Cardiac MRI To Assess The Cardioprotective Efficacy Of Remote Ischaemic Perconditioning In Patients With ST-Elevation Myocardial Infarction.

MD (Res) Thesis

UCL

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2013

Declaration

I, Jonathan Michael Hasleton, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis

Signature.....

Name: Jonathan Michael Hasleton Date 2/7/2013

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ACKNOWLEDGMENTS

I would like to thank the subjects who agreed to take part in this research study despite their intercurrent illness.

I wish to thank my supervisors, Professor Derek Yellon, Dr Derek Hausenloy and Dr James Moon for their support and guidance during my research and subsequent writing of this thesis.

Additional thanks go to my colleagues, Dr Girish Babu, Dr Edney Boston-Griffiths, Dr Andrew Flett and Dr Dan Sado who have offered immeasurable help and encouragement.

Thank you to my parents, Sandi and Philip Hasleton and also my parents-in-law, Avril and Tony Blasebalk, for their ongoing wisdom and kindness.

אחרון אחרון חביב, thanks are long overdue to my wife, Emma and children, Otti, Meital, Rayut, Sivan and Naftali who offer continuing support, delight and ongoing forbearance.

Abstract

The optimal treatment for acute myocardial infarction (AMI) requires the rapid restoration of flow in the infarct related artery and reperfusion of the myocardium at the tissue level through angioplasty. Coronary angioplasty, in the setting of AMI, is aimed at reducing myocardial infarct size, preserving left ventricular function and improving long term outcomes. Paradoxically, the opening of an occluded vessel following a period of ischaemia can result in further myocyte death. This is called lethal reperfusion injury. Current treatments for AMI have failed to attenuate reperfusion injury and combined morbidity and mortality rates remain high. There is an urgent need for the development of novel cardioprotective therapies in the clinical arena.

Remote ischaemic perconditioning describes the cardioprotective effect evoked by applying brief episodes of ischaemia and reperfusion applied to a limb with a blood pressure cuff prior to reperfusion but following the onset of ischaemia. Such strategies have been utilised in a number of proof of concept studies demonstrating the ability of remote ischaemic conditioning to attenuate reperfusion injury and reduce infarct size.

As therapies for AMI improve, it has become increasingly difficult to demonstrate incremental improvements in currently used biomarkers. Cardiovascular magnetic resonance (CMR) imaging is an important imaging technique that is able to assess traditional imaging parameters with a high degree of accuracy and reproducibility. CMR is also provides supplementary information due to its unique ability to characterise the myocardium and identify the components of reperfusion injury. The

identification and quantification of these adverse elements of reperfusion offer further prognostic information beyond traditional imaging biomarkers and show promise as novel endpoints in clinical cardiovascular research.

Data is presented on the use of remote ischaemic perconditioning in patients undergoing primary angioplasty. CMR endpoints have been utilised to assess the efficacy of this conditioning protocol in a high-risk patient group.

1. INTRODUCTION

1.1 Ischaemic Heart Disease

1.1.1 Epidemiology of Ischaemic Heart Disease

Ischaemic heart disease (IHD) is the leading cause of morbidity and mortality worldwide, particularly in middle to high-income countries. Over seven million people every year die from coronary artery disease, accounting for 12.8% of all deaths.[1] Cardiovascular death rates in England have been falling but cardiovascular disease remains the main cause of death with up to 88,000 deaths attributable to IHD in 2008.[2] It also remains the most common cause of premature death (death before the age of 75) and heart failure in the UK with over 28,000 premature deaths in the UK in 2008.[2]

The decline in IHD mortality rates does not necessarily mean a decline in IHD absolute number of deaths and the absolute number of IHD deaths may continue to increase due to ageing of the population.[3] At the same time, the decline in death rates does not translate into a decline in the global burden of disease caused by IHD, which remains extremely high. Looking at health care costs in isolation, IHD cost the UK £3.2 billion in 2006.[2]

Whilst there has been a reduction in the incidence of ST-elevation myocardial infarction (STEMI) over the last decade,[4] there has been an associated increase in the incidence of non-ST elevation myocardial infarction (NSTEMI) as well as an increase in the number of patients with heart failure.[5] The age- and sex-adjusted incidence of acute myocardial infarction (AMI) has decreased over the last decade.[6] Changes in the prevalence of emerging cardiovascular risk factors, such as diabetes and obesity, or changing demographic characteristics of patients hospitalised with acute myocardial infarction, may have differentially promoted the development of NSTEMI.[7] At the same time the introduction of cardiac biomarkers to aid the diagnosis of myocardial necrosis will have increased the pick-up rate of infarction in patients with otherwise normal electrocardiograms.

The mortality of STEMI is influenced by many factors, among them: age, Killip class, time delay to treatment, mode of treatment, history of prior myocardial infarction, diabetes mellitus, renal failure, number of diseased coronary arteries, ejection fraction, and treatment.[8] Observational data suggests that this reduction in mortality in STEMI patients is due in part to a marked increase in interventional therapy (primary angioplasty) and changes in pharmacological therapy, including increases in use of β-blockers, statins, ACE inhibitors (or ARBs), and thienopyridines in patients with an acute coronary syndrome (ACS), and Glycoprotein IIb/IIIa inhibitors.[9] These improvements will have been driven by increased use of evidence-based treatments, better adherence to treatment guidelines, and reduced variation across hospitals.[10]

1.1.2 Definition of Acute Myocardial Infarction

IHD is a manifestation of coronary artery disease or atherosclerosis. Atherosclerosis is a chronic and multifocal immune-inflammatory, fibro-proliferative disease of medium-sized and large arteries mainly driven by lipid accumulation.[11] Build up of lipid in the coronary arteries over time leads to the development of a coronary atherosclerotic fibrolipid plaque. As plaques develop they can encroach into the arterial lumen. Plaques will often therefore disrupt blood flow in the coronary vessels by causing luminal obstruction leading to myocardial ischaemia - an imbalance between myocardial perfusion and demand. Pathological, imaging, and biological observations have demonstrated that atherosclerotic plaques rupture or erode, with differing degrees of superimposed thrombosis and distal embolisation, resulting in myocardial hypoperfusion, forming the basic pathophysiological mechanisms in most conditions of ACS.[12] Thrombotic occlusion of an epicardial coronary artery causes ischaemia and subsequent myocardial cell death if epicardial patency is not promptly restored. Acute myocardial infarction is defined as a clinical (or pathologic) event caused by myocardial ischaemia in which there is evidence of myocardial injury or necrosis. ESC and AHA guidelines define criteria for acute myocardial infarction as a rise and/or fall of cardiac biomarkers, along with supportive evidence in the form of typical symptoms, suggestive electrocardiographic changes, or imaging evidence of new loss of viable myocardium or new regional wall motion abnormality.[13]

1.2 Ischaemia – Reperfusion Injury

Despite an almost 50% reduction in the number of deaths from IHD in the UK over the last fifty years,[14] the one year cardiovascular death rate is approximately 9% with hospitalisation for heart failure at 10%. Reperfusion is essential to salvage viable myocardium but paradoxically reperfusion itself can lead to further damage to the myocardium, the so-called “double-edged sword” of reperfusion or lethal reperfusion injury.[15] Protecting the heart against lethal reperfusion injury through the application of interventional strategies at the onset of reperfusion provides a novel approach to cardioprotection. It is necessary, therefore, to discover novel treatment strategies which will provide further protection to the myocardium from ischaemia-reperfusion injury, and can be used as adjunctive therapy to the current reperfusion strategies to further reduce morbidity and mortality.

Although the prompt restoration of blood flow, by restoring epicardial patency, to the ischaemic myocardium is the optimal endpoint for patients suffering an STEMI, the onset of reperfusion itself is associated with myocardial injury itself. This injury, entitled ‘Reperfusion injury’, attenuates the benefits of reperfusion therapies such as primary angioplasty.

There are four types of reperfusion injury:

a. Reperfusion-induced arrhythmias

b. Myocardial Stunning

c. Microvascular Obstruction (MVO)

d. Lethal Reperfusion Injury

1.2.1 Reperfusion induced arrhythmias

Reperfusion induced arrhythmias are frequently seen in patients receiving reperfusion therapies, such as primary angioplasty. The most commonly associated arrhythmia seen is an accelerated idioventricular rhythm (AIVR). Ventricular tachycardia and fibrillation remain the most important causes of sudden death following restoration of antegrade coronary flow. AIVR was previously thought to be a marker of coronary artery patency following thrombolysis.[16] However, although reperfusion arrhythmias are thought to be treatable and reversible, recent data suggests that their presence is a signal of adverse cellular response to infarct artery recanalisation, as indicated by larger infarct size.[17]

1.2.2 Myocardial Stunning

Myocardial stunning is prolonged post ischaemic contractile dysfunction of the myocardium salvaged by reperfusion. This dysfunction can persist for several hours after transient non lethal ischaemia but eventually is followed by full functional recovery.[18]

1.2.3 Microvascular obstruction

Microvascular obstruction manifests in the catheter laboratory as coronary no-reflow. However the cause of coronary no reflow is microvascular obstruction - the inability to reperfuse a previously ischaemic region. The actual pathogenesis of this is unclear but has been attributed to a variety of factors such as capillary damage, endothelial and cardiomyocyte swelling, compression of capillaries, oxidative stress and inflammation.

In 1966 Krug *et al*.[19] noted a prolonged disturbance of blood supply following reperfusion of a region supplied by a temporarily ligated coronary artery. Using a feline heart they demonstrated abnormalities of the 'deep' myocardial circulation after episodes of ischaemia and reperfusion. The group showed interesting changes on histological sections with interstitial oedema and dilated capillaries, which were partially ruptured and packed with erythrocytes. The interstitial space was noted to be wider than normal with recognisable haemorrhage.

The ultrastructural features were further characterised in the canine model by Kloner and colleagues in 1974.[20] The group used Thioflavin S which is a fluorescent vital stain for endothelium and allows for the measurement of the qualitative distribution of coronary flow following ischaemia-reperfusion. Intravenous Thioflavin S was injected simultaneously with the release of coronary occlusion or after reperfusion. After 40 minutes of ischaemia and reperfusion, staining revealed a homogenous distribution of Thioflavin S indicating successful reperfusion into previously ischaemic tissue. However, after 90 minutes of circumflex occlusion and reperfusion, there were subendocardial areas noted which did not take up the stain indicating a lack of capillary perfusion or no-reflow. Electron microscopy showed that the major features within the no-reflow areas were ultrastructural evidence of MVO postulated to be due to due to oedema and endothelial disruption.[21]

Radioactive and fluorescent microspheres have also been used in the experimental setting to demonstrate the presence of MVO.[22,23] Microsphere methods provide information on regional perfusion within organs and are an essential tool in cardiovascular research.[24] Unfortunately, both the use of microspheres and Thioflavin S staining require post-mortem evaluation, therefore precluding the serial examination of MVO in the same animal and its application to humans.

Essentially, multiple factors (Tissue oedema, endothelial disruption, plugging of capillaries by neutrophils and microthrombi, inflammation due to the generation of oxygen-free radicals and activation of complement components, and contracture of neighbouring myocytes)[25,26] converge to reduce perfusion at a microvascular level.

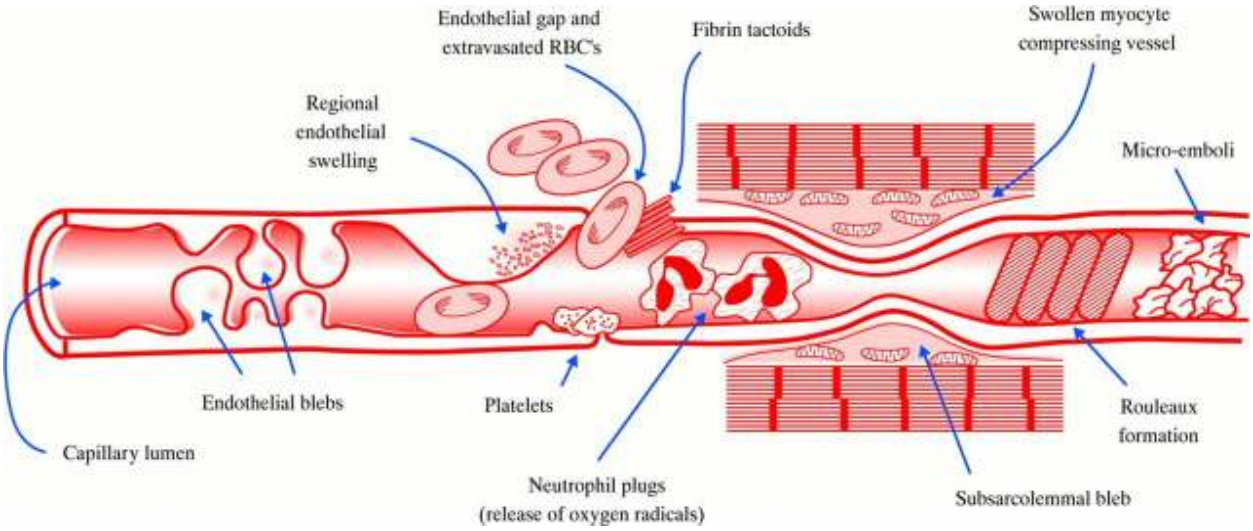

Figure 1 Schematic Summarising different mechanisms of no-reflow and accompanying ultrastructural alterations of the microvascular bed (RBC, red blood cell)

Reprinted with permission from Reffelmann T, Kloner RA Heart 2002;87:164.

The consequences of coronary ligation of a non-atherosclerotic coronary artery, however, cannot be directly extrapolated to the human situation, where myocardial infarction is caused by occlusive coronary thrombosis superimposed onto an atherosclerotic unstable plaque.[27] Angioplasty leads to distal coronary microembolisation of atherosclerotic debris or thrombotic material that is responsible for a substantial part of clinically observed MVO.[28] Microemboli are composed of platelets, leukocytes, erythrocytes, and atherosclerotic material, including cholesterol crystals and; platelet-leukocyte aggregates that contribute to impaired microcirculation.[29] The theory of microembolisation of friable material released from atherosclerotic plaques both at the point of plaque disruption prior to coronary occlusion and also following angioplasty would appear logical in humans. However in experimental models no-reflow occurs in the absence of atherosclerosis and thrombi; whereas human hearts with acute myocardial infarction are likely to have both.

Therefore human hearts that undergo reperfusion therapy for an acute myocardial infarction have the potential for microvascular obstruction due to a number of causes.[30]

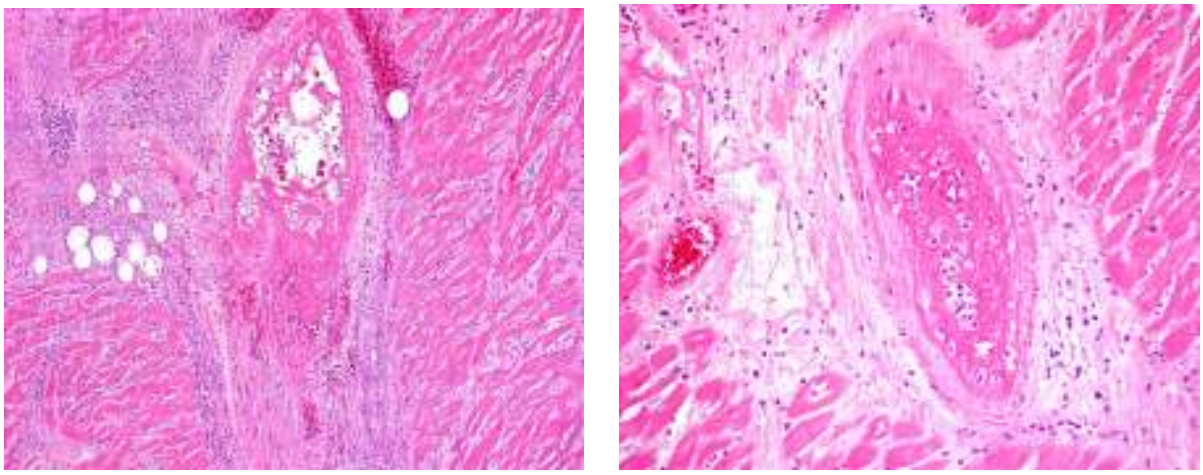

Figure 2 Histopathological slides showing a cross section through a coronary artery with evidence of microvascular obstruction
Images Courtesy of Dr P Gallagher, Department of Pathology, University of Southampton.

A number of techniques can be employed alone or in combination to demonstrate the presence of microvascular obstruction. It is clear that the presence of microvascular obstruction is a predictor of poorer outcomes. However, the ability to demonstrate a worse prognosis does not necessarily enable a quantitative approach. The ideal modality would not only identify the presence of MVO and therefore add to our knowledge of its incidence but also allow us to quantify the amount of MVO. The ability to quantify MVO in turn enables its use as an endpoint in clinical trials for assessing the efficacy of novel cardioprotective therapies, reduce sample size necessary for such studies and will further aid risk stratification in patients following acute infarction.

1.2.3.1 Coronary Angiography and No Reflow

Coronary angiography is often the first point in the evolution of a myocardial infarction that 'no-reflow' is identified and a number of indices are used to identify the 'no-reflow' syndrome. The first, and most basic is TIMI (Thrombolysis in Myocardial Infarction) flow. This is a widely used scale from 0-3, referring to blood flow seen down a coronary artery on angiography during percutaneous coronary intervention. There are also limitations of using coronary angiography to assess for capillary reperfusion. Although TIMI grades 1 and 2 flow are known to indicate poor tissue perfusion, TIMI grade flow 3 has generally been thought to represent successful reperfusion. Studies have however shown that other markers of tissue perfusion have shown that 10-20% of patients with TIMI grade flow 3 have poor perfusion at a myocardial level despite epicardial patency.[31]

For those patients who after primary angioplasty appear to have TIMI 3 flow, up to 40% of these patients will still have microvascular obstruction that often remains undiagnosed unless different forms of imaging are used to delineate it. So up to 40% of patients with TIMI 3 flow on coronary angiography at the end of primary angioplasty procedure will still not be perfusing there myocardium and this patient group fares worse post STEMI.

There are other clinical indicators that we can use post STEMI including:

1.2.3.2 The ECG and No Reflow

The ECG is the most simple and commonly used diagnostic test for identifying ischaemia and infarction. ECGs are routinely performed before and after primary angioplasty for acute infarction. The resolution of ST-elevation has been taken as a marker of success of reperfusion in this setting. A number of methods exist to quantify the extent of ST-segment resolution (STR). The majority of studies use the sum of STR across a 12-lead ECG. The sum of ST segment deviation is calculated before and after fibrinolysis or angioplasty. STR has also been looked at in a single lead by examining the lead with the maximum ST segment deviation. The sum STR has been shown to correlate well with infarct related artery (IRA) patency and TIMI flow yet these do not necessarily translate into complete microvascular reperfusion. The interpretation of STR data suffers from variability between studies, in that methodology, definitions and time points of measurements differ between institutions.[27] It is also important to note that differences exist between anterior and inferior MI with regard to ST segment resolution. Patients with anterior infarction develop significantly less ST resolution than those with inferior infarction, despite only small differences in epicardial blood flow, therefore suggesting that ST segment resolution is a less accurate predictor of epicardial reperfusion among patients with anterior than inferior MI.[32]

Just as the absence of ST-segment resolution does not always exclude the occurrence of reperfusion, albeit at a slower pace, ST-segment elevation also eventually resolves in acute infarction even in the absence of reperfusion.[31]

1.2.3.3 Myocardial Contrast Echocardiography (MCE)

Contrast echocardiography has been recommended to assess the myocardium for the presence of no-reflow. There are many benefits to echocardiography including the availability, transportability and price. Contrast echocardiography utilises gas-filled microbubbles that are very effective in scattering of ultrasound.[33] Furthermore the microbubbles are generally small in size (< 5μm) and should not block capillaries whilst being retained in the vascular space, so can be can used to define microvascular perfusion.[34] MCE likely underestimates the incidence and extent of MVO with studies using MCE suggesting the presence of no-reflow in 25-30% patients with AMI.[35] Cardiovascular Magnetic Resonance (CMR) studies suggest that the incidence of no-reflow is a lot higher at 50%.[36] Safety concerns exist with administration of contrast particularly in the setting of potentially unstable patients including acute coronary syndromes. Theoretically microbubbles bigger than the capillary may exacerbate no-reflow through mechanical obstruction of the vessel. [37]

1.2.3.4 Cardiac Magnetic Resonance Imaging (CMR)

The use of CMR to assess microvascular obstruction will be discussed at length later further on in this thesis.

1.2.4 Lethal Myocardial Reperfusion Injury

Lethal myocardial reperfusion injury is the most harmful form of reperfusion injury and is defined as the death of cardiomyocytes, which were viable at the end of the ischaemic phase.[38] At the point of reperfusion where you have ischaemically damaged but viable cardiomyocytes, the process of reperfusion tips them into necrosis, irreversibly damaging them. The main evidence that reperfusion injury exists, in animal studies, is that by giving an intervention at reperfusion, you can reduce the infarct size by up to 40-50%. This suggests that 40-50% of the final infarct is determined by reperfusion itself. The existence of lethal reperfusion injury (particularly in man) as a separate entity distinct from cellular damage initiated by ischaemia and then exacerbated by reperfusion has caused controversy. Given the difficulty in predicting the onset of an ischaemic insult it was difficult to investigate the effect of reperfusion compared to that of ischaemia alone. Becker demonstrated larger numbers of irreversibly injured cells after reperfusion than before reperfusion in cardiac biopsy specimens.[39] Further evidence and confirmation of the existence of reperfusion injury came from Zhao *et al*. Their group demonstrated in a canine model that applying an intervention at the point of reperfusion (three cycles of thirty second I/R) was as effective as pre-conditioning in reducing reperfusion injury in the form of myocardial oedema and infarct size.[40] They were therefore able to show that reperfusion induced the death of myocytes that were viable (or reversibly damaged) at the transition point from ischaemia to reperfusion. The first demonstration that reperfusion injury existed in man was published by Staat *et al*. in 2005. The group showed how a postconditioning protocol (four balloon inflations of 60 seconds each, interrupted by 60 seconds of reperfusion) after direct stenting in STEMI patients

undergoing PPCI significantly reduced infarct size (measured by reduction in CK over 72 hours).[41]

Effective therapies for treating reperfusion injury that have translated into mortality benefit are however lacking.

A number of potential mechanisms have been postulated to act as mediators of lethal reperfusion injury.

1.2.4.1 Reactive Oxygen Species (ROS)

Reoxygenation of the myocardium following ischaemia causes production of ROS with a burst of oxidative stress. While low levels of oxygen radicals and oxidants are normally formed in cells and play important roles in cellular homeostasis, mitosis, differentiation, and signalling, following ischaemia and reperfusion radical formation is greatly increased triggering myocardial injury.[42]

The main source of oxidative stress in the reperfused myocardium is inflammatory cells. At the same time the mitochondria also remain an important site of ROS formation. Active neutrophils can release significant amounts of superoxide and hypochlorous acid from their nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and myeloperoxidase systems, respectively. The superoxide anion is broken

down to hydrogen peroxide (H_2O_2) by superoxide dismutase, and then to hydroxyl radical. In addition, the myeloperoxidase (MPO) system, converts H_2O_2 to hypochlorous acid (HOCl) in the presence of halides. HOCl oxidizes various amines to toxic chloramines.[38] The accumulation of ROS in cells can lead to reversible and irreversible damage to cells and tissue through a number of actions including oxidative modifications of proteins (denaturation of proteins that form enzymes and ion channels), lipids (lipid peroxidation leading to membrane damage and calcium overload), DNA, Sarcoplasmic reticulum (leading to calcium dyshomeostasis and contractile dysfunction) along with impairment of metabolism and activation of adhesion molecules on the vascular endothelium. These actions eventually lead to loss of the cells molecular functions.[43,44]

ROS have also been shown to have deleterious effects on the cell survival by reducing the bioavailability of nitric oxide (NO). The role of NO in cardioprotection is multifaceted although endogenous NO-synthase derived NO does not appear to be directly involved in triggering or mediating the early phase of ischaemic preconditioning protection.[45] The overall role as to whether NO acts as a cardioprotective agent or promotes cell death remains unclear. Certain groups have demonstrated NO as a mediator of cardioprotection[46] whereas others have shown it to induce apoptosis.[47] Bell *et al.* have suggested a dose dependent effect where low dose NO can protect the myocardium through mitochondrial depolarisation and subsequent inhibition of mitochondrial Ca^{2+} overload.[48] Higher concentrations of NO appear to lead to loss of protection and when combined with superoxide, contribute to cell death.[49]

Oxidative stress is also implicated in the opening of the mitochondrial permeability transition pore (mPTP), whose irreversible opening at the onset of myocardial reperfusion is a critical mediator of lethal myocardial reperfusion injury. Excessive ROS generation not only facilitates cell death through mPTP opening but prolonged pore opening also leads to an increase in mitochondrial ROS formation in cardiac myocytes[50] creating a vicious cycle to promote lethal reperfusion injury.[51]

1.2.4.2 Calcium Overload

Following the advent of ischaemia, there is a shift to anaerobic glycolysis with the subsequent production of H^+ ions secondary to the breakdown of ATP molecules. The lack of ATP leads to an intracellular build up of Na^+ as the energy dependent Na^+ / K^+ -ATPase fails to export Na^+ from the cell. At the same time the drop in intracellular pH leads to the activation of the Na^+ / H^+ exchanger with subsequent influx of Na^+ ions. The ischaemia-induced inhibition of the Na/K ATPase and the failure of intracellular Na^+ to recover completely on reperfusion have been shown to be important determinants of electrical and contractile dysfunction in the ischaemic /reperfused myocardium.[52] The intracellular rise in Na^+ is linked with the rise in Ca^{2+} as Na^+ ions compete with calcium for extrusion via the Na^+/Ca^{2+}exchanger (NCX), resulting in a build up of intracellular calcium.[53] The consequences of Ca^{2+} overload during ischaemia include cell-to-cell uncoupling, opening of connexin hemichannels and mitochondrial Ca^{2+} overload.[54]

Reperfusion of the ischaemic and already Ca^{2+} overloaded myocardium exacerbates cellular injury further with worsening of the intracellular Ca^{2+} load. The further influx of Ca^{2+} ions is mediated by the already present intracellular Na^{+} overload with reversal of the sarcolemmal Na^{+}/Ca^{2+}-exchanger. Restoration of the mitochondrial membrane potential at reperfusion also allows entry of Ca^{2+} into the mitochondria. Other mechanisms involve the rapid normalisation of pH, which enables the reactivation of proteins that were inhibited during the acidotic ischaemic phase which are involved in Ca^{2+} regulation. Excess of Ca^{2+} induces cardiomyocyte death by causing hypercontracture of the heart cells (representing a significant shortening of the cell, which is caused by strong contractile activation but becomes irreversible by disruption of cytoskeletal structures.),[55] MPTP opening and caplain mediated proteolysis.

1.2.4.3 Restoration of Physiological pH.

As noted above, the onset of ischaemia is associated with a drop in intracellular pH due to a switch to anaerobic metabolism and the ensuing build-up of lactate and accumulation of H^{+} ions following the breakdown of ATP. The acidotic environment is not conducive to a number of noxious cellular activities which become inhibited at the abnormally low pH. These include the hypercontracture of myocytes, calpain mediated proteolysis and opening of the mPTP. Yet whilst acidosis has been shown to protect against cell death during ischaemia, the rapid normalisation of pH contributes significantly to reperfusion injury and loss viability of cardiac myocytes.[56]

Reperfusion of the myocardium prompts the activation of the Na^+/H^+ exchanger and the $Na^+/HCO3^-$ symporter to restore physiological pH,[57] with a reduced intracellular H^+ load but increased Na^+ load (favouring Ca^{2+} accumulation within the cell). Normalisation of pH contributes to reperfusion injury through hypercontracture and MPTP opening.[42,58]

1.2.4.4 The Mitochondrial Permeability Transition Pore (mPTP).

Whilst discussing different mediators of lethal reperfusion injury above it becomes clear that the mPTP plays a central role in facilitating cell death. Cardiac mitochondria play an important role in all three major pathways of cell death (which will be discussed later): apoptosis, necrosis and autophagy, with mPTP opening facilitating in particular necrotic and apoptotic cell death. The mPTP is a voltage dependent high conductance channel located in the mitochondrial inner membrane. The exact molecular composition of the pore remains to be elucidated. Three main proteins were previously thought to be involved including a mitochondrial peptidyl-prolyl cis–trans isomerase known as cyclophilin-D, the adenine nucleotide translocase (ANT) and the mitochondrial phosphate carrier (PiC).[59] It has since been shown that mPTP opening can occur without the involvement of cyclophilin-D or ANT and that cyclophilin-D is the mitochondrial receptor for cyclosporin and modulates the PTP rather than being a structural pore component.[60] ANT is also likely a regulatory component of the pore whereas the role of PiC is less well understood.[61]

The mPTP was first described in 1979 by Haworth and Hunter who demonstrated how the permeability of mitochondria which have lost their endogenous protection could be modulated over a wide range by adjusting the level of Ca^{2+}. They surmised that the 'simplest molecular mechanism to account for the phenomena' was the presence of a 'trans-membrane hydrophilic channel' where the response to Ca^{2+} was interpreted to be a gating effect at the internal end of hydrophilic channels. These channels spanned the inner mitochondrial membrane.[62] A decade later it was shown that Ca^{2+} and oxidative stress synergistically promoted the reversible opening of the pore lending credence to its facilitation of ischaemia-reperfusion injury.[63] The pore remains closed under ischaemic conditions with hypoxia de-energising the mitochondria which are therefore unable to accumulate large amounts of Ca^{2+}, and the low intracellular pH, caused by the large increase in glycolytic lactic acid production, causes powerful inhibition of pore opening.

Pore opening is therefore enhanced with reperfusion and the reversal of these conditions.[64]

Pore opening results in a number of processes, the end point of which are usually cell death. Firstly the increased permeability to protons leads to dissipation of the two components of the proton motive force (pmf), the pH gradient and the membrane potential. In the absence of a pmf, mitochondria cannot synthesise ATP via oxidative phosphorylation and the ATPase goes into reverse and starts to breakdown the ATP produced by glycolysis. Therefore, myocytes cannot maintain ATP levels and the consequent disruption of metabolism and ionic homeostasis leads to uncoupling of the mitochondria and necrotic cell death.[65]

Secondly the opening of the pore also results in rapid exchange of solutes up to 1.5 kDa in size, with redistribution of nicotinamide adenine dinucleotide (NADH) to the cytosol and an influx of cytosolic water. This is because the matrix protein concentration is very high, exerting a colloidal osmotic pressure leading to swelling of the matrix compartment, causing matrix expansion. The inner mitochondrial membrane can accommodate this increase in volume since the cristae can unfold, whereas the outer mitochondrial membrane (which has a smaller surface area) cannot and following rupture releases the contents of the inter-membranous space. These contents include pro-apoptotic factors, such as cytochrome c and apoptosis-inducing factor, which are released into the cytosol, where the process of apoptosis is initiated.[65] The catastrophic mitochondrial swelling often leads to cell death before apoptosis can proceed, due to energetic collapse and uncontrolled enzymatic processes.[53,66]

Whilst oxidative stress promotes mPTP opening and damage to the mitochondria, this can also lead to the generation of mitochondrial ROS, setting up a vicious cycle of impaired mitochondrial function and the exacerbation of ROS induced cardiomyocyte damage. This has been labelled ROS-induced ROS release (RIRR), and ROS generation in only small numbers of mitochondria can affect neighbouring mitochondria, eventually propagating the ROS surge to the whole cell through this positive feedback loop.[67]

1.3 Mechanisms of myocyte cell death

Three main mechanisms of myocyte cell death are notable during ischaemia reperfusion injury including necrosis, apoptosis and autophagy. Parameters that indicate unequivocal cell death include loss of plasma membrane integrity, cellular fragmentation, and phagocytosis by neighbouring cells. Although necrosis and apoptosis are distinct forms of cell death with clearly distinguishing morphological and biochemical features, the two types of death can occur simultaneously in tissues or cell cultures exposed to the same stimulus with the intensity of the same initial insult deciding the prevalence of either apoptosis or necrosis.[68]

1.3.1 Necrosis

Necrosis was previously felt to be an unregulated process but more recent data has suggested that the events are actively regulated occurring in a programmed manner-termed necroptosis or programmed cell necrosis.[69] The distinctive features of necrosis include loss of plasma membrane integrity, cellular and organelle swelling, and marked inflammation. ATP levels are markedly reduced in necrotic cells, due to severe mitochondrial damage that disables ATP generation as well as unrestrained energy expenditures.[70] Apoptosis was first described in 1971 by Kerr as a form of 'shrinkage necrosis' in an experimental model of hepatic ischaemia.[71]

1.3.2 Apoptosis

Apoptosis is a structured form of cell death in which cells commit to a suicide program, which leads to the elimination of the cell without inducing an inflammatory response. It is characterised by cell shrinkage, chromatin condensation, DNA fragmentation, membrane blebbing, formation of membrane -enclosed apoptotic bodies and phagocytosis of these corpses by macrophages, or occasionally, neighbouring cells.[72] ATP levels in apoptotic cells are well preserved due to continued production and decreased expenditures.[70] Apoptosis therefore will result in the death of individual cells with little effect on surrounding, often healthy, tissue. In necrosis on the other hand, the dead cell expels its contents into the interstitial space causing damage to all the surrounding cells. Hence when cells die by necrosis in the context of myocardial ischaemia and reperfusion surviving cells are rarely found in the infarct zone with all cardiomyocytes being killed up until the border zone of the infarct.[73] Programmed myocyte cell death is the major form of myocardial damage produced by occlusion of an epicardial coronary artery, whereas necrotic myocyte cell death follows apoptosis and contributes to the progressive loss of cells with time after infarction. Apoptosis representing the major independent form of myocyte cell death at 4.5 hours and myocyte necrosis peaking at one day.[74]

1.3.3 Autophagy

Autophagy, or cellular self-digestion, is a cellular pathway involved in protein and organelle degradation. Although autophagy is primarily a protective process for the cell, it also plays a role in cell death. On a basic level, autophagy represents the cells own response to starvation[75] with an intracellular recycling process in which organelles, proteins, and lipids are catabolised by lysosomal degradation providing cells deprived of nutrients with amino acids, free fatty acids, and energy.[76] Although autophagy occurs at low levels in a normal heart, the process is enhanced in a number of pathophysiological conditions including ischaemia and reperfusion. Enhancing autophagy can also promote survival in response to brief hypoxia and lower levels of oxidative stress by removing damaged and harmful organelles and the recycling of macromolecules to maintain energy levels and protein synthesis. Concurrently severe stress (prolonged hypoxia and subsequent reperfusion), results in excessive and/or long-term up regulation of autophagy causing cell death by excessive self-digestion of essential organelles and proteins.[77]

1.4 Cardioprotection

Kubler and Hass defined cardioprotection as encompassing 'all mechanisms and means that contribute to the preservation of the heart by reducing or even preventing myocardial damage'.[78] The definition has been refined further to take into account the specific pathophysiological process through which the myocardial injury is incurred. Focusing on acute ischaemia-reperfusion injury (IRI), Hausenloy uses the term 'cardioprotection' to refer to the endogenous mechanisms and therapeutic strategies that reduce or prevent myocardial damage incurred as a consequence of acute IRI.[79] One strategy that has been shown to protect the heart against acute IRI is the utilisation of the endogenous mechanisms through which the heart protects itself from outside insult. The term used to describe these strategies (pharmacological or mechanical) is ischaemic conditioning. This is a broad heading given to the concept that brief episodes of non-lethal ischaemia and reperfusion applied to an organ or tissue bestow powerful protection against a subsequent episode of sustained, lethal IRI. The conditioning stimulus can be applied at a number of time points including (i) prior to the index myocardial ischaemic event (Ischaemic preconditioning or IPC), (ii) after the advent of ischaemia but before reperfusion (Ischaemic perconditioning or IPerC) and (iii) following reperfusion (Ischaemic postconditioning or IPost). At the same time the conditioning stimulus can be applied directly to the heart or to organs/tissue remote from the heart (remote ischaemic conditioning).

1.4.1 Ischaemic Preconditioning (IPC)

IPC was first described by Murry *et al.*[80] in a seminal paper in 1986. The group demonstrated that by subjecting the canine myocardium to four 5 minute cycles of ischaemia (induced by ligation of the circumflex artery) followed by reperfusion, infarct size was reduced by 75% when the heart was then subjected to a sustained episode of 40 minutes of ischaemia. This protection disappeared when the investigators extended the period of ischaemia from 40 minutes to 3 hours.[63] Following Murry's work, ischaemic preconditioning has been reproducibly demonstrated in a number of animals including rabbits[81], rats[82], mice[83], pigs[84] and in man.

IPC stimulates a biphasic pattern of cardioprotection with an early (classical or acute IPC) phase, which provides protection almost immediately lasting for approximately 2-3 hours. This is followed by a delayed or late IPC phase. The delayed phase is often described as a second window of protection (SWOP) and provides protection after 12-24 hours, lasting up to 72 hours.[85,86] The cardioprotection conferred by delayed IPC remains significant but is not as powerful as protection provided by early IPC.[87]

1.4.1.1 Triggers of IPC

Cardioprotection induced by preconditioning is dependent on multiple complex intracellular signalling pathways. Triggers of cardioprotection exist that act before the onset of the index ischaemic episode and activate downstream signalling mechanisms which appear to display a 'memory' effect.[88] In 1991 Liu *et al.* demonstrated in a rabbit model that the build up of adenosine (from hydrolysis of ATP) stimulated A1 adenosine receptors triggering the protection afforded by IPC. During the same study they also showed that preconditioning protection could be blocked by two dissimilar adenosine receptor blocking agents. Similarly a 5-minute intracoronary infusion of either adenosine or the A,-specific agonist was as effective in limiting infarct size as a 5-minute period of regional ischaemia.[81] Similar studies were undertaken by looking at bradykinin and opioids which likewise demonstrated that an infusion of bradykinin limited infarct size in a rabbit model (similar to one cycle of ischaemic preconditioning) whereas pre-treatment with a bradykinin receptor antagonist or naloxone (opioid receptor antagonist) abolished the infarct size reduction achieved by IPC.[89,90] These studies and others have shown that protection through IPC can be reproduced by pharmacological agents, acting as triggers of preconditioning , and that endogenous activation of a G-protein-coupled receptor (GPCR) is an essential component of IPC-induced protection in that IPC is a receptor-mediated phenomenon.[91] Subsequent studies demonstrated that protection provided by the trigger substances could be abolished by inhibitors of protein kinase C (PKC). Furthermore, ligands to other GPCR in the heart are able to mimic preconditioning through PKC.[92] These studies therefore suggest the role of PKC as a common downstream mediator of IPC (with mediators referring to factors that act

following the onset of ischaemia and 'mediate' the protective effects of IPC.) GPCR ligands activate PKC via stimulation of phospholipase C, which breaks down membrane lipids to diacylglycerol, contributing to PKC activation.

1.4.1.2 Mediators of IPC / Survival Kinases

Multiple mediators of IPC have been identified in the form of pro-survival anti-apoptotic protein kinases (coined 'survival kinases'). There is significant interplay or 'cross talk' between the survival kinases. 'Cascades' or pathways in which different kinases are dependent on the activation of others have been described. There is also considerable overlap in the end effect of the individual kinases and cascades.

The main mediators of IPC include PKC, tyrosine kinase, PI3K - Akt cascade, mitogen activated protein kinases (MAPK) and the Janus kinase (JAK)-signal transducers and activators of transcription (STAT) pathway.

Phosphatidylinositol 3-kinase (PI3K) is a key signalling enzyme implicated in cell survival and metabolic control with downstream targets including Akt, endothelial nitric oxide synthase (eNOS) and different isoforms of PKC. The PI3K-Akt pathway has been shown to act both prior to the ischaemic event following IPC[93] and at the time of reperfusion with reactivation of the ischaemic kinase cascades (as part of the RISK pathway).[94] Akt activates or inhibits downstream signalling molecules including

metabolic enzymes, apoptotic molecules and transcription factors resulting in the inhibition of MPTP opening. Key downstream signalling molecules of Akt are Glycogen synthase kinase-3β (GSK-3β) and Bcl-2-associated death promoter (BAD). Akt phosphorylates and inhibits both GSK-3β and BAD promoting cell survival by increasing glycogen synthesis, increasing the threshold for mPTP opening and inhibiting apoptosis.[95]

Tyrosine kinases can be divided into: (1) receptor tyrosine kinases which may act as triggers of IPC by activating PKC and (2) cytosolic receptor tyrosine kinases which may act as mediators of IPC by acting downstream or in parallel with PKC.[96]

The MAPK family includes four major serine/threonine protein kinase subfamilies. A number of these subfamilies are well described, including c-Jun NH2-terminal kinases (JNKs), extracellular signal-regulated kinase-1/2 (ERK1/2, also known as p42/p44 MAPK), p38 MAPKs, and the big MAPK-1 (BMK1/ERK5). They are activated by a diverse range of stimuli including ischaemia and reperfusion.[97] MAPK activation therefore has been shown to take place during the trigger phase of IPC and also at reperfusion.[98] The kinases convey their extracellular signals to their intracellular targets through the activation of various intracellular signalling pathways thereby controlling a diverse array of cellular processes. Activation ultimately leads to promotion of cell survival by reducing apoptosis, gap junction conduction, intracellular pH, calcium and oxidative stress and inhibition of mPTP opening.

