

Improving mechanistic understanding of left ventricular dysfunction and risk in stable coronary artery disease.

Thesis submitted for the degree of *Doctor of Philosophy*

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Abstract

Background: There remains a need for better mechanistic understanding of myocardial dysfunction and adverse clinical outcomes in patients with stable coronary artery disease (CAD).

Methods and Results:

- i) Serial cardiovascular magnetic resonance (CMR) was performed on 43 patients undergoing coronary artery bypass grafting (CABG). Visual ischaemic burden (median: 18.0% [10.5-21.0] vs 42.0% [27.0-51.0], $P<0.001$) and global myocardial perfusion reserve (median: 2.40 [1.72-2.75] vs 1.71 [1.36-2.22], $P=0.002$) improved post-operatively. Change in patient-reported health status correlated with change in visual ischaemic burden.
- ii) From 33 patients undergoing CABG, 63 left ventricular (LV) biopsies were acquired. The global myocardial ATP/ADP ratio was reduced in CAD patients as compared to donor tissue, with increased expression of oxidative phosphorylation genes encoding electron transport chain complexes. Paired analyses of biopsies obtained from LV segments with or without inducible ischaemia revealed no difference in the regional metabolic or single-cell transcriptomic landscape.
- iii) The relevance of genetic and CMR features of dilated cardiomyopathy (DCM) in individuals with CAD was assessed. Rare variants in DCM-associated genes were associated with LV remodelling and outcomes in individuals with CAD in the UK Biobank. Non-infarct pattern myocardial scar in patients with CAD recruited to a London registry was associated with adverse remodelling but was not a predictor of outcome and had no rare genetic basis.
- iv) Late gadolinium enhancement (LGE) quantification, alongside computational image analysis, was performed in 437 patients with CAD. Core infarct mass and peri-infarct zone mass were independently associated with sudden cardiac death (SCD), improving risk stratification beyond LV ejection fraction. Several shape-based scar microstructure features associated with SCD.

Conclusions: My results suggest that ischaemia has a global effect on the myocardium, with a potential role for targeting mitochondrial redox biology. Adverse cardiac remodelling in CAD is complex with ischaemic myocardial scar an important determinant of outcomes, particularly arrhythmic.

Thesis structure

Research aim: To assess the utility of cardiovascular magnetic resonance (CMR), alongside human left ventricular tissue analysis, to provide mechanistic insight and improve risk stratification in stable coronary artery disease (CAD)

Chapter 1: Background - Contemporary understanding of stable CAD and unmet research questions

Each chapter includes:

- Introduction
- Methods
- Results
- Discussion
- Conclusions

Chapter 2: Serial phenotyping with multiparametric CMR in patients undergoing coronary artery bypass grafting.

Evaluating the use of multiparametric CMR to assess for dynamic myocardial disease in CAD.

Chapter 3: Assessing mitochondrial bioenergetics in coronary artery disease: A translational multiomic tissue study in humans.

Assessing the multiomic profile of left ventricular tissue from patients with stable CAD.

Chapter 4: Assessing for genetic and CMR features of dilated cardiomyopathy in patients with CAD.

Evaluating for overlapping features of dilated cardiomyopathy and CAD.

Chapter 5: Comprehensive phenotypic characterisation of late gadolinium enhancement predicts sudden cardiac death in CAD.

Evaluating the utility of in-depth scar phenotyping by CMR to predict sudden cardiac death in patients with stable CAD.

Chapter 6: Overview of thesis and summary of future work.

Statement of originality

I declare that the work presented in this thesis is my own except where appropriately referenced or acknowledged otherwise.

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List of abbreviations and acronyms

ACEi = angiotensin-converting enzyme inhibitor
ARB = angiotensin II receptor blocker
AD = adipocytes
ADP = adenosine diphosphate
AF = atrial fibrillation
ASCD = aborted sudden cardiac death
ATP = adenosine triphosphate
BMI = body mass index
bSSFP = balanced steady-state free precession
CABG = coronary artery bypass grafting
CAD = coronary artery disease
CCS = Canadian Cardiovascular Society
CI = confidence interval
CMR = cardiovascular magnetic resonance
DBP = diastolic blood pressure
EC = endothelial cells
ECV = extracellular volume
ETC = electron transport chain
FA = fatty acid
FB = fibroblasts
FC = fold change
FDR = false discovery rate
GCS = global circumferential strain
GLS = global longitudinal strain
GRS = global radial strain
HEP = high energy phosphate
IQR = interquartile range
LAD = left anterior descending artery
LAVi = indexed left atrial volume
LAX = long axis
LC-MS = Liquid chromatography–mass spectrometry
LCx; left circumflex artery
LGE = late gadolinium enhancement
LV = left ventricular
LVEDVi = indexed left ventricular end-diastolic volume

LVEF = left ventricular ejection fraction
 LVESVi = indexed end-systolic LV volume
 LVMi = indexed left ventricular mass
 MBF = myocardial blood flow
 MnSOD = manganese-dependent superoxide dismutase
 MOLLI = Modified Lock-Locker inversion recovery
 MI = myocardial infarction
 MPR = myocardial perfusion reserve
 NADH = nicotinamide adenine dinucleotide
 NI-LGE = non-infarct pattern late gadolinium enhancement
 ns = non-significant
 NTproBNP = N-terminal-pro B-type natriuretic peptide
 NYHA = New York Heart Association
 OXPHOS = oxidative phosphorylation
 PC = pericytes
 PCA = principal component analysis
 PCI = percutaneous coronary intervention
 PIZ = peri-infarct zone SCD
 PPP = pentose phosphate pathway
 PSIR MOCO = phase-sensitive inversion recovery motion-corrected
 RAVi = indexed right atrial volume
 RCA = right coronary artery
 RVEDVi = indexed right ventricular end-systolic volume
 RVEF = right ventricular ejection
 RVSVi = indexed right ventricular stroke volume
 SAQ-7 = Seattle Angina Questionnaire-7
 SAX = short axis
 SBP = systolic blood pressure
 SCD = sudden cardiac death
 sHR = subdistribution hazard ratio
 SMC = smooth muscle cells
 snRNAseq = single-nuclei ribonucleic acid sequencing
 TCA = tricarboxylic acid
*TTN*tv = titin truncating variant
 vCM = ventricular cardiomyocytes

Personal Contribution to the work

In 2018, I successfully applied for a Clinical Research Fellowship at Imperial College London, funded by the National Heart and Lung Institute Foundation. Under the supervision of Professor Sanjay Prasad, Dr Ali Vazir, Professor Mike Murphy and Dr Brian Halliday I designed, led and performed the work detailed in this thesis.

Alongside the guidance of my supervisors, I developed the hypotheses, protocol, study documents and ethical submission for the AMBITION study (Chapters 2-3). I initially met with the Royal Brompton and Harefield Patient Advisory Committee to gauge feedback on the study plan. I subsequently attended the Regional Ethics Committee to answer questions relating to the study. With aid from my supervisors, I ensured all analysis pipelines with University of Cambridge and Imperial College London were robust, deliverable and would answer the study questions. I also organised multiple meetings with key stakeholders at Royal Brompton and Harefield Hospital to ensure the study protocol of serial cardiovascular magnetic resonance and acquisition of myocardial biopsies during coronary artery bypass grafting (CABG) was feasible. I recruited every patient in the study, supervised all cardiovascular magnetic resonance (CMR) scans and was present during every CABG operation to process the tissue biopsies. I designed the research database, inputted all data and coordinated transfer of the tissue samples to the relevant laboratories. Unfortunately, due the restrictions in place during the COVID-19 pandemic, my involvement with the wet lab aspects was restricted beyond the pilot patients and subsequently focussed on the analytical components. Dr Anja Gruszczyk conducted the luciferase-based assay, Dr Ming Yang performed the liquid chromatography–mass spectrometry, and Mr Sam Barnett and Ms Rebecca Toscano-Rivalta prepared the single-nuclei preparation. Following receipt of the data, I conducted the analysis alongside Dr Christina Schmidt (bioinformatician for the metabolomics), Mr Michael Lee (bioinformatician for the transcriptomics) and Ms Ruth Owen (medical statistician).

Under the guidance of my supervisors, I generated the hypotheses and led the studies included in Chapters 4-5. I curated the study cohort of patients with stable coronary artery disease from the large Royal Brompton Hospital CMR registry. I reviewed the data for every patient with a potential diagnosis of CAD, performing quality control on the baseline demographic information and excluding patients who did not meet my inclusion/exclusion criteria. With the support of team of medical student (recruited by myself and Dr Daniel Hammersley over the Summer of 2019), I coordinated the long-term follow up of all patients retrieving General Practitioner, Hospital and patient questionnaire data. I inputted all follow up data on to the research database and organised multiple outcome adjudication sessions. With regards to the CMR analysis, I coordinated the late gadolinium enhancement (LGE) quantification, performed independently by Dr Suzan Hatipoglu for scientific robustness. Additionally, I coordinated the independent assessment of LGE patterns used in Chapter 4, performed by Dr John Baksi and Dr Brian Halliday. The genetic analysis was performed with support from Dr Sean Zheng, Dr Kathryn McGurk, Mr Pantazis Theotokis and Professor James Ware. For the scar microstructure analysis used in Chapter 5, I reviewed and performed quality control of all LGE slices with Mr Hassan Zaidi. I performed the remaining analysis for Chapters 4-5 with assistance from Ms Ruth Owen (medical statistician).

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Next, my supervisors. Dr Ali Vazir has provided guidance throughout. It is unfortunate that our planned involvement with the commercial trial didn't quite play out as either of us expected. The experience of working with industry, alongside the exposure to setting up a randomised control trial, was invaluable and provided me with some of the most useful learning during my PhD. Professor Mike Murphy has given me unrivalled exposure and teaching in the field of mitochondrial biology. The AMBITION study would not have been possible without his generous time and enthusiastic support during both conception and throughout delivery. Next, Dr Brian Halliday. The direct line to his brilliant academic mind was of immeasurable help. His rapid feedback and critique on all projects helped not only shape my hypotheses and manuscripts, but more importantly my understanding of academic medicine. And finally, my primary supervisor, Professor Sanjay Prasad. Throughout the entire journey, the unparalleled support and dedication that he has given me has been phenomenal. I doubt any other supervisor gives so much one-to-one time to their students. All project ideas were encouraged, and doors opened whenever needed. The breath of this PhD is a testament to Sanjay's talent, drive, knowledge and desire to progress both his students, and the field of cardiovascular research. Thank you – I owe so much of my achievements throughout the last 3 years, and I predict the next 30 years, to yourself.

My family have always championed my desire to dip my feet in academia. My parents worked tirelessly to promote my education and fully supported my protracted decision to revert back to student life for a few years. Thank you, Mum, Dad, Robert and Bethan.

And finally, Katherine, Otilie and more recently, baby Clementine. Katherine, during this (joint) PhD journey, we have moved house on 5 occasions, got married, had two babies, all within the disruption

of a global pandemic. Your support of the early starts, late nights and weekend work has been amazing, in addition to your tolerance of the repetitive notion that ‘it’s just one more busy week and then it should ease off’ didn’t always materialise. Thank you. Without you, this PhD would not have started, progressed or been as thoroughly enjoyable.

Chapter 1

1 Background - Contemporary understanding of stable coronary artery disease and unmet research questions

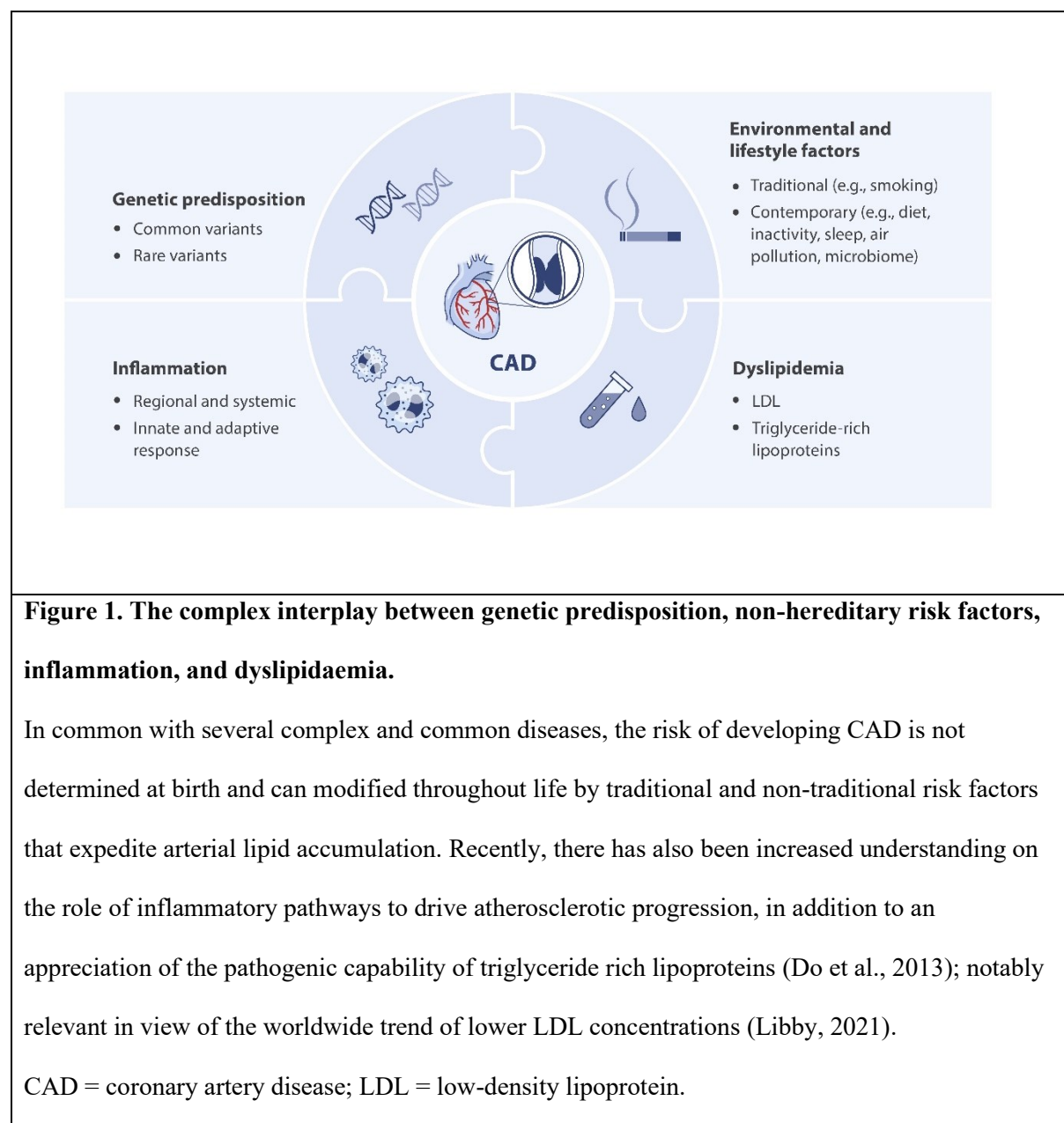
The results of ISCHEMIA and REVIVED-BCIS demonstrate the limited prognostic role of coronary revascularisation in stable ischaemic heart disease. Despite these trials investigating the utility of invasive management to improve clinical outcomes, discussion has pivoted towards the value of non-invasive ‘ischaemia and viability’ testing in these populations. This is misguided; the results in fact highlight the importance of the phenotypes recruited in these trials. In ISCHEMIA, a trial of patients with at least moderate ischaemia, nearly 13% of patients experienced a cardiovascular event (Maron et al., 2020). In REVIVED-BCIS, 38% of participants, all of whom had a significant bulk of viable myocardium, met the primary endpoint of death from any cause or hospitalisation for heart failure (Perera et al., 2022). It thus remains valid, at the cellular and patient level, that the identification of inducible myocardial ischaemia and the characterisation of dysfunctional (yet viable) myocardium, has scientific and prognostic relevance. The challenge is to interrogate *why* these phenotypes are important; both to facilitate improved risk prediction but also to permit the identification of novel and more effective therapies, beyond coronary revascularisation, that target the myocardial consequences of ischaemia.

My thesis will explore these themes, focussing on myocardial pathology *downstream* from stable coronary atherosclerosis that can drive ventricular remodelling (e.g., repetitive episodes of ischaemia and impaired myocardial perfusion reserve) and precipitate adverse clinical outcomes (e.g., complex scar patterns in patients with life-threatening arrhythmia).

1.1 Defining coronary artery disease

Coronary artery disease (CAD) encompasses the pathological process of atherosclerotic plaque accumulation within the epicardial coronary tree. It is characterised by the complex interplay between

dyslipidaemia, inflammation, genetic susceptibility, environmental risk factors and lifestyle; resulting in infiltration of lipid particles into the arterial intima (Figure 1) (Libby, 2021). Common variant association studies have identified over 50 loci that are associated with risk of CAD development, with polygenic risk scores demonstrating the predictive nature of aggregated risk alleles (Khera et al., 2016). Additionally, aligning with many common and complex diseases, the risk of developing CAD is not solely determined by genetic predisposition, but further modified by comorbidities in tandem with environmental stressors (e.g., pollution) and lifestyle factors (e.g. diet, smoking and inactivity) (Khera & Kathiresan, 2017; Libby, 2021) .



1.2 Epidemiology of coronary artery disease

There are many factors influencing the demographic of CAD. Importantly, the absolute number of individuals living with CAD is rising (Dai et al., 2022), with European data showing a continent-wide prevalence approaching 35 million people (Timmis et al., 2020). This increase in burden can be apportioned to 3 broad drivers: i) an aging and growing population; ii) improved survival following acute myocardial infarction by widespread adoption of rapid reperfusion strategies and advancements in medical therapies and; iii) the global diabetes and obesity epidemics (Dai et al., 2022; Sorbets et al., 2020; Timmis et al., 2018). Paralleling these direct causes, societal adaptations have also reduced mortality by targeting modifiable risk factors (O’Flaherty et al., 2013), thus arguably also driving a rise in the prevalence of people living with CAD. Specifically, legislated smoking bans (Mackay et al., 2010), in addition to dietary policy and reductions in cholesterol (primarily not attributed to statins), the most notable societal policy changes to have affected mortality associated with CAD (O’Flaherty et al., 2013). The combined impact of therapeutic advancements and improved risk factor management has led to global shifts in the epidemiology patients with CAD. Developing countries now harbour a significant proportion of the CAD burden and previously underrepresented subgroups (e.g., younger women and geriatric cohorts) are becoming increasingly prominent (Libby, 2021). Despite the decrease in case fatality rates, CAD remains the largest global cause of death globally (Nowbar et al., 2019), notably in older adults (GBD 2019 Diseases and Injuries Collaborators, 2020).

1.3 Clinical presentations in coronary artery disease

1.3.1 Acute coronary syndromes

Superficial erosion of atherosclerotic plaque, leading to thrombosis formation and a rapid decrease in vessel patency (Heusch & Gersh, 2016; Libby, 2021) characterise Type 1 myocardial infarction. This cascade of events is associated with dynamic circulating cardiac troponin levels and are typically heralded by ischaemic chest pain at rest with varying degrees of electrocardiographic and imaging evidence of myocardial hypoperfusion. An important subset of patients with CAD can also present with acute myocardial ischaemic injury (depicted by the rise and fall of cardiac troponin) without

compelling evidence of evolving atherosclerotic plaque erosion (DeFilippis et al., 2019). These events, labelled Type 2 myocardial infarction, are caused by the disparity between available oxygen supply and required myocardial oxygen demand secondary to mechanisms other than disrupted coronary plaque (e.g., decreased oxygen carry capacity in acute blood loss or increased oxygen demand in tachyarrhythmia) (Sandoval & Jaffe, 2019). Importantly however, such events can signify the presence of underlying coronary atheroma and consideration of further investigation should be considered on a personalised basis to attempt to reduce the high-risk of subsequent coronary and heart failure events.

1.3.2 Chronic coronary artery disease

Patients can also commonly present with clinical features representative of non-acute coronary atherosclerotic disease. The 2019 European Society of Cardiology guidelines detailed the range of these potential scenarios, employing the moniker chronic coronary syndromes (CCS) (Knuuti et al., 2020). Central to the concept of CCS, is that although typified by periods of stability, coronary atherosclerosis is progressive and dynamic (Knuuti et al., 2020). The recently reported CLARIFY registry of over 32,000 patients aimed to describe the phenotype and outcomes of individuals with CCS (Sorbet et al., 2020). Despite the impressive adoption of present-day medical therapies, the study highlighted that both angina and history of prior myocardial infarction remain prognostically relevant. Angina (from the Greek *ankhon* , ‘a strangling’) describes the visceral manifestation of myocardial ischaemia and is typically due to a transient increase in oxygen demand in the presence of coronary and/or myocardial disease that is unable to meet this requirement. This is commonly experienced on physiological stress during exercise, commonly representing obstructive atheromatous disease or microvascular dysfunction. These repetitive and cumulative episodes of myocardial hypoperfusion not only impact quality of life and risk of future cardiovascular events but are also proposed to drive the intracellular adaptations that can lead to dysregulation of left ventricular (LV) contractile performance (Canty & Fallavollita, 2001; Depre & Vatner, 2005). Indeed, an overarching paradigm in CCS is that although a disease of the vasculature, the downstream consequences on the myocardium lead to the debilitating symptoms and drive a significant proportion of the increased

morbidity and mortality associated with condition (e.g., heart failure and life-threatening ventricular arrhythmia).

1.4 Myocardial dysfunction in patients with chronic coronary syndromes.

Pathology of the myocardium drives an important degree of symptomatology and outcomes in patients with stable CAD. Elucidating the key abnormalities within these regions may afford the potential to both improve risk prediction and identify novel drug therapies directly targeting the dysfunctional myocardial unit. Impairment of LV systolic performance in the presence of *chronic* epicardial CAD is traditionally attributed to two pathophysiological processes; 1) Adverse ventricular remodelling secondary to prior myocardial infarction and 2) Dysfunctional, yet potentially viable, myocardium in the presence of upstream obstructive coronary atherosclerosis.

1.4.1 Myocardial metabolism in health

Normal human cardiac function relies on the constant delivery of oxygen to maintain metabolism. This supply of oxygen permits oxidative phosphorylation within mitochondria, generating around 90-95% of energy, in the form of the adenosine triphosphate (ATP)/adenosine diphosphate (ADP) ratio, required for cardiac contraction and relaxation. The other 5-10% of ATP is derived from glycolysis. To drive cellular respiration, the heart relies on several differing substrates, exhibiting flexibility to shift between fuels as needed. The primary sources are fats (both long-chain fatty acids and triglycerides), carbohydrates (including oxidation of pyruvate, the end-product of both glycolysis and lactate metabolism) and ketones (namely acetoacetate and β -hydroxybutyrate) (Figure 2). The proportion of each substrate used is complex, in part influenced by oxygen delivery, nutritional status, circulating substrate plasma levels and transporter numbers (e.g., the fatty acid translocase CD36 for fats) (Barry, 2004; Zuurbier et al., 2020).

Fatty acids are the predominant substrate during normoxia, responsible for ~60-90% of ATP production (Barry, 2004). After cellular uptake, fatty acids are first esterified to form fatty acyl-CoA. The fatty acid moiety is subsequently transferred to carnitine by carnitine palmitoyl transferase I in the outer mitochondrial membrane (a critical regulatory rate-limiting step) to produce long chain

acylcarnitine (Kerner & Hoppel, 2000; Murthy & Pande, 1987). This long chain acylcarnitine is then transported into the mitochondrial matrix and is converted back to long-chain acyl-CoA by carnitine palmitoyl transferase II. The fatty acyl-CoA can then enter beta-oxidation to produce acetyl-CoA, NADH and FADH₂.

Glucose, another key source of energy for the heart, is an efficient substrate in terms of ATP produced relative to atoms of oxygen consumed. ATP generation can occur from either mitochondrial oxidation of glycolysis-derived pyruvate or cytoplasmic glycolysis. Glucose enters the cardiomyocyte via the GLUT1 and GLUT4 transporters with GLUT4 being responsible for insulin-dependent uptake.

Following uptake into the cardiomyocyte, glucose is rapidly phosphorylated by hexokinase to produce glucose 6-phosphate; this intermediate is then directed towards several pathways, namely to generate pyruvate from glycolysis or to synthesise glycogen. The pyruvate produced from glycolysis can be converted to lactate (within the cytosol) or be transported into the mitochondria through the mitochondrial pyruvate carrier, the latter permitting oxidation to acetyl-CoA by the multi-enzyme complex pyruvate dehydrogenase (Lopaschuk et al., 2021).

Ketone bodies, lactate and branch chain amino acid are other substrates with robust data describing their utility as key fuels in cellular respiration. Lactate enters the cardiomyocyte through the monocarboxylic anion transporter, with subsequent conversion to pyruvate by lactate dehydrogenase.

This pyruvate is then handled in similar fashion to the pyruvate derived from glucose. Ketones, predominantly β -hydroxybutyrate, enter the heart via SLC16A1 and are transported to mitochondria. They subsequently undergo oxidation via β -hydroxybutyrate dehydrogenase 1 to acetoacetate which is then converted to acetoacetyl-CoA before undergoing a thiolysis reaction, generating acetyl-CoA (Lopaschuk et al., 2021). Finally, branch chain amino acids metabolism consists of several steps involving transamination and oxidative decarboxylation, again resulting in the production of acetyl-CoA (Lopaschuk et al., 2021).

Irrespective of which substrate is used, each is ultimately metabolised to form acetyl-CoA, itself then directed towards the citrate acid cycle where electrons are produced from the oxidation of NADH and FADH₂. These electrons are then transferred along the electron transport chain in a series of redox reactions which, in combination with two shuttles, eventually expel hydrogen ions into the

intermembrane space. This process generates a transmembrane electrochemical potential gradient (the movement of electrons is coupled to the expulsion of protons at complex I, III and IV) which permits ATP production at F_0F_1 -ATP synthase.

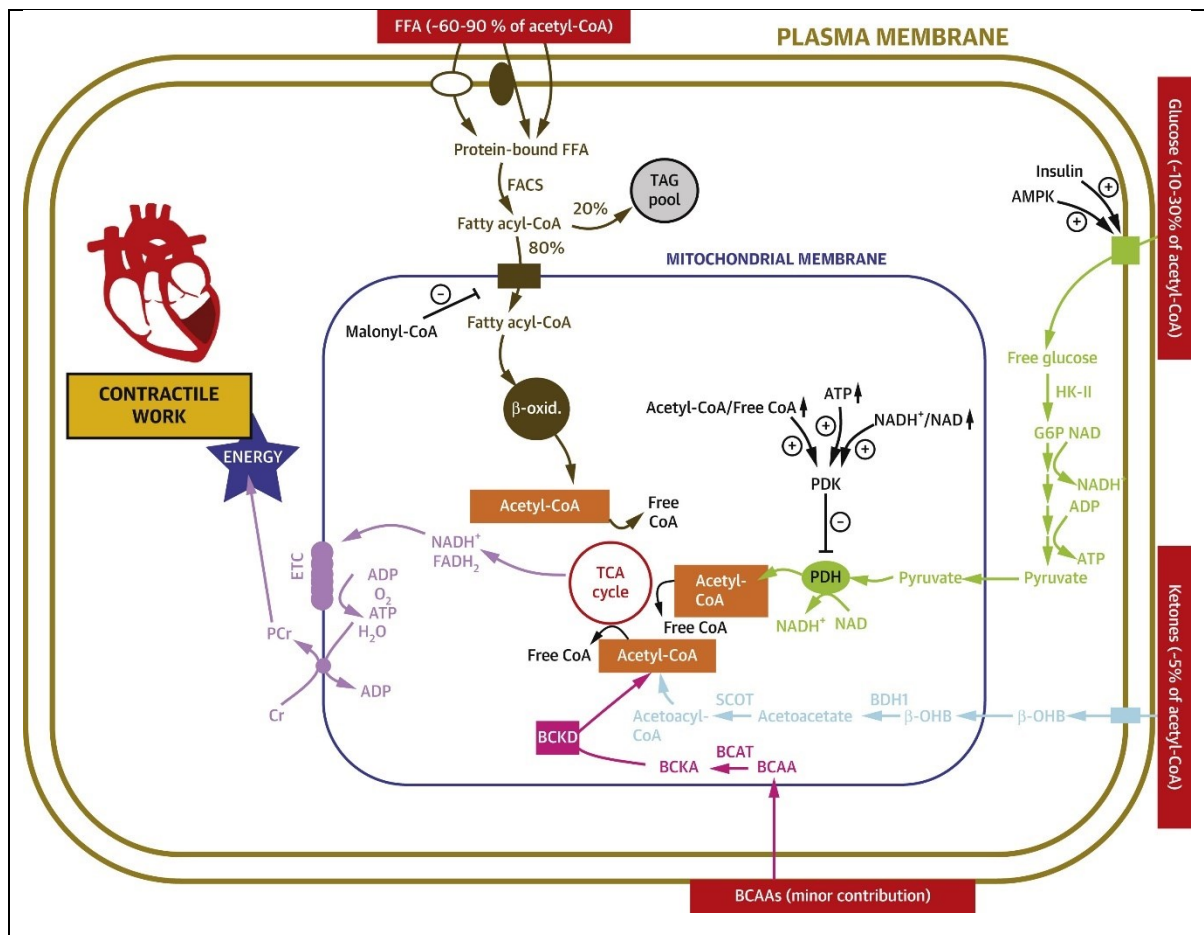


Figure 2. Traditional understanding of cardiac metabolism in health.

The predominant source of fuel in healthy subjects is fatty acids, with smaller contribution from carbohydrates, ketone bodies and branch chain amino acids.

β -OHB = β -hydroxybutyrate; AMPK = adenosine monophosphate-activated protein kinase; ATP = adenosine triphosphate; BCAA = branched-chain amino acid; BCAT = branched-chain amino acid aminotransferase; BCKA = branched-chain ketoacid; BDH1 = β -hydroxybutyrate dehydrogenase-1, CoA = coenzyme A; ETC = electron transport chain; FACS = fatty acyl-CoA synthetase; FFA = free fatty acids; G6P = glucose-6-phosphate; HK-II = hexokinase-II; PCr = phosphocreatine; PDH = pyruvate dehydrogenase; PDK = pyruvate dehydrogenase kinase; SCOT = succinyl-CoA:3-oxoacid CoA transferase; TAG = triglyceride. Reproduced with permission from *JACC*, (Honka et al., 2021).

1.4.2 Myocardial metabolism in acute ischaemia

During an evolving myocardial infarction, the blood supply to the myocardium is dramatically reduced. This results in rapidly hypoxic, or anoxic, conditions and decreased supply of the nutrients required for normal cellular respiration. During anaerobic periods, maintenance of the ATP/ADP ratio is provided by anaerobic glycolysis to permit the functioning of vital cellular processes to ensure survival; in part facilitated by elevated glucose extraction from the circulation, increased translocation of GLUT transporters to the sarcolemma and augmented glycogenolysis (Zuurbier et al., 2020). This however triggers accumulation of lactate, decreasing pH and cytosolic calcium overload (Pell et al., 2016; Steenbergen et al., 1993). Maintenance of cellular viability requires the continued activity of the ATP-using Sodium/Potassium and Calcium ion pumps. When these are inhibited by a significantly lowered ATP/ADP ratio during persisting ischaemia, tissue swelling and plasma membrane rupture can lead to cell death (Karmazyn, 1999; Murphy & Steenbergen, 2008). Importantly, during ischaemia, levels of several metabolic intermediates accumulate, including xanthine, hypoxanthine and succinate (Chouchani et al., 2014); the latter a universal signature of ischaemia which primes the cardiomyocyte for a significant release of pathological reactive oxygen species generation should reperfusion occur.

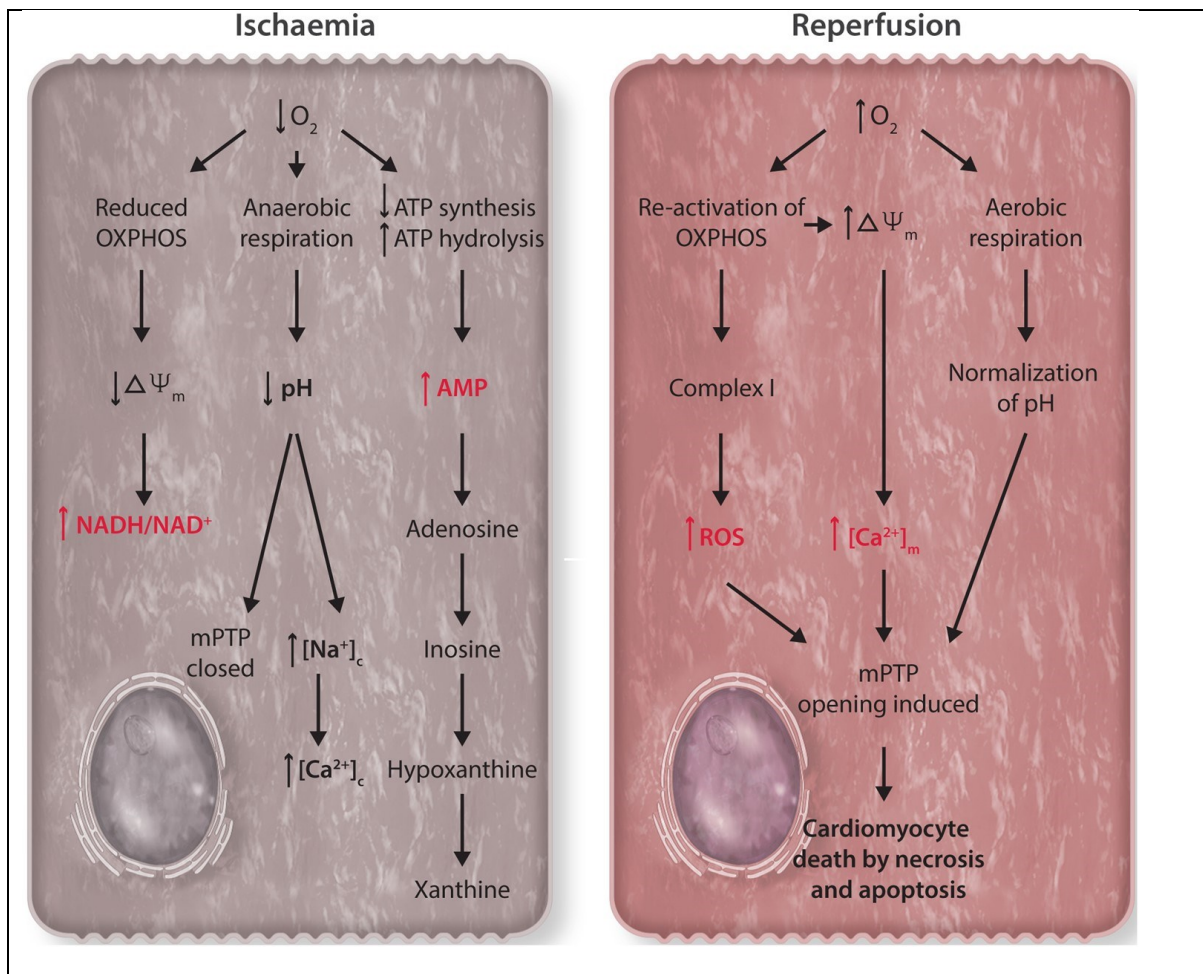
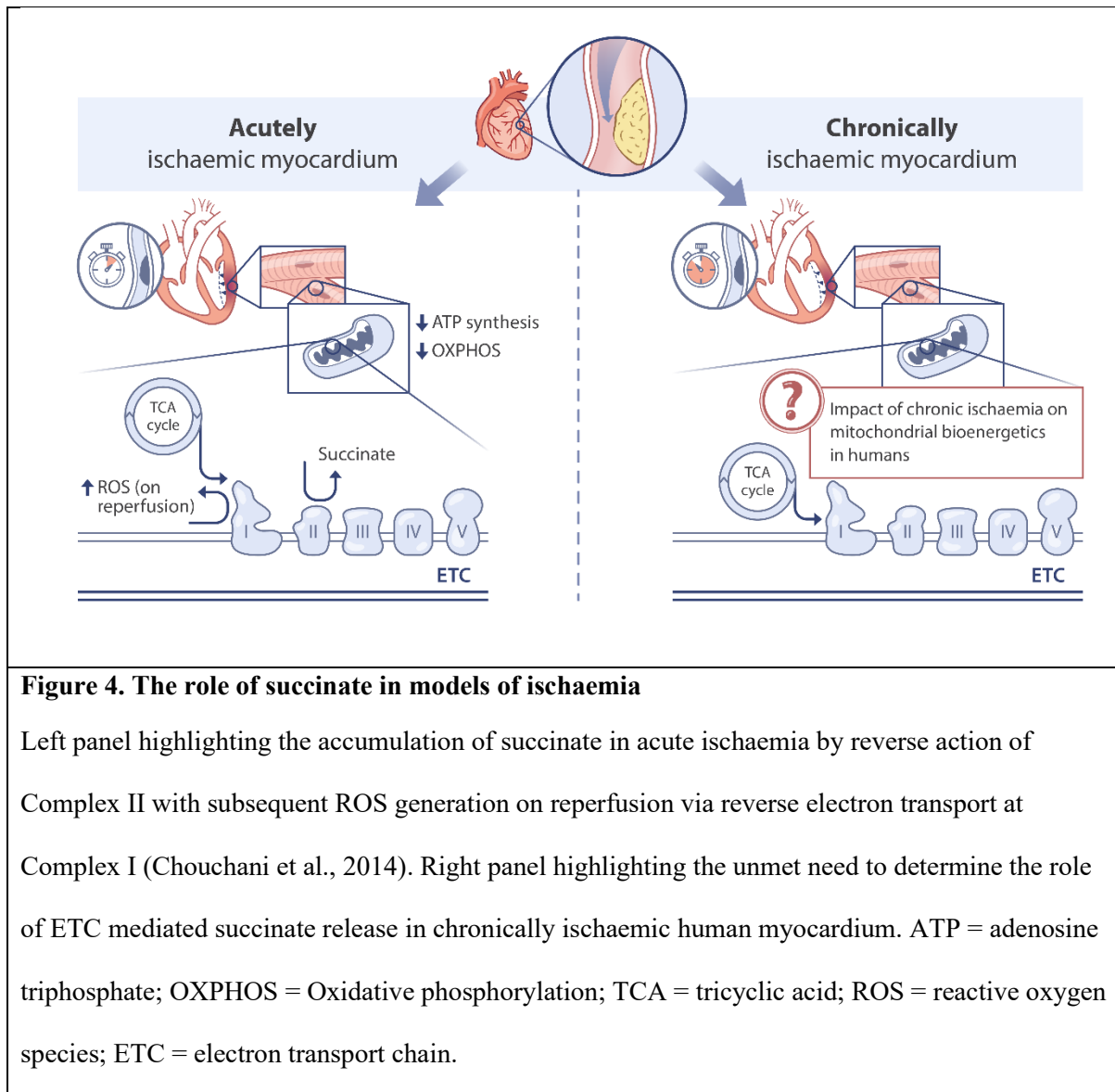


Figure 3. Illustration summarising the key components of ischaemia/reperfusion injury.

The paucity of oxygen during myocardial ischaemia drives the shift to anaerobic respiration, resulting in lactate generation, a decrease in intracellular pH and of overload of sodium and calcium ions. The low pH also prevents the opening of the mPTP. Oxidative phosphorylation is disrupted and there is elevation of the NADH/NAD⁺ ratio. ATP stores are depleted as it is hydrolysed to AMP to maintain the mitochondrial membrane potential. Following reperfusion, the electron transport chain is restarted, leading to the normalisation of both the intracellular pH and the mitochondrial membrane potential. There is also a large influx of calcium ions into the mitochondrion. Complex I is quickly reactivated, generating ROS. The opening of the mPTP subsequently results in the collapse of the mitochondrial membrane potential, leading to apoptosis and cell death. Reproduced with permission from *Cardiovascular Research*, (Pell et al., 2016).

1.4.3 *Electron transport chain and reactive oxygen species during acute ischaemia/reperfusion injury (IR)*

Reactive oxygen species (ROS) are a class of small reactive molecules that are produced at several sites within human cells. At physiological quantities, they regulate several cardiovascular processes including signalling pathways and mitochondrial quality control systems. ROS are however implicated in many cardiovascular diseases when production overwhelms endogenous scavenging mechanisms and compensatory signalling pathways (Brown et al., 2017). The subsequent increased ROS levels can disrupt macromolecules (e.g., lipids and proteins) and can lead to a breakdown of cellular energy production and cell death (Brown et al., 2017). Mitochondria are a key source of ROS during IR injury, with a key species, superoxide, predominantly generated at Complex I and Complex III of the electron transport chain. The Murphy lab have comprehensively investigated the cellular mechanisms implicated in mitochondrial superoxide release during IR injury. Chouchani and colleagues demonstrated that succinate accumulation, driven by reverse action of Complex II, is a universal marker of ischaemia in animal models of cardiac disease (Chouchani et al., 2014). Furthermore, they reported that this accrued succinate is re-oxidised upon reperfusion which in turn, leads to superoxide generation via reverse electron transport at Complex I (Chouchani et al., 2014). Subsequent translational research within their wider group investigated the circulating metabolic profile in patients with acute coronary syndromes. They performed paired blood sampling from the aortic root and coronary sinus, reporting ‘cardiac-specific’ succinate release that may echo the accumulation of succinate during ischaemia with subsequent release from the myocardium on reperfusion (Kohlhauer et al., 2018). Additionally, Martin and colleagues demonstrated elevated succinate levels in human myocardium exposed to *ex vivo* ischaemia, reporting for the first time the role of this key TCA cycle intermediate in a human model of acute ischaemia (Martin et al., 2019). Whether succinate accumulation occurs in chronically ischaemic human myocardial tissue is unknown but could represent a novel drug target in the treatment of non-acute CAD (Figure 4).



1.4.4 Myocardial metabolism in post myocardial infarction models

Alongside the inflammation-mediated fibrotic response, there are several perturbations to metabolism (including changes in substrate utilisation and oxidative phosphorylation) as the heart failure syndrome develops (Figure 5). In non-ischaemic phenotypes, human biopsy research from Nascimben and colleagues demonstrated a 25-30% reduction in myocardial ATP levels phenotypes (Nascimben et al., 1996; Starling et al., 1998). These results were later confirmed using ^{31}P NMR spectroscopy, again in patients without an ischaemic aetiology (Beer et al., 2002). Aligned to these findings, there is also broad agreement of altered myocardial substrate utilisation in heart failure. Most studies report an increase in cardiac glucose uptake, with a compensatory switch to the more energy efficient glycolysis

in the absence of elevated levels of glucose oxidation (Bertero & Maack, 2018). Again however, these results are not always mirrored in models of ischaemic heart failure with no change in glucose oxidation reported in infarcted rat hearts with systolic dysfunction (Amorim et al., 2010). Data pertaining to changes in fatty acid oxidation also vary, however studies (including in the post myocardial infarction setting) have reported reduced fatty acid utilisation (Heather et al., 2006; Rosenblatt-Velin et al., 2001). These changes may in part be due to a decrease in PPAR alpha signalling in addition to reduced activity of the transcription factor responsible for mitochondrial biogenesis, PPAR- γ co-activator 1 α (PGC-1 α) (Brown et al., 2017). Ketone body metabolism also appears to be altered in heart failure with data supporting elevated ketone utilisation in human phenotypes (Bedi et al., 2016).

Aligned to these metabolic alterations, there are also broader changes to mitochondrial function with increased mitochondrial ROS generation, coupled to downstream ROS related damage reported in clinical studies of heart failure (Ide et al., 2000, Ide et al., 2001). Early animal data demonstrated increased oxidative stress in pre-clinical myocardial infarction studies (Hill & Singal, 1997; Shiomi et al., 2004), with subsequent data identifying the protective role of increased endogenous antioxidant expression in these models (Matsushima et al., 2006). Additionally, human biopsy research in individuals with CAD has demonstrated increased ROS production within chronically ischaemic myocardial segments (Stride et al., 2013). An elegant human tissue study by Ait-Aissa and colleagues also showed dysregulated electron transport chain (ETC) activity in patients with CAD (Ait-Aissa et al., 2019).

1.4.5 *Dysfunctional viable myocardium*

Myocardial hibernation represents another phenotype that is commonly encountered in ischaemic LV impairment. Early discovery of patients with chronic CAD who have areas of ventricular hypokinesia in the context minimal replacement myocardial fibrosis led to a paradigm shift in clinical practice and research activity. This entity describes myocardium that is deemed to be dysfunctional, but viable, with the potential for left ventricular reverse remodelling following therapy, traditionally revascularisation. This drove 3 decades of research with the generation of two (competing) concepts attempting to detail the underlying pathophysiological adaptations (Figure 6). First, Rahimoola popularised the theory that ‘hibernating’ myocardium resulted from adaptive downregulation of contractile function and metabolism, driven by reduced *resting* myocardial blood flow, to prevent overt ischaemia and myocyte death (Rahimtoola, 1989). This proposes a state of equilibrium where reduced resting myocardial oxygen supply matches demand from the, albeit sacrificed, hypocontractile ventricle (Ryan & Perera, 2018). The temporal nature of these events was later contested, initially by Canty and Klocke (Canty & Klocke, 1987) followed by experiments by Vantner and colleagues and later Canty and Fallavollita. Studies in the mid 1990’s by Vantner and team noted preservation of baseline blood flow in a progressive coronary stenosis pig model that induced ventricular hypokinesia (Shen et al., 1996; Shen & Vantner, 1995) and led to the conclusion that hibernating myocardium is due to repetitive episodes of myocardial ischaemia with cumulative myocardial stunning (Wijns et al., 1998). Canty and Fallavollita and colleagues subsequently generated pig models with increasing stenosis severity again demonstrating normal resting baseline blood flow during short-term hibernation, additionally documenting a transition to a reducing resting flow state after 2 months (Fallavollita & Canty, 1999). These experiments led to the new central idea that reduced resting myocardial blood flow is the result, not the cause, of impaired myocardial function, with impairment of coronary flow reserve defining hibernating myocardium and driving contractile impairment (Heusch, 2021). Importantly, during longer follow up periods, reduction in the resting blood flow is noted, reconciling the two theories and providing evidence for models of both short- and long-term hibernation (Fallavollita & Canty, 1999; Heusch, 2021).

Overview of the ‘competing’ theories of hibernating myocardium

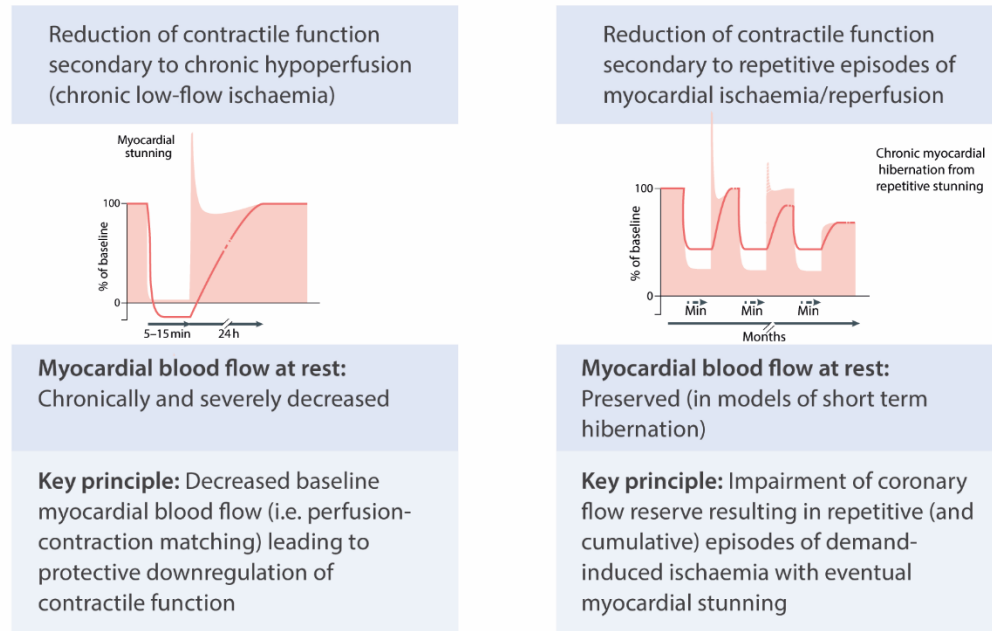


Figure 6. Schematic illustration detailing the two proposed theories for hibernating myocardium.

Left: The ‘smart’ heart hypothesis proposing that chronic reductions to baseline coronary flow resulted in adaptive, protective, downregulation of cardiac function and bioenergetic pathways to match the reduced supply, resulting in conserved energy needs and resisting irreversible cardiac injury. Right: Repetitive stunning hypothesis proposing that impairment in coronary flow reserve (due to the coronary atherosclerotic disease) results in cumulative and repetitive episodes of demand ischaemia leading stunning and eventual persistent impairment of systolic function. Central images adapted from *Springer Nature* with permission, (Heusch, 2021).

1.4.6 Myocardial metabolism in dysfunctional viable myocardium

Since the discovery of the hibernating myocardium, there has been concerted effort to detail the bioenergetic profile underpinning the phenotype, with pre-clinical models predominantly demonstrating preservation of regional energetic capacity. McCalls et al assessed ATP and phosphocreatine levels from swine LV tissue biopsies taken at an average of 5 weeks after partial occlusion of the LAD. They demonstrated preservation of energetic reserve in chronically ischaemic

myocardium compared to non-LAD regions, concluding that the myocardium adapts to chronic hypoperfusion (McFalls et al. 1997). Hu et al again utilised a swine model, collecting myocardial biopsies at a later timepoint of 3 months following induced stenosis of the LAD. They documented similar results with preservation of the ATP/ADP ratio between areas of dysfunctional viable segments and remote myocardium within the same heart (Hu et al., 2009). Human studies in patients with hibernating myocardium undergoing CABG have revealed comparable findings. Wiggers et al combined positron emission tomography with cardiac tissue high-energy phosphate analysis in 25 patients undergoing CABG. They demonstrated that metabolic energy stores in recoverable myocardium are maintained, compared to remote myocardium (Wiggers et al., 2001). Conversely, however, there are a limited number of studies highlighting the presence of regional differences in metabolomic status. Elsässer et al assessed the energetic landscape of hibernating myocardium in 16 patients undergoing CABG, acquiring transmural LV biopsies during surgery. They reported a reduction in high-energy phosphate concentrations in hibernating myocardium and concluded that this phenotype is indeed an energy depleted state (Elsässer et al., 2002); the disparate results comparing to the prior human studies potentially relating to more advanced degrees of adverse remodelling and myocardial fibrosis.

Animal studies have also investigated serial changes in mitochondrial function following revascularisation in models of stable CAD. Holley and colleagues detailed residually elevated expression of uncoupling protein-2 within hibernating swine myocardium post revascularisation (Holley et al., 2015). Kelly et al demonstrated continuing abnormalities in electron transport chain protein expression in the revascularised swine heart (Kelly et al., 2011). Finally, Page and colleagues again noted residual abnormalities in contractile and metabolic protein expression following revascularisation in a hibernating swine model (Page et al., 2015). These studies all highlighted residual myocardial pathology following restoration of epicardial blood supply in animal models of chronic coronary artery disease.

1.5 A novel cause for LV systolic impairment in patients with CAD; exploring for shared features with dilated cardiomyopathy

Alongside the traditional causes for LV impairment in chronic CAD (hibernating myocardium and prior MI), there is an increasing appreciation of patients with an apparent dual pathology with both coronary artery disease and non-ischaemic ventricular dysfunction. Dilated cardiomyopathy (DCM) describes a heterogenous group of diseases, characterised by LV dilatation and impairment of function in the absence of obstructive CAD or abnormal loading pathologies (e.g. hypertension or valvular disease) (Pinto et al., 2016). DCM is an important cause of heart failure and sudden cardiac death and remains the leading cause of heart transplantation worldwide (Halliday et al., 2017; Lund et al., 2016). The heterogeneity of the diagnosis is highlighted by its definition, essentially describing a syndrome of exclusion, incorporating a diverse range of underlying diseases and barring just a few (encapsulated by the common phrase “non-ischaemic dilated cardiomyopathy”). There have, however, been notable advances regarding our understanding of common risk factors for DCM, with particular focus on the complex interplay between rare and common genetic variants and lifestyle factors. There are currently around 12 genes with robust evidence describing their causal role in DCM (Mazzarotto et al., 2020), with rare Titin truncating variants (*TTN*tv) implicated in up to 25% of patients (Herman et al., 2012; Roberts et al., 2015). Research from our wider group has also reported the shared genetic predisposition between DCM and cardiomyopathy related to alcohol consumption, chemotherapy and pregnancy. The typical analogy depicting a ‘two-hit’ phenomenon requiring a genetic variant (e.g., *TTN*tv) and an environmental insult or stressor (e.g., chemotherapy). Another potential modifier of LV structure in patients with an underlying predisposition to genetic cardiomyopathy could be the presence of obstructive CAD or prior myocardial infarction (Figure 7). This is important as the diagnosis of CAD may preclude the physician considering a concomitant cardiomyopathic process, negating risks associated with specific genetic aetiologies and excluding family and genetic screening. Indeed, there has been an increasingly appreciation of the coexistence of key DCM genes in patients with CAD. The Goldstein lab assessed the genetic profile of close to 6,000 individuals with heart failure, reporting that in patients with a rare pathogenic variant in known cardiomyopathy gene, 60%

had a diagnosis of ischaemic heart disease (Povysil et al., 2021). Aligned to this finding, multiple studies have reported the presence of non-ischaemic patterns of myocardial scar in patients with CAD (Almehmadi et al., 2014; Nucifora et al., 2021) with unanswered questions pertaining to whether these individuals have a dual pathology with DCM or if the phenotype solely represents the common end-stage of the heart failure syndrome.

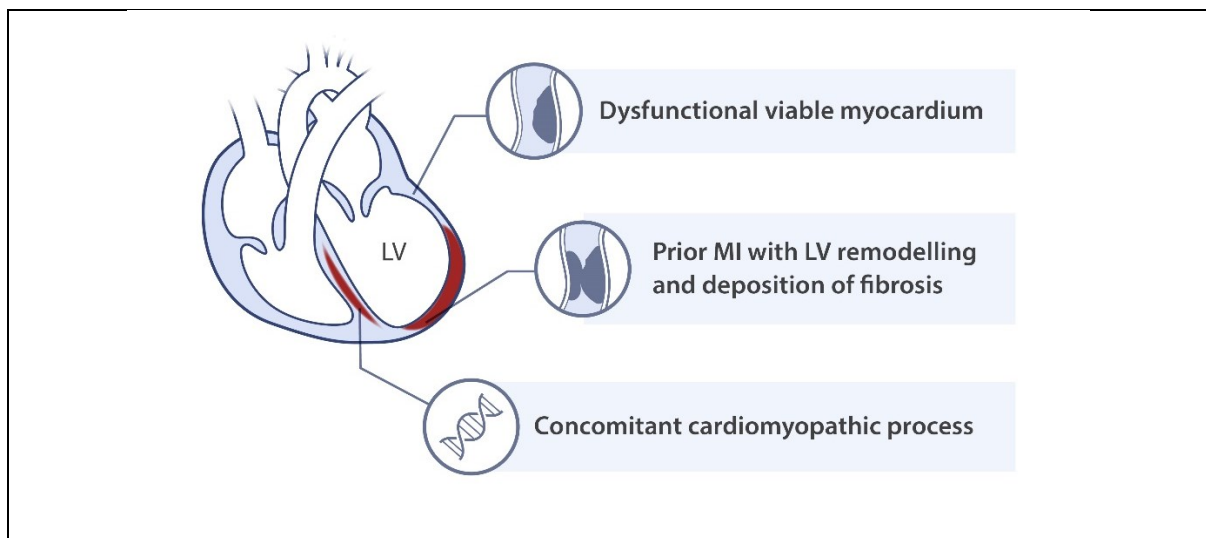
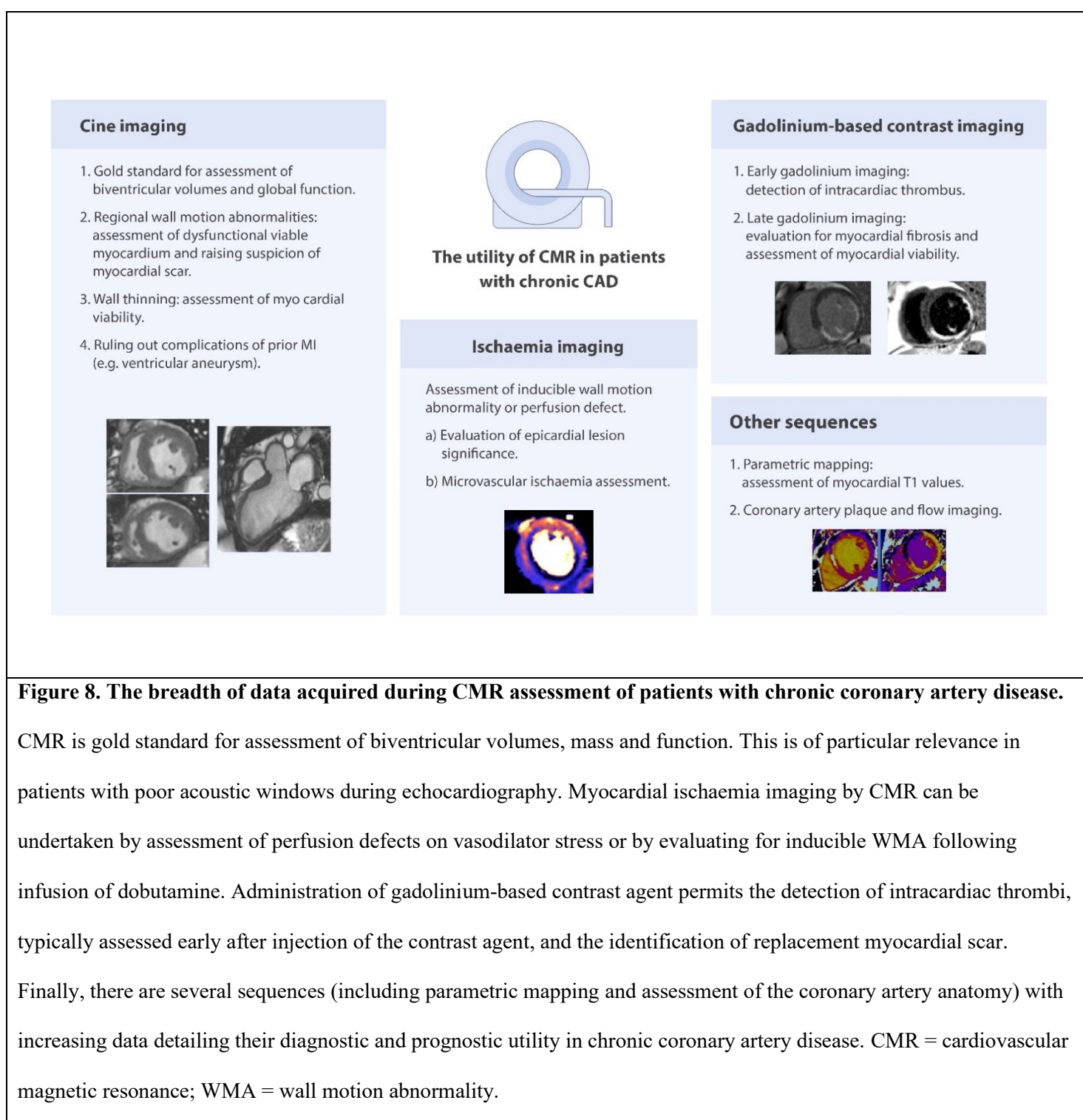


Figure 7. Extending the possible mechanisms associated with LV impairment in patients with chronic coronary artery disease.

