and adherent to a dedicated standardised protocol (see Appendix 9.1.7).

2.1.10 Electrocardiography

A standard 12-lead electrocardiogram (ECG) was performed for all patients prior to echocardiography. Cardiac rhythm was recorded and the ECG was checked to ensure no features (see below) were present precluding adenosine administration during CMR (also see Appendix 9.1.6).

- 2nd or 3rd degree AV block
- Atrial Flutter with heart block (≥3:1)
- Sinus bradycardia (heart rate < 40 b.p.m)

All ECGs were further assessed by *PK* and *AMM* for the presence of Q waves, as surrogates of MI[229] (of relevance to Chapter 3).

Transthoracic echocardiography

In all subjects, 2-dimensional TTE was performed as per American Society of Echocardiography guidelines (ASE)[230] using an iE 33 system with S5-1 transducer (frequency transmitted 1.7 MHz, received 3.4 MHz; Philips Medical Systems, Best, The Netherlands). The scans were performed by 2 British Society of Echocardiography (BSE) accredited sonographers (*AMM*, *JM*).

The TTE protocol is detailed in Appendix 9.1.8. A 3-lead ECG with clearly displayed QRS complexes was attached to all subjects. The number of recorded beats for image acquisition was adjusted: in sinus rhythm, 3 beats; in AF, 5 beats; in fast AF (heart rate > 90 b.p.m), a

10 beat acquisition was considered. Routine 2D, colour and Doppler images were acquired in conventional parasternal long-axis, short-axis and apical 4-, 2- and 3-chamber views.

Six minute walk test

2.1.10.1 Rationale for use as an outcome measure

The gold standard method for assessing functional capacity in HF is by measuring oxygen consumption during maximal stress – the cardiopulmonary exercise test (CPET)[231]. However, the 6MWT is a recognised sub-maximal alternative that is simple, inexpensive and has become widely utilised in HF populations. Since its initial evaluation as a prognostic aid in the SOLVD study[232] (of HFrEF), further studies have highlighted the 6MWT as a powerful predictor of outcomes in individual cohorts of HFrEF[233-235], HFpEF[236] and in one study comprising both HF phenotypes[237].

The 6-MWT was first validated for use in HF patients in 1985 in a small study[238] comprising both HF (n = 18) and subjects with lung disease (n = 25). This study demonstrated reproducibility of results when a standardized protocol was applied. A subsequent systematic review[239] of 14 studies addressed the ability of the 6MWT in determining functional exercise capacity, specifically in HF. The review focused on reproducibility (n = 9 studies), validity compared to peak VO2 (n = 12 studies) and predictive value of the 6MWT (n = 5 studies). The authors concluded that the 6MWT has good reliability, moderate validity (compared to peak VO2) and a significant ability to predict functional capacity in HF patients (provided distances walked were < 490 m). Overall, reproducibility assessments (intra-class correlation coefficient [ICC]) have ranged from 0.78 to 0.921. Correlations with peak VO2 have ranged from 0.579 to 0.88. Reasonable correlation has been also been shown with other measures of activities of daily living (including NYHA and quality of life [QOL] questionnaires).

Furthermore, the 6 MWT has satisfactory long-term reproducibility[240] (agreement when test repeated at 1 year - ICC 0.80) and is sensitive to changes in quality of life[240-242]. In

addition, since exercise incapacity typifies HF, the 6MWT has been proposed as an appropriate outcome measure[242] and been trialed as a clinical endpoint in recent HF studies[236,243]. A summary of the major studies highlighting the applicability of the 6MWT in HF are shown below in Table 2.2.

Table 2.2 Key studies of reproducibility, validity and predictive value of the six minute walk test in heart failure

Study	Year	n =	Population	Main findings
Guyatt[238]	1985	N = 18 EF unknown	NYHA II – IV Mean age 65	ICC = 0.921 Moderate correlation with bicycle ergometer test (r = 0.579) Moderate correlation with QOL questionnaires (r = 0.473 –
Lipkin[241]	1986	N = 26 HFrEF and HFpEF	NYHA II/III Mean age 58	Curvilinear relationship with peak Vo2 In patients with low peak VO2, 6MWT distance varied considerably; with high peak VO2, the 6MWT distance
Riley[244]	1992	N = 16 HFrEF	NYHA II - IV Mean age 65	Good reproducibility: CoV = 6.71% Strongly correlated with peak VO2 r = 0.88
Cahalin[233]	1996	N = 45 HFrEF	NYHA III/IV Mean age 49	Moderate correlation with peak VO2 (r = 0.64) Predicts prognosis if distance < 300m
Roul[234]	1998	N = 121 HFrEF	NYHA II/III Mean age 59	In those walking < 300 m, moderate-good correlation with peak VO2 (r = 0.65) Predicts prognosis if distance < 300m
O'Keefe[245]	1998	N = 60 EF unknown	NYHA I - IV Mean age 82	ICC = 0.91 Good correlation with CHQ score r = 0.79
Zugck[235]	2000	N = 113 HFrEF	NYHA I - III Mean age 54	Moderate-good correlation with peak VO2 (r = 0.68) Predictor of prognosis: distance < 300m Provided prognostic information similar to peak VO2
Demers[246]	2001	N = 768 HFrEF	NYHA I - IV Mean age 63	Baseline ICC 0.90 Weakly correlated with MLHF r = -0.26 Moderately inversely correlated with NYHA r = -0.43
Ingle[240]	2005	N = 1077 HFrEF	NYHA II – IV Age > 60 years	Reproducibility at 1 year: ICC 0.80 (in patients with unchanged symptoms and unchanged 6MWT distance At 1 year: strong inverse correlation of Δ 6MWT distance
Ingle[32]	2008	N = 672 HFrEF and HFpEF	Patient - NYHA I – IV Age > 68	Close relation between 6MWT distance with patient perceived NYHA class irrespective of HFrEF or HFpEF
Guazzi[247]	2009	N = 253 HFrEF and HFpEF	NYHA II/III Mean age 62	Reproducibility: ICC 0.78 Strong correlation with peak VO2 r = 0.788

2.1.10.2 Test procedure

All tests were supervised by *PK* (vast majority), *MH*, *SM*, *AMM* or *JM*. The 6MWT was performed based on published American Thoracic Society Guidelines (2002)[248], which provide a standardized approach to testing. Patients were informed that beta-blockers should be withheld for at least 48 hours prior to the study visit (also to ensure adequate response to adenosine stress for CMR). A stepwise sequence paying attention to the checklist detailed below was followed (see Appendix 9.1.9):

1. Check for contraindications

- Absolute
    - Unstable angina within preceding month
    - Myocardial Infarction within preceding month
- Relative
    - Resting HR > 120 beats per minute (b.p.m)
    - Systolic BP > 180 mmHg
    - Diastolic BP > 100 mmHg

2. Patient preparation

The patients were advised to:

- wear comfortable clothing
- wear appropriate shoes for walking
- use usual walking aids during the test e.g. walking stick, Zimmer frame
- not undertake vigorous exercise within 2 hours prior to the test
- rest seated in a chair near the starting position for a minimum of 10 minutes prior to the test
- use supplemental Oxygen as per prescribed regime if on long-term therapy

3. Equipment check

- Stopwatch

- CRF containing the 6MWT proforma to document results
- Wheelchair to transport patients to test location
- Pulse oximeter to record oxygen saturations
- A source of oxygen if required
- Automated BP and pulse rate measurement tool (OMRON)
- 2 Cones

4. Location & course

- Indoors in the same long, flat, straight corridor within the hospital building
- Course length was 20 metres, demarcated by 2 cones (serving as the turn-around points) connected to a piece of string
- The test was commenced at a point marked on the floor
- The length of the course was marked every 3 metres
- There was a chair positioned next to the start point
- The location had a telephone next to the designated testing area in case of emergency

5. Reasons for test termination

The test was terminated once 6 minutes had elapsed

OR

If any of the following were present:

- Chest pain
- Intolerable breathlessness
- Leg cramps
- Patient was staggering
- Patient was diaphoretic
- Patient was pale/ “ashen faced”

OR

If patients were unwilling to continue (if so, the reason was documented)

6. Safety

The test was supervised by health care professionals with either Basic Life Support (*AMM*, *JM*, *MH*, *SM*) or Advanced Life Support (*PK*) certification.

7. Measurements recorded

- Before and after test
    - BP
    - Pulse rate
    - Oxygen saturations (pulse oximetry)
    - Fatigue & dyspnoea using the Borg scale
- During the test
    - Total number of laps = completed laps + final partial lap
    - *Note - A partial lap was measured using the 3 metre markers and rounded up to nearest metre*
    - Total distance covered in metres was recorded

8. Instructions

Patients were instructed as follows:

"The object of this test is to walk as far as possible for 6 minutes. You will walk back and forth in this hallway. Six minutes is a long time to walk, so you will be exerting yourself. You will probably get out of breath or become exhausted. You are permitted to slow down, to stop, and to rest as necessary. You may lean against the wall while resting, but resume walking as soon as you are able.
You will be walking back and forth around the cones. You should pivot briskly around the cones and continue back the other way without hesitation. Now I'm going to show you. Please watch the way I turn without hesitation."

The test supervisor then demonstrated this by walking one lap themselves.

"Are you ready to do that? Remember that the object is to walk as far as possible for 6 minutes, but don't run or jog. Start now, or whenever you are ready."

Patients were positioned near the start line. The supervisors also stood nearby but did not walk with the patients. As soon as the patients started to walk, the timer was started. Patients were not spoken to during the test other than using standard phrases of encouragement.

"You are doing well. You have x minutes to go."

Each lap was recorded every time the patient returned to the start line. Patients were informed after each minute of the number of minutes outstanding. If they needed to stop and rest during the test, they were reminded to continue to walk as soon as they were able. The timer was not paused during rest periods. If patients stopped before the stipulated 6 minutes and refused to continue, the test was discontinued and the reasons were recorded.

Patients were advised 15 seconds prior to test completion:
"In a moment I am going to tell you to stop. When I do, just stop where you are and I will come to you."
Upon test completion, patients were congratulated and offered a drink of water.

9. Borg score

Patients were asked to grade their perceived level of exertion (i.e. breathlessness and fatigue) pre- and post-6MWT according to the Borg scale (see Table 2.3) which was printed onto an A4 size laminated paper.

Table 2.3 The Borg scale

Score	Perceived breathlessness/fatigue
0	Nothing at all
0.5	Very, very slight (just noticeable)
1	Very slight
2	Slight (light)
3	Moderate
4	Somewhat severe
5	Severe (heavy)
6	
7	Very severe
8	
9	
10	Very, very severe (maximal)

10. Variability

Measures to minimise variabilty included:

- Following the standardised protocol detailed in the instructions as above
- Using only the same standardised phrases for encouragement as detailed above
- Using the same corridor to perform the test
- Using the same proforma as part of the CRF to document results (see Appendix 9.1.6)

Minnesota living with heart failure questionnaire

2.1.10.3 Description and administration

The Minnesota living with heart failure (MLHF) questionnaire[249] comprises 21 items (see Table 2.4) that enquire about patients' perceptions of the impact of HF and therapies upon QOL across three sub-domains: physical aspects of daily life (9); emotional/psychological functioning (5) and socio-economic impact (7). Questions assess the impact of typical physical symptoms of HF e.g. shortness of breath, fatigue, ankle swelling. Other questions assess aspects of physical and social well being e.g. climbing stairs, household work, recreational activities and hobbies. Additionally, mental and emotional functions such as loss of concentration, memory, worry and being a burden to others is evaluated.

Each item is rated on a 6-point Likert scale (0 = no limitation to 5 =maximal limitation). The total score reflects overall QOL and ranges from 0 (best) to 105 (worst). The questionnaire typically takes 10 minutes to be completed.

According to guidelines established by the authors, the total score best reflects the impact of HF on QOL. In addition, summation of responses to the sub-group of questions for the physical and emotional domains may also be used to describe the physical and psychological effects of HF on QOL.

In our study, the MLHF was administered to all HF patients in the interval between the six-minute walk test and CMR, in a quiet area in the BRU (in accordance with prescribed guidance). http://178.23.156.107:8085/Instruments_files/USERS/mlhf.pdf.
A 'non-profit research project user license' was granted prior to use from the University of Minnesota.

Table 2.4 The Minnesota Living With Heart Failure Questionnaire

The following questions ask how much your heart failure (heart condition) affected your life during the past month (4 weeks). After each question, circle the 0, 1, 2, 3, 4 or 5 to show how much your life was affected. If a question does not apply to you, circle the 0 after that question.						
Did your heart failure prevent you from living as you wanted during the past month (4 weeks) by -	**No**	**Very little**				**Very much**
1. causing swelling in your ankles or legs?	0	1	2	3	4	5
2. making you sit or lie down to rest during the day?						
3. making your walking about or climbing stairs difficult?						
4. making your working around the house or yard difficult?						
5. making your going places away from home difficult?						
6. making your sleeping well at night difficult?						
7. making your relating to or doing things with your friends or family difficult? ?						
8. making your working to earn a living difficult?						
9. making your recreational pastimes, sports or hobbies difficult?						
10. making your sexual activities difficult?						
11. making you eat less of the foods you like?						
12. making you short of breath?						
13. making you tired, fatigued, or low on energy?						
14. making you stay in a hospital?						
15. costing you money for medical care?						
16. giving you side effects from treatments?						
17. making you feel you are a burden to your family or friends?						
18. making you feel a loss of self-control in your life?						
19. making you worry?						
20. making it difficult for you to concentrate or remember things?						
21. making you feel depressed?						

2.1.10.4 Rationale for use

A range of QOL measures specific to HF are available including the Chronic Heart Failure Questionnaire (CHQ)[250] and Kansas City Cardiomyopathy Questionnaire (KCCQ)[251]. In

our study, we utilised the MLHF questionnaire[249] for multiple reasons. It is the most commonly used, evidence based HF-specific patient reported outcome measure in clinical studies to date and has been extensively validated[252]. Furthermore, it has been shown to be a robust assessment tool across a range of desirable performance criteria (see Table 2.5)[253].

High test-retest reliability[254] as assessed by ICC (0.89) and internal consistency reliability[255] as assessed by Cronbach's alpha (0.91) have been reported previously. Supportive evidence for content validity for the MLHF was shown in a descriptive study of patients' perceptions of quality of life using open-ended interviewing[256].

Following a comparison of the content analysis of interviews with the content of MLHF, the authors concluded that both measures fully addressed the QOL issues identified by the patient sample. Statistically significant correlations between MLHF scores and NYHA[254] grade and fatigue[257] provide supportive evidence for construct validity. Furthermore, in a study comparing QOL in elderly subjects with and without HF, both the overall MLHF scores and sub-domain scores reliably discriminated between both groups[258]. MLHF was also used in a prior echocardiographic observational study[86] aimed at characterising cohorts of HFpEF and HFrEF in comparison with healthy controls.

The responsiveness of the MLHF questionnaire has also been demonstrated across various interventional clinical trials (e.g. biventricular pacing[259] and drug therapies[249,260]) in HFrEF and in HFpEF[261] (exercise training). Across the spectrum of HFpEF and HFrEF in the CHARM study[262], MLHF had high acceptability; 88% of subjects answered all items and 98% completed at least 75% of all items.

Table 2.5 Desirability criteria for patient reported outcome measure

Criteria	Definition/Test
Reliability	
Test-retest reliability	The stability of the tool over time; assessment method – administering the questionnaire to subjects on two different occasions and examining the correlation between test and re-test scores
Internal consistency	How closely related a set of items are as a group i.e. measure the same construct in the scale; assessed by Cronbach's alpha
Validity	
Content validity	The extent to which the items in the scale are representative of the conceptual domain it is intended to cover; assessed by expert opinion and review of the literature
Construct validity	Evidence that the scale correlates well with other measures of similar constructs in the hypothesised direction; assessed by correlations between the measure and other similar measures **Or** The ability of the scale to discriminate between known-groups; assessed by comparing scores for sub-groups who are expected to differ on the construct being measured (e.g diseased group versus control group)
Responsiveness	Ability of the scale to detect significant change over time; assessed by comparing scores before and after an intervention of known efficacy e.g. with a t-test
Practicality	
Acceptability	Subjects' willingness to complete questionnaire (impacts on data quality); assessed by levels of incomplete data or non-response

CMR imaging

Scans were predominantly supervised by *PK* and in a handful of cases by *AS*. All CMR scans were performed on a 3-Tesla scanner with an 18-channel cardiac coil (Siemens, Erlangen, Germany). Pre-CMR screening was performed to ensure no contraindications to scanning or adenosine stress (see CMR protocol in Appendix 9.1.10). Two cannulae were inserted (one in each arm) to allow ease of intravenous contrast and adenosine administration. The blood results from earlier samples processed by the hospital during the study visit were checked for eGFR (to ensure eligibility) and haematocrit (for subsequent ECV calculations).

Only those imaging sequences acquired and subsequently analysed for the purpose of this thesis are discussed in detail below. A summary of the CMR protocol is shown in Table 2.6. Scan duration was typically 1 hour. All images were acquired with retrospective ECG gating unless arrhythmia was present, in which case prospective gating was employed. Typically, patients were imaged in breath-held end-expiration. Parallel imaging (factor 3 for cine, factor 2 for stress and LGE) was used to shorten breath-holds. When breath-holding was difficult and image-quality was degraded as a consequence, free breathing images were acquired.

The total dose of contrast administered was 0.15 mmol/kg (Gadovist, Bayer Healthcare, Berlin, Germany). The first dose (0.04 mmol/kg) was given during stress perfusion imaging. The second dose (same quantity) was given during rest perfusion imaging, followed immediately after the sequence by a ‘top-up’ dose of 0.07 mmol/kg.

Table 2.6 Summary of the CMR study protocol

Order	Sequence
1	Localisers
2	Repeat localisers in magnet isocentre
3	HASTE black blood anatomical axial images
4	Further localisers: vertical long-axis, horizontal long-axis, short-axis
5	Cine imaging: 4-chamber, 2-chamber, 3-chamber
6	Pre-contrast short-axis *MOLLI* images: basal, mid and apical
7	Stress perfusion
8	Cine imaging: complete LV short-axis stack
9	Cine imaging: complete LA short axis stack
10	Rest perfusion
11	Cine imaging: sagittal oblique of the aorta
12	Phase contrast & magnitude imaging: aortic flow at pulmonary artery level
13	Late gadolinium enhancement imaging
14	Post-contrast short-axis *MOLLI* images: basal, mid and apical

2.1.10.5 Cine imaging

Following initial localisers, balanced steady state free precession (SSFP) cine images were acquired in conventional long-axis orientations: 4-, 2- and 3-chamber (see Figure 2.1). The field of view (FOV) was altered to a minimum, dependent upon patient size. Segments were amended according to heart rate: < 70 b.p.m, 15 segments; 70 – 80 b.p.m, 12 segments; 80 – 100 b.p.m, 11 segments. Thirty phases were used for image construction (40 phases for the 3-chamber view). All images were acquired with a slice thickness of 8 mm and a distance factor of 25%. The image matrix was set at 256 x 204. The FOV was optimised to achieve in-plane resolution between 1.1 to 1.7 mm x 1.3 to 1.9 mm.

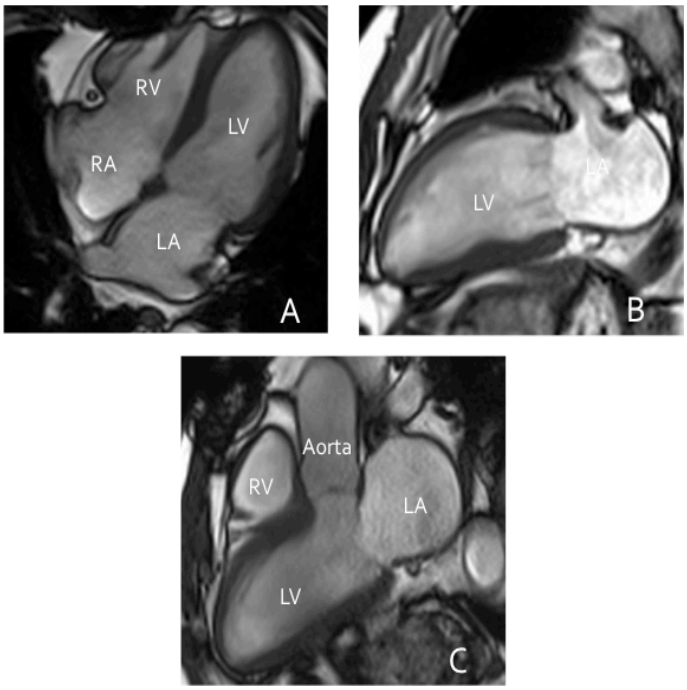


Figure 2.1 CMR long-axis cine images

Immediately after stress perfusion, further cine imaging was performed in contiguous short-axis slices covering the entire LV and RV, from base to apex (Figure 2.2). The first (basal) slice was planned at the level of the mitral valve annulus perpendicular to the interventricular septum, to minimise partial volume at the atrial-ventricular interface. The same process was repeated (beginning again at the basal slice) downwards for acquisition of the short-axis LA stack (Figure 2.2 Panel B).

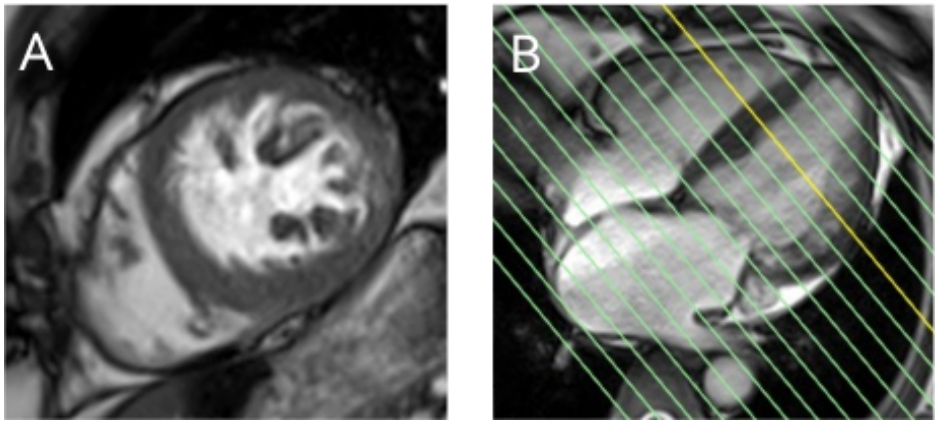


Figure 2.2 CMR short-axis LV cine images and planning for LV/RV/LA stack

2.1.10.6 *MOLLI* sequence

The Modified Inversion Recovery Look Locker (*MOLLI*)[263] sequence was performed with the following parameters: breath-held or free breathing, single-shot sequence, 3(3)3(3)5 sampling pattern, 8 mm slice thickness, 300 x 400 mm FOV, 50° flip angle, 120 ms minimum TI, 80 ms increments of inversion time. Pre-contrast short-axis slices were acquired at the basal, mid-ventricular and apical levels. Post-contrast imaging was also undertaken copying the same slice positions.

As reported previously by our research group[164], in order to minimise artefacts, acquisitions were performed with the region of interest at magnet isocentre. In addition, a small shim volume was applied around the myocardium with a larger FOV (≥400 mm). Despite the above measures, if artefacts persisted, imaging was repeated following a change in either the phase-encode direction or resonance offset frequency.

2.1.10.7 Perfusion imaging

The same three short-axis slice positions used in the *MOLLI* sequences were copied for perfusion imaging. Fast Low Angle Shot (FLASH), a saturation recovery gradient echo

sequence was used for imaging. Other perfusion-specific imaging parameters were: 224 x 179; parallel imaging factor x2; if heart rate < 70 b.p.m, 40 acquisitions; if heart rate 70 – 90 b.p.m, 50 acquisitions, if heart rate > 90 b.p.m, 60 acquisitions, if heart rate > 110 b.p.m, a default setting of 2 beat trigger and reduced matrix size (192 x 154). Prior to stress, test acquisitions were performed to ensure the smallest FOV without any wrap.

For pharmacological stress vasodilation, 140-210 mcg/kg/min of adenosine (depending on haemodynamic and symptomatic response) was infused for a minimum of 3 minutes and during image acquisition. A radiographer or research nurse (*MH*, *SM*) was present with the patient in the scanning room during stress. Oxygen saturations were monitored throughout. Blood pressure, heart rate and symptoms were recorded before and during stress at one-minute intervals. Image acquisition was commenced, typically 5 seconds after the injection of contrast at a rate of 5 mls/second, followed by 20 mls of saline flush. Rest perfusion imaging was performed approximately 10 minutes after stress imaging using identical imaging parameters and contrast dosage as per stress perfusion.

2.1.10.8 Late gadolinium enhancement imaging

LGE imaging was performed in the same slice positions as the cine images, at least 10 minutes following the final injection of contrast. A segmented, phase-sensitive inversion recovery (PSIR) gradient echo sequence with a 2 beat trigger was used. The TR was set approximately 100 milliseconds less than the RR interval. In patients in with progressively worsening breath-holding or with diminishing image quality, single-shot imaging was undertaken to speed up scan times.

A Look Locker inversion time (TI) scout was performed on the mid-ventricular cine imaging slice position to determine the optimal TI to null unaffected myocardium. The TI was progressively adjusted by 10 ms approximately every 1-2 slices to ensure adequate nulling was maintained throughout image acquisition. In the event of an image showing doubtful enhancement, the acquisition was repeated with the phase encoding direction swapped.

Two-stage strategy for imaging analysis

For both TTE and CMR, all image analysis was undertaken in a two-stage strategy. Additional methodology pertaining to each imaging parameter analysed in this thesis are detailed in the relevant results Chapters.

2.1.11 Initial un-blinded imaging analysis

The first stage (detailed in results Chapter 3) involved the generation of clinical reports for both modalities i.e. TTE and CMR, using routinely practiced methods in clinical practice. Scans were reported un-blinded to patient demographics and clinical history i.e. history of hypertension. Importantly, readers reporting the scans were blinded to data from the alternate modality i.e. readers reporting TTE were blinded to results from CMR and vice versa.

The rationale for this approach was in order to test the hypothesis that clinical CMR detects new, and important clinical pathologies in HFpEF, not readily apparent with routine clinical TTE. The second stage of analysis (used in results Chapters 4 onwards) would thus permit analysis of a 'purer' cohort of HFpEF. During stage one, image quality was graded as detailed in (Table 2.7).

Table 2.7 Assessment criteria for image quality grade - during stage one of analysis (un-blinded)

Grade	Parameter/Criteria
X	Not performed
0	Non-analysable
1	Sub-optimal but analysable; poor
2	Fair
3	Good

2.1.12 Subsequent blinded imaging analysis

The second stage of image analysis was performed blinded to all clinical data following scan anonymysation. All scans were given two different (one each for TTE and CMR), random, computer generated 5-digit imaging codes, to facilitate blinded analysis. These codes were kept locked during image analysis until the final un-blinding process, prior to a database lock. Only pre-designated study personnel had access to these codes throughout.

Initial un-blinded transthoracic echocardiography analysis

Standard TTE clinical reports were generated following analysis performed in routine clinical practice by 2 BSE accredited sonographers (*AMM*, *JM*) as per existing ASE guidelines for performing[230] and interpreting[264] scans. LVEF for study inclusion was calculated using the biplane method[77] or estimated visually in cases of poor endocardial border definition. For borderline cases, final consensus required review by a third observer (*PK*). Further echocardiographic analysis details are provided in Chapter 3.

Initial un-blinded CMR analysis

CMR clinical reports were generated following analysis (by *PK*) performed in routine clinical practice by 2 Consultant Cardiologists (GPM, *ASHC*) with expertise in CMR as per Society for Cardiovascular Magnetic Resonance guidelines for performing and interpreting

scans[265]. LVEF, volumes, wall thickness and perfusion were assessed using commercially available clinical software (Argus, Siemens Medical Solutions, Erlangen, Germany). Further CMR analysis details are provided in Chapter 3.

Subsequent blinded transthoracic echocardiography analysis

All TTE scans were analysed off-line by *AMM* and *JM*, using QLAB Xcelera CMQ (cardiac myocardial quantification) software. The primary purpose of blinded TTE analysis was to identify diastolic dysfunction as per ESC guidelines[20], in conjunction with BNP values and the presence or absence of AF. The following parameters were calculated:

1. E/E'

Diastolic function[53] was assessed using the transmitral inflow velocity and mitral annular velocity. Pulse wave Doppler was used to measure the early and late LV diastolic inflow (E and A waves). Tissue Doppler was used to measure septal and lateral diastolic velocities (E'). The respective septal and lateral E/E' ratios were then calculated and averaged to provide an overall measure of diastolic function.

2. LV mass

LV mass was calculated from LV linear dimensions (at end-diastole) based on the prolate ellipse method recommended by the American Society of Echocardiography[77]. The formula[266] used was as follows:

LV mass = 0.8 x {1.04 [(LV internal diameter + posterior wall thickness + septal wall thickness)^3 – (LV internal diameter)^3]} + 0.6 g.

3. LA volume

Analogous to LV volumetric measurement, LA volume was measured using the modified Simpson's method[77] in the apical 2- and 4-chamber views. LA planimetry was performed with care taken to exclude the pulmonary veins from from contours as illustrated previously in Figure 1.8. More detailed methodology is provided in Chapter 5.

Subsequent blinded CMR analysis

CMR42 software, version 5.0.3 (Circle Cardiovascular Imaging, Calgary, Canada) was used to carry out all CMR analysis. All CMR scans were analysed off-line by *PK*. Overall image quality was graded as shown in the Table below.

Table 2.8 Assessment criteria for image quality grading during blinded analysis

Grade	Parameter/Criteria
X	Not performed
0	Non-analysable
1	Minor artefact may affect analysis; still analysable
2	Minimal artefact; does not affect analysis
3	Good
4	Excellent

2.1.12.1.1 Ventricular volumes, function and mass analysis

From the short-axis cine stack, manual contours (Figure 2.3) were drawn for the LV endocardium and epicardium at end-diastole and end-systole. This allowed calculation of LV end-diastolic volume (LVEDV), LV end-systolic volume (LVESV), LV stroke volume (LVSV), LV ejection fraction (LVEF) and LV end-diastolic mass (LVEDM). Papillary muscles and trabeculations were excluded from contours since this method has shown the better reproducibility for mass assessment[267].

Similar to the LV, RV end-diastolic and end-systolic contours were drawn to allow calculation of RV end-diastolic volume (RVEDV), RV end-systolic volume (RVESV), RV stroke volume (RVSV) and RV ejection fraction (RVEF). RV mass assessment was not performed. All volumetric data and mass were indexed for body surface area (BSA), using

Mosteller's method[268] and denoted by the suffix 'i' e.g. LVMI for left ventricular mass indexed.

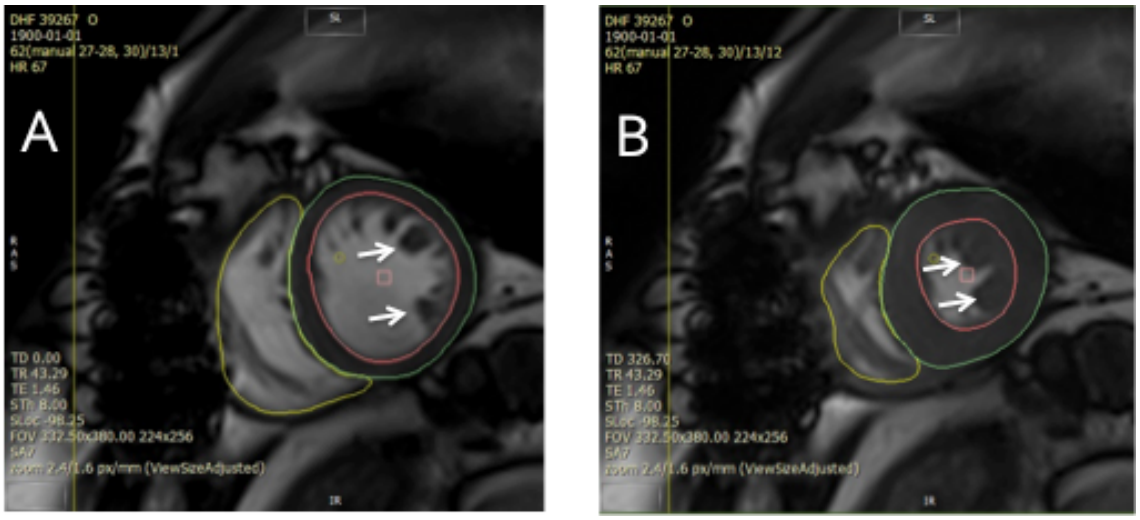


Figure 2.3 CMR calculation of ventricular volumes and left ventricular mass

End-diastolic (A) and end-systolic (B) frames illustrating manually drawn contours of the LV endocardium (pink), LV epicardium (green) and RV endocardium (yellow) for volumetric and mass analysis. Note – (white arrows) papillary muscles and trabeculations were excluded from LV mass calculations.

2.1.12.1.2 Left atrial volumes and function

A detailed description of LA volumetric and functional quantification is provided in Chapter 5. Although, the short-axis LA stack was analysed, only the results of the biplane area-length method are discussed in this thesis. All scans were analysed, irrespective of cardiac rhythm.

2.1.12.1.3 Fibrosis assessment

Focal fibrosis

Both qualitative and quantitative assessments of LGE images were undertaken and are described in greater detail in Chapter 4.

Analysis of diffuse fibrosis

Although 3 short-axis slices were acquired as part of the CMR protocol, only the mid-ventricular slice *MOLLI* images were chosen for analysis due to concerns regarding partial volume effects afflicting basal and apical slices [163,269]. Further methodological details are also provided in Chapter 4.

Study outcomes

The overall study was comprised of the following outcome measures:

- Number of new clinical diagnoses detected up by CMR (see Chapter 3)
- Composite end-point
- Exercise capacity as assessed by 6MWT
- QOL as assessed by MLHF Questionnaire

The composite endpoint was either all-cause mortality or repeat hospitalisation for HF (defined as a hospital admission for which HF was the primary reason and which required diuretic, inotropic or intravenous nitrate therapy). Hospital databases and patient records were sourced to obtain composite outcome data. Patient follow-up was for a minimum of 6 months post-study entry.

Data capture, storage and handling

Initial data from the patients' study visit including patient demographics, clinical details, 6MWT results and MLHF questionnaire was recorded onto a paper CRF. This data was subsequently manually entered into an Excel spreadsheet, version 14.6.5 (by *PK*). The

results of blinded TTE (by *AMM*, *JM*) and CMR analysis (by *PK*) were entered into separate Excel spreadsheets, until data merge at the time of unblinding. All data was stored on University, password protected computers. Raw TTE images were anonymysed and archived onto the Excelera workstation. Raw CMR images were anonymysed (by *MH* or *SM*) and archived onto a Siemens, Syngovia CMR workstation in accordance with a dedicated study operating protocol (SOP - see Appendix 9.1.11). A data lock of the final study database was carried out on the 28th of October 2015.

Sample size calculation

The overall study was designed with the primary intention of developing plasma biomarkers for HFpEF and powered at 80% ($p < 0.05$) to detect a standardised difference of 0.45 between HFpEF and the other groups. However, for the CMR parameters analysed in this thesis, statistical power remains adequate. CMR affords superior reproducibility and precision compared to TTE and hence a much smaller sample size is required to detect between group differences compared with TTE[132]. This is illustrated by a study[147] comparing the assessment of LV mass by TTE and CMR. The authors concluded that to detect a 10 gram difference in LV mass, with a power of 90% and an alpha error of 0·05, 505 patients would be required by TTE and only 14 for CMR. Furthermore, in HF patients and using a two group design, the estimated sample size to detect ECV change of 0.038 or λ change of 0.063 (corresponding to ~3% increase of histological myocardial fibrosis) with a power of 80% and an alpha error of 0.05 was 27 in each group, respectively[270].

Statistical analysis

SPSS version 22, IBM Corp., Armonk, New York) was used to conduct all statistical analyses. Continuous data was assessed for normality using the Shapiro-Wilk test, histograms and Q-Q plots. Normally distributed data are expressed as mean ± standard deviation (SD). Non-parametric data are expressed as median (25 - 75% interquartile range [IQR]). Categorical data are expressed as absolute numbers or percentages. BNP, creatinine, 6MWT distance and the MLHF score were log10 transformed before analysis.

2.1.13 Group comparisons

For group differences, a p value of <0.05 was deemed to be statistically significant, with p values given to 3 decimal places. Comparisons of means of 2 groups were performed using the independent samples t test. For comparing 3 groups, and if data was normally distributed, one way-ANOVA with Bonferroni correction was employed. For similar comparison of non-normally distributed data, the Kruskal-Wallis test was used. The Chi-square or Mann-Whitney U tests were used to compare categorical data, as appropriate.

2.1.14 Survival analysis

Kaplan-Meier analysis was undertaken to calculate cumulative event-free rates. The difference between stratified Kaplan-Meier plots or curves was assessed using the Log-Rank test. Cox proportional hazard and multiple regression analyses were performed to determine which variables were related significantly to the composite endpoint of death and/or re-hospitalisation with HF. In patients with 2 different events, the time to first event only was used. To identify independent predictors, covariates with univariable Cox regression association with the endpoint at $p < 0.10$ were then entered, to prevent model over-fitting into subsequent multivariable analysis, using both backwards and forwards stepwise elimination methods. Additional measures to prevent model over-fitting were the exclusion of highly correlated variables ($r > 0.7$) and ensuring that the final multivariable model comprised of approximately one variable per ten events. To provide clinical context, smaller multivariable models were also created to separately assess the impact of clinical factors, functional parameters, imaging markers etc. To assess the incremental benefits and prognostic strengths of some CMR markers tested in this thesis (in Chapters 5 and 6), such markers were then added to these smaller multivariable models to ascertain if they still remained significant ($p < 0.05$). Continuous variables were Z-standardized to enable comparison of hazard ratios based upon one SD increase in the predictor variable.

2.1.15 Biomarker testing

For biomarker testing, receiver operating characteristic (ROC) analysis was undertaken to evaluate the optimal threshold and discriminatory power of variables for binary classification e.g. HFpEF group versus healthy controls, composite end-point group versus no end-points. By plotting sensitivity (y-axis) versus 1-specificity (x-axis), ROC curves were generated. Maximal sensitivity and specificity were chosen to define the optimal thresholds. Discriminatory power was assessed by calculating the area under the curve (AUC) as described by the method of Hanley and McNeil[271]. An AUC of 0.5 was deemed to have zero discriminatory power whereas an AUC of 1 had absolute discriminatory power. As a guide, the utility of biomarkers based on AUC were graded as follows: 0.9-1 = excellent; 0.8-0.9 = good; 0.7-0.8 = fair; 0.6-0.7 = poor and 0.5-0.6 = fail. Category-free net reclassification index (NRI) was further used to assess biomarker performance in up or down classifying subjects into the correct groups (e.g. HFpEF versus controls)[272].

2.1.16 Associations

Pearson's (r) or Spearman's (r_s) correlations were performed to check for potential associations with other continuous variables. Further linear regression modeling was undertaken to identify the strongest independent associations.

2.1.17 Inter-modality agreement, reproducibility, intra-observer and inter-observer assessments

Cohen's Kappa (K) was used to test for agreements of similarities in image grading between CMR and TTE ($p > 0.05$ was considered significant). For CMR, assessments of intra-observer and inter-observer variability were undertaken a minimum of 4 weeks apart (by *PK* and *JRA*). The coefficient of variation (CoV)[273] and two-way mixed-effect intra-class correlation coefficient (ICC)[274] for absolute agreement were used to assess reproducibility. Agreement was defined as excellent if ICC was ≥ 0.75.

The Bland-Altman method[275] was used to define the limits of agreement for inter-observer and intra-observer variability.

3 NEW DIAGNOSES IDENTIFIED BY CMR IN HFpEF AND THEIR PROGNOSTIC RELAVENCE

Published:

Kanagala P, Cheng ASH, Singh A, McAdam J, Marsh AM, Arnold JR, Squire IB, Ng LL, McCann GP. *Diagnostic and prognostic utility of cardiovascular magnetic resonance imaging in heart failure with preserved ejection fraction – implications for clinical trials*. J Cardiovasc Magn Reson. 2018 Jan 11;20(1):4. doi: 10.1186/s12968-017-0424-9.

Young Investigator Runner-up. *Diagnostic And Prognostic Utility Of Cardiovascular Magnetic Resonance Imaging In Heart Failure With Preserved Ejection Fraction.* Annual Meeting - British Society for Heart Failure, London, UK 2016

Abstract

Aims

HFpEF is a poorly characterised condition. We aimed to phenotype patients with HFpEF using multiparametric stress CMR and to assess the relationship to clinical outcomes.

Methods and Results

One hundred and fifty four patients (51% male, mean age 72 ± 10 years) with a diagnosis of HFpEF underwent both TTE and CMR during a single study visit. The CMR protocol comprised cine, stress/rest perfusion and late gadolinium enhancement imaging on a 3-Tesla scanner. Follow-up outcome data (death and heart failure hospitalisation) were captured after a minimum of 6 months. CMR detected previously undiagnosed pathology in 42 patients (27%), who had similar baseline characteristics to those without a new diagnosis. These diagnoses consisted of: coronary artery disease (n = 20, including 14 with 'silent' MI), microvascular dysfunction (n = 11), probable or definite hypertrophic cardiomyopathy (n = 10) and constrictive pericarditis (n = 5). Four patients had dual pathology. During median follow-up of 623 days, those with a new CMR diagnosis were at higher risk of adverse outcome for the composite endpoint (Log-Rank test: p = 0.047). In Cox regression multivariable analysis, a new CMR diagnosis was the strongest independent predictor of adverse outcome (HR: 1.92; CI: 1.07-3.45; p = 0.03).

Conclusions

CMR diagnosed new pathology in a significant minority of HFpEF (27%). These patients were at increased risk of death and/or heart failure hospitalisation.

Background

HFpEF presents with marked clinical heterogeneity and accounts for approximately half of all HF cases. It is projected to be the predominant phenotype in the near future[6,35]. While interventions have improved outcomes in HFrEF, similar therapies have been ineffective in HFpEF and there remain no specific, evidence-based treatments[113]. Furthermore, a wide range of pathologies such as silent MI and ischaemia due to CAD, HCM and constrictive pericarditis may masquerade as HFpEF[19,111,140]. These 'phenocopies' may share many features of HFpEF, such as preserved ejection fraction (EF), left ventricular hypertrophy (LVH), diastolic dysfunction, atrial dilatation and elevated natriuretic peptides. Hence, focus has shifted to studying 'purer' forms of HFpEF by excluding such conditions from contemporary clinical trials[114].

At present, TTE remains the primary diagnostic tool for HFpEF[20] and is the main gatekeeper for entry into clinical trials of this entity[113,114]. However, cardiovascular magnetic resonance imaging (CMR) is the recognised gold standard for assessment of the majority of parameters that make up the latest HFpEF guidelines[20,130,132,145]. The superior diagnostic capabilities of CMR across the spectrum of aforementioned 'phenocopies' is also well established[140-143]. However, no reports in the literature detail the systematic use of CMR in patients with suspected HFpEF. We aimed to establish the proportion of new clinical diagnoses in HFpEF patients identified with CMR, and to assess their impact upon clinical outcome.

Methods

3.1.1 Study population

The study design, rationale, inclusion and exclusion criteria and ethics have been previously detailed in the general methods Chapter. The study population described and analysed in this Chapter pertains to those who initially attended with *suspected* HFpEF.

Potentially eligible patients were invited to participate following screening of the hospital database, outpatient clinics and wards. All enrolled patients underwent comprehensive clinical assessment (including patient reporting of angina symptoms and previous MI or revascularisation), venepuncture, 12-lead ECG recording and TTE followed by CMR (provided no contraindications) during the same visit. The clinical reports of all scans were disseminated to the responsible physician(s) to inform patient management.

3.1.2 Blood samples

Blood was sampled for BNP (immunoassay (Siemens, Erlangen, Germany) and other biochemical markers (sodium, urea and creatinine). Estimated GFR was calculated from the Modification of Diet in Renal Disease formula[228].

3.1.3 ECG

The 12-lead ECGs performed were assessed (by *PK* and *AMM*) for the presence of pathological Q waves as surrogates of transmural MI[229].

3.1.4 Imaging

Clinical reports were generated for TTE and CMR scans with knowledge of patient demographics and past medical history (e.g. history of hypertension). All subsequent quantitative and qualitative analyses used to generate the reports were performed independently with readers blinded to data from the other scan. Image quality was graded as: 0 = non-interpretable; 1 = poor; 2 = fair; 3 = good.