JAK proteins are cytosolic tyrosine kinases associated with the intracellular domain of membrane-bound receptors, which transduce signals from extracellular ligands to the nucleus to orchestrate the appropriate cellular response. All four members of the JAK family (JAK1, JAK2, JAK3 and tyrosine kinase 2) transduce their signal through recruitment of STAT transcription factors. The STAT family consists of seven members are structurally similar proteins but functionally heterogeneous.[99,100] IPC activates STAT1 and STAT3 leading to the up regulation of the inducible nitric oxide synthase (iNOS) gene.[101] The JAK-STAT pathway is mainly implicated in delayed preconditioning.[80]

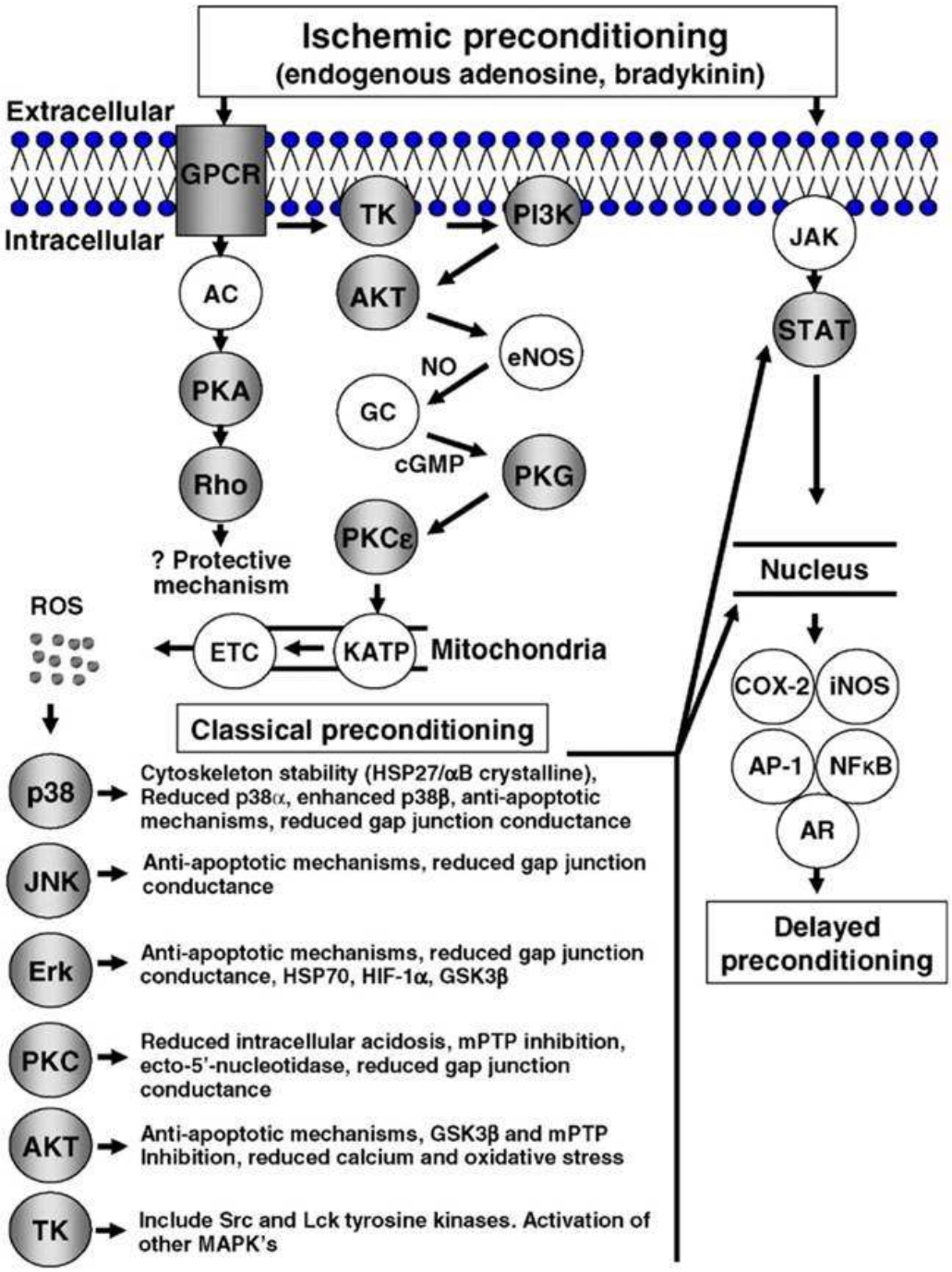

Figure 3 Overview of mediators/survival kinases in ischaemic preconditioning.
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1.4.1.3 Effector Mechanisms of IPC

The end point of the different cascades is to recruit the effector mechanisms of cardioprotection which are thought to be the mitochondria.[75] The role of the mitochondria in IPC is multifaceted including;

1. Opening of the mitochondrial K_{ATP} channel thereby preserving mitochondrial energy by increasing ATP synthesis and reducing ATP hydrolysis.

2. Reduction in intracellular and mitochondrial Ca^{2+} accumulation

3. Attenuation of mitochondrial production of ROS at reperfusion whilst at the same time generation of signalling ROS prior to ischaemia.

4. Inhibition of mPTP opening.

The major limitation of ischaemic preconditioning is the necessity of applying or introducing the preconditioning stimulus before the onset of the index ischaemic event. This, therefore, limits the potential clinical application to planned settings of myocardial ischaemia-reperfusion injury such as coronary artery bypass graft surgery, cardiac transplantation and elective percutaneous coronary intervention.

1.4.2 Ischaemic Postconditioning (IPost)

IPost was first described in 2003 by Zhao *et al*[40] who demonstrated in a canine model that interrupting reperfusion with short lived episodes of myocardial ischaemia could reduce infarct size comparable to that observed with preconditioning. By applying a conditioning stimulus at the point of reperfusion, one is therefore able to circumvent the problem of pre-empting the onset of ischaemia and apply the intervention of 'conditioning' to patients presenting following the onset of ischaemia e.g. acute myocardial infarction. As will become relevant later, the group also showed that the percentage of tissue oedema in both the pre- and postconditioning groups was significantly less when compared with the respective region in the control group, suggesting that both interventions will modify the degree of oedema as well as infarction.[86] The ability of IPost to reduce infarct size has since been reproduced in multiple models including mouse,[102] rat,[103] rabbit,[104] pig[105] and humans.

The mechanism of postconditioning-induced protection also comprises signal transduction pathways that involve the protein kinases described above. These two cardioprotective phenomena appear to recruit common signalling pathways at the time of myocardial reperfusion thereby offering a potentially common target for cardioprotection.[80,88] The main trigger for cardioprotection invoked via IPost is thought to be intravascular adenosine with endogenous activation of adenosine receptors, in particular the A2A and A3AR subtypes.[106] Survival kinases implicated in IPost include PKC,[107] PKG (via NO - cGMP pathway),[108] the activation of p70S6k and eNOS via the PI3K - Akt pathway[87] and the MEK 1/2 - ERK 1/2 pathway.[109]

Two common pathways through which IPC and IPost mediate protection have been elucidated and are named the RISK (reperfusion injury salvage kinase) and SAFE (Survivor Activating Factor Enhancement) pathway.

The RISK pathway describes a group of survival protein kinases [the PI3K-Akt pathway and Erk1/2 mitogen-activated protein kinase (MAPK)], which when activated at the time of myocardial reperfusion confers powerful cardioprotection.[110] The SAFE pathway involves Tumour necrosis factor alpha (TNFα) that activates the JAK-STAT pathway and thereby promoting cell survival.[111] These two pathways relay the cardioprotective signal underlying IPC and IPost, from cell membrane receptors to the mitochondria where protective mechanisms subsequently occur such as mitochondrial permeability transition pore (MPTP) inhibition, mitochondrial connexin-43 channel activation, and mitochondrial ATP-dependent potassium channel opening.[112]

Unsurprisingly given the shared pathways involved in IPC and IPost, IPost has been reported to target many of the proponents of lethal reperfusion injury, such as oxidative stress, calcium accumulation, inflammation, and mitochondrial permeability transition pore (mPTP) opening.[113]

The widespread use of IPost in the clinical arena is limited by both its invasive nature and the fact that it is restricted to patients undergoing PCI. A more amenable

approach would be to mimic IPost, using pharmacological agents that target the RISK or SAFE pathways, thereby obviating the need for such an invasive IPost protocol.[114] Another way to obviate the invasive nature of IPost is to harness the intriguing finding that cardioprotection can be elicited from applying the preconditioning or postconditioning stimulus to an organ or tissue remote from the heart offers an innovative treatment strategy for protecting the heart.[115]

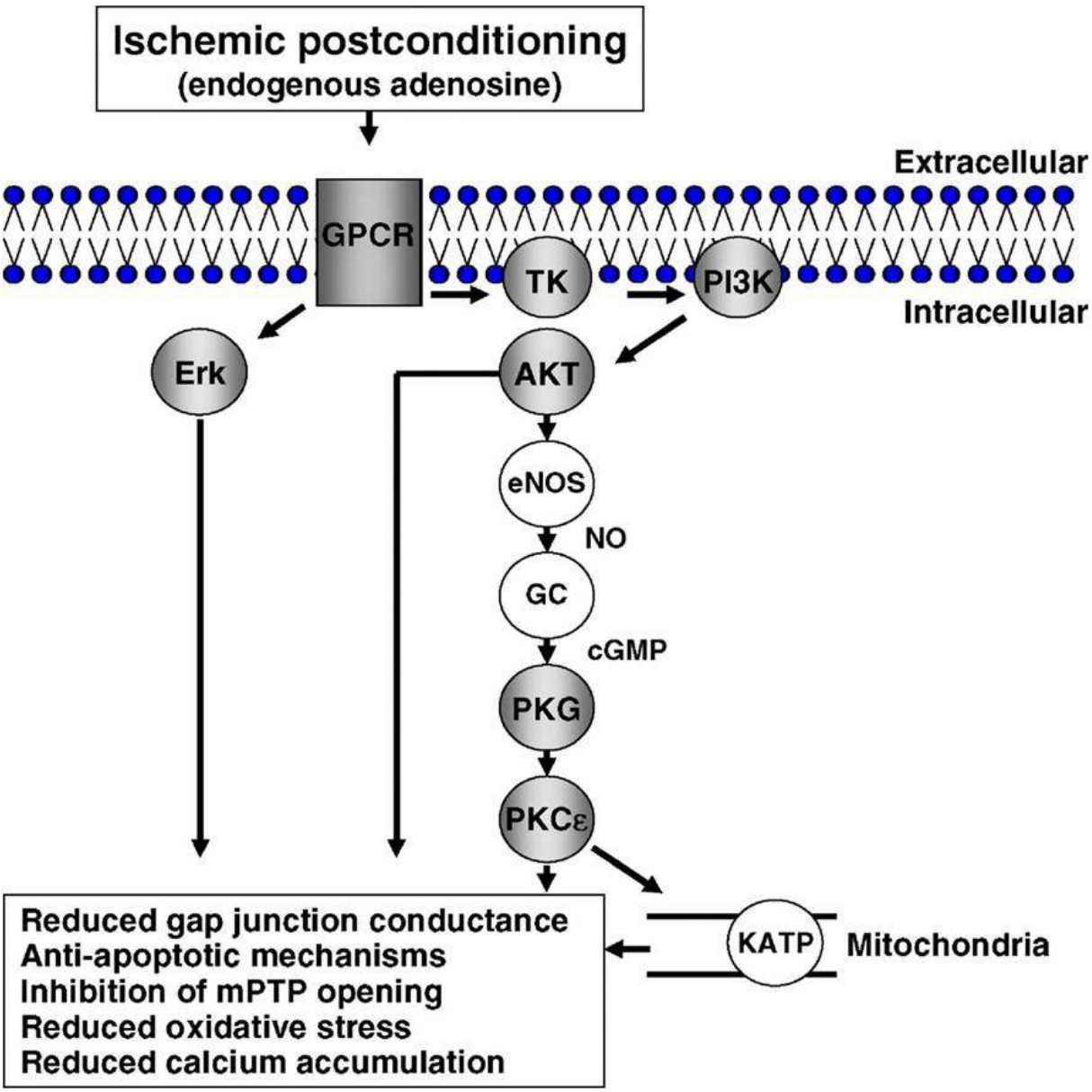

Figure 4 Overview of survival kinases in ischaemic postconditioning
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In the case of AMI, it has been difficult to utilise cardioprotective strategies firstly given the inability to predict the onset of ischaemia and secondly concerns over further invasive balloon inflations in an already unstable patient. The application of remote ischaemic conditioning has obviated these issues providing a cardioprotective strategy that can be applied following the onset of ischaemia and is delivered in a non-invasive manner.

1.4.3 Remote Ischaemic Conditioning (RIC)

RIC describes the cardioprotective effect elicited from applying one or more cycles of non-lethal ischaemia-reperfusion to an organ or tissue remote from the heart. It was originally described by Przyklenk[116] who showed that brief episodes of ischaemia in one vascular bed (Circumflex territory) protects remote, virgin myocardium from subsequent sustained coronary artery occlusion (Left anterior descending artery) in a canine model reducing infarct size. This form of intramyocardial cardioprotection across coronary territories was then extended beyond the heart such that the latter could be protected by applying the RIC stimulus to organs and tissues remote from the heart.[117]

Soon after the discovery by Przyklenk *et al*. it was demonstrated that the heart could be protected by a RIC stimulus applied to organs remote from the heart including kidney,[118] small intestine,[119] brain[120] and lower limb.[121] The aim of moving the conditioning stimulus outside of the heart was to alleviate the need for an interventional approach which is not the case if one needs to apply an ischaemic stimulus to these remote organs.

The breakthrough away from an interventional approach (and therefore enabling its clinical application) was started by Oxman[122] who induced ten minutes of lower limb ischaemia by applying a thin elastic tourniquet around the upper third of the rats' hind extremity in a tight position, closed by a clamp to stop the arterial blood supply in the leg. This was followed by ten minutes of reperfusion prior to thirty minutes of ischaemia in the LAD territory. The group demonstrated a significant reduction in the frequency of reperfusion ventricular arrhythmias between the control group and the group treated with RIC via limb ischaemia. The frequency of arrhythmias was similar between the groups treated with 'classical preconditioning' and the group treated with RIC.[122]

RIC by limb ischaemia can be achieved by either tourniquet application to the limb or direct occlusion of the femoral artery, and has been shown to be effective and reproducible in reducing injury of other organs in both animals and humans.[117]

Kharbanda *et al.*[123] subsequently demonstrated that transient limb ischaemia [Remote IPC before the ischaemic insult was induced by 3 cycles of ischaemia (5 minutes of cuff inflation and deflation) of the contralateral arm] in humans could protect against endothelial IRI of an opposite limb. The ability to induce remote ischaemic conditioning (RIC) using limb ischaemia and reperfusion has facilitated the translation of RIC into the clinical setting. Importantly, the timing of the RIC stimulus can accommodate most clinical settings of acute IRI, as it has been reported to protect the organ or tissue whether applied prior to (termed remote ischaemic preconditioning, RIPC),[116] after the onset of ischaemia (termed remote ischaemic perconditioning, RIPerC),[124] or even at the time of reperfusion (termed remote ischaemic postconditioning, RIPost).[117,125]

1.4.3.1 Mechanisms of RIPC

The actual mechanism through which an episode of brief ischaemia and reperfusion in an organ or tissue exerts protection against a subsequent sustained insult of IRI in a remote organ is unclear. Although signal transduction pathways recruited within the protected organ are thought to be similar to those in IPC and IPost, the mechanistic pathway linking the remote organ / tissue to the heart is yet to be fully elucidated.[91] There appears to be an intervening communication or transfer step between these triggers from the remote site to the target organ that is not required by either direct IPC or IPost.[126] In general, the mechanisms underlying RIC are thought of as three inter-related events (1) the initial events occurring in the remote organ or tissue in response to the RIC stimulus. The application of brief episodes of IR to the remote organ or tissue generates endogenous factors which protect the target organ or tissue from injury.[127] (2) The protective signal is conveyed from the remote organ or tissue to the target organ or tissue. The transmission of the protective signal is multifactorial comprising blood-borne factor(s), neuronal mechanisms, and/or systemic responses.[128] (3) The events occurring in the target organ or tissue which confer the protective effect.[129] It is hypothesised that Step two / conveying of the signal from the remote organ to its intended target takes place either by a humoral or neuronal process.

1.4.3.2 Humoral Hypothesis

It has been shown that RIPC requires a period of reperfusion of the remote organ or tissue suggesting that cardioprotection requires wash-out of cardioprotective factor(s) into the circulation.[129] The humoral hypothesis proposes that the endogenous substance or some other as yet unidentified humoral factor generated in the remote organ or tissue enters the bloodstream activating its respective myocardial receptor thereby stimulating the various intracellular pathways implicated in ischaemic preconditioning.[130] McClanahan *et al.* showed that ischaemic preconditioning of non-cardiac tissue (by intermittent left renal artery occlusion) was as protective of the myocardium as classical myocardial preconditioning.[118] Dickson *et al.* postulated that if the trigger signal responsible for preconditioning at a distance is humoral in nature, then it should be transferable from one animal to another of similar species. The group then demonstrated through a number of studies that the effect of ischaemic preconditioning was transferable from a preconditioned donor heart to a 'virgin non-preconditioned acceptor heart' either through blood transfusion into an untreated animal[131] or through perfusion of coronary effluent into an untreated isolated rabbit heart.[132] Both studies demonstrated a significant reduction in infarct size of 77% and 69% respectively suggesting the presence of a humoral trigger signal for distant preconditioning.[133] In order to exclude an ongoing afferent neurogenic mechanism, Kostantinov *et al.* showed that RIPC by lower limb ischaemia reduced infarct size in a pig donor (denervated) heart following orthotopic heart transplantation from a brain dead donor.[134] The study could not however rule out the possibility of a humoral effect on local, intramyocardial, neurones.[134] A

number of endogenous substances have been investigated for their role in humoral pathways of RIC. These include adenosine, bradykinin, opioids, calcitonin gene-related peptide and endocannabinoids.[131] There is, however, no consensus regarding the identities of the trigger/triggers and cardiac signalling components required to reduce infarct size with RIC.[135]

1.4.3.3 Neural Hypothesis

The neural theory proposes that preconditioning the remote organ or tissue generates an endogenous substance such as adenosine, bradykinin or calcitonin gene-related peptide (CGRP), which then activates a local afferent neural pathway. This in turn recruits an efferent neural pathway terminating at the heart and mediating cardioprotection.[136] Gho *et al.* presented evidence for a neurally mediated pathway in rats when they demonstrated that the reduction in MI size induced by brief ischaemia and reperfusion of the anterior mesenteric artery could be abolished in the presence of a ganglion blocker, hexamethonium. Interestingly ganglion blockade abolished protection by mesenteric artery ischaemia but not by coronary artery ischaemia.[137] The same group postulated that bradykinin released locally during ischaemia-reperfusion in the mesenteric bed stimulates sensory nerves projecting on efferent nerves to the heart and demonstrated cardioprotection conferred by local administration of bradykinin to the mesenteric artery could be abolished by hexamethonium.[138]

A recent human healthy volunteer study, however, demonstrated that the bradykinin-2 inhibitor HOE-140 had no effect on RIPC. Although this human study investigated

the ability of RIPC to abolish the reduction in acetylcholine-mediated, endothelium-dependent vasodilatation induced by IRI it does raise the possibility of a different mechanism in humans and animals.[139]

The role of adenosine released from a remotely preconditioned organ to stimulate afferent nerve fibres has also been extensively investigated. Pell *et al.* used the non-specific adenosine receptor antagonist, 8-sulphophenyltheophylline (8-SPT) delivered before the conditioning stimulus to successfully block the cardioprotective effects of RIPC induced by renal ischaemia.[140] A similar study by Takao *et al.* showed that when 8-SPT was given following the RIPC stimulus (induced by renal artery ischaemia and reperfusion), infarct size reduction could again be abolished in rabbits.[141] Evidence for the role of the neuronal pathway has also been presented in a number of studies that have demonstrated the loss of RIC protection in animals subjected to resection of different nerves.[114,142,143]

Evidence also exists for the role of a systemic response in the cardioprotective effect of RIC. RIC is thought to induce profound changes in gene expression and cellular function, including mitochondrial adaptation to metabolic stress and leukocyte activation.[144]

Given proof for the role of both a neuronal and humoral pathway it is likely that there is interplay/cross over between both pathways in order to mediate cardioprotection. Indeed in a study by Lim *et al.* using limb ischaemia in a mouse model required both the neural and humoral pathways to limit myocardial infarct size. [129]

After the cardioprotective signal has been relayed from the remote preconditioned organ to the heart intracellular signal transduction mechanisms are recruited within cardiomyocytes which are similar to those that participate in IPC and IPost.[91] These include the ligand binding to G-protein cell surface coupled receptors including adenosine, bradykinin, opioids, angiotensin, and endocannabinoids. The binding to these cell surface receptors activates intracellular kinases such as PKC, and other signalling components such as reactive oxygen species, nitric oxide and the mitochondrial KATP channel.[145]

1.4.4 Clinical Applications of Cardioprotective Strategies

From what we have written above it is clear that the cardioprotective strategies of IPC, IPost and RIC lend themselves not just to the arena of bench-side laboratory work but belong crucially in the realm of clinical medicine where there is a real need to translate these effects into reductions in peri-procedural injury (whether it be PCI or cardiothoracic surgery) and infarct size, thereby improving outcomes in patients with coronary artery disease.

1.4.4.1 Cardioprotection in Cardiac Surgery

In 1993, Yellon's group were the first to show that IPC could be used as a therapeutic intervention for cardioprotection in the clinical setting. Fourteen patients were randomised prior to coronary artery bypass surgery (CABG) to either a

preconditioning protocol or placebo. The preconditioning protocol involved two 3 minute periods of cross-clamping the aorta interspersed with 2 minutes of reperfusion prior to 10 minutes of cross-clamping with electrical ventricular fibrillation (causing acute global ischaemic injury) while the distal aorto-coronary anastomoses were done (and subsequent acute global reperfusion injury). The group were able to show a slowing of the rate of ATP depletion, measured from myocardial biopsy, in patients treated with IPC.[146] In order to evaluate the effect of IPC throughout the whole course of the operation as opposed to just metabolic changes occurring at a single time point, Yellon's group went on to use the same preconditioning protocol in patients undergoing CABG and measured serum Troponin-T (TnT) as a marker of ischaemic injury. They found that patients in the IPC group had less perioperative myocardial injury (PMI) as evidenced by lower serum TnT concentrations.[147] A meta-analysis of IPC in cardiac surgery was compiled by Walsh in which the majority of trials used a similar IPC protocol to that of Yellon's group. Other protocols included the use of different stimuli, such as a single cross-clamp application of 5 minutes duration, or simultaneous aortic and caval clamping and one group used a single 1 minute period of aortic cross-clamping followed by 5 minutes of reperfusion. These various protocols of intermittent aortic cross clamping showed benefit in the form of fewer ventricular arrhythmias , less inotropic requirements and a shorter intensive care unit stay (by three hours).[148]

However, due to the invasive nature of this particular IPC protocol and the risk of arterial thrombo-embolism from cross-clamping and declamping an atherosclerotic aorta, it has been difficult to warrant performing larger prospective clinical studies that would be necessary to determine definitively whether IPC induced by

intermittent aortic cross-clamping can improve clinical outcomes in patients undergoing CABG surgery.[149]

1.4.4.2 RIC in Cardiac Surgery

The phenomenon of RIC obviates the need for such an invasive approach to cardioprotection. Gunaydin *et al.* were the first to utilise RIPC in the clinical setting of CABG in a small trial of only eight patients with half being randomised to RIPC. Their protocol involved a tourniquet wrapped around the right upper extremity of the patient (right upper limb ischaemia) that was inflated to 300mmHg and deflated twice to perform 3 minutes of ischaemia separated with 2 minutes of reperfusion in the preconditioning group.[150] This protocol failed to demonstrate a difference in cardiac enzymes (CPK, CK-MB and LDH) between the two groups when they were measured 5 minutes after declamping the aorta. Given that the study size is so small and also the failure to measure cardiac enzymes at any other time points beyond 5 minutes of aortic declamping makes the data difficult to interpret.[149] Six years later Cheung *et al*. were the first to demonstrate positive results with the use of RIC in cardiac surgery. 37 children undergoing surgical repair of congenital heart defects were randomised to RIPC induced by four 5 minute cycles of lower limb ischaemia and reperfusion using a blood pressure cuff inflated to 15mmHg above systolic pressure or control. The group showed that this RIPC protocol was effective in reducing Troponin I, postoperative inotropic requirements and airway resistance at six hours.[151] In adults, undergoing CABG, Hausenloy *et al.* randomised 57 patients undergoing elective surgery to RIPC induced by three 5-min cycles of right upper

limb ischaemia, using a blood pressure cuff inflated to 200 mmHg, with an intervening 5 minutes of reperfusion.[152] RIPC reduced Troponin-T release at multiple time points postoperatively and the total area under the curve for concentration was reduced by 43% in the RIPC group. Given that cross-clamp fibrillation was the predominant method used to protect the heart in this trial, the group repeated the study with a new cohort of patients undergoing elective CABG receiving cold-blood cardioplegia alone in order to determine whether RIPC using the same protocol conferred cardioprotection in a setting that is was clinically applicable with cold-blood cardioplegia having become the standard of care. This study confirmed the results of its forerunner demonstrating a 42% reduction in Troponin-T release over a 72 hour postoperative period,[153] confirming the use of RIPC to reduce PMI in man. A secondary analysis of these two studies showed beneficial effects of RIPC outside the heart with a reduction in the incidence of acute kidney injury (AKI) in the preconditioned group.[154]

Trial	N	Clinical Setting	Intervention	Outcome
Gunaydin (2000)[155]	8	Elective CABG +/- Valve	Upper Limb 2 x 3min	↓LDH
Cheung (2006)[152]	37	Paediatric Congenital Heart Defect Repair	Lower Limb 4 x 5min	↓TnI ↓Ionotropic req. ↓ Airway resistance
Hausenloy (2007)[153]	57	Elective CABG	Upper Limb 3 x 5min	↓TnT (AUC/72hrs) ↓ AKI[155]
Frassdorf (2009)[156]	30	Elective CABG	Sevoflurane	↓TnI
Venugopal (2009)[154]	45	Elective CABG +/- Valve	Upper Limb 3 x 5min	↓TnT (AUC/72hrs) ↓AKI[155]
Zhou (2010)[157]	60	Paediatric Congenital Heart Defect Repair	Upper Limb 3 x 5min Pre-Op (1 & 24hrs)	↓Respiratory Index ↓Ionotropic req. ↓TnT ↓CK-MB
Thielman (2010)[158]	53	Elective CABG	Upper Limb 3 x 5min	↓Peak/Mean TnI ↓TnI (AUC/72hrs)
Rahman (2010)[159]	162	Elective/Urgent CABG	Upper Limb 3 x 5min	No reduction in TnT
Wagner (2010)[160]	101	Elective CABG	Upper Limb 3 x 5min Pre-Op (18hrs)	↓TnI
Hong (2010)[161]	130	Off Pump CABG	Upper Limb 4 x 5min	No significant reduction (↓TnI AUC by 26%)
Ali (2010)[162]	100	CABG	Upper Limb 3 x 5min	↓CK-MB
Karuppasamy (2011)[163]		CABG	Upper Limb 3 x 5min	No significant reduction TnI
Hong (2012)[164]	70	Off Pump CABG	Upper Limb 4 x 5min (RIPC & RIPost)	↓TnI AUC

Table 1 Table showing Clinical studies investigating therapeutic interventions which have shown benefit in the setting of cardiac bypass

Adapted from Hausenloy and Yellon. Preconditioning in the Heart. J.M. Gidday et al. (eds.), Innate Tolerance in the CNS: Translational Neuroprotection by Pre- and Post-Conditioning. 2013. DOI 10.1007/978-1-4419-9695-4_4. [91]

1.4.4.3 RIC in Coronary Intervention

Angioplasty is also an appealing target in which to attempt to ameliorate IRI. PMI has been noted during PCI with raised cardiac enzymes including CK-MB and troponin elevations but also by the presence of late gadolinium enhancement on cardiac MRI (CMR).[165] PMI in this setting can be caused by a number of factors including myocardial ischaemia-reperfusion injury arising from the angioplasty balloon inflations, coronary embolisation and distal branch occlusions.[166] The initial study looking at the role of IPC in angioplasty was disappointing. Illiodromitis *et al.* reported that no myocardial protection was conferred when forty one patients with stable angina and single vessel coronary artery disease underwent elective PCI. RIPC was induced by three 5 minute cycles of bilateral upper-limb ischaemia (cuffs inflated to 200mmHg) immediately before PCI but in contrast to what would be expected the group observed that RIPC appeared to exacerbate cardiac enzyme release after angioplasty.[167] The study is small and the failure to demonstrate protection by RIPC could be explained by the inclusion of a large proportion of patients with diabetes mellitus, a group where IPC is thought to be less effective[168] and the high incidence of nitrate use in the control arm which has been shown to have preconditioning-mimetic properties.[169] The study also fails to state the actual number of patients who had lesions predilated before stenting or the number of patients with the presence of collateral supply. If predilatation (or post-dilatation) did take place it would be helpful to know the number of times the lesion was predilated, to what pressure and for what duration, all factors which are likely to contribute to PMI or paradoxically effect IPC.

Hoole *et al.* randomised 202 patients awaiting elective PCI to either RIPC induced by upper limb ischaemia with three cycles of cuff inflations to 200mmHg for 5 minutes or control. Interestingly they excluded patients taking preconditioning-mimetic and preconditioning-blocking medication. In the same vein participants were instructed to avoid any strenuous activity that could provoke angina (which could also act as a preconditioning mimetic) before their procedure. The group showed that RIPC attenuated PCI-related troponin-I (TnI) release at 24 hours with a significantly lower median troponin in the RIPC group and no detectable troponin release in 48% of patients in the RIPC group compared with 29% of patients in the control group. Patients in the RIPC group also reported less chest pain during the procedure, demonstrated fewer ST-segment changes on ECG during balloon inflation, and, above all, appeared to show fewer major adverse cardiac and cerebral events at 6 months (mostly driven by repeat admission for an acute coronary syndrome).[170] Six year follow up showed a reduction in the rate of major adverse cardiac and cerebral events.[171]

A smaller study by Ghaemian *et al.* was able to confirm the findings of Hoole's study albeit with a different RIPC protocol. In this study of 80 patients, RIPC was induced by 2 cycles of lower-limb ischaemia and reperfusion with cuff pressure being elevated to above systolic pressure. The group showed that significantly fewer patients in the RIPC group had biochemical evidence of myocardial injury (as measured by TnT) at 12 and 24 hours compared to the control group.[172] The use of the lower limb / thigh deserves a mention given that the majority of modern day RIC trials have used the upper limb in view of its accessibility and the fact that upper limb arteries are less prone to atherosclerotic disease than the lower limb thereby

avoiding potential complications of distal arterial embolisation or venous thromboembolism. The use of the lower limb, given the significantly larger bulk of skeletal muscle to deliver the RIC stimulus, may confer greater cardiac protection if the phenomenon is dose dependent.[172] It is interesting then that Ghaemian *et al.* were able to see a significant reduction in TnT with just two cycles of RIPC when using lower limb ischaemia with the cuff being inflated to lower pressures when compared with other studies, which have used three or four cycles. The time advantage of two cycles over the usual three becomes crucial in the setting of acute myocardial infarction where longer door-to-balloon or call-to-balloon times are thought to be strong predictors of infarct size and outcome.

Prasad *et al.* also investigated the use of RIC in a seemingly similar trial but failed to demonstrate a cardioprotective effect with no difference noted in peak TnT or CK-MB.[173]The group used three cycles of RIC induced by three minutes of upper limb ischaemia (Cuff to 200mmHg) and three minutes of reperfusion. The authors point out a number of differences between their study that failed to show protection and that of Hoole et al., which did demonstrate a cardioprotective effect. The study by Prasad *et al.* (a) had a smaller sample size making it possible that the negative result is a false negative, (b) used a shorter RIC cycle (cycles of 3 minutes as opposed to 5 minutes) meaning the stimulus may not have been enough to induce cardioprotection with the protocol being applied immediately before angioplasty as opposed to an hour by before angioplasty (c) 33-36% presented with an acute coronary syndrome requiring ‘urgent’ revascularisation and over 50% were noted to have unstable angina as opposed to the CRISP Stent study in which all cases were elective and would therefore be labelled as having stable angina (d) TnT was used as opposed to TnI (e) patients were older and had a higher frequency of diabetes

mellitus, these both being characteristics in which RIC is more difficult to induce,[43,];[173] and (f) no exclusion of patients with recent preceding angina as a possible preconditioning mimetic or patients on oral hypoglycaemic agents (particularly glibenclamide) which can block preconditioning.

In a recent study more closely matching that of Hoole *et al.* of 205 patients, Lou *et al.*confirmed the findings that an RIPC protocol of three cycles of 5 minutes of ischaemia (Cuff to 200mmHg) and 5 minutes of reperfusion significantly reduced PMI as measured by high sensitivity TnI.[174]

With the studies above in cardiac surgery and elective PCI, one is able to pre-empt the episode of index ischaemia, yet this is obviously not the case with an STEMI. For this reason the conditioning stimulus can only be applied either after the onset of ischaemia but before reperfusion (per-conditioning) or following reperfusion with IPost. A number of mechanical and pharmacological strategies have been shown to be of benefit in patients presenting with an acute myocardial infarction who undergo PPCI. Yet at the same time, a number of strategies that have shown promise in animal models have not translated into positive clinical studies in humans.

Trial	N	Setting	Intervention	Outcome
Illiodromitis[1][67] **(2006)**	41	Elective Angioplasty	Bilateral Upper Limb 3 x 5min	↑CK-MB ↑ TnI
Hoole/Davies (2009/2013)[170,171]	242	Elective Angioplasty	Upper Limb 3 x 5min	↓TnI ↓Chest pain ↓ST segment deviation ↓MACCE at 6 yrs
Prasad[173] **(2010)**	95	Elective Angioplasty	Upper Limb 3 x 5min	No difference in periprocedural myonecrosis (TnT)
Ghaemian[172] **(2012)**	80	Elective Angioplasty	Upper Limb 2 x 5min	↓TnT ↓Chest pain ↓ST segment deviation
Lou[174] **(2013)**	205	Elective Angioplasty	Upper Limb 3 x 5min	↓hscTnI ↓type4a MI

Table 2 Table of the use of RIC in the setting of elective angioplasty

1.4.4.4 Pharmacological Conditioning in STEMI

Pharmacological agents are appealing in the setting of an STEMI as they can be administered quickly prior to reperfusion via a number of different access routes including the intracoronary route allowing rapid perfusion into the myocardium.

A large Japanese study (J-Wind) by Kitakaze *et al.* assessed the administration of a three-day infusion of atrial natriuretic peptide (ANP) in patients with an STEMI. The infusion was started following reperfusion. In this study of 569 patients, ANP significantly reduced biochemical infarct size as measured by CK but did not significantly reduce troponin levels. The authors also noted a significant improvement in ejection fraction at 6 months, and decreased the incidence of cardiac death and admission to hospital because of heart failure in the ANP group.[175]

Although not commented on in the paper, it is important to note the large number of patients in the control group who were labelled as 'Rescue' in whom one may expect a poorer outcome whilst paradoxically a more judicious use of intra-aortic balloon pumps in the treatment arm.

Piot *et al.*[176] randomised 58 patients to an intravenous bolus of cyclosporine (which has been shown to be an inhibitor of mPTP opening) or placebo to patients prior to PPCI with direct stenting and demonstrated a significant reduction in CK. In a sub-group of 27 patients, a reduction in infarct size measured by CMR within the first week was demonstrated. As with any clinical trial it is difficult to control for all variables and it was noted that more patients (although not significant) in the control group underwent facilitated or rescue PCI having failed thrombolysis. Further CMR studies at six months in 28 showed to assess post infarct remodeling showed a sustained reduction in infarct size detrimental effect on LV remodeling. The study was a proof on concept study, which was not powered to address remodelling. A larger multi-centre study (looking to recruit over 900 patients) is underway to address whether cyclosporine can improve clinical outcomes in STEMI patients.[177]

Our own group administered erythropoietin (which has shown promise in preclinical studies) to patients prior to PPCI, which failed to reduce infarct size as measured by CMR. Interestingly this study, through the use of CMR, noted a significant increase in the incidence of MVO. This finding turned the trial from a neutral trial to a negative trial. Whereas traditional biomarkers did not show any difference between the two groups, imaging biomarkers assessed by CMR were able to note the increase in MVO.[178]

Lønborg *et al*. showed how exenatide increased myocardial salvage (Measured by CMR using T2 weighted STIR imaging) when administered prior to PPCI.[179] A *post hoc* analysis showed that exenatide treatment was associated with a 30% decrease in final infarct size only in patients with an ischaemic time of < 132 minutes.[180]

1.4.4.5 RIC in STEMI

Rentoukas *et al*. were the first to study the role of RIC in STEMI. 96 patients were randomised to either a protocol that would seem to be a mixture of per-conditioning and IPost in that it was a twenty-minute protocol that started ten minutes before the first balloon inflation/reperfusion.[181] RIC was induced by inflating a blood pressure cuff placed on the upper limb to 20mmHg above systolic pressure for 4 minutes and 4 minutes of reperfusion for three cycles. Two other groups were randomised including the control group and a third group, which were treated with the per-conditioning protocol as well as the initiation of a morphine infusion at the onset of reperfusion. In the RIPC groups, a significantly higher proportion of patients achieved full ST-segment resolution but this did not translate into a significant reduction in TnI. A *post-hoc* analysis of the data only showed significance when the two RIPC groups were aggregated and compared to the control. These findings do however suggest a potential role of morphine as a mediator of cardioprotection, which is supported by recent observations, indicating that pre-treatment with the opiate receptor antagonist naloxone can interfere with cardioprotection.[182,183]

A recent study by Bøtker *et al.* randomised 333 patients presenting with a STEMI to a per-conditioning protocol of four cycles of alternating 5-min inflation and 5-min deflation of a standard upper-arm blood-pressure cuff to 200mmHg or control prior to primary angioplasty. The protocol was started by ambulance staff on route to hospital. Analysis of 251 patients in the 'intention to treat analysis' showed that myocardial salvage (assessed by myocardial nuclear scanning) as a percentage of left ventricle was significantly higher in the intervention group. Comparison of mean salvage index did not however reach significance. No difference was noted either in final infarct size, LV function or troponin release. Per-protocol analysis of the 142 patients who completed follow up demonstrated, that the primary endpoint of salvage index and percentage salvage was significantly higher in the remote conditioning group than in the control group.[184] The difference appeared to be driven by those patients presenting with anterior myocardial infarctions with the largest areas at risk therefore having the greatest proportion of potentially salvageable myocardium. This confirms previous cardioprotection studies in STEMI patients, which suggest that the patients most likely to benefit from cardioprotective strategies are those with the largest area at risk (AAR). Therefore, in studies in which all patients were included, any cardioprotective effect may be diluted accounting for some of the negative clinical cardioprotection studies.[112] For this reason, some studies will only recruit patients with occluded LAD arteries, yet this moves our work away from real world scenarios in which patients present with occlusions of any of the three major coronary arteries.

In Bøtker's study,[184] the treatment protocol was completed before arrival at the cardiac centre in all but 16 patients which is likely a reflection of the long transfer

times in Denmark. Mean symptom to balloon times was 190 minutes although call to balloon time is not recorded. For those 16 patients the protocol was continued in tandem with the angioplasty i.e. following the advent of reperfusion. In the same vein reperfusion had also begun prior to arrival at the hospital in a significant proportion of patients with 40% of patients having a patent coronary artery on initial angiography (>TIMI 0 flow), indicating that at least for some patients the protective arm ischaemia was applied after the recommended time-window, possibly lowering the expected benefit of the RIPC. This would explain the subgroup analysis findings of higher levels of myocardial salvage in patients with a fully occluded artery on arrival (TIMI 0 flow).[185] At the same time this also likely represents a real world analysis of patients presenting with acute myocardial infarction as vessel occlusion is a dynamic and multifactorial process and efficient anti-platelet and antithrombotic therapies instituted early can lead to spontaneous reperfusion prior to PPCI.[112] TIMI 0 flow on admission does not exclude the possibility of stuttering reperfusion and reocclusion having taken place prior to angiography or visa-versa where a patent artery on admission does not exclude a significant period of complete occlusion prior to angiography. Stuttering reperfusion and occlusion is likely to have a conditioning effect but leaves us unable to accurately assess whether we are giving our intervention as an IPC, IPost or as a per conditioning strategy.