Outside of acute myocardial stunning, there are traditionally 2 broad pathological states associated with LV impairment in patients with CAD. First, prior myocardial infarction with LV wall thinning and deposition of replacement of myocardial fibrosis. Second, obstructive coronary atherosclerotic disease resulting in the development of dysfunctional, yet potentially viable, myocardium. A third category may also exist; patients with CAD that have a co-existing vulnerability to the development of a non-ischaemic cardiomyopathy. CAD = coronary artery disease; LV = left ventricular.

1.6 Imaging the myocardium using Cardiovascular Magnetic Resonance.

Despite echocardiography being the first line investigation to describe cardiac structure and function, the utility of cardiovascular magnetic resonance (CMR) to capture this data more accurately, alongside granular characterisation of tissue composition, is widely adopted in stable CAD (Figure 8).



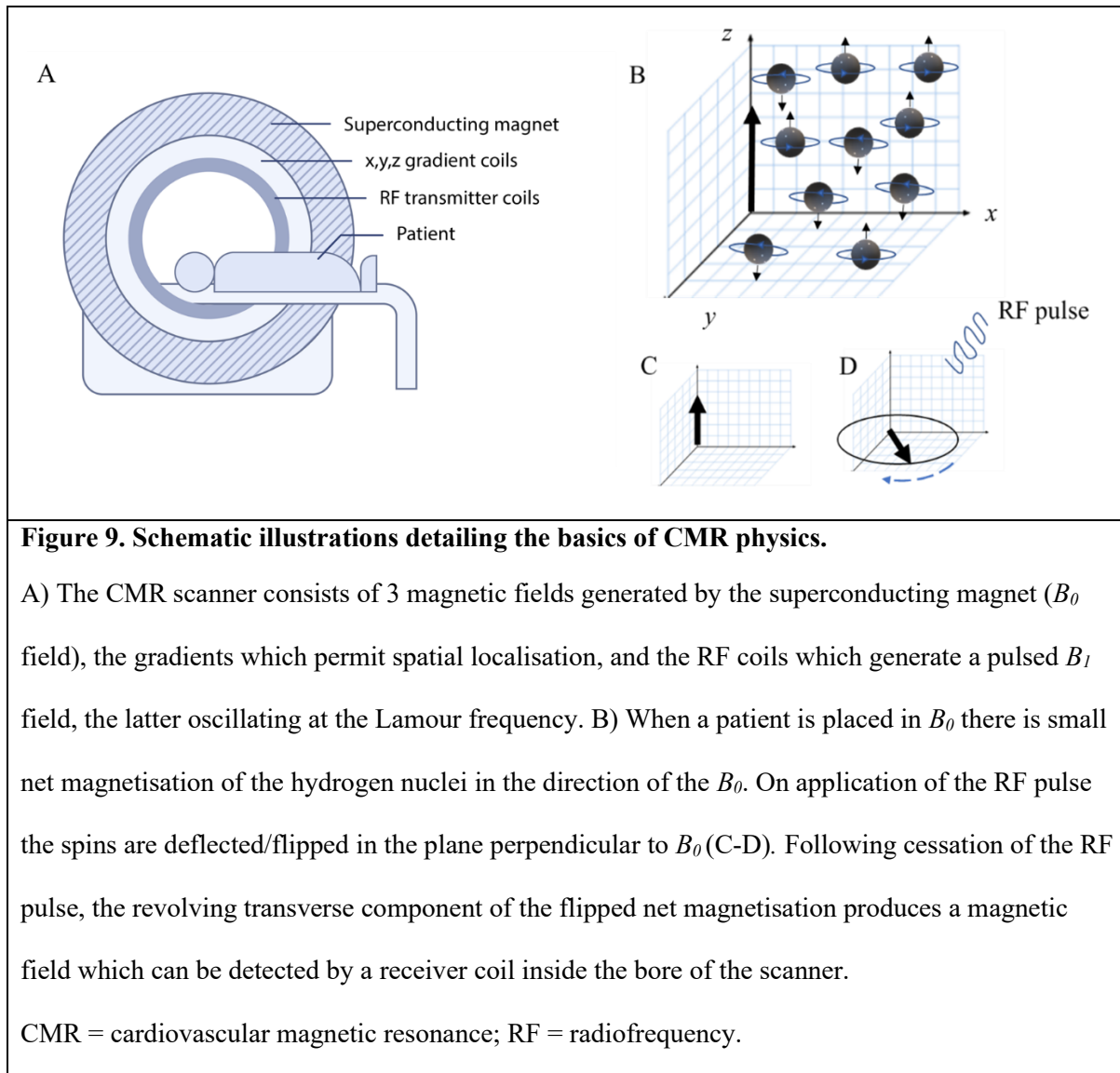
1.6.1 Introduction to CMR physics

CMR scanners typically apply 3 magnetic fields to aid generation of the signal used to form the recognisable medical image (Figure 9):

1. The main superconducting magnet producing a strong, uniform, and constant magnetic field, in a horizontal plane (B_0).
2. The 3 gradient coils installed on the inner bore of the main magnet that rapidly switch on and off to generate magnetic fields in the x , y and z directions. This function permits the resultant signal to be encoded in 3 dimensions, allowing for oblique cross-sectional imaging to be undertaken.
3. A radiofrequency magnetic field used to generate the crucial magnetic resonance signal in the xy plane.

The key principle of CMR is the use of the magnetic resonance properties of (typically) hydrogen protons within free water or lipids. These nuclei have magnetic moments ('spins') which are usually randomly orientated but when exposed to a large magnet (B_0), produce a bulk net magnetisation in the direction of the magnetic field (termed M_z). This is not however sufficient to generate medical images and thus the radiofrequency (RF) coil is applied to deliver energy to the protons at a specific frequency, known as the Larmour frequency. This RF pulse deflects the spins in a plane perpendicular to B_0 with varying degrees of flip angle. Following this excitation pulse, the spins begin to return to the equilibrium state inside B_0 , with regrowth of M_z (T1 relaxation or spin-lattice relaxation) and decay of the transverse component of the magnetisation, M_{xy} (T2* and T2 relaxation, spin-spin relaxation). Importantly, the T1, T2 and T2* of different tissue vary, a crucial aspect that provides the "contrast" seen in final images. Spatial localisation is performed using slice selection, frequency encoding and phase encoding gradients, alongside the generation of *echoes* (fundamentally either spin echo or gradient echo) used to generate the measured signal; a step principally undertaken in view of the dephasing caused by the gradient coils which impacts the decay induced by the initial RF pulse. The frequency-encoded data acquired during each phase-encoding step is termed the *k-space*, signal

which is subsequently converted into a recognisable MR image using Fourier transformation (Manning & Pennell, 2019).



1.6.2 Vasodilator stress perfusion

The examination relies on the administration of a vasodilator agent (e.g., adenosine, regadenoson or dipyridamole) to abolish/exceed the compensatory mechanisms present at *rest* in the myocardium of patients with upstream significant coronary stenoses. With decreasing vessel diameter, coronary flow is maintained at rest by microvascular dilatation and recruitment of collateral arteries (de Waard et al., 2018). Achieving complete hyperaemia results in coronary flow becoming proportional to perfusion

pressure, thus flow distal to coronary stenosis is proportionally (in addition to relatively compared to remote myocardial segment) reduced (de Waard et al., 2018; Dewey et al., 2020). In the context of a severe stenosis, coronary steal, typified by a myocardial perfusion reserve of <1 , can also occur as blood is redirected to the remote myocardium.

Following infusion or injection of the vasodilatory agent, a saturation recovery (90°) preparation pulse is typically applied followed by a sequence permitting rapid readout of the acquired data, commonly either with echo planar imaging, spoiled gradient echo or balanced steady state free precession.

Coverage of the ventricle is usually at 3 levels; basal, mid-cavity and apex with the notable limitation that assessment of whole heart ischaemia is not conducted in routine clinical practice. Assessment of hypoperfusion is typically performed using visual analysis of the raw perfusion image during the first pass of a gadolinium-based contrast agent. Myocardial segments supplied by a significant coronary stenosis should appear persistently (e.g., ≥ 3 frames) hypointense as compared to regions subtended by an unobstructed coronary artery. Signal loss due to susceptibility artefacts, Gibbs ringing at the subendocardial interface, or spatial resolution issues needs to be considered during the assessment.

The performance of stress CMR to diagnose haemodynamically significant CAD has been shown in multiple single centre observational studies with meta-analysis data demonstrating a per-patient sensitivity and specificity of 0.90 (0.75–0.97) and 0.94 (0.79–0.99) respectively (Danad et al., 2017).

CE-MARC assessed 752 patients with suspected CAD; participants underwent CMR, single-photon emission computed tomography (SPECT) and traditional invasive coronary angiography. For

multiparametric CMR, sensitivity was 87%, specificity 83%, positive predictive value 77% and negative predictive value 91% for detecting significant coronary heart disease. In comparison, the sensitivity of SPECT was 67%, specificity 83%, positive predictive value 71%, and negative predictive value 79% (Greenwood et al., 2012). The prognostic utility of stress CMR also been

demonstrated; the recent retrospective multicentre SPINS registry of 2,349 patients reporting an odds ratio of 4.2 for acute myocardial infarction ($P<0.001$) and 2.4 for cardiovascular death ($P=0.004$)

during the first 4 years of follow-up in patients with documented ischaemia (Kwong et al., 2019). A

further prospective study of 9,151 patients detailed the independent prognostic role for stress CMR in patients with known or suspected stable CAD, importantly improving both prediction of mortality and

risk reclassification (net improvement: 11.4%; 95% CI 7.3-13.6, $P<0.001$) (Heitner et al., 2019). The MR inform trial also demonstrated the utility of stress CMR to guide invasive coronary revascularisation, demonstrating non-inferiority to a fractional flow reserve based strategy with respect to major adverse cardiac events (Nagel et al., 2019).

Increasingly, quantitative approaches that permit the accurate, automated, calculation of myocardial blood flow are being adopted in CMR. These techniques have the prerequisite requirement of quantifying the arterial input function driving the delivery of the contrast agent and typically use either a dual-sequence or dual-bolus approach. Quantification of myocardial blood flow is then typically undertaken using either Fermi function deconvolution (Jerosch-Herold et al., 1998) or a blood tissue exchange model (Kellman et al., 2017). Fully quantitative CMR can improve differentiation of epicardial multivessel disease from microvascular disease (Kotecha et al., 2019), in addition to improving the accuracy of CMR to diagnose multivessel disease, relative to standard visual assessment of perfusion defects (Kotecha et al., 2020). Automated MBF quantification has also been shown to have prognostic utility with Knott and colleagues reporting the independent association between each 1ml/g/min decrease in stress MBF and adverse cardiac events in CAD patients (Knott et al. 2020).

1.6.3 Tissue characterisation

A further key strength of CMR is the ability to characterise the extra- and intra- cellular composition of LV myocardium. Traditionally, this relates to sequences that use the T1 shortening properties of gadolinium, in tandem with inversion recovery gradient echo sequences, to evaluate for myocardial scar. Following myocardial infarction there is expansion of the intercellular space with the resulting deposition of collagen-rich fibrosis. Gadolinium-based contrast agents accumulate in the extracellular space and shorten T1 relaxation times of adjacent molecules, showing increased signal intensity of T1 weighted imaging assessed at a time point of approximately 10mins. The pattern of scarring following myocardial infarction typically follows a subendocardial to transmural course, mirroring the wavefront of ischaemic myocardial cell death. Research by Kim and colleagues demonstrated the accuracy of LGE imaging to characterise the extent of myocardial infarction in a canine model of disease (Kim et al., 1999). Our group subsequently demonstrated the utility of LGE imaging to differentiate non-ischaemic cardiomyopathy from ischaemic left ventricular dysfunction (McCrohon et al., 2003), potentially highlighting the role of the modality as a gatekeeper to coronary angiography (Assomull et al., 2011). LGE imaging also provides important prognostic information with infarct size associated with all-cause mortality across 7 randomised primary percutaneous coronary intervention trials using CMR (Stone et al., 2016). Additionally, Lee and colleagues reported the association between LGE extent and adverse cardiac events, independent of the LV function, in patients after CABG (Lee et al., 2016). LGE-CMR can also inform on long term arrhythmic risk prediction in cardiac disease (Disertori et al., 2016) with an evolving role in the characterisation of dense core infarction and the adjacent peri-infarct zone. More recently, LGE-CMR has been harnessed to evaluate complex scar geometry, predominantly in tandem with electroanatomical voltage mapping or computational modelling techniques (Arevalo et al., 2016). One issue with traditional LGE imaging in patients with prior myocardial infarction is the potential for poor contrast between the hyperenhanced scar and the relatively bright blood pool on T1 weighted imaging. Dark blood late enhancement sequences have circumvented this issue, typically by applying an inversion recovery T2 preparation aimed at improving the detection of subendocardial enhancement (Figure 10) (Kellman 2017).

More recently, a new era of tissue characterisation sequences have been developed that detail a spatial, pixel-by-pixel, representation of the absolutely denominated numerical T1 or T2 properties, presented in units of time (typically milliseconds) (Ferreira & Piechnik, 2020). Parametric mapping can detail intracellular abnormalities of the cardiomyocyte (e.g., iron overload or accumulation of glycosphingolipid in Anderson-Fabry disease), in addition to extracellular interstitial changes (e.g., myocardial fibrosis or oedema). Importantly, distinct from T1-, T2-*weighted* imaging, mapping allows the visualisation and quantification of the disease process, irrespective of the presence of focal or diffuse disease (Messroghli et al., 2017).

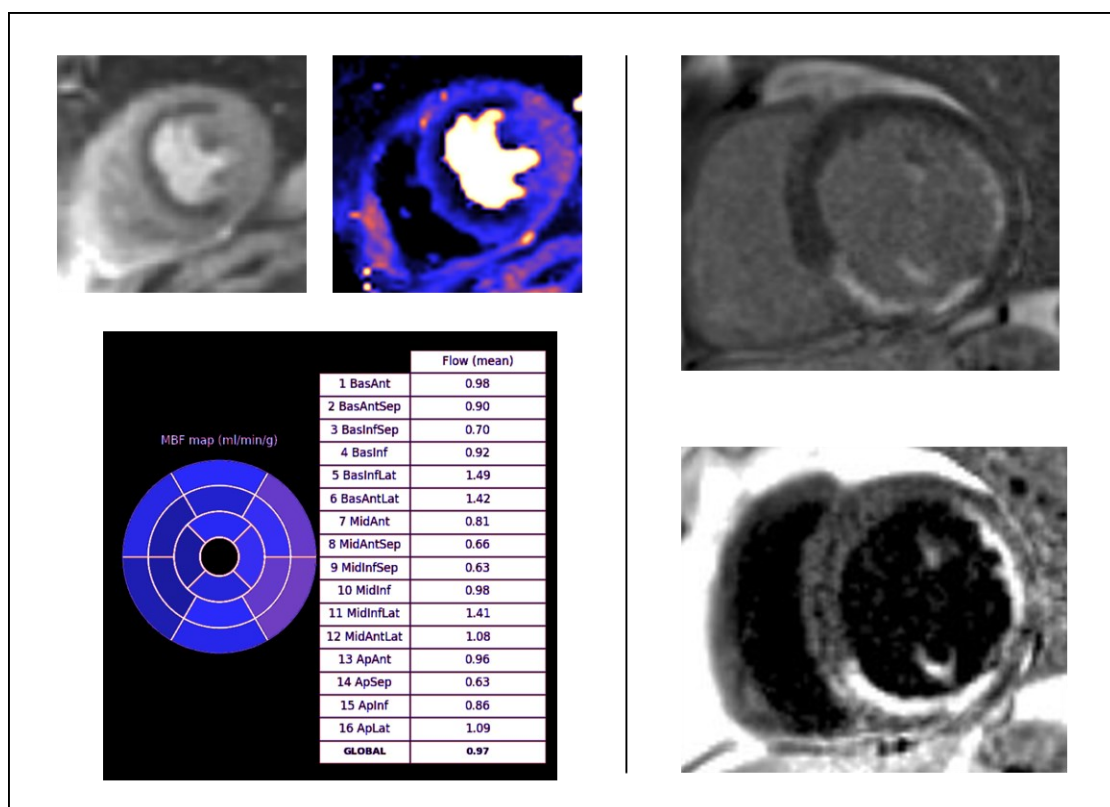


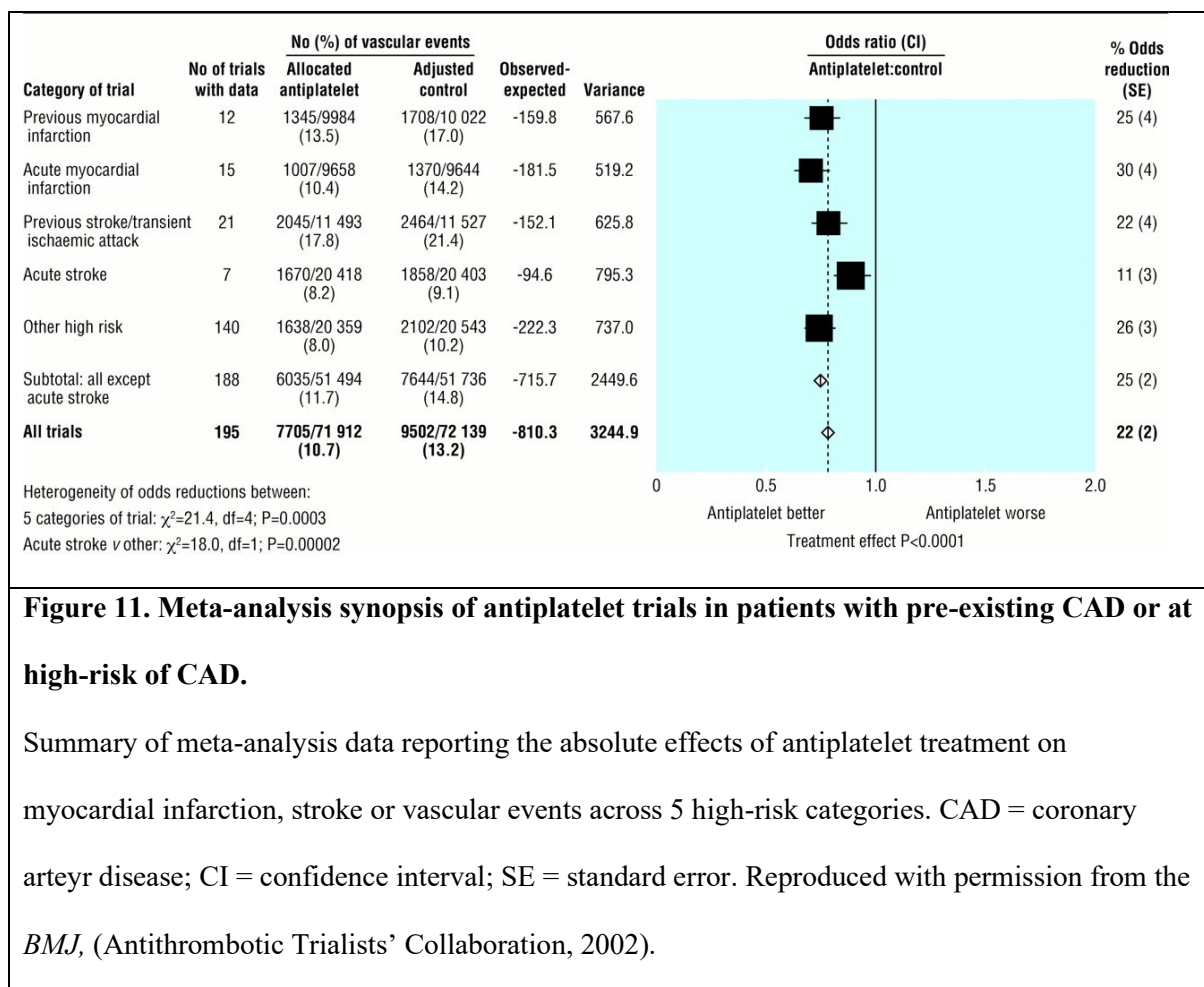
Figure 10. Examples of CMR sequences assessing inducible myocardial ischaemia and LGE.

Left: Raw perfusion imaging (left) demonstrating a perfusion defect in anterior wall, ventricular septum and inferior wall. Perfusion mapping (right) permits quantification of segmental myocardial blood flow in these regions, enabling more granular appreciation of inducible hypoperfusion and removing the need for a relative difference in hypo intensity to be present to define a positive test result. Right: Typical bright blood LGE imaging (top) demonstrating inferior and lateral wall subendocardial infarctions. Dark blood LGE imaging (bottom), emphasising the presence of subendocardial infarction in view of the improved contrast between scar and the blood pool. LGE = late gadolinium enhancement.

1.7 The management of patients with stable coronary artery disease

1.7.1 Lifestyle and medical therapy to improve symptoms and outcomes

The cornerstone of treatment aims to reduce symptom burden, enhance quality of life, and improve event-free survival. Lifestyle management is key, including guidance to stop smoking (cessation associated with 36% risk reduction in mortality (Critchley & Capewell, 2003)), and promotion of a healthy diet and weight, alongside appropriate physical activity; all with a Class 1 indication in ESC and AHA guidelines (Fihn et al., 2012; Knuuti et al., 2020). In parallel, medical therapy aims to reduce anginal symptoms and prevent cardiovascular events. In patients with known stable CAD, low-dose aspirin reduces future events by nearly a quarter in high-risk patients (Antithrombotic Trialists' Collaboration, 2002), notably in individuals with occlusive CAD and prior myocardial infarction, Figure 11). In patients with non-obstructive CAD, data for long-term benefit is less clear with a recent prospective observational study of 6,386 patients reporting no association between baseline aspirin use and major adverse cardiovascular events (10.9% vs 14.7%, $P=0.06$) or death (9.6% vs 10.9%, $P=0.47$) (Indraratna et al., 2022). Additionally, a prior metanalysis of primary prevention randomised control trials reported a significant, yet only modest (0.07% per year) absolute risk reduction risk in apparently healthy patients prescribed aspirin (Antithrombotic Trialists' (ATT) Collaboration et al., 2009) .



Conversely, lipid lowering medications consistently show prognostic utility with metanalyses (including both primary and secondary prevention trials) reporting all-cause mortality reductions with statin therapy for both women (RR 0.91, 99% CI 0.84-0.99) and men (RR 0.90, 99% CI 0.86-0.95) (Cholesterol Treatment Trialists' (CTT) Collaboration et al., 2015). Additionally, compared with less intensive statin regimens, more intensive treatment programmes lead to a 15% (95% CI 11-18, $P<0.0001$) greater reduction in major adverse vascular events (Cholesterol Treatment Trialists' (CTT) Collaboration et al., 2010). As such, international guidelines mandate that statins should be started in all patients with confirmed CAD irrespective of low-density lipoprotein cholesterol (LDL-C) levels with a goal to reduce LDL-C by 50% from index evaluation to a level of $<1.4\text{mmol/L}$ (Mach et al., 2020). In patients where their individualised treatment goal is not achieved on the maximum tolerated statin, addition of ezetimibe should be considered. Randomised control data showing an absolute risk difference of 2% with ezetimibe therapy compared to patients treated with simvastatin alone (HR

0.94, 95% CI, 0.89-0.99; P=0.016) (Cannon et al., 2015). Finally, proprotein convertase subtilisin–kexin type 9 (PCSK9) inhibitors should be added to the treatment regime in patients with inadequate LDL-C control with statin and ezetimibe therapy; both evolocumab and alirocumab leading to a 15% reduction in major cardiovascular events, relative to placebo (Sabatine et al., 2017; Schwartz et al., 2018).

Alongside anti-platelet and lipid lowering therapy, control of symptoms is paramount with the presence of angina being associated with a poor health status (Manolis et al., 2019) and an increased risk of adverse clinical events (Mesnier et al., 2021). Short acting nitrates for the immediate relief of angina remains first line in ESC and AHA guidelines with oral beta-receptor antagonists and calcium channel blockers recommended to control symptoms and heart rate if required (Fihn et al., 2012; Knuuti et al., 2020). Additionally, meta-analysis data shows that betablockade can reduce long term morbidity and mortality by nearly a quarter in patients with prior myocardial infarction (pooled odds ratio 0.77 [0.69-0.85]) (Freemantle et al., 1999). Furthermore, in patients with LV systolic dysfunction, multiple randomised control trials, primarily using carvedilol and bisoprolol, have demonstrated the prognostic benefit of betablockade, including in patients post myocardial infarction. A metanalysis of the placebo-controlled CIBIS and CIBIS II trials (53% of patients with prior myocardial infarction) reporting a nearly 30% relative reduction in death associated with bisoprolol (Leizorovicz et al., 2002). Renin-angiotensin-aldosterone blockers also reduce all-cause death and cardiovascular morbidity in ischaemic heart disease; results consistent across cohorts with LV systolic impairment (risk reduction of 19% for all-cause mortality with captopril relative to placebo (Pfeffer et al., 1992) and patients without apparent heart failure (20% risk reduction for major adverse cardiovascular events with perindopril relative to placebo (Fox & EUROPA Investigators, 2003)).

The first-in-class single pill combining a neprilysin inhibitor angiotensin receptor blocker, sacubitril-valsartan, has additionally been shown to reduce cardiovascular mortality and heart failure hospitalisations, compared to enalapril therapy, in patients with symptomatic heart failure (43% with prior myocardial infarction) and reduced LVEF (McMurray et al., 2014). In patients with reduced LVEF in the context of acute myocardial infarction however, sacubitril-valsartan was not associated

with all-cause mortality or incident heart failure compared to ramipril therapy (HR 0.9, 95% CI 0.78-1.04, P=0.17) (Pfeffer et al., 2021).

Another class of drug with robust outcome data in patients post myocardial infarction are the aldosterone antagonists spironolactone and eplerenone. In the prematurely stopped EMPHASIS-HF trial, eplerenone was shown to reduce cardiovascular death and hospitalisation for heart failure, compared to placebo, in patient with the LVEF $\leq 35\%$ post myocardial infarction (HR 0.63, 95% CI, 0.54-0.74, P<0.001) (Zannad et al., 2011).

More recently, inhibitors of the sodium-glucose cotransport 2 isoform have been shown to be both safe and cardioprotective for patients with atherosclerosis and diabetes mellitus, the EMPA-REG OUTCOME trial reporting a reduced hazard ratio in the empagliflozin group, relative to placebo (HR 0.86, 95% CI 0.74-0.99) (Zinman et al., 2015). Subsequent placebo-controlled trials in heart failure cohorts with and without reduced LVEF have reported similar findings. Metanalysis from the two large trials using dapagliflozin (DAPA-HF, n=56% ischaemic cardiomyopathy) and empagliflozin (EMPEROR-Reduced, n=52% ischaemic cardiomyopathy) in patients with systolic heart failure reported a 13% reduction in all-cause mortality (pooled HR 0.87, 95% CI 0.77–0.98, P=0.018) and 14% reduction in cardiovascular death (0.86, 0.76–0.98, P=0.027) (Zannad et al., 2020).

1.7.2 Promoting left ventricular reverse remodelling in coronary artery disease

Left ventricular reverse remodelling (LVRR) is an important concept in cardiovascular disease and describes the capability of the failing heart to recover systolic performance, resulting in favourable long-term outcomes (Kalogeropoulos et al., 2016). Predicting LVRR in heart failure cohorts remains challenging, with an ischaemic aetiology a key metric that historically associates with failure of LV recovery (Wilcox et al., 2012). Accordingly, patients with recovered LVEF are less likely to have a prior diagnosis of CAD (Kalogeropoulos et al., 2016). Prospective studies in advanced disease cohorts have also shown that patients with CAD and reduced LVEF experience less LVRR, as compared to dilated cardiomyopathy patients, following cardiac resynchronisation therapy (St John Sutton et al.,

2006) and mechanical circulatory support (Wever-Pinzon et al., 2016). Importantly, only modest improvement in global LV performance was observed in the largest randomised control trial of surgical treatment for ischaemic heart failure (LVEF $27.8 \pm 7.5\%$ to $30.3 \pm 10.8\%$ over follow-up in patients with evidence of myocardial viability) (Panza et al. 2019). In clinical practice, a key driver of this lack of LVRR is replacement myocardial fibrosis, with transmural deposition typically preventing localised improvements in LV contractility post restoration of epicardial blood flow (Kim et al., 2000). Importantly, LVRR has also been demonstrated by targeting both the beta-adrenoceptors (Australia/New Zealand Heart Failure Research Collaborative Group., 1997; Doughty et al., 1997) and renin-angiotensin-aldosterone system (B. Greenberg et al., 1995; Konstam et al., 1992; Solomon et al., 2005; Wong et al., 2002) in ischaemic heart disease. Conversely, a more recent randomised control trial on 93 patients with prior myocardial infarction reported that sacubitril-valsartan did not have a significant effect on left ventricular reverse remodelling, compared to valsartan (Docherty et al., 2021).

1.7.3 The role of coronary revascularisation.

The role of epicardial coronary revascularisation in CAD cohorts continues to evolve. In the setting of ST elevation myocardial infarction, primary percutaneous coronary intervention (PPCI) reduces mortality, reinfarction and stroke if it can be provided within 12 hours of symptom onset; meta-analysis data demonstrating short-term reductions in death (7% vs 9%, $P=0.0002$), non-fatal reinfarction (3% vs 7%, $P<0.0001$) and a composite of death, non-fatal reinfarction and stroke (8% vs 14%, $P<0.0001$) driven by PPCI relative to thrombolysis (Keeley et al., 2003). The data becomes less clear in acute coronary syndrome patients without persistent ST elevation with the current guidelines recommending selective invasive coronary angiography dependent on the presence of high-risk clinical features (e.g., haemodynamic instability, persistent chest pain, acute heart failure, dynamic ST changes, resuscitated cardiac arrest, GRACE score >140), with limited evidence for an early invasive strategy amongst stable patients (Collet et al., 2021). In chronic coronary heart disease, there is increasingly even less evidence detailing the symptomatic and prognostic role for restoration of epicardial vessel patency (Figure 12).

2007	PARR-2: n=450 patients with suspected CAD and LVEF $\leq 35\%$ - PET-guided care (incl coronary revascularisation if viability determined) vs standard of care - At 1 and 5 years follow up $\rightarrow$ no difference in CV death/MI (PET arm HR 0.78, 95% CI 0.58-1.1, P=0.15)
	COURAGE: n=2287 patients with stable CAD and inducible ischaemia - PCI with OMT versus OMT - Over median 4.6 years $\rightarrow$ no difference in CV death/non fatal MI (HR for the PCI group 1.05, 95% CI 0.87-1.27, P=0.62)
2009	BARI-2D: n=2368 DM patients with stable CAD - CABG or PCI versus OMT - Over mean of 5.3years $\rightarrow$ no difference in mortality or death/MI/stroke
2012	FAME 2: n=888 patients with stable CAD - FFR guided PCI versus OMT - Over mean of 213 days $\rightarrow$ Difference in urgent revascularisation/death/ non-fatal MI (4.3% vs 12.7%, HR with PCI 0.32, 95% CI 0.19-0.53, P<0.001) - Death/nonfatal MI HR 0.61 (0.28-1.35, P=0.22)
2016	STICHES: n=1212 patients with stable CAD and LVEF $\leq 35\%$ - CABG versus OMT (extension trial of STICH) - Over a median of 9.8years $\rightarrow$ HR for CABG 0.84, 95% CI 0.73-0.97; P=0.02
2018	ORBITA: n=200 with stable CAD and single vessel disease - Double blind, placebo controlled, PCI vs OMT - No significant difference in Δ exercise time increment (PCI minus placebo 16.6 s, 95% CI -8.9-42.0, P=0.2)
2020	ISCHEMIA: n=5179 patients with stable CAD and moderate or severe inducible ischaemia - PCI or CABG vs OMT - MACE: Invasive strategy, HR 0.93, 95% CI 0.80-1.08, P=0.34
2022	REVIVED-BCIS: n=700 with stable CAD and LVEF $\leq 35\%$ - PCI vs OMT - Over median of 41months $\rightarrow$ No significant difference in death/hospitalisation for HF (PCI group HR 0.99, 95% CI 0.78-1.27, P=0.96)

Figure 12. Summary of the key randomised control trials assessing the utility of coronary revascularisation and/or non-invasive imaging in patients with stable CAD.

CABG = coronary artery bypass grafting; CAD =coronary artery disease; CI = confidence interval; DM =diabetes mellitus; LVEF = left ventricular ejection fraction; HF = heart failure; HR = hazard ratio; OMT = optimal medical therapy; MACE = major adverse cardiovascular events; MI = myocardial infarction; PCI = percutaneous coronary intervention. Trials used in synopsis (Al-Lamee et al., 2018; BARI 2D Study Group et al., 2009; Beanlands et al., 2007; de Bruyne et al., 2012; Maron et al., 2020; Perera et al., 2018; Shaw et al., 2008; Velazquez et al., 2016)

1.7.3.1 Improving outcomes in patients with stable coronary artery disease and evidence of inducible myocardial ischaemia

Observational data from the Cedars-Sinai Medical Center using stress myocardial perfusion single photon emission computed tomography (MPS) suggested a survival benefit afforded by coronary revascularisation, as compared to medical therapy, in patients with a significant ischaemic burden. Two seminal studies were conducted. First, 10,627 consecutive patients with no prior myocardial infarction or coronary revascularisation underwent MPS and short term follow up (mean 1.9 years). Cardiac mortality was increased in patients with a greater burden of stress induced hypoperfusion, with a cross over falling between 10-12.5% following which revascularisation appeared beneficial over medical therapy (Hachamovitch et al., 2003). Subsequently the same team reported on 13,969 individuals; including patients with known CAD, extending follow up (mean 8.7 years) and broadening the primary endpoint to all-cause death. In this analysis they again demonstrated the association between improved survival in patients with an extensive ischaemic burden (and without extensive scar) treated invasively as compared to with medical therapy (Hachamovitch et al., 2011). Despite being unable to infer treatments effects from observational data, these 2 studies drove the paradigm that coronary revascularisation improves outcomes in stable patients with a substantial ischaemic burden. Next followed a set of randomised controlled trials aiming to conclusively address this notion. First, the COURAGE trial randomised 2,287 patients (post angiography) with evidence of inducible myocardial ischaemia and at least one epicardial coronary stenosis (>70% or >80% depending on vessel). Over a median of 4.6 years, PCI did not reduce the primary endpoint of death or non-fatal myocardial infarction when added to medical therapy (Boden et al., 2007). The COURAGE investigators also reported on 2 nuclear sub studies. First, 314 (of the 2287) patients underwent serial MPS before and 6-18months following treatment. This sub study demonstrated a significant decrease in ischaemic burden in patients treated with PCI, compared to optimal medical therapy alone (33% versus 19% respectively, $P=0.0004$) (Shaw et al., 2008). Additionally, they reported on hypothesis generating data suggesting a lower adverse clinical event rate in patients with a serial reduction in ischaemia, although all the associations were attenuated in multivariable models (Shaw et al., 2008). Second, 1,381 randomised patients underwent baseline MPS and long term follow up. In this sub

study, the presence of moderate to severe ischaemia on index MPS did not predict adverse events (Shaw et al., 2012).

The subsequent ISCHEMIA trial was conducted with an attempt to address the perceived limitations of the COURAGE trial, notably by randomising patients prior to coronary angiography (ensuring a reduced impact of clinician bias) and aiming to recruit patients with moderate to severe ischaemia. Of 5,179 randomised patients (followed for a median of 3.2 years), an initial invasive strategy, as compared to a conservative approach, did not reduce the risk of death or major adverse cardiovascular events (Maron et al., 2020). Additionally, sub study analysis of the ISCHEMIA trial also failed to show an association between the degree of ischaemia and clinical outcomes in either treatment arm; demonstrating that relative to mild or no ischaemia, neither moderate ischaemia nor severe ischaemia was associated with increased all-cause mortality (moderate ischaemia HR, 0.89, 95% CI 0.61–1.30; severe ischaemia HR, 0.83, 95% CI, 0.57–1.2, $P=0.33$) (Reynolds et al., 2021).

1.7.3.2 Revascularisation in patients with ischaemic heart failure

Another area of intense research interest is the use of coronary revascularisation to improve LV systolic function and outcomes in patients with ischaemic heart failure. The only prospective randomised trial assessing the utility of CABG in this setting was the STICH trial (Surgical Treatment of Ischaemic Heart Failure). STICH was a multicentre, unblinded, randomised trial in 1,212 patients with ischaemic cardiomyopathy and LVEF <35%. Over extended 10-year follow-up, the trial reported reduced all-cause mortality and cardiovascular mortality in patients in the CABG arm, compared to patients treated with medical therapy (HR 0.84, 95% CI 0.73-0.97, $P=0.02$ and HR 0.79, 95% CI 0.66-0.93, $P=0.006$ respectively). Interestingly, there was minimal incremental benefit of CABG on LVRR and no relation was noted between improvement in LV function and long-term outcomes (Panza et al. 2019); potentially highlighting that the improved outcomes associated with CABG may not be solely attributed to improvement in LV function. Alongside research evaluating the role of coronary revascularisation in ischaemic cardiomyopathy, the use of myocardial viability testing to guide these decisions is intensely debated. This notion stems from hypothesis that dysfunctional but

viable myocardium can recover systolic performance upon restoration of the upstream coronary blood flow, driving improved long-term outcomes.

Viability testing can be undertaken using different modalities, each assessing different aspects of cellular and/or extracellular morphology and integrity. SPECT uses radionuclide-labelled tracers that accumulate inside myocytes with an intact sarcolemma membrane. SPECT has a mean sensitivity of 84% (95% CI 80-88) and mean specificity of 77% (95% CI 73-81) for predicting recovery of global LV function following revascularisation (Camici et al., 2008). Dobutamine stress echocardiography is another frequently used technique to assess myocardial viability. This modality evaluates for augmentation of myocardial contractility following infusion of low-dose dobutamine (typically 5mcg.kg/min-20mcg/kg/min). Baseline resting wall thinning (<0.5-0.6cm) details myocardium that is unlikely to recover. Dobutamine stimulates adrenoreceptors and demonstration of contractile reserve has a reported mean sensitivity of 76% (95% CI 71-80%) and specificity of 81% (95% CI 77-85%) in predicting segmental recovery post revascularisation (Camici et al., 2008). Positron emission tomography using F18-fluorodeoxyglucose evaluates for a mismatch between perfusion and metabolism and has good sensitivity (81%, 95% CI 75-86%) for predicting functional recovery after revascularisation (Camici et al., 2008). Finally, CMR can assess myocardial viability using three techniques: 1) Describing the transmural extent of LGE; 2) Detailing the degree of wall thinning; 3) Investigating for contractile reserve on low dose dobutamine infusion. Comparing across these CMR techniques, a meta-analysis of prospective trials demonstrated that LGE-CMR had the highest sensitivity and negative predictive value, with low dose dobutamine CMR having the highest specificity and positive predictive value to accurately diagnose myocardial viability (Romero et al. 2012) (Figure 13).

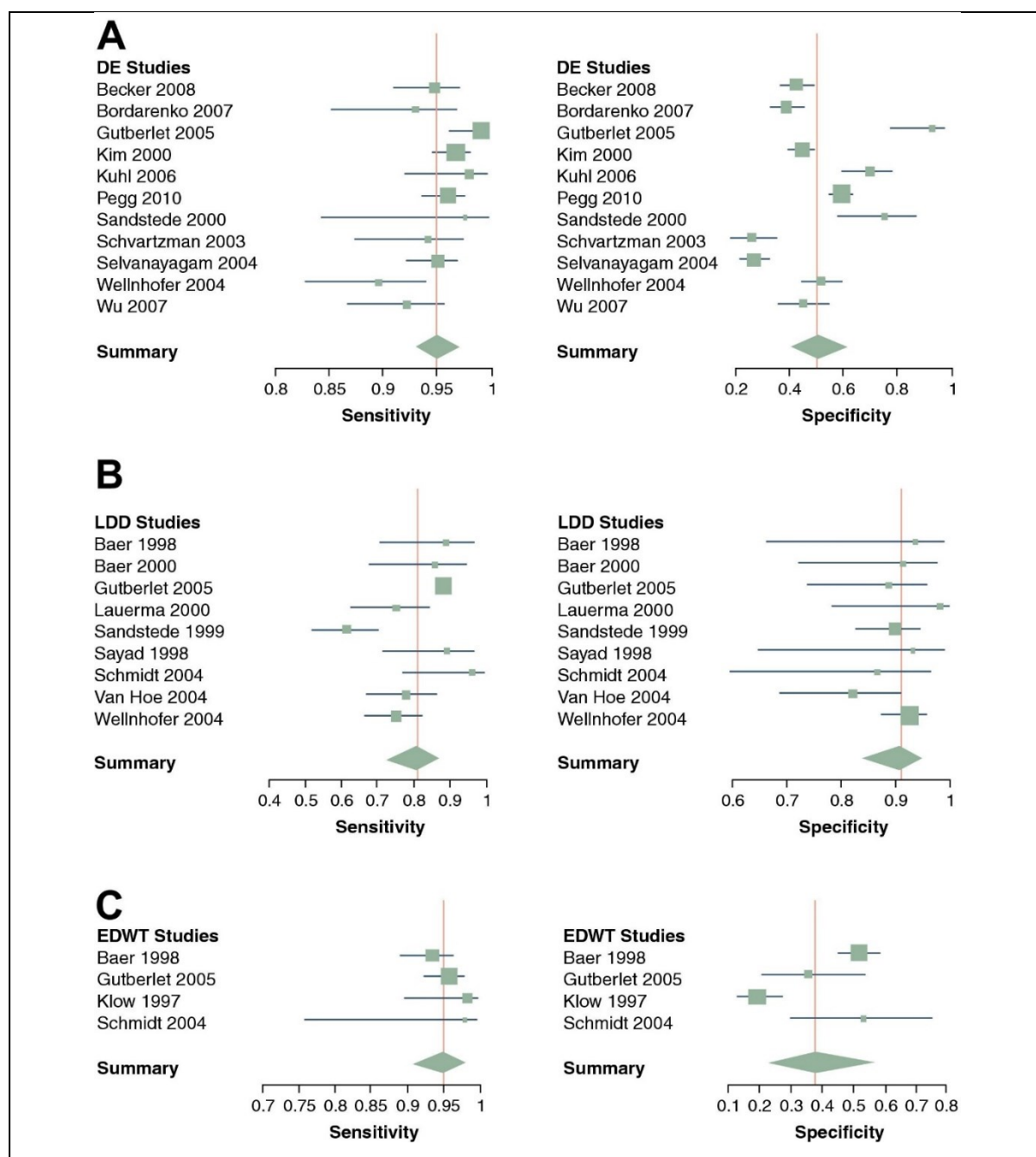


Figure 13. Forest plots of sensitivity and specificity of CMR to assess myocardial viability.

Section A showing the pooled results from delayed-enhancement CMR studies. Section B detailing the pooled results from low dose dobutamine CMR studies. Section C reporting the pooled analysis from CMR studies assessing end-diastolic wall thickness. The size of the square symbol is proportional to number of participants in each study. Horizontal lines are the 95% confidence intervals. CMR = cardiac magnetic resonance; DE = contrast delayed enhancement; EDWT = end-diastolic wall thickness; LDD = low-dose dobutamine. Reproduced with permission from *JACC: Cardiovascular imaging*, (Romero et al., 2012).

The prognostic importance of identifying myocardial viability has also been thoroughly investigated. Meta-analyses from predominantly observational studies using SPECT, DSE and FDG-PET demonstrated the seemingly strong association between coronary revascularisation and reduced all-cause mortality in CAD patients with reduced LVEF and evidence of myocardial viability; one study detailing an 80% reduction in annual mortality associated with revascularisation (16% vs. 3.2%, $P<0.0001$) (Allman et al. 2002) (Figure 14). The signal from this (and other) retrospective studies have not been replicated in subsequent prospective trials. In patients with severely reduced LVEF and suspected CAD, an FDG-PET guided strategy did not improve cardiovascular outcomes at 1 year; the cumulative proportion of patients experiencing the composite event was 30% in the PET arm versus 36% in the standard of care arm (relative risk 0.82, 95% CI 0.59-1.14, $P=0.16$) (Beanlands et al., 2007). From the 601 patients recruited to the non-randomised STICH viability sub study, no significant interaction was identified between the presence or absence of myocardial viability and the advantageous effect of CABG plus optimal medical therapy (OMT) over OMT alone on long term follow-up ($P=0.34$ for interaction) (Panza et al. 2019). More recently, the REVIVED-BCIS trial randomised 700 patients with LVEF $\leq 35\%$, extensive CAD suitable for PCI, and evidence of myocardial viability to PCI alongside OMT or OMT alone. Over a median of 41 months, the addition of PCI to OMT did not lead to reduction in death or hospitalisation for heart failure (HR 0.99, 95% CI 0.78-1.27, $P=0.96$) (Perera et al., 2022).

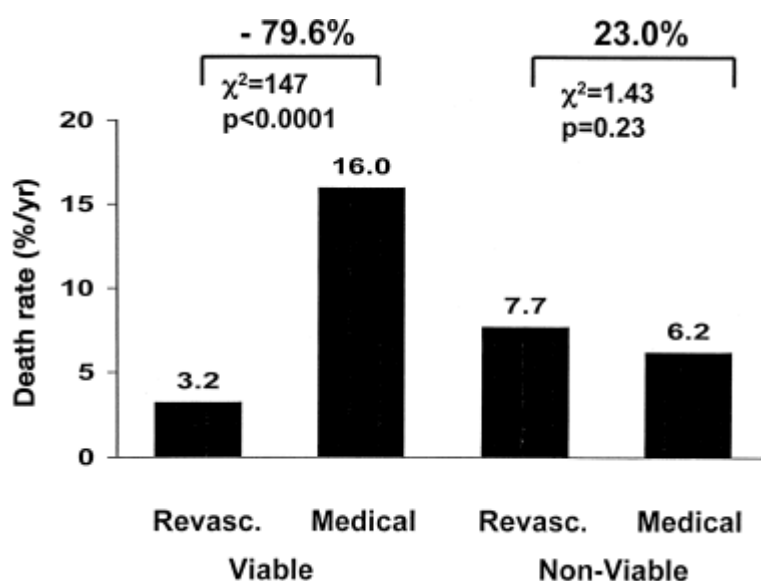


Figure 14. Meta-analysis evaluating the association between myocardial viability testing, coronary revascularisation, and clinical outcomes in patients with CAD and reduced LVEF.

Rates of all-cause mortality for patients with and without myocardial viability treated by revascularisation versus medical therapy. The results demonstrating an approximately 80% decrease in mortality for patients with viability treated by revascularisation ($P<0.0001$). In patients without myocardial viability, there was no significant difference in mortality with revascularisation compared to medical therapy. CAD = coronary artery disease; LVEF = left ventricular ejection fraction. Reproduced with permission from *JACC*, (Allman et al., 2002).

1.7.4 Preventing sudden cardiac death

Coronary atherosclerosis remains the most prevalent cause of sudden cardiac death (SCD). The largest autopsy study of 5,859 cases reported nearly three quarters of the patients had post-mortem evidence of CAD (Haukilahti et al., 2019). Conversely, SCD accounts for around 50% of mortality in CAD cohorts (Myerburg & Junttila, 2012). Arrhythmia is the presumed mechanism underpinning most cases (Tseng et al., 2018), with aetiology broadly attributed to evolving/recent acute coronary syndrome (with myocardial ischaemia driving a significant proportion of events), and scar-related arrhythmia. During transient ischaemia, and early after myocardial infarction, abnormal and enhanced

automaticity (principally related to ionic imbalances) are the presumed mechanisms driving arrhythmia generation (Gräni et al., 2020). Conversely, in the chronic setting, myocardial scar provides the substrate required for circus movement re-entry. This mechanism is typified by the need for an anatomical obstacle (i.e., scar), an area of unidirectional block, and a length of circuit that permits resolution of the refractory period in the cells adjacent to the site of block (Antzelevitch & Burashnikov, 2011; Gräni et al., 2020). Seminal work by De Bakker and colleagues in the late 1980's and early 1990's detailed the milieu capable of generating life threatening arrhythmia in infarcted myocardium. Their results supported the ideology that re-entrant ventricular tachycardia is the presumed mechanism of arrhythmia generation, highlighting the role of surviving myocardium interspersed with core infarction (de Bakker et al., 1988). Additionally, they described a "Zigzag" course of activation facilitated by the branching and merging of viable myocytes within collagen bundles (de Bakker et al. 1993).

Implantable cardioverter defibrillators (ICD) can treat ventricular arrhythmia with increasingly robust outcome data of over 20 years. Initially, the MADIT II investigators demonstrated the role of prophylactic ICD insertion in 1,232 patients with prior myocardial infarction and LVEF <30%. The hazard ratio for the risk of death from any cause in the ICD group, relative to the conventional-therapy group was 0.69 (95% CI 0.51-0.93, P=0.016) (Moss et al., 2002). The subsequent SCD-HeFT trial randomised 2,521 symptomatic patients to ICD therapy, amiodarone or placebo therapy; ICD therapy being associated with a 23% decreased risk of death (HR 0.77; 97.5% CI, 0.62-0.96, P=0.007) (Bardy et al., 2005).

Decisions regarding primary prevention ICD implantation thus still remain centred around evaluation of symptom class alongside dichotomous assessment of LV systolic function using a LVEF cut-off of 30-35%. Typically assessed at a solitary timepoint, this fails to take into account the dynamic nature of LVEF (Goldberger et al., 2011; Myerburg & Goldberger, 2017). The ever-increasing demand on clinical services represents a parallel issue and indeed a comprehensive assessment of SCD risk at a singular timepoint (remote from acute myocardial infarction) is desirable. Less dynamic features which characterise the arrhythmogenic substrate therefore offer promise. Additionally, recent

improvements in drug therapy may have attenuated the benefit of ICDs and new trials assessing traditional (and novel) predictors of SCDs in patients on present-day, guideline-directed, medication are required.

1.8 Unmet research questions in patients with chronic coronary artery disease

Whilst there are many important research questions, recent evidence has highlighted key areas that warrant attention. First, international guidelines have emphasised the need to evaluate the role of non-invasive imaging to improve risk stratification in cardiac disease; notably to identify patients with CAD at high personalised risk of sudden cardiac death (Al-Khatib et al. 2018; Priori et al. 2015). Second, there is a need to investigate the role *myocardial* pathology in patients with chronic coronary syndromes; aiming to identify newer and more effective therapies for patients with inducible myocardial ischaemia and dysfunctional viable myocardium.

1.8.1 Improving risk prediction of sudden cardiac death.

International guidelines have highlighted the gap in evidence surrounding the use of LGE-CMR to aid risk stratification in chronic CAD (Zeppenfeld et al., 2022). The presence of CAD represents an intermediate-risk trait for SCD (Fishman et al., 2010), supporting the need for continued research aiming to identify novel prevention strategies for these individuals. In patients without prior life-threatening arrhythmia, present day assessment of SCD risk centres on the binary assessment of LV ejection fraction alongside ordinal evaluation of heart failure class (Al-Khatib et al., 2018; Priori et al., 2015). Both the European Society of Cardiology and American Heart Association guidelines are primarily based on the ground-breaking SCD-HeFT and MADIT II trials (Bardy et al., 2005; Moss et al., 2002), consecutively reporting the survival benefit afforded by primary prevention ICD insertion as compared to conventional therapy. Inclusion criteria for these randomised trials mandated a severely reduced LVEF, supported by observational data describing the association between impairment of LV systolic function and mortality (Bigger et al., 1984; Gradman et al., 1989). This practice however fails to incorporate crucial aspects of SCD risk prediction (Figure 15). First, there

remains a lack of evidence for direct causal link between LVEF calculation and ventricular arrhythmia (Wellens et al., 2014). Conversely, other metrics (e.g., myocardial scar, ECG repolarisation abnormalities, autonomic dysfunction) have been shown to be integral in arrhythmogenesis (Deyell et al., 2015). Second, an appreciation of the dynamicity of LVEF is absent, thereby negating the potential for fluctuating SCD risk. Although this typically relates to the potential for LV reverse remodelling with attenuation of arrhythmogenic potential, a diagnosis of ischaemic cardiomyopathy is a risk factor for a decrease in LVEF over follow up (Zhang et al., 2015) with the hypothetical serial increase in SCD risk. Third, the gradient risk of SCD is not appreciated when employing a dichotomous cut-off of LVEF (Myerburg & Goldberger, 2017); a patient with a LVEF of 36% is unlikely have a significantly different risk profile to an individual with an LVEF just below the threshold. Forth, the competing risk of death from a non-sudden cause is not appreciated (Goldberger et al., 2011). Patients with a low LVEF have concomitant increased risk of ‘pump’ failure death, a cause of mortality not afforded protection by ICD therapy.

Limitations with the current LVEF-centric approach to SCD risk prediction

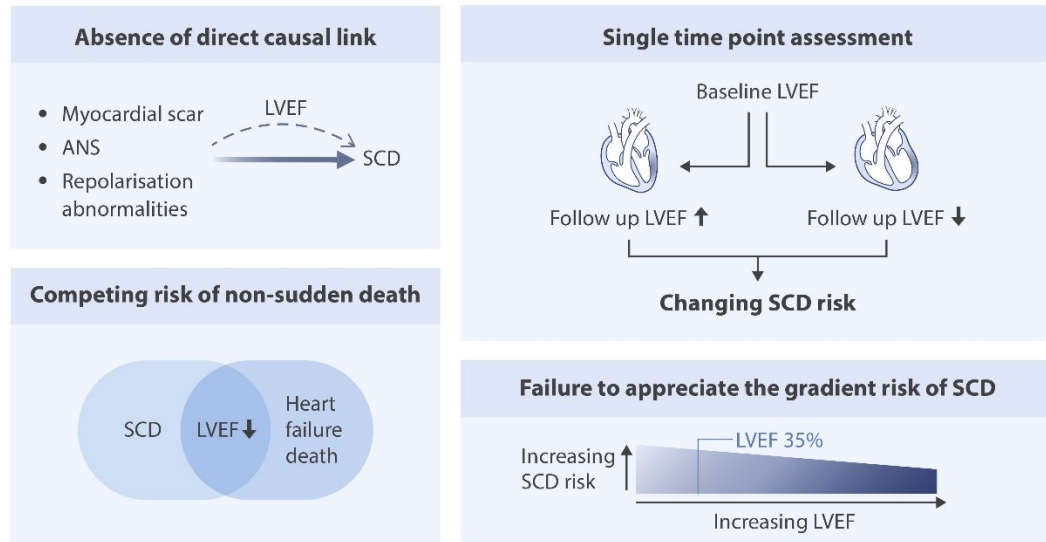


Figure 15. Limitations to the present-day approach to SCD risk prediction in patients with CAD.

There are 4 key limitations to the current approach of stratifying the risk of future life-threatening arrhythmia. First, there remains a lack of evidence detailing a direct causal link between LVEF and SCD risk. Conversely, deposition of extracellular fibrosis had robust evidence describing its role in entry ventricular tachycardia in infarcted myocardium. Second, patients with LV systolic impairment can have fluctuating LVEF, with the potential to experience both an improvement and deterioration of contractile function. Single time point assessment of LV function can negate this phenomenon, thus failing to appreciate the potential change to SCD risk. Third, patients with LV systolic failure have increased risk of death by non-sudden cause, alongside their risk of arrhythmic mortality. The competing risk of non-sudden death is not captured by routine clinical (and research) approaches to risk stratification. Finally, the risk of SCD cannot be dichotomised around a single LVEF threshold. A gradient approach, potentially incorporating the non-linear relationship between LV function and SCD, may represent a more appropriate method to define a patient's personalised risk of SCD.

ANS = autonomic nervous system; LVEF = left ventricular ejection fraction; SCD = sudden cardiac death.

One key area of research, aiming to target the issues described above, is the role of CMR to better characterise SCD risk. Clinical CMR scanners have the required spatial resolution to identify myocardial infarction, with good correlation between LGE on CMR and histological evidence fibrosis (Kim et al., 1999). Additionally, LGE imaging has been used to characterise both dense infarction and the adjacent peri-infarct zone by exploiting the differing signal intensities of these regions. The presence of LGE is a powerful independent predictor of ventricular arrhythmia (Disertori et al., 2016) and importantly, assessment of the peri-infarct zone has also been shown to associate with inducibility of VT (Schmidt et al., 2007) and appropriate ICD therapy (Roes et al., 2009). More recently, evaluation of complex scar geometry (e.g., entropy) (Androulakis et al., 2019) and 3D patient-specific ventricular models (Arevalo et al., 2016) has further demonstrated the potential prognostic utility of CMR in chronic CAD (Figure 16). Importantly, many questions remain pertaining to the utility of complex scar characterisation and prediction of SCD in prospectively recruited cohorts with a broad range of LVEF.

