Myocardial Haemorrhage Revealed by Magnetic Resonance Imaging Mapping in Acute ST-elevation Myocardial Infarction: Relationships with Heart Function and Health Outcomes

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Abstract

ST-elevation myocardial infarction (STEMI) management has evolved dramatically, with improved pharmacological treatment, rapid achievement of reperfusion with percutaneous coronary intervention (PCI) and advanced secondary prevention programmes, resulting in a decline in morbidity and mortality. However, it is well recognised that myocardial perfusion remains compromised in up to 50% of STEMI patients, despite rapid and successful mechanical revascularisation of the epicardial artery. This occurrence is called the "no-reflow" phenomenon and as a result, a substantial proportion of acute STEMI patients develop chronic cardiac failure, owing to poor microvascular function and myocardial perfusion. Although pathological and clinical observations initially seemed to support the theory that no-reflow was a consequence of microvascular obstruction (predominantly from distal embolisation of athero-thrombotic debris), irreversible microvascular injury and subsequent intramyocardial haemorrhage (IMH) are now also thought to play important factors in this process.

T2*-CMR is the reference diagnostic method for imaging myocardial haemorrhage *in-vivo,* however technical issues have limited T2* imaging in clinical practice. The largest cohort studies of myocardial haemorrhage in STEMI patients to date, have not used T2* CMR, but instead used qualitative T2-weighted imaging methods to detect haemorrhage, which are hampered by image artefact. Because of the different CMR techniques, uncertainties have arisen surrounding the pathophysiology and clinical significance of myocardial haemorrhage, and its relationships with microvascular obstruction (MVO). In some studies, myocardial haemorrhage is associated with adverse remodelling and adverse clinical outcome, however other studies have shown that myocardial haemorrhage does not have prognostic significance beyond MVO.

Recent developments in CMR imaging techniques have enabled clinically feasible, rapid parametric mapping, which allows direct determination of myocardial magnetic relaxation times (T1, T2 and T2*). These quantitative, novel mapping methods, address many of the inherent limitations associated with dark blood T2-weighted techniques, for a more objective assessment of the infarct core.

The principal aim of this thesis is to define the clinical significance of myocardial haemorrhage using quantitative CMR mapping techniques and to determine whether

detection of haemorrhage might improve risk stratification in STEMI survivors. In addition, I aim to characterise the evolution and inter-relationships between IMH and MVO in STEMI survivors to inform and implement targeted therapeutic interventions.

Methods

(1) Natural history study: We performed a single centre cohort study in 324 reperfused STEMI patients treated predominantly by emergency percutaneous coronary intervention (PCI) (*The BHF MR-MI study; Clinicaltrials.gov NCT02072850*). The index of microcirculatory resistance (IMR), a prognostically validated invasive microcirculatory biomarker, was measured acutely in the culprit coronary artery at the end of PCI using guidewire based-thermodilution. Infarct zone IMH and MVO were delineated as hypointense zones on T2* mapping CMR (T2* value <20 ms) and contrast-enhanced-CMR at 1.5 Tesla, respectively, 2 days and 6 months post-MI. T1- and T2-mapping techniques were also used to assess the infarct core and evaluate IMH.

(2) Time-course study: 30 patients underwent serial CMR at 4 time-points: < 1 day (4 to 12 hours), 3 days, 10 days and 6-7 months post-reperfusion. Adverse remodelling was defined as an increase in left ventricular end-diastolic volume (LVEDV) ≥ 20% at 6 months. Adverse cardiovascular events were pre-specified and defined according to internationally accepted criteria. All-cause death or heart failure were independently assessed during follow-up blind to other data.

(3) Randomised proof-of-concept trial: We hypothesised that brief deferral of stenting after initial reperfusion, associated with the benefits of normal coronary flow and anti-thrombotic therapies, would reduce microvascular injury and increase myocardial salvage. We implemented a randomised proof-of-concept clinical trial of deferred PCI vs. immediate stenting (NCT01717573) (Carrick et al., 2014).

In summary, the main findings of this thesis are:

- Myocardial haemorrhage (defined by T2* CMR) is an independent predictor of adverse remodelling and all cause death or heart failure in the longer-term post STEMI.

- Myocardial hemorrhage occurs in primary and secondary phases within the first 10 days post-MI and is a secondary phenomenon to the initial occurrence of microvascular obstruction.

- Myocardial haemorrhage peaked at day 3 post-MI in reperfused STEMI patients, and the temporal changes in oedema may be a secondary process.

- A hypointense infarct core on T2-mapping always occurred in the presence of microvascular obstruction and commonly in the absence of myocardial haemorrhage within 12 hours and 3 days post-MI, indicating that the presence of T2-core is more closely associated with microvascular obstruction than myocardial haemorrhage.

- Infarct core pathology revealed by T2 (ms) was independently associated with all-cause death or heart failure hospitalisation during longer term follow-up.

- Native T1 values (ms) within the infarct core were independently associated with adverse remodelling and adverse clinical outcome and had similar prognostic value when compared to microvascular obstruction.

- IMR measured in the culprit coronary artery after reperfusion is more strongly associated with myocardial haemorrhage than microvascular obstruction in STEMI survivors 2 days later.

- The proof-of-concept pilot deferred stenting trial showed that compared with standard of care with immediate stenting, brief deferral of stenting after initial reperfusion; reduced angiographic no-reflow, tended to reduce IMH and MVO, and increased myocardial salvage.

The findings of this PhD are novel and have important clinical implications. Firstly, we found that myocardial haemorrhage occurs commonly and is a biomarker for prognostication in STEMI survivors. Secondly, IMR adds early prognostic information at the time of emergency reperfusion and has potential to stratify patients at risk of IMH for more intensive therapy. Thirdly, our results confirm that infarct pathologies are evolving dynamically and potentially, may be amenable to targeted therapeutic interventions.

Finally, IMR has the potential to stratify STEMI patients acutely and deferred PCI is a simple intervention that could be practice changing, if the planned Phase 3 trial DEFER-STEMI confirms the hypothesis.

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Finally a huge debt of gratitude is owed to the patients who volunteered for this study, many of whom travelled far afield to return for a follow-up MRI scan.

Declaration

I declare that, except where reference is made to the contribution of others, this thesis is a result of my own work, written entirely by myself and has not been submitted for any other degree at the University of Glasgow or any other institution.

David Carrick, May 2015

List of Presentations Publications and Prizes

Publications

Carrick D, Berry C. Prognostic importance of myocardial infarct characteristics. Eur Heart J Cardiovasc Imaging. 2013 Apr;14(4):313-5.

Ahmed N, **Carrick D**, Layland J, Oldroyd KG, Berry C. Role of cardiac magnetic resonance imaging (MRI) in acute myocardial infarction (AMI). Heart Lung Circ. 2013 Apr;22(4):243-55.

Carrick D, Oldroyd KG, McEntegart M, Haig C, Petrie MC, Eteiba H, Hood S, Owens C, Watkins S, Layland J, Lindsay M, Peat E, Rae A, Behan M, Sood A, Hillis WS, Mordi I, Mahrous A, Ahmed N, Wilson R, Lasalle L, Généreux P, Ford I, Berry C. A Randomized Trial of Deferred Stenting versus Immediate Stenting to Prevent No-or Slow Reflow in Acute ST-Elevation Myocardial Infarction (DEFER-STEMI). J Am Coll Cardiol. 2014 May 27;63(20):2088-98.

Carrick D, Haig S, Rauhalammi S, Ahmed N, Mordi I, McEntegart M, Petrie MC, Eteiba H, Lindsay M, Watkins S, Hood S, Davie A, Mahrous A, Sattar N, Welsh P, Tzemos N, Radjenovic A, Ford I, Oldroyd KG, Berry C. Pathophysiology of left ventricular remodeling in survivors of ST-elevation myocardial infarction: inflammation, remote myocardium and prognosis. JACC Cardiovasc Imaging. 2015 April; in press.

Presentations

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- *Oral presentation, EuroPCR, Paris, May 2013*

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Prizes

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- Scottish Cardiac Society, Kerry Hogg Memorial Research Prize, Glasgow, 2013
- Society for Cardiovascular Magnetic Resonance Early Career Award Finalist, Nice 2015
- British Society of Cardiovascular Magnetic Resonance Young Investigator Award, runner-up prize, London 2015

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List of Abbreviations

AAR: Area at risk

ACD: All cause death

A.D.: Dr Andrew Davie

A.D.N.: Dr Adelle Dawson

AIC: Akaike information criterion

A.M.: Ahmed Mahrous

A.R.: Dr Alan Rae

A.S.: Dr Arvind Sood

AUC: Area-under-the-curve

BHF: British Heart Foundation

C.B.: Professor Colin Berry

CCU: Coronary care unit

CE-CMR: Contrast-enhanced cardiac magnetic resonance imaging

CFR: Coronary flow reserve

CI: Confidence interval

CMR: Cardiac magnetic resonance imaging

C.O.: Dr Colum Owens

CoV: Coefficient of variation

CRP: C-reactive protein

CTFC: Corrected TIMI frame count

D.C.: Dr David Carrick

ECG: Electrocardiogram

ECV: Extracellular volume fraction

EGE: Early gadolinium enhancement

FFR: Fractional flow reserve

GRE: Gradient-echo

H.E.: Dr Hany Eteiba

HF: Heart failure

HR: Hazard ratio

I.M.: Dr Ify Mordi

IMH: Intra-myocardial haemorrhage

IMR: Index of microcirculatory resistance

IQR: Interquartile range

IV: Intravenous

J.I.: Dr John Irving

K.G.O.: Professor Keith Oldroyd

LGE: Late gadolinium enhancement

LV: Left ventricle

LVEDV: Left ventricular end-diastolic volume

MACCE: Major adverse cardiovascular events

MACE: Major adverse cardiac event

M.B.: Dr Miles Behan

MBG: Myocardial blush grade

MI: Myocardial infarction

M.M.: Dr Margaret McEntegart

M.M.L: Dr Mitchell Lindsay

MOLLI: Modified look-locker inversion-recovery

M.P.: Dr Mark Petrie

MVO: Microvascular obstruction

MRI: Magnetic resonance imaging

N.A.: Dr Nadeem Ahmed

NPV: Negative predictive value

N.T.: Dr Niko Tzemos

NT-proBNP: N-terminal-pro-brain natriuretic peptide

OR: Odds ratio

PCI: Percutaneous coronary intervention

PPV: Positive predictive value

PSIR: Phase sensitive inversion recovery

R.N.: Dr Robin Northcote

ROC: Receiver operator characteristic

R.W.: Rebekah Wilson

SAE: Serious adverse event

SD: Standard deviation

S.H.: Dr Stuart Hood

S.H.T: Dr Stuart Hutcheson

S.R.: Mr Sam Rauhalammi

SSFP: Steady state free precession

STEMI: ST-segment elevation myocardial infarction

STIR: Short tau inversion recovery

S.W.: Dr Stuart Watkins

T1: Longitudinal relaxation time

T2: Transverse relaxation time

T2*: T2-star relaxation time

TE: Echo time

TI: Inversion time

TIMI: Thrombolysis in myocardial infarction

TR: Repetition time

TSE: Turbo spin echo

W.S.H.: Professor Stuart Hillis

1 Chapter 1: Introduction

1.1 Background

Acute ST-segment elevation myocardial infarction (STEMI) is a leading global cause of premature morbidity and mortality (Steg et al., 2012). STEMI management has evolved dramatically, now encompassing dedicated STEMI networks, potent antithrombotic drugs, rapid achievement of reperfusion, and advanced secondary prevention programmes, which has resulted in a decline in morbidity and mortality in STEMI patients (Widimsky et al., 2010, McManus et al., 2011, Jernberg et al., 2011, Fox et al., 2007). Despite this, mortality remains substantial with approximately 12% of patients dead within 6 months (Fox et al., 2006), but with higher mortality rates in high-risk patients (Fox et al., 2010), which justifies continued research to improve therapeutic strategies and outcome.

Early restoration of myocardial perfusion is the most important goal of treating patients with acute STEMI and has been shown to be effective at reducing mortality (Steg et al., 2012). However, it is well recognised that myocardial tissue perfusion remains compromised in up to 50% of STEMI patients, despite rapid restoration of epicardial patency (Hombach et al., 2005, Wu et al., 1998b, Carrick and Berry, 2013). This phenomenon, referred to as "no-reflow", is associated with larger post-infarction myocardial necrosis, which is a major determinant of morbidity and mortality in STEMI survivors.

Although pathological and clinical observations initially seemed to support the notion that "no-reflow" was the result of microvascular obstruction (MVO), assumed to be due to distal embolisation of epicardial thrombotic and atheromatous debris; irreversible microvascular injury and subsequent intramyocardial haemorrhage (IMH) are now also thought to be important factors in this process. Understanding the role of intramyocardial haemorrhage in the no-reflow phenomenon and myocardial injury, as well as its evolution and relationships with MVO, is crucial to the development of novel reperfusion therapeutic strategies to treat acute MI.

There is conflicting evidence in the literature about the clinical significance of IMH, partly because of non-standardised methods to detect IMH *in vivo*, with most studies to date not using haemorrhage sensitive sequences. Also, in acute MI patients, the inter-relationships

between myocardial haemorrhage and other infarct pathologies, such as MVO, and their temporal evolution are uncertain.

In this chapter, I shall provide background to the pathophysiology of ischaemic-reperfusion haemorrhage and its relationship with other infarct characteristics such as MVO, before providing details on the techniques for detection of IMH. Finally, I shall review the current evidence regarding the clinical significance of IMH.

1.2 Pathophysiology of myocardial haemorrhage in acute reperfused myocardial infarction

1.2.1 Reperfusion injury

Early restoration of myocardial perfusion, with either thrombolytic therapy or primary percutaneous intervention (PCI), is the main therapeutic objective in patients with ST-elevation myocardial infarction (STEMI) (Steg et al., 2012, Windecker et al., 2014). Whilst prompt reperfusion increases myocardial salvage and improves clinical outcome (Steg et al., 2012), the restoration of flow to ischaemic myocardium can induce injury, paradoxically reducing the beneficial effects of myocardial reperfusion (Yellon and Hausenloy, 2007). This phenomenon, termed "myocardial reperfusion injury" leads to four main types of cardiac dysfunction: (1) myocardial stunning, which is reversible post-ischaemic contractile dysfunction; (2) lethal reperfusion injury as an independent mediator of cardiomyocyte death; (3) microvascular obstruction (MVO) (also referred to as "no-reflow") and intramyocardial haemorrhage (IMH) and (4) reperfusion arrhythmias (Frohlich et al., 2013, Yellon and Hausenloy, 2007).

The concept of myocardial reperfusion injury as an independent mediator of cardiomyocyte death, distinct from ischaemic injury is contentious. The evidence for the existence of myocardial reperfusion injury as a distinct entity, has been indirect and relied upon the demonstration that an intervention used at the beginning of myocardial reperfusion can reduce infarct size (Yellon and Hausenloy, 2007). The postulated major components of myocardial reperfusion injury include: oxidative stress, intracellular calcium overload, rapid restoration of physiological pH within the cell and inflammation

(neutrophil infiltration). These factors mediate cardiomyocyte death by opening the mitochondrial permeability transition pore and inducing cardiomyocyte hypercontracture (Frohlich et al., 2013, Hausenloy and Yellon, 2003) (figure 1-1).

1.2.2 Pathophysiology of microvascular obstruction

Success of coronary reperfusion in STEMI is often limited by failed tissue perfusion, as might be indicated by persistent ST-elevation on the electrocardiogram. Patients with the no-reflow phenomenon have a poor clinical prognosis (Ito et al., 1996, Wu et al., 1998b, Eitel et al., 2014, van Kranenburg et al., 2014). The focus of primary PCI has extended from merely achieving epicardial artery patency towards preserving the integrity of the coronary microcirculation.

The pathophysiology of IMH is inextricably associated with MVO (Driesen et al., 2012, Fishbein et al., 1980, Kumar et al., 2011, O'Regan et al., 2010, Robbers et al., 2013, van den Bos et al., 2006). The no-reflow phenomenon was originally described in 1974 by Kloner *et al.* (Kloner et al., 1974). No-reflow or MVO is characterised by small vessel changes that prevent adequate tissue perfusion despite a revascularised and patent epicardial coronary artery (Basso and Thiene, 2006, Kloner et al., 1974). MVO is thought to be due to both luminal obstruction (i.e. neutrophil plugging, platelets, athero-thrombotic embolisation and endothelial swelling) and external compression (oedema, haemorrhage) (Kloner et al., 1980, Manciet et al., 1994). The release of inflammatory, thrombogenic and vasoconstrictor substances have also been implicated in the aetiology (Kleinbongard et al., 2011).

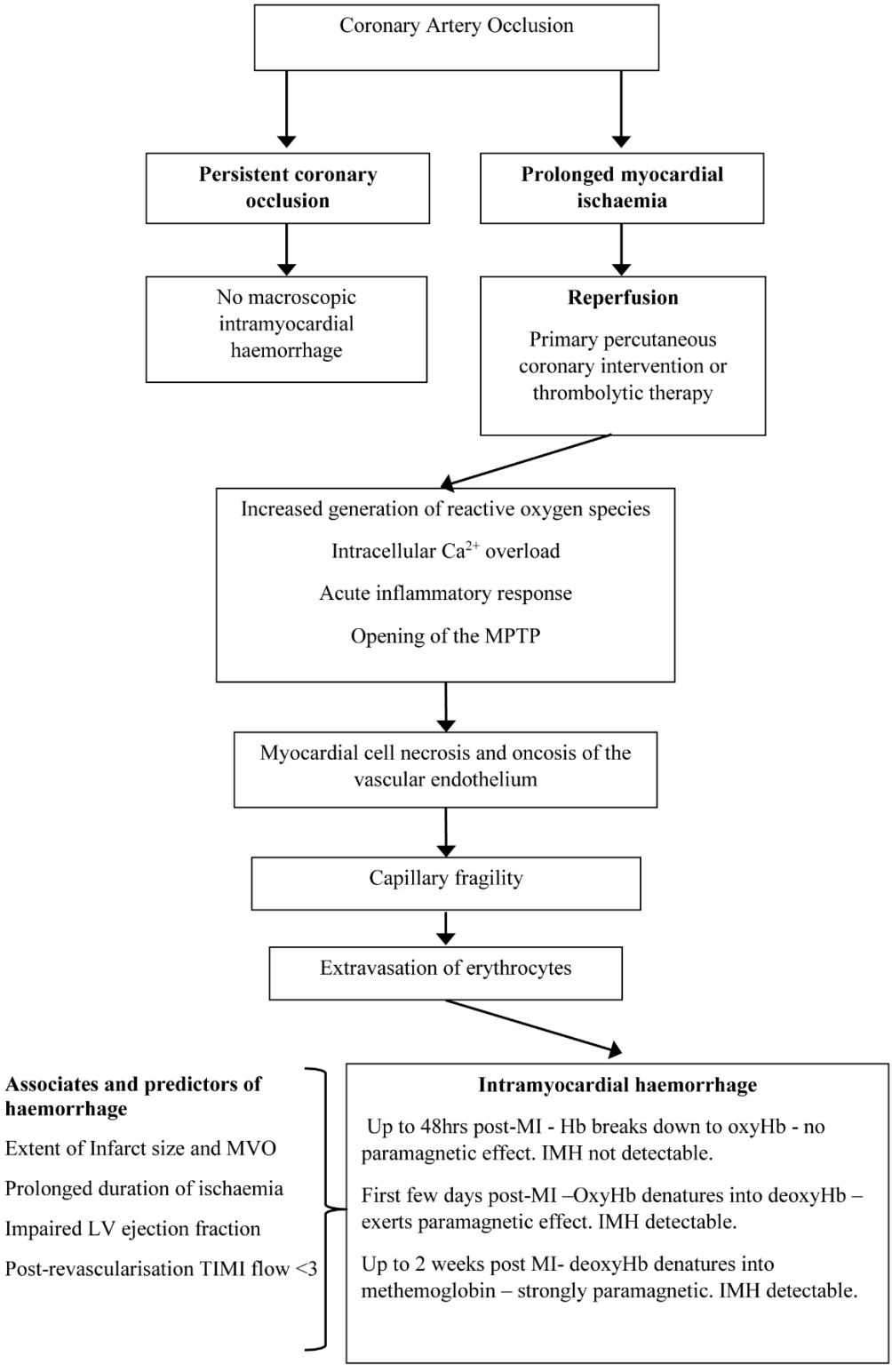
These complex pathophysiological mechanisms of MVO can lead to it being classified as “structural” or “functional” (Galiuto, 2004). Functional MVO may have potentially reversible components (e.g. microembolisation inducing microvascular spasm (Wilson et al., 1989) and extrinsic oedema) and structural MVO reflects irreversible damage to the microvascular bed with endothelial disruption.

1.2.3 Pathological basis of IMH and its anatomical distribution

Reperfusion is a prerequisite for macroscopic haemorrhage and it does not occur in the presence of a persistent coronary occlusion (Garcia-Dorado et al., 1990, Pislaru et al.,

1997). The extent of IMH after acute MI is highly correlated with infarct size and the duration of ischaemia, but interestingly has been found to be independent of thrombolytic therapy (Kloner and Alker, 1984, Basso and Thiene, 2006, Garcia-Dorado et al., 1990). The occurrence of IMH after severe microvascular injury can be explained by loss of endothelial integrity. Reperfusion of myocardium after a prolonged period of ischaemia leads to oncosis (cell death) of the vascular endothelium and thus to a breakdown of the microvascular barrier resulting in capillary fragility and extravasation of erythrocytes into the reperfused myocardium i.e. haemorrhage (Garcia-Dorado et al., 1990, Higginson et al., 1982) (figure 1-1). Due to the wavefront of myocardial necrosis (Reimer et al., 1977), the endocardium is the most vulnerable area for ischaemic damage. In animal models it is observed that IMH is confined to the region of most severe microvascular injury, in the infarct core and that it lags behind the no-reflow process (Fishbein et al., 1980, Higginson et al., 1982, Payne et al., 2011a, Robbers et al., 2013, Kumar et al., 2011). In contrast to the infarct core, no haemorrhage is seen in the border zone of the infarct (McNamara et al., 1981, Reimer et al., 1977, Robbers et al., 2013), an area which is potentially salvageable. Other recent clinical studies using T2* CMR to define haemorrhage, also observed that haemorrhage only occurred within regions of MVO, whereas MVO could occur without the presence of haemorrhage (Kali et al., 2013b, Kumar et al., 2011, Zia et al., 2012, Kidambi et al., 2013, O'Regan et al., 2010), supporting the hypothesis that MVO precedes haemorrhage.

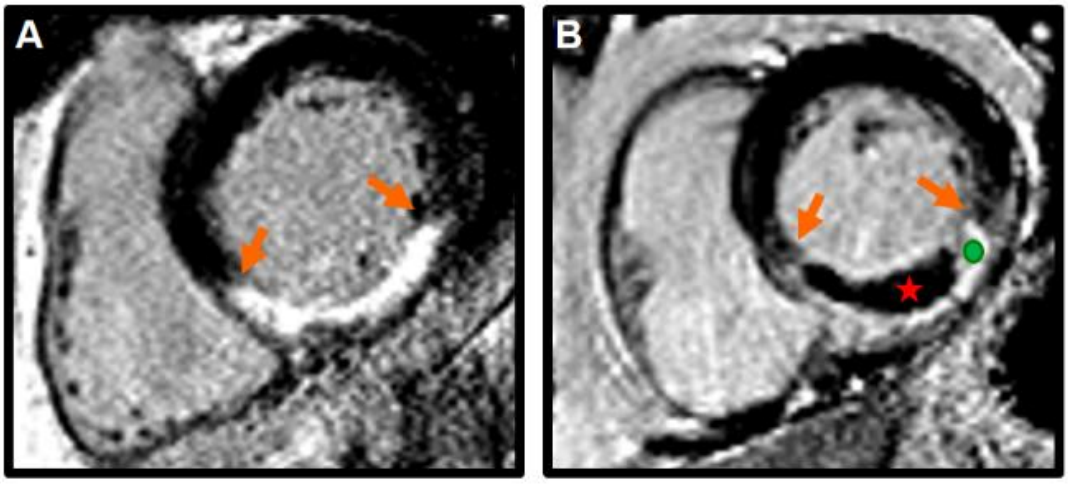
Figure 1-1 A schematic diagram of the pathophysiology of intramyocardial haemorrhage



A schematic figure of the mechanisms which underlie and predict the development of intramyocardial haemorrhage following coronary artery occlusion (MPTP, mitochondrial permeability transition pore; Hb, Haemoglobin; oxyHb, oxyhaemoglobin; deoxyHb, deoxyhaemoglobin).

Initial pathological and clinical observations seemed to support the hypothesis that distal embolisation of epicardial thrombotic and atheromatous material was the main mechanism for precipitating MVO and no-reflow. This notion led to the general assumption that the contrast-devoid core of gadolinium-enhanced CMR images represented MVO. This terminology reflects the original hypothesis of microvascular blockage as the underlying cause of no-reflow. However, in 2013, new evidence emerged from a comprehensive CMR translational study using a porcine STEMI model (Robbers et al., 2013) that indicated that the areas of the MVO and IMH largely overlap, and together indicate myocardial tissue with vascular damage and extravasation of erythrocytes, rather than microvascular occlusion. The assumption of obstruction was found to be true for the border zone of the infarcted myocardium (corresponding to the hyperenhanced region on late gadolinium enhancement), where intact microvessels were identified that contained microthrombi. Whereas the contrast-devoid core of the infarcted tissue was shown to represent IMH secondary to microvascular destruction, rather than obstruction. Figure 1-2 shows contrast-enhanced CMR images from reperfused STEMI patients (day 2 post-PCI), one with and one without MVO. Highlighted is the contrast-devoid infarct core ("microvascular destruction") and the hyperenhanced border zone in the patient with MVO.

Figure 1-2 Late gadolinium enhancement images from acute reperfused STEMI patients, 2 days post-PCI



(A) late gadolinium enhancement (LGE) image showing extensive hyperenhancement of the inferior wall, representing near transmural infarction, with no evidence of MVO. (B) LGE image again showing transmural hyperenhancement with a central contrast-devoid core (red star), representing the area known as "microvascular obstruction". The hyperenhanced border of the infarct is denoted by the green circle.

1.2.4 Summary

IMH reflects severe reperfusion injury in acute myocardial infarction involving the structural and functional integrity of the microcirculation. Aggressive antithrombotic regimens dominate pharmacological adjunctive strategies in PPCI, due to consideration of distal embolisation of thrombus fragments as the main determinant of MVO or no-reflow. Presumably, excessive antiplatelet therapy could be involved in the development/ aggravation of IMH. In support of this theory, use of glycoprotein IIb/IIIa inhibitors in addition to bivalirudin in a porcine model, showed significant increase in the frequency of IMH compared to bivalirudin alone (Buszman et al., 2012). In contrast, the administration of low-dose intracoronary streptokinase immediately following PPCI, was shown to improve microvascular function, limit infarct size and preserve LV function (Sezer et al., 2009, Sezer et al., 2007).

It remains unclear, whether haemorrhage represents an unintended consequence of evidence-based anticoagulant and antiplatelet therapies, or more likely is a manifestation of more severe MI. Based on pathological studies (Fishbein et al., 1980, Garcia-Dorado et al., 1990, Robbers et al., 2013) microvascular obstruction is a precursor to the development of intramyocardial haemorrhage, which is confined to the most severe area of microvascular injury in the infarct core. However, the inter-relationships between haemorrhage and other infarct pathologies, including MVO, and their temporal evolution in the early reperfusion period remain uncertain.

1.3 Detection of myocardial haemorrhage

1.3.1 Introduction

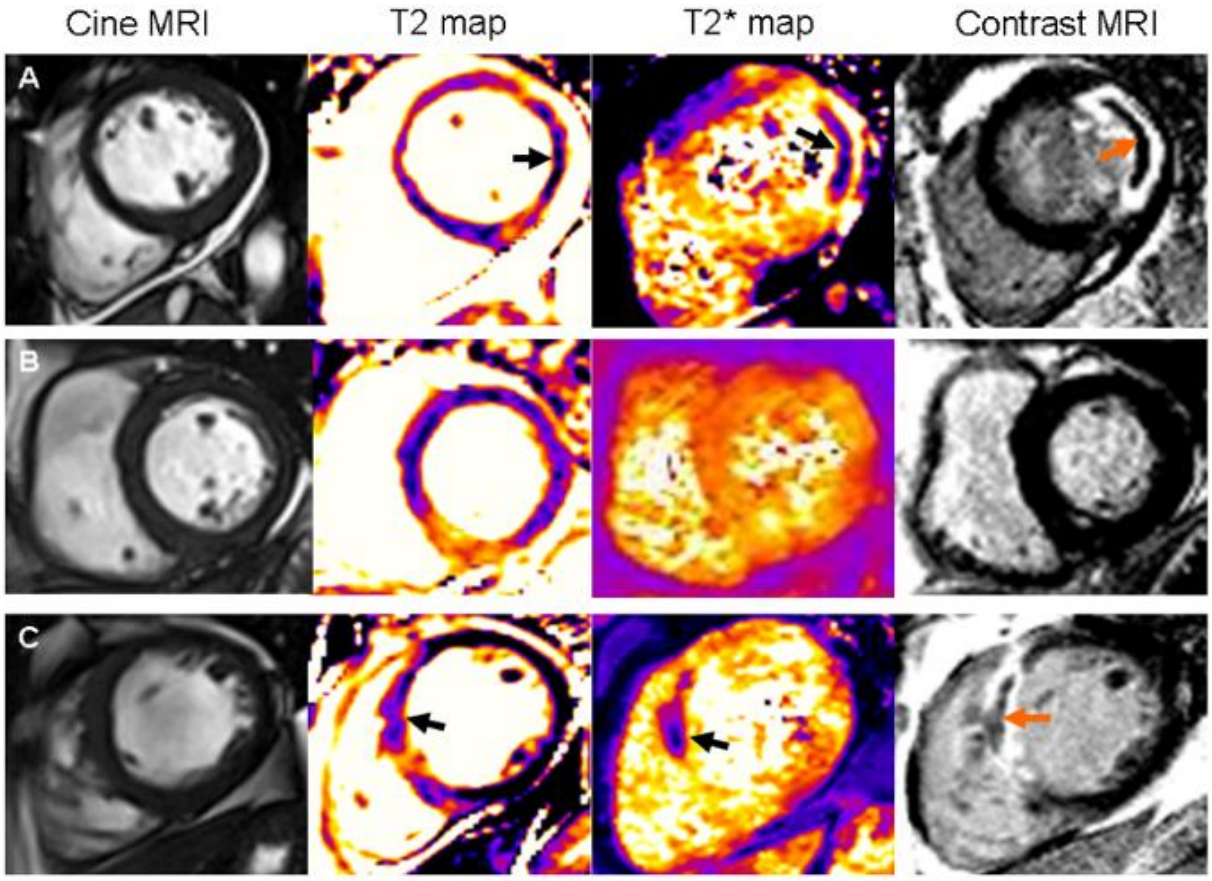
Alternative methods to CMR that can be used to assess microvascular injury and the risk of intramyocardial haemorrhage following coronary reperfusion include: persistent ST-segment elevation on the ECG (Nijveldt et al., 2008), angiographic measures (such as myocardial blush grade and corrected TIMI frame count) (Marra et al., 2010, Vicente et al., 2009), myocardial contrast echo (Wu et al., 1998a), PET, SPECT (Schofer et al., 1985), and recently multidetector cardiac CT (Gerber et al., 2006). However, these techniques assess myocardial perfusion rather than haemorrhage and CMR is considered to be the gold-standard method of assessment, which is able to specifically detect IMH and has been

validated histologically (Ghugre et al., 2011, Kumar et al., 2011, Payne et al., 2011a, Robbers et al., 2013). Haemorrhage can be detected with T1, T2 and T2* sequences and have been validated with histopathological findings. The vast majority of studies to date, both clinical and experimental, have used T2 or T2* sequences rather than T1 sequence to detect haemorrhage (Eitel et al., 2011, Ganame et al., 2009, Kali et al., 2013b, Kandler et al., 2014, Kidambi et al., 2013, Mather et al., 2011b, O'Regan et al., 2010). However, the standardised imaging method or protocol for assessment of IMH is still debated, as is the timing of image acquisition post-reperfusion.