1.4.5 Limitations of Conditioning

There are a number of limitations that have impeded the translation of cardioprotective strategies from preclinical research into mainstream clinical practice.

One of the main limitations of a conditioning strategy, as mentioned above, was the need to directly condition the heart. The advent of RIC, obviated the need to directly condition the myocardium although techniques used often remained invasive in nature. The move to use limb ischaemia with a blood pressure cuff or tourniquet as well as investigation of pharmacological agents has further facilitated the translation of ischaemic conditioning into various clinical settings involving IRI. The protocol of inflating a blood pressure cuff on a human limb is safe, cheap and involves a protocol that can be initiated in a pre-hospital setting without significant technical ability. It is interesting to note however, that although concerns exist with regard to conditioning the heart directly, there have been no reported adverse events in humans that are directly attributable to an IPost strategy.[186]

Another of the limitations of cardioprotection has been the failure of clinical cardioprotective studies to replicate the findings of their preclinical predecessors. This has been the focus of much interest and a number of reasons appear to exist.

(i) The majority of clinical trials have been small proof of concept studies, which may allow for type II statistical errors. The need to demonstrate actual clinical benefit in larger scale multicentre, randomised, double-blind, controlled studies has been taken on board by a number of working groups and larger human cardioprotective studies are now underway.[187]

(ii) There appears to be little consensus as to which protocol is best used for RIC and hence different groups are applying stimuli to different limbs for differing lengths of time.

The question of whether RIC is an all-or-none stimulus or is dose dependent is yet to be elucidated but will be essential in helping to standardise protocols across clinical studies in an aim to gain reproducible results.

(iii) There are major differences between animal models used in conditioning studies and the typical patient undergoing PPCI for an acute myocardial infarction. Some of these differences will be difficult to overcome but others need to be adjusted for in future preclinical studies.

(a) Age and health of the animal - Animal models will likely involve the use of young, healthy animals without the co-morbidities that a patient may be expected to have such as diabetes mellitus or hypertension. Patients with co-morbid conditions are chronically treated with multiple different medications, which may mimic or block conditioning effects whereas animal models will be treatment naive.

(b) Animal *vs.* Human models of IRI - Animal models of myocardial ischaemia differ from that of the human in that they involve the ligation of a non-atherosclerotic vessel and are therefore unlikely to stimulate the inflammatory settings of acute infarction. At the same time, removal of the ligature, to restore flow will not mimic the conditions of reperfusion induced by angioplasty with the subsequent embolisation and MVO. Patients undergoing PPCI are likely to have residual disease at first presentation that may not be 'dealt' with when focus is placed on the culprit lesion in the setting of acute infarction. Experimentally induced IRI will be fixed, continuous and often, particularly in small animals, relatively short whereas in humans ischaemia and reperfusion is a dynamic and possibly intermittent / stuttering process with varying but often prolonged periods of ischaemia and reperfusion.

(iv) Study endpoints – Major differences exist in how groups design their studies including how they assess their chosen endpoints. A wide range of endpoints exist that are utilised within cardiovascular research including both biochemical and imaging biomarkers. Our study focuses on the use of CMR as a novel non-invasive imaging strategy to assess the efficacy of cardioprotective strategies. The use of CMR will be discussed in detail in the coming sections.

1.5 Cardiac MRI

The role of imaging within acute infarction is multifaceted. It helps to accurately establish the diagnosis, can aid in risk stratification and therapeutic decision making and can also be used to monitor response to different therapies. In the setting of acute myocardial infarction CMR is able to provide a host of information including assessment of global and regional wall motion, calculation of left (and right) ventricular volumes, calculation of stroke volume and hence ejection fraction. The ability to perform tissue characterisation is a key feature that makes CMR unique when compared to other imaging modalities. It enables accurate definition of abnormal myocardium thereby advancing myocardial phenotyping from a binary quantitative approach (how much myocardium is either alive dead), to a more sophisticated assessment of myocardial quality with a multi-parametric assessment. This approach promises both clinical utility and new insights into the fundamental biological mechanisms of cardioprotective interventions. CMR allows assessment of myocardium at risk either by T2-weighted imaging or infarct endocardial surface area (Infarct-ESA), imaging of microvascular damage (MVO or haemorrhage) and accurate assessment of the transmurality of infarction as well as the amount of myocardial necrosis.

1.5.1 Cardiac Volumes

Accurate assessment of cardiac volumes, mass and function has important prognostic and therapeutic implications; hence the accuracy of the data can significantly affect the appropriateness of certain diagnostic or therapeutic approaches in an individual patient. Serial measurements are important to evaluate treatment and changes in volumes and function over time. Cardiac volumes and function are therefore intrinsic to cardiovascular research and are used as surrogate endpoints in a large proportion of cardiovascular studies. Most imaging modalities can measure cardiac volumes. CMR is considered the gold standard[188] for the measurement of cardiac volumes and function. The ability of CMR to delineate between blood pool and myocardium allows measurements to be made with a high degree of accuracy and reproducibility.[189] Other imaging modalities that can be used to assess cardiac volumes include echocardiography and ventriculography (angiography or radionuclide).

Echocardiography is widely used post myocardial infarction to assess LV function, as it is cheap, easily available, portable and non-invasive. M-mode and 2-D echocardiography are acoustic window and operator dependent and rely on geometric assumptions that do not hold true in patients with dilated, remodelled ventricles[190] IHD is a regional or segmental process, so that volume measurement is more important than assessing LV end-diastolic diameter (LVEDD), which is readily measured by transthoracic or transoesophageal echocardiogram but only rises following global ventricular stretch. Echocardiography is thereby less useful in

ischaemic disease for measuring ventricular volumes because of its inaccuracy when regional asynergy is present.[191]

Ventriculography only allows for a single two-dimensional assessment of volumes. Imaging is reliant on heart rhythm and ectopy (or patient movement) can often render non-diagnostic images. Should the images be non-diagnostic there is little scope for repeating the cine run given the risk of contrast nephropathy with increasing contrast load.

Radionuclide ventriculography is used to assess cardiac function yet suffers from similar limitations to echocardiography including poor acoustic windows or spatial resolution and geometric assumptions. At the same time it lacks the accessibility of echocardiography and involves exposure to ionising radiation. Radionuclide angiography has been shown to underestimate left ventricular function

A significant advantage of CMR for evaluation of cardiac indices is its reproducibility and accuracy compared with 2D planar or projection techniques that depend on geometric assumptions in order to define mass and volume determinations. This allows small changes in myocardial mass and/or volume to be detected over time or as a result of therapy. This is particularly useful for determining the impact of therapy or for research purposes in clinical trials.[192] The high reproducibility of CMR suggests that a smaller sample size is required to detect a change in volumes, mass[193], and function in comparison with published values for techniques using LV geometric

assumptions.[194] CMR LV size and systolic function are precisely determined with standard errors of about 5%.[194]

1.5.2 Infarct Size

Myocardial infarct size is a major determinant of prognosis following an acute myocardial infarction (AMI). Infarct size has been correlated with malignant arrhythmias,[195] heart failure and mortality[196] hence the need for therapeutic strategies to limit the amount of infarcted myocardium.

Infarct size, as a direct measure of damage in myocardial infarction, has long been used as a surrogate measure in cardiovascular trials. A number of imaging and non-imaging biomarkers have been used to express infarct size including ECG measurements, cardiac enzymes (CK, CK-MB and Troponin) measured as an 'area under the curve' or peak enzyme release in serial measurements, Technetium-sestamibi SPECT and Cardiac MRI.

These markers are 'surrogate' or 'replacement' measures used in trials of cardiovascular disease. The real endpoint relevant for definitive evaluation of a treatment typically is survival. For this reason a significant number of trials will use primary endpoints in clinical cardiovascular studies of death, reinfarction or heart failure. The use of these so called hard endpoints can prove difficult given that incidence of the event is often low during short - or medium - term follow-up. Furthermore, some events might not be linked to atherosclerosis resulting in low sensitivity. As a consequence, large sample sizes and long follow-up periods are

required absorbing time and financial resources. Missing data and noncompliance are also more likely in longer-lasting studies.[197,198]

The use of surrogate endpoints was initially based on an established association between the presumed surrogate and the corresponding true endpoint. However, the mere existence of an association between a possible surrogate endpoint and the true endpoint is not sufficient for using the former as a surrogate. What is required is that the effect of the treatment on the surrogate endpoint reliably predicts the effect on the true endpoint.[187]

1.5.2.1 Determinants of Infarct size

There are multiple determinants of final infarct size. The main determinants include;

(i) Duration of coronary occlusion

Reimer and Jennings identified the wavefront phenomenon of ischaemic cell death as a function of the duration of ischaemia.[199] Their seminal papers demonstrated that necrosis occurs first in the subendocardial myocardium and with longer durations of coronary occlusion involves progressively more of the transmural thickness of the ischaemic zone (area of myocardium subtended by the occluded coronary artery which we will describe as the 'area at risk'). Microvascular injury, evidenced by interstitial haemorrhage, also progresses from subendocardial to subepicardial

zones but the time scale is slower than that for myocyte necrosis.[199] Hence the maxim of “time is muscle” and the drive to constantly improve on pain-to-balloon and door-to-balloon times to halt the ‘wavefront’/progression of necrosis in a bid to salvage myocardium. The time between symptom onset and the provision of reperfusion therapy (ideally by PPCI) reflects total ischaemic time and minimising delays is associated with improved outcomes.[8] Longer ischaemic times mean less salvageable myocardium and delay is linked with larger infarct size and increased mortality.[200] In this regard little has changed in 35 years as Reimer and Jennings concluded in 1977 that since the amount of potentially salvageable myocardium progressively decreases as the period of ischaemia is prolonged, time is the most critical variable involved in delaying or preventing ischaemic myocardial cell death.[199]

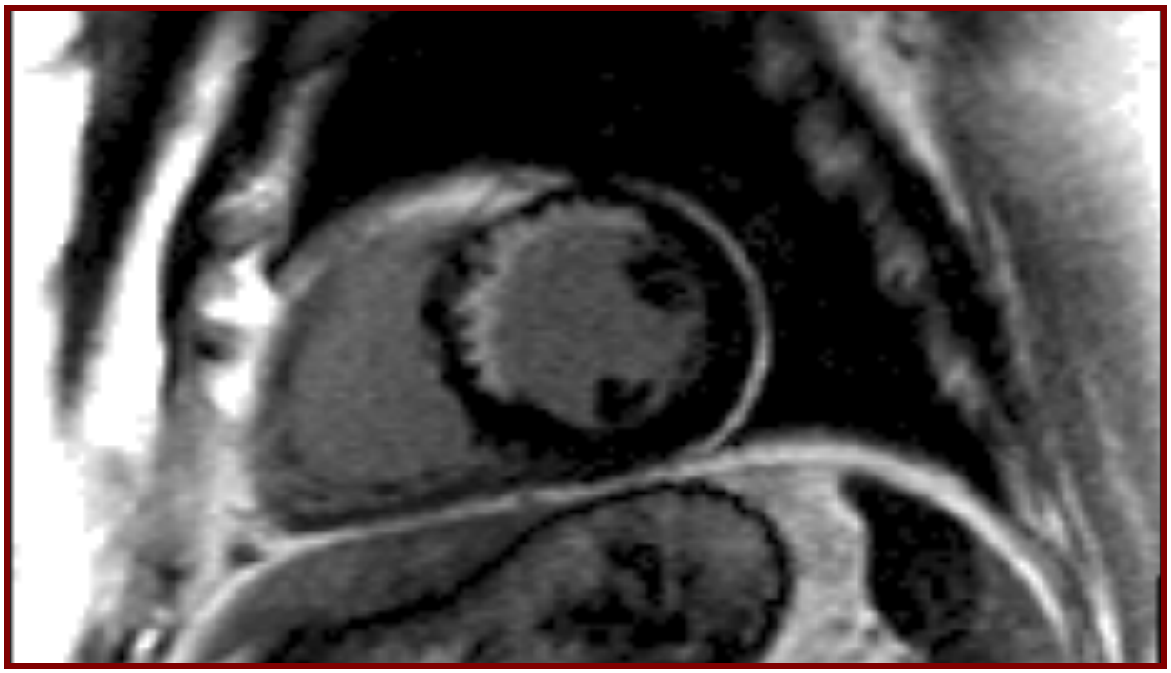


Figure 5 Wavefront Phenomenon - CMR short axis image demonstrating anteroseptal LGE

The infarction can be seen to be spreading out from the endocardium to epicardium. The progression of spread having been halted by PPCI demonstrating myocardial salvage.

(ii) Area at risk (AAR)

The area at risk (AAR) refers to the myocardium subtended by a particular coronary artery that once occluded is at risk of infarction. The size of the AAR is a major determinant of the final infarct size. Reimer and Jennings again showed that the quantity of myocardium made ischaemic by occluding a particular coronary artery varied from dog to dog and that the AAR correlated with the amount of necrosis.[201] Given that the amount of necrosis can only ever be as large as the AAR then the comparison of infarct size from a particular coronary bed cannot be made between subjects. Small differences in the AAR may result in a significant variation of infarct size, underscoring the fact that most of the infarct size variability is due to the extent of the myocardium at risk.[202] In order to standardise measures infarct size can be expressed as a percentage of the AAR. This also enables the quantification of myocardial salvage which is the potential infarct size (AAR) minus the actual infarct size. The assessment of myocardial salvage may be a more important measure of outcome than traditional measures including biomarker release or EF.[203]

The ability to measure the AAR is particularly ideal in STEMI patients, where the size of the AAR can differ (from 10 to 50% of the LV) depending on which the coronary territory is affected. [112]

The AAR can be estimated from a number of different methods including:

(a) The extent of ST-segment elevation on presenting 12-lead ECG. Although theoretically a 12-lead ECG is easy to perform and allows estimation of the AAR prior to reperfusion, in the era of PPCI the diagnosis of STEMI is often made from a limited Paramedic ECG prior to the angioplasty. A routine 12-lead ECG, in the presence of a diagnostic, would unnecessarily increase door-to-balloon times. The ECG only allows for estimation of the AAR and not infarct size so, cannot be used to assess myocardial salvage.[204]

(b) The number of regional wall motion abnormalities present on echocardiography or LV angiography. The circumferential extent of the abnormally contracting segments are calculated and expressed as a percentage of the internal circumference of the LV in end-diastole. This offers an indirect and often inaccurate measure of the AAR.[97]

(c) Coronary angiography using the BARI or APPROACH scores.

These markers, however, do not very accurately or even quantitatively reflect myocardial salvage. Therefore, research on reperfusion strategies, although highly sought, has been debilitated by a requirement for large sample sizes and prolonged follow-up periods.[205]

The current gold-standard non-invasive imaging technique for measuring the AAR is myocardial perfusion imaging (single-photon emission computerized tomography - SPECT) where an intravenous injection of technetium is given to the STEMI patient prior to PPCI. The patient then has a perfusion scan within 6-8 hours and the radioisotope is not taken up by the myocardium in the territory of the infarct-related coronary artery - this is the AAR. The patient then has a repeat scan 7-10 days later which delineates the resultant infarction.

Despite the need for two separate studies, SPECT has the advantage of offering the assessment of AAR and infarct size using the same modality. Myocardial salvage as assessed by SPECT has been shown to correlate with mortality.[206] SPECT suffers from a number of limitations including the need to image the patient within six hours of reperfusion (with ideally a 24 hour nuclear imaging service to enable to administration of the isotope - ^{99}Tc-sestamibi), which is usually impractical. The patient will have to undergo two studies with exposure to ionising radiation (which would need to be added to the patients exposure for PPCI) to provide relatively limited spatial resolution. SPECT scans have been shown to have difficulty in evaluating the inferior wall of the left ventricle adjacent to the diaphragm, and also lack penetration in obese patients.[207] The poor spatial resolution of SPECT is reflected in the fact that CMR systematically detects subendocardial infarcts that are missed by SPECT.[208] In those patients enrolled in trials with a patent coronary artery at admission, ^{99}Tc-sestamibi may escape into the ischaemic bed therefore underestimating the AAR.[184]

CMR, with high temporal and spatial resolution, allows for the quantification of AAR and infarct size therefore allowing for the assessment of myocardial salvage in a single scan which can safely be performed early following PPCI. The use of CMR to assess infarct size and AAR will be discussed below.

(iii) Residual flow in the territory at risk (Collaterals and Antegrade flow in the IRA). As mentioned earlier, acute infarction can often be a stuttering process with dynamic changes in flow ranging from TIMI 0 to TIMI III flow. In a number of studies between 40 - 50% of STEMI patients presented to the hospital already having significant coronary flow (TIMI flow >1) within the infarct related artery.[183,209]

The presence of antegrade flow makes it difficult to retrospectively assess the area at risk. For example, using ^{99}Tc-sestamibi in those patients with a patent coronary artery at admission, may lead to an underestimation of the AAR in view of the chance that the trace may escape into the ischaemic bed.[184] It is also important to note that antegrade flow down the IRA will confound conditioning studies as mechanical or pharmacological mimetics need to be applied prior to the opening of the occluded artery. Once the artery is open (TIMI flow >1) and the cascade of reperfusion injury begun, it is too late to apply pre- or perconditioning strategies. Similarly the presence of the coronary collateral circulation has been noted to be a confounding factor in such studies for identical reasons. Collateral arteries are usually only evident in 10–40% of MI patients undergoing PCI, yet most MI studies have suggested that myocardial perfusion may be maintained and LV function preserved in patients with an adequate coronary collateral circulation.[210] Hence, with

continuous flow to the AAR, through the collateral circulation the degree of IRI will be diminished. Collateral flow supplying the myocardium (that likely develop as an adaptation to ischaemia) will thereby reduce the AAR and infarct size.[211] Depending on the exact amount of collateral flow recruitable during a brief coronary occlusion, long-term cardiac mortality has been shown to be reduced by up to one fourth compared with the same situation without collateral supply.[212]

In the presence of collateral blood supply the precise timing of IRI will not be easy to establish and therefore one cannot reliably apply a conditioning strategy. Studies in cardioprotection will often therefore either exclude patients with collateral flow on initial angiography or attempt to measure it. The assessment of coronary collateral blood flow is very difficult. It can only be assessed during the occlusion of the collateral-receiving artery by the measurement of intracoronary occlusive pressure or velocity derived collateral flow index, expressed as a fraction of flow during vessel patency.[213] The majority of collateral vessels in humans may not be angiographically detectable;[214] therefore any grading system provides only an estimate of collateral flow.[215]

The presence of collateral flow has caused difficulty in translating cardioprotective studies from animals to humans. The collateral circulation in humans differs from that of animals with a wide spectrum of collateral flow exists between various mammalian species.[216] The canine coronary circulation contains a well-developed native system of functional, preformed epicardial collaterals, which results in the persistence of 15–30% of baseline blood flow during coronary occlusion. In the normal human heart, a native system of preformed collaterals is also present, but is much less developed, located primarily in the subendocardium.[217] It has been suggested that significant coronary collateralisation to the AAR may in part explain the negative findings of the

clinical cardioprotection studies[112] and that patients with collateral flow should be excluded from such studies.[218]

(iv) Hemodynamic factors: Haemodynamic factors including blood pressure and heart rate at the time of coronary artery occlusion also impact on infarct size. Tachycardia and/or hypotension occurring in a STEMI patient will likely increase infarct size through increased myocardial oxygen consumption.[219] Preclinical studies have demonstrated that augmenting coronary flow and therefore reducing myocardial oxygen demand prior to reperfusion reduces infarct size and increases myocardial salvage.[220] Disappointingly clinical studies have failed to demonstrate benefit in human studies with the use of intra-aortic balloon counterpulsation (IABC) as an adjunct to revascularization in STEMI patients.[221]Interestingly for a study (CRISP-AMI) setup to examine one of the determinants of infarct size, it failed to take coronary collateralisation (not recorded) or evidence of anterograde flow (TIMI > 0 = 30% patients) on initial angiography into account. In the same vein, the patients in the study had ischaemic times of longer than 3 hours which will likely limit the amount of salvageable myocardium.

1.5.2.2 Cytoplasmic Markers (CK and CK-MB) of Myocardial Necrosis

Measurement of cardiac biomarker levels is routinely performed in clinical practice, as well as in clinical trials after myocardial infarction to estimate the extent of myocardial necrosis. Venous sampling of creatine kinase (CK) and CK-MB

concentrations have been shown to correlate with histopathological measures of infarct size[222] as well as with functional and clinical outcomes in the setting of STEMI.[223] Concerns with regard to relying on cytoplasmic markers to predict infarct size include (i) uncertainty as to which method (peak concentrations, area under time-concentration curve [AUC], or single time-point measurements) is most reliable, (ii) the fact that both CK and CK-MB are not cardiospecific, being found in both skeletal and myocardial tissue[224] and (iii) reperfusion therapy is thought to alter the kinetics of these enzymes leading to a higher peak value at an earlier time point.[225]

Troponins are more sensitive and specific for detection of myocardial necrosis and are currently the gold standard for the diagnosis of myocardial infarction. Troponin measurements at differing time points have been shown to correlate with myocardial function measured by echocardiography[226] and function and infarct size measured by SPECT[227] imaging and CMR.[228]

1.5.2.3 Late Gadolinium Enhancement (LGE) Imaging

Late gadolinium enhancement (LGE) imaging by CMR is a highly sensitive and specific technique for imaging infarct size post myocardial infarction offering high resolution images which have only previously been achievable by the histopathological analysis of postmortem specimens.

Contrast enhancement using gadolinium (Gd) agents is based on T1-shortening and the distribution of contrast agent within the tissue. The mechanism of early and late enhancement relates to the different wash-in and wash-out kinetics of normal myocardium and tissue with myocardial infarction or fibrosis.[229] Gadolinium-based

contrast agents rapidly diffuse into the extracellular space but, because of chemical charge and molecular size, contrast is unable to cross intact cell membranes. In essence, these agents cannot normally enter the intracellular space in the presence of intact myocytes and the volume of distribution is small. In the acute phase following infarction, myocyte cell membranes rupture with loss of sarcomere integrity (88% of myocytes in infarcted regions have sarcolemmal tears, compared with 0% in normal regions)[230], allowing the extracellular tracers to be passively distributed into the intracellular spaces of irreversibly injured myocardial cells. Hence the volume of distribution of Gd-DTPA is increased in the setting of acute infarctions in humans.[231] Additionally to an increased volume of distribution, an altered wash-in and wash-out kinetic, where Gd-DTPA becomes “trapped” in the infarcted tissue due to the severely reduced wash-out is a contributory factor to an increased concentration and hyperenhancement.[232] Changes in kinetics could be due to a number of factors, including changes in coronary flow rates, capillary permeability, or functional capillary density.[229]

The principles in chronic infarction are similar in that the distribution of Gd-DTPA is dependent on the degree of architectural disruption of the myocardium. Within the infarcted zone, myocytes are replaced by collagenous scar. Fibrosis (scar) has a larger extracellular space (hence larger concentration and volume of distribution) than does normal myocardium. Multiple studies analysing myocardial histology showed a significantly elevated proportion of fibrosis in scar compared to viable myocardium (25–49% vs. 9–25%, respectively). Therefore, the elevated signal intensity on LGE imaging in scar tissue can be explained by an increased volume of

distribution due to the higher degree of fibrosis secondary to myocyte loss and fibrous reorganisation.[233]

Following the administration of Gd-DTPA (which shortens the T1 relaxation time) an inversion recovery sequence is applied which suppresses the normal myocardium resulting in the infarcted myocardium appearing bright.

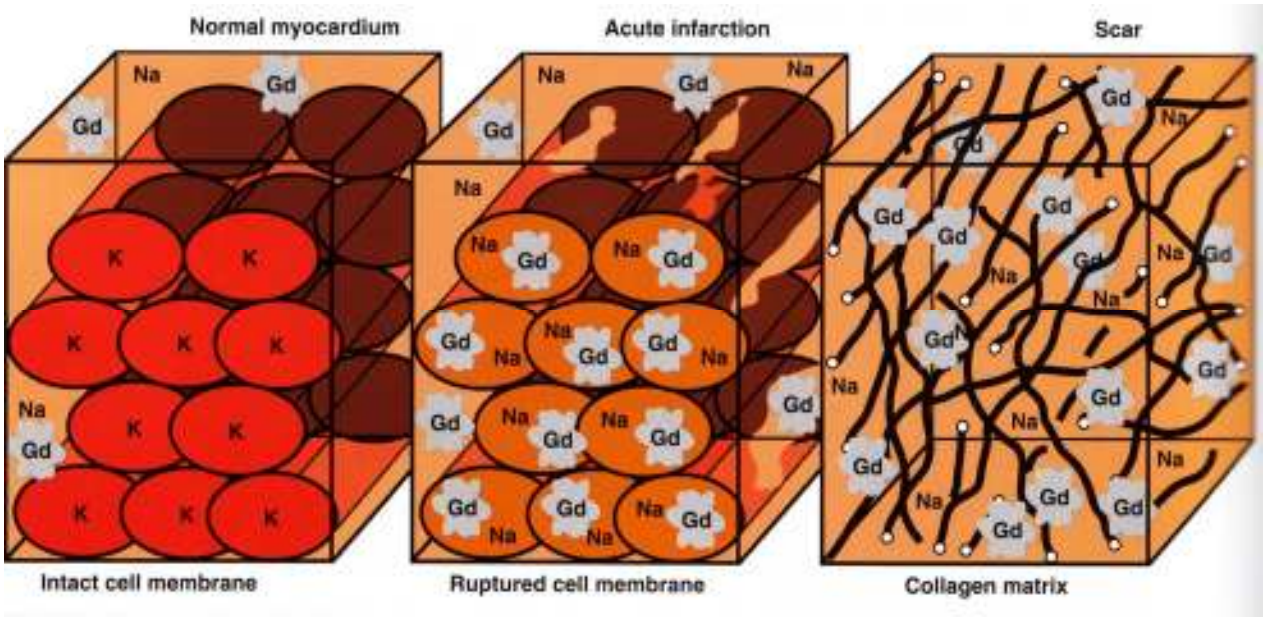


Figure 6 Mechanism of Late enhancement imaging and distribution of Gd-DTPA in normal myocardium, acute and chronic infarction

Reprinted with permission from Kim RJ *et al*. Assessment of myocardial viability by contrast enhancement. In: Higgins CB, de Roos A, eds. Cardiovascular MRI and MRA. Philadelphia, PA: Lippincott Williams and Wilkins; 2003; 209– 237..[234]

Kim *et al.*[235] demonstrated in a canine model that late enhancement is seen with both (reperfused and non-reperfused) acute and chronic infarction with the location and spatial extent of necrosis showing excellent correlation with macroscopic slices

stained with TTC. However severe, transient ischaemia was not shown to lead to hyperenhancement despite the presence of myocardial stunning. Other important findings from their study included infarct shrinkage over time (8 days to 3 weeks) and partial volume effect of infarct imaging in the use of late enhancement technique.

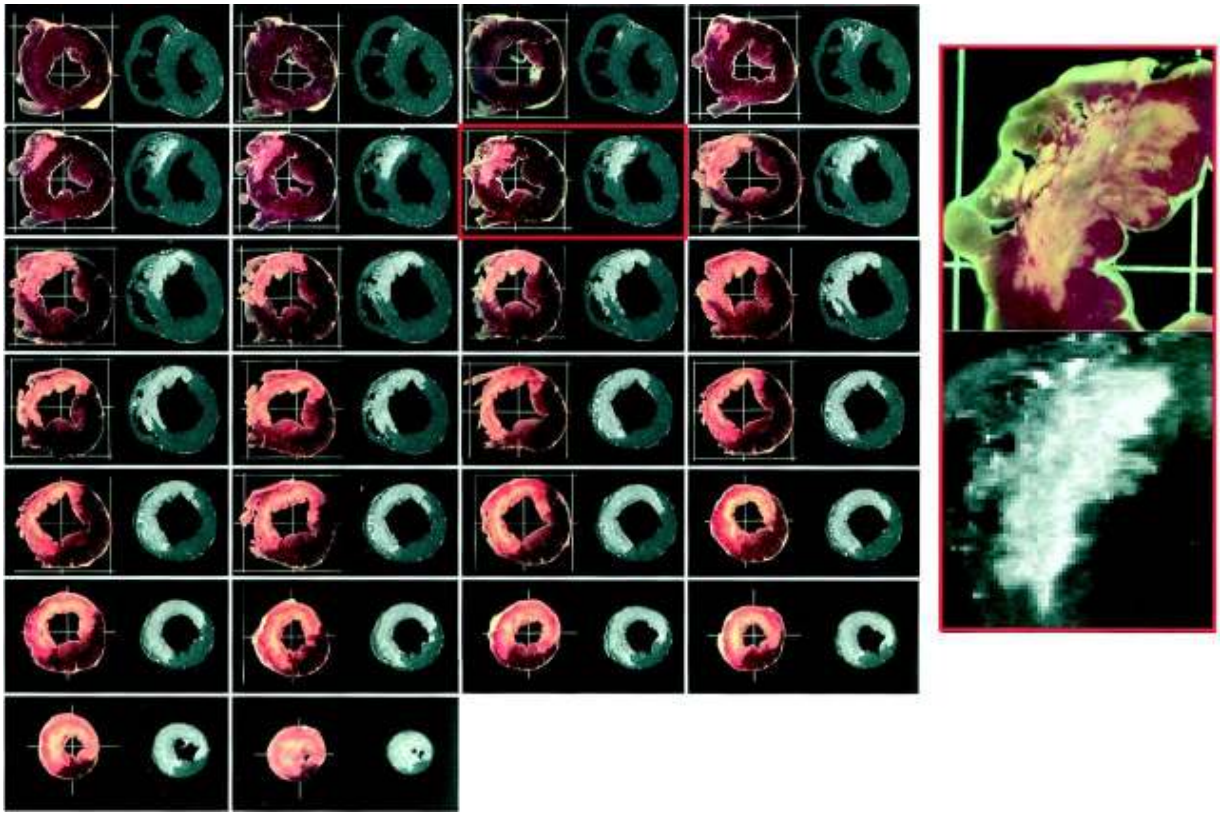


Figure 7 The comparison of ex-vivo MR images with TTC-stained slices in 1 animal at 3 days after infarct

Reprinted with permission from Kim R J et al. Circulation 1999;100:1992-2002

From a clinical perspective the results demonstrated the ability of CMR to distinguish between acute myocardial infarction (hyperenhancement and evidence of regional wall motion abnormality), injured but viable myocardium (no hyperenhancement but evidence of regional or global contractile dysfunction), and normal myocardium (no

hyperenhancement with normal function). In the setting of chronic myocardial infarction, late enhancement imaging and cine MRI may also distinguish between myocardial necrosis, hibernating myocardium, and normal myocardium.[187]

Mahrholdt *et al*.[236] further demonstrated the high sensitivity and specificity of detection of infarction by CMR as compared to the previous gold standard of SPECT imaging. Whereas transmural infarction was picked up at similar rates by both modalities, SPECT imaging missed subendocardial infarction that was picked up by CMR and confirmed histologically on TTC staining. This confirmed the findings of Kim *et al*. above that subendocardial infarcts detected by CMR corresponded to infarcts defined histologically.[235]

Wu *et al.* showed that initial infarct burden as delineated by infarct size (measured on delayed enhancement images on CMR done at day 2 post PPCI) was the strongest predictor of persistent LV enlargement (ESVi) and future cardiac events (cardiac death, non-fatal re-infarction and symptomatic congestive heart failure requiring hospitalisation.).[237] Infarct size was more predictive of adverse cardiovascular events than ejection fraction (EF) or ESVi measured in the acute phase post MI. ESVI and EF calculated within 7 days of a STEMI can be inaccurate measures of the extent of irreversible myocardial injury because of ischaemic stunning or previous areas of chronic hibernation.[238] Unlike LVEF and left ventricular volumes, infarct size is not dependent on preload / afterload or stunning. Wu's group showed that patients with an infarct size measured at 18.5% were unlikely to develop adverse events with a negative predictive value of over 90%.[237] This high negative predictive value demonstrates the importance of quantifying the amount of infarcted myocardium suggesting that CMR is able to identify patients who are at higher risk of

adverse LV remodelling and MACE and therefore may warrant more aggressive treatment.[236,237]

The additive value of quantifying the amount of LGE was more recently shown by Larose *et al*. who demonstrated that the occurrence of LV dysfunction at 6 months invariably increased with greater LGE: a cut-off of ≥23% LGE measured on CMR very early following PPCI (within 12 hours) showed the best accuracy for late LV dysfunction (sensitivity 89%, specificity 74%) beyond traditional risk factors such as infarct territory, maximum CK-MB rise, pain-to-balloon time, presence of Q waves, and LVEF during STEMI. LGE volume incurred the strongest association to LV function change, beyond infarct transmurality, MVO, and salvaged myocardium (measured using T2-weighted STIR imaging).[239]

The study by Larose also addresses the question of when should infarct size be measured in order to best risk stratify patients. Infarct healing occurs early after reperfused infarction and is characterised by infarct shrinkage, infarct and adjacent wall thinning and loss in the myocardial mass. Most infarct healing is finished at 4 months post-MI suggesting that this is a reasonable time point to study the morphological consequences of infarct repair.[240] Early studies by Pfeffer *et al*. suggested that although left ventricular volumes were increased in relation to infarct size measured at four months, no such relationship existed early (24 hours) after infarction.[241] A small study of 22 patients demonstrated a reduction in infarct size (as measured by LGE imaging) in patients treated with PPCI of 31% between week one and five months post infarction. Infarct size decreased relatively to the same extent in small and large infarctions and shrinkage appeared to be independent of the

territory affected.[242] The follow-up measurements by Larose's group averaged 27% less than those made within 12 hours after reperfusion and match the follow measurements of other studies using CMR.[243] These reductions in infarct size likely reflect resolution of acute inflammatory changes and replacement of resorbed tissue by scar. The relative magnitude of any changes is apparently insufficient to negate the value of measurements during the early post infarction period.[244] Ganame *et al*. showed that although LV remodelling continued up to 1 year after the acute event, infarct size when measured at baseline (median 72 hours post PPCI) remained a powerful predictor powerful predictor of late LV remodelling.[240] Therefore LGE imaging early after PPCI may overestimate myocardial necrosis when the volume of the infarcted tissue tends to increase due phenomena such as hyperaemia, oedema and inflammatory cells infiltration.[245] Despite this early measurement of infarction remains a strong independent predictor of adverse outcomes.

Engblom *et al.* reported a rapid initial reduction in the degree of LGE during the first week following reperfusion for AMI. The group explained their findings firstly based on the original studies of Reimer and Jennings suggesting that resorption of oedema, haemorrhage, and irreversibly injured myocytes within the region of infarction lead to the reduction in LGE at one week. The group also postulated that the loss of hyperenhancement of a viable peri-infarction zone surrounding the irreversibly injured core of myocytes early after infarction contributed to the early reduction in LGE.[246]

Multi centre data now exists both for the assessment of CMR to detect infarction and also to link CMR endpoints with mortality. One international multi centre double blinded randomised control trial looked at the performance of delayed enhancement imaging for the detection and assessment of infarction using CMR. 566 patient scans were done across 26 centres. The sensitivity of detecting MI in the acute setting was 99% and 96% in the setting of chronic MI. The accuracy was also extremely high when assessing for the presence of hyperenhancement in the correct infarct related artery territory, as determined by coronary angiography[247] (Interestingly this in itself has limitations as the possibility of infarction with angiographically normal coronary arteries exists in which CMR will determine the presence of infarction).

Importantly, this study represented the first multicenter trial designed to evaluate an imaging approach for detecting infarction. Although several multicenter trials had previously used infarct size measurements by SPECT as a surrogate endpoint to assess the efficacy of an investigative therapy, these trials were not designed to evaluate SPECT, and limited multicenter data on the sensitivity or accuracy of radionuclide imaging for detecting or localizing infarction have been reported.[248] With regard to SPECT, there is minimal data on chronic MI and NSTEMI as the majority of the data with SPECT comes from STEMI.[249] Yet even in patients with STEMI up to 25% of patients with clinically defined infarction appear to have no evidence of infarction of SPECT imaging in that SPECT appears to miss the infarct. For example the EMERALD trial investigated distal embolus protection in acute MI using MIBI. The data showed that the inter quartile range of infarct size in the control arm was 0 - 23%.[250] The results therefore suggest that at least a quarter of patients had infarction missed by SPECT in that particular trial.

In a further multi centre trial Kim *et al.* showed that both LV volumes and extent of infarct (number of segments with evidence of scar) measured by CMR in a non-selected (n=1561) population, demonstrated incremental prognostic value over a clinical model. Survival curves showed that the extent of scar predicted outcome not only in all patients but also in patients with preserved ejection fraction.[251]

Given that the presence and extent of infarction measured by CMR predicts adverse cardiovascular events, the ability to accurately and reproducibly measure LGE becomes important. A number of methods have been have been used to measure the amount of LGE in acute infarction as well as other cardiovascular pathologies that manifest focal fibrosis. The different techniques will be discussed later within this thesis. The techniques include;

(i) Visual Assessment

(ii) Planimetry

(iii) Semi-automated methods

(iv) Full Width Half maximum

Controversy exists as to when is the most appropriate time to image and measure infarct size. Reimer and Jennings talk of the changing anatomic reference base of the evolving myocardial infarction[252] and timing of infarct imaging should therefore be viewed under the prism of the underlying pathophysiology of infarction.[248] The infarct zone is heterogeneous and the extent of infarction is dynamic due to oedema,

haemorrhage and inflammation. The infarct zone may therefore appear to extend without any further myocyte death. What follows is subsequent reduction in the extent of infarction as oedema subsides, haemorrhage is reabsorbed and dead myocytes are removed and replaced by scar.[253] There are advantages to both scanning patients early after AMI and also opting to wait a number of weeks. In scanning patients early, one is able to assess multiple components of infarction including oedema, haemorrhage and MVO, which not be present at a later date. Leaving CMR to a later stage means that resolution of regional wall motion abnormalities or stunning may have resolved giving an accurate assessment of overall LV function. This enables assessment of patients for devices such as implantable cardiac defibrillators. Assessment of IS by CMR as an inpatient soon after PPCI is safe and obviates the need for the patient to return at a later stage. In terms of clinical trials, given that measurement of CMR indices early after PPCI offers prognostic information, it would seem reasonable to use this data as a clinical endpoint.