3.1.4.1 TTE

Images were acquired and reported as per American Society of Echocardiography guidelines using an iE 33 system with S5-1 transducer (Philips Medical Systems, Best, The Netherlands)[230]. TTE studies were performed and reported by two BSE accredited sonographers (*AMM*, *JM*). LVEF for study inclusion was calculated using the biplane method (see Figure 3.1) or estimated visually in cases of poor endocardial border definition. For borderline cases, final consensus required review by a third observer (*PK*).

Any regional wall motion abnormalities (RWMA) were reported according to established nomenclature[264]: hypokinetic, akinetic, dyskinetic, scar/thinning. All patients with suspected HCM[141] or constrictive pericarditis[276] based upon recognised TTE criteria were to be excluded from the study and were not intended to have undergone subsequent CMR

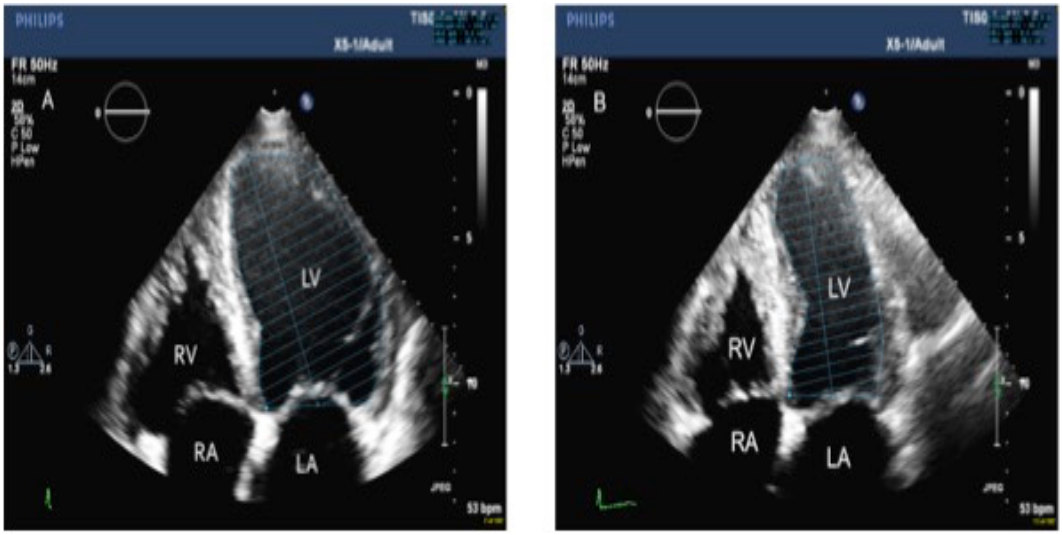


Figure 3.1 Echocardiographic measurement of left ventricular volumes and ejection fraction using Simpson's method

(A) End-diastolic frame and (B) End-systolic frame for ejection fraction calculation from the apical 4-chamber view

3.1.4.2 CMR

CMR scans were performed on a 3-Tesla scanner (Siemens Skyra Erlangen, Germany) with an 18-channel cardiac coil. The protocol was previously reported by our group[227]. Cine imaging was performed in three long axes and a short axis cine stack was performed in the interval between stress and rest perfusion acquisitions. For pharmacological stress, 140-210 mcg/kg/min adenosine (depending on haemodynamic and symptomatic response) was infused for at least 3 minutes. Stress and rest perfusion images at the basal, mid-ventricular and apical levels were acquired after injection of 0.04 mmol/kg of contrast (Gadovist, Bayer Healthcare, Berlin, Germany). Following rest perfusion, a 'top-up' bolus of 0.07

mmol/kg was given to make a total contrast dose of 0.15 mmol/kg. LGE was performed 10-15 minutes after the final injection of contrast.

CMR analyses were undertaken and clinical reports generated by two experienced imaging cardiologists (*GPM*, *ASHC*), with cases randomly split between them. LVEF and volumes, wall thickness and perfusion were analysed using commercially available software (Argus, Siemens, Erlangen, Germany). LV contours were drawn manually (excluding papillary muscles) to derive end-diastolic and end-systolic volumes and LVEF from the short-axis cine stack as reported by our group previously with excellent intra-observer and inter-observer variability[174]. Volumetric data were indexed to BSA.

3.1.5 Definitions of 'new diagnoses' from CMR

MI was defined as high signal intensity area(s) on LGE involving at least the sub-endocardium in a coronary artery distribution and the segmental extent and transmurality were described. For ischaemia evaluation, in conjunction with LGE images, stress and rest perfusion images were semi-quantitatively assessed for reversible perfusion defects. The defects were categorised into ischaemia likely to be due to epicardial CAD or microvascular dysfunction[277]. Criteria taken into account to define ischaemia were defects: appearing first when contrast entered LV myocardium, persisting beyond peak myocardial enhancement (typically > 4 seconds), > one pixel width, most prominent in the sub-endocardial layer, demonstrating a transmural gradient across affected segments which regressed to the sub-endocardium over time, present at stress but not at rest and in coronary arterial territorial distribution[277]. Circumferential, sub-endocardial perfusion defects seen at least on one ventricular level or crossing coronary territories were reported as suggestive of microvascular dysfunction, albeit with the caveat that significant CAD could not be reliably excluded.

Constrictive pericarditis (e.g. diastolic septal bounce, pericardial effusion, thickening and hyperenhancement on LGE) and HCM were diagnosed based on established CMR parameters[140,141,143,276]. A diagnosis of HCM was considered in all patients with LV wall

thickness of ≥ 15 mm[141]. In such cases, the degree and pattern of LVH and medical history (including hypertension, blood pressure control, anti-hypertensive medications) were considered to gauge whether wall thickness was proportionate or disproportionate. A characteristic spade-like configuration of the LV cavity and apical:basal wall thickness ratio ≥ 1.3 was used to diagnose apical HCM[143]. The overall likelihood of HCM was categorised as definite or probable.

3.1.6 Follow-up and endpoints

Patients were followed up for a minimum of 6 months post-study entry. The primary endpoint was the combination of hospitalisation for HF (defined as a hospital admission for which HF was the primary reason and which required diuretic, inotropic or intravenous nitrate therapy) or all-cause mortality. Hospital databases and patient records were sourced to obtain outcome data.

3.1.7 Statistical analysis

Statistical analyses were performed using SPSS version 22, IBM Corp., Armonk, New York). Probability (p) values < 0.05 were considered statistically significant. Normality was assessed using the Shapiro-Wilk test, histograms and Q-Q plots. Normally distributed data are expressed as mean ± SD. Non-parametric data are expressed as median (25 - 75% IQR). Categorical data are expressed as absolute numbers or percentages. Comparisons of means of 2 groups were performed using the independent samples *t* test. The Chi-square test was used to compare categorical data. Cohen's Kappa (K) was used to test for agreements of similarities in image grading between CMR and TTE ($p > 0.05$ was considered significant). Cox proportional hazard and multiple regression analyses were performed to determine which variables were related significantly to the composite endpoint of death and/or hospitalisation with HF. BNP levels were $\log_{10}$ transformed and hazard ratios for subsequent analysis refer to 1 SD increment of the transformed BNP. Only variables with a univariable p value < 0.10 were entered into subsequent multivariable analysis. Kaplan-Meier survival curves were used to demonstrate cumulative event-free

rates in patients stratified into 2 CMR groups (‘no new diagnoses’ versus ‘new diagnoses’). The Log-Rank test was used to test for statistical significance.

Results

A summary of the study overview, patients excluded and results are presented in Figure 3.2. One hundred and ninety six patients attended for screening. The presence of severe lung disease was the most common reason for exclusion. One hundred and eighty patients met the initial study inclusion criteria. The majority of patients who did not undergo subsequent CMR evaluation were either claustrophobic or had pacemakers. A total of 154 patients underwent CMR, of whom 5 did not undergo stress perfusion imaging.

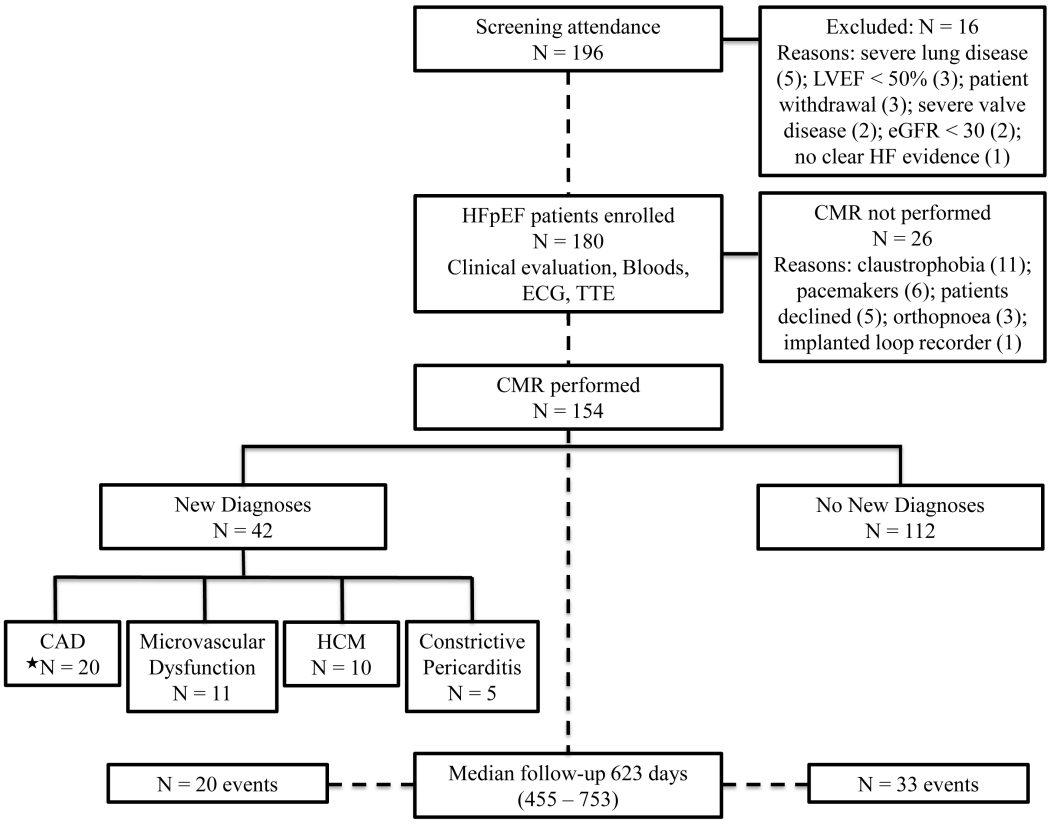


Figure 3.2 Study overview

*Of the 20 patients with newly diagnosed coronary artery disease (CAD), 4 patients had concomitant hypertrophic cardiomyopathy (HCM)

Baseline characteristics of the CMR population stratified by the presence or absence of new CMR diagnoses are summarized in Table 3.1. Patients with and without new diagnoses on CMR had similar baseline characteristics, including LV volumes and EF. The cohort had a wide age range (37 – 97 years) with the majority of patients over 65 years. Nearly one-third were in NYHA class III or IV. There was a high prevalence of obesity and hypertension and nearly half the patients had a history of AF and a similar proportion of diabetes. Approximately a fifth had chronic lung disease. At baseline, CAD was present in 21%, including 15 patients with known MI.

Table 3.1 Baseline characteristics of HFpEF patients who underwent CMR

	All	No new diagnoses (n = 112)	New diagnoses (n = 42)	p value
Age, years	72±10	73±9	72±12	0.61
Male	78 (50.6)	54 (48.2)	24 (57.1)	0.32
Atrial fibrillation	72 (46.8)	50 (44.6)	24 (52.4)	0.42
Heart rate (b.p.m)	70±14	70±14	72±16	0.57
SBP (mmHg)	143±25	144±25	146±26	0.61
DBP (mmHg)	74±12	74±12	74±13	0.99
BMI (kg/m^2)	34±7	34±7	33±9	0.66
NYHA I/II	106 (68.8)	77 (68.8)	29 (69.0)	0.97
NYHA III/IV	48 (31.2)	35 (31.3)	13 (31.0)	
Known CAD	32 (20.8)	-	-	-
Hypertension	139 (90.3)	111 (89.3)	39 (92.9)	0.60
Diabetes	75 (48.7)	54 (48.2)	21 (50.0)	0.88
COPD/Asthma	27 (17.5)	17 (15.2)	10 (23.8)	0.21
Pulm. oedema	110 (71.4)	79 (70.5)	31 (73.8)	0.69
Aspirin	54 (35.1)	42 (37.5)	12 (28.6)	0.30
Beta-blocker	99 (64.3)	74 (66.1)	25 (59.5)	0.45
ACEi or ARB	130 (84.4)	97 (86.6)	33 (78.6)	0.22
Statin	97 (63.0)	70 (62.5)	27 (64.3)	0.84
Loop diuretic	125 (81.2)	91 (81.3)	34 (81.0)	0.97
Sodium (mmol/L)	139±3.4	139±3.6	140±2.6	0.39
Urea (mmol/L)	8.7±3.8	8.8±4.0	8.3±3.5	0.46
eGFR	66±19	66±19	64±19	0.46
BNP ng/L	145 (66 – 259)	134 ± (57.5 – 251)	175 ± (111 – 263)	0.12
LVEF (%)	57±6	57±6	57±7	0.98
LVEDVI (ml/m^2)	74±18	73±17	77±21	0.26
LVESVI (ml/m^2)	33±11	32±10	34±13	0.30

ACEi = angiotensin converting enzyme inhibitor; ARB = angiotensin II receptor blocker; BNP = B-type natriuretic peptide; CMR = cardiovascular magnetic resonance imaging; DBP = diastolic blood pressure; GFR = estimated glomerular filtration rate; LVEF = left ventricular ejection fraction; LVEDVI = left ventricular end-diastolic volume indexed to BSA; LVESVI = left ventricular end-systolic volume indexed to BSA; SBP = systolic blood pressure

3.1.8 Imaging

Overall, image quality was better for CMR compared to TTE (median grade: 2 vs 1 respectively). In those with a new diagnosis on CMR, this difference was also maintained and statistically significant (kappa statistic [-0.021], p = 0.72).

3.1.9 'New diagnoses' from CMR

CMR identified previously unknown diagnoses in 42 patients (27%). The following new pathologies (see Figure 3.2) were noted: epicardial CAD based on MI or ischaemia (n = 20), microvascular dysfunction (n = 11), HCM (n = 10) and constrictive pericarditis (n = 5). Three patients with HCM had co-existent CAD (2 with new MI and 1 with ischaemia). One patient with constrictive pericarditis also had concurrent MI. Examples of such new diagnoses are shown below.

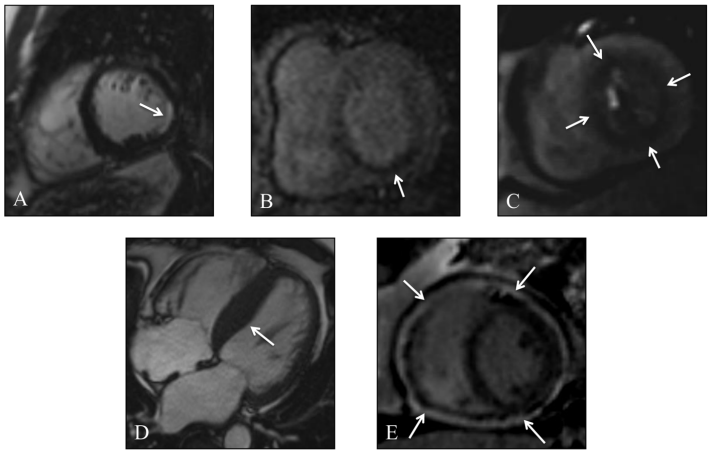


Figure 3.3 Examples of typical findings in the 'new diagnoses' group

CMR images of: A) sub-endocardial, inferolateral MI of 25-50% transmurality on LGE; B) inferoseptal and inferior perfusion defect consistent with right coronary artery territory ischaemia; C) global, concentric perfusion defect consistent with microvascular dysfunction; D) horizontal long axis cine demonstrating asymmetrical septal hypertrophy in HCM; E) constrictive pericarditis with circumferential pericardial hyperenhancement on LGE; white arrows point towards pathology; LGE = late gadolinium enhancement imaging

3.1.10 CAD

Fourteen patients had LGE indicating 'silent' MI (affecting 37 segments). Of these, 3 patients had known CAD at baseline but no prior known MI or pathological Q waves on ECG. On segmental analysis (see Figure 3.4), infarcts were typically small, in a territory not subtended by the left anterior descending coronary artery (95%) and of < 50% transmurality (68%). Corresponding RWMAs on TTE were only reported in 38%. As expected, the ability to diagnose MI by RWMA detectable by TTE worsened with diminishing transmurality of MI (0-50% [24%] versus 51-100% [67%]). On review of the corresponding ECGs, only one case fulfilled the Q wave criterion for MI.[229]

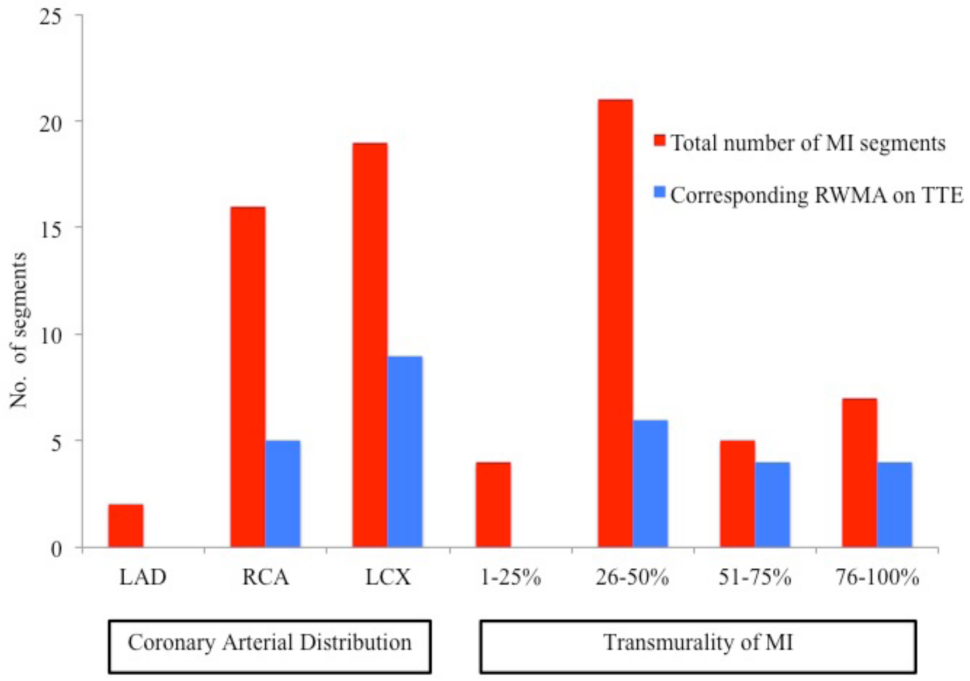


Figure 3.4 Characteristics of newly diagnosed myocardial infarction according to coronary arterial distribution and transmurality

LAD = left anterior descending artery; RCA = right coronary artery; LCX = left circumflex artery; % transmurality of MI is illustrated as 1-25, 26-50, 51-75, 76-100; RWMA = regional wall motion abnormality.

There were 31 patients with reversible perfusion defects and in 11 of these, the pattern suggested microvascular dysfunction. In the remaining 20 patients with ischaemia in an

epicardial CAD pattern, 13 had no prior known CAD. Of these 13 patients, CMR detected a new diagnosis of MI in 4 patients. Accounting for these, CAD was newly diagnosed in 20 patients (new MI in 11 and new ischaemia in 9).

3.1.11 HCM

Findings consistent with HCM (definite: n = 4; probable: n = 6) were reported in CMR studies of 10 patients. Individual patient characteristics are shown in Table 3.2. The main CMR phenotypic patterns of HCM were: asymmetrical septal hypertrophy (70%), focal fibrosis on LGE (70%) and maximal hypertrophy at the basal anteroseptum (50%). With TTE, measured wall thickness was significantly lower (mean difference 2.3 ± 2.2 mm, $p < 0.05$), compared to CMR. Echocardiographic criteria for diagnosis of HCM were reported in only 50% of cases of new CMR diagnosis of HCM. The pattern of LVH on TTE was primarily concentric.

Table 3.2 Characteristics of newly diagnosed hypertrophic cardiomyopathy patients

Patient	Age	HTN	Image modality	Image grade	Maximal wall		Hypertrophy		SAM	LVOTO	LGE		Likelihood of HCM
					mm	region	ASH	Concentric			Mid-	Insertion	
A	71	+	TTE	2	15	Basal	-	+	-	-	NA		Definite
			CMR	2	19	Basal	+	-	-	-	+	+	
*B	85	-	TTE	3	12	Apical	-	+	-	-	NA		Definite
			CMR	3	10	Apical	-	-	-	-	-	-	
C	79	+	TTE	1	15	Basal	-	+	+	-	NA		Probable
			CMR	2	15	Basal	+	-	-	-	-	+	
D	37	-	TTE	1	17	Basal	u/a	u/a	u/a	u/a	NA		Definite
			CMR	3	22	Basal	-	+	-	-	+	+	
E	68	+	TTE	2	16	Basal	-	+	-	-	NA		Definite
			CMR	2	21	Basal	+	-	-	-	+	-	
F	87	+	TTE	2	12	Basal	-	+	+	+	NA		Probable
			CMR	3	15	Basal	+	-	+	+	+	+	
G	62	+	TTE	2	13	Basal	-	+	+	-	NA		Probable
			CMR	2	15	Basal	+	-	+	-	-	-	
H	70	+	TTE	1	14	Basal	-	+	-	-	NA		Probable
			CMR	2	15	Mid	+	-	-	-	+	-	
I	74	-	TTE	1	14	Basal	-	+	-	-	NA		Probable
			CMR	3	17	Basal	-	+	-	-	-	-	
J	72	+	TTE	1	16	Basal	-	+	-	-	NA		Probable
			CMR	3	18	Basal	+	-	-	-	+	-	

Abbreviations: ASH = asymmetrical septal hypertrophy; CMR = cardiac magnetic resonance; HTN = hypertension; LVOTO = left ventricular outflow tract obstruction; NA = not applicable; SAM = systolic anterior motion of the mitral valve; TTE = transthoracic echocardiography; u/a = unable to assess; - = absent; + = present. Image grade: 1 = poor; 2 = fair; 3 = good

3.1.12 Constrictive pericarditis

Constrictive pericarditis was identified in 5 patients, with at least 3 out of the 4 main diagnostic parameters for CMR present in all cases (see Table 3.3). Whilst pericardial thickening on CMR was universally reported in patients with constrictive pericarditis, this finding was not identified in any of the TTE reports. Furthermore, in 3 out of 4 patients, TTE failed to identify septal bounce that was observed with CMR.

Table 3.3 Imaging characteristics of newly diagnosed constrictive pericarditis patients

Patient	**Image grade**		**Pericardial thickening**		**Pericardial effusion**		**Septal bounce**		**Septal E' ≥ 9 cm/s**	**Pericardial enhancement**
	TTE	CMR	TTE	CMR	TTE	CMR	TTE	CMR	TTE	CMR
A	2	2	-	+	+	+	-	-	-	+
B	2	3	-	+	-	+	-	+	+	+
C	1	3	-	+	-	-	-	+	+	+
D	1	2	-	+	-	+	-	+	+	-
E	1	3	-	+	+	+	+	+	-	+
Abbreviations: CMR = cardiac magnetic resonance; TTE = transthoracic echocardiography; - = absent; + = present Image grade: 1 = poor; 2 = fair; 3 = good										

3.1.13 Clinical outcomes

During a median follow-up of 623 days (IQR 455 – 753), there were a total of 53 events (19 deaths, 34 hospitalisations with HF). Of these, 'the new CMR diagnoses group' accounted for 20 events (8 deaths, 12 hospitalisations with HF). Event-free rates (Figure 3.6) were significantly lower in the 'new CMR diagnoses' group (52.4% vs 70.5%, Log-Rank test: p <0.05). The results of univariable and multivariable Cox proportional hazards analysis to predict events are shown in Table 3.4. On multivariable analysis, a new CMR diagnosis (hazard ratio [HR]: 1.92; 95% confidence interval [CI]: 1.06 to 3.45; p < 0.05), log BNP (HR: 1.44; CI: 1.03 to 2.02; p < 0.05, and urea (HR: 1.10; CI: 1.01 to 1.21; p < 0.05) were predictors of the primary endpoint.

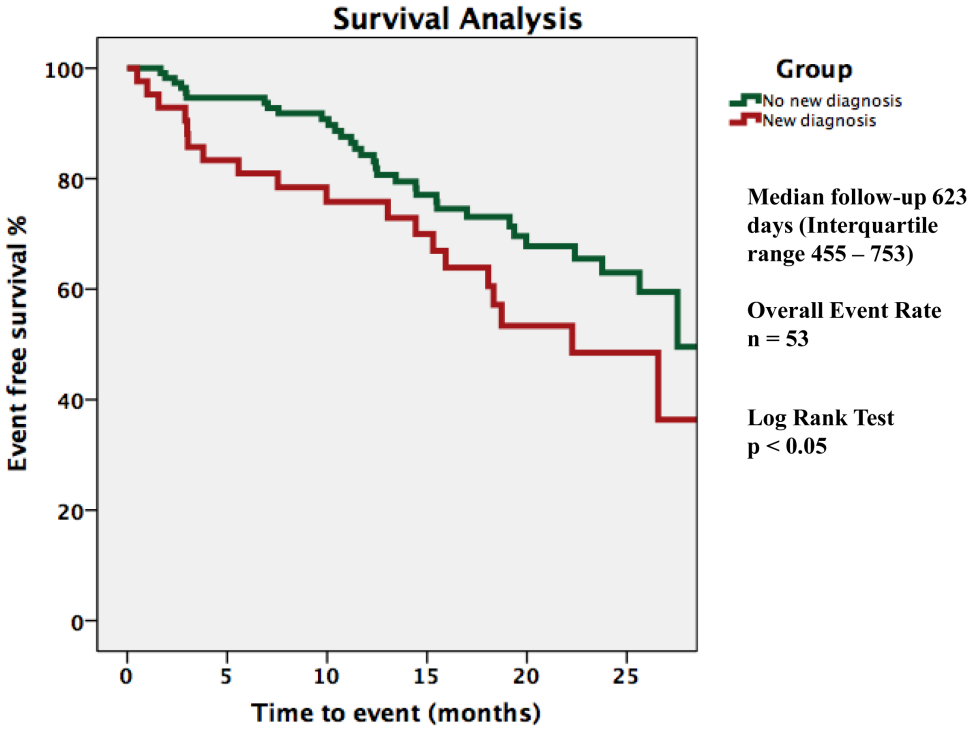


Figure 3.5 Kaplan Meier analysis stratified according to the presence or absence of new CMR diagnoses

Table 3.4 Cox regression in HFpEF inclusive of new CMR diagnoses

	Univariable model, HR (95% CI)	p	Multivariable model, HR (95% CI)	p
Age	1.01 (0.99–1.05)	0.34		
Gender	1.48 (0.84–2.60)	0.17		
Heart rate (b.p.m)	1.00 (0.98–1.02)	0.64		
Systolic Blood Pressure (mmHg)	1.00 (0.98–1.01)	0.38		
Diastolic Blood Pressure (mmHg)	0.97 (0.95–1.00)	0.03	0.99 (0.97–1.02)	0.48
NYHA III/IV	1.80 (1.02–3.17)	0.04	1.55 (0.83–2.89)	0.17
Hypertension	2.40 (0.58–9.87)	0.23		
Diabetes	1.03 (0.59–1.79)	0.91		
Sodium (mmol/L)	0.97 (0.90–1.05)	0.45		
Urea (mmol/L)	1.09 (1.02–1.15)	0.01	1.10 (1.01–1.21)	0.04
eGFR (ml/min)	0.99 (0.97–1.00)	0.07	1.01 (0.99–1.03)	0.37
ZLog BNP (ng/L)	1.47 (1.08–2.01)	0.02	1.44 (1.03–2.02)	0.03
New diagnoses group	1.75 (1.00–3.07)	0.05	1.92 (1.06–3.45)	0.03
Abbreviations as per previous Tables and: HR = hazard ratio; CI = confidence interval				

Discussion

The principal finding in our study is that stress CMR unmasks potentially clinically relevant undiagnosed cardiac pathology in a significant proportion of patients (27%) labelled as HFpEF after echocardiography. A clinically relevant proportion of our patients was identified as having hitherto unknown CAD or microvascular dysfunction. Moreover, despite being part of the TTE-based exclusion criteria at study entry, new cases of HCM and constrictive pericarditis were identified during subsequent CMR evaluation. Our observations suggest that previous intervention trials in HFpEF are likely to have included patients meeting one or more exclusion criteria, thereby possibly influencing treatment response. These additional pathologies, when grouped together in our cohort, were associated with adverse outcomes.

3.1.14 'New CMR diagnoses'

The reasons for the higher pick-up rate of new clinical diagnoses with CMR are multiple. Firstly, the overall image quality for TTE in our study was poor, reflecting the clinical profile of our challenging population, with a high prevalence of obesity, lung disease and AF[278]. These comorbidities are typical of HFpEF as reported in the literature[35]. Furthermore, the low feasibility (inadequate endocardial border definition in nearly one-third) and diagnostic utility of TTE in HF has previously been reported and is subject to wider limits of agreement compared with CMR[132,133]. The ability of CMR to interrogate any imaging plane and perform in vivo tissue characterisation (e.g. by LGE) makes this the reference standard for detection of new diagnoses in our cohort[130,132,145].

Previous reports quote a wide range for the prevalence of CAD in HFpEF, comprising primarily data from epidemiological studies and registries. Furthermore, the presence of CAD was variably based on patient reporting, use of insensitive and non-specific investigations (e.g. ECG, exercise treadmill tests), inconsistent diagnostic cut-offs for angiographic disease severity, and did not incorporate CMR[167]. In this study, CMR increased the overall proportion of significant CAD (silent MI and/or ischaemia) from 21% to 34%, equivalent to a relative increase of 63%. These findings (and microvascular

dysfunction) might be expected, given the proportion of elderly, hypertensive and diabetic patients in our cohort[279]. Furthermore, these greater number of 'new' CAD diagnoses is perhaps unsurprising given that CAD was not part of our exclusion criteria. We used a practical definition of HFpEF and current clinical guidelines[3] for HF do not mandate routine investigation for CAD unless accompanied by anginal symptoms recalcitrant to medical therapy. Additionally, the higher numbers of 'silent' CAD could also be explained by the inability of some patients to provoke clinical symptoms due to limited exercise capacity owing to co-morbidities. Conversely, exertional breathlessness may represent angina equivalent. The typical patterns of infarction (small number of segments and ≤ 50% transmurality) in our study are in keeping with overall preservation of LVEF. In such cases, the diagnostic accuracies of both ECG (Q wave) and TTE (RWMAs) are low in concordance with published literature[170].

Diagnosing HCM represents an imaging challenge in this cohort of patients. The latest HCM diagnostic guidelines[141] advocate a morphological description of imaging in suspected subjects. These guidelines are also more inclusive of considering HCM as a diagnosis in any patients whereby increased LV wall thickness cannot solely be explained by abnormal loading conditions. CMR features supportive of HCM in hypertensive patients include a more asymmetric pattern of LVH and LGE at the insertion points and in segments of maximal LV wall thickening[280,281]. Furthermore, LGE is reportedly present in 65% with HCM, similar to our cohort[141]. HCM is characterised by non-specific diverse patterns of hypertrophy with or without left ventricular outflow tract obstruction or systolic anterior motion of the mitral valve[141,143,282]. In HFpEF, LVH is a common finding[35] and co-existing conditions such as ageing, obesity and hypertension are additional confounders[283]. Furthermore, hypertensive heart disease classically presents with concentric hypertrophy and wall thickness rarely exceeds 15-16 mm[281]. Deciphering the pattern of LVH according to mass and relative wall thickness calculations traditionally used in TTE is fraught with intrinsic methodological limitations[72]. These factors along with sub-optimal image quality[282] and the very high prevalence of hypertension (90%) may explain the underreporting of HCM by TTE in our cohort. In our study, patients who met wall thickness criteria for HCM on TTE were not reported as likely HCM most probably due to a predominant concentric pattern of LVH. Whilst TTE traditionally risks overestimating

wall thickness (e.g. oblique cuts)[141], underestimation has been noted in a small (12%) proportion, especially if confined to the inferolateral, anterolateral or apical segments. In contrast, the superior endocardial definition afforded by CMR allows a more precise measurement of LV wall thickness and hypertrophy[282].

Current TTE diagnostic criteria for constrictive pericarditis have lower sensitivities compared to CMR (pericardial thickening: 36% vs 88%, septal bounce: 62% vs 81%)[140,276]. In our cohort, the majority of these TTE parameters were not detected, which again is a likely reflection of poor image quality.

3.1.15 Implications

Our CMR findings reinforce the marked clinical heterogeneity in HFpEF[35] and provide alternative explanations for symptoms in a significant minority of patients. These findings may also explain in part the poor outcomes seen in HFpEF clinical trials whereby TTE remains the primary entry tool for enrolment. Furthermore, CMR refines the diagnosis and sub-categorises HFpEF into 'purer forms' and alternative pathologies, enabling disease-specific tailored therapies, and provides prognostic data. Survival following silent MI is comparable to known MI[284]. Importantly, diagnosis by CMR enables initiation of effective secondary prevention treatment and guides revascularisation, given that most affected myocardial segments identified in our cohort were viable[142]. Our data suggest that screening for significant CAD should be undertaken in patients with suspected HFpEF. A diagnosis of HCM has implications for both patients and relatives. CMR improves risk stratification and may enable earlier initiation of therapies such as implantable defibrillator devices[141]. Constrictive pericarditis is potentially curable and pericardial enhancement on LGE may predict treatment response[140].

The routine use of stress CMR in HFpEF patients should refine diagnosis and treatment strategies as we move towards an era of precision medicine. However, further randomised trails are needed to assess the wider impact of CMR in terms of clinical outcome, resource utilization and cost-effectiveness.

3.1.16 Limitations

The definition of HFpEF used in our study was not in accordance with latest ESC guidelines[3,20]. However, we took a pragmatic approach to reflect a real world setting. In particular, the presence of diastolic dysfunction was not a pre-requisite for study entry since recent contemporary clinical trials have highlighted normal diastolic function at rest in approximately a third of such patients[114]. Although all patients meeting inclusion criteria were invited, 26 out of 180 (14%) did not undergo CMR, which might raise concerns about its applicability to the wider HFpEF population. Whilst chronic obstructive pulmonary disease (COPD) is quite prevalent in the clinical scenario of HFpEF, we only excluded patients with severe disease (and likewise severe valvular disease) to minimise the contribution from alternate causes of HF symptoms. Besides, our cohort still comprised COPD subjects in nearly one-fifth who underwent CMR. Six patients with pacemakers did not undergo CMR: at the time the study was conducted, our centre was not implanting MR conditional devices.

Discriminating microvascular dysfunction from global coronary ischaemia can be challenging with CMR and raises the possibility of under-reporting of CAD. Furthermore, patients did not have stress echocardiography, which may have identified more patients with ischaemia. In this cohort of patients with multiple risk factors for LVH, ultimately the imaging diagnosis of HCM is one of exclusion. However, the most recent ESC guidelines recommend defining HCM in patients with LVH ≥ 15mm not solely explained by loading conditions[141]. Our CMR reports were generated using a clinical protocol exclusive of T1 and T2 mapping which were not routinely used at the time of study conduct. T1 mapping may have unmasked further hypertrophic phenotypes[141] such as cardiac amyloid and Anderson Fabry's disease, and T2 mapping may have been helpful in cases of constrictive pericarditis[140].

While the CMR reports were generated by *GPM* and *ASHC*, clinical endpoints were collated by *PK* who was not blind to CMR results. However, the HF hospitalisation events were clearly objectively defined (see methods section) and assessment of vital status is robust. Some patients may have had hospitalisations exclusive of our hospital. However,

there should be no systematic bias for those with or without ‘new’ diagnoses.

Conclusions

In HFpEF, CMR identifies previously undetected pathology in a significant proportion of patients. This group of additional diagnoses is associated with worse outcomes and is an independent predictor of death and/or re-hospitalisation due to HF.

4 FOCAL AND DIFFUSE FIBROSIS IN HFpEF

Manuscript accepted for publication:

Kanagala P, Cheng ASH, Singh A, Khan JN, Gulsin GS, Patel P, Gupta P, Arnold JR, Squire IB, Ng LL, McCann GP. *Relation of focal and diffuse fibrosis assessed by cardiovascular magnetic resonance imaging to clinical outcome in heart failure with preserved ejection fraction.* (Accepted by JACC CVI)

Abstract

Aims

Myocardial fibrosis has been implicated in the pathophysiology of HFpEF. We aimed to assess the presence and extent of focal and diffuse fibrosis in HFPEF compared to asymptomatic controls, and their relation to clinical outcome.

Methods and Results

In this prospective, observational study, 140 age- and sex-matched subjects (HFpEF n=96; controls n=44, age 73±8, males 49%) underwent cardiovascular magnetic resonance imaging. LGE and T1 mapping to calculate myocardial extra-cellular volume indexed to BSA (iECV) were used to assess fibrosis.

Patients with HFpEF had more concentric remodeling and worse diastolic function. Focal fibrosis was more frequent in HFpEF (overall n=49; infarction n=17; non-ischaemic n=36; mixed pattern n=4) compared to controls (overall n=3). Diffuse fibrosis was also greater in HFpEF than controls (iECV: 13.7±4.4 ml/m^2 versus 10.9±2.8ml/m^2, $p < 0.0001$).

During median follow-up (517 days), there were 25 composite events (4 deaths, 21 heart failure hospitalisations) in HFpEF. MI on LGE was a predictor of outcomes on univariable analysis only. With multivariable analysis, iECV (HR 2.157; CI 1.326–3.507; $p = 0.002$) was an independent predictor of outcome along with E/E' (HR 1.942; CI 1.258–2.999; $p = 0.003$). iECV also significantly correlated with left ventricular end-diastolic volume indexed ($r = 0.582$, $p < 0.0001$), BNP ($r = 0.371$, $p = 0.007$), maximal left atrial volume indexed ($r = 0.267$, $p = 0.010$) and creatinine ($r = 0.258$, $p = 0.013$).

Conclusions

Both focal and diffuse myocardial fibrosis are more prevalent in HFpEF compared to age- and sex-matched controls. iECV significantly correlates with indices of LV remodeling, diastolic function and renal function and is an independent predictor of adverse outcome in HFpEF.

Background

HFpEF accounts for up to half of all heart failure patients in the community and outcomes remain poor[6]. Current prognostic markers in HFpEF largely relate to clinical and echocardiographic parameters[10,12]. However, CMR is the recognised gold standard for the majority of imaging parameters that comprise latest guidance on HFpEF[3]. Both focal fibrosis (MI and 'non-ischaemic' fibrosis) and interstitial myocardial fibrosis have been implicated in the pathophysiology of HFpEF by promoting adverse ventricular remodeling, increasing myocardial stiffness and in turn causing diastolic dysfunction[131]. Focal fibrosis[135] including MI[151] can be detected by LGE and pre- and post- contrast T1 mapping allows calculation of myocardial extra-cellular volume (ECV), a surrogate marker of interstitial fibrosis[153,163]. LGE is associated with reduced survival across a range of clinical conditions[135], including HFrEF, hypertrophic cardiomyopathy and, in a single study with small sample size, HFpEF[157].

In HFpEF however, the pattern of interstitial fibrosis tends to be more diffuse, which cannot be detected using the LGE technique[135,163]. CMR T1 parametric mapping techniques enable quantification of the extra-cellular matrix[163], a surrogate marker of diffuse fibrosis, and have been validated histologically[153]. To date, only 2 small prospective HFpEF outcome studies, both lacking phenotyped-reference healthy control groups have evaluated diffuse fibrosis utilising either post-contrast T1 times[161] or ECV[285]. Recently, in a further refinement, iECV (ECV indexed to BSA) was related to outcomes in patients with aortic stenosis[286] but this has not been studied in HFpEF and related to clinical outcomes.

We aimed to: 1) evaluate whether there were differences in the presence and extent of both focal and diffuse fibrosis between HFpEF and matched controls without heart failure and 2) whether fibrosis provided additional prognostic value beyond traditional clinical and echocardiographic indices.

Methods

4.1.1 Patient population

HFpEF patient recruitment, study inclusion and exclusion criteria and ethics were previously outlined in the general methods Chapter. For comparison, asymptomatic age- and sex-matched controls without known cardiac disease were also recruited. We did not exclude hypertensive controls (n = 19) since hypertension is widely implicated in the pathophysiology of HFpEF and we wanted to account for this potential confounder. All subjects underwent history and review of medical notes, blood sampling, TTE and CMR during the same visit as described earlier (see Chapters 2 and 3).

4.1.2 Functional measures

Exercise capacity was assessed using NYHA class and standardized six minute walk test (6MWT)[287] and quality of life metrics were derived from the Minnesota Living with Heart Failure (MLHF) Questionnaire[249].

4.1.3 Transthoracic echocardiography

TTE was performed as per American Society of Echocardiography guidelines [230]. All TTE scans were analysed off-line by *AMM* and *JM*, using QLAB Xcelera CMQ (cardiac myocardial quantification) software. Echocardiographic E/E' was derived as described in the general methods Chapter (also see Figure 1.7).

4.1.4 CMR protocol

The CMR protocol has previously been described in Chapters 2 and 3. *MOLLI* images were acquired pre- and post-contrast in basal, mid-ventricular and apical short axis slices. The *MOLLI* sequence[263] was performed with the following parameters: breath-held or free breathing, single-shot sequence, 3(3)3(3)5 sampling pattern, 8 mm slice thickness, 300 x 400 mm field of view, 50° flip angle, 120 ms minimum TI, 80 ms increments of inversion time. In order to minimise artefacts, prior to *MOLLI* image acquisition, We employed similar techniques to minimise artefacts as previously reported by our group (also see Chapter 2, section 2.1.9.6) whereby T1 times were calculated from motion corrected

parametric maps (MOCO) with excellent reproducibility[164]. The MOCO images were generated from the *MOLLI* sequence using a built-in post-processing image registration technique from Siemens software (Syngo MR D13) which accounts for image mis-registration caused by mis-triggering, patients' breathing or movements during the scan[166].

LGE was performed at least 10 minutes after injection of 0.15 mmol/kg contrast (Gadovist, Bayer Healthcare, Berlin, Germany) in the same slice positions as the cine images. A 2D phase-sensitive inversion recovery (PSIR) gradient echo sequence was used and the optimal TI determined following a standard Look-Locker sequence. Single-shot multi-slice acquisitions were obtained in patients with poor breath-hold technique or arrhythmia.

4.1.5 CMR image analysis

Images were analysed by a single observer (*PK*) using *CVI42* software (Circle Cardiovascular Imaging, Calgary, Canada) and blinded to all clinical data. Ventricular volumes, EF and LV mass (excluding papillary muscles) were calculated from the short-axis cine stack[174]. Left atrial volumes were calculated from the biplane method excluding the appendage and pulmonary veins[288]. All volumetric and mass data were indexed to BSA. Indexed LV end-diastolic mass was divided by 1.05, the specific density of myocardial tissue to derive myocardial volume[286].

4.1.6 LGE analysis of focal myocardial fibrosis

As described previously[164], qualitative assessment of LGE images was first undertaken by two experienced observers (*PK*, *ASHC*) to achieve consensus for identifying the presence and pattern of LGE i.e. ischaemic versus non-ischaemic. If there was disagreement, a third observer (*GPM*) adjudicated. Fibrosis was considered present if LGE was visualised on both short- and orthogonal long-axis LGE images. Insertion point fibrosis was included in our analysis. The full width half maximum technique (FWHM) was then used to quantify fibrosis[149].