1.3.2 MRI and the detection of haemorrhage

MRI characterises ischaemic injury based on water content and fundamental nuclear magnetic properties of tissue: longitudinal (T1) and transverse (T2) relaxation times. Ischaemia causes oedema in the area-at-risk, which increases T2/ T1 relaxation times and this is depicted by increased signal intensity (i.e. hyperenhancement) on CMR (Berry et al., 2010, Payne et al., 2011b, Aletras et al., 2006, Garcia-Dorado et al., 1993). The appearance of haemorrhage on MRI is based upon the paramagnetic effects of haemoglobin degradation products (Bradley, 1993). These breakdown products produce different signal intensities on CMR and therefore imaging of intramyocardial haemorrhage will depend on the time post-reperfusion and the particular sequence used. IMH may initially consist of oxyhaemoglobin which lacks paramagnetic properties. Subsequently, in the hours and days after reperfusion, oxyhaemoglobin is denatured to deoxyhaemoglobin, which exerts paramagnetic effects, in turn depleting T2/ T2* signal. Deoxyhaemoglobin is later converted into methaemoglobin which is strongly paramagnetic with respect to both T1- and T2-magnetisation. After about two weeks methaemoglobin is further converted into haemosiderin which is contained within macrophages and results in low T2/ T2* values (Wu, 2012). Therefore, in the early reperfusion period, myocardial haemorrhage can be visualised on CMR as a hypointense region (T2/ T2* sequences), within the infarct core, surrounded by high signal intensity from oedema (figure 1-3).

Figure 1-3 Appearances of IMH on T2 and T2* sequences



CMR images from 3 acute reperfused STEMI patients, acquired day 2 post-MI. (A) Transmural lateral infarction, with MVO present (orange arrow) on contrast-enhanced image. There is a hypointense core shown on both the T2 and T2 map, with a surrounding area of elevated signal form oedema; this area corresponds to the contrast-devoid core on the late gadolinium enhancement (LGE) image. (B) Subendocardial infero-septal infarct, with no evidence of MVO. The elevated T2 signal from oedema can be appreciated (middle left). (C) Similar to (A), images reveal a haemorrhagic antero-septal infarction. The hypointense core on T2 and T2* maps is due to the paramagnetic effects of intramyocardial haemorrhage.*

1.3.3 T2 weighted MRI

Technical differences exist between several proposed cardiac MRI techniques in detecting IMH. Most studies to date have used dark-blood inversion recovery T2-weighted MRI methods either solely (Amabile et al., 2012, Beek et al., 2010, Bekkers et al., 2010a, Eitel et al., 2011, Ganame et al., 2009, Husser et al., 2013), or in combination with T2* imaging (Ghugre et al., 2011, Kali et al., 2013b, Kandler et al., 2014, Kidambi et al., 2013, Kumar

et al., 2011, Mather et al., 2011b, O'Regan et al., 2010). Dark blood T2-weighted MRI is known to be hampered by poor image quality, partly due to the low contrast-to-noise ratio between normal and abnormal myocardium (Kellman et al., 2007, Wince and Kim, 2010). This method is also prone to artefact from motion and from blood stasis at the left ventricular wall, causing subendocardial bright rim artefacts. A further limitation is the qualitative nature of this technique, which gives rise to diagnostic uncertainty.

Bright-blood T2-weighted MRI techniques are potential alternatives to dark blood T2-weighted MRI that may offer a more robust image quality (Aletras et al., 2008, Kellman et al., 2007). A pre-clinical validation study in swine (n=15) found bright-blood T_2-weighted MRI to have a high diagnostic accuracy for IMH with positive and negative predictive values for pathological evidence of haemorrhage of 94% and 96% respectively (Payne et al., 2011a)

A hypointense core within the hyperintense infarct zone revealed by T2-weighted CMR is a common observation that in some (Basso et al., 2007, Payne et al., 2011a), but not all (Cannan et al., 2010, Jackowski et al., 2006), studies corresponds with histology evidence of myocardial haemorrhage. Therefore it has been proposed that a T2 hypointense core may simply represent the presence of MVO without IMH. This may be due to reduction in perfusion to the infarct core secondary to obstructed capillary flow, resulting in reduced oedema and thus lower T2-signal (Wu, 2012, Verhaert et al., 2011). Consequently, it does not seem possible to differentiate if a T2 hypointense core represents MVO, IMH or both, by using exclusively T2-weighted sequences.

1.3.4 T2 imaging*

T2*-weighted CMR is the reference diagnostic method for myocardial haemorrhage *in vivo* (Basso et al., 2007, Kali et al., 2013b, Kumar et al., 2011), however technical issues have limited T2* imaging in clinical practice. Despite the sensitivity of T2* imaging for iron (Carpenter et al., 2011), it is also sensitive to off-resonance artefacts arising from bulk magnetic susceptibility differences at the heart-lung interface, particularly affecting the infero-lateral LV wall. Because T2* loss from off-resonance artefacts and IMH arise from opposite sides of the myocardial wall (i.e. epicardial wall for off-resonance artefacts and endocardial wall for IMH), it is possible to carefully discriminate between haemorrhage and off-resonance artefacts.

T2* imaging is more specific in detecting myocardial haemorrhage in comparison to T2-weighted imaging because the paramagnetic effects of haemoglobin products are stronger on T2* than T2, causing greater signal depletion within the infarct core (Kali et al., 2013b, Kumar et al., 2011). Since T2 imaging is known to be highly sensitive to oedema, which commonly accompanies acute reperfusion haemorrhage, the appearance of haemorrhage may be masked or reduced on T2 images, whereas T2* is relatively insensitive to oedema (Lotan et al., 1992). The role of T2* weighted sequences for detecting IMH has been extensively validated by histology, one such study using a canine model (Kumar et al., 2011), showed a strong correlation between haemorrhage sizes assessed *in vivo* with T2* and assessed *ex vivo* by triphenyltetrazoliumchloride (TTC). Kali *et al.* (Kali et al., 2013b) more recently compared T2 versus T2* imaging for the detection of IMH in both humans and canines. They observed that T2* was more suitable for the detection and characterisation of reperfusion haemorrhage. Furthermore, a clinical study by Kandler *et al.* (Kandler et al., 2014) in 151 STEMI patients, demonstrated that hypointense core on T2* were also present on T2 imaging but not vice versa, suggesting that T2* sequences were more accurate for IMH detection than T2.

1.3.5 Quantitative T2 mapping

Recent developments in CMR imaging techniques are enabling clinically-feasible rapid parametric mapping of myocardial magnetic relaxation properties (T1, T2, and T2* relaxation times). There is a growing body of evidence for the clinical utility of quantitative assessment of relaxation times. To generate a parametric map of relaxation times, multiple images of the same region of the myocardium are acquired with different sensitivity to the parameter of interest, and the signal intensities of these images are fit to a model that describes the underlying physiology or relaxation parameters. The parametric map is an image of the fitted perfusion parameters or relaxation times. These mapping techniques hold great promise for quantitative assessment of infarct characteristics, but to date there has been few studies, with small patient numbers in this field.

Quantitative T2 mapping, which allows direct determination of T2 relaxation times, overcomes many of the inherent limitations associated with dark blood T2-weighted CMR and may allow for a more objective assessment of the infarct core (Giri et al., 2009, Verhaert et al., 2011, Ghugre et al., 2011, Zia et al., 2012, Ugander et al., 2012, Nassenstein et al., 2014, Park et al., 2013). Verhaert *et al. (Verhaert et al., 2011)*

compared quantitative T2 mapping to dark-blood T2 STIR, in a cohort of 27 acute MI patients. They showed that T2 mapping was more accurate and robust than T2 STIR in detecting myocardial oedema. However, to date there has been no experimental animal studies or clinical trials using this novel methodology to detect and quantify IMH.

1.3.6 T1 weighted sequences

In a swine model, Pedersen *et al*. (Pedersen et al., 2012) investigated whether IMH could be detected by exploiting the T1-shortening effect of methaemoglobin. They demonstrated for the first time, a higher diagnostic sensitivity and specificity of T1-weighted inversion recovery sequences (T1WIR), compared to T2 short tau inversion recovery sequences (T2-STIR) and to T2* weighted sequences, for the detection of reperfusion IMH. This was validated with pathology and T1WIR sequences depicted IMH as an area of hyperintense signal, instead of the usual hypointense signal appreciated with T2 and T2* CMR.

One small study by Dall'Armellina *et al.* (Dall'Armellina et al., 2012), including 32 STEMI patients, used T1 mapping to assess myocardial injury 24 hours post-MI. They found that MVO resulted in a hypointense core on T1 maps and showed that the T1 values in the injury zone were associated with functional recovery at 6 months. However, segments with MVO/ T1 core were excluded from this analysis and therefore no conclusions could be drawn regarding intramyocardial haemorrhage.

1.3.7 Clinical significance of myocardial haemorrhage in STEMI

The clinical significance of IMH is still unclear because of non-standardised methods to detect IMH *in vivo*. The largest cohort studies of myocardial haemorrhage in STEMI patients to date have not used T2* imaging (Amabile et al., 2012, Ganame et al., 2009, Husser et al., 2013, Robbers et al., 2013, Bekkers et al., 2010a, Beek et al., 2010), although some smaller studies have used T2* CMR (Kandler et al., 2014, Mather et al., 2011b, O'Regan et al., 2010, Kidambi et al., 2013) (table 1-1). Because of these different CMR techniques, uncertainties have arisen around the pathophysiology and clinical significance of myocardial haemorrhage, and its relationships with microvascular obstruction.

Ganame *et al.* showed, in a multivariate analysis, that intramyocardial haemorrhage detected on T2-weighted images is an independent predictor of adverse LV remodelling at

4 months, regardless of infarct size (Ganame et al., 2009). The largest prospective study to date was conducted by Eitel *et al.* and included 346 STEMI survivors (Eitel et al., 2011). They demonstrated that the presence of haemorrhage, defined as a hypointense core on T2-weighted imaging, occurred in 35% of patients and was associated with larger infarcts, greater extent of MVO, less myocardial salvage and reduced LV ejection fraction. They were the first to demonstrate the prognostic significance of a T2 hypointense core, since it was a strong predictor of adverse outcome at 6 months. In a similar sized cohort (n=304), Husser *et al*. (Husser et al., 2013) also showed that a T2-weighted hypointense core after STEMI predicted MACE and adverse remodelling. However, due to the strong interrelation with MVO, the addition of T2 imaging did not improve the predictive value of contrast-enhanced CMR.

On the contrary, two smaller studies demonstrated that T2 hypointense core was not an independent predictor of adverse LV remodelling, nor had prognostic significance beyond that of MVO (Beek et al., 2010, Bekkers et al., 2010a). Of note, the studies in table 1-1 that showed an association between IMH and adverse remodelling were those in which baseline LV ejection fraction was significantly reduced and therefore patients were more likely to remodel adversely over time.

Using a combination of T2-weighted and T2* imaging, Mather *et al.* (Mather et al., 2011b) described an association between IMH and prolonged QRS duration on signal-averaged ECG, a marker of arrhythmic risk. IMH was also observed to be associated with adverse remodelling.

In summary, previous studies examining the significance of myocardial haemorrhage post-STEMI have been limited by their qualitative techniques, not including haemorrhage sensitive sequences, together with small sample sizes (table 1-1). I propose a multi-parametric MRI protocol, including parametric mapping techniques, T2* imaging and late enhancement imaging for a comprehensive, quantitative assessment of severe reperfusion injury. The key question remains: whether or not myocardial haemorrhage has independent predictive value for adverse LV remodelling and health outcomes in the longer term, and this question can only be answered through a reasonably large cohort study.

Table 1-1 Clinical studies on the prognostic significance of CMR defined IMH

Study	MRI method for IMH detection	Imaging time post-reperfusion	Incidence IMH (%)	Findings
Ganame *et al.* (Ganame et al., 2009) (n = 98)	T2-weighted	1 week and 4 months	24	IMH is an independent predictor of adverse LV remodelling at 4 months
Bekkers *et al. (Bekkers et al., 2010a) (n = 90)*	T2-weighted	5 days and 103 days	43	Only infarct size was an independent predictor of LV remodelling
Beek *et al.* (Beek et al., 2010) (n = 45)	T2-weighted	2-9 days and 4 months	49	IMH was not an independent predictor of functional changes at follow-up, and had no prognostic value beyond MVO
O' Regan *et al.* (O'Regan et al., 2010) (n = 50)	T2*	3 days	58	IMH was closely associated with the development of MVO and was associated with infarct transmurality
Mather *et al.* (Mather et al., 2011b) (n = 48)	T2* and T2-weighted	2 days and 3 months	25	IMH is an independent predictor of adverse remodelling and associated with prolonged fQRS duration; a marker of arrhythmic risk
Eitel *et al.* (Eitel et al.,	T2-weighted	3 days	35	Presence of IMH is a strong predictor of MACE at 6 month

2011) (n = 346)				follow up
Amiable *et al.* (Amabile et al., 2012) (n = 114)	T2-weighted	4-8 days	10	IMH associated with larger infarct sizes and worse clinical outcome
Husser *et al.* (Husser et al., 2013) (n = 304)	T2-weighted	1 week and 6 months	34	IMH is a predictor of MACE and correlates strongly with MVO; the addition of T2-imaging does not improve predictive value beyond LGE CMR
Zia *et al.* (Zia et al., 2012) (n = 62)	T2* and T2-weighted	2 days, 3 weeks and 6 months	32	Presence of IMH resulted in a trend towards LV remodelling at 6 months
Kali *et al.* (Kali et al., 2013b, Kali et al., 2013a) (n = 15)	T2* and T2-weighted	3 days and 6-months	73	Patients with IMH are at risk of developing chronic iron deposits within the infarct zone, which can be a source of prolonged inflammatory burden
Kidmabi *et al.* (Kidambi et al., 2013) (n = 39)	T2* and T2-weighted	Day 2, 7, 30 and 90	36	MVO and IMH are greater independent predictors of infarct zone contractile recovery than infarct volume or transmural extent
Kandler *et al.* (Kandler et al., 2014) (n = 151)	T2* and T2-weighted	3 days	50	IMH was associated with impaired LV function and larger infarct size

1.4 Coronary pressure wire to assess microvascular dysfunction at the time of emergency PCI

1.4.1 Role of Microcirculation

Myocardial blood flow comprises the epicardial circulation, smaller branches of the coronary tree collectively referred to as the microcirculation as well as the contribution from collateral flow. An important determinant of myocardial blood flow involves the modulation of vascular tone within the microcirculation with the epicardial circulation fulfilling a conduit vessel function only (Camici and Crea, 2007). Therefore specifically focusing on the microcirculation rather than solely the epicardial circulation may provide a further path for prognostic improvements in patients with IHD, particularly in the acute setting.

1.4.2 Coronary microvascular resistance

Resistance equals pressure gradient divided by flow. In the case of the coronary circulation, the mean aortic to distal coronary back pressure gradient divided by total sinus blood flow over time yields total coronary resistance (mmhg/ml/min). Under normal conditions the epicardial arteries which run over the surface of the heart do not create any significant resistance to blood flow. Even at high flow rates only a negligible pressure difference exists between the central aorta and the most distal part of the angiographically smooth epicardial artery (Marcus et al., 1990).

Under normal physiological conditions, resistance is principally determined by vasomotor regulation of the arterioles with a diameter of less than 400 μm and flow is kept constant over a wide level of perfusion pressures by auto-regulation (Chilian, 1997, Marcus et al., 1990). Therefore, under baseline conditions the knowledge of coronary resistance reflects basal metabolism, but when auto-regulation is exhausted, as in under pharmacological hyperaemia, minimal resistance can be calculated.

1.4.3 Thermodilution

Thermodilution is a method based on the indicator dilution principle (Stewart, 1897, Meier and Zierler, 1954), which states that by injecting a certain amount of indicator into the bloodstream and measuring the concentration of indicator over time distal to the injection site, volumetric flow can be quantified. It is based on the following basic relationship: Flow = volume/mean transit time. Coronary blood flow and volume can be quantified by thermodilution. For this purpose, a coronary guidewire is used with a shaft that acts as a proximal thermistor and a combined pressure/temperature microsensor mounted close to the tip (De Bruyne et al., 2001, Pijls et al., 2002, Fearon et al., 2003b).

There are some technical limitations and practical issues however to be considered when quantifying coronary blood flow and volume using this method. First, stable positioning of the catheter is required, which might be challenging as the catheter should be kept in the same place during baseline and hyperaemic measurements (De Bruyne et al., 2001). Also it is recommended to maintain a distance of at least 6 cm between the guiding catheter and the temperature sensor at the tip in order to allow adequate mixing of blood and saline (De Bruyne et al., 2001). Disappearing of saline into side branches might lead to overestimation of blood flow. Injection of saline mainly during systole or diastole can be misleading, so measurements should be performed in triplicate to correct for this error which can occur especially in patients with bradycardia (De Bruyne et al., 2001). Also the volume of injected saline itself should be sufficiently low as to not influence coronary blood flow.

1.4.4 Thermodilution derived coronary flow reserve

Human coronary artery blood flow can increase three to fourfold in response to ischaemia (Vassalli and Hess, 1998). This property has been formalised as a concept known as the coronary flow reserve (CFR). CFR is defined as the ratio of hyperaemic flow to baseline flow. In determining CFR, pharmacological agents such as adenosine and papaverine are used to induce maximal hyperaemia (Vassalli and Hess, 1998). CFR was originally developed to assess the severity of epicardial coronary disease and used as a marker of PCI adequacy. However, the use of CFR in this manner has been shown to be somewhat limited as CFR not only assesses the epicardial compartment but also reflects microvascular function (Knaapen et al., 2009).

Under resting conditions, coronary blood flow is dependent on determinants of myocardial oxygen demand, namely heart rate, contractility and ventricular load. However when myocardial oxygen demand is constant and within the realms of autoregulation, coronary blood-flow is independent of perfusion pressure. During maximal hyperaemia when resistance vessels are maximally dilated, blood flow is no longer autoregulated and varies linearly with perfusion pressure. Thus, as CFR is the ratio of peak hyperaemic-to-resting flow it is affected by determinants of resting coronary blood flow a fact that can affect the reproducibility of the ratio (Ng et al., 2006). It is also influenced significantly by epicardial vessel disease and is therefore an invalid method of quantifying microvascular disease in the majority of patients presenting to cardiology practices.

The validity of the thermodilution principle to demonstrate CFR on a commercially available guide-wire (PressureWire 3, Radi Medical Systems) was first validated in an experimental dog model by De Bruyne et al in 2001 (De Bruyne et al., 2001). In this in-vitro model, absolute flow was compared with the inverse mean transit time (1/Tmn) of a thermodilution curve obtained after a bolus of 3ml saline at room temperature. A very close correlation ($r>0.95$) was found between absolute flow and 1/Tmn. In the canine model a significant correlation was found between CFR, calculated from the ratio of hyperaemic to resting flow velocities using a Doppler flow wire, and the CFR derived from the ratio of resting to hyperaemic Tmn ($r=0.76; p = <0.001$)

Therefore thermodilution derived CRF is calculated as follows (De Bruyne et al., 2001). Coronary flow reserve

(CFR) is defined as the ratio of peak hyperaemic to resting flow (F) (Gould et al., 1974).

1. CFR = F at hyperaemia / F at rest

Flow is the ratio of the volume (V) divided by Tmn. Thus, CFR can be expressed as follows.

2. CFR = (V/Tmn) at hyperaemia / (V/Tmn) at rest

Assuming the epicardial volume (V) remains unchanged, CFR can be calculated as follows.

3. CFR = Tmn at rest / at hyperaemia

However, CFR has two well-recognised limitations when used to assess coronary microvascular resistance: (1) its inability to distinguish between relative epicardial and microvascular contribution to total resistance (Kern, 2000) and (2) its dependence upon haemodynamic factors (ie, blood pressure, heart rate, etc) (Ng et al., 2006), affecting its reproducibility negatively. To circumvent these limitations, a novel index of microvascular resistance (IMR) was proposed by Fearon *et al*. (Fearon et al., 2003a).

1.4.5 Index of Microvascular Resistance

The index of microvascular resistance (IMR) is a well-validated method of measuring microvascular resistance and function. Like thermodilution derived CFR, it utilises the temperature pressure sensitive guidewire to simultaneously measure transit time and distal coronary pressure during maximal hyperaemia. IMR is the product of these two parameters.

IMR = Pd x Tmn

A fundamental assumption in the theory is that Tmn is inversely proportional to hyperaemic blood flow. Because

F = V/ Tmn

Where flow (F) equals the ratio of epicardial vascular volume (V) and mean transit time (Tmn). Because true microvascular resistance (TMR) equals distal perfusion pressure divided by flow:

TMR = Pd/F

And because the vascular volume (V) may be assumed to remain constant at maximal hyperaemia by combining equations 1 and 2, can be derived that TMR is proportional to the product of distal coronary pressure and Tmn:

TMR = Pd.Tmn

Using an open chest swine model, Fearon et al compared true microvascular resistance (TMR) defined at the distal LAD pressure divided by absolute coronary flow derived from the use of an ultrasonic flow probe, with IMR (Fearon et al., 2003a) with and without microvascular dysfunction. Microvascular dysfunction was artificially generated using microspheres injected into the coronary arteries. The investigators found a reasonable correlation between IMR and TMR ($r = 0.54$ $p<0.0001$. The investigators also found that IMR increased with worsening microvascular function independent of epicardial stenosis. Work performed by Arnoudse et al confirmed the utility of IMR in assessing microvascular resistance. Using an in-vitro model the group demonstrated an excellent correlation between TMR and IMR (r2 0.94) and suggested that the tool is independent of epicardial stenosis (Aarnoudse et al., 2004b).

In the presence of severe stenoses, some investigators have shown that neglecting the increasing contribution of collateral flow may lead to an overestimation of microvascular resistance (Aarnoudse et al., 2004a, Fearon et al., 2004). Thus in the presence of epicardial stenosis the IMR equation is modified as follows:

IMR = PaTmn(Pd-Pw/Pa-Pw)

where Pa is the hyperemic aortic pressure, Pd the hyperemic distal pressure beyond a stenosis and Pw the coronary wedge pressure defined as the mean distal coronary pressure in the target vessel during balloon occlusion (Aarnoudse et al., 2004a).

IMR has been compared with CFR in patients without significant epicardial stenosis. In a small study, Ng and colleagues compared CFR and IMR in the same patients under different haemodynamic conditions (Ng et al., 2006). The group examined the effects of increasing heart rate with the use of temporary pacing, afterload reduction with the use of sodium nitroprusside and increasing contractility with the use of dobutamine. Compared with CFR, IMR demonstrated superior reproducibility and significantly less influence of the underlying haemodynamic environment (Ng et al., 2006). Accordingly IMR appears to be a specific measure of microvascular integrity independent of epicardial stenosis and is thus perhaps more applicable to the general catheter laboratory population of patients with IHD.

1.4.6 IMR in STEMI

In a few clinical studies with patients with acute myocardial infarction, IMR measured directly after primary PCI was linked to myocardial damage after MI (Fearon et al., 2008, Lim et al., 2009, McGeoch et al., 2010). IMR predicted infarct size, as shown by biomarkers including peak CK (Fearon et al., 2008, Lim et al., 2009) and troponin I (McGeoch et al., 2010), as well as severity of myocardial infarction, as shown by infarct volume (McGeoch et al., 2010) and LV function (Fearon et al., 2008, Lim et al., 2009, McGeoch et al., 2010). Moreover, McGeoch *et al*. (McGeoch et al., 2010) showed that patients who displayed MVO, as measured by contrast-enhanced CMR, had higher IMR after PCI than patients in whom MVO did not occur.

A recent study by Payne *et al*. (Payne et al., 2012) confirmed the ability of IMR to predict infarct size, LV function and MVO determined by contrast-enhanced CMR at 3-month follow-up in a larger group of patients with acute STEMI. IMR predicted myocardial salvage was linked to the presence of MVO as well as the extent of MVO as assessed by CMR.

In a landmark recent study, 253 acute STEMI patients with IMR >40 had a higher rate of the primary endpoint of death or rehospitalisation for heart failure at one year than patients with an IMR ≤40 (17.1% versus 6.6%; p=0.027) (Fearon et al., 2013). This marker has the potential to identify those patients who may require closer follow-up and more aggressive medical management to avoid poorer outcome.

The potential advantages of IMR are that it is readily available in the catheterisation lab, specific for the microvasculature, quantitative and reproducible, in addition to being a predictor of outcomes in STEMI. Therefore, the direct quantitative measure of microvascular function during primary PCI, in combination with MRI, may potentially enhance our understanding of severe ischaemic-reperfusion injury and provide a more comprehensive assessment for the characterisation of myocardial haemorrhage.

1.5 Aims of thesis

The hypothesis that this thesis will test is whether the detection of intramyocardial haemorrhage by CMR has prognostic value in STEMI survivors. This thesis will also examine the evolution and inter-relationships between myocardial haemorrhage and microvascular obstruction, to inform and implement novel therapeutic interventions. Furthermore, it will evaluate the prognostic significance of infarct core tissue characteristics using parametric mapping techniques in survivors of acute STEMI. A full outline of the aims of this thesis are stated below:

- To detect myocardial haemorrhage using T2* mapping in a large relatively unselected STEMI population and re-evaluate its clinical associates and prognostic significance.

- To study the time-course of myocardial haemorrhage evolution with serial CMR after reperfusion and assess the temporal relationships between myocardial haemorrhage versus microvascular obstruction.

- To assess the clinical associates and prognostic significance of a T2 hypointense core, revealed by T2 mapping and determine the relationship with myocardial haemorrhage revealed by T2* CMR.

- To assess the evolution of myocardial haemorrhage and oedema using quantitative T2 and T2* methods at serial time-points post-MI.

- To assess the clinical associates and prognostic significance of native T1 measured within the hypointense infarct core, using T1 mapping and determine the relationship with myocardial haemorrhage revealed by T2* CMR.

- To assess whether IMR measured at the end of primary percutaneous coronary intervention (PPCI) might discriminate STEMI patients at risk of subsequent intramyocardial haemorrhage.

- To assess whether during primary PCI, brief deferral of stenting after initial coronary reperfusion, might reduce the occurrence of angiographic no-reflow,

microvascular obstruction and myocardial haemorrhage, compared to usual care with immediate stenting.

2 Chapter 2: Methods

2.1 Preamble

In this section I will describe the CMR and statistical methods used that were common to studies in this thesis. Detailed study specific methods are described within the relevant chapters.

2.2 Setting and recruitment

This prospective CMR cohort study was conducted at the Golden Jubilee National Hospital, Clydebank between 11 May 2011 and 22 November 2012. This hospital is a regional referral centre for primary and rescue percutaneous coronary intervention (PCI). The hospital provides clinical services for a population of 2.2 million. A screening log was recorded, including patients who did not participate in the cohort study. Near consecutive patients were screened and consented predominantly by myself or by the consultant cardiologist on-call who was performing the PCI if I was not in the hospital. All consultant interventional cardiologists in our institution (KGO, CB, AD, SH, MP, MM, MML, CO, SW, HE, AR, RN) enrolled patients into the study and recruitment took place round the clock. A breakdown of study recruitment by day of week, to the nearest hour and proportion undertaken out of hours is shown in figures 2-1, 2-2 and 2-3. The study was publically registered (ClinicalTrials.gov identifier is NCT02072850).