1.5.3 Area at Risk assessed by CMR

CMR has been used to delineate the AAR on standard LGE imaging with:

(i) Infarct Endocardial Surface Area (Infarct-ESA) measurement on LGE imaging

The measurement of the lateral boundaries of the endocardial surface area of infarction is based on the wavefront phenomenon. The Infarct-ESA technique relies on two principles;

(a) The subendocardial lateral boundaries of the infarct are established within the first forty minutes of coronary occlusion. Infarction then spreads out towards the epicardium. [254] Subsequent increases in infarct size are dependent on the transmural wave-front progression of necrosis from endocardium to epicardium, modulated by the presence of collateral flow and time-to-reperfusion.[255] Taking a short-axis stack through the LV both the endocardial surface area and the endocardial surface area of the hyperenhanced region (infarct-ESA) are traced. The infarct-ESA from all slices is summed and divided by the total endocardial surface area. This is expressed as a percentage of the LV to define the area at risk:

$$\%\ Infarct - ESA = \left(\frac{Summed\ Endocardial\ Infarct\ Length}{Total\ LV\ endocardial\ length}\right) x\ 100$$

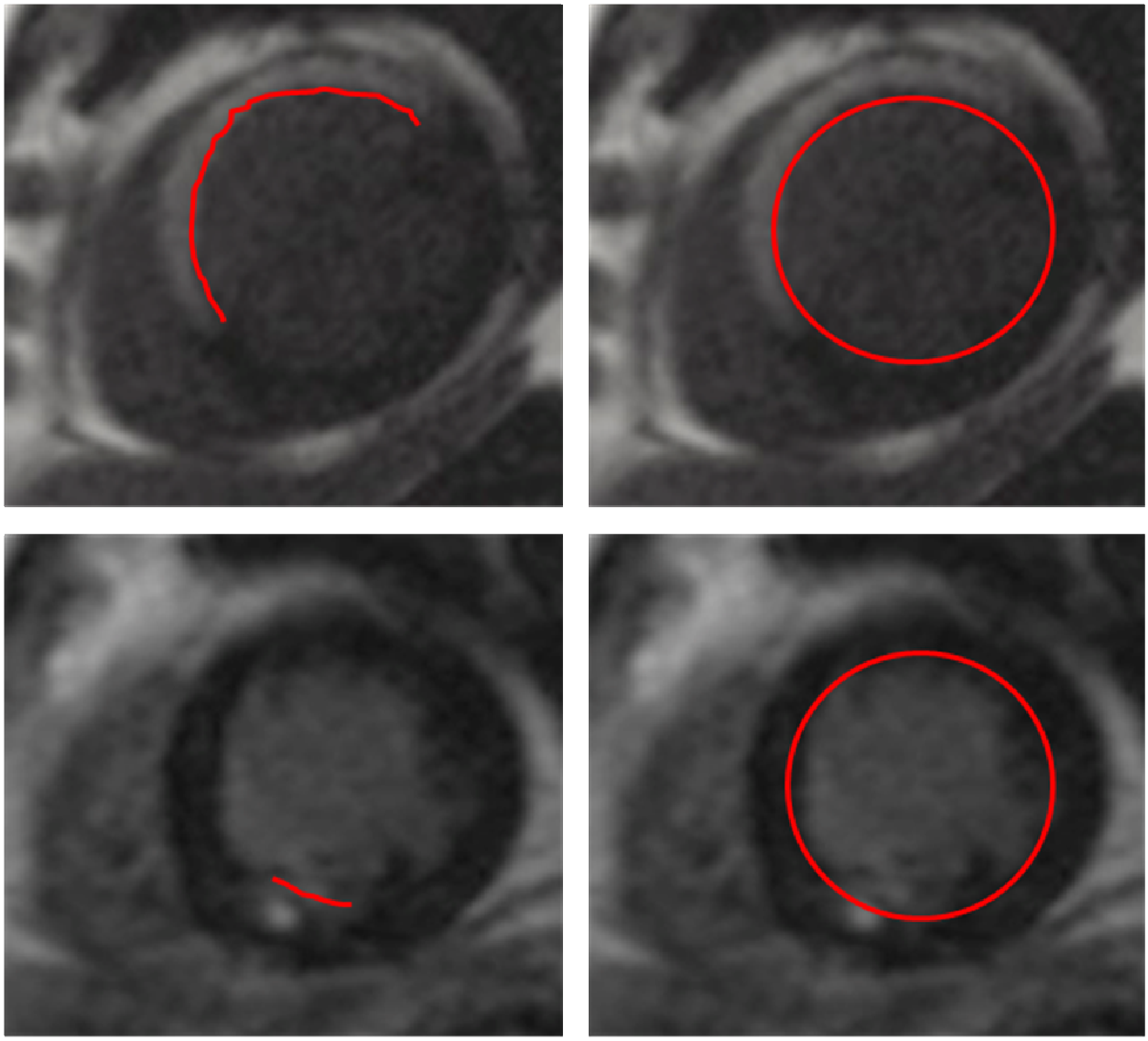


Figure 8 Illustration of the Infarct ESA technique

Note is made of how this technique will underestimate the AAR in situations of small degrees of infarction.

(b) LGE imaging by CMR can detect even very small subendocardial infarction.

In a study of 78 patients, Versteylen *et al.* compared the performance of angiographic, electrocardiographic and CMR techniques to assess the AAR. The group demonstrated that CMR techniques agreed best with the physiological

concepts, followed by the angiographic methods, with the electrocardiographic scores showing the worse agreement. Between both CMR techniques, the ESA method demonstrated a better degree of agreement than T_2-weighted imaging.[256] Unfortunately the study did not compare this technique with the current reference standard of SPECT. The group sensibly point out the risks inherent in using different techniques to quantify the infarct size and AAR. Even though measuring infarct size and AAR on CMR (whether with T2-weighted images or Infarct-ESA) may use different measurements, less variation is likely to occur when comparing one CMR measurement with another CMR measurement than with a non-CMR measurement. Two different techniques may be highly correlated but one may always be consistently underestimating or overestimating the other.[255]

In another small study, looking at only CMR techniques, Ubachs *et al.* compared Infarct-ESA to T2-weighted imaging (used as a 'reference standard'). The group demonstrated Infarct-ESA technique underestimated AAR in comparison to T2-weighted imaging.[257] The use of T2-weighted imaging as a reference standard for AAR assessment may be slightly premature. At the same time the fact that one technique may underestimate a variable does not preclude the comparator from overestimating the same variable. The limitations of T2-weighted imaging will be discussed below. The group do however point out a significant limitation of the infarct-ESA technique whereby it does underestimate the AAR. When ischaemic time is very short or in essence a STEMI is aborted, the absence of any necrosis or late enhancement makes it difficult to define the AAR on LGE images. The result of this is twofold - one will be unable to normalise groups with regards to the AAR, yet the calculation of myocardial salvage can proceed unimpeded. If there is no identifiable necrosis, then irrelevant of the size of the AAR, the amount of myocardial

salvage will approach 100% (MSI=1). Yet when the degree of necrosis does not appear to reach the lateral borders of the risk zone both the AAR and the resultant degree of salvage will be underestimated.

(ii) Oedema imaging using T2 weighted CMR

Oedema is a basic element of tissue response to acute injury. In this sense the myocardium is no different to other tissues and organs in the body, in that myocardial oedema occurs in reaction to many cardiovascular conditions (both acute and chronic) and following clinical interventions to address these disease processes.

The term myocardial oedema refers to both fluid accumulation in the cardiac interstitium (vasogenic) and to myocyte swelling (cytogenic). Usually there is only a very small interstitial fluid component, contained within a gel of collagen, elastic fibres and glucosamine glycans, which drains from the subendocardium to subepicardium via the lymphatic system.[258] Myocardial fluid balance is determined by fluid filtration rate out of the coronary microvascular exchange vessels into the cardiac interstitium and its removal rate from the interstitium via myocardial lymphatic vessels.[259] Under normal conditions, however, the rate at which fluid enters the cardiac interstitium is equal to the myocardial lymph flow rate, and, thus, myocardial water content remains relatively constant.

Experimental studies in canine and pig hearts suggest that myocardial oedema which develops in the AAR following a reperfused infarct can be visualised and

measured using a T2-weighted imaging sequence which detects oedema as an increased signal intensity.

In 1983 Higgins *et al.* showed that T2 weighted nuclear magnetic imaging techniques were sensitive to myocardial oedema that had formed as a result of acute myocardial infarction in a dog.[260] Signal intensity on T2-weighted images was noted to be increased in the infarcted segment. The same group used a similar model but imaged canine hearts earlier and in-vivo demonstrating that the increased signal intensity was noted as early as 30 minutes following reperfusion and uniformly increased at three hours. The suggestion therefore was that the area of increased signal intensity identified not just infarcted myocardium but also the AAR.[261,262]McNamara *et al.* brought T2-weighted imaging in acute infarction into the clinical arena in a small study demonstrating similar results to earlier animal experiments by detecting myocardial infarction as a region of high signal intensity relative to that of adjacent normal myocardium.[263]

The ability to detect infarcted myocardium does not necessarily imply the ability to detect acutely infarcted myocardium. Yet the capability to determine acutely ischaemic or infarcted myocardium represents a significant step in characterising the metabolic condition of the myocardium beyond simple indices of regional strain or contractile function.[264] Most imaging techniques that are used to image the myocardium are unable to differentiate acute from chronically infarcted tissue. Echocardiography and cine MRI will show a regional wall motion abnormality both in the acute and chronic setting. Wall thinning although mostly pathognomonic for chronic remodelled infarcted myocardium, will not necessarily be present in a non-

transmural infarction. Necrotic tissue will show up as bright on late enhancement MRI imaging in both acute and chronic setting (although literature exists that suggests that the signal intensity in acutely infarcted tissue is greater than that of chronic infarction).

A small canine study by Aletras *et al.* demonstrated that regional hyperintense areas on T2-weighted images obtained two days post infarction accurately define the AAR in both reperfused and non-reperfused infarctions when compared with microsphere area at risk measurements.[265] An elegant study by Mewton *et al*. assessed the 'post reperfusional' AAR with two T2-weighted techniques (STIR and ACUTE2) and infarct-ESA against the reference pathological AAR (Uniperse blue dye) in an *in vivo* pig model of acute reperfused myocardial infarction. Arterial enhanced CT was also used to assess AAR prior to reperfusion. The group demonstrated that both T2-weighted techniques and Infarct-ESA correlated well with pathology yet overestimated the AAR. At the same time the best correlation was obtained with pre-reperfusion CT.[266] The authors hypothesise that the overestimation in size of AAR compared to other studies may be an issue of timing. Abdel-Aty *et al.* reported that transmural increased signal on T2-weighted images were noted within 30 minutes following experimental occlusion in open-chest dogs. Increased T2 signal preceded necrosis (LGE or troponin release) and was augmented by reperfusion.[267] Abdel-Aty *et al.* studied 73 patients with acute or chronic infarction and found that T2-weighted imaging had a high specificity (96%) to distinguish one from the other.[268] The finding of a transmural area of oedema that was larger than the actual area of infarction helped reawaken interest in the use of T2-weighted imaging to delineate the AAR.

A number of studies have since attempted to validate the use of T2-weighted oedema imaging for measuring the area at risk in humans. These are listed below:

Study Author	N	Day	Sequence	Comparator
Oritz-Perez[255]	83		N/A	BARI *vs* Infarct ESA (r = 0.90, p<0.001) APPROACH *vs* Infarct ESA (r = 0.87, p<0.001)
Wright[209]	108		STIR (8mm)	BARI *vs* Infarct ESA (r = 0.42, p<0.001) Infarct ESA *vs* T2W (r = 0.77, p<0.001)
Carlson[269]	16	7	STIR (8-10mm)	SPECT *vs* T2W (r = 0.70, p<0.001)
Sörensson[260]	16	7	CE SSFP cines (8mm)	SPECT *vs* T2W (r = 0.78, p<0.001)
Hadamitzky [261]	207	4	TSE (8mm)	SPECT *vs* T2W (r = 0.80, p<0.0001)
Ubachs[248]	37	4	TSE (8-10mm)	Infarct ESA *vs* T2W (r = 0.17, p=0.002)
Berry[270]	50	4	T2-SSFP (6mm)	APPROACH (r = 0.74, p<0.0001) DUKE (r = 0.54, p=0.0001)
Payne[271]	54	2	STIR ACUTE2	APPROACH *vs* STIR (r = 0.44, p<0.01) APPROACH *vs* ACUTE2 (r = 0.68, p<0.01)
Fuernau[271]	197	3	STIR (N/A)	APPROACH (r = 0.87, p<0.001) Infarct ESA *vs* T2W (r = 0.56, p<0.001) APPROACH *vs* Infarct ESA (r = 0.44, p<0.001)
Lønborg[272]	91	2	STIR (8-15mm)	T2W *vs* T2W (TE 65msec; slice thickness 15 mm *vs* TE 100 msec; slice thickness 8 mm)
Moral[273]	75	4	STIR (8mm)	APPROACH (r = 0.69, p<0.001) BARI (r = 0.72, p<0.001) Infarct ESA *vs* T2W (r = 0.87, p<0.001) APPROACH *vs* BARI (r=0.91, p<0.001) BARI *vs* Infarct ESA (r=0.70, p<0.001) APPROACH *vs* Infarct ESA (r = 0.72, p<0.001)

Table 3 Table showing studies attempting to validate the use of T2-weighted oedema imaging for measuring the area at risk in humans

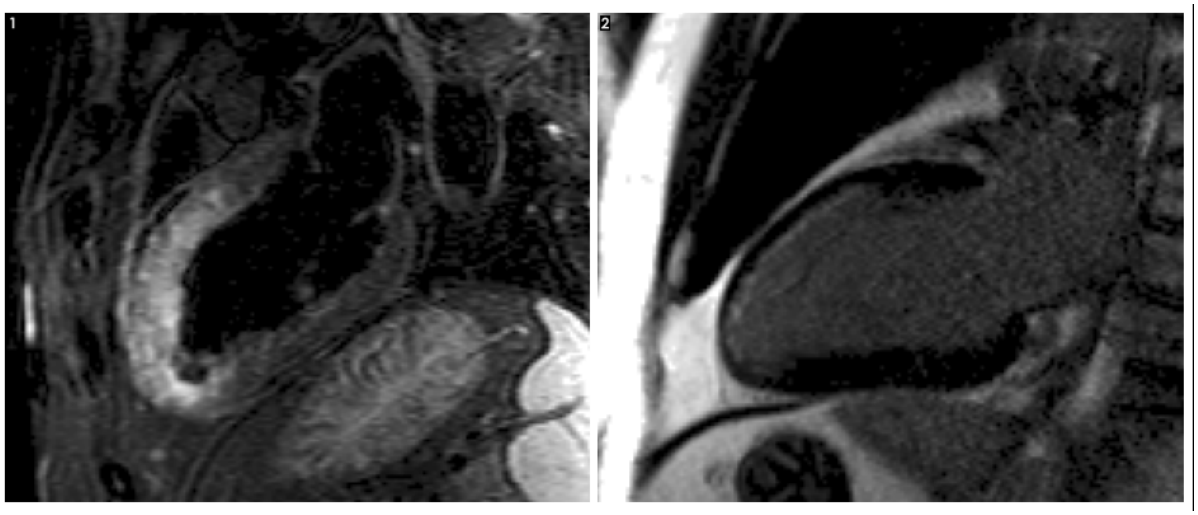


Figure 9 CMR Long axis images of a study patient two days post PPCI

Image 1 is an IR sequence demonstrating a long segment of subendocardial LGE. Image 2 is a T2-weighted (TIRM) sequence demonstrating increased signal (myocardial oedema) throughout the anterior wall. Images are consistent with significant myocardial salvage.

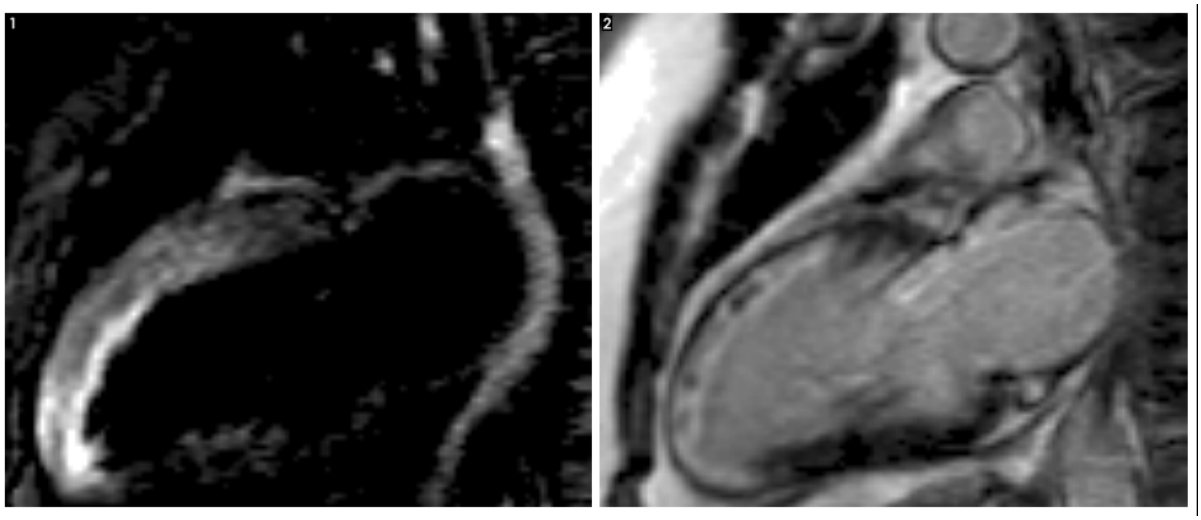


Figure 10 CMR Long axis images of a study patient three days post PPCI

Image 1 is an IR sequence demonstrating extensive/transmural anterior LGE with the presence of MVO. Image 2 is a T2-weighted sequence showing as in the previous patient in the figure above increased signal (myocardial oedema) throughout the anterior wall. There is however no evidence of myocardial salvage.

Carlsson *et al*. showed in a small series that the AAR measured by T2-weighted imaging correlated well with SPECT. The group also showed that the AAR when measured by CMR was similar between day one and day seven with the area of oedema disappearing by six weeks.[269] The study limitations include its small sample size and the fact that the scans were done across two different platforms (Siemens and Philips) with varying sequences. Another small study by Sörensson *et al.* achieved similar results using a different T2-weighted (post-contrast) sequence. The CMR studies were again performed across two different CMR platforms (GE and Philips) yet enrolment was limited to patients with TIMI 0 flow on initial angiography.[274] A study by Hadamitzky *et al.*, although showing a good correlation between SPECT and T2-weighted CMR for AAR assessment is hampered by a number of limitations.[275] Over 50% of patients included in the trial had TIMI flow ≥ 1 meaning that SPECT will likely underestimate the AAR given that ^{99}Tc-sestamibi may escape into the ischaemic bed. Despite the overall good correlation between CMR and SPECT, the authors note substantial differences in individual cases. These include (i) patients in whom the calculated infarct size was larger than the AAR for both modalities and (ii) patients with a short ischaemic time demonstrating a signal intensity too low to be completely detected automatically. It is also important to note that over 10% of patients were excluded due to poor image quality on T2-weighted images. Bland-Altman plots for this study showed wide limits of agreement for measurement of AAR (as well as scar, and salvage area) suggesting that these modalities should not be used interchangeably on serial testing.[276]

In summary studies that have attempted to validate the use of T2-weighted imaging for the assessment of the myocardium of risk are varied and suffer from a number of limitations including;

(i) Sample sizes are often small

(ii) Patients scanned on multiple platforms within single studies

(iii) No inter-study reproducibility data

(iv) Variation exists in the time from reperfusion to CMR imaging

(v) Failure to compare risk zones to reference standard

(vi) No consensus exists on the most suitable T2-weighted sequence (or parameters) to use with multiple sequences being harnessed within a single study.

(vii) No consensus on most appropriate threshold to define abnormal (oedematous) myocardium. By using a lower threshold for quantifying oedema, one increases the likelihood that the measured AAR will be substantially larger than infarct size simply from partial volume arguments.[277]

Aside from the lack of consensus on the use of T2-weighted sequences, controversy exists as to whether the T2-weighted imaging can be appropriately used to measure the AAR. These concerns include;

(a) T2-weighted imaging delineates the area of infarction rather than the AAR - A number of small pre-clinical studies have suggested that T2-weighted imaging highlights the area of acute infarction as opposed the area at risk.[278,279,280]

(b) Delineation of reversible *vs.* irreversible myocardial injury - In theory T2-weighted imaging will delineate the AAR including the area of infarction but will not be able to

delineate between the two. Croisille *et al*. point to the dramatic changes in the myocardium of patients with irreversible injury *vs.* irreversible injury. This includes significant differences in wall thickness, electrolytes, ultrastructural changes and in total water content (88% *vs* 0-10%). The significant difference in total water content between reversibly injured myocardium and irreversibly injured myocardium, lead Croisille *et al*. to question the oft cited finding of transmural increase in signal intensity.[281]

(c) The effect of cardioprotective strategies on myocardial oedema - the greatest challenge over the use of T2-weighted CMR to assess the AAR is the suggestion that a cardioprotective strategy may be able to reduce the amount of myocardial oedema and hence reduce the measured AAR as delineated by the oedematous region. The underlying principle of the AAR is that it is a fixed amount of myocardium subtended by a particular artery that would not be affected by any particular intervention. As mentioned earlier, Zhao *et al.* demonstrated in a canine model that both pre- and post-conditioning protocols were able to significantly reduce the percentage of tissue oedema (as assessed by the *ex vivo* measurement of water content in a tissue sample of the area at risk).[40] Thuny *et al.* were also able to show in humans that ischaemic post-conditioning reduces the extent myocardial oedema (as assessed by T2 weighted imaging - STIR, slice thickness 7mm) in STEMI patients undergoing PPCI.[282]

Studies using T2-weighted imaging have been hampered by technical difficulties. Dark-blood methods are subject to artefacts such as posterior wall signal loss due to cardiac motion, and bright subendocardial rims due to stagnant blood.[283] Sequences and image quality are also limited by arrhythmias and difficulty breath holding. Newer sequences such as the T_2-prepared SSFP bright blood approach,[283] ACUT$_2$E TSE-

SSFP (a hybrid between steady state free precession (SSFP) and turbo spin echo (TSE) for bright-blood T2-weighted imaging)[284] go someway towards addressing these issues with a higher diagnostic accuracy than dark-blood T2-weighted MRI.[285] T2-mapping offers a quantitative approach increased accuracy in the detection of myocardial oedema whilst at the same time addressing the technical issues of previous sequences.

T2-weighted imaging clearly advances our understanding of the complex pathophysiology of AMI, yet is associated with many unanswered questions. The use of these sequences in the assessment of the AAR needs to be undertaken with caution. Such a caveat should not limit research into the attenuation of (and hence imaging of) myocardial oedema as a modifiable component of reperfusion injury.

Newer techniques such as T2 mapping offer advantages over standard T2 weighted images both in terms of their diagnostic accuracy but also in the ability of the technique to quantify the T2 signal of the myocardium.[286] T1 mapping techniques are also being utilised to image myocardial oedema.[287]

1.5.4 Microvascular Obstruction (MVO) assessed by CMR

Anatomic no-reflow was first recognised in a canine model of reperfused myocardial infarction and was described as the failure to restore normal myocardial blood flow despite the removal of the coronary obstruction.[20] Therefore restoration of coronary flow by PPCI to remove the epicardial obstruction does not guarantee adequate perfusion at a myocardial level. Given that the presence of MVO is associated with a larger infarct size, impaired (LV) ejection fraction, adverse LV remodelling and poorer clinical outcomes, the ability to identify MVO is crucial. CMR is currently the best technique to identify microvascular obstruction. João *et al.* demonstrated the ability of CMR to identify MVO in humans and described how large infarcts, associated with prolonged obstruction of the infarct-related artery, were characterised by central dark zones surrounded by hyperenhanced regions.[288] Microsphere blood flow in areas of hypoenhancement, following reperfused infarction, are significantly reduced (>50%) when compared with myocardium remote from infarcted tissue.[289] This helps explain the regional differences in signal intensity within infarcted tissue (hypo- and hyperenhancement) which are secondary to impaired wash-in (secondary to impaired perfusion) and wash-out kinetics of Gd-DTPA. Slow wash-in of gadolinium in reperfused, infarcted regions leads to early, low tissue contrast concentrations, whereas slow washout eventually leads to higher contrast concentrations compared with normal tissue.[290] Gadolinium is not a pure intravascular agent and extravasates into the interstitium within minutes following administration, it will gradually diffuse into the initially hypoenhanced zone. Therefore, the size of MVO will decrease over time from contrast administration. Given that MVO is often imaged using three different techniques (post-contrast), the

earliest technique used will demonstrate a larger area of MVO than the last technique used in a particular study which may underestimate the extent of MVO.[291]

MVO can be imaged by a number of techniques using CMR. These include:

1. Gadolinium enhanced first-pass myocardial perfusion tracks the first minute of gadolinium arrival and distribution within the myocardium. This sequence should be the most sensitive technique for detecting MVO[292,293] as it allows less time (than the other later imaged sequences) for diffusion of gadolinium to artefactually diminish MVO volume. The sequence does however afford only half the spatial resolution of the inversion recovery sequences and typically only covers three slices of the myocardium.[294]

2. Late gadolinium enhancement imaging (LGE). As with standard LGE imaging, pictures are taken 10 - 15 minutes following administration of contrast using inversion recovery sequences. LGE technique offers information on a more equilibrated distribution of the contrast agent in the infarct area with high-resolution images but is limited by a longer examination time because it requires at least 10 minutes after contrast agent injection to obtain equilibration. Multiple breath holds to fully cover the LV.[293] Signal intensity in the area of hypoenhancement rises slowly in the first 5 to 10 minutes after contrast injection. The extent of hypoenhancement may vary with the time after injection, as it is unlikely that the degree of microvascular damage is identical throughout the entire infarct. Some areas may have only mild

damage and allow slow contrast wash-in by diffusion from surrounding regions with intact microcirculation. Other areas may have extensive microvascular damage, resulting in persistent hypoenhancement.[295] In essence areas of MVO may be missed at differing times following contrast agent administration when using LGE imaging techniques are used to identify it.

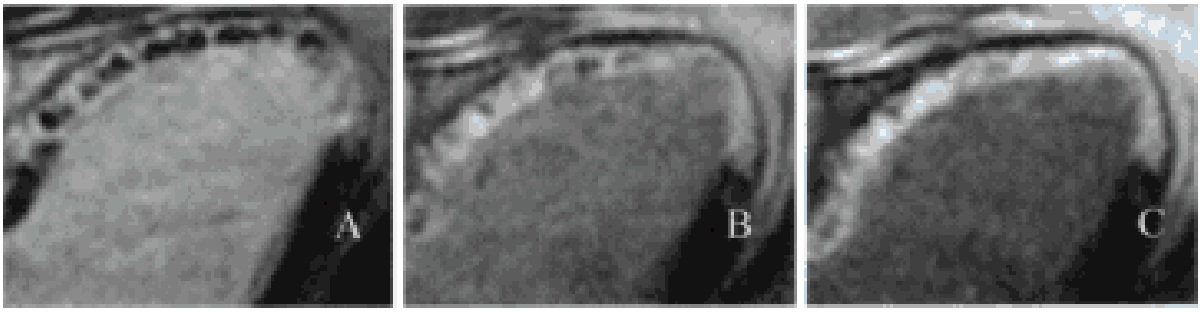


Figure 11 Figure showing Temporal changes in signal intensity after 0.2 mmol/kg gadolinium-DTPA administered intravenously

Temporal changes in signal intensity after 0.2 mmol/kg gadolinium-DTPA administered intravenously in the same patient as Figure 2. Magnified view of three-chamber view at 2 min (A), 15 min (B), and 30 min (C).

Reproduced with permission from: Beek *et al*. Delayed contrast-enhanced magnetic resonance imaging for the prediction of regional functional improvement after acute myocardial infarction. J Am Coll Cardiol. 2003;42(5):895-901. doi:10.1016/S0735-1097(03)00835-0

3. Early gadolinium enhancement imaging (EGE) Imaging within the few minutes after contrast injection and using similar inversion recovery sequences as for LGE imaging but with a fixed and long inversion time. Advantages include complete ventricular coverage, optimal signal-to-noise (SNR) and spatial resolution which combined may overcome the shortcomings mentioned above.[296] Full coverage of the left ventricle means that EGE images can then be compared to late enhancement images.

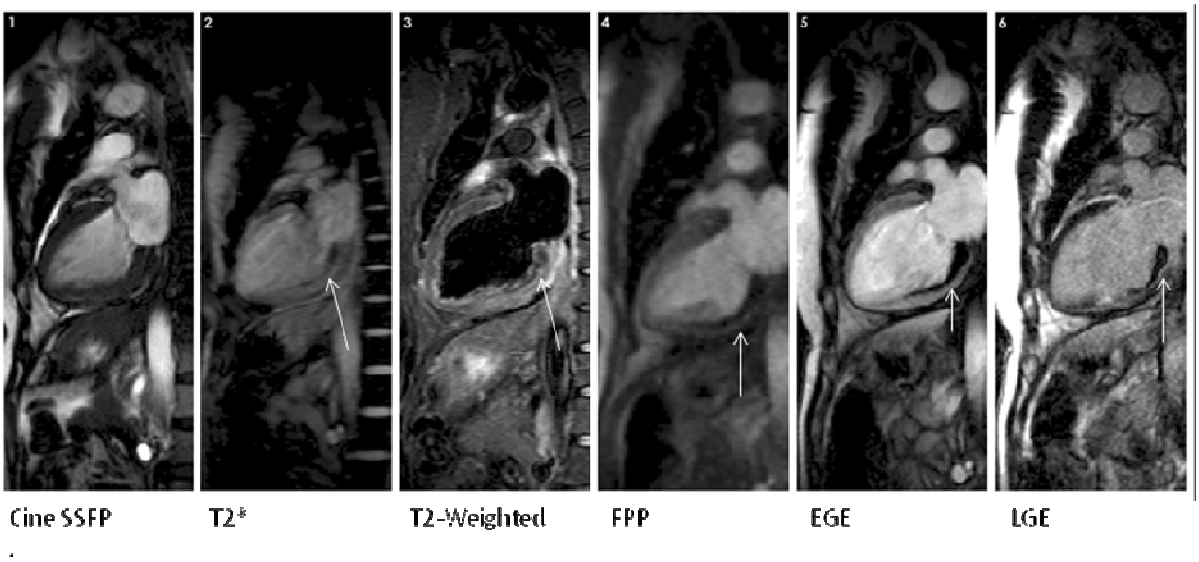


Figure 12 Figure of a study patient showing two chamber images showing the different techniques available to image MVO and haemorrhage

1.Two chamber Cine for reference, 2. T2 Gradient Echo 3. T2-weighted (TIRM) with increased signal in the inferior wall with an area of signal void in the subendocardium. 4. FFP, 5. EGE with fixed T1 440 and 6. LGE

As mentioned above the presence of MVO appears to be an independent predictor of major adverse cardiovascular events. It should be taken into account that for the studies investigating the relationship between MVO and clinical outcomes, significant variability exists between how the MVO is imaged and at what time point following infarction that the imaging takes place.

Wu *et al.*[297] demonstrated that the presence of MVO as assessed by gadolinium enhanced first-pass myocardial perfusion imaging at one minute post contrast was strongly correlated with increased major adverse cardiovascular events when compared to patients without MVO. The group also demonstrated the relationship between infarct size measured by CMR and the risk of adverse cardiac events post

infarction - patients with larger infarcts having worse outcomes. Although the presence of MVO is related to larger areas of late enhancement, MVO is a strong (independent) predictor of adverse cardiovascular events, even after the extent of total myocardial damage is controlled for.[297] The study by Wu *et al*. also cast doubt on the ability of LV indices (LV volumes, mass and ejection fraction) to predict outcome post infarction and at the same time highlighting the merits of methods that directly assess myocardial injury. The poor predictive value of ejection fraction following AMI has been attributed to a number of factors including;

1. Myocardial stunning following AMI can lead to an initial underestimation of LVEF. Restoration of function cannot be expected to occur immediately when reperfusion or other protective interventions are applied after several hours of severe myocardial ischaemia. Therefore, evaluation of cardiac performance several hours or even days after relief of ischaemia will not provide an adequate assessment of eventual function.[298]

2. Hyperkinesis of remote non-infarcted myocardium may be present in the first hours after MI.[299] This may in turn normalise the measured global ejection fraction despite dysfunction within the infarcted zone. This hyperkinesis of the remote myocardium will likely subside leading to a deterioration in the ejection fraction when measured at a later date possibly despite an improvement in function of the infarcted segment. [300]

3. The ability of ejection fraction to accurately reflect the extent of myocardial damage early after acute infarction is limited by its dependence on global pre- and afterload conditions.[169]

One potential mechanisms for the negative effect of the presence of MVO on outcomes is the adverse effect on LV remodeling

Summary of CMR studies which that have assessed the functional and clinical outcome of the presence of MVO

Study Author, Journal	Number (n)	MVO assessment	Quantification	Time point post-MI	Findings
Functional outcome and LV remodelling					
Wu[291]	44	EGE	Present/absent	4–16 days 16 months	MVO predicted LV remodelling
Hombach[301]	110	LGE	Present/absent	6–8 days 7.5 months	Late MVO (PMO) and infarct size predicted LV remodelling
Nijveldt[36]	60	FPP LGE	Present/absent	2–9 days 4 months	Late MVO strongest predictor of LV remodeling
Orn[302]	42	FPP	Present/absent	2 days 1 week 2 months 1 year	MVO detectable at 1 week predicted LV remodeling
Weir[303]	100	FPP EGE LGE	Present/absent	4 days 12 weeks 24 weeks	Late MVO better predicted remodeling at 6 months
Wong[304]	40	FPP LGE	Present/absent	3 days 90 days	Late MVO strongest predictor of LV ejection fraction at 90 days

Clinical Outcome					
Wu[291]	44	EGE	Present/absent	4–16 days	MVO predicted MACE at 16 months, independent of infarct size
Hombach[301]	110	LGE	Present/absent	6–8 days	MVO predicted MACE at 7.5 months, independent of LVEF, LVEDV, or infarct size
Bruder[305]	67	EGE (by SS SSFP-IR)	Quantified	5 days	MVO >0.5 % independently predicted MACE at 1 year
Cochet[306]	184	FPP LGE	Present/absent	3–7 days	Late MVO predicted MACE at 1 year
De Waha[307]	438	EGE LGE	Quantified	3 days	Presence and extent of late MVO are independent prognosticators and better predicted MACE at 19 months
Note: FPP: first pass perfusion; EGE: early gadolinium enhancement technique; LGE: late gadolinium enhancement technique; MVO: microvascular obstruction; MACE: major adverse cardiovascular events; LVEDV: left ventricular end-diastolic volume.					
Late MVO describes MVO detectable by the LGE technique at a minimum of 10 mins post-gadolinium administration, and can be otherwise described as persistent MVO or 'PMO'					

Table 4 Table showing the summary of CMR studies which have assessed the functional and clinical outcome of the presence of MVO

Adapted from Wu, K.: 'Functional and clinical impact of microvascular obstruction in acute MI'. Presented at the SCMR Scientific Sessions. Nice, France; 2011.

Further studies have confirmed that the presence of MVO, as assessed by CMR is a key determinant in prognosis following AMI. A large study on the impact of MVO on long term outcomes after STEMI followed up 438 patients over a median of nine months. de Waha *et al.* demonstrated that MVO on LGE (as opposed to EGE) MVO was independently associated with mortality and the occurrence of the combination of death, myocardial re-infarction, and congestive heart failure. The extent of MVO measured on LGE images showed a graded relationship with the occurrence of this composite endpoint and presence and extent of late MO provided an incremental prognostic value above the traditional prognostic markers.[307] The same group went on to demonstrate that the analysis of a ratio of MVO to infarct size adds incremental prognostic value. The ratio was determined to be an even stronger predictor for long-term prognosis after STEMI than either MVO or infarct size alone.[308] Similar studies extended follow up out to five years (median 52 months) but with a much smaller sample size suggested that the ratio of infarct size to MVO measured on first pass (as opposed to LGE measured by de Waha's group) imaging added superior prognostic value compared to the extent of MVO alone.[309] Malek *et al.* in a small study postulated that the presence of MVO but not its extent corresponded to larger infarct size in STEMI. Disappointingly the method used to quantify the amount of MVO was an arbitrary allocation of 'small' or 'large'.[310]

Given its adverse effect on outcome, it is reasonable to try and direct cardioprotective strategies towards the treatment/prevention of MVO. If we can attenuate MVO, or this form of reperfusion injury, then we can expect to improve clinical outcomes in our patients. Treating MVO may allow better healing, less adverse ventricular remodeling, continued downstream access to drug levels and

allow for better collateral flow.[311] Currently there is no effective therapy for doing this. Numerous pharmacological agents have been tried including adenosine, nitrates, calcium channel blockers and all of them may improve some of the perfusion indices but have not been shown to actually improve clinical outcomes.

Clearly the presence of MVO pertains to a worse outcome in patients following STEMI being a marker of severe microvascular destruction. The presence of MVO has been shown to have incremental prognostic value above traditional prognostic markers including cardiac biomarkers and LV function. It is a promising marker to help evaluate new strategies to reduce reperfusion injury.[312] Historically, clinical trials have used less sensitive surrogates for identifying MVO including ECG, angiographic measures and MCE. MVO may be missed unless more precise assessments (using CMR) are employed in clinical trials of cardioprotective agents in AMI. There are currently few human clinical trials in cardioprotection that have employed MVO, as assessed by CMR, as an endpoint, but they are beginning to increase in number.[313]

Study	N	Intervention	MVO Assessment	Quantification	Time Point Post MI days	Conclusion
Thiele[314]	154	IV *vs.* IC Abciximab	EGE LGE	Quantified %LV Mass	2	IC reduced IS and MVO
Atar[315]	234	FX06	LGE	Quantified %LV Mass	5	Reduced necrotic core zone
Ludman[316]	51	Erythropoietin	EGE or LGE	Presence or Absence	2	Increased incidence MVO
Zia[317]	60	Thrombectomy	LGE	Quantified %LV Mass	2	Reduction in degree MVO
Prunier [318]	110	Erythropoietin	LGE	Presence or Absence	5	Reduced incidence MVO
Wöhrle[319]	51	Bivalirudin *vs* unfractionated heparin plus abciximab	LGE	Quantified	2	No difference in MVO
Desmet[320]	112	Adenosine	LGE	Quantified	2-3	No difference in MVO

Table 5 Table showing clinical studies that have assessed MVO as a secondary endpoint

Further validation and prognostic studies would help define the role of MVO in future CMR endpoint clinical trials and help answer the numerous unresolved including (i) at which time point post STEMI is best to image for MVO, (ii) which CMR sequence (and hence what time point post contrast administration) provides the primary

prognostic information and (iii) does the amount/extent of MVO impact on prognosis or is the poor prognostication derived solely from it's presence irrelevant of size.

1.5.5 Intramyocardial Haemorrhage assessed by CMR

Intramyocardial haemorrhage (IMH) is by no means a new concept in the setting of acute myocardial infarction. Haemorrhagic infarction has been demonstrated both in infarcts treated by mechanical and pharmacological therapy (with the extent of the haemorrhage not increased by the lytic state).[321] However, IMH occurs frequently in a reperfused infarct but only rarely in the case of a non-reperfused infarction.[322] Passoti *et al.* describe haemorrhagic infarction as a reperfusion-associated pathology in that it was rarely documented in the pre-reperfusion era, but its reported incidence markedly increased after the start of the thrombolytic era and it continues to be seen with mechanical intervention.[323]

Previously IMH could only be identified *in-vitro* in experimental studies or post mortem. CMR has allowed for the *in-vivo* identification of IMH, therefore reawakening interest in this phenomenon. The inability to image IMH has meant that the clinical implications were generally unknown. The interplay between haemorrhage and other components of infarction (e.g. oedema, MVO etc.) also remains unclear. Reperfused acute infarctions have been noted macroscopically to have a reddish appearance. IMH is thought to be due to vascular cell damage and leakage of erythrocytes out of the injured vessels and into the extravascular space.[324] IMH is imaged by CMR by the use of two different sequences:

(i) T2-weighted Imaging of cerebral haemorrhage over time has shown that its appearance on MRI depends on the age of the haemorrhage and whether T1 or T2 weighted imaging is used. The effects of haemorrhage on T2 relaxation are complex and depend on the presence of blood degradation products (ferritin and haemosiderin), the effects of cellular compartmentalisation and the form of hemoglobin present. As a hematoma ages, the haemoglobin passes through several forms (oxyhaemoglobin, deoxyhaemoglobin, and methaemoglobin) prior to red cell lysis and breakdown into ferritin and haemosiderin.[325,326] Early imaging of haemorrhage causes a shortening of T2 consistent with the paramagnetic effects of deoxyhaemoglobin associated with IMH. This manifests itself as a signal void (or hypointense region) within an area of high signal intensity that represents oedema.[327] In an observational study of only two patients Basso *et al.* showed excellent overlap among the location, spatial extent, and shape of the hypointense regions by ex vivo T_2 CMR images and haemorrhage by pathologic investigation.[328]

Early studies showed that the presence of IMH was associated with severe microvascular injury.[329] There is however some disagreement as to the prognostic significance of IMH beyond that of MVO. Using T2-weighted MRI to image myocardial haemorrhage, a recent study by Ganame *et al.* demonstrated that in the acute phase, the presence of IMH was associated with larger infarct size and transmurality, larger LV volumes and lower LV ejection fraction, and more impaired contractility in the infarcted and peri-infarct territory. Evidence of adverse remodelling was shown at four months with haemorrhagic infarcts showing pronounced increase

in end-diastolic and end-systolic LV volumes with lack of functional recovery (globally and regionally in the infarcted area), and significant wall thinning in the infarcted, adjacent, and remote areas.[330] Beek *et al.* studied 45 patients with T2 weighted imaging and similarly found that the presence of IMH to be associated with larger infarcts, presence of MVO, higher left ventricular volumes and lower ejection fraction, and the lack of improvement at follow-up.[331] Their findings differed from Ganame's group in that they were unable to demonstrate that haemorrhage was an independent predictor of functional change at follow up and essentially showed that IMH had no prognostic significance beyond MVO. Bekkers *et al.* undertook a similar study and found that IMH and MVO were strongly related yet only infarct size was an independent predictor of LV remodelling.[332]

As with T2-weighted imaging for oedema, studies on IMH are limited by differences in protocol used to investigate the phenomenon. Such alternatives in protocols may account for some of the differences in outcomes. The approach of Beek *et al.* was slightly atypical in that they imaged in the long axis and one short axis view at what is described at the core of the infarct (short axis slice noted to have the most significant regional wall motion abnormality on cine imaging). In all three studies, although MVO was observed in all patients found to have IMH, the converse was not shown - with IMH not occurring in the absence of MVO. Also when IMH was absent, the degree of MVO was noted to be significantly smaller.