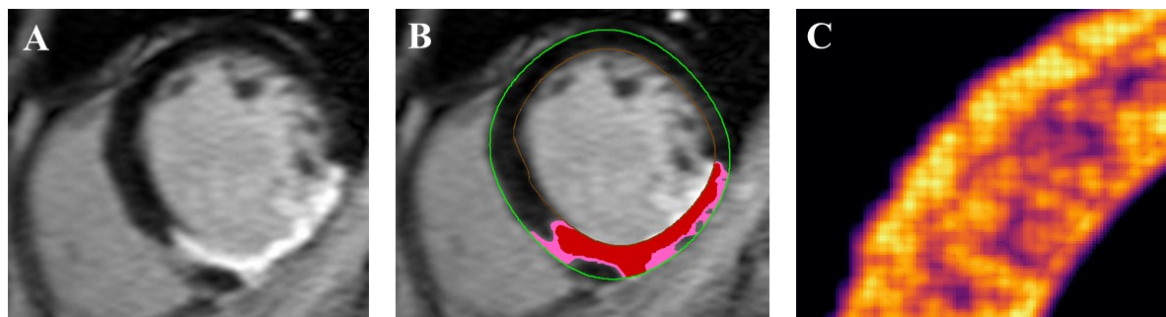


Figure 16. The utility of scar characterisation by CMR to predict life-threatening arrhythmia

A) LGE-CMR slice showing a near transmural myocardial infarction of the inferior wall, extending into the ventricular septum and lateral segments. B) Signal intensity-based quantification using the Full-Width at Half Maximum methodology highlighting the core infarction (red) and adjacent peri-infarct zone (pink). C) Illustration (from a separate LGE-CMR scan) detailing a high entropy value of the depicted scar region; suggesting a high degree of heterogeneity within the infarcted segment. LGE-CMR = late gadolinium enhancement cardiovascular magnetic resonance.

1.8.2 Deciphering the key cellular changes within the myocardium of patients with CAD.

The presence of a significant bulk of dysfunctional cardiomyocytes downstream from obstructive CAD represents an attractive target for novel therapies aiming to promote ventricular recovery and improve outcomes. Additionally, the recent negative ISCHEMIA and REVIVED-BCIS trials have demonstrated the need to focus on alternative therapies (beyond coronary revascularisation) and targets. Importantly however, there remains a paucity of human data detailing the key cellular abnormalities with impaired, but viable, myocardium. This is partly in view of the limited access to human left ventricular tissue, but also due to traditional tissue studies employing a reductionist analytical approach, limited by tools that permitted the evaluation of a small spectrum of molecular changes. More recently, with the advent of omics technology, the ability to undertake discovery, data-driven research, has allowed broad, unbiased, and in-depth analysis of the cellular changes within cardiac disease. Omics based research aims to characterise the pathways in which genetic variation (genomics), drives alterations in gene expression (transcriptomics), that in turn modulates protein expression (proteomics) and levels of the end-products of metabolism (metabolomics) (McGarrah et al., 2018). Additionally, integrating these platforms in a systems biology approach affords the potential for increased understanding of the complex biological networks within cardiovascular disease, importantly allowing the interaction between genetics and the environment to be explored.

1.8.2.1 Genomics

The field of genetics has seen great advances during the first quarter the 21st century. From the publication of the draft human genome in 2004 (International Human Genome Sequencing Consortium, 2004), a multi-national project costing billions of dollars, to the present-day ability to map an individual's genome at a fraction of the price and time. Within clinical cardiovascular medicine, the role of genetics continues to expand, notably in the domain of DCM. Initially using Sanger sequencing of candidate genes, with the subsequent adoption of next-generation sequencing (Figure 17), there are now more than 50 genes hypothesised to contribute or cause DCM (Japp et al., 2016; Tayal et al., 2017). Titin, the largest human protein, is implicated in approximately 15-25% of

DCM cases. Additionally, there is increasing data describing the prognostic value of identifying certain variants in specific genes (e.g., *LMNA* in view of its proarrhythmogenic tendency). More recently, whole exome sequencing, a technique that maps protein coding regions, has been used to identify putative new disease genes, with the notable success of identifying the cytoskeletal gene, *FLNC*, as an important cause of heritable cardiomyopathy (Valdés-Mas et al., 2014). The role of whole genome sequencing, the unbiased mapping of all genes, continues to be explored, including in the ongoing genetic study of 2,000 patients with DCM from our wider group (NCT03843255).

The genetic basis of CAD is debatably more complex than DCM. Albeit seen in clusters of families, CAD is a common disease and is typically not a Mendelian disorder. Common variant association studies assessing the frequency of common polymorphisms in individuals with and without coronary atherosclerosis have been explored; identifying approximately 60 genetic loci associated with the disease (Khera & Kathiresan, 2017). The first common variant association study in CAD was published in 2007, with multiple separate groups detailing the association between variants at the 9p21 locus and CAD (Helgadottir et al., 2007; McPherson et al., 2007; Samani et al., 2007). This finding has further been replicated by other institutions yet the mechanism underpinning this association remains unclear, a frequent finding in genome wide association studies.

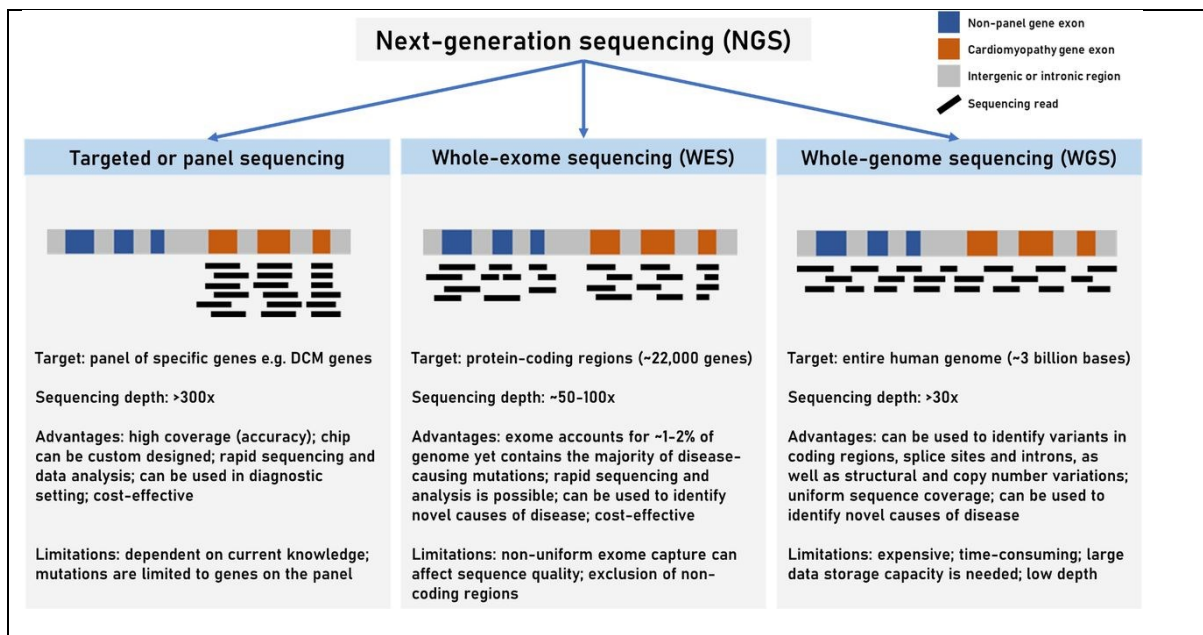


Figure 17. A summary of targeted next generation sequencing, whole-exome sequencing and whole-genome sequencing.

A synopsis of the current approaches in next generation sequencing. DCM = dilated cardiomyopathy. Reproduced with permission from the *BMJ*, (Spracklen et al., 2022)

1.8.2.2 Transcriptomics

Transcriptomics describes the study of how genes are regulated and expressed in a tissue of interest; the field aiming to characterise the link between the genome and cell function (Pedrotty et al., 2012). The human transcriptome is a complex set of pathways comprising both coding and non-coding RNAs (Figure 18) (Robinson et al., 2022) and can be characterised using gene expression microarrays (Quackenbush, 2006) or RNA sequencing (Wang et al., 2009). Additionally, the growth of single-cell technology has permitted the transcriptomic characterisation of the different cell types within an experimental sample, in addition to identifying the heterogeneity of cells of the same type.

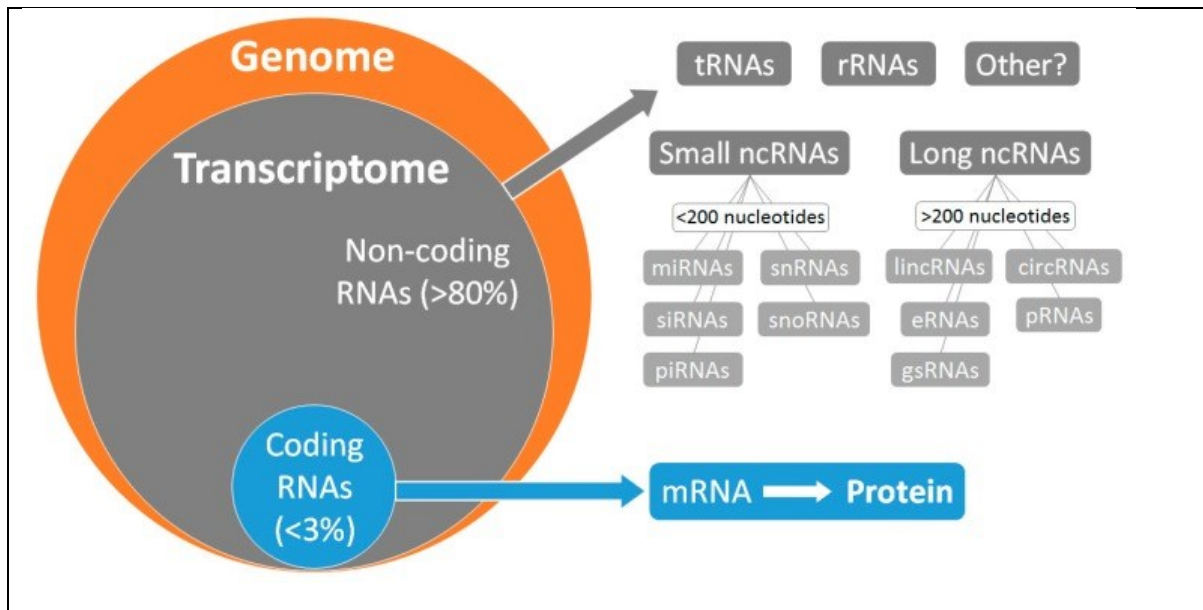


Figure 18. Coding and non-coding RNAs in the human genome.

tRNAs = transfer RNAs; rRNAs = ribosomal RNAs; ncRNAs = noncoding RNAs; miRNAs = microRNAs; siRNAs = small-interfering RNAs; snRNAs = small nuclear RNAs; snoRNAs = small nucleolar RNAs; piRNAs = piwi-interacting RNAs; lincRNAs = long intergenic RNAs; eRNAs = extracellular RNAs; gsRNAs = germline small RNAs; circRNA = circular RNAs; pRNAs = promoter-associated RNAs; mRNA = messenger RNA. Reproduced under the open access Creative Common CC BY license.

As gene expression varies across different tissue types, there is a compelling argument to perform cardiac transcriptomic research on myocardial biopsies. In view of the limited access to human myocardium however, data is usually confined to advanced phenotypes in patients undergoing surgical procedures.

Reichart et al evaluated the single-cell transcriptomic landscape of 61 patients with end-stage non-ischaemic cardiomyopathy, comparing to 18 controls. They reported cell types and states and molecular signals, in addition to describing predicted intercellular communications that characterise DCM and arrhythmogenic cardiomyopathy (Reichart et al., 2022). This included the report of previously unknown subpopulations of cardiomyocytes and fibroblasts.

Wehrens al assessed the single-cell transcriptomic profile of 5 individuals with hypertrophic cardiomyopathy (HCM) undergoing myomectomy. They highlighted the heterogeneity within

cardiomyocytes identifying multiple different subpopulations, including a subset with enriched *NPPA* expression (Wehrens et al., 2022). Additionally, they reported enrichment of traditional marker genes for cardiac stress within cardiomyocytes, relative to controls, but also demonstrated increased expression genes that previously had not been functionally linked to HCM, including *MB* which encodes myoglobin (Wehrens et al., 2022). Ranjbarvaziri et al employed a multiomic approach in the assessment of LV tissue from 27 patients with advanced HCM, comparing to 13 donor hearts. Alongside altered metabolite levels suggestive of bioenergetic decompensation, there was broad dysregulation of genes associated with fatty acid metabolism and the tricyclic acid cycle (Ranjbarvaziri et al., 2021).

Research within CAD populations is more limited. Kittleson et al used gene expression profiling of 48 myocardial samples in patients with advanced heart failure of mixed aetiology undergoing left ventricular assist device insertion or heart transplantation; demonstrating that transcriptomic analysis was able to accurately predict disease aetiology (Kittleson et al., 2004). More recently, Kuppe et al assessed the human transcriptome (including both single nuclear sequencing and spatial transcriptomics) in 23 patients with acute myocardial infarction (Kuppe et al., 2022). This novel dataset was able to add spatial context to intercellular interaction, again demonstrating notable cell heterogeneity and novel subtypes of cardiomyocytes, endothelial cells, myeloid cells and fibroblasts (Kuppe et al., 2022).

1.8.2.3 Proteomics

Proteomics describes the study of the complete protein complement of a genome, aiming to map the dynamic molecular milieu more closely related to phenotype relative to the genomic and transcriptomic fingerprint (Arrell et al., 2001). Indeed, proteomics can provide data beyond these upstream experiments, notably as disease processes can be both mediated and modulated at the post-transcriptomic and post-translational layer (following protein synthesis), or leaked from damaged cells; thus not predicted by genetic information (Lam et al., 2016). Proteomic experiments can broadly be undertaken using a targeted or nontargeted approach, the latter aiming to provide an

unbiased assessment of the protein architecture within a sample. There are multiple technological platforms, each suited to particular research questions, however the majority of cardiovascular proteomics is undertaken on either mass spectrometry or affinity-based platforms (the latter including the Aptamer based approach, Olink). Importantly each platform and approach have their own advantages and limitations pertaining to scope, sensitivity, specificity and quantification potential (Figure 19). Importantly, discovery proteomics has been central to the identification of key circulating biomarkers used in every day cardiovascular practice, including NTproBNP (de Lemos et al., 2003), Troponin I (Adams et al., 1993) and Troponin T (Reichlin et al., 2009).

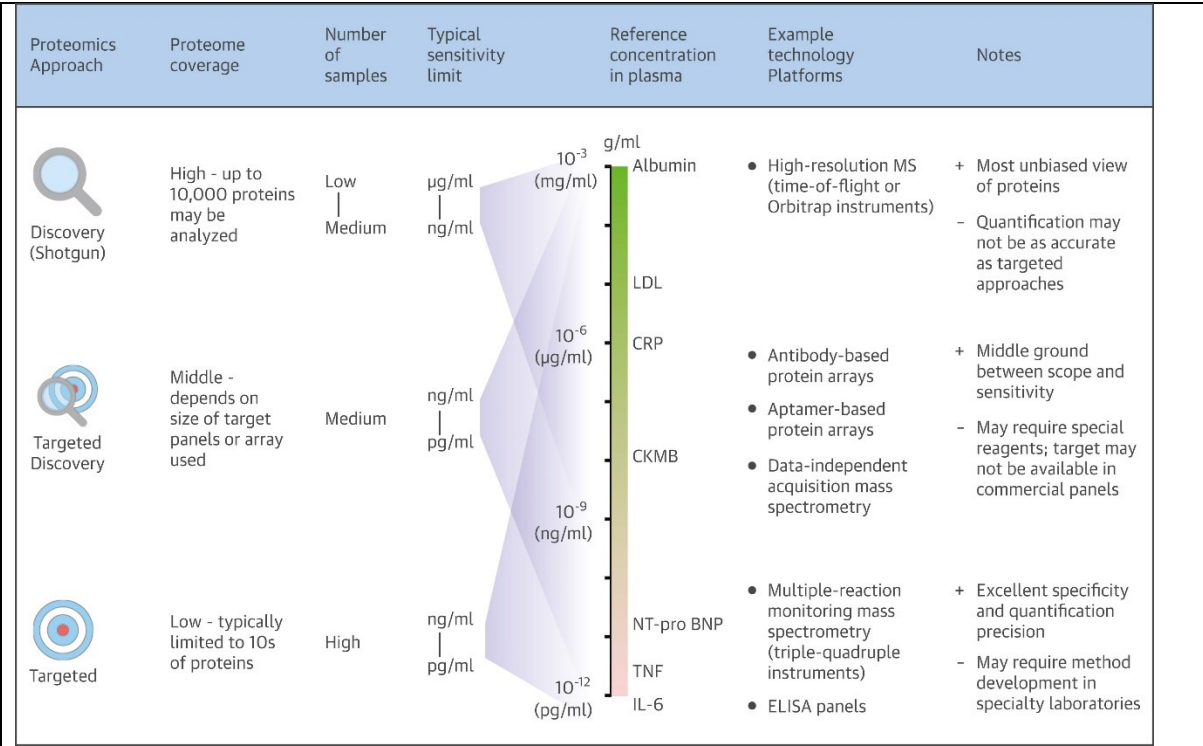


Figure 19. Sensitivity and coverage of differing proteomic experimental strategies.

Synopsis of the three common approaches to proteomic study design: Discovery (shotgun), targeted, and targeted discovery proteomics. CKMB = creatine kinase-myocardial band; CRP = C-reactive protein; ELISA = enzyme-linked immunosorbent assay; LDL = low-density lipoprotein; MS = mass spectrometry; NT-proBNP = N-terminal pro-B-type natriuretic peptide. Reproduced with permission from *JACC*, (Lam et al., 2016)

Aligned with transcriptomic research however, there has been limited proteomic studies using human myocardial biopsies, again in view of the difficult access to relevant cardiac tissue. Coats et al performed proteomic analysis on 11 patients with HCM, identifying 151 differentially expressed proteins (namely associated with metabolism, calcium regulation and oxidative stress) in patients with HCM, relative to control hearts (Coats et al., 2018). Yi et al assessed the proteomic landscape of LV samples from 6 patients with ischaemic cardiomyopathy, comparing to 7 donor hearts. They reported upregulation of 104 proteins, and downregulation of 63 protein, notably associated with metabolism and muscle contraction (Yi et al., 2019).

1.8.2.4 Metabolomics

Metabolomics describes the field measuring the end products of metabolism, metabolites. The technique permits the phenotypic fingerprinting of an organism, integrating the consequences of upstream multiomic alterations with environmental modulators (e.g., diet and gut microbiota) (McGarrah et al., 2018). Similar to proteomics, there are two monikers under which metabolomics can be performed: non-targeted and targeted. Non-targeted approaches permit the broad, unbiased, evaluation of the metabolome, with targeted approaches analysing a limited number of preselected intermediates, usually from the same metabolic pathway. There are two prevalent platforms for cardiac metabolomics analysis, both permitting the automated identification and quantification of a range of intermediates. First, nuclear magnetic resonance identifies metabolites using chemical shifts in resonance frequency (Müller et al., 2021). The technique is robust and low cost, but only allows the quantification of a relatively low number of abundant metabolites. Second, mass spectrometry requires the ionisation of analytes, with subsequent measurement of the intensity of ions generated, recorded as a mass-to charge ratio (m/z). Preceding the MS, analyte separation using liquid chromatography or gas chromatography is typically undertaken. See Figure 20 for a summary of the commonly applied instrumentation in metabolomic studies.

	NMR	GC-MS	LC-MS/MS (Triple Quad)	LC-MS/MS (Q-TOF/Orbitrap)
Metabolomics Applications	Nontargeted/targeted	Nontargeted/targeted	Targeted	Nontargeted/targeted
Metabolome coverage	Low 100s	100s	100s	100s–1000s
Throughput	10–30 min	20–60 min	15–60 min	15–60 min
Platform cost	Comparable or greater than high-end MS platforms	Least expensive MS	Midrange MS cost	Most expensive MS
Benefits	Noninvasive/nondestructive and suitable for in vivo/in situ Quantitative High-salt compatibility Isotope selectivity (^1H , ^{13}C) Structural information and positional ^{13}C -label Detailed libraries	Mature technology Robust and reproducible metabolite separation Quantitative NIST database aids in metabolite identification Precise isotopomer measurement	Most sensitivity MS and high selectivity Quantitative Easily transferrable methods Can be performed direct/flow injection No derivatization is needed for most applications Flexible and wide choice of LC columns and mobile phase	High resolution Accurate mass determination Good metabolite coverage Suitable for small macromolecule analysis Fragment fingerprinting with MS ⁿ experiments No derivatization is needed for most applications Flexible and wide choice of LC columns and mobile phase
Challenges	Poor sensitivity, but can be improved with HP-methods Large physical footprint Large sample requirement Co-resonances limit 1-dimensional NMR applications	Requires volatile or derivatized samples Not amenable to all metabolite classes Unknown identification difficult Retention/spectral libraries required for nontargeted	Time-consuming to establish methods Unknown identification difficult Lacks novel discovery Unsuitable for nontargeted analysis Variable chromatographic performance	Limited, but growing, spectral databases makes unknown identification difficult Intra and interbatch variability because of baseline drift Variable chromatographic performance Requires greater expertise for operation and analysis

Figure 20. Comparison of the commonly used instruments for metabolomics studies.

Summary table of the popular instruments used in cardiovascular metabolomic studies. GC-MS = gas chromatography-mass spectrometry; HP = high-performance; LC-MS/MS = liquid chromatography-mass spectrometry/mass spectrometry; NIST = National Institute of Standards and Technology; NMR = nuclear magnetic resonance; Q-TOF = Quad Time-of-Flight. Reproduced with permission from *Circulation Research*, (McGarrah et al., 2018).

In cardiovascular research, the majority of human metabolomic studies have assessed circulating markers of disease, including the paired collection of blood from the aortic root and coronary sinus. There are again minimal human LV tissue studies, in part due to the need for rapid sample freezing for most assays, with prior research limited to patients with advanced disease phenotypes. Bedi et al assessed LV biopsies from 15 non-diabetic patients with DCM undergoing LVAD insertion or heart transplantation. They reported the surprising finding of decreased myocardial levels of key lipid intermediates, alongside increased abundance of ketogenic β -hydroxybutyryl CoA and increased myocardial utilisation of β -hydroxybutyrate; concluding that ketone utilisation may be a crucial metabolic adaptation in the failing human heart (Bedi et al., 2016). Diakos et al assessed myocardial metabolism in paired biopsies from 31 patients pre- and post- LVAD insertion, comparing to 11 normal donors. They found increased glycolysis, in combination with pyruvate mitochondrial oxidation mismatch in individuals after LVAD therapy, the results suggestive of ongoing mitochondrial dysfunction despite mechanical unloading (Diakos et al., 2016).

Overall, a common theme across all multiomic studies in cardiovascular medicine is the paucity of data detailing the key abnormalities within the myocardium of patients with less advanced phenotypes. Additionally, the heterogeneity of studies limits the generalisability to specific disease aetiologies, e.g., ischaemic heart disease. Future studies assessing the left ventricular multiomic profile in CAD patients with a substantial bulk of viable myocardium may identify early changes in the molecular landscape that could represent attractive drug targets aimed at preventing progressive adverse cardiac remodelling.

1.9 Conclusions

The presence of inducible ischaemia is a prognostically relevant finding. Critics will state the phenomenon is solely a corollary for plaque burden, but it remains valid that the investigation of the end-organ effects of this plaque may be scientifically relevant. In parallel, the identification of large regions of dysfunctional, yet viable, myocardium represents an attractive target for therapies aiming to promote left ventricular reverse remodelling. Importantly, animal models of stable CAD have demonstrated persisting abnormalities of myocardial cellular function following coronary revascularisation (Holley et al., 2015; Kelly et al., 2011; Page et al., 2015), suggesting the need for alternative or adjunctive therapeutic approaches to target the myocardial consequences of ischaemia. At the other end of the spectrum, investigation of the complex structures within *infarcted* myocardium is a promising approach to improve SCD risk prediction.

CMR can interrogate all these parameters with high spatial and temporal resolution and affords the potential drive translational research in these fields – aiding the discovery of key cellular and extracellular processes downstream from coronary atherosclerosis. My thesis will explore these concepts.

1.10 Thesis hypotheses

1.10.1 Hypotheses for Chapter 2

1. Quantitative stress perfusion CMR can identify serial changes in LV myocardial blood flow (MBF) in patients undergoing CABG.
2. Baseline and change in patient reported health status, assessed using the Seattle Angina Questionnaire-7 summary score, associate with:
 - a. Baseline and change in CMR derived LV visual perfusion defect burden.
 - b. Baseline and change in CMR derived LV MBF.
3. CMR markers of interstitial fibrosis regress following CABG.

1.10.2 Hypotheses for Chapter 3

1. LV myocardium in patients with stable coronary artery disease has a different multiomic profile as compared to human donor myocardium.
2. Human LV myocardium with and without inducible ischaemia have differing multiomic profiles.
3. LV myocardium from patients with impaired LVEF has a different multiomic profile compared to LV tissue from patients with preserved LVEF.

1.10.3 Hypotheses for Chapter 4

1. The presence of non-infarct pattern LGE (NI-LGE) in patients with stable CAD is associated with adverse LV remodelling.
2. There is increased burden of rare variants in DCM-associated genes in CAD patients with NI-LGE as compared to CAD patients without this imaging biomarker.
3. In individuals with CAD, rare variants in DCM-associated genes are associated with adverse LV modelling.
4. In individuals with CAD, rare variants in DCM-associated genes are associated with adverse clinical outcomes.

1.10.4 Hypotheses for Chapter 5

1. Core infarct mass and peri-infarct zone mass predict sudden cardiac death beyond LVEF calculation in prospectively recruited patients with stable CAD.
2. Morphological and texture-based scar microstructure features have independent and incremental association with SCD beyond LGE mass.

Chapter 2

2 Serial phenotyping with multiparametric cardiovascular magnetic resonance **in patients undergoing coronary artery bypass grafting.**

2.1 Abstract

Background: Serial multiparametric cardiovascular magnetic resonance (CMR) in patients undergoing coronary artery bypass grafting (CABG) may provide mechanistic insight into dynamic, and persistent, abnormalities of the myocardium in patients with stable coronary artery disease (CAD).

Methods: Patients with CAD awaiting elective CABG were prospectively recruited into an observational cohort study. Baseline evaluation including bloods, Seattle Angina Questionnaire-7, and adenosine stress perfusion CMR was undertaken. Automated, fully quantitative stress and rest myocardial blood flow was calculated alongside assessment of the visual ischaemic burden. Native and post-contrast septal T1 indices were also measured. Repeat evaluation at 6-12 months post CABG was performed.

Results: Of 43 patients who underwent serial evaluation with CMR (mean age 62.1 ± 9.3 , median LVEF 68% [IQR: 62-73%]), there was improvement in the median visual ischaemic burden (18% [IQR: 11-21] vs 42% [IQR: 27-51], $P < 0.001$), mean global stress myocardial blood flow (1.5 ± 0.5 ml/min/g vs 1.3 ± 0.5 ml/min/g, $P = 0.002$) and median global myocardial perfusion reserve (2.4 [IQR: 1.7-2.8] vs 1.7 [IQR: 1.4-2.2], $P = 0.002$) following CABG. Greater improvement in the SAQ-7 summary score was associated with a larger visual ischaemic burden at baseline ($\rho = 0.44$, $P = 0.004$) and a greater decrease in the visual ischaemic burden following CABG ($\rho = -0.38$, $P = 0.02$).

Quantitative MBF metrics did not associate with baseline or change in SAQ-7 summary score. Mean LV extracellular matrix volume decreased following CABG (16.6 ± 3.6 ml/m² vs 18.2 ± 4.6 ml/m², $P = 0.009$).

Conclusion: Multiparametric CMR, including fully automated quantification of MBF, identifies dynamic changes in myocardial perfusion and interstitial fibrosis in patients following CABG. Visual assessment of inducible myocardial hypoperfusion may identify patients who will derive the most symptomatic benefit from CABG. The results, however, suggest an important degree of residual inducible ischaemia and an unclear clinical role for CMR-derived MBF calculations.

2.2 Introduction

Coronary artery bypass grafting (CABG) permits, at least theoretically, protection from repetitive myocardial ischaemia and prevention from the sequelae of upstream native vessel plaque rupture (Newby et al., 2021). Predicting which patients will benefit from CABG though is challenging with the risk of adverse outcomes following surgery remaining high, alongside an important cohort of patients who remain symptomatic post operatively. Comprehensive phenotyping with multiparametric cardiovascular magnetic resonance (CMR) affords the potential to assess key changes in myocardial geometry, function and perfusion; interrogating the downstream consequences of coronary atherosclerosis that may drive symptoms and clinical outcomes in patients undergoing CABG.

First, fully automated assessment of myocardial blood flow (MBF) permits objective and reproducible investigation of inducible ischaemia. This may have utility beyond the spatial assessment of visual perfusion defects, notably when correlating with symptoms (Patel et al., 2022), investigating multivessel disease, or within the framework of blinded placebo-controlled trials (Iskandrian et al., 2014). The role of quantitative stress perfusion CMR in patients with bypass grafts remains, however, unclear. Traditional qualitative approaches have reduced diagnostic accuracy in patients with prior CABG (Bernhardt et al., 2009) and currently there is only single timepoint studies assessing the accuracy (Seraphim et al., 2021) and prognostic utility (Seraphim et al., 2022) of fully-quantitative techniques. Serial datasets may enhance our understanding of the utility of MBF calculation by CMR to assess dynamic, and persistent, changes to myocardial perfusion following CABG.

Second, CMR also permits the concomitant characterisation of both replacement and interstitial myocardial fibrosis with recent data demonstrating dynamic changes to the extracellular matrix in patients following aortic valve surgery (Treibel et al., 2018). Whether changes to the interstitium occur in patients following CABG is unclear but could represent a modifiable myocardial process in patients with CAD.

In Chapter 2, I assess the utility of serial multiparametric CMR to investigate the association between symptoms, inducible myocardial hypoperfusion and markers of fibrosis in patients undergoing CABG.

2.3 Hypotheses

1. Quantitative stress perfusion CMR can identify serial changes in LV myocardial blood flow (MBF) in patients undergoing CABG.
2. Baseline and change in patient reported health status, assessed using the Seattle Angina Questionnaire-7 summary score, associate with:
 - a. Baseline and change in CMR derived LV visual perfusion defect burden.
 - b. Baseline and change in CMR derived LV MBF.
3. CMR markers of interstitial fibrosis regress following CABG.

2.4 Methods

2.4.1 Study design

Patients with stable CAD awaiting CABG were prospectively recruited into an observational cohort study between September 2019 and April 2021 (The AMBITION study). All patients provided written informed consent (Appendix Figure 55). The study was approved by the National Research Ethics Service (19/EE/0166) and complied with Declaration of Helsinki. Blood was drawn for assessment of NT-proBNP and high sensitivity Troponin I levels. Functional status was assessed using the New York Heart Association (NYHA) functional score, Canadian Cardiovascular Society (CCS) Angina grade and Seattle Angina Questionnaire-7. All patients underwent comprehensive CMR with fully automated quantitative stress perfusion, parametric mapping and late gadolinium enhancement (Figure 21). Transmural myocardial tissue biopsies were acquired from regions with and without inducible myocardial ischaemia in a subset of patients, results detailed in Chapter 3. At 6-12 months post CABG, serial evaluation with functional assessment, bloods and CMR was undertaken.

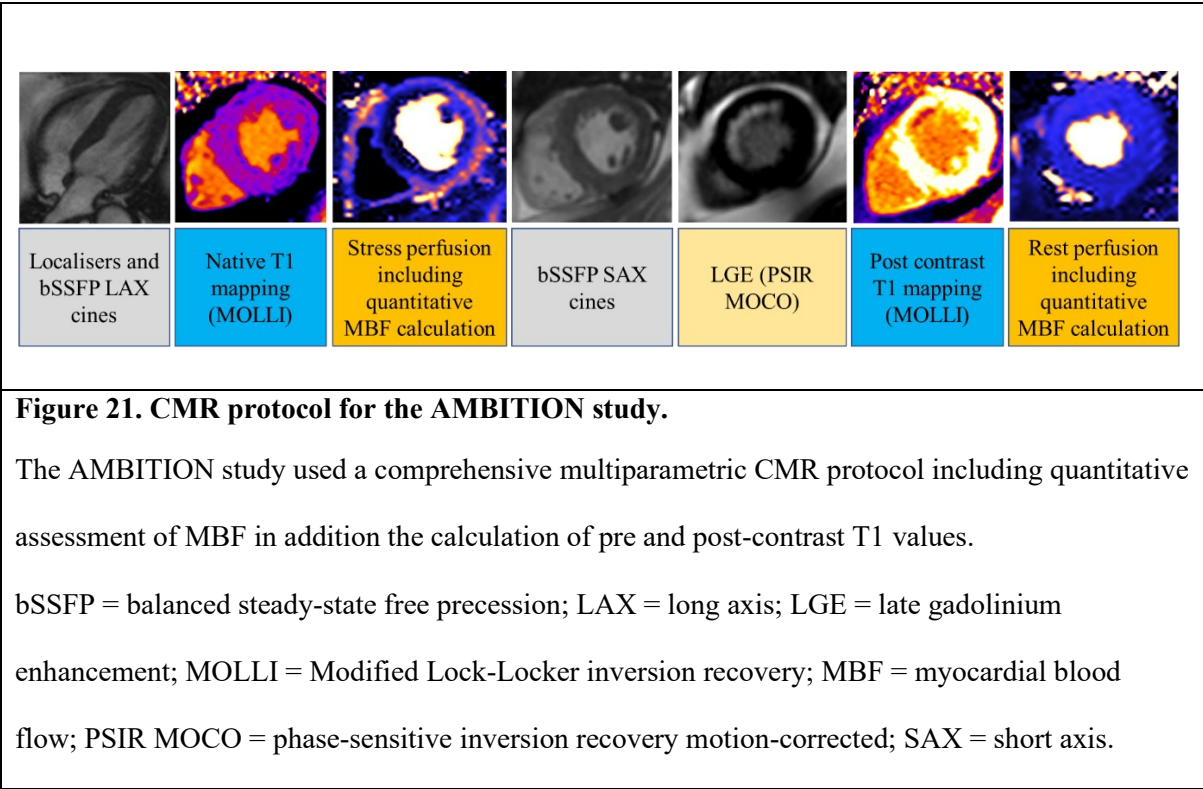
2.4.2 Inclusion and exclusion criteria.

Patients with CAD awaiting clinically indicated CABG with evidence of inducible myocardial ischaemia on CMR were enrolled. Exclusion criteria were: a) valve disease requiring intervention during surgery; b) evidence of concomitant non-ischaemic cardiomyopathy; c) liver failure (e.g. INR >2); d) contraindication to CMR; e) inability to give consent.

2.4.3 CMR protocol.

All scans on a 1.5 Tesla Siemens Aera scanner. In brief, localisers were followed by balanced steady-state free precession (bSSFP) cine long axis imaging. Native myocardial T1 maps using a modified Look-Locker inversion recovery (MOLLI) 5(3)3 sequence at the basal and mid-ventricular level and four-chamber view was undertaken. Intravenous adenosine was then infused for 3-5 minutes at a rate of 140-210mcg/kg/min in a stepwise protocol, ensuring a symptomatic and hemodynamic response. Short axis slices at the basal, mid-ventricular and apical levels were acquired during the first pass of a 0.05mmol/kg IV Gadobutrol bolus (Gadovist, Sweden). Automated perfusion maps were produced using inline perfusion software contained within the Gadgetron online reconstruction framework

together with pixel-wise myocardial blood volume maps as described previously (Kellman et al., 2017). This approach uses a dual-sequence technique with distinct pulse sequences for the arterial input function and myocardial tissue. A top-up of 0.05mmol/kg of IV Gadobutrol was then administered followed by bSSFP short-axis cine imaging. After 10minutes, late gadolinium enhancement (LGE) imaging was performed using a free-breathing phase-sensitive inversion recovery (PSIR) motion-corrected (MOCO) sequence. Post-contrast T1 mapping was undertaken approximately 20minutes following the top-up Gadobutrol injection followed by rest perfusion imaging. At 6-12 months post CABG, patients underwent the same CMR protocol, importantly using the same adenosine dose and infusion duration.



2.4.4 CMR analysis

All CMR analysis was undertaken by an independent Level 3 CMR Operator (Dr Suzan Hatipoglu) on CVI42 (Circle Cardiovascular Imaging, Calgary, Canada). The analysis was performed in batches with the pre- and post-CABG cases presented in a differing order. Ventricular volumes were indexed to body surface area. Native T1 values were calculated at the mid-ventricular septum, avoiding regions of LGE. Visual perfusion defect burden was determined per segment by the identification of reduced relative signal intensity. A semi-quantitative approach was used to detail visual perfusion defect burden, aligning to methodology used in the MR INFORM trial to build a 32-segment model (Hussain et al., 2012). The quantitative analysis of myocardial perfusion was fully automated with no human operator adjustment. MBF (ml/min/g) was calculated on a pixel-wise basis in high-resolution images using a blood tissue exchange model and partial differential equations, including estimation of the arterial time delay between arrival of the bolus in the LV cavity and the pixel of interest. Global stress and rest MBF were derived, with the subsequent calculation of the myocardial perfusion reserve. LGE quantification was performed using the Full-Width at Half Maximum methodology. The LV extracellular volume fraction (ECV), indexed extracellular matrix volume and indexed cell volume were calculated as follows:

$$ECVfraction = (1 - haematocrit) \times \frac{\left(\frac{1}{T1_{myo\ post}} - \frac{1}{T1_{myo\ pre}} \right)}{\left(\frac{1}{T1_{blood\ post}} - \frac{1}{T1_{blood\ pre}} \right)}$$

$$Indexed\ LV\ cell\ volume = (1 - ECV\ fraction) \times (Indexed\ LV\ mass/1.05)$$

$$Indexed\ LV\ extracellular\ matrix\ volume = ECV\ fraction \times (Indexed\ LV\ mass/1.05)$$

2.4.5 Statistical analysis

Continuous variables are reported as mean (standard deviation) or median (interquartile range [IQR]). Categorical variables are reported as N (%). Characteristics were compared between patients with a global MPR <median versus those with a global MPR ≥median using the 2-sample t-test for

continuous variables and chi-squared test or Fisher's exact test for categorical variables. Differences in symptoms, blood markers and CMR metrics were summarised as mean or median (95% confidence interval) and compared pre and post CABG using the paired t-test or Sign test depending on the distribution of the *changes* in each variable (i.e., not the distribution of the variable at each visit). Spearman correlation coefficients were calculated for the associations between baseline or change in patient-reported health status and CMR metrics. All tests used a 2-sided statistical significance of $P < 0.05$. Analyses were performed using Stata v17.0.

2.5 Results

From 45 patients, 43 individuals were enrolled and underwent serial examination with multiparametric CMR (mean age 62.1 ± 9.3 , median LVEF 68% [IQR: 62-73%], median MPR 1.7 [1.4-2.2]). Figure 22 details the derivation of the cohort and recruitment timeline. Of the 2 patients that did not complete the study, 1 patient was lost to follow up and 1 patient died. The median difference between baseline and follow up CMR was 238 days (IQR 201-387).

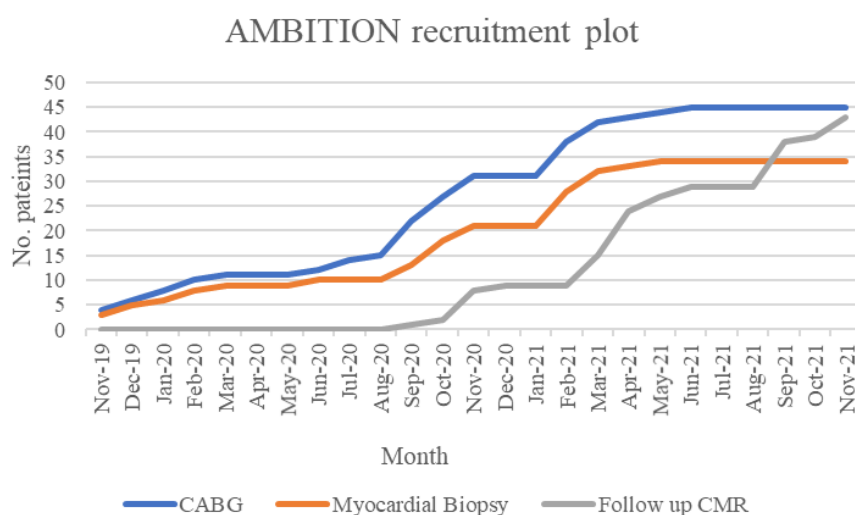
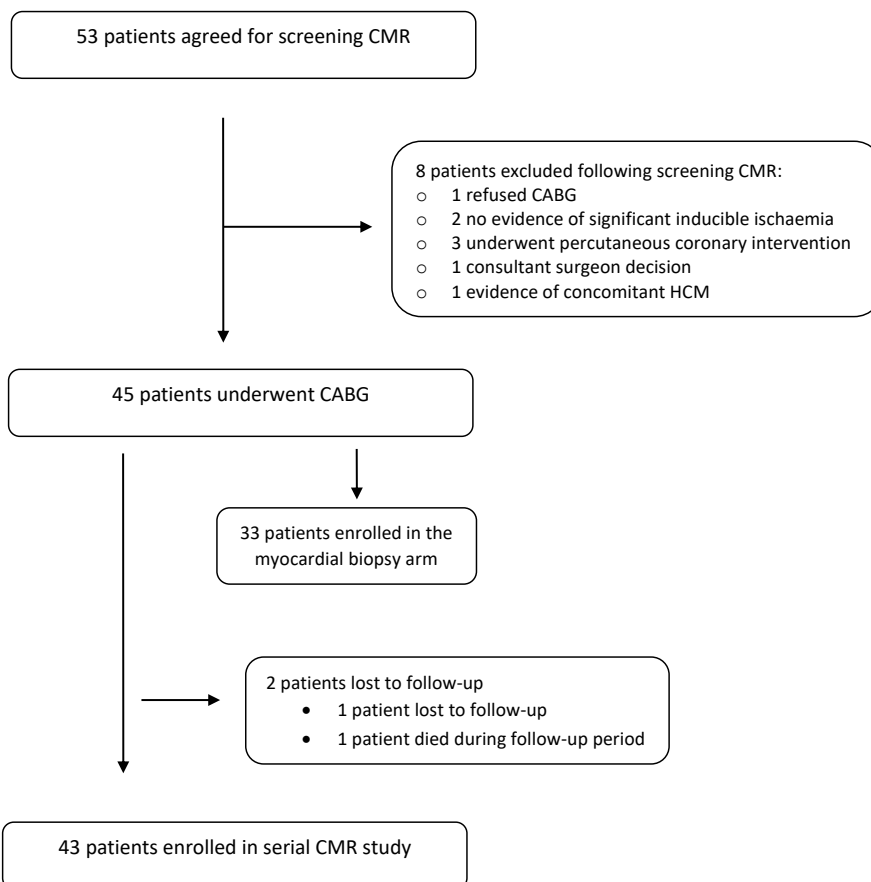


Figure 22: AMBITION study cohort

Top: Derivation of the study cohort. Bottom: Recruitment timeline for the AMBITION study. Enrolment was impacted by the COVID-19 pandemic. CABG = coronary artery bypass grafting; CMR = cardiovascular magnetic resonance; PCI = percutaneous coronary intervention.

At baseline, the median NYHA class, CCS class and SAQ-7 Summary score (0-100, higher = less symptomatic) were 2 (IQR: 1-2), 2 (IQR: 1-3) and 66.0 (IQR: 43.1-89.7). The median indexed end-diastolic LV volume, indexed end-systolic LV volume and LVEF at enrolment were 70.6ml/m² (IQR: 65.3-80.3 ml/m²), 22.7 ml/m² (IQR: 18.2-29.3 ml/m²), 68.4% (IQR: 62.2-73.0%). On quantitative perfusion imaging, the mean global stress MBF, mean global rest MBF and median global MPR at baseline were 1.27±0.47ml/min/g, 0.71±0.14ml/min/g and 1.71 [IQR: 1.36-2.22] respectively. The median visual perfusion defect burden was 42.0% [IQR: 27.0-51.0%] prior to CABG. Tissue characterisation at baseline demonstrated a mean native septal T1, ECV, indexed LV cell volume and indexed LV extracellular matrix volume fraction of 1004.3±39.1ms, 0.28±0.04, 47.3±9.2ml/m², 18.2±4.6ml/m² respectively. Patients with a lower global MPR was more likely to have history of hypertension (P=0.03).

Table 1: Baseline characteristics, stratified by global MPR

	All patients	Global MPR		P-value
		≤1.7	>1.7	
Demographics	N=43	N=24	N=19	
Age (years)	62.1 (9.3)	62.6 (10.4)	61.5 (8.0)	0.69
Sex				0.69
Male	41 (95.3)	23 (95.8)	18 (94.7)	
Female	2 (4.7)	1 (4.2)	1 (5.3)	
Heart rate (bpm)	67.3 (11.8)	69.8 (11.3)	64.3 (11.9)	0.13
SBP (mmHg)	135.2 (20.4)	140.3 (20.6)	128.7 (18.8)	0.07
DBP (mmHg)	73.8 (12.1)	75.2 (13.5)	72.1 (10.1)	0.41
Number of severely diseased vessels				0.59
1	1 (2.3)	1 (4.2)	0 (0.0)	
2	14 (32.6)	6 (25.0)	8 (42.1)	
3	28 (65.1)	17 (70.8)	11 (57.9)	
Prior MI	13 (30.2)	7 (29.2)	6 (31.6)	0.86
Prior revascularisation	12 (27.9)	5 (20.8)	7 (36.8)	0.25
Hypertension	30 (69.8)	20 (83.3)	10 (52.6)	0.03
Diabetes mellitus	14 (32.6)	10 (41.7)	4 (21.1)	0.15
Hypercholesterolemia	34 (79.1)	19 (79.2)	15 (78.9)	0.99
Current smoker	3 (7.0)	1 (4.2)	2 (10.5)	0.41
Number of vessels grafted				0.08
1	5 (11.6)	1 (4.2)	4 (21.1)	
2	22 (51.2)	12 (50.0)	10 (52.6)	
3	16 (37.2)	11 (45.8)	5 (26.3)	
Number of arterial grafts used				0.52
0	1 (2.3)	1 (4.2)	0 (0.0)	
1	28 (65.1)	14 (58.3)	14 (73.7)	
2	13 (30.2)	8 (33.3)	5 (26.3)	
3	1 (2.3)	1 (4.2)	0 (0.0)	
Medications				
Betablocker	32 (74.4)	17 (70.8)	15 (78.9)	0.54
ACEi/ARB	29 (67.4)	17 (70.8)	12 (63.2)	0.59
Lipid-lowering medication	42 (97.7)	23 (95.8)	19 (100.0)	0.56
Calcium channel blocker	14 (32.6)	9 (37.5)	5 (26.3)	0.44
Long-acting nitrate	10 (23.3)	4 (16.7)	6 (31.6)	0.22
ACEi = angiotensin-converting enzyme inhibitor, ARB = angiotensin II receptor blocker, DBP = diastolic blood pressure, MI = myocardial infarction, SBP = systolic blood pressure. Continuous variables are reported as mean (standard deviation). Categorical variables are reported as N (%). Characteristics were compared between patients with a Global MPR <median versus those with a Global MPR ≥median using 2-sample t-tests for continuous variables and Fisher's exact tests for categorical variables.				

2.5.1 Serial changes to health status and circulating markers of myocardial wall stress and injury.

There were improvements in NYHA class (1 [IQR: 1-1] vs 2 [IQR: 1-2], $P<0.001$), CCS angina grade (1 [IQR: 1-1] vs 2 [IQR: 1-3], $P<0.001$) and SAQ-7 Summary score (100.0 [IQR: 87.5-100.0] vs 66.0 [IQR: 43.1-89.7], $P<0.001$) after CABG. Levels of NT-proBNP (204 [IQR: 130.0-357.0] vs 147.0ng/L [73.0-360.0], $P=0.28$) and hsTroponin I (5.9 [IQR: 3.4-8.5] vs 6.9ng/L [IQR: 3.6-13.1], $P=0.35$) were similar post operatively.

2.5.2 Serial changes to CMR assessment of myocardial perfusion.

Overall, 40 (93%) patients underwent serial perfusion imaging. After CABG, the median burden of visual perfusion defect decreased (18.0% [10.5-21.0] vs 42.0% [27.0-51.0] vs, $P<0.001$) (Figure 23A). All patients, however, had evidence of inducible ischaemia following CABG, with 30 (75%) patients having a residual global perfusion burden of $>10\%$. On quantitative perfusion imaging, there was an increase in mean global stress MBF ($1.54\pm0.50\text{ml/min/g}$ vs $1.27\pm0.47\text{ml/min/g}$, $P=0.002$) and median global MPR (2.40 [IQR: 1.72-2.75] vs 1.71 [IQR:1.36-2.22], $P=0.002$) following CABG (Figure 23B). Mean global rest MBF trended towards a decrease post CABG ($0.68\pm0.25\text{ml/min/g}$ vs $0.71\pm0.14\text{ml/min/g}$, $P=0.17$), but this did not reach statistical significance (Figure 23B). There was improvement in median global MPR across all 3 coronary territories and at all LV levels (base, mid-cavity and apex) (Table 3). Mean global stress endocardial/epicardial ratio increased following CABG (0.80 ± 0.08 vs 0.77 ± 0.08 , $P=0.03$).

No baseline characteristics were associated with a visual perfusion defect burden of $>10\%$ at follow-up (Table 2).

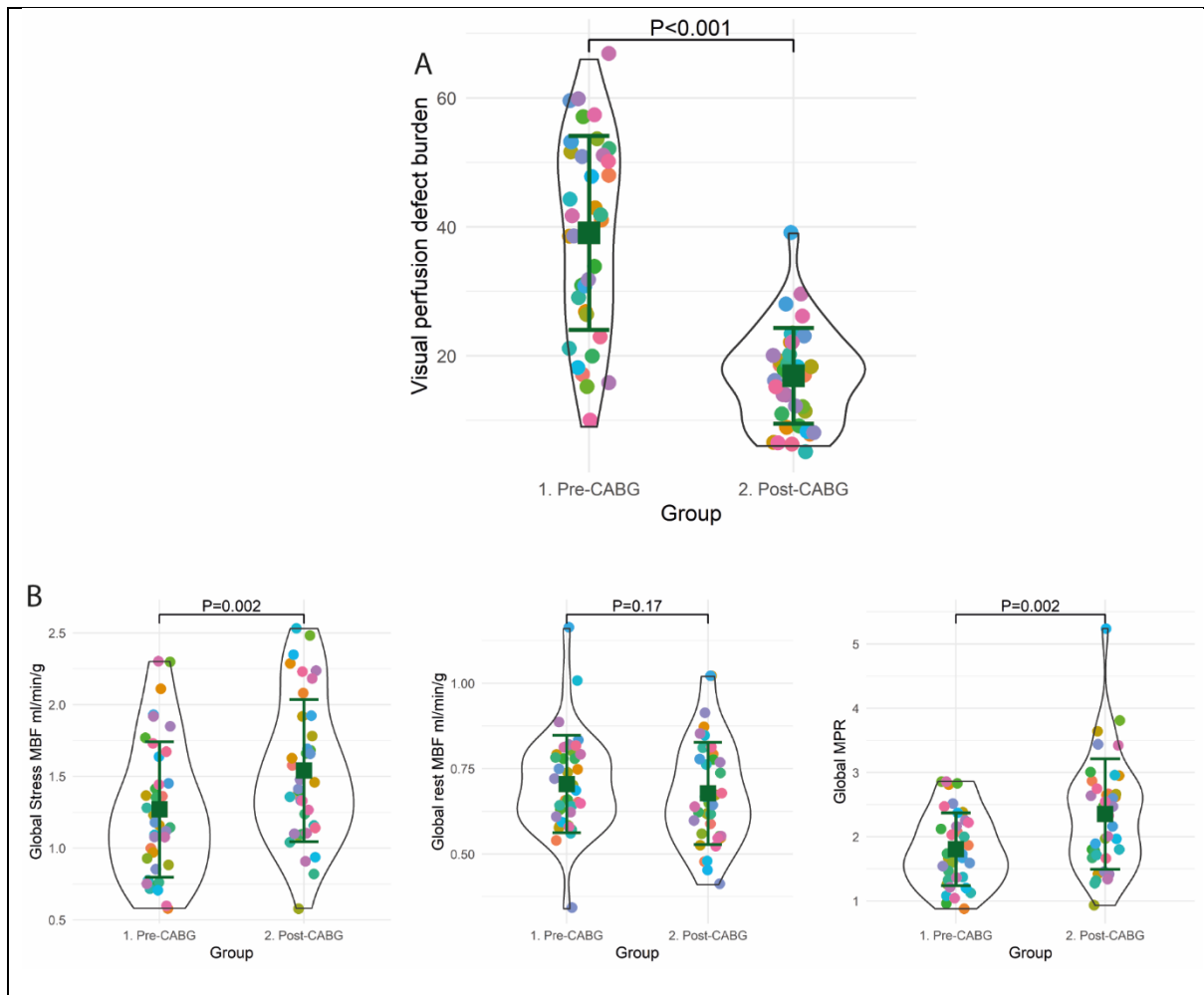


Figure 23. Serial CMR myocardial perfusion data in patients undergoing CABG

A. Plot demonstrating the serial reduction in visual perfusion burden following CABG. B. Plots demonstrating the increase in global stress MBF and MPR following CABG. There was no significant change in global rest MBF.

CABG = coronary artery bypass grafting; CMR = cardiovascular magnetic resonance; MBF = myocardial blood flow; MPR = myocardial perfusion reserve.

Table 2. Predictors of significant inducible ischaemia at follow-up

Variable	Univariable	
	OR (95% CI)	P-value
Age (per 10 years)	0.98 (0.45, 2.13)	0.96
BMI (kg/m²)	0.86 (0.71, 1.04)	0.13
Diabetes mellitus	2.00 (0.36, 11.23)	0.43
Hypertension	0.43 (0.08, 2.41)	0.34
Current smoker	--	
NYHA		0.90
1	Reference group	
2	1.41 (0.25, 7.90)	
3	0.87 (0.13, 5.82)	
CCS		0.73
1	Reference group	
2	1.33 (0.22, 8.22)	
3	0.75 (0.13, 4.36)	
SAQ-1 summary score	1.00 (0.97, 1.03)	0.86
LVEF (%)	1.07 (0.98, 1.16)	0.11
LGE mass	0.93 (0.85, 1.02)	0.11
Number of arterial grafts used		0.87
0	Reference group	
1	1.29 (0.26, 6.37)	
2	--	
3	--	
Univariable associations between baseline characteristics and residual inducible ischaemia, defined as global visual perfusion defect burden >10% at follow-up. BMI = body mass index; CCS = Canadian Cardiovascular Society; LGE = late gadolinium enhancement; LVEF = left ventricular ejection fraction; NYHA = New York Heart Association; SAQ-7 = Seattle Angina Questionnaire-7.		

2.5.3 *Serial changes in tissue characterisation, myocardial structure and function*

There was no change in mean native septal T1 (1000.8 ± 45.9 ms vs 1004.3 ± 39.1 ms, $P=0.53$), ECV fraction (0.26 ± 0.03 vs 0.28 ± 0.04 , $P=0.64$), or indexed LV cell volume (46.2 ± 9.2 ml/m² vs 47.3 ± 9.2 ml/m², $P=0.26$) after CABG. There was a decrease in mean indexed LV extracellular matrix volume after CABG (16.6 ± 3.9 ml/m² vs 18.2 ± 4.6 ml/m², $P=0.009$).

There was no significant change in median indexed LV end-diastolic volume (69.8 ml/m² [IQR 60.5-81.7 ml/m²] vs 70.6 ml/m² [IQR: 65.3-80.3ml/m²], $P=0.07$), indexed LV-end-systolic volume (24.1 ml/m² [IQR; 18.2-30.5 ml/m²] vs 22.7 ml/m² [IQR: 18.2-29.3 ml/m²], $P=0.32$) or LVEF (65.2% [IQR: 57.3-72.9%] vs 68.4% [IQR: 62.2-73.0%], $P=0.07$) after CABG. Post CABG, indexed left atrial volume increased (39.6 ± 11.1 ml/m² vs 34.4 ± 11.6 ml/m², $P<0.001$). All results are detailed in Table 3.

Table 3: Serial changes in symptoms, CMR metrics and circulating markers of myocardial wall stress and injury following CABG.