Figure 2-1 Study recruitment broken down by day of the week

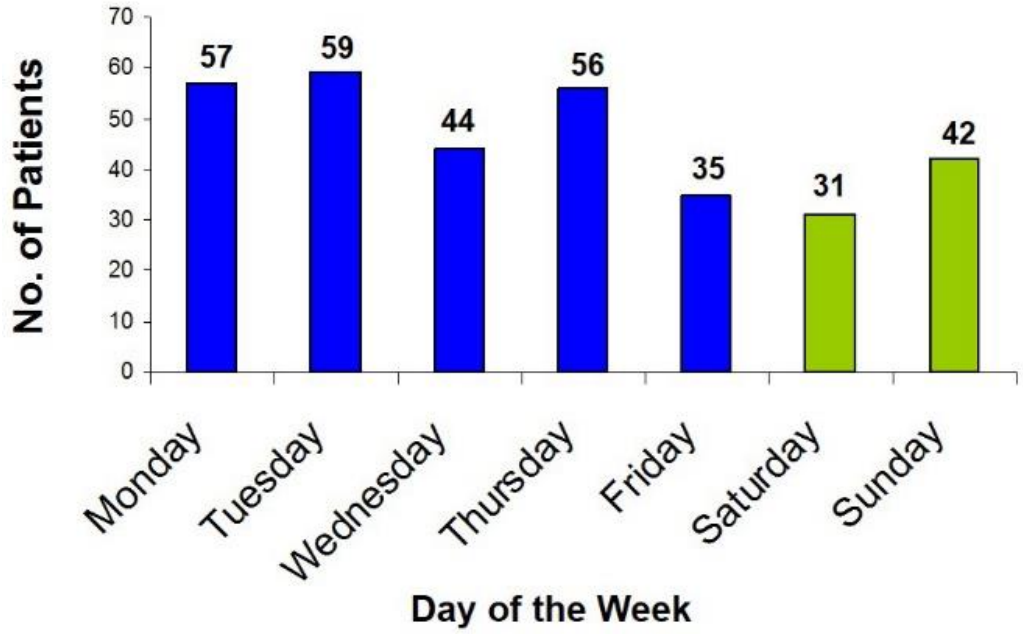


Figure 2-2 Study recruitment broken down to the nearest hour

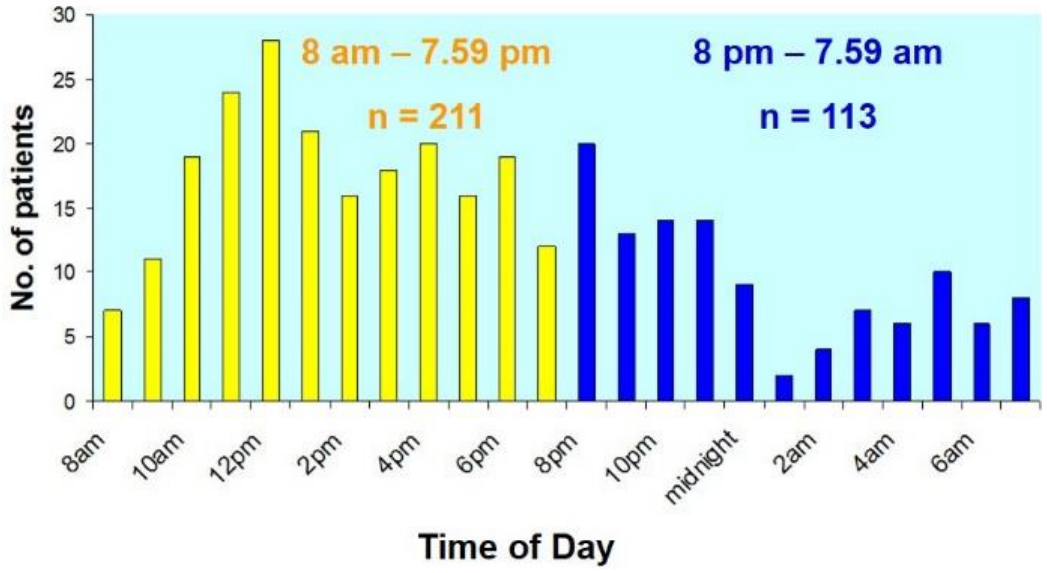
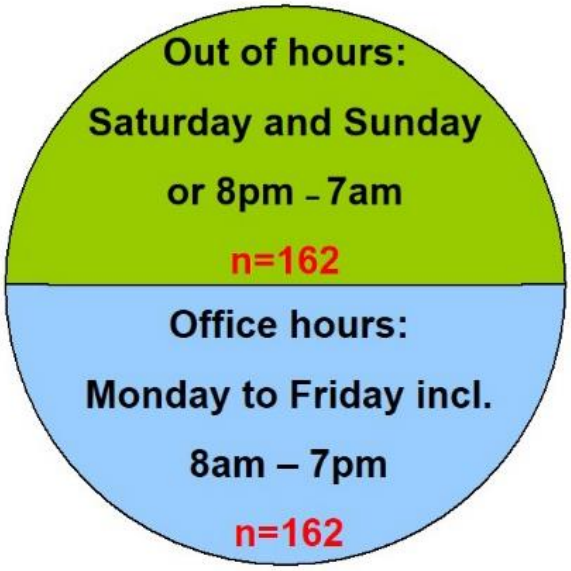


Figure 2-3 Proportion of study recruitment undertaken outside "normal working hours"



2.3 Study populations

2.3.1 STEMI patients

Three hundred and seventy two STEMI patients provided written informed consent. The eligibility criteria included an indication for primary PCI or thrombolysis for acute STEMI due to a history of symptoms consistent with acute myocardial ischemia and with supporting changes on the electrocardiogram (ECG) (i.e. ST-segment elevation or new left bundle-branch block) (O'Gara et al., 2013). Exclusion criteria represented standard contra-

indications to contrast CMR, including a pacemaker and an estimated glomerular filtration rate < 30 ml/min/1.73 m^2.

2.3.2 Serial imaging sub-study

Thirty STEMI patients underwent serial CMR in order to characterise the evolution of myocardial haemorrhage by T2 and T2* quantification, and evaluate the temporal relationship with microvascular obstruction. Each patient was imaged at 4 time points, with the identical imaging protocol that was used in the main STEMI cohort: 4 to 12 hours, 3 days, 10 days and 6-7 months post-reperfusion.

2.3.3 Deferred stenting sub-study

One hundred and one STEMI patients were enrolled in a prospective randomised controlled parallel group trial of deferred PCI versus immediate stenting during primary PCI, to assess whether deferred stenting might reduce no-reflow and salvage myocardium in primary PCI. This trial was a proof-of-concept trial nested in the main prospective cohort study. This will be described in detail in Chapter 9.

2.3.4 Healthy volunteers

CMR was also performed in 50 healthy volunteers of similar age and gender in order to obtain local reference values for myocardial T1, T2 and T2*. Patients and healthy volunteers underwent the same imaging protocol except that healthy volunteers <45 years did not receive gadolinium contrast. Thirteen healthy volunteers were aged <45 years and therefore did not receive contrast.

2.4 Coronary angiogram acquisition and analyses

Coronary angiograms were acquired during usual care with cardiac catheter laboratory X-ray (Innova®) and IT equipment (Centricity®) made by GE Healthcare. The coronary anatomy and disease characteristics of study participants were described based on the clinical reports of the attending cardiologist.

2.4.1 TIMI coronary flow grade

Coronary blood flow can be described based on the visual assessment of coronary blood flow revealed by contrast injection into the coronary arteries (TIMI-Study-Group, 1985) (table 2-1).

Table 2-1 TIMI coronary flow grade definition.

TIMI Coronary Flow Grade	
0	No flow
1	Minimal flow past obstruction
2	Slow (but complete) filling and slow clearance
3	Normal flow and clearance

2.5 Percutaneous coronary intervention

During ambulance transfer to the hospital, the patients received 300 mg of aspirin, 600 mg of clopidogrel and 5000 IU of unfractionated heparin (O'Gara et al., 2013, Steg et al., 2012). The initial primary PCI procedure was performed using radial artery access. A conventional approach to primary PCI was adopted in line with usual care in our hospital (O'Gara et al., 2013, Steg et al., 2012). Conventional bare metal and drug eluting stents were used in line with guideline recommendations and clinical judgement. The standard transcatheter approach for reperfusion involves minimal intervention with aspiration thrombectomy only or minimal balloon angioplasty (e.g. a compliant balloon sized according to the reference vessel diameter and inflated at 4-6 atmospheres 1-2 times). During PCI, glycoprotein IIbIIIa inhibitor therapy was initiated with high dose tirofiban (25 μg/kg/bolus) followed by an intravenous infusion of 0.15 μg/kg/min for 12 hours, according to clinical judgement and indications for bail-out therapy (O'Gara et al., 2013, Steg et al., 2012). No reflow was treated according to contemporary standards of care with intra-coronary nitrate (i.e. 200 μg) and adenosine (i.e. 30 – 60 μg) (O'Gara et al., 2013, Steg et al., 2012), as clinically appropriate. In patients with multivessel coronary disease, multivessel PCI was not recommended, in line with clinical guidelines (O'Gara et al., 2013, Steg et al., 2012). The subsequent management of these patients was symptom-guided.

2.6 Invasive coronary physiology protocol

In this study, a commercially available 0.014 inch floppy pressure guide wire (PressureWire-6, St Jude Medical) was used with the appropriate software and interface (Radi-Analyzer, RADI Medical Systems). This wire has a micro-sensor at a location 3 cm from the floppy tip, which enables simultaneous recording of coronary pressure (referred to as Pd i.e. distal coronary pressure) measurement as well as temperature measurement at the location of that sensor, with an accuracy of 0.02°C. The shaft of this wire, acting as additional electric resistance, can be used as a second thermistor, providing the input signal at the coronary ostium of any fluid injection with a temperature is different from blood. All signals can be displayed and recorded on the commercially available analyser for future off-line analysis.

2.6.1 Pressure wire preparation

In the majority of cases the coronary pressure/temperature sensitive guidewire was used as the primary guide-wire. The guide-wire was calibrated outside the body, equalised within the guide catheter, with the pressure sensor positioned at the ostium of the guide catheter, and then advanced into the distal segment of the culprit artery. Meticulous attention was taken to ensure appropriate catheter engagement and only guide catheters without side holes were used in the study. Study numbers were entered into the analyser unit so that de-identified recording and storage of coronary physiological data could be made.

2.6.2 Hyperaemic agent used during pressure wire studies

In this study we used intravenous adenosine administered through an anti-cubital vein at a dose of 140/micrograms/kg/min via a volume controlled infusion pump. The patient was then assessed for a symptomatic and physiological response to adenosine. When this occurred the physiological measurements were taken. This route of adenosine administration was chosen to allow a hyperaemic “steady state” to occur allowing time to take the appropriate measurements. Prior to administration of the intravenous infusion we administered a bolus of intracoronary glyceryl tri-nitrate into the coronary artery to minimise the potential effects of arterial spasm on the readings.

Central venous infusion of adenosine through the femoral vein has been the gold standard method of hyperaemia induction (Pijls et al., 1996). However, it requires an additional procedure for femoral vein access and is less convenient to use during transradial coronary catheterisation procedures, which is the preferred method of arterial access, particularly in the emergency setting.

In a study by Seo et al, involving 71 patients, no difference was found in the hyperaemic efficiency of intravenous administration of adenosine via the forearm compared to the femoral vein (Seo et al., 2012). There was no difference between the hyperaemic mean transit time and index of microcirculatory resistance between the two routes of adenosine infusion suggesting minimal resistance and thus maximal hyperaemic response was achieved with both forms of intravenous access. Consistent with these findings, De Bruyne et al showed that the hyperaemic efficacy of adenosine was similar between central and peripheral venous infusions, and increasing the dose to >140 µg/kg/min did not improve the vasodilatory action of adenosine (De Bruyne et al., 2003).

2.6.3 Thermodilution curves

Thermodilution curves were generated following stenting in the infarct related artery. We used guide catheters without side holes to allow accurate delivery of a saline bolus into the coronary ostium. Care was also taken to flush the catheter with saline thereby removing contrast that could potentially interfere with the measurements. Thermodilution curves in the culprit coronary artery were obtained by short manual injections of 3 ml of room temperature saline. The average of the 3 values was taken as the mean baseline transit time (TmnBase), shown previously to be inversely proportional to coronary blood flow (De Bruyne et al., 2001). Care was taken to obtain consistent and reproducible curves with superimposed envelopes as shown in figure 2-4. We were also careful not to advance or pull back the wire during these measurements. Following the attainment of hyperemia the injection protocol was repeated to derive the hyperemic transit time (TmnHyp). Simultaneous measurement of mean aortic and distal coronary pressure under resting and hyperaemic conditions was also undertaken (PaBase, PdBase, PaHyp PdHyp respectively).

2.6.4 Measurement of coronary wedge pressure (Pw)

This was measured by balloon inflation within the area of the stented segment. When the delivery balloon was inflated, occluding antegrade flow, mean pressure distal to the stenosis was recorded as the coronary wedge pressure (Pw) in millimetres of mercury (mmHg).

2.6.5 Coronary Flow Reserve

Coronary Flow Reserve was also measured during physiological assessment and was defined as: CFR = TmnBase / TmnHyp.

CFR interrogates both the epicardial and microvascular compartments providing a comprehensive assessment.

2.6.6 Measurement of the index of microcirculatory resistance (IMR)

IMR is calculated as the product of simultaneously measured distal coronary pressure (Pd) and thermodilution-derived mean transit time (Tmn) of a bolus of Saline injected at room temperature into the coronary artery during maximal hyperaemia induced by continuous intravenous infusion of adenosine (140mcg/kg/min) (figure 2-4). The inverse of Tmn has been shown to correlate with absolute coronary blood flow. In the absence of any stenosis in the epicardial artery IMR is equal to Pd x Tmn at maximal hyperaemia. When an epicardial stenosis is present accurate determination of IMR requires knowledge of coronary wedge pressure and can be represented by the following equation:

$$IMR = Pa.Tmn\ [(Pd-Pw)/(Pa-Pw)]$$

where Pa represents the aortic pressure measured by the guiding catheter and Pw is the coronary wedge pressure measured by the pressure wire during balloon occlusion as described previously.

Figure 2-4 Typical output from RADI analyser with thermodilution curves at rest and hyperaemia, with simultaneous Pa and Pd recording

Thermodilution curves under resting conditions (blue lines) and during hyperaemia induced by intravenous adenosine infusion (yellow lines).

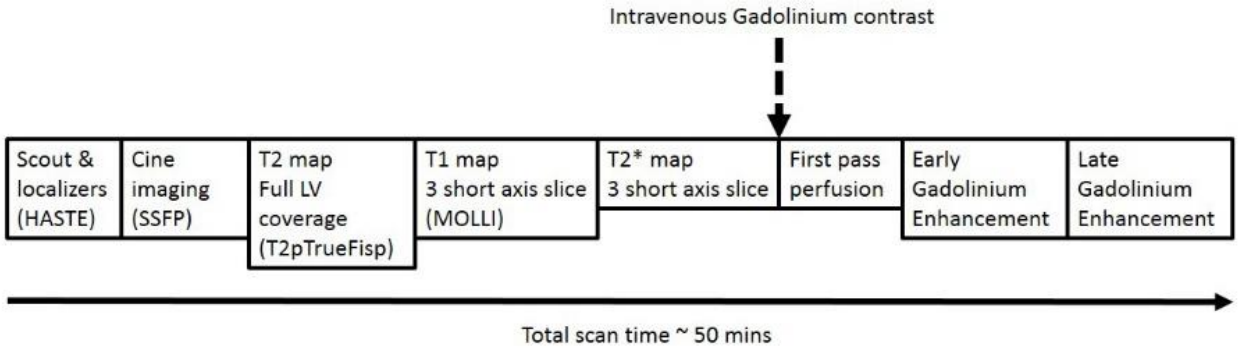
2.7 Consent and ethics

The study was approved by the West of Scotland Research Ethics Committee, reference 10-S0703-28 (appendix 1) and informed consent was obtained from each patient. Given the time constraints involved recruiting patients in the acute setting, verbal consent was obtained once the patient was stabilised following emergency PCI, prior to conducting pressure wire studies. Once the patient was transferred to the coronary care unit and before CMR, patients were given a detailed patient information sheet (appendix 2). After a period for consideration and discussion, patients were asked to sign the consent form (appendix 3).

2.8 CMR acquisition

CMR was performed on a Siemens MAGNETOM Avanto (Erlangen, Germany) 1.5-Tesla scanner with a 12-element phased array cardiac surface coil (Kramer et al., 2013, Moon et al., 2013). All patients underwent a standard protocol (including the healthy volunteer and serial imaging sub-study patients) and had ECG monitoring during the CMR exam. The imaging protocol included cine CMR with steady-state free precession (SSFP), T2*-mapping, T2-mapping (Giri et al., 2009, Verhaert et al., 2011), native T1 mapping (Messroghli et al., 2007a, Messroghli et al., 2004) and delayed-enhancement phase-sensitive inversion-recovery pulse sequences (Kellman et al., 2002). The scan acquisitions were spatially co-registered and also included different slice orientations to enhance diagnostic confidence. The standard CMR protocol is outlined in figure 2-5.

Figure 2-5 Standard CMR protocol

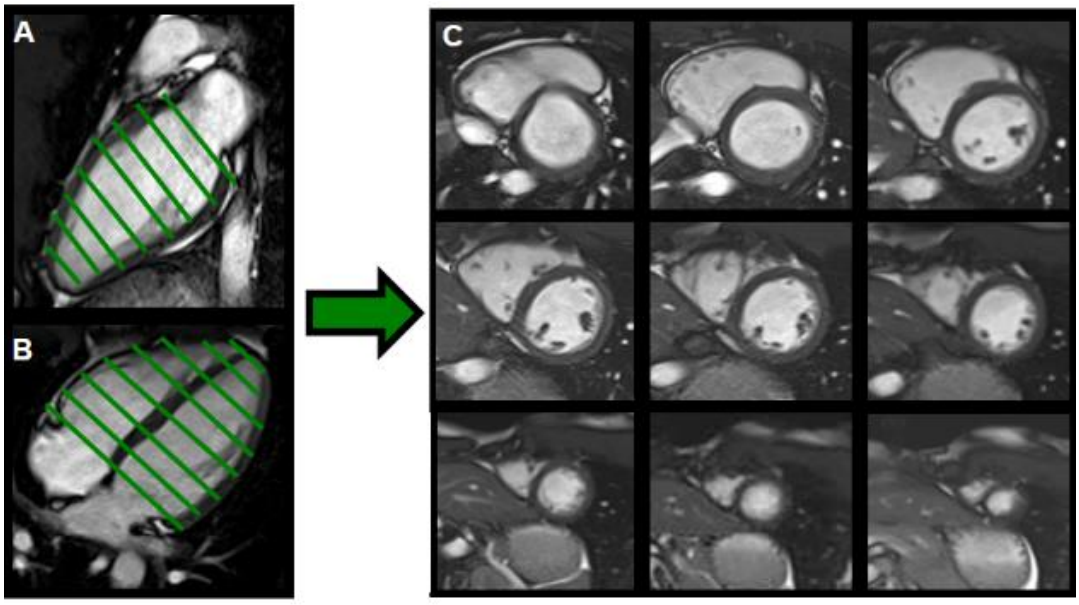


I shall now proceed to describe each sequence in more detail.

2.8.1 Steady-state free precession (SSFP) – "Cine" imaging

SSFP cine imaging (using multi-slice single-shot breath-hold true fast imaging – trueFISP) was used for functional assessment and a short-axis cine stack of the LV from base to apex was acquired, consisting of 7 mm thick slices, with a 3-mm interslice gap. Cine images were also obtained in the 3-chamber, horizontal long-axis and vertical long-axis planes (figure 2-6). Typical sequence parameters were TE 1.2 ms, TR 3.3 ms, flip angle 70º, field of view 340x27 0mm, matrix size 256x180 .

Figure 2-6 Cine imaging - assessment of LV function and volumes



(A) Vertical long axis (VLA) cine, (B) horizontal long axis (HLA) cine, and (C) short axis cine stack. Using the diastolic frame from both the HLA and VLA cines, parallel cines are acquired until the whole ventricle has been covered, at 1cm intervals (e.g. a slice thickness of 7mm with a 3mm interslice gap). This forms the basis for LV volumetric, mass and quantitative functional analysis.

2.8.2 T2 mapping*

T2*-maps were obtained using an investigational prototype T2* map sequence (multi-echo GRE) acquired in 3 short-axis slices (basal, mid and apical). Typical imaging parameters were: bandwidth ~814 (x8) Hz/pixel; flip angle 18°; matrix 256x115; spatial resolution 2.6 x 1.6 x 10 mm; slice thickness 8 mm. Eight echoes were acquired with TE ranging from 1.9 to 15.7 ms.

2.8.3 T2 mapping

T2 maps were acquired in contiguous short axis slices covering the whole ventricle, using an investigational prototype T2-prepared (T2P) TrueFisp sequence (Giri et al., 2009, Verhaert et al., 2011). T2 maps were generated from 3 images, acquired during a single breath hold, at different echo times (0 ms, 24 ms, 55 ms) represented by the T2P. A motion correction algorithm was applied, that minimised the misregistration between individual T2P images (Xue et al., 2008). Finally, the three acquired images were automatically

processed to fit the T2 decay curve at each pixel to generate a T2 map. Typical imaging parameters were: bandwidth ~947 Hz/pixel; flip angle 70°; T2 preparations: 0 ms, 24 ms, and 55 ms respectively; matrix 160 x 105 pixels; spatial resolution 2.6 x 2.1 x 8.0 mm; slice thickness 8 mm.

2.8.4 T1 mapping

Native T1 maps were acquired in 3 short-axial slices (basal, mid and apical), using an optimised modified look-locker inversion-recovery (MOLLI) investigational prototype sequence (Messroghli et al., 2007a, Messroghli et al., 2004) before contrast administration. MOLLI merges images from three consecutive inversion-recovery experiments into one data set. After the inversion pulse, the recovery of longitudinal magnetization is repetitively sampled. Three experiments are combined within one protocol (3 (3) 3 (3) 5) (Messroghli et al., 2007a), with slightly shifted inversion times (TI), thereby enabling a pixel-based T1 quantification in the myocardium, during one breath-hold. Between experiments, there are a number of heart cycles without any data acquisition to allow for full recovery of magnetization. The following typical parameters were common to all acquired studies: band width ~1090 Hz/pixel; flip angle 35°; echo time (TE) 1.1 ms; T1 of first experiment 100 ms; TI increment 80 ms; matrix 192 x 124 pixels; spatial resolution 2.1 x 1.1 x 8.0 mm; slice thickness 8 mm; scan time 17 heartbeats.

2.8.5 Early and late gadolinium enhancement

Early gadolinium enhancement (EGE) imaging was acquired 1, 3, 5 and 7 minutes post-contrast injection using a TrueFISP readout and fixed inversion time (TI) of 440 ms. Late gadolinium enhancement images covering the entire LV were acquired 10-15 minutes after IV injection of 0.15 mmol/kg of gadoterate meglumine (Gd^{2+}-DOTA, Dotarem, Guebert S.A., Villepinte, France) using segmented phase-sensitive inversion recovery (PSIR) turbo fast low-angle shot sequence (Kellman et al., 2002). Typical imaging parameters were: matrix = 192 x 256, flip angle = 25°, TE = 3.36 ms, bandwidth = 130 Hz/pixel, echo spacing = 8.7ms and trigger pulse = 2. The voxel size was 1.8 x 1.3 x 8 mm^3. A Look-Locker scout scan was undertaken to determine the inversion times associated with optimal nulling of the myocardial signal. The inversion times were in the range 240 to 350 ms.

2.9 Healthy volunteers

The purpose of including healthy volunteers was to collect normative reference data for myocardial native T1, T2 and T2* in individuals without prior cardiovascular disease or therapy and who were reasonably representative of the population of individuals from whom the STEMI patients were drawn. Second, the reference native T1, T2 and T2* values were required to be measured on the same CMR scanner and with the same protocol that was used for the STEMI patients including during the same time-period.

Healthy volunteers were invited to participate by placing adverts in public buildings (e.g. hospital, University) and through personal contacts of the researchers. Matching and selection of the healthy volunteers was done by the researchers in order to reflect the age and gender distribution of the STEMI patients. The healthy volunteers were resident in the same catchment area as the STEMI population. Fifty age- and gender-matched healthy volunteers who had a normal ECG and no prior history of cardiovascular disease or therapy underwent CMR during the same time period. The absence of late gadolinium enhancement (myocardial fibrosis or scar) was determined qualitatively by visual assessment, and the absence of late gadolinium enhancement was a requirement for inclusion of the volunteer in this analysis.

The rationale for including healthy volunteers in this study is as follows: firstly, native T1, T2 and T2* values may vary between CMR scanners and so a local reference range is recommended in CMR guidelines (Kramer et al., 2013, Moon et al., 2013). Secondly, native T1, T2 and T2* values may vary spatially in the heart. Myocardial native T1, T2 and T2* values were regionally segmented in regions-of-interest and summarised according to the AHA model (Cerqueira et al., 2002).

Two of the healthy volunteers had abnormal CMR scans with evidence of hyper-enhancement on contrast imaging and were therefore excluded from analyses. One volunteer had evidence of recent myocardial infarction, with a clinical history consistent with crescendo angina and was referred for coronary angiography and subsequent PCI. The other volunteer had CMR findings consistent with dilated cardiomyopathy and was referred to the appropriate cardiology services for assessment.

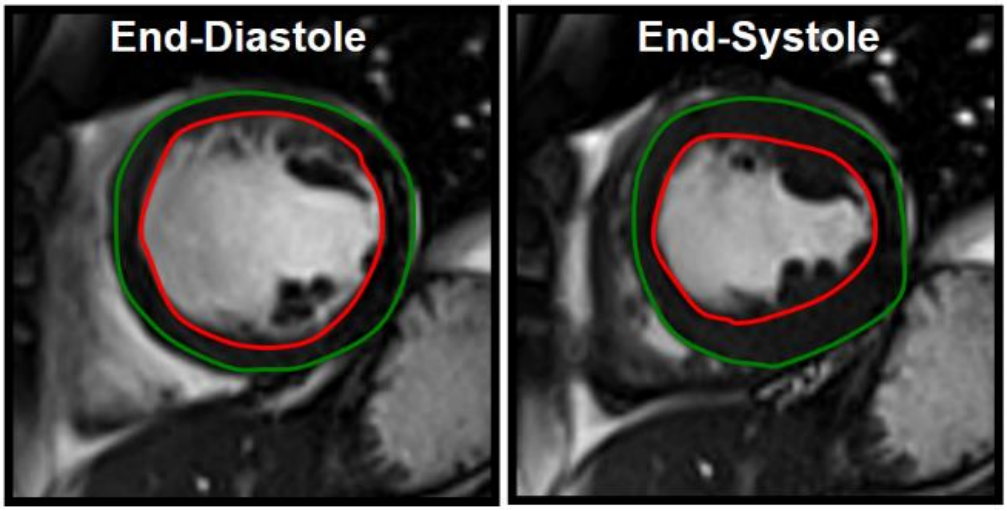
2.10 CMR image analyses

The images were analysed on a Siemens work-station by observers with at least 2 years CMR experience. D.C. did the majority of analysis, N.A. and I.M. assisted with infarct analysis, S.R. assisted with healthy volunteer sub-group analysis. LV dimensions, volumes and ejection fraction were quantified using computer assisted planimetry (syngo MR®, Siemens Healthcare, Erlangen, Germany). All scan acquisitions were spatially co-registered. The late gadolinium enhancement images were analysed for infarct size and microvascular obstruction by observers (N.A., I.M.) who were blinded to all of the other data. In healthy volunteers, the absence of LGE was determined qualitatively by visual assessment.

2.10.1 Assessment of LV mass and function

Post-processing was performed using commercially-available Argus software (Siemens, Erlangen). The number of slices required to cover the LV in end-diastole and end-systole varied from scan to scan dependent on the long axis diameter of the LV. End-systole was chosen as the point where the total LV blood pool was smallest and end-diastole as the point where it was largest. The most basal LV slice at both end-systole and end-diastole was defined as that in which the blood pool was surrounded by 50% or more of ventricular myocardium. Once selected, the endocardial and epicardial borders were manually outlined. Papillary muscles were included as part of the myocardial blood pool. Following tracing of the myocardial borders for each slice, an automated calculation was carried out by the Argus software to obtain left ventricular mass, end-systolic volume, end-diastolic volume and left ventricular ejection fraction using a sum of discs method (figure 2-7).

Figure 2-7 Manual planimetry for LV volume and mass analysis



Endocardial (red boarder) and epicardial (green boarder) boarders are traced at end-systole and end-diastole. LV volumes are calculated by contouring the endocardial boarders, then summing the endocardial surface area measured from each slice, multiplied by the inter-slice distance.