Study	N	Patients IMH	CMR Platform	Day	T2W Sequence (Thickness,TE)	IMH without MVO	Outcome
Ganame[330]	98	25%	Philips	2	STIR (8mm,100ms)	No	IMH independent predictor adverse remodelling
Beek[311]	45	49%	Siemens	5	STIR (7mm, 64ms)	No	IMH related to infarct size, MVO and function
Bekkers[332]	90	43%	Siemens	5	STIR (8mm, 100ms)	No	Infarct size independent predictor of adverse remodelling.
Amabile[333]	114	11%	Siemens	5	TSE (8mm, 52ms)	No	Independent predictor of adverse clinical events
Eitel[334]	346	35%	Philips Siemens	3	STIR (8mm, 80ms) (10mm, 61ms)	No	IMH predicts MACE

Husser[335]	304	34%	Siemens	6	STIR (8mm, 33ms) TSE (8mm, 100ms)	Yes	IMH predicts MACE and adverse Remodelling
Mather[294]	53	33% 25%	Philips	2	STIR (10mm, 100ms) T2*	No	IMH independent predictor adverse remodelling
O'Regan[326]	15	40%	Philips	3	STIR (10mm, 100ms) T2*	No	IMH indicates reduced salvage

Table 6 List of studies assessing IMH and it's relationship to outcomes

Eitel *et al*. demonstrated that the presence of a hypointense core within areas of oedema carried independent prognostic value being a strong indicator of MACE at 6 months. The occurrence of IMH was found to be associated with the presence and extent of late MVO, larger infarct size and LV dysfunction.[334] A similarly sized trial by Husser *et al*. suggested the presence of IMH is a small proportion of their cohort who displayed no MVO. The group found that although the presence of IMH was an independent prognosticator of adverse prognosis and remodelling, the addition of information from T2-weighted information did not improve predictive value beyond infarct size and cardiac volumes.[335]

(ii) T2* sequence is a well used and validated sequence in the clinical context to quantify the severity of myocardial (and hepatic) iron loading in patients with thalassaemia major.[336] O'Regan *et al.* applied the T2* sequence in the setting of reperfused AMI to image IMH. The group also showed the possible effect of IMH on the AAR measured with T2-weighted imaging. At higher threshold levels, signal from oedema can be masked by presence of haemorrhage in infarct core leading to underestimation of AAR. Pedersen *et al.* assessed the T1 and two T2 techniques (T2-STIR and T2*) for the assessment of IMH and compared them to *ex-vivo* pathological samples. The T1 weighted imaging yielded a higher sensitivity and specificity for IMH as well as a better agreement with pathology compared to T2-STIR and T2* imaging. In a limited number of pigs T2 weighted imaging was either unable to delineate haemorrhage or demonstrated an area of hypo-enhancement on T2-weighted images that was not present on the corresponding pathological slices.[337]

1.5.6 Diffuse fibrosis

There is a significant structural component in the gaps between the cells. The ratio of cells to interstitium is important and will vary dependent on different disease processes. The importance of the interstitium was noted in 1854 by Claude Bernard when he described the "Le Milieu intérieur" or 'the environment within'.[338] This refers to the extracellular environment, and its physiological capacity to ensure protective stability for the tissues and organs of multicellular living organisms.[339] In essence the interstitium provides the myocardium with its architectural structure. Disease processes lead to disruption of this architectural structure with expansion of the

interstitial space usually through the development of fibrosis (excessive deposition of extracellular matrix material in addition to myocyte degeneration leads to expansion of the interstitial space).[340] This is strongly associated with left ventricular remodelling and adverse outcomes such as heart failure, arrhythmia and death.[341] The pathophysiology interstitial expansion varies with different disease process with different subtypes of fibrosis being described. The final common pathway is the formation of focal fibrosis which is usually irreversible. Diffuse (interstitial) fibrosis which has been described in a number of disease states (present in the aging heart, diabetes, systemic hypertension, in idiopathic dilated cardiomyopathy, and in left ventricular (LV) pressure-overload and volume-overload states induced by chronic aortic valve regurgitation and stenosis.) and has shown to be reversible with specific therapies.[342]

Assessment and quantification of fibrosis has generally been achieved through endomyocardial biopsy. Biopsy constitutes an invasive procedure with a low but definite risk of complications and is prone to sampling error especially in the setting of diseases with patchy myocardial involvement.[343]

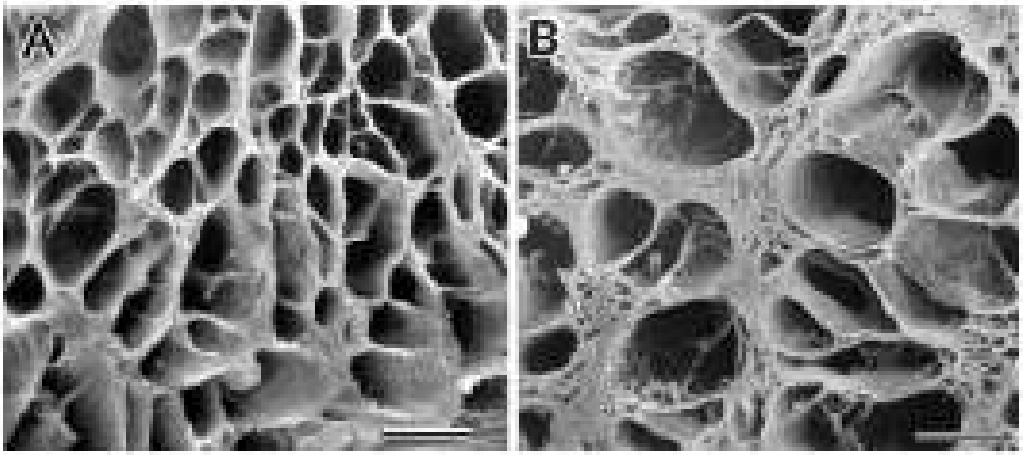


Figure 13 Three Dimensional remodelling of Cardiomyocytes in a patient with Aortic Stenosis
From Kanzaki, Y., et al., Three-Dimensional Remodeling of Cardiomyocytes in a Patient With Aortic Stenosis: Scanning Electron Microscopy. Circulation, 2009. **119**(2): p. e10-e10.

Gaps between the cells are imaged using CMR with contrast agents. Late gadolinium enhancement imaging enables us to image the gaps between cells. We are therefore able to identify focal fibrosis using inversion recovery (IR) sequences following a bolus of contrast. The IR technique results in a high sensitivity to regional T1 variation but sacrifices absolute quantification meaning that differing degrees of focal interstitial expansion will look the same (bright).[344] The LGE technique cannot be used to visualize diffuse fibrosis because “normal” myocardium with diffuse fibrosis is “nulled” to highlight focal scar, thereby losing all information of any background interstitial expansion.[345] Newer CMR imaging techniques, where a primed contrast infusion is used to eliminate contrast kinetic effects offer the ability to non-invasively measure the myocardial extracellular volume.[346] T1 mapping measures myocardial longitudinal magnetic relaxation. Measurements can be performed before and after contrast administration. Chelated gadolinium contrast distributes between cells embedded in the interstitium between cells (extracellular space) and blood plasma such that the relative pre- and post-contrast signal changes measure the myocardial extracellular volume fraction (ECV).[347] These techniques have been shown to correlate with histological techniques. Such changes in the extracellular volume (as measured by the volume of distribution of gadolinium) have shown to with markers of disease severity.

1.6 AIMS AND OBJECTIVES

In this thesis we have investigated the role of CMR in STEMI patients treated by PPCI. Specifically, we have investigated its role in assessing the cardioprotective efficacy of remote ischaemic preconditioning in this patient group.

1.6.1 Hypothesis

Cardiac MRI can be used to assess whether Remote Ischaemic Perconditioning has any beneficial effect in patients undergoing PPCI for STEMI.

1. To determine the most reliable and robust CMR method for quantifying myocardial infarction in STEMI patients undergoing PPCI (chapter 3).
2. To investigate the beneficial effects of remote ischaemic preconditioning in STEMI patients undergoing PPCI as assessed by CMR (chapter 4)
3. To investigate the use of CMR for tissue characterising the myocardial infarction in terms of myocardial oedema, diffuse interstitial volume, and intra-myocardial haemorrhage (chapter 5)

2 Methods

2.1 Overview

We conducted a two centre, open label randomised control study to assess the effect of remote ischaemic perconditioning induced by transient upper limb ischaemia on myocardial injury in consecutive patients presenting with STEMI who subsequently underwent PPCI.

2.2 Ethical Approval and Informed Consent.

The protocol for this study was written in accordance with the International Conference on Harmonisation - Good Clinical Practice (ICH-GCP) guidance, and the study was subject to approval by the joint University College London (UCL)/University College London Hospitals (UCLH) committees for the ethics of human research. Approval for the study was sought on the standard application form initially provided by the NHS COREC (Central office for the Research Ethics Committees), and subsequently integrated with the National Research Ethics Service (NRES), affiliated to the National Patient Safety Agency (NPSA). In addition to the study protocol, the patient information sheet, consent form, and a letter to the patient's general practitioner, were also approved by the ethical committee.

Once ethical approval was obtained, a separate application was made to the research and development (R&D) department UCLH, who also acted as sponsors of

the study. Any amendments to the protocol were classed as major or minor, as per advice from the ethical committee. I made the amendments and submitted the documents, annotated with appropriate dates and version numbers for approval for each amendment.

Any serious adverse event or reaction during the course of the study had to be reported to the R&D department as per guidelines. No serious adverse events or reactions occurred during the course of the study.

2.3 Patient Selection

Patients admitted to either of the two centres with a STEMI and a plan to undergo PPCI were approached with regard to taking part in the study. An abbreviated form of written consent was taken from the patient. The patient was then revisited following PPCI and full informed consent was taken.

2.3.1 Inclusion Criteria

Patients were included if they were aged over 18 years and presented to one of the two centres within 12 hours of onset of symptoms, fulfilled ECG criteria for STEMI (>2mm ST-elevation in chest leads, >1mm ST elevation in contiguous limb leads or new onset left bundle branch block) with a plan to undergo PPCI. Other inclusion criteria were the finding of a single vessel culprit lesion, Thrombolysis in Myocardial

Infarction (TIMI) grade of 0-1 and Rentrop collateral grade of 0 on coronary angiography.

2.3.2 Exclusion Criteria

Exclusion criteria included abnormal renal function (eGFR <35 ml/min/1.73m^2), known thromboembolic disease, peripheral vascular disease affecting the upper limbs, malignancy, multi-vessel coronary artery disease, cardiogenic shock and cardiac arrest. Patients who were claustrophobic or had metal implants which meant they could not enter an MRI scanner were excluded from the CMR scan. Patients with pre-infarct angina or known to be taking pre-conditioning mimetics were also excluded.

2.3.3 Randomisation

Patients were randomised to either the control group or the Remote ischaemic Perconditioniing (RIPerC) group, using electronic randomisation methods of random sequence generation for the two treatments. A simple randomisation method was used to allocate patients to receive either RIPerC or a placebo. (http://www.random.org/sequences/)

2.4 Remote ischaemic Perconditioning (RIPerC)

2.4.1 Control group

These patients had an un-inflated blood pressure cuff placed on the arm for 20 minutes prior to myocardial reperfusion.

2.4.2 RIPerC group

RIPerC was induced by transient upper limb ischaemia using a standard 9 inch blood pressure cuff. Patients received 2 cycles of 5 minutes of upper limb ischaemia (with the cuff inflated to 200 mmHg), separated by 5 minutes of reperfusion between each cuff inflation prior to myocardial reperfusion. A similar protocol of three cycles was first used by Kharbanda *et al.*,[123] to induce RIPC and was shown to protect against endothelial IRI of an opposite limb in normal human volunteers.

2.5 Primary Percutaneous Coronary Intervention (PPCI)

Patients were taken directly for PPCI. The procedure was carried out according to the interventional consultants preference with no restriction placed on vascular access site, choice of stent or method of stenting (Direct/Predilatation/Post dilatation). Any patients who following angiography did not undergo primary angioplasty were excluded from continuation in the study. Patients who underwent intervention to a non-culprit vessel (following angioplasty to the culprit artery) were also excluded. The use of medication including glycoprotein IIb/IIIa inhibitors was

also left to the interventional consultant's discretion but patients receiving thrombolytics or adenosine were excluded.

2. 6 Study Endpoints

2.5.1 Blood Tests

Blood samples were taken for TnT, CK-MB and CK levels at the time of arterial sheath insertion (0 hour), and at 6 hours, 12 hours, and 24 hours post PCI procedure. Full blood count, renal function and glucose levels were also checked at baseline.

In both recruiting Centres, TnT was measured quantitatively by a one-step enzyme immunoassay based on electrochemiluminescence technology (Elecsys 2010; Roche). The Elecsys troponin T assay consists of two monoclonal antibodies specifically directed against human cardiac troponin T, and detects free cardiac troponin T, as well as its binary and ternary complexes. The lower detection limit of troponin T concentration that can be distinguished from zero is 0.01 µg/l, and the lowest troponin T concentration that meets a 10% coefficient of variation requirement is 0.03 µg/l.[348,349,350]

CK-MB mass assay in both the recruitment centres were measured using Elecsys 2010 CKMB STAT assay (Roche Diagnostics). The Elecsys CK-MB STAT assay[351] employs two different monoclonal antibodies directed against human CK-MB. Increased CKMB value is defined as a measurement above the 99th percentile URL, which is designated as the decision level for the diagnosis of MI. Sex-specific values

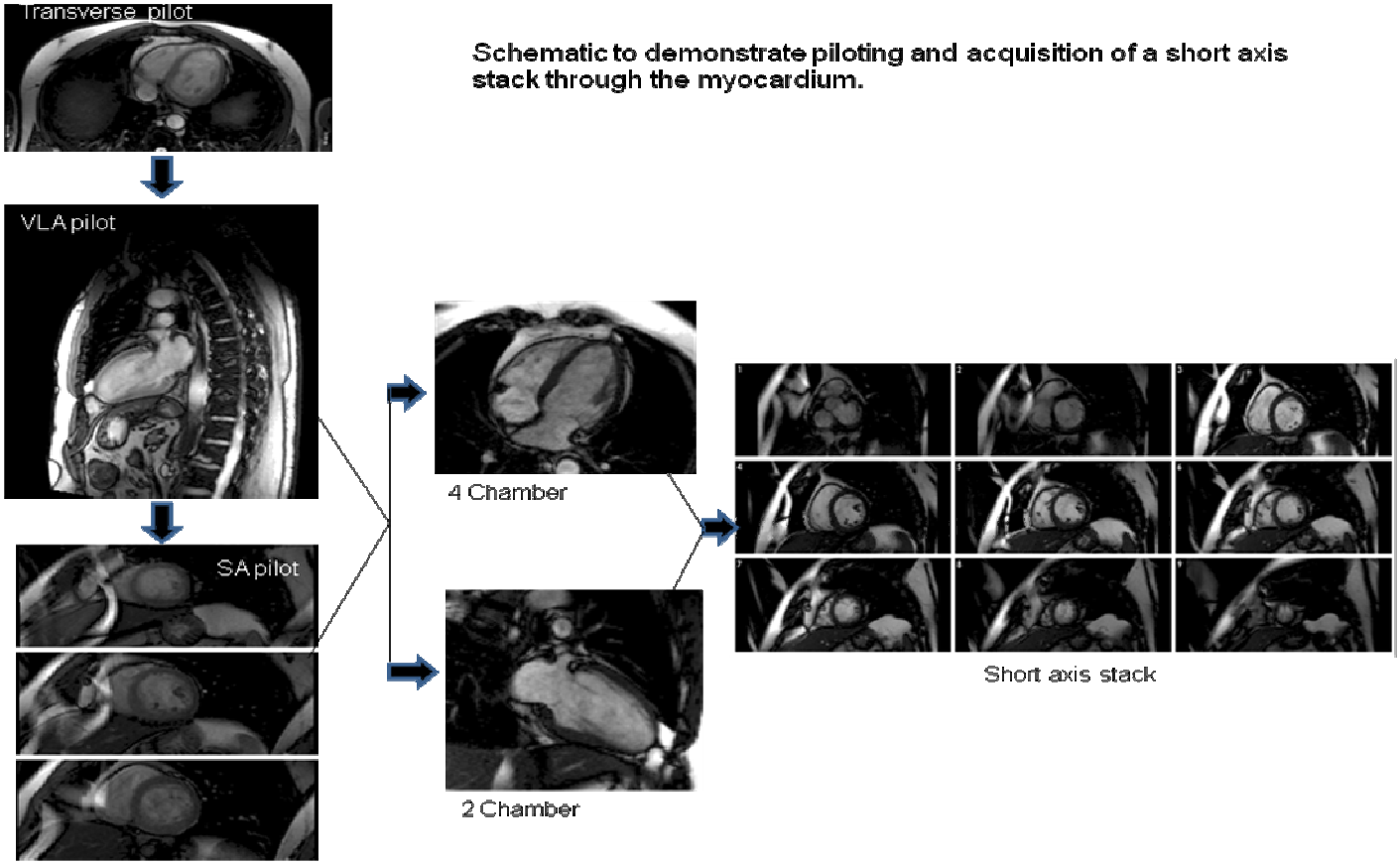
should be employed are recommended to be used in clinical practice.[13] The lower detection limit of this assay is 0.1µg/L with the 99th percentile limit varying between 3.7-6.7µg/L dependent on gender.[352,353]

2.5.2 Cardiac MRI

Cardiac MRI was carried out prior to discharge. Patients were scanned on one of two 1.5 Tesla Siemens AVANTO scanner (Erlangen, Germany) either at The Heart Hospital or Great Ormond Street Hospitals, London, UK. Patients were transferred using a critical ambulance and medical escort. Prospective ECG gating was using along with standard imaging acquisition.[354] Free breathing pilot scout images were obtained in three orthogonal planes - transaxial, coronal and sagittal - to align the true long axis of the left ventricle. Typical imaging parameters were as follows: repeat time (TR) = 3.39ms, echo time (TE) 1.7ms, slice thickness, 5mm, field of view 360 x 360mm, read matrix 256, flip angle 60º.

To enable analysis of cardiac volumes, SSFP cine images were obtained with breath holding at end expiration. A perpendicular two chamber pilot view (Horizontal long axis/HLA) was obtained through a perpendicular plane passing through the apex and centre of the mitral valve. Four perpendicular short axis pilot views were then acquired either side of the atrioventricular groove. The four chamber SSFP cine was obtained from the four short axis pilot views by cutting through the centre of the left ventricle below the aorta. Using the two chamber pilot view and the four chamber cine, a two chamber long axis SSFP cine is obtained. Left ventricular short axis SSFP cines were obtained using the two and four chamber cines. Initial

perpendicular short-axis slice is placed on the atrioventricular groove through the back of the left and right ventricles.[354,355] A short axis stack of slices from the atrioventricular ring to apex was then acquired with a slice thickness of 7mm and a 3mm gap between each slice. The number of cardiac phases per acquisition was 80-90% of the RR interval divided by the temporal resolution (typically 48ms) with 8 to 12 slices to cover the whole LV. Typical FISP imaging parameters were TE 1.6ms, TR 3.2 ms, in plane pixel size 2.3 x 1.4mm, slice thickness 7mm, flip angle 60º, acquired in 12 heart beats, with 15 to 20 phases. Parameters were optimised to take into consideration arrhythmias and inability of the patient to hold their breath.



Schematic to demonstrate piloting and acquisition of a short axis stack through the myocardium.

Figure 14 Schematic to demonstrate Piloting and Acquisition of a Short Axis Stack through the Myocardium

The ventricular volume is the sum of the endocardial areas multiplied by the interslice distance, and ventricular mass is the area occupied between the endocardial and epicardial border multiplied by the interslice distance.[189] Analysis of cardiac volumes was performed on a personal computer using the software package CMRtools (Imperial College©) and the plug-in LV tools (an automatic algorithm that allows for user-independent myocardial border delineation). First the short axis stack and two long axis perpendicular slices that pass through the centre of the mitral valve (four chamber and two chamber) are selected along with the short axis stack. The apex is then manually selected and the axis of the LV manually adjusted so that slices are aligned correctly. The endocardial and epicardial borders are semi automatically segmented in both systole and diastole and a thresholding tool is used to differentiate blood pool from myocardium (to include papillary muscle) enabling the calculation of the LV mass. The long axis images are then used to identify the position and orientation of the mitral valve leaflets at end-diastole and end-systole to exclude regions that belong to the left atrium. The stroke volume (SV) is calculated from the difference between the end diastolic volume (EDV) and the end systolic volume (ESV).

Stroke Volume (SV) = EDV – ESV

The ejection fraction can then also be calculated using the following equation:

$$E_f = \frac{SV}{EDV} = \frac{EDV - ESV}{EDV}$$

The calculation of LV mass can be calculated manually, after planimetry of the epicardial and endocardial borders in end-diastole, by a summation of the discs technique and multiplying the myocardial muscle volume by the density of myocardial tissue (1.05 g/cm^3).[356]

0.1mmol/kg Gadoteric acid/gadolinium (Dotarem®; Guerbet SA, Paris, France) was administered via an antecubital vein followed by a 20ml of saline. Early gadolinium enhancement images were obtained early after administration gadolinium (1-2 minutes) with coverage of the left ventricle in the short axis with slices matching those of the original SSFP short axis stack with no interslice gap. A fixed inversion time (TI) of 440ms was used. Other imaging parameters were TR 7.0ms, TE 4.9ms, flip-angle 23°.

Late gadolinium images were taken ten minutes following administration of contrast. Standard breath-hold, segmented inversion recovery gradient echo sequences were used with image parameters : slice thickness 8 mm, TR 9.8 ms, TE 4.6ms, Flip angle 21º, field of view 340 x 220 mm (transverse plane), sampled matrix size 256 x 115-135, 21 k–space lines acquired every other RR interval (21 segments with linear reordered phase encoding), spatial resolution 1.3 x 2.1 x 8 mm, no parallel imaging. Images were obtained in the same planes as SSFP cine images. The inversion time was adjusted to null the myocardium. To avoid cerebrospinal fluid (CSF - normal CSF has long T1 and long T2 times that manifest as dark signal on T1-weighted images and bright signal on T2-weighted images.)[357] Ghosting a presaturation band was placed over the CSF. In the event of arrhythmias or the patient having difficulty with breath holding, sequences were optimised to obtain the best images including

the use of prospective gating or single shot multi-slice non breath hold imaging respectively.[358]

The extent of infarction/infarct size was then calculated. In order to determine the optimal method to quantify infarction using LGE images a study was carried out comparing the different techniques described to assess infarct size. SNR and CNR are assessments of image quality.[359] SNR was calculated as mean signal intensity of enhanced area/SD of noise. CNR was calculated as (mean signal intensity of enhanced area − mean signal intensity of unenhanced area)/1.5 × SD air. Myocardial volume and mass analysis were carried out on cine images using standard techniques.[354] Short-axis images were manually segmented for epicardial and endocardial borders (excluding papillary muscles) to obtain the myocardial volume. Where more than one image of the same slice position was present, the optimal image was selected for analysis. All further quantification occurred on the presegmented images. LGE was quantified manually by tracing around the borders of infarction or fibrosis. Semi automated analysis were then carried out using purpose-written ImageJ (National Institutes of Health, Bethesda, MD) macros to determine FWHM and 6-, 5-, 4-, 3-, and 2-SD (above the mean remote myocardial signal) derived volumes. This involved the manual delineation of ROIs, firstly in the remote myocardium used to generate mean and SD for 6-, 5-, 4-, 3-, and 2-SD thresholds. Secondly, an ROI was drawn around hyperintense myocardium and used to define maximal signal for the FWHM threshold. Manual corrections were then required and done twice for all automated ROIs: first, MVO (defined as a hypointense core within a hyperintense region in patients with acute myocardial infarction[337]) was manually adjusted to be included as LGE. Second, any blood pool

or pericardial partial voluming and artifact (which occurred only rarely) were manually removed from the ROI. LGE volume as a percent of myocardium was quantified per slice, twice by 2 readers (J.H. and A.S.F., analysis blinded with 1-month temporal separation between repeat analyses) in all patients, with interobserver and intraobserver reproducibility and LGE volume assessed.[360]

3 Quantification of Myocardial Infarct Size Using Late Gadolinium Enhancement CMR

3.1 Preface

A version of this chapter was written with Dr Andrew Flett and published in Journal of the American College of Cardiology: Cardiovascular Imaging 2011.[360]

3.2 Introduction

The Objective of this sub-study was to determine the most reproducible technique to measure focal fibrosis using CMR that could be translated into a research tool for our subsequent study. Myocardial scar represents the replacement of 'normal' cardiomyocytes with those injured or destroyed by varying disease processes that affect the myocardium. Late gadolinium enhancement (LGE) imaging by CMR has become the reference standard for imaging myocardial scar. Following contrast administration and with the correct settings during an inversion recovery sequence, normal myocardium will appear black (nulled) whilst damaged myocardium will appear bright ('enhancement'). The use of LGE imaging to identify myocardial scar has been validated in both animals and humans. In animal models LGE has been shown to distinguish between reversible and irreversible ischaemic injury. Whilst with *ex-vivo* imaging in models of acute and chronic infarction, the location, spatial extent, and 3D shape of the regions of increased signal intensity have been shown to

correlate with those of the irreversibly injured regions defined by TTC.[235] CMR techniques are able to identify tiny areas of infarction. Assuming a myocardial specific gravity of 1.05 g/cm3, CMR has been able to visualise discrete areas of infarction with measured volumes of <1g.[361] The extent and degree of myocardial injury after an acute ischaemic event are strong predictors of patient outcome, and interventions that reduce injury significantly improve prognosis.[235] In a recent meta-analysis of 4,438 patients, the overall hazard ratio for MACE was 2.65 for the presence of any LGE. Furthermore, there was a continuous relationship between risk and the amount of LGE detected - for every 10% of the left ventricular mass with LGE, the risk of MACE increased by 56%.[362] Therefore the quantification of LGE offers further prognostic information beyond the simple presence of infarction. The ability to accurately prognosticate in the setting of AMI allows for the tailoring of therapy to the patients who are most likely benefit from it the most such as the implantation of implantable cardiac defibrillators.[363] Different groups have used varying amounts of LGE as cut-offs for predicting adverse outcomes in patients suffering AMI. Given the effectiveness of PPCI and current anti-platelet therapy, the ability to demonstrate incremental reductions in mortality in the setting of AMI is increasingly difficult and necessitates studies with larger sample size. This forces logistical and financial barriers on the testing of novel therapeutic strategies, and also limits the number of treatments that can be evaluated.[248] Given the high sensitivity of LGE and the reproducibility of measurements of infarct size, the use of LGE imaging as a surrogate endpoint in clinical trials has become increasingly appealing. CMR provides the possibility of the detection of smaller changes in infarct size and therefore the possibility of a reduction of sample size populations required to demonstrate benefit (or otherwise) from therapy. Sample size is dependent on

estimated measurement variability. This parameter is represented by the expected SD in the measurements made within a cohort. As statistical variability increases, the sample size needed to detect the minimum difference increases. Visa-versa, as measurement variability decreases, the sample size needed to detect a minimum difference decreases.[364]

The accuracy of a measurement system is the degree of closeness of measurements of a quantity to that quantity's actual (true) value. The precision of a measurement system, also called reproducibility or repeatability, is the degree to which repeated measurements under unchanged conditions show the same results.[365] Ideally, quantification of infarction should be accurate and precise with measurements close to and tightly clustered around a known value or reference standard.

A number of different techniques exist for the quantification of myocardial scar from LGE images. The gold standard quantification technique is not clear. Current techniques rely on the fact that LGE imaging makes scarred myocardium appear brighter than normal myocardium and as such can be defined as a specific signal intensity above normal myocardium. Regions of hyperenhancement have been described when the signal intensity is higher than the mean signal intensity plus 2 standard deviations (SD) of the signal intensity of the remote myocardium.

Previous SCMR reporting guidelines recommended a threshold of more than 2SD as a cut off to report scar area on LGE images.[354] More recent guidance confirms the

controversy and uncertainty around the most accurate way to quantify myocardial scar. The task force for the standardisation of post processing in CMR refrained from making a dedicated statement regarding the optimal method for the quantitative assessment of scar.[366] Different groups have used different techniques including;

(i) Manual planimetry - drawing round the region of scar as perceived by the operator. The size of the hyperenhanced regions is influenced by the image window settings (centre and width), that reflect the personal preference of the person undertaking the analysis.[367]

(ii) Standard deviations of differing signal intensities - Mean signal intensities plus 2,3,4,5 and 6 SD above the remote myocardium have been used with semi-automated algorithms to threshold out the hyperenhanced segment. Obviously the potential exists for lower signal intensity cut offs to overestimate infarct size and higher cut offs to underestimate in the same way.

(iii) Full width half maximum - an initial region is determined to grow to include all pixels with SI >50% of a selected point. The maximum Si inside this initial region is then determined, and the final MI extent is defined as the area presenting with a signal intensity 50% above the maximum of the initial region. In the canine model Amado *et al.* demonstrated that the FWHM technique was able to accurately and reproducibly quantify the amount of infarction that correlated with post mortem infarct size measured by TTC staining.[368]

Our objective was to compare the seven quantification techniques across the spectrum of disease in which LGE has been linked to outcome: AMI, CMI and hypertrophic cardiomyopathy (HCM), assessing the LGE volumes obtained, the reproducibility of each method and any associated effects on future study design. We hypothesised that the techniques would yield significantly different LGE volumes; that LGE quantification would vary with LGE aetiology; and that the 7 techniques would have statistically different reproducibility.

3.3 Methods

The 7 techniques (thresholding at 2, 3, 4, 5, 6 SD from remote, FWHM and manual quantification, see general methods section) were compared in a retrospective manner in 60 consecutive patients referred for CMR with the confirmed clinical diagnosis of AMI (n=20,), CMI (n=20) and HCM (from a tertiary referral cardiomyopathy clinic n=20,), who had evidence of LGE. MI was defined by clinical presentation compatible with a STEMI, angiographic confirmation of coronary artery disease in the appropriate territory and a raised troponin. In AMI CMR was performed within one week and in CMI the scan was performed no less than 3 months from the AMI. No other inclusion/exclusion criteria were used. Mean LGE volume, intra- and inter-observer variability were assessed and implications for sample size calculations derived. CMR protocol and analysis was carried out as previously described in the methods section.

3.3.1 Statistical Methods

LGE volume by each method was compared using a one-way repeated measure ANOVA with Bonferroni post-hoc analysis; reproducibility by intra-class correlation coefficient[369] (ICC) and the significance of reproducibility differences using a Wilcoxon Rank comparison of the squared differences.[370] Bland-Altman[371] testing was performed in order to assess systematic offsets and the relation of observed differences to LGE extent. In addition, the reproducibility data was graphically displayed showing the variability attributed to intra and inter-observer effects as a proportion of total variability for each method (1-ICC).[194] The sample size required to detect a clinically important change in LGE (5% was used illustratively) was calculated for each method for both paired and unpaired data by estimating the total patient level variability using standard variance components analysis.[372] This modelling technique includes as variables both intra-observer and inter-observer effects which accounts for any observed systematic differences in the LGE.

3.4 Results

Patient demographics and CMR data are shown in the table below (table 7 and table 8 for each of the individual conditions studied.

Patient Characteristics by disease			
	AMI	**CMI**	**HCM**
N	20	20	20
Age (median, IQR)	58, 48-61	60, 52-66	50, 37-57
Gender (M:F)	16:4	17:3	14:6
Peak Troponin	7.5±5.2	7.0±4.7	-
Infarct LAD:RCA:Cx*	10:7:3	10:5:5	-
MI to scan time (days)	2.1±1.2	132±14.1	-
*** LAD – Left anterior descending, RCA – Right coronary artery, Cx – Circumflex**			

Table 7 Patient Characteristics by Disease

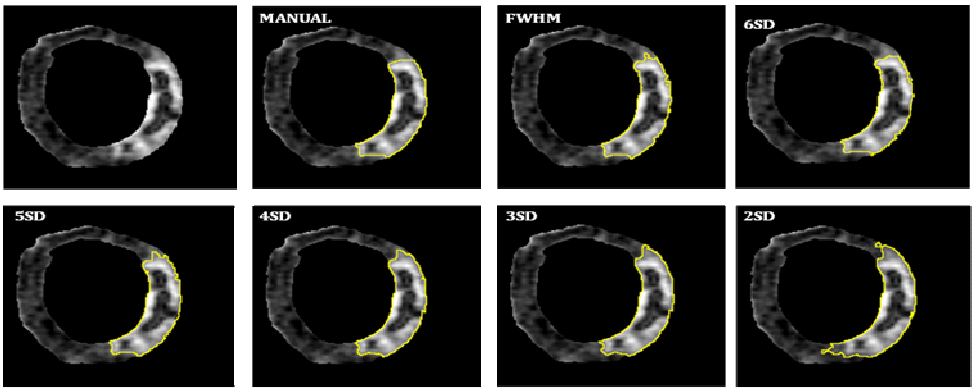
CMR Characteristics by Disease			
	AMI	**CMI**	**HCM**
EDV (mls)$	147±27	172±51	136±33
ESV (mls)$	67±19	82±41	34±13
SV (mls)$	80±2	90±25	102±27
EF %$	55±10	54±13	76 ±9
Mass (g)	175±35	175±46	236 ±74
Mean TI Range	359-384	355-405	333-411
SNR (mean±SD)Ŧ	22±10	31±21	27±14
CNR (mean±SD) Ŧ	12±6	16±11	12±7
Max WT (mm)	-	-	19±4
$ EDV – End diastolic volume, ESV – End systolic volume, EF – Ejection Fraction			
Ŧ SNR – Signal to Noise Ratio, CNR – Contrast to Noise Ratio*			

Table 8 CMR Characteristics by Disease

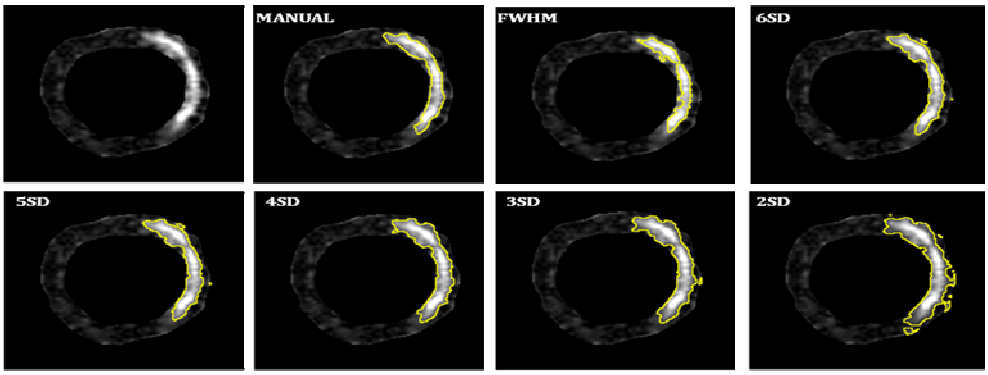
3.4.1 LGE Extent

Calculated LGE volume varied substantially dependent on the quantification method used (Figure 15). Comparing all methods against the manual technique, there was no significant difference between manual and 6SD in the three conditions. There was no significant difference between manual and FWHM in HCM and between manual and 6 or 5SD in CMI. The 2SD technique generated LGE volumes up to 2x as high as FWHM, 6SD and manual techniques ($p<0.001$). The difference was most marked in HCM (Figure 15). The most extreme mean LGE difference in each disease was from 22% to 30% (AMI); 14% to 23% (CMI) and 11% to 29% (HCM).

Acute Myocardial Infarction



Chronic Myocardial Infarction



Hypertrophic Cardiomyopathy

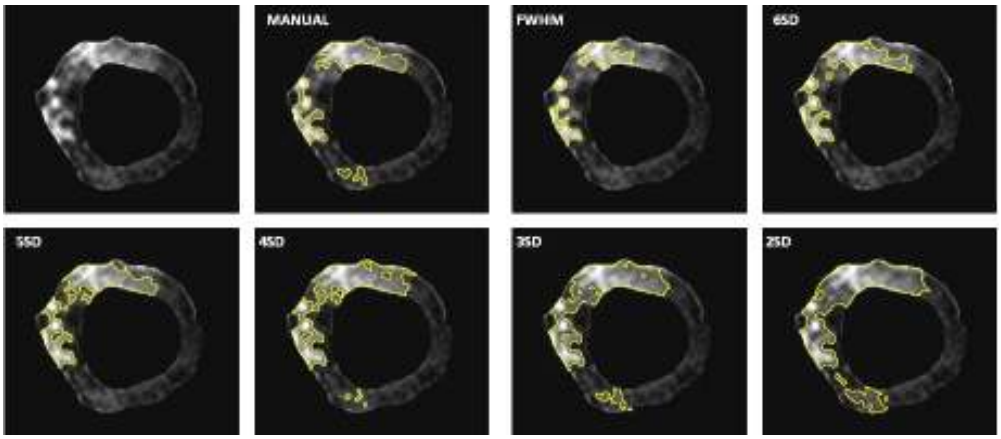


Figure 15 LGE Quantification using Seven Techniques

Late gadolinium enhancement quantification using seven techniques in hypertrophic cardiomyopathy, chronic and acute myocardial infarction and: Example analysis of a

single short axis LGE slice in hypertrophic cardiomyopathy, chronic and acute myocardial infarction using the 7 quantification techniques.

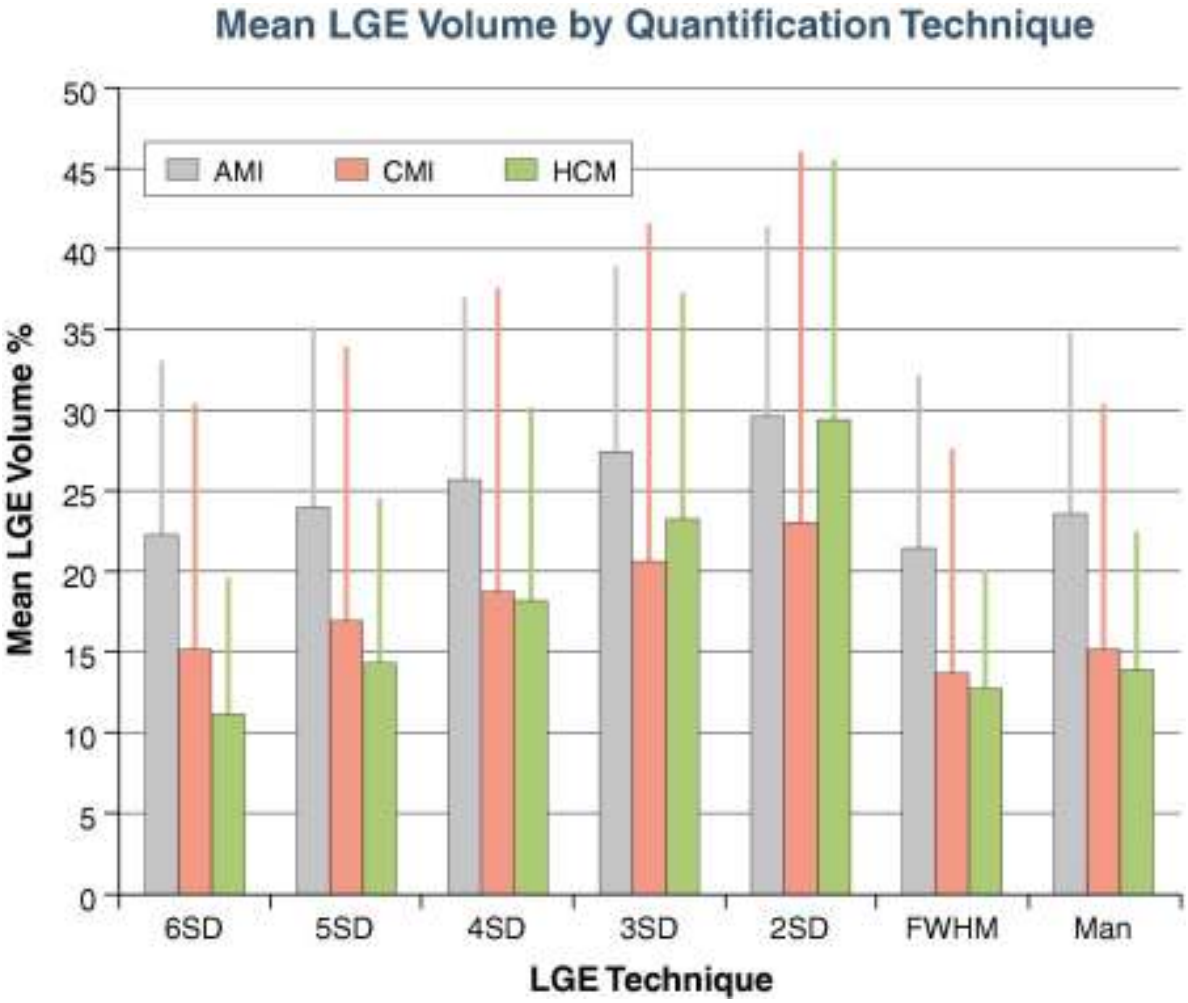


Figure 16 Mean LGE Volume By the seven Techniques

3.4.2 Bland-Altman Analysis

Bland-Altman analysis (see supplemental data for the 42 Bland-Altman plots performed) confirmed minimal inter/intra-observer bias (offset) for all methods except in 5 HCM assessments (SD2 through 6) where a small systematic offset appears to

have arisen from the 2 observers drawing their remote myocardium ROIs differently. Additionally, it was confirmed that observed differences in LGE were not related to the mean.