	Pre-CABG	Post-CABG	Mean/median* difference (95% CI)	P-value
Symptom class				
NYHA functional score	2.0 (1.0-2.0)	1.0 (1.0-1.0)	-1.0 (-1.0, 0.0)*	<0.001
CCS angina grade	2.0 (1.0-3.0)	1.0 (1.0-1.0)	-1.0 (-1.0, -1.0)*	<0.001
SAQ-7 Summary score	66.0 (43.1-89.7)	100.0 (87.5-100.0)	26.0 (8.3, 35.7)*	<0.001
CMR characteristics				
LVEDVi (ml/m2)	70.6 (65.3-80.3)	69.8 (60.5-81.7)	-4.3 (-7.0, 0.2)*	0.07
LVESVi (ml/m2)	22.7 (18.2-29.3)	24.1 (18.2-30.5)	0.7 (-0.8, 2.2)	0.32
LVEF (%)	68.4 (62.2-73.0)	65.2 (57.3-72.9)	-2.2 (-5.7, 0.3)*	0.07
LAVi	34.4 (11.6)	39.6 (11.1)	5.3 (2.6, 8.0)	<0.001
LVMi	68.7 (12.9)	65.9 (12.5)	-2.7 (-4.8, -0.7)	0.009
3D GLS	-12.6 (3.5)	-13.1 (4.0)	-0.2 (-1.4, 0.2)*	0.13
3D GCS	-19.0 (-20.4-- 15.5)	-17.9 (-19.9--13.8)	0.5 (-0.2, 1.1)*	0.16
3D GRS	29.3 (9.0)	30.1 (11.0)	1.0 (-1.3, 2.8)*	0.36
Septal T1	1004.3 (39.1)	1000.8 (45.9)	7.0 (-9.0, 18.0)*	0.53
ECV fraction	0.28 (0.04)	0.26 (0.03)	-11.0 (-22.9, 12.2)*	0.64
LV extracellular matrix volume	18.2 (4.6)	16.6 (3.9)	-0.01 (-0.02, 0.01)*	0.009
LV cell volume	47.3 (9.2)	46.2 (9.2)	-1.5 (-2.6, -0.4)	0.26
Global stress MBF (ml/min/g)	1.27 (0.47)	1.54 (0.50)	-1.1 (-3.0, 0.8)	0.002
Global rest MBF (ml/min/g)	0.71 (0.14)	0.68 (0.15)	0.23 (0.16, 0.42)*	0.17
Global MPR	1.71 (1.36-2.22)	2.40 (1.72-2.75)	-0.03 (-0.07, 0.01)	0.002
Global visual perfusion defect burden (%)	42.0 (27.0-51.0)	18.0 (10.5-21.0)	0.5 (0.2, 0.8)*	<0.001
MPR by coronary territory:				
LAD	1.75 (1.28-2.27)	2.36 (1.71-2.74)	0.6 (0.1)	<0.001

LCx	1.82 (1.42-2.45)	2.42 (1.69-3.00)	0.5 (0.0-1.1)	0.002
RCA	1.65 (1.22-2.17)	2.31 (1.49-2.75)	0.4 (0.1-0.9)	<0.001
MPR by LV level:				
Base	1.95 (1.34-2.43)	2.30 (1.78-3.02)	0.3 (0.0-0.9)	0.003
Mid-cavity	1.64 (1.26-2.15)	2.17 (1.67-2.63)	0.5 (0.1)	<0.001
Apex	1.73 (1.27-2.03)	2.46 (1.54-2.94)	0.7 (-0.1-1.2)	0.01
Global stress endocardial/epicardial ratio	0.77 (0.08)	0.80 (0.08)	0.01 (-0.03-0.07)	0.03
Extent of MI scar (%)	7.6 (2.6-15.7)	7.0 (3.8-15.4)	1.0 (-1.5-2.0)	0.46
Blood results				
NTproBNP (ng/L)	147.0 (73.0-360.0)	204.0 (130.0-357.0)	45.5 (-73.0-128.0)	0.28
hsTroponinI (ng/L)	6.9 (3.6-13.1)	5.9 (3.4-8.5)	-0.2 (-4.9-1.4)	0.35
Continuous variables are reported as mean (standard deviation) or median (IQR). Categorical variables are reported as N (%). Serial characteristics were compared using the paired t-test for parametric variables and the Sign test for non-parametric data. The mean/median difference (95% CI) between the pre- and post-operative results is also detailed. The median differences are represented with *. CABG = coronary artery bypass grafting; CCS = Canadian Cardiovascular Society; ECV = extracellular volume; GCS = global circumferential strain; GLS = global longitudinal strain; GRS = global radial strain; IQR = interquartile range; LAD = left anterior descending artery; LCx; left circumflex artery; LAVi = indexed left atrial volume; LVEDVi = indexed left ventricular end-diastolic volume; LVEF = left ventricular ejection fraction; LVESVi = indexed end-systolic LV volume; LVMi = indexed left ventricular mass; MBF = myocardial blood flow; MPR = myocardial perfusion reserve; NYHA = New York Heart Association; RCA = right coronary artery; SAQ-7 = Seattle Angina Questionnaire-7.				

2.5.4 Assessing the association between health status, myocardial perfusion and tissue characterisation metrics

Increased baseline global visual perfusion defect burden was associated with a lower baseline SAQ-7 summary score ($\rho=-0.33$, $P=0.04$, Figure 24A). Additionally, a greater improvement in the SAQ-7 summary score was associated with a larger myocardial ischaemic burden at baseline ($\rho=0.44$, $P=0.004$, Figure 24B). Finally, a greater improvement in SAQ-7 summary score was associated with a

larger decrease in global myocardial ischaemic burden following CABG ($\rho=-0.38$, $P=0.02$, Figure 24C).

Baseline global stress MBF, global rest MBF and global MPR were not associated with baseline SAQ-7 summary score ($\rho=-0.22$, $P=0.19$; $\rho=-0.19$, $P=0.25$; $\rho=-0.14$, $P=0.45$, respectively, Figure 25A). Change in SAQ-7 summary score was not associated with baseline global stress MBF ($\rho=0.11$, $P=0.51$, Figure 25B), global rest MBF ($\rho=0.07$, $P=0.70$) or global MPR ($\rho=0.08$, $P=0.64$). Change in SAQ-7 summary score was not associated with change in global stress MBF ($\rho=0.04$, $P=0.79$, Figure 25C), global rest MBF ($\rho=0.18$, $P=0.28$) or global MPR ($\rho=-0.08$, $P=0.65$). There was no significant association between baseline global MPR and LVEF ($\rho=0.20$, $P=0.22$) or change in global MPR and LVEF ($\rho=0.22$, $P=0.18$).

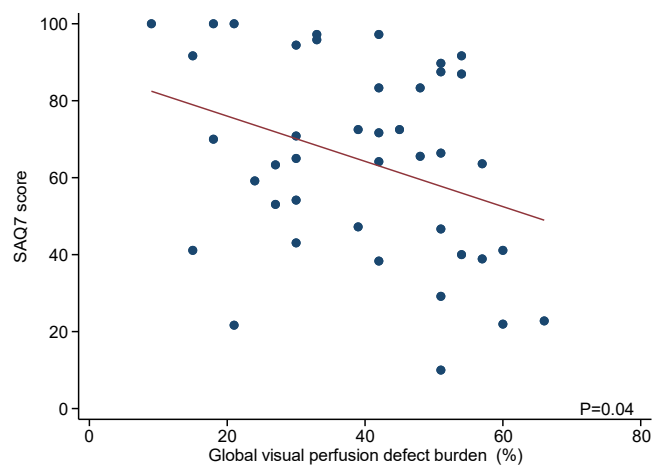
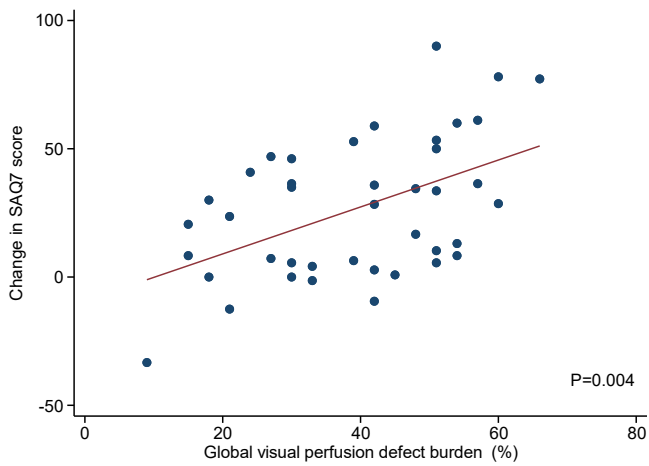
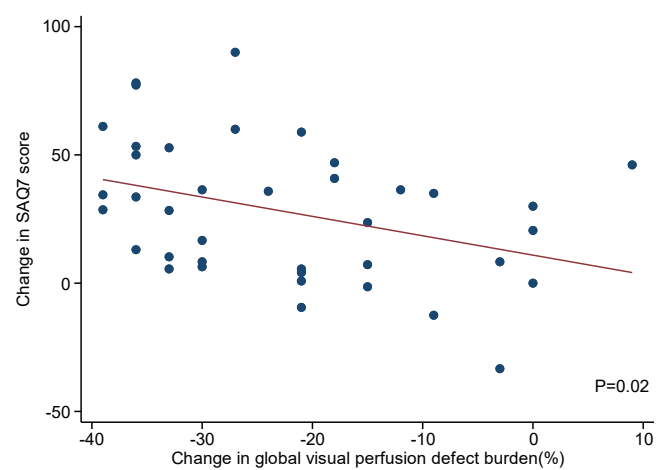
A**B****C**

Figure 24: Associations between visual perfusion defect burden and SAQ-7 summary score.

A: Plot demonstrating the association between greater baseline visual perfusion defect burden and lower baseline SAQ-7 summary score. B: Plot demonstrating the association between higher baseline global visual perfusion defect burden and greater change in SAQ-7 summary score. C: Plot demonstrating the association between change in global visual perfusion defect burden and change in SAQ-7 summary score. SAQ = Seattle Angina Questionnaire.

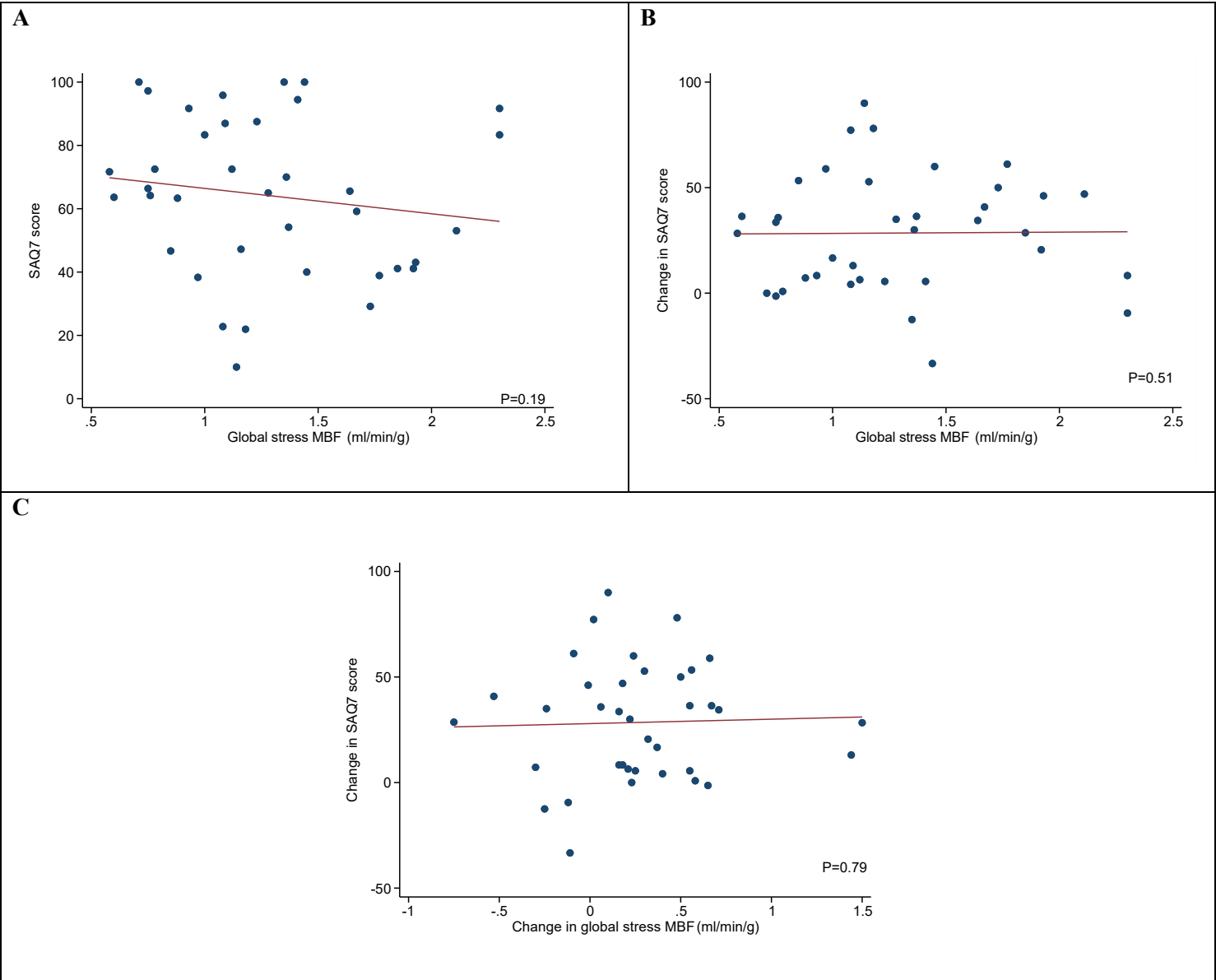


Figure 25: Associations between stress MBF and SAQ-7 summary score.

A: Plot demonstrating the lack of association between SAQ-7 summary score and baseline stress MBF. B: Plot demonstrating the lack of association between change in SAQ-7 summary score and baseline stress MBF. C: Plot demonstrating the lack of association between change in SAQ-7 summary score and change in stress MBF. MBF = myocardial blood flow; SAQ = Seattle Angina Questionnaire.

There was no significant association between change in LV extracellular matrix volume and baseline global myocardial ischaemic burden ($\rho=-0.24$, $P=0.14$, Figure 26A). Additionally, there was no significant association demonstrated between change in LV extracellular matrix volume and change in global myocardial ischaemic burden ($\rho=0.25$, $P=0.13$, Figure 26B).

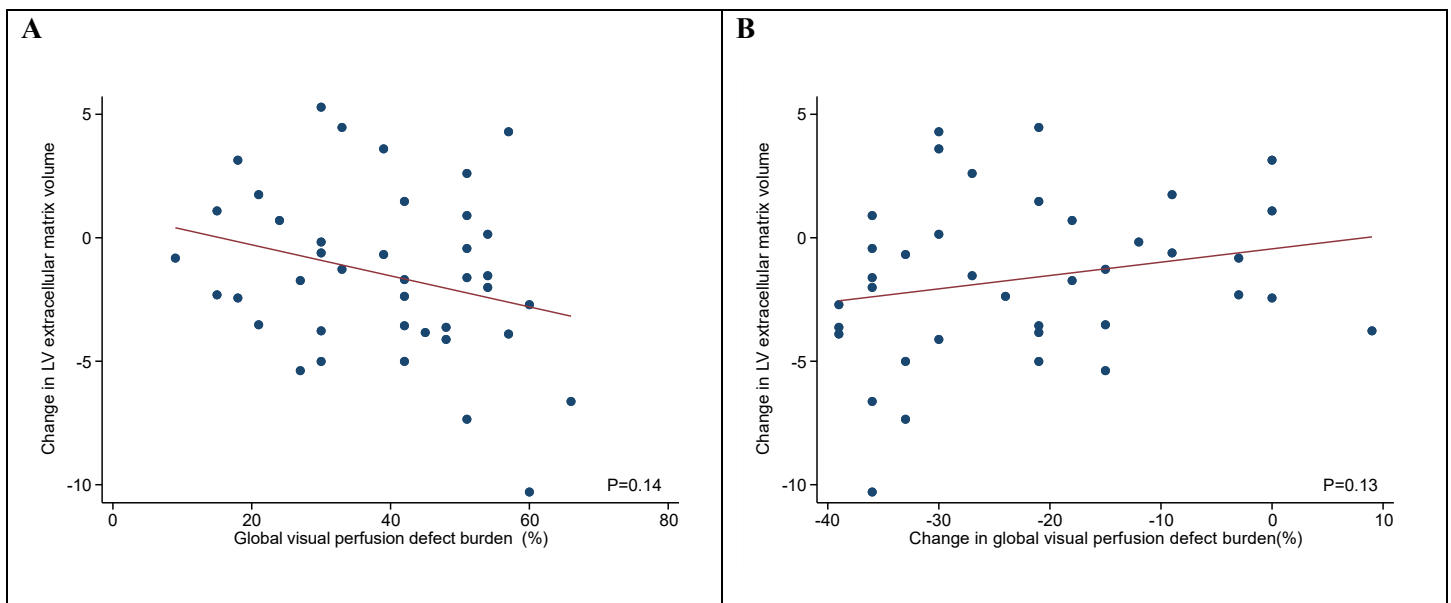


Figure 26: Association between visual perfusion defect burden and LV extracellular matrix volume.

A: Plot demonstrating the lack of significant association between change in LV extracellular matrix volume and baseline visual perfusion defect burden. B: Plot demonstrating the lack of significant association between change in LV extracellular matrix volume and change in visual perfusion defect burden. LV = left ventricular.

2.6 Discussion

In Chapter 2, I assess the utility of multiparametric CMR to evaluate dynamic changes in myocardial structure, function and perfusion in patients undergoing CABG. The main findings are: i) CMR assessment of visual perfusion defect burden, global stress MBF and global MPR improve after CABG; ii) Postoperatively, 75% of patients had a residual ischaemic burden of >10%; iii) Decrease in visual perfusion defect burden, but not stress MBF, associated with improvement in patient-reported health status following CABG; iv) LV extracellular matrix volume decreases following CABG, potentially highlighting regression of interstitial fibrosis.

2.6.1 *Dynamic myocardial perfusion and tissue characterisation indices following CABG*

Multiple nuclear studies have demonstrated the marked, albeit expected, improvement in myocardial perfusion indices following coronary revascularisation (Iskandrian et al., 2014). There remains, however, a paucity of data detailing the dynamic assessment of MBF in CABG cohorts; prior studies either excluding (Patel et al., 2020), or poorly representing (Sammut et al., 2018), post graft individuals. Chapter 2 details novel data, describing the serial improvement in CMR-derived stress MBF and MPR in patients undergoing CABG. First, the results suggest that quantitative stress perfusion CMR can detail myocardial blood flow during vasodilator stress in the presence of bypass grafts. This builds on prior mechanistic single-timepoint research that demonstrated the limited impact of left internal mammary grafts on MBF (Seraphim et al., 2021). Second, the data also continues to highlight the important degree of residual inducible myocardial ischaemia in patients post CABG. Persisting ischaemia following coronary revascularisation has previously been detailed using semi-quantitative perfusion CMR (Arnold et al., 2011). Recent retrospective data using the same quantitative approach as in our study has also demonstrated the independent association between CMR-derived MBF and clinical outcomes in patients with prior CABG (Seraphim et al., 2022). The mean post-operative global stress MBF from my results is similar to the mean value reported in the recent outcome study in CABG patients (Seraphim et al., 2022); and unsurprisingly is lower than in

healthy controls (Kotecha et al., 2019) and patients with native single vessel CAD (Kotecha et al., 2020), all studies using the same CMR technique.

Finally, multiple tissue characterisation parameters of intracellular and extracellular composition were assessed by serial CMR analysis. The decrease LV extracellular matrix volume suggests the regression of interstitial fibrosis, potentially highlighting a modifiable parameter in patients with stable CAD and could represent a target of novel drug therapies. This result aligns with research demonstrating dynamic changes in CMR derived extracellular indices in patients following aortic valve surgery (Treibel et al., 2018) and in individuals with DCM during treatment withdrawal (Halliday et al., 2021).

2.6.2 Association between patient reported health status and myocardial perfusion

The association between improvement in the SAQ-7 summary score and decreasing visual perfusion defect burden post CABG supports (the biologically plausible) strategy of restoring epicardial blood supply to reduce angina. This is of interest considering the ORBITA trial data showing an important placebo competent to symptomatic improvement following single vessel percutaneous coronary intervention (Al-Lamee et al., 2018). Overall, my results suggest a role for ischaemia testing by CMR, along with health status assessment, to refine escalation of therapy decisions in patients with multivessel disease. Placebo-controlled myocardial revascularisation trials exploring the association between visual perfusion burden and health status in multivessel disease cohorts would be of value. The lack of association between the quantitative perfusion metrics and SAQ-7 summary score is, however, interesting and could reflect an important degree of microvascular dysfunction in these patients; an entity which may associate less well with symptoms and is less easily appreciated on assessment of the raw first-pass perfusion imaging. Importantly however, my results are discordant with prior data, a recent observational study demonstrating the strong association between quantitative MBF on positron emission tomography and patient-reported angina (Patel et al., 2022).

2.6.3 Limitations

The study includes a small number of patients, enrolled in a single centre study. The data should therefore be viewed as hypothesis generating. Graft patency was not assessed at follow up and therefore the aetiology of the persisting perfusion defects could not be determined (e.g., graft failure, progressive native vessel disease or microvascular dysfunction). Additionally, the use of adenosine stress perfusion in the context of bypass grafts has challenges, although prior studies evaluating the use of perfusion imaging in patients with prior CABG are encouraging (Knott et al. 2020; Kotecha et al. 2020; Kotecha et al. 2019; Seraphim et al. 2022; Seraphim et al. 2021). Larger studies assessing the serial association between symptoms, quantitative CMR and graft patency (using CT coronary angiography) would be of value. Finally, the patients underwent unblinded CABG and therefore the potential for the improvement in symptoms to be attributed, at least in part, to placebo effect is possible. The CMR analysis was however performed by an independent reader performing the analysis in batches with patients presented in a differing order.

2.7 Conclusion

In Chapter 2, I demonstrate that although quantitative perfusion CMR can identify serial changes in myocardial hypoperfusion following CABG, the derived MBF metrics do not associate with patient-reported health status. Conversely, visual assessment of perfusion defect burden predicts change in symptoms, suggesting that the traditional assessment of CMR ischaemia may have a more clinically relevant role compared to quantitative perfusion modules.

Additionally, my results detail an important degree of post-operative inducible ischaemia in patients following CABG. Translational studies aiming to identify adjunctive therapeutic approaches that target the consequences of myocardial ischaemia would be of interest.

Chapter 3

3 Assessing mitochondrial bioenergetics in coronary artery disease: A translational multiomic tissue study in humans (The AMBITION study).

3.1 Abstract

Background: Severe or recurrent myocardial ischaemia can lead to chronic left ventricular (LV) dysfunction and heart failure in patients with coronary artery disease (CAD). Better understanding of regional and global myocardial bioenergetics in patients with inducible myocardial ischaemia might provide insight into mechanisms and potential therapeutic targets.

Objectives: To assess the multiomic profile of LV myocardium in patients with stable CAD.

Methods: Patients undergoing coronary artery bypass grafting (CABG) had preoperative quantitative stress perfusion cardiovascular magnetic resonance. During surgery, paired transmural LV biopsies were acquired on the beating heart from a region of inducible ischaemia, and a remote LV segment. LV samples from human organ donors were used as controls. Myocardial biopsies underwent high-energy phosphate (HEP) quantification (adenosine triphosphate [ATP] and adenosine diphosphate [ADP]), alongside metabolite analysis by liquid chromatography-mass spectrometry (LC-MS). Differential expression of key bioenergetic genes was also assessed by single-nuclei ribonucleic acid sequencing (snRNAseq) in a subset of patients.

Results: From 33 patients, 63 LV biopsies were acquired on the beating heart during CABG (mean age 60 ± 9 years, median LV ejection fraction 67% [IQR: 61-71%]). Analysis of LV samples from 11 essentially healthy donor hearts were included. The global myocardial ATP/ADP ratio was reduced in CAD patients as compared to donor LV tissue (median [IQR]: 2.2 [1.5-2.8] versus 7.4 [6.8-8.6], $P=0.001$), with increased expression of oxidative phosphorylation (OXPHOS) genes encoding the electron transport chain and ATP synthase complexes across multiple cell types. Paired analyses of biopsies obtained during CABG from LV segments with or without inducible ischaemia revealed no significant difference in the ATP/ADP ratio ($P=0.36$), broader metabolic profile or expression of ventricular cardiomyocyte genes implicated in OXPHOS.

Conclusions: My results suggest that viable human myocardium in patients with stable CAD has global alterations in bioenergetic and transcriptional profile without large regional differences between areas with or without inducible ischaemia. These findings suggest a global myocardial energetic deficit in chronic CAD that might be a therapeutic target.

3.2 Introduction

Despite successful implementation of population level strategies to reduce the burden of coronary artery disease (CAD), recent increases in obesity and diabetes are driving a surge in the incidence of atherosclerosis (Timmis et al., 2018). Additionally, the advent of rapid reperfusion strategies for acute myocardial infarction, alongside the adoption of evidence-based medical therapies, has resulted in declining case fatality rates and a subsequent increase in the global burden of chronic coronary syndromes (Timmis et al., 2018). Compounding the issue of rising disease burden, there remain gaps in evidence regarding the optimal treatment strategy for patients with stable CAD.

The recent International Study of Comparative Health and Effective With Medical and Invasive Approaches (ISCHEMIA) trial randomised more than 5,000 patients with stable CAD and evidence of inducible myocardial ischaemia to invasive or conservative management (Maron et al., 2020). The trial reported a lack of prognostic benefit from invasive therapy, reigniting debate over the role of coronary revascularisation for patients with chronic coronary syndromes. A key objective of restoring epicardial blood flow is to increase tissue perfusion during periods of increased demand in order to improve symptoms and, at least conceptually, to protect the myocardium from the deleterious effects of ischaemia that might lead to myocardial dysfunction and heart failure. Importantly however, there remains a lack of human data detailing abnormalities in myocardial cellular function associated with myocardial ischaemia. This includes changes to energy availability (in the form of ATP/ADP ratio) and alterations in oxidative phosphorylation (OXPHOS). Furthermore, it remains unclear whether metabolites that drive the maladaptive response to severe hypoxia (e.g. the tricarboxylic acid cycle intermediate, succinate (Chouchani et al., 2014; Martin et al., 2019)) accumulate in human models of chronic ischaemia. The paucity of data arises, in part, from the lack of access to relevant human cardiac tissue. Previous studies in advanced heart failure patients, where tissue is more readily available, have highlighted impairment of myocardial energetics (Bedi et al., 2016; Nascimben et al., 1996; Starling et al., 1998). However, whether this generalises to the larger group of patients with relatively preserved left ventricular (LV) function is unclear.

To explore this issue further, I integrated information from quantitative perfusion cardiovascular magnetic resonance prior to elective coronary artery bypass grafting (CABG) with metabolomic and transcriptomic analyses of LV biopsies from myocardial regions with and without inducible ischaemia obtained at the time of surgery.

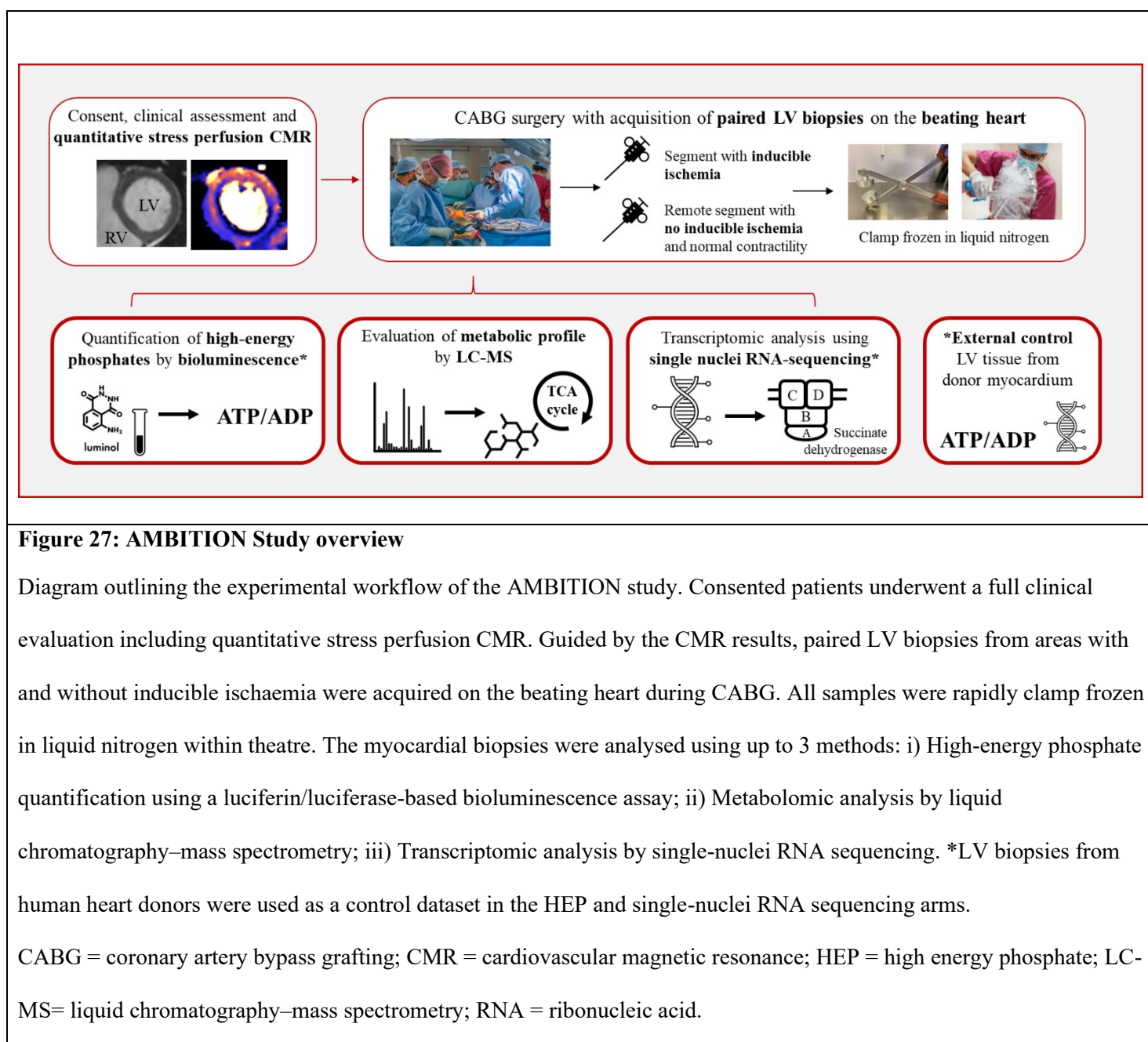
3.3 Hypotheses

1. LV myocardium in patients with stable coronary artery disease has a different multiomic profile as compared to human donor myocardium.
2. Human LV myocardium with and without inducible ischaemia have differing multiomic profiles.
3. LV myocardium from patients with impaired LVEF has a different multiomic profile compared to LV tissue from patients with preserved LVEF.

3.4 Methods

3.4.1 Study overview

AMBITION, a single-centre, observational cohort study, was approved by the National Research Ethics Service (19/EE/0166) and complied with the Declaration of Helsinki. Patients with CAD awaiting clinically indicated CABG were invited to participate and provide written informed consent. Exclusion criteria for the study were: a) valve disease requiring intervention during surgery; b) evidence of concomitant non-ischaemic cardiomyopathy; c) liver failure (e.g. INR >2); d) contraindication to CMR; e) inability to give consent. Patients underwent pre-operative CMR with quantitative stress perfusion and late gadolinium enhancement imaging. During CABG, transmural LV biopsies were acquired. Previously published data from donor LV tissue were used in the external control analysis (Litviňuková et al., 2020; Martin et al., 2019). The study workflow is summarized in Figure 27.



3.4.2 CMR protocol

Patients underwent CMR on a 1.5 Tesla Siemens Aera scanner. Localisers were followed by balanced steady-state free precession (bSSFP) cine long axis imaging. Intravenous adenosine was subsequently infused for 3-5 minutes at a rate of 140-210µg/kg/min in a stepwise protocol, ensuring a symptomatic and hemodynamic response. Short axis slices at the basal, mid-ventricular and apical levels were acquired during the first pass of a 0.05 mmol/kg IV Gadobutrol bolus (Bayer, Germany). Automated perfusion maps were produced using inline software contained within the Gadgetron online

reconstruction framework together with myocardial blood volume maps as described previously (Kellman et al., 2017). A top-up of 0.05 mmol/kg of IV Gadobutrol was then administered followed by bSSFP short-axis cine imaging. After 10 minutes, late gadolinium enhancement (LGE) images were acquired followed by rest perfusion imaging (Captur et al., 2019).

3.4.3 CMR analysis

CMR analysis was performed after each scan to identify areas of inducible myocardial ischaemia and provide volumetric and viability evaluation prior to surgery. The final CMR analysis was undertaken on CVI42 (Circle Cardiovascular Imaging, Calgary, Canada) by an independent Level 3 accredited operator (Dr Suzan Hatipoglu) blinded to the biopsy data. Biventricular volumetric analysis was performed followed by calculation of ischaemic myocardial burden. The extent of myocardial infarction was assessed by LGE quantification using the Full Width at Half Maximum technique (Flett et al., 2011).

3.4.4 CABG tissue collection

LV tissue from patients undergoing CABG was sampled from two or more pre-determined areas (as guided by CMR) on the beating heart during surgery: 1) a region of viable myocardium with inducible ischaemia ['ischaemic' biopsy]; 2) a region of remote myocardium with normal contractility, no qualitative evidence of inducible hypoperfusion on perfusion imaging, and without infarct pattern LGE ['remote' biopsy]. The *a priori* plan was to acquire paired biopsies, permitting high energy phosphate (HEP) quantification and LC-MS for each individual patient. Surgical discretion, however, resulted in some patients not undergoing paired tissue collection. Conversely, where deemed safe, extra tissue was acquired for single-nuclei RNA sequencing. The CABG biopsies were performed on the beating heart (prior to aortic cross-clamp, cardioplegia and hypothermia in the cases using cardiopulmonary bypass) with a Tru-Cut® needle or scalpel by the consultant cardiac surgeon. The majority of operations were performed off-pump without cardiopulmonary bypass. Samples were immediately clamp-frozen in theatre using a Wollenberg clamp (manufactured by Josh Firman, LMB Workshop, UK) which had been pre-cooled in liquid nitrogen until tissue acquisition. The clamps

were then reopened, and the tissue rapidly transferred into Eppendorf tubes (pre-cooled in dry ice) before storage at -80°C awaiting further analysis.

3.4.5 Donor tissue acquisition

For the ATP and ADP analysis, LV biopsies were acquired from normoxic, beating, human hearts at the time of donation after brainstem death (DBD) from donors who were considered unsuitable for cardiac transplantation (Martin et al., 2019). There was no documented history of CAD in the donors and all individuals died from intracranial haemorrhage. Informed consent was provided by a family member. This study was approved by the National Research Ethics Service (Regional Ethics Committee Reference 15/EE/0152). The LV tissue for the single nuclei ribonucleic acid sequencing (snRNAseq) data was acquired from DBD (n=6) or donation after circulatory death (n=1) from individuals with an unremarkable cardiovascular history (Litviňuková et al., 2020). Tissue sample HCA_D11 was processed at the Wellcome Sanger Institute (Hinxton, UK) under the Regional Ethics Committee Reference 15/EE/0152. Tissue samples HCA_H2-H7 were processed at the Harvard Medical School (Boston, Massachusetts, USA) under the Human Research Ethics Board approval Pro00011739 (University of Alberta, Edmonton, Canada). Informed consent was obtained from the donor families.

3.4.6 Statistical analysis

Baseline characteristics for the CAD patients are summarized as frequency (%) for categorical variables and mean (standard deviation, σ) or median (interquartile range, IQR) for continuous variables. The multiomic results are presented as mean (standard deviation, σ) or median (interquartile range, IQR) as appropriate. Arbitrary levels for the LC-MS metabolites are used to aid description and visualisation. Univariate and multivariable analyses were undertaken to assess for a significant difference in the LV multiomic profile of 1) CAD patients versus donor controls; 2) ischaemic versus remote myocardium from within the same heart; 3) CAD patients with and without reduced LV ejection fraction. A full description of the statistical analyses is detailed in the relevant tissue analysis sections below. Specifically, when comparing against donor control myocardium, the mean values from the ischaemic and remote myocardium were used. The univariate HEP and metabolomic results are plotted without

adjustment for multiple testing, aligning with prior metabolomic studies harnessing human LV tissue (Bedi et al., 2016). All other p-values are adjusted for multiple testing using the Benjamini-Hochberg method. Analysis was performed in *R* (v4.1) and Scanpy Jupyter Lab (Wolf et al., 2018) based on Python.

3.4.7 Tissue analysis for high energy phosphates

ATP and ADP concentrations were determined using a luciferase-based assay (Strehler, 1974). Frozen LV samples (~1-5mg) were homogenized in ice-cold perchloric acid extractant (3% vol/vol HClO₄, 2 mM Na₂EDTA, 0.5% Triton X-100). The supernatant was diluted to a concentration of 1 mg frozen tissue per ml. Samples, ATP and ADP standards were pH neutralized using a potassium hydroxide solution (2 M KOH, 2 mM Na₂EDTA, 50 mM MOPS), vortexed until the formation of a white precipitate (KClO₄), and then subsequently centrifuged (17,000 × g for 1 min at 4 °C). For ADP measurements, 250 µl neutralized sample supernatant was mixed with 250 µl ATP sulfurylase assay buffer (20 mM Na₂MoO₄, 5 mM GMP, 0.2 U ATP sulfurylase (New England Biolabs) and Tris-HCl buffer (100 mM Tris-HCl, 10 mM MgCl₂ (pH 8.0)), with subsequent incubation for 30 min at 30 °C with shaking (500 r.p.m.), and finally heated at 100 °C for 5 min and then cooled on ice. Standards (100 µl), samples for ATP measurement (100 µl) or samples for ADP measurement (200 µl) (in duplicate) were added to 400 µl Tris-acetate (TA) buffer (100 mM Tris, 2 mM Na₂EDTA, 50 mM MgCl₂, pH 7.75 with glacial acetic acid) in luminometer tubes. 10 µl pyruvate kinase solution (100 mM PEP, 6 U pyruvate kinase suspension (Sigma # P1506)) was added to 1 set of samples for ADP measurement and incubated for 30 min at 25 °C in the dark to convert ADP to ATP. The additional duplicate tube (without addition of pyruvate kinase solution) served as an ADP ‘blank’ value. Next, all samples were then assayed for ATP content in a Berthold AutoLumat Plus luminometer LB953 by addition of 100 µl Luciferase/Luciferin Solution (7.5 mM DTT, 0.4 mg ml⁻¹ BSA, 1.92 µg luciferase ml⁻¹ (SIGMA no, L9506), 120 µM D-luciferin (SIGMA no. L9504), made in TA buffer (25% vol/vol glycerol)), delivered via auto injection and protected from light. Bioluminescence of the ATP-dependent luciferase activity was determined for 45 seconds post injection and the results quantified against standard curves.

The HEP data solely underwent univariate analysis (without adjustment for multiple testing). First, I assessed the HEP content of LV myocardium from CAD patients, comparing against donor controls. In this experiment, the mean HEP values from the remote and ischaemic samples in the CAD patients were used. Analysis was performed using Welch's t-test for normally distributed data and the Wilcoxon-Mann Whitney U test for non-normally distributed data. Second, I performed paired analysis of the HEP content in 'ischaemic' versus 'remote' myocardium from within the same LV. Analysis was performed using the paired t-test for normally distributed data and the Wilcoxon-signed-rank test for non-normally distributed data. Third, I assessed the LV HEP levels of CAD patients with impaired LVEF, comparing to the CAD patients with preserved LVEF. In this experiment, both 'ischaemic' and 'remote' biopsies were included as biological replicates. All analysis was conducted in the *R* environment (including the *rstatix* package), generating plots using *ggplot2* (Wickham, 2016) and *ggsignif*.

3.4.8 *Tissue extraction and metabolite analysis by LC–MS*

Frozen LV tissue samples (~1-5mg) were weighed into Precellys tubes (Stretton Scientific Ltd., Derbyshire, UK), and an exact volume of extraction solution (50% methanol, 30% acetonitrile and 20% water) was added to obtain 40 mg specimen per ml of extraction solution, permitting comparisons between experimental conditions for the same metabolite. The samples were subsequently lysed after the addition of 3 ceramic beads using a Precellys 24 tissue homogenizer (Bertin Corp, Rockville, MD 20850, USA. 5500 r.p.m for 15 seconds × 2) and finally centrifuged ($16,162 \times g$ for 10 min at 4 °C). The supernatant was transferred into glass vials (Microsolv Technology Corp., Leland, NC 28451, USA) and stored at –80 °C until LC–MS analysis.

HILIC chromatographic separation of metabolites was achieved using a Millipore Sequant ZIC-pHILIC analytical column (5 µm, 2.1 × 150 mm) equipped with a 2.1 × 20 mm guard column (both 5 mm particle size) with a binary solvent system. Solvent A was 20 mM ammonium carbonate, 0.05% ammonium hydroxide; Solvent B was acetonitrile. The column oven and autosampler tray were held at 40 °C and 4 °C, respectively. The chromatographic gradient was run at a flow rate of 0.200 mL/min as follows: 0–2 min: 80% B; 2-17 min: linear gradient from 80% B to 20% B; 17-17.1 min: linear gradient

from 20% B to 80% B; 17.1-22.5 min: hold at 80% B. Samples were randomised and analysed with LC-MS in a blinded manner with an injection volume was 5 μ l. Pooled samples were generated from an equal mixture of all individual samples and analysed interspersed, at regular intervals, within the sample sequence as a quality control. Each sample was analysed with three analytical replicates.

Metabolites were measured with a Thermo Scientific Q Exactive Hybrid Quadrupole-Orbitrap Mass spectrometer (HRMS) coupled to a Dionex Ultimate 3000 UHPLC. The mass spectrometer was operated in full-scan, polarity-switching mode, with the spray voltage set to +4.5 kV/-3.5 kV, the heated capillary held at 320 °C, and the auxiliary gas heater held at 280 °C. The sheath gas flow was set to 55 units, the auxiliary gas flow was set to 15 units, and the sweep gas flow was set to 0 unit. HRMS data acquisition was performed in a range of $m/z = 70-900$, with the resolution set at 70,000, the AGC target at 1×10^6 , and the maximum injection time (Max IT) at 120 ms. Metabolite identities were confirmed using two parameters: (1) precursor ion m/z was matched within 5 ppm of theoretical mass predicted by the chemical formula; (2) the retention time of metabolites was within 5% of the retention time of a purified standard run with the same chromatographic method. Each sample underwent 3 analytical repeats with subsequent peak annotation and chromatogram review and peak area integration were performed using the Thermo Fisher software Tracefinder 5.0. The peak area for each detected metabolite was subjected to the "Filtering 80% Rule", half minimum missing value imputation, and normalized against the total ion count (TIC) to correct any variations introduced from sample handling through instrument analysis. Samples were excluded after performing testing for outliers based on geometric distances of each point in the PCA score analysis as part of the muma package (v.1.4)(Gaude et al., 2013).

The normalised LC-MS results were first explored by principal component analysis (PCA) to assess for clusters within the CAD patients, with subsequent labelling of the 'ischaemic' and 'remote' samples. PCA analysis was performed using the R base package stats (v.4.0.5) (<https://www.r-project.org/>) with the function `prcomp` and visualized using the `autoplot` function of `ggplot2` (v.3.3.5) (Wickham, 2016) after loading the `ggfortify` package (v.0.4.14) (Tang et al., 2016). Next, differential metabolite analysis was performed to investigate patterns of metabolite changes within patients (e.g. between 'ischaemic' and 'remote' samples) that were consistent across the cohort. Differential metabolomics analysis was

performed using the R package „gtools“ (v.3.9.2) (Warnes et al., 2021) to calculate the Log2FC using the functions „foldchange“ and „foldchange2logratio“ (parameter base=2). The corresponding p-value was calculated using the R base package stats (v.4.1.3) with the function “wilcox.test” or “t.test” and adjusted using Benjamini Hochberg “BH”. Volcano plots were generated using the EnhancedVolcano package (v. 1.12.0) (Blighe & Rana, 2020) and correlation plots using corrplot (v.0.92). Univariate analysis of specific metabolites of interest was conducted, comparing ‘ischaemic’ and ‘remote’ segments. In this experiment, statistical analysis was conducted on the mean values of the analytical repeats and no adjustment for multiple testing was plotted. Analysis was performed using the paired t-test for normally distributed data and the Wilcoxon-signed-rank test for non-normally distributed data. Finally, the LV metabolic landscape of CAD patients with impaired LVEF was assessed, comparing to the CAD patients with preserved LVEF. In this experiment, both ‘ischaemic’ and ‘remote’ biopsies were included as biological replicates. Analysis was performed using the Welch’s t-test for normally distributed data and the Wilcoxon-Mann Whitney U test for non-normally distributed data.

3.4.9 Single-nuclei preparation and RNA sequencing

Clamp-frozen myocardial samples (~3-5 mg) were processed for nuclei isolation and snRNAseq as previously described (Litviňuková et al., 2020; Nadelmann et al., 2021). Briefly, samples were mechanically digested using dounce homogenisation and nuclei sorted based on NucBlue (Thermo Fisher Scientific) positive staining using the FACS Aria Fusion Cell Sorter (BD Biosciences). Sorted nuclei were visually inspected under microscope to assess integrity and manually counted using a haemocytometer. Nuclei were loaded onto the Chromium Controller (10x Genomics), and in view of the limited mass of each biopsy, 500 nuclei were targeted per reaction. 3’ gene expression libraries were generated using Chromium Next GEM Single Cell v3.1 3’ GEX kits (10x Genomics) as per manufacturer’s instructions. Quality control of cDNA and libraries was performed using Bioanalyser High Sensitivity DNA Analysis (Agilent). Sequencing was performed using NovaSeq (Illumina) at a sequencing depth of 50,000 reads per nuclei. The raw sequence reads were mapped to the human reference genome (GRCh38 v2020-A) provided by 10X Genomics, and using the CellRanger suite (v5.0.1) with default parameters recommended for single-nuclei samples. Data for control samples

were extracted from the healthy heart atlas study (Litviňuková et al., 2020), taking samples that came from the LV free wall and used the V3 10X chemistry in order to match the region and chemistry used for the CABG samples. The snRNAseq data were processed using Scanpy toolkit (v1.8.2), using the QC thresholds and approach previously described (Litviňuková et al., 2020). Batch correction was performed using Harmony (Korsunsky et al., 2019) in order to correct for patient variation. Cell type annotation was performed by looking for enrichment of previously described marker genes in specific clusters (Litviňuková et al., 2020). Differentially expressed genes between i) CAD patients and controls and ii) 'ischaemic' and 'remote' myocardial segments, were compared by converting snRNAseq counts to pseudobulk per sample and per cell type followed by analysis using edgeR (M. D. Robinson et al., 2010). Enrichment of biological process Gene Ontology terms was performed using ShinyGO (v0.75)(Ge et al., 2020), with plots created in R using ggplot2. P-values were adjusted for multiple testing using FDR.

3.5 Results

From 33 patients (mean age 60 ± 9 years, 31 men (94%), median LVEF 67% [IQR: 61-71%]), 63 LV biopsies were obtained during CABG from sites with or without inducible ischaemia (Table 4).

Surgical discretion resulted in 3 patients not having paired biopsies. A summary of the experiments is outlined in Figure 28A and a full breakdown of the analyses performed on each patient is detailed in Table 5.

Table 4: Baseline characteristics of the patients undergoing CABG.	
Variable	
Demographics	
Patients, n	33
Biopsied segments, n	63
Off-pump CABG*, n	26 (79%)
Age (y), mean (sd)	60 (9)
Men, n	31 (94%)
Body mass index, mean (sd)	30 (4)
Heart rate (bpm), mean (sd)	67 (12)
SBP (mmHg), mean (sd)	130 (20)
DBP (mmHg), mean (sd)	73 (13)
Prior MI, n	9 (27%)
Prior revascularisation, n	8 (24%)
Hypertension, n	21 (64%)
Diabetes mellitus, n	11 (33%)
Hypercholesterolemia, n	25 (76%)
Current smoker, n	2 (6%)
Medications	
Beta-blocker, n	24 (73%)
ACEi/ARB, n	21 (64%)
Lipid-lowering medication, n	32 (97%)
Calcium channel blocker, n	11 (33%)
Long-acting nitrate, n	7 (21%)
CMR characteristics	
LVEDVi (ml/m ²), median (IQR)	73 (65-80)
LVESVi (ml/m ²), median (IQR)	23 (19-29)

LVEF (%), median (IQR)	67 (61-71)
Global stress MBF (ml/min/g), mean (sd)	1.3 (0.4)
Global rest MBF (ml/min/g), mean (sd)	0.7 (0.2)
Global MPR, mean (sd)	1.8 (0.5)
Global myocardial ischaemic burden (%), mean (sd)	38 (15)
Ischaemic biopsy site MPR, mean (sd)	1.3 (0.5)
Remote biopsy site MPR, mean (sd)	2.3 (0.8)
LV extent of myocardial infarction pattern scar (%), median (IQR)	6.4 (1.3-15.7)
Blood results	
NTproBNP (ng/L), median (IQR)	220 (83-360)
hsTroponin-I, (ng/L), median (IQR)	7.5 (4.1-13.5)
ACEi = angiotensin-converting enzyme inhibitor; ARB = angiotensin II receptor blocker; CABG = coronary artery bypass grafting; CMR = cardiovascular magnetic resonance; DBP = diastolic blood pressure; IQR = interquartile range; LV = left ventricular; LVEDVi = indexed left ventricular end-diastolic volume; LVEF = left ventricular ejection fraction; LVESVi = indexed left ventricular end-systolic volume; MBF = myocardial blood flow; MPR = myocardial perfusion reserve; MI = myocardial infarction; NTproBNP = N-terminal-pro B-type natriuretic peptide; SBP = systolic blood pressure. Continuous variables are reported as mean (standard deviation) or median (interquartile range). Categorical variables are reported as N (%). *Off-pump CABG not requiring cardiopulmonary bypass.	

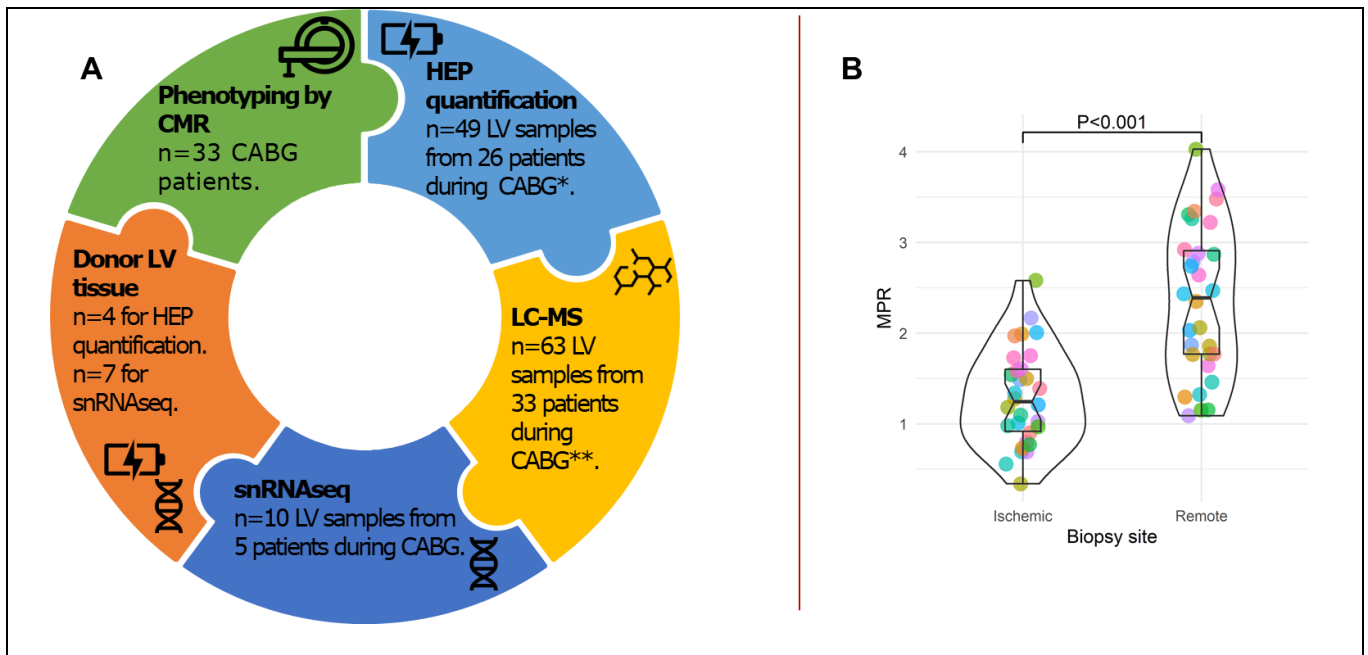


Figure 28. Overview of the AMBITION study workflow and CMR results.

A. Summary of the laboratory analyses undertaken on the myocardial biopsies. The *a priori* aim was to acquire paired LV biopsies from regions with ('ischaemic' biopsy) and without ('remote' biopsy) inducible myocardial ischaemia, permitting HEP quantification and LC-MS in all patients. Surgical discretion and insufficient sample resulted in a lack of HEP and LC-MS data in some patients. Where it was deemed safe, additional myocardium was acquired from the same sites for single-nuclei RNA sequencing. **B.** Violin box-plot demonstrating a significant difference in the biopsy site MPR of the ischaemic and remote segments, calculated by quantitative stress perfusion CMR. Each colour represents an individual patient.

*For the paired analysis of ischaemic versus remote biopsies, 46 LV samples from 23 patients are presented in the HEP results. **For the paired analysis of ischaemic versus remote biopsies, 58 LV samples from 29 patients are presented in the LC-MS results. CABG = coronary artery bypass grafting; CMR = cardiovascular magnetic resonance; HEP = high energy phosphate; LC-MS = Liquid chromatography–mass spectrometry; LV = left ventricular; MPR = myocardial perfusion reserve; snRNAseq = single-nuclei ribonucleic acid sequencing

Table 5: Breakdown of the experiments performed on each patient.			
Patient	HEP quantification	LC-MS	RNA sequencing
Patient 120	✓	✓	✗
Patient 089	✓	✓	✗
Patient 094	✓	✓	✓
Patient 080	✓	✓	✓
Patient 088	✗	✓	✗
Patient 054	✗	✓	✗
Patient 093	✓	✓	✗
Patient 039	✓	✓	✗
Patient 064	✓	✓	✗
Patient 101	✓	✓	✗
Patient 103	✓	✓	✗
Patient 075	✗	✓	✗
Patient 036	✓*	✓*	✗
Patient 099	✓	✓	✗
Patient 071	✓	✓	✗
Patient 073	✗	✓	✗
Patient 050	✓	✓	✗
Patient 081	✗	✓*	✗
Patient 090	✗	✓	✗
Patient 065	✓	✓	✗
Patient 085	✓	✓	✓
Patient 055	✓*	✓	✗
Patient 038	✓	✓	✗
Patient 076	✓	✓	✗
Patient 045	✗	✓	✗
Patient 083	✓	✓	✓
Patient 100	✓	✓	✗
Patient 040	✓	✓ _‡	✗
Patient 068	✓	✓	✗
Patient 091	✓	✓	✗
Patient 102	✓	✓	✓
Patient 043	✓	✓	✗
Patient 048	✓*	✓ _∞	✗
CAM H1	✓	✗	✗
CAM H2	✓	✗	✗
CAM H3	✓	✗	✗
CAM H4	✓	✗	✗

HCA_D11	×	×	✓
HCA_H2	×	×	✓
HCA_H3	×	×	✓
HCA_H4	×	×	✓
HCA_H5	×	×	✓
HCA_H6	×	×	✓
HCA_H7	×	×	✓
The donor LV myocardium was acquired from the “CAM” and “HCA” patients. ‡ Remote sample excluded from final analysis following outlier assessment. ∞ Remote sample excluded from final analysis following outlier assessment and no ischaemic biopsy acquired. * No paired sample available. HEP = high-energy phosphate; LC-MS = Liquid chromatography–mass spectrometry; LV = left ventricular; RNA = ribonucleic acid			

Overall, 30 (91%) patients underwent a research protocol quantitative stress perfusion cardiovascular magnetic resonance (2 patients had 6-slice quantitative perfusion during a clinical scan, 1 patient had failed injection of gadolinium contrast during the stress perfusion module). The mean global myocardial perfusion reserve was 1.8 ± 0.5 with a significant difference in segmental myocardial perfusion reserve of LV regions with and without inducible ischaemia (mean+SD: 1.3 ± 0.5 and 2.3 ± 0.8 respectively, $P < 0.001$, Figure 28B). Of the 33 patients, 26 (79%) CABG operations were performed off-pump without cardiopulmonary bypass, cardioplegia or therapeutic hypothermia. The remaining 7 (21%) patients underwent biopsy on the beating heart prior to aortic cross clamp and cardioplegia. The metadata for the human donors is detailed in Table 6.

Table 6. Metadata for the donor control myocardium					
	Age	Sex	BMI	Cause of death	Diagnosis
CAM_H1	30-40	F	25-30	DBD	CVA/ICH
CAM_H2	50-60	M	30-35	DBD	CVA/ICH
CAM_H3	60-70	F	25-30	DBD	CVA/ICH
CAM_H4	60-70	F	30-35	DBD	CVA/ICH
HCA_D11	60-65	F	25-30	DCD	Trauma
HCA_H2	50-55	M	20-25	DBD	CVA/ICH
HCA_H3	50-55	M	25-30	DBD	Suicide
HCA_H4	55-60	M	25-30	DBD	CVA/ICH
HCA_H5	50-55	F	20-25	DBD	Trauma
HCA_H6	40-45	F	20-25	DBD	Suicide
HCA_H7	45-50	F	35-30	DBD	CVA/ICH
CVA = cerebrovascular accident; DBD = donation after brainstem death;					
DCD = donation after circulatory death; ICH = intracranial hemorrhage.					