Various methods to calculate the extent of focal fibrosis have been studied previously including manual contouring around regions of fibrosis and standard deviations of signal

intensities in the order of 2 SD, 3 SD, 4 SD, 5 SD and 6 SD above 'normal' myocardium. However, the FWHM technique was chosen in our study to semi-quantitatively delineate the extent of focal fibrosis since it has been shown to be the most reproducible and accurate in comparison with the other techniques and across both ischaemic and non-ischaemic fibrosis[149]. Following consensus on the qualitative presence of fibrosis, endocardial and epicardial contours were manually drawn in each short-axis LGE image. Regions of interest (ROI) were then drawn in 'normal' myocardium and in the core of the most 'hyperintense' areas of fibrosis. The software within CVI42 automatically highlighted and calculated the extent of focal fibrosis throughout the myocardium using half the maximal signal in the fibrosis 'core' as the new threshold (see Figure 4.1). Focal fibrotic size expressed as grams or a percentage of total LV myocardial mass was derived.

Examples of focal fibrosis and measurement are shown in Figure 4.2. In some cases, both patterns of fibrosis were evident in the same subject and were therefore re-analysed separately i.e. MI was quantified first and non-ischaemic fibrosis was analysed subsequently after drawing exclusion zone contours around MI areas.

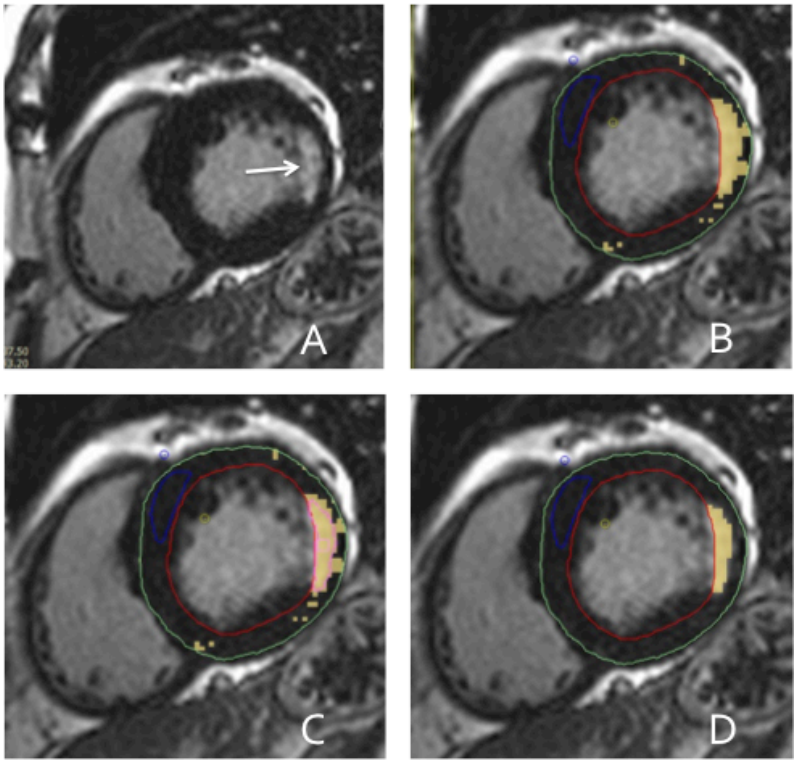


Figure 4.1 Quantification of LGE focal fibrosis using the full width half maximum technique

A. sub-endocardial hyperenhancement consistent with a MI (white arrow) B. endocardial (red) and Epicardial (green) manual contours showing corresponding highlighted (yellow) areas of hyperenhancement C. regions of interest in 'normal' myocardium (blue) and within the core of hyperenhancement (pink) D. New highlighted area of hyperenhancement based upon the full width half maximum threshold

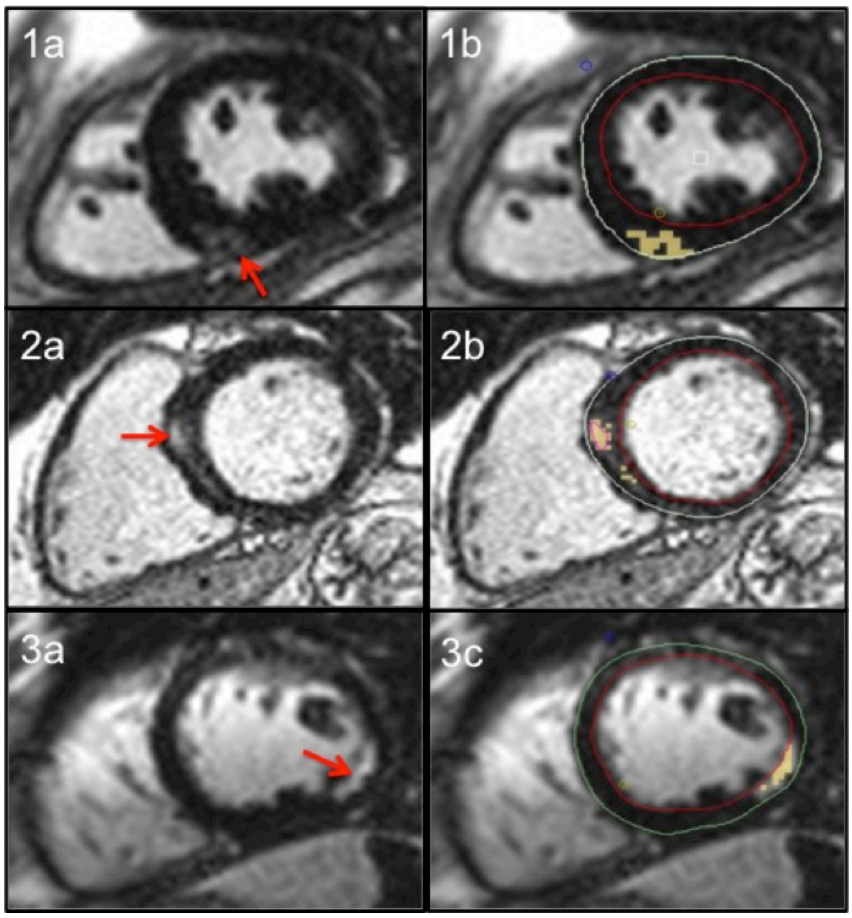


Figure 4.2 Examples of differing patterns of focal fibrosis

Late gadolinium enhancement images demonstrating focal fibrosis (red arrows) and corresponding, quantified burden (highlighted in yellow) using the full width half maximum technique: 1 – insertion point fibrosis; 2 – mid-wall fibrosis; 3 – sub-endocardial myocardial infarction

4.1.7 Analysis of diffuse myocardial fibrosis

Quantitative analyses were performed on T1 MOCO parametric maps (pre- and post-contrast), whereby T1 values were encoded within pixel intensities of the *MOLLI* images. As described by our group previously, ROIs were manually drawn in the myocardium and blood pool in the centre of the LV cavity to generate native and post-contrast T1 values[164]. For the myocardial ROI, endocardial and epicardial contours were drawn in the mid-myocardium to ensure papillary muscles, epicardial fat and blood pool were avoided since they affect T1 values and subsequent ECV derivation[163]. An example of such manual contouring is illustrated below in Figure 4.3.

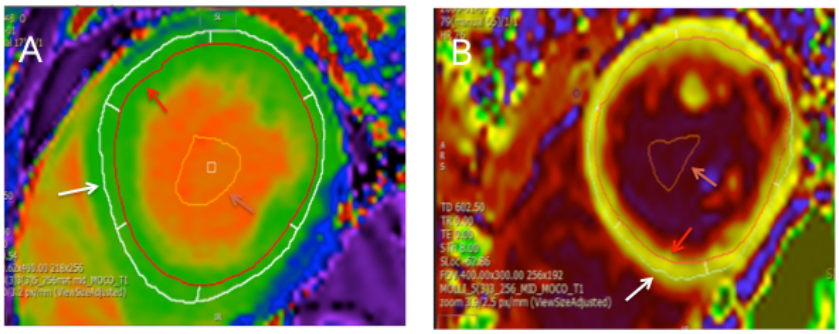


Figure 4.3 LV myocardial contours to enable calculation of T1 values and ECV

Mid-myocardial (white and red arrows] and left ventricular cavity (orange) contours to enable derivation of T1 values and ECV, performed on pre- (A) and post-contrast (B) *MOLLI* images

Only the mid-ventricular slice *MOLLI* images were chosen for analysis to further reduce potential errors from partial volume effects in the apex (thinner myocardium) and also the basal slices (left ventricular outflow tract)[163,289,290]. The software provided T1 results for 6 mid-ventricular segments (7-12) corresponding to the American Heart Association nomenclature after using the anterior RV insertion point as a reference marker. After inputting blood haematocrit values, the software generated segmental ECV values [164]. Segments with MI or artefact (see Figure 4.4) were excluded from final T1 and ECV calculation, and segmental values were then averaged. Regions of focal non-ischaemic fibrosis were included in our ECV calculations, consistent with other reported studies[163,289].

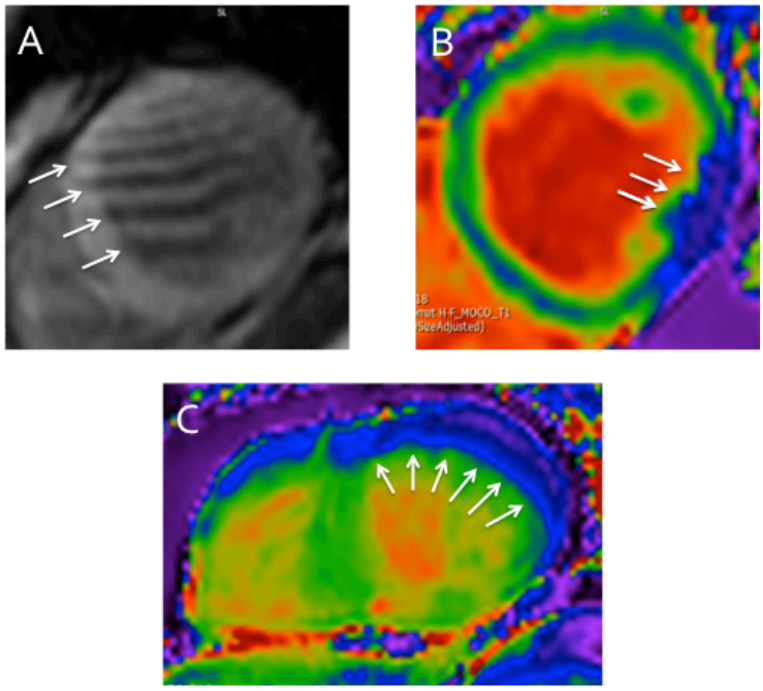


Figure 4.4 Examples of artefacts encountered during ECV analysis

White arrows above point towards artefacts

The inbuilt formulas used to compute ECV[291] were as follows:

a) R1 = 1/T1

b) Partition coefficient (λ) = (R1 myocardium post-contrast – R1 myocardium pre-contrast) ÷ (R1 blood post-contrast – R1 blood pre-contrast)

c) ECV = λ (1 – haematocrit)

iECV was derived using the formula: ECV (%) x left ventricular end-diastolic myocardial volume indexed to BSA[286].

4.1.8 Outcome data

The clinical endpoint was a composite of mortality or repeat hospitalisation for HF. Hospital databases and patient records were sourced to obtain outcome data. Patient follow-up was for a minimum of 6 months post-study entry.

4.1.9 Statistical analysis

Statistical tests were performed using SPSS V22. Continuous data were assessed for normality using histograms, Q-Q plots and the Shapiro-Wilk test. Summary data are presented as mean (± SD) or median (25 – 75% IQR). Between group differences were compared using the t-test, Mann-Whitney U test and the Chi-square test as appropriate. BNP, creatinine, 6MWT distance and the MLHF score were $\log_{10}$ transformed before analysis.

Kaplan-Meier analysis was undertaken to calculate event rates. Differences in survival curves were tested using the Log-Rank test. Univariable Cox regression modeling was initially performed to identify variables associated with outcome. Variables tested were those shown to have prognostic importance from the literature with the intention of preventing model over-fitting[10,12]. Those covariates associated with the endpoint at $p < 0.1$ were then entered into subsequent multivariable analysis to identify independent predictors using both backwards and forwards stepwise elimination methods. Continuous variables were Z-standardized to enable comparison of hazard ratios based upon one SD increase in the predictor variable. The combined accuracy of the independent variables to predict events was then tested by ROC analysis.

Pearson's and Spearman's correlations were performed to check for potential associations of iECV with other variables. Further linear regression modeling was undertaken to identify the strongest independent associations. In cases of collinearity, the variable with the highest coefficient was entered into multivariable analysis. A p value of less than 0.05 was considered significant. Assessments of intra-observer and inter-observer variability for focal fibrosis and ECV calculation were undertaken on 10 randomly selected patients, a minimum of 4 weeks apart (by *PK* and *JRA*).

Results

Two hundred and thirty-two subjects were enrolled (HFpEF n = 182, controls n = 50) of whom 96 patients with HFpEF and 44 controls had complete datasets including T1 maps. Reasons for exclusion are shown in Figure 4.5.

Baseline clinical and imaging characteristics are summarized in Table 4.1 and Table 4.2. In the HFpEF group, there was a high burden of obesity, hypertension, diabetes and AF. Nearly one-fifth had a history of angina or lung disease. Over two-thirds (71%) had prior evidence of pulmonary congestion on chest radiography and a significant minority (29%) were NYHA class III/IV at the time of assessment. Compared to controls, HFpEF patients had worse exercise capacity, renal function, increased LV mass (LVMI) but not LV volumes, and more concentric remodeling (mass/volume). HFpEF patients also had higher filling pressures (E/E') and maximal left atrial volume indexed [LAVImax]) consistent with worse diastolic function.

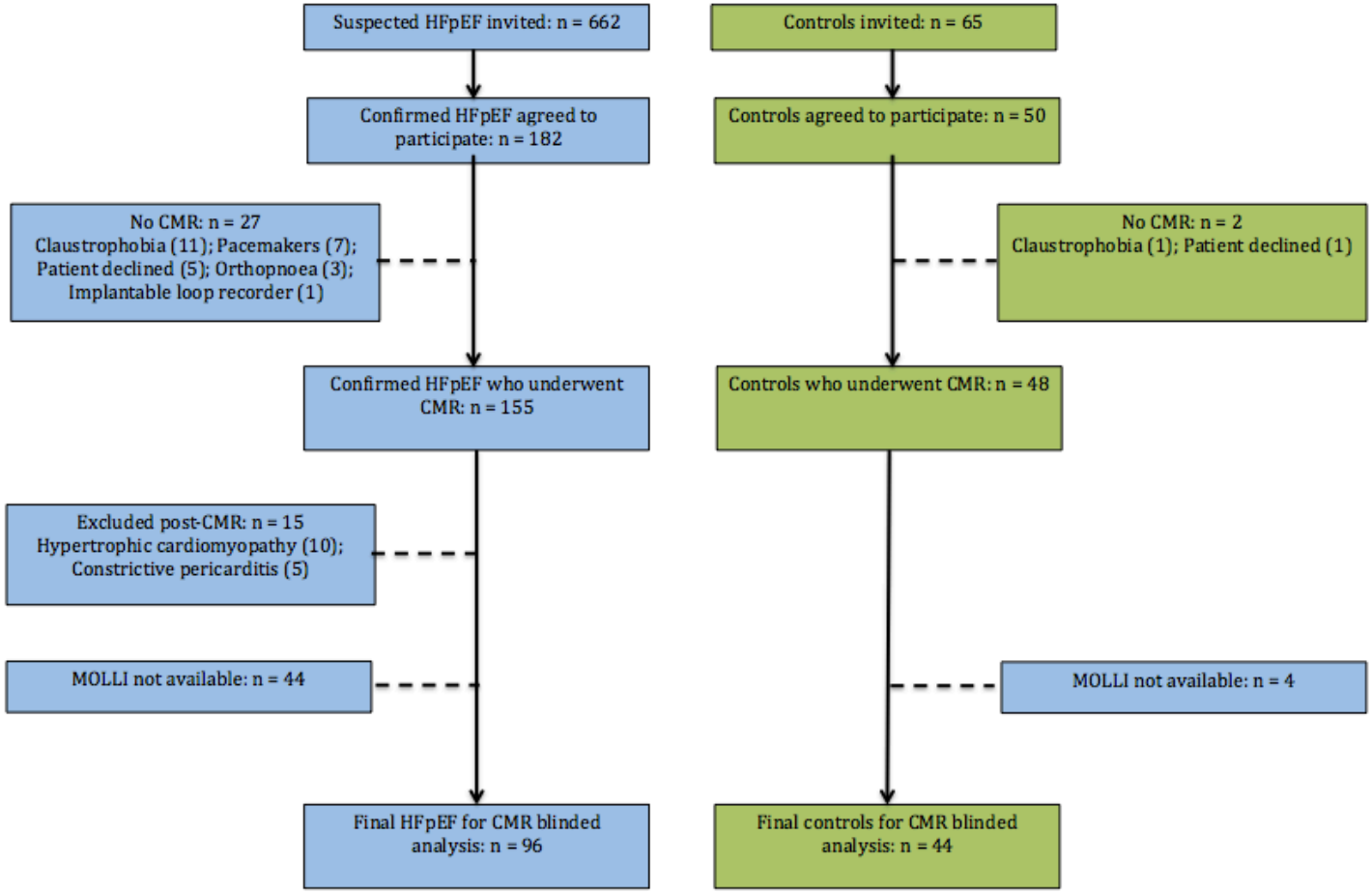


Figure 4.5 Study recruitment overview

Table 4.1 Baseline clinical characteristics

	HFpEF n = 96	Controls n = 44	p value
Demographics			
Age, years	73 ± 9	73 ± 5	0.784
Male (%)	46 (48)	21 (48)	0.983
BMI(kg/m^2)	34 ± 7	25 ± 3	< 0.0001
Clinical Findings			
Heart rate (b.p.m)	69 ± 14	68 ± 11	0.614
Systolic BP (mmHg)	146±25	151 ± 24	0.282
Diastolic BP (mmHg)	75 ± 12	80 ± 11	0.025
Atrial fibrillation (%)	32 (33)	0 (0)	< 0.0001
Medical History			
Diabetes (%)	48 (50)	0 (0)	< 0.0001
Hypertension (%)	86 (90)	19 (43)	< 0.0001
Angina (%)	19 (20)	0 (0)	0.002
Known MI (%)	13 (14)	0 (0)	0.010
Asthma or COPD (%)	18 (19)	2 (5)	0.026
Smoking (%)	52 (54)	16 (36)	0.050
Hypercholesterolaemia (%)	45 (47)	16 (36)	0.244
PVD (%)	2 (2)	0 (0)	0.335
TIA or CVA (%)	9 (9)	1 (2)	0.006
Medication			
Betablocker (%)	68 (71)	1 (2)	< 0.0001
ACEi or ARB (%)	82 (85)	9 (20)	< 0.0001
MRA (%)	31 (32)	0 (0)	< 0.0001
Loop Diuretic (%)	76 (79)	0 (0)	< 0.0001
Functional Status			
NYHA I/II (%)	68 (71)	NA	NA
NYHA III/IV (%)	28 (29)	NA	NA
6MWT distance (m)	190 (120 – 270)	380 (350 –	< 0.0001
MLHF score	46 (22 – 61)	NA	NA
Bloods			
Sodium (mmol/L)	139.5 ± 3.4	140.2 ± 1.8	0.084
Urea (mmol/L)	8.5 ± 3.6	6.1 ± 1.5	< 0.0001
Creatinine (mmol/L)	87 (71 – 113)	69 (56 - 85)	< 0.0001
Haemoglobin (g/L)	129 ± 19	140 ± 14	< 0.0001
Haematocrit (%)	38 ± 5	41 ± 4	0.013
BNP (ng/L)	144 (66 – 250)	33 (24 – 44)	< 0.0001

Table 4.2 Baseline imaging characteristics

	HFpEF n = 96	Controls n = 44	p value
Previous chest radiography			
Pulmonary congestion (%)	68 (71)	NA	NA
Raised cardiothoracic ratio	65 (68)	NA	NA
Pleural effusion (%)	33 (34)	NA	NA
TTE			
E/E'	12.8 ±4.8	9.0 ± 2.9	< 0.0001
CMR			
Volumes, function and LV mass			
LVEF (%)	56 ± 6	58 ± 5	0.406
LVEDVI (ml/m^2)	78 ± 18	81 ± 14	0.409
LVESVI (ml/m^2)	34 ± 11	34 ± 8	0.708
LVMI (g/m^2)	51 ±13	46 ± 9	0.004
LV mass /LVEDV	0.68 ± 0.15	0.57 ± 0.09	< 0.0001
RVEF (%)	54 ± 10	56 ± 6	0.090
RVEDVI (ml/m^2)	79 ± 20	83 ± 15	0.307
RVESVI (ml/m^2)	37 ± 14	37 ± 9	0.922
LAVImax (ml/m^2)	54 ± 27	35 ± 12	< 0.0001
LGE - Focal fibrosis			
Total focal fibrosis (%)	49 (51)	3 (7)	< 0.0001
Total focal fibrosis (g)	3.6 (2.0–6.4)	2.5 (0.5–2.6)	< 0.0001
Total focal fibrosis (% of LV	3.0 (2.0–6.3)	2.0 (0.8–3.0)	< 0.0001
Ischaemic pattern (%)	17 (18)	0 (0)	< 0.0001
Ischaemic pattern (% of LV	3.0 (2.2–4.6)	NA	NA
Non-ischaemic pattern (%)	36 (38)	3 (7)	< 0.0001
Non-ischaemic pattern (% of	2.9 (1.4–6.5)	2.0 (0.8–3.0)	< 0.0001
T1 mapping - Diffuse Fibrosis			
Native Myocardial T1 (ms)	1234 ± 73	1197 ± 91	0.021
Post-Contrast Myocardial T1	461 ± 63	495 ± 85	0.011
ECV (%)	27.8 ± 4.6	25.3 ± 3.2	< 0.0001
iECV (ml/m^2)	13.7 ± 4.4	10.9 ±2.8	< 0.0001

4.1.10 Focal fibrosis

Results are shown in Table 4.2 above. Approximately half (n = 49, 51%) of the HFpEF cohort had evidence of focal fibrosis (versus n = 3, 7% in controls; $p < 0.0001$). The predominant pattern of fibrosis in HFpEF was non-ischaemic in 36 (38%). MI was present in 17 (18%) of patients, including 7 patients with previously unknown MI, but in none of the controls. Both MI and non-ischaemic fibrosis was present in 4 patients. In those with HFpEF exhibiting LGE hyperenhancement, the quantified fibrotic burden was relatively small: 3% of LV mass (ischaemic 3% and non-ischaemic 2.9%).

4.1.11 Diffuse fibrosis

Native T1, post contrast T1, ECV and iECV (13.7 vs 10.9 ml/m^2; $p < 0.0001$) were all significantly different between HFpEF and controls.

4.1.12 Intra-observer and inter-observer assessments

Data for quantification of T1 mapping and LGE are shown in Table 4.3. Intra-observer and inter-observer variability were excellent for all measures (intraclass correlation coefficients > 0.95).

Table 4.3 Intra-observer and inter-observer variability for assessment of focal and diffuse fibrosis

Parameter	Mean ± SD value	Coefficient of variation (%)	Mean ± SD difference	Intraclass correlation coefficient	95% Bland Altman Limits of agreement
Intra-observer variability					
Total focal fibrosis (g)	3.1±2.1	6.6	0±0.2	0.997	-0.4 to 0.4
ECV (%)	27.7±4.3	4.6	-0.5±1.3	0.960	-2.9 to 2.1
Myocardial Native T1 (ms)	1233±114	1.1	7±13.7	0.992	-19.8 to 33.8
Post-contrast T1 (ms)	467±50	0.6	-1.9±2.8	0.998	-7.4 to 3.5
Inter-observer variability					
Total focal fibrosis (g)	3.2 ±2.1	8.6	- 0.1±0.3	0.991	- 0.4 to 0.7
ECV (%)	27.8±4.4	4.6	-0.3±1.3	0.960	-2.8 to 2.2
Myocardial Native T1 (ms)	1238±110	2.5	16.4±31	0.954	-44.4 to 77.1
Post-contrast T1 (ms)	468±51	0.8	-0.5±3.6	0.998	-7.5 to 6.5

4.1.13 Outcomes

During median follow-up of 517 days (range 356 – 756), there were 25 events (4 deaths, 21 HF hospitalisations) in patients with HFpEF. There were no events in the control group.

4.1.14 Predictors of clinical outcome

On univariable analysis (Table 4.4), 7 variables were associated with adverse outcomes: lung disease, haemoglobin, Log BNP, E/E', right ventricular end-systolic volume indexed (RVESVI), MI and iECV. Quantified focal fibrosis (total) was not associated with outcome (AUC = 0.520, p = 0.704). On multivariable analysis, the only independent predictors of outcome were iECV (hazard ratio [HR] 2.157; 95% confidence interval [CI] 1.326 – 3.507; p = 0.002) and E/E' (HR 1.942; CI 1.258 – 2.999; p = 0.003). The multivariable model predicted outcomes with an area under the ROC curve of 0.764 (sensitivity 63%, specificity 90%, p = 0.001). Kaplan Meier survival curves stratified according to quartiles of iECV are shown in Figure 4.6. The highest quartile of iECV (> 16.8 ml/m^2) was associated with greatest risk of adverse outcome (Log-Rank p = 0.002).

Table 4.4 Cox regression in subjects who underwent CMR extra-cellular volume assessment

	Univariable analysis		Multivariable analysis	
	Hazard Ratio (CI 95%)	**p value**	**Hazard Ratio (CI 95%)**	**p value**
Clinical				
Asthma or COPD	2.569 (1.095 – 6.026)	0.030		NS
Bloods				
Haemoglobin (g/L)	0.640 (0.409 – 1.002)	0.051		NS
Log BNP (ng/L)	1.933 (1.086 – 3.442)	0.025		NS
Imaging				
E/E’	1.866 (1.243 – 2.802)	0.003	1.942 (1.258 – 2.999)	0.003
RVESVI (ml/m2)	1.436 (0.941 – 2.191)	0.094		NS
Presence of MI	2.445 (1.001 – 5.974)	0.050		NS
iECV (ml/m2)	1.677 (1.137 – 2.473)	0.009	2.157 (1.326 – 3.507)	0.002
For all continuous data, Hazard Ratios refer to one standard deviation increase in Z-standardized values; CI = confidence interval; NS = not significant				

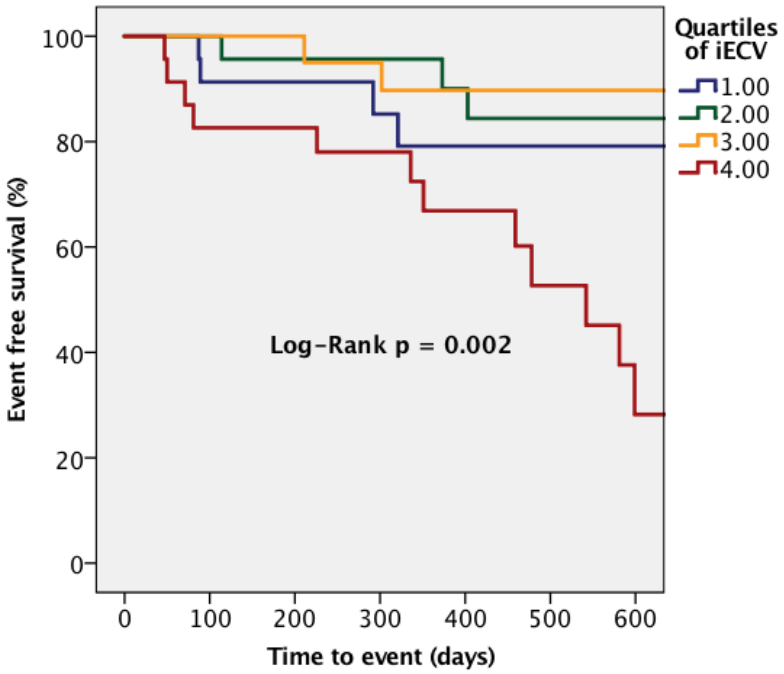


Figure 4.6 Kaplan-Meier analysis stratified according to quartiles of indexed extra-cellular volume (iECV)

4.1.15 Associations of iECV

iECV in HFpEF was not associated with measures of exercise capacity or quality of life: NYHA $r_s = 0.032$, $p = 0.765$; 6MWT distance $r_s = -0.013$, $p = 0.908$; MLHF score r_s -0.172, $p = 0.111$. Univariable associations of iECV in HFpEF are shown in Table 4.5. Due to collinearity, LV and RV end-systolic volume and LVEF were excluded from the multivariable analysis. Heart rate, systolic blood pressure, serum creatinine, BNP, left ventricular end-diastolic volume indexed (LVEDVI) and LAVImax remained significant on multivariable analysis (also see Figure 4.7). Of those independently associated with iECV, the strongest correlations were with: LVEDVI (Pearson's $r = 0.582$, $p < 0.0001$), BNP ($r = 0.371$, $p = 0.007$), LAVImax ($r = 0.267$, $p = 0.010$) and serum creatinine ($r = 0.258$, $p = 0.013$).

Table 4.5 Univariable and multivariable linear regression models for the associations with iECV

	Univariable analysis		Multivariable analysis	
	Standardized coefficients (Beta)	**P value**	**Standardized coefficients (Beta)**	**P value**
Clinical				
Heart rate (b.p.m)	-0.144	0.095	0.136	0.048
Systolic Blood Pressure (mmHg)	0.241	0.005	0.156	0.016
Bloods				
Creatinine (mmol/L)	0.321	< 0.0001	0.233	0.001
Haemoglobin (g/L)	-0.264	0.002		NS
BNP (ng/L)	0.508	< 0.0001	0.258	0.001
CMR				
*LVEDVI (ml/m2)	0.525	< 0.0001	0.474	< 0.0001
*LVESVI (ml/m2)	0.510	< 0.0001		
*LVEF (%)	-0.175	0.095		
*RVEDVI (ml/m2)	0.405	< 0.0001		NS
*RVESVI (ml/m2)	0.300	0.004		
LAVImax (ml/m2)	0.372	< 0.0001	0.150	0.035
* Variables which exhibited significant collinearity; of these LVEDVI (ml/m2) and RVEDVI (ml/m2) were entered into multivariable analysis				

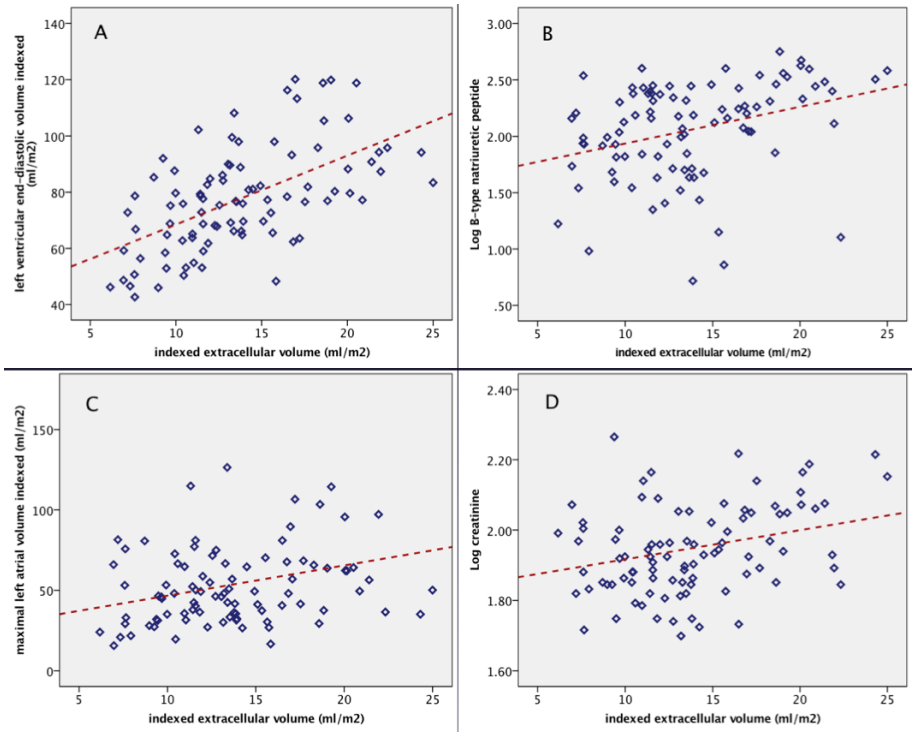


Figure 4.7 Scatter plots of important associations with indexed extra-cellular volume

Discussion

This is the first outcome study to systematically evaluate fibrotic burden in a well-phenotyped cohort of HFpEF and age- and sex-matched control populations using CMR. Furthermore, we evaluated iECV as a newer marker of diffuse fibrosis for the first time in HFpEF and related this to clinical outcome. The principal findings in our study are that: (1) both focal and diffuse fibrosis are elevated in HFpEF compared to asymptomatic controls; (2) diffuse fibrosis as assessed by iECV independently predicted prognosis in HFpEF; and (3) iECV was associated with LVEDVI, markers of LV diastolic dysfunction (BNP, LAVImax) and renal function.

4.1.16 Focal fibrosis

Overall, there was more focal fibrosis (ischaemic and non-ischaemic) in HFpEF than controls. We also detected new cases of previously unknown MI, which were generally

small, in keeping with overall preservation of LVEF. Unlike the only study previously to demonstrate independent prediction of outcomes with LGE-quantified focal fibrosis in HFpEF[157], our patients had less burden of non-ischaemic fibrosis and LGE was not related to outcomes. The reason for this difference might be due to the quantification method used for focal fibrosis. Although various semi-automated quantification methods exist, we used the FWHM technique, which is the most reproducible across the spectrum of both ischaemic and non-ischaemic etiologies[149]. The previous study used a threshold of > 2 SDs of signal intensity above remote myocardium to define fibrosis, which can result in over-estimation and measurement errors from partial volume effects[149] and in addition, as the ECV in these patients is diffusely increased, defining normal myocardium is problematic.

4.1.17 Diffuse fibrosis

Recently, iECV has been proposed as novel marker of diffuse fibrosis [286]. In a cohort of aortic stenosis, iECV correlated well with histological fibrosis, discriminated between disease and healthy controls and was the only T1 mapping parameter to differentiate between differing grades of valve stenosis. Furthermore, it demonstrated association with clinical outcomes[286].

ECV was quantifiable in 97% in our subjects and with a high degree of reproducibility, which is of clinical relevance. Recently, ECV was shown to predict outcome in a large retrospective study (n = 1172), encompassing all-comers referred for CMR[289]. In that study, ECV analysis was similar to our method and predicted outcomes independently across the whole cohort, irrespective of EF. Similar to our results, diffuse fibrosis was more strongly associated with poor outcomes than non-ischaemic focal fibrosis. However, as well as the selection bias in only recruiting patients referred for clinical CMR, the proportion of subjects with clinical HF was small and it is unclear how many patients had HFpEF.

In our study, although native T1 was also significantly increased, iECV was the only marker of diffuse fibrosis to provide prognostic value in multivariable analysis. Native T1 values reflect both intra- and extra-cellular changes whilst post-contrast T1 is subject to a variety of confounders[153]. On the other hand, ECV is effectively a ratio, taking into account both pre- and post-contrast values and cancelling out systematic biases in T1

measurements. ECV and iECV are therefore more likely to provide a better reflection of diffuse fibrosis[163]. This is further supported by evidence showing better correlation of ECV with histologically-measured fibrosis than for native or post-contrast T1 values[153,163].

To date, only 2 prior prospective studies have demonstrated the association of diffuse fibrosis with clinical outcomes in HFpEF. The first study[161] utilised post-contrast T1 times as a measure of diffuse fibrosis in a much smaller cohort of HFpEF (n = 61) and had intrinsic limitations as outlined above. In the second study of 117 HFpEF subjects, ECV was also associated with adverse events [285]. In contrast to our study, focal fibrosis was defined as myocardial signal intensity of > 5 SDs above the mean intensity of healthy myocardium and such regions were excluded from ECV calculations. Furthermore, ECV was associated with outcomes when confined to CMR parameters only but not in a combined multivariable model including clinical variables, unlike our study utilising iECV.

4.1.18 Importance of fibrotic assessment in HFpEF

Our findings of increased fibrosis shed further insight into the pathophysiology of HFpEF. The link between myocardial fibrosis, ventricular stiffness and structural chamber modification is well known and likely explains the greater adverse remodeling and diastolic dysfunction seen in our predominantly hypertensive HFpEF cohort[131,292]. Previous studies have highlighted the association of ECV and strain measures of both systolic and diastolic dysfunction in hypertensive LVH subjects at risk of developing HFpEF[293]. Furthermore, in a recent small study, ECV was the imaging parameter that best discriminated between HFpEF (n = 62) and hypertensive (n = 22) heart disease subjects[294]. ECV also independently predicts invasive catheter derived measures of LV stiffness in HFpEF[292] and significantly correlates with peak LV filling rate assessed by cine CMR[162].

The independent associations between iECV and variables in our study are perhaps unsurprising. The relationship between LV remodeling (LVEDVI, LVESVI and LVEF as surrogates) and fibrosis is well established. Prior studies of heart failure with reduced ejection fraction have shown that both focal (LGE) and diffuse fibrosis (post contrast T1 times) independently predict LV remodeling[295,296]. Furthermore, the associations of iECV in our study are similar to results from the PARAMOUNT study of HFpEF[297]. In this trial

ST2, galectin-3, matrix metalloproteinase-2 and collagen III N-terminal propeptide as surrogate plasma markers of fibrosis (and the extra-cellular matrix) also correlated strongly with natriuretic peptides (NT-proBNP), left atrial volume, E/E' and eGFR. Interestingly, iECV did not correlate with E/E' in our study: which may in part be explained by the differing fluid status of subjects, the majority of whom invariably had a period of offloading with diuretics prior to CMR as part of routine clinical care[53,292]. Renal dysfunction likely accelerates fibrosis and this association with diffuse fibrosis (native T1 times and ECV) was previously highlighted in a cross sectional study of chronic kidney disease (stages 2-4) subjects devoid of heart failure[298]. Furthermore, in HFpEF, renal disease is highly prevalent[12].

iECV appears to detect diseased myocardium not readily apparent with LGE, which was not associated with outcome. Unlike irreversible replacement fibrosis identified by LGE, diffuse fibrosis may be reversible and therefore a potential therapeutic target[289]. Our work lends further support to a growing body of evidence highlighting ECV (and iECV) as promising biomarkers across a spectrum of cardiac pathologies. Furthermore, their association with outcomes appears stronger compared to traditional LGE assessment which has historically been more extensively studied[163,289].

4.1.19 Limitations

As discussed in Chapter 3, we took a pragmatic appraoach to define HFpEF to reflect a real world setting. The presence of diastolic dysfunction based upon echocardiography was not required for study entry since contemporary HFpEF clinical trials have also reported its absence in nearly one-thirds[114]. Conversely, diastolic dysfunction has also been noted in a significant proportion of asymptomatic elderly subjects devoid of cardiac disease[199]. As the iECV data have been acquired only once, we cannot infer causality between causes of increased iECV.

A proportion of consecutive trial subjects (nearly 24%) who underwent CMR did not undergo *MOLLI* imaging due the sequence not being available. However, a comparison of the HFpEF group who underwent *MOLLI* imaging versus those who did not (see Appendix Table 9.1) revealed no major differences in baseline clinical characteristics, providing

strong supportive data that our results are likely representative across the whole cohort. Furthermore, although the overall numbers undergoing *MOLLI* imaging was reduced, a sample size of only 54 has been proposed for a two-group design to detect differences in ECV when tested across HF subjects previously[270].

Conclusion

Focal and diffuse fibrosis are more prevalent in HFpEF compared to age- and sex- matched healthy controls. Diffuse fibrosis as assessed by iECV in HFpEF, correlates with left ventricular volume, markers of diastolic dysfunction (BNP and left atrial volume) and serum creatinine. iECV is an independent predictor of adverse outcomes in HFpEF.

5 LEFT ATRIAL DYSFUNCTION IN HFpEF

Manuscript under review:

Kanagala P, Cheng ASH, Singh A, Khan JN, Patel P, Gupta P, Arnold JR, Squire IB, Ng LL, McCann GP. *Left atrial ejection fraction: a novel diagnostic and prognostic biomarker in heart failure with preserved ejection fraction.* (Submitted to JCMR)

Early Career Award (clinical) – Finalist.

Kanagala P, Cheng ASH, Singh A, Khan JN, Patel P, Gupta P, Arnold JR, Squire IB, Ng LL, McCann GP. *Left atrial ejection fraction: a novel imaging biomarker for diagnosis and prognosis in heart failure with preserved ejection fraction.* The joint EuroCMR/ Society for Cardiovascular Magnetic Resonance (SCMR) Meeting – Barcelona, Spain 2018

Abstract

Aims

Left atrial contractile function, as assessed by echocardiography is impaired in HF. We aimed to investigate the diagnostic and prognostic utility of left atrial ejection fraction (LAEF) quantified with CMR in HFpEF.

Methods and Results

As part of our single-centre, prospective, observational study, 188 subjects (HFpEF n = 140, controls n = 48) underwent CMR. LAEF was calculated using the biplane method. The diagnostic potential of LAEF was tested using ROC analysis and the net reclassification index (NRI) to discriminate between HFpEF and controls. Cox regression analysis was performed to identify independent predictors of outcome.

Atrial fibrillation (AF) was present in 43 (31%) of HFpEF subjects. Overall, LAEF < 44% differentiated HFpEF from controls (receiver operator characteristic-area under curve [ROC-AUC] in all = 0.794; sinus rhythm = 0.777). Adding LAEF to a model comprising existing European Society of Cardiology markers including B-type natriuretic peptide, E/E', maximum left atrial volume indexed to BSA and left ventricular mass improved both the ROC-AUC and NRI in all subjects (ROC-AUC 0.892 to 0.918, p = 0.073; NRI 56.8%, 95% confidence interval (CI) 22.4 – 91.1, p = 0.001) and in sinus rhythm alone (ROC-AUC 0.860 to 0.894, p = 0.138; NRI 53.8%, 95% CI 17.9 – 89.7, p = 0.003).

During median follow-up (616 days), there were 44 composite events (8 deaths, 36 HF hospitalisations) in HFpEF. LAEF was an independent predictor of outcome in all subjects (HR 0.703; CI 0.501 – 0.986; p = 0.041) and in sinus rhythm alone (HR 0.392; CI 0.206 – 0.744; p = 0.004).

Conclusions

CMR-derived LAEF provides incremental value to current diagnostic markers and is an important prognostic biomarker in HFpEF.

Background

Left atrial (LA) remodeling and dysfunction have been implicated in the pathophysiology of HF and are associated with poorer outcomes across a range of pathologies[299]. To date, the evidence base for such observations has largely been derived from echocardiography which is reliant upon adequate LA endocardial border definition for both volumetric and strain assessments[188]. CMR however, affords superior spatial resolution, has excellent reproducibility and is the current gold standard for LA volumetric[146] and functional assessment in sinus rhythm[189] or AF[190]. To date, prospective CMR studies assessing LA dysfunction in HF are lacking. Furthermore, no studies have evaluated both the diagnostic and prognostic capabilities of left atrial ejection fraction (LAEF) in HFpEF.

Recently, CMR measures of LA function identified subjects from the general population at heightened cardiovascular risk[191] as well as those who developed incident HF[192]. In a further study of patients with predominant HFrEF, CMR-measured LAEF was also associated with adverse outcomes[193].

In this prospective, observational study of a well-characterised cohort with HFpEF we aimed to assess whether CMR-derived LAEF may improve upon current diagnostic criteria and is of prognostic value. The relation of LAEF to markers of exercise capacity and quality of life measures was also evaluated. The feasibility of LAEF (and LA volume) calculation by CMR and TTE was also compared.

Methods

5.1.1 Study population

The study population, recruitment, ethics, inclusion and exclusion criteria have been detailed in Chapter 2.

For comparison with HFpEF, 48 asymptomatic controls (age and sex-matched) were recruited. Hypertensive subjects were included in this group (n = 22) since hypertension is so prevalent in this age group of patients.

All subjects underwent comprehensive clinical assessment and blood sampling, TTE and CMR during the same visit. A standardized six minute walk test (6MWT) was used to assess exercise capacity[287] and quality of life metrics were derived from the Minnesota Living with Heart Failure (MLHF) Questionnaire[249].

5.1.2 Transthoracic echocardiography

E/E' was derived as described in the general methods Chapter 2. The feasibility of measuring LA volumes and LAEF by TTE was recorded. LA planimetry was performed in the apical 2- and 4-chamber views and LA volumes were derived using the modified Simpson's method[77]. The plane of the mitral annulus served as the inferior border (see Figure 1.8).