2.10.2 T1, T2 and T2 - standardised measurements in myocardial regions of interest*

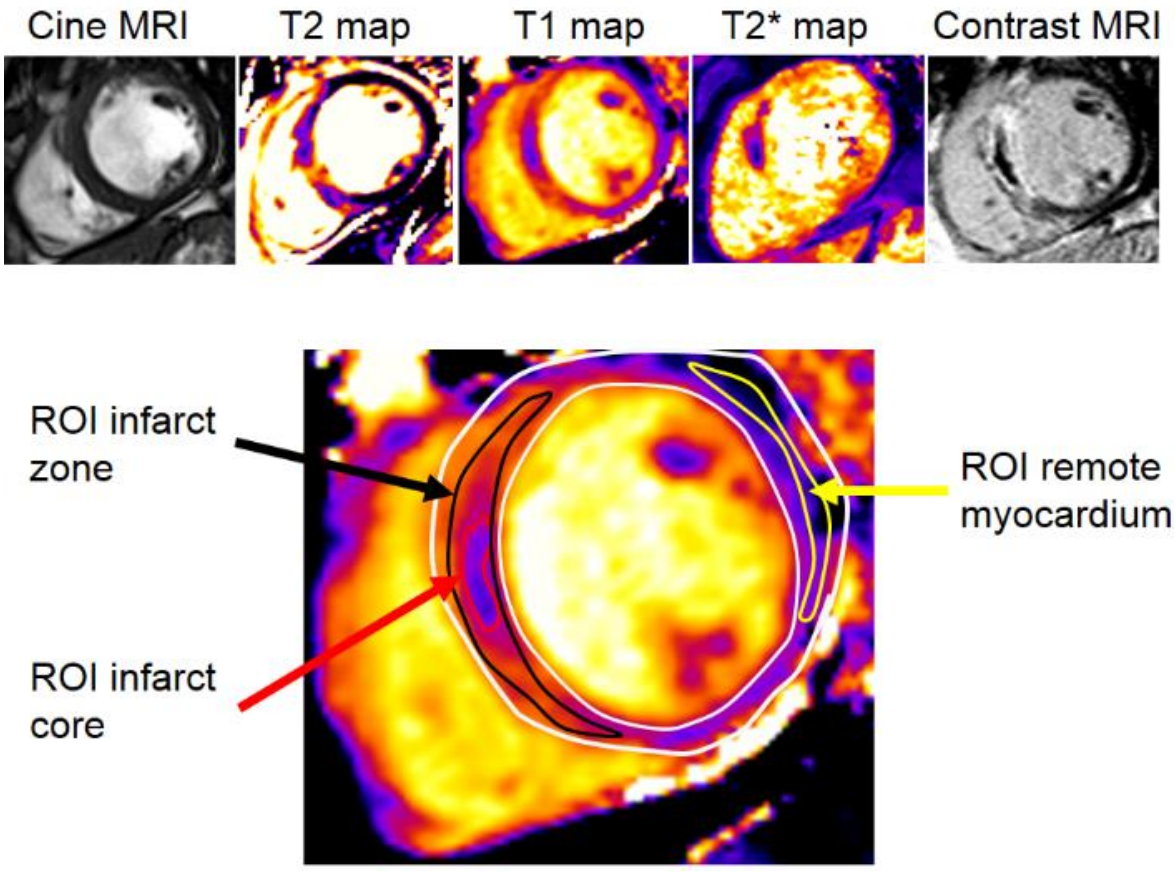
LV contours were delineated with computer assisted planimetry on the raw T2* image and the last corresponding T2 raw image, with echo time of 55 ms (Wassmuth et al., 2013). Contours were then copied onto the colour-encoded spatially co-registered maps and corrected when necessary by consulting the SSFP cine images. Apical segments were not included because of partial volume effects. Particular care was taken to delineate regions of interest with adequate margins of separation from tissue interfaces prone to partial volume averaging such as between myocardium and blood. T1 maps were analysed in a similar fashion to the T2 and T2* maps, with delineation of the LV contours on the raw images and then copying these onto the colour-encoded maps, in keeping with contemporary guidelines (Moon et al., 2013). Each T1/ T2/ T2* map image was assessed for the presence of artefacts relating to susceptibility effects or cardio-respiratory motion. Each map was evaluated against the original images. When artefacts occurred, the affected segments were not included in the analysis.

T1/ T2/ T2* values were segmented spatially and regions of interest were defined as (1) remote myocardium, (2) injured myocardium and (3) infarct core. The regions-of-interest were planimetered to include the entire area of interest with distinct margins of separation from tissue interfaces to exclude partial volume averaging (figure 2-8). The remote myocardial region-of-interest was defined as myocardium 180º from the affected zone with no visible evidence of infarction, oedema or wall motion abnormalities (assessed by inspecting corresponding contrast enhanced T1-weighted, T2-weighted and cine images, respectively). The infarct zone region-of-interest was defined as myocardium with pixel values (T2) >2 SD from remote myocardium on T2-weighted CMR (Giri et al., 2009, Verhaert et al., 2011). The infarct core was defined as an area in the centre of the infarct territory having a mean T1/ T2/ T2* value of at least 2 standard deviations (SDs) below the T1/ T2/ T2* value of the periphery of the area-at-risk. The assessment of T1/ T2/ T2* maps and adjudication (present/absent) of a hypointense core was performed independently by D.C.

Healthy volunteers

In healthy volunteers, the mid-ventricular T1-, T2- and T2*- colour-encoded maps were segmented into 6 equal segments, using the anterior right ventricular-left ventricular insertion point as the reference point (Cerqueira et al., 2002). T1, T2 and T2* were measured in each of these segments, and regions of interest were planimetered distinct and separate from blood-pool and tissue interfaces. These segmental values were also averaged to provide one value per subject. Results are presented as average values for segments and slices.

Figure 2-8 Quantitative parametric mapping analysis with defined regions-of-interest



MRI images from an acute reperfused antero-septal STEMI patient, acquired on day 2 post-MI. The regions-of-interest (ROI) were planimetered to include the entire area of interest with distinct margins of separation from tissue interfaces, to directly measure T1, T2 and T2 relaxation times.*

2.10.3 Myocardial haemorrhage

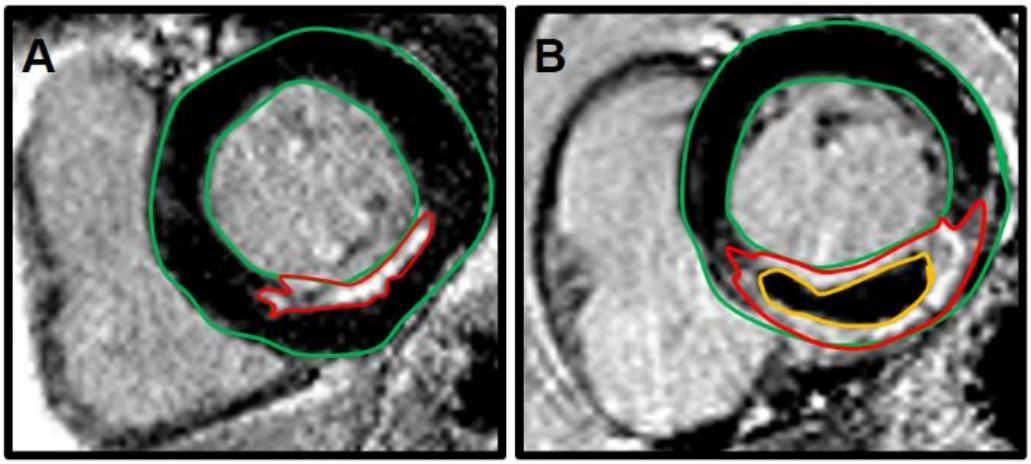
On the T2* maps, a region of reduced signal intensity within the infarcted area, with a T2* value of <20 ms (Ghugre et al., 2011, Kandler et al., 2014, O'Regan et al., 2010, Anderson et al., 2001), was considered to confirm the presence of myocardial haemorrhage.

2.10.4 Infarct definition and size

The presence of acute infarction was established based on abnormalities in cine wall motion, rest first-pass myocardial perfusion, and delayed-enhancement imaging in two imaging planes. In addition, supporting changes on the electrocardiogram and coronary angiogram were also required. Acute infarction was considered present only if late gadolinium enhancement was confirmed on both the axial and long axis acquisitions. The

myocardial mass of late gadolinium (grams) was quantified using computer assisted planimetry and the territory of infarction was delineated using a signal intensity threshold of >5 standard deviations above a remote reference region and expressed as a percentage of total LV mass (Kramer et al., 2013, Flett et al., 2011) (figure 2-9). Infarct regions with evidence of microvascular obstruction were included within the infarct area and the extent of microvascular obstruction LV ventricular mass was also measured.

Figure 2-9 Quantification of infarct size on late gadolinium enhancement imaging



(A) Subendocardial infarct with no MVO - manual planimetry of the region of hyperenhancement (red) with contrast thresholding set to signal intensity 5SD above remote myocardium. This was repeated in every short-axis left ventricular slice and the infarct size was expressed as a percentage of total LV mass ((area of LGE/total myocardial area) x 100). (B) Transmural infarct with MVO (orange) – area of MVO was included within infarct area and the extent of MVO was also measured.

2.10.5 Microvascular obstruction

Microvascular obstruction was defined as a dark zone on EGE imaging 1, 3, 5 and 7 minutes post-contrast injection that remained present within an area of LGE at 15 minutes (figure 2-9). Identification of microvascular obstruction was performed independently by I.M. and N.A.

2.10.6 Area-at-risk

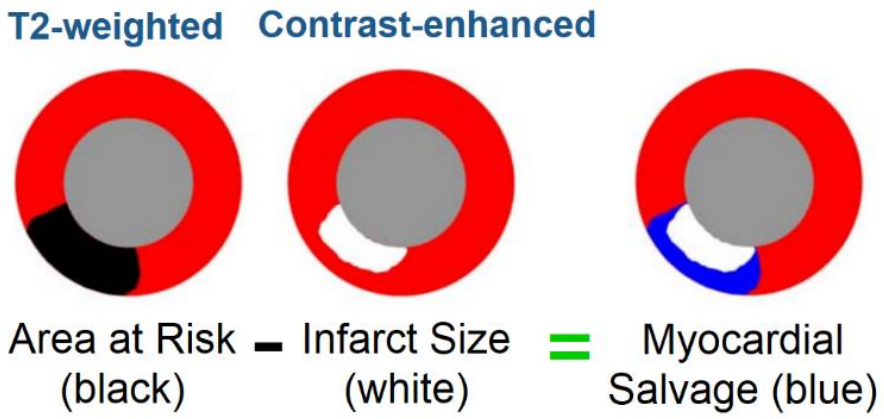
Area-at-risk was defined as LV myocardium with pixel values (T2) >2 standard deviations from remote myocardium (Eitel et al., 2010, Dall'Armellina et al., 2011, Ugander et al.,

2012, Berry et al., 2010, Payne et al., 2011b, Payne et al., 2012). In order to assess the area-at-risk the epicardial and endocardial contours on the last corresponding T2-weighted raw image with an echo time of 55 ms were planimetered (Giri et al., 2009, Wassmuth et al., 2013). Contours were then copied to the computed T2 map and corrected when necessary by consulting the SSFP cine images.

2.10.7 Myocardial salvage

Myocardial salvage was calculated by subtraction of percent infarct size from percent area-at-risk (Eitel et al., 2010, Berry et al., 2010, Payne et al., 2011b, Payne et al., 2012) (figure 2-10). The myocardial salvage index was calculated by dividing the myocardial salvage area by the initial area-at-risk.

Figure 2-10 Myocardial salvage analysis



2.10.8 Adverse remodelling

A number of definitions of remodelling have been used in different studies, including increase in LV end-diastolic and end-systolic volumes (White et al., 1987). In this thesis we defined adverse remodelling as an increase in LV end-diastolic volume ≥ 20% at 6 months from baseline, in keeping with recent studies in the same field (van Kranenburg et al., 2014, Hombach et al., 2005).

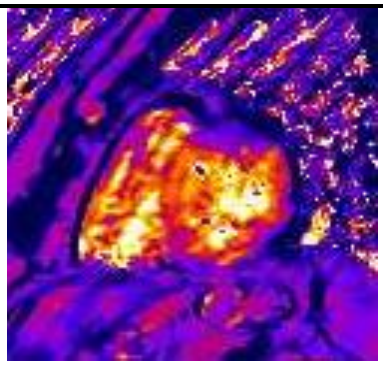
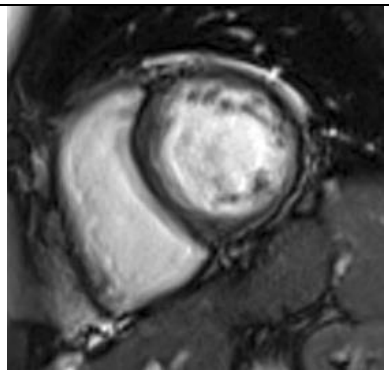
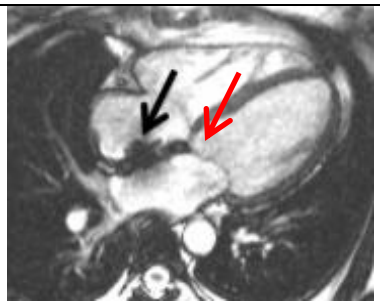
2.10.9 Reference ranges

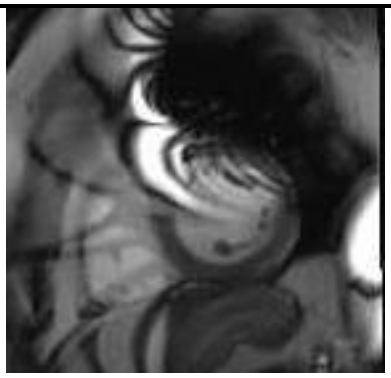
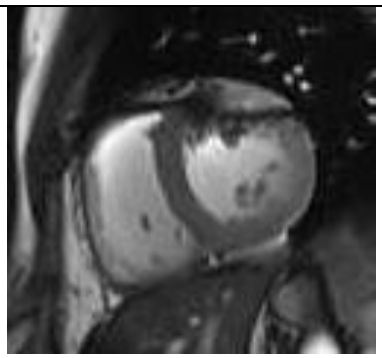
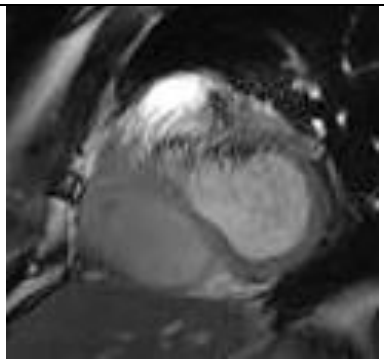
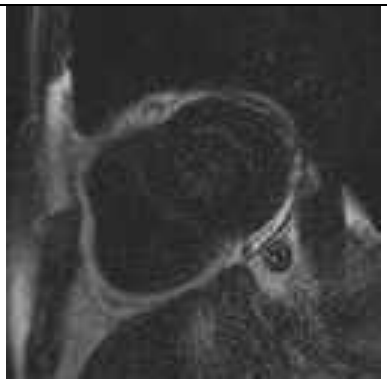
Reference ranges used in the laboratory were 105 – 215 g for LV mass in men, 70 – 170 g for LV mass in women, 77 – 195 ml for LV end-diastolic volume in men, 52 – 141 ml for LV end-diastolic volume in women, 19 – 72 ml for LV end-systolic volume in men and 13 – 51 ml for LV end-systolic volume in women.

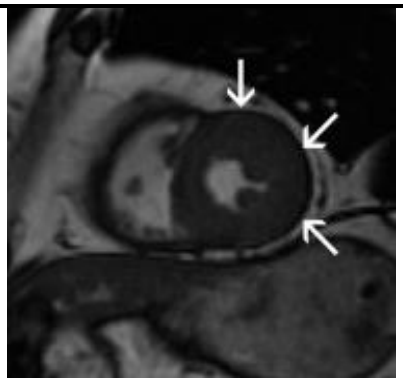
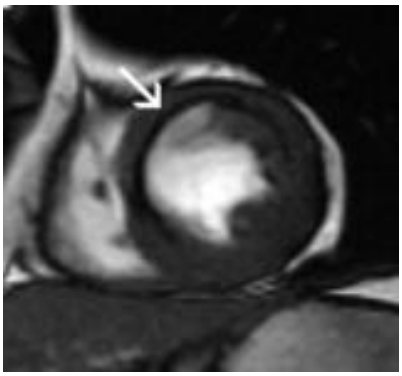
2.10.10 Assessment of artefacts

I was present for all of the scans to assess for artefacts pertaining to the different imaging sequences and if necessary instructed repeat acquisition. I created a quality control chart to inform, the radiographers acquiring scans and physicians performing analyses, of the type of artefacts to expect.

Figure 2-11 Image quality assessment

Artefact		Description	Example
Motion	M	Ghosting in the phase-encoding direction due to patient movement/breathing – ***relevant to all images***	
Gating	G	Blurred cardiac border – ***relevant to all images***	
Susceptibility	S	Distortion due to the presence of metallic objects within the field of view – ***relevant to all images***	

Artefact		Description	Example
Wrap	W	Superimposition of anatomy due to incorrect field of view – ***relevant to all images, but only if the <u>segment of interest</u> is affected***	
SSFP off-resonance	SSFP	Banding artefact passing through the myocardium – ***relevant to cine, T1 and T2 maps***	
Flow	F	Blurring/distortion due to blood flow – ***relevant to cine, T1 and T2 maps***	
LGE Contrast	LC	Sub-optimal contrast between normal myocardium, infarcted myocardium and blood pool, due to sub-optimal timing of image acquisition, inappropriate choice of TI or pathology – ***relevant to LGE***	

Artefact		Description	Example
Chemical shift	CS	Bright or black line at fat-tissue interface. In patients with MI, subencodcardial chemical shift artefacts can be observed in SSFP images (cine, T1 & T2 maps) due to the presence of lipomatous metaplasia	 
Partial volume	PV	Reduced definition due to large voxel size	

2.11 Electrocardiogram

A 12 lead electrocardiogram (ECG) was obtained before coronary reperfusion and 60 minutes afterwards with Mac-Lab® technology (GE Healthcare) in the catheter laboratory and a MAC 5500 HD recorder (GE Healthcare) in the Coronary Care Unit. The ECGs were acquired by trained cardiology staff. The ECGs were de-identified and transferred to the local ECG management system. The ECGs were then analysed by the University of Glasgow ECG Core Laboratory which is certified to ISO 9001: 2008 standards as a UKAS Accredited Organization.

The extent of ST-segment resolution on the ECG assessed 60 minutes after reperfusion compared to the baseline ECG before reperfusion (O'Gara et al., 2013) was expressed as complete (≥70%), incomplete (30% to < 70%) or none (≤30%).

2.12 Biochemical and haematological laboratory analyses

2.12.1 Biochemical assessment of infarct size

Troponin T was measured (Elecsys Troponin T, Roche) as a biochemical measure of infarct size. The high sensitive assay reaches a level of detection of 5 pg/ml and achieves less than 10% variation at 14 pg/ml corresponding to the 99th percentile of a reference population. A blood sample was routinely obtained 12 – 24 hours after hospital admission.

2.12.2 Biochemical markers of inflammation and adverse remodelling

Serial systemic blood sample were obtained immediately after reperfusion in the cardiac catheterisation laboratory, and subsequently between 0600 - 0700 hours each day during the initial in-patient stay in the Coronary Care Unit. C-reactive protein (CRP) was measured in an NHS hospital biochemistry laboratory using a particle enhanced immunoturbimetric assay method (Cobras C501, Roche),) and the manufacturers calibrators and quality control material, as a biochemical measure of inflammation. The high sensitive assay CRP measuring range is 0.1-250 mg/L. The expected CRP values in a healthy adult are < 5 mg/L, and the reference range in our hospital is 0 - 10 mg/L. A blood sample was routinely obtained in the cardiac catheter laboratory immediately following revascularization and then again at 0700 hrs on the first and second days after admission to hospital.

NT-proBNP, a biochemical measure of LV wall stress, was measured in a research laboratory using an electrochemiluminescence method (e411, Roche) and the manufacturers calibrators and quality control material. The limit of detection is 5 pg/ml. Long-term coefficient of variations of low and high controls are typically <5%, and were all within the manufacturers range.

2.12.3 Haematological measures of inflammation

Leucocyte count and leucocyte sub-populations were measured as a hematological measure of inflammation using sheath flow technology incorporating semi-conductor laser beam, forward and side scattered light (Sysmex XT200i and XT1800i for white blood cell and differential white blood cell counts, respectively). The linearity ranges for white blood

cells was 0.00-440.0 x10(9) /L. The following are the normal ranges for full blood count parameters:

Figure 2-12 Normal ranges for full blood count parameters.

	MALE	**FEMALE**
WBC x 10^9/L	4.0 - 11.0	4.0 - 11.0
RBC x 10^12/L	4.50 - 6.50	3.80 - 5.80
Hgb g/L	130 - 180	115 - 165
HCT L/L	0.400 - 0.540	0.370 - 0.470
MCV fL	78 - 99	78 - 99
MCH Pg	27.0 - 32.0	27.0 - 32.0
MCHC g/L	310 - 360	310 - 360
PLATELETS x 10^9/L	150 - 400	150 - 400
NEUTROPHILS x 10^9/L	2.5 - 7.5	2.5 - 7.5
LYMPHOCYTES x 10^9/L	1.5 - 4.0	1.5 - 4.0
MONOCYTES x 10^9/L	0.2 - 0.8	0.2 - 0.8
EOSINOPHILS x 10^9/L	0.0 - 0.4	0.0 - 0.4
BASOPHILS x 10^9/L	0.01 - 0.10	0.01 - 0.10

A blood sample was routinely obtained in the cardiac catheter laboratory, immediately following revascularization and then again at 0700 on the first and second days after admission to hospital.

2.13 Pre-specified health outcomes

A comprehensive definition of adverse events and their adjudication is detailed in the Clinical Event Charter (appendix 4). We pre-specified adverse health outcomes that are pathophysiologically linked with STEMI. We defined adverse events as:

1) Major Adverse Cardiovascular Events (MACE) is the composite of 'cardiovascular death, non-fatal MI, unplanned hospitalization for transient ischemic attack or stroke.'

2) 'Major Adverse Cardiac Events' are defined as 'cardiac death, or unplanned hospitalization for myocardial infarction or heart failure'.

The serious adverse events (SAEs) were independently assessed by an accredited cardiologist (A.M.) who was not a member of the research team. This cardiologist followed an agreed charter (appendix 4) and he was blinded to all of the other clinical data. The SAEs were defined according to standard guidelines (appendix 4) and categorised as having occurred either during the index admission or post-discharge. All study participants were followed up by patient contacts through telephone calls, clinic visits and review of the electronic medical records for a minimum of 18 months after discharge.

2.14 Statistical methods

2.14.1 Sample size calculation for the whole cohort

The sample size of 300 was predetermined based on the incidence of infarct pathology (e.g. myocardial haemorrhage or microvascular obstruction) affecting at least one third of the cohort. With an estimated haemorrhage incidence of 33% at 48 h post-STEMI, 100 subjects would have evidence of myocardial haemorrhage and 200 subjects would not. The study would have 90% power at a 5% level of significance using a two sided two sample t-test to detect a between-group difference in mean LV end-systolic volume index of 4.65 ml/m^2 equivalent to three eighths of a common standard deviation (or an effect size of 0.375). We predicted a between-group difference in mean LVESVI of 4.65 ml/m2 equivalent to three eighths of a common standard deviation (or an effect size of 0.375). This number of patients will be sufficiently large to determine whether the occurrence of haemorrhage might predict the occurrence of adverse remodelling represented by an

LVESVI > 15% ULN which occurs in about 40% of all STEMI patients We also estimated that at least 30 MACE events would occur based on a conservative estimate of the event rate (10-12%) at 18 months. The sample size calculation was performed using nQuery version 7.0.

2.14.2 Statistical analysis

Categorical variables are expressed as number and percentage of patients. Most continuous variables followed a normal distribution and are therefore presented as means together with standard deviation. Those variables that did not follow a normal distribution are presented as medians with interquartile range. Differences in continuous variables between groups were assessed by the Student's t-test or analysis of variance (ANOVA) for continuous data with normal distribution, otherwise the nonparametric Wilcoxon rank sum test or Kruskal-Wallis test. Differences in categorical variables between groups were assessed using a Chi-square test or Fisher's test, as appropriate. Correlation analyses were Pearson or Spearman tests, as indicated. Random effects models were used to compute inter-rater reliability measures (intra-class correlation coefficient (ICC)) for the reliability of CMR parameters measured independently by 2 observers in 20 randomly selected patients from the whole cohort. Outcome analysis was performed using Receiver operating curve (ROC), Cox logistic regression and time-to event curves constructed using the Kaplan-Meier method. All p-values were 2-sided, and a p-value > 0.05 indicated the absence of a statistically significant effect. Statistical analyses was performed on MINITAB 17.1.0 software or SAS version 9.3.

2.15 Funding of the study

This research was supported by the British Heart Foundation Grant (Project Grant PG/11/2/28474), the National Health Service, and the Chief Scientist Office. Professor Berry was supported by a Senior Fellowship from the Scottish Funding Council

3 Chapter 3: Patient characteristics, index admission data, angiographic and CMR results

3.1 Patient screening and recruitment

A total of 372 STEMI patients provided written informed consent, between 11th May 2011 and 22nd November 2012, to undergo CMR 2 days and 6 months post-MI. Of these 372 patients referred for emergency reperfusion therapy, 324 (87%) underwent CMR at 1.5 Tesla, 2.2±1.9 days post-revascularisation. The reasons for not undergoing CMR are shown in figure 3-1. 289 (89%) of the 324 patients with a baseline CMR, had pressure wire studies performed at the time of primary PCI (35 patients enrolled into the deferred stenting sub-study did not have pressure wire studies carried out due to operator discretion). 30 of the 324 STEMI patients were enrolled into a serial imaging sub-study (figure 3-1); all of these patients attended for all of the CMR scans at the 4 time-points (described in chapter 2). In addition, 101 of the 324 patients were enrolled into the deferred stenting sub-study (described in chapters 2 and chapter 9; a randomised controlled proof-of-concept trial nested into the larger prospective cohort study (figure 3-1).

Overall 300 (93%) patients had repeat CMR 6 months post-MI (figure 3-1). All patients (n=324) with CMR had vital status assessed at least 18 months after enrolment (figure 3-1).

3.2 Patient characteristics

The characteristics of the patients are shown in table 3-1. Of the 324 patients with a baseline CMR scan, 237 (73%) were male and mean (standard deviation (SD)) age was 59 (11). Within the cohort, cardiovascular risk factors included current smoking at the time of admission in 196 (61%) patients, hypertension in 105 (32%) patients and diabetes mellitus (defined as a history of diet-controlled or treated diabetes) in 34 (11%) patients.

3.2.1 Presenting characteristics at index admission and haemodynamic instability

The mean time from symptom onset to reperfusion was 253 (212) minutes. 21 (7%) patients had successfully cardioverted ventricular fibrillation from time of initial presentation or during emergency PCI. The majority of patients were Killip heart failure class I or II at presentation, but 23 (7%) patients were Killip heart failure class III or IV. 34 (11%) patients had a systolic blood pressure recorded at < 90 mmHg at some point during primary PCI procedure or on the coronary care unit (CCU) in the early reperfusion period.

4 (1%) patients required haemodynamic support with an intra-aortic balloon pump following reperfusion and 3 (1%) patients required intravenous inotropes whilst on CCU.

3.2.2 Mode of reperfusion

The reperfusion strategy in the study population (n=324), was primary PCI in 302 (93%) patients and 22 (7%) patients received thrombolysis whilst en route to our tertiary referral centre. Of the 22 patients that received thrombolysis, 14 (4%) failed to reperfuse and underwent emergency rescue PCI. 8 (3%) patients had successful thrombolysis and went to the cath lab within 24 hours of presentation.

Figure 3-1 Study flow diagram

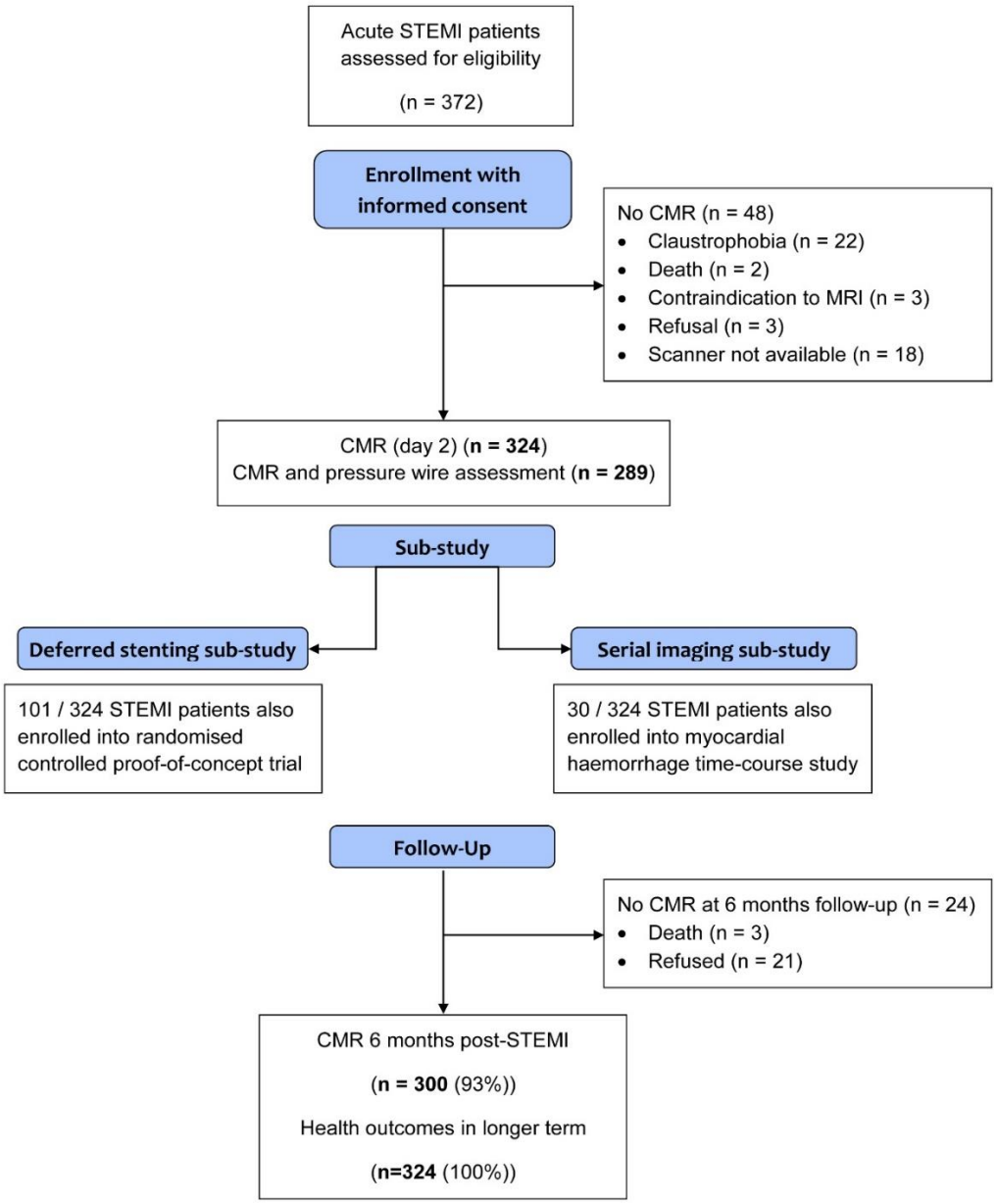


Table 3-1 Baseline clinical and angiographic characteristics of patients with acute STEMI and a CMR at baseline.