3.5.1 Global metabolic profile of LV tissue from patients with stable CAD.

Global HEP content of viable LV myocardium from 26 CAD patients was assessed, comparing with results of 4 control LV samples acquired on the beating heart following brainstem death (Martin et al., 2019). On univariate analysis, there was no significant difference in myocardial ATP content between groups (median [IQR], nmol/mg: 13.3 [8.5-16.9] and 14.9 [13.0-16.3] respectively, $P=0.58$, Figure 29A), however there was a significant increase in myocardial ADP concentrations in the CAD patients versus donor controls (median [IQR], nmol/mg: 5.0 [4.5-7.9] and 0.9 [0.8-1.4] respectively, $P<0.001$, Figure 29A). Consequently, the ATP/ADP ratio was significantly reduced in patients with CAD as compared to donor control samples (median [IQR]: 2.2 [1.5-2.8] versus 7.4 [6.8-8.6], $P<0.001$, Figure 29A). To ensure this result was not due to the inclusion of patients with reduced LV systolic function undergoing CABG, I did a subgroup analysis including only patients with normal LVEF according to the normal ranges adjusted for age, sex and body surface area ($n=20$) (Maceira et al., 2006). In this analysis, there remained a significant difference in the LV ATP/ADP ratio between CAD patients and donors (median [IQR]: 2.6 [1.8-3.0] and 7.4 [6.8-8.6], respectively, $P<0.001$). Single-nuclei RNA sequencing was performed on 10 LV samples from 5 patients to assess patterns of gene expression, comparing to data from 7 donor LV samples acquired following brainstem ($n=6$) or circulatory ($n=1$) death (Litviňuková et al., 2020). UMAP representation of the isolated nuclei is

presented in Figure 29B. Gene Ontology analysis highlighted upregulation of several biological processes associated with normal cellular respiration and OXPHOS in patients with CAD compared to donor myocardium (Figure 29C). Specifically, genes associated with the mitochondrial electron transport chain complex I (including *NDUFA13*, *NDUFA9*, *NDUFB8*), complex II (*SDHAF2*, *SDHD*), complex III (including *UQCRI0*, *UQCRI1*, *UQCRB*), complex IV (including *COX16*, *COX6A1*, *COX8A*) and ATP synthase (including *ATP5F1A*, *ATP5MF*, *ATP5PO*) had increased expression within left ventricular cardiomyocytes of CAD patients compared to donor control samples (Figure 29D). Furthermore, expression of these key genes broadly aligned across the other cell types, including fibroblasts, endothelial cells, pericytes and smooth muscle cells (Figure 29D). There was increased expression of *SOD2*, the gene encoding manganese-dependent superoxide dismutase (MnSOD), in CAD patients compared to controls across all cell types (Figure 29E). Expression of *SLC2A4*, the gene encoding the insulin-sensitive glucose transporter GLUT4, was significantly reduced across multiple cell types in CABG samples compared to donor myocardium (Figure 29E). There was also reduced expression of *PPARA*, the gene encoding PPAR- α in ventricular cardiomyocytes of CAD patient comparing to controls (Figure 29E). Expression of *CPT1A* and *CPT2*, the genes encoding carnitine palmitoyltransferase 1A and carnitine palmitoyltransferase 2 respectively, were similar between CAD and controls (Figure 29E). Gene specificity and cell abundance plots are presented in Figure 30A-B.

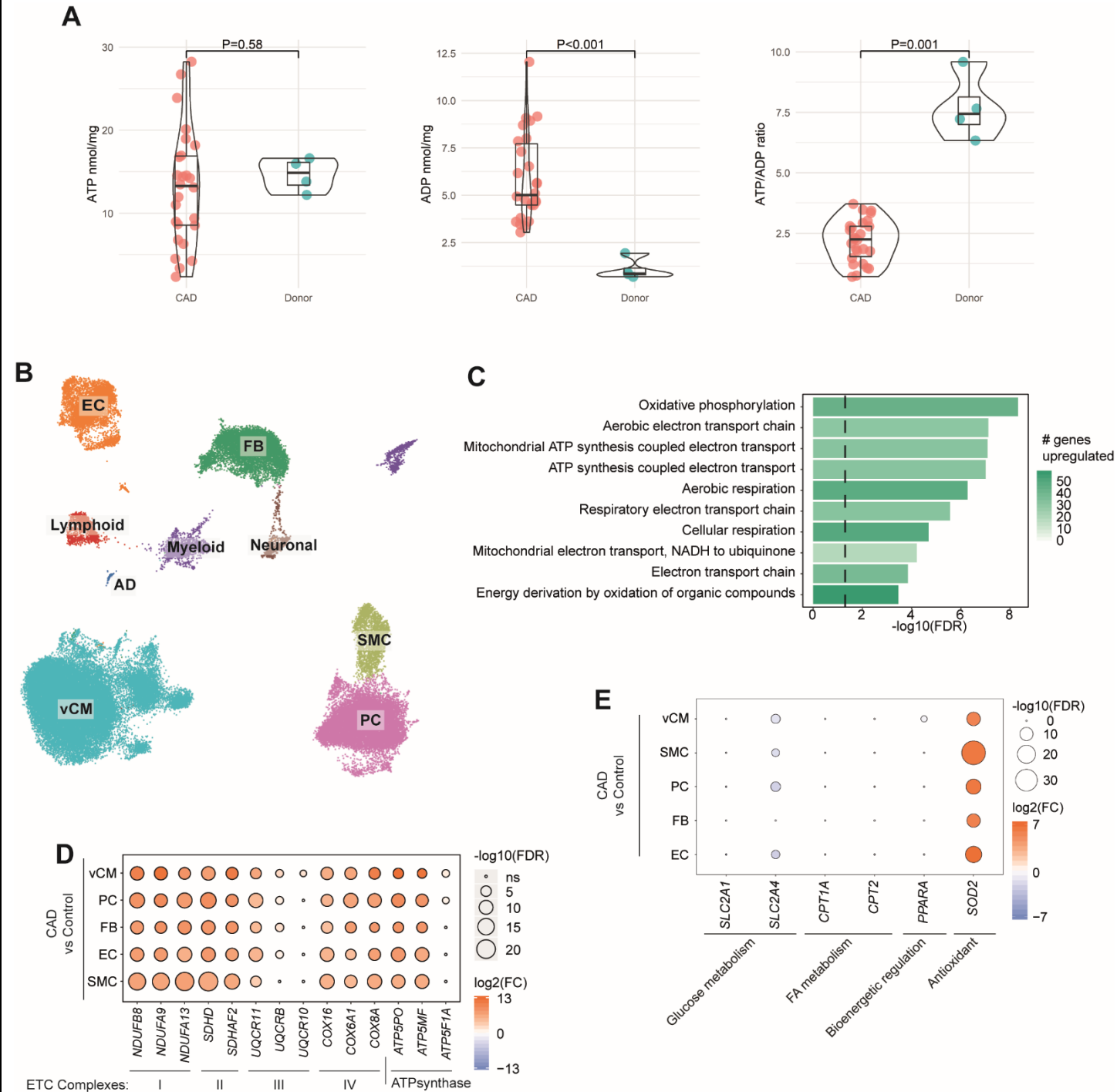
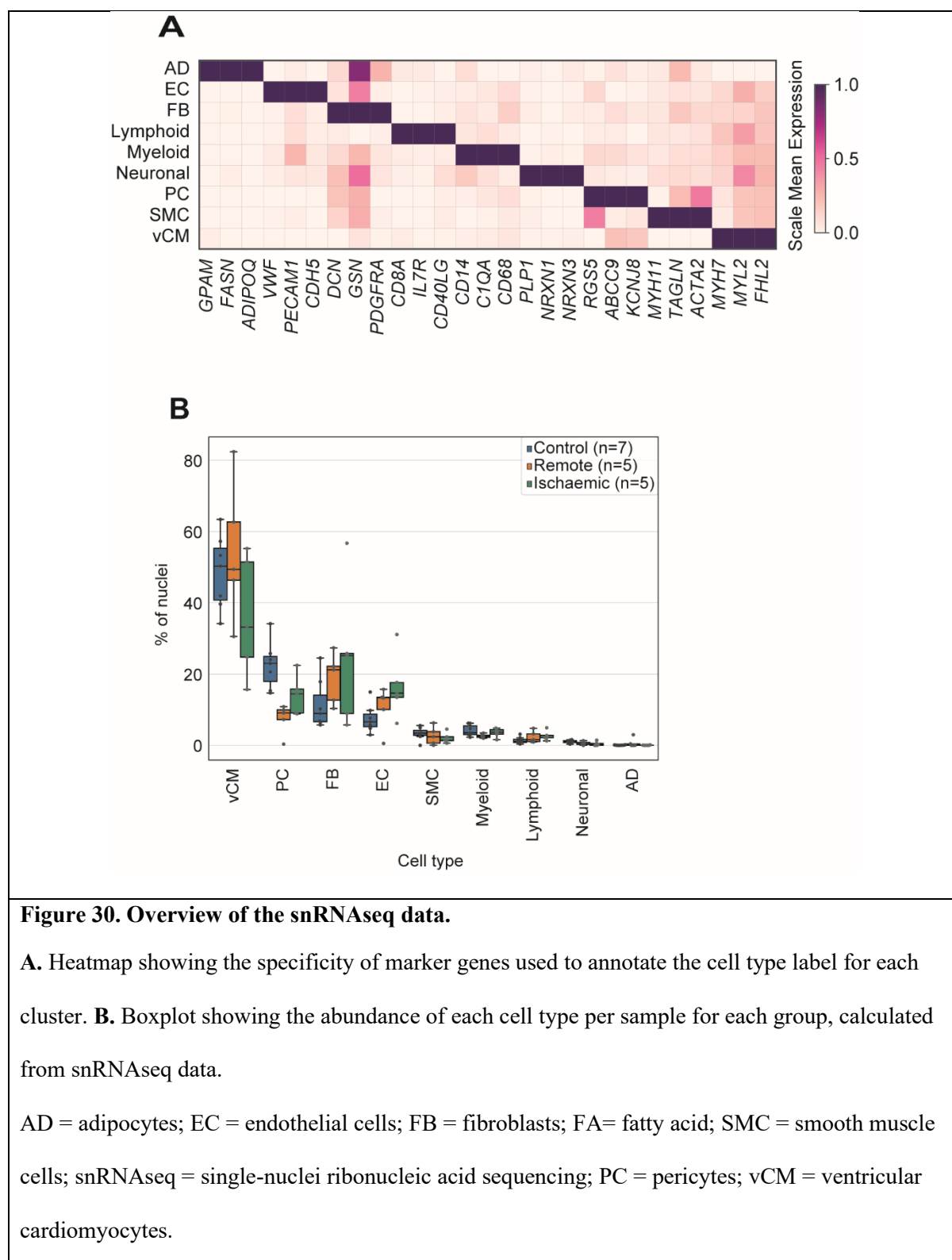


Figure 29. Global alterations in LV high energy phosphate content and single-nuclei transcriptomic landscape between CAD patients and control donor myocardium.

A. Violin box-plots demonstrating the myocardial high energy phosphate content of patients with stable CAD versus donor control myocardium, analysed and quantified via a luciferase-based bioluminescence assay. The results demonstrate that the ATP/ADP ratio is significantly lower in the CAD biopsies as compared to the donor heart samples, driven by the differing ADP levels. **B.** UMAP representation of 65,886 nuclei from 5 pairs of CABG samples and 7 donor controls, colour coded by cell type. **C.** Gene ontology analysis of the snRNAseq data demonstrating significant upregulation of several biological processes associated with normal cellular bioenergetic function within ventricular cardiomyocytes in patients undergoing CABG compared to control donor myocardium. The x-axis shows the significance level, represented as $-\log_{10}(\text{FDR})$, and the colour indicates the number of upregulated genes that match the gene ontology term. The dashed line indicates the significance threshold of FDR 0.05. **D.** Differential single-nuclei transcriptomic expression of key genes encoding the ETC and ATP synthase between CABG patients and controls, stratified by cell type. The results detailing increased expression of genes encoding all ETC complexes and ATP synthase in CAD patients compared to controls, broadly aligned across cell types. **E.** Differential expression of key genes, identified by snRNAseq, implicated in glucose metabolism (*SLC2A1*, *SLC2A4*), fatty acid metabolism (*CPT1A*, *CPT2*), bioenergetic regulation (*PPARA*) and endogenous antioxidant mechanisms (*SOD2*); CABG versus control samples stratified by cell type. There was increased expression of *SOD2* (the gene encoding MnSOD) across all cell types in patients with CAD compared to controls. There was reduced expression of *SLC2A4* (the gene encoding GLUT4) across the majority of cell types in patients with CAD compared to controls.

AD = adipocytes; ADP = adenosine diphosphate; ATP = adenosine triphosphate; CABG = coronary artery bypass grafting; CAD = coronary artery disease; EC = endothelial cells; ETC = electron transport chain; NADH = nicotinamide adenine dinucleotide; FA = fatty acid; FB = fibroblasts; FC = fold change; FDR = false discovery rate; MnSOD = manganese-dependent superoxide dismutase; ns = non-significant; SMC = smooth muscle cells; snRNAseq = single-nuclei ribonucleic acid sequencing; PC = pericytes; vCM = ventricular cardiomyocytes.



3.5.2 Regional metabolic profile of LV tissue from patients with stable CAD.

Overall, 46 paired LV samples (i.e., 'ischaemic' and 'remote' LV biopsies from the same heart) from 23 patients were included for the regional assessment of HEP content. Paired univariate analysis revealed no significant difference in concentrations of ATP (median [IQR], nmol/mg: 13.9 [7.6-20.4] versus 12.9 [5.4-19.2], $P=0.48$) or the ATP/ADP ratio (median [IQR]: 2.2 [1.5-2.5] versus 2.3 [1.1-3.9], $P=0.36$) between regions with and without inducible ischaemia (Figure 31A). The myocardial ADP content was higher in regions with inducible ischaemia compared to regions without inducible ischaemia (median [IQR], nmol/mg: 6.2 [4.2-8.1] versus 4.7 [3.3-6.6] respectively, $P=0.03$, Figure 31A).

On metabolite profiling by LC-MS, 58 paired LV samples from 29 patients were included in the final analysis. PCA revealed no clear patient clusters, nor a separation of samples originating from myocardium with and without inducible ischaemia (Figure 31B). Furthermore, differential metabolite analysis of each patient (comparing 'ischaemic' versus 'remote' myocardium) revealed that individual alterations in metabolic landscape do not correlate well between patients, with a resultant paucity of strong patient clusters (Figure 31C).

Specifically assessing metabolites known to accumulate in acute ischaemia (Chouchani et al., 2014; Martin et al., 2019), there was no significant difference in the levels of succinate (median [IQR], arbitrary units: 2.9 [2.5-4.0] versus 2.9 [2.2-3.9], $P=0.97$, Figure 31D), lactate (median [IQR], arbitrary units: 12.3 [8.3-19.3] versus 12.1 [7.8-18.4], $P=0.62$, Figure 32A) or hypoxanthine (median [IQR], arbitrary units: 2.2 [1.3-4.2] versus 2.2 [1.2-3.3], $P=0.54$, Figure 32A) between regions with or without inducible ischaemia. On assessment of substrate content between regions, there was no significant difference in levels of glucose ($P=0.44$) and the glycolytic intermediates dihydroxyacetone phosphate ($P=0.69$) and phosphoenolpyruvate ($P=0.88$), Figure 32B.

In the single-nuclei RNA sequencing arm, 10 paired samples from 5 patients were included. Gene Ontology analysis revealed no significant differential expression of biological processes pertaining to OXPHOS between 'ischaemic' and 'remote' regions within ventricular cardiomyocytes (Figure 31E). Additionally, there was no significant difference in the expression of genes implicated in glucose

transport (*SLC2A1*, *SLC2A4*), fatty acid metabolism (*CPT1A*, *CPT2*), or regulation of cellular bioenergetic function (*PPARA*, *PRKAA2*, *PPARGC1A*) between regions with or without inducible ischaemia in ventricular cardiomyocytes.

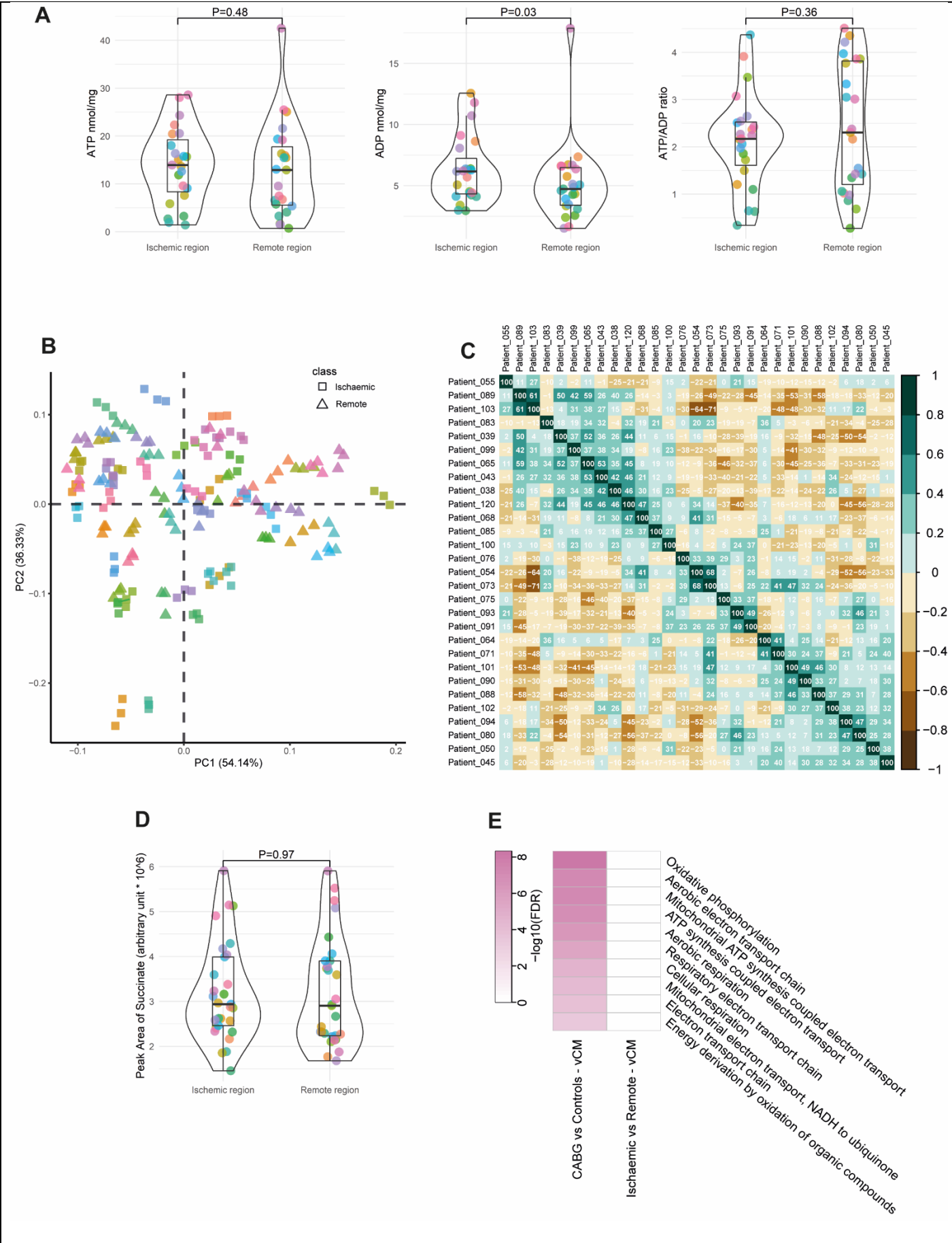


Figure 31. Regional differences in LV metabolic profile in patients with stable CAD.

A. Violin box-plots demonstrating paired analysis of the myocardial HEP content in patients with evidence of regional myocardial ischaemia, analysed and quantified using a luciferase-based bioluminescence assay. Each colour represents an individual patient. The results show that ATP levels and the ATP/ADP ratio are not significantly different between chronically 'ischaemic' and 'remote' myocardium from within the same LV. There is a small, yet significant, increase in myocardial ADP levels from regions with inducible ischaemia compared to regions without inducible ischaemia from the same LV. **B.** PCA of the LC-MS data revealing no clusters based on ischaemic (square) or remote (triangle) biopsies and no clear clusters of patients. Each colour represents an individual patient, with the 3 analytical repeats from each patient included in the plot. **C.** Log2FC of the LC-MS differential metabolite analysis (comparing ischaemic versus remote samples) with hierarchical clustering. The metabolite correlation between patients within those clusters is relatively minor (predominantly 20-50%); suggesting that the patterns of regional metabolite changes within individuals are not significantly replicated across the cohort. **D.** Violin box plots detailing paired univariate analysis of succinate (assessed by LC-MS), comparing regions of chronic ischaemia and remote myocardium. The result demonstrating no significant difference in the level of this key intermediate between regions. **E.** Gene Ontology analysis of the snRNAseq data demonstrating no significant differential expression of OXPHOS genes in ventricular cardiomyocytes between ischaemic and remote segments from within the same LV. CABG versus control sample results are also included as a reference.

ADP = adenosine diphosphate; ATP = adenosine triphosphate; CABG = coronary artery bypass grafts; NADH = nicotinamide adenine dinucleotide; FA = fatty acid; FC = fold change; FDR = false discovery rate; LC-MS = Liquid chromatography–mass spectrometry; LV = left ventricular; OXPHOS = oxidative phosphorylation; PCA = principal component analysis; snRNAseq = single-nuclei ribonucleic acid sequencing; vCM = ventricular cardiomyocytes.

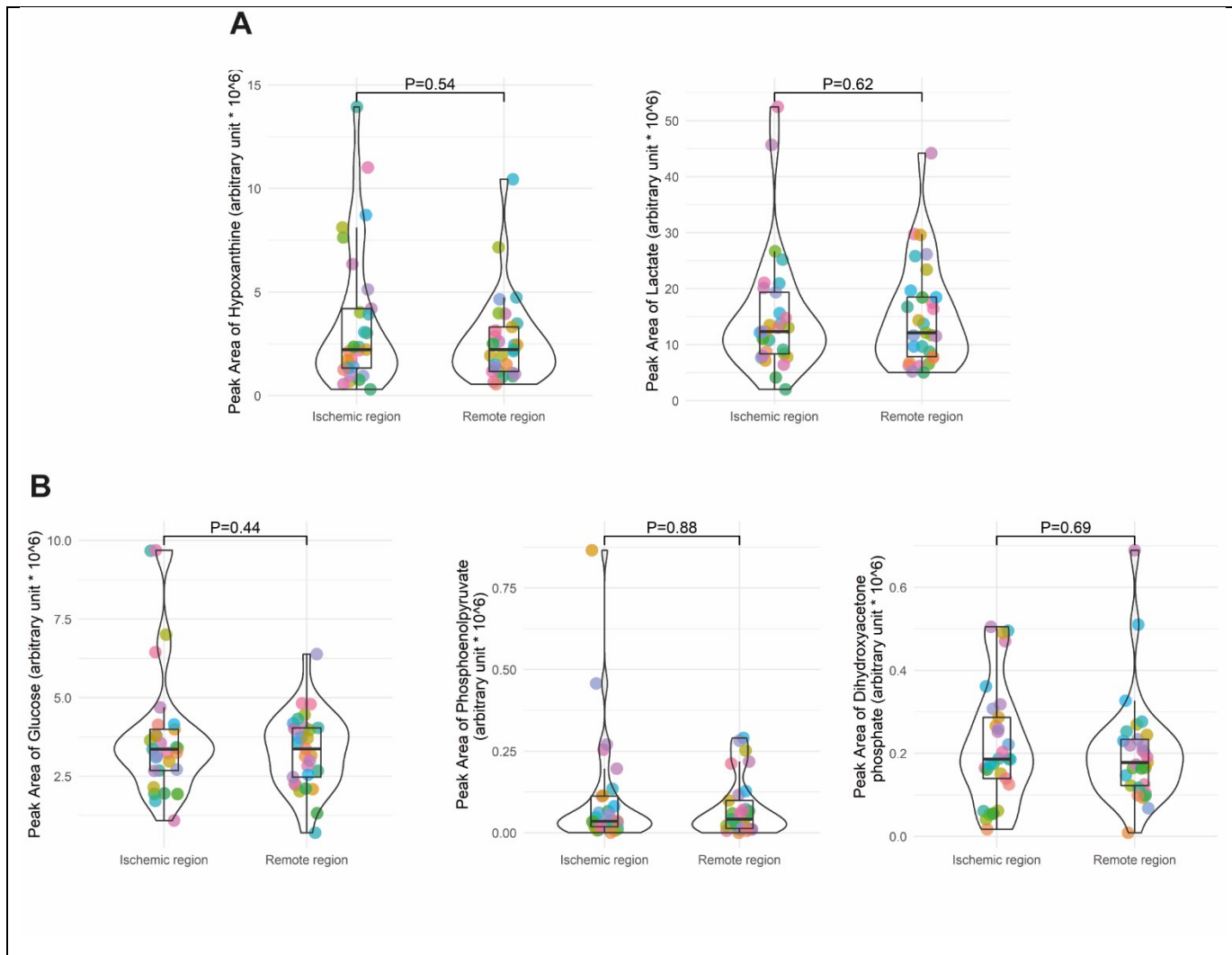


Figure 32. Univariate LC-MS results of metabolites implicated in glucose and fatty acid metabolism, comparing across ‘ischaemic’ and ‘remote’ regions.

A. Violin box plots detailing paired univariate analysis of myocardial hypoxanthine and lactate content (assessed by LC-MS) in chronically ischaemic versus remote LV regions from the same heart. The results demonstrating no significant difference in the levels of these metabolites between regions. **B.** Violin box plots detailing paired univariate analysis of myocardial glucose content and glycolytic intermediate levels (assessed by LC-MS) in chronically ischaemic and remote LV regions in the same heart. The results demonstrating no significant difference in the levels of these metabolites between regions.

LC-MS = Liquid chromatography–mass spectrometry; LV = left ventricular.

3.5.3 Exploratory analysis assessing LV metabolism stratified by LV systolic function.

Subgroup analysis was performed on the HEP and LC-MS results, stratifying patients by left ventricular ejection fraction (LVEF). In this exploratory analysis, patients with paired biopsies had both samples included as biological replicates. Of the 26 patients in the HEP analysis, 6 (23%) patients had reduced LVEF according to the normal ranges adjusted to age, sex and body surface area (median LVEF [IQR]: 47% [43-54%] vs 68% [66-71%]) (Maceira et al., 2006). There was a significant reduction in global myocardial ATP levels (median [IQR], nmol/mg: 9.2 [3.3-13.5] versus 15.1 [6.8-20.4], $P=0.04$) and the ATP/ADP ratio (median [IQR], 1.5 [1.1-2.0] versus 2.4 [1.4-3.5], $P=0.03$) in patients with reduced LVEF as compared to patients with preserved LVEF (Figure 33A). There was no significant difference in myocardial ADP content in patients with impaired LV function compared to patients with preserved function (median [IQR], nmol/mg: 5.3 [3.6-6.7] versus 5.1 [4.2-6.6] respectively, $P=0.78$).

Metabolomic analysis included 6 (23%) patients with reduced LVEF (median LVEF [IQR]: 43% [41-49%] vs 68% [66-72%]). Differential metabolite analysis revealed 17 metabolites that were significantly upregulated ($\text{Log}_2\text{FC} > 0.5$ and $p\text{-value} < 0.05$) in patients with impaired compared to preserved LVEF; including multiple amino acids (e.g. taurine, hypotaurine and isoleucine) in addition to several TCA cycle metabolites (Figure 5B). Additionally, 8 metabolites were significantly downregulated ($\text{Log}_2\text{FC} < -0.5$ and $p\text{-value} < 0.05$) in patients with impaired LVEF compared to preserved LVEF (Figure 5B); notably several short chain and medium chain acylcarnitines.

Given my *a priori* hypotheses pertaining to metabolites that accumulate in acute ischaemia (Chouchani et al., 2014), I performed further analysis on these intermediates. In this analysis, there was a significant increase in myocardial succinate (median [IQR], arbitrary units: 3.8 [3.0-4.9] versus 2.7 [2.3-3.7], $P=0.04$) and a similar trend for lactate (median [IQR], arbitrary units: 15.5 [13.5-21.0] versus 11.7 [7.8-18.4], $P=0.08$) in CAD patients with a reduced compared to preserved LVEF (Figure 33C).

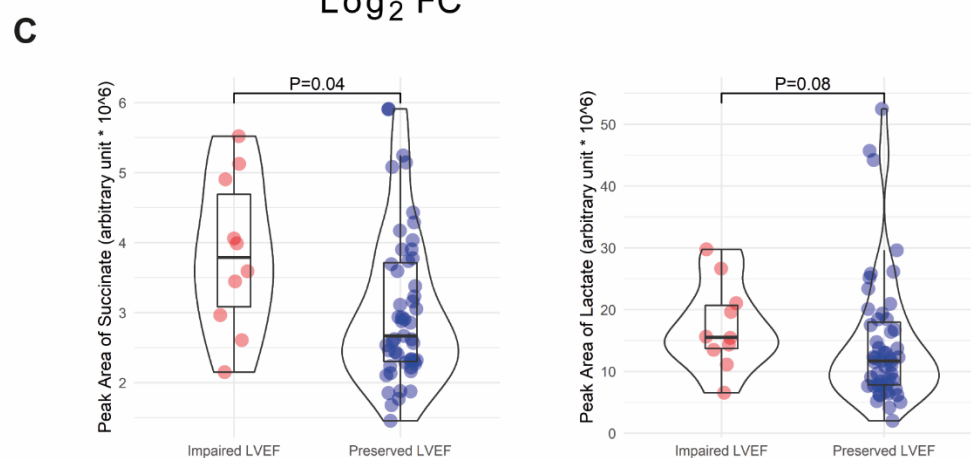
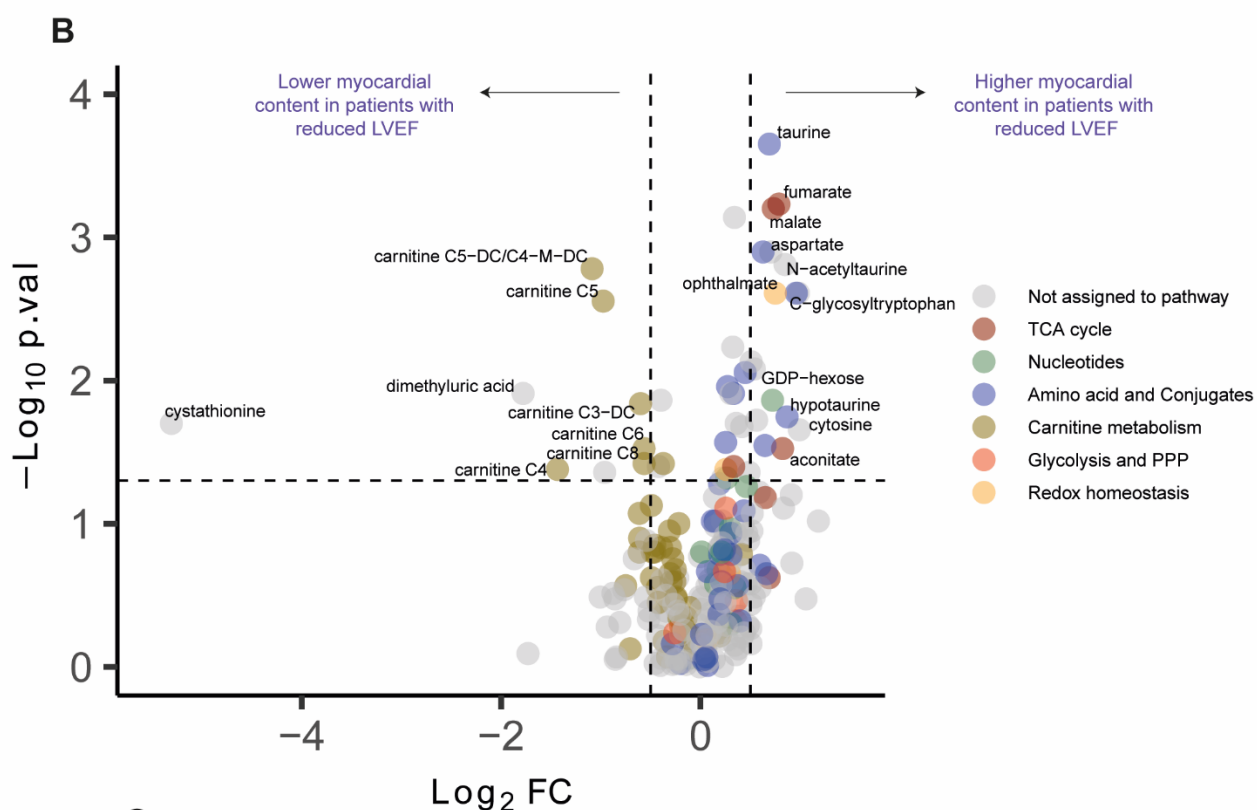
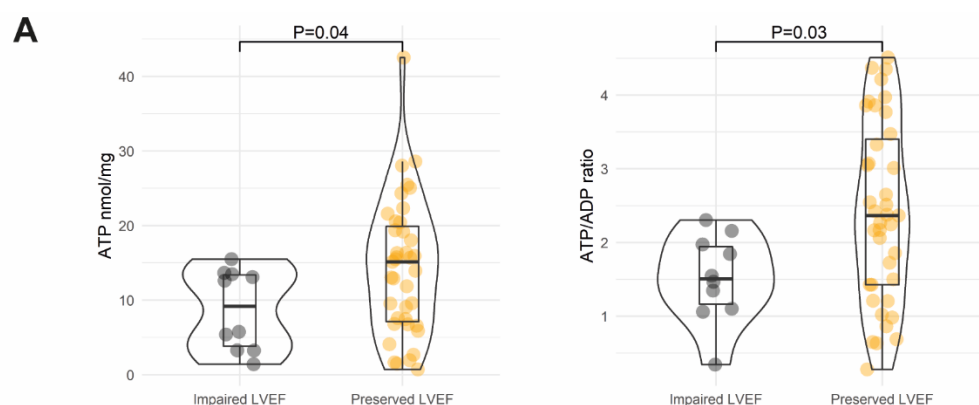


Figure 33. Exploratory analysis comparing the bioenergetic profile of CAD patients with preserved LVEF versus impaired LVEF.

A. Exploratory univariate analysis of myocardial HEP content in patients with CAD, stratifying by LV function; analysed and quantified using a luciferase-based bioluminescence assay. The results demonstrate a significant reduction in ATP levels and the ATP/ADP ratio in patients with impaired LVEF compared to preserved LVEF. **B.** Volcano plot of the LC-MS data highlighting metabolites that were significantly altered between patients with and without reduced LVEF; P-value of <0.05 with $\log_2\text{FC} > 0.5$ or $\log_2\text{FC} < -0.5$. **C.** Exploratory univariate analysis of the LC-MS data highlighting the alterations in metabolites known to accumulate in acute ischaemia (succinate and lactate), comparing CAD patients with reduced LVEF and preserved LVEF.

ADP = adenosine diphosphate; ATP = adenosine triphosphate; CAD = coronary artery disease; DC = dicarboxy; FC = fold change; HEP = high energy phosphate; LVEF = left ventricular ejection fraction; PPP = pentose phosphate pathway; TCA = tricarboxylic acid.

3.6 Discussion

In this chapter, I integrated phenotyping by cardiovascular magnetic resonance (CMR) with LV tissue analysis to assess the multiomic myocardial profile of patients with stable CAD. The central findings are: i) LV myocardium from patients with chronic CAD is energetically deficient as compared to non-failing human donor myocardium, despite increased expression of biological processes associated with OXPHOS; ii) There is no substantial regional difference in the energetic, metabolic or transcriptomic profile of myocardium from regions with or without inducible ischaemia from the same LV; iii) Viable LV myocardium from patients with CAD and LV systolic impairment is energetically impaired with a different metabolic landscape compared to myocardium from patients with CAD and preserved LVEF.

3.6.1 The myocardium of patients with stable CAD has an altered bioenergetic profile compared to non-failing donor myocardium.

CAD is a leading global cause of morbidity and mortality in adults (GBD 2017 Causes of Death Collaborators, 2018; GBD 2019 Diseases and Injuries Collaborators, 2020b; Ziaicani & Fonarow, 2016). Although primarily a vascular disease, the downstream impact of CAD on ventricular structure and function can precipitate heart failure (Cleland et al., 2003). Despite advances in therapy, there is a need to improve outcomes in this large patient population, with growing consensus that future treatments need to directly target myocardial cellular dysfunction (Wilcox et al., 2015).

Analysis of the HEP data showed an increase in myocardial ADP, despite preservation of ATP, in patients with chronic CAD as compared to donor hearts leading to a lower ATP/ADP ratio, suggesting impaired energy availability. Accumulation of ADP is a marker of tissue ischaemia (Saeb-Parsy et al., 2021), driven by reduced ATP production and increased consumption, leading to disruption of the ATP/ADP ratio. Second, single-nuclei RNA sequencing highlighted multiple biological processes associated with OXPHOS that were upregulated in the myocardium of patients with CAD compared to heart donors, including upregulation of several genes encoding electron transport chain complexes in multiple cell types. Integrating these results with the HEP data suggests that in the presence of severe epicardial CAD, myocardial mitochondria fail to meet the bioenergetic needs of the cell and

thus upregulate OXPHOS gene expression, a compensatory response aiming to increase energetic availability. However, as the key driver of pathophysiology is repetitive episodes of myocardial hypoxia, this increase in mitochondrial capacity does not translate to an improved ATP/ADP ratio potentially because of the lack of oxygen and disrupted substrate utilisation. Additionally, reduced expression of *SLC2A4*, the gene encoding the GLUT4 transporter, may signify a transition towards a fetal metabolic program; a finding previously reported in studies characterizing LV tissue from patients with heart failure (Razeghi et al., 2001, 2002).

Finally, there was increased expression the gene encoding MnSOD across multiple cell types in CAD patients comparing to controls. MnSOD is an important endogenous antioxidant and the increased *SOD2* expression may be in response to augmented levels of mitochondrial reactive oxygen species (ROS). This may represent a potential therapeutic target in stable CAD, notably for mitochondria-targeted antioxidants.

3.6.2 There are no regional differences in bioenergetic profile between chronically ischaemic and remote areas of myocardium within the same left ventricle.

On assessment of the regional bioenergetic profile of the CAD patients, there was no significant difference in the myocardial ATP content or ATP/ADP ratio between regions with or without inducible ischaemia, despite differences in myocardial perfusion reserve. These results align with animal models (Hu et al. 2009; McFalls et al. 1997) and prior human studies (Flameng et al., 1987; Wiggers et al., 2001) suggesting a lack of regional difference in myocardial energy availability in stable CAD. Furthermore, the broad metabolic profiling did not identify regional alterations in the bioenergetic landscape of patients with evidence of localized ischaemia. Importantly, there was no substantial regional difference in the content of metabolites that accumulate in acute ischaemia (e.g., succinate, lactate and hypoxanthine) (Chouchani et al., 2014). This can be explained as although regions subtended by epicardial CAD are susceptible to intermittent episodes of altered perfusion during vasodilator stress, the metabolite imbalances seen in severe hypoxia are absent at rest.

Additionally, in acute ischaemia, there is increased reliance on anaerobic glycolysis to generate ATP and maintain the ATP/ADP ratio. I demonstrate that this branch of glucose metabolism is similar in

segments with and without inducible ischaemia, with no increase in glycolytic intermediates. Finally, the single-nuclei RNA sequencing data provided additional evidence that metabolism is similar between LV regions with and without inducible ischaemia, including expression of genes encoding the electron transport chain complexes, glucose metabolism (including GLUT1 and GLUT4 transporters) and bioenergetic regulation (including AMP-activated protein kinase and PGC-1 α). Integrating these findings with the control data suggests that, for patients with CAD, energetic impairment of the myocardium is a global, rather than regional, phenomenon. These results provide further support for exploring therapies that target myocardial bioenergetic alterations in patients with CAD. Such therapies might complement coronary revascularisation as animal models show persistent abnormalities in myocardial mitochondrial function following restoration of epicardial blood flow (Holley et al., 2015; Kelly et al., 2011).

3.6.3 Energetic impairment is greater in patients with impaired LV function.

There is a paucity of research on myocardial metabolomic abnormalities in humans with CAD and mild to moderately reduced LV contractile function. Stratifying the patient cohort by LV dysfunction, I demonstrated that patients with LV systolic impairment had a significant reduction in global ATP/ADP ratio as compared to patients with preserved LVEF. These results are consistent with previous studies showing decreased adenosine nucleotide concentrations in models of reduced LV function (Nascimben et al., 1996; Starling et al., 1998) and support the paradigm that the failing heart is depleted of energy (Neubauer, 2007). Additionally, metabolomic analysis revealed increased myocardial content of several metabolites when LV function was impaired. One metabolite, taurine, is implicated in the regulation of calcium homeostasis and reduction of oxidative stress (Oudit et al., 2004). Accumulation of myocardial taurine has been previously reported in pre-clinical models of ischaemic LV dysfunction (McKirnan et al., 2019) and offers a potential therapeutic target. I also found an increase in myocardial succinate in patients with LV dysfunction. Succinate accumulation is a marker of acute ischaemia, driven either by the reverse action of complex II in the electron transport chain, or its inhibition by a reduced CoQ pool in conjunction with glutaminolysis (Chouchani et al., 2014). The accumulated succinate is rapidly oxidized on reperfusion, causing

reverse electron transport at complex I and generation of pathological ROS (Chouchani et al., 2014). Data on the role of succinate in human myocardial ischaemia is more limited, primarily based on assessment of paired blood samples in patients with acute coronary syndrome (Kohlhauer et al., 2018) or tissue analysis from donor myocardium (Martin et al., 2019). I demonstrate myocardial succinate may be another therapeutic target in patients with chronic ischaemic LV dysfunction.

3.6.4 Limitations

Firstly, I acknowledge that the CABG samples were exposed to a degree of *ex vivo* ischaemia following biopsy. However, all samples were clamp frozen in liquid nitrogen in cardiac theatres, within 60 seconds of being removed from the beating heart. Second, I acknowledge the limitations of using control donor myocardium in view of the inevitable ischaemia that occurs following brainstem or circulatory death, tissue acquisition and sample processing. Importantly, 10/11 (91%) of the control LV tissue was acquired from donation after brainstem death donors with all biopsies in the metabolomic analyses occurring on the normoxic beating heart. There is also increasing data highlighting the stability of the transcriptome following short periods of cold ischaemia (Madisson et al., 2019). Additionally, I acknowledge that the control population was not well matched to the patient population. It is possible that the observed changes may therefore reflect differences in age and co-morbidity rather than recurrent myocardial ischaemia. I also recognise the lack of external control data for the LC-MS results. The paired biopsy technique, however, permits important analysis in human studies, controlling for the innate heterogeneity of patients with chronic CAD (e.g., comorbidities and drug therapy). Finally, not all experiments were performed on each patient. The amount of tissue available was primarily driven by safety concerns and therefore it was not possible to run all assays in each patient. This reflects the challenges of undertaking a beating-heart myocardial biopsy study in humans.

3.7 Conclusions

This chapter highlights the feasibility and utility of integrating advanced phenotyping by CMR with multiomic LV tissue analysis in patients with stable CAD. The results suggest that the myocardium of patients with CAD is energetically disrupted despite upregulation of OXPHOS pathways.

Additionally, I demonstrate a lack of compelling regional difference in the LV metabolic and transcriptomic landscape of stable CAD; suggesting global myocardial dysregulation of mitochondrial bioenergetics. Proof-of-mechanism trials of interventions that might improve myocardial energetics for patients with CAD (e.g., mitochondria-targeted antioxidants), with or without concomitant coronary revascularisation should be considered.

Chapter 4

4 Assessing for genetic and cardiovascular magnetic resonance features of dilated cardiomyopathy in patients with coronary artery disease.

4.1 Abstract

Background: Patients with a predisposing vulnerability for dilated cardiomyopathy (DCM) who develop coronary artery disease (CAD) may be at increased risk of adverse cardiac remodelling and poor clinical outcomes.

Objective: To examine the relevance of genetic and cardiovascular magnetic resonance (CMR) features of DCM in individuals with CAD.

Methods: The chapter includes 2 independent cohorts. First, patients with stable CAD were recruited into a registry (London Cohort); all of whom underwent late gadolinium enhancement (LGE)-CMR on clinical grounds, genetic sequencing as part of the research protocol, and long-term adjudicated follow-up. Second, individuals with CAD recruited into the UK Biobank (UKB) were evaluated.

Results: Of 453 patients (mean left ventricular ejection fraction [LVEF] $47\pm 17\%$) recruited into the London cohort, 63 (14%) patients had non-infarct pattern LGE (NI-LGE). Patients with NI-LGE had a lower LVEF ($38\pm 18\%$ vs $48\pm 16\%$, $P<0.001$) compared to patients without NI-LGE, despite a lower amount of infarct scar (12g [IQR 0g-23g] vs 19g [IQR 8g-32g], $P=0.001$, respectively). There was no significant enrichment of rare protein altering variants in DCM-associated genes in patients with NI-LGE compared to patients without NI-LGE (9.7% vs 6.7%, odds ratio 1.5, 95% confidence interval [CI] 0.5-4.4, $P=0.41$). Over a median of 6.4 years, NI-LGE was not associated with major adverse cardiovascular events on multivariable analysis. Of 1,619 individuals with CAD included from the UKB CMR substudy, participants with a rare variant in a DCM-associated gene had lower LVEF compared to genotype negative individuals (mean $47\pm 10\%$ vs $57\pm 8\%$, $P<0.001$). Across 26,892 individuals with CAD recruited to UKB, rare pathogenic variants in DCM genes were associated with increased risk of adverse clinical events on multivariable analysis (Hazard ratio 1.54, 95% CI 1.21-1.96, $P=0.004$).

Conclusions: Rare pathogenic variants in DCM genes impact LV remodelling and outcomes in stable CAD. NI-LGE is associated with adverse remodelling but is not an independent predictor of outcome and had no rare genetic basis in our study.

4.2 Introduction

The utility of both cardiovascular magnetic resonance (CMR) and genetic sequencing to enhance characterisation of cardiac disease is well documented and increasingly performed (McDonagh et al., 2021). A key strength of CMR is the ability to provide diagnostic clarity, assigning cardiac phenotype to discrete disease subgroups. Late gadolinium enhancement (LGE) sequences can identify patterns of myocardial scar felt to be characteristic for a specific pathology; notably mid-wall/subepicardial LGE in dilated cardiomyopathy (DCM) and subendocardial LGE in coronary artery disease (CAD). In addition, the presence and distribution of myocardial fibrosis informs long term prognosis in multiple cardiovascular pathologies, thus adding an additional layer of pertinent clinical data (Disertori et al., 2016; Halliday et al., 2019). Equally, identification of key genetic variants in patients with cardiomyopathy can increase diagnostic clarity, convey prognostic information (e.g., increased arrhythmogenic burden with *LMNA* mutations) and guide patient management (McDonagh et al., 2021).

Interestingly, clinical features typically associated DCM are increasingly being recognised in CAD populations. First, patterns of myocardial scar associated with DCM have been identified in patients with a primary diagnosis of CAD (Figure 34) (Almehmadi et al., 2014; Soriano et al., 2005). In parallel, there is broad heterogeneity in the degree of adverse remodelling for equivalent levels of infarct scar in patients with stable CAD. In clinical practice, these patients are often labelled as having a ‘dual pathology’ of coronary heart disease and DCM with the physician commonly assigning a predominant disease process based on the extent of CAD and myocardial infarction. This subjective diagnostic label can alter downstream management decisions including appropriateness for revascularisation and recommendations for family and genetic screening. Second, genetic variants known to cause DCM have also been identified in CAD cohorts. The Goldstein group detailed that up to 50% of heart failure patients with Titin truncating variants (*TTN*trvs) had a diagnosis of ischaemic heart disease, collating data from across 3 large populations (Povysil et al., 2021). Whether a ‘second hit’ effect of CAD results in greater adverse remodelling and worse outcomes in individuals with variants in DCM-associated genes remains unclear.

In Chapter 4, I seek to determine the prevalence and prognostic significance of key DCM markers (genetic and phenotypic) in patients with stable CAD and to understand their impact on the degree of adverse remodelling.

4.3 Hypotheses

1. The presence of non-infarct pattern LGE (NI-LGE) in patients with stable CAD is associated with adverse LV remodelling.
2. There is increased burden of rare variants in DCM-associated genes in CAD patients with NI-LGE as compared to CAD patients without this imaging biomarker.
3. In individuals with CAD, rare variants in DCM genes are associated with adverse LV modelling.
4. In individuals with CAD, rare variants in DCM genes are associated with adverse clinical outcomes.

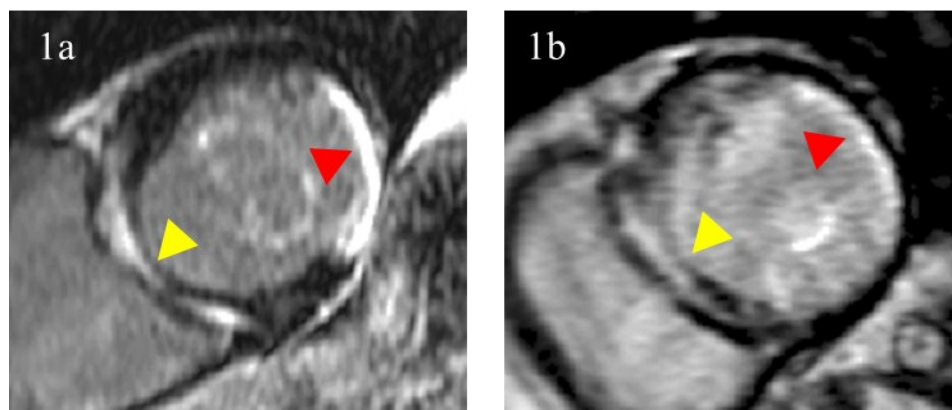


Figure 34. Examples of NI-LGE in patients with known CAD.

LGE-CMR images from unique patients with CAD. 1a) Individual with previous MI and coronary revascularisation, LVEF 37% on CMR. Transmural infarction (red arrow) of the lateral wall and mid-wall fibrosis of the ventricular septum (yellow arrow); b) Individual with known three vessel disease and LVEF 36% on CMR. Subendocardial infarction (red arrow) of the lateral wall and mid-wall fibrosis of the ventricular septum (yellow arrow).

CMR = cardiovascular magnetic resonance; NI-LGE = non-infarct pattern late gadolinium enhancement; LVEF = left ventricular ejection fraction; MI = myocardial infarction.

4.4 Methods

4.4.1 London cohort

Patients were prospectively recruited into a CMR registry from August 2009 to January 2016, following informed written consent. A CMR scan at 1.5T (Siemens Aera) was either undertaken on the day of recruitment or, in a minority of patients, had been performed at an earlier date within the institution. In brief, balanced steady-state free precession sequences were performed to acquire long and short-axis cine images. Gadopentetate dimeglumine or gadobutrol (0.1mmol/Kg) was injected intravenously (IV) and an inversion recovery gradient echo sequence was undertaken to obtain the LGE dataset as described previously (Assomull et al., 2006). Consenting patients also underwent biobanking of whole blood for genetic analysis.

CAD was confirmed by either the presence of: a) severe epicardial CAD; b) previous coronary revascularisation or ;c) history of prior myocardial infarction verified on CMR. Severe epicardial CAD was defined as $\geq 75\%$ stenosis in the left main stem or proximal left anterior descending artery, or $\geq 75\%$ in any other 2 epicardial coronary arteries (Felker et al., 2002). The exclusion criteria were myocardial infarction within 40 days prior to CMR, severe primary valve disease (or prior valvular intervention) or a primary diagnosis of dilated, hypertrophic or infiltrative cardiomyopathy.

4.4.1.1 Assessment of imaging traits by Cardiovascular Magnetic Resonance

Ventricular volumes and LV mass were calculated using a dedicated platform (CMRtools, Cardiovascular Imaging Solutions, London, United Kingdom) as previously described (Halliday et al., 2017, Gulati, et al., 2017). The presence of non-infarct pattern LGE was confirmed by two independent Level 3 accredited CMR operators (Dr John Baksi and Dr Brian Halliday) blinded to the clinical and outcome data. NI-LGE was confirmed if present in: i) long- and short-axis planes; ii) 2 phase-encoding directions; and iii) extending beyond the localized ventricular insertion points and left ventricular outflow tract. NI-LGE was defined as linear mid-wall, subepicardial or in multiple patterns. The location of NI-LGE was categorised as ventricular septum, LV free wall or in both locations. Scar extending from myocardial infarction was not considered to be NI-LGE. LGE quantification of ischaemic and non-ischaemic pattern fibrosis was performed by an independent

Level 3 CMR operator (Dr Suzan Hatipoglu) using the full-width at half maximum method on specialised software (CVI42, Circle Cardiovascular Imaging Inc, Calgary, Canada).

4.4.1.2 Clinical endpoints and follow up data collection

Patients were followed up using serial health questionnaires alongside general practitioner and hospital documentation. Implantable cardioverter defibrillator (ICD) reports, death certificates and post-mortem results were retrieved where necessary. The length of follow-up was defined from the date of CMR prior to consent until an endpoint was met or until the most recent contact date. Event times were determined from the date of the preceding CMR date for ≤ 10 years follow-up. Clinical outcomes were adjudicated by a panel of experienced cardiologists blinded to the CMR data. The primary endpoint was composite of cardiovascular mortality, life threatening arrhythmia or major heart failure event. Life threatening arrhythmia was defined as either a i) sudden cardiac death (death that occur unexpectedly, including situations where symptom duration was ≤ 1 hr, following a confirmed arrhythmia/unsuccessful resuscitation or in circumstances where the patient was witnessed alive ≤ 24 hr prior to death and no another identifiable cause of death) was noted (Hicks et al. 2015); ii) major arrhythmic event defined as appropriate ICD shock for a ventricular tachyarrhythmia, successful resuscitation following ventricular fibrillation or haemodynamically unstable VT (Buxton et al., 2006). Major heart failure (HF) event was defined as i) HF hospitalisation (admission to hospital of ≥ 24 hr/encompassing 1 calendar day with the initiation or optimisation of HF therapies) ii) HF death (death in the context of worsening clinical features of HF) or iii) cardiac transplantation or left ventricular assist device implantation.

4.4.1.3 Assessment for rare protein altering variants in DCM-associated genes

DNA extraction was performed on available whole blood (273/453 cases) using automated platforms followed by targeted sequencing on the Solid 5500xl or Illumina NextSeq platforms. Samples from both platforms were jointly analysed, annotated and filtered according to a customised bioinformatics pipeline. Additionally, sequencing data from a healthy volunteer cohort and a reference population (Genome Aggregation database [gnomAD]) were used as additional comparator groups (Karczewski et al., 2020). In brief, next generation sequencing reads underwent quality control per sample before

being aligned against a reference human genome (hg19) using BWA (v0.7.17). For the Illumina platform any reads aligned with low mapping quality ($Q < 8$) were filtered out. Aligned reads were pre-processed to identify any duplicates and recalibrate quality scores using the genome analysis toolkit (GATK v4) (van der Auwera, G. A; O'Connor, 2020). The GATK HaplotypeCaller was used for variant calling, and genomic vcf files were used for joint-genotyping following the GATK best practices (van der Auwera et al., 2013). Stringent hard-filtering was performed to all SNP and INDEL variants following GATK recommendations for germline short variant discovery. The Ensembl Variant Effects Predictor (VEP v104) was used for annotation of genomic consequence (McLaren et al., 2016). The annotated multi-sample VCF was taken forward for quality control and burden testing analysis. Analysis was performed on genes robustly associated with DCM. Protein altering variants (PAVs) in 11 DCM genes were assessed (*BAG3*, *DSP*, *DES*, *LMNA*, *MYH7*, *PLN*, *RBM20*, *SCN5A*, *TNNC1*, *TNNT2* and *TTN*). We restricted analyses to variant classes known to cause disease and implicated in mechanisms of pathogenesis. *TTN* variants were only considered if they reside in constitutively expressed exons with $PSI > 0.9$ (Roberts et al., 2015). Data on *FLNC* was not available as a proportion of patients were sequenced prior to Filamin C variants being recognised as a cause of DCM. Rare variants in CAD cases and healthy controls were defined with overall minor allele frequency (MAF) < 0.0001 in the highest disease-specific maximum credible population in gnomAD exomes (v2.1) (Popmax filtering AF (95% CI)) (Whiffin et al., 2017).

4.4.2 UK Biobank cohort

The UK Biobank (UKB) is a nationwide biomedical cohort study that has recruited $> 500,000$ participants (Bycroft et al., 2018; Sudlow et al., 2015). The study was reviewed by the National Research Ethics Service (11/NW/0382, 21/NW/0157) and written informed consent was required from all participants. The study adheres to the principles set out in the Declaration of Helsinki and the data was de-identified.