5.1.3 CMR protocol

The CMR protocol has been described in previous Chapters (2, 3 and 4). Prospective ECG gating was employed in cases of arrhythmia (e.g. AF, ectopics).

5.1.4 CMR analysis

All images were analysed by a single operator (*PK*) blinded to clinical data, using *CVI42* software (Circle Cardiovascular Imaging, Calgary, Canada). Ventricular volumes, EF and LV mass were calculated from the short-axis cine stack excluding papillary muscles and trabeculations as previously described[174]. RV performance and analysis is detailed in the next Chapter. Qualitative assessment of LGE images was undertaken by two experienced observers (*PK*, *ASHC*) to achieve consensus for identifying the presence of MI. The mid-short axis *MOLLI* images were analysed for ECV calculation as described in Chapter 4.

5.1.5 Analysis of LA parameters

The biplane area-length method (excluding the appendage and pulmonary veins – see Figure 5.1) was employed for LA volumetric[300] and functional analysis[193]. The LA endocardial border was manually contoured in both the 2- and 4-chamber views with the

mitral annulus serving as the anterior border. The maximum LA area was contoured in the frame immediately prior to mitral valve opening. The minimum LA area was contoured in the frame immediately after mitral valve closure. LA volumes (LAV) were calculated using the area-length method, where: volume = (0.85 x area^2)/length. LAEF was derived as follows: LAEF = (LAVmax – LAVmin) / LAVmax. Surrogates of LA reservoir function i.e. reservoir volume ([LAVmax – LAVmin]) and LA conduit function i.e. conduit volume ([LV stroke volume – LA reservoir volume]) were also calculated. All volumetric and mass data were indexed to BSA.

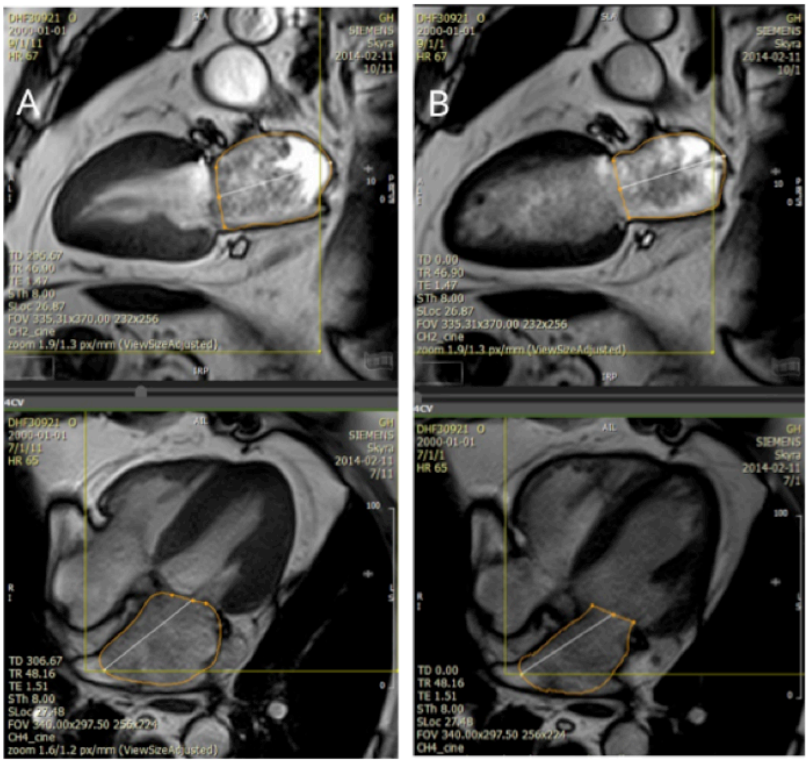


Figure 5.1 Calculation of CMR derived left atrial ejection fraction

Cine 2- and 4-chamber images illustrating contoured maximum (A) and minimum (B) left atrial areas for volume (and ejection fraction) derivation

5.1.6 Follow-up and endpoints

The primary endpoint was the composite of all-cause mortality or first HF hospitalisation. Hospital databases and patient records were sourced to obtain outcome data. Patient follow-

up was for a minimum of 6 months post-study entry. Only the first event was included in the outcome analysis.

5.1.7 Statistical analysis

Statistical tests were performed using SPSS v22. Normality for continuous data was assessed using histograms, Q-Q plots and the Shapiro-Wilk test. Summary data are presented as mean (± SD) or median (25 – 75% IQR). Between group differences were compared using the t-test, Mann-Whitney U test and the Chi-square test as appropriate. BNP, creatinine, MLHF score and 6MWT distance were $\log_{10}$ transformed before analysis. Pearson's and Spearman's correlations were performed to check for potential associations of LAEF with other variables.

The differences in feasibility of performing LA volumetric and LAEF measurements between CMR and TTE was assessed using the one-sample T test. Intermodality agreements between CMR and TTE were tested using the Bland-Altman method. Assessments of intra-observer and inter-observer variability for CMR measured LA function were undertaken on 10 randomly selected patients, a minimum of 4 weeks apart (by *PK* and *JRA*). ROC analysis was performed for LAEF and other traditional diagnostic markers as per ESC HFpEF guidelines (i.e. BNP, E/E', LAVImax and LV mass) to discriminate between HFpEF and controls. The net reclassification index (NRI) was used to evaluate the incremental benefit of LAEF when added to existing diagnostic markers.

Kaplan-Meier analysis was undertaken to calculate event rates. Differences in survival curves were tested using the Log-Rank test. Covariates with univariable Cox regression association with the endpoint at $p<0.1$ were then entered (to prevent model overfitting) into subsequent multivariable analysis, using both backwards and forwards stepwise elimination methods, to identify independent predictors. Continuous variables were Z-standardized to enable comparison of hazard ratios based upon one SD increase in the predictor variable. The accuracy of the final multivariable model to predict events was then tested by further ROC analysis. A p value of less than 0.05 was considered significant. To further assess the incremental strength of LAEF as a prognostic marker, it was added to smaller, clinically

meaningful, separate multivariable models incorporating univariable predictors such as clinical parameters, functional and imaging markers.

Results

5.1.8 Comparison of HFpEF and controls

Following CMR, 15 HFpEF patients were diagnosed with either hypertrophic cardiomyopathy (n = 10) or constrictive pericarditis (n = 5) and excluded from further analysis (see Chapters 2 and 3). Our final cohort thus comprised a total of 188 participants (see Figure 5.2). Baseline demographics and imaging characteristics are summarized in Table 5.1 and Table 5.2.

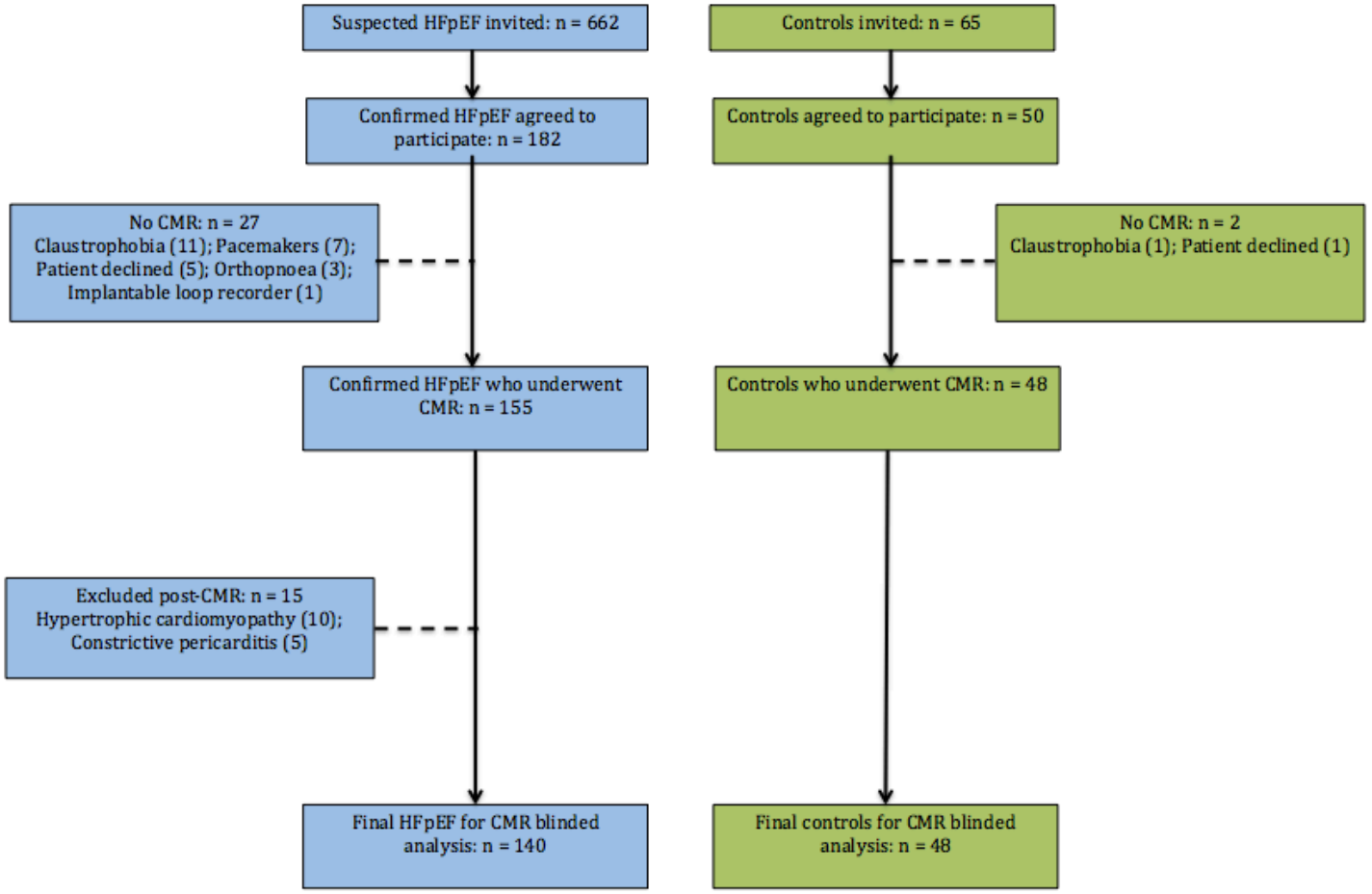


Figure 5.2 Study recruitment overvie

Table 5.1 Baseline clinical characteristics

	HFpEF n = 140	Controls n = 48	p value
Demographics			
Age (years)	73 ± 9	73 ± 5	0.820
Male (%)	68 (49)	24 (50)	0.977
Clinical			
Heart rate (b.p.m)	70 ± 14	68 ± 10	0.308
Systolic BP (mmHg)	145 ± 25	151 ± 24	0.001
Diastolic BP (mmHg)	74 ± 12	79 ± 10	0.006
BMI (kg/m^2)	34 ± 7	25 ± 3	<0.0001
Sinus rhythm (%)	97 (69)	48 (100)	<0.0001
Atrial Fibrillation	43 (31)	0 (0)	<0.0001
Prior HF hospitalisation	92 (66%)	NA	NA
Diabetes (%)	70 (50)	0 (0)	<0.0001
Hypertension (%)	127 (91)	22 (46)	<0.0001
Angina (%)	23 (16)	0 (0)	0.003
Known MI (%)	16 (11)	0 (0)	<0.0001
Coronary artery disease (%)	31 (22)	0 (0)	<0.0001
Asthma or COPD (%)	24 (17)	3 (6)	0.134
Smoking (%)	75 (54)	17 (35)	0.033
Hypercholesterolaemia (%)	69 (49)	18 (38)	0.367
PVD (%)	3 (2)	0 (0)	0.120
TIA or CVA (%)	19 (14)	1 (2)	0.025
Medications			
Betablocker (%)	95 (68)	2 (4)	<0.0001
ACEi or ARB (%)	120 (86)	10 (21)	<0.0001
MRA (%)	43 (31)	0 (0)	<0.0001
Loop Diuretic (%)	113 (81)	0 (0)	<0.0001
Functional status			
NYHA I/II (%)	97 (69)	NA	NA
NYHA III/IV (%)	43 (31)	NA	NA
6MWT distance (m)	180 (120 – 250)	380 (350 – 440)	<0.0001
MLHF score	49 (25 – 65)	NA	NA
Bloods			
Sodium (mmol/L)	139 ± 4	140 ± 2	0.098
Urea (mmol/L)	9 ± 4	6 ± 1	<0.0001
Creatinine (mmol/L)	89 (73 – 115)	71 (56 – 85)	<0.0001
Haemoglobin (g/L)	129 ± 22	140 ± 15	0.003
BNP (ng/L)	136 (66 – 254)	33 (24 – 44)	<0.0001

Table 5.2 Baseline imaging characteristics excluding LA parameters

	HFpEF n = 140	Controls n = 48	p value
Previous Chest Radiography			
Pulmonary oedema (%)	97 (69)	NA	-
Raised cardiothoracic ratio (%)	101 (72)	NA	-
Pleural effusion (%)	49 (35)	NA	-
Echocardiography			
E/E'	13 ± 6	9 ± 3	<0.0001
CMR LV parameters			
LVEF (%)	56 ± 5	58 ± 5	0.019
LVEDVI (ml/m2)	79 ± 18	81 ± 14	0.409
LVESVI (ml/m2)	35 ± 10	34 ± 8	0.541
LVMI (g/m2)	52 ± 15	46 ± 9	<0.0001
LV mass/LVEDV	0.68 ± 0.16	0.57 ± 0.09	<0.0001
ECV (%)	28 ± 4.6	25 ± 3.2	<0.0001
Presence of MI	23 (16)	0 (0)	<0.0001

HFpEF and healthy controls were well matched for age (73 years) and sex. Approximately two-thirds of HFpEF patients had experienced prior hospital admissions for decompensated HF or had radiographic evidence of pulmonary congestion. Consistent with prior studies, HFpEF was frequently associated with co-morbidities including obesity, diabetes, hypertension, AF. HFpEF patients had worse renal function and lower haemoglobin. A significant minority of HFpEF also had known ischaemic heart disease (22%, MI noted in 16%) and lung disease (17%). Furthermore, HFpEF patients had dramatically poorer exercise capacity (shorter 6MWT distance) and nearly one-third were in New York Heart Association (NYHA) III/IV.

5.1.9 Imaging data

Indices of diastolic dysfunction as per ESC guidelines i.e. BNP, E/E', LAVImax and LV mass were significantly higher in HFpEF. LV volume was marginally lower in HFpEF but there was evidence of concentric remodeling with increased mass/volume and higher ECV in the HFpEF cohort. RV volumes and function were similar in HFpEF and controls.

5.1.10 LA parameters

Overall, HFpEF subjects had larger atria and lower LAEF compared to controls (Table 5.3). Within HFpEF, AF was present in 31% and was associated with significantly higher LA volumes and lower LAEF (LAVImax 76 mls, LAVImin 66mls, LAEF 14%) compared to sinus rhythm (LAVImax 43mls, LAVImin 26mls, LAEF 41%, $p < 0.0001$). Nearly one-third of HFpEF had normal LA size [300] using a cut-off of $\leq 40 ml/m^2$. LA dysfunction (LAEF < 44%) was present in approximately three-quarters of HFpEF overall and in nearly half of those with HFpEF in spite of normal-sized LA.

In HFpEF, LAEF was not significantly associated with NYHA class ($rs = -0.042$, $p = 0.622$), 6MWT distance ($rs = 0.056$, $p = 0.524$) or MLHF questionnaire score ($rs = -0.058$, $p = 0.506$). There were strong negative correlations between LAEF and LA volumes (see Figure 5.3). As LAEF diminished, LA volumes increased (LAVImax Pearson's $r = -0.602$, $p < 0.0001$; LAVImin $r = -0.762$, $p < 0.0001$). An inverse exponential (curvilinear) fit best demonstrated the relationship between LAEF and both LAVImax ($r2 = 0.378$, $p < 0.0001$) and LAVImin ($r2 = 0.612$, $p < 0.0001$).

Table 5.3 Baseline imaging characteristics including LA parameters

	HFpEF	Controls	p value
Overall - all subjects including AF			
LAEF (%)	32 ± 16	51 ± 11	<0.0001
LAEF < 44%	103 (74)	10 (21)	<0.0001
Normal-sized LA	50 (36)	33 (69)	<0.0001
LAEF < 44% in normal-sized LA	24 (48)	6 (18)	0.006
LAVImax (ml/m2)	53 ± 25	35 ± 12	<0.0001
LAVImin (ml/m2)	38 ± 26	17 ± 8	<0.0001
LA reservoir volume indexed	15 ± 7	17 ± 6	0.025
LA conduit volume indexed (ml/m2)	29 ± 9	30 ± 9	<0.677
AF subjects only			
LAEF (%)	14 ± 7	NA	-
LAEF < 44%	42 (98)	NA	-
Normal-sized LA	2 (5)	NA	-
LAEF < 44% in normal-sized LA	1 (50)	NA	-
LAVImax (ml/m2)	76 ± 27	NA	-
LAVImin (ml/m2)	66 ± 25	NA	-
LA reservoir volume indexed	10 ± 5	NA	-
LA conduit volume indexed (ml/m2)	32 ± 10	NA	-
Sinus rhythm subjects only			
LAEF (%)	41 ± 12	51 ± 11	<0.0001
LAEF < 44%	60 (62)	10 (21)	<0.0001
Normal-sized LA	49 (51)	33 (69)	<0.037
LAEF < 44% in normal-sized LA	23 (47)	6 (18)	0.008
LAVImax (ml/m2)	43 ± 17	35 ± 12	<0.001
LAVImin (ml/m2)	26 ± 13	17 ± 8	<0.0001
LA reservoir volume indexed	17 ± 6	17 ± 6	0.791
LA conduit volume indexed (ml/m2)	28 ± 8	30 ± 9	0.136

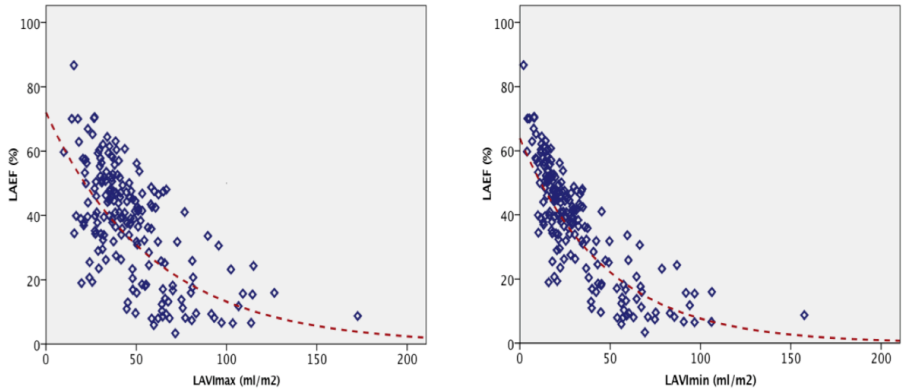


Figure 5.3 Scatter plot illustrating the relationship between left atrial ejection fraction and indexed left atrium volumes

LAVImax (left panel); LAVImin (right panel)

5.1.10.1 Feasibility and intermodality agreements for LA parameters

LA volumetric and LAEF measurements were feasible in all HFpEF patients who underwent CMR. In contrast, TTE feasibility was significantly lower (78%, $p < 0.001$). TTE also significantly underestimated LA volumes compared to CMR irrespective of cardiac rhythm (overall mean difference 23 ± 27mls; $p < 0.001$). This difference was more evident with increasing LA size. There were no statistically significant differences in LAEF between CMR and TTE. Intermodality agreements for LA volumes and LAEF are shown in Table 5.4 and Figures 5.4 – 5.6.

5.1.11 Intra-observer and inter-observer assessments

Intra-observer and inter-observer variability agreements for LA volumes and LAEF were excellent (intra-class correlation coefficients 0.95-0.99). Results are shown in Table 5.5. Bland-Altman plots are illustrated in Figures 5.7 – 5.9.

Table 5.4 Inter-modality agreements for LA volumes and LAEF between CMR and Echocardiography

Parameter	CMR Mean ± SD	Echocardiography Mean ± SD	Mean difference ± SD	95% Limits of Agreement	P value
All patients					
LAV min (ml)	70 ± 48	53 ± 34	17 ± 24	-30 to 64	<0.0001
LAV max (ml)	100 ± 48	78 ± 34	23 ± 27	-30 to 75	<0.0001
LAEF (%)	35 ± 17	35 ± 17	0.1 ± 13	-26 to 26	0.933
Sinus rhythm					
LAV min (ml)	52 ± 30	41 ± 21	11 ± 19	-26 to 48	<0.0001
LAV max (ml)	85 ± 35	68 ± 24	17 ± 25	-32 to 66	<0.0001
LAEF (%)	42 ± 13	41 ± 13	-0.6 ± 14	-27 to 26	0.556
Atrial fibrillation					
LAV min (ml)	134 ± 46	95 ± 36	39 ± 28	-15 to 93	<0.0001
LAV max (ml)	152 ± 49	112 ± 40	40 ± 26	-11 to 92	<0.0001
LAEF (%)	12 ± 6	15 ± 10	3 ± 12	-20 to 25	0.138

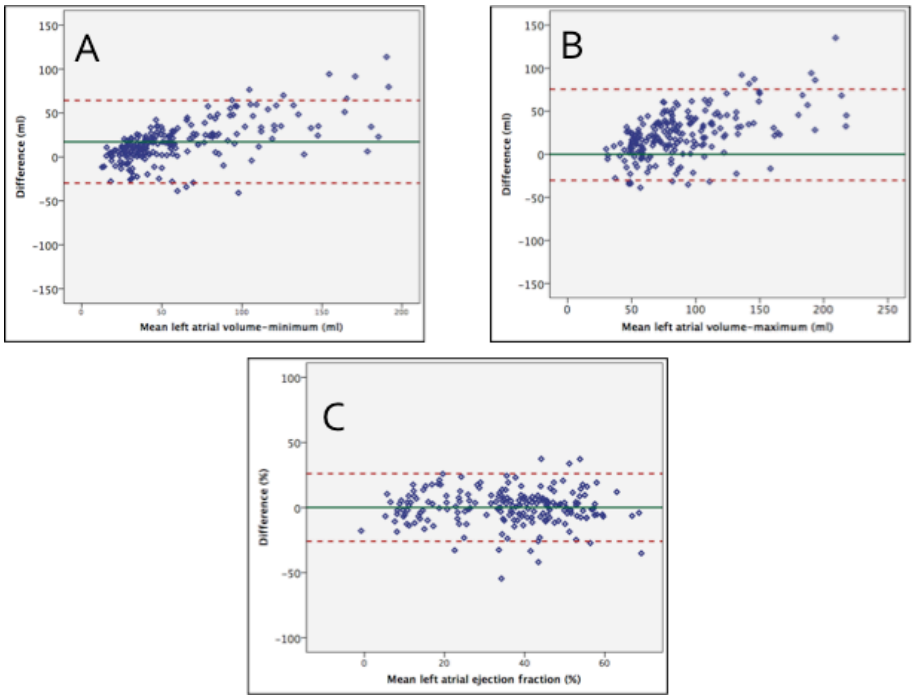


Figure 5.4 Bland-Altman plots for Echocardiography versus CMR quantification of left atrial volumes and function in all subjects

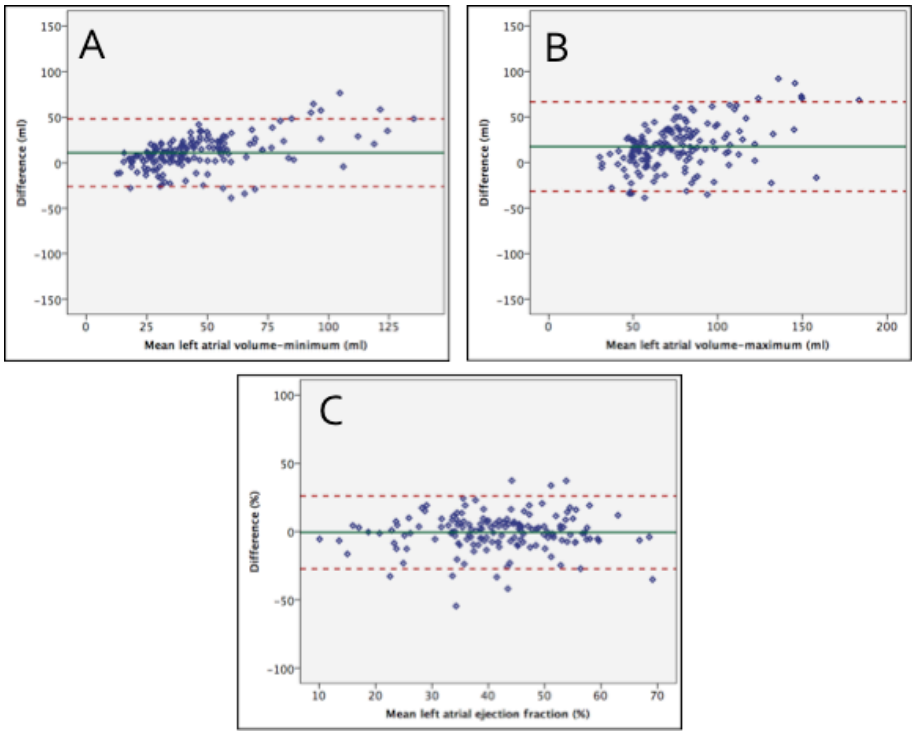


Figure 5.5 Bland-Altman plots for Echocardiography versus CMR quantification of left atrial volumes and function in sinus rhythm

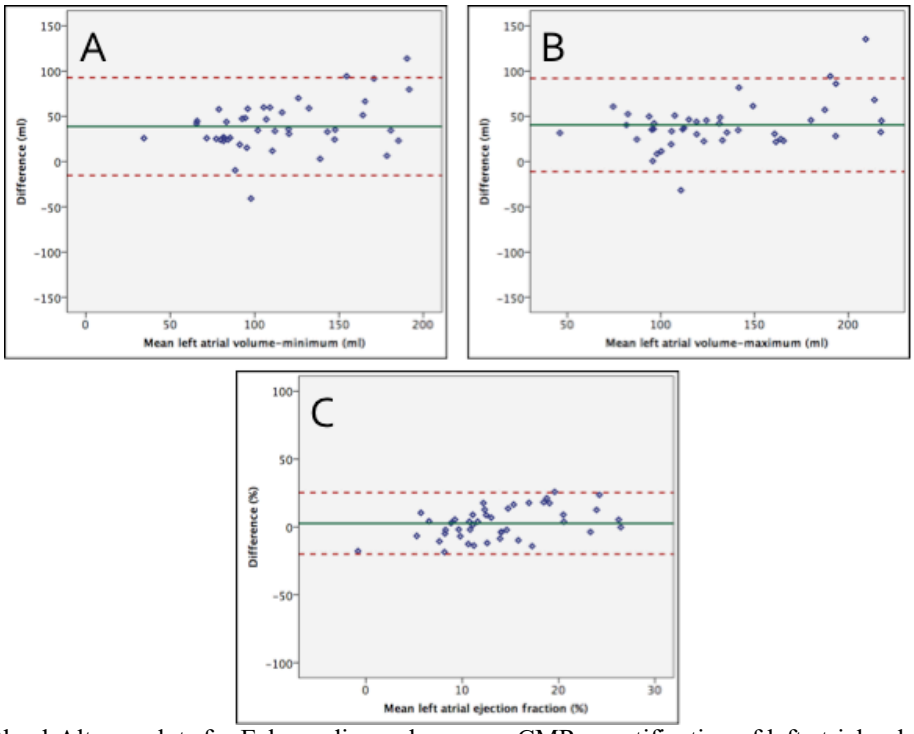


Figure 5.6 Bland-Altman plots for Echocardiography versus CMR quantification of left atrial volumes and function in AF

Table 5.5 Intra-observer and inter-observer assessments for left atrial volumes and left atrial ejection fraction

Parameter	Observer 1 Mean ± SD	Observer 2 Mean ± SD	Mean difference ± SD	ICC	Variability (1 – ICC)	Co-efficient of variation	95% Limits of Agreement
Intra-observer							
LAV min (ml)	70 ± 45	71 ± 44	1 ± 4	0.99	0.01	5.4	-7 to 8
LAV max (ml)	99 ± 48	101 ± 49	2 ± 5	0.99	0.01	4.8	-7 to 12
LAEF (%)	33 ± 13	33 ± 13	0.1 ± 3	0.98	0.02	9.4	-6 to 6
Inter-observer							
LA min (ml)	70 ± 45	71 ± 46	0.7 ± 5	0.99	0.01	6.8	-9 to 10
LA max (ml)	99 ± 48	102 ± 47	3 ± 6	0.99	0.01	6.3	-10 to 15
LAEF (%)	33 ± 13	35 ± 16	2 ± 4	0.95	0.05	12.2	-6 to 10

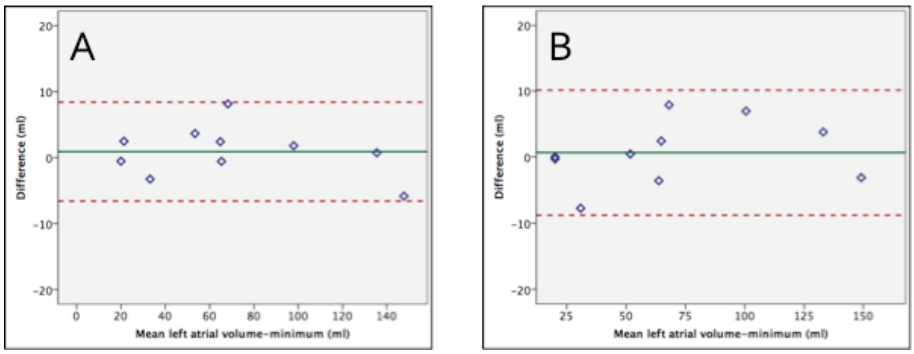


Figure 5.7 Bland-Altman plots for CMR intra-observer (A) and inter-observer (B) assessments of left atrial volume-minimum

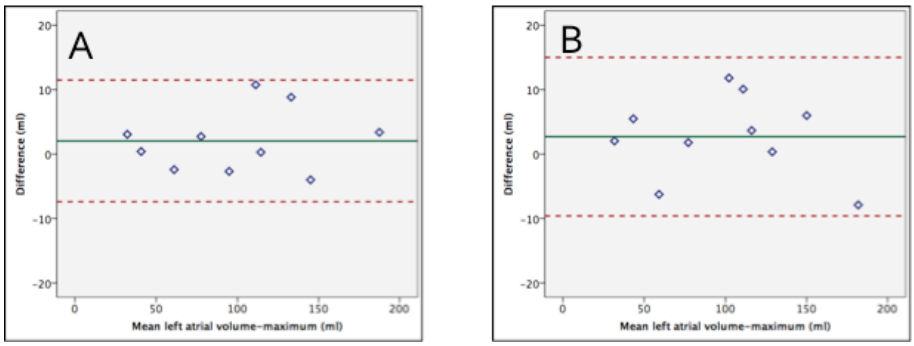


Figure 5.8 Bland-Altman plots for CMR intra-observer (A) and inter-observer (B) assessments of left atrial volume-maximum

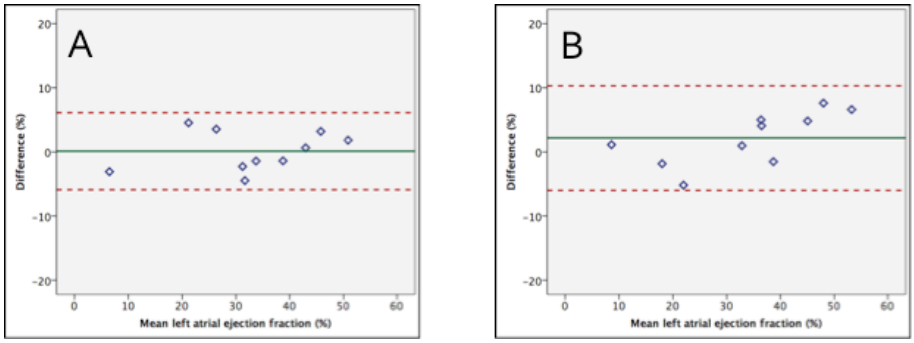


Figure 5.9 Bland-Altman plots for CMR intra-observer (A) and inter-observer (B) assessments of left atrial ejection fraction

5.1.12 Markers discriminating HFpEF from controls

In the whole cohort (Table 5.6), a LAEF threshold below 44% best discriminated HFpEF from controls using maximal sensitivity-specificity analysis; ROC-AUC 0.794, sensitivity 70%, specificity 80%, positive predictive value (PPV) 90%, negative predictive value (NPV) 51%, $p < 0.00001$. In sub-group analysis of sinus rhythm subjects, the same LAEF threshold yielded a ROC-AUC of 0.727, sensitivity 60%, specificity 80%, PPV 85%, NPV 51% and $p < 0.00001$. Overall, LAEF had a higher ROC-AUC than all current ESC diagnostic biomarkers ($p < 0.00001$) except BNP. When LAEF was added to a model containing all the ESC biomarkers, the ROC-AUC improved from 0.892 to 0.918 in all subjects ($p = 0.0729$) and from 0.860 to 0.894 in sinus rhythm ($p = 0.1378$).

When net reclassification was performed (see Table 5.7), adding LAEF significantly improved the combined ESC model: NRI in all subjects 56.8%, 95% confidence interval (CI) 22.4 – 91.1, $p = 0.001$; NRI in sinus rhythm 53.8%, 95% CI 17.9 – 89.7, $p = 0.003$. The model comprising LAEF predominantly reclassified healthy controls misclassified by the ESC model as having HFpEF into the truly healthy group (overall $p = 0.005$, sinus rhythm $p = 0.025$). Conversely, in those with HFpEF who were misclassified as healthy controls, there was a trend towards improved reclassification as HFpEF utilising LAEF but this did not reach statistical significance (overall $p = 0.116$, sinus rhythm $p = 0.055$).

Table 5.6 ROC analysis for diagnosis of HFpEF

	ROC-AUC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	p value
Overall - all subjects including AF						
BNP	0.861	80	91	96	63	< 0.0001
E/E'	0.760	56	84	90	42	< 0.0001
LAVImax	0.723	58	76	86	41	< 0.0001
LV mass	0.664	51	78	86	38	0.001
LAEF	0.794	70	80	90	51	< 0.0001
ESC diagnostic markers combined	0.892	79	91	96	62	< 0.0001
ESC diagnostic markers combined **+ LAEF**	0.918	72	100	100	58	< 0.0001
Sinus rhythm subjects only						
BNP	0.821	75	91	94	65	< 0.0001
E/E'	0.749	84	56	79	64	< 0.0001
LAVImax	0.646	68	58	76	48	0.006
LV mass	0.660	50	76	80	44	0.003
LAEF	0.727	60	80	85	51	< 0.0001
ESC diagnostic markers combined	0.864	73	91	94	63	< 0.0001
ESC diagnostic markers combined **+ LAEF**	0.890	78	84	91	67	< 0.0001
ESC = European Society of Cardiology; NPV = negative predictive value; PPV = positive predictive value; ROC-AUC = receiver operator characteristics-area under curve						

Table 5.7 Net reclassification using left atrial ejection fraction

HFpEF diagnosis	Net Reclassification Index (95%CI)	P value
Overall - all subjects including AF		
Yes	14.5 (-3.6 – 32.7)	0.116
No	42.2 (13 – 71.4)	0.005
Total	56.8 (22.4 – 91.1)	0.001
Sinus rhythm subjects only		
Yes	20.5 (-0.4 – 41.3)	0.055
No	33.3 (4.1 – 62.6)	0.025
Total	53.8 (17.9 – 89.7)	0.003

5.1.13 Parameters associated with outcomes

During median follow-up of 616 days (range 455 – 761), there were a total of 44 composite events (31.4%, 8 deaths, 36 HF hospitalisations) in patients with HFpEF, The event rate was higher in the AF sub-group than in sinus rhythm (39.5% versus 28.9%). There were no events in the control group. No subjects were lost to follow-up.

Kaplan-Meier survival plots according to LAEF for all patients and for those in sinus rhythm only are shown in Figure 5.10. When stratified into 2 groups (above and below median LAEF), lower LAEF was associated with increased risk of death or HF hospitalisation (all patients Log-Rank p = 0.002; sinus rhythm Log-Rank p = 0.009).

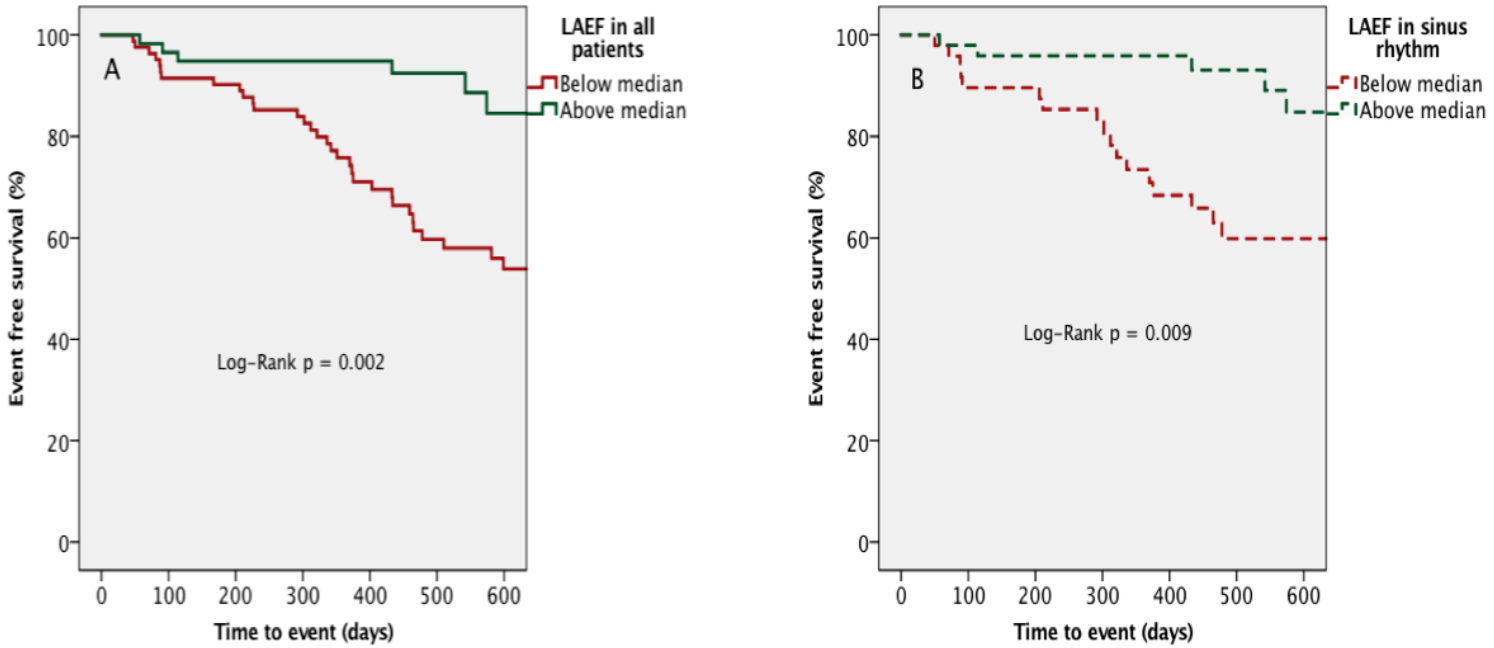


Figure 5.10 Kaplan-Meier analysis stratified according to median left atrial ejection fraction in (A) all subjects and in (B) sinus rhythm only

On univariable Cox regression analysis comprising all HFpEF subjects, 17 clinical and imaging variables showed association with adverse outcomes (Table 5.8). Following multivariable analysis, independent predictors of outcome were: prior HF hospitalisation, lung disease, Log BNP and LAEF (hazard ratio [HR] 0.703; 95%CI 0.501 – 0.986; p = 0.041). Since AF patients had lower LAEF, we assessed the prognostic value of LAEF in the presence and absence of AF. Importantly, AF was not significantly associated with outcomes on univariable analysis (p = 0.139). When the analyses were repeated for those in sinus rhythm only, LAEF remained an independent predictor of adverse outcomes (HR 0.392 0.406; 95%CI 0.206 – 0.744; p = 0.004) along with prior HF hospitalisation and Urea. The final multivariable models for predicting the composite endpoint yielded ROC-AUCs of 0.781 in all subjects and 0.834 in sinus rhythm (p < 0.0001 for both).

In separate analysis, when added to several clinically relevant, smaller multivariable models and the strongest predictors overall, LAEF remained an independent predictor of outcomes (see Table 5.9).

Table 5.8 Cox regression in subjects who underwent CMR left atrial ejection fraction assessment

	All patients				Sinus rhythm			
	Univariable		Multivariable		Univariable		Multivariable	
	Hazard ratio	P value	Hazard ratio	P value	Hazard ratio	P value	Hazard ratio	P value
Age	1.445	0.026			1.195	0.372		
Average DBP	0.673	0.017			0.580	0.109		
Prior HF hospitalisation	3.547	0.004	2.983	0.015	5.882	0.004	5.313	0.007
Asthma or COPD (%)	2.374	0.012	3.330	0.001	1.754	0.205		
NYHA III/IV	1.781	0.066			2.150	0.054		
6MWT distance	0.678	0.030			0.536	0.103		
MLHF score	1.324	0.099			1.570	0.045		
Urea (mmol/L)	1.282	0.048			1.479	0.009	1.421	0.023
Log Creatinine (mmol/L)	1.317	0.051			1.509	0.017		
Haemoglobin (g/L)	0.741	0.055			0.711	0.083		
Log BNP (ng/L)	1.622	0.008	1.718	0.019	1.755	0.008		
E/E’	1.427	0.014			1.446	0.039		
LV mass	1.328	0.051			1.608	0.030		
LAVImax	1.330	0.047			1.398	0.217		
LAEF	0.674	0.012	0.703	0.041	0.455	0.004	0.392	0.004
ECV	1.474	0.050			1.363	0.386		
Presence of MI on CMR	1.891	0.079			2.061	0.120		

Table 5.9 Clinically relevant multivariable modeling assessing the incremental prognostic impact of left atrial ejection fraction

Multivariable models	Hazard ratio	P value
Model 1 (clinical)		
Age	1.251 (0.855 – 1.831)	0.249
AverageDBP	0.698 (0.493 – 0.988)	0.043
Prior HF hospitalisation	2.957 (1.209 – 7.233)	0.018
Asthma or COPD (%)	2.438 (1.222 – 4.861)	0.011
+ **LAEF**	0.647 (0.462 – 0.907)	0.012
Model 2 (functional)		
NYHA III/IV (%)	1.265 (0.536 – 2.983)	0.592
6MWT distance	0.934 (0.562 – 1.553)	0.792
MLHF score	1.342 (0.959 – 1.878)	0.087
+ **LAEF**	0.674 (0.495 – 0.918)	0.012
Model 3 (blood tests)		
Urea (mmol/L)	1.272 (0.984 – 1.644)	0.066
Lg Creatinine (mmol/L)	1.119 (0.750 – 1.670)	0.582
Haemoglobin (g/L)	0.666 (0.475 – 0.935)	0.019
Lg BNP (ng/L)	1.303 (0.878 – 1.932)	0.189
+ **LAEF**	0.628 (0.457 – 0.863)	0.004
Model 4 (imaging markers of diastolic dysfunction)		
E/E’	1.440 (1.081 – 1.920)	0.013
LVMI	1.249 (0.937 – 1.665)	0.130
LAVImax	0.919 (0.588 – 1.436)	0.712
+ **LAEF**	0.594 (0.423 – 0.833)	0.003
Model 5 (imaging markers of fibrosis)		
ECV	1.195 (0.785 – 1.818)	0.407
MI on LGE	1.714 (0.837 – 3.511)	0.141
+ **LAEF**	0.691 (0.508 – 0.941)	0.019
Model 6 (strongest parameters combined)		
Prior HFhospitalisation	3.275 (1.359 – 7.895)	0.008
Asthma or COPD (%)	2.726 (1.370 – 5.424)	0.004
Haemoglobin (g/L)	0.785 (0.529 – 1.165)	0.229
E/E’	1.332 (0.995 – 1.784)	0.054
+ **LAEF**	0.680 (0.492 – 0.941)	0.020

Discussion

This is the first study to prospectively evaluate both the diagnostic and prognostic potential of CMR-derived LAEF in well-phenotyped cohorts of HFpEF and healthy subjects. The principal findings from our study are that (a) LAEF is lower in HFpEF compared to age- and sex-matched healthy controls, (b) irrespective of cardiac rhythm, LAEF reliably identifies HF with good diagnostic accuracy and outperformed conventional imaging biomarkers of HFpEF, (c) LAEF additionally provides incremental diagnostic value compared to existing ESC guidelines (d) LAEF improved reclassification of subjects when added to a model containing standard markers of HFpEF and (e) importantly, our study is the first to demonstrate that CMR-LAEF is also an independent predictor of outcomes in HFpEF.