Characteristics*	All STEMI patients n=324
Clinical	
Age, years	59 (11)
Male sex, n (%)	237 (73)
BMI, (kg/m^2)	29 (5)
History	
Hypertension, n (%)	105 (32)
Current smoking, n (%)	196 (61)
Hypercholesterolemia, n (%)	94 (29)
Diabetes mellitus‡, n (%)	34 (11)
Previous angina, n (%)	40 (12)
Previous myocardial infarction, n (%)	25 (8)
Previous PCI, n (%)	18 (6)
Presenting characteristics	
Heart rate, bpm	78 (17)

Systolic blood pressure, mmHg		132 (25)
Diastolic blood pressure, mmHg		79 (14)
Time from symptom onset to reperfusion, min		253 (212)
Ventricular fibrillation†, n (%)		21 (7)
Heart failure, Killip class at presentation, n (%)	I	233 (72)
	II	68 (21)
	III	17 (5)
	IV	6 (2)
Medications at discharge		
Betablockers, n (%)		308 (95)
Statins, n (%)		324 (100)
ACE-inhibitors, n (%)		320 (99)
Aspirin, n (%)		324 (100)
Clopidogrel, n (%)		324 (100)
ECG		
ST segment elevation resolution post PCI, n (%)		
Complete, ≥70 %		148 (46)

Partial, 30% to < 70%		127 (39)
None, ≤30%		48 (15)
Coronary angiography		
Reperfusion strategy, n (%)		
Primary PCI		302 (93)
Rescue PCI (failed thrombolysis)		14 (4)
Successful thrombolysis		8 (3)
Number of diseased arteries¥, n (%)	1	174 (54)
	2	105 (32)
	3	45 (14)
Culprit artery, n (%)	Left anterior descending	121 (37)
	Left circumflex	59 (18)
	Right coronary	144 (44)
TIMI coronary flow grade pre-PCI, n (%)	0/1	236 (73)
	2	58 (18)
	3	30 (9)
TIMI coronary flow grade post-PCI, n (%)	0/1	4 (1)

	2	15 (5)
	3	305 (94)

Footnote: TIMI = Thrombolysis in Myocardial Infarction grade, PCI = percutaneous coronary intervention. Killip classification of heart failure after acute myocardial infarction: class I - no heart failure, class II - pulmonary rales or crepitations, a third heart sound, and elevated jugular venous pressure, class III - acute pulmonary oedema, class IV - cardiogenic shock. * Data are given as n (%) or mean (SD). ‡ Diabetes mellitus was defined as a history of diet-controlled or treated diabetes. † Successfully electrically cardioverted ventricular fibrillation at presentation or during emergency PCI procedure. ¥ Multivessel coronary artery disease was defined according to the number of stenoses of at least 50% of the reference vessel diameter, by visual assessment and whether or not there was left main stem involvement. Missing data: Heart rate, n=3; Time from symptom onset to reperfusion, n=17; ST-segment resolution, n=1.

3.2.3 Angiographic data

236 (73%) patients had an occluded culprit artery (TIMI coronary flow grades 0/1) at initial angiography (table 3-1). Following primary PCI, 305 (94%) patients had TIMI grade 3 flow in the culprit artery at the end of the procedure. The culprit artery was the left anterior descending artery (LAD) in 121 (37%) cases, right coronary artery (RCA) in 144 (44%) cases and the left circumflex artery (LCX) in 59 (18%) cases. Of note, 297 (92%) patients received intravenous glycoprotein IIbIIIa inhibitor therapy, initiated at bolus dose in the catheterisation lab, followed by continuous infusion for 12 hours.

3.3 Pressure wire assessment following emergency reperfusion

The methodology of these measurements is described in chapter 2. 289 (89%) of the 324 patients with baseline CMR had pressure wire studies at the end of the PCI procedure. There were no procedural related complications/ adverse events, whilst conducting invasive assessment of coronary physiology. A summary of the pressure wire data is shown in table 3-2. Index of microvascular resistance (IMR) was available in all 289 patients. Coronary flow reserve (CFR) data was missing in 6 patients because the operator only acquired hyperaemic thermodilution measurements and did not acquire during resting conditions. Fractional flow reserve (FFR) was missing in 46 patients because the operator never opened the arterial pressure transducer port on the manifold and therefore the hyperaemic aortic pressure was not recorded.

Table 3-2 Summary of invasive coronary physiology assessment, measured immediately following PCI.

Invasive coronary physiological parameter*	All STEMI patients with pressure wire assessment after PCI (n=289)
FFR (units)	0.92 (0.87, 0.97)
CFR (units)	1.6 (1.1, 2.1)
IMR (units)	24 (14, 44)

*Footnote: IMR = Index of microvascular resistance; CFR = coronary flow reserve; FFR = fractional flow reserve. *Data are given as median (IQR). Missing data: FFR, n=46; CFR, n=6.*

3.4 CMR data

3.4.1 Completeness of data acquisition

The MRI protocol is described in the methods section. Of the 324 patients that had baseline CMR, all patients had cine MRI, T2 mapping and late gadolinium enhancement imaging acquired. All patients had evaluable T2 maps. LV function / volume analysis was not possible in 3 patients due to artefact on cine images, related to arrhythmia. Delayed enhancement imaging was of insufficient quality for analysis in 2 cases, due to severe cardio-respiratory motion artefact. The investigational prototype MOLLI T1 mapping was only made available to us after 24 patients had already been recruited and was not acquired in a further 8 patients, due to them poorly tolerating the scan. T1 maps from 4 patients were non-evaluable due to motion artefact and therefore we had evaluable T1 maps in 288 patients at baseline. The investigational prototype T2* mapping technique was also only made available at a later date, after 26 patients had been recruited and was not acquired in a further 12 patients due to them poorly tolerating the scan. T2* maps were insufficient quality for analysis in 41 patients due to severe motion artefact and therefore we had evaluable T2* maps in 245 patients at day 2 CMR.

300 (93%) patients attended for 6 month follow-up CMR. The reasons for not attending for follow-up CMR were refusal in 21 cases and death in 3 cases. LV function / volume analysis was not possible in 5 cases, due to artefact on cine images, related to arrhythmia. Delayed enhancement imaging was insufficient quality for analysis in 2 patients and was not acquired in a further 2 patients because they exited the CMR scanner early due to poorly tolerating the scan.

3.5 CMR findings at baseline and follow-up in STEMI patients

The results of baseline and follow-up CMR findings for the main STEMI cohort (n=324) are shown in table 3-3. Overall, the mean (SD) acute infarct size was 18.0 (13.5) %; 51%

of patients had evidence of late microvascular obstruction and 41% of patients had evidence of myocardial haemorrhage.

Table 3-3 CMR findings in STEMI patients at day 2 and 6 month follow-up.

CMR parameters	CMR day 2 post-MI (n=324)	CMR 6 month post-MI (n=300)
LV ejection fraction, % (SD)	55.0 (9.6)	61.9 (9.4)
LV end-diastolic volume, ml (SD)		
Men	161.3 (33.3)	168.6 (42.0)
Women	125.0 (25.4)	127.3 (28.6)
LV end-systolic volume, ml (SD)		
Men	75.3 (26.6)	68.0 (34.2)
Women	55.1 (18.0)	46.3 (17.5)
LV mass, g (SD)		
Men	144.54 (32.7)	127.5 (26.5)
Women	99.1 (23.3)	92.0 (19.6)
Oedema and infarct characteristics		
Area at risk, % LV mass (SD)	31.9 (11.9)	-
Infarct size, % LV mass (SD)	18.0 (13.5)	12.8 (10.1)
Myocardial salvage, % of LV mass (SD)	13.9 (8.8)	-
Myocardial salvage index, % of LV mass (SD)	49 (30)	-

Early microvascular obstruction present, n (%)	186 (57)	
Late microvascular obstruction present, n (%)	164 (51)	-
Late microvascular obstruction, % LV mass (SD)	2.9 (5.0)	-
Myocardial native T2 values‡*		
Myocardial haemorrhage, n (%)	101 (41)	-
T2* remote myocardium (all subjects), ms (SD)	31.5 (2.4)	-
Men, ms	31.5 (2.1)	-
Women, ms	31.5 (3.1)	-
T2* infarct zone (all subjects), ms (SD)	31.8 (7.7)	-
Subjects with core, ms	25.3 (5.2)	
Subjects without core, ms	36.3 (5.7)	
T2* hypointense infarct core, ms (SD)	14.1 (3.7)	-
Myocardial native T2 values		
T2 remote myocardium (all subjects), ms (SD)	49.7 (2.1)	-
Men, ms	49.6 (2.0)	-
Women, ms	50.1 (2.1)	-
T2 infarct zone (all subjects), ms (SD)	62.9 (5.1)	-
Subjects with core, ms	62.8 (5.4)	

Subjects without core, ms	63.1 (4.7)	
T2 hypointense core present, n (%)	197 (61)	-
T2 hypointense infarct core, ms (SD)	53.9 (4.8)	-
Myocardial native T1 values¥		
T1 remote myocardium (all subjects), ms (SD)	961 (25)	-
Men, ms	959 (25)	-
Women, ms	968 (25)	-
T1 infarct zone (all subjects), ms (SD)	1097 (52)	-
Subjects with core, ms	1093.1 (51.9)	
Subjects without core, ms	1102.6 (51.1)	
T1 hypointense core present, n (%)	160 (56)	-
T1 hypointense infarct core, ms (SD)	997 (57)	-

*Footnote: * Data are given as n (%) or mean (SD). Abbreviations: LV = left ventricle T2 maps were acquired with full LV coverage. In one patient, area at risk could not be measured due to SSFP off-resonance artefact, otherwise all T2 maps were suitable for analysis. ¥ T1 maps were acquired with 3 short-axis slices (n=288 with evaluable T1 maps at baseline). ‡ T2* maps were acquired with 3 short-axis slices (n=245 with evaluable T2* maps at baseline). Myocardial haemorrhage was defined as T2* infarct core <20 ms.*

3.5.1 T2, T2 and T2* values in STEMI patients*

T2*, T2 and T1 values measured in the: remote zone, injury zone and infarct hypointense core were independent of the culprit artery territory (tables 3-4, 3-5 and 3-6).

Table 3-4 Differences in T2 values measured in the infarct hypointense core, remote- and injury zones by culprit artery territory.

Myocardial region of interest	Culprit artery	T2 value, ms (SD)	P-value
Remote zone	LAD	49.8 (2.1)	0.240
	LCX	50.1 (2.3)	
	RCA	49.6 (1.9)	
Injury zone	LAD	62. 7 (5.1)	0.407
	LCX	62.3 (6.0)	
	RCA	63.3 (4.8)	
Infarct core	LAD	53.3 (4.9)	0.238
	LCX	53.6 (5.4)	
	RCA	54.6 (4.5)	

Footnote: P-values from one way ANOVA. LAD = left anterior descending artery; LCX = left circumflex artery; RCA = right coronary artery.

Table 3-5 Differences in T2* values measured in the infarct hypointense core, remote- and injury zones by culprit artery territory.

Myocardial region of interest	Culprit artery	T2* value, ms (SD)	P-value
Remote zone	LAD	31.8 (2.2)	0.340
	LCX	31.4 (2.1)	
	RCA	31.3 (2.6)	
Injury zone	LAD	31.5 (7.8)	0.882
	LCX	31.4 (8.0)	
	RCA	32.0 (7.5)	
Infarct core	LAD	14.3 (3.7)	0.854
	LCX	14.4 (3.6)	
	RCA	13.9 (3.8)	

Footnote: P-values from one way ANOVA. LAD = left anterior descending artery; LCX = left circumflex artery; RCA = right coronary artery.

Table 3-6 Differences in T1 values measured in the infarct hypointense core, remote- and injury zones by culprit artery territory.

Myocardial region of interest	Culprit artery	T1 value, ms (SD)	P-value
Remote zone	LAD	963.90 (23.66)	0.059
	LCX	965.60 (26.92)	
	RCA	957.49 (25.09)	
Injury zone	LAD	1088.73 (48.38)	0.084
	LCX	1105.09 (57.72)	
	RCA	1101.44 (51.26)	
Infarct core	LAD	991.8 (64.5)	0.592
	LCX	995.6 (62.6)	
	RCA	1002.1 (47.9)	

Footnote: P-values from one way ANOVA. LAD = left anterior descending artery; LCX = left circumflex artery; RCA = right coronary artery.

3.5.2 Intra- and inter-observer agreement of T1, T2 and T2 measurements*

Native T1 in regions-of-interest in remote zones, injured zones and infarct core in a subgroup of 20 randomly chosen patients were independently measured by two observers. The intra-class correlation coefficient for reliability of remote T1, infarct zone T1 and infarct core T1 were 0.92 (95% confidence interval (CI): 0.80, 0.97), 0.93 (0.84, 0.97) and 0.92 (0.71, 0.97); all $p<0.001$, respectively. Bland-Altman plots showed no evidence of bias (figures 3-2 to 3-4).

T2 values in regions-of-interest in remote zones, injured zones and infarct core, in a subgroup of 20 randomly chosen patients were also independently measured by two

observers. The intra-class correlation coefficients for reliability of remote T2, infarct zone T2 and infarct core T2 were 0.93 (95% confidence interval (CI): 0.82, 0.97), 0.89 (0.74, 0.95) and 0.86 (0.68, 0.94); all p<0.001, respectively. Bland-Altman plots showed no evidence of bias (figures 3-5 to 3-7).

In addition, T2* values in regions-of-interest in remote zones, injured zones and infarct core, in a subgroup of 20 randomly chosen patients were also independently measured by two observers. The intra-class correlation coefficients for reliability of remote T2*, infarct zone T2* and infarct core T2* were 0.69 (95% confidence interval (CI): 0.37, 0.87), 0.75 (0.47, 0.89) and 0.90 (0.77, 0.96); all p<0.001, respectively. Bland-Altman plots showed no evidence of bias (figures 3-8 to 3-10).

Figure 3-2 Bland-Altman plot for inter-observer agreement of myocardial remote zone T1 values

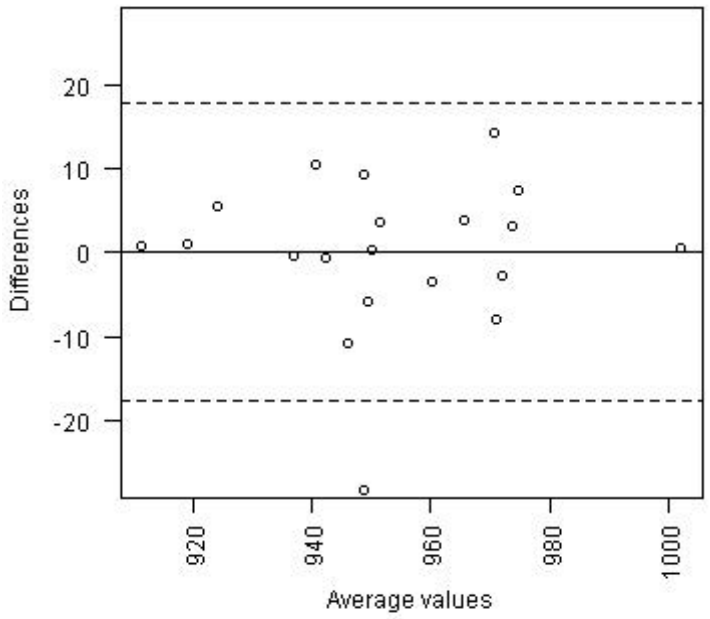


Figure 3-3 Bland-Altman plot for inter-observer agreement of myocardial injury zone T1 values

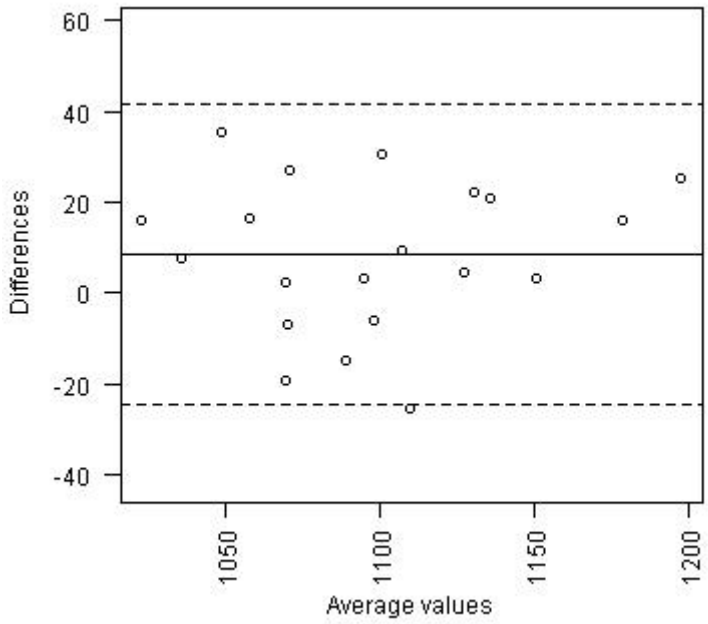


Figure 3-4 Bland-Altman plot for inter-observer agreement of myocardial infarct core T1 values

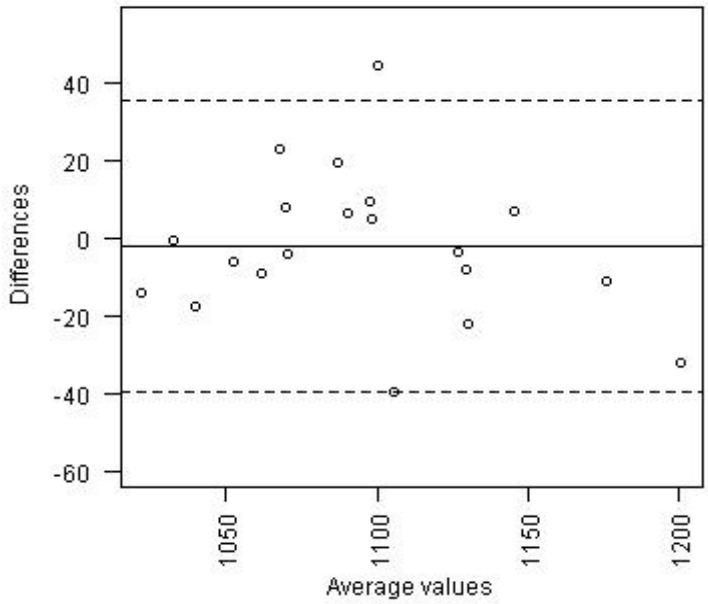


Figure 3-5 Bland-Altman plot for inter-observer agreement of myocardial remote zone T2 values

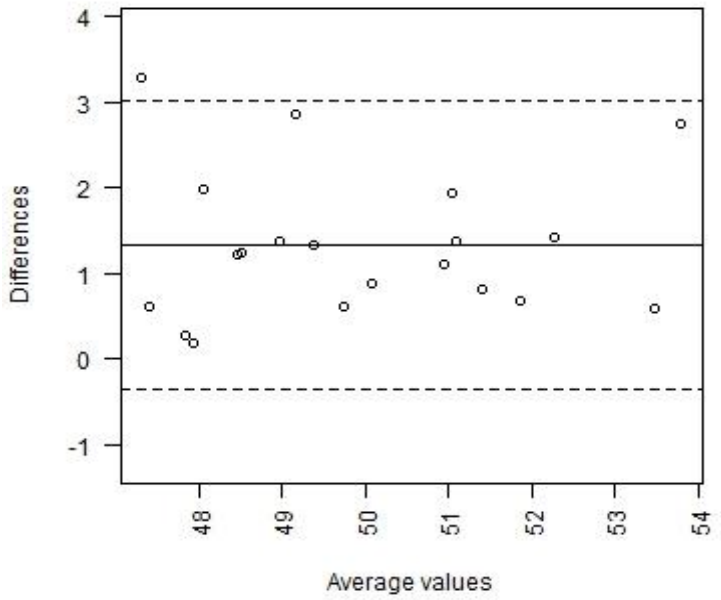


Figure 3-6 Bland-Altman plot for inter-observer agreement of myocardial injury zone T2 values

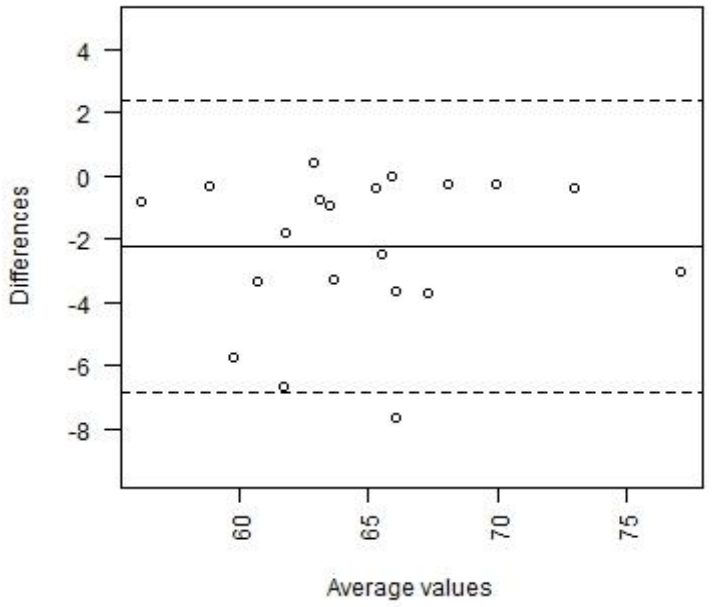


Figure 3-7 Bland-Altman plot for inter-observer agreement of infarct core T2 values

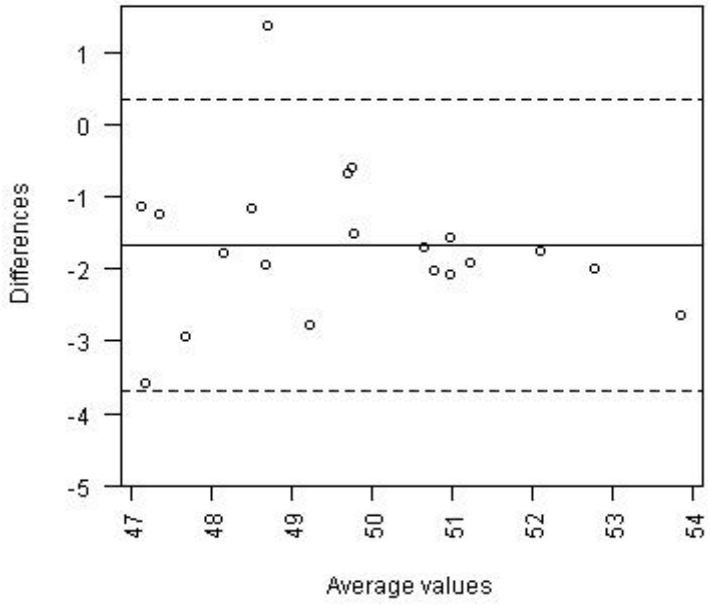


Figure 3-8 Bland-Altman plot for inter-observer agreement of myocardial remote zone T2* values

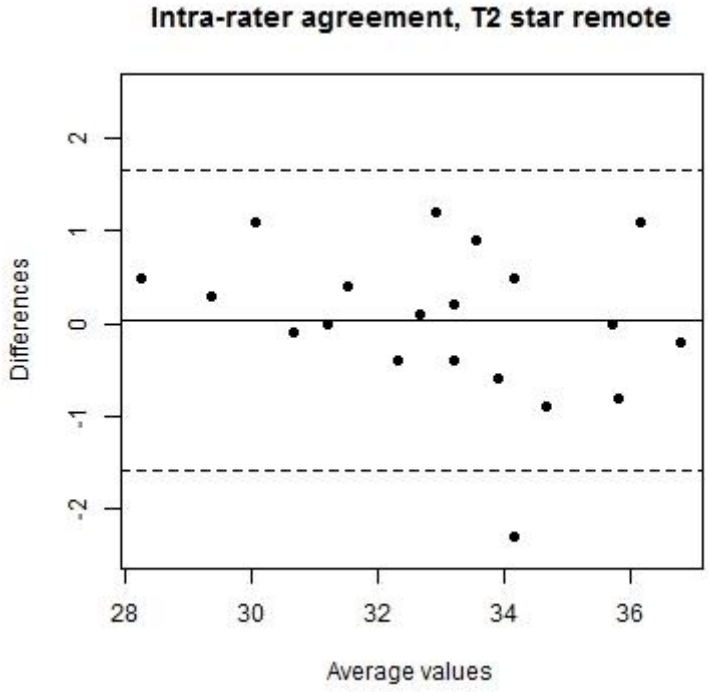


Figure 3-9 Bland-Altman plot for inter-observer agreement of myocardial injury zone T2* values

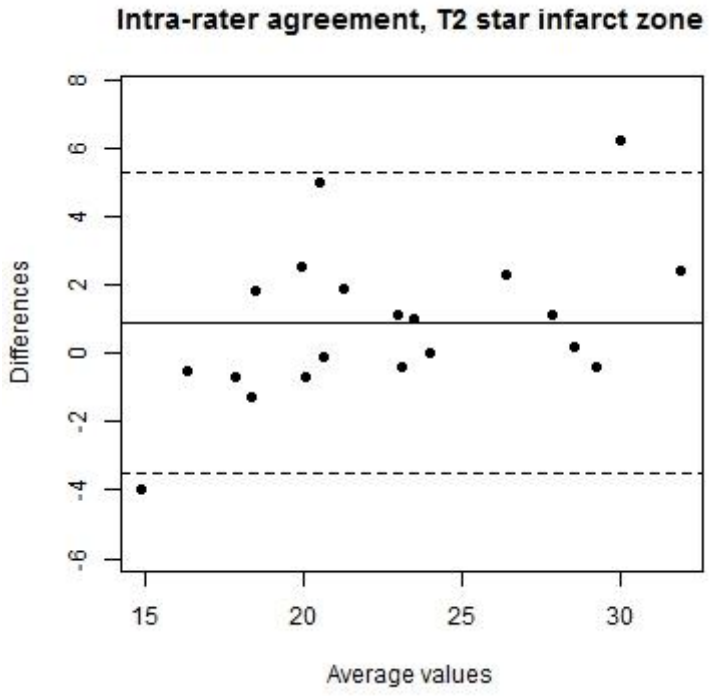
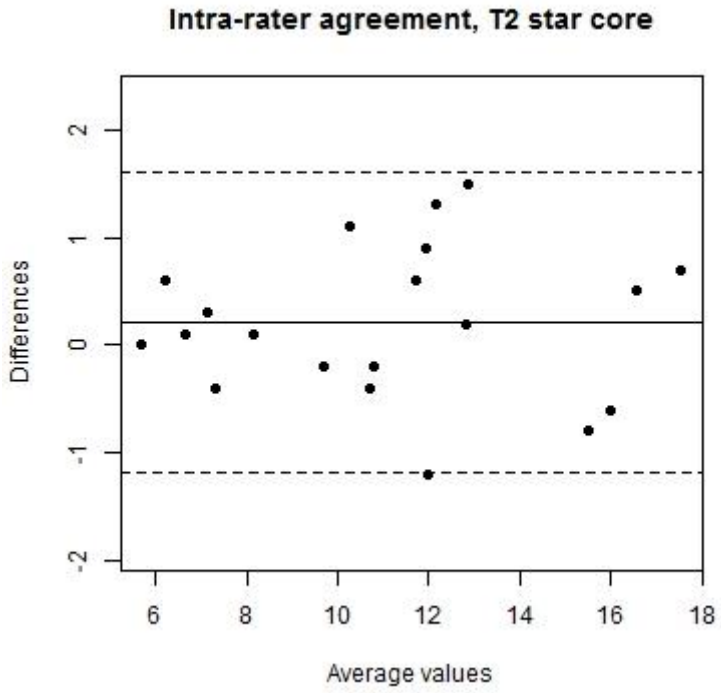


Figure 3-10 Bland-Altman plot for inter-observer agreement of infarct core T2* values



3.6 Healthy volunteer CMR results

Fifty healthy volunteers from the same geographical region (52% male, mean (SD) age 54 (13) years) without a history of cardiovascular disease or therapy and who had a normal electrocardiogram were enrolled during the same time period as the STEMI patients. The volunteers were scanned using the same 1.5 Tesla MRI scanner (Siemens AVANTO) as the STEMI patients, and the approach to image analysis was the same as for STEMI patients also, including regional segmentation of the left ventricle according to the American Heart Association model (Cerqueira et al., 2002) . A summary of the CMR findings for healthy volunteers are shown in table 3-7.

Table 3-7 CMR findings in 50 age- and sex-matched healthy volunteers.

CMR parameters	Healthy volunteers (n=50)
LV ejection fraction, %	67.2 (4.5)
LV end-diastolic volume, ml (SD)	
Men	167.8 (31.6)
Women	134.1 (23.0)
LV end-systolic volume, ml (SD)	
Men	56.8 (14.9)
Women	43.6 (12.3)
LV mass, g (SD)	
Men	124.5 (22.7)
Women	92.0 (20.4)
T2 of myocardium (all subjects), ms (SD)	49.5 (2.5)

Men, ms	48.5 (2.1)
Women, ms	50.5 (2.5)
T1 of myocardium (all subjects), ms (SD)	958 (24)
Men, ms	948 (20)
Women, ms	968 (25)
T2* of myocardium (all subjects), ms (SD)	31.0 (2.1)
Men, ms	30.8 (2.1)
Women, ms	31.3 (2.1)

3.6.1 T1, T2 and T2 values in healthy volunteers compared to STEMI patients*

At the mid-ventricular level, mean remote zone native T1 was similar in STEMI patients (961 (25) ms) and healthy volunteers (958 (24); p=0.314). Remote zone native T1 was higher in male STEMI patients than in male volunteers (959 (25) vs. 948 (20) ms, respectively; p=0.024), but similar in female STEMI patients (968 (25) ms) and volunteers (968 (23) ms). In healthy subjects, mid-ventricular T1 values were lower in males than in females (948 (20) ms vs. 968 (23) ms; p=0.003). In both men and women, the infero-lateral segment had the highest T1 compared to the antero-septal segment (960 (28) ms vs. 939 (26) ms and 978 (32) ms vs. 961 (34) ms, respectively; p<0.001 and p=0.011).

The coefficients of variation (CoV) for native T1 in the mid-ventricular level with regions-of-interest within myocardial regions were: anterior segment CoV = 2.35; antero-lateral segment CoV = 2.98; antero-septal segment CoV = 3.35; inferior segment CoV = 2.49; infero-lateral segment CoV = 3.22; infero-septal segment CoV = 2.90.