The current study was conducted under Application Numbers 47602 and 40616; 2 groups were curated. The first group comprised individuals with CAD recruited to UKB with both genetic and CMR evaluation (UKB Group 1). The second group comprised all individuals recruited to UKB with

CAD who underwent genetic evaluation (UKB Group 2). UKB Group 1 is a subset of UKB Group 2. CAD was determined by a history of myocardial infarction or coronary revascularisation based on self-reported, hospital, and Death Register record data. The UKB fields used to determine the diagnosis of CAD were as follows:

Self-reported	“heart attack/myocardial infarction”, “coronary artery bypass grafts (CABG)”, “triple heart bypass”, “coronary angioplasty (PTCA) +/- stent”
ICD-10 codes	I210, I211, I212, I213, I214, I219, I220, I221, I228, I229, I252
ICD-9 codes	410, 411, 412
OPCS4 codes	K483, K491, K492, K493, K494, K498, K499, K751, K752, K753, K754, K758, K759, K401, K402, K403, K404, K408, K409, K411, K412, K413, K414, K418, K419, K421, K422, K423, K424, K428, K429, K431, K432, K433, K434, K438, K439, K441, K442, K448, K449, K451, K452, K453, K454, K455, K456, K458, K459

4.4.2.1 Assessment for rare pathogenic variants in DCM-associated genes

From the UKB whole exome sequencing data, carriers of rare variants (minor allele frequency <0.1% and filtering allele frequency <0.00004 in gnomAD) with appropriate disease-causing mechanisms in 12 definitive or strong evidence DCM genes (*BAG3*, *DES*, *DSP*, *FLNC*, *LMNA*, *MYH7*, *PLN*, *RBM20*, *SCN5A*, *TNNC1*, *TNNT2*, and *TTN*) were identified. For *TTN*, only cardiac expressed exons with PSI>0.9 were included (Roberts et al., 2015). Variants were then filtered to identify those that would be considered pathogenic or likely pathogenic in DCM (excluding variants with evidence for pathogenicity in HCM), using CardioClassifier (Whiffin et al., 2018) and Clinvar. UKB whole-exome

sequencing data was processed using VEP (version 105 (McLaren et al., 2010)) with plugins for Genome Aggregation database (gnomAD) (Karczewski et al., 2020), LOFTEE (Karczewski et al., 2020) and SpliceAI (Jaganathan et al., 2019)

4.4.2.2 Assessment of imaging traits by Cardiovascular Magnetic Resonance

UKB participants recruited in the imaging sub study underwent CMR at 1.5T. Segmentation of the cine imaging was undertaken using a deep learning neural network with subsequent calculation of biventricular volumes, left atrial volume and strain indices as previously described (de Marvao et al., 2021).

4.4.2.3 Clinical endpoints

For survival analysis, first occurrence outcomes data was used. In brief, UKB mapped: i) primary care data; ii) ICD-9 and ICD-10 codes from hospital inpatient data; iii) ICD-10 codes from Death Register records and; iv) self-reported medical conditions to ICD-10 codes. The earliest occurrence of each event in a participant's lifetime was reported. The primary outcome for this study was a composite of death, stroke, arrhythmic event (cardiac arrest, ICD implantation, atrial fibrillation [AF]) or major heart failure event (left ventricular assist device implantation, cardiac transplantation). The secondary outcomes were i) all-cause mortality; ii) AF and iii) heart failure. For the non-fatal secondary outcomes, competing risk analysis was performed to account for death.

4.4.3 Statistical analysis

Baseline characteristics were summarised as frequency (%) for categorical variables and mean (standard deviation, SD) or median (interquartile range, IQR) for continuous variables.

In the London cohort, continuous variables were compared with Student t test or Mann-Whitney U tests based on normality of data. Categorical variables were compared with χ^2 test or the Fisher exact test where applicable. Kaplan-Meier curves were fitted to describe the cumulative incidence of the primary outcome stratified by presence or absence of NI-LGE and were compared using the logrank test. Cox regression analyses were performed to explore the association between NI-LGE presence and distribution and the primary outcome. The multivariable analyses were adjusted for age, sex and LVEF (Model A) and a subset of covariables from Table 1 (Model B). To select the covariables in

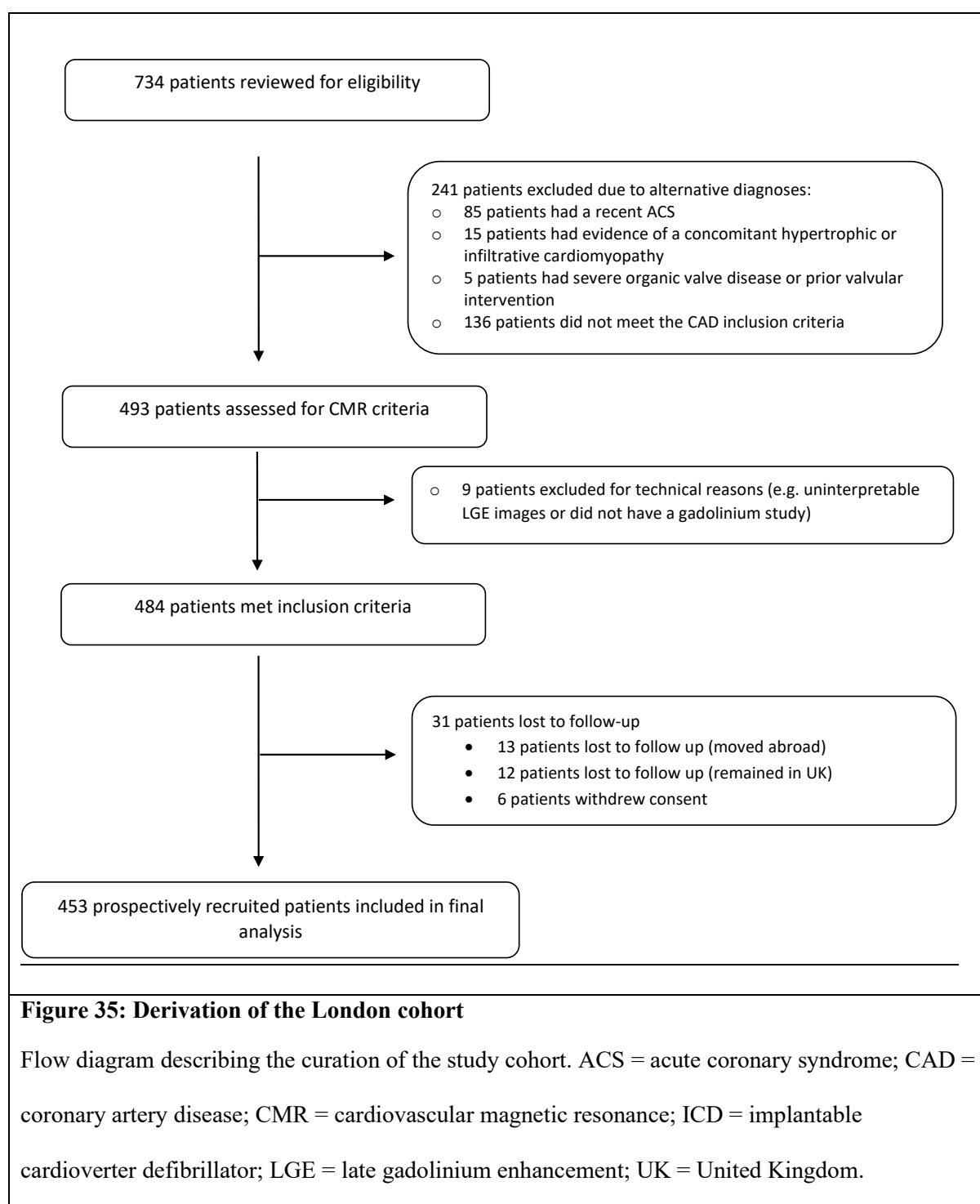
Model B, a forward stepwise procedure was applied with $p > 0.10$ as the criterion for exclusion. In the genetic analysis, we tested whether CAD patients with NI-LGE had a higher mutation burden in rare PAVs (including a separate analysis for *TTN*ts) compared to (i) CAD patients without NI-LGE, (ii) healthy volunteers (HVOLs) and (iii) unrelated individuals from gnomAD.

In the UKB, comparison of CMR traits in CAD participants with and without a rare variant in a DCM-associated gene was performed using analysis of covariance, adjusting for age, age², sex, systolic blood pressure and for the non-indexed traits, body surface area. Clinical outcomes were analysed in participants with CAD, stratified by presence or absence of a rare variant in a DCM-associated gene. Cox proportional hazards were calculated for lifetime risk and incident risk of clinical events, adjusting for age, age², sex, and genetic principal components 1-10. For the non-fatal secondary outcomes, competing risk analysis was performed using the cause-specific survival method. Time-to-event was censored at first event for each outcome, death, or last recorded follow-up.

4.5 Results

4.5.1 *The London cohort*

From 734 patients with suspected CAD, 453 were included in the final London cohort (Figure 35, Table 7). The median follow-up was 6.4 years (IQR: 5.1-7.9 years) and the median number of days between CMR and recruitment was 0 days (IQR: 0-0 days). The mean age was 64 ± 10 years, mean LVEF $47 \pm 17\%$ and 426 (95%) had angiographic evidence of severe CAD. Overall, 363 (80%) patients were referred for assessment of myocardial ischaemia/viability with 43 (9%) patients undergoing CMR for diagnostic uncertainty. Additional indications for CMR included evaluation of valvular disease, assessment for myocardial fibrosis or prior to cardiac device implantation. The proportion of patients referred for ischaemia/viability assessment was similar in those with and without NI-LGE (47/63 [74.6%] versus 316/390 [81.0%] respectively, $P=0.24$). DNA sequencing was performed on 273/453 (60%) patients on research grounds; 234 cases using the Solid platform and 39 cases using the Illumina platform.



4.5.1.1 Cardiac phenotype of CAD patients with non-infarct pattern LGE on CMR

NI-LGE was confirmed in 63 (14%) patients with involvement of the ventricular septum in 55 (12%) individuals. Patients with NI-LGE had increased indexed LV end-diastolic volumes ($131 \pm 48 \text{ ml/m}^2$ vs $103 \pm 38 \text{ ml/m}^2$, $P < 0.001$) and a lower LVEF ($38 \pm 18\%$ vs $48 \pm 16\%$, $P < 0.001$) compared to patients

without NI-LGE (Table 7). Patients with NI-LGE had a similar number of severely diseased coronary vessels ($p=0.46$) but a decreased prevalence of prior clinical myocardial infarction (56% vs 76%, $P<0.001$), lower number of infarcted myocardial segments (median, [IQR]: 3 [0-6] vs 5 [3-8], $P<0.001$) and reduced infarct LGE mass (median, [IQR]: 12g [0g-23g] vs 19g [8g-32g], $P=0.001$, Table 7) as compared to individuals without NI-LGE.

4.5.1.2 Genotype of CAD patients

Across the whole London cohort, rare PAVs in DCM-associated genes were identified in 14 patients; including *TTN*tvts that were detected in 4 patients. There was no significant difference in age ($P=0.17$), sex distribution ($P=0.62$), proportion with prior myocardial infarction ($P=0.28$) or the presence of significant CAD ($P=0.81$) in patients with and without *TTN*tv (Supplemental Table 2). The mean LVEF of patients with and without a *TTN*tv was $33\pm 15\%$ vs $41\pm 16\%$ respectively, $P=0.37$. The median infarct pattern mass of patients with and without a *TTN*tv was 8.6g [IQR 0.0-20.9g] vs 21.9g [IQR 9.9-35.1g] respectively, $P=0.09$. There was no significant enrichment of PAVs in patients with NI-LGE as compared to patients without NI-LGE (9.7% vs 6.6%, odds ratio 1.5, 95% CI 0.5-4.4, $P=0.41$), HVOLs (9.7% vs 9.4%, odds ratio 1.0, 95% CI 0.4-2.5, $P=0.82$) or the reference population gnomAD (6.5% vs 4.0%, OR 1.7, 95% CI 0.7-3.4, $P=0.16$). Specifically evaluating *TTN*, there was not a significantly increased burden of *TTN*tv in patients with NI-LGE as compared to patients without NI-LGE (3.2% vs 1.0%, odds ratio 3.5, 95% CI 0.2-48.5, $P=0.22$), HVOLs (3.2% vs 0.7%, odds ratio 4.7, 95% CI 0.5-22.0, $P=0.09$) or gnomAD (1.6% vs 0.3%, odds ratio 5.4, 95% CI 0.6-20.0, $P=0.06$).

Additionally, the burden of *TTN*tv in patients with and without a LVEF $<35\%$ was 2.7% vs 0.6%, odds ratio 4.5, 95% CI 0.4-240.0, $P=0.3$. The burden of *TTN*tv in patients with LVEF $<35\%$ compared to HVOLs was 2.7% vs 0.7%, odds ratio 3.9, 95% CI 0.7-15.1, $P=0.06$ respectively. There was increased burden of *TTN*tv in patients with LVEF $<35\%$ as compared to the reference population gnomAD (1.4% vs 0.3%, odds ratio 4.6, 95% CI 0.9-13.6, $P=0.03$ respectively).

There was no significant difference in the burden of rare synonymous DCM variants between: i) CAD patients with and without NI-LGE (9.5% vs 13.8%, odds ratio 0.7, 95% CI 0.2-1.7, P=0.5); ii) CAD patients with and without LVEF <35% (14.5% vs 11.7%, odds ratio 1.3, 95% CI 0.6-2.8, P=0.6); iii) CAD patients with LVEF <35% and HVOLs (14.5% vs 13.9%, odds ratio 1.1, 95% CI 0.6-1.8, P=0.9); iv) CAD patients with LVEF <35% and the reference population gnomAD (7.7% vs 5.9%, odds ratio 1.3, 95% CI 0.8-2.2, P=0.3).

Table 7. Baseline characteristics of the London cohort.

Variable	Non-infarct LGE		P-value
	No (N=390)	Yes (N=63)	
Demographics			
Age (years)	64.1 (9.9)	66.8 (9.5)	0.04
Female	62 (15.9)	1 (1.6)	0.002
Caucasian	322 (82.6)	50 (79.4)	0.54
BMI (kg/m2)	27.9 (5.1)	27.5 (4.2)	0.63
Significant CAD*	366 (95.1)	60 (95.2)	0.95
CAD type			0.46
Single vessel	118 (32.2)	17 (28.3)	
2 vessels	108 (29.5)	15 (25.0)	
3 vessels	140 (38.3)	28 (46.7)	
Prior MI	295 (75.6)	35 (55.6)	<0.001
Hypertension	204 (52.3)	35 (55.6)	0.63
Diabetes mellitus	112 (28.7)	18 (28.6)	0.98
Documented hypercholesterolemia	317 (81.3)	51 (81.0)	0.95
Prior PCI	202 (51.8)	26 (41.3)	0.12
Prior CABG	108 (27.7)	18 (28.6)	0.89
Atrial fibrillation	66 (16.9)	10 (15.9)	0.84
NYHA			0.09
I	140 (36.1)	14 (22.2)	
II	167 (43.0)	32 (50.8)	
III or IV	81 (20.9)	17 (27.0)	
Medication history			
Antithrombotic	378 (96.9)	57 (90.5)	0.02

Diuretic	174 (44.6)	36 (57.1)	0.06
Beta-blocker	308 (79.0)	44 (69.8)	0.11
ACE-i/ARB	332 (85.1)	49 (77.8)	0.14
Lipid-lowering drug	344 (88.2)	56 (88.9)	0.88
Aldosterone antagonist	90 (23.1)	21 (33.3)	0.08
CMR volumetric measurements			
LVEF (%)	48.3 (16.0)	38.4 (18.2)	<0.001
LV mass indexed (g/m ²)	76.7 (22.5)	93.6 (30.3)	<0.001
LVEDVi (ml/m ²)	103.2 (38.2)	131.2 (48.2)	<0.001
RVEF (%)	59.1 (11.9)	51.9 (15.3)	<0.001
RVEDVi (ml/m ²), median (IQR)	72.6 (61.9-85.4)	78.0 (68.0-89.8)	0.01
CMR LGE characteristics			
Any LGE present	349 (89.5)	63 (100.0)	0.007
Total LGE mass (g), median (IQR)	19.2 (7.8-31.7)	17.8 (8.9-31.6)	0.83
Infarct pattern LGE present	349 (89.5)	45 (71.4)	<0.001
Infarct pattern LGE mass (g), median (IQR)	19.2 (7.8-31.7)	11.5 (0.0-23.3)	0.001
No. infarcted segments, median (IQR)	5.0 (3.0-8.0)	3.0 (0.0-6.0)	<0.001
NI-LGE location			.
Septal	0 (.)	37 (58.7)	
LV free-wall	0 (.)	8 (12.7)	
Both	0 (.)	18 (28.6)	
NI-LGE pattern			.

Linear midwall	0 (.)	46 (73.0)	
Sub-epicardial	0 (.)	5 (7.9)	
Multiple patterns	0 (.)	12 (19.0)	
Non-infarct pattern LGE mass (g), median (IQR)	0.0 (0.0-0.0)	5.2 (2.7-9.2)	<0.001
Non-infarct pattern LGE mass (% of LV mass), median (IQR)	0.0 (0.0-0.0)	3.5 (1.5-4.6)	0.02
DNA sequencing**	N=210 sequenced	N=63 sequenced	
Rare protein altering variant	9 (4.3)	5 (8.0)	0.32
Titin truncating variant	2 (1.0)	2 (3.2)	0.22
ACEi = angiotensin-converting enzyme inhibitor; AF = atrial fibrillation; ARB = angiotensin II receptor blocker; BMI = body mass index; CABG = coronary artery bypass grafting; CAD = coronary artery disease; CMR = cardiovascular magnetic resonance; DBP = diastolic blood pressure; IQR = interquartile range; LGE = late gadolinium enhancement; LVEDVi = indexed left ventricular end-diastolic volume; LVEF = left ventricular ejection fraction; MI = myocardial infarction; NI-LGE = non-infarct pattern late gadolinium enhancement; NYHA = New York Heart Association; RVEDVi = indexed right ventricular end-systolic volume; RVEF = right ventricular ejection fraction; PCI = percutaneous coronary intervention; SBP = systolic blood pressure. *Patients with evidence of severe CAD or a history of prior coronary revascularisation. **Genetic sequencing was performed on 273/453 patients within the London cohort. Continuous variables are reported as mean (standard deviation) or median (interquartile range). Categorical variables are reported as N (%).			

Table 8. Demographics of patients in the London cohort, stratified by TTNtv

	TTNtv		
Variable	Absent (N=269)	Present (N=4)	P-value
Age (years), mean (sd)	65.1 (10.0)	69.8 (5.3)	0.17
Male, n (%)	238 (88.5)	4 (100.0)	0.62
Significant CAD, n (%)	251 (93.3)	4 (100.0)	0.81
Prior MI, n (%)	200 (74.3)	2 (50.0)	0.28
LVEF, % (sd)	40.8 (15.8)	33.0 (14.7)	0.37
Infarct pattern LGE mass, g (IQR)	21.9 (9.9-35.1)	8.6 (0.0-20.9)	0.09
Non-infarct pattern LGE, n, (%)	61 (22.7)	2 (50.0)	0.23
CAD = coronary artery disease; LGE = late gadolinium enhancement; LVEF = left ventricular ejection fraction; MI = myocardial infarction; TTNtv = titin truncating variant			

4.5.1.3 Association between the presence of NI-LGE and cardiovascular mortality, life threatening arrhythmia or major heart failure event

Over a median follow up of 6.4 years, 147 (32%) patients met the primary endpoint of cardiovascular mortality, life threatening arrhythmia or major heart failure event. Figure 36A shows the cumulative incidence of the primary composite endpoint stratified by presence or absence of NI-LGE illustrating that patients with CAD and NI-LGE had no increased risk of the primary endpoint on univariable analysis. On univariate and multivariable Cox regression analysis, the presence of NI-LGE was not associated with an increased risk of the primary outcome (Table 9).

4.5.1.4 Association between the distribution of NI-LGE and cardiovascular mortality, life threatening arrhythmia or major heart failure event

From 453 patients, 55 (12%) had NI-LGE that was present in the ventricular septum with 8 (2%) patients demonstrating LGE that was isolated to the LV free wall. Cumulative incidence of the

primary endpoint stratified by presence or absence of septal NI-LGE demonstrates that patients with this location of myocardial fibrosis had increased risk of the primary endpoint (Figure 36B). On multivariable Cox regression analysis however, septal NI-LGE did not predict the primary composite endpoint after adjustment for covariates in both Model A (age, sex and LVEF) and Model B (covariates selected via a forward stepwise selection) (Table 9). Stratifying by NI-LGE burden, cumulative incidence of the primary endpoint according to extent of NI-LGE suggests that patients with increased extent had increased risk of the primary endpoint (Figure 36C). On multivariable Cox regression analysis however, extent of NI-LGE was not significantly associated with the primary endpoint over follow-up (Table 9). Finally, of the 453 patients, 58 (13%) had NI-LGE that included the ventricular mid-wall. Only 5 (1%) patients had myocardial scar limited to the subepicardium. On univariate and multivariable analyses, mid-wall LGE was not significantly associated with the primary endpoint (Figure 36D and Table 9).

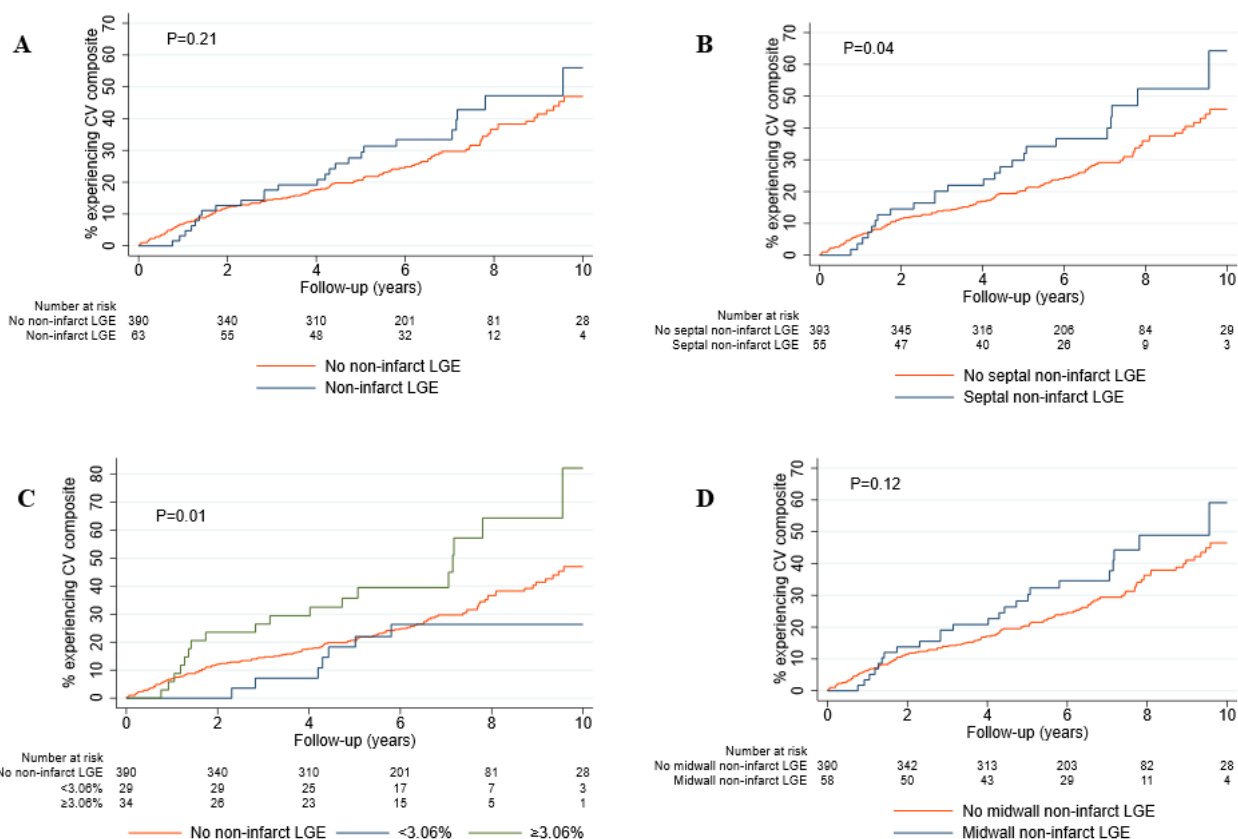


Figure 36. Kaplan-Meier plots for the primary composite endpoint of CV mortality, major heart failure event and life-threatening arrhythmia in the London cohort.

A: Kaplan-Meier plots for the composite CV endpoint, stratified by presence or absence of NI-LGE. The plots demonstrating that patients with NI-LGE had no increased risk of the primary endpoint.

B: Kaplan-Meier plots for the composite CV endpoint, stratified by presence or absence of NI-LGE within the ventricular septum. The plots demonstrating that patients with septal NI-LGE had increased risk of the primary endpoint.

C: Kaplan-Meier plots for the composite CV endpoint, stratified by NI-LGE extent. The plots demonstrating that patients with increased extent of NI-LGE had increased risk of the primary endpoint.

D: Kaplan-Meier plots for the composite CV endpoint, stratified by presence or absence of mid-wall NI-LGE. The plots demonstrating that patients with mid-wall NI-LGE had no increased risk of the primary endpoint.

CV = cardiovascular; NI-LGE = non-infarct pattern late gadolinium enhancement.

Table 9. Univariable and multivariable Cox regression analyses for the primary endpoint in the London cohort

	Unadjusted		Adjusted Model ¹		Adjusted Model 2 ²	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Non-infarct LGE						
0-2 years	1.03 (0.49, 2.18)	0.94	0.52 (0.24, 1.14)	0.10	0.60 (0.28, 1.31)	0.2
2-10 years	1.50 (0.89, 2.55)	0.13	0.77 (0.44, 1.35)	0.36	0.92 (0.53, 1.59)	0.76
Midwall non-infarct LGE						
0-2 years	1.18 (0.56, 2.50)	0.67	0.56 (0.25, 1.23)	0.15	0.64 (0.29, 1.40)	0.27
2-10 years	1.56 (0.91, 2.68)	0.11	0.73 (0.41, 1.30)	0.28	0.88 (0.50, 1.54)	0.65
Septal non-infarct LGE						
0-2 years	1.26 (0.59, 2.66)	0.55	0.56 (0.26, 1.24)	0.16	0.65 (0.29, 1.42)	0.28
2-10 years	1.78 (1.04, 3.05)	0.04	0.74 (0.42, 1.32)	0.31	0.91 (0.61, 1.60)	0.74
Extent of non-infarct LGE						
0-2 years		0.20		0.99		0.94
<3.06% (vs 0%)	--		--		--	
≥3.06% (vs 0%)	1.99 (0.94, 4.21)		0.95 (0.43, 2.06)		1.15 (0.53, 2.49)	
2-10 years		0.14		0.55		0.68
<3.06% (vs 0%)	1.13 (0.52, 2.46)		0.64 (0.28, 1.45)		0.73 (0.33, 1.62)	
≥3.06% (vs 0%)	1.95 (1.01, 3.77)		0.88 (0.45, 1.74)		1.11 (0.57, 2.18)	

Univariate and multivariable Cox regression analysis investigating the association between presence, pattern, location and extent of NI-LGE with the composite endpoint of CV mortality, life threatening arrhythmia or major heart failure event. The Kaplan-Meier plots cross at approximately 2 years and thus the regression analysis was separated for 0-2 years and 2-10 years. ¹Model adjusted for a subset of variables in Table 1 identified in a forward stepwise procedure (LVEF, age, diabetes, NYHA functional score, LVEDVi and history of prior revascularisation). ²Model adjusted for LVEF, age and sex.

CV = cardiovascular; LGE = late gadolinium enhancement; HR = hazard ratio. NI-LGE = non-infarct pattern LGE; LVEF = left ventricular ejection fraction.

4.5.1.5 Association between the presence and distribution of NI-LGE and all-cause mortality

Over a median follow up of 6.4 years, 142 patients (31%) died. Cumulative incidence of all-cause mortality stratified by presence or absence of NI-LGE demonstrated that patients with CAD and NI-LGE had an increased risk of death on univariable analysis (Figure 37). The Kaplan-Meier plots begin to separate at approximately 4 years and thus subsequent analysis for this endpoint was assessed before and after this timepoint. On univariable Cox regression analysis, the presence of NI-LGE was associated with an increased risk of death after 4 years (HR 2.06, 95% CI 1.24-3.45, P=0.006). After adjusting for the variables in both Model A and Model B the presence of NI-LGE was not significantly associated with death, both from 0-4 years and between 4-10 years (Table 10). On multivariable Cox regression analysis, the location, pattern or extent of NI-LGE was not associated with all-cause mortality (Table 10).

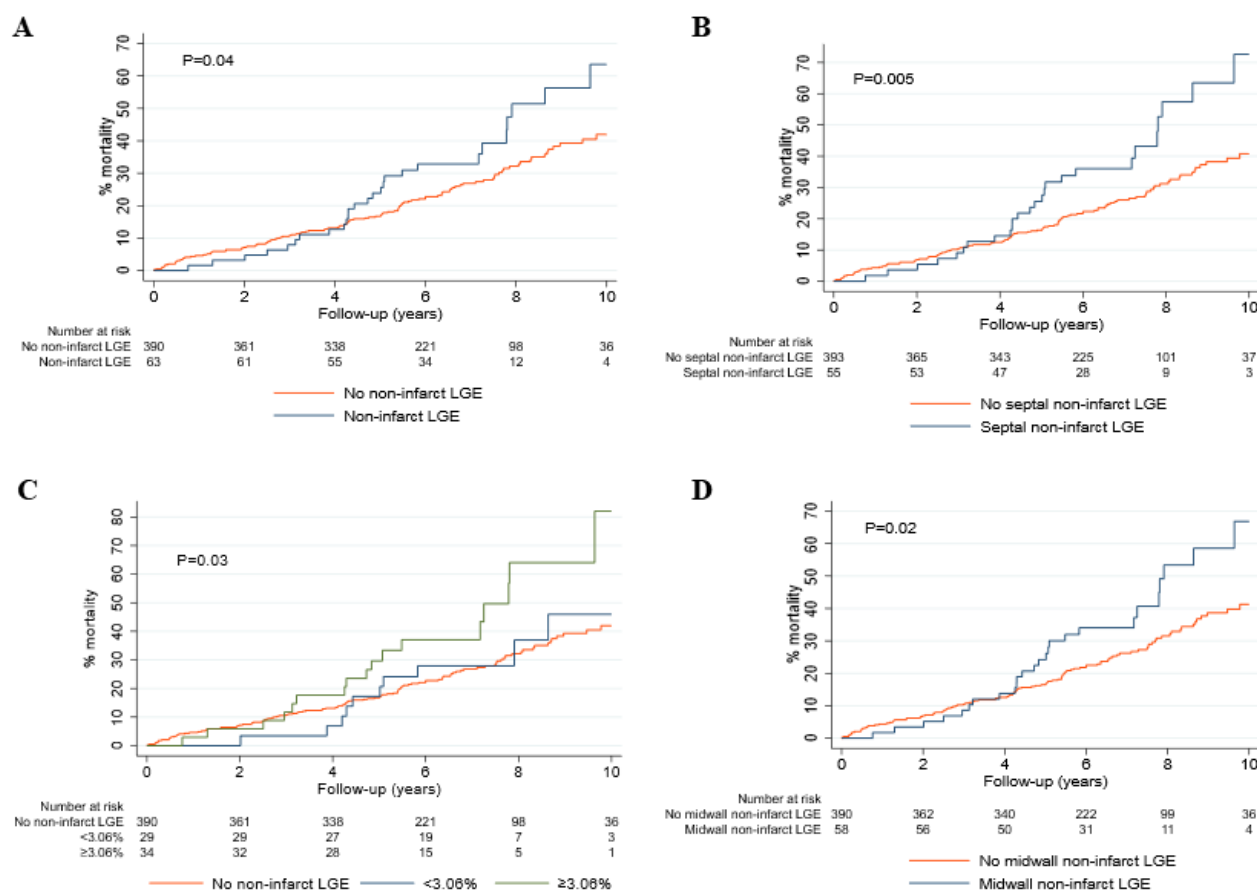


Figure 37. Kaplan-Meier plots for all-cause mortality in the London cohort, stratified by presence, pattern, location and extent of NI-LGE

A: Kaplan-Meier plots for all-cause mortality, stratified by presence or absence of NI-LGE. The plots suggesting that patients with NI-LGE had an increased risk of death.

B: Kaplan-Meier plots for all-cause mortality, stratified by presence or absence of NI-LGE within the ventricular septum. The plots suggesting that patients with septal NI-LGE had increased risk of death.

C: Kaplan-Meier plots for all-cause mortality, stratified by NI-LGE extent. The plots suggesting that patients with increased extent of NI-LGE had increased risk of death.

D: Kaplan-Meier plots for all-cause mortality, stratified by presence or absence of mid-wall NI-LGE. The plots suggesting that patients with NI-LGE had increased risk of death.

CV = cardiovascular; NI-LGE = non-infarct pattern LGE.

Table 10. Univariable and multivariate Cox regression analysis for all-cause mortality in the London cohort.

	Unadjusted		Adjusted Model 1 ¹		Adjusted Model 2 ²	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Non-infarct LGE						
0-4 years	0.94 (0.45, 1.99)	0.88	0.50 (0.23, 1.08)	0.08	0.54 (0.25, 1.18)	0.12
4-10 years	2.06 (1.24, 3.45)	0.006	1.48 (0.86, 2.53)	0.16	1.46 (0.85, 2.50)	0.17
Midwall non-infarct LGE						
0-4 years	1.07 (0.51, 2.27)	0.85	0.54 (0.25, 1.18)	0.13	0.59 (0.27, 1.29)	0.18
4-10 years	2.16 (1.28, 3.64)	0.004	1.46 (0.84, 2.55)	0.18	1.45 (0.83, 2.51)	0.19
Septal non-infarct LGE						
0-4 years	1.14 (0.54, 2.42)	0.72	0.55 (0.25, 1.19)	0.13	0.60 (0.27, 1.31)	0.2
4-10 years	2.46 (1.46, 4.16)	0.001	1.56 (0.88, 2.75)	0.12	1.57 (0.89, 2.76)	0.12
Extent of non-infarct LGE						
0-4 years:		0.48		0.18		0.22
<3.06% (vs 0%)	0.50 (0.12, 2.05)		0.31 (0.07, 1.30)		0.29 (0.07, 1.23)	
≥3.06% (vs 0%)	1.34 (0.58, 3.13)		0.63 (0.26, 1.49)		0.75 (0.32, 1.79)	
4-10 years:		0.008		0.27		0.61
<3.06% (vs 0%)	1.58 (0.76, 3.29)		1.24 (0.57, 2.68)		1.21 (0.57, 2.58)	
≥3.06% (vs 0%)	2.66 (1.40, 5.05)		1.69 (0.88, 3.26)		1.71 (0.88, 2.58)	

Univariable and multivariable Cox regression analyses investigating the association between the presence, pattern, location and extent of NI-LGE for all-cause mortality. Results stratified for 0-4 years and 4-10 years. ¹Model adjusted for the significant variables identified in a forward stepwise procedure (LVEF, age, diabetes, atrial fibrillation and NYHA functional score). ²Model adjusted for LVEF, age and sex.

NI-LGE = non-infarct pattern LGE; LVEF = left ventricular ejection fraction. LGE = late gadolinium enhancement; HR = hazard ratio.

4.5.2 The UK Biobank cohort

4.5.2.1 Cardiac phenotype of individuals with CAD and carriers of rare pathogenic variants in DCM-associated genes.

The UKB was initially used to investigate the association between rare pathogenic variants in DCM-associated genes and cardiac imaging traits in individuals with known CAD (UKB Group 1). In total, 36,470 participants had data from CMR and whole-exome sequencing. In 1,619 participants with CAD, the presence of a pathogenic rare variant (n=8 individuals, 7 with *TTN*ts) was associated with reduced biventricular ejection fraction (LVEF: $46.7 \pm 10.3\%$ vs $56.7 \pm 7.9\%$, $P=0.0003$; RVEF: $49.8 \pm 8.7\%$ vs $55.8 \pm 6.9\%$, $P=0.01$), reduced indexed stroke volumes (LV stroke volume: $39.5 \pm 9.2 \text{ ml/m}^2$ vs $47.0 \pm 9.0 \text{ ml/m}^2$, $P=0.01$; RV stroke volume: $37.8 \pm 11.1 \text{ ml/m}^2$ vs $47.5 \pm 9.7 \text{ ml/m}^2$, $P=0.003$) and increased indexed end-systolic LV volume ($47.3 \pm 18.3 \text{ ml/m}^2$ vs $36.9 \pm 13.5 \text{ ml/m}^2$, $P=0.03$) compared to individuals without a rare pathogenic variant (n=1611) (Table 11). Additionally, rare variant carrier status was associated with decreased LV global strain patterns (radial: $31 \pm 10\%$ vs $42 \pm 9\%$, $P<0.001$; circumferential: $-15 \pm 4\%$ vs $-21 \pm 4\%$, $P<0.001$; longitudinal: $-14 \pm 4\%$ vs $-18 \pm 3\%$, $P=0.005$) (Table 11). No significant difference was seen for indexed LV or RV end-diastolic volumes or indexed RV end-systolic volume (Table 11).

Table 11. Imaging traits in individuals with CAD recruited into the UKB

Variable	No rare variant (n=1611)	With rare variant (n=8)	P-value
LV ejection fraction (%)	56.7 (7.9)	46.7 (10.3)	<0.001
LVEDVi (ml/m2)	83.9 (17.7)	86.8 (23.8)	0.75
LVESVi (ml/m2)	36.9 (13.5)	47.3 (18.3)	0.03
LVSVi (ml/m2)	47.0 (9.0)	39.5 (9.2)	0.01
LV mass (g)	99.5 (22.9)	111.0 (30.2)	0.19
LAVi (ml/m2)	42.5 (14.2)	52.2 (28.7)	0.11
LA ejection fraction (%)	56.5 (11.3)	45.4 (18.4)	0.02
RV ejection fraction (%)	55.8 (6.9)	49.8 (8.7)	0.01
RVEDVi (ml/m2)	85.2 (15.6)	76.2 (17.7)	0.05
RVESVi (ml/m2)	37.7 (9.5)	38.4 (11.3)	0.99
RVSVi (ml/m2)	47.5 (9.7)	37.8 (11.1)	0.003
RAVi (ml/m2)	47.2 (15.5)	50.6 (21.9)	0.72
RA ejection fraction (%)	43.7 (9.8)	34.8 (12.5)	0.03
LV global radial strain (%)	42.1 (9.3)	31.0 (9.7)	<0.001
LV global circumferential strain (%)	-20.7 (4.2)	-15.1 (4.4)	<0.001
LV global longitudinal strain (%)	-17.7 (3.3)	-13.9 (4.1)	0.005
Cardiac imaging traits among 1,619 CAD UKB participants with and without a rare variant in a DCM-associated gene. All individuals with <i>TTN</i>tv were heterozygous. Values are mean (standard deviation). Analyses were adjusted for age, sex, systolic blood pressure and for non-indexed values, body surface area, using analysis of covariance test. LAVi = indexed left atrial volume; LVEDVi = indexed left ventricular end diastolic volume; LVESVi = indexed left ventricular end systolic volume; LVSVi = indexed left ventricular stroke volume; RAVi = indexed right atrial volume; RVEDVi = indexed right ventricular end diastolic volume; RVESVi = indexed right ventricular end systolic volume; RVSVi = indexed right ventricular stroke volume.			

4.5.2.2 *Clinical outcomes in individuals with CAD and carriers of rare pathogenic variants in DCM-associated genes*

In the overall UKB cohort (UKB Group 2), there were 31,957 individuals with a history of CAD. In this group, the presence of a pathogenic rare variant (n=183, n=170 patients with *TTN*tv) was associated with increased lifetime risk of the composite endpoint (HR 1.72, 95% CI 1.44-2.05, $P<0.001$, Figure 38A), heart failure (HR 1.94, 95% CI 1.52-2.48, $P<0.001$) and atrial fibrillation (HR 2.18, 95% CI 1.74-2.74, $P<0.001$), but not with survival (HR 1.19, 95% CI 0.87-1.61, $P=0.3$). In 26,892 participants with a known CAD diagnosis date, pathogenic rare variants (n=132 patients) were associated with time to the composite endpoint after diagnosis (HR 1.54, 95% CI 1.21-1.96, $P=0.004$) (Figure 38B), time to heart failure (HR 1.83, 95% CI 1.36-2.45, $P<0.001$) and time to atrial fibrillation (HR 1.96, 95% CI 1.47-2.62, $P<0.001$), but not with time to death (HR 1.19, 95% CI 0.84-1.66, $P=0.3$). Sensitivity analysis restricting only to carriers of *TTN*tv showed similar associations with outcomes (Figure 39).

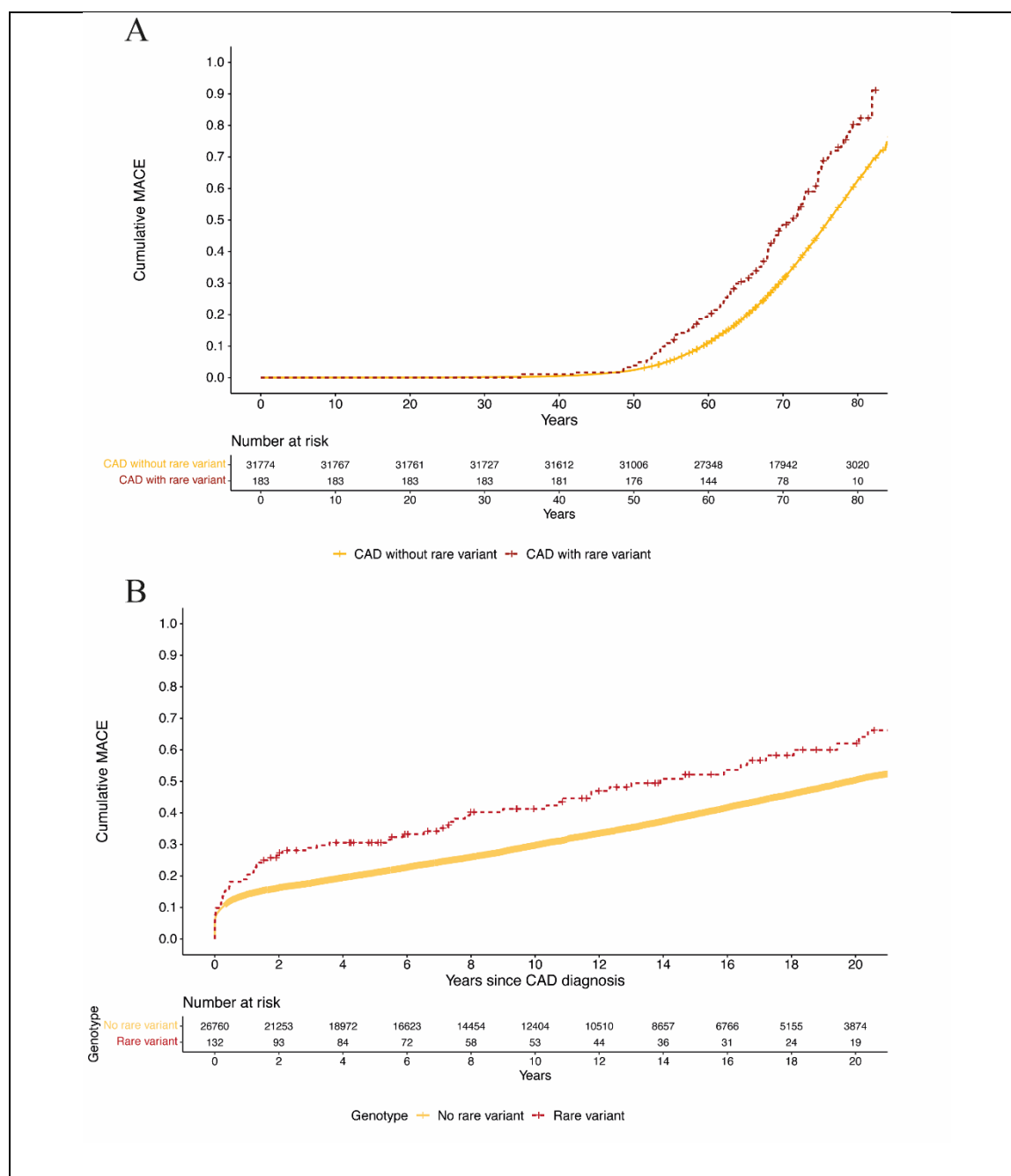
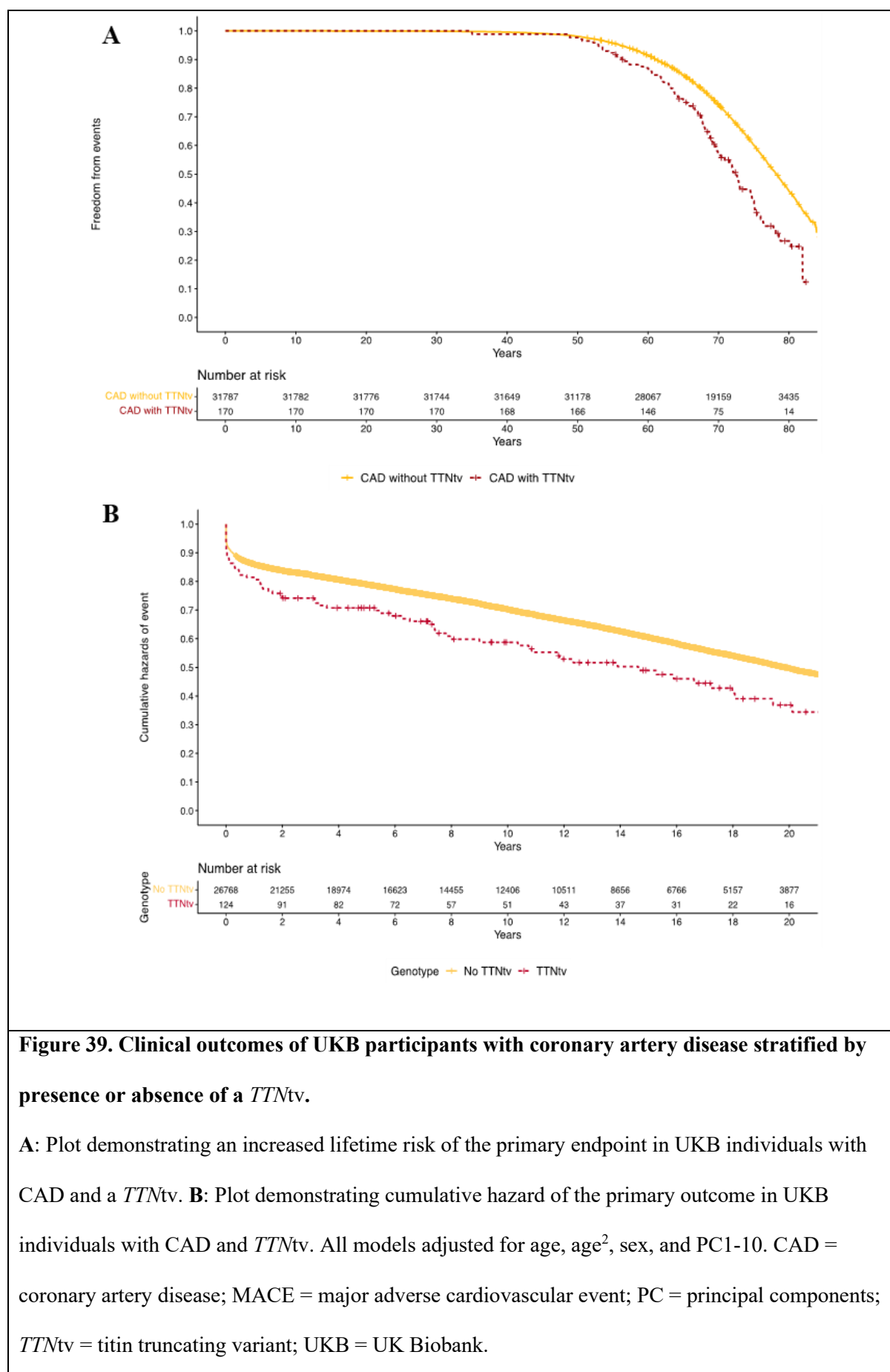


Figure 38. Clinical outcomes of UKB participants with coronary artery disease stratified by presence or absence of a rare pathogenic variant in DCM-associated gene.

A: Plot demonstrating an increased lifetime risk of the primary endpoint in UKB individuals with CAD and a rare pathogenic variant in a DCM-associated gene. **B:** Plot demonstrating cumulative hazard of the primary outcome in UKB individuals with CAD and a rare pathogenic variant in a DCM-associated gene. All models adjusted for age, age², sex, and PC1-10. CAD = coronary artery disease; MACE = major adverse cardiovascular event; PC = principal components; UKB = UK Biobank.



4.6 Discussion

In this chapter, I explored whether rare variants in DCM-associated genes and non-ischaemic patterns of myocardial fibrosis are associated with adverse remodelling and outcomes in patients with CAD. Such association may explain the broad variation in LV dimensions and function for equivalent levels of infarct scar seen in this population. My results support the notion that genetic variation contributes to LV dysfunction and clinical outcomes in CAD. I additionally report that NI-LGE is a marker of adverse remodelling in CAD but was not independently associated with cardiovascular outcomes and in the majority of patients, had no rare genetic basis.

4.6.1 *NI-LGE in patients with CAD.*

One key objective of my study was to explore the possibility that there is a rare genetic basis for non-ischaemic myocardial scar in patients with stable CAD; a putative explanation that has been offered in prior commentary (Nucifora et al., 2021). Distinguishing cause from effect regarding the aetiology of NI-LGE in patients with CAD is, however, challenging to definitively resolve. Arguably, the association of NI-LGE with a more advanced heart failure phenotype (as found in the current study) aligns to the key pathophysiological mechanism underpinning the deposition of replacement fibrosis. LGE is present in approximately 30% of DCM and is likely a marker of significant cardiac remodelling with the presence of mid-wall fibrosis associated with more marked LV dilatation and lower LVEF (Gulati et al., 2013; Halliday et al., 2019). The progressive ventricular dilatation is presumed to be an important driver of this extracellular remodelling with the resulting increased wall stress activating key fibrogenic networks including the renin-angiotensin-aldosterone system, adrenergic stimuli, inflammatory cascades and redox signalling (Frangogiannis, 2019; Halliday & Prasad, 2019). In patients with extensive CAD and adverse LV remodelling, the increase in wall stress could theoretically promote similar LGE patterns as seen in non-ischaemic pathologies. However, an alternative explanation is that patients with significant LV dilatation in the context of limited CAD or myocardial infarction burden may have a predisposing vulnerability to developing DCM. It is important to consider that both CAD and DCM are common conditions, with an estimated population prevalence of

approximately 1:60 and 1:250 respectively (Hershberger et al., 2013; Khan et al., 2020), and on this basis and that of their independent pathophysiological drivers, it is entirely biologically plausible that the co-existence of both pathologies may occur in some individuals. My results suggest that rare protein altering variants in genes associated with DCM (including *TTN*ts) are uncommon in CAD patients with NI-LGE and therefore not a predominant driver of this imaging biomarker. Importantly however, the greater LV dilatation and lower LVEF in patients with NI-LGE could not be explained by more severe CAD extent or a greater burden of myocardial infarction, raising the possibility of a second intercurrent pathological process. Future research assessing for polygenic basis for NI-LGE would be of value.

The results also demonstrate that NI-LGE is not a strong independent predictor of outcomes in patients with CAD, potentially as the risk of adverse clinical events associated with the heart failure syndrome is captured by other variables. These results deviate from prior studies. Almekhadi et al evaluated the prognostic relevance of differing patterns of myocardial fibrosis in patients with reduced LVEF of either ischaemic or non-ischaemic aetiology (Almekhadi et al., 2014). They noted the presence of mid-wall LGE in 15% of patients with CAD, demonstrating its independent association with appropriate implantable cardioverter defibrillator therapy over a median of 15 months (Almekhadi et al., 2014). More recently, Nucifora et al investigated a similar hypothesis in a prospectively investigated group of patients with stable CAD and reduced LVEF. As with my study, they demonstrated an association between this imaging biomarker and LV dilatation (Nucifora et al., 2021). Conversely however, they found a significant association between mid-wall LGE and adverse cardiovascular outcomes over median of 13 months (Nucifora et al., 2021). The divergence of these results from ours may hinge on their finding of similar LVEF values between patients with and without mid-wall LGE; although it remains unclear whether a potential difference in LVEF may have been attenuated by their inclusion criteria of only patients with LVEF<50% as it would be unusual to have LV dilatation alone without a downstream effect on LVEF. In the London cohort of unselected patients with chronic CAD, there was a ten-percentage point gap in LVEF between groups, in part driving the neutral outcome results after adjustment for the LVEF was made on multivariable analysis. Indeed, the lack of an independent

association between NI-LGE and outcome in this study supports the concept that NI-LGE is simply a marker of adverse remodelling in patients with CAD.

4.6.2 The impact of rare DCM variants on cardiac phenotype and clinical outcomes in individuals with CAD

There is an increasing body of work describing a ‘two hit’ phenomena where environmental insults modulate expression of an underlying genetic susceptibility to cardiomyopathy; notably in peripartum cardiomyopathy and chemotherapy associated cardiomyopathy where an association between *TTN*ts and lower LVEF has previously been demonstrated (Garcia-Pavia et al., 2019; Ware et al., 2016). Whether *TTN*ts impact cardiac phenotype in individuals with CAD is less clear. This could have clinical utility in patients where there is an apparent discrepancy between the extent of myocardial infarction and the degree of ventricular remodelling. My study demonstrates that rare pathogenic variants in DCM-associated genes are associated with impaired biventricular systolic function in CAD, the result driven by *TTN*ts. This suggests that *TTN*ts can modify cardiac morphology in ischaemic heart disease, already the largest cause of LV systolic dysfunction globally, and builds on prior data highlighting the broader role of titin in heart failure (Povysil et al., 2021). Finally, I demonstrate that rare pathogenic variants in DCM-associated genes predict adverse outcomes in individuals with CAD, which may inform risk stratification. These novel results align with increasing data detailing the prognostic relevance of rare variants across not only DCM cohorts (Corden et al., 2019; Haggerty et al., 2019; Tayal et al., 2017), but also in individuals without phenotypic evidence of cardiomyopathy (de Marvao et al. 2021).

4.6.3 Limitations

Despite robust inclusion criteria for CAD in the London cohort, there is the potential of referral bias for patients with diagnostic uncertainty (e.g., higher suspicion of a DCM). Importantly however, 80% of the patients in the London cohort were referred for ischaemia/viability testing with only 9% undergoing CMR for diagnostic uncertainty. The London cohort was predominantly male with only 1

female having NI-LGE. This does therefore limit the generalisability of the results for female patients. Albeit that I found no significant difference in the burden rare DCM variants in patients with NI-LGE in the London cohort, the possibility of a false negative result is raised given the sample size and larger prospective studies would be of value. Finally, the absence of a control group in the London cohort limits interpretation, notably as non-infarct patterns of scar may be related to comorbidity and aging, rather than DCM.

4.7 Conclusions

In patients with stable CAD, NI-LGE is associated with adverse cardiac remodelling but is not an independent predictor of outcome. Additionally, NI-LGE had no rare genetic basis in my study. More likely, in patients with CAD and adverse LV remodelling, the increase in wall stress promotes similar LGE patterns as seen in non-ischaemic pathologies. Finally, I demonstrate that rare pathogenic variants in DCM-associated genes impact LV remodelling and outcomes in stable CAD; suggesting the existence of a high-risk subgroup of patients with a dual pathology of DCM and ischaemic heart disease. These results highlight the need for further research exploring the prognostic relevance of genetic and imaging markers of DCM in stable CAD.

Chapter 5

- 5 Comprehensive phenotypic characterisation of late gadolinium enhancement predicts sudden cardiac death in coronary artery disease.**

5.1 Abstract

Background: Late gadolinium enhancement (LGE) cardiovascular magnetic resonance (CMR) offers the potential to non-invasively characterise the phenotypic substrate for sudden cardiac death (SCD).

Objectives: To assess the utility of infarct characterisation by CMR, including scar microstructure analysis, in predicting SCD in patients with coronary artery disease (CAD).

Methods: Patients with stable CAD were prospectively recruited into a CMR registry. LGE quantification of core infarction and the peri-infarct zone (PIZ) was performed alongside computational image analysis to extract morphological and texture scar microstructure features. The primary outcome was SCD or aborted SCD.

Results: Of 437 patients (mean age 64 years, mean left ventricular ejection fraction [LVEF] 47%) followed for a median of 6.3 years, 49 patients (11.2%) experienced the primary outcome. On multivariable Cox regression analysis, PIZ mass and core infarct mass were independently associated with the primary outcome (per gram: hazard ratio [HR] 1.07, 95% CI 1.02-1.12, $P=0.002$ and HR 1.03, 95% CI 1.01-1.05, $P=0.01$ respectively) and the addition of both parameters improved discrimination of the model (C-statistic 0.64 to 0.79). Severely reduced LVEF did not predict the primary endpoint after adjustment for scar mass. On scar microstructure analysis, the number of LGE islands in addition to scar transmural, radiality, interface area and entropy were all associated with the primary outcome after adjustment for severely reduced LVEF and raised NYHA functional class. No scar microstructure feature remained associated with the primary endpoint when PIZ mass and core infarct mass were added to the regression models.

Conclusions: Comprehensive LGE characterisation independently predicted SCD risk beyond conventional predictors used in implantable cardioverter defibrillator (ICD) implantation guidelines. These results signify the potential for a more personalised approach to determining ICD candidacy in CAD.

5.2 Introduction

Current tools to identify patients at high risk of sudden cardiac death (SCD) are limited. Specifically, left ventricular ejection fraction (LVEF) is an imprecise metric and innovative approaches are required to identify arrhythmogenic substrate beyond this measure (Myerburg & Goldberger, 2017). SCD risk prediction is of notable importance for patients with coronary artery disease (CAD) as their underlying aetiology alone confers an enhanced risk profile (Fishman et al., 2010). It is therefore crucial to evaluate the utility of novel prediction tools to identify high-risk patients within this cohort. In patients with chronic CAD, re-entrant ventricular tachycardia (VT) is the presumed mechanism underpinning the majority of SCD cases (Sramko et al., 2019). Septa of replacement extracellular fibrosis (resultant from necrosing myocytes) perforating bundles of surviving myocytes can provide an arrhythmogenic milieu capable of facilitating a re-entry circuit (de Bakker et al. 1993). These areas of heterogeneous tissue, more recently termed the ‘peri-infarct’ or ‘gray’ zone, are typically located at the transition point between viable myocardium and compact scar and are hypothesised to contain the substrate for slow conduction and fixed/functional block that initiate and maintain VT (Sramko et al., 2019). Implantable cardioverter defibrillators (ICD) can treat re-entrant arrhythmia and have been shown to protect against a high proportion of SCD (H. Greenberg et al., 2004). Decisions regarding primary prevention ICD implantation currently centre around evaluation of NYHA class alongside dichotomous assessment of LV systolic function using a LVEF cut-off of 30-35% (Al-Khatib et al., 2018; Priori et al., 2015). Typically assessed at a solitary timepoint, this fails to take into account the dynamic nature of LVEF (Goldberger et al., 2011; Myerburg & Goldberger, 2017). The ever-increasing demand on clinical services represents a parallel issue and a comprehensive assessment of SCD risk at a singular timepoint (remote from acute myocardial infarction) is desirable. Less dynamic features which characterise the arrhythmogenic substrate therefore offer promise.