3.4.3 Reproducibility in LGE measurements.

For both intra- and inter-observer, the FWHM technique was the most reproducible in all 3 conditions ($p<0.001$) when compared to the other techniques studied. Inter- and intra-observer reproducibility of all LGE techniques were worse in HCM than AMI and CMI. In CMI and HCM, manual quantification was the least reproducible technique. For HCM, the FWHM technique was the only statistically acceptable quantification method (ICC >0.7)[373] (Figure 17).

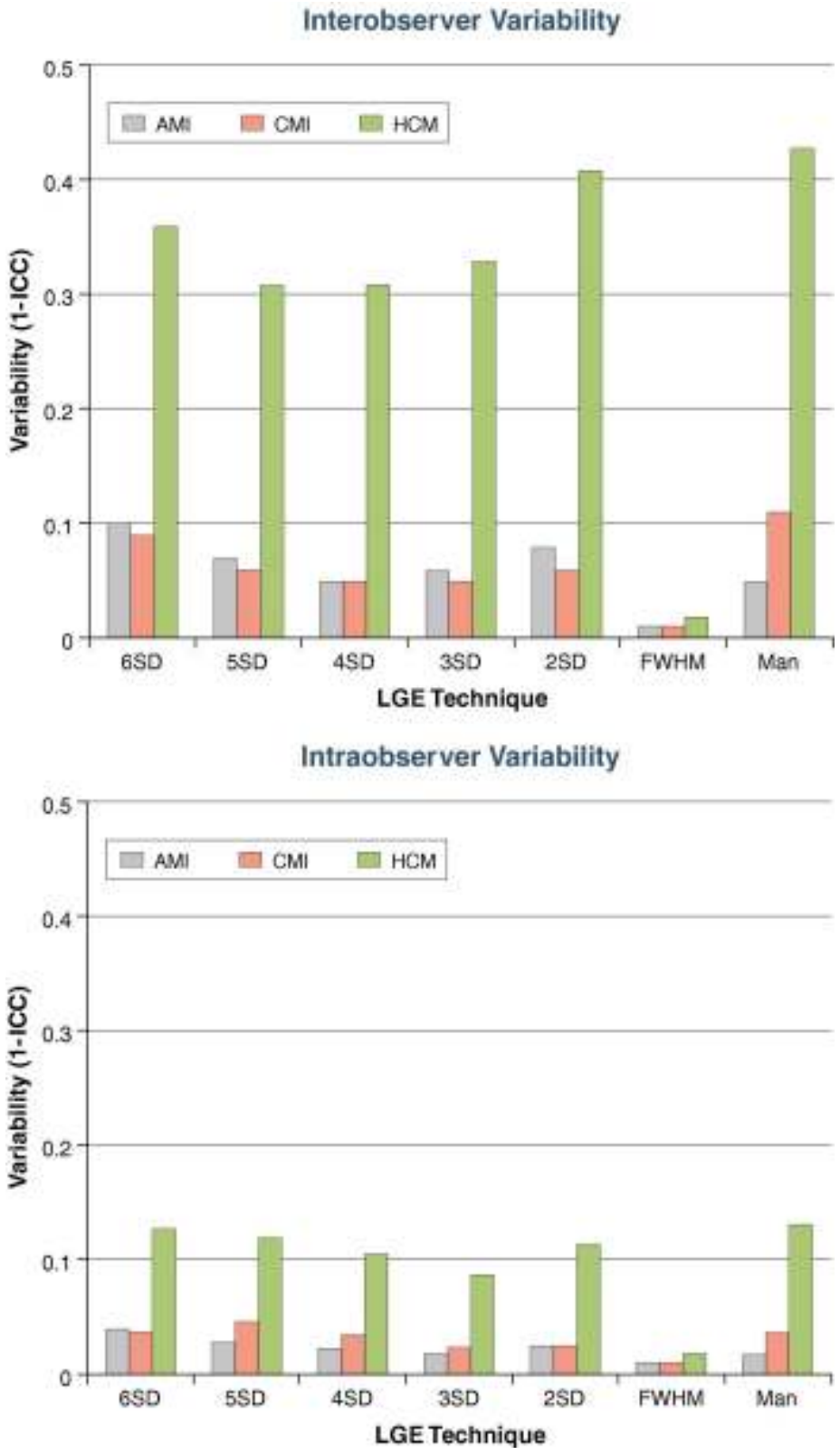


Figure 17 Intra And Interobserver variability by LGE quantification technique

3.4.4 Impact of Reproducibility on Sample Size Calculations

The improved reproducibility of the FWHM technique has a significant impact on sample sizes needed to demonstrate a change in the number of patients required for a trial with the same power. On the basis of a difference in paired comparisons of slices between two observers, the FWHM technique inter-observer reproducibility compared to the next best method reduces required sample size by 69% (CMI), 60% (AMI) and 91% (HCM) for equivalent power. On the basis of unpaired analysis, taking into account an estimate of the total variability of each method on a patient basis, the calculated sample sizes using the FWHM compared to the next best method is reduced by 17%(AMI), 7% (CMI), and 56% (HCM).

3.5 Discussion

Quantification of LGE has important implications for cardiovascular research and also clinical practice across a spectrum of cardiovascular disease. Our study demonstrates how in three cardiovascular disease processes, 7 different techniques used to quantify LGE can provide significantly different results. The FWHM technique provided the most reproducible method across all three conditions proposing the ability to significantly reduce sample size in clinical trials that use LGE as a surrogate endpoint. Uses of alternative techniques to quantify LGE have the potential to more than double the size of a given clinical trial with ensuing cost and time implications.

Our study confirms the findings of Amado *et al*. that the FWHM criterion is a reproducible segmentation method to define infarct size when they compared it to TTC stained segments. Speiwak *et al*.[374] compared differing quantification techniques but just in patients with HCM. Although their group found as we did (albeit in a smaller sample) that the FWHM technique was the most reproducible, they rejected it on the basis that results of FWHM technique did not correlate with visual analysis. The group concluded that 6SD above the mean SI of the remote myocardium provided the best agreement with visual assessment in patients with HCM. The assumption being that visual assessment and manual planimetry provide a gold standard for quantification of LGE when we have demonstrated that the manual technique is not reproducible, especially in HCM, where it is statistically unacceptable (ICC <0.7). Bondarenko *et al.* in 2005 showed significant overestimation of LGE with 2–4SD techniques in CMI. The same group in 2009 demonstrated the ability of 6SD to predict segmental functional improvement after revascularization over 2-5SD and FWHM.[375],[367] The difference between the other techniques was, however, small and non significant. No reproducibility assessments were provided by the group for their second study.

A number of limitations exist within the different thresholding techniques. Firstly, no real gold standard exists to compare these techniques with. TTC staining is the most popular method for measuring infarct size ex-vivo. TTC staining in itself has been shown to underestimate infarct size in certain scenarios.[376] It cannot be used in-vivo and therefore is only of use in post-mortem human studies. Although TTC staining is common place in animal studies, the histological approach is strongly affected by the precise sequence and conditions in which the individual steps are carried out.

Therefore, the quality of histological images varies widely and is often poor in terms of contrast and border delineation introducing significant variability to quantification.[377] Dehydration of myocardial tissue may also lead to variability between LGE extent (expanded by myocardial oedema) and *ex-vivo* sizing. It is also important to note that the pathophysiology underlying infarct measurement using TTC staining and LGE are different with the techniques being reliant on the release of NADH or increased volume of distribution respectively.

Secondly, for the thresholding techniques, the signal intensity is highly dependent on the selected area of the remote myocardium used to calculate the threshold. In turn, each of the above techniques requires the operator to select a region of interest (whether it be remote myocardium, LGE or both), which adds subjectivity to the approach and may affect sizing. The standard deviation in remote myocardium primarily depends on the signal-to-noise ratio of the acquisition, which is influenced by imaging protocol, contrast media, time from administration to imaging, contrast dose, RF receiver coil setup and aetiology of scar. [366,367]

Thirdly, thresholding techniques are only semi-automated, relying on the user to accurately trace the myocardial borders (Epicardial and Endocardial) and exclude artefact. Artefact can be found both within the myocardium or more importantly at the subendocardium from where the infarction is likely to spread. It is at the interface between the subendocardium and blood pool that it becomes difficult to distinguish artefact from infarction as there may be minimal difference in the signal intensity between the two regions. Regional wall motion abnormalities can lead to stasis of blood which in turn can cause increased signal intensity at the subendocardial

border,[378] further confounding the quantification of the infarction. In addition, *in vivo* imaging is complicated by blurring from associated cardiac motion. This results in significantly more partial volume effects compared with *ex vivo* imaging, creating voxels with intermediate (gray) signal intensity containing an admixture of viable and nonviable myocytes—particularly at the infarct boundary.[379]

With regards to the FWHM technique, authors have raised concerns that the method assumes a bright infarct core and may not be accurate in homogeneously gray scars. In addition, multiple separate islands of scar may cause difficulties with this analysis method.[248] In our experience however, cases like this were rare and when they did occur, all methods, rather than the FWHM alone, were more prone to errors. LGE is frequently less well defined in HCM than in infarction and as such the identification of myocardium with normal signal (for use in the thresholding techniques based on mean and SD of signal intensity) is often difficult. The identification of an area of high signal however for the FWHM technique is more straightforward. This is reflected in our results with impaired reproducibility of all techniques in HCM compared to infarcts, but the FWHM relatively preserved.

Assessment was made of intraobserver and interobserver reproducibility but our study is limited by lack of assessment of interstudy reproducibility (the SD of the mean difference between 2 repeated studies). Inter-study reproducibility of a technique is important where repeated examinations are required such as to assess the effect of a therapy over time. The absence of ionising radiation means that CMR lends itself well to repeated testing.[380] High interstudy reproducibility (low SD) leads to greater reliability of observed changes in a parameter under measure, and smaller

sample sizes. The ratio of the SD of interstudy reproducibility between techniques is related to sample size by a square function, therefore small improvements in reproducibility magnify effects on sample size.[381, 382] Little data exists on the interstudy reproducibility of LGE in patients with IHD or HCM. Analysis of interstudy reproducibility may have demonstrated lower ICCs and decrease the sample size reduction suggested above. Yet the FWHM method would likely retain the optimal reproducibility of quantification techniques. At the same time lack of interstudy variability limits the extension of our findings to paired studies. Our study of RIC in acute infarction is, however, an unpaired study with one CMR scan being performed following randomisation to treatment.[379]

As mentioned above, the relative SNR of scar tissue versus normal myocardium can vary dependent on the dose of the contrast media used. We used a single dose of gadolinium at 0.1 mmol/kg. Other groups have used 0.2 mmol/kg as well as differing time points after contrast to image acquisition. There is the potential for these issues to affect reproducibility. From a practical point of view this lower dose might underestimate the presence of LGE especially in patients with HCM or myocarditis. This would be an issue of CNR and SNR, which was felt to be outside of the remit of a study based on post-processing techniques. All the LGE images used in this study were optimised, but our consecutive patient recruitment still reflects real-world scanning.

New methods for thresholding and quantification continue to be developed. The Otsu-Auto-Threshold (OAT) method is an automatic method for the calculation of an optimal threshold for quantifying tissue characteristics in CMR. The algorithm

automatically divides an SI histogram into two classes: normal and enhanced. The threshold is determined using an exhaustive search for values that minimize the intra-class variance between two populations of signal intensities.[383] OTSU is an automated technique requiring no user defined ROI - unlike FWHM and SD techniques. OTSU overcomes the limitation of an observer wrongly defining the abnormal tissue as normal (or visa-versa) as only epicardial and endocardial borders have to be defined.

3.6 Conclusion

The FWHM technique for LGE quantification is the most reproducible, regardless of underlying aetiology, across the spectrum of cardiac disease in which LGE quantification is known to be important. Reproducibility of the FWHM method allows for study sample size on the basis of scar as an end point to be reduced for equivalent power.

4 Effect of Remote Ischaemic Conditioning on Myocardial Infarct Size in STEMI patients

4.1 Introduction

Primary percutaneous coronary intervention is the treatment of choice in the setting of acute myocardial infarction.[384] Despite prompt restoration of flow in the infarct related artery and continual improvements in anti-platelet[385] and anticoagulant[386] therapy mortality remains high. Paradoxically the restoration of flow to the ischaemic myocardium can induce further damage through myocardial reperfusion injury.[387] The presence of reperfusion injury attenuates the benefits of angioplasty in patients suffering a myocardial infarction, yet there is currently no therapy to protect the heart against reperfusion injury. As such, novel therapeutic strategies for protecting the heart against ischaemia–reperfusion injury (IRI) are urgently needed to: reduce myocardial injury, preserve cardiac function, prevent the development of heart failure, and improve clinical outcomes in the setting of acute myocardial infarction.[388]

Conditioning the heart is an endogenous protective strategy. Pre- and post-conditioning have shown promise as strategies to minimise cardiac damage in settings where ischaemia can be anticipated.[80,41] Acute myocardial infarction is however an unpredictable event which does not allow for the application of strategies to pre-empt the index ischaemic event.

Remote Ischaemic Conditioning (RIC) describes the cardioprotective effect elicited by applying brief episodes of non-lethal ischaemia and reperfusion to an organ or

tissue remote from the heart.[116] In the clinical setting, RIC has been be achieved using a non-invasive virtually cost-free protocol in which a blood pressure cuff to the upper arm or leg is inflated to supra-systolic pressures to induce one or more brief episodes of ischaemia and reperfusion[124] In animal[124] and human[184] studies an RIC protocol has been applied following the onset of ischaemia (and prior to reperfusion) called Remote Ischaemic *Per*conditioning. Such a strategy has shown benefit in STEMI patients following the measurement of traditional surrogate markers of infarction.

We aimed to investigate whether RIC reduces infarct size in the setting of an acute myocardial infarction using cardiac MRI indices as surrogate endpoints.

4.2 Methods

The study methodology is described in the general methods chapter earlier

4.3 Statistical Methods

Patient demographics were compared between the two groups. Fisher's exact test was used for the analysis of demographics measured on a categorical scale. The unpaired t-test was used for continuous variables found to be normally distributed, whilst the Mann-Whitney test was used for continuous variables not found to be normally distributed.

Primary outcomes were measures obtained from the cardiac MRI scans. These outcomes were all measured on a continuous scale, and an examination of the distribution of these variables, suggested that all were approximately normally distributed. As a result, the unpaired t-test was used to compare between groups. Further analyses of CMR indices are described below.

The secondary outcomes were the three cardiac biomarkers, which were measured at baseline (0 hours), and then at three further time points. Data from all time points were included in single analysis for each outcome. To allow for the repeated measurements from each patient, the analysis was performed using multilevel linear regression. Two level models were used with individual measurements nested within patients. The 6, 12 and 24 hour time points were considered as outcomes, with the baseline values a covariate in the analyses. An examination of the data indicated that all three biomarker outcomes had positively skewed distributions, and thus the outcomes were given a log transformation before analysis. The interaction between time and treatment group was examined. A significant interaction would indicate that the differences between groups varied at the different time points. If no significant interaction was found, this term was omitted from the analysis, and a constant difference between groups at all time points was assumed.

Further analyses of the biomarkers, calculated the area under the curve (AUC) for each patient. The AUC values were found to have skewed distributions, and so the Mann-Whitney test was used to compare between the two groups. This analysis was restricted only to patients who provided data at all four time points, as the AUC could not be reliably calculated for patients with missing data (e.g. patients with CMR indices but not complete biomarker data).

4.4 Results

Patients were screened for inclusion in the study with patients immediately recognised as ineligible. At this stage patients were consented for the study prior to coronary angiography. Following angiography a further patients were excluded due to further exclusion criteria. 19 patients were excluded following initial angiography in view of TIMI flow >1(n=10), angiographically normal coronary arteries (n=4), three vessel coronary artery disease (n=2) and significant collateralisation (n=5). Two patients had angioplasty to more than one vessel and were therefore excluded. In view of pre-infarct angina and also patients taking either Nicorandil or Glibenclamide, 6 further patients were excluded. 1 patient withdrew consent following PPCI. Therefore 61 patients were successfully randomised, to receive either placebo (n=33) or RIC (n=28).

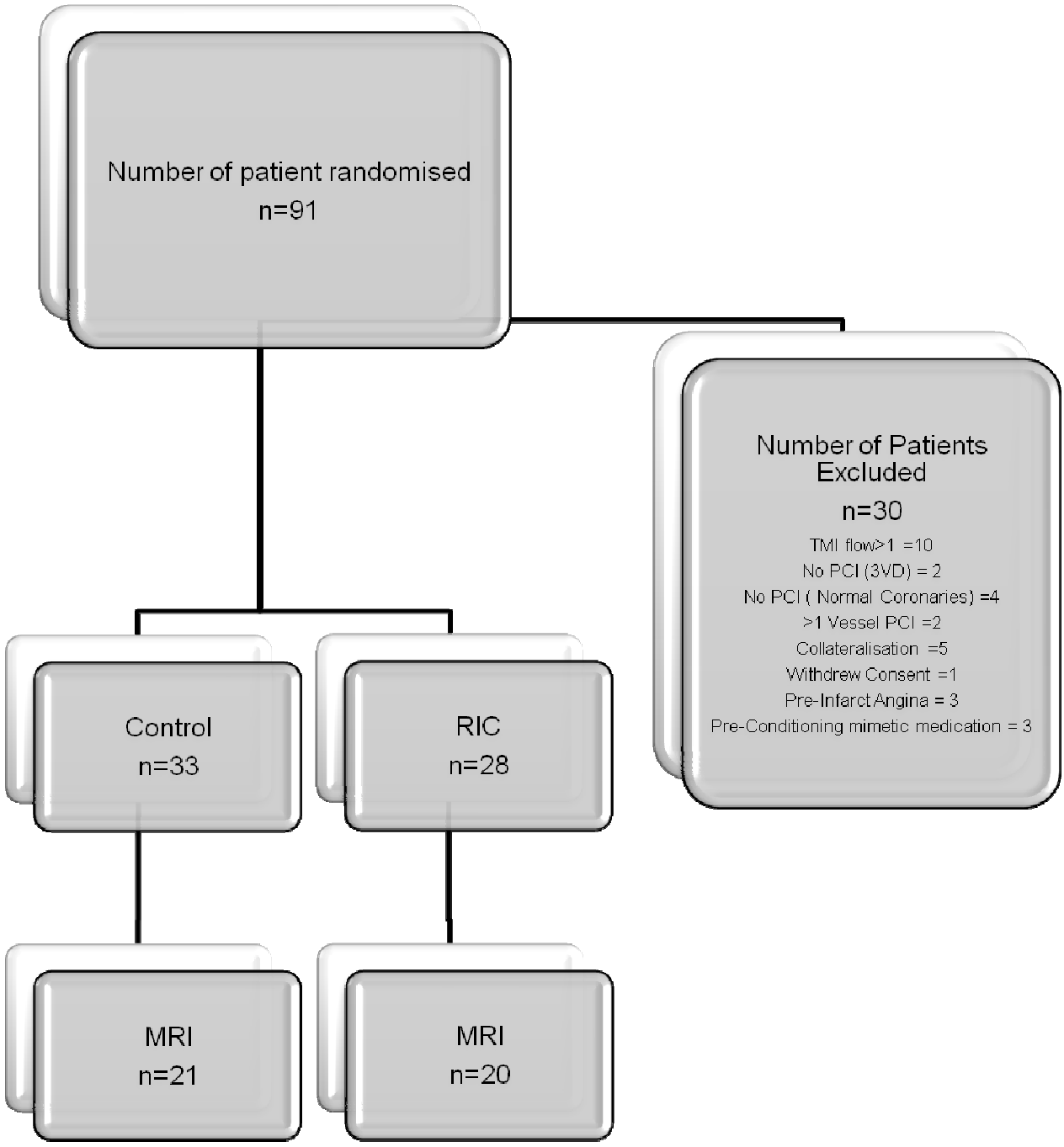


Figure 18 Schematic of Recruitment for RIC Trial

4.4.1 Patient Demographics

The groups were all well balanced with regards to demographic and angiographic variables. Comparisons of patient demographics between groups were made, and the results are summarised in the following table. Categorical variables are summarised by the percentage in each category. Continuous variables found to be normally distributed are summarised by the mean and standard deviation, whilst continuous variables not found to be normally distributed are summarised by the median and inter-quartile range. P-values indicating the significance of the results are also reported. None of the patient demographics were found to vary significantly between the two study groups.

Variable	Control (n=33)	RIPC (n=28)	P-value
Age - Mean (SD)	57.3 (9.9)	57.7 (10.4)	0.88
Weight - Mean (SD)	86.5 (21.6)	83.0 (16.7)	0.53
Vessel: LAD (%)	47	32	0.50
Circumflex (%)	16	14	
RCA (%)	38	54	
TIMI flow: TIMI 0 (%)	82	85	1.00
TIMI 1(%)	18	15	
No. balloon inflations - Median (IQR)	3 (2, 5)	4 (3, 6)	0.20
Duration balloon inflations - Median (IQR)	60 (35, 110)	76 (42, 109)	0.53
Drug eluting stent (%)	53	48	0.80
Bare Metal stent:(%)	47	52	1.00
Diabetes: (%)	25	11	0.19
Metformin: (%)	12	0	0.12
Hypertension: (%)	38	46	0.60
Hyperlipidaemia: (%)	36	50	0.31
Creatinine - Mean (SD)	79.5 (18.1)	85.4 (22.1)	0.27
Ischaemia time - Median (IQR)	180 (141, 239)	181 (130, 344)	0.66

Table 9 Table showing Patient Demographics

4.4.2 Cardiac MRI endpoints

With regard to the primary outcome, myocardial infarct size assessed by late enhancement imaging on CMR showed no statistically significant difference between the RIC group and the placebo group. Findings were similar for all CMR indices measured and the results are summarised in Table 10 with no significant differences found been the groups.

4.4.2.1 *Volumes and Infarct Size*

Outcome	Control Mean (SD) (n=21)	RIPC Mean (SD) (n=20)	Difference Mean (95% CI)	P-value
Infarct size (g)	2.7 (1.9)	2.3 (1.7)	-0.4 (-1.6, 0.8)	0.49
EDV (mls)	69 (12)	72 (14)	3 (-5, 12)	0.39
ESV (mls)	29 (12)	35 (14)	6 (-2, 14)	0.15
SV (mls)	39 (8)	37 (7)	-2 (-7, 2)	0.31
EF (%)	59 (12)	53 (12)	-6 (-13, 2)	0.12
Mass	88 (15)	90 (20)	2 (-10, 13)	0.79
EDV – End diastolic volume, ESV – End systolic volume, SV – Stroke volume, EF – Ejection fraction				

Table 10 Table showing Cardiac Volumes and Infarct Size

4.4.2.2 *Area at risk and Myocardial salvage*

The AAR (calculated by Infarct-ESA) was compared between the two study groups. This variable was found to be approximately normally distributed, and so the unpaired t-test was used for the analysis. Analyses were performed for all patients combined, and also for two of the vessel subgroups separately. Finally the association between the area at risk and infarct size was examined using Pearson

correlation. The analysis results are summarised in the next table. The figures presented are the mean and standard deviation in each group, along with the mean difference between groups, and confidence interval for this difference. The difference was calculated as value for RIPC group minus value for control group.

Patient group	Control Mean (SD)	RIPC Mean (SD)	Difference Mean (95% CI)	P-value
All patients	28.5 (13.8)	28.4 (14.5)	-0.1 (-9.6, 9.3)	0.97
LAD vessel only	37.3 (11.8)	39.2 (15.0)	1.9 (-13.1, 16.8)	0.79
RCA vessel only	16.9 (7.4)	20.8 (9.4)	3.9 (-5.1, 13.0)	0.37

Table 11 Table comparing the Area at Risk between groups and between IRA

The results suggested no differences in AAR between the two groups, either for all patients, or when the groups were split into their component infarct related artery.

The association between AAR and infarct size was also examined. Pearson correlation gave a correlation coefficient of 0.83, with an associated p-value of <0.001. This suggests strong evidence of a positive association between the two variables. In other words greater infarct sizes were associated with higher AAR values.

We then calculated the extent of myocardial salvage and compared this between the two groups. The salvage Index (Area at risk – final infarct size / area at risk) was measured on a continuous scale, and was not found to be normally distributed. Therefore, the Mann-Whitney test was used to compare between groups. No difference was found in the degree of salvage between the two groups.

Variable	Control (n=33)	RIPC (n=28)	P-value
Salvage Index - Median (IQR)	0.24 (0.08, 0.53)	0.39 (0.10, 0.59)	0.39

Table 12 Table demonstrating the salvage Index between study groups

The association between the salvage index, ischaemia time and infarct as continuous parameters was examined using Spearman's rank correlation. The correlation coefficients and associated p-values are summarised in the next table

Variable 1	Variable 2	Correlation Coefficient	P-value
Salvage index	Ischaemia time Infarct size	0.04 -0.59	0.83 **<0.001**

Table 13 Table showing the association between the Salvage Index, Ischaemia time and Infarct

There was no significant association between ischaemia time and infarct size. Ischaemia time was also not significantly associated with salvage index. However, there was a statistically significant association between salvage index and infarct size. There was, as expected, a negative correlation suggesting that the salvage index was lower for patients with a larger infarct size.

4.4.2.3 MVO

The variables related to the incidence of MVO were measured on a categorical scale, and so Fisher's exact test was used for the analysis of these variables. The association between various continuous variables was also examined. However, due to the skewed distribution of these variables, Spearman's rank correlation was used for the analyses.

Incidence of MVO per CMR technique applied	**Control (n=33)**	**RIPC (n=28)**	**P-value**
MVO (FPP): N (%)	13 (62%)	12 (63%)	1.00
MVO (EGE): N (%)	11 (52%)	12 (57%)	1.00
MVO (LGE): N (%)	11 (52%)	12 (57%)	1.00

Table 14 Table showing the Incidence of MVO per CMR technique applied

There was no significant difference in the incidence of MVO between the two study groups. Hence the treatment of two cycles of RIPC failed to reduce the incidence of MVO in STEMI patients having undergone PPCI.

We examined the difference between the CMR methods to identify MVO in terms of the presence of MVO. As each method was assessed on the same patients, the paired exact test was used to compare between pairs of MVO methods. Patients in

whom all three techniques were not employed were excluded from the analysis. The results are presented in the following table.

MVO Method	MVO present N (%)	P-value
FPP	25/40 (63%)	0.63
EGE	23/40 (58%)	
FPP	25/40 (63%)	0.63
LGE	23/40 (58%)	
EGE	23/42 (55%)	1.00
LGE	23/42 (55%)	

Table 15 Table to compare Imaging methods to assess MVO

Although slightly more patients with MVO were identified using the FPP perfusion technique there was no significant difference between the three techniques in their ability to detect MVO. This analysis will be open to bias in that the operator was not blinded between the individual techniques and will have identified MVO on the technique used which will have been the FPP in the majority of cases.

Finally we examined how ischaemia time and infarct size varied between patients who displayed evidence of MVO on CMR. MVO was defined using the FPP method for these analyses. Although the previous analyses suggested no significant differences in the presence of MVO between techniques, this method had the highest occurrence of MVO.

The results are summarised in the next table.

Variable	MVO absent	MVO present	P-value
Ischaemia time - Median (IQR)	208 (124, 434)	192 (164, 296)	0.96
Infarct size – Mean (SD)	0.98 (1.04)	3.52 (1.59)	**<0.001**

Table 16 Table to demonstrate how Ischaemia time and Infarct size varied between patients with MVO on CMR

The results suggest that ischaemia time did not significantly affect the presence or absence of MVO. However, there was a large difference in infarct size between patients with MVO and those without. Infarct size was much larger in the presence of MVO, with a mean infarct size of 3.5g compared to a mean of infarct size of 1.0g when MVO was absent.

4.4.3 Cardiac Biomarker Endpoints

Analysis of the cardiac biomarkers also failed to show any significant difference between the two groups. A summary of the results of these outcomes at each point in the study are given in the next table. Due to the skewed distribution of the outcomes, the values were summarised by the median and inter-quartile range in each group.

Outcome	Time point	Control Median (IQR)	RIPC Median (IQR)
Troponin	0 hours	0.0 (0.0, 0.2)	0.2 (0.0, 0.9)
	6 hours	4.7 (1.6, 6.4)	3.2 (1.0, 6.3)
	12 hours	3.1 (1.6, 5.8)	3.2 (1.7, 5.0)
	24 hours	2.6 (1.1, 3.4)	2.6 (1.7, 3.3)
CK	0 hours	105 (81, 297)	169 (102, 505)
	6 hours	1485 (470, 2764)	1280 (640, 2364)
	12 hours	1225 (586, 1915)	1335 (880, 2223)
	24 hours	751 (317, 1199)	885 (501, 1258)
CK-MB	0 hours	7 (3, 36)	21 (5, 42)
	6 hours	143 (57, 257)	188 (99, 279)
	12 hours	102 (60, 195)	142 (80, 215)
	24 hours	42 (21, 110)	67 (33, 97)

Table 17 Table showing analysis of Cardiac Biomarkers between the two groups studied

The subsequent graphs demonstrate the differences between groups with regards to the different biomarkers measured. The graphs show the median and 95% confidence interval for the median at each time point for each of the two groups. Visually the graphs appear to show similar values in each of the two groups at each time point for all three outcomes.

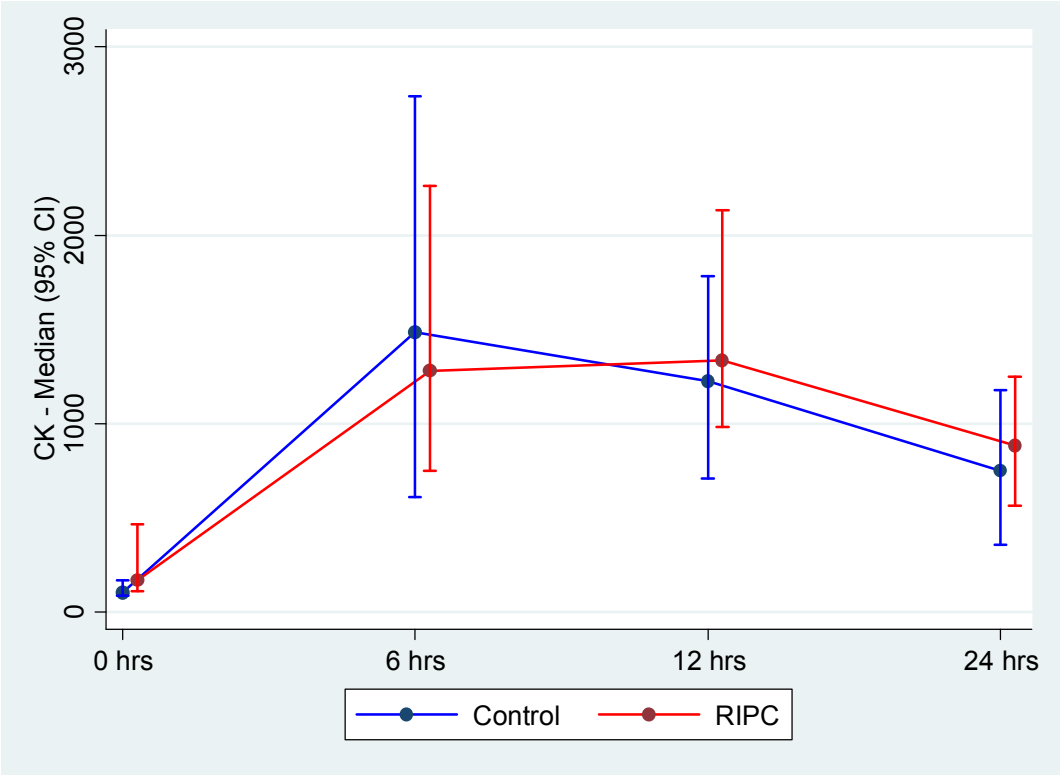


Figure 19 Graph showing the difference between groups with regard to different biomarkers

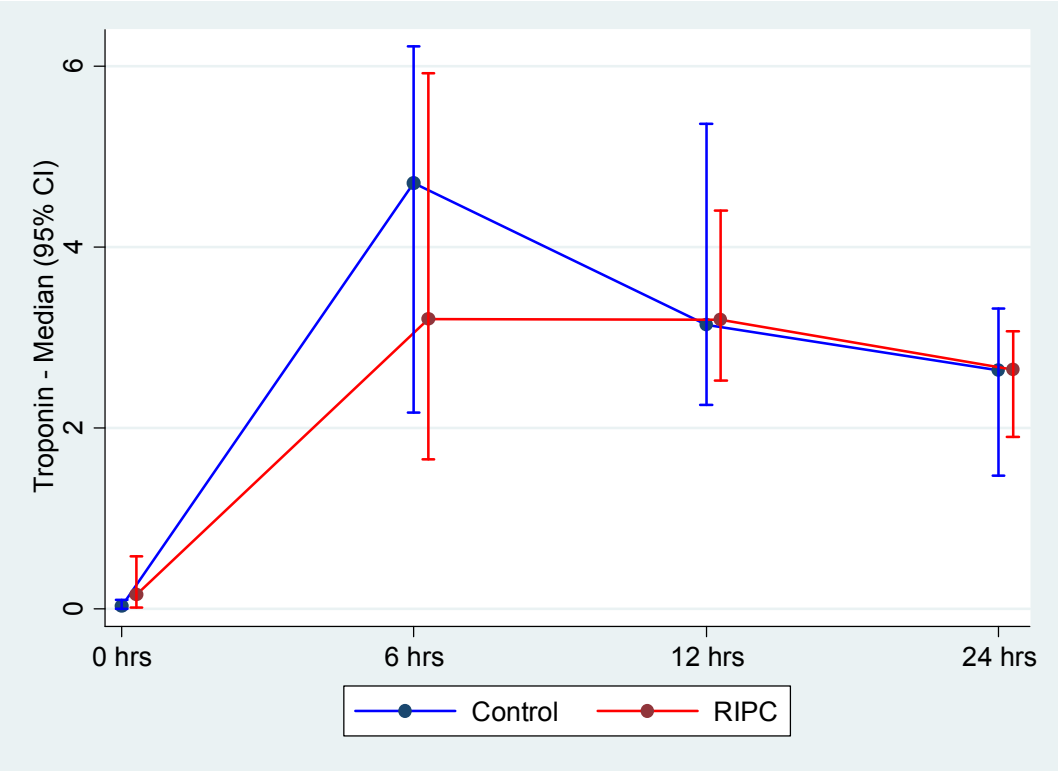


Figure 20 Graph showing the difference between groups with regard to different biomarkers

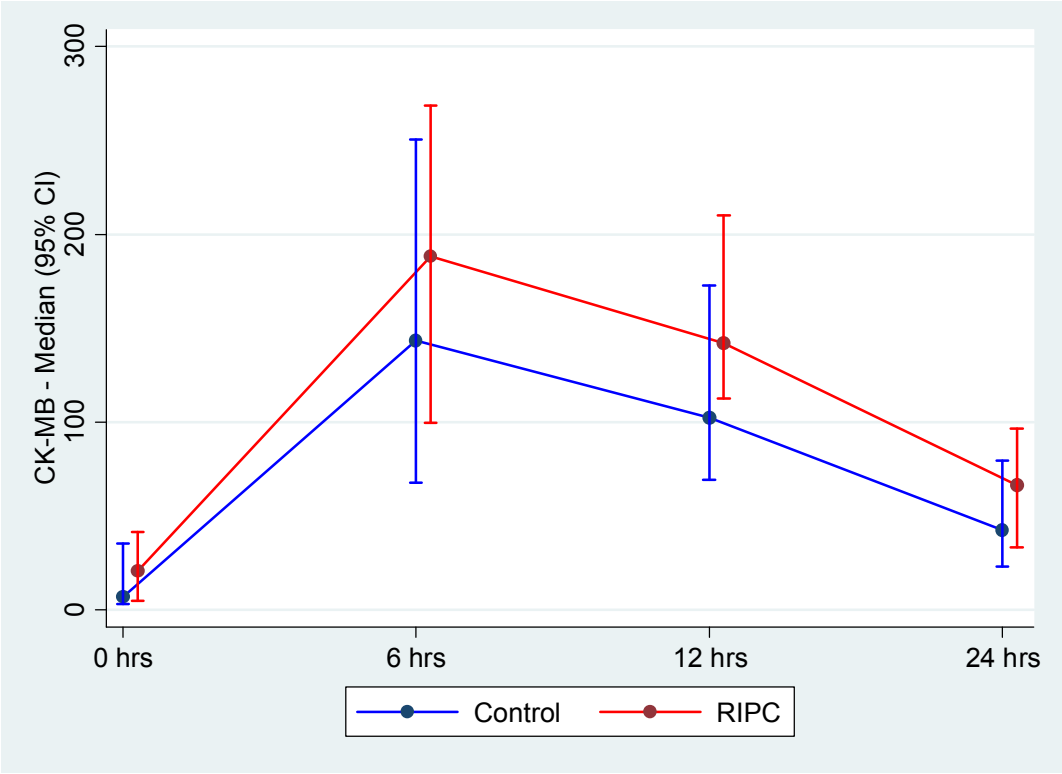


Figure 21 Graph showing the difference between groups with regard to different biomarkers

Multilevel regression methods were then used to compare the difference in outcomes between the groups, and the results are summarised in the next table. The first column gives the p-value from the time by group interaction. This indicates if the differences between groups varied at the different post-baseline time points. The final two columns give the difference between groups assuming a constant group different at all time points. As the outcomes were analysed on the log scale, the size of difference between groups was reported as a ratio, along with corresponding confidence interval. This gives the ratio of outcome in the RIPC group relative to the control group. The final p-values give the significance of these group differences.

Assuming a constant different between groups, the further analyses indicated no significant differences in any of the three outcomes between the two patient groups.

Outcome	Interaction P-value	Ratio (RIPC/control) (95% CI)	P-value
Troponin	0.31	0.91 (0.66, 1.26)	0.57
CK	0.58	1.26 (0.67, 1.97)	0.60
CK-MB	0.61	1.32 (1.72, 2.43)	0.37

Table 18 Table showing no significant differences in the outcome of the three biochemical biomarkers measured

The results suggest that there is no significant interaction between groups and time for any of the three biomarkers. This suggests that the differences between groups were not statistically different at the post-baseline time points. It can therefore be assumed that there was a constant difference between biomarker groups at all three subsequent time points.

Analyses calculated the area under the curve (AUC) for those patients with measurements at all four time points. A comparison of the AUC values between the two groups was made and the results are summarised in the next table. The figures reported are the median and inter-quartile range in each group, along with the median difference and confidence interval for this difference.

Outcome	Control Median (IQR)	RIPC Median (IQR)	Difference Median (95% CI)	P-value
Troponin	62 (34, 110)	82 (55, 110)	11 (-25, 46)	0.53
CK (*)	15 (9, 33)	28 (15, 50)	9 (-6, 25)	0.23
CK-MB (*)	2.2 (1.3, 3.6)	2.7 (1.6, 3.6)	0.4 (-1.1, 1.7)	0.54

Table 19 Table showing the comparison of the AUC values between the two groups
(*) AUC values recorded in 1000s

The AUC analysis results suggest that the area under the curve values did not differ significantly between the two groups for any of the three biomarkers examined.

The association between the different biomarkers was examined, with analyses performed at each time point. The results are summarised in the next table. Due to the skewed distribution of the blood test results, Spearman's rank correlation was used for the analyses. The figures presented are the correlation coefficients, along with their corresponding p-values indicating the significance of the results.