In healthy subjects, mid-ventricular T2 values were lower in males than females (48.5 (2.1) ms vs. 50.6 (2.5) ms; p=0.003). Overall, the inferior segment had the highest T2 value

compared to the anterior segment (50.0 (2.8) ms vs. 49.1 (3.0), respectively; p=0.031). At the mid-ventricular level, mean remote zone native T2 was similar in STEMI patients (49.7 (2.1) ms) and healthy volunteers (49.5 (2.5) ms; p=0.511).

The coefficients of variation CoV for native T2 in the mid-ventricular level with regions-of-interest within myocardial regions were: anterior segment CoV = 6.00; antero-lateral segment CoV = 6.49; antero-septal segment CoV = 6.25; inferior segment CoV = 5.50; infero-lateral segment CoV = 4.58; infero-septal segment CoV = 5.36.

T2* values were similar in healthy volunteers, irrespective of gender or location of measurement. At the mid-ventricular level, mean remote zone T2* values were similar in STEMI patients (31.5 (2.4) ms) and healthy volunteers (31.0 (2.1) ms; p=0.162).

3.7 Discussion – patient characteristics, admission data, CMR findings and angiographic results

3.7.1 Patient characteristics

The mean age of patients in this study was 59 years, with the majority being male (73%), anterior infarction in 37% of cases and 73% of patients with TIMI ≤ 1 flow at initial angiography, which is in keeping with contemporary studies in this field (Bekkers et al., 2010a, Eitel et al., 2011, Ganame et al., 2009, Husser et al., 2013, Kandler et al., 2014). Eitel *et al*. (Eitel et al., 2011), using T2-weighted methods to detect haemorrhage, included 346 acute STEMI patients median age 64 years, 70% male, 46% anterior location infarction and 71% with an occluded artery (TIMI flow ≤1) at initial angiography, which is similar to the population in this study. Also, Husser et al. (n=304), again using qualitative T2-weighted methods, had a similar study population to this study, with mean age of 60 years, 80% male, 55% anterior infarction and mean time of symptom onset to reperfusion of 269 (190) minutes (symptom onset to reperfusion in this study 253 (119) minutes). The largest study to date to include T2* imaging for the detection of IMH (n=151), again had a similar study population to this study, with mean age 61 years, 75% male, 61% with an occluded artery pre-PCI, 42% anterior infarction and mean time of symptom onset to reperfusion of 263 (196) minutes.

Our study population is inhomogeneous, and includes patients treated with PCI, thrombolysis and both (rescue PCI). Our study is one of the few in the CMR STEMI literature to include information on patients who have received thrombolysis. On sub-group analysis there was no interaction between thrombolysis and CMR findings and therefore we decided to include these patients.

My study population represents a well-treated cohort, given the high rates of secondary preventive medications on discharge and patients with TIMI grade 3 flow post-PCI (table 3-1), and is in keeping with contemporary studies in the field.

3.7.2 Pressure wire data in comparison with previous studies

There have been many recent publications looking at IMR in acute STEMI patients, although there are variations in the timing of pressure wire assessment post-PCI and some studies limited IMR measurement to anterior infarcts. The median IMR in this study was 24, comparable to previous published studies of similar patient groups (summarised in table 3-8). Our study was at the lower end of IMR values and this may be due to the relatively lower proportion of anterior infarcts included (37% of patients), compared with for example: 49% of cases in the study by McGeoch et al. (McGeoch et al., 2010) and 55% of cases in the study by Fearon et al. (Fearon et al., 2013).

Table 3-8 Median IMR values in acute STEMI studies with similar patient groups to this study.

Authors	Year	Sample size	MI culprit territory	Timing	Median IMR
Fearon et al. (Fearon et al., 2008)	2007	29	All	At PCI	32
Ito et al. (Ito et al., 2010)	2009	40	All	At PCI	26
Lim et al. (Lim et al., 2009)	2009	40	Anterior only	At PCI	33

Sezer et al. (Sezer et al., 2010)	2010	35	All	At 48 hours	29
McGeoch et al. (McGeoch et al., 2010)	2010	57	All	At PCI	35
Payne et al. (Payne et al., 2012)	2012	108	All	At PCI	26
Fearon et al. (Fearon et al., 2013)	2013	253	All	At PCI	31
Cuculi et al. (Cuculi et al., 2014)	2014	82	All	At PCI	(mean) 42

3.7.3 CMR findings in comparison with previous studies

There is considerable variability in the reported incidence of IMH and MVO in acute reperfused STEMI patients and much of this is likely related to the heterogeneity in methods used to detect IMH/ MVO and the lack of standardisation with regard to when temporally these infarct characteristics are measured. The incidence if IMH/ MVO in our study (41% and 54% respectively, in cohort with evaluable T2* map at baseline) are in keeping with contemporary studies, in similar groups of patients (table 3-9).

Table 3-9 Incidence of IMH and MVO in acute reperfused STEMI patients, in contemporary studies.

Authors	Year	Sample size	Methodology to detect IMH	Methodology to detect MVO	Incidence IMH (%)	Incidence MVO (%)
Ganame et al. (Ganame et al., 2009)	2009	98	T2-weighted	Early GE	24	64

Bekkers et al. (Bekkers et al., 2010a)	2010	90	T2-weighted	LGE	43	54
Beek et al. (Beek et al., 2010)	2010	45	T2-weighted	LGE	49	60
O'Regan et al. (O'Regan et al., 2010)	2010	50	T2*	LGE	58	58
Mather et al. (Mather et al., 2011b)	2011	48	T2* and T2-weighted	Early GE	25	63
Eitel et al. (Eitel et al., 2011)	2012	346	T2-weighted	LGE	35	66
Amiable et al. (Amabile et al., 2012)	2012	114	T2-weighted	LGE	10	55
Husser et al. (Husser et al., 2013)	2012	304	T2-weighted	LGE	34	36
Zia et al. (Zia et al., 2012)	2012	62	T2-weighted and T2*	LGE	32	62
Kali et al. (Kali et al., 2013b)	2013	14	T2-weighted and T2*	LGE	50	50
Kidambi et al. (Kidambi et al., 2013)	2013	39	T2-weighted and T2*	LGE	36	56

Robbers et al. (Robbers et al., 2013)	2013	26	T2-weighted	LGE	55	59
Kandler et al. (Kandler et al., 2014)	2014	151	T2-weighted and T2*	LGE	50	66

Footnote: LGE = late gadolinium enhancement; Early GE = early gadolinium enhancement

There are very few clinical studies, with only small patient numbers, that have used novel T1 mapping (Dall'Armellina et al., 2012, Messroghli et al., 2004) or T2 mapping (Giri et al., 2009, Nassenstein et al., 2014, Park et al., 2013, Verhaert et al., 2011) methods, in acute reperfused STEMI patients. These studies have generally concentrated on area-at-risk measurement and excluded the hypointense infarct core on parametric maps (corresponding to the area of MVO) from analysis. In contrast, we have focussed on the hypointense infarct core on mapping sequences, in an effort to better evaluate IMH and its relationship with MVO. Our mean T2* value (14.1 ms) within the infarct core of haemorrhagic infarcts is consistent with previous findings (Kali et al., 2013b, O'Regan et al., 2010).

The native T1 values of healthy subjects in the current study (men = 949±20 ms, women = 968±23 ms) were similar to T1 values reported by Piechnik *et al*. (men = 947±20 ms and women = 974±25 ms) (Piechnik et al., 2010), although lower than the study by Liu *et al.* (men = 962±37 and women = 984±47 ms) (Liu et al., 2013); however the cohort studied by Liu *et al.* (Liu et al., 2013) had several cardiovascular risk factors and hypertension and diabetes were prevalent. T2* values of remote myocardium in STEMI patients and values in healthy volunteers in this current study, were consistent with previous studies (Kali et al., 2013b, O'Regan et al., 2010). As were our T2 values (Nassenstein et al., 2014, Verhaert et al., 2011, Wassmuth et al., 2013).

It can therefore be seen that although natural variations will apply, the CMR analysis data for my study cohort is in keeping with contemporary work in this area.

3.8 Conclusion

The raw data on which this thesis is based, patient population, invasive coronary physiology data and CMR findings are consistent with contemporary work in these fields.

4 Chapter 4: Myocardial haemorrhage after acute reperfused ST-elevation myocardial infarction: temporal evolution, relation to microvascular obstruction and prognostic significance

4.1 Preamble

As discussed in chapter 1, T2* CMR is the gold standard technique to detect myocardial haemorrhage, although most studies in acute STEMI patients have not used T2* imaging to detect haemorrhage, but instead used black-blood T2-weighted methods, which are known to be hampered by artefacts and less specific for haemorrhage. In this chapter, I will use T2* imaging to define myocardial haemorrhage and therefore report the results of the patients with an evaluable T2*map at baseline.

4.2 Introduction

The success of emergency coronary reperfusion therapy in ST-elevation myocardial infarction (STEMI) is commonly limited by failed tissue perfusion (Yellon and Hausenloy, 2007). This disconnect is mainly due to two pathologies: microvascular obstruction (Kloner et al., 1974, Jaffe et al., 2008) and intramyocardial haemorrhage (Higginson et al., 1982). Based on morphological (Kloner et al., 1974) and functional studies (Wilson et al., 1989), microvascular obstruction may have structural and functional components (Galiuto, 2004), which may reflect irreversible (i.e. endothelial disruption) and reversible (e.g. microvascular spasm, extrinsic oedema) components. Myocardial haemorrhage manifests because of aggregation and extravasation of erythrocytes (Higginson et al., 1982, Fishbein et al., 1980, Payne et al., 2011a, Robbers et al., 2013). The time-course of these pathologies and when diagnostic imaging might be most appropriate is yet to be defined.

T2*-weighted CMR is the reference diagnostic method for myocardial haemorrhage *in vivo* (Basso et al., 2007, Kali et al., 2013b, Kumar et al., 2011), however technical issues have limited T2* imaging in clinical practice. The largest cohort studies of myocardial haemorrhage in STEMI patients to date have not used T2* imaging (Amabile et al., 2012, Ganame et al., 2009, Husser et al., 2013, Robbers et al., 2013, Bekkers et al., 2010a, Beek et al., 2010), although some smaller studies have used T2* CMR (Kandler et al., 2014, Mather et al., 2011b, O'Regan et al., 2010, Zia et al., 2012). Because of these different CMR techniques, uncertainties have arisen around the pathophysiology and clinical significance of myocardial haemorrhage, and its relationships with microvascular obstruction. In some studies, myocardial haemorrhage is associated with adverse remodelling (Beek et al., 2010, Ganame et al., 2009, Husser et al., 2013, Kandler et al., 2014, O'Regan et al., 2010), persistent LV systolic dysfunction (Kidambi et al., 2013), late

arrhythmic risk (Mather et al., 2011b) and adverse clinical outcome (Amabile et al., 2012, Eitel et al., 2011), however, other studies have shown that myocardial haemorrhage does not have prognostic significance beyond microvascular obstruction (Beek et al., 2010, Bekkers et al., 2010a, Husser et al., 2013).

In this study we aimed to: (1) detect myocardial haemorrhage using T2* mapping in a large relatively unselected STEMI population and re-evaluate its clinical associates and prognostic significance, (2) study the time-course of myocardial haemorrhage evolution with serial CMR early after reperfusion, and (3) assess the temporal relationships between myocardial haemorrhage versus microvascular obstruction.

To this end, we used quantitative T2* mapping which potentially offers increased accuracy for the detection of myocardial haemorrhage than T2-weighted methods because T2* relaxation times are measured directly (Ghugre et al., 2011, Zia et al., 2012, Kali et al., 2013b, O'Regan et al., 2010).

4.3 Methods

4.3.1 Study population and STEMI management

Recruitment for the main cohort study was between 11th May 2011 and 22nd November 2012. However, the investigational prototype T2* map sequence was only made available to us on 17th July 2011, after 24 patients had already been recruited. Therefore the cohort of patients that had T2* imaging acquired were recruited between 17th July 2011 and 22 November 2012. Three hundred and forty three STEMI patients provided written informed consent. The eligibility criteria included an indication for primary percutaneous coronary intervention (PCI) or thrombolysis for acute STEMI as described in chapter 2.

4.3.2 CMR acquisition

Al patients underwent the CMR protocol described in detail in chapter 2. In brief this included cine CMR with steady-state free precession (SSFP), T2*-mapping acquired in 3 short-axis slices, T2-mapping in contiguous short-axis slices covering the whole ventricle (Giri et al., 2009, Verhaert et al., 2011), and delayed-enhancement phase-sensitive

inversion-recovery pulse sequences (Kellman et al., 2002). I will report on the T1 mapping sequences in chapter 7.

Serial imaging sub-study

Thirty STEMI patients underwent serial CMR in order to characterise the evolution of myocardial haemorrhage by T2 and T2* quantification, and evaluate the temporal relationship with microvascular obstruction. Each patient was imaged at 4 time points, with the identical imaging protocol as above: 4 to 12 hours, 3 days, 10 days and 6-7 months post-reperfusion.

Healthy volunteers

CMR was also performed in 50 healthy volunteers of similar age and gender in order to obtain local reference values for myocardial T2 and T2*.

4.4 CMR analyses

The CMR analyses are described in chapter 2. In the serial imaging sub-study, standardised measurements of T2 and T2* in myocardial regions of interest (remote myocardium, injured myocardium and infarct core) were performed. In the main STEMI cohort, myocardial haemorrhage was reported in a binary fashion, defined as a region of reduced signal intensity within the infarcted area on T2* maps, with a T2* value of <20 ms (Ghugre et al., 2011, Kandler et al., 2014, O'Regan et al., 2010, Anderson et al., 2001).

4.5 Statistical analyses

As described in chapter 2, categorical variables are expressed as number and percentage of patients. Most continuous variables followed a normal distribution and are therefore presented as means together with standard deviation. Those variables that did not follow a normal distribution are presented as medians with interquartile range. Differences between groups were assessed using one-way ANOVA, Kruskal-Wallis test or Fisher's where appropriate. Univariable and multivariable logistic regression analyses were performed to identify predictors of myocardial haemorrhage. Binary logistic regression models were used to identify predictors of adverse remodelling at 6-month follow-up. In stepwise linear

regressions, the Akaike information criterion (AIC) was used a measure of the relative quality of the models for this dataset, and the model with the minimum AIC value was reported.

Receiver operating curve (ROC), Kaplan-Meier and Cox proportional hazards methods were used to identify potential clinical predictors of all-cause death/heart failure events and MACE, including patient characteristics, CMR findings and myocardial haemorrhage. A p-value > 0.05 indicates the absence of a statistically significant effect.

4.6 Results

Of 343 STEMI patients referred for emergency PCI, 300 underwent serial CMR at 1.5 Tesla 2.1±1.8 days and 6 months after hospital admission (Figure 4-1). 286 STEMI patients had T2* maps acquired. 245 (86%) patients had evaluable T2* data (figure 4-1) and all of these patients had evaluable T2 maps. CMR follow-up at 6 months was achieved in 228 (93%) of the patients with T2* mapping performed and all (n=245) patients had health outcomes assessed at minimum of 18 months after enrolment.

4.6.1 Myocardial haemorrhage time-course study

30 STEMI patients underwent serial CMR on 4 occasions. The first CMR examination was performed at a mean of 8.6±3.1 hours following emergency PCI, the second at 2.9±1.5 days, the third at 9.6±2.3 days and the fourth 213±27 days following PCI. 102 (85%) CMR scans had evaluable T2* data and 117 (98%) had evaluable T2 data.

4.6.2 Patient characteristics

The clinical characteristics are shown in table 4-1. Based on T2*-CMR, 101 (41%) patients had IMH. Male sex, anterior infarction, TIMI flow ≤1 before PCI and inflammation were more common in patients with myocardial haemorrhage. Heart failure during index admission was also more common in patients with myocardial haemorrhage, indicated by the higher NT-proBNP level and more Killip heart failure class >2 at presentation. In addition, patients with myocardial haemorrhage had less resolution of ST-segment elevation post-PCI.

The characteristics of the serial imaging cohort were similar to the main study population and are described in table 4-2.

4.6.3 Myocardial haemorrhage is associated with myocardial infarct characteristics

The CMR findings at 2 days post-MI and 6 month later are shown in table 4-3. Clinical cases are shown in figure 4-2. Compared to patients without myocardial haemorrhage, patients with myocardial haemorrhage had a larger LV mass, larger LV volumes, and lower LV ejection fractions early post-MI and at 6 months. The initial area-at-risk, infarct size and microvascular obstruction were also larger and there was less myocardial salvage in patients with myocardial haemorrhage, ($p<0.001$, respectively, table 4-3).

Figure 4-1 Study flow diagram

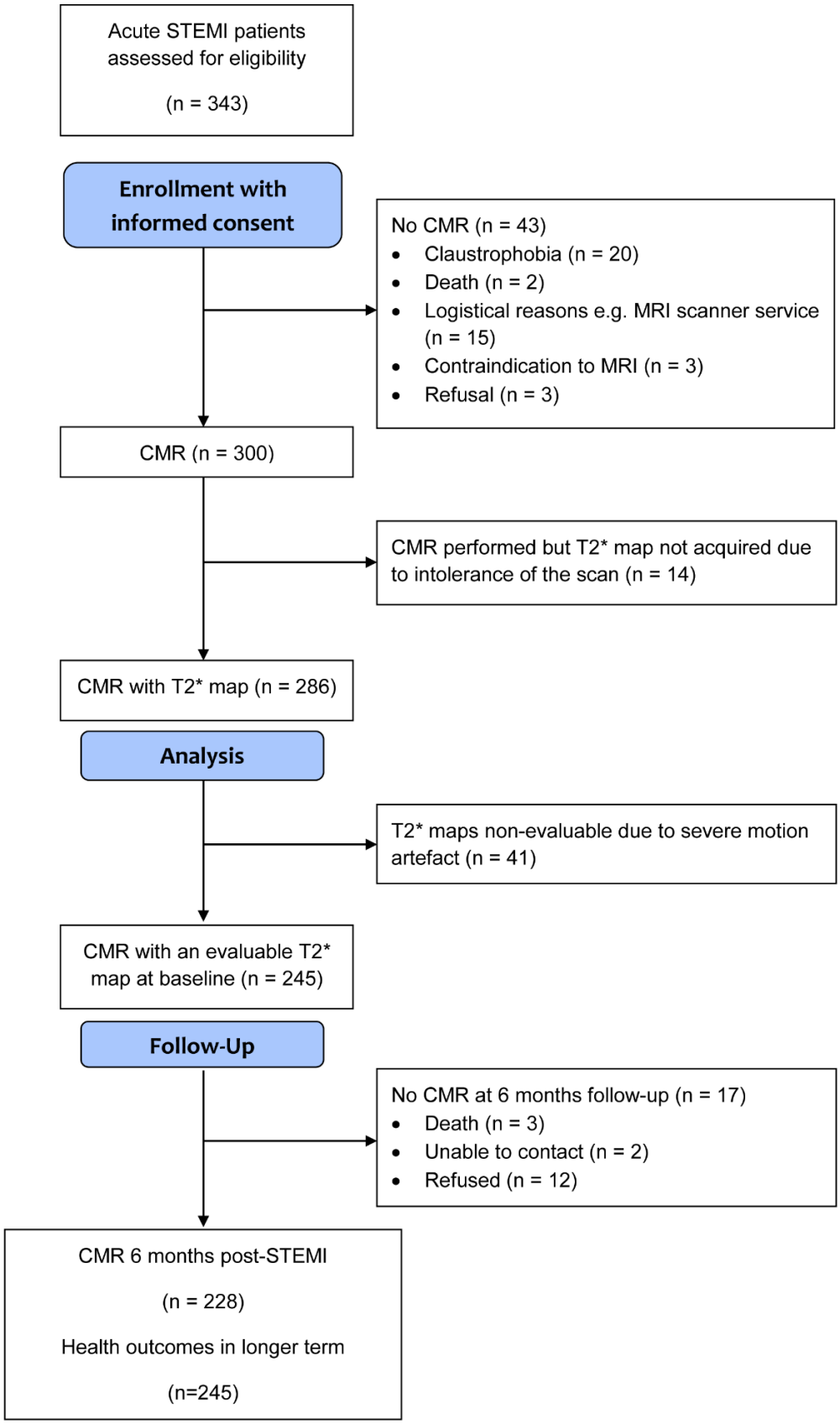


Table 4-1 Clinical and angiographic characteristics of 245 patients with acute STEMI who had CMR at baseline with evaluable T2* maps.

Characteristics*	All Patients n = 245	Haemorrhagic infarct (T2*core +) n = 101	Non-haemorrhagic infarct (T2*core -) n = 144	p value
Clinical				
Age, years	58 (11)	59 (12)	58 (11)	0.745
Male sex, n (%)	187 (76)	84 (83)	103 (72)	0.047
BMI, (kg/m^2)	28 (5)	28 (5)	28 (5)	0.848
History				
Hypertension, n (%)	77 (31)	37 (37)	40 (28)	0.163
Current smoking, n (%)	153 (62)	70 (69)	83 (58)	0.081
Hypercholesterolemia, n (%)	68 (28)	31 (31)	37 (26)	0.469
Diabetes mellitus‡, n (%)	28 (11)	15 (15)	13 (9)	0.220
Previous angina, n (%)	31 (13)	15 (15)	16 (11)	0.437
Previous myocardial infarction, n (%)	17 (7)	8 (8)	9 (6)	0.619
Previous PCI, n (%)	13 (5)	10 (10)	3 (2)	0.009
Presenting characteristics				

Heart rate, bpm		78(16)	80 (17)	77 (16)	0.202
Systolic blood pressure, mmHg		136 (25)	136 (23)	136 (26)	0.887
Diastolic blood pressure, mmHg		80 (14)	82 (14)	79 (14)	0.074
Time from symptom onset to reperfusion, min		176 (123, 324)	207 (125, 364)	171 (124, 303)	0.161
Ventricular fibrillation†, n (%)		15 (6)	7 (7)	8 (6)	0.788
Heart failure, Killip class at presentation, n (%)	I	171 (70)	59 (58)	112 (78)	
	II	57 (23.3)	26 (25.7)	31 (21.5)	<0.001
	III/IV	17 (7)	16 (16)	1 (1)	
Electrocardiogram					
ST segment elevation resolution post PCI, n (%)					
Complete, ≥70 %		107 (44)	31 (31)	76 (53)	
Partial, 30% to < 70%		99 (41)	48 (48)	51 (36)	0.001
None, ≤30%		38 (16)	22 (22)	16 (11)	
Coronary angiography					
Reperfusion strategy, n (%)					
Primary PCI		229 (94)	92 (91)	137 (95)	0.173
Rescue PCI (failed thrombolysis)		10 (4)	7 (7)	3 (2)	

Successful thrombolysis		6 (2)	2 (2.0)	4 (3)	
Number of diseased arteries¥, n (%)	1	132 (54)	58 (57)	74 (51)	
	2	70 (29)	25 (25)	45 (31)	0.714
	3	37 (15)	16 (16)	21 (15)	
	LM	6 (2)	2 (2)	4 (3)	
Culprit artery, n (%)	Left anterior descending	96 (39)	46 (46)	50 (35)	
	Left circumflex	48 (20)	26 (26)	22 (15)	0.003
	Right coronary	101 (41)	29 (29)	72 (50)	
TIMI coronary flow grade pre-PCI, n (%)	0/1	180 (74)	88 (87)	92 (64)	<0.001
	2/3	65 (27)	13 (13)	52 (36)	
TIMI coronary flow grade post-PCI, n (%)	0/1	3 (1)	2 (2)	1 (1)	
	2	11 (5)	5 (5)	6 (4)	0.658
	3	231 (94)	94 (93)	137 (95)	
Initial blood results on admission					
C-reactive protein, (mg/L)		3.0 (2.0, 7.0)	3.0 (2.0, 7.0)	3.0 (2.0, 7.0)	0.116
Leucocyte cell count ($x10^9L$)		12.5 (3.5)	13.7 (3.8)	11.6 (3.1)	<0.001

Neutrophil count (x10^9L)	9.7 (3.3)	11.0 (3.5)	8.8 (2.9)	<0.001
Monocytes (x10^9L)	0.9 (0.4)	1.0 (0.5)	0.8 (0.3)	<0.001
NT-proBNP index admission, pg/mL	767 (363, 1635)	1117 (646, 1647)	606 (300, 1414)	0.007

Table 4-2 Clinical and angiographic characteristics of the 30 patients in the longitudinal clinical study stratified by the presence of haemorrhage on day 3 CMR.

Characteristics*	All Patients n = 30	Myocardial hemorrhage (T2*core +) n = 13 (43%)	No myocardial hemorrhage (T2*core -) n = 17 (57%)	p value
Clinical				
Age, years	54 (10)	53 (11)	55 (9)	0.602
Male sex, n (%)	25 (83)	10 (77)	15 (88)	0.628
BMI, (kg/m^2)	28 (5)	27 (5)	29 (4)	0.257
History				
Hypertension, n (%)	8 (27)	3 (23)	5 (29)	1.000
Current smoking, n (%)	21 (70)	10 (77)	11 (65)	0.691
Hypercholesterolemia, n (%)	13 (43)	6 (46)	7 (41)	1.000
Diabetes mellitus‡, n (%)	2 (7)	1 (8)	1 (6)	1.000
Previous angina, n (%)	3 (10)	2 (15)	1 (6)	0.565
Previous myocardial infarction, n (%)	1 (3)	0 (0)	1 (6)	1.000
Previous PCI, n (%)	1 (3)	0 (0)	1 (6)	-

Presenting characteristics					
Heart rate, bpm		77 (17)	81 (14)	75 (19)	0.340
Systolic blood pressure, mmHg		141 (26)	143 (16)	139 (33)	0.712
Diastolic blood pressure, mmHg		84 (12)	86 (11)	83 (13)	0.472
Time from symptom onset to reperfusion, min		156 (112, 243)	137 (112, 274)	161 (118, 206)	0.837
Ventricular fibrillation†, n (%)		1 (3)	0 (0)	1 (6)	1.000
Heart failure, Killip class at presentation, n (%)	I	22 (74)	9 (69)	13 (76)	
	II	7 (23)	3 (23)	4 (24)	0.811
	III/IV	1 (3)	1 (8)	0 (0)	
Electrocardiogram					
ST segment elevation resolution post PCI, n (%)					
Complete, ≥70 %		15 (50)	1 (8)	1 (6)	
Partial, 30% to < 70%		13 (43)	6 (46)	7 (41)	1.000
None, ≤30%		2 (7)	6 (46)	9 (53)	
Number of diseased arteries¥, n (%)	1	14 (47)	4 (31)	10 (58)	
	2	11 (37)	6 (46)	5 (29)	0.298
	3	5 (17)	3 (23)	2 (12)	

	LM	0 (0)	0 (0)	0 (0)	
Culprit artery, n (%)	LAD	9 (30)	5 (38)	4 (24)	
	LCX	10 (33)	6 (46)	4 (24)	0.112
	RCA	11 (37)	2 (15)	9 (53)	
TIMI coronary flow grade pre-PCI, n (%)	0/1	24 (80)	12 (92)	12 (71)	0.196
	2/3	6 (20)	1 (8)	5 (29)	
TIMI coronary flow grade post-PCI, n (%)	0/1	0 (0)	0 (0)	0 (0)	
	2	2 (7)	1 (8)	1 (6)	1.000
	3	28 (93)	12 (92)	16 (94)	
Initial blood results on admission					
Neutrophil count ($x10^9L$)		10.1 (3.1)	11.3 (3.0)	9.3 (3.0)	0.083
NT-proBNP, pg/mL		588 (306, 1541)	864 (655, 1637)	529 (301, 1254)	0.841

*P-values were obtained from t-tests or Mann-Whitney test as appropriate for continuous variables, and Fisher's tests for categorical variables.

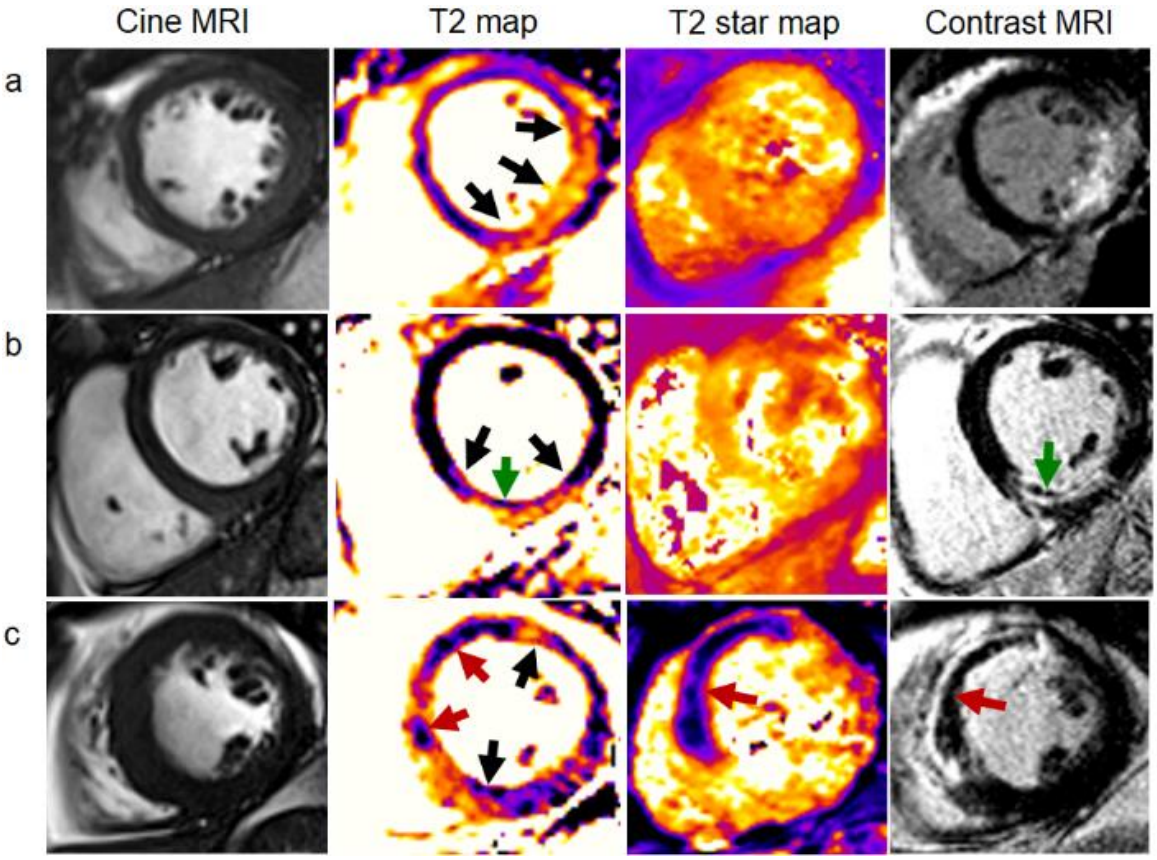
Table 4-3 Baseline and 6-month CMR findings of the entire patient population and according to the presence of myocardial haemorrhage.