Late gadolinium enhancement (LGE) cardiovascular magnetic resonance (CMR) non-invasively identifies dense myocardial fibrosis with high spatial resolution and has good histological correlation in CAD models (Kim et al., 1999). Additionally, quantification of the peri-infarct zone (PIZ) by LGE-

CMR has been shown to associate with all-cause mortality (Hagbayan et al., 2019; Kwon et al., 2014; Watanabe et al., 2014; Yan et al., 2006) and ICD therapy (Roes et al., 2009) with more recent studies also evaluating the role of scar microstructure (e.g. entropy) in ventricular arrhythmia (Androulakis et al., 2019). There remains, however, a paucity of data describing the application of complex scar analysis to predict SCD in prospectively recruited cohorts with a broad range of LVEF. Indeed, the utility of non-invasive imaging to predict SCD remains a key research need highlighted by both the American Heart Association and European Society of Cardiology (Al-Khatib et al., 2018; Priori et al., 2015). In this chapter, LGE quantification was performed, in combination with bespoke computational analysis of scar microstructure features, to provide novel mechanistic interrogation into the drivers of SCD in prospectively investigated patients with CAD.

5.3 Hypotheses

1. Core infarct mass and peri-infarct zone mass predict sudden cardiac death beyond LVEF calculation in prospectively recruited patients with stable CAD.
2. Morphological and texture-based scar microstructure features have independent and incremental association with SCD beyond LGE mass.

5.4 Methods

5.4.1 Study design

Patients referred to our centre for evaluation of ischaemic heart disease with LGE-CMR were recruited into a registry between August 2009 and January 2016. These patients were referred from local cardiology clinics in addition to a broad network of specialist and non-specialist hospitals. The registry complied with the Declaration of Helsinki and the National Research Ethics Service approved the protocol. All patients provided informed written consent. CMR was undertaken on a 1.5 Tesla scanner (Sonata/Avanto, Siemens) on the day of recruitment or, in a minority of patients, at a prior date during disease work up. In brief, the LGE datasets were acquired following intravenous injection of gadopentetate dimeglumine or gadobutrol (0.05mmol/Kg). An inversion recovery gradient echo

sequence was subsequently undertaken at 10mins, typically in two phase encoding directions with parameters as described previously (Assomull et al., 2006). The slice thickness was 8mm with a 2mm gap, resulting in an in-plane resolution of $\sim 2.2\text{mm} \times \sim 1.6\text{mm}$. Inversion times were optimised to ensure adequate nulling of normal myocardium (characteristically between 280ms and 400ms).

The inclusion criteria for the study was: a) severe epicardial CAD; b) prior coronary revascularisation; or c) documented history of prior myocardial infarction (confirmed on CMR). Severe epicardial CAD was defined as $\geq 75\%$ stenosis in the left main stem/proximal left anterior descending artery or $\geq 75\%$ in 2 epicardial coronary arteries. Exclusion criteria were Class I indication for a secondary prevention ICD, myocardial infarction (MI) within 40 days prior to CMR, severe primary valvular disease, previous valvular intervention, or a primary diagnosis of dilated cardiomyopathy, active myocarditis, cardiac sarcoidosis, hypertrophic cardiomyopathy or infiltrative cardiomyopathy. The 40-day post MI window was chosen in view of the impact of acute myocyte necrosis on LGE and international guidelines mandating this period of convalescence before consideration of primary prevention ICD therapy (Al-Khatib et al., 2018). No patients had an ICD implanted prior to CMR.

5.4.2 CMR analysis for quantification of the core infarct mass and PIZ mass

LGE quantification of the core infarct and adjacent PIZ was performed by a Level 3 accredited CMR operator (Dr Suzan Hatipoglu) blinded to the clinical outcomes. Analysis was undertaken on CVI42 (Circle Cardiovascular Imaging Inc, Calgary, Canada) using the full width at half maximum method (FWHM). Magnitude reconstructions of the gradient echo sequences were analysed. Initial quality control of each LGE study was undertaken and patients with suboptimal scans were excluded.

Epicardial and endocardial LV contours were drawn from the short-axis LGE slices and a region of interest (ROI) was created within an area of infarct pattern hyperenhancement. Core infarct was then classified as any LV region with a signal intensity (SI) of $\geq 50\%$ of the maximal SI found within the reference ROI. The PIZ was defined as LV myocardium (adjacent to core infarct) with SI between 35%-50% of the maximally detected SI within the reference ROI. Exclusion zones were applied to disregard regions that were erroneously identified as LGE. In the slices where no overt LGE was present, core mass and PIZ mass were imputed as 0g.

5.4.3 CMR analysis of the scar microstructure

The LGE slices were reviewed (Dr Richard Jones, Mr Hassan Syed Zaidi) and contours optimised for computational analysis where necessary (e.g. to ensure the contours were not interrupted at the LV outflow tract). Harnessing both the contoured LGE images and corresponding slice masks, we extracted morphological and texture scar microstructure features using similar methodology as previously described by our group (Balaban et al., 2019, 2021). The calculated scar microstructure features were i) transmural; ii) radiality; iii) number of LGE components; iv) interface area; v) entropy (see Table 12 for list of feature descriptions). Specifically, interface area describes the extent of the interface/border between either core infarct or the PIZ and adjacent tissue or total scar (core infarct + PIZ) and adjacent myocardium, in both cases excluding the bounding epicardial/endocardial surfaces. Entropy represents the degree of complexity within an area of interest. In LGE analysis, entropy quantifies the heterogeneity in SI across a scar region; a uniform area of scar would have minimal difference in SI across the region, with a relatively low entropy value. A heterogenous region of scar would have a wider SI range, resulting in a higher entropy value.

Each scar microstructure feature was computed separately for: a) core infarct; b) PIZ; c) total scar (i.e. core infarct + PIZ). The individual slice results were then aggregated across the short-axis stack to better represent LGE topology throughout the LV, where applicable.

Table 12: Scar microstructure features.

Scar feature	Feature description
Transmurality*	The extent of spread of LGE emanating outwards from the endocardium to epicardium, calculated using a ray tracing method.
Radiality*	Quantification of the circumferential spread of LGE in relation to the centre of the LV blood pool.
Number of components*	The number of distinct LGE components in a slice (e.g. number of islands of core or PIZ scar).
Interface area*	The extent (i.e. area) of the borderzone between myocardium and adjacent LGE.
Entropy [‡]	The level of disorder or heterogeneity within the LGE. Calculated by applying standard Shannon entropy(Androulakis et al., 2019)
The 5 scar microstructure features that were extracted from the LGE images. *morphological feature, [‡]texture feature. LGE = late gadolinium enhancement; LV = left ventricle; PIZ = peri-infarct zone.	

5.4.4 Follow up data collection and clinical endpoints

Patients were followed up using health questionnaires alongside primary care and hospital documentation. ICD reports, death certificates and post-mortem results were requested as appropriate. Survival status was confirmed via the UK NHS Digital service to ensure that no deaths were omitted. The duration of follow-up was determined from the date of CMR until an endpoint was confirmed or until the most recent patient contact date. Event times were calculated from the date of the preceding CMR up until a maximum of 10 years. All clinical outcomes were adjudicated by an independent panel of experienced cardiologists blinded to the LGE data. The a priori primary endpoint was a composite of SCD or aborted SCD. SCD was defined as a death that occurred unexpectedly, including scenarios where symptom duration was ≤ 1 hr, following an identified arrhythmia/unsuccessful resuscitation or in circumstances where the patient was witnessed alive ≤ 24 hr prior to death and without another identifiable cause of death (Hicks et al. 2015). Aborted SCD was defined as

appropriate ICD shock for a ventricular tachyarrhythmia, effective resuscitation following ventricular fibrillation or haemodynamically unstable VT requiring electrical cardioversion (Buxton et al., 2006). The prespecified secondary endpoints included: i) major heart failure (HF) composite of HF hospitalisation (admission to hospital of ≥ 24 hr/encompassing 1 calendar day requiring initiation or escalation of HF therapies), HF death (in the context of progressive clinical features of HF), or cardiac transplantation/left ventricular assist device insertion, ii) all-cause mortality and iii) SCD or aborted SCD in patients with a LVEF $\geq 35\%$.

5.4.5 Statistical analysis

Baseline characteristics were summarised in the total cohort as frequency (%) for categorical variables and mean (standard deviation, σ) or median (interquartile range, IQR) where appropriate for continuous variables. Kaplan-Meier curves were plotted to describe the cumulative incidence of the primary outcome by tertiles of PIZ mass and core infarct mass over follow-up, compared using the logrank test.

To investigate the utility of LGE quantification in the prediction of the primary outcome, univariable and multivariable Cox regression models were generated. The primary multivariable model was adjusted using binary cut-offs of LVEF $< 35\%$ and NYHA class > 1 to align with current clinical guidelines for ICD implantation (Model A) (Priori et al., 2015). Additionally, competing risk analysis was performed using Fine-Gray subdistribution hazard modelling. A secondary Cox regression model was also fitted using baseline covariates associated with the primary outcome (Model B). To select the covariables in Model B, a forward stepwise procedure was applied using a subset of variables in Table 2 with $p < 0.10$ as the criterion for inclusion, forcing in known predictors of the outcome (age, sex and LVEF). To prevent creating an overly complex model, not all variables that were associated with the primary endpoint on univariable analysis were used in the multivariable model (e.g., indexed LV mass and RVEF). In all models, core infarct mass and PIZ mass were subsequently added to assert whether either metric was independently associated with the primary outcome. Model performance was assessed using Harrel's C-statistic. The secondary endpoints were assessed using multivariable

Cox regression modelling, selecting variables using a forward stepwise approach (as described in Model B). Subgroup analysis of patients with LVEF $\geq 35\%$ was solely adjusted for age, sex and LVEF. Forest plots were generated using core infarct mass and PIZ mass per 10 grams to aid visual representation. The scar results are presented in grams reflecting the utility of absolute scar mass to predict SCD highlighted in a large, but retrospective, CMR study (Zegard et al., 2021). In addition, sensitivity analyses were conducted to: i) Assess the association between relative scar mass (expressed as a percentage of LV mass) and the primary outcome; ii) Evaluate the impact of excluding patients without infarct pattern LGE on the primary outcome; iii) Evaluate the association between total fibrosis mass (sum of core infarct mass and PIZ mass) and the primary endpoint; iv) Assess the association between scar mass and the primary endpoint after adjusting for future MI or subsequent coronary revascularisation as time-varying covariates.

To assess the association between the scar microstructure features and the primary outcome, univariable and multivariable Cox regression models were fitted, the latter adjusted for LVEF $< 35\%$ and NYHA class > 1 . Core infarct mass and PIZ mass were then added to each model to assess whether any microstructure feature was independently associated with the primary outcome following inclusion of the traditional LGE quantification results.

All statistical analysis was performed on Stata version 17 (StatCorp) and Python v3.7.4.

5.5 Results

At baseline, 734 patients were assessed for eligibility with the final cohort consisting of 437 patients (Figure 40).

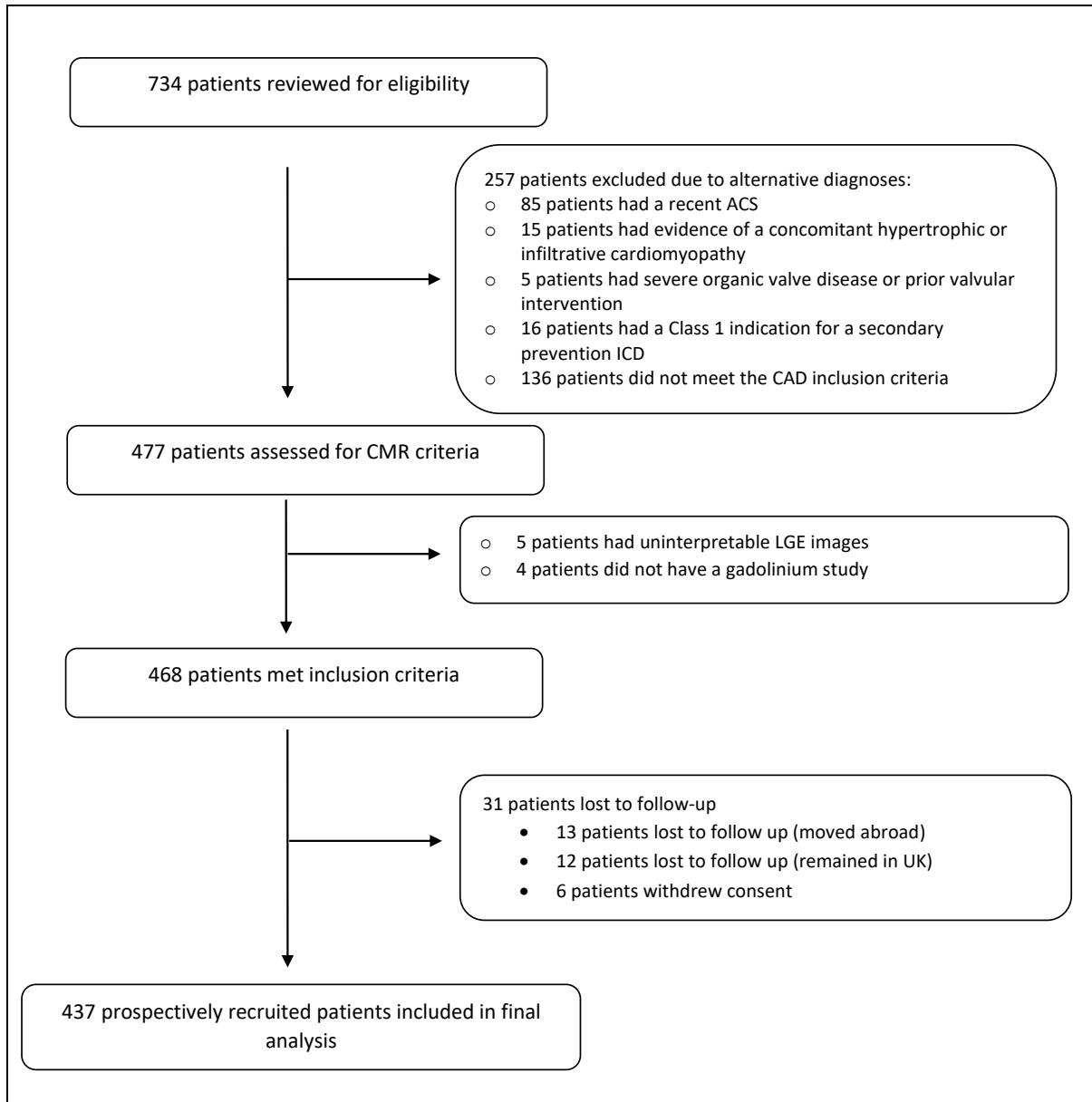


Figure 40: Study cohort.

Flow diagram describing the curation of the study cohort. ACS = acute coronary syndrome; CAD = coronary artery disease; CMR = cardiovascular magnetic resonance; ICD = implantable cardioverter defibrillator; LGE = late gadolinium enhancement; UK = United Kingdom.

The median interval between CMR and recruitment was 0 days (IQR 0-0 days), mean age was 64±9.9 years, mean LVEF 47%±16.8% and 95% of patients had significant CAD or previous coronary revascularisation. All 25 (5%) patients without documented significant CAD/previous coronary revascularisation had a history of prior myocardial infarction confirmed on CMR. Overall, 412 (94%) patients underwent CMR as an ambulatory outpatient visit, with the assessment of cardiac function and scar burden as the primary indication in 364 (83%) patients (Table 13).

Table 13. Scan indication	
Scan indication	Patients
Assessment of cardiac function and scar burden, n	364 (83%)
Diagnostic uncertainty, n	42 (10%)
Prior to device insertion, n	18 (4%)
Other (e.g., valve assessment), n	13 (3%)

During the study, 113 patients underwent ICD implantation (102 patients for primary prevention and 11 patients who developed a secondary indication during follow-up). Patients were followed up for a median of 6.3 years (IQR: 5.0-7.9 years). Baseline characteristics are described in Table 14 and Table 15. Extended baseline data is detailed in Appendix Table 19 and Appendix Table 20.

Table 14: Baseline characteristics, stratified by median PIZ mass

Variable	All patients	PIZ<8.8g	PIZ≥8.8g	P-value
Demographics				
Age (years)	64.4 (9.9)	65.2 (9.5)	63.6 (10.2)	0.10
Female	61 (14.0)	41 (18.8)	20 (9.1)	0.004
Caucasian	357 (81.7)	178 (81.7)	179 (81.7)	0.98
BMI (kg/m2)	27.8 (5.0)	27.6 (4.7)	28.0 (5.2)	0.34
Heart rate (bpm)	69 (13.3)	69 (14.1)	69 (12.6)	0.92
SBP (mmHg)	127 (19.3)	131 (19.4)	123 (18.3)	<0.001
DBP (mmHg)	73 (11.7)	75 (11.5)	71 (11.7)	<0.001
Significant CAD*	412 (95.4)	198 (91.2)	214 (99.5)	<0.001
CAD type				0.15
<i>Single vessel</i>	131 (31.8)	72 (36.4)	59 (27.6)	
<i>2 vessels</i>	117 (28.4)	51 (25.8)	66 (30.8)	
<i>3 vessels</i>	164 (39.8)	75 (37.9)	89 (41.6)	
History of MI	316 (72.3)	141 (64.7)	175 (79.9)	<0.001
Prior PCI	225 (51.5)	119 (54.6)	106 (48.4)	0.20
Prior CABG	121 (27.7)	58 (26.6)	63 (28.8)	0.61
Hypertension	231 (52.9)	116 (53.2)	115 (52.5)	0.88
Diabetes mellitus	128 (29.3)	59 (27.1)	69 (31.5)	0.31
Documented hypercholesterolemia	356 (81.5)	171 (78.4)	185 (84.5)	0.10
Documented family history of premature CAD	95 (21.7)	50 (22.9)	45 (20.5)	0.54
Smoking status				0.01
<i>Yes</i>	45 (10.3)	19 (8.8)	26 (11.9)	
<i>Ex-smoker</i>	242 (55.5)	109 (50.2)	133 (60.7)	
<i>No</i>	149 (34.2)	89 (41.0)	60 (27.4)	
Baseline AF	73 (16.7)	33 (15.1)	40 (18.3)	0.38
NYHA functional class				0.17
<i>I</i>	145 (33.3)	81 (37.3)	64 (29.4)	
<i>II</i>	197 (45.3)	95 (43.8)	102 (46.8)	
<i>III or IV</i>	93 (21.4)	41 (18.9)	52 (23.9)	
Medications				
Antithrombotic therapy	420 (96.1)	210 (96.3)	210 (95.9)	0.81

Beta-blocker	338 (77.3)	158 (72.5)	180 (82.2)	0.02
ACEi/ARB	368 (84.2)	168 (77.1)	200 (91.3)	<0.001
Lipid-lowering drug	386 (88.3)	188 (86.2)	198 (90.4)	0.17
CMR volumetric measurements				
LVEF (%)	47 (16.8)	55 (16.0)	39 (13.3)	<0.001
Indexed LV mass (g/m ²)	79 (24.6)	72 (20.9)	86 (26.0)	<0.001
LVEDVi (ml/m ²)	107 (40.8)	89 (28.9)	124 (43.5)	<0.001
RVEF (%)	58 (12.7)	59 (11.2)	57 (13.9)	0.03
CMR LGE characteristics				
Presence of infarct pattern LGE	378 (86.5)	159 (72.9)	219 (100.0)	<0.001
Predominant territory				0.010
<i>Anterior</i>	172 (45.5)	60 (37.7)	112 (51.1)	
<i>Lateral</i>	54 (14.3)	31 (19.5)	23 (10.5)	
<i>Inferior</i>	152 (40.2)	68 (42.8)	84 (38.4)	
PIZ mass (g), median (IQR)	8.8 (4.2-14.4)	NA	NA	NA
Infarct core mass (g), median (IQR)	17.6 (6.5-30.2)	6.4 (0.0-12.8)	29.0 (20.8-39.6)	<0.001

ACEi = angiotensin-converting enzyme inhibitor; AF = atrial fibrillation; ARB = angiotensin II receptor blocker; BMI = body mass index; CABG = coronary artery bypass grafting; CAD = coronary artery disease; CMR = cardiovascular magnetic resonance; DBP = diastolic blood pressure; IQR = interquartile range; LGE = late gadolinium enhancement; LVEDVi = indexed left ventricular end-diastolic volume; LVEF = left ventricular ejection fraction; MI = myocardial infarction; NA = not applicable; NYHA = New York Heart Association; RVEDVi = indexed right ventricular end-systolic volume; RVEF = right ventricular ejection fraction; PIZ = peri-infarct zone; PCI = percutaneous coronary intervention; SBP = systolic blood pressure. *Patients with evidence of severe CAD or a history of prior coronary revascularisation.

Continuous variables are reported as mean (standard deviation) or median (interquartile range).

Categorical variables are reported as N (%). Characteristics were compared between patients with a PIZ mass <median versus those with a PIZ mass ≥median using 2-sample t-tests or Mann-Whitney U tests for continuous variables and χ^2 test or Fisher's exact tests for categorical variables.

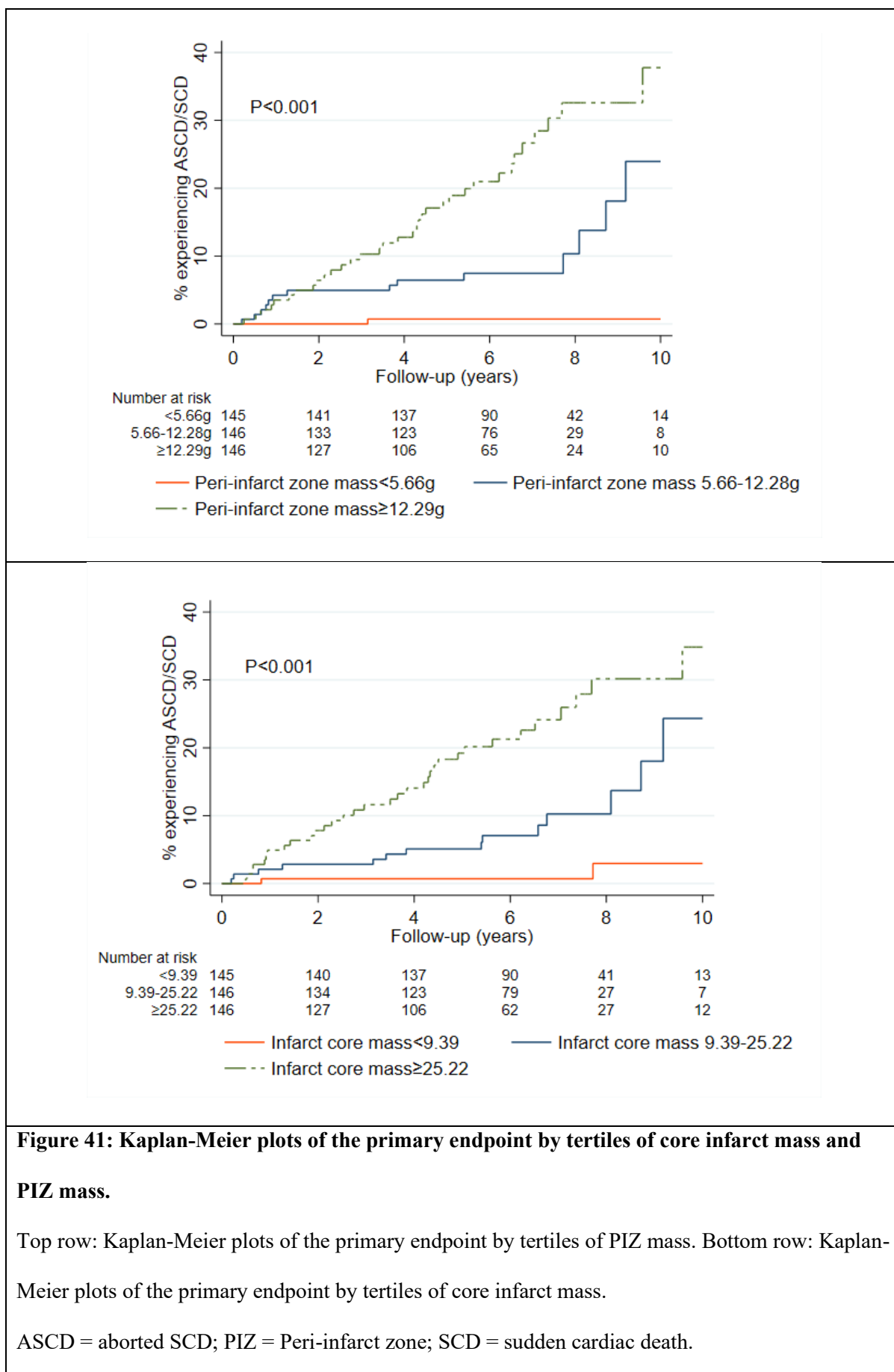
Table 15: Descriptive statistics for the scar microstructure features, stratified by the primary outcome.				
Scar feature	All Patients	No SCD/aSCD	SCD/aSCD	P-value
Interface area – total LGE interface area across all slices				
Core infarct interface area	92.0 (85.8 - 98.2)	84.5 (78.3 - 90.7)	151.4 (132.1 - 170.7)	<0.001
PIZ interface area	139.9 (130.0 - 149.8)	129.6 (119.7 - 139.6)	220.8 (187.2 - 254.5)	<0.001
Core infarct & PIZ combined interface area	107.0 (99.8 - 114.2)	99.6 (92.3 - 106.8)	166.1 (143.7 - 188.5)	<0.001
Entropy – mean LGE entropy across all slices				
Core infarct entropy	2.3 (2.2 - 2.4)	2.2 (2.1 - 2.3)	2.9 (2.8 - 3.1)	<0.001
PIZ entropy	1.6 (1.5 - 1.7)	1.5 (1.5 - 1.6)	2.0 (1.9 - 2.1)	<0.001
Core infarct & PIZ combined entropy	2.5 (2.4 - 2.6)	2.4 (2.3 - 2.5)	3.2 (3.0 - 3.3)	<0.001
Transmurality – mean transmuralty of LGE across all slices				
Core infarct transmuralty	0.3 (0.3 - 0.4)	0.3 (0.3 - 0.3)	0.5 (0.4 - 0.5)	<0.001
PIZ transmuralty	0.2 (0.2 - 0.2)	0.2 (0.2 - 0.2)	0.3 (0.2 - 0.3)	<0.001
Core infarct & PIZ combined transmuralty	0.4 (0.4 - 0.4)	0.4 (0.4 - 0.4)	0.6 (0.5 - 0.6)	<0.001
Number of components – total number of LGE components across all slices				
Number of core infarct components	4.0 (3.7 - 4.3)	3.8 (3.5 - 4.1)	5.6 (4.8 - 6.4)	<0.001
Number of PIZ components	12.8 (12.0 - 13.7)	12.0 (11.1 - 12.9)	19.3 (16.8 - 21.8)	<0.001
Number of core infarct & PIZ combined components	4.7 (4.4 - 5.0)	4.5 (4.2 - 4.8)	6.5 (5.5 - 7.6)	<0.001
Radiality – mean radiality of LGE across all slices				
Core infarct radiality	0.2 (0.2 - 0.2)	0.2 (0.2 - 0.2)	0.3 (0.2 - 0.3)	<0.001
PIZ radiality	0.3 (0.2 - 0.3)	0.2 (0.2 - 0.3)	0.4 (0.3 - 0.4)	<0.001
Core infarct & PIZ combined radiality	0.2 (0.2 - 0.3)	0.2 (0.2 - 0.2)	0.3 (0.3 - 0.4)	<0.001
Mean value (95% confidence interval) for the scar microstructure features; all patients and stratified for the primary outcome. PIZ = peri-infarct zone.				

5.5.1 Primary outcome

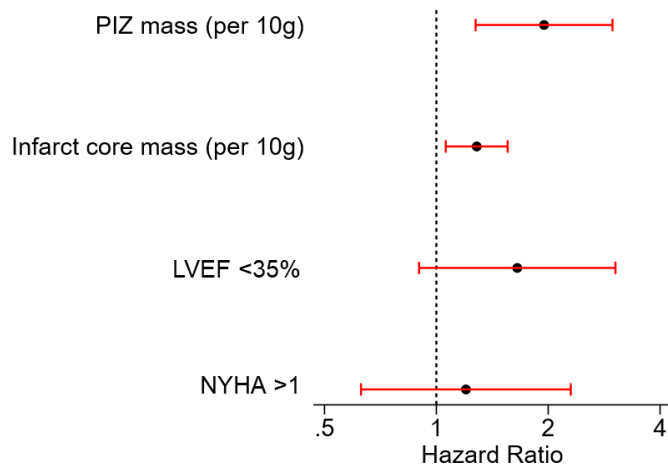
5.5.1.1 Composite of SCD or aborted SCD: Core infarct mass and PIZ mass

At 10 years follow up, 49 patients (11.2%) had experienced the primary outcome (20 patients experiencing SCD and 29 patients experiencing aborted SCD). Autopsy results were obtained for 12 of the deaths assigned as SCD. In cases where autopsy data was not available, SCD was diagnosed by the independent panel of cardiologists using standard endpoint definitions (Hicks et al. 2015).

Cumulative incidence of the primary outcome by tertiles of PIZ mass suggest that patients in higher tertiles had an increased risk of the primary outcome (10-year risk 0.7%, 24.0% and 37.8% for patients with PIZ mass <5.66g, 5.66-12.28g and $\geq$ 12.29g respectively, $P<0.001$, Figure 41). Similarly, patients in the higher tertiles of core mass had an increased risk of the primary outcome (10-year risk 3.7%, 24.0% and 34.6% for patients with core mass <9.39, 9.39-25.21g and $\geq$ 25.22g respectively, $P<0.001$, Figure 41). On univariable analysis, an increase in PIZ mass and core infarct mass was significantly associated with an increased risk of the primary outcome (per gram: HR 1.12, 95% CI 1.09-1.15, $P<0.001$ and HR 1.05, 95% CI 1.04-1.06, $P<0.001$ respectively). Additionally, a decrease in LVEF was significantly associated with an increased risk of the primary outcome on univariable analysis (per %: HR 0.96, 95% CI 0.94-0.97, $P<0.001$).



After adjustment for the variables in Model A (LVEF <35% and NYHA Class >1), both PIZ mass and core infarct mass remained independently associated with the primary outcome (per gram: HR 1.07, 95% CI 1.02-1.12, P=0.002 and HR 1.03, 95% CI 1.01-1.05, P=0.01 respectively, Figure 42) and improved the ability of the model to predict the primary outcome (C-statistic from 0.64 to 0.79). Severely impaired LVEF was not significantly associated with the primary endpoint on multivariable analysis (HR 1.65, 95% CI 0.90-3.03, P=0.11, Figure 42). Indeed, removing LVEF from the final model resulted in no change in the C-statistic (0.79 to 0.79). Additionally, PIZ mass and core infarct mass remained independently associated with the primary endpoint after accounting for the competing risk of non-sudden death (per gram: subdistribution HR 1.07, 95% CI 1.03-1.12, P=0.001 and subdistribution HR 1.03, 95% CI 1.01-1.04, P=0.003 respectively, Figure 43). Model performance was similar with either the addition of core infarct mass or PIZ mass alone or the simultaneous addition of both scar metrics (Figure 42). On sensitivity analyses, the estimated HRs were similar after adjusting for LVEF <35%, NYHA>1 and: i) relative PIZ mass and core infarct mass expressed as a percentage of LV mass (per %: HR 1.11, 95% CI 1.06-1.17, P<0.001 and HR 1.05, 95% CI 1.02-1.08, P<0.001 respectively); ii) PIZ mass and core infarct mass when only the 378 (87%) patients with infarct pattern LGE were included (per gram: HR 1.06, 95% CI 1.02-1.11, P=0.005 and HR 1.02, 95% CI 1.00-1.04, P=0.02 respectively); iii) total fibrosis mass as a sum of core infarct mass and the PIZ mass (per gram: HR 1.04, 95% CI 1.03-1.05, P<0.001); iv) PIZ mass and core infarct mass after adjusting for subsequent MI (n=32) as a time-varying covariate (per gram: HR 1.07, 95% CI 1.03-1.12, P=0.002 and HR 1.03, 95% CI 1.01-1.05, P=0.009 respectively); v) PIZ mass and core infarct mass after adjusting for future revascularisation (n=161) as a time-updated covariate (per gram: HR 1.07, 95% CI 1.02-1.11, P=0.002 and HR 1.03, 95% CI 1.01-1.05, P=0.009 respectively).



Variable	HR (95% CI)	P-value
LVEF<35%	3.09 (1.75, 5.45)	<0.001
NYHA >1	1.33 (0.70, 2.52)	0.39
Harrell's C-statistic = 0.64		

Variable	HR (95% CI)	P-value
Infarct core mass (g)	1.04 (1.03, 1.06)	<0.001
LVEF<35%	1.98 (1.10, 3.58)	0.02
NYHA >1	1.14 (0.60, 2.19)	0.69
Harrell's C-statistic = 0.79		

Variable	HR (95% CI)	P-value
PIZ mass (g)	1.11 (1.07, 1.14)	<0.001
LVEF<35%	1.70 (0.92, 3.13)	0.09
NYHA >1	1.21 (0.63, 2.32)	0.57
Harrell's C-statistic = 0.78		

Variable	HR (95% CI)	P-value
PIZ mass (g)*	1.07 (1.02, 1.12)	0.002
Infarct core mass (g)*	1.03 (1.01, 1.05)	0.01
LVEF<35%	1.65 (0.90, 3.03)	0.11
NYHA >1	1.20 (0.63, 2.30)	0.58
Harrell's C-statistic = 0.79		

Figure 42: Multivariable Cox regression analysis (Model A) for the primary endpoint with subsequent addition of the LGE quantification data.

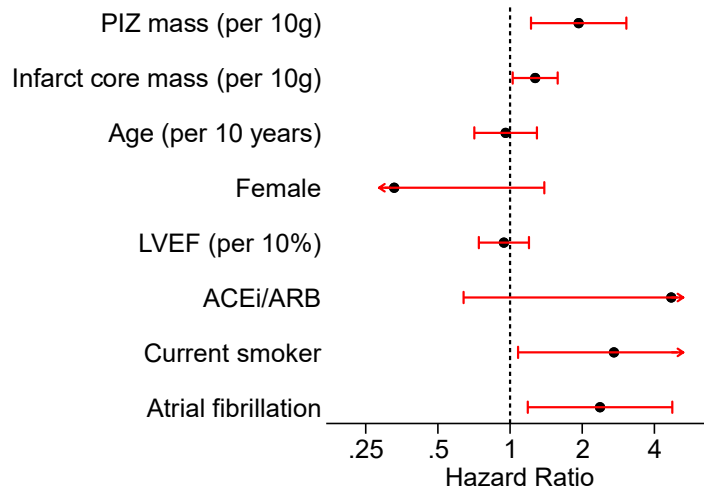
Top row: Forest plot of the final multivariable Cox model using LVEF <35%, NYHA class >1, core infarct mass and PIZ mass. Data presented using LGE results per 10grams. Middle and bottom rows: Multivariable Cox models using binary cut-offs of LVEF <35% and NYHA class >1 with subsequent addition of the LGE quantification data. *Results per 10g: PIZ mass HR 1.95, 95% CI 1.28-2.98, P=0.002 and core infarct mass HR 1.28, 95% CI 1.06-1.56, P=0.01. CI = confidence interval; HR = hazard ratio; LVEF = left ventricular ejection fraction; NYHA = New York Heart Association; PIZ = peri-infarct zone.

Variable	sHR (95% CI)	P-value	Variable	sHR (95% CI)	P-value
LVEF<35%	2.66 (1.51, 4.69)	0.001	PIZ mass (g)	1.07 (1.03, 1.12)	0.001
NYHA >1	1.20 (0.64, 2.27)	0.57	Infarct core mass (g)	1.03 (1.01, 1.04)	0.003
			LVEF<35%	1.42 (0.80, 2.53)	0.23
			NYHA >1	1.09 (0.56, 2.11)	0.80

Figure 43: Competing risk analysis.

Competing risk survival analysis using Fine-Gray subdistribution Hazard modelling; SCD/aborted SCD versus non-sudden death. CI = confidence interval; sHR = subdistribution hazard ratio; LVEF = left ventricular ejection fraction; NYHA = New York Heart Association; PIZ = peri-infarct zone.

Using Model B, PIZ mass and core infarct mass remained independently associated with the primary outcome after adjusting for baseline covariates (per gram: HR 1.07, 95% CI 1.02-1.12, P=0.005 and HR 1.02, 95% CI 1.00-1.05, P=0.03 respectively, Figure 44) and improved the discrimination ability of the model to predict the primary outcome (C-statistic 0.76 to 0.82). LVEF was not associated with the primary endpoint on multivariable analysis (per %: HR 0.99, 95% CI 0.97-1.02, P=0.63, Figure 44). Model performance was similar with either the addition of core infarct mass or PIZ mass alone or the simultaneous addition of both scar metrics (Figure 44). A summary of univariable and multivariable Cox regression analyses is detailed in Appendix Table 21.



Variable	HR (95% CI)	P-value
Age (per 10 years)	0.76 (0.57, 1.02)	0.07
Female	0.31 (0.07, 1.28)	0.11
LVEF (%)	0.96 (0.95, 0.98)	<0.001
ACE-i/ARB	6.32 (0.87, 46.15)	0.07
Smoking status		0.04
Non-smoker	Reference group	
Ex-smoker	1.57 (0.77, 3.17)	
Current smoker	2.61 (1.05, 6.44)	
Atrial fibrillation	1.98 (1.01, 3.90)	0.05
Harrell's C-statistic = 0.76		

Variable	HR (95% CI)	P-value
Infarct core mass (g)	1.04 (1.02, 1.06)	<0.001
Age (per 10 years)	0.89 (0.66, 1.21)	0.46
Female	0.37 (0.09, 1.53)	0.17
LVEF (%)	0.99 (0.96, 1.01)	0.23
ACE-i/ARB	5.24 (0.71, 38.47)	0.10
Smoking status		0.06
Non-smoker	Reference group	
Ex-smoker	1.54 (0.77, 3.12)	
Current smoker	2.36 (0.94, 5.92)	
Atrial fibrillation	2.51 (1.25, 5.03)	0.009
Harrell's C-statistic = 0.81		

Variable	HR (95% CI)	P-value
PIZ mass (g)	1.10 (1.06, 1.14)	<0.001
Age (per 10 years)	0.94 (0.70, 1.27)	0.69
Female	0.25 (0.06, 1.08)	0.06
LVEF (%)	0.99 (0.97, 1.01)	0.33
ACE-i/ARB	5.05 (0.69, 37.05)	0.11
Smoking status		0.06
Non-smoker	Reference group	
Ex-smoker	1.20 (0.59, 2.46)	
Current smoker	2.75 (1.10, 6.88)	
Atrial fibrillation	2.04 (1.04, 3.99)	0.04
Harrell's C-statistic = 0.81		

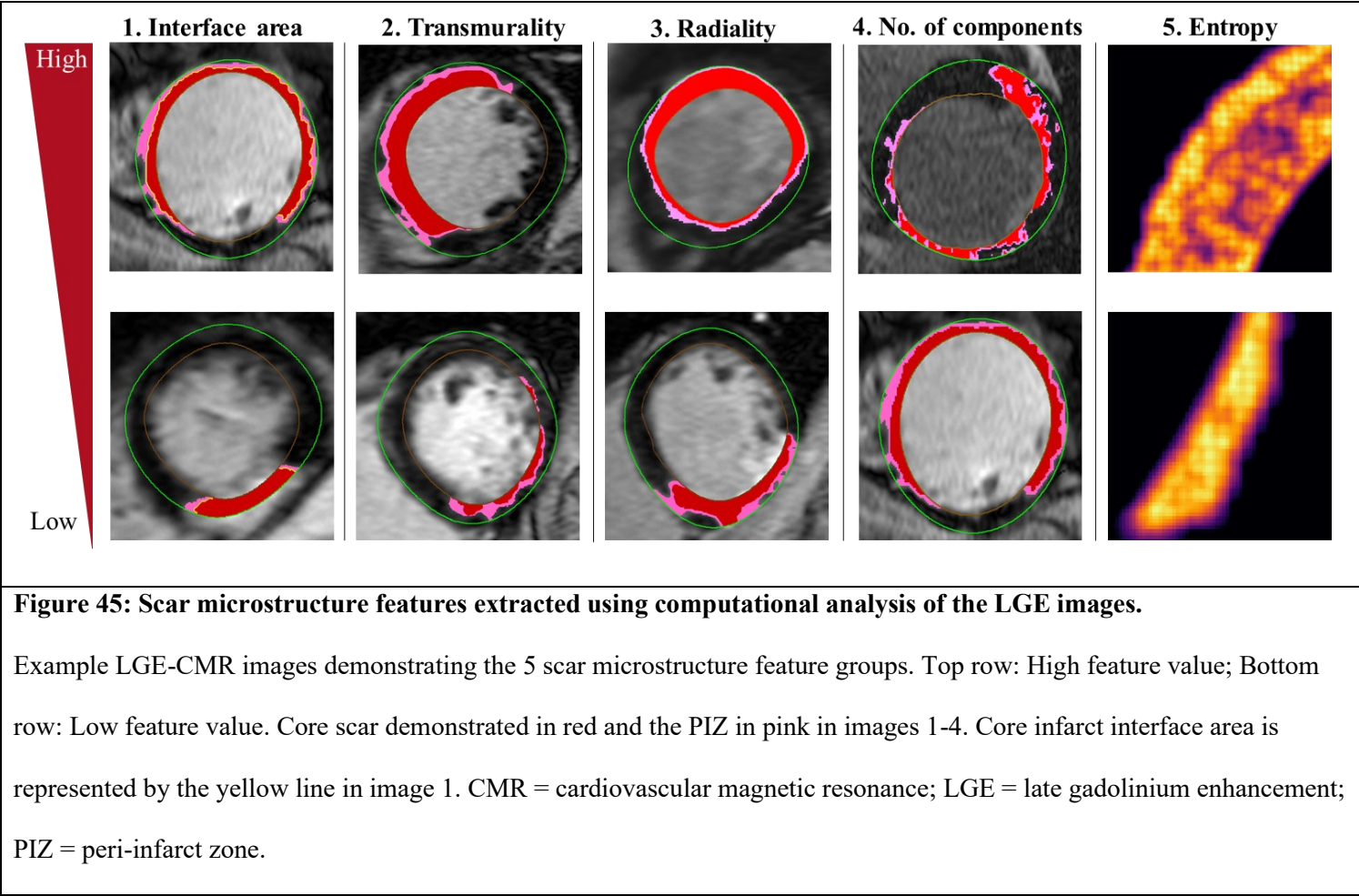
Variable	HR (95% CI)	P-value
PIZ mass (g)*	1.07 (1.02, 1.12)	0.005
Infarct core mass (g)*	1.02 (1.00, 1.05)	0.03
Age (per 10 years)	0.96 (0.71, 1.29)	0.78
Female	0.33 (0.08, 1.39)	0.13
LVEF (%)	0.99 (0.97, 1.02)	0.63
ACE-i/ARB	4.70 (0.64, 34.57)	0.13
Smoking status		0.04
Non-smoker	Reference group	
Ex-smoker	1.30 (0.64, 2.65)	
Current smoker	2.71 (1.08, 6.80)	
Atrial fibrillation	2.37 (1.19, 4.75)	0.02
Harrell's C-statistic = 0.82		

Figure 44: Multivariable Cox regression analysis (Model B) for the primary endpoint with subsequent addition of the LGE quantification data.

In Model B, a forward stepwise procedure was applied using a subset of variables in Table 2 (with $p < 0.10$ as the criterion for inclusion), forcing in recognised predictors of the outcome (age, sex and LVEF) and with the subsequent addition of LGE quantification data. Top row: Forest plot of the final multivariable Cox regression model showing the simultaneous addition of the LGE quantification data. Middle and bottom rows: Multivariable Cox models for the primary endpoint with progressive addition of the LGE quantification data. *Results per 10g: PIZ mass HR 1.93, 95% CI 1.22-3.06, $P=0.005$ and core infarct mass HR 1.27, 95% CI 1.03-1.58, $P=0.03$. ACEi = angiotensin-converting enzyme inhibitor; ARB = angiotensin II receptor blocker; CI = confidence interval; HR = hazard ratio; LVEF = left ventricular ejection fraction; PIZ = peri-infarct zone.

5.5.1.2 Composite of SCD or aborted SCD: Scar microstructure analysis

In examining the scar microstructure, each of the 5 features in Table 12 was separately calculated for; a) core infarct; b) PIZ; c) total scar (core infarct + PIZ). Figure 45 shows example images highlighting LGE slices with high and low feature values.



All scar microstructure features were significantly associated with the primary endpoint on univariable and multivariable Cox regression analysis, the latter after adjustment for LVEF <35% and NYHA Class >1 (Table 16). Figure 46 shows the scar microstructure features with the largest effect estimates. After the addition of core infarct mass and PIZ mass to the Cox regression models, no scar microstructure feature remained significantly associated with the primary endpoint (Table 17).

Table 16: Multivariable analysis of the scar microstructure features, adjusted for LVEF <35% and NYHA >1		
Scar feature	Scar feature HR and 95% CI	Scar feature P-value
Interface area – total LGE interface area across all slices		
Core infarct interface area	2.0 (1.52 - 2.62)	<0.001
PIZ interface area	1.64 (1.29 - 2.09)	<0.001
Core infarct & PIZ combined interface area	1.59 (1.26 - 2.0)	<0.001
Entropy – mean LGE entropy across all slices		
Core infarct entropy	2.4 (1.53 - 3.77)	<0.001
PIZ entropy	2.07 (1.38 - 3.09)	<0.001
Core infarct & PIZ combined entropy	2.29 (1.46 - 3.58)	<0.001
Transmurality – mean transmuralty of LGE across all slices		
Core infarct transmuralty	1.89 (1.37 - 2.6)	<0.001
PIZ transmuralty	1.69 (1.23 - 2.33)	0.001
Core infarct & PIZ combined transmuralty	2.23 (1.54 - 3.22)	<0.001
Number of components – total number of LGE components across all slices		
Number of core infarct components	1.39 (1.08 - 1.8)	0.012
Number of PIZ components	1.61 (1.27 - 2.03)	<0.001
Number of core infarct & PIZ combined components	1.46 (1.14 - 1.86)	0.002
Radiality – mean radiality of LGE across all slices		
Core infarct radiality	1.55 (1.18 - 2.03)	0.001
PIZ radiality	1.65 (1.24 - 2.2)	0.001
Core infarct & PIZ combined radiality	1.58 (1.19 - 2.09)	0.002
Multivariable Cox regression models of the primary endpoint for each scar microstructure feature, adjusted for LVEF <35% and NYHA >1. CI = confidence interval; HR = Hazard ratio; LGE = late gadolinium enhancement; PIZ = peri-infarct zone		

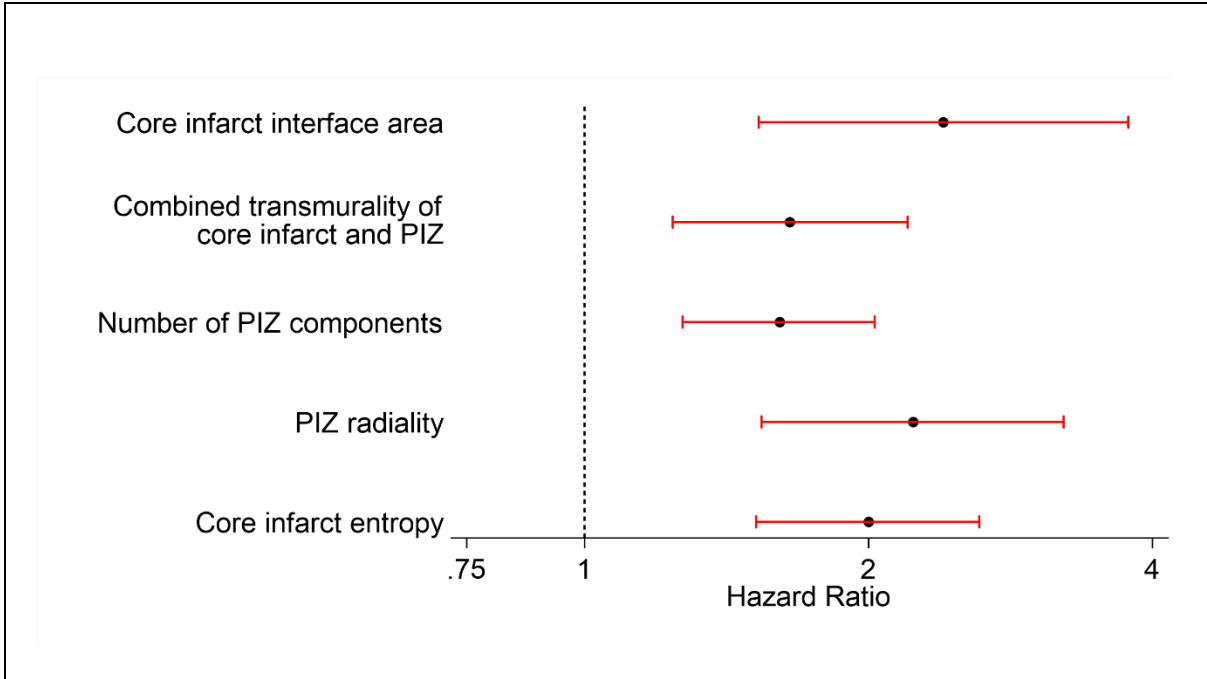


Figure 46: Multivariable analysis of the scar microstructure features for the primary outcome.

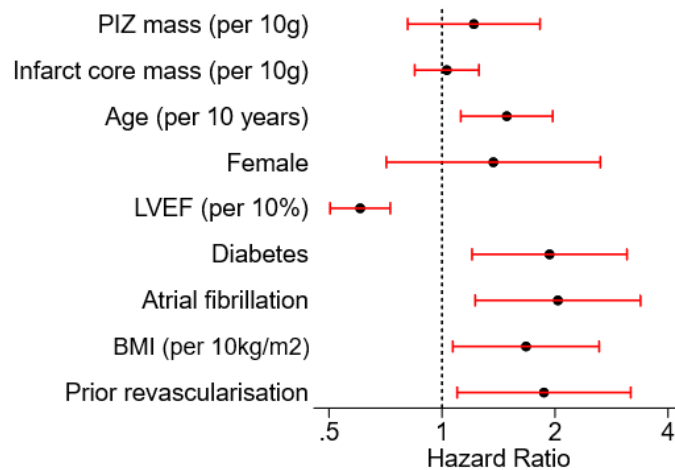
Individual Cox regression models for the scar microstructure features with the largest effect estimates within each group. Each model adjusted for LVEF <35% and NYHA Class >1. PIZ = peri-infarct zone.

Table 17: Multivariable analysis of the scar microstructure features, adjusted for LVEF <35%, NYHA >1, core infarct mass and PIZ mass		
Scar feature	Scar feature HR and 95% CI	Scar feature P-value
Interface area – total LGE interface area across all slices		
Core infarct interface area	0.92 (0.63 - 1.34)	0.648
PIZ interface area	0.9 (0.66 - 1.25)	0.544
Core infarct & PIZ combined interface area	0.8 (0.56 - 1.15)	0.232
Entropy – mean LGE entropy across all slices		
Core infarct entropy	1.29 (0.74 - 2.26)	0.369
PIZ entropy	1.05 (0.64 - 1.73)	0.833
Core infarct & PIZ combined entropy	1.19 (0.67 - 2.09)	0.555
Transmurality – mean transmural of LGE across all slices		
Core infarct transmural	1.42 (0.9 - 2.24)	0.135
PIZ transmural	0.97 (0.63 - 1.5)	0.892
Core infarct & PIZ combined transmural	1.43 (0.84 - 2.42)	0.185
Number of components – total number of LGE components across all slices		
Number of core infarct components	0.76 (0.53 - 1.09)	0.139
Number of PIZ components	0.9 (0.64 - 1.29)	0.577
Number of core infarct & PIZ combined components	0.88 (0.6 - 1.29)	0.517
Radiality – mean radiality of LGE across all slices		
Core infarct radiality	0.73 (0.47 - 1.13)	0.154
PIZ radiality	0.75 (0.48 - 1.18)	0.218
Core infarct & PIZ combined radiality	0.65 (0.41 - 1.03)	0.069
Multivariable Cox regression models of the primary endpoint for each scar microstructure feature, adjusted for LVEF <35%, NYHA >1, core infarct mass and PIZ mass. CI = confidence interval; HR = Hazard ratio; LGE = late gadolinium enhancement; PIZ = peri-infarct zone		

5.5.2 Secondary outcomes

5.5.2.1 Major heart failure event

During follow-up, 78 (17.9%) patients experienced a major heart failure event. On multivariable analysis, there was no significant association between either scar metric and this secondary endpoint (PIZ mass per gram: HR 1.02, 95% CI 0.98-1.06, P=0.35 and core infarct mass per gram HR 1.00, 95% CI 0.98-1.02, P=0.77, Figure 47).



Variable	HR (95% CI)	P-value
Age (per 10 years)	1.42 (1.07, 1.87)	0.01
Female	1.35 (0.70, 2.61)	0.37
LVEF (%)	0.95 (0.93, 0.96)	<0.001
Diabetes	1.93 (1.20, 3.11)	0.007
Atrial fibrillation	1.98 (1.20, 3.25)	0.007
BMI (kg/m ²)	1.06 (1.01, 1.11)	0.01
Prior revascularisation	1.94 (1.14, 3.30)	0.02
Harrell's C-statistic = 0.79		

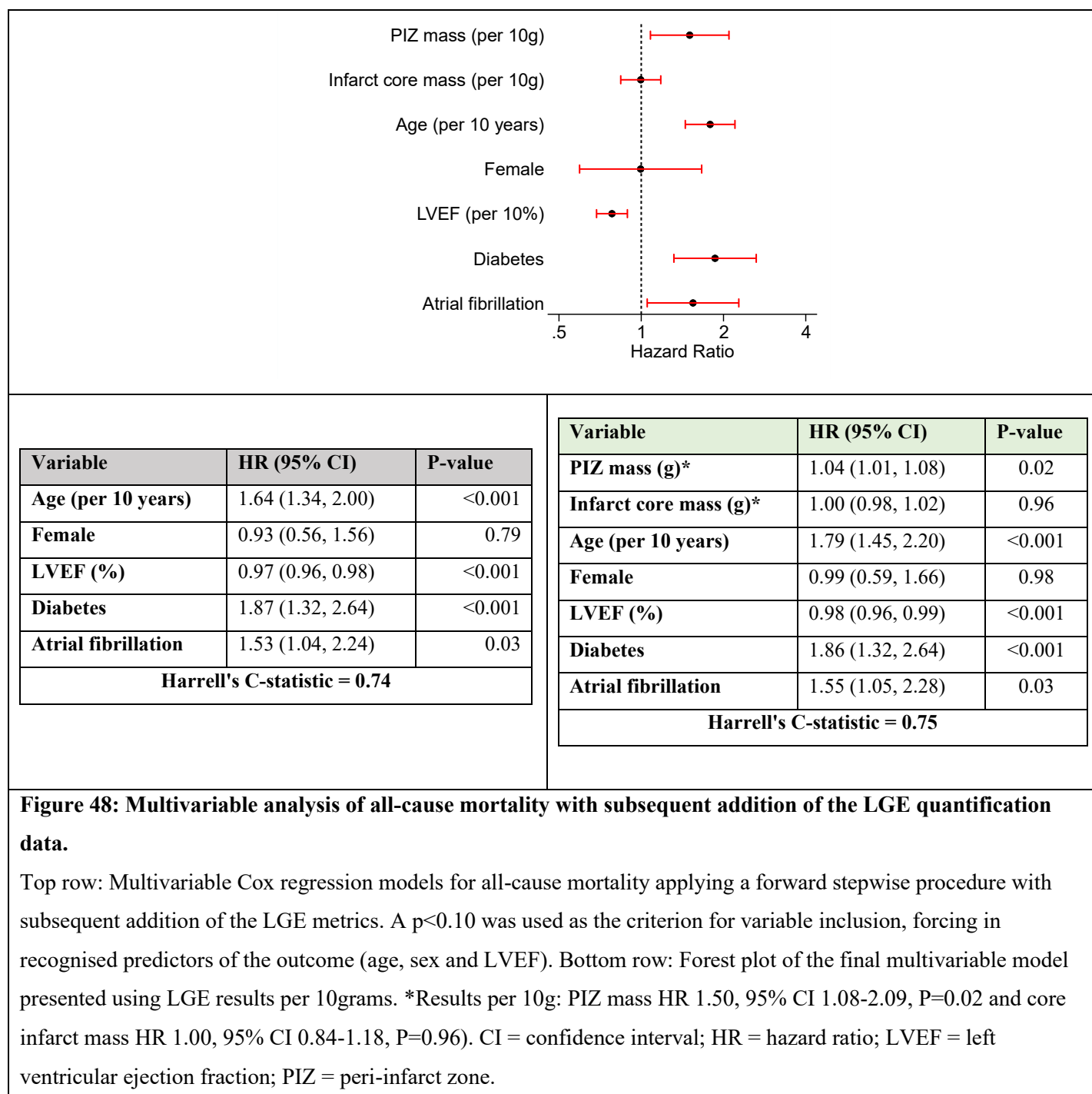
Variable	HR (95% CI)	P-value
PIZ mass (g)*	1.02 (0.98, 1.06)	0.35
Infarct core mass (g)*	1.00 (0.98, 1.02)	0.77
Age (per 10 years)	1.49 (1.12, 1.97)	0.006
Female	1.37 (0.71, 2.64)	0.35
LVEF (%)	0.95 (0.93, 0.97)	<0.001
Diabetes	1.93 (1.20, 3.11)	0.007
Atrial fibrillation	2.04 (1.23, 3.38)	0.006
BMI (kg/m ²)	1.05 (1.01, 1.10)	0.02
Prior revascularisation	1.87 (1.10, 3.18)	0.02
Harrell's C-statistic = 0.79		

Figure 47: Multivariable analysis of major heart failure events with subsequent addition of the LGE quantification data.