Our study also reinforces the superior feasibility of CMR for LA volumetric and LAEF calculations compared to TTE. Furthermore, LAEF was measured with a high degree of reproducibility. These are added strengths when considering its role as a potential imaging biomarker.

5.1.14 LAEF as a potential diagnostic biomarker

Our work adds to a growing body of evidence implicating LA remodeling and dysfunction in HF[299]. Impaired LA function has previously been noted in conditions associated with HFpEF (e.g. diabetes, hypertension) even in the presence of a normal LA size[185]. Furthermore, LAEF is reportedly lower in HFpEF compared to hypertensive subjects with LVH, corroborating with our findings[127]. Diminished LA contractile reserve as a marker of exercise incapacity has also been shown in subjects with preserved LV ejection fraction with[183] and without heart failure[301].

Current ESC guidelines advocate the measurement of LA volumes and LV mass in all subjects with suspected HFpEF[3]. However, these measures are reliant on image quality and adequate endocardial border definition, unfortunately lacking in a third of HF cases when assessed with TTE[132]. Excellent spatial resolution and the ability to scan in any image plane make CMR the current imaging gold standard[132]. Current imaging diagnostic criteria

provide cut-offs for LAVImax and LV mass that are echocardiography-based and do not routinely incorporate CMR[3]. In our study, we performed a robust analysis comparing the diagnostic utility of these markers utilising gold-standard CMR measurements. ROC analyses and net reclassification indices confirmed the strong signal from LAEF.

Overall, LAEF outperformed E/E', LAVImax and LV mass in discriminating between HFpEF and healthy controls despite the increased age and prevalence of hypertension in both cohorts, factors known to increase LV mass and LA size[302]. The reasons for the strong discriminatory capabilities of LAEF are likely multiple. Firstly, LAEF reduction might be a more precise reflection of elevated filling pressures than the other traditional surrogate imaging markers of chronic diastolic dysfunction[181]. Similar to our study, published literature has demonstrated normal-sized LA in approximately one-third of HFpEF subjects[119]. Our findings of reduced LAEF even in the presence of normal-sized atria reaffirms prior observations that LA dysfunction likely precedes overt LA remodeling in HFpEF[180]. Towards the other end of the spectrum, with worsening LA dilatation (and likely chronic LV&LA pressure overloading), we have also demonstrated a close relationship between LA systolic function and volumes akin to the Frank-Starling mechanism i.e. LAEF reduces significantly more at higher volumes as contractile reserve becomes exhausted[186,193]. In our subjects, more specific derangements in both reservoir (increased LAVmax and reservoir volume) and booster pump (increased LAVmin) function were also noted. LA reservoir function may be compromised by reduced LA compliance and LV longitudinal dysfunction typical of HFpEF[303]. In addition, LV diastolic dysfunction and concomitant elevated filling pressures further contribute to ineffective LA active emptying through increasing LA afterload and wall tension[187]. Compensatory improvements in conduit function may in part explain the lack of difference in conduit volume between HFpEF and controls in our study[302,304].

5.1.15 LAEF as a potential prognostic biomarker

This is the first prospective study that shows CMR-derived LAEF is an independent prognostic marker in HFpEF, inclusive of AF subjects or in sinus rhythm alone. Previously, TTE-based observational studies[305] and HFpEF clinical trials[180,187] have highlighted perturbed LA function as a marker of adverse outcomes. Using indices of LA strain measured by speckle tracking, LA dysfunction was independently associated with either

prior[180] or subsequent[187] HF hospitalisations and death[305]. In a further retrospective TTE study involving both HFpEF and HFrEF, LAEF was independently associated with death only in HFpEF[186]. However, in the latter study, the groups were not evenly matched and the controls comprised subjects referred for cardiac catheterisation and were perhaps not truly representative of a healthy comparator group. In the one published CMR study to date evaluating the role of LA function in HF (heterogeneous population primarily comprising HFrEF), LAEF independently predicted mortality and incident AF. However, this retrospective study was again limited by referral bias, lacking a control group and excluding subjects who were in AF (nearly one-third)[193].

The potential value of LAEF as a prognostic biomarker may not be confined to HF alone. In a previous study of 312 subjects free of HF, who were in sinus rhythm and of a similar age group to our cohort, LAEF and LA strain were independent predictors of outcomes including future development of AF, HF and cardiovascular death[306]. All of the aforementioned studies however share intrinsic limitations of TTE[189].

Beyond HF, CMR data also further support LA dysfunction as a mediator of outcomes. Similar to our findings, the incremental prognostic value of LA function beyond LAVImax has previously been shown in a prospective study of asymptomatic subjects from the general population[191] and in chronic hypertensives without prevalent cardiovascular disease[307]. These findings suggest that LAEF also reflects a more advanced state of LA remodeling than LA dilation alone[299]. In another population study, LA strain using CMR feature tracking was independently associated with future development of incident heart failure[192].

5.1.16 LAEF and AF

The association between LA dilation and AF and their attendant cardiovascular risk is well recognised[299]. In HF, AF risk is also known to increase with diminishing LAEF[193]. AF occurs in approximately two-thirds of HFpEF patients at some point during their lifetime[308]. Interestingly, in our study, AF was not associated with adverse outcomes even though event rates were higher in this sub-group and LAEF was significantly lower compared to those in sinus rhythm. This suggests that LAEF exerts its influence on

outcomes through alternate mechanisms, either directly or indirectly[191,302]. LA dysfunction as a mediator of pulmonary vascular damage, RV dysfunction and progressive biventricular failure has also been proposed[186]. Additional reports have also highlighted that LA dysfunction in the presence of AF has incremental thromboembolic and mortality risk, beyond the CHADS2 score. Furthermore, LA dysfunction (using echo strain measures) predicts the success of restoring and maintaining sinus rhythm following either direct-current cardioversion or AF ablation[299].

5.1.17 Potential implications of our study

Our study reaffirms the pathophysiological role of LA dysfunction in HFpEF. CMR-measured biplane LAEF is simple, reproducible and provides both diagnostic and prognostic information which are strengths for consideration as a potential biomarker[89]. In contrast, traditional 2-D TTE measures of LA function are less reliable, time-consuming, subject to greater measurement errors and require greater operator skill, especially speckle tracking and strain measures. CMR is becoming increasingly accessible and may more reliably discriminate breathless individuals with equivocal BNP levels[136] and suboptimal echocardiographic imaging windows (especially HFpEF)[132]. Recent data from small studies have suggested that LA dysfunction may also be a potential therapeutic target. Maintenance or restoration of sinus rhythm in patients following AF catheter ablation has shown improved LA function and less arrhythmia recurrence[309]. In a small pilot study of HFpEF, insertion of a mechanical inter-atrial septal device was associated with reduced LA pressure and improved symptom status[310].

5.1.18 Limitations

This is a single-centre study and the results should be confirmed in additional populations. We used a pragmatic approach to define our HFpEF population to reflect a real world setting as opposed to latest ESC guidelines[3]. Importantly, the presence of diastolic dysfunction was not a pre-requisite for study inclusion since recent contemporary clinical trial data have highlighted normal diastolic function in approximately a third of such patients[114]. Our control group also included hypertensive subjects and was therefore not totally free of cardiovascular disease. However, if anything, this is likely to have potentially

underestimated the differences between HFpEF and control groups. Our study is the largest prospective CMR study to date evaluating LAEF in well-phenotyped cohorts of HFpEF and age- and sex-matched healthy controls.

We recognise that this is not a screening study. The primary purpose of performing ROC and NRI analysis was to highlight the discriminatory capabilities of LAEF in HFpEF compared to controls. Furthermore, NRI use in our study is limited by the absence of a "true gold standard" for diagnosing HFpEF. For robust diagnostic biomarker evaluation, further testing in unselected populations is needed.

Conclusions

CMR-derived LAEF is highly feasible, reproducible and provides incremental diagnostic value beyond existing ESC guidelines in HFpEF. Furthermore, it also independently predicts outcomes.

6 RIGHT VENTRICULAR SYSTOLIC DYSFUNCTION IN HFpEF

Manuscript written for journal submission:

Kanagala P, Cheng ASH, Singh A, Khan JN, Patel P, Gupta P, Arnold JR, Squire IB, Ng LL, McCann GP. *Prevalence of right ventricular dysfunction and prognostic significance in heart failure with preserved ejection fraction.*

Abstract

Aims

There is a paucity of data characterising right ventricular performance in HFpEF using the gold standard of CMR. We aimed to assess the proportion of right ventricular systolic dysfunction (RVD) in HFpEF and assess its relation to clinical outcomes.

Methods and Results

As part of a single-centre, prospective, observational study, 183 subjects (135 HFpEF, and 48 age- and sex-matched controls) underwent CMR. RVD (defined as right ventricular ejection fraction < 47%) based on our own reference controls was present in 19% of HFpEF. Patients with RVD had lower systolic blood pressure, more frequent AF, radiographic evidence of pulmonary congestion and raised cardiothoracic ratio and larger right ventricular volumes.

During median follow-up of 615 days (range 455 – 761), 30% of HFpEF subjects experienced the composite endpoint of death or hospitalisation with HF. Kaplan-Meier survival curves demonstrated association of RVD with an increased risk of the composite endpoint (Log-Rank p = 0.001). In Cox regression analysis, in addition to prior HF hospitalisation, chronic lung disease and Log BNP, RVD was an independent predictor of outcomes (HR 2.439, 95% CI 1.201-4.953 p = 0.014).

Conclusion

Right ventricular systolic dysfunction as assessed by CMR is prevalent in nearly one-fifth of HFpEF patients and is independently associated with death and/or hospitalisation with HF.

Background

The importance of RV function and its impact upon functional status[195] and outcomes[196] in HFrEF is well established. However, HFpEF currently accounts for approximately half of all cases of heart failure[6] and the role of right ventricular systolic dysfunction (RVD) in this setting is less well studied. To date, the majority of evidence for RVD is largely derived from echocardiographic data[197]. Moreover, the reported prevalence of RVD in HFpEF varies depending upon the choice of RV assessment tool and differing diagnostic thresholds (e.g. tricuspid annular plane systolic excursion, fractional area change, RVEF)[311].

CMR is the recognised imaging gold standard for RV volumetric and functional assessment, providing excellent accuracy and reproducibility[204,312]. However, only 2 CMR studies[205,206] have assessed RV function in HFpEF, again with differing thresholds for RVD and both lacked reference control groups. All of the above observations were recently recognised in a position statement from the Heart Failure Association of the European Society of Cardiology, proposing further prospective outcome studies to identify clear cut-off values for RVD that are prognostically and clinically relevant[197]. In this prospective, observational study we aimed to assess the prevalence of RVD in HFpEF compared to age- and sex-matched healthy subjects and explore the relation to clinical outcome.

Methods

6.1.1 Study population

The study population including HFpEF and control subjects, recruitment, ethics, inclusion and exclusion criteria have been detailed in earlier Chapters. During a single study visit, subjects underwent comprehensive clinical assessment, blood sampling, TTE and CMR and completed the MLHF questionnaire and 6MWT.

6.1.2 Chest radiography

The radiology reports of latest chest X-rays were sourced from the hospital computerized reporting system. The presence of pulmonary congestion and an enlarged cardiothoracic ratio were recorded. All reporting was done by Radiologists prior to study enrolment.

6.1.3 CMR protocol

The CMR protocol has been described in earlier Chapters.

6.1.4 CMR image analysis

Cine images were analysed using semi-automated *cvi42* software (Circle Cardiovascular Imaging, Calgary, Canada) by a single experienced observer (*PK*), blinded to all clinical data. All volumetric data were indexed to BSA. Ventricular volumes, ejection fraction and LV mass (excluding papillary muscles) were calculated from the short-axis cine stack as previously described[146,174] and also illustrated in Figure 2.3. The biplane method, excluding the appendage and pulmonary veins was used to calculate left atrial volumes and LAEF[288].

RVD was defined as RVEF < 47% based upon normative data from the published literature utilising the same technique as in our study[146] and our own healthy controls whereby the lower limit of RVEF was also 47%. MI was defined following qualitative assessment of LGE images by two experienced observers (*PK*, *ASHC*) as per standard criteria[277]. In cases of disagreement, a third observer (*GPM*) adjudicated. MI was deemed to be present if sub-endocardial enhancement was visualised on both short- and orthogonal long-axis LGE images. In the sub-group who had *MOLLI* imaging performed ECV was analysed as described in Chapter 4.

6.1.5 Outcome data

Hospitalisation for HF was defined as a hospital admission for HF which required diuretic, inotropic or intravenous nitrate therapy. Hospital databases and patient records were sourced to obtain outcome data. The composite endpoint was a composite of mortality or hospitalisation for HF. Patients were followed up for a minimum of 6 months post-study entry.

6.1.6 Statistical analysis

Statistical tests were performed using SPSS V22. A p value of less than 0.05 was considered significant. Normality for continuous data was assessed using histograms, Q-Q plots and the Shapiro-Wilk test. Summary data are presented as mean (± SD) or median (25 – 75% IQR or range). Between group differences were compared using the t-test, Mann-Whitney U test and the Chi-square test as appropriate. BNP, creatinine, 6MWT distance and MLHF score were $\log_{10}$ transformed before analysis.

Univariable Cox regression modeling was initially performed to identify variables associated with outcome. Parameters associated with endpoints at $p < 0.1$ were entered into multivariable analysis to identify independent predictors using both backwards and forwards stepwise elimination methods. In cases of collinearity, the variable with the highest coefficient was entered into multivariable analysis. Continuous variables were Z-standardized to enable comparison of hazard ratios based upon one SD increase in the predictor variable. The accuracy of the final Cox models to predict events was then tested by ROC analysis. To further assess the incremental strength of RVD as a prognostic marker, RVD was added to smaller, clinically meaningful, separate multivariable models incorporating factors related to outcomes such as clinical parameters, functional and imaging markers.

Kaplan-Meier survival analysis was undertaken to calculate event rates. The Log-Rank test was used to test differences in survival curves. Assessments of intra-observer and inter-observer variability for RV parameters were undertaken (by *PK* and *JRA*) a minimum of 4 weeks apart, on 10 randomly selected patients.

Results

Two hundred and thirty two subjects were enrolled (HFpEF n = 182, controls n = 50), of whom 49 were excluded from the analysis. Of these, RV assessment could not be performed in 5 patients due to degraded image quality. Additional reasons for exclusion are shown in Figure 6.1. Our final cohort who underwent RV analysis comprised 183

participants (HFpEF n = 135, controls n = 48). Baseline demographics and imaging characteristics are summarized in Table 6.1 and Table 6.2.

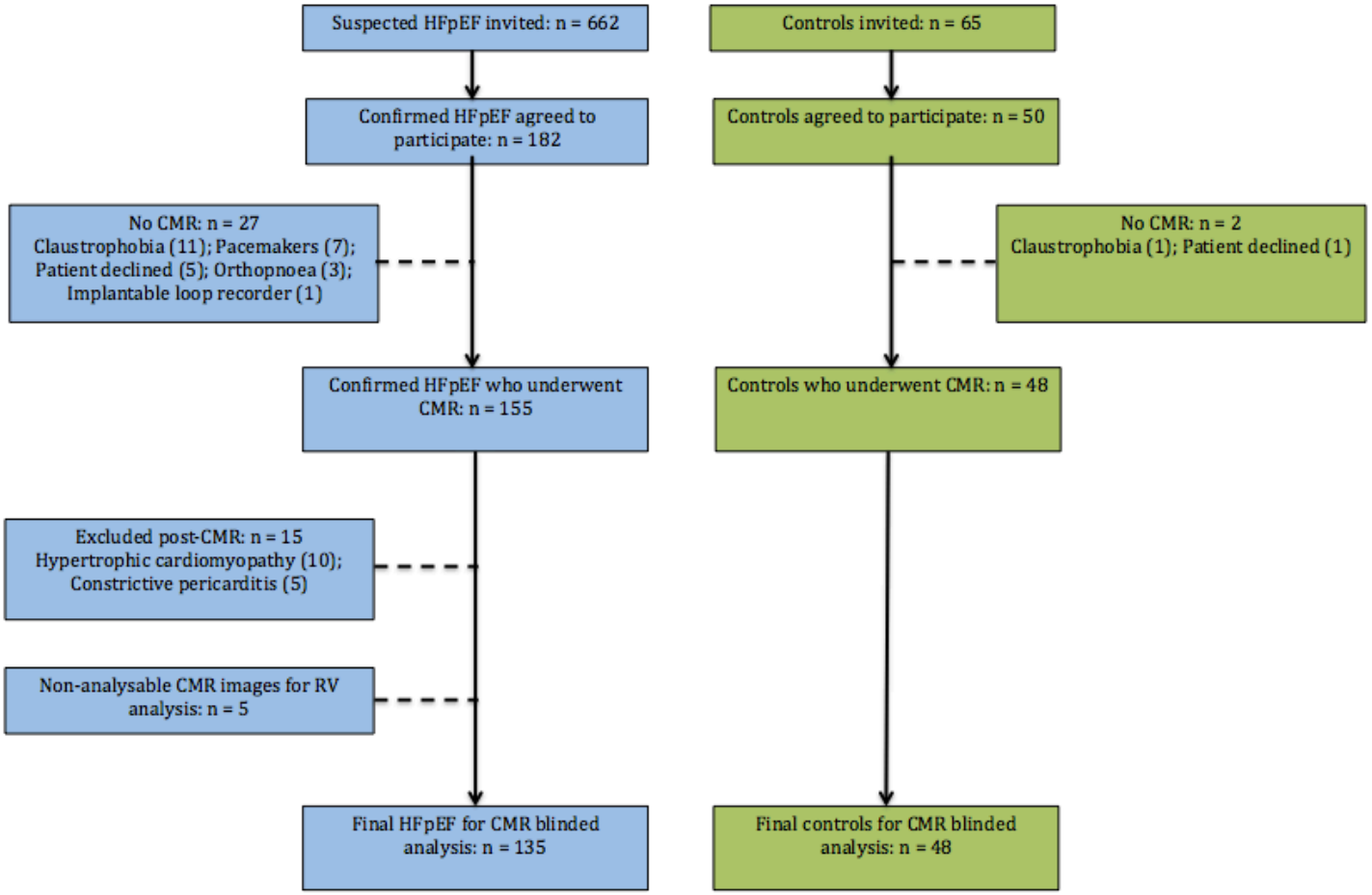


Figure 6.1 Study recruitment overview

Table 6.1 Baseline clinical characteristics of the study population

	Controls n = 48	HFpEF n = 135	p	HFpEF No RVD n = 110	HFpEF RVD n = 25	p
Age (years)	73±5	72±9	0.521	72±9	75±11	0.183
Male (%)	24 (50)	66 (49)	0.895	51 (46)	15 (60)	0.218
Heart rate (b.p.m.)	68±10	70±14	0.195	70±14	70±14	0.991
SBP (mmHg)	151±24	145±25	0.193	147±25	136±26	0.042
DBP (mmHg)	79±10	74±12	0.016	74±12	74±14	0.924
BMI (kg/m2)	25±3	34±7	<0.0001	34±7	33±7	0.623
AF (%)	0 (0)	41 (30)	<0.0001	28 (25)	13 (52)	0.009
Prior HF hospitalisation	NA	89 (66)	NA	67 (61)	22 (88)	0.010
Diabetes (%)	0 (0)	67 (50)	<0.0001	54 (49)	13 (52)	0.793
Hypertension (%)	22 (46)	122 (90)	<0.0001	97 (88)	25 (100)	0.071
Angina (%)	0 (0)	22 (16)	0.003	18 (16)	4 (16)	0.965
Known MI (%)	0 (0)	15 (11)	0.016	13 (12)	2 (8)	0.583
Known CAD (%)	0 (0)	30 (22)	<0.0001	24 (22)	6 (24)	0.813
Lung disease (%)	3 (6)	21 (16)	0.101	16 (15)	5 (20)	0.497
Betablocker (%)	2 (4)	93 (69)	<0.0001	72 (65)	21 (84)	0.071
ACEi or ARB (%)	10 (21)	116 (86)	<0.0001	95 (86)	21 (84)	0.759
MRA (%)	0 (0)	42 (31)	<0.0001	32 (29)	10 (40)	0.288
Loop Diuretic (%)	0 (0)	108 (80)	<0.0001	86 (78)	22 (88)	0.268
NYHA III/IV (%)	NA	40 (30)	NA	30 (27)	10 (40)	0.208
6MWT distance (m)	394±73	190 (120-250)	<0.0001	190 (130-250)	180 (100-273)	0.579
MLHF score	NA	49 (25-65)	NA	48 (24-64)	60 (29-68)	0.244
Sodium (mmol/L)	140±2	139±4	0.007	139±3	140±4	0.661
Urea (mmol/L)	6±2	8±3	<0.0001	8±3	8±4	0.613
Creatinine (mmol/L)	71 (56–85)	88 (73 – 113)	<0.0001	90 (73 – 116)	84 (70 – 108)	0.283
Haemoglobin	140±15	129±22	<0.0001	129±22	127±21	0.658
BNP (ng/L)	33 (24 – 44)	136 (65 – 256)	<0.0001	134 (54 – 269)	170 (84 – 245)	0.428

Table 6.2 Baseline imaging characteristics of the study population

	Controls n = 48	HFpEF n = 135	p	HFpEF No RVD n = 110	HFpEF RVD n = 25	p
Previous Chest Radiography						
Pulmonary congestion (%)	NA	93 (69)	NA	71 (65)	22 (88)	0.025
Raised CTR (%)	NA	98 (73)	NA	75 (68)	23 (92)	0.018
Pleural effusion (%)	NA	48 (36)	NA	36 (33)	12 (48)	0.159
Echo						
E/E'	9±3	13±5	<0.0001	13±5	13±6	0.723
CMR						
LVEF (%)	58±5	56±5	0.019	56±5	55±6	0.449
LVEDVI (ml/m2)	81±14	79±18	0.409	79±19	77±16	0.493
LVMI (g/m2)	46±9	52±15	<0.0001	52±16	52±10	0.886
LV mass/LV volume	0.57±0.09	0.68±0.16	<0.0001	0.67±0.16	0.70±0.15	0.447
RVEF (%), median, range	55 (47 – 70)	54 (4 – 73)	0.090	44.2 (4.3 – 46.7)	41±9	<0.0001
RVEDVI (ml/m2)	83±15	80±19	0.307	76±16	98±20	<0.0001
RVESVI (ml/m2)	37±9	37 ±14	0.849	33 ± 10	57 ± 15	<0.0001
LAVImax (ml/m2)	35±12	53±35	<0.0001	51±23	62±31	0.054
LAEF (%)	51±11	32±16	<0.0001	35±16	22 ±12	0.001
MI on LGE	0 (0)	23 (17)	0.002	17 (15)	6 (24)	0.305

6.1.7 Comparison of HFpEF and controls

Overall, HFpEF and healthy controls were well matched for age (73±9 years) and sex. Approximately two-thirds of HFpEF patients had experienced prior hospital admission for decompensated HF or had radiographic evidence of pulmonary congestion. HFpEF was frequently associated with co-morbidities including obesity, diabetes, hypertension, AF, renal dysfunction and anaemia. A significant minority of HFpEF also had known ischaemic heart disease (22%) and lung disease (16%). Furthermore, HFpEF patients had worse exercise capacity (shorter 6MWT distance) and nearly a third were in NYHA III/IV. Metrics of diastolic dysfunction (BNP, E/E', maximal left atrial volume indexed [LAVImax] and LV mass) were higher in HFpEF. As reported in the previious Chapter, LAEF was also lower in HFpEF. LVEF was lower in HFpEF ($p = 0.019$), albeit preserved overall. More concentric remodeling was also evident in HFpEF. The control group tended to have a higher RVEF with a narrow range (median 55, 47 – 70) in contrast to HFpEF (median 54, 4 – 73), although the difference between the groups did not reach statistical significance ($p = 0.090$).

6.1.8 Intra-observer and inter-observer assessments

Intra-observer and inter-observer variability were excellent for all RV parameters including RVEF (see Table 6.3.). Bland-Altman plots are illustrated in Figures 6.2 – 6.4.

Table 6.3 Intra-observer and inter-observer variability for assessment of right ventricular parameters

Parameter	Observer 1 Mean ± SD	Observer 2 Mean ± SD	Mean difference ± SD	Intra-class correlation coefficient (ICC)	Variability (1 – ICC)	Coefficient of variation (%)	95% Bland Altman Limits of agreement
Intra-observer							
RVEF (%)	49 ± 10	50 ± 11	0.8 ± 3	0.95	0.05	6.8	-6 to 7
RVEDV (ml)	201 ± 67	200 ± 69	- 2 ± 7	0.99	0.01	3.5	-16 to 12
RVESV (ml)	108 ± 66	106 ± 68	-2 ± 6	0.99	0.01	5.9	-14 to 10
Inter-observer							
RVEF (%)	49 ± 10	53 ± 10	4 ± 6	0.79	0.21	11.1	-7 to 15
RVEDV (ml)	201 ± 67	198 ± 74	-3 ± 15	0.98	0.02	7.6	-33 to 27
RVESV (ml)	108 ± 66	98 ± 62	-10 ± 17	0.96	0.04	16.4	-43 to 23

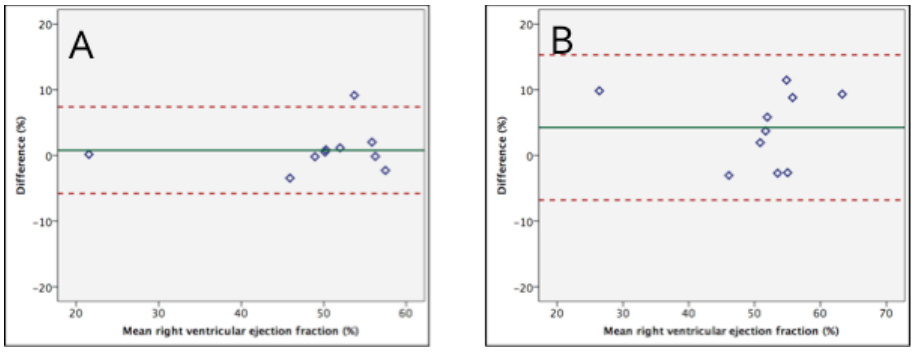


Figure 6.2 Bland-Altman plots for CMR intra-observer (A) and inter-observer (B) assessments of right ventricular ejection fraction

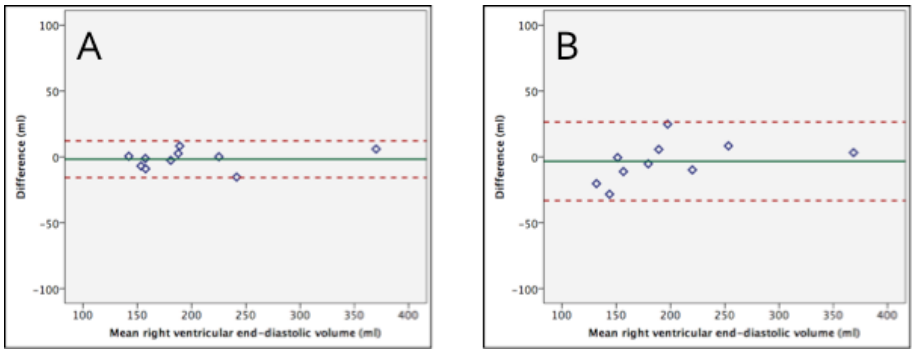


Figure 6.3 Bland-Altman plots for CMR intra-observer (A) and inter-observer (B) assessments of right ventricular end-diastolic volume

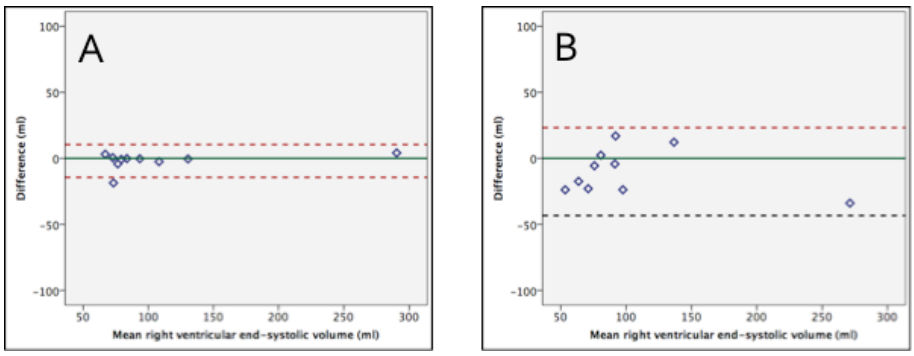


Figure 6.4 Bland-Altman plots for CMR intra-observer (A) and inter-observer (B) assessments of right ventricular end-systolic volume

6.1.9 Comparison of HFpEF with and without RVD

RVD (was present in nearly one-fifth (19%) of patients with HFpEF. The RVD group presented more frequently with lower systolic blood pressure, AF, radiographic evidence of pulmonary congestion and elevated cardiothoracic ratio, larger right ventricular volumes, lower LAEF and of borderline significance with increased LAVImax. Furthermore, prior hospitalisation with decompensated HF was also more prevalent in this sub-group. There were no significant differences between groups in terms of medical history, biochemical profiles and prescribed cardiac pharmacotherapies. Although measures of functional status and quality of life were worse in the RVD group (greater NYHA III/IV, shorter 6 MWT distance, higher scores on the MLHF questionnaire), these differences did not reach statistical significance.

6.1.10 RVD and outcomes

During median follow-up of 615 days (455 – 761), 30% of HFpEF subjects (n = 41) experienced the composite endpoint of death (n = 13) or re-hospitalisation with HF (n = 28). There were no events in the control group. Kaplan-Meier survival curves stratified according to the presence or absence of RVD in HFpEF are shown in Figure 6.5. Patients with RVD had significantly higher event rates (48% versus 26%, Log-Rank p = 0.001).

On univariable Cox regression analysis (Table 6.4), eighteen parameters were associated with adverse outcomes. RVEDVI and RVESVI were not entered into subsequent multivariable analysis due to co-linearity and interaction with RVD. During multivariable analysis, RVD remained an independent predictor of outcomes (HR 2.439, 95% CI 1.201-4.953 p = 0.014), in addition to prior HF hospitalisation (HR 2.904, 95% CI 1.106 – 7.623, p = 0.030), lung disease (HR 2.932, 95% CI 1.329 – 6.467, p = 0.008) and Log BNP (HR 1.833, 95% CI 1.147 – 2.928, p = 0.011). Since lung disease is historically known to be associated with RVD, we checked for any interaction between these variables. There was no statistical correlation (p = 0.497). The final Cox model incorporating these independent variables to predict outcome yielded an area under the ROC curve of 0.772 (sensitivity 95%, specificity 51%, p < 0.0001; Figure 6.6).

In separate analysis, when added to several clinically relevant, smaller multivariable models and the strongest predictors overall, RVD remained an independent predictor of outcomes (see Table 6.5).

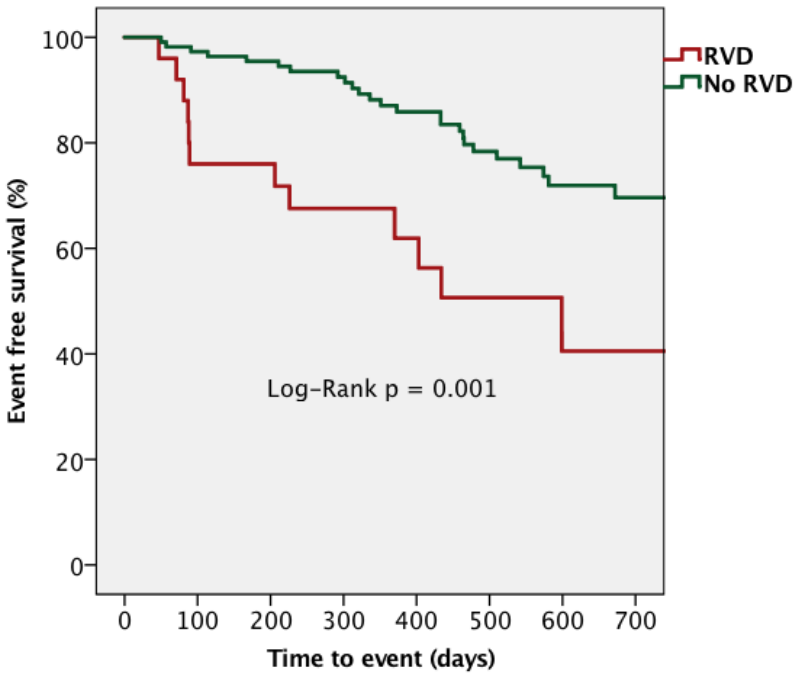


Figure 6.5 Kaplan-Meier analysis stratified according to the presence or absence of right ventricular dysfunction

Table 6.4 Cox regression in subjects who underwent CMR right ventricular assessment

	Univariable analysis		Multivariable analysis	
	Hazard Ratio (CI 95%)	**p value**	**Hazard Ratio (CI 95%)**	**p value**
Age, years	1.367 (1.014 – 1.843)	0.040		
Diastolic BP (mmHg)	0.647 (0.450 – 0.929)	0.018		
Prior HF hospitalisation	3.947 (1.534 – 10.160)	0.004	2.904 (1.106 – 7.623)	0.030
Lung disease	2.035 (0.961 – 4.305)	0.063	2.932 (1.329 – 6.467)	0.008
NYHA III/IV	1.766 (0.930 – 3.353)	0.082		
6MWT distance (m)	0.615 (0.384 – 0.984)	0.043		
MLHF score	1.363 (0.965 – 1.927)	0.079		
Haemoglobin (g/L)	0.736 (0.538 – 1.009)	0.057		
Log BNP (ng/L)	1.781 (1.165 – 2.722)	0.008	1.833 (1.147 – 2.928)	0.011
Imaging				
E/E’	1.427 (1.046 – 1.947)	0.025		
LVMI (g/m^2)	1.380 (0.999 – 1.905)	0.051		
*RVEDVI (ml/m^2)	1.365 (0.995 – 1.871)	0.054		
*RVESVI (ml/m^2)	1.419 (1.069 – 1.885)	0.016		
LAVImax (ml/m^2)	1.348 (1.024 – 1.776)	0.033		
LAEF (%)	0.658 (0.472 – 0.917)	0.014		
ECV (%)	1.711 (1.037 - 2.824)	0.036		
Presence of MI	1.972 (0.961 – 4.045)	0.064		
Presence of RVD	2.970 (1.489 – 5.925)	0.002	2.439 (1.201 – 4.953)	0.014
* These variables were not entered into subsequent multivariable analysis due to co-linearity and strong interaction with RVD.				

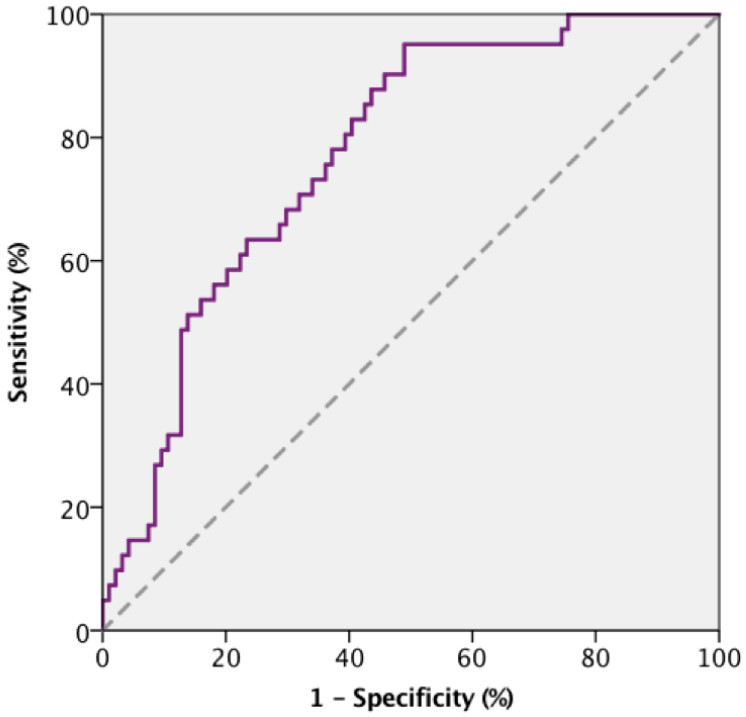


Figure 6.6 ROC analysis of the final multivariable Cox regression model inclusive of right ventricular dysfunction to predict outcomes

Table 6.5 Clinically relevant multivariable modeling assessing the incremental prognostic impact of right ventricular dysfunction

Multivariable models	Hazard ratio	P value
Model 1 (clinical)		
Age	1.212 (0.858 – 1.711)	0.276
AverageDBP	0.840 (0.568 – 1.242)	0.382
Prior HF hospitalisation	3.315 (1.239 – 8.867)	0.017
Asthma or COPD (%)	2.255 (1.025 – 4.962)	0.043
+ RVD	2.100 (1.017 – 4.338)	0.045
Model 2 (functional)		
NYHA III/IV (%)	1.193 (0.487 – 2.924)	0.699
6MWT distance	0.990 (0.515 – 1.900)	0.975
MLHF score	1.312 (0.878 – 1.960)	0.185
+ RVD	2.818 (1.324 – 5.996)	0.007
Model 3 (blood tests)		
Haemoglobin (g/L)	0.826 (0.596 – 1.146)	0.253
Lg BNP (ng/L)	1.595 (1.021 – 2.492)	0.040
+ RVD	2.767 (1.385 – 5.528)	0.004
Model 4 (imaging markers of diastolic dysfunction)		
E/E’	1.459 (1.052 – 2.022)	0.023
LVMI	1.440 (0.991 – 2.094)	0.056
LAVImax	0.885 (0.576 – 1.359)	0.575
LAEF	0.637 (0.394 – 1.030)	0.066
+ RVD	2.859 (1.303 – 6.269)	0.009
Model 5 (imaging markers of fibrosis)		
ECV	1.421 (0.837 – 2.411)	0.193
Presence of LGE - MI	2.199 (0.831 – 5.819)	0.112
+ RVD	3.463 (1.406 – 8.531)	0.007
Model 6 (strongest parameters combined)		
Prior HF hospitalisation	2.488 (0.926 – 6.686)	0.071
Asthma or COPD (%)	2.567 (1.060 – 6.218)	0.037
Log BNP	2.024 (1.206 – 3.397)	0.008
E/E’	1.186 (0.850 – 1.654)	0.316
+ RVD	2.846 (1.334 – 6.076)	0.007

Discussion

This is the first prospective study to evaluate the prevalence of RVD in a well-phenotyped group of HFpEF and age- and sex-matched control populations using CMR. The principal findings in our study are that in HFpEF: (1) RVD is present in a significant minority (19% and (2) RVD is independently associated with the risk of death or hospitalisation with HF.

6.1.11 Prevalence of RVD

To date, the reportedly wide range of prevalence of RVD in HFpEF of 4 to 44% has been derived almost exclusively from echocardiographic data[311]. Factors implicated in this variation in prevalence include the differing populations studied (community based, registry data, clinical trials) and variable definitions of both HFpEF (LVEF ≥ 45% and LVEF > 50%) and RVD[197,311]. In addition, the complex geometry of the RV renders it a difficult chamber to assess with traditional 2D echocardiography, especially in the context of HFpEF when imaging may be more challenging due to co-morbidites such as lung disease, obesity and AF[197]. CMR is the established gold standard for RV assessment[204,312]. To date, only one study[205] has reported prevalence (19%) of RVD quantified by CMR in HFpEF, using a RVEF cut-off of < 45%, primarily based upon ARVC guidelines[313]. We observed a similar prevalence of RVD using a slightly higher RVEF cut-off of RVEF < 47% based on our own internal reference controls, a particular strength of our study.

6.1.12 Significance of RVD in HFpEF

In HFrEF, the presence of RVD is associated with poorer functional status, exercise capacity[194,195] and worse prognosis[196]. However, a similar association of RVD with outcomes in HFpEF has not been observed consistently. In echocardiographic studies of community[203] and hospital based HFpEF subjects referred for invasive right heart catheterisation[200], RVD was independently predictive of mortality. To the contrary, in a larger observational study[202] comprising outpatient HFpEF recruits and in the TOPCAT clinical trial[314], RVD did not adversely impact upon prognosis. The likely explanation for these differences include: variable HFpEF LVEF cut-offs, use of different parameters to define RVD as described earlier and more stringent exclusion criteria in clinical trials

compared to community settings such as renal dysfunction or CAD which have been shown to be associated with RVD [197]but are also independently associated with poorer outcome[12].

Our work however adds to findings from the only 2 CMR-based HFpEF outcome studies to date[205,206] and clearly implicates RVD as an important mediator of outcomes in HFpEF. In the first study[206], all surrogates of RVD, irrespective of modality (CMR, echocardiography and invasive right heart catheterisation) were independently associated with death and or HF hospitalisation. In the above study, a much lower RVEF cut-off (<35%) on CMR was related to outcomes. In the second study[205], RVD measured by CMR outperformed echocardiographic-derived measures of RVD as a prognostic marker. The RVEF cut-off to define RVD (< 45%) was also chosen based upon ROC analysis to detect end-points. In contrast to both of the aforementioned studies, our follow-up times were longer, the presence of RVD below the lower limit of EF in our controls and not just more severe RVD was significantly associated with worse outcomes in our cohort.

In line with previous studies, RVD in our HFpEF cohort was also associated with lower systolic blood pressure, AF, adverse RV remodeling (RV enlargement), HF hospitalisations[203] and more prevalent pulmonary congestion[315]. Moreover, RVD was an independent marker of subsequent HF hospitalisations. The likely reasons for this are multiple. Firstly, RVD is associated with increased venous congestion[315] as also demonstrated by the higher rates of congestive radiographic changes in our RVD subjects. Our RVD subjects and previous studies[205,206] also demonstrated an association with increased left atrial (LA) size and echocardiographic E/e', surrogate markers of high LA pressure, which likely further contributes to congestion. Furthermore, the RVD group also had a greater proportion of AF, which is known to further exacerbate RV contractile dysfunction[200], increasing the likelihood of hospitalisation[179,194].

Previously reports have suggested a clear relationship between RVD and the severity of left heart disease as reflected by NYHA class, natriuretic peptides or LV systolic function[311]. However, in our study, these parameters were not different between those with and without RVD. This finding may suggest that RVD may be part of the natural aetiological profile in HFpEF whereby biventricular remodeling often co-exists, even in early stages[316].

6.1.12.1 Causes of RVD in HFpEF and mechanisms implicated in outcomes

Although the observational nature of our study precludes determination of causation, AF was significantly associated with RVD, suggesting a contributory role. Our findings of a higher AF prevalence are consistent with similar reports from previous HFpEF studies[200,203,205,314]. However, it remains unclear whether AF is a cause or consequence of RVD in HF[194]. In HFrEF, RVD reportedly predicts future AF development[317]. Irrespective of HF subtype or aetiology, AF in the setting of RVD is associated with haemodynamic instability and with poorer outcomes[194,318]. In the HFpEF population at large, development of AF confers a poorer quality of life[179], increases hospitalisation rates and worsens mortality[179,308].

Other authors have previously implicated male gender, lung disease, CAD, diastolic dysfunction and pulmonary hypertension in the aetiology of RVD, and as drivers of risk in HFpEF[200,203,311]. However, these factors were not consistently associated with RVD in our cohort.

6.1.13 Potential implications of our study

Identifying RVD is potentially important for multiple reasons. HF hospitalisations are associated with significant morbidity and are a drain on healthcare resources.[14] Importantly, the prevalence of HFpEF is rising[6]. Understanding the mechanistic triggers for decompensation in HFpEF may also enable targeted therapies (eg. RV focused, management of AF). Whilst treatments in unselected HFpEF patients have been neutral at best[113], one small study addressing pulmonary hypertension and RVD using a phosphodiesterase-5 inhibitor showed significant improvements in both cardiac haemodynamics and RV function[319].