Timepoint	Variable 1	Variable 2	Correlation Coefficient	P-value
0 hours	Troponin	CK	0.61	**<0.001**
	Troponin	CK- MB	0.64	**<0.001**
	CK	CK-MB	0.69	**<0.001**
6 hours	Troponin	CK	0.83	**<0.001**
	Troponin	CK- MB	0.74	**<0.001**
	CK	CK-MB	0.73	**<0.001**
12 hours	Troponin	CK	0.78	**<0.001**
	Troponin	CK- MB	0.66	**<0.001**
	CK	CK-MB	0.71	**<0.001**
24 hours	Troponin	CK	0.82	**<0.001**
	Troponin	CK- MB	0.66	**<0.001**
	CK	CK-MB	0.86	**<0.001**

Table 20 Table showing the association between the different biomarkers

The analyses indicated that, as would be expected, there were strong correlations between each pair of biomarker outcomes at all the time points. All correlations were positive in nature, suggesting that the different blood tests were all positively related.

The association between the biomarker results and both infarct size and ischaemia time was examined using Spearman's rank correlation. The maximum blood test result from all time points was used in the analyses. The results are summarised in the following table.

Variable 1	Variable 2	Correlation Coefficient	P-value
Ischaemia time	Troponin	0.15	0.27
	CK	0.08	0.59
	CK-MB	0.19	0.18
Infarct size	Troponin	0.72	**<0.001**
	CK	0.73	**<0.001**
	CK-MB	0.44	**0.01**
Ischaemia time	Infarct size	-0.16	0.33

Table 21 Table showing the association between the biomarker results and both infarct size and ischaemia time

Interestingly the results suggested that there were no statistically significant association between ischaemia time and the biomarkers sampled. However, all three biomarkers were found to be significantly associated with infarct size with the positive correlations suggesting that a larger infarct size was associated with higher values for all three biomarkers.

There was no significant association between ischaemia time and infarct size. Ischaemia time was also not significantly associated with salvage index. However, there was a statistically significant association between salvage index and infarct size. There was a negative correlation suggesting that, as expected, the salvage index was lower for patients with a larger infarct size.

4.5 Discussion

The main finding of this proof of concept study, is that the use of two cycles of RIC prior to PPCI failed to reduce infarct size in STEMI patients. These findings differ from those of Rentoukas *et al.*[181] and Bøtker *et al.*[184] who in larger studies with differing endpoints demonstrated the benefit of an RIC protocol prior to PPCI (with three and four cycles respectively). At the same time our findings are consistent with numerous other trials of cardioprotective strategies that have failed to show benefit in different clinical settings. A recent meta-analysis of 23 studies of the use of RIC in different clinical settings showed no evidence that RIC reduces mortality or MACE associated with ischaemic events, yet there was a reduction in peak troponin release. [389]

It is also important to comment on the yet unpublished work by Crimi *et al.* who used an RIC protocol of three cycles of 5 minute inflations up to 200mmHg. Interestingly the group applied the protocol to the lower limb and the intervention was begun at the point of reperfusion (first device time) making it a IPost protocol. The patient cohort was selected with the inclusion of patients with LAD infarcts with TIMI 0-1 flow on initial angiography without evidence of significant collateralisation. The study showed a 20% reduction in enzymatic infarct size as measured by CK-MB. Whilst there was a trend to improvement in infarct size when measured by late enhancement imaging on CMR, no statistically significant difference was found. Crimi's hypothesis was that this may be due to the fact that only 80% underwent late enhancement imaging. Yet at the same time it should be noted that the AAR was not measured and manual planimetry was used to quantify the size of the infarction. The

other interesting aspect of the study is the use of myocardial oedema as a marker of reperfusion injury and were able to show the oedema as measured by T2-weighted imaging was significantly reduced in the RIC group.

Our study differs from those of Rentoukas *et al.*[181] and Bøtker *et al.*[184] in a number of important aspects, which are reflective of the failure to translate cardioprotective strategies in pre-clinical work.

Differences in the study protocols is likely to be reflected in the results. Differences in the protocols included the point at which the protocol was initiated, the number of cycles used and the length of each cycle

(a) Number of Cycles: Each group (including our own) used a different study protocol varying from two to four cycles of upper limb ischaemia. Our study was limited by the inability to apply RIC prior to arrival at the cardiac centre, along with short door-to-balloon times. This meant that only two cycles could be applied prior to the deployment of the first device leading to the advent of reperfusion. Bøtker's group on the other hand had the benefit of longer transfer times (symptom to balloon times of >3hrs) with the protocol being initiated by the Paramedics. Given the simplicity and safety of the protocol, the recruitment of the ambulance service to instigate RIC is to be recommended. However, time for a longer protocol is a double edged sword as it will imply a longer ischaemic time for the patient. The protocol of Rentoukas *et al.* differs again. This group began the application of three cycles 10 minutes before the estimated time of first balloon inflation. Even if we use the inflation of the first balloon as the index of reperfusion, this would imply that the protocol continued well after

reperfusion given that three cycles of ischaemia and reperfusion will last 24 minutes. Yet reperfusion will often begin following the successful passing of the first wire down the occluded artery. This protocol is therefore more of a post-conditioning protocol rather than one of per-conditioning.

Early studies of preconditioning revealed an ischaemic threshold below which preconditioning provides no protection and that increasing the ischaemic trigger increases the protection until the effect saturates.[390] Loukogeorgakis *et al*[391] compared RIPC and RIPostC in an *in-vivo* model of endothelial IR injury. They were able to demonstrate that the degree of protection by RIPostC against endothelial IR injury in conduit vessels is similar to that achieved by RIPC. This experimental study also gives us a number of insights into the degree of stimulus required to cross the preconditioning threshold and provide protection. Three cycles of upper limb RIPC was shown to induce protection, yet shortening the stimulus to just two cycles failed to induce protection. At the same time the group demonstrated that the amount of tissue exposed to the preconditioning stimulus was a determinant of protection. Whereas two cycles of RIPC was ineffective against endothelial IR injury when applied to the upper limb, the same protocol applied to the leg was shown to induce protection.

There is however divergent data with Li *et al*. demonstrating in a canine model that multiple cycles (six or twelve) offered no more protection than a single cycle.[392]

Although there is no consensus on the number of cycles required to induce protection, it is conceivable that our protocol of just two cycles was below this

threshold and hence failed to provide protection. We may hypothesise that we been afforded more time to apply a longer protocol we may have seen a difference between the groups. At the same time, conclusions based on certain known data, when they are ampliative in nature, i.e. when they are extended to unknown areas, can have validity only on the assumption of everything else being equal.[393] Further research in the clinical domain is clearly needed to define the optimum number of cycles needed to induce protection.

(b) Cycle Length and Inflation: Cycle length also varied between the protocols and there remains no consensus as to how long a cuff should remain inflated for, nor to what pressure the cuff should be inflated to. Bøtker's group protocol was similar to ours and cuff inflation was to 200mmHg for 5 minutes. Whereas with Rentoukas's group the cuff was inflated to 20mmHg above systolic arterial pressure for 4 minute cycles. The group do not supply any data on the average BP of their different patient groups. Just as the volume of tissue to which the conditioning stimulus is exposed is a determinant of the degree of protection, so to the length of time to which it is exposed to the stimulus is also likely to influence the protective effect. In preconditioning the ischaemic time induced by the stimulus appears to be the determining factor of the conditioning effect.[394] The period of reperfusion requires a minimum duration of between 30 seconds to one minute.[395] Protection has been demonstrated when the period of reperfusion has been extended out to one hour.[396] It is unknown whether it is the ischaemic period, the reperfusion period, or the number of cycles that is the critical factor in postconditioning.[390]

(c) Patient Selection: Patient selection is obviously a significant determinant of outcome in any clinical trial. Randomised controlled trials (RCTs) have long been the gold standard research design because of their potential to offer unbiased estimates of an intervention's' effectiveness. Evidence however suggests that trial participants are often unrepresentative of the target population, which can introduce bias in the measures of effect.[397] Trials tend to recruit highly selected, unrepresentative populations; therefore the results cannot reliably be translated to real world scenarios. Multiple factors have been shown to affect IRI and the response of patients to cardioprotective strategies. These factors include;

(i) Age where structural and functional changes during ageing render the heart more susceptible to cell death from ischaemia/reperfusion. Cardioprotective manoeuvres such as ischaemic pre and postconditioning appear to lose their effectiveness with ageing.[398]

(ii) Gender. Both experimental and clinical studies have indicated that female gender influences favourably aids the remodeling and the adaptive response to myocardial infarction.[399] In some studies, particularly in the rat, females show less IRI; however, in many animal studies no gender difference in IRI injury is observed.[400]

Data on age and gender are easily collected and further work is needed to elucidate the actual effects of these aspects on cardioprotective strategies.

Our own study (as with that of Bøtker and Rentoukas) excluded essentially the sickest patients who were deemed to be unstable (e.g. cardiac arrest prior to

admission or cardiogenic shock). Interestingly mechanical cardioprotective strategies, such as RIC, may be particularly beneficial in this patient group, as they have the potential to mediate multiorgan protection.[112]

(d) Concomitant medication - A number of medications have been shown to either disrupt or augment cardioprotection. Nicornadil is thought to provide cardioprotection through inhibition of ROS formation.[401] Controversy exists as to whether the diabetic heart is amenable to cardioprotective strategies.[402] At the same time certain oral diabetic medication has been shown to lower the threshold required to protect the diabetic heart from IRI.[403] Unfortunately different anti-diabetic agents appear to provide differing levels of protection. Whilst not all confounding variables can be controlled for, at the very least the pre-treatment of patients with such medication needs to be recorded. In an ideal scenario patients taking these medications would be excluded from clinical studies to ensure that any possible confounding effect is removed.

In the same vein patients with IHD are likely to have multiple co-morbidities. How these co-morbidities either in isolation or indeed in combination may modify responses to IRI and the cardioprotection conferred by IPC and IPost is not clear.[404]

(e) Endpoints. Different surrogate endpoints have been used for each of the three trials which will also influence outcomes. As therapy for acute MI advances, it becomes increasingly difficult to demonstrate incremental benefit of cardioprotective strategies. The selection of appropriate endpoints in clinical trials is of central importance in the assessment of the effects of cardioprotective interventions.

Meaningful end points should ideally (i) link the treatment effect to the underlying pathophysiology; (ii) be clinically relevant and provide prognostic information to subsequent patient outcome; and (iii) show high specificity and sensitivity, as well as high reproducibility.[405,406] Imaging with cardiac MRI would appear to fulfil all three criteria, providing information on both infarct size and the AAR as well as ongoing insight into the pathophysiology of acute infarction through imaging of the different components of reperfusion injury. CMR is an almost 'one-stop' shop for the evaluation of patients post PPCI. However, variability in end point definitions, creates a significant barrier to the understanding of results across clinical trials[407] or the pooling of results for the purpose of meta-analysis.

In our study, the failure of RIC to reduce myocardial infarct size in PPCI patients is in conflict with the pre-clinical data reporting reductions in myocardial infarct size in a swine model of ischemia-reperfusion injury, with RIC applied prior to reperfusion.[129] The failure to translate cardioprotective strategies from animal models to human studies is discussed earlier on.

	Hasleton	Bøtker	Rentoukas	Crimi
N	61	251	96	96
Ischaemic Time (min)	180	189	194	180
Diabetes (%)	18	9	31.5	12
Pre Infarct Angina	Excluded	Not Excluded	Not excluded	Not excluded
Conditioning Mimetics	Excluded	Not excluded	Not excluded	Not excluded
Conditioning	Prior to reperfusion	Prior to reperfusion	Protocol completed during reperfusion	Post Reperfusion
Limb	Arm	Arm	Arm	Leg
Cycles	2	4	3	3
Cycle Length (min)	5	5	4	5
Cuff Inflation (mmHg)	200	200	20 above systolic pressure	200
Anterior MI only	No	No	No	Yes
TIMI flow < 1 only	Yes	No	No	Yes
Collateral vessels	Excluded	Included	Included	Excluded
AAR measured	Yes (Infarct ESA)	Yes (SPECT)	No	No
Endpoint	Infarct Size (CMR)	Myocardial Salvage (SPECT)	ST Segment Resolution (ECG)	AUC CK-MB

Table 22 Table of studies investigating the application of RIC in the setting of acute Myocardial Infarction

4.6 Conclusion

In summary we have demonstrated that two cycles of remote ischaemic preconditioning failed to reduce myocardial infarct size or the incidence of MVO in the setting of PPCI for acute myocardial infarction. However, RIC remains a safe adjunct to PPCI and with additional cycles of ischaemia and reperfusion and larger sample sizes promises to show benefit in future studies.

5 Tissue Characterisation with Cardiovascular Magnetic Resonance

Myocardial tissue composition holds important information regarding disease-specific patterns of injury, current activity of disease-related mechanisms, and the reversibility of changes.[408] CMR provides information beyond simple geometry and functional indices of cardiovascular structure. By making use of the magnetic properties of H^+ found in each cell, CMR allows for an almost *in-vivo* histopathological assessment of the myocardium. To this end CMR sequences are now available that enable the assessment of additional components of reperfusion injury and infarction including myocardial oedema, intramyocardial haemorrhage and diffuse (as opposed to focal) myocardial fibrosis. Additional indices allow for a more detailed profiling of individual patients as well as more accurate assessment of future risk. To this end treatment, in the form of pharmacotherapy, device-based therapy or surgery, can be tailored to individuals needs. Within the research arena, identification of further markers (beyond function and LGE) of adverse prognosis adds to surrogate endpoints for clinical trials. The use of ventricular volumes, contractility and even infarct size can be misleading in certain settings with the measurements of only complementary markers showing differences between trial groups. CMR is therefore unique in its ability to identify and assess numerous markers of disease that can be utilised for both patient and research benefit.

We aimed to characterise the myocardium in the setting of an acute myocardial infarction employing CMR sequences to identify myocardial oedema, intramyocardial haemorrhage and diffuse fibrosis.

5.1 Myocardial Oedema

Approximately 75% of the myocardium is water. This is mainly contained within the cells and to a lesser extent within the vasculature and depending on the disease state, the interstitium. A small amount of water is contained within the interstitial space. Control of the amount of fluid within the interstitial space is governed by the hydrostatic pressures within the microvascular exchange vessels and within the interstitial space and colloid osmotic pressures within the microvessels and within the interstitium. Normally the same amount of water that enters the interstitial space would be removed from the interstitium *via* the myocardial lymphatic system[409] meaning that fluid does not build up within this compartment and it does not become oedematous. Myocardial oedema is therefore the accumulation of an excess of water within the interstitial space due to the disruption of water homeostasis within the myocardium. Myocardial oedema develops when the rate at which fluid entering the cardiac interstitium (microvascular filtration rate) is greater than the rate at which it is taken away.[410] Myocytes themselves will swell with water during ischaemia as part of the process of necrosis. The massive inflow of water into the cell by the osmotic imbalance ultimately leads to cell swelling and rupture of the plasma membrane.[411] This in turn leads to the release of cellular contents (including water) into the expanding extracellular space. Ischaemia with or without infarction can induce myocardial oedema.

Within the clinical arena, different imaging techniques have been employed for the assessment of myocardial oedema.

Assessment of LV mass and ventricular wall measurements by echocardiography has been proposed as a surrogate for myocardial oedema. Whilst oedema will increase wall thickness and myocardial mass measurements, these indices are not specific to oedema. The application of echocardiography in this setting is therefore limited.

CT has also been used to demonstrate myocardial oedema in canine and swine models.[412] Early experiments were hampered by limited temporal and spatial resolution. Mahnken *et al*. reported good agreement between unenhanced CT and T2-weighted CMR. The pictures presented by the group in their paper show an area of enhancement on CT that appears to match and is certainly no bigger than the area of infarction on the LGE images. CT, as always, is limited by radiation. With unenhanced CT imaging for oedema, the difference in the CT values is low, resulting in the need to apply high tube-current time products to keep the image noise in acceptable ranges and to provide an acceptable CNR. This therefore results in the same radiation exposure as a coronary CT angiogram.[413] Whilst dual source CT with ECG gating offers the potential to reduce radiation doses[414], serious concerns continue to exist as to the use of even smaller doses of radiation and the causation of malignancy.[415]

CMR offers a more robust ability to image myocardial water content. A number of different sequences have been used to assess the myocardium for oedema. These sequences take advantage of the long T2 relaxation times of the water - logged myocardium. Long T2 values produce high signal and hence oedema appears as bright on T2 weighted sequences. Higgins *et al*. was the first to demonstrate the correlation between myocardial water content and increased T2 relaxation times in a canine model of acute infarction.[260] The relationship between myocardial water content and relaxation times was, however, noted a decade earlier by Kiricuta *et al.* who documented that T2 relaxation times decreased as tissues (including the heart) matured and their water content decreased.[416] T2 weighted sequences have therefore been employed across a range of cardiovascular pathologies to demonstrate acute and chronic myocardial oedema with the demonstration of a linear relationship between water content and T2 relaxation times. Settings include acute infarction (with studies demonstrating the lack of increased signal in chronic infarction), myocarditis[417], pulmonary hypertension and transplant rejection.[418]

A number of groups have suggested the use of T2 weighted imaging to elucidate the myocardium at risk in acute infarction. At the very least, T2 weighted imaging in the setting of AMI identifies oedema as a product of reperfusion injury.

Popular techniques include;

1. Turbo Spin Echo (TSE) is a multi echo sequence using several phase encoded echoes
2. Turbo Inversion Recovery Magnitude (TIRM) is the combination of a basic TSE and an inversion pulse for the preparation of longitudinal magnetisation.
3. Short T_I TAU Inversion Recovery (STIR) (Triple inversion recovery) is a TIRM sequence using a short inversion time suitable for suppressing the signal from fat.
4. T_2-SSFP is an alternative to TSE sequences. This is a bright blood sequence that helps eliminate bright endocardial surface artefacts. T_2-prepared SSFP is reported to have higher diagnostic accuracy than T_2-prepared dark-blood TSE with respect to determining the coronary artery distribution and determining whether a patient had an acute or chronic MI.[283]
5. ACUT(2)E TSE-SSFP is a hybrid between steady state free precession (SSFP) and TSE for bright-blood T2-weighted imaging with signal-to-noise ratio (SNR) and contrast-to-noise ratio (CNR) similar to dark-blood TSE.[284]

We aimed to apply T2-weighted sequences to a subgroup of patients with acute myocardial infarction who had been treated with PPCI who had enrolled in the RIC trial. Given that T2-weighted images have previously been used to calculate the AAR, we sought to compare the AAR as measured by the infarct-ESA and T2-weighted CMR techniques. During our trial period a number of other sequences became available (ACUT(2)E TSE-SSFP and STIR) that we also applied in selected patients.

5.1.1 Methods

Consecutive patients recruited to the RIC STEMI trial and without contraindication to CMR underwent standard scan as laid out in the protocol earlier. All scans were carried out within a week of PPCI. Oedema imaging was carried out prior to the administration of contrast. Following cine imaging, assessment was made of the short axis slice exhibiting the highest degree of regional wall motion abnormality. If no regional wall motion abnormality was detected then a mid-ventricular slice was selected. A Turbo Inversion Recovery Magnitude T2-weighted sequence was applied with typical parameters of TR 3.1ms, TE 67ms, slice thickness 8mm, field of view 340×450mm and sampled matrix size 256×192. A short axis stack was then acquired with the application of the T2-weighted sequence matching the cine short axis stack slice positions.

Patients who had difficulty tolerating the scan whether in view of ability to breath hold or lie in one position for a fixed time were excluded so as not to unnecessarily prolong their scan. Patients in whom we were unable to visually identify a region of raised signal intensity were excluded.

Images were analysed as per the methods stated earlier. For this cohort of patients, the epicardial and endocardial surfaces were manually segmented. Using a visual assessment and windowing of the short axis T2-weighted images, the area of high signal was manually segmented and expressed as a percentage of the left ventricle. Artefact was noted within the blood pool in a number of patients. This was removed through comparison with cine images at the same level. Areas of hypointensity that corresponded with microvascular obstruction either on FPP, EGE or LGE was included in the segmented area of high signal intensity and classified as

intramyocardial haemorrhage. The infarct-ESA was calculated as previously described.

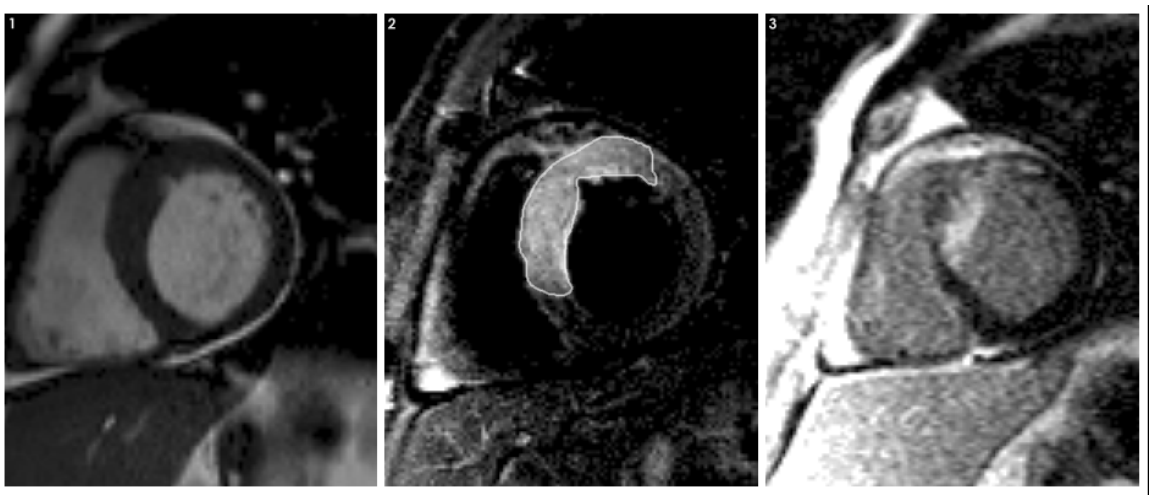


Figure 22 Short axis section of the LV following PPCI for an anterior STEMI
Short axis section of the LV from a study patient following PPCI for an anterior STEMI with 1. Cine SSFP for reference, 2. T2-weighted TIRM image demonstrating increased signal in the anterior and antero-septal wall and 3. is the corresponding LGE image.

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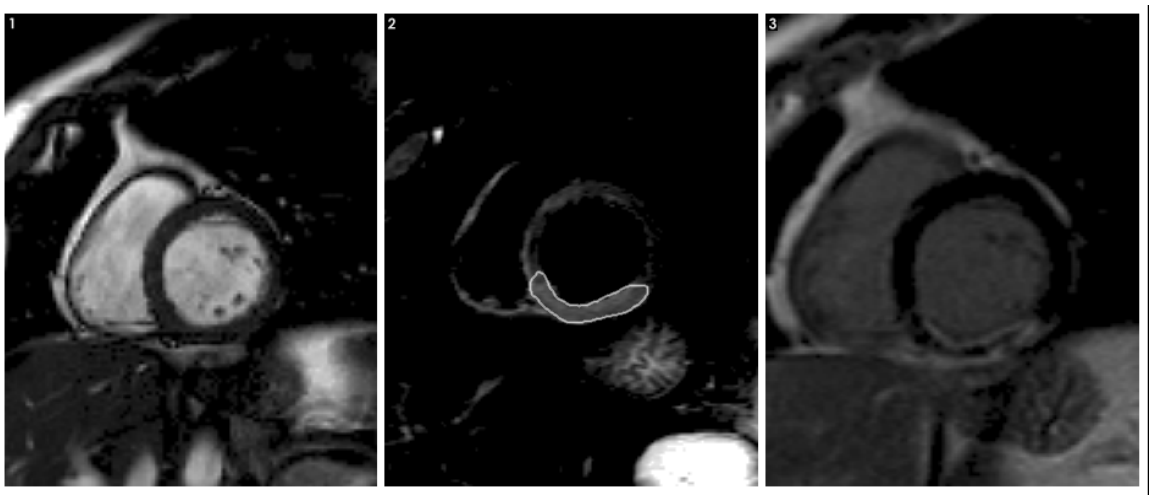

Figure 23 Short Axis Section of the LV following PPCI for an Inferior Infarction
Short axis section of the LV from a study patient following PPCI for an inferior infarction. Note that in panel 3 below there is extensive MVO but no signal void is appreciated on the T2-weighted image

5.1.2 Statistical Methods

We compared two techniques (Infarct-ESA and T2-weighted oedema imaging) used to quantify the AAR. Initially Pearson correlation was used to examine if there was any agreement between the two methods. However, a high correlation does not necessarily imply good agreement between the two methods, and thus the agreement between the two methods was also assessed using the Bland-Altman limits of agreement method.

5.1.3 Results

Six patients were excluded in view of restlessness and difficulty with breath-holding. In two patients there was no discernable increased signal on T2-weighted images and in two separate patients there was no evidence of infarction on LGE imaging. Yet review of the clinical history, ECG, angiography and cardiac biomarkers all confirmed an AMI. The two patients without evidence of increased signal on T2-weighted imaging had evidence of LGE in the territory of the infarct related artery without evidence of wall thinning in that area, also suggesting an acute event. All patients who were included had evidence of increased signal within the myocardium. In one patient the identified area of increased signal did not fit with the territory supplied by the infarct related artery and did not match up with the area of infarction as identified on LGE. The patient was subsequently excluded.

This gave a correlation coefficient of 0.85, with associated p-value of <0.001. This suggests a highly significant association between the two variables, and this association is shown in the next plot, along with the line of best fit.

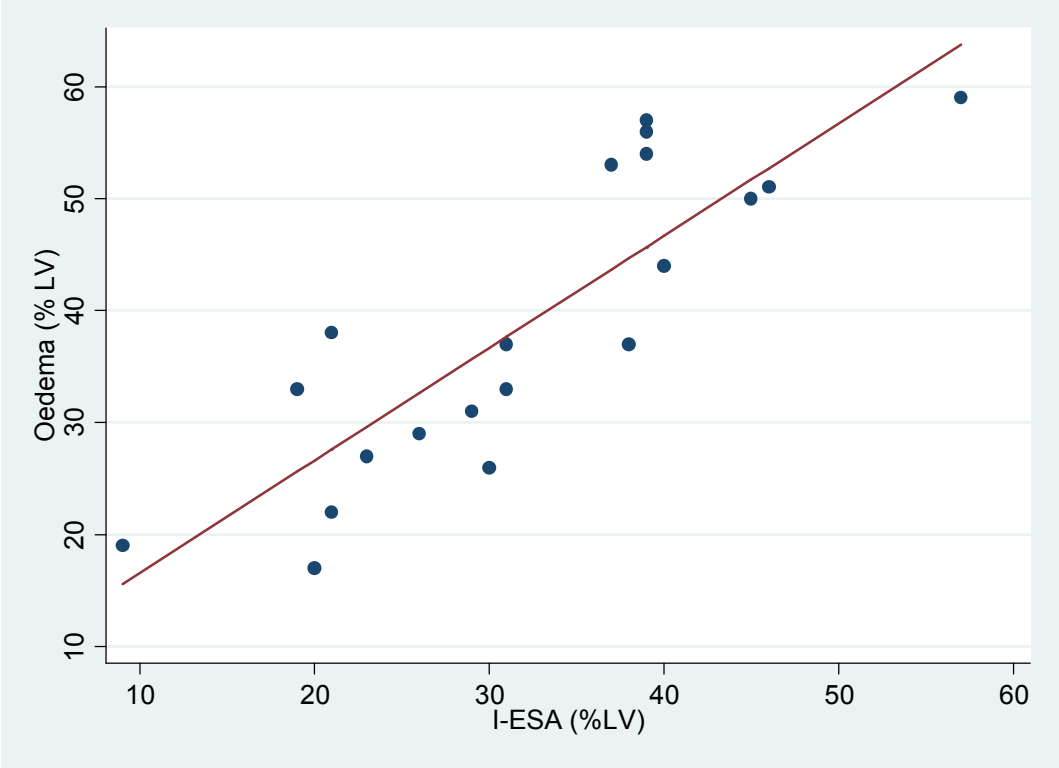

Figure 24 Graph showing the Correlation and Line of best fit for Oedema and Infarct ESA measurements.

As the two methods were measuring the same variable, it would be expected that there is a strong association between them. However, a high correlation does not necessarily imply good agreement between the two methods.

As a result, the agreement between the two methods was assessed using the Bland-Altman method. This analysis indicated that, considering the differences as oedema minus I-ESA, there was a mean difference of 6.7 between methods, with a 95% Bland-Altman limits of agreement of -7.3 to 20.6. This result firstly suggests that the oedema method gives slightly higher values, on average, than the I-ESA method. The key result for this method is the width of the limits of agreement. The limits suggested that the majority of differences between the two methods are between -7

to 21. A difference between measurement methods as extreme as this would likely introduce bias into any AAR or salvage calculations.

A graphical illustration of the results is given in a Bland-Altman plot below, which plots the difference between methods against the average of the two methods. The lines presented are the mean difference between methods (in red) and the limits of agreement (in blue).

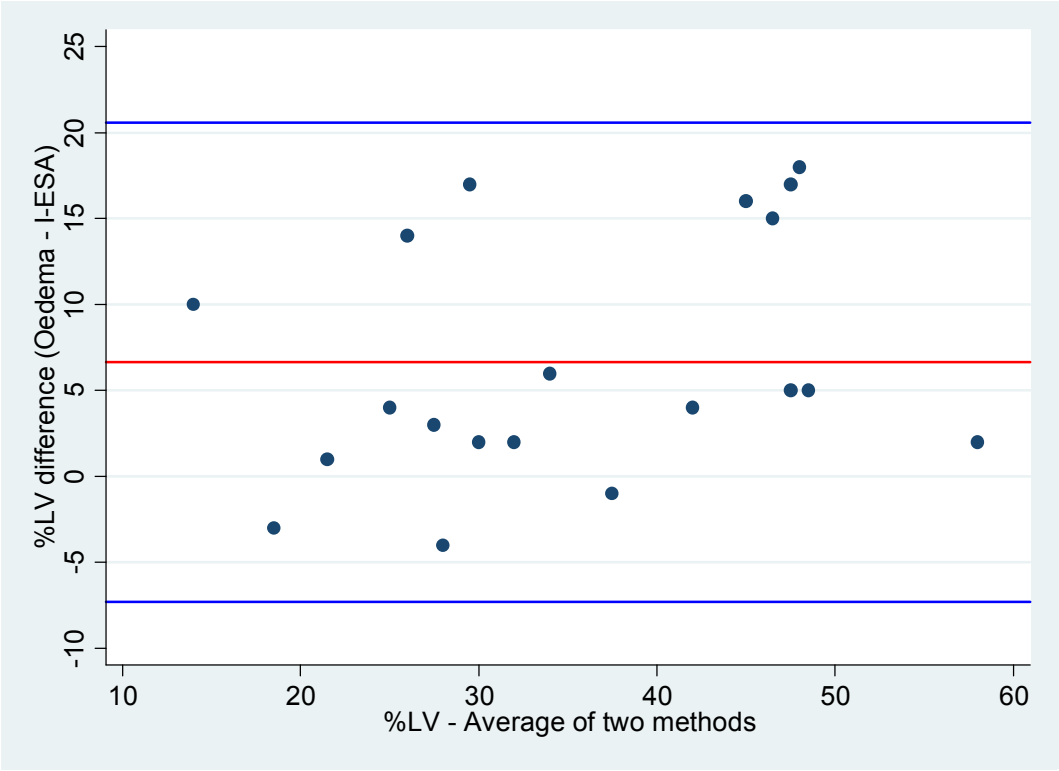

Figure 25 Bland-Altman plot comparing Oedema and Infarct ESA

5.1.4 Discussion

T2-weighted imaging is able to identify the territory subtended by the infarct - related artery through the presence of myocardial oedema. Oedema is a marker of acute injury and would not be expected to be seen in a healthy individual. We did however not scan any 'normal' patients to test for this. Oedema imaging although in theory is

simple to implement does not necessarily easily produce the desired results. Motion (cardiac and respiratory), patient compliance and arrhythmia all impact on image quality and signal intensity. Newer sequences continue to be developed which attempt to overcome these practical failings with signal drop off[283] and motion.[419]
The use of T2 weighted sequences has been limited by comparatively long sampling (and breath hold) times. Images are sensitive to cardiac motion (and to this end imaging is ideally done during mid-diastolic diastasis, the period of minimal wall motion)[420] which can cause image degradation.

Image quality (particularly with black blood imaging) is also impaired by insufficient through plane flow for the blood nulling preparation to be completely effective. This is compounded in the setting of stunning and regional wall impairment with complex myocardial mechanics, arrhythmias and blood flow patterns. The dark-blood techniques are therefore prone to residual high signal static blood artefacts next to the endocardial border due to inadequate suppression of the signal from the blood pool and are also limited by posterior wall signal losses associated with improper timing.[284]

The choice of slice thickness varies between investigative groups. Thicker slices allow for a reduction in signal dropout and less artefact at the endocardial border. Increased slice thickness addresses some issues of image quality but comes at a cost. Ideally when one is calculating myocardial salvage between T2-weighted images and LGE images the slice thickness. Variation in slice thickness (sometimes up to 100%) will render salvage calculations void given that the extent of LGE can vary significantly between individual slices.

The reproducibility of T2-weighted imaging has been questioned. Our experience is similar to other groups in that we found it difficult to reproduce T2-weighted images not only between patients but also, more concerningly, in between individual scan sequences in the same myocardial slice with the application of the same sequence. Difficulties in obtaining consistent images have pushed users away from standard T2-weighted sequences in favour of quantitative T2 mapping, which offers precise cut offs for differentiating normal from abnormal myocardium.[421]

Just as the question as to which is the 'best' sequence to image myocardial oedema is yet to be answered, so to the question as to how to best quantify the extent of oedema also is not clear. A number of groups have set out to evaluate the most appropriate technique to quantify oedema. The ability to delineate the extent of oedema will likely depend on the T2-weighted sequence used although no group has yet applied different quantification techniques to images produced by different sequences. McAlindon *et al.* evaluated the techniques we applied (with the addition of the OTSU method) in LGE imaging to STIR images. Importantly the group investigated the inter- and intra- observer and inter-scan reproducibility. They found that manual quantification provided the best inter-, intra- observer and best inter-scan agreement, with the lowest variability.[422]

The major limitation of T2 weighted imaging is the leap from being able to image myocardial oedema to the certainty that the area of increased signal intensity identified by CMR is a direct representation of the AAR. As we have noted earlier, a number of studies have set out as our own to correlate areas of increased signal on

T2 weighted images with other surrogates of the AAR including infarct-ESA. The correlation of one surrogate with another surrogate does not prove the actual relationship of the extent of oedema to the actual AAR. Pathological studies measures of the AAR via the measurement of oedema are also limited by techniques that involve removal and dehydration of myocardial tissue. Pre-clinical (and more recently clinical) studies have however provided the greatest threat to the idea that the extent of oedema is directly related to the AAR. The AAR is of a predefined, fixed size that can only be changed by the development of collateral vasculature and not by interventions introduced around the time of reperfusion. Findings that novel interventions are able to reduce the extent of oedema mean that it is unable to reliably serve as a surrogate for the AAR unless we are willing to undertake a non-scientific approach of using the 'best of a bad bunch' in regard to available techniques. This does not infer the end for T2-weighted imaging in the setting of AMI, as oedema appears to be a product of the complex cascade of IRI. The assessment of myocardial oedema in the setting of AMI will help to further our knowledge of the pathophysiology of reperfusion injury and act as an endpoint in clinical studies alongside other indices including MVO and infarction.

5.1.5 Study Limitations

The small sample size is the most significant limiting factor of the study. There is also no assessment made of inter- or intra-observer variability. Calculating the extent of the area with increased signal intensity, involved the subjective process of manually segmenting out the epicardial and endocardial surface layers. This was followed by the operator driven manual delineation of the area of increased signal

intensity. Manual segmentation is a long and tedious task that is prone to intra- and inter-observer variability.[423] The segmentation of abnormal myocardium on T2-weighted images is technically more demanding, particularly because of the comparatively reduced signal to noise levels associated with this method. This involves adjustment of window and level settings and a threshold approach to delineate the area of increased signal intensity based on a difference in signal intensity (decided by the eye of the operator) from the mean signal intensity of an normal area of myocardium.[424] Therefore to further manually delineate the area increased signal may serve only to introduce further variability.

The assessment of myocardial oedema adds to the armamentarium of CMR. In the setting of acute myocardial infarction sheds light on the pathophysiology of IRI whilst enabling us to quantify the extent of the ischaemic phenomenon. Attenuation of myocardial oedema provides a further target for novel cardioprotective techniques. The ability of CMR to delineate the AAR needs to be judged against the pitfalls of the techniques that went before it.

5.2 Intramyocardial Haemorrhage (IMH)

CMR also has the ability to delineate intramyocardial haemorrhage, another by product of IRI. IMH appears to be intrinsically linked with MVO. Studies investigating IMH are not clear whether it occurs as an independent phenomenon or whether IMH is only seen in the context of severe microvascular destruction. It is likely that the phenomenon of MVO occurs in a range of severity with those patients who exhibit haemorrhage lying at the more severe end of the spectrum carrying with them a worse prognosis. To this end the identification of IMH provides further prognostic information for patients who have suffered an AMI. A number of techniques exist to image IMH with both T2-weighted images and T2* imaging being employed in the identification of haemorrhage.

In a small group (n=8) of patients we used both T2-weighted imaging (TIRM) and T2* techniques to assess IMH.

5.2.1 Methods

Patients were scanned as per the protocols described earlier for assessment of LV volumes, myocardial oedema and LGE. As previously short axis slice that was felt to have a significant area of regional wall motion abnormality was chosen. Prior to the administration of contrast, a T2* with typical parameters as follows; eight echoes obtained; change in echo time, 4.8 msec; voxel size, 1.7 × 3.7 × 10 mm; flip angle, 20°; section thickness, 10 mm. T2* analysis was performed with regions of interest drawn in the area of MVO and also separately in an area of remote myocardium. The

signal intensity of this region was measured for each image, and this was then plotted against the echo time to form an exponential decay curve as has been previously described.[425]

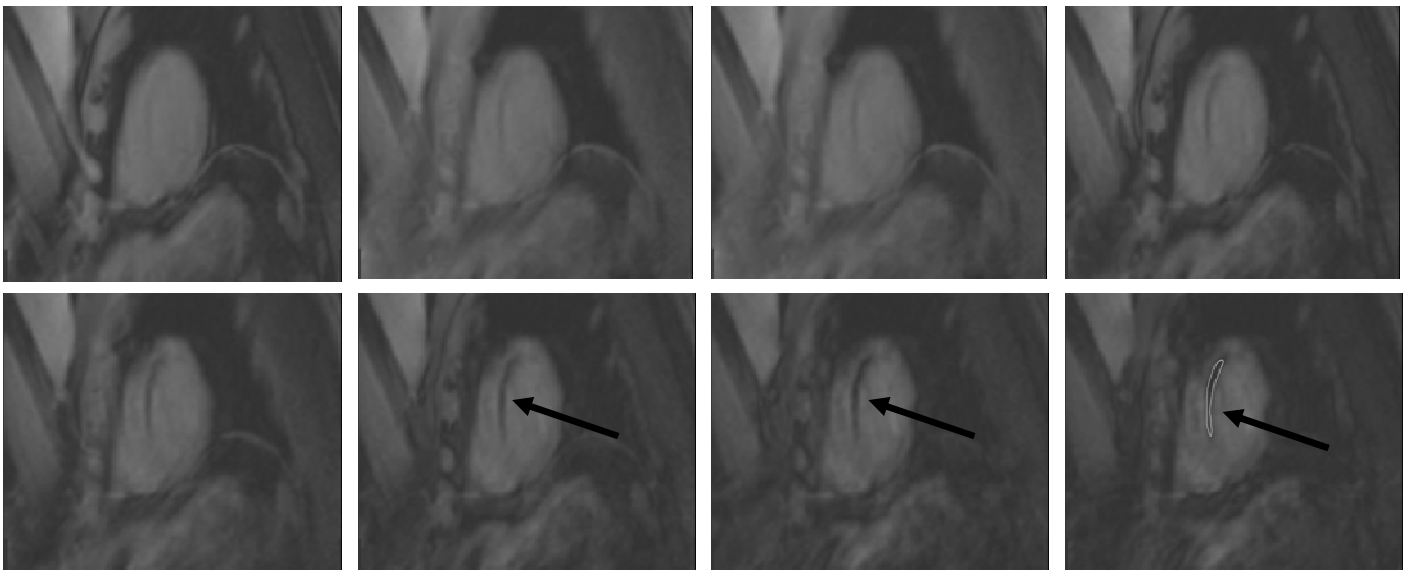

Figure 26 T2* Images showing decay constant of less than 20ms with evidence of reperfusion haemorrhage

5.2.2 Statistical Methods

Analysis of the T2* variables between the area of infarction and the area of infarction was performed using the paired t-test. Additionally, Pearson correlation was used to examine the association between the two sets of measurements.