Top row: Multivariable Cox regression models for major heart failure events applying a forward stepwise procedure with subsequent addition of the LGE metrics. A $p < 0.10$ was used as the criterion for variable inclusion, forcing in recognised predictors of the outcome (age, sex and LVEF). Bottom row: Forest plot of the final multivariable model presented using LGE results per 10grams. *Results per 10g: PIZ mass HR 1.22, 95% CI 0.81-1.82, $P = 0.35$ and core infarct mass HR 1.03, 95% CI 0.85-1.25, $P = 0.77$. BMI = body mass index; CI = confidence interval; HR = hazard ratio; LVEF = left ventricular ejection fraction; PIZ = peri-infarct zone.

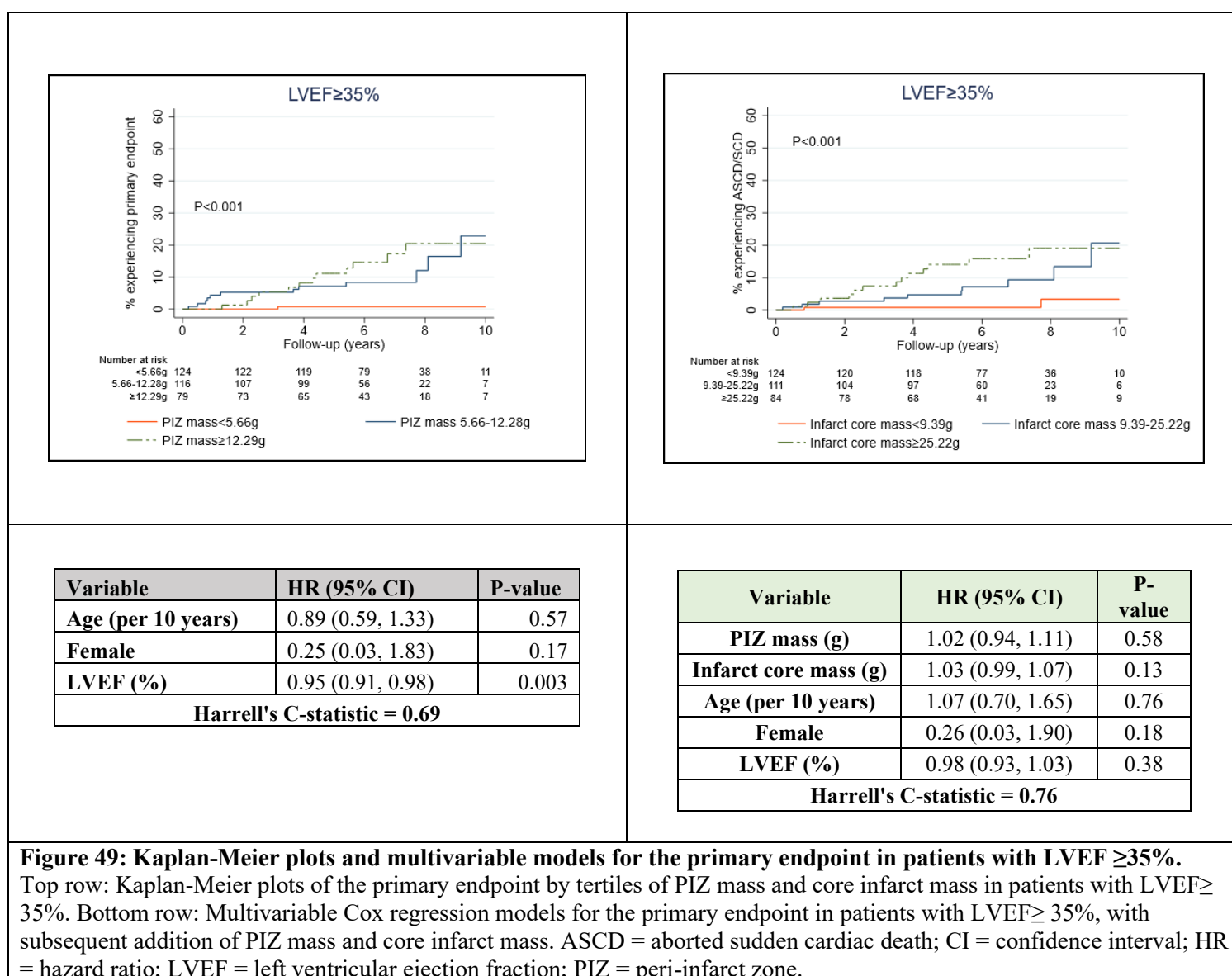
5.5.2.2 All-cause mortality

There were 138 (31.6%) deaths during the follow-up period (92 cardiovascular deaths, 46 non-cardiovascular deaths). On multivariable analysis, PIZ mass was independently associated with mortality (per gram: HR 1.04, 95% CI 1.01-1.08, $P=0.02$, Figure 48). Core infarct mass was not significantly associated with the endpoint when both scar metrics were included in the model (per gram: HR 1.00, 95% CI 0.98-1.02, $P=0.96$, Figure 48).



5.5.2.3 Composite of sudden cardiac death or aborted sudden cardiac death in patients with LVEF $\geq 35\%$.

25 out of 319 patients with LVEF $\geq 35\%$ experienced SCD or aborted SCD. Cumulative incidence of the outcome by tertiles of both PIZ mass and core infarct mass suggest that patients in higher tertiles had an increased risk of the primary outcome (Figure 49). On univariable analysis, both PIZ mass and core infarct mass were associated with the endpoint (per gram: HR 1.09, 95% CI 1.04-1.14, $P < 0.001$ and HR 1.04, 95% CI 1.02-1.06, $P < 0.001$ respectively). After adjustment for age, sex and LVEF, neither PIZ mass nor core infarct mass remained significantly associated with the primary endpoint when both were included in the multivariable model (per gram: HR 1.02, 95% CI 0.94-1.11, $P = 0.58$ and HR 1.03, 95% CI 0.99-1.07, $P = 0.13$ respectively, Figure 49).



Scar microstructure analysis in patients with LVEF $\geq 35\%$ highlighted 3 features that were significantly associated with the primary endpoint on multivariable analysis (adjusted for age, sex and LVEF): Transmurality, interface area and entropy (Table 18). After the addition of core infarct mass and PIZ mass to the Cox regression models, 1 specific scar microstructure feature remained significantly associated with the primary endpoint: Combined transmural area of core infarct and the PIZ (HR 2.29, 95% CI 1.04-5.01, P=0.04).

Table 18: Multivariable analysis of the scar microstructure features in patients with LVEF $\geq 35\%$, adjusted for age, sex and LVEF.		
Scar feature	Scar feature HR and 95% CI	Scar feature P-value
Interface area – total LGE interface area across all slices		
Core infarct interface area*	1.52 (1.02 - 2.27)	0.039
PIZ interface area	1.51 (1.0 - 2.28)	0.052
Core infarct & PIZ combined interface area	1.27 (0.87 - 1.85)	0.213
Entropy – mean LGE entropy across all slices		
Core infarct entropy*	2.49 (1.16 - 5.33)	0.019
PIZ entropy*	2.04 (1.07 - 3.88)	0.029
Core infarct & PIZ combined entropy*	2.84 (1.31 - 6.17)	0.008
Transmurality – mean transmural area of LGE across all slices		
Core infarct transmural area	1.58 (0.9 - 2.79)	0.113
PIZ transmural area*	2.16 (1.17 - 3.98)	0.014
Core infarct & PIZ combined transmural area*	2.89 (1.41 - 5.94)	0.004
Number of components – total number of LGE components across all slices		
Number of core infarct components	1.27 (0.85 - 1.89)	0.237
Number of PIZ components	1.18 (0.78 - 1.8)	0.432
Number of core infarct & PIZ combined components	1.14 (0.73 - 1.79)	0.564
Radiality – mean radiality of LGE across all slices		
Core infarct radiality	1.22 (0.77 - 1.91)	0.396
PIZ radiality	1.19 (0.73 - 1.93)	0.491
Core infarct & PIZ combined radiality	1.11 (0.69 - 1.77)	0.676
Multivariable Cox regression models of the primary endpoint for each scar microstructure feature in patients with LVEF $\geq 35\%$. Each model adjusted for age, sex and LVEF. *Scar microstructure features reaching statistical significance. CI = confidence interval; HR = Hazard ratio; LGE = late gadolinium enhancement; PIZ = peri-infarct zone		

5.6 Discussion

To my knowledge, this is the first study assessing the utility of multiscale myocardial scar characterisation by CMR to predict SCD in prospectively investigated patients with CAD. The cohort represents a real-world dataset of typical patients with chronic coronary artery disease and a full spectrum of LV systolic function. The principal findings are: i) PIZ mass and core infarct mass independently predict SCD after adjusting for clinical parameters used in ICD implantation decisions, including on competing risk analysis; ii) Reduced LVEF does not predict SCD when LGE quantification data is included in the multivariable models; iii) Neither PIZ mass nor core infarct mass associates with major heart failure events on multivariable analysis; iv) Computational analysis identified a group of clinically plausible scar microstructure features that associate with SCD; however, these metrics did not independently predict SCD after adjustment for total scar mass.

5.6.1 LGE-CMR predictors of SCD in stable CAD

Multiple observational studies in CAD cohorts have demonstrated the role of PIZ quantification by LGE-CMR to identify patients at increased risk of all-cause mortality (Hagbayan et al., 2019; Kwon et al., 2014; Watanabe et al., 2014; Yan et al., 2006), inducibility of VT during electrophysiology study (Schmidt et al., 2007) and appropriate ICD therapy (Acosta et al., 2018; Roes et al., 2009; Sánchez-Somonte et al., 2021; Wu et al., 2012). The majority of these studies either restricted recruitment to patients with impaired LVEF or those with prior ICD implantation. Additionally, in the studies including individuals with an existing secondary prevention ICD indication, the novel CMR metrics were unlikely to alter management decisions. Zegard et al recently published a retrospective study assessing the association between LGE and SCD in a cohort of CAD patients with a broad range of LVEF (Zegard et al., 2021). In my study, using a prospectively recruited cohort, I build on the signal that they described in their retrospective registry. I show that both core infarct mass and PIZ mass independently predict SCD with PIZ mass demonstrating the stronger association with the primary endpoint.

Reduction in LVEF was not associated with the primary endpoint following addition of the core infarct mass and PIZ mass to the multivariable models. This aligns with prior data in CAD cohorts detailing the limitation of LVEF to predict clinical outcomes after adjusting for LGE (Roes et al., 2007). These results are clinically relevant as LVEF calculation remains the central measure used in ICD implantation decisions (Al-Khatib et al., 2018; Priori et al., 2015), driven by inclusion criteria of the seminal trials assessing the utility of primary prevention ICD therapy (Bardy et al., 2005; Moss et al., 2002). It is well appreciated however that impaired LVEF does not directly identify arrhythmogenic myocardial substrate. Up to 70% of SCD cases in CAD populations occur in subjects without severely reduced LVEF (Dagres & Hindricks, 2013) and there remains a paucity of evidence identifying a convincing causal relationship between LVEF and SCD (Wellens et al., 2014).

Conversely, myocardial fibrosis is a mechanistically plausible metric in SCD prediction and may also represent a relatively static parameter in patients who do not suffer a subsequent MI.

Finally, neither core infarct mass nor PIZ mass predicted major heart failure events on multivariable analysis. Additionally, there was a significant association between both scar metrics and the primary endpoint after accounting for the competing risk of non-sudden death. These results highlight the potential utility of LGE quantification as a precision tool in event prediction; hypothetically targeting ICD implantation to CAD patients with high future arrhythmic risk and lower risk of death from non-sudden causes. Importantly, the discrimination ability of the multivariable models were similar following the addition of core infarct mass or PIZ mass alone or the simultaneous addition of both scar metrics; potentially suggesting that quantification of only one scar parameter may be needed to improve ICD candidacy. A randomised control trial addressing this question would be of interest.

5.6.2 Scar microstructure analysis

Computational analysis of the LGE images permitted mechanistic interrogation of the key morphological and texture related scar features that predicted SCD, providing an additional layer of granularity above the raw quantification data.

Specifically, two of the morphological features have a growing evidence base describing their association with ventricular arrhythmia. First, interface area describes the extent of the border

between LGE and adjacent tissue. This parameter has been found to associate with major arrhythmic events in dilated cardiomyopathy with simulation modelling describing a plausible mechanism of action to promote unidirectional conduction block (Balaban et al., 2021). I show for the first time a significant association between scar interface area and SCD in patients with CAD. Second, critical VT isthmus sites have been found in close proximity to areas of increasingly transmural scar (Piers et al., 2014). My study demonstrates the novel utility of scar transmural to predict SCD in patients with CAD.

Recent studies have also demonstrated the association between the texture related feature, scar entropy, and ventricular arrhythmia (Androulakis et al., 2019). Scar entropy describes the level of disorder or heterogeneity within a region of myocardial fibrosis (Androulakis et al., 2019). My study is the first to demonstrate the utility of this microstructure feature to predict SCD in prospectively investigated patients with a broad range of LVEF.

Inclusion of both core infarct mass and PIZ mass in the multivariable models resulted in no scar microstructure feature remaining independently associated with the primary outcome. This may be explained by individual microstructure features scaling together, resulting in a combined signal that is adequately captured by raw quantification of core infarct and the PIZ. Importantly however, in patients with LVEF $\geq 35\%$, high combined transmural of the core infarct and PIZ identified patients at increased risk of SCD after inclusion of the LGE quantification data to the Cox regression model. Further mechanistic studies are required to fully dissect the role of this microstructure feature in arrhythmogenesis.

5.6.3 Limitations

This is a single centre study and thus the generalisability of the results may be questioned. However, the results align with previous prospective studies assessing all-cause mortality (Hagbayan et al., 2019) and the patient cohort represents a real-world dataset, referred from local cardiology clinics and a broad base of secondary and tertiary hospitals. The study contains a high percentage of male and Caucasian patients and thus the results may not be applicable to female patients and non-Caucasian populations. I did not assess myocardial ischaemia however the *a priori* hypothesis was to evaluate

the utility of LGE characterisation to predict SCD in stable CAD patients. Reflecting this, the cohort predominantly contained patients with mild ischaemic symptoms (64% were Canadian Cardiovascular Society [CCS] Angina Class I with 26% of patients being CCS Class 2).

Concerns regarding the ability of 2-dimensional LGE to accurately characterise the PIZ have been raised. This principally surrounds the issue of partial voluming where imaged voxels contain both cleanly demarcated core scar and adjacent normal myocardium. This results in an intermediate signal due to the limited spatial resolution and not because of a region of viable myocytes interspersed with collagen. Future studies harnessing 3-dimensional LGE would allow for whole-heart coverage with reduced voxel dimensions and improved spatial resolution. The elimination of slice gaps would permit more detailed characterization of the relationship of LGE features along the slice direction (e.g. increasing the confidence to describe potential VT conduction channels).

LGE quantification was only performed using the FWHM method and recent retrospective studies have highlighted the association between LGE quantification by standard deviation approaches and SCD (Zegard et al., 2021). The FWHM approach, however, has the highest degree of reproducibility compared to other methods for quantifying LGE (Flett et al., 2011) and the majority of PIZ outcome studies have incorporated this methodology (Haghighyan et al., 2019). Additionally, I only performed LGE quantification on a single platform and therefore the generalisability to other vendors is potentially limited.

5.7 Conclusions

Chapter 5 provides novel prospective data demonstrating the value of myocardial fibrosis characterisation by CMR to predict SCD in a cohort of stable CAD patients. I also highlight the limitation of LVEF calculation in SCD risk prediction. Multicentre trials should now be considered utilising appropriate cut-offs for these LGE metrics above which patients are randomised to ICD implantation or conventional medical therapy.

6 Overview of thesis and future work

Despite great advances in the field of acute myocardial injury, there is a need for more effective treatments and risk stratification criteria in stable CAD cohorts. Importantly, the role of novel therapeutic approaches that target the consequences of myocardial ischaemia and fibrosis need investigation. My thesis focused on these gaps in evidence, detailing 4 interrelated chapters.

6.1 Chapter 2: Comprehensive serial phenotyping of patients with stable coronary artery disease undergoing coronary artery bypass grafting.

Question:

- Is multiparametric CMR, including quantitative stress perfusion, able to detect changes in MBF and tissue characterisation parameters following CABG and is there a relationship between symptoms and inducible ischaemia?

Findings:

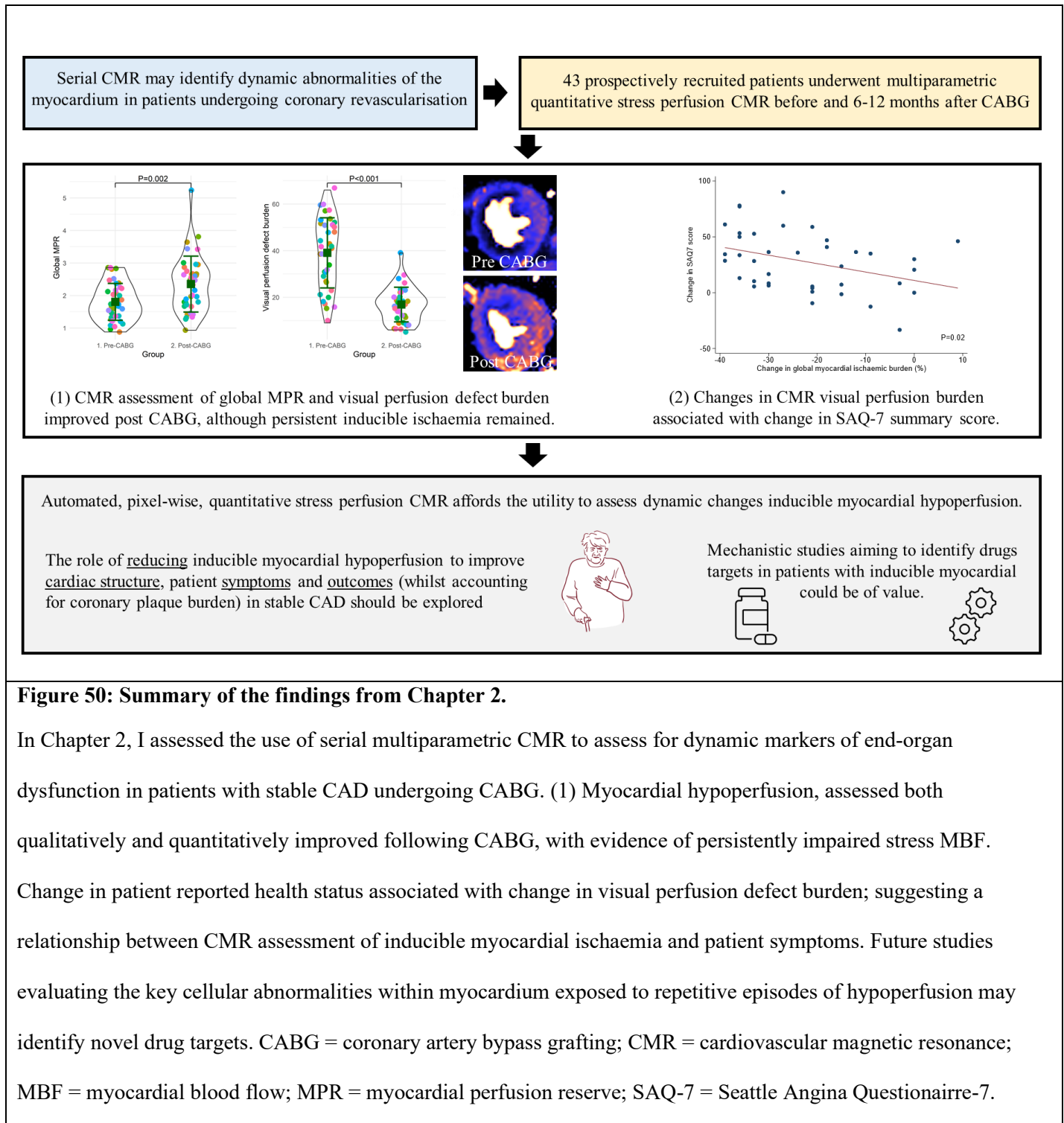
- Qualitative and fully-quantitative assessment of myocardial perfusion improves following CABG, with evidence of an important degree residual inducible ischaemia.
- There is an association between improvements in patient reported health status and a decrease in the ischaemic burden by visual assessment. Fully quantitative measures of MBF by CMR do not associate symptoms in patients undergoing CABG.
- LV extracellular matrix volume decreases following CABG.

Meaning:

- Visual assessment of ischaemic myocardial burden associates with cardiac symptoms and may be better suited, over quantitative measures of MBF, to monitor efficacy of treatments aimed at reducing the burden of inducible ischaemia.
- Interstitial fibrosis may regress following CABG and further research assessing the prognostic utility of targeting the extracellular matrix volume should be explored.

Future work:

- Research studies aiming to provide mechanistic insight into the aetiology of persistent inducible myocardial hypoperfusion following CABG.
- Randomised controlled trial using a novel mitochondrial-targeted antioxidant to reduce CMR markers of inducible myocardial ischaemia in patients with stable CAD.



6.2 Chapter 3: Assessing mitochondrial bioenergetics in coronary artery disease: A translational multiomic tissue study in humans (The AMBITION study).

Question:

- What is multiomic landscape of LV myocardium in patients with stable CAD?

Findings:

- Viable LV myocardium from humans with stable CAD has an altered bioenergetic profile compared to donor LV myocardium, with evidence of altered OXPHOS and upregulation of genes encoding endogenous antioxidants.
- The metabolic and transcriptomic profile of LV myocardium with and without inducible ischaemia from the same heart is similar.

Meaning:

- Patients with CAD may have a global myocardial deficit in mitochondrial bioenergetic function that might be a novel therapeutic target.

Future work:

- The next phase will involve a proof-of-mechanism trial with a novel therapy (e.g., a mitochondrial-targeted antioxidant), using CMR and myocardial biopsies to assess drug target engagement and changes to cardiac structure, function and bioenergetics (Figure 52).

Better understanding of myocardial bioenergetics in patients with stable CAD might provide insight into mechanisms of LV dysfunction.

From 33 patients with evidence of inducible ischemia on CMR, 63 LV biopsies were acquired on the beating heart during CABG.

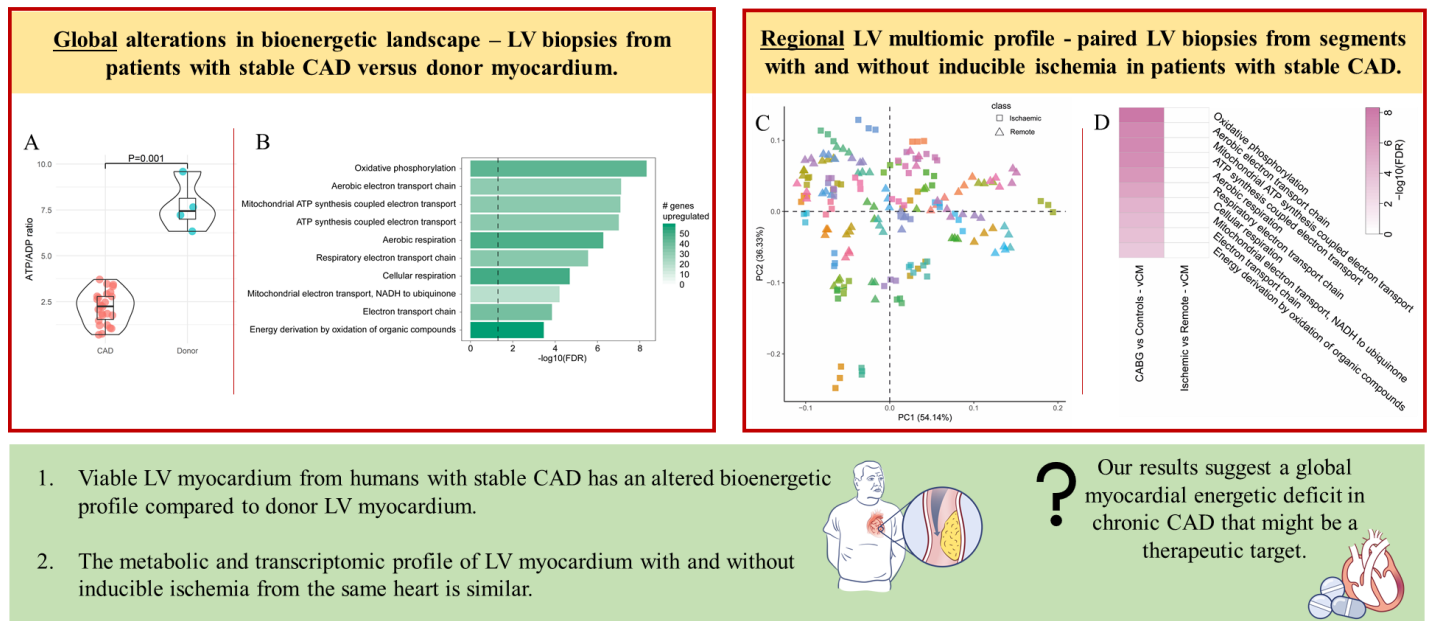


Figure 51: Summary of the findings from Chapter 3

Patients undergoing CABG had LV biopsies acquired on the beating heart. LV samples from donor hearts were included as controls. First, global alterations between CAD patients and control donor myocardium were assessed. **A:** Violin box-plot demonstrating the lower ATP/ADP ratio in CAD biopsies as compared to donor samples. **B:** Gene ontology analysis demonstrating upregulation of several biological processes associated with normal cellular function within ventricular cardiomyocytes (vCM) of CAD patients compared to donor myocardium. Second, regional differences in LV metabolic profile were assessed, using paired biopsies from segments with ('ischaemic') and without ('remote') inducible ischaemia (as determined by CMR). **C:** Principal component analysis of the liquid chromatography–mass spectrometry data revealing no clusters based on ischaemic (square) or remote (triangle) biopsies and no clear clusters of patients. **D:** Gene Ontology analysis demonstrating no significant differential expression of oxidative phosphorylation genes in ventricular cardiomyocytes between segments with or without inducible ischaemia.

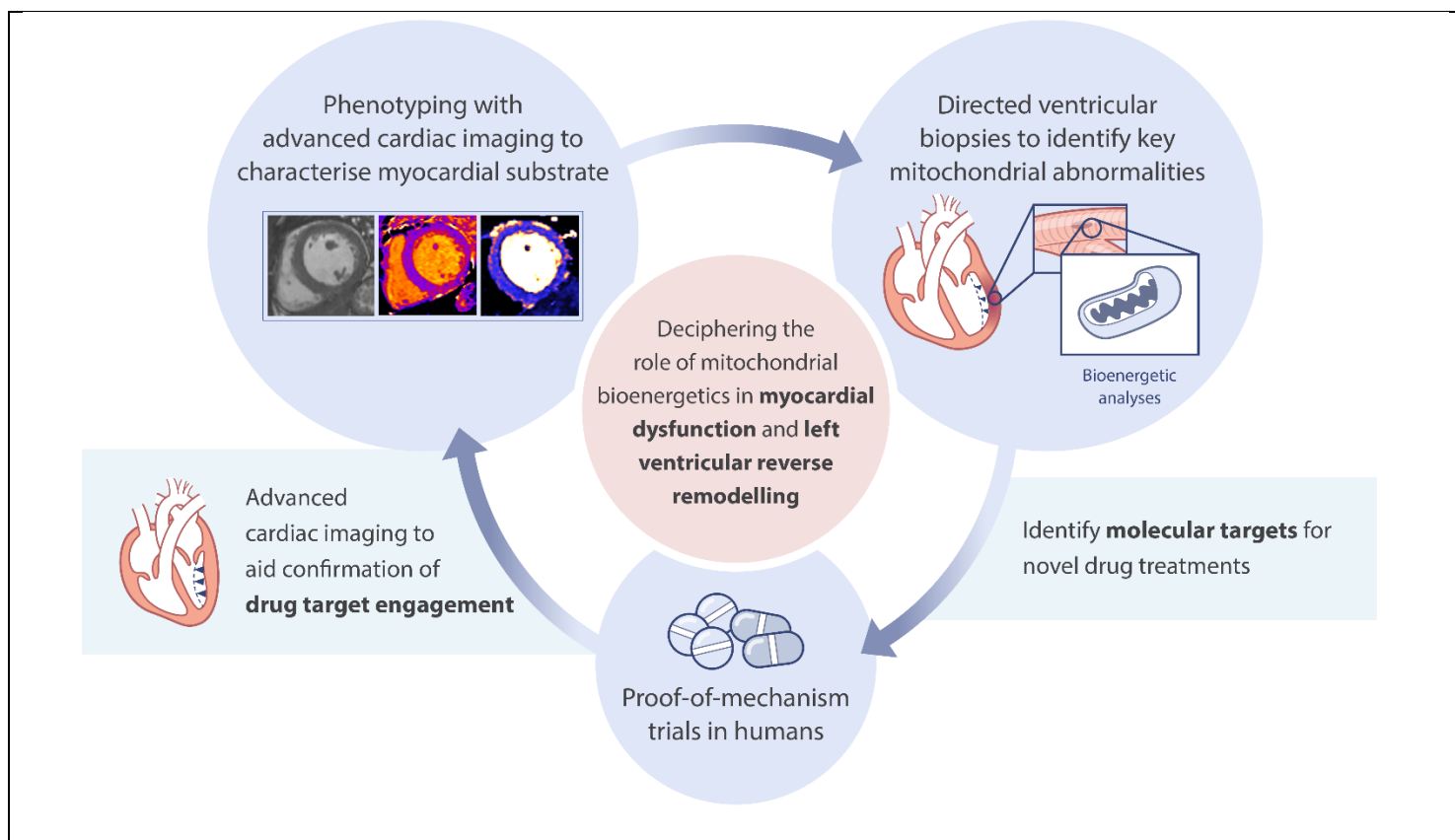


Figure 52: Proposed second phase of the AMBITION study.

In chapter 3, I utilised advanced cardiac imaging (e.g. CMR) to direct cardiac biopsies with the aim of determining the key bioenergetic aberrations in human ischaemic myocardium. In the next phase, proof-of-mechanism trials using a novel therapy will be conducted; targeting the abnormalities identified in Chapter 3 and using advanced cardiac imaging to assess drug target engagement.

6.3 Chapter 4: Assessing for genetic and cardiovascular magnetic resonance features of dilated cardiomyopathy in patients with coronary artery disease.

Question:

- What is the relevance of genetic and cardiovascular magnetic resonance (CMR) feature of DCM in individuals with CAD?

Findings:

- Non-infarct pattern myocardial scar and rare pathogenic variants in DCM genes are both associated with adverse cardiovascular remodeling in individuals with CAD.
- In individuals with CAD, the presence of a rare variant in a DCM-associated gene predicts future adverse clinical events.

Meaning:

- Rare pathogenic variants in DCM-associated genes modulate LV remodelling and are associated with adverse clinical outcomes in patients with stable CAD. Such patients may represent a subgroup that benefit from enhanced medical surveillance.
- NI-LGE is similarly associated with adverse cardiac remodelling but, by contrast, is not an independent predictor of clinical outcomes in patients with stable CAD. NI-LGE was not found to have a rare genetic basis in this study and is likely to be primarily driven by increased wall stress in the setting of progressive systolic dysfunction.

Future work:

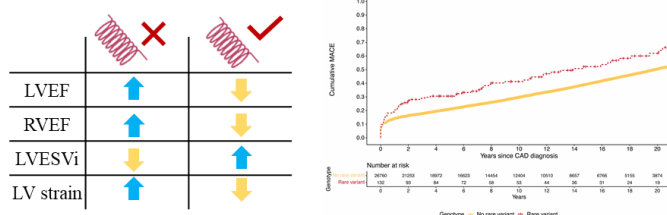
- Assess the use of polygenic risk scores to better target patients with CAD for enhanced surveillance and novel therapies.

Assessment for shared genotypic and phenotypic features of DCM and CAD in 2 independent cohorts using CMR and genetic sequencing

Individuals with CAD recruited into the UK Biobank

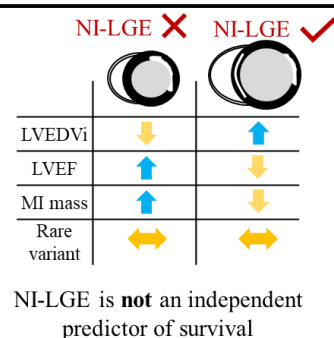
Rare pathogenic variants in DCM genes were associated with **adverse cardiac remodelling** and **worse outcomes** in individuals with CAD

CMR phenotyping (n=1,1619, rare pathogenic variant=8) **Outcome data** (n=26,892, rare pathogenic variant=132)



453 patients with stable CAD recruited into a LGE-CMR registry

NI-LGE is associated with **greater adverse LV remodelling**, despite lower MI burden



Rare DCM variants
NI-LGE

?Subgroup of CAD patients with features of DCM

LVEF ↓



? Prognostic significance of genetic variants known to cause DCM in patients with CAD and reduced LVEF



Figure 53: Summary of the findings in Chapter 4

In Chapter 4, 2 independent cohorts were used to investigate for overlapping features of DCM and CAD. Initially, the prevalence, distribution and prognostic relevance of NI-LGE in patients prospectively recruited into a CMR and genetics registry was assessed (The London Cohort). NI-LGE was associated with adverse LV remodelling which could not be explained by increased burden of myocardial infarction. There was no enrichment in rare variants in DCM-associated genes in patients with NI-LGE compared to patients without this imaging biomarker. Next, the UKB was used to assess for the association between rare variants in DCM-associated genes, phenotype and outcomes in individuals with CAD. Individuals with rare pathogenic variants in DCM-associated genes had greater adverse cardiac remodelling and worse clinical outcomes as compared to genotype negative individuals; a result suggesting the presence of a high-risk subgroup of patients with CAD and concomitant vulnerability to DCM.

CAD = coronary artery disease; CMR = cardiovascular magnetic resonance; DCM = dilated cardiomyopathy; NI-LGE = non-infarct pattern LGE; UK = UK Biobank.

6.4 Chapter 5: Comprehensive Phenotypic Characterisation of Late Gadolinium Enhancement Predicts Sudden Cardiac Death in Coronary Artery Disease.

Question:

- What is the utility of in-depth myocardial scar characterisation to predict SCD in prospectively recruited patients with stable CAD?

Findings:

- Comprehensive LGE characterisation can predict SCD in prospectively recruited patients with stable CAD and a broad range of LVEF.
- Numerous shape-based morphological and texture scar microstructure features associate with SCD; providing a degree of mechanistic interrogation beyond standard LGE characterisation.

Meaning:

- LGE characterisation offers the potential to replace severe LV systolic impairment as the central metric used to identify primary prevention ICD candidates in patients with stable CAD.

Future work:

- We are currently assessing the role of 3D scar microstructure assessment, LV shape and paced simulation models to predict SCD in patients with stable CAD.

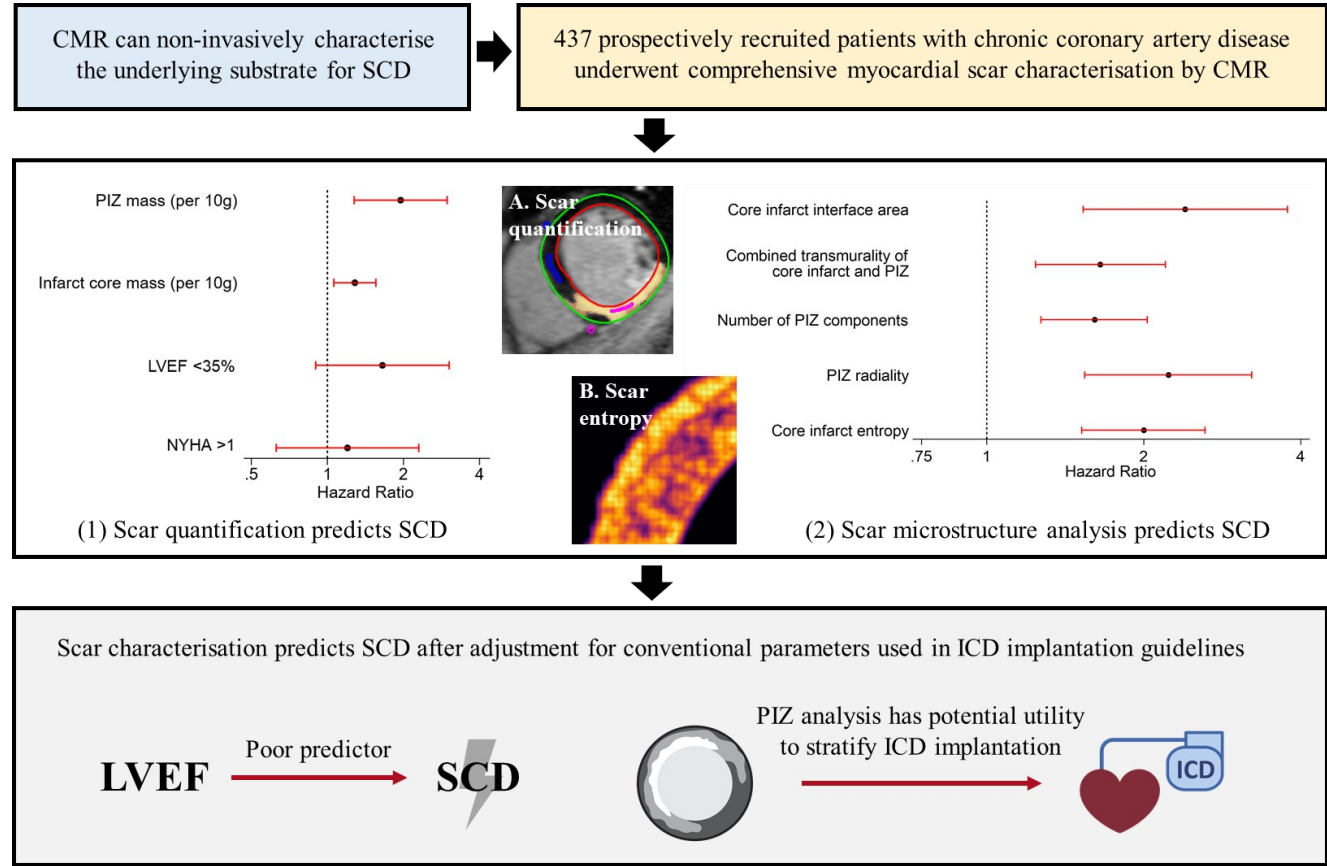


Figure 54: Summary of the findings in Chapter 5.

Core infarct mass and PIZ mass (illustrated in image A) independently predicted SCD after adjusting for parameters used in ICD implantation decisions; PIZ mass and core infarct mass presented per 10g (1). Computational LGE analysis extracted a set of morphological and texture scar features (e.g., entropy, depicted in image B) that associated with SCD on multivariable analysis (each model adjusted for LVEF <35% and NYHA >1) (2). CMR = cardiovascular magnetic resonance; ICD = implantable cardioverter defibrillator; LGE = late gadolinium enhancement; LVEF = left ventricular ejection fraction; NYHA = New York Heart Association; PIZ = peri-infarct zone; SCD = sudden cardiac death.

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Appendix

Selected awards and publications

Prizes

European Society of Cardiology

Winner Young Investigator of the Year (Spotlight of the Congress) ESC Congress 2022.

Selected publications related to work in this thesis

Amin, R. J., Morris-Rosendahl, D., Edwards, M., Tayal, U., Buchan, R., Hammersley, D. J., **Jones, R. E.**, Gati, S., Khalique, Z., Almogheer, B., Pennell, D. J., Baksi, A. J., Pantazis, A., Ware, J. S., Prasad, S. K., & Halliday, B. P. (2022). The addition of genetic testing and cardiovascular magnetic resonance to routine clinical data for stratification of etiology in dilated cardiomyopathy. *Frontiers in Cardiovascular Medicine*, 9. <https://doi.org/10.3389/fcvm.2022.1017119>

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Selected book chapters related to work in this thesis

Hammersley D.J., **Jones R.E.**, Halliday B.P., Dilated Cardiomyopathy. Sex and Cardiac Electrophysiology: Differences in Cardiac Electrical Disorders Between Men and Women. 2020

Selected abstracts related to work in this thesis

Hammersley D.J., **Jones R.E.**, Mach L., Owen R., Lota A.S., Khalique Z., de Marvao A., Gulati A., Baruah R., Guha K., Ware J.S., Cleland J.G., Pennell D.J., Tayal U., Halliday B.P., Prasad S.K. The impact of diabetes mellitus on clinical phenotype and cardiovascular mortality in patients with non-ischaemic dilated cardiomyopathy. AHA 2022

Hammersley D.J., Zaidi H.A., **Jones R.E.**, Hatipoglu S., Androulakis E., Mach L., Lota A.S., Tayal U., Khalique Z., de Marvao A., Baruah R., Guha K., Pennell D.J., Halliday B.P., Bishop M.J., Prasad S.K. Myocardial fibrosis entropy is associated with life-threatening arrhythmia in non-ischaemic cardiomyopathy. BSCMR 2022

Zaidi H.A., **Jones R.E.**, Hammersley D.J., Hatipoglu S., Balaban G., Mach L., Lamata P., Halliday B.P., Bishop M.J., Prasad S.K. Cardiovascular Magnetic Resonance Myocardial Scar Pattern Predicts Major Cardiovascular Events in Coronary Artery Disease. HRS 2022

Jones R.E., Gruszczyk A.V., Schmidt C., Hammersley D.J., Wong J., Mach L., Lee M., Barnett S., Toscano-Rivalta R., Raja S., De Robertis F., Vazir A., Griffin J., Dumas M-E., Cleland J.G.F., Pennell D.J., Halliday B.P., Bhudia S., Nosedà M., Frezza C., Murphy M.P., and Prasad S.K., Assessing Mitochondrial Bioenergetics in stable coronary artery disease: A Translational study Integrating multiOmic analysis of human left ventricular tissue and quaNtitative stress perfusion cardiovascular magnetic resonance (The AMBITION study). NHLI Away day 2022.

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Hammersley D.J., Halliday B.P., Gulati A., Ali A., Ismail T., **Jones R.E.**, Tayal U., Lota A.S., Hsu L., Wage R., Gatehouse P., Firmin D., Owen R., Pennell D.J., Arai A., Prasad S.K. Lower myocardial perfusion reserve is associated with adverse cardiovascular outcomes in patients with dilated cardiomyopathy. AHA 2019

Informed Consent Form for Adults with capacity

**Study title: Assessing mitochondrial bioenergetics in coronary heart disease (The AMBITION study)
Group A – Coronary Artery Bypass Grafting group**

The study has been explained to me by Dr/Mr/Mrs/Ms _____

Participants should complete the whole of this form themselves.

(please <u>initial</u> each statement as appropriate)	
I confirm that I have read and understand the attached patient information sheet entitled 'Assessing mitochondrial bioenergetics in coronary heart disease (The AMBITION study), Group A – Coronary Artery Bypass Grafting group' (Version 1.5, Dated: February 10 th 2021). I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	☐
I agree to biopsies of my heart muscle being taken for detailed analysis (including for genetic/DNA/RNA testing).	☐
I agree to the storage of blood and heart tissue for current and future research (including for genetic/DNA analysis); including storage in the Royal Brompton and Harefield NHS Foundation Trust Cardiovascular Research Centre Biobank at the end of the study.	☐
I give permission for my anonymised blood, heart tissue samples, and medical information to be passed to researchers in <u>universities</u> , <u>research institutes</u> , or <u>commercial organisations</u> within the UK. (delete as appropriate)	☐
I give permission for my anonymised blood, heart tissue samples, and medical information to be passed to researchers in <u>universities</u> , <u>research institutes</u> , or <u>commercial organisations</u> outside the UK; including outside the EU, where data protection laws may differ (delete as appropriate)	☐
I agree to anonymous images or data from the study being published or used for educational purposes.	☐
I give permission for relevant sections of my medical notes and data collected during the study to be disclosed to authorised individuals from the Royal Brompton and Harefield NHS Foundation Trust, Imperial College London and regulatory authorities in the strictest confidence.	☐
I agree that a letter will be sent to my consultant and general practitioner informing them of my participation in this study.	☐
I agree to being contacted in the future by phone, fax or post in case further medical information is required or to arrange appointments.	☐
I agree to my consultant, general practitioner or hospital being contacted for updates on my progress and for information relating to my medical conditions and progress to be released.	☐
I agree to take part in this study.	☐

Name of Patient

Date

Signature

Name of Person taking consent
(if different from Principal Investigator)

Date

Signature

Principal Investigator

Date

Signature

Figure 55. AMBITION study consent form

The Seattle Angina Questionnaire-7

1. The following is a list of activities that people often do during the week. Although for some people with several medical problems it is difficult to determine what it is that limits them, please go over the activities listed below and indicate how much limitation you have had **due to chest pain, chest tightness or angina over the past 4 weeks**.

Place an X in one box on each line.

Activity	Extremely limited	Quite a bit limited	Moderately Limited	Slightly limited	Not at all limited	Limited for other reasons or did not do the activity
a. Walking indoors on level ground	☐	☐	☐	☐	☐	☐
b. Gardening, vacuuming or carrying groceries	☐	☐	☐	☐	☐	☐
c. Lifting or moving heavy objects (e.g. furniture, children)	☐	☐	☐	☐	☐	☐

2. Over the **past 4 weeks**, on average, how many times have you had **chest pain, chest tightness or angina**?

I have had **chest pain, chest tightness or angina**...

4 or more times per day	1-3 times per day	3 or more times per week but not every day	1-2 times per week	Less than once a week	None over the past 4 weeks
☐	☐	☐	☐	☐	☐

3. Over the **past 4 weeks**, on average, how many times have you had to take nitroglycerin (nitroglycerin tablets or spray) for your **chest pain, chest tightness or angina**?

I have taken nitroglycerin...

4 or more times per day	1-3 times per day	3 or more times per week but not every day	1-2 times per week	Less than once a week	None over the past 4 weeks
☐	☐	☐	☐	☐	☐

4. Over the **past 4 weeks**, how much has your **chest pain, chest tightness or angina** limited your enjoyment of life?

It has extremely limited my enjoyment of life	It has limited my enjoyment of life quite a bit	It has moderately limited my enjoyment of life	It has slightly limited my enjoyment of life	It has not limited my enjoyment of life at all
☐	☐	☐	☐	☐

5. If you had to spend the rest of your life with your **chest pain, chest tightness or angina** the way it is right now, how would you feel about this?

Not satisfied at all	Mostly dissatisfied	Somewhat satisfied	Mostly satisfied	Completely satisfied
☐	☐	☐	☐	☐

Figure 56. Seattle Angina Questionnaire-7

Appendix Table 19: Baseline characteristics, stratified by the primary outcome.

Variable	All patients	No SCD/aSCD	SCD/aSCD	P-value
Demographics				
Age (years)	64.4 (9.9)	64.7 (10.0)	62.0 (8.5)	0.07
Female	61 (14.0)	59 (15.2)	2 (4.1)	0.03
Caucasian	357 (81.7)	178 (81.7)	179 (81.7)	0.98
BMI (kg/m ²)	27.8 (5.0)	27.6 (4.9)	29.1 (5.2)	0.05
Heart rate (bpm)	69 (13)	69 (13)	71 (15)	0.52
SBP (mmHg)	127 (19)	127 (19)	123 (21)	0.20
DBP (mmHg)	73 (12)	74 (12)	72 (12)	0.50
Significant CAD*	412 (95.4)	365 (95.1)	47 (97.9)	0.37
CAD type				0.15
<i>Single vessel</i>	131 (31.8)	118 (32.3)	13 (27.7)	
<i>2 vessels</i>	117 (28.4)	102 (27.9)	15 (31.9)	
<i>3 vessels</i>	164 (39.8)	145 (39.7)	19 (40.4)	
History of MI	316 (72.3)	275 (70.9)	41 (83.7)	0.06
Prior PCI	225 (51.5)	205 (52.8)	20 (40.8)	0.11
Prior CABG	121 (27.7)	103 (26.5)	18 (36.7)	0.13
Hypertension	231 (52.9)	205 (52.8)	26 (53.1)	0.98
Diabetes mellitus	128 (29.3)	111 (28.6)	17 (34.7)	0.38
Documented hypercholesterolemia	356 (81.5)	314 (80.9)	42 (85.7)	0.42
Documented family history of premature CAD	95 (21.7)	85 (21.9)	10 (20.4)	0.66
Smoking status				0.05
<i>Yes</i>	45 (10.3)	36 (9.3)	9 (18.4)	
<i>Ex-smoker</i>	242 (55.5)	213 (55.0)	29 (59.2)	
<i>No</i>	149 (34.2)	138 (35.7)	11 (22.4)	
Baseline AF	73 (16.7)	60 (15.5)	13 (26.5)	0.05
NYHA functional class				0.47
<i>I</i>	145 (33.3)	132 (34.2)	13 (26.5)	
<i>II</i>	197 (45.3)	171 (44.3)	26 (53.1)	
<i>III or IV</i>	93 (21.4)	83 (21.5)	10 (20.4)	
Medications				

Antithrombotic therapy	420 (96.1)	371 (95.6)	49 (100.0)	0.14
Beta-blocker	338 (77.3)	299 (77.1)	39 (79.6)	0.69
ACEi/ARB	368 (84.2)	320 (82.5)	48 (98.0)	0.005
Lipid-lowering drug	386 (88.3)	340 (87.6)	46 (93.9)	0.20
CMR volumetric measurements				
LVEF (%)	47 (17)	49 (17)	37 (14)	<0.001
Indexed LV mass (g/m ²)	79 (24)	78 (24)	91 (27)	<0.001
LVEDVi (ml/m ²)	107 (41)	103 (38)	136 (50)	<0.001
RVEF (%)	58 (13)	59 (12)	55 (15)	0.08
CMR LGE characteristics				
Presence of infarct pattern LGE	378 (86.5)	329 (84.8)	49 (100.0)	0.003
Predominant territory				0.75
<i>Anterior</i>	172 (45.5)	152 (46.2)	20 (40.8)	
<i>Lateral</i>	54 (14.3)	47 (14.3)	7 (14.3)	
<i>Inferior</i>	152 (40.2)	130 (39.5)	22 (44.9)	
PIZ mass (g), median (IQR)	8.8 (4.2-14.4)	7.9 (3.4-13.1)	15.8 (10.8-23.1)	<0.001
Infarct core mass (g), median (IQR)	17.6 (6.5-30.2)	15.6 (5.0-27.5)	34.2 (23.6-44.2)	<0.001
ACEi = angiotensin-converting enzyme inhibitor; AF = atrial fibrillation; ARB = angiotensin II receptor blocker; aSCD = aborted sudden cardiac death; BMI = body mass index; CABG = coronary artery bypass grafting; CAD = coronary artery disease; CMR = cardiovascular magnetic resonance; DBP = diastolic blood pressure; IQR = interquartile range; LGE = late gadolinium enhancement; LVEDVi = indexed left ventricular end-diastolic volume; LVEF = left ventricular ejection fraction; MI = myocardial infarction; NA = not applicable; NYHA = New York Heart Association; RVEDVi = indexed right ventricular end-systolic volume; RVEF = right ventricular ejection fraction; PIZ = peri-infarct zone; PCI = percutaneous coronary intervention; SCD = sudden cardiac death; SBP = systolic blood pressure. *Patients with evidence of severe CAD or a history of prior coronary revascularisation. Continuous variables are reported as mean (standard deviation) or median (interquartile range). Categorical variables are reported as N (%).				

Appendix Table 20: Reproducibility analysis for the scar quantification data

	Intraobserver ICC	Interobserver ICC	ICC between serial scans*
Core infarct mass	0.85	0.85	0.77
PIZ mass	0.90	0.77	0.87
Reproducibility data for core infarct mass and PIZ mass quantification using intraclass correlation coefficient calculation. * calculated in patients who had 2 clinical scans without documented evidence of an incident myocardial infarction between studies. ICC = intraclass correlation coefficient; PIZ = peri-infarct mass			

Appendix Table 21: Summary of univariable and multivariable Cox regression analysis for the primary endpoint.

	Univariable analysis		Multivariable analysis			
			Model A ⁺		Model B ⁺⁺	
Variable	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Demographics						
Age (per 10 years)	0.83 (0.63, 1.10)	0.19	-	-	0.96 (0.71, 1.29)	0.78
Female	0.24 (0.06, 0.99)	0.05	-	-	0.33 (0.08, 1.39)	0.13
Caucasian	0.88 (0.45, 1.72)	0.71	-	-	-	-
BMI (kg/m2)	1.05 (1.00, 1.11)	0.04	-	-	-	-
Heart rate (per 10 bpm)	1.05 (0.86, 1.30)	0.62	-	-	-	-
SBP (per 10 mmHg)	0.88 (0.75, 1.03)	0.11	-	-	-	-
DBP (per 10 mmHg)	0.88 (0.69, 1.12)	0.30	-	-	-	-
Significant CAD*	2.32 (0.32, 16.84)	0.40	-	-	-	-
CAD type		0.65	-	-	-	-
<i>Single vessel</i>	0.77 (0.38, 1.57)					
<i>2 vessels</i>	1.08 (0.55, 2.13)					
<i>3 vessels</i>	Reference group					
History of MI	2.08 (0.97, 4.43)	0.06	-	-	-	-
Prior PCI	0.59 (0.34, 1.05)	0.07	-	-	-	-
Prior CABG	1.62 (0.91, 2.90)	0.10	-	-	-	-
Hypertension	1.00 (0.57, 1.75)	1.00	-	-	-	-
Diabetes mellitus	1.48 (0.82, 2.67)	0.19	-	-	-	-
Documented hypercholesterolemia	1.32 (0.59, 2.94)	0.50	-	-	-	-
Documented family history of premature CAD	0.87 (0.62, 1.22)	0.42	-	-	-	-
Smoking status		0.01	-	-		0.04
<i>Yes</i>	Reference group				Reference group	
<i>Ex-smoker</i>	1.74 (0.87, 3.50)				1.30 (0.64, 2.65)	
<i>No</i>	3.24 (1.34, 7.82)				2.71 (1.08, 6.80)	

Baseline AF	1.95 (1.04, 3.69)	0.04	-	-	2.37 (1.19, 4.75)	0.02
NYHA functional class			-	-	-	-
<i>II, III or IV</i>	1.60 (0.85, 3.02)	0.15	1.20 (0.63, 2.30)	0.58	-	-
Medications						
Antithrombotic therapy	6.2e+14 (0.00, .)	1.00	-	-	-	-
Beta-blocker	1.24 (0.62, 2.48)	0.55	-	-	-	-
ACEi/ARB	10.06 (1.39, 72.93)	0.02	-	-	4.70 (0.64, 34.57)	0.13
Lipid-lowering drug	1.94 (0.60, 6.23)	0.27	-	-	-	-
CMR volumetric measurements						
LVEF (%)	0.96 (0.94, 0.97)	<0.001	-	-	0.99 (0.97, 1.02)	0.63
LVEF <35%	3.22 (1.84, 5.65)	<0.001	1.65 (0.90, 3.03)	0.11	-	-
Indexed LV mass (g/m2)	1.02 (1.01, 1.03)	<0.001	-	-	-	-
LVEDVi (ml/m2)	8.25 (3.92, 17.36)	<0.001	-	-	-	-
RVEF (%)	0.98 (0.96, 1.00)	0.03	-	-	-	-
CMR LGE characteristics						
Presence of infarct pattern LGE	7.6e+14 (0.00, .)	1.00	-	-	-	-
Predominant territory		0.71	-	-	-	-
<i>Anterior</i>	Reference group					
<i>Lateral</i>	1.16 (0.49, 2.75)					
<i>Inferior</i>	1.29 (0.70, 2.36)					
PIZ mass (g)	1.12 (1.09, 1.15)	<0.001	1.07 (1.02, 1.12)	0.002	1.07 (1.02, 1.12)	0.005
Infarct core mass (g)	1.05 (1.04, 1.06)	<0.001	1.03 (1.01, 1.05)	0.01	1.02 (1.00, 1.05)	0.03

ACEi = angiotensin-converting enzyme inhibitor; AF = atrial fibrillation; ARB = angiotensin II receptor blocker; BMI = body mass index; CABG = coronary artery bypass grafting; CAD = coronary artery disease; CI = confidence interval; CMR = cardiovascular magnetic resonance; DBP = diastolic blood pressure; HR = hazard ratio; LGE = late gadolinium enhancement; LVEDVi = indexed left ventricular end-diastolic volume; LVEF = left ventricular ejection fraction; MI = myocardial infarction; NYHA = New York Heart Association; RVEF = right ventricular ejection fraction; PIZ = peri-infarct zone; PCI = percutaneous coronary intervention; SBP = systolic blood pressure. *Patients with evidence of severe CAD or a history of prior coronary revascularisation. [†]Model A: To align with current clinical guidelines for ICD implantation, a multivariable model using binary cut-offs of LVEF <35% and NYHA class >1 was generated with subsequent addition of the LGE quantification data. ^{††}Model B: To select this model, a forward stepwise procedure was applied with $p < 0.10$ as the criterion for inclusion, forcing in recognised predictors of the outcome (age, sex and LVEF) with simultaneous addition of the LGE quantification data. A limited number of robust variables were chosen to prevent creating an overly complex model. Not all variables that were associated with the primary endpoint on univariable analysis were used in the multivariable model (e.g indexed LV mass and RVEF).

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