Characteristics*	All Patients n = 245	Haemorrhagic infarct (T2*core +) n = 101	Non-haemorrhagic infarct (T2*core -) n = 144	p value
CMR findings 2 days post-MI				
LV ejection fraction, %	55 (10)	51 (10)	57 (8)	<0.001
LV end-diastolic volume, ml	153 (34)	164 (34)	145 (32)	<0.001
Men	161 (32)	171 (31)	153 (32)	<0.001
Women	124 (23)	127 (26)	123 (22)	0.534
LV end-systolic volume, ml	71 (26)	82 (27)	64 (22)	<0.001
Men	76 (26)	86 (27)	68 (23)	<0.001
Women	55 (15)	61 (16)	53 (14)	0.037
LV mass, g	135 (38)	147 (39)	127 (35)	<0.001
Men	146 (34)	154 (38)	140 (30)	0.006
Women	99 (24)	111 (26)	94 (21)	0.01
Oedema and infarct characteristics				
Area at risk, % LV mass	33 (12)	39 (11)	29 (10)	<0.001
Infarct size, % LV mass	19 (14)	29 (12)	12 (10)	<0.001
Myocardial salvage, % of LV mass	19 (9)	18 (8)	20 (10)	0.064
Myocardial salvage index, %	61 (24)	46 (17)	71 (17)	<0.001
Late microvascular obstruction present, n (%)	133 (54 %)	101 (100%)	32 (22 %)	<0.001
Late microvascular obstruction, % LV mass	0.5 (0.0, 4.2)	5.3 (2.1, 9.5)	0.0 (0.0, 0.0)	<0.001
T2 hypointense core present, n (%)	161 (66%)	101 (100%)	60 (42%)	<0.001
CMR findings 6 months post-MI (n = 228)				
LV ejection fraction, %	61 (9)	56 (10)	65 (7)	<0.001
Change in LV ejection fraction at 6 months from baseline, %	7 (8)	5 (7)	8 (8)	0.005
LV end-diastolic volume, ml	160 (42)	180 (49)	146 (30)	<0.001

Men	169 (42)	188 (48)	154 (29)	<0.001
Women	128 (23)	133 (20)	127 (24)	0.066
Change in LV end-diastolic volume at 6 months from baseline, ml	6 (28)	15 (30)	1 (22)	<0.001
LV end-systolic volume, ml	64 (33)	82 (40)	52 (20)	<0.001
Men	69 (35)	86 (42)	55 (21)	<0.001
Women	48 (17)	62 (13)	43 (15)	<0.001
Change in LV end-systolic volume at 6 months from baseline, ml	-7 (22)	0 (26)	-12 (18)	<0.001
LV mass, g	119 (30)	127 (30)	114 (28)	0.001
Men	128 (27)	133 (2)	123 (26)	0.007
Women	91 (18)	95 (17)	90 (18)	0.270
Infarct size, % LV mass	13 (10)	21 (10)	8 (7)	<0.001

**P-values were obtained from t-tests or Mann-Whitney test as appropriate for continuous variables, and Fisher's tests for categorical variables.*

Figure 4-2 Examples of acute reperfused STEMI patients with and without evidence of myocardial haemorrhage on day 2 CMR



Three patients with acute STEMI treated by primary PCI using the same anti-thrombotic strategies. Each patient had normal TIMI grade 3 flow at the end of PCI. Cardiac MRI was performed for each patient 2 days post-reperfusion. **(a)** ***Patient with no evidence of myocardial haemorrhage or microvascular obstruction****. T2 within the injury zone (middle left) measured 67.8 ms. T2* within the injury zone (middle right) measured 39 ms. Acute infarct size revealed by late gadolinium enhancement (LGE) (right) was 24%. The LVEF and LV end-diastolic volume were 51% and 132 ml, respectively. Analysis of the repeat MRI scan after 6 months follow-up indicated that the final infarct size was 18% of LV mass and the LV end-diastolic volume had reduced to 109 ml. This patient had an uncomplicated clinical course.* **(b)** ***Patient with T2 hypointense core and microvascular obstruction, in the absence of haemorrhage****. T2 mapping (middle left) revealed a hypointense region within the infarct core (green arrow), corresponding to the the area of microvascular obstruction (MVO) on contrast-enhanced MRI (right; green arrow). T2 within the infarct core measured 53 ms, which was substantially lower than the T2 value measured at the periphery of the infarct zone (72 ms). T2* within the injury zone measured 36 ms (middle right). Acute infarct size revealed by LGE (right) was 19%. The LVEF and end-diastolic volume were 52% and 158 ml, respectively. Six month follow-up CMR revealed infarct size was 15% of left ventricular mass and there was an increase in the LV*

end-diastolic volume to 171 ml. This patient had no adverse events during follow-up. **(c)** ***Patient with myocardial haemorrhage****. T2 mapping (middle left) revealed a hypointense region within the infarct core (red arrow), corresponding to the hypointense region on T2* map (middle right; red arrow) and the area of MVO on contrast-enhanced MRI (right; red arrow). T2 within the infarct core measured 44 ms, which was substantially lower than the T2 value measured at the periphery of the infarct zone (61 ms). T2* within the infarct core measured 9 ms. Acute infarct size revealed by LGE (right) was 38%. MVO depicted as the central dark zone within the infarct territory (red arrow) was 13% of LV mass. The LVEF and end-diastolic volume were 40.8% and 190 ml, respectively. The final infarct size at 6 months was 32% of LV mass and the LV end-diastolic volume had increased to 231 ml. This patient was re-hospitalised for new onset heart failure during follow-up and ICD implantation (after 8 months), following a deterioration in LVEF.*

4.6.4 Comparison of myocardial haemorrhage (T2 core), T2 hypointense core and microvascular obstruction*

A hypointense infarct core was detected with T2 mapping in 161 (66%) STEMI patients. Microvascular obstruction with early gadolinium- and late gadolinium enhancement CMR was revealed in 151 (62%) and 133 (54%) patients, respectively. All patients with myocardial haemorrhage, as defined by T2* imaging, had late microvascular obstruction and a hypo-intense core on T2 imaging. In contrast, 32 (13%) patients had late microvascular obstruction in the absence of myocardial haemorrhage and all of these patients had a hypo-intense core on T2 imaging. 28 (11%) patients had a T2 hypo-intense core without evidence of late microvascular obstruction or myocardial haemorrhage.

The results of intra- and inter-observer agreement of T2 and T2* core measurements are shown in chapter 3, section 3.5.2.

4.6.5 Myocardial haemorrhage and associations with clinical characteristics

101 STEMI survivors had evidence of myocardial haemorrhage revealed by T2* mapping with CMR 2 days post-MI. The clinical characteristics that were univariably associated with the presence of myocardial haemorrhage, from binary logistic regression were: male gender (odds ratio (95% confidence interval (CI)): (1.97 (1.04, 3.71); p=0.037), history of previous PCI (5.16 (1.38, 19.27); p=0.015, current smoker (1.66 (0.97, 2.84); p=0.064),

Killip heart failure classification >2 (30.37 (3.93, 234.69); p=0.001), TIMI coronary flow 2/3 pre-PCI (0.26 (0.13, 0.51); p<0.001), and ≤30% ST-segment resolution post-PCI (3.37 (1.56, 7.26); p=0,002).

In stepwise logistic regression using AIC, myocardial haemorrhage was independently associated with sex, smoking, history of previous PCI, TIMI coronary flow grade at initial angiography, ECG evidence of reperfusion injury and Killip class (all p<0.03) (table 4-4).

Table 4-4 Associates of myocardial haemorrhage, as defined by T2* CMR, in multivariable stepwise regression analyses (n=245).

Multiple stepwise regression	Odds ratio (95% CI)	p value
A. Including patient characteristics and angiographic data		
Male	2.36 (1.15, 4.85)	0.019
Previous PCI	5.92 (1.23, 28.56)	0.027
Smoker	2.45 (1.21, 4.96)	0.013
Killip class >2	15.13 (1.86, 123.12)	0.011
TIMI flow >1 at initial angiography	0.27 (0.13, 0.56)	<0.001
≤30% ST-segment resolution post-PCI	3.08 (1.27, 7.50)	0.013

Footnote: The odds ratio (95% confidence intervals) indicates the magnitude and direction for myocardial haemorrhage.

4.6.6 Myocardial haemorrhage and adverse remodelling at 6-months

At 6 months, LV end-diastolic volume increased on average (SD) by 6 (27) ml in 224 patients with evaluable data (table 4-3). The average increase in LV end-diastolic volume at 6 months was greater in patients with myocardial haemorrhage compared to those without (15 (30) vs. 1 (22); p<0.001). Adverse remodelling, defined as an increase in LV end-diastolic volume by ≥20%, occurred in 27 (12%) patients and 17 (63%) of these patients had myocardial haemorrhage at baseline.

The clinical characteristics that were univariably associated with adverse remodelling and their p-values that were included in the multivariable model were: age (p=0.804), male gender (p=0.811), body mass index (p=0.693), previous MI (p=0.306), diabetes mellitus (p=0.816), previous PCI (p=0.469), cigarette smoking (p=0.500), history of hypertension

(p=0.329), history of hypercholesterolaemia (p=0.774), history of angina (p=0.816), heart rate (p=0.167), systolic blood pressure at initial angiography (p=0.511), Killip class II vs. Killip class I (reference category) (p=0.046), Killip class III/IV vs. Killip class I (reference category) (p=0.031), symptom onset to reperfusion time (p=0.355), TIMI flow grade 2/3 vs. grade 1 (reference category) at initial angiography (p=0.529), ST segment resolution (none vs. complete (reference category), and p=0.343; incomplete vs. complete (reference category), p=0.064).

The presence of myocardial haemorrhage (binary) was multivariably associated with adverse remodelling, independent of baseline LV end-diastolic volume (odds ratio (95% CI): 2.64 (1.07, 6.49); p=0.035) (table 4-5). Patients with myocardial haemorrhage on MRI had significantly higher NT-proBNP results at 6 month follow-up, compared to patients without evidence of haemorrhage (247 (158, 570) vs. 108 (61, 226) pg/mL; p<0.001).

In multivariable regression, T2* core (continuous, ms) was not associated with adverse remodelling.

Table 4-5 Multivariable predictors of adverse LV remodelling at 6 months post-STEMI.

Multiple stepwise regression	Odds ratio (95% CI)	p value
*Patient characteristics and angiographic findings**		
Myocardial haemorrhage	**2.60 (1.16, 5.86)**	**0.021**
Patient characteristics, angiographic findings and LV end-diastolic volume		
Myocardial haemorrhage	**2.64 (1.07, 6.49)**	**0.035**
Killip class 2	2.62 (1.04, 6.62)	0.041
LV end-diastolic volume at baseline, ml	0.99 (0.97, 1.00)	0.043

Footnote: The odds ratio (95% confidence intervals) indicates the magnitude and direction for adverse LV remodelling.

4.6.7 Myocardial haemorrhage, microvascular obstruction, T2 hypointense core and LV outcomes at 6 months

The relationships for the presence of myocardial haemorrhage, T2 map core and microvascular obstruction for LV outcomes, including LV end-diastolic volumes and LV

ejection fraction are shown in table 4-6. Myocardial haemorrhage is consistently associated with worse LV outcomes 6 months post-MI.

Table 4-6 Relationships for the presence of myocardial haemorrhage (T2* core), T2 map core and microvascular obstruction, and left ventricular outcomes at baseline and follow-up.

	LVEDV baseline	LVEDV 6 months	Change LVEDV	Adverse remodelling	LVEF at baseline	LVEF 6 months	LVEF change
T2* core (binary)	<0.001	<0.001	<0.001	0.021	<0.001	<0.001	0.006
Direction of relationship	(+)ve	(+)ve	(+)ve	(+)ve	(-)ve	(-)ve	(-)ve
T2 core (binary)	0.001	<0.001	0.003	0.023	<0.001	<0.001	0.010
Direction of relationship	(+)ve	(+)ve	(+)ve	(+)ve	(-)ve	(-)ve	(-)ve
MVO (binary)	<0.001	<0.001	<0.001	0.048	<0.001	<0.001	0.010
Direction of relationship	(+)ve	(+)ve	(+)ve	(+)ve	(-)ve	(-)ve	(-)ve

4.6.8 Myocardial haemorrhage and longer term health outcomes

245 (100%) patients had longer term follow-up completed. The median duration of follow-up was of 827 days. 8 (3.3%) patients died or experienced a heart failure event post-discharge. The presence of myocardial haemorrhage (binary) was associated with cardiovascular cause of death or heart failure hospitalisation post discharge (hazard ratio 12.9, 95% CI 1.6, 100.8; p=0.015).

T2* core (continuous, ms) was not associated with health outcome.

4.6.9 Temporal evolution of myocardial haemorrhage and microvascular obstruction from acute reperfusion through to 6 months

Intramyocardial haemorrhage occurred in 7 (23%), 13 (43%), 11 (33%), and 4 (13%) patients, versus microvascular obstruction in 18 (60%), 17 (57%), 10 (33%) and 0 patients at 4 - 12 hours, 3 days, 10 days and 7 months, respectively (table 4-7). The amount of microvascular obstruction (% LV mass) in patients with haemorrhagic infarction was at its greatest at 4 – 12 hours post-reperfusion and remained similar at day 3 CMR, then reduced by day 10 (table 4-8). In contrast, the amount of myocardial haemorrhage progressively increased from 4 – 12 hours with a peak at day 3 and decreased by day 10 (p=0.001) (table 4-8). The amount of T2 hypointense core (% LV mass) followed a similar pattern to haemorrhage. At 7 months, 4 (13%) patients had evidence of persisting haemorrhage, but none of the patients had microvascular obstruction.

Table 4-7 CMR findings of serial imaging sub-group (n=30) at 4 time intervals post-reperfusion.

	4 < 12 hours n = 30	3 Days n = 30	10 Days n =30	6-7 months n =30
LV ejection fraction, %	52 (9)	56 (9)	59 (8)	59 (8)
LV end-diastolic volume, ml	158 (135, 184)	165 (129, 181)	164 (132, 194)	161 (120, 196)
Area at risk, % LV mass	34 (10)	39 (12)	31 (12)	-
Infarct size, % LV mass	19 (13)	20 (13)	14 (10)	14 (10)
Late microvascular obstruction present, n (%)	18 (60)	17 (57)	10 (33)	0
Early microvascular obstruction, n (%)	20 (67)	17 (57)	15 (50)	-
T2 hypointense core present, n (%)	19 (63)	18 (60)	14 (47)	0
Myocardial haemorrhage				

present, n (%)	7 (23)	13 (43)	11 (37)	4 (13)

Table 4-8 The temporal evolution of amount (% LV mass) of microvascular obstruction, T2 hypointense core and myocardial haemorrhage in acute reperfused STEMI patients (n=13).

	4 < 12 hours	3 Days	10 Days	6-7 months
Late microvascular obstruction, % LV mass	5.3 (2.1, 10.5)	5.4 (2.7, 8.4)	1.3 (0.1, 4.2)	-
T2 hypointense core, % LV mass	6.0 (4.6, 8.7)	9.9 (6.6, 11.3)	3.3 (1.5, 6.4)	-

Footnote: the amount of microvascular obstruction and T2 hypointense were calculated using full LV coverage, whereas myocardial haemorrhage is the average of basal, mid and apical slice acquisitions.

4.6.10 Persistence of microvascular obstruction in relation to the presence of myocardial haemorrhage

Microvascular obstruction resolved by day 10 in 8 (44%) patients, 2 (25%) of whom had evidence of myocardial haemorrhage. Whereas microvascular obstruction persisted at day 10 in 10 (56%) patients, all (100%) of whom had evidence of haemorrhage.

4.7 Discussion

We have undertaken the largest clinical study to date of myocardial haemorrhage using diagnostic T2* CMR mapping in a relatively unselected STEMI population following emergency invasive management. We have also reported for the first time a serial imaging analysis for the evolution and time-course of myocardial haemorrhage and microvascular obstruction in the early reperfusion period.

Our main findings are 1) the incidence of myocardial haemorrhage occurred in 41% of STEMI patients and the presence of myocardial haemorrhage was associated with the

clinical severity of MI and was associated with adverse LV remodelling, although when analysed in a continuous format, there was no relationship between T2* and remodelling. IMH was also associated with cardiovascular death and first hospitalisation for heart failure, 2) considering the time-course of myocardial haemorrhage in a sub-group of STEMI patients, CMR within 12 hours of emergency PCI revealed myocardial haemorrhage in approximately one quarter of the patients and the incidence nearly doubled by day 3, implying a hyper-acute phase followed by secondary haemorrhage, 3) myocardial haemorrhage was a secondary event, which dynamically increased, following the initial occurrence of microvascular obstruction, 4) the severity of microvascular obstruction affected its degree of persistence and the presence of haemorrhage differentiated persistent, structural microvascular destruction from functional, potentially reversible microvascular obstruction, 5) a hypointense infarct core on T2-mapping always occurred in the presence of microvascular obstruction and commonly in the absence of myocardial haemorrhage within 12 hours and 3 days post-MI, indicating that the presence of T2-core is more closely associated with microvascular obstruction than myocardial haemorrhage.

Studies to date of reperfusion haemorrhage, have been limited by either the subjective nature of qualitative evaluation of haemorrhage or not using haemorrhage sensitive CMR sequences. Most clinical studies have used dark blood T2-weighted imaging to detect haemorrhage (Ganame et al., 2009, Beek et al., 2010, Bekkers et al., 2010a, Eitel et al., 2011, Amabile et al., 2012, Husser et al., 2013), however, this qualitative technique is hampered by imaging artefact (Wince and Kim, 2010) and false-positive effects of microvascular obstruction (Cannan et al., 2010, Jackowski et al., 2006). Thus, it does not seem feasible to differentiate microvascular obstruction and haemorrhage based solely on T2-weighted imaging.

T2-weighted imaging is also strongly influenced by oedema and the hyperintense signal from oedema may mask the hypointense signal from haemorrhage (Lotan et al., 1992), on the contrary T2* techniques are relatively insensitive to the effects of oedema (Kali et al., 2013b). Quantitative T2 mapping addresses the limitations associated with T2-weighted techniques (Wince and Kim, 2010, Cannan et al., 2010, Jackowski et al., 2006, Lotan et al., 1992), offers increased accuracy in the detection of myocardial oedema and may provide a more objective assessment of the infarct core because it directly measures T2 relaxation times (Giri et al., 2009, Verhaert et al., 2011, Ghugre et al., 2011, Zia et al., 2012, Ugander

et al., 2012, Hammer-Hansen et al., 2014, Nassenstein et al., 2014, Park et al., 2013), and T2*-mapping holds greater promise.

The temporal evolution of myocardial haemorrhage and relationship with microvascular obstruction, in the early post-infarct period is incompletely understood. Experimental studies have inferred that haemorrhage occurs as a consequence of reperfusion (Pislaru et al., 1997), whereas other studies have implied that haemorrhage may be a secondary phenomenon due to progressive capillary breakdown (Fishbein et al., 1980, Payne et al., 2011a, Robbers et al., 2013, Kumar et al., 2011). A recent experimental study by Robbers et al (Robbers et al., 2013) indicated that microvascular obstruction might be a modifiable precursor of haemorrhage, which represented irreversible microvascular destruction. Our serial imaging data support the notion that haemorrhage occurs as a complication of microvascular obstruction, since microvascular obstruction was at its greatest extent from the outset, while haemorrhage progressively increased from <12 hours to day 3 post-reperfusion. Also, in accordance with other recent studies using T2* imaging to define haemorrhage (Kali et al., 2013b, Kumar et al., 2011, Zia et al., 2012, Kidambi et al., 2013, O'Regan et al., 2010, Mather et al., 2011b), we observed that haemorrhage only occurred within regions of microvascular obstruction.

Previous studies using dark blood T2-weighted imaging to define haemorrhage showed that microvascular obstruction occurred commonly in the absence of a T2 hypointense core (Amabile et al., 2012, Eitel et al., 2011, Ganame et al., 2009, Kandler et al., 2014). In contrast, we observed that all patients with microvascular obstruction had a hypointense core on T2-mapping. Our results concur with the findings of one of only a few histologically confirmed postmortem analyses (Jackowski et al., 2006), which showed that a T2 hypointense core always represented microvascular obstruction, with or without haemorrhage.

We also observed that a T2 hypointense core was more closely related to early microvascular obstruction, than late microvascular obstruction or haemorrhage. The occurrence of a T2 hypointense core on T2 maps in the absence of haemorrhage likely represents a reduction in the effective tissue water to the infarct core due to associated obstructed capillary flow (e.g. cellular debris and extrinsic oedema) and microvascular spasm, thereby reducing the supply of protons and subsequent reduction in T2 signal.

There are conflicting data regarding the temporal change in size of microvascular obstruction in the early reperfusion period. Canine studies (Wu et al., 1998a, Rochitte et al., 1998) have shown that the amount of microvascular obstruction increases in the first 48 hours after reperfusion and then remains stable between 2 and 9 days. However there have not been any confirmatory studies in humans to demonstrate expansion of microvascular obstruction at any time-point post-reperfusion. Our findings indicate that the extent of microvascular obstruction remains stable between 4 – 12 hours and day 3, then decreases to day 10, which is in agreement with other clinical data, demonstrating that microvascular obstruction appears small at one week (Orn et al., 2009, Mather et al., 2011a).

Persistent microvascular obstruction at 1 week appears to be a different entity to microvascular obstruction resolving the first week. Our study may explain the variability in time-course data of resolution of microvascular obstruction, since T2* imaging is able to differentiate structural microvascular destruction (i.e. haemorrhage) from potentially reversible, functional microvascular obstruction.

Our results have important clinical implications. Reperfusion haemorrhage related effects on LV end-diastolic volume and LV ejection fraction occur early and significant changes can be observed within the first 10 days post-MI. These early changes result in long-term adverse remodelling and this could represent a high risk group that should be targeted for anti-remodelling therapy. In addition haemorrhage is a non-contrast CMR biomarker with potential to reflect the efficacy of novel therapeutic interventions in STEMI patients.

4.7.1 Limitations

A main limitation of our study is lack of pathological correlation of our imaging results. As a result of time constraints we only acquired 3 short-axis slices using T2* mapping and therefore minor degrees of haemorrhage could have been missed. We could also not compare the total amount of haemorrhage to total amount of microvascular obstruction or T2 hypointense core due to the limited T2* slice acquisition. In addition, the inclusion of thrombolysed patients may represent a confounder and the sample size of this sub-group is too small to draw any conclusions. There was a substantial proportion of artefacts with the T2* sequence, limiting quantification of haemorrhage in a high number of patients. The use of high-pass filtered processing may have helped to overcome these limitations (Goldfarb et al., 2013). In addition, a technique has been proposed for applying an

automated truncation method to pixel wise T2* mapping to improve image quality (Sandino et al., 2015). Despite the technical limitations, T2* seems to be the most sensitive cardiac MRI sequence to detect haemorrhage.

4.7.2 Conclusion

We found that myocardial haemorrhage occurs commonly and is a biomarker for prognostication in STEMI survivors. The severity of MVO affects its degree of persistence and T2* imaging differentiates persistent, structural microvascular injury from functional, potentially reversible MVO. Haemorrhage occurs in primary and secondary phases within the first 10 days post-MI and is a secondary phenomenon to the initial occurrence of microvascular obstruction.

5 Chapter 5: Myocardial haemorrhage after acute reperfused ST-elevation myocardial infarction evolves dynamically and contributes to the early bimodal pattern in myocardial oedema: advanced imaging and clinical significance

5.1 Introduction

In acute ST-elevation myocardial infarction (STEMI), myocardial haemorrhage is a complication that is associated with the duration of ischemia and reperfusion (Betgem et al., 2014, Kloner et al., 1974, Jaffe et al., 2008, Higginson et al., 1982), and is an adverse prognostic factor in the longer term (Ganame et al., 2009, Amabile et al., 2012, Eitel et al., 2011, Husser et al., 2013). Myocardial haemorrhage is potentially a therapeutic target for novel interventions however the temporal evolution of myocardial haemorrhage and its association with other MI characteristics early post-MI are uncertain.

Myocardial oedema is a consequence of ischemia and infarction and has functional importance, since oedema impairs myocyte contractility (Bragadeesh et al., 2008). The extent of oedema revealed by cardiac magnetic resonance (CMR) is a retrospective marker of the ischemic area-at-risk (Aletras et al., 2006, Berry et al., 2010, Garcia-Dorado et al., 1993), which in turn is a prognostic determinant post-MI (Califf et al., 1985). For oedema to be taken as a retrospective marker of the area-at-risk, its initial size should be stable. Dall'Armelina *et al* (Dall'Armellina et al., 2011) reported that the area-at-risk was maximal and constant in size within the first 5 - 7 days post-MI but then decreased in size subsequently. Recently, Fernández-Jiménez *et al* (Fernandez-Jimenez et al., 2015) assessed myocardial oedema in a swine model of MI (with or without reperfusion) at 2 hours, 24 hours, 4 days or 7 days (n=5 per group) using CMR and quantification of myocardial water content by post-mortem tissue desiccation. They found a bimodal pattern in myocardial oedema with high water content peaks at 2 hours and 7 days post-reperfusion and an intervening decrease in myocardial water content at 24 hours.

Tissue haemorrhage is typically characterised by an acute primary phase and then potentially secondary haemorrhagic transformation in the sub-acute phase hours – days later (Fishbein et al., 1980, Alvarez-Sabin et al., 2013), and deoxyhemoglobin has paramagnetic effects that enable myocardial haemorrhage to be detected using T2- and T2*-weighted CMR (Anzalone et al., 2004, Payne et al., 2011a). Since myocardial haemorrhage is virtually universal in swine after 40 minutes of ischemia the observations by Fernández-Jiménez *et al* (Fernandez-Jimenez et al., 2015) could be explained by myocardial haemorrhage, however, the time-course and relationships between myocardial oedema and haemorrhage early post-MI in STEMI survivors are uncertain.

We hypothesized that 1) myocardial haemorrhage evolves progressively after acute STEMI with incident haemorrhage occurring in some patients immediately after reperfusion followed by a secondary phase of haemorrhage, 2) T2 and T2* signals within the infarct zone follow similar time-courses and 3) T2 signal is inversely associated with the amount of haemorrhage, whereas the extent of oedema (area-at-risk) is stable.

5.2 Methods

5.2.1 Study population and STEMI management

To examine these hypotheses, we performed a comprehensive longitudinal CMR study of myocardial haemorrhage and oedema in a cohort of reperfused STEMI survivors in a single regional cardiac centre between 3 November 2011 and 18 September 2012. Thirty STEMI patients provided written informed consent and the eligibility criteria and acute STEMI management are as described in detail in chapter 2.

5.2.2 CMR acquisition

CMR was performed on 4 occasions (4 to 12 hours and approximately 3 days, 10 days and 7 months) post-reperfusion as described in chapter 2. The imaging protocol which was the same between scans was performed as described in chapter 2. Briefly, this included cine CMR with steady-state free precession (SSFP), T2*-mapping, T2-mapping (Giri et al., 2009, Verhaert et al., 2011), and delayed-enhancement phase-sensitive inversion-recovery pulse sequences (Kellman et al., 2002).

CMR was also performed in 50 healthy volunteers of similar age and gender in order to obtain local reference values for myocardial T2 and T2* (chapter 2 and 3).

5.2.3 CMR analyses

The images were analysed on a Siemens work-station by observers with at least 3 years CMR experience (N.A., D.C., I.M., and S.R.). All of the images were reviewed by an experienced CMR cardiologist (C.B.). LV dimensions, volumes and ejection fraction were quantified using computer assisted planimetry (syngo MR®, Siemens Healthcare, Erlangen, Germany). The late gadolinium enhancement images were analysed by observers (N.A., I.M.) who were blinded to all of the other data.

T2 and T2* standardised measurements in myocardial regions of interest (defined as (1) remote myocardium, (2) injured myocardium and (3) infarct core) were performed as described in chapter 2.

5.2.4 Myocardial Haemorrhage

Myocardial haemorrhage was scored visually. On the T2* maps, a region of reduced signal intensity within the infarcted area, with a T2* value of <20 ms (Ghugre et al., 2011, Kandler et al., 2014, O'Regan et al., 2010, Anderson et al., 2001), was considered to confirm the presence of myocardial haemorrhage.