5.2.3 Results

The mean T2* decay constant within the area of infarction was 16.9msec, as compared to 33.1msec within the area of remote myocardium. The results indicate that the T2* decay in the area of infarction were significantly different from the values, taken from the remote myocardium, a difference that was strongly statistically significant ($p<0.001$). The analysis results are summarised in the next table.

Measurement	**N**	**Mean (SD)** **(T2* decay constant -msec)**	**P-value**
Remote **Infarct**	8 8	33.1 (6.1) 16.9 (4.9)	**<0.001**

Table 23 Mean T2* values in areas of IMH and Remote Myocardium

The individual data values are shown graphically in the next plot.

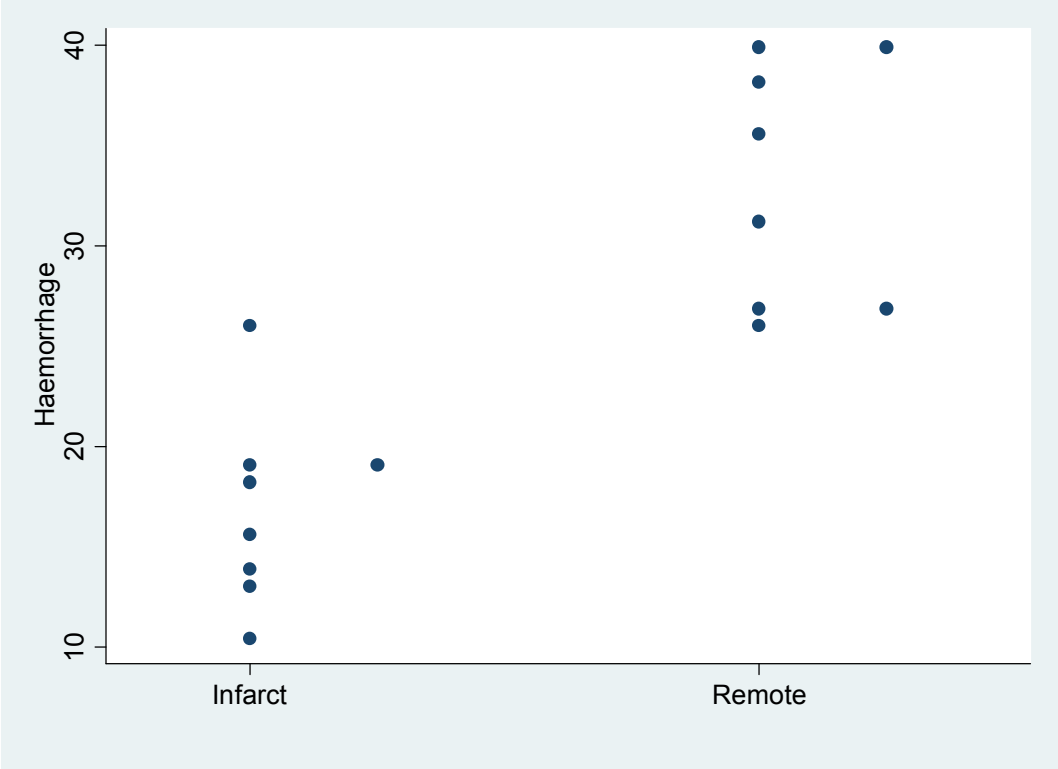

Figure 27 Dot Plot showing T2* values by Region of interest

5.2.4 Discussion

The results of this small study demonstrate the ability of CMR to assess reperfusion haemorrhage in the setting of AMI. All the patients with evidence of haemorrhage, had evidence of MVO (on all three imaging techniques – FPP, EGE and LGE), suggesting that MVO and haemorrhage are intrinsically linked. Our results are consistent with those of O'Regan[326] showing that T2* decay measurements of less than 20msec appeared to correlate with areas of IMH. In all but one of the infarcts studied was the T2* decay constant < 20msec. We were however not able to demonstrate any significant correlation (negative or otherwise) between the presence or degree of haemorrhage and other CMR indices including cardiac volumes and EF. Other studies have been able to demonstrate correlation between

the presence of IMH, as assessed by either T2* or T2-weighted images, and adverse outcomes (See table 6 in Introduction). Further studies are however necessary to assess the IMH to answer a number of uncertainties surrounding the assessment of IMH with CMR. The most robust technique to assess IMH is not yet clear with both T2* and T2-weighted imaging able to provide on IMH. The time course of the development and resolution of IMH in humans has yet to be elucidated and is necessary to guide appropriate imaging in clinical trials post AMI. Clinical studies are already listing IMH as a secondary endpoint in the assessment of novel cardioprotective agents (Study Evaluating the Use of Vasodilators to Reduce Infarct Size and Microvascular Obstruction in ST-elevation MI, NCT01747174). Such studies will be limited by the fact that the incremental value provided by IMH as assessed by CMR over and above traditional imaging biomarkers (infarction and MVO) is not yet clear and further validation is needed. However, IMH appears to represent a further imaging biomarker that is likely in the future to be of prognostic benefit and value as a clinical trial endpoint to aid the assessment of novel cardioprotective therapies.

5.3 Diffuse Fibrosis

The myocardial extracellular matrix (ECM) is a complex microenvironment that plays a fundamental role in the myocardial remodelling process. The ECM however only takes up a small proportion of the cardiac structure in a healthy heart of approximately 6%.[426] The ECM serves anchor cardiac myocytes and therefore determines the structural architecture of the heart. It therefore plays an integral role in the cardiovascular mechanics and electrical impulse propagation.[427] With advancing age and as part of the pathophysiology of cardiovascular disease interstitial expansion takes place with an increase in ECM of up to 35%.[426] Interstitial tissue volume is primarily determined by the amount of extracellular matrix.[428] Fibrosis invariably follows oedema, if long-standing. Fibrosis with interstitial expansion and increasing extracellular volume is end point of most processes that lead to irreversible or reversible tissue damage in the heart. With IHD, the extent of fibrosis is the most accurate marker of injury severity.[408] The gold standard for the assessment of myocardial fibrosis is myocardial biopsy, which carries significant risk with the possibility of sampling an area of the myocardium that may not be representative of the disease state.[429] A number of pharmacological agents having been investigated in animal models with the aim of reducing the degree of diffuse fibrosis. At present however there are no human studies aimed at reducing diffuse fibrosis that have been reported.[430]

Imaging of focal fibrosis using late gadolinium enhancement imaging is now a well validated technique that equates closely to macroscopically identified scar on postmortem analysis. This has been shown to be the case across the spectrum of

cardiovascular diseases and has sparked interest in the effect of non-cardiovascular disease processes on the heart. Imaging of focal fibrosis, however, only takes us so far with regards to mechanistic insights into particular pathologies. Diffuse myocardial fibrosis is difficult to distinguish using LGE as the myocardial signal intensity is nulled to enhance to heterogeneity of abnormal myocardium. Therefore homogeneously diffuse tissue will be nearly isointense and therefore globally "nulled" becoming indistinguishable from normal tissue.[431] Inversion recovery sequences utilised for LGE therefore delineate focal fibrosis but miss diffuse fibrosis. Measurement of the extracellular volume fraction using CMR offers the ability to identify and quantify the degree of extent fibrosis within the myocardium. Myocardial fibrosis has long been linked with adverse remodelling with the extrapolation that this translates to adverse outcomes in certain patient groups. Data is now emerging on the ability of ECV measurement by CMR to predict mortality as well as other cardiovascular composite end points.[432,433] Quantification of the myocardial interstitial space may therefore represent an important diagnostic and prognostic biomarker as well as a possible further endpoint in clinical trials in cardiovascular disease.[434]

The technique of T1-mapping in acute myocardial infarction was first described by Messroghli *et al*. in a small study of eight patients and reported focal changes in T1 measurements.

Given that in the setting of an MI the size of the extracellular space will be dependent on the amount of myocyte necrosis (myocyte wall rupture) one might hypothesise that the greater the degree of necrosis the larger the extracellular space. In acute infarction extracellular volume is increased by the presence of

myocardial oedema. Hence with the 'resolution' of myocardial oedema, the extracellular volume will shrink. Extracellular volume may reflect the extent of structural destruction of the myocardial architecture - the greater the extracellular volume the greater the degree of destruction. Imaging of the extracellular space and calculation of the extracellular volume therefore adds to our ability to characterise (anatomically and pathologically) the myocardium *in vivo* and enhances our understanding of the process of infarction and the differing degrees of disruption wrought by the different components of an MI and reperfusion injury.

5.3.1 Methods

We sought to characterise the myocardium in patients with an AMI who had undergone reperfusion with PPCI by calculating the degree of fibrosis within the reperfused myocardium. The research was granted approval from the local research ethics committee, and all participants provided written informed consent. Fifteen consecutive patients who had undergone successful PPCI and had no contraindication to CMR were recruited to the study. Patients had a full blood count taken at baseline for measurement of their haematocrit and subsequent calculation of the volume of distribution of contrast in the blood.

An Equilibrium CMR technique was used as described by Flett *et al*. in validation studies in patients with aortic stenosis and hypertrophic cardiomyopathy.[23] The equilibrium CMR technique is based on the concept that at a point of steady state the volume of distribution of contrast in the blood ($Vd_{(b)}$) will equal the volume of

distribution of contrast in the myocardium ($Vd_{(m)}$). The $Vd_{(b)}$ is calculated by the calculation of 1 - haematocrit.

The $Vd_{(m)}$ can then be calculated with the formula:[435]

$$Vd(m) = (1 - haematocrit)\, x \left(\frac{\left(\frac{1}{T1}\right) myo\ post\ - \left(\frac{1}{T1}\right) myo\ pre}{\left(\frac{1}{T1}\right) blood\ post - \left(\frac{1}{T1}\right) blood\ pre} \right)$$

The equilibrium technique removes uncertainties regarding contrast kinetics in heart failure or renal impairment and does not require an assumption of pseudo-equilibrium.[436] Patients were scanned according to standard protocols described above. Following cine imaging of the short axis stack, the slice felt to have the most marked region of wall motion abnormality was selected. Multiple T1 measurements were then made at this chosen short axis slice position using a multi–breath-hold, spoiled gradient echo inversion recovery (IR) sequence prior to the administration of contrast and following equilibrium with a primed infusion of contrast. Typical parameters were: slice thickness 8 mm, TR 9.8 ms, TE=4.6 ms, field of view 315×420 mm, sampled matrix size 256×130, 21 k–space lines acquired every other RR interval. T1 measurement were performed before and after equilibrium contrast in a single short axis slice in a single phase using increasing inversion times (TI) per breath-hold of 140 ms, then 200 to 800 ms in increments of 100-ms. Equilibrium was achieved through an initial bolus of Gadoteric acid (0.1 mmol/kg, Dotarem; Guerbet SA). After standard EGE and LGE imaging patients were commenced on a primed

infusion (0.0011 mmol/kg/min) for approximately 45 minutes (the majority of which the patient spent out of the scanner. Patients were then returned to the scanner and following re piloting further T1 measurements were performed in the same short axis slice. Three regions of interest were then drawn including the blood pool, the area of infarction (as noted on later LGE images) and remote myocardium. Mean signal intensities were plotted with restoration of phase and a curve-fitting technique was used to find the null point: TI′.[345]

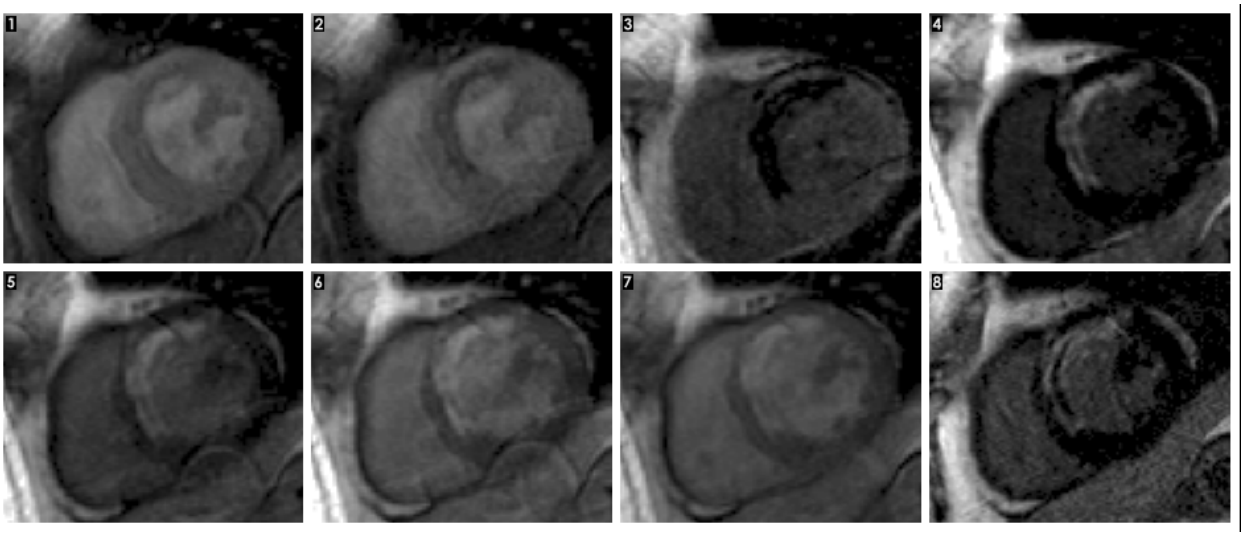

Figure 28 Figure showing an example of a post contrast T1 map (The last image is a standard LGE for reference).

5.3.2 Statistical Methods

The first set of analyses made comparisons between the various regions of interest /volume of distribution measurements. The paired t-test was used to compare between the measurements. These analyses were restricted only to patients with adequate pre and post contrast maps. Associations between the different volume distribution measurements and other study parameters were examined. All variables

were measured on continuous scales, and thus Pearson correlation was used for all analyses.

5.3.3 Results

The overwhelming majority of patients studied were male (92%). The median age was 57 years. Three patients were excluded. The first refused to remain on the infusion. The second was excluded in view of poor quality images that were felt to be un-interpretable. With the third the chosen slice for pre-contrast analysis subsequently turned out to have no evidence of LGE on later imaging and was subsequently excluded. In order to prevent this in future scans, further imaging in the form of T2* and T2-weighted imaging was applied to attempt to select an appropriate slice. Such a strategy is not full proof given the issues with T2-weighted imaging and the absence of MVO. The area of LGE correlated with the occluded artery at presentation. 6 patients presented with an occluded LAD, 6 with an occluded RCA and 1 with an occluded Circumflex. 7 patients had evidence of MVO and 6 patients with LGE without MVO (on either FPP, EGE or LGE images). The mean myocardial ECV in the remote myocardium was 0.26+/-0.08. This compares well with studies that have measured ECV in healthy patients.[346] The mean ECV in the area of infarction was (0.585+/-0.079) significantly higher than the remote myocardium ($p<0.001$). This difference remained highly significant when comparing the remote myocardium separately with areas of MVO and regions of infarction without MVO. There was however no significant difference ($p=0.3$) between the ECV measured in areas of infarction without evidence of MVO (0.598+/-0.086) and areas of MVO

(0.574+/-0.07). No correlation was found between the myocardial ECV and peak troponin or the other indices measured by CMR including ESV, EDV and EF.

Graphical illustrations of the results are shown in the next graphs. The first graph shows the mean values, along with corresponding 95% confidence intervals, whilst the second graph shows the individual data values.

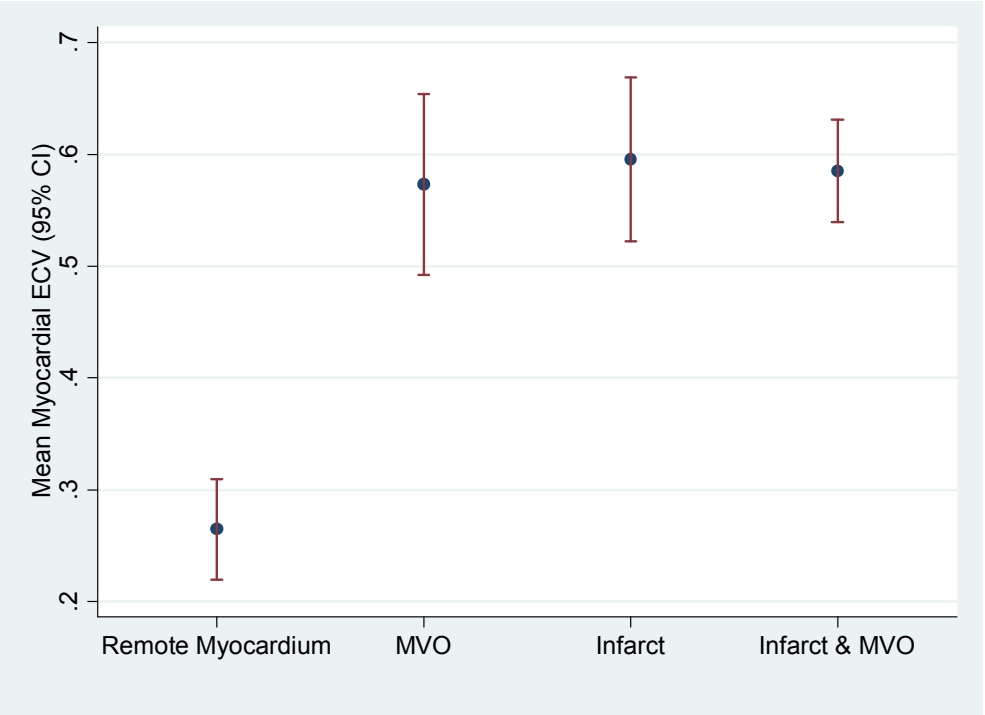

Figure 29 Box plot for measured ECV values

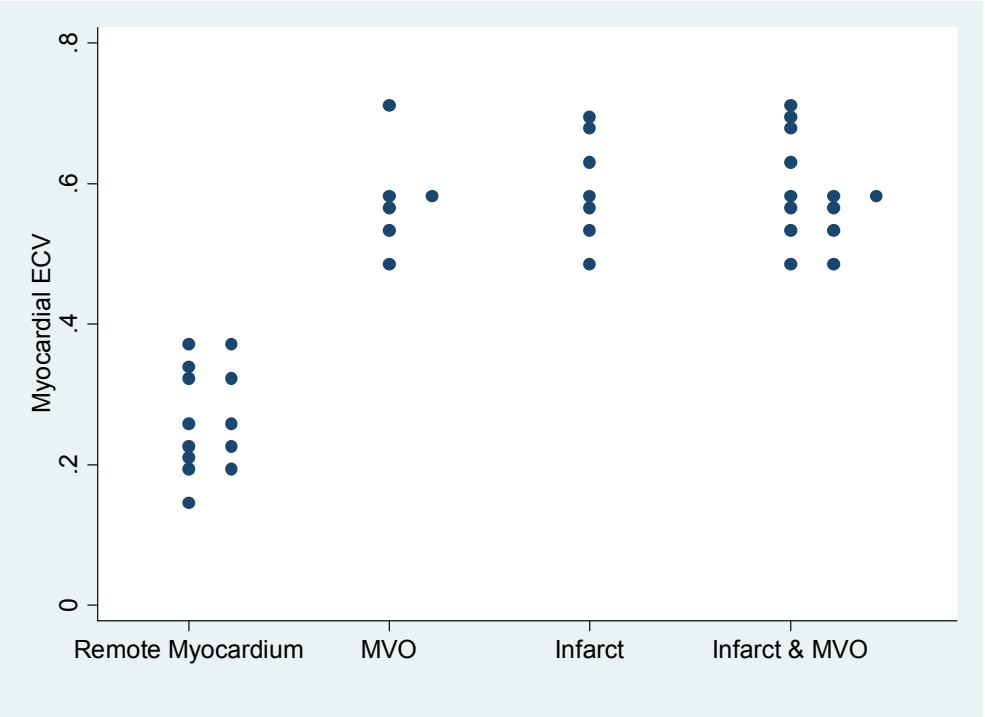

Figure 30 Dot Plot for Measured ECV values

The next table shows the correlation analyses between the ECV measurement and the other CMR indices. The correlation coefficients and associated p-values from these analyses are summarised in the next table.

Volume of Distribution (Vd)	Variable 2	Correlation Coefficient	P-value
$Vd_{(M)}$/Remote Myocardium	EDV	-0.27	0.40
	ESV	-0.34	0.29
	SV	-0.06	0.86
	EF	0.30	0.35
$Vd_{(MVO)}$/MVO	EDV	-0.54	0.5
	ESV	-0.41	0.49
	SV	-0.09	0.89
	EF	0.37	0.54
$Vd_{(I)}$/ Infarct	EDV	-0.36	0.43
	ESV	-0.41	0.36
	SV	-0.24	0.60
	EF	0.37	0.41
ECV MVO & Infarct	EDV	-0.35	0.27
	ESV	-0.34	0.28
	SV	-0.20	0.53
	EF	0.30	0.34

Table 24 Table showing correlation between Vd measurements and cardiac volumes.

5.3.4 Discussion

Our study points out the significant degree of interstitial expansion within an acute myocardial infarct as opposed to normal levels within the remote myocardium. ECV measurement furthers our ability to investigate infarction by CMR. Whilst the imaging of focal fibrosis with LGE enables the *in vivo* macroscopic identification of necrosis, it is clear that the visual (or even semi-automated) assessment of LGE is limited by the arbitrary use of thresholds. We are essentially assessing relatively crude macroscopic reflections of inherently minor microscopic pathological changes. Viable myocytes have been shown to be present within (and on the periphery of) infarct zones but may not be appreciated on clinical LGE images.[437] ECV measurement allows for *in vivo* imaging of fibrosis (both diffuse and focal) at an almost microscopic level.

The low measurements of ECV fit with those found in the hearts of otherwise healthy individuals. This is somewhat at odds with what we might have expected. It has previously been reported that compensatory contractile and structural changes take place in the remote uninvolved myocardial regions following myocardial infarction.[438] It would appear that increases in contractility in the remote myocardium is compensatory, possibly being triggered by the Frank-Starling mechanism[439] rather than processes that we may expect to lead to an increase in the ECV. Although this adds to our understanding of the state of the myocardium in the acute phase it would be informative to collate information about the remote myocardium in the chronic phase following a period of remodelling when one may expect changes in the ultrastructural architecture of the remote myocardium. In the acute setting myocardial oedema will exacerbate extracellular expansion. In order to get a clear picture of the

remote myocardium we chose an area as far away from the infarct zone as possible. It would be interesting therefore to investigate areas of myocardium that have been salvaged or on the peripheries of the infarct, in that they show evidence of oedema without infarction. One may therefore be able to quantify the incremental contribution of oedema to the ECV on top of the infarction. Further work is clearly needed to assess the contribution of the different components of infarction / reperfusion injury to interstitial expansion. It is likely that as ischaemia, infarction and remodelling are dynamic processes and that the degree of interstitial expansion will change over time through to the stage of chronic infarction following the resolution of oedema, MVO and haemorrhage but with wall thinning. We were unable to demonstrate a difference in the ECV when comparing regions of infarction and regions of MVO. This is not necessarily the case with other groups who have noted differences in the nature of the different components of reperfusion using newer T1 mapping techniques. Azarisman *et al*. reported on a pre-contrast T1 mapping study of 23 patients following AMI showing differences between infarction and MVO. They demonstrated that mean segmental T1 values differed significantly according to the extent of ischaemic injury.[440] Ugander *et al*. showed in a canine model that the salvaged myocardium has a post-contrast T1 which is approximately 50 ms less than remote myocardium suggesting a higher ECV in the area of salvaged myocardium as compared to the remote myocardium.[441] At 3T, Dall'Armellina noted how T1 values could predict improvement of segmental function at six months following an acute coronary syndrome. In a subgroup of 7 patients they demonstrated that T1 values in the MVO regions are significantly lower than in the surrounding LGE-positive myocardium.[442]

Our study has a small sample size with a preponderance of male subjects. Sado *et al*. showed that healthy females have a higher myocardial ECV than male counterparts although the reason for this is not clear.[346] The group also demonstrated in a larger cohort across the spectrum of cardiovascular disease the range of extracellular expansion with AMI showing the largest ECV. The group did not split their AMI patients into those with MVO and those without.

The equilibrium technique is a robust way of assessing the ECV within the myocardium. It does however have limitations. From the point of view of running a clinical service, the technique adds on considerable time (in the form of the primed infusion) and is likely cumbersome for the patient. We applied the technique to only one chosen short axis slice. Whilst all the slices analysed had evidence of LGE – the quantification of one slice is unlikely to be representative of the whole myocardium. This may explain the lack of correlation between other CMR indices measured.

Newer, faster sequences for T1 mapping techniques promise whole heart coverage and improved clinical utility over significantly fewer breath holds. ECV quantification by single breath-hold ShMOLLI T1 mapping can measure ECV across the spectrum of interstitial expansion. It appears to be better tolerated, more reproducible and better correlates with histology compared to the older multibreath-hold FLASH techniques.[443] The use of a bolus of contrast rather than a prolonged infusion also appears to show promising results with regard to reducing the length of the scan although such a technique may overestimate the ECV in conditions of greater interstitial expansion.[347]

The measurement of ECV by CMR shows significant differences in the myocardium beyond those detected by standard CMR technique. In the setting of AMI, the technique is able to distinguish between injured and normal myocardium with a high degree of accuracy. Further work, in larger groups is needed to further characterise the infarcted myocardium into the individual components of reperfusion injury to assess the additive value of each component.

5.4 Conclusion

We have a number of methods that we can use to detect the different components of reperfusion injury in both animals and humans. CMR stands out with its ability to assess numerous parameters with prognostic relevance in one sitting although some techniques are as yet less validated than others. The need to assess the AAR for clinical research in acute infarction is clear yet questions remain about the ability of different techniques to accurately do this. Oedema, haemorrhage and measurement of interstitial expansion measured by CMR as products of reperfusion injury and infarction offer exciting potential as imaging biomarkers in future clinical research.

6 Discussion and Conclusion

Remote ischaemic conditioning has been shown to be a safe and effective form of cardioprotection across a number of clinical and preclinical cardiovascular settings. We specifically investigated the application of remote ischaemic perconditioning in the setting of AMI as an adjunct to PPCI. PPCI remains the treatment of choice for patients with AMI. The availability of PPCI is increasing across the UK as regional programmes are rolled out to treat patients suffering with AMI as quickly as possible. The number of PPCI procedures in the UK continues to increase, yet the mortality and morbidity from AMI remains high. It is therefore clear that further measures are needed to improve patient outcome in this setting. Protection in the setting of AMI has always been more difficult to demonstrate given the inability to predict the onset of ischaemia, yet relatively small studies showing benefit (through reduction in different biochemical and imaging biomarkers) in this setting are beginning to emerge.

Our study failed to demonstrate the benefit of a perconditioning protocol using two cycles of upper limb ischaemia prior to PPCI. The reason for the failure of this protocol to show protection has been discussed above. We were able to show that two cycles of upper limb ischaemia, although ineffective in inducing cardioprotection, is a safe, well tolerated and easy to apply protocol. There were no adverse events associated with the treatment protocol. The three other groups who have investigated cardioprotective strategies using limb ischaemia have used differing protocols. This emphasises the need for further studies into cardioprotective strategies in the setting of AMI. There is a need to clarify the most efficacious

method (whether upper or lower ischaemia) of applying the cardioprotective stimulus. In the same vein, given the time imperative associated with reperfusion, it is paramount to elucidate the threshold required to induce cardioprotection. Learning the lessons from our original study above, our institute therefore proposed further studies to elucidate the most effective protocol for inducing cardioprotection in the setting of AMI and these projects are now ongoing. Such studies will not only investigate the ideal number of cycles necessary to induce protection but also whether cardioprotection in the lower limb can be induced with fewer cycles than an upper limb protocol or visa-versa.

Larger multicentre randomised controlled studies are necessary to determine the impact of RIC on short-term and long-term clinical outcomes in STEMI patients undergoing PPCI by assessing hard clinical endpoints such as cardiovascular death and hospitalisation for heart failure. The table below lists on going clinical trials (as searched for on http://clinicaltrials.gov/) that assess cardioprotective strategies in the setting of AMI using CMR endpoints. Only three trials employ RIC. Only two of the three RIC trials are using CMR endpoints. Interestingly two of the trials are adopting a novel approach of applying an RIC protocol daily for a month following PPCI.

Our study has demonstrated the importance of the selection of biomarkers and measurement technique employed as endpoints in cardiovascular trials. We have shown how the use of differing measurement techniques can lead to statistically different results in endpoints. The standardisation of measurement techniques across different groups would be an important step in enabling the comparison of

results across experiments and hence the application to real world populations. It is possible that the demonstration of a statistically significant result with a 'softer' endpoint will not show the same significance with a 'harder' endpoint. Visa versa, negative studies utilising softer endpoints may later be found to be positive with the use of harder endpoints.

Recent statements by both Working Group of Cellular Biology of the Heart of the European Society of Cardiology[113] and the NIH Consortium for Preclinical Assessment of Cardioprotective Therapies (CAESAR)[444] have emphasised the importance of study design including the choice of MI-limiting endpoint selection.

We have shown how cardiovascular magnetic resonance imaging can be harnessed for a multi parametric approach to assessing the efficacy of novel cardioprotective therapies such as RIC. Our study has confirmed the safety of CMR in a cohort of patients soon after PPCI. There were no adverse events associated with the CMR studies in our trial and was well tolerated by the majority of patients. CMR offers the ability to assess traditional endpoints such as cardiac volumes, ejection fraction and infarct size with a high degree of accuracy and reproducibility. It also allows for the identification of a range of pathologies that were initially identified on microscopic examination in preclinical research such as oedema, haemorrhage, microvascular obstruction and diffuse interstitial fibrosis. Research in AMI has taken great strides with the use of CMR and forty years after seminal work by the likes of Krug, Kloner and Reimer and Jennings who identified the products of reperfusion injury, CMR has enabled the investigation of these phenomenon in-vivo that were previously limited to only *in-vitro* / post-mortem examination. Whilst these phenomena require further

validation they appear to offer exciting novel imaging biomarkers that not only add to our ability to understand the pathophysiology of reperfusion injury but can also act as robust endpoints for clinical cardiovascular research trials.

Following on from these small studies our research institute is undertaking further research to optimise and develop CMR sequences for faster and more reproducible acquisition, determine interstitial volume in different components of reperfusion injury and remote myocardium whilst investigating the effects of RIC on the different components of the acute myocardial infarction treated with PPCI.

Remote ischaemic conditioning offers the potential to provide protection in the setting of AMI in which attention is needed to improve outcomes. The use of CMR techniques to characterise the myocardium serves to complement and advance research into novel cardioprotective therapies.

	Intervention	Primary Endpoint	CMR Endpoints	T2W	AAR
CONDI2 NCT01857414	RIC (4 x 5min cycles)	MACE	Nil	-	-
CRIC-RCT NCT01423929	RIPerC (4 x 5min cycles) Repeated for 28days	Volumes by CMR	Volumes	No	No
DREAM NCT01664611	Daily RIC	EF by CMR	Volumes IS	No	No
ASSIST-MI NCT01818960	Multivessel PCI *vs* Culprit vessel PCI	IS by CMR	Volumes IS	No	No
Vasodilators NCT01747174	IC Adenosine IC Sodium Nitroprusside	IS by 3T CMR	Volumes Salvage Haemorrhage MVO IS (48-72hrs)	Yes STIR	Yes
NITRITE-AMI NCT01584453	IC Sodium Nitrite	CK (AUC/48hrs)	Volumes Salvage MVO IS (48hrs & 6months)	Yes STIR	Yes
Allogeneic Mesenchymal Bone Marrow Cells NCT01770613	IV Allogeneic Mesenchymal Bone Marrow Cells	Safe Tolerability MACE	IS by CMR	No	No
MINIMISE STEMI NCT01882179	MRA	IS by CMR (12 weeks)	Volumes Salvage MVO IS (3days & 12wks)	Yes	Yes

PrepRAMSES **NCT01493037**	Intermittent Coronary Sinus Occlusion (PICSO)	Delivery Feasability	IS (3 & 120days)	No	No
EMBRACE **NCT01572909**	Bendavia	CK-MB (AUC/72hrs)	Volumes Salvage IS (>2SD) (4 & 30days)	Yes STIR	Yes
Postconditioning **NCT01324453**	IPost 4x30sec Inflation	IS by CMR Volumes Salvage MVO (3 days)	Volumes Salvage IS MVO (3days & 12month)	N/A	N/A
DANAMI-3 **NCT01435408**	IPost Deferred DES	MACE	IS Salvage (3 months)	N/A	Yes
BB3 **NCT01539590**	BB3	IS by CMR (30 days)	Volumes IS (1 & 6 months)	No	No
PRIME **NCT01483755**	IPost 4x60sec (Delayed)	IS by CMR (5 days)	IS	No	No
MICROS **NCT01382472**	Rosuvastatin	IS by CMR (2 days)	IS MVO (FPP,LGE) (2days & 2months)	No	No
GIPS-III **NCT01217307**	Metformin	EF by CMR (4 months)	Volumes IS (4 months)	No	No
DETO$_2$X-AMI **NCT01787110**	Oxygen	IS by CMR	IS (3-5 days)	No	No
ROSEMARY **NCT01153334**	Rosuvastatin	IS by CMR (3 - 7 days)	IS (3 - 7 days)	No	No
VELOCITY **NCT01655433**	Hypothermia	SAE	Volumes Salvage IS (3 days)	N/A	Yes
AVOID **NCT01272713**	Oxygen	TnI/CK	Salvage IS (4days & 6months)	Yes	Yes

SOCCER NCT01423929	Oxygen	Salvage by CMR (4 - 6 days)	Volumes Salvage IS MVO (4 - 6 days)	N/A	Yes
NOMI NCT01398384	Nitric Oxide (Inhaled)	IS by CMR (2 - 3 days)	Volumes Salvage IS MVO	N/A	Yes
NIAMI NCT01388504	Sodium Nitrite	IS by CMR (10 - 14 days)	Volumes IS (12days & 6 months)	N/A	Yes
DanShock NCT01633502	VAD	Death	Volumes Salvage IS (6 months)	No	Yes
ADVANCE NCT01216995	Adipose-Derived Regenerative Cells	IS by CMR (6 months)	IS	No	No
OPTIMA-STRATEGY NCT01462188	Delayed stenting	MBG	IS MVO (6 months)	No	No
THROMBUS ASPIRATION NCT01379248	Export Catheter	MVO by CMR (1 - 4 days)	MVO	No	No

Table 25 Table showing ongoing trials in acute coronary syndromes utilising CMR endpoints

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Addendum

1. Please include a description of the sample size calculation for chapter 4

   The primary endpoint of the study was MI size as measured by late gadolinium enhancement CMR. Sample size calculations were based on two previous studies that used CMR to assess the cardioprotective effect of different novel therapeutic agents. A sample size of at least 40 patients in total was determined based on the following assumptions: (a) a 20% reduction in mass of MI as measured on late enhancement imaging; (b) power of at least 80%; (c) significance declared at the two-sided 5% level.[1],[2]

2. Infarct size was assessed by a single scan. This needs to be justified, as it would have been usual to have a later scan to measure the mature infarct size.

   The primary outcome for our study was the measurement of infarct size by Cardiac MR (CMR). The scan was undertaken acutely within 72hrs of reperfusion by primary angioplasty. No follow up scan was undertaken.

   Controversy exists as to when is the ideal time to assess cardiac indices including the assessment of infarct size with late gadolinium enhancement (LGE) imaging following an acute myocardial infarction (AMI). Consistent with the original work of Reimer and Jennings, CMR studies have shown a reduction in infarct size within the first week following AMI. Contributory factors likely include the resorption of the products of reperfusion injury including myocardial oedema and haemorrhage which will cause an expansion in the interstitial volume of the myocardium and the over estimation of infarct size. On going remodelling with shrinkage and thinning of the infracted myocardium will lead to further changes in the infarct size.

   Given the change in infarct size over time the question remains of when to best utilise CMR following PPCI and how to interpret early measures of infarct size in the context of clinical trials.

   From a practical point of view the assessment of infarct size by CMR prior to discharge means the patient does not have to make a return visit. Early CMR will also allow for the visualisation of mechanical complications such as LV pseudoaneurysm, ventricular septal defect, mitral regurgitation, and ventricular thrombus. At the same time products of reperfusion injury such as myocardial oedema, microvascular obstruction and intra myocardial haemorrhage can be appreciated but are likely to have resolved at later time points when infarct size is thought to be stable.

   According to the definition published by a working group of the National Institutes of Health, a surrogate endpoint is ‘a biomarker that is intended to substitute for a clinical endpoint that is expected to predict clinical benefit, harm, or lack of benefit or harm.’ The group describe the a biomarker as ‘a characteristic that is objectively measured and evaluated as an indicator of

normal biological processes, pathogenetic processes or pharmacological responses to a therapeutic intervention.[3] According to the International Conference on Harmonisation (ICH) the strength of the evidence for surrogacy depends upon (i) the biological plausibility of the relationship, (ii) the demonstration in epidemiological studies of the prognostic value of the surrogate for the clinical outcome and (iii) evidence from clinical trials that treatment effects on the surrogate correspond to effects on the clinical outcome.[4,5]

Whilst the use of LGE to measure infarct size late after AMI fulfils these criteria to harness it as a surrogate endpoint in clinical trials, evidence for the use of measurement of infarct size acutely has been lacking.

Bodi *et al*.[6] studied 214 patients with CMR within one week of STEMI with the aim to test the prognostic value of a comprehensive CMR assessment compared with known clinical risk profiles. Median follow up was 1.5 years. They concluded that the only variables that independently predicted outcome seemed to be the number of segments with wall motion abnormality and transmural necrosis. Larose *et al.*[7] studied 103 patients following successfully reperfused acute STEMI with all patients undergoing CMR within 12 hours of PPCI. The amount of LGE was quantified using the FWHM technique. Patients were followed up for a median of 33 months and underwent repeat CMR at 6 months. The group demonstrated that quantification of LGE early after STEMI was able to predict late heart failure and adverse events beyond traditional risk factors. Eitel *et al.*[8] studied 208 patients who underwent CMR early after successful PPCI (median 3 days). The group used T2 weighted imaging along with LGE to calculated the myocardial salvage index (MSI) and demonstrated that salvage index was able to predict major adverse cardiac events including death, reinfarction and heart failure at six months. The authors therefore suggest that the ability to quantify the extent of salvage after revascularisation might serve as a novel strong endpoint for clinical trials investigating the success of reperfusion strategies. Long term follow up (median 18.5 months) of the same cohort confirmed the use of salvage index to predict long-term clinical outcome in acute reperfused STEMI.[9]

Hadamitzky *et al.*[10] have recently shown the prognostic value of LGE and MVO early (median of 4.9 days) in 281 patients after reperfused STEMI. This retrospective observational study compared CMR indices to the current reference standard of SPECT with all patients having both scans. The group showed with follow up to three years that the best predictor of adverse outcomes (All cause mortality, recurrent MI and congestive cardiac failure requiring hospitalisation) was MVO followed by infarct size assessed by CMR.

The largest body of evidence for the use of CMR early after PPCI comes from the CMR sub-study of the AIDA-STEMI trial. This sub study confirmed the findings of the main trial that there was no benefit of intracoronary versus intravenous abciximab administration on myocardial damage and/or reperfusion injury. At the same time the sub study demonstrated that infarct size determined by CMR was significantly associated with major adverse cardiac events at 12 months. CMR was carried out in 795 patients at a median of 3 days post PPCI.[11]

Further long-term data is needed to cement the use of CMR indices early after reperfused STEMI as surrogate endpoints in clinical trials. There is, however, a growing body of evidence that infarct size assessed acutely by CMR carries important prognostic information and is likely to strengthen our understanding not only of reperfusion injury but also the therapeutic effect of novel cardioprotective agents.

3. Please describe your contribution to chapter 3 in more detail

Chapter 3 was written in collaboration with Dr Andrew Flett and a version of this chapter was published in JACC Cardiovascular Imaging in 2011.[12] The macro used for analysis was created by Dr Flett. All analyses carried out using the Image J macro were carried out both by Dr Flett and myself with both readers independently analysing all scans. Statistical analysis was aided by Cono Ariti from the Department of Medical Statistics, London School of Hygiene and Tropical Medicine. I contributed towards the writing of the manuscript although the introduction, discussion and conclusions of Chapter 3 of this thesis vary from that published in JACC Cardiovascular Imaging whilst the methodology and results sections are similar.

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