6.1.14 Limitations

Since we excluded severe lung disease (which can cause RVD), our reported prevalence of RVD is probably lower than in the general HFpEF population at large. We did not measure pulmonary artery pressures (PAP) using echocardiography to establish the contribution of

pulmonary hypertension to RVD and to outcomes. However, Doppler echocardiographic measures of pulmonary hypertension are often inaccurate in comparison to the gold standard of invasive right heart catheterisation[320]. Besides, a prior CMR study[205] has already shown no association of RVD with invasively measured PAPs. Although, RVEF measurement is reportedly more reproducible using axial slice orientations[321], we deliberately assessed RV function from the short axis orientation since this is the method used routinely in clinical practice and our normative data were also derived using the same methodology[146]. Importantly, our technique yielded excellent reproducibility for RVEF measurement.

RV contractile function was dichotomised into either the presence or absence of RVD in order to present our findings into more clinically meaningful data, relevant to clinicians. However, we recognise that such an approach is laden with pitfalls compared to continuous variables. These include the loss of statistical power, increased risk of false positives and underestimation in the extent of variation in outcomes between groups.

Conclusions

RVD as assessed by CMR is present in a significant proportion of HFpEF and is independently associated with death and/or HF hospitalisations.

7 STRUCTURAL AND FUNCTIONAL DIFFERENCES BETWEEN HFpEF, HFrEF AND CONTROLS

Background

HFpEF represents a growing clinical entity that remains incompletely understood. Unlike HFrEF, which has been extensively studied and for which a compelling evidence base exists, similar data is sadly lacking in HFpEF. Furthermore, the notion that HFpEF and HFrEF exist as part of the same syndrome or as separate entities remains subject to debate[35]. The majority of epidemiological and clinical trial data on HFpEF are largely echocardiography derived. CMR enables the measurement of LV/LA volumes and LV mass with excellent accuracy and reproducibility[130,132,145]. Furthermore, CMR remains the gold standard for RV volumetric and functional assessment and provides unique tissue characterisation properties to enable surrogate assessments of the extra-cellular space (e.g. LGE assessment of focal fibrosis and T1 mapping to enable ECV quantification). To date, only a few CMR studies comparing HFpEF with HFrEF and controls have been undertaken. We aimed to assess the structural and functional differences between all 3 age- and sex-matched groups.

Methods

7.1.1 Study population

The overall study description including screening & recruitment, inclusion & exclusion criteria, protocols and investigations were as previously detailed in the Methods Chapter. In brief, HFpEF was defined as clinical or radiographic evidence of HF and LVEF > 50% on TTE. HFrEF was defined as clinical or radiographic evidence of HF and LVEF < 40% on TTE. During a single study visit, subjects underwent comprehensive clinical assessment, blood sampling, TTE and CMR and completed the MLHF questionnaire and 6MWT. All chest X-ray reports performed by Radiologists prior to study enrolment were collated.

7.1.2 CMR protocol

A detailed description of the CMR protocol is provided in the general Methods Chapter. In brief, scans included: cines in conventional long- and short-axis imaging planes, pre- and post-contrast short-axis *MOLLI* imaging and LGE imaging copying the cine slice positions, at least 10 minutes following the final administration of contrast (Gadovist, Bayer Healthcare, Berlin, Germany).

7.1.3 CMR analysis

All CMR analysis was performed by *PK* using semi-automated *cvi42* software (Circle Cardiovascular Imaging, Calgary, Canada) and blinded to clinical data. The calculation of LV and RV volumes, LVEF, RVEF and LV mass has also been described in earlier Chapters. Volumes and mass were indexed to BSA. RVD was defined as RVEF < 47%. LA volumetric analysis and LAEF calculation was as per Chapter 5. LA dysfunction was defined as LAEF < 44% based upon our earlier study findings (also from Chapter 5). LA dilation was reported if LAVImax was greater than 40 ml/m^2. LGE analysis was undertaken (by *PK* and *ASHC*) to qualitatively detect the presence of focal fibrosis in either an ischaemic or non-ischaemic pattern according to established criteria. ECV was measured from pre- and post-contrast T1 parametric maps as a marker of diffuse fibrosis (see Chapter 4).

7.1.4 Statistical analysis

SPSS (version 22, IBM Corp., Armonk, New York) was used to conduct all statistical analyses. Continuous data was assessed for normality using the Shapiro-Wilk test, histograms and Q-Q plots. Normally distributed data are expressed as mean ± SD. Non-parametric data are expressed as median (25 - 75% IQR). Categorical data are expressed as absolute numbers or percentages. For comparison of normally distributed data between the 3 groups, the one way-ANOVA with Bonferroni correction was used to detect differences. For similar comparison of non-normally distributed data, the Kruskal-Wallis test was

employed. The Chi-square or Mann-Whitney U tests were to compare categorical data, as appropriate. CMR assessments of intra-observer and inter-observer variability were undertaken a minimum of 4 weeks apart (by PK and *JRA*), on a subset of 10 randomly selected patients. The coefficient of variation (CoV)[273] and two-way mixed-effect intra-class correlation coefficient (ICC)[274] for absolute agreement were used to assess reproducibility. Agreement was defined as excellent if ICC was ≥ 0.75. The Bland-Altman method[275] was used to define the limits of agreement for inter-observer and intra-observer variability.

7.1.4.1 Image quality grading

Overall image quality was graded on a Likert scale as previously described. The differences in image grade between echocardiography and CMR were assessed using Cohen's Kappa (K); a p value > 0.05 was considered significant.

7.1.5 Follow-up and endpoints

The whole cohort follow-up was for a minimum of 6 months post-study entry. The primary endpoint was the composite of all-cause mortality or first HF hospitalisation. Hospital databases and patient records were sourced to obtain outcome data. Only the first event was included in the outcome analysis.

Results

7.1.6 Study recruitment overview

All subjects were recruited over a period of 26 months. The final participant was enrolled in April 2015. The overall study consort diagram is shown in Figure 7.1.

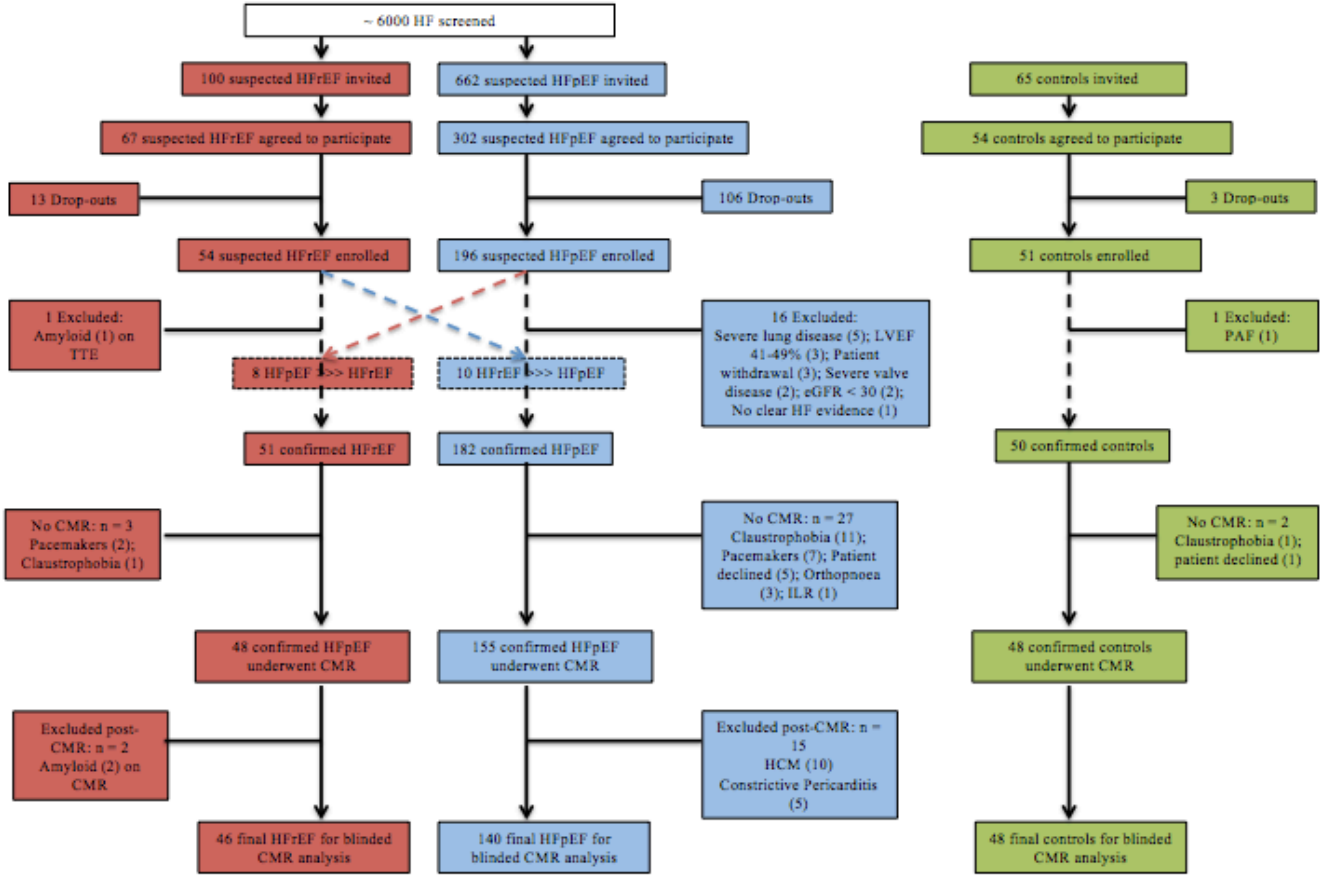


Figure 7.1 Overall study consort diagram

ILR = implantable loop recorder; PAF = paroxysmal atrial fibrillation

7.1.6.1.1 HFpEF recruitment

Approximately 6000 patients with HF were screened. Six hundred and sixty two patients with *suspected* HFpEF were invited to participate. Of these, 196 patients eventually enrolled into the study (n = 302 agreed to participate; n = 106 dropped out before study visit). From those enrolled, 16 were deemed ineligible due to study exclusion criteria. Eight patients were noted to have LVEF < 40% at echocardiographic assessment and were thus recruited into the HFrEF arm. However, an additional 10 patients with *suspected* HFrEF who had LVEF > 50% on the study visit day were recruited into the HFpEF arm. Therefore, a total of 182 patients with confirmed HFpEF were recruited. Of these, 27 patients did not undergo CMR evaluation, predominantly due to either claustrophobia or having permanent pacemakers. In the remainder, following CMR, a further 15 patients with either HCM or constrictive pericarditis as described in Chapter 3 were excluded. The final HFpEF cohort that underwent blinded CMR analysis thus comprised 140 patients.

7.1.6.1.2 HFrEF recruitment

Out of 100 *suspected* HFrEF invited, 54 patients enrolled (n = 67 agreed to participate; n = 13 dropped out before the study visit). As reported above, 10 of these patients were noted to have preserved LVEF on the day and were switched to the HFpEF arm. Conversely however, a further 8 patients were acquired to the HFrEF arm from the *suspected* HFpEF arm.

One patient with suspected amyloid was excluded on the basis of echocardiography. Of the 51 patients with confirmed HFrEF, 3 did not undergo CMR. Following CMR, a further 2 patients with amyloid not previously detected by echocardiography were excluded. The final HFrEF cohort that underwent blinded CMR analysis thus comprised 46 patients.

7.1.6.1.3 Recruitment of healthy controls

Out of 65 controls invited, 51 were enrolled (n = 54 agreed to participate; n = 3 dropped out before the study visit). One subject was noted to have AF on the day and was hence

excluded. CMR was not performed in 2 subjects. The final healthy control cohort that underwent CMR comprised 48 subjects.

7.1.6.2 Missing data and feasibility of analysis

CMR left and right ventricular volumes, EF and LV mass were not analysed in 5 patients with HFpEF due to degraded image quality. Across the whole cohort of 234 subjects, *MOLLI* imaging sequence was not available in a small but significant proportion (n = 55, 24%) of consecutive CMR scans (44 HFpEF, 7 HFrEF, 4 controls) and therefore no T1 images were acquired in these subjects. Of those who did undergo *MOLLI* imaging, a further 4 patients with HFpEF had non-analysable images. Analysis of LGE and LA parameters were feasible in all subjects.

The 6MWT was performed in 223 (95%) subjects acroos the whole cohort, with 11 HF patients (HFpEF n = 7, HFrEF n = 4) declining to undertake the test. The MLHF questionnaire was completed in 174 out of 186 HF patients (94%), with missing data in 8 HFpEF and 4 HFrEF patients.

7.1.6.3 Image quality

Overall, image quality was better for CMR compared to TTE (median grade: 3 vs 2; kappa statistic [-0.021], p = 0.72). This difference was maintained irrespective of patient group (see Table 7.1).

7.1.7 Population profiles

The baseline clinical characteristics of the study population are detailed in Table 7.2. Imaging characteristics are shown in Table 7.3. All 3 groups were well matched for age (mean 73 years) and sex (equal male: female).

Table 7.1 Overall and sub-group image quality in the study (Echocardiography and CMR)

	Overall cohort					HFpEF					HFrEF					Healthy				
	Median	**1**	**2**	**3**	**4**	**Median**	**1**	**2**	**3**	**4**	**Median**	**1**	**2**	**3**	**4**	**Median**	**1**	**2**	**3**	**4**
TTE	2	7 (3)	225 (96)	2 (1)	-	2	5 (3)	135 (97)	-	-	2	2 (4)	44 (96)	-	-	2	-	46 (96)	2 (4)	-
CMR	3	5 (2)	77 (33)	140 (60)	12 (5)	3	5 (3)	57 (41)	77 (55)	1 (1)	3	-	18 (39)	20 (44)	8 (17)	3	-	2 (4)	43 (90)	3 (6)

Table 7.2 Overall study baseline clinical characteristics

	HFrEF N = 46	HFpEF N = 140	Controls N = 48	p value
Age (years)	72±8	73±9	73±5	0.820
Male (%)	23 (50)	68 (49)	24 (50)	0.977
Heart rate (b.p.m)	67±16	70±14	68±10	0.308
Systolic BP (mmHg)	132±24 Δ*	145±25	151±24	0.001
Diastolic BP (mmHg)	71±17 Δ	74±12 Δ	79±10	0.006
BMI (kg/m2)	28±6*	34±7 Δ	25±3	<0.0001
Atrial Fibrillation	9 (20)	43 (31) Δ	0 (0)	<0.0001
Diabetes (%)	18 (39) Δ	70 (50) Δ	0 (0)	<0.0001
Hypertension (%)	25 (54) *	127 (91) Δ	22 (46)	<0.0001
Angina (%)	11 (24) Δ	23 (16) Δ	0 (0)	0.003
Known MI (%)	19 (41) Δ *	16 (11) Δ	0 (0)	<0.0001
CAD (%)	23 (50) Δ *	31 (22) Δ	0 (0)	<0.0001
Asthma / COPD (%)	9 (20)	24 (17)	3 (6)	0.134
Smoking (%)	28 (61) Δ	75 (54) Δ	17 (35)	0.033
High cholesterol (%)	21 (46)	69 (49)	18 (38)	0.367
PVD (%)	3 (7)	3 (2)	0 (0)	0.120
TIA or CVA (%)	5 (24) Δ	19 (14) Δ	1 (2)	0.025
Betablocker (%)	41 (89) Δ *	95 (68) Δ	2 (4)	<0.0001
ACEi or ARB (%)	36 (78) Δ	120 (86) Δ	10 (21)	<0.0001
MRA (%)	19 (41) Δ	43 (31) Δ	0 (0)	<0.0001
Loop Diuretic (%)	37 (80) Δ	113 (81) Δ	0 (0)	<0.0001
NYHA III/IV (%)	12 (26)	43 (31)	NA	0.551
6MWT distance (m)	210 (165 – 290) Δ*	180 (120 -250) Δ	380 (350 – 440)	<0.0001
MLHF score	36 (22 – 59)	49 (25 – 65)	NA	0.096
Sodium (mmol/L)	140±3	139±4	140±2	0.098
Urea (mmol/L)	9 ± 4 Δ	9±4Δ	6±1	<0.0001
Creatinine (mmol/L)	97 (77 – 128) Δ	89 (73 – 114.8) Δ	71 (56.3 – 84.5)	<0.0001
Haemoglobin (g/L)	134±24	129±22Δ	140±15	0.003
Haematocrit (%)	40±7	38±6	41±4	0.071
BNP (ng/L)	387 (178 – 634) *	135.6 (65.5 – 254.4) Δ	33 (24 – 44)	<0.0001
Δ p < 0.05 for HFpEF or HFrEF vs controls; * p < 0.05 vs HFpEF				

Table 7.3 Overall study baseline chest radiography and echocardiography characteristics

	HFrEF N = 46	HFpEF N = 140	Controls N = 48	p value
Previous Chest Radiography				
Pulmonary congestion (%)	31 (67)	97 (69)	NA	0.933
Raised CTR (%)	35 (76)	101 (72)	NA	0.362
Pleural effusion (%)	21 (46)	49 (35)	NA	0.138
Echo				
E/E'	15±5 Δ*	13±6Δ	9±3	<0.0001
Δ p < 0.05 for HFpEF or HFrEF vs controls; * p < 0.05 vs HFpEF				

7.1.7.1.1 HF vs controls

Compared to controls, HF patients had a significantly greater prevalence of CAD and diabetes, poorer renal function, and evidence of higher LV filling pressures (i.e. higher E/E' and BNP). Exercise capacity was significantly diminished in HF patients.

7.1.7.1.2 HFpEF vs controls

Approximately two-thirds of HFpEF patients had experienced prior hospital admission for decompensated HF or had radiographic evidence of pulmonary congestion. As reported in previous studies, HFpEF was frequently associated with co-morbidities including obesity, diabetes, hypertension, AF, renal dysfunction and anaemia. A significant minority of HFpEF also had known ischaemic heart disease (22%) and lung disease (17%).

7.1.7.1.3 HFpEF vs HFrEF

Compared to HFrEF, patients with HFpEF had a lower proportion of known ischaemic heart disease, higher BMI and a lesser severity of diastolic dysfunction (lower BNP and E/E'). AF was more prevalent in HFpEF (31%) compared to HFrEF (20%). HFpEF patients

also had a significantly lower 6MWT distance (180 m versus 210 m; p = 0.038). The MLHF score tended to be higher in HFpEF, although not reaching statistical significance was not reached and NYHA class was similar between groups.

7.1.8 CMR structural and functional differences between the groups

7.1.8.1 LV parameters

Parameters are shown in Table 7.4. Compared to controls, LVEF was marginally lower in HFpEF, albeit preserved overall (p = 0.019). LV volumes were similar but HFpEF patients exhibited higher LV mass and a greater degree of concentric remodeling (higher mass/volume ratio).

The prevalence of both focal (ischaemic and non-ischaemic) and diffuse fibrosis was also higher in HFpEF (ECV 28% vs 25%, p <0.0001). The predominant pattern of focal fibrosis was non-ischaemic in HFpEF (31% overall).

In comparison to HFpEF, patients with HFrEF had marked reductions in LVEF and substantially higher LV volumes. LV mass was even higher in HFrEF but with a reduction in mass/volume ratio indicative of adverse eccentric remodeling. Overall, the burden of both focal (89% vs 47%) and diffuse fibrosis (ECV 31% vs 28%) was even greater in HFrEF. In HFrEF, the predominant pattern of focal fibrosis was ischaemic (57%) and in such cases, the size of MI expressed as a percentage of LV mass, was larger compared to those seen in HFpEF (9.8% vs 3%, p < 0.0001). Similarly, non-ischaemic fibrosis was also more prevalent (41% in HFrEF vs 33% in HFpEF, p < 0.0001). However, there was no statistical difference in terms of non-ischaemic fibrotic size (p = 0.179).

Table 7.4 Overall study baseline CMR LV structural and functional characteristics

	HFrEF N = 46	**HFpEF N = 140**	**Controls N = 48**	**p value**
LV function, volumes, mass and remodeling				
LVEF (%)	28±9 Δ*	56±5 Δ	58±5	<0.0001
LVEDVI (ml/m^2)	142±44 Δ*	79±18	81±14	<0.0001
LVESVI (ml/m^2)	106±44 Δ*	35±10	34±8	<0.0001
LVMI (g/m^2)	64±22 Δ*	52±15 Δ	46±9	<0.0001
LV mass/LV volume	0.47±0.15 Δ*	0.68±0.16 Δ	0.57±0.09	<0.0001
LV focal and diffuse fibrosis				
LGE present	41 (89) Δ*	66 (47) Δ	4 (8)	<0.0001
LGE present - MI	26 (57) Δ*	23 (16) Δ	0 (0)	<0.0001
If MI, size of infarct	9.8 (4.2 – 20.6) *	3.0 (1.3 – 4.6)	NA	<0.0001
LGE present – non-MI	19 (41) Δ*	49 (33) Δ	5 (10)	<0.0001
If non-MI, size of scar	3.9 (2.2 – 7.7)	2.9 (1.4 – 6.5)	2.4 (0.6 – 3.6)	0.179
ECV (%)	31±8 Δ*	28±5 Δ	25±3	<0.0001
Δ $p < 0.05$ for HFpEF or HFrEF vs controls; * $p < 0.05$ vs HFpEF				

7.1.8.1.1 RV parameters

RV baseline parameters are shown in the Table below. RVD was more prevalent in HF. In HFpEF, RVD was present in 19%. In these subjects, there were no differences in RV volumes compared to controls. Overall, RV contractile function as assessed by RVEF was lower in HFrEF compared to the other groups. RVD was more prevalent in HFrEF (46%) compared to HFpEF and was also associated with greater remodeling (increased RV end-systolic volumes) compared to both HFpEF and controls.

Table 7.5 Overall study baseline CMR RV structural and functional characteristics

	HFrEF N = 46	HFpEF N = 140	Controls N = 48	p value
RVEF (%), median, range	49 (20 -72) Δ*	54 (4 – 74)	55 (47 -70)	<0.0001
RVD (%)	21 (46) Δ*	25 (19) Δ	0 (0)	<0.0001
RVEDVI (ml/m2)	86±27	80±20	83±15	0.212
RVESVI (ml/m2)	53±33 Δ*	37±14	37±9	<0.0001
Δ $p < 0.05$ for HFpEF or HFrEF vs controls; * $p < 0.05$ vs HFpEF				

7.1.8.1.2 LA parameters

LA baseline parameters are shown in Table 7.6. Across the cohort and irrespective of whether AF was present or not, HF patients had higher LA volumes, a greater proportion of dilated atria and worse LAEF compared to controls ($p < 0.0001$). Even when LA size was normal, LA dysfunction (defined by LAEF < 44%) was more prevalent in the HF groups. HFrEF patients had worse LA dysfunction and higher LA volumes compared to HFpEF, also irrespective of cardiac rhythm status.

Table 7.6 Overall study baseline CMR LA structural and functional characteristics

	HFrEF N = 46	HFpEF N = 140	Controls N = 48	p value
Overall including AF subjects				
LAEF	29±14 Δ	32±16 Δ	51±11	<0.0001
LAEF < 44%	40 (87) Δ*	103 (74) Δ	10 (21)	<0.0001
Dilated LA	38 (83)	90 (64%)	15 (31)	<0.0001
LAVImax (ml/m2)	59±24 Δ	53±25 Δ	35±12	<0.0001
LAVImin (ml/m2)	44±24 Δ	38±26 Δ	17± 8	<0.0001
LA reservoir volume indexed (ml/m2)	15±7	15±7	17±6	0.087
LA conduit volume indexed (ml/m2)	23±9Δ*	29±9	30±9	<0.0001
Sinus rhythm subjects only				
LAEF (%)	33 12 Δ*	41 ± 12 Δ	51 ± 11	<0.0001
LAEF < 44%	31 (84) Δ*	60 (62) Δ	10 (21)	<0.0001
LAEF < 44% in normal-sized LA	6 (75) Δ*	23 (47) Δ	6 (18)	<0.0001
Dilated LA	29 (78) Δ*	48 (49) Δ	15 (31)	<0.0001
LAVImax (ml/m2)	55±19 Δ*	43±17 Δ	35±12	<0.0001
LAVImin (ml/m2)	38±18 Δ*	26±13 Δ	17±8	<0.0001
LA reservoir volume indexed (ml/m2)	17±6	17±6	17±6	0.957
LA conduit volume indexed (ml/m2)	22±9 Δ*	28±8	30±9	0.001
Δ $p < 0.05$ for HFpEF or HFrEF vs controls; * $p < 0.05$ vs HFpEF				

7.1.8.2 Intra-observer and inter-observer agreements of CMR parameters

The results for CMR intra-observer and inter-observer assessments of LV EF, volumes and mass are shown in Figures 7.2 – 7.5 and Table 7.7. Data for fibrotic assessments, LA and RV parameters have been disclosed in the relevant Chapters previously. All intra-observer agreements were excellent (CoVs < 10%) and universally better than for inter-observer agreements. The majority of inter-observer agreements remained excellent albeit LVESV, RVEF and RVESV fared worse (but still good).

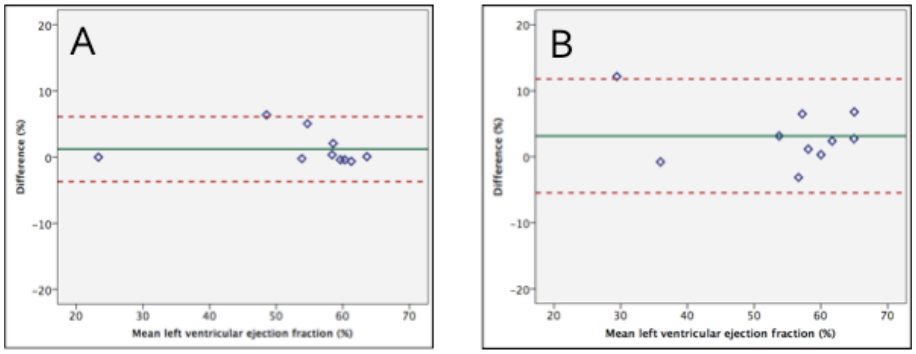


Figure 7.2 Bland-Altman plots for CMR intra-observer (A) and inter-observer (B) assessments of left ventricular ejection fraction

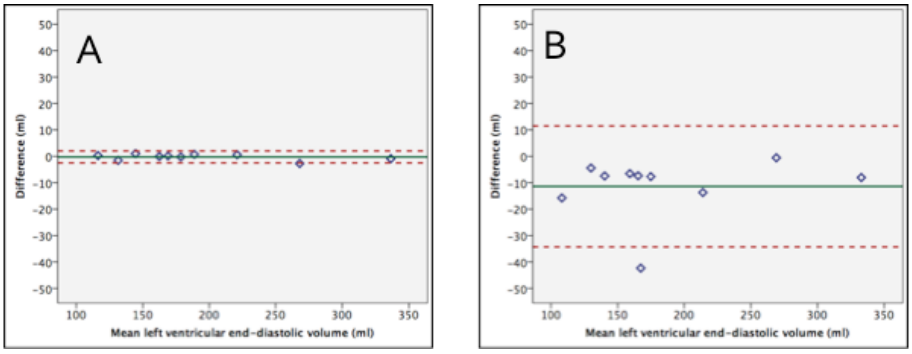


Figure 7.3 Bland-Altman plots for CMR intra-observer (A) and inter-observer (B) assessments of left ventricular end-diastolic volume

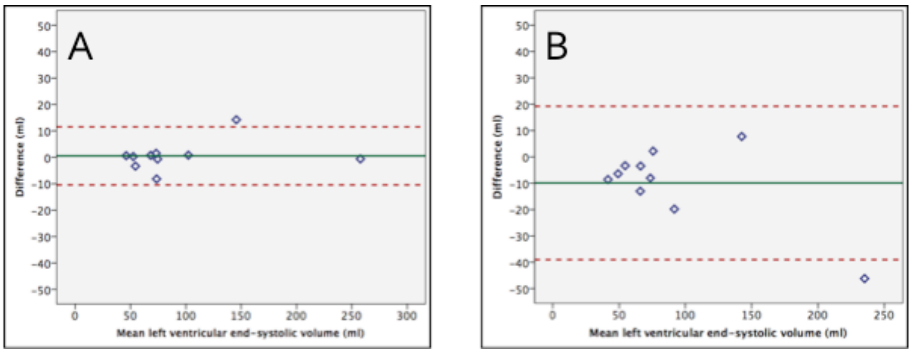


Figure 7.4 Bland-Altman plots for CMR intra-observer (A) and inter-observer (B) assessments of left ventricular end-systolic volume

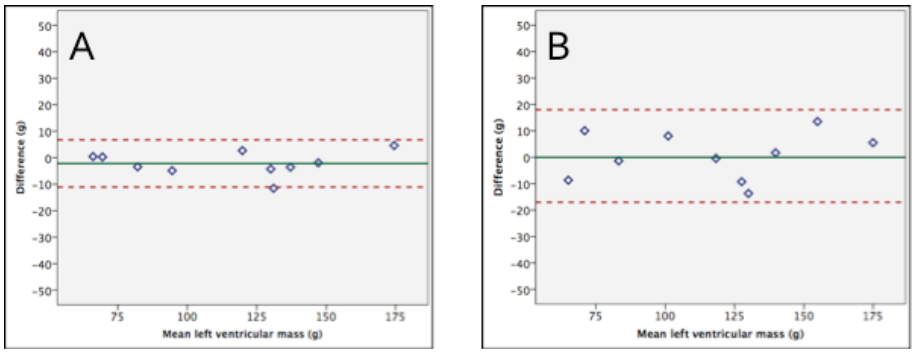


Figure 7.5 Bland-Altman plots for CMR intra-observer (A) and inter-observer (B) assessments of left ventricular mass

Table 7.7 Intra-observer and inter-observer variability for assessment of left ventricular parameters

Parameter	Observer 1 Mean ± SD	Observer 2 Mean ± SD	Mean difference ± SD	ICC	Variability (1 – ICC)	CoV	95% Limits of Agreement
Intra-observer							
LVEF (%)	53 ± 13	54 ± 12	1 ± 3	0.98	0.02	4.7	-4 to 6
LVEDV (ml)	192 ± 68	192 ± 67	-0.3 ± 1	0.99	0.01	0.4	-3 to 2
LVESV (ml)	95 ± 63	95 ± 65	0.5 ± 6	0.99	0.01	5.9	-11 to 12
LV mass (g)	116 ± 36	114 ± 36	-2 ± 5	0.99	0.01	3.9	-11 to 7
Inter-observer							
LVEF (%)	53 ± 13	56 ± 12	3 ± 4	0.91	0.09	8.1	-6 to 12
LVEDV (ml)	192 ± 68	180 ± 70	-11 ± 12	0.97	0.03	6.3	-34 to 12
LVESV (ml)	95 ± 63	85 ± 54	-10 ± 15	0.96	0.04	16.6	-39 to 19
LV mass (g)	116 ± 36	117 ± 37	0.6 ± 9	0.97	0.03	7.7	-17 to 18

7.1.9 Follow-up and endpoints

Overall, the median follow-up was 518 days (356 - 725). Follow-up was longer in HFpEF (616 days [455 – 761]) compared to HFrEF (364 days [267 – 416]). Composite end-point event-rates were similar in HFpEF (n = 44 [31%], 8 deaths, 36 HF hospitalisations) and HFrEF (n = 14 [30%], 2 deaths, 12 HF hospitalisations). There were no events in the control group.

Discussion

The results from our study provide important insights into the clinical and pathophysiological profiles of HFpEF, relative to HFrEF and controls. Firstly, our study re-affirms the clinical heterogeniety of HFpEF evident from large-scale epidemiological studies. Secondly, striking differences in imaging parameters common to both HF groups were noted when compared to controls. Finally, these disturbances in both cardiac structure and function occurred to differing degrees in HFpEF and HFrEF.

7.1.10 Clinical phenotypes and characterisation

Our HFpEF cohort was characterized by a high prevalence of both cardiovascular and non-cardiovascular co-morbidity consistent with prior clinical trial[314] and epidemiological data[12]. Such studies also observed a similar burden of hypertension, obesity, CAD, diabetes, AF, renal dysfunction, lung disease and anaemia[4,9,13,47,322,323].

Compared to HFrEF, HFpEF patients had higher BMI, greater proportion of hypertension and AF but less CAD. These findings are also similar to published literature[4,9,13,47,322,323]. Both HF groups also displayed marked reductions in exercise capacity and poor quality of life, commensurate with a previous study[86]. Unlike, that study however, exercise capacity was lower in HFpEF compared to HFrEF in our cohort. Possible explanations for this include the contribution of a greater co-morbidity burden seen in HFpEF[237], as well as vascular stiffening and reduced aortic distensibilty[121] which were not assessed in our study.

Event rates between both HF groups were also similar, consistent with prior observational[9] and registry[47] data which also revealed comparable mortality[9,47] and HF rehospitalisation[47].

7.1.11 Imaging phenotypes and characterisation

Our study also reinforces the marked pathophysiological heterogeneity evident in HFpEF. In addition to the clear presence of diastolic dysfunction, a pre-requisite for HFpEF diagnosis according to latest guidelines, we observed differences in the at the chamber level afflicting the LV, RV and LA. Furthermore, we noted alterations at the tissue level in terms of the extra-cellular matrix.

The lower LVEF in HFpEF (albeit preserved overall) compared to controls is likely a reflection of mildly reduced overall contractile function or indeed subtle systolic abnormalities[48]. Furthermore, MI or non-ischaemic fibrosis may cause regional disturbances in systolic performance and were evident in nearly half of HFpEF patients in our cohort. Impaired longitudinal systolic function has also previously been observed in multiple HFpEF studies[48,96,98]. In contrast, LVEF was markedly reduced in HFrEF and was associated with higher focal fibrotic burden.

In HFpEF, the predominant pattern of concentric remodeling (or hypertrophy) is a description of increased relative wall thickness and normal chamber dimension[73] and has been shown succinctly by several investigators previously compared to controls and hypertensive subjects without HF[86,127].

At the structural level, these changes are intuitively linked to increased cardiomyocyte hypertrophy (and stiffness) and elevated interstitial collagen content (also observed by the surrogate measure of increased ECV in our cohort)[107,162]. Furthermore, cardiomyocytes in HFpEF grow in a transverse direction keeping cell length constant. In HFrEF, cardiomyocytes grow proportionally in both transverse and longitudinal directions i.e narrow and elongated[107]. However, reinforcing the heterogeneity in HFpEF, large scale epidemiological[74,75] and registry[76] data have also revealed that concentric remodeling/hypertrophy is not the sole pattern evident[74-76] and eccentric patterns (up to

16%) and normal LV geometry (nearly one-third) can also be present in a significant minority[74].

7.1.11.1.1 RVD

RVD may be part of the natural aetiological profile in HFpEF whereby biventricular remodeling often co-exists, even in early stages[316] or as a marker of prognosis[197]. The reported prevalence of RVD in HFpEF is variable (4 to 44%) and has primarily been derived from TTE data across differing populations (clinical trials, community based and registry data), utilising variable definitions of both HFpEF (LVEF ≥ 45% and LVEF > 50%) and different diagnostic thresholds (TAPSE, FAC and RVEF).[311] There has also been conflicting data as to whether the prevalence of RVD is similar[324] or different[198,325] between HFpEF and HFrEF.

In HFpEF, only 2 CMR studies have analysed RV performance and both lacked control groups[204,312]. In the first study[206] (n = 142) significant RVD was defined semi-quantitatively as at the presence of least moderate RV systolic dysfunction (prevalence 12%). The second study[205] (n = 171), a RVEF cut-off of < 45%, primarily based upon ARVC guidelines defined RVD (prevalence 19%) [313]. To the best of our knowledge, no prospective CMR studies have compared RVD in HFpEF and HFrEF.

Our study confirms that RVD is indeed present in a significant minority of HFpEF and that it is also more prevalent in HFrEF compared to HFpEF, based upon our own internal reference controls. As reported in Chapter 6, AF was significantly associated with RVD in HFpEF, suggesting a contributory role and is backed up by similar findings from numerous other studies[200,203,205,314]. Other authors have also implicated the higher burden of lung disease, CAD, diastolic dysfunction and pulmonary hypertension in the aetiology of RVD in HFpEF[200,203,311]

Previous TTE data have provided conflicting evidence on the comparative prevalence of RVD between HFpEF and HFrEF. Whilst some authors[198,325] have reported a greater presence of RVD in HFrEF as in our study, others[324] have shown a similar prevalence between groups. Our findings are likely explained in part by the higher proportion of CAD

(ischaemia and MI) in our HFrEF group which is intrinsically linked to impaired RV contractility[311]. Furthermore, impaired LV contractility is also known to indirectly contribute to RV underperformance[326].

7.1.11.1.2 LA dysfunction and remodeling

In our study both HF groups displayed diminished LA function and more adverse LA remodeling (increased LA volumes), irrespective of AF. Our work is additive to the growing evidence base implicating these parameters in HF[176]. LA dysfunction identifies subjects from the general population at heightened cardiovascular risk[191] as well as those who develop incident HF[192]. Impaired LA function has also been noted in antecedent conditions of HF (e.g. diabetes, hypertension) even in the presence of a normal LA size[185].

Similar to our findings, lower LAEF has been previously shown in HFpEF compared to hypertensive subjects with LVH[127]. Furthermore, a trend towards worse LAEF in HFrEF when compared to HFpEF has also been reported, when imaged with TTE[186]. In that study, analogous to structural changes in the LV, HFrEF appears to display more eccentric LA remodeling whilst HFpEF was characterised by higher LA wall stress. In our study, worsening LA dilation was observed in the HFrEF group compare to HFpEF. In this setting, the greater reductions seen in LAEF is commensurate with the relationship between chamber systolic function and volumes explained by the Frank-Starling mechanism i.e. LAEF reduces to a greater degree at higher volumes as contractile reserve becomes exhausted[186,193].

7.1.12 Implications

Previous authors[327] have questioned whether HFpEF truly exists or whether it is just a collection of co-morbidities in elderly subjects that ultimately drive symptoms and outcomes[12,35]. Firstly, the HFpEF group was characterized by a significant event rate whilst controls did not have any events, providing supportive evidence against this notion. Our study confirms that whilst HFpEF is indeed laden with co-morbidity, it has clear pathophysiological disturbances compared to HFrEF and controls, confirming its existence as a separate entity even when accounting for the influence of age. Whilst both HF groups

shared abnormalities in LV systolic and diastolic function as also reported previously[102], the degree of derangements are however markedly different between the groups.

The structural and functional changes observed in our HFpEF cohort also carry prognostic relevance and have important implications for future study design and therapies. Worse outcomes have been shown previously with regards to LV remodeling[119], focal[157] and diffuse fibrosis[161,285], RVD[200,203,205,206] and LA dysfunction[180,186,187,193,305]. These parameters may represent alternative treatment targets in HFpEF.

The clear distinct patterns of LV remodeling seen in HFpEF (concentric) and HFrEF (eccentric) may in part also explain the differing responses to vasodilator therapy in the 2 groups. The slope of the ESPVR (or end-systolic LV elastance), a measure of contractility is influenced by chamber size[101]. In HFpEF, elastance is increased[57,107] and heightens sensitivity to volume changes resulting in substantial BP drops with vasodilators. In HFrEF however, elastance is diminished[107] and similar therapy improves stroke volume without concurrent BP drops[328].

Spironolactone in mice models of HFpEF has shown attenuation of LV remodeling and diffuse fibrosis (ECV) when assessed with CMR[329]. Identification of focal ischaemic fibrosis may guide appropriate revascularization[142,330]. Restoration of sinus rhythm in patients undergoing catheter ablation improves LA function[309]. Ivabradine albeit in HFrEF, was associated with improved LA mechanics[331]. Insertion of a mechanical inter-atrial septal device in a small pilot study of HFpEF was associated with reduced LA pressure and improved symptom status[310].

At present, treatment options in HFpEF are directed at alleviating symptoms and are limited to diuretic therapy by minimizing volume over-loading and addressing co-morbidity[3]. Outcome based clinical trial data have been neutral at best partly owing to the marked clinical heterogeneity displayed in HFpEF, as also demonstrated in our study. This has led to growing calls to shift focus on therapies targeting specific pathophysiological derangements based on imaging biomarkers (sub-types) of HFpEF[35,330].

The ability of CMR to scan in any imaging plane, its increasing availability, superior spatial resolution and tissue characterisation properties with better reproducibility and repeatability compared to TTE across existing ESC diagnostic markers, as well as those studied in our study suggest that CMR is probably best placed to deliver such studies. This

is further supported by the observation in our overall cohort comprising predominantly elderly subjects (with and with out HF) whereby TTE imaging can be challenging[130,132], of inferior image quality of TTE compared to CMR.

7.1.13 Limitations

We observed a very small minority of HF patients who appeared to transition from HFpEF to HFrEF (4%) and vice versa (19%). This provides a counter argument that HFpEF exists as part of a single HF syndrome and migrates across the HF spectrum in a continuum. Similar instances have also been reported in the literature, primarily in longitudinal studies of hypertensive heart disease[90-93] where interval rates of MI were largely unknown. Recovery of LVEF in HFrEF is also a recognised phenomenon, accounting for nearly 1 in 4 HFrEF patients[332]. These small subsets may contaminate (or cloud) our data. Overall, our single-centre, cross-sectional observational study results are strongly supportive of a separate syndrome hypothesis for HFpEf and HFrEF. Ultimately however, larger scale, longitudinal studies are needed to corroborate this finding.

Conclusions

HFpEF exists as a distinct clinical and pathophysiological entity compared to age- and sex-matched controls. HFpEF is characterised by (mild) reductions in LV function, concentric pattern of remodeling, more myocardial fibrosis (focal and diffuse), LA remodeling and dysfunction and more prevalent RVD when compared with controls. Compared to HFpEF, HFrEF has worse LV, LA and RV contractile function and more prevalent fibrosis (focal and diffuse).

8 CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

Summary of Findings

Our study addresses several gaps in knowledge and hypotheses identified in the introductory Chapter. The principal findings from our intensively phenotyped-HFpEF patients who underwent both CMR and TTE are summarized below.

CMR detected new, alternative clinical diagnoses in a significant proportion of patients labeled as HFpEF by TTE. These alternative diagnoses comprised CAD, MVD, hypertrophic cardiomyopathy and constrictive pericarditis which may masquerade as HFpEF. When added to standard evaluation, CMR increased the true proportion of underlying CAD in HFpEF, compared to baseline. When grouped together, overall such newer diagnoses detected by CMR, were independently associated with clinical end-points.

Following exclusion of HCM and constrictive pericarditis, we undertook further unblinded analysis of a 'purer' cohort of HFpEF. We observed that focal (LGE assessment) fibrosis was more prevalent in HFpEF compared to age- and sex-matched controls. Furthermore, we found that a new marker of diffuse fibrosis studied for the first time in HFpEF, iECV (ECV indexed to BSA) was also greater in HFpEF compared to controls and significantly correlated with indices of LV remodeling, diastolic function and renal function. iECV was also strongly associated with adverse outcome in HFpEF.

LA function and remodeling was assessed using LAEF and LA volumetric measurements. LA dilation was greater and volumes were higher in HFpEF, compared to controls. LAEF was lower in HFpEF and was strongly related to LA volumes. LAEF reliably discriminated patients from controls and higher AUCs than other imaging markers for HFpEF diagnosis (E/E', LVMI, LAVImax). Furthermore, LAEF provided incremental value to current ESC diagnostic markers and was also strongly related to prognosis in HFpEF. Using our own internal controls as a reference, we detected RVD in a significant minority (19%) of HFpEF. RVD was an independent predictor of outcomes in HFpEF and was a more powerful predictor than iECV or LAEF in our cohort.
HFpEF and HFrEF had similar event rates during follow-up. Abnormalities in LV systolic and diastolic function were noted in both HFpEF and HFrEF. However, in comparison to

HFpEF, HFrEF patients had worse LV, LA and RV contractile function and more prevalent fibrosis (focal and diffuse).

Overall, the image quality of TTE was inferior to CMR in our cohort of predominantly elderly subjects. Finally, all the CMR parameters tested in this thesis were analysed with a high degree of feasibility and with excellent intra-observer and inter-observer variability results.