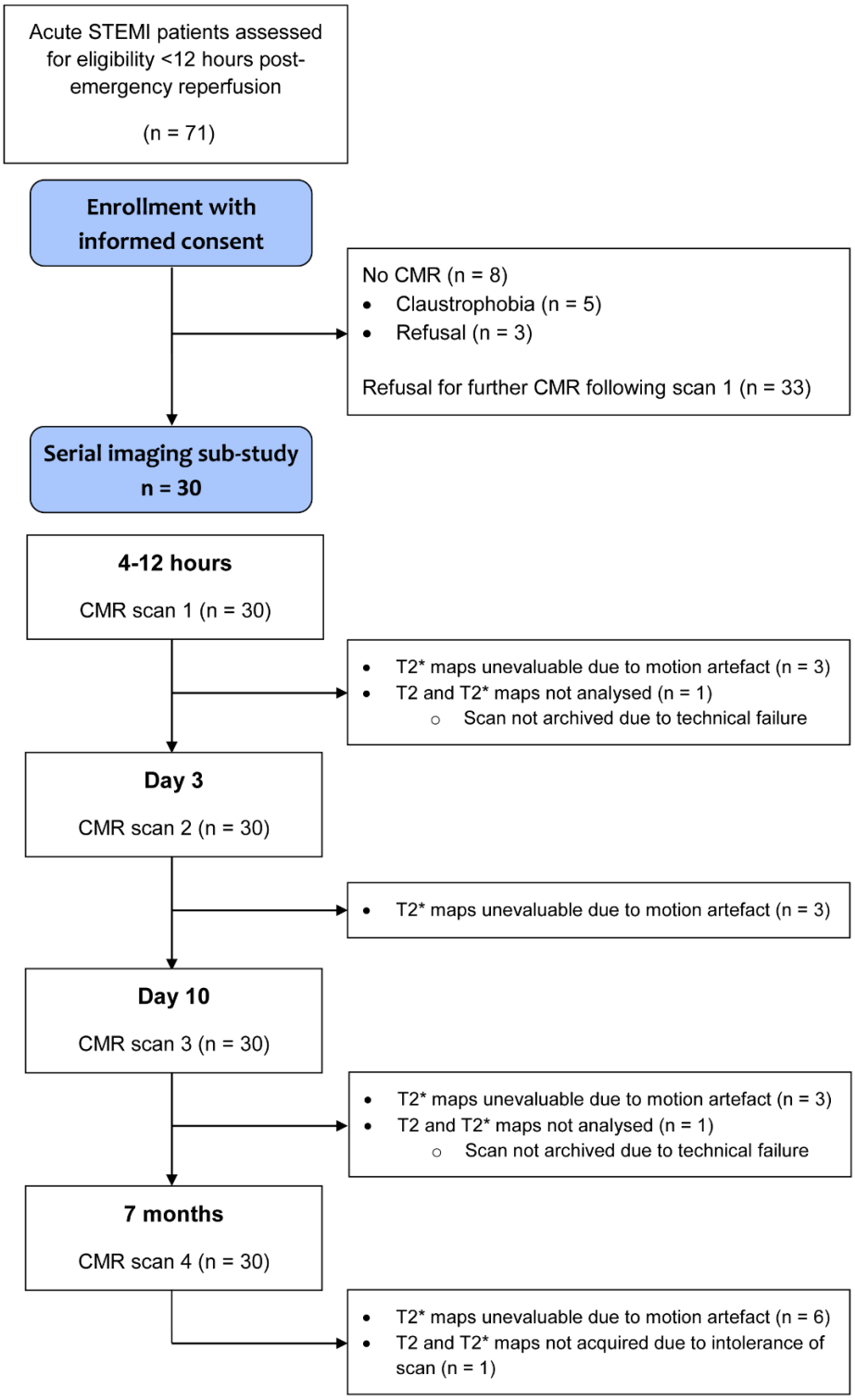
5.3 Statistical analyses

Categorical variables are expressed as number and percentage of patients. Most continuous variables followed a normal distribution and are therefore presented as means together with standard deviation. Those variables that did not follow a normal distribution are presented as medians with interquartile range. Differences between independent groups were assessed using t-tests, Mann-Whitney tests, or Fisher's tests where appropriate. Changes over time were assessed using generalized linear mixed effects models with subject ID as the only random effect. A p-value > 0.05 indicates the absence of a statistically significant effect. Random effects models were used to compute inter-and intra- rater reliability measures (intra-class correlation coefficient (ICC)) for the reliability of remote zone, infarct zone and infarct core T2 and T2* values measured independently by 2 observers in 20 randomly selected patients from the cohort.

5.4 Results

Thirty STEMI patients (mean age 54 years, 83% male) (table 5-1) gave informed consent and underwent serial CMR at 1.5 Tesla on 4 occasions (figure 5-1). The CMR examinations were performed (mean±SD) 8.6±3.1 hours, 2.9±1.5 days, 9.6±2.3 days and 213±27 days following primary PCI. Evaluable T2 and T2* data were available in 117 (98%) and 102 (85%) CMR scans, respectively (figure 5-1). Information on vital status and SAEs were available in all of the participants.

Figure 5-1 Study flow diagram



5.4.1 Temporal evolution of myocardial haemorrhage following ischemia/reperfusion

Myocardial haemorrhage occurred in 7 (23%), 13 (43%), 11 (33%), and 4 (13%) patients at 4 - 12 hours, 3 days, 10 days and 7 months, respectively (table 5-2). Clinical case examples are shown in figure 2. In patients with myocardial haemorrhage, the amount of haemorrhage (% LV) increased progressively from 4 – 12 hours with a peak at 3 days and then a decrease at 10 days (p=0.001) (table 5-2 and figure 3). The opposite pattern was seen with T2* core values, with a nadir at day 3 CMR (p=0.004) (table 5-3).

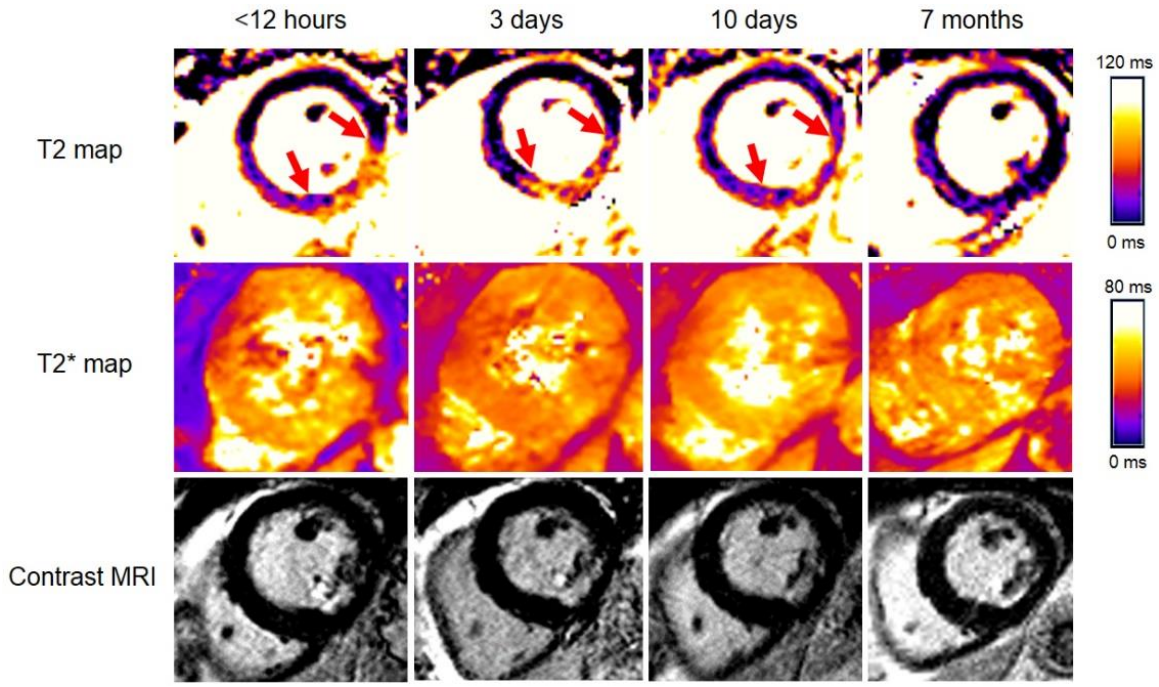
5.4.2 Temporal evolution of myocardial oedema and the area-at-risk

The extent of myocardial oedema (% LV) increased from the initial CMR scan 4 – 12 hours post-MI to a maximum 3 days post-MI and then reduced at 10 days and 7 months post-MI. The AAR fluctuated in size in both groups with an increase from 4 – 12 hours to a maximum at 3 days followed by a modest reduction in size by day 10 and a marked reduction by 7 months when the oedema could not be reliably measured (table 5-2).

The increase in the extent of oedema at 3 days mirrored the increase in haemorrhage at this time-point. In addition, the end-diastolic wall thickness measured in the infarct zone, followed the same temporal changes (table 5-4)

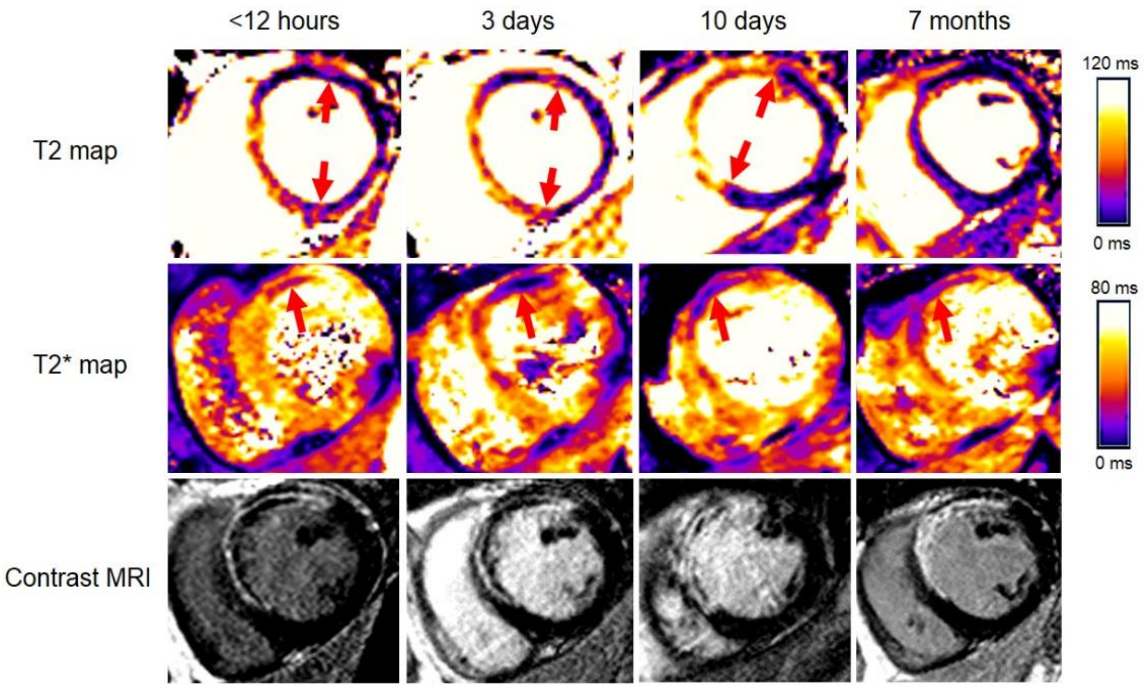
Figure 5-2 CMR T2 mapping, T2* mapping and contrast enhanced images at 4 time-points post-reperfusion, from patients with and without myocardial haemorrhage, following emergency percutaneous coronary intervention (PCI).

(a) Case 1 (no hemorrhage)



Patient with no myocardial haemorrhage. *T2 value within infarct zone measured 59 ms at 8 hours post-reperfusion, 65 ms at day 3, 76 ms at day 10 and 50 ms 7 months post-MI. Late gadolinium enhancement (LGE) imaging revealed a sub-endocardial infero-lateral infarct, with no evidence of microvascular obstruction. There was no hypointense core on T2* maps. LV ejection fraction progressively increased from 56% to 69% and LV end-diastolic volumes progressively reduced from 151 ml to 129 ml at 7 months.*

(b) Case 2 (hemorrhage)



Patient with myocardial haemorrhage. *T2 maps reveal a hypo-intense infarct core, which corresponded to the hypointense core on T2* maps and to the region of microvascular obstruction. T2* within the infarct core measured 18 ms at 11 hours post-reperfusion, 7 ms at day 3, 13 ms at day 10 and 18 ms at 7-months post-MI. Corresponding T2 core measurements were 55 ms at 11 hours, 46 ms at day 3 and 58 ms at day 10. LGE imaging revealed a transmural antero-septal infarct, with extensive microvascular obstruction. LV ejection fraction did not improve and measured 46% on day 3 vs. 45% at 7 months. LV end-diastolic volumes progressively increased from 165 ml to 210 ml at 7 months.*

Table 5-1 Clinical and angiographic characteristics of the 30 patients in the longitudinal clinical study.

Characteristics*	All Patients n = 30	No myocardial hemorrhage (T2*core -) n = 17 (57%)	Myocardial hemorrhage (T2*core +) n = 13 (43%)	p value
Clinical				
Age, years	54 (10)	55 (9)	53 (11)	0.602
Male sex, n (%)	25 (83)	15 (88)	10 (77)	0.628
BMI, (kg/m^2)	28 (5)	29 (4)	27 (5)	0.257
History				
Hypertension, n (%)	8 (27)	5 (29)	3 (23)	1.000
Current smoking, n (%)	21 (70)	11 (65)	10 (77)	0.691
Hypercholesterolemia, n (%)	13 (43)	7 (41)	6 (46)	1.000
Diabetes mellitus‡, n (%)	2 (7)	1 (6)	1 (8)	1.000
Previous angina, n (%)	3 (10)	1 (6)	2 (15)	0.565
Previous myocardial infarction, n (%)	1 (3)	1 (6)	0 (0)	1.000
Previous PCI, n (%)	1 (3)	1 (6)	0 (0)	-

Presenting characteristics					
Heart rate, bpm		77 (17)	75 (19)	81 (14)	0.340
Systolic blood pressure, mmHg		141 (26)	139 (33)	143 (16)	0.712
Diastolic blood pressure, mmHg		84 (12)	83 (13)	86 (11)	0.472
Time from symptom onset to reperfusion, min		156 (112, 243)	161 (118, 206)	137 (112, 274)	0.837
Ventricular fibrillation†, n (%)		1 (3)	1 (6)	0 (0)	1.000
Heart failure, Killip class at presentation, n (%)	I	22 (74)	13 (76)	9 (69)	
	II	7 (23)	4 (24)	3 (23)	0.811
	III/IV	1 (3)	0 (0)	1 (8)	
Electrocardiogram					
ST segment elevation resolution post PCI, n (%)					
Complete, ≥70 %		15 (50)	1 (6)	1 (8)	
Partial, 30% to < 70%		13 (43)	7 (41)	6 (46)	1.000
None, ≤30%		2 (7)	9 (53)	6 (46)	
Number of diseased arteries¥, n (%)	1	14 (47)	10 (58)	4 (31)	
	2	11 (37)	5 (29)	6 (46)	0.298
	3	5 (17)	2 (12)	3 (23)	

	LM	0 (0)	0 (0)	0 (0)	
Culprit artery, n (%)	LAD	9 (30)	4 (24)	5 (38)	
	LCX	10 (33)	4 (24)	6 (46)	0.112
	RCA	11 (37)	9 (53)	2 (15)	
TIMI coronary flow grade pre-PCI, n (%)	0/1	24 (80)	12 (71)	12 (92)	0.196
	2/3	6 (20)	5 (29)	1 (8)	
TIMI coronary flow grade post-PCI, n (%)	0/1	0 (0)	0 (0)	0 (0)	
	2	2 (7)	1 (6)	1 (8)	1.000
	3	28 (93)	16 (94)	12 (92)	
Medical therapy					
ACE-inhibitor or ARB		30 (100)	17 (100)	13 (100)	-
Beta-blocker		30 (100)	17 (100)	13 (100)	-
Initial blood results on admission					
Neutrophil count ($x10^9L$)		10.1 (3.1)	9.3 (3.0)	11.3 (3.0)	0.083
NT-proBNP, pg/mL		588 (306, 1541)	529 (301, 1254)	864 (655, 1637)	0.841

*Footnote: *P-values were obtained from t-tests or Mann-Whitney test as appropriate for continuous variables, and Fisher's tests for categorical variables.*

Table 5-2 Comparison of CMR findings in patients with myocardial haemorrhage (day 3) vs. patients without myocardial haemorrhage (day 3). CMR scans were obtained < 12 hours, 3 days, 10 days, and 7 months post-reperfusion.

	4 - 12 hours		3 days		10 days		7 months		P-value*		
Myocardial haemorrhage	Yes	No	Yes	No	Yes	No	Yes	No	All	Yes	No
LV ejection fraction, %	50 (7)	54 (10)	52 (8)	58 (8)	56 (9)	61 (7)	55 (8)	62 (7)	<0.001	<0.001	<0.001
LV end-diastolic volume, ml	160 (36)	160 (31)	163 (33)	161 (30)	169 (35)	160 (31)	176 (35)	154 (31)	0.698	0.001	0.377
Area at risk, % LV mass	39 (9)	31 (9)	44 (8)	35 (13)	36 (9)	28 (13)	-	-	<0.001	<0.001	0.029
Infarct size, % LV mass	29 (13)	12 (8)	30 (12)	12 (7)	22 (9)	9 (5)	22 (9)	8 (4)	<0.001	<0.001	<0.001
T2 hypointense core, n (%)	7 (100)	12 (52)	13 (100)	5 (29)	10 (91)	4 (21)	1 (25)	0			
Myocardial haemorrhage, n (%)	7 (100)	23 (100)	13 (100)	17 (100)	11 (100)	19 (100)	4 (100)	26 (100)			

*Footnote: *Generalised linear mixed effects models were used to obtain p-values. P-values are not presented for categorical data, since the model is not supported for this function.*

Table 5-3 T2 and T2* relaxation times in the ischemic and remote zones for the serial imaging subset (n=30), at multiple time intervals post-reperfusion, stratified by the presence of haemorrhage on day 3.

Timing of MRI	4 < 12 hours		3 days		10 days		7 months				
	n = 30		n = 30		n =30		n =30				
IMH (day 3)*	Yes	No	Yes	No	Yes	No	Yes	No	All	Yes	No
T2* infarct zone, ms	29.2 (5.8)	37.7 (3.3)	26.6 (4.8)	39.6 (3.5)	28.6 (3.3)	37.0 (4.3)	29.2 (4.0)	32.7 (2.0)	0.018	0.095	<0.001
T2* infarct core, ms	17.8 (6.0)	-	14.1 (4.1)	-	16.7 (5.9)	-	18.9 (6.2)	-	-	<0.001	-
T2* remote zone, ms	31.9 (2.0)	32.4 (1.8)	32.9 (1.9)	32.3 (2.0)	32.6 (1.6)	32.0 (1.3)	32.4 (2.3)	32.3 (1.6)	0.478	0.361	0.876
T2 infarct zone, ms	62.8 (6.7)	62.1 (2.9)	61.4 (4.1)	64.4 (4.9)	68.1 (3.7)	65.9 (5.3)	54.0 (2.8)	52.0 (3.2)	<0.001	<0.001	<0.001
T2 infarct core, ms	55.5 (6.9)	54.2 (2.6)	51.8 (4.6)	54.4 (4.5)	59.2 (3.6)	59.2 (4.4)	-	-	<0.001	0.008	0.057
T2 remote zone, ms	48.5 (2.5)	48.5 (2.0)	49.3 (1.7)	48.7 (2.1)	50.5 (2.4)	49.2 (2.1)	50.3 (1.6)	50.0 (1.4)	<0.001	0.002	0.003

Footnote: The T2 infarct core values are given for only those patients that had a T2* hypointense core to measure (n = 7 at < 12 hours, n = 13 at day 3, n = 11 at day 10 and n = 4 at 7 months).*

Table 5-4 Temporal change in infarct zone end-diastolic wall thickness at serial time-points post-MI.

Timing of MRI	4 < 12 hours		3 days		10 days		7 months				
	n = 30		n = 30		n =30		n =30				
IMH (day 3)	Yes	No	Yes	No	Yes	No	Yes	No	All	Yes	No
Infarct zone, end-diastolic wall thickness, mm	1.14 (0.39)	1.10 (0.18)	1.22 (0.36)	1.17 (0.17)	1.08 (0.31)	1.09 (0.20)	0.78 (0.22)	0.91 (0.18)	<0.0001	0.007	0.001

Footnote: Data given as mean (standard deviation). P-values obtained from ANOVA.

5.4.3 Temporal evolution of T2 relaxation times and myocardial haemorrhage

The temporal evolution of T2 values within the infarct zone varied in association with T2* values. In patients with myocardial haemorrhage a bimodal time-course in T2 values was observed within the area-at-risk (p=0.006) and the infarct core (p=0.008) (table 5-3, figure 5-3 and 5-4). By contrast, this pattern differed in patients without myocardial haemorrhage in whom T2 values increased progressively up to 10 days post-MI (p=0.042). By 7 months, T2 values had fallen in both groups, however, in patients with myocardial hemorrhage, T2 values still remained higher in the infarct zone compared to T2 values in the remote zone (p=0.001) and those of healthy volunteers (p<0.001). They also tended to be higher than T2 values in the infarct zone of patients without haemorrhage (p=0.059; table 5-3).

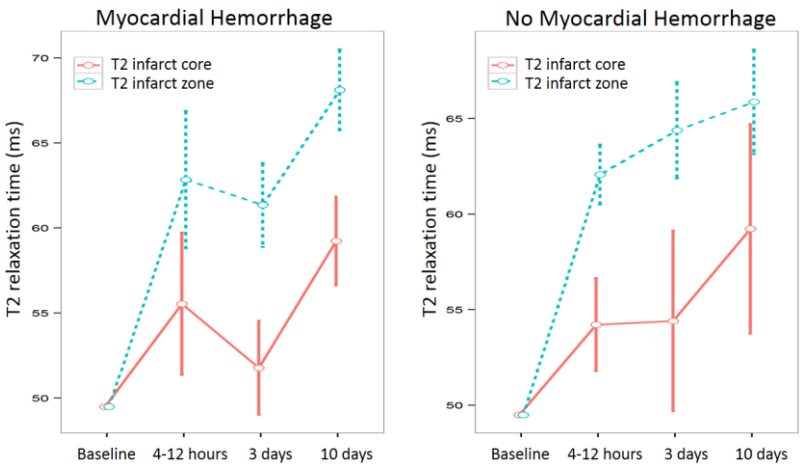
T2 values in the myocardial remote zone increased over time in both patients with and without myocardial haemorrhage (p=0.002 and p=0.003, respectively), but to a greater extent in patients with haemorrhage (table 5-3).

5.4.4 Intra- and inter-observer agreement of T2 and T2 measurements*

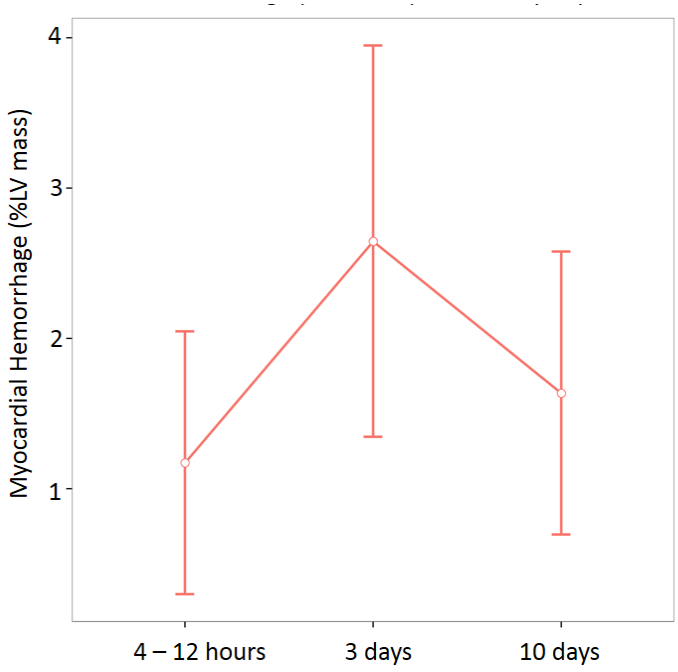
The results for intra-class correlation coefficient for reliability of T2 and T2* measurements and Bland-Altman plots are shown in chapter 3, section 3.5.2.

Figure 5-3 Time course of T2 values in the early reperfusion period in patients with and without myocardial haemorrhage and evolution of haemorrhage (% LV mass) in the early reperfusion period.

A

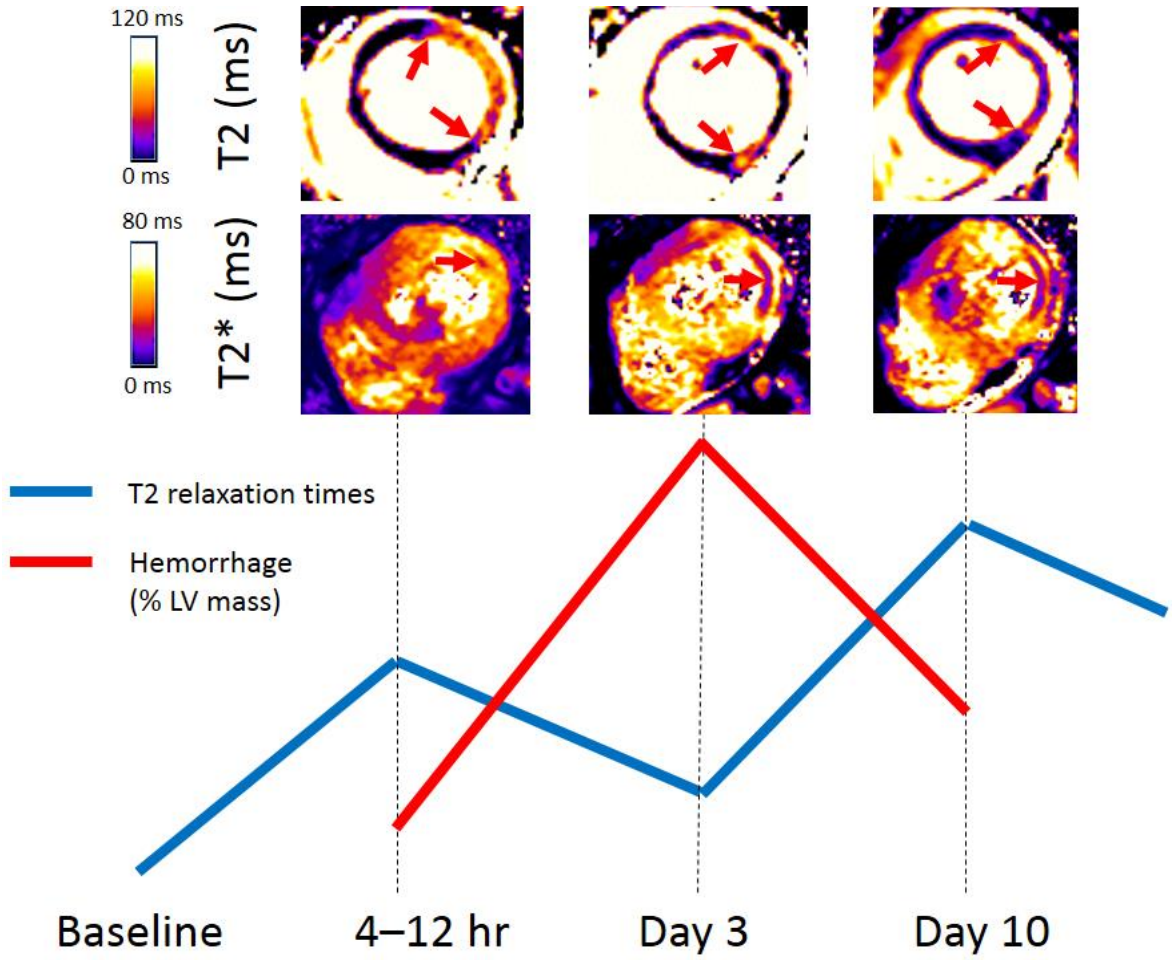


B



Time-course of CMR T2 relaxation times and myocardial haemorrhage (% LV mass) during the first 10 days after ischemia/reperfusion. Patients without haemorrhage have a progressive rise in T2 relaxation times during the first 10 days post-reperfusion, whereas patients with haemorrhage have a bimodal pattern. Myocardial haemorrhage peaks on day 3 post-reperfusion. Baseline T2 values are taken from age and sex-matched healthy volunteers (n = 50).

Figure 5-4 T2 values within the infarct core and infarct zone follow a bimodal pattern with the nadir associated with peak haemorrhage



The bimodal pattern in T2 relaxation times in the early reperfusion period is explained by the evolution of myocardial haemorrhage.

5.4.5 Temporal relationships between intra-myocardial haemorrhage and left ventricular outcomes from < 12 hours to 7 months post-reperfusion

Overall, LV mass decreased from 140±26 g 3 days post-MI to 119±26 g 7 months post-MI (p=0.003). By 7 months post-MI, LV ejection fraction tended to increase (56±9% vs. 59±8 %, p=0.061) and infarct size (% of LV mass) tended to be less (20±13% vs. 14±10%; p=0.10).

Three days post-MI, compared to patients without hemorrhage, patients with hemorrhage had a larger initial area-at-risk (46 (36, 50) vs. 31 (26, 44); p=0.007) and a lower LV ejection fraction (52 (47, 55) vs. 55 (54, 64); p=0.042) (table 5-2).

LV end-diastolic volume increased over time in patients with myocardial haemorrhage (p=0.001; table 5-2). By contrast, LV end-diastolic volume reduced over time in patients without haemorrhage (table 5-2). Day 3 post-MI, infarct size was greater in the patients with haemorrhage compared to patients without haemorrhage (p<0.001; table 5-2).

5.4.6 T2 relaxation times in the myocardial remote zones and in healthy volunteers*

T2* values in the remote zone did not change over time (p=0.361 for patients with myocardial haemorrhage; p=0.876 for patients without haemorrhage) and these values were similar to T2* values in healthy controls (chapter 3, table 3-7). At 7 months, in patients with myocardial haemorrhage 3 days post-MI, T2* values in the infarct zone remained reduced compared with the remote zone, whereas in patients without haemorrhage, T2* values in the infarct zone were similar to T2* values in the remote zone (table 5-3).

5.5 Discussion

We have undertaken the first longitudinal study of myocardial haemorrhage in the early reperfusion period involving serial CMR on 4 occasions in STEMI survivors.

Our main findings are (1) the incidence of myocardial haemorrhage was 43%, (2) approximately one quarter of the patients had evidence of myocardial haemorrhage 4 - 12 hours post-MI and the incidence nearly doubled by 3 days, (3) T2* values within the haemorrhagic core followed a similar pattern to