Table 8.1 Summary of structural and functional differences between HFpEF, HFrEF and controls as assessed by CMR

	HFrEF	HFpEF	Healthy
Left ventricle			
LVEF	Down++	Normal	Normal
LV volumes	Dilated	Normal	Normal
LV mass	Increased ++	Increased	Normal
LV mass/volume ratio	Decreased	Increased	Normal
LV Remodeling	Eccentric	Concentric	Normal
Filling pressures	Increased ++	Increased	Normal
Fibrosis			
Focal fibrosis	Increased ++	Increased	Normal
Focal ischaemic fibrosis proportion	Increased ++	Increased	
Focal ischaemic fibrosis size	Increased ++	Increased	
Focal non-ischaemic fibrosis proportion	Increased	Increased	
Focal non-ischaemic fibrosis size	Increased	Increased	
Diffuse fibrosis	Increased ++	Increased	Normal
Left atrium			
LA volumes	Increased ++	Increased	Normal
LAEF	Decreased ++	Decreased	Normal
Right ventricle			
RV dysfunction	Increased ++	Increased	Nil

Implications of study findings

Our study confirms that HFpEF is characterized by clinical and pathophysiological heterogeneity but exists as an entity distinct from both controls and HFrEF. We observed key differences in cardiac structure and function based upon CMR which are possible therapeutic targets.

CMR refines the clinical diagnosis of HFpEF, risk stratifies subjects and sub-categorises patients into both clinical and imaging phenotypes which may enable disease-specific or mechanism focused tailored therapies for e.g. CAD, HCM, fibrosis-targeted or LAEF-targeted. Further, larger scale studies are first needed to validate (and corroborate) our findings as well as assessing the wider impact of CMR in terms of clinical outcome, resource utilization and cost-effectiveness.

Limitations

Specific limitations pertaining to each results Chapter have been disclosed previously. In addition, the single-center study design means that a center-specific bias cannot be excluded. Our study population was highly selected and our results need to be confirmed in larger multi-center studies of unselected cohorts. Outcome data was captured based upon events computerized or from medical records locally in our center. Therefore, some events may have potentially been missed in cases whereby patients presented to other regions. However, there was no systematic bias in detecting outcomes.

The observational nature of our study means that it is ultimately hypothesis generating and causality cannot be inferred. As described previously, the overall study was designed with the primary aim of developing plasma biomarkers for HFpEF and powered at 80% ($p < 0.05$) to detect a standardised difference of 0.45 between HFpEF and the other groups. We initially aimed to recruit: n = 200 HFpEF, n = 50 HFrEF and n = 50 controls. The study was therefore not powered to detect smaller differences between the groups. Furthermore, since overall target recruitment was not achieved, the overall power was slightly reduced. The power calculations were also not based upon outcomes. Therefore, the results should be considered exploratory. Nonetheless, our study comprises one of the largest HFpEF

cohorts to date to have undergone extensive plasma biomarker and CMR profiling.

The demographic data presented in this Thesis are exclusive of details pertaining to patient ethnicity. In subjects devoid of cardiovascular risk, ethnicity is known to be an important factor associated with cardiac structural and functional changes including LV mass, LV and LA volumes[333,334]. Furthermore, although the risk of developing HFpEF is reportedly not different across different ethnicities[335], in hospitalised patients with HFpEF, ethnicity differences have previously shown association with mortality and readmission rates[336]. We did not account for the potential influence of ethnicity on outcomes in our study cohort. Future work assessing the impact of ethnicity obtained from our source data in HFpEF may shed further insights.

Future work and potential developments in HFpEF

Ultimately, the overall aim of our study was to develop both imaging and plasma biomarkers to better phenotype HFpEF. Plasma samples collected as part our study were stored to enable batch analysis at a future stage. As part of an agreement with Bristol-Myers-Squibb, small sample volumes of plasma were transported and analysed (blinded) in the United States of America to test for some of those novel markers described in my published review article[158]. Integrating these plasma markers with imaging parameters to better phenotype could be of significant importance in our understanding, characterising and monitoring of treatment of HFpEF[297,330].

Recently, CMR LA strain measures assessed with feature-tracking software independently predicted incident HF in the general population[192]. As part of our CMR protocol, short-axis LA cine imaging was undertaken in a substantial sub-set. For these, LA volumetric (and LAEF) analysis was also undertaken (by PK). CMR-measured LA strain in long-axis and short-axis cines may provide further insights into HFpEF pathophysiology and assess whether this is related to outcome measures.

In recent studies of phenotypically similar AS patients, myocardial perfusion reserve (MPR) was related to exercise capacity[174] and outcomes[337]. Perfusion analysis of both

stress and rest images has already been undertaken (by *PK*) but absolute blood quantification has not yet been completed, which is dependent upon our collaborator.

As previously mentioned, while similar neurohormonal therapies used in HFrEF[3] have shown clear benefits, the results from HFpEF clinical trials[113] have largely been disappointing. However, further scrutiny of subgroup analyses provides grounds for some optimism. While the Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist (TOPCAT) trial[114] comparing Spironolactone with placebo showed a non-significant (11%) reduction in the composite end-point of cardiovascular death, aborted cardiac arrest or HF re-hospitalisation, significant reductions in overall HF hospitalisations were also noted. Furthermore, following data un-blinding, substantial regional variations in event rates were recorded with markedly lower events in Russia and Georgia compared to the Americas, suggesting that some patients in the Eastern European countries may not truly have had HF[338]. Recent data also suggest that HFpEF patients towards the lower end of the 'preserved EF' spectrum might benefit to a greater extent from neurohormonal therapies that block the renin-angiotensin-aldosterone system than those at the higher end[339]. Finally, since the inception of this Thesis, a landmark study of Sacubitral/Valsartan in HFrEF[340] has shown clear benefits in outcomes compared to standard therapy with ACE inhibition (Elanapril). Similar therapy in a recent phase II trial of HFpEF (n = 301) has also shown promise with reductions in NT-proBNP at 12 weeks, improvements in both LA function and NYHA class at 36 weeks[341]. This has provided the basis for a full outcome study of Sacubitral/Valsartan in HFpEF (n = 4800) for which recruitment is underway and the results are keenly anticipated[342].

9 APPENDICES AND SUPPLEMENTARY DATA

9.1.1 Original advert for healthy volunteers

Volunteers Wanted for Heart

- **Are you over 18 years old?**
- **Do you have heart failure?**
- **Would you like to have a heart MOT?**

If you can answer yes to the above questions you may be able to help and take part in a research study at Glenfield Hospital!

The research study is investigating if there is any way to tell if a person has diastolic heart failure from the chemicals in their blood or urine. It is hoped that the results of this study will in the future improve the diagnosis and treatment of patients.

If you wish to participate or find out more information, please contact:

Research Nurse: Mary Harrison
Glenfield Hospital Leicester
- **(0116) 2583385** • email: **mary.harrison@uhl-tr.nhs.uk**

NHS
National Institute for Health Research

Poster Advertisement:
Biomarkers in Diastolic Heart Failure

Version 1, 13.01.2014

9.1.2 Original Study Information Sheet – for patients

University Hospitals of Leicester NHS
NHS Trust

INFORMATION SHEET FOR PATIENTS WITH WEAKENED HEART FUNCTION

"Sample and data collection for Diastolic Heart Failure Study" (DHF)

Chief Investigator: Professor Leong Ng, Professor & Honorary Consultant in Medicine & Therapeutics.

Invitation to participate
We would like to invite you to take part in a research project. Before you decide we would like you to understand why the research is being done and what it would involve. **We will go through this information with you, please ask any questions you have.**

What is the purpose of the study?

Heart failure is a common problem affecting the health and wellbeing of many individuals. It causes various symptoms ranging from slight shortness of breath, to severe breathlessness, fluid retention, fatigue and reduced ability to carry out day to day activites.

Systolic heart failure means that the ventricles of the heart do not contract properly during each heartbeat so blood is not adequately pumped out of the heart. In some cases there is only a slight reduction in the power of the ventricle, which causes mild symptoms. If the power of the pumping action is more reduced then symptoms become more severe.

Diastolic Heart Failure occurs when the ventricles do not fill up with blood enough when the heart rests in between each heartbeat. This can sometimes be due to the wall of the ventricle being stiffer than usual. This makes it more difficult to stretch.

We want to find out if there is any way to tell if a person has diastolic heart failure from the chemicals in their blood or urine and if these chemicals can help us assess the risk the illness poses to the patient.

Why have I been invited to take part?
You have been invited to take part because you **might** have diastolic heart failure. We need to compare the blood, urine and data of people who might have heart failure to those who do not have heart failure. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form.

Do I have to take part?
No, participation is voluntary and it is up to you to decide if you want to take part. You can withdraw at any time, without giving a reason. This would not affect the standard of care you receive.

What will happen to me if I decide to take part?
If you agree to participate, we will take some blood (up to 25 ml, approximately 5 teaspoons) and urine (up to 200 ml) and store them for investigation into various proteins, chemicals and genetic material that are associated with heart failure. We will ask you about your health, your family history of illness, take measurements of your height and weight, measure your blood pressure, take a tracing of your heart (ECG), monitor your heart rate, ask you to walk for 6 minutes and measure your lung capacity by asking you to blow into a tube. The study also involves 2 types of heart scan, for which you need to have two cannulae (small plastic tubes) inserted into two separate veins so we can administer a 'dye' (contrast agent) to obtain clear pictures. The cannulae are left in during the scans and removed immediately after.

ECG: This is a simple **painless** test used to measure the electrical activity of the heart. You will have 10 small stickers placed on your chest, arms and legs which will be connected to an ECG machine that records a tracing of the heart. The test will only take a few minutes.

AMBULATORY ECG: similar to the ECG, your heart rate will be monitored whilst you are resting and walking. We will connect small stickers on your chest to a lightweight recording box attached to a belt around your waist or hanging loosely around your neck. This will be on worn for approximately two hours.

BLOOD PRESSURE MEASUREMENT: This is again, a simple **painless** test using an inflatable cuff to measure the pressure of blood in the vessels of the arms. In addition, we would measure the pressure in the aorta (major blood vessel leaving the heart) and the speed of blood flow around your body using the same cuff on the thigh and a light and gentle inflatable cuff around the neck.

ECHOCARDIOGRAM: This takes about 30 minutes. Gel is put on to the chest, and pictures of the heart are taken using an ultrasound probe.

CARDIAC MRI: Magnetic Resonance Imaging (MRI) are safe- no radiation is used for this scan. There are no known risks from the technique. Some people may experience claustrophobia. Our MRI staff will do all that they can to make you feel comfortable during the scan, and will be monitoring you via a video camera and audio link. If we are unable to make you feel comfortable in the scanner, we will not go ahead with the scan, you can still participate in the rest of the study. We will need to insert two small tubes (cannulae) into your arms for the contrast dye and the adenosine medication. The contrast medication we use during the scan is very safe but, as with any injection, reactions may occur. These include

a warm sensation at the injection site, nausea or vomiting and transient skin rash. These effects usually only last for a few minutes. People with a history of allergy are more likely to suffer a more severe reaction, but this is rare (less than 1 in 3000). The department is equipped to cope with allergic reactions if they happen. Adenosine, the medication we use to increase the blood flow to the heart, can cause flushing, breathlessness and chest discomfort. However, all of these feelings usually subside within one or two minutes or even more quickly when the medication is stopped. If you are sensitive to Adenosine, Dobutamine may be used instead.

This is usually all done during one study visit which will last 2-3 hours. Occasionally, we *may* ask to split your visit in order to make your participation as efficient and pleasant as possible. The research staff will make sure you are comfortable during your visit and can provide you with drinks and snacks. After the research visit the researchers will periodically look at your medical records to source certain information for up to 20 years.

Will I be paid for taking part?
You will not be paid for taking part in the research, but reasonable travel expenses can be reimbursed and free parking will be made available for you.

What will I have to do?
Other than attending the study visit you do not have to do anything.

What are the possible disadvantages and risks of taking part?
This is a very safe research project. You will need to attend The NIHR Biomedical Research Unit at Glenfield Hospital for a 2-3 hour study visit. You will have a needle to take blood, and a cannula (plastic tube in the blood vessel) during the scans. These can cause bruising and slight pain. For the scans we will need to inject a contrast 'dye' to obtain the pictures, and this rarely causes side effects except occasionally mild nausea and flushing which quickly stops.

What are the possible benefits of taking part?
We cannot promise the study will directly benefit you, but the information we get from this study might help the treatment of future patients. If you take part in a study you will have more contact with us during your visit, and have more opportunities to ask questions and be informed about your health, which some patients find helpful.

What if there is a problem during the study visit?
This is a very safe project and you are unlikely to be harmed. Medical support, indemnity against negligent harm, and the NHS complaints mechanism are available to you.

Will my taking part in the study be kept confidential?
Yes, we will follow legal and ethical guidelines to keep your participation confidential.
<u>If you might like to participate please read the detailed information below.</u>

What will happen if I don't want to carry on taking part in the study?
You can contact the research team to let them know that you no longer wish to take part in the research, or parts of the research and you do not need to give a reason.

What if there is a problem?
If you feel unwell or are injured during the study then one of the study staff will provide you with appropriate medical care. If you want to make a complaint about the project you can speak to the research team, or use the NHS complaints mechanism. The Patient Information and Liaison Service can help with this; their number is 08081 788337. NHS indemnity applies to this project and you can take legal action in the event that you are harmed through negligence but you may have to pay for legal support to do so.

Will my participation be kept confidential?
Yes, we will keep your information confidential and secure in compliance with the Data Protection Act 1998.

We ask for your permission to tell your GP if we find anything wrong with your heart. We will also talk to you about anything we find that is wrong and may decide to make a referral to the hospital services.

We will not be using your blood or urine for quite a while, and so we will not tell you about results we get from your samples. We will protect your identity by giving your samples a study ID number. This will be linked to your personal data but only senior researchers will be able to access the list linking these bits of information. We protect your medical data in the same way and store it in a secure database.

The data and sample collection will be managed by the NIHR Cardiovascular Biomedical Research Unit, Leicester. If you have consented to have your samples stored in their tissue bank, and for researchers to have access to information in your medical records you can be confident that they will only be used in high quality, ethical research and that they will not be able to access information that identifies you. The Unit will send you invitations to participate in research on behalf of researchers so they will not disclose your contact details or identity.

It is also very helpful if we can track your health condition throughout the study using national records and we will ask for your permission to do this. The Medical Research Information Service (MRIS) and Hospital Episode Statistics (HES) allows us to access health information about you. In order to do this, we are seeking your permission to provide these services with some of your personal details (including your name, date of birth, address and NHS number). With this information, we will be able to access simple health information about you for the duration of the study (up to 20 years) to allow us to understand the long-term progress of patients with heart failure. Information will be provided in strict confidence and will be kept securely. You **will not** be contacted by these services at any time.

Sometimes, research projects and tissue banks are inspected to ensure that they are run well and the people conducting inspections will be able to access your data and samples and identity but are legally bound to keep that confidential.

Will my GP know that I am taking part?

We will not routinely tell your GP that you are taking part in the research but we request your permission to contact them if we think there might be something wrong with your heart. We will always discuss this with you so that you know we have done so.

What will happen to any samples that I provide?
You will provide us with a sample of blood and a sample of urine. They will be analysed in a laboratory so we can decide if there are any differences between the blood and urine of people who do and do not have diastolic heart failure. This will include genetic testing. If you have agreed to it, the samples will be part of a tissue bank and analysed for all sorts of different heart research projects.

Samples are stored securely and are accessible only to those authorised to access them including laboratory staff who process, store and analyse them and the researchers working on this project (and other authorised projects if you agreed to have the samples placed in the tissue bank).

What will happen to the results of the research?
Results of the research will be published in journals, or shared at conferences or poster presentations. These will not identify you individually. Research is used to guide doctors, nurses and other healthcare professionals when they diagnose and treat their patients so ultimately the results will help them provide healthcare.

We will send you a summary explaining what we found out by doing this research project if you consent to us doing so.

If you would like to keep up to date about the work happening in the NIHR Cardiovascular Biomedical Research Unit, Leicester you can contact the Research Governance and PPI Officer on 0116 258 3473 and ask to receive the newsletter.

Who is organising and funding this research?
This research is organised and funded by the NIHR Cardiovascular Biomedical Research Unit at the University Hospitals of Leicester NHS Trust and University of Leicester. The Unit is funded by the National Institute for Health Research which is part of the Department of Health.

There is a lot of heart research taking place through the NIHR Cardiovascular Biomedical Research Unit at Leicester, so we are also asking for your permission to include you samples and data in a tissue bank for use in other heart research. Tissue banks mean we can do a lot of research without having to keep asking patients for samples and information for individual research projects so they are very useful and can

1. Use your blood and urine in other heart research projects
2. Allow the tissue bank to source information from your medical records for heart research
3. Allow staff from this tissue bank to contact you with invitations to participate in other research projects (you would be under no obligation to agree to take part in them).

You can still take part in the research project without giving these additional permissions. Who has reviewed the project?

All research in the NHS is looked at by independent group of people, called a Research Ethics Committee, to protect your interests. The project has been given a favourable opinion by East Midlands (Nottingham) Research Ethics Committee.

How do I contact the research team?
The research team can be contacted through the NIHR Cardiovascular Biomedical Research Unit, Leicester.
Mary Harrison (Research Nurse)
NIHR Cardiovascular Biomedical Research Unit
Glenfield Hospital
Groby Road
Leicester
LE3 9QP
0116 2583385
bruadmin@leicester.ac.uk

Thank you for taking the time to consider participating in this project.

9.1.3 Ethics approval

NHS
Health Research Authority

NRES Committee East Midlands - Nottingham 1
The Old Chapel
Royal Standard Place
Nottingham
NG1 6FS

Telephone: 0115 8839309
Facsimile: 0115 8839924

24 July 2012

Professor Leong L Ng
Professor of Medicine & Therapeutics
University of Leicester
Cardiovascular Sciences
Clinical Sciences Building
Leicester Royal Infirmary
LE2 7LX

Dear Professor Ng

Full title of study:	**Sample and data collection for diastolic heart failure study: Biomarkers for Diastolic Heart Failure**
REC reference number:	**12/EM/0222**

Thank you for your letter of 10th July 2012. I can confirm the REC has received the documents listed below as evidence of compliance with the approval conditions detailed in our letter dated 12 June 2012. Please note these documents are for information only and have not been reviewed by the committee.

Documents received

The documents received were as follows:

Document	*Version*	*Date*
Covering Letter		10 July 2012
Advertisement	2	10 July 2012
Participant Consent Form: Consent Form for Healthy Controls	1.0	10 July 2012
Participant Information Sheet: Healthy Volunteer Information Sheet	2.0	10 July 2012
Participant Information Sheet: Information Sheet for Patients with Weakened Heart Function	2.0	10 July 2012

You should ensure that the sponsor has a copy of the final documentation for the study. It is the sponsor's responsibility to ensure that the documentation is made available to R&D offices at all participating sites.

12/EM/0222	**Please quote this number on all correspondence**

Yours sincerely

W Rees

Wendy Rees
Assistant Committee Co-ordinator

9.1.4 Consent Form for patients

Insert patient study identification number here

NHS
National Institute for Health Research

CONSENT FORM FOR PATIENTS
Sample and data collection for diastolic heart failure study

		Please initial		
1.	I confirm that I have read and understood the Patient Information Sheet, Version 4 dated 21.3.13 for the Diastolic Heart Failure research, and have had the opportunity to ask questions.	☐		
2.	I understand that my participation is voluntary and that I can withdraw at any time. This will not affect the medical care I receive.	☐		
3.	I agree to donate blood and urine samples to allow their use in this research project. I understand that I will not receive any individual feedback or benefit from any intellectual property that could result from the use of the samples.	☐		
			YES	NO
4.	OPTIONAL I consent to my samples being stored in a tissue bank for use by other researchers managed by the NIHR Cardiovascular Biomedical Research Unit, Leicester	☐	☐	☐
5.	I agree to the researchers creating a database that contains information sourced from my medical records over the next 20 years.	☐		
			YES	NO
6.	OPTIONAL I consent to information being obtained from my medical records and stored in the NIHR Cardiovascular Biomedical Research Unit data and tissue bank for use in other heart research.	☐	☐	☐
7.	OPTIONAL I consent to some of my personal information including name, date of birth, address and NHS number being shared in strict confidence with the NHS Medical Research Information Serice (MRIS) and Hospital Episode Statistics (HES) which will provide information about my health condition to the researchers over the next 20 years.	☐	YES ☐	NO ☐
8.	OPTIONAL I agree to be contacted in future if there are research projects I might wish to participate in.	☐	YES ☐	NO ☐
9.	I consent to the researcher contacting my GP if my scans show there is a problem with my heart.	☐		
10.	OPTIONAL I would like to receive updates about the research project.	☐	YES ☐	NO ☐
11.	I agree to my records being looked at by representatives of the sponsor or the bodies which govern research in the UK where it is relevant to the conduct of the project e.g. audit	☐		
12.	I agree to take part in the study and understand what it involves.	☐		

Patient name:	Name of person taking consent:
Signature	Signature
Date	Date

9.1.5 Consent Form for controls

Insert patient study identification number here

NHS
National Institute for Health Research

CONSENT FORM FOR HEALTHY VOLUNTEERS
Sample and data collection for diastolic heart failure study

Please initial

1. I confirm that I have read and understood the Information Sheet for Healthy Volunteers, Version 4 dated 21.3.13 for the Diastolic Heart Failure research, and have had the opportunity to ask questions. ☐
2. I understand that my participation is voluntary and that I can withdraw at any time. This will not affect the medical care I receive. ☐
3. I agree to donate blood and urine samples to allow their use in this research project. I understand that I will not receive any individual feedback or benefit from any intellectual property that could result from the use of the samples. ☐

YES NO

4. OPTIONAL I consent to my samples being stored in a tissue bank for use by other researchers managed by the NIHR Cardiovascular Biomedical Research Unit, Leicester ☐ ☐
5. I agree to the researchers creating a database that contains information sourced from my medical records over the next 20 years. ☐

YES NO

6. OPTIONAL I consent to information being obtained from my medical records and stored in the NIHR Cardiovascular Biomedical Research Unit data and tissue bank for use in other heart research. ☐ ☐
7. OPTIONAL I consent to some of my personal information including name, date of birth, address and NHS number being shared in strict confidence with the NHS Medical Research Information Serice (MRIS) and Hospital Episode Statistics (HES) which will provide information about my health condition to the researchers over the next 20 years. YES ☐ NO ☐
8. OPTIONAL I agree to be contacted in future if there are research projects I might wish to participate in. YES ☐ NO ☐
9. I consent to the researcher contacting my GP if my scans show there is a problem with my heart. ☐
10. OPTIONAL I would like to receive updates about the research project. YES ☐ NO ☐
11. I agree to my records being looked at by representatives of the sponsor or the bodies which govern research in the UK where it is relevant to the conduct of the project e.g. audit ☐
12. I agree to take part in the study and understand what it involves. ☐

Name of volunteer:	Name of person taking consent:
Signature	Signature
Date	Date

9.1.6 Copy of the original study paper case record form

DHF Trial initial assessment protocol & checklist (i.e. patient CRF)

Patient ID No: ☐☐☐☐☐☐☐☐☐☐

Patient Tel No: ☐☐☐☐☐☐☐☐☐☐

Screening Date: ☐☐-☐☐-☐☐☐☐

Assessment Date: ☐☐-☐☐-☐☐☐☐

Recruitment setting *(tick)*:

Out-patient: HF ☐ General Cardiology ☐ Hypertension ☐

In-patient: (specify) ward _____

Other: (specify) _____

Demographics:

Age: ☐☐ Gender: M ☐ F ☐

Ethnicity: Caucasian ☐ South Asian ☐ Black ☐ Other ☐

Inclusion Criteria:

Heart failure (signs & symptoms / radiography/ known diagnosis) Yes ☐ No ☐

Aged 18 & above Yes ☐ No

☐

LVEF <40% Yes ☐ No

☐

Or

LVEF >50% Yes ☐ No

☐

Exclusion criteria:

Patient unable to give consent Yes ☐ No

☐

Non-cardiovascular life expectancy < 6 months Yes ☐ No

☐

Recent MI (< 6 months) Yes ☐ No

☐

Greater than moderate valve disease Yes ☐ No

☐

Known severe lung disease (or FEV_1 < 30% or FVC <50% predicted) Yes ☐ No

☐

eGFR <30 Yes ☐ No

☐

Absolute contraindication to CMR: *(Please circle list if yes)* Yes ☐ No

☐

Permament pacemaker or ICD

Brain Aneurysm Clip

Implanted neural stimulator

Cochlear implant (specific implant must be checked that it is MR safe)

Ocular foreign body (e.g. metal shavings) - unless removed

Other implanted medical devices: (e.g. Swan Ganz catheter, temporary pacing wire)

Insulin pump

Metal shrapnel or bulle

Renal dysfunction (eGFR <30ml/min)

Absolute contraindication to Adenosine: *(Please circle list if yes)* Yes ☐ No

☐

2nd or 3rd degree AV block

Atrial Flutter with heart block (≥3:1)

Severe asthma

Unstable angina pectoris

Known hypersensitivity to adenosine

Sinus bradycardia (heart rate < 40 b.p.m)

Systolic BP < 90 mmHg

Note: If absolute contraindications to CMR, patients can still volunteer for the other investigations. If absolute contraindication to Adenosine, Dobutamine may be used for stress.

Heart Failure Diagnosis:

Clinical features must include at least **one** typical HF symptom/ more specific sign

OR

If less typical symptom/ less specific sign, must be accompanied by radiographic evidence (raised CTR/ pulmonary oedema)

Typical HF symptoms:

Breathlessness Yes ☐ No

☐

Orthopnoea Yes ☐ No

☐

PND Yes ☐ No

☐

Reduced Ex Tolerance Yes ☐ No

☐

Fatigue/tiredness/increased time to recover following exercise Yes ☐ No

☐

Ankle swelling Yes ☐ No

☐

Less typical HF symptoms:

Nocturnal cough Yes ☐ No

☐

Wheeze Yes ☐ No

☐

Weight gain > 2kg/week Yes ☐ No

☐

Weight loss (in advanced HF) Yes ☐ No

☐

Bloated feeling Yes ☐ No

☐

Loss of appetite Yes ☐ No

☐

Confusion Yes ☐ No

☐

Depression Yes ☐ No

☐

Palpitations Yes ☐ No

☐

Syncope Yes ☐ No

☐

***More specific HF signs*:**

Raised JVP Yes ☐ No

☐

Hepatojugular reflux Yes ☐ No

☐

S3 gallop Yes ☐ No

☐

Laterally displaced apex Yes ☐ No

☐

Cardiac murmur Yes ☐ No

☐

Less specific HF signs:

Peripheral oedema (ankle/sacral/scrotal) Yes ☐ No

☐

Pulmonary crepitations Yes ☐ No

☐

Reduced air entry at lung bases/effusion Yes ☐ No

☐

Tachycardia Yes ☐ No

☐

Irregular pulse Yes ☐ No

☐

Tachypnoea (>16 b.p.m) Yes ☐ No

☐

Hepatomegaly Yes ☐ No

☐

Ascites Yes ☐ No

☐

Cachexia (tissue wasting) Yes ☐ No

☐

CXR:

Cardiothoracic ratio ≥ 50%: Yes ☐ No

☐

Pulmonary oedema: Yes ☐ No

☐

Aetiology of Heart Failure:

IHD ☐ Hypertension ☐ Valve disease ☐ Idiopathic

DCM ☐ Restrictive ☐ Unknown ☐ Other ☐

Examination:

Heart rate (b.p.m): 1 ☐☐☐ 2 ☐☐☐ 3 ☐☐☐ Average

☐☐☐

Systolic BP (mmHg): 1 ☐☐☐ 2 ☐☐☐ 3 ☐☐☐ Average

☐☐☐

Diastolic BP (mmHg): 1 ☐☐☐ 2 ☐☐☐ 3 ☐☐☐

Average ☐☐☐

Note: Patient should be seated and resting for 10 minutes before measuring HR & BP, use dominant arm, measure x3, document average readings

Respiratory Rate (b.p.m): ☐☐☐ Temp (^{0}C) ☐☐☐

Height: ☐☐☐cm & Feet ☐☐☐/inches ☐☐☐

Weight (kg): ☐☐☐ BMI (kg/m^{2}): ☐☐

CVS Risk Factors:

DM □ Hypertension □ Raised lipids □ Smoker □

Ex-smoker □ Family History of IHD □

Medical History:

Chronic HF □ Angina □ MI □ AF □ CAD □

PCI □ CABG □ CKD □ Asthma □ PVD □ CVA □

TIA □ Gout □ Valvular disease □

Other medical / surgical history:

Medication:

Functional assessment:

NYHA class: I ☐ II ☐ III ☐ IV ☐

EQ-5D questionnaire: Yes ☐ Date _____

(tick & date)

MLHF questionnaire: Yes ☐ Date _____

(tick & date)

ECG: Yes ☐ Date _____

(tick & date)

Spirometry: Yes ☐ Date _____

(tick & date)

FEV_1 (% predicted normal) ☐☐

FVC (% predicted normal) ☐☐

FEV_1/FVC ratio ☐☐

Consent Form: Yes ☐ Date _____

(tick & date)

Consent Form for reproducibility: Yes ☐ Date _____

(tick & date)

Bloods & Urine sample: Yes ☐ Date _____

(tick & date)

6 minute walk test: Yes ☐ Date _____

(tick & date)

Echo: Yes □ Date _____

(tick & date)

CMR: Yes □ Date _____

(tick & dat

9.1.7 Copy of the original Spirometry standard operating protocol

DHF Trial Spirometry Protocol

Patient preparation

Ensure patient is comfortable

Explain the purpose of the test

- To see if they are eligible for the DHF trial
- To ensure there is no abnormality to suggest that symptoms are related to possible lung problems

Performing hand-held spirometry

Demonstrate correct technique yourself (first)

Attach a **new**, clean, disposable, one-way mouthpiece to the spirometer

Ask the patient to breathe in as deeply as possible (full inspiration)

Ask the patient to breathhold just long enough to seal their lips around the mouthpiece

*Note: The patient should **not** purse their lips as if blowing a trumpet*

Ask the patient to pinch their nose or wear a nose clip

Ask the patient to now blow the breath out, forcibly, as hard and fast as possible, until there is nothing left to breathe out

Note: for COPD patients, this can take upto 15 secs, encourage the patient to keep blowing out

Perform a total of 3

The best 2 recordings should ideally be within 100mls or 5% of each other

Document the **best** of the 3 recordings for analysis

Interpretation

Compare FEV_1 to the predicted normal values and calculate the percentage of the predicted value

Compare FVC to the predicted normal values and calculate the percentage of the predicted value

Record & exclude if:

FEV1 < 30% predicted
FVC < 50% predicted

9.1.8 Copy of the original Echocardiography protocol

DHF STUDY

ECHOCARDIOGRAPHY STUDY PROTOCOL

General Information

1. *Patient information*

a) The patient information should be entered by the study name DHF (entered into the surname field) and the study imaging code generated (into the Subject ID field)

b) Do not enter the patient name, hospital number or date of birth

c) Input height and weight

2. *ECG gating*

All cine loops & still frames must contain a clearly displayed QRS complex

3. *Number of recorded beats in all the views listed below*

In sinus rhythm, 3 beat acquisition is mandatory

In atrial fibrillation, 5 beat acquisition is mandatory

In fast atrial fibrillation (heart rate > 90 b.p.m), consider taking a 10 beat acquisition

4. *Recordings and analysis of transmitral flow, pulmonary venous flow and tissue Doppler* measurements must be performed at the end of non-forced expiration

4. *Recording images*

Please record images without measurements (these will be performed off-line)

5. *Image storage*

All echocardiographic studies will be stored on the Xcelera storage system for off-line analysis

Study Personnel

Image acquisition and analysis should be performed independently by echocardiographers with full BSE accreditation for adult transthoracic echocardiography.

Named personnel eligible:

Dr Anna Marie Marsh

Mr John Macadam

Dr Prathap Kanagala

Equipment

Echocardiographic studies will be performed using the commercially available iE33 Philips machine equipped with a broadband transducer S5-1 (frequency transmitted 1.7 MHz, received 3.4 MHz) or another with equivalent specifications.

Echo study protocol

View	Modality	Structures visualised & assessed
Parasternal long axis (PLAX)	2D	LV cavity
		Interventricular septum
		Posterior wall
		LVOT (measure on zoom)
		Aortic valve, aortic root
		Right ventricular cavity
		Left atrium

		Mitral valve
	M-mode	Left ventricle, right ventricle
		Mitral valve
		Aortic root, aortic valve, left atrium
	CFM	Mitral valve (MR)
		Aortic valve & LVOT (AR)
		Interventricular septum (VSD)

Parasternal short axis (PSAX) *AV level*	2D	Aortic valve
		Tricuspid valve
		Right ventricle
		Pulmonary valve
		Pulmonary artery
	CFM	Atrial septum (ASD)
		Aortic valve (AR)
		Tricuspid valve (TR)
		RVOT
		Pulmonary valve
		Pulmonary artery
	CW	Tricuspid valve
		Pulmonary valve

Parasternal short axis (PSAX) *Base*	2D	Left ventricle
		Right ventricle
		Mitral valve
	CFM	Mitral valve (MR)

Parasternal short	2D	Left ventricle

axis (PSAX) *Mid*		
		Right ventricle

Parasternal short axis (PSAX) *Apex*	2D	Left ventricle
		Right ventricle

Apical four chamber (A4C)	2D	Left ventricle (including zoom)
		Right ventricle
		Left atrium
		Right atrium
		Mitral valve
		Tricuspid valve
		Pulmonary vein (Right upper)
	M-mode	Lateral mitral annulus - MAPSE
		Tricuspid annulus – TAPSE
	CFM	Mitral valve
		Tricuspid valve
		Pulmonary vein (Right upper)
	CW	Mitral valve
		Tricuspid valve
	PW	Mitral valve tips (E, A)
		Pulmonary vein (Right upper)
	TDI	Lateral wall (mitral annulus)
		Septum (mitral annulus)

Apical five chamber (A5C)	2D	LVOT
		Aortic valve
		Aortic root

	CFM	LVOT, aortic valve and aortic root
	CW	LVOT, aortic valve and aortic root
	PW	LVOT (Images to enable measurement of IVRT)

Apical two chamber (A2C)	2D	Left ventricle
		Left atrium
		Mitral valve
	CFM	Mitral valve

Apical three chamber (A3C)	2D	Left ventricle
		LVOT
		Aortic valve
		Mitral valve
	CFM	LVOT, aortic valve, aortic root
		Mitral valve
	CW	LVOT, aortic valve, aortic root
	PW	Mitral valve tips (E, A), LVOT

Subcostal	2D	All 4 cardiac chambers / structures
		Atrial septum
		IVC
	M-mode	IVC
	CFM	Atrial septum

Suprasternal	2D	Arch

	CFM	Arch, aorta
	CW	Ascending and descending aorta
	PW	Descending aorta (if there is flow reversal AR)

General measures to optimize image quality for the regions of interest

2D

Use the narrowest sector width possible

Use the smallest depth possible (do not display structures outside the regions of interest)

Focus should be set to middle of the image

Greyscale & dynamic range should be adjusted accordingly to produce marked contrast between echodense & echolucent areas

Adjust gain (and time-gain compensation) to eliminate background noise and enable clear blood-tissue boundaries

Use harmonic imaging with maximal outputs from the machine

CFM

Use the smallest sector width possible

Use a velocity range of approximately 60 cm/sec

Tissue Doppler

Position sample volume at or 1 cm within the lateral & septal mitral annulus insertion sites

Use low gain settings

Set aliasing velocity between 15-20 cm/sec

Set sweep speed to 50-100 mm/sec

Set frame rates between 40-80 frames/sec (ensure adequate endocardial and epicardial border definitions)

Optimise baseline to maximize the velocity excursion

Minimise the angle between the direction of cardiac motion and sampling to < 20%

Use a sample volume of 5-10mm

Pulmonary vein sampling

In the A4C view, angulate the transducer probe slightly superiorly

Use CFM to guide location (initially start with standard velocity range of approximately 60cm/sec & reduce as required to visualize low velocity flow

Use a sample volume of 2-3mm

Place sample volume > 0.5 cm into the pulmonary vein

Set wall filters low enough to visualise onset & cessation of atrial reversal (AR)

Set sweep speed between 50-100 mm/sec

Mitral valve inflow (PW)

Use a sample volume of 1-3 mm

Place sample volume at the level of the mitral valve tips

Optimise gain and wall filter settings to ensure a clear spectral display

Set sweep speed between 50-100 mm/sec

LA volume

Use Simpson's rule (method of discs)

Use A4C & A2C views

After optimizing the image using 2D as above, set focal zones distally to improve lateral resolution in the far field

Increase gain just to the point at which image drop-outs in the atrial walls have disappeared

Obtain image with maximal LA size (to ensure no foreshortening)

Select adequate frames for planimetry (for maximal volume – select end-systolic frame just before mitral valve opening at the end of the T-wave on ECG)

Planimeter LA area

- the mitral annular plane is the inferior border
- exclude the appendage and pulmonary veins

LA long axis measurement should be orthogonal to mitral annular plane from its midpoint to superior margin of LA

LVEF

Use Simpson's rule (method of discs)

Use A4C & A2C views

Optimise image as per LA volumes

Obtain image with maximal LV length (to ensure no foreshortening & move a rib space down if necessary)

Select adequate frames for planimetry (end-systolic frame at the end of the T-wave & end-diastolic frame at the end of the R wave on the ECG)

The aortic valve should be out of the image plane

The LV apex should be visible at the top of the sector

Planimeter LV area

- The mitral annual plane is the superior border
- Exclude the papillary muscles

LV long axis measurement should be orthogonal to mitral annular plane from its midpoint to the apex

Calculations / assessment tools / references for ECHO CRF

LV mass

LV mass calculation will be used as per the cubic method (built in software package)

Measurements required are: LVIDd, LVISDd, LVPWd

Devereux RB, Alonso DR, Lutas EM, Gottlieb GJ, Campo E, Sachs I, Reichek N. Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings.1986 Feb 15;57(6):450-8.

LVEF/LVESV/LVEDV - indexed

Calculation will be using Simpson's rule (method of discs)

Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise JS, Solomon SD, Spencer KT, Sutton MS, Stewart WJ; Chamber Quantification Writing Group; American Society of Echocardiography's Guidelines and Standards Committee; European

Association of Echocardiography.Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr. 2005 Dec;18(12):1440-63.

LA volume/Mitral inflow/Pulmonary venous flow/Tissue Doppler annular velocities

Use to reference normal values and grading of severity (LA size & diastolic dysfunction)

Recommendations for the evaluation of left ventricular diastolic function by echocardiography.
Nagueh SF, Appleton CP, Gillebert TC, Marino PN, Oh JK, Smiseth OA, Waggoner AD, Flachskampf FA, Pellikka PA, Evangelista A. Recommendations for the evaluation of left ventricular diastolic function by echocardiography. J Am Soc Echocardiogr. 2009 Feb;22(2):107-33.

Software used to generate data will be from the QLAB Xcelera CMQ (cardiac myocardial quantification)

9.1.9 Copy of the original six minute walk test protocol

DHF Trial Six minute walk test Protocol

Check for Contraindications

Absolute

Unstable angina within preceding month

Myocardial Infarction within preceding month

Relative

Resting HR > 120 b.p.m

Systolic BP > 180 mmHg

Diastolic BP > 100 mmHg

Note: If any of the above please discuss with named personnel (doctor) on DHF trial

Patient preparation

The patient should:

- wear comfortable clothing
- wear appropriate shoes for walking
- use usual walking aids during the test e.g. walking stick, Zimmer frame
- not have exercised strenuously within 2 hours prior to the test
- sit at rest in a chair near the starting position for a minimum of 10 minutes prior to the test
- use supplemental Oxygen as per prescribed regime if on long-term therapy

Location & course

- Indoors

- Long, flat, straight course (100-feet hallway)
- Course length but be 30 metres
- Start the test at a point marked on the floor
- Turn around points should be marked by a cone (x2 in total)
- The length of the course should be marked every 3 metres

Reasons for termination

Once 6 minutes have elapsed

OR

If any of the following are present:

- Chest pain
- Intolerable breathlessness
- Leg cramps
- Patient is staggering
- Patient is sweaty
- Patient is pale/ “ashen faced”

OR

- If patient unwilling to continue (if so, document reason)

Safety

If the test is terminated early due to any of the above points, the patient should sit/ lie supine as appropriate.

Following measures should be taken:

- Blood pressure
- Pulse rate
- Oxygen saturations
- Discussion with medical personnel involved in the DHF trial

The technician responsible for supervising the test should be:

- Familiar with the location of the nearest cardiac arrest trolley
- certified in Basic Life Support

Recordings

Before and after test

- BP
- Pulse rate
- Oxygen saturations (pulse oximetry)
- Fatigue & dyspnea using Borg scale

Total number of laps = completed laps + final partial lap

Note: 1 completed lap = 60 metres, measure partial lap using the 3 metre markers and round up to nearest metre

Total distance walked in 6 minutes (in metres)

Test instructions

Before

Instruct the patient as follows:

"The object of this test is to walk as far as possible for 6 minutes. You will walk back and

forth in this hallway. Six minutes is a long time to walk, so you will be exerting your- self. You will probably get out of breath or become ex- hausted. You are permitted to slow down, to stop, and to rest as necessary. You may lean against the wall while rest- ing, but resume walking as soon as you are able.

You will be walking back and forth around the cones. You should pivot briskly around the cones and continue back the other way without hesitation. Now I'm going to show you. Please watch the way I turn without hesitation."

Demonstrate by walking one lap yourself. Walk and pivot around a cone briskly.

"Are you ready to do that? I am going to use this counter to keep track of the number of laps you complete. I will click it each time you turn around at this starting line. Remember that the object is to walk AS FAR AS POSSI- BLE for 6 minutes, but don't run or jog.

Start now, or whenever you are ready."

<u>During</u>

Position the patient at the starting line. You should also stand near the starting line during the test. Do not walk with the patient. As soon as the patient starts to walk, start the timer.

Do not talk to anyone during the walk. Use an even tone of voice when using the standard phrases of encourage- ment. Watch the patient. Do not get distracted and lose count of the laps. Each time the participant returns to the starting line, click the lap counter once (or mark the lap on the worksheet). Let the participant see you do it. Ex- aggerate the click using body language, like using a stop- watch at a race.

After the first minute, tell the patient the following (in even tones): "You are doing well. You have 5 minutes to go."

When the timer shows 4 minutes remaining, tell the pa- tient the following: "Keep up the good work. You have 4 minutes to go."

When the timer shows 3 minutes remaining, tell the pa- tient the following: "You are doing well. You are halfway done."

When the timer shows 2 minutes remaining, tell the pa- tient the following: “Keep up the good work. You have only 2 minutes left.”

When the timer shows only 1 minute remaining, tell the patient: “You are doing well. You have only 1 minute to go.”

Do not use other words of encouragement (or body lan- guage to speed up).

If the patient stops walking during the test and needs a rest, say this: “You can lean against the wall if you would like; then continue walking whenever you feel able.” Do not stop the timer. If the patient stops before the 6 minutes are up and refuses to continue (or you decide that they should not continue), wheel the chair over for the patient to sit on, discontinue the walk, and note on the worksheet the distance, the time stopped, and the reason for stopping pre- maturely.

When the timer is 15 seconds from completion, say this: “In a moment I’m going to tell you to stop. When I do, just stop right where you are and I will come to you.”

When the timer rings (or buzzes), say this: “Stop!” Walk over to the patient. Consider taking the chair if they look exhausted. Mark the spot where they stopped by placing a bean bag or a piece of tape on the floor.

<u>After</u>

Ask the patient “What if anything, kept you from walking farther?”

Congratulate the patient on good effort and offer a drink of water.

9.1.10 Copy of the original CMR standard operating protocol

The cardiac MRI protocol will require the acquisition of functional, stress/rest perfusion and late gadolinium enhancement (LGE) images on the **3T** research scanner. All study subjects should screened for the presence of **contraindications** to MRI as per normal departmental policies:

Permament pacemaker or ICD
Brain Aneurysm Clip
Implanted neural stimulator
Cochlear implant (specific implant must be checked that it is MR safe)
Ocular foreign body (e.g. metal shavings) Unless removed
Other implanted medical devices: (e.g. Swan Ganz catheter)
Insulin pump
Metal shrapnel or bullet
Renal dysfunction (eGFR <30ml/min)

Patient preparation

Patients with baseline eGFRs between 30-40 ml/min/m^2 must have their eGFR checked on the same day prior to the CMR and documented in the CRF. If the eGFR is < 30 ml/min/m^2, gadolinium should not be administered.

Ensure intravenous access (2 cannulae)
Abstinence from caffeine for >12hours

Imaging

All images will use retrospective ECG gating unless arrhythmias are present in which case prospective gating can be used. Parallel imaging (factor 3 for cine, factor 2 for stress and LGE) will be used to shorten the breath hold.

3T Magnetic Resonance Study Protocol

	Localisers.
	To repeat localisers with the patient in the isocentre.
	HASTE 30 slices free breathing
	Further localisers: VLA, HLA, SAX
	Cine imaging using trueFISP. Matrix 256 x 80% All images to be slice thickness of 8 mm, 25% distance factor. Field of view altered to minimum, according to patient's size in all scans. Segments altered according to heart rate: <70 beats per minute, 14 segments, 70 to 80 beats per minute, 12 segments, 80 to 100 beats per minute, 11 segments. Number of phases for image construction=30. 4 chamber view. 2 chamber view. 3 chamber view with temporal resolution **11 segments**. Number of calculated phases 40.
	MOLLI/T1 Mapping 3 SAX Slices copying B, M and A slices from tagging. NB _adjust shim box before running *MOLLI*. Bring box close to LV in on tagged images
	FLASH Stress perfusion. Adenosine is commenced (140 mcg per kg per minute for 3 minutes). Check blood pressure, prior to adenosine infusions and at one-minute intervals. Oxygen saturations to be monitored throughout. Check perfusion scan without contrast for optimised field of view/artefact. Smallest field of view without any wrap. Contrast to be injected at 0.04mmol/kg, 5 ml per second, followed by a 20 ml flush. Injection to commence after giving breathe IN instruction. Inject, then breathe out. Acquisition starts. 3 short axis slices depending on heart rate: Matrix 224 x 80%, parallel imaging factor x 2

	<70 beats per minute 40 acquisitions 70-90 beats per minute 50 acquisitions >90 beats per minute 60 acquisitions. **NB If HR > 110 may default to 2 beat trigger- reduce matrix to 192** The patient is instructed to breathe quietly, when they can no longer hold their breath.
	Complete LV and LA short axis coverage. First slice planned at mitral valve annulus, perpendicular to inter-ventricular septum. Ensure complete coverage.
	Rest perfusion. Ensure 10 min between rest and stress Further 0.04 mmol/kg Gadolinium. Identical parameters to those used in stress perfusion. 3 short axis slices. Further 0.07 mmol/kg Gadolinium (total dose 0.15mmol/kg)
	Sagittal oblique to include ascending aorta, aortic arch and descending aorta If artefact switch to FLASH/gradient echo
	High temp aortic cine at PA bifurcation same sequence (aortic compliance). Perpendicular to SaO. **Simultaneous BP and document pulse pressure**.
	High temporal resolution Aorta flow measured at pulmonary artery bifurcation, descending aorta. Copy slice position 14. Venc 150cm/s. Reconstruct to 120 phases
	Delayed contrast imaging inversion recovery flash sequence. TI scout copying mid sax image position. Complete short axis coverage copy in image positions from SAX cines. Use phase-sensitive sequence. Capture RR interval. Set TR ~100 msec less than RR interval. Alter TI by 10 msec approximately every 1 to 2 slices. If slice shows doubtful enhancement, repeat slice, and swap phase encoding direction. Also plan modified 2- chamber through inferior insertion point and anterior insertion point of RV to septum if LGE seen here 4 chamber, 2 chamber, 3 chamber, SAX stack.

	MOLLI/T1 Mapping 3 Sax slices as per pre contrast
	Additional sequences may be undertaken should other pathology be identified eg AoV valve disease (aov cine, LVOT views)

Adenosine stress MRI guidance

- Patients will have a 12 lead ECG & 2 cannulae inserted. If the patient only has 1 limb, has had a mastectomy etc, both cannulae can be put in one arm.
- The entire visit takes up to 1.5 hours but scanning time is about 45 minutes.
- The drug used for pharmacological stress is called Adenosine which is a potent vasodilator. This makes the patient feel like they are exercising and it is common to experience flushing, awareness of heart beat /palpitation, mild shortness of breath. Adenosine is very safe if the contraindications are observed. Occasionally patients may develop severe chest pain in which case the drug should be stopped. BP normally drops 5-15mmHg. During adenosine infusion we recommend a healthcare practioner remains in the scanning room with the patient to reassure them and maintain constant communication.
- Adenosine is administered at 140 μg/kg body weight/min for 3 minutes and during first pass perfusion. At the discretion of the supervising physician, if there is no haemodynmic response and/or if the patient does not experience any effects, the infusion may be prolonged or the dose may be increased to 210/μg/kg body weight/min.

- **Contraindications to adenosine**
    - 2nd or 3rd degree AV block
    - Atrial Flutter with heart block (≥3:1)
    - Severe asthma (see below)
    - Unstable angina pectoris
    - Known hypersensitivity to adenosine
    - Sinus bradycardia (heart rate < 40 b.p.m)
    - Systemic arterial hypotension (< 90 mmHg)

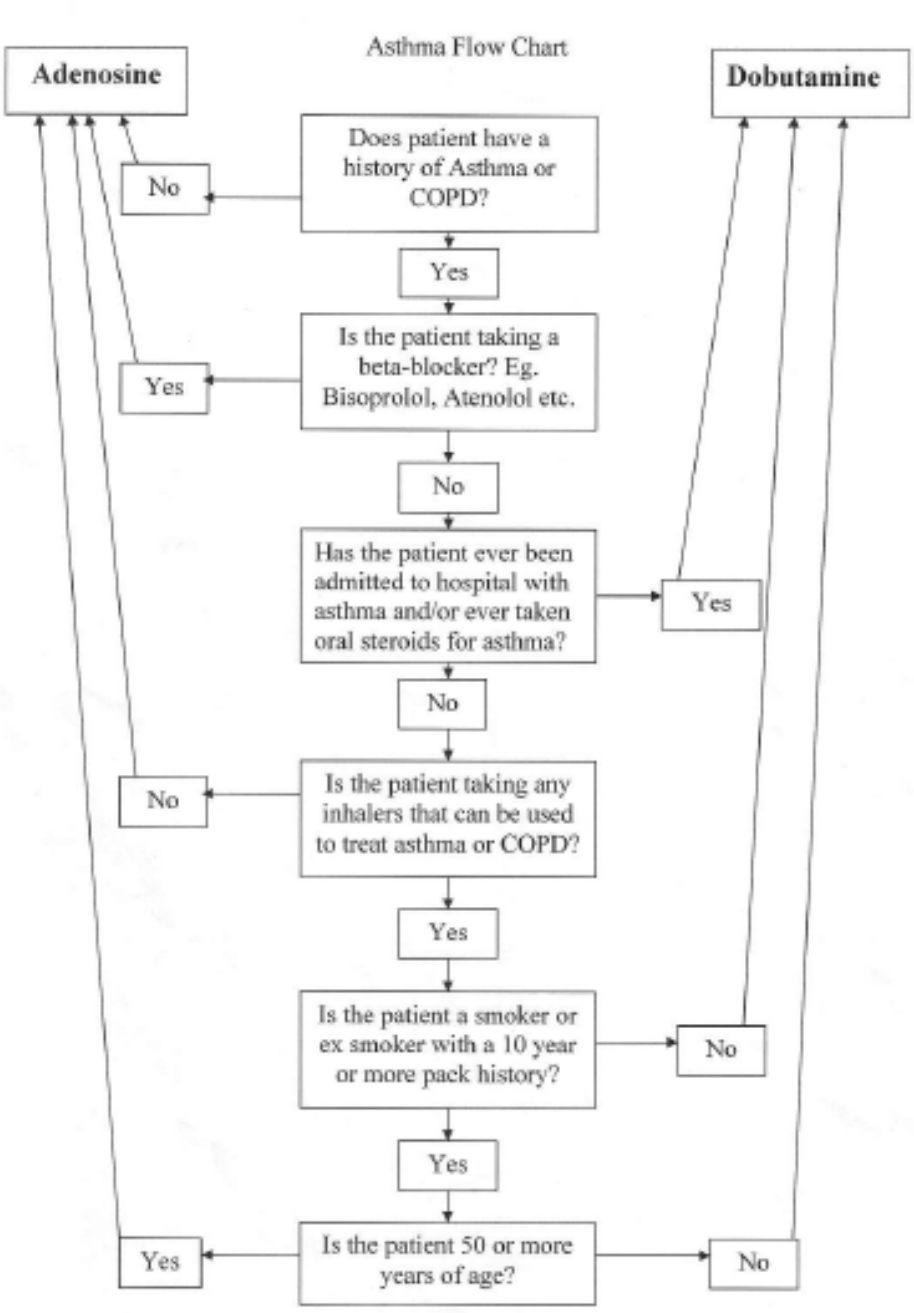
- **Indications to discontinue adenosine infusion**
    - Persistent heart block (despite coughing)

    - Severe chest pain
    - If SBP drops below 90mmHg.
    - At patient's request.

- Patients MUST abstain from caffeine for 12 hours prior to the test. If they have had caffeine within 6 hours of the test please cancel the appointment. If they have had caffeine within 12 hours but not within 6 hours please consult the doctor supervising the test.
- Patients should only drink water, squash or fruit juice. No coffee, tea, decaffeinated coffee or tea, herb teas, chocolate, hot chocolate, chocolate ice cream, fizzy drinks, etc.
- Patients may eat as usual (no chocolate)
- Patients should take their medication including anti-anginals as usual but:
    - **dipyridamole (Persantin) must be discontinued for 48** hours prior to the test.

There is no special preparation for diabetics.

- Patients may have sedation if required but they must not drive for the rest of the day and should have someone accompany them for 12 hours


Asthma Flow Chart
Adenosine
Dobutamine
Does patient have a history of Asthma or COPD?
No
Yes
Is the patient taking a beta-blocker? Eg. Bisoprolol, Atenolol etc.
Yes
No
Has the patient ever been admitted to hospital with asthma and/or ever taken oral steroids for asthma?
Yes
No
Is the patient taking any inhalers that can be used to treat asthma or COPD?
No
Yes
Is the patient a smoker or ex smoker with a 10 year or more pack history?
No
Yes
Is the patient 50 or more years of age?
Yes
No

Drugs to avoid in Adenosine stress testing:

Over the counter headache, allergy, cold & cough remedies and nasal decongestants:

Lemsip
Wigraine
Cafergot
Esgic
Fioricet
Fiorinal
Norgesic
Synalgos-DC

Drugs containing theophylline:

Aerolate
Constant-T
Elixophyllin
Franol plus
Nuelin SA
Quibron
Phyllocontin continus
Respbid
Slo-bid
Slo-phyllin
Tedral SA
Theo-24
Theoclear
Theo-Dur
Theolair
Theo-Organidin
Theo-Sav
Theostat
TheoX
T-PHYL
Uniphyllin Continus

Remember: Herb Tea (especially the green varieties eg mint) contain theophylline)

Drugs containing Dipyridamole:

Persantin;
Persantin retard;
Asasantin retard

This list is not exhaustive. Please add appropriately

9.1.11 CMR scan anonymisation protocol

Standard Operating Procedure:

Anonymising Imaging data for blinded analysis.

University of Leicester/
NIHR Leicester Cardiovascular Biomedical Research Unit
Glenfield Hospital, Leicester

Prepared by	Dr Sheraz Nazir
Reason for amendment	Conversion to generic SOP
Approved by	Dr G McCann
Siganature:	
Date:	

SOP: Anonymising of Coronary angiograms and CMR scans

For 'blinded' studies all imaging studies to be analysed for outcome measures need to be anonymised prior to analysis.

There are 4 main steps involved in this process:

1) Generate unique study identification code (USIC)
2) Import study (from PACS server or CD/DVD from other centres)
3) Anonymise study using USIC
4) Transfer anonymised study to analysis computer

1. HOW TO GENERATE USICs

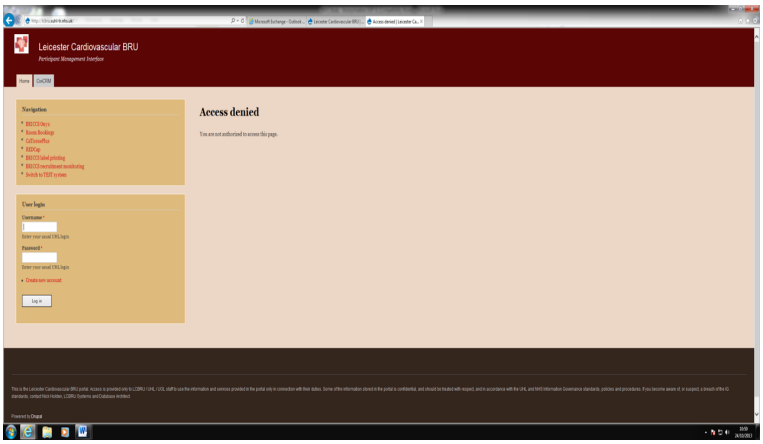
1.1 Access to anonymisation module

- An anonymisation module, or 'widget', is created by Nick Holden (Cardiovascular BRU Systems and Database Architect). For the MVO study this was made live on 16th July 2013 and available on the Leicester Cardiovascular BRU website to the MVO access group; this consisted of users to be involved in generating USICs and must never include any individuals responsible for directly analysing studies for outcome measures.

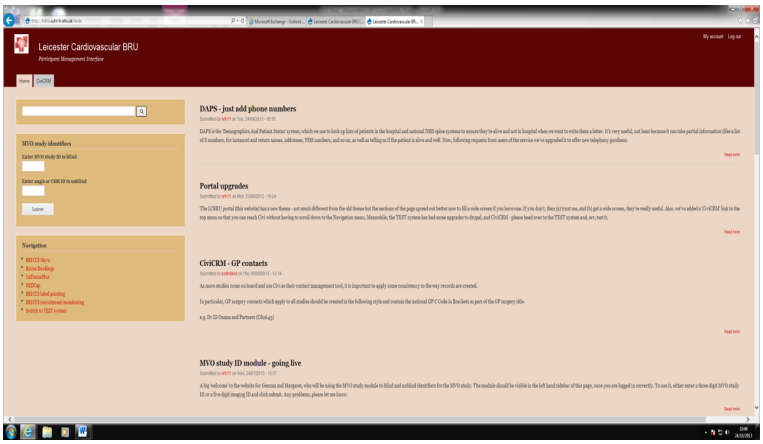
Research staff wanting access to the anonymisation module should email Nick Holden [Nick.Holden@uhl-tr.nhs.uk] who will grant access and maintain the list of authorised users.

1.2 Generating USICs

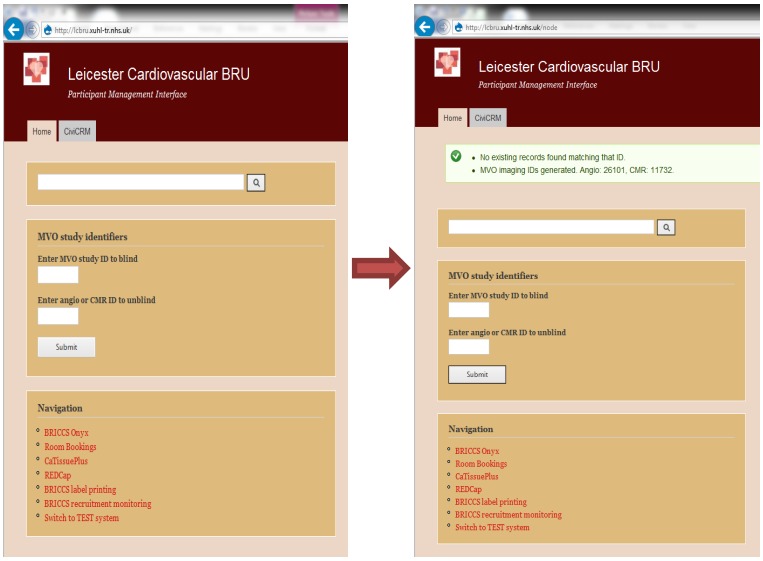
- Log into Leicester Cardiovascular BRU website on http://lcbru.xuhl-tr.nhs.uk/ using your University Hospitals of Leicester (UHL) login credentials



- If you have access to the MVO anonymisation module you will see the following screen upon successful login. If you do not see this page, and you believe you should have access, then please contact Nick Holden (see previous page).



- Enter the MVO study randomisation number in the box provided and click Submit to generate USICs for the cardiovascular magnetic resonance (CMR) scan and coronary angiogram.



You will then see a pop-up message informing you:

a) Whether previous records exist for the MVO randomisation number you have entered

b) The USICs (5 digits) for both the 'Angio' and 'CMR'-these will replace the patient details and be used to anonymise the imaging studies

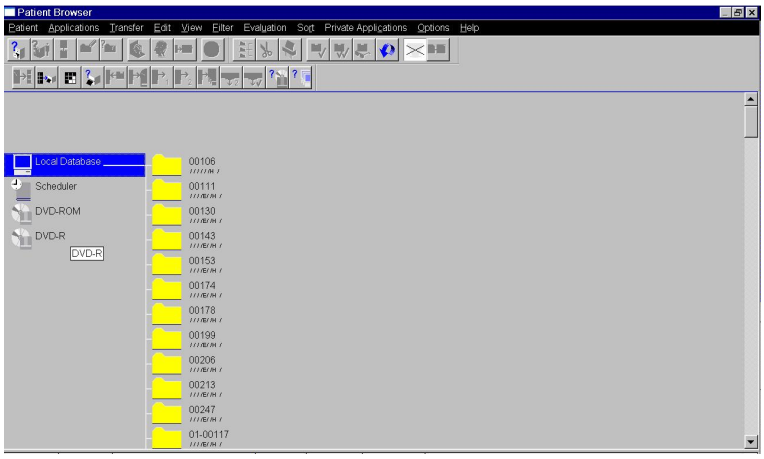
- The anonymisation can be reversed once the study has been analysed, to facilitate data entry, by entering the USIC for either the 'angio' or 'CMR' and clicking Submit

The 'unblinded' study ID (i.e. the original study randomisation number) will then be displayed in a pop-up box.

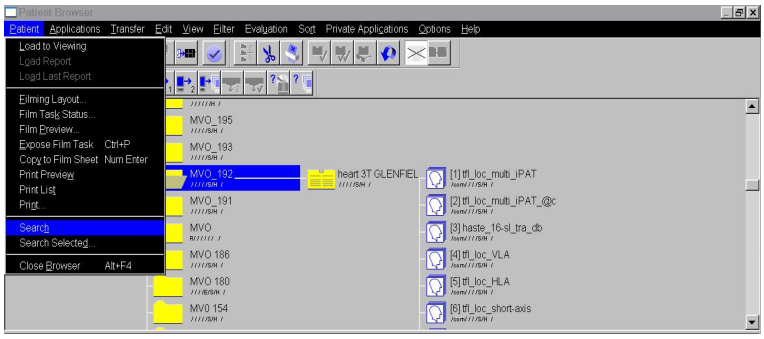
2. HOW TO IMPORT A STUDY

2.1 Importing from server

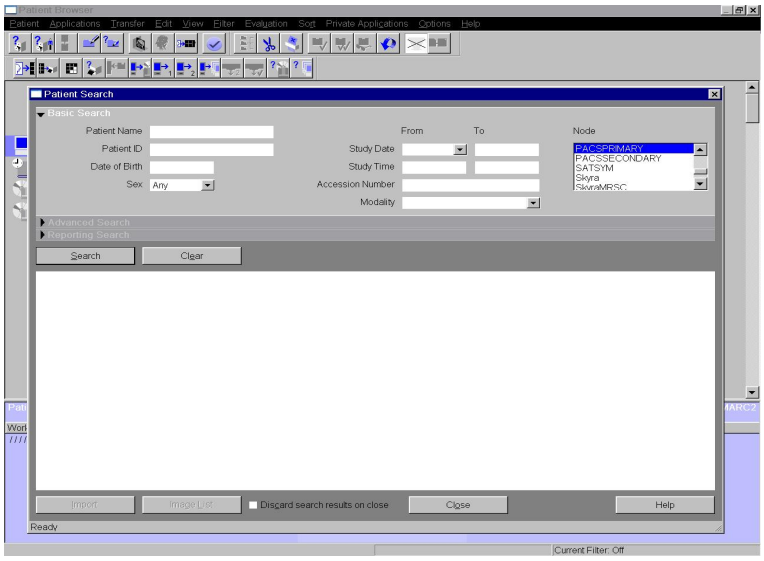
1. Start-up workstation (need to use the main workstation used by the radiographers in 1.5T or 3T control room or the 'LeoSkyra' workstation in the 'analysis room' adjacent to F26 – 3T scanner) for anonymisation and transfer of data. The other workstations in Radiology will allow you to transfer but not anonymise the studies.



2. On the top toolbar select '**Patient**' → '**Search**' → opens *Search* screen.



3. Enter patient details (*name, ID, sex*) → under '**Modality**' tab select *Magnetic Resonance* → under '**Node**' (list of workstations at right hand side of screen) select *PACSPRIMARY* (this is the main hospital server where all CMRs are stored) → **'Search'**.



4. All of the MRIs that the patient has had will then come up in the results list at the bottom of the screen (white box). Each CMR scan will be shown as a *yellow folder icon*.

5. Select the appropriate study by clicking once on it – it will turn blue when selected.

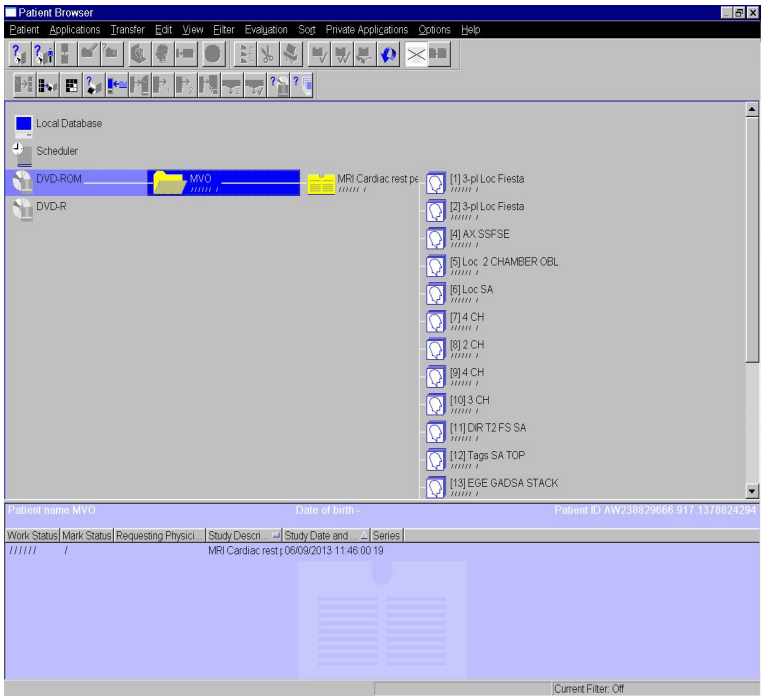
6. Select **'Import'** at the bottom of the screen.

7. The imported study will then appear under the left-hand side tab **'Local Database'.**

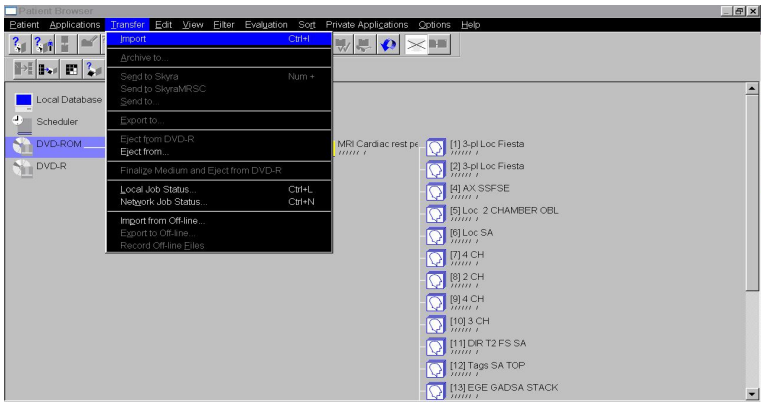
2.2 Importing from CD/DVD from other centres

1. Insert CD/DVD from outside centre in to DVD-ROM drive on workstation PC.

2. The study will appear under the left-hand side tab **'DVD-ROM'** once it has loaded. Click the study once (it will turn *blue*) to select it.



3. Once the study is selected, click **'Transfer'** in the main toolbar and then click **'Import'.**

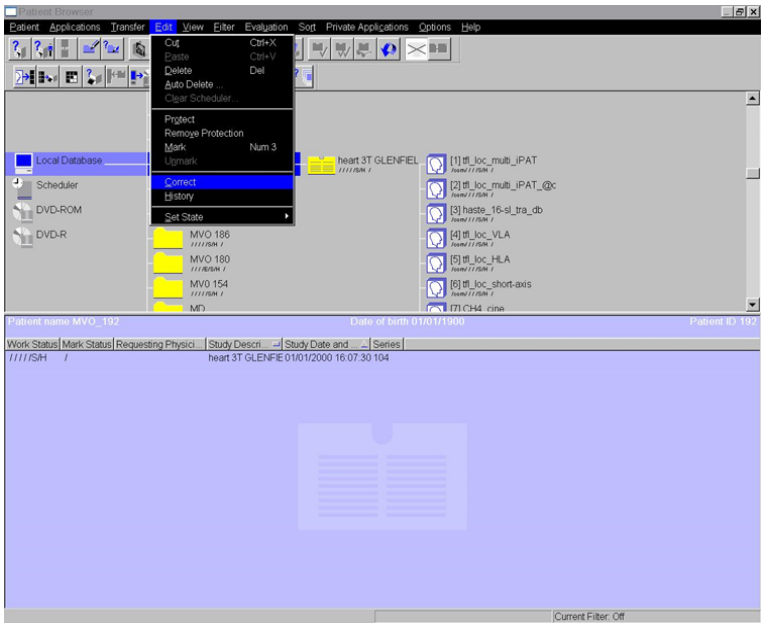


4. The imported study will then appear under the left-hand side tab **'Local Database'.**

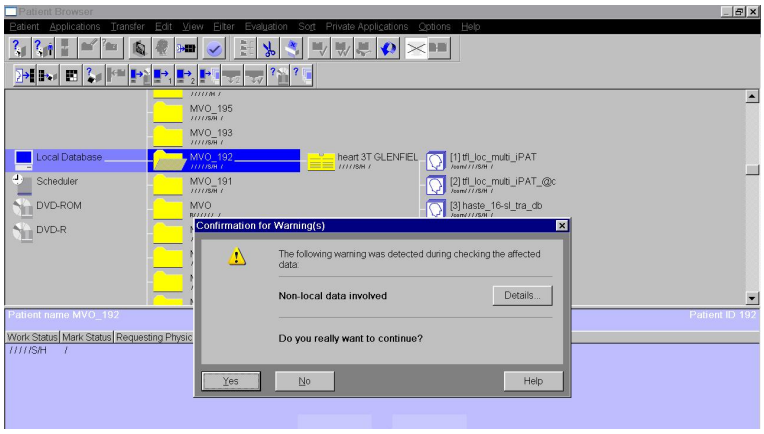
3. ANONYMISING THE STUDY

1. Under the tab **'Local Database'**, click once on the study to be anonymised (it will highlight blue when selected).

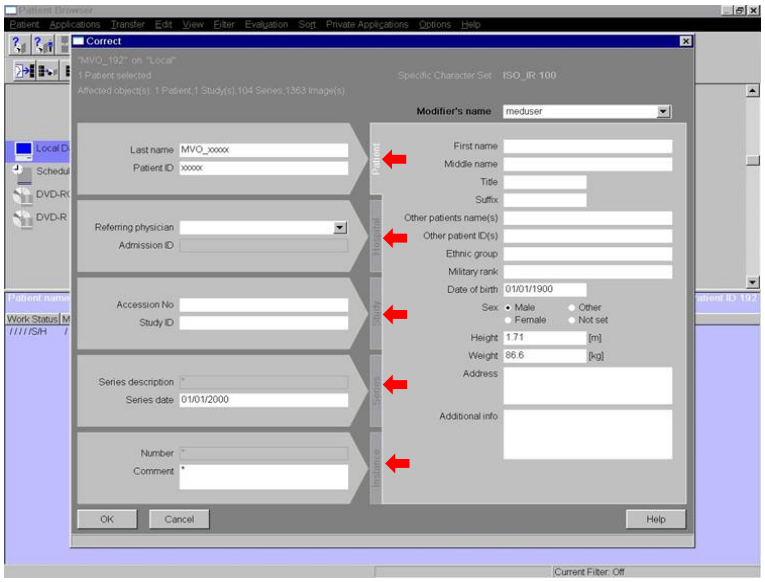
2. Click **'Edit'** on the main toolbar and then click **'Correct'**.



3. A warning box will appear asking if you are sure you want to continue – Click **'Yes'.**



4. Scroll through the vertical tabs (*red arrows*) and remove all patient identifiable data: patient name, DOB, address, hospital, study date and time, series, instance. In the **'Last name'** field enter '**study name_xxxxx'** where xxxxx is the 5 digit USIC generated earlier. Once process completed click **'Ok'**.



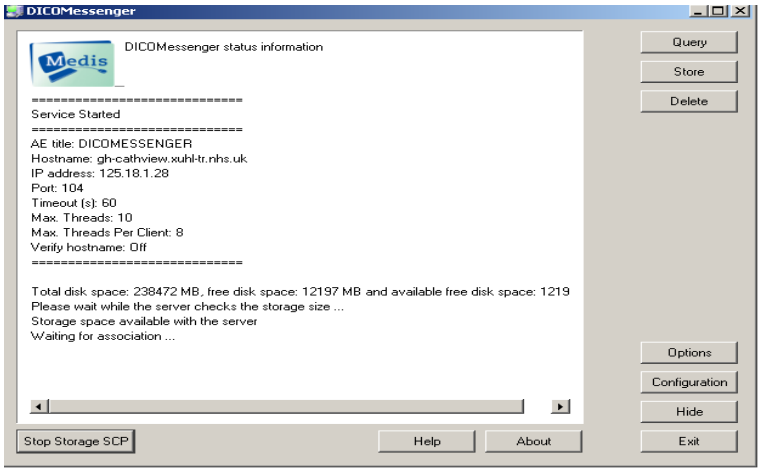
5. The study will now appear in the browser window (under **'Local** database') with the anonymised (USIC) details

4. TRANSFERRING THE STUDY

1. Ensuring that the research computer in the analysis room next to the 3T scanner is ready to receive studies (computer in the corner of this room, top left). This computer designated the node name **'MESSENGER'**.

2. Click on the icon '**DICOMMessenger**' on the desktop. This opens a program that connects the *MESSENGER* computer to the CMR workstations and should be left open (it is not possible to transfer studies unless this is open).

3. Once *DICOMessenger* is loaded it can be 'minimised' but DO NOT CLOSE.



4. Go back to **'BROWSER'** window and click on anonymised study in **'Local database'** – it will highlight blue once selected.

5. Click **'Transfer'** on main toolbar and then click **'Send to…'**. A box will appear with a list of nodes/workstations. Scroll down to, and then select, the node **'Messenger'**. Once selected (highlighted blue) click 'Send'. The study will now be transferred.

6. Any studies that you send to *MESSENGER* will go into a directory on the U:/drive called **'DICOM MESSENGER FOLDER'**, which you can access through **'My Computer'**.

7. Check the *DICOM MESSENGER FOLDER* to ensure that the anonymised study has come across, and once confirmed, cut and paste it into its final destination folder from where it can be analysed.

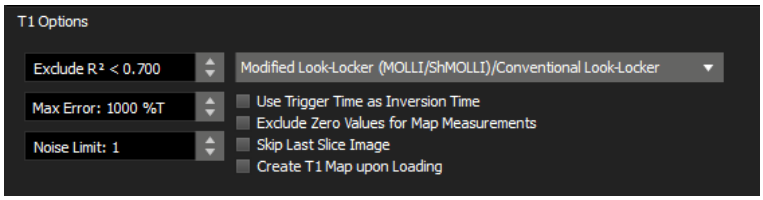
9.1.12 Copy of the T1 mapping analysis standard operating protocol

CMR SOP for T1 colour map analysis

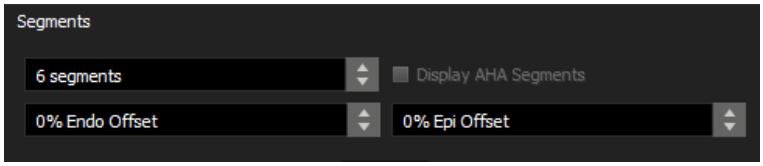
1. Click on T1 mapping module: 

2. Designated settings for protocol

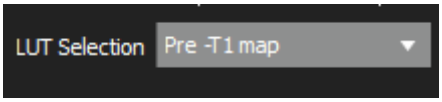
a. Ensure T1 settings as follows:



b. For mid-ventricular slice regional/segmental analysis, set segments:

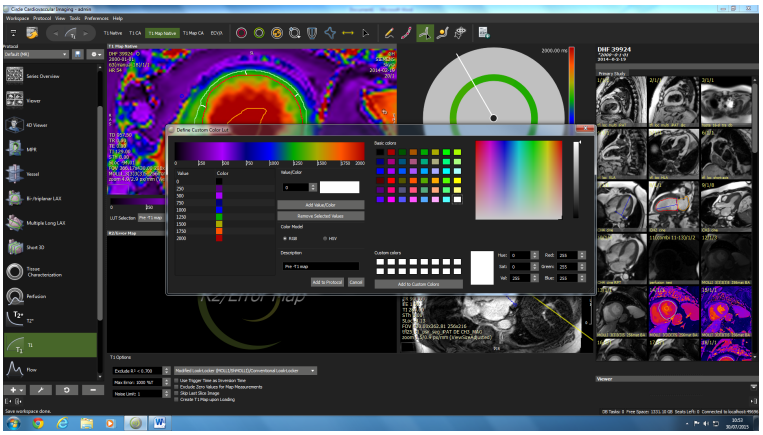


c. Choose appropriate colour scale of your choice (bear in mind that native/pre-contrast T1 rarely exceed 1500ms, that pre-contrast T1 is > post-contrast T1 and T1 values for 3T is > 1.5 T

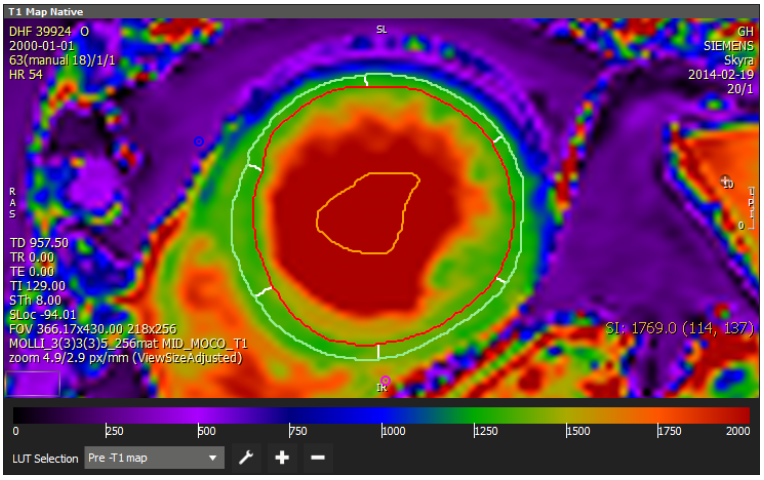
For pre-contrast T1 colour scale, click 

Click 

Choose designated colour for stipulated T1 values:



The colour scale and range shows up underneath the main analysis window

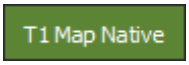


To save settings to the protocol, select 

and click 

3. Repeat step 2. For post-contrast T1 map analysis

4. Contours for analysis:

Select 

Drag the *MOLLI* into the main analysis window

Zoom to ensure optimal contours

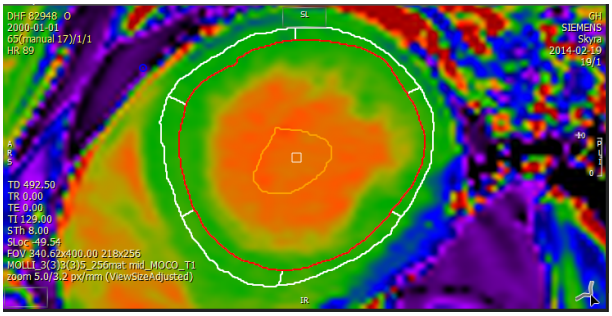
Select  for endocardial contours and draw

Select  for epicardial contours and draw

Select  for blood-pool contours and draw

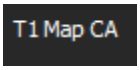
Note- For LV/ epicardial contours: avoid trabeculae, epicardial fat, blood pool since they significantly affect T1 values. Exclude partial volume and artefacts (see examples at the end of the SOP). Typically, contours are a lot narrower compared to conventional LV volumetric and mass analyses

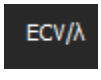
For an example of good contours see below :



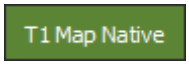
Drag long axis LGE image into LAX reference window and define LV extent as per LV volumetric analysis SOP using 

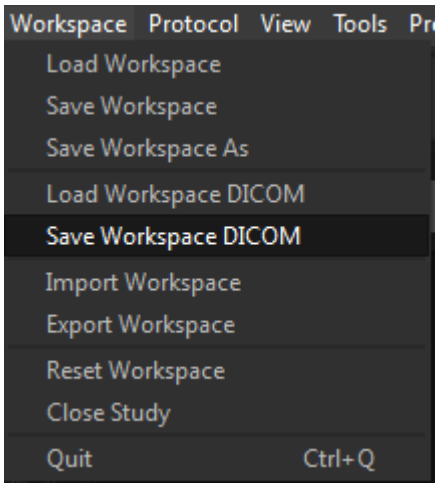
For segmentation, use  and click at the anterior LV/RV insertion point

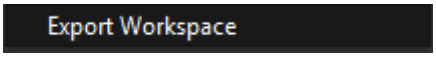
5. Repeat step 4. for post-contrats T1 map analysis by selecting 

6. The system automatically generates ECV per segment after clicking 

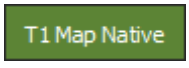
7. Saving contours:

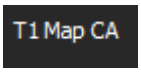
a. Click 

b. Click Workspace and then select save workspace dicom 

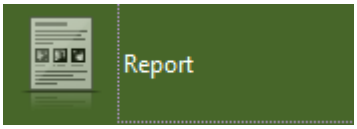
c. Also export workspace for future review into a designated folder by clicking 

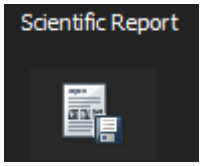
8. Generating and saving a report:

a. Click  and 

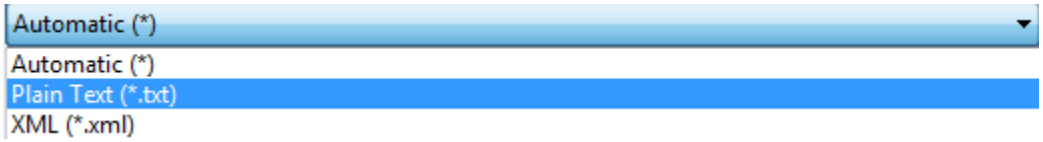
b. Click  and 

c. Click  and 

d. Click 

e. Click 

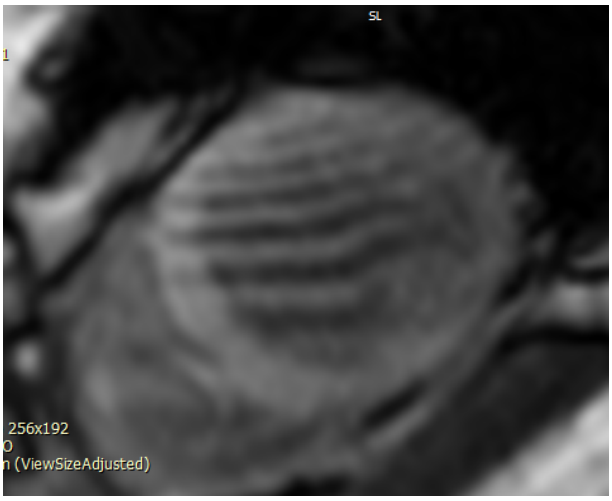
f. Save as a text file

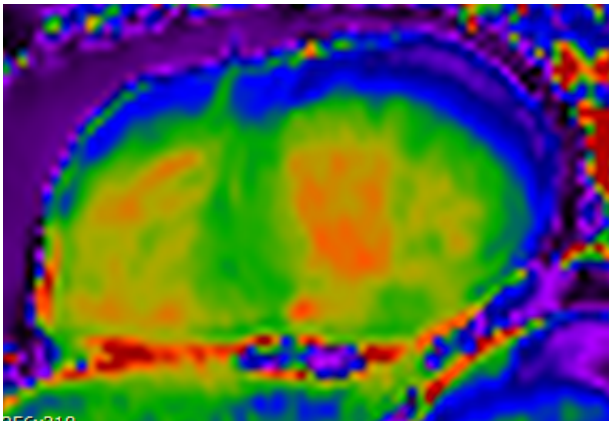


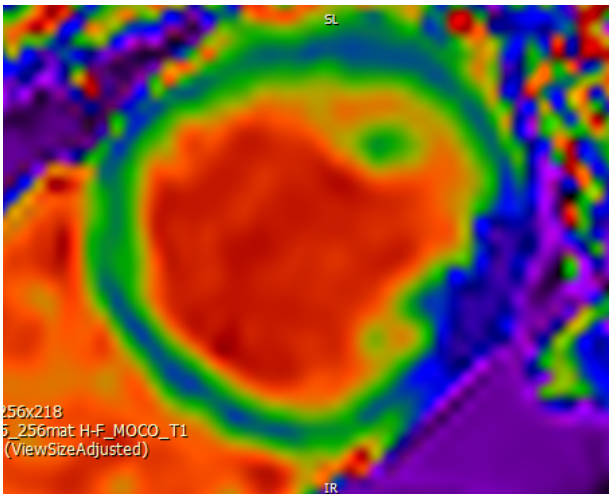
9. Additional tips and examples:

a. Before commencing T1 analyses, review images in the viewer for areas of LGE

b. Review *MOLLI* series of images to ensure no artefacts on the non-colour maps as these will affect T1 values







c. Make a note of segments with artefacts since these can be excluded from your text files subsequently when calculating the average ECV per slice

SOP generated by Dr P Kanagala

Signed: Dr G P McCann _____

Date: _____

9.1.13 Data for HFpEF subjects who did not undergo *MOLLI* imaging

Table 9.1 Baseline clinical characteristics stratified according to HFpEF subjects who underwent *MOLLI* imaging versus no *MOLLI* imaging

	HFpEF Had *MOLLI* n = 96	**HFpEF No *MOLLI* n = 44**	**p value**
Age, years	73±9	72±10	0.809
Male (%)	46 (48)	22 (50)	0.819
Clinical Findings			
Heart rate (b.p.m)	69±14	73±13	0.065
Systolic BP (mmHg)	146±25	142±25	0.329
Diastolic BP (mmHg)	75±12	73±12	0.440
Body mass index (kg/m2)	34±7	33±7	0.293
Sinus rhythm (%)	64 (67)	33 (75)	0.321
Medical History			
Diabetes (%)	48 (50)	22 (50)	1.000
Hypertension (%)	86 (90)	41 (93)	0.496
Angina (%)	19 (20)	4 (9)	0.113
Known MI (%)	13 (14)	3 (7)	0.246
Asthma or COPD (%)	18 (19)	6 (14)	0.456
Smoking (%)	52 (54)	23 (52)	0.835
Hypercholesterolameia (%)	45 (47)	24 (55)	0.399
PVD (%)	2 (2)	1 (2)	0.943
TIA or CVA (%)	9 (9)	10 (23)	0.039
Medication			
Beta blocker (%)	68 (71)	27 (61)	0.265
ACEi or ARB (%)	82 (85)	38 (86)	0.882
MRA (%)	31 (32)	12 (27)	0.550
Loop Diuretic (%)	76 (79)	37 (84)	0.493
Functional Status			
NYHA III/IV (%)	28 (29)	15 (34)	0.558
Bloods			
Sodium (mmol/L)	139.5±3.4	138.8±3.7	0.233
Urea (mmol/L)	8.5±3.6	8.7±3.9	0.706
Creatinine (mmol/L, median, IQR)	87 (71 – 113)	92 (79 – 123)	0.509
Haemoglobin (g/L)	129±19	127±28	0.563
Haematocrit (%)	38±5	38±8	0.820
BNP (ng/L, median, IQR)	144 (66 – 250)	134 (63 – 267)	0.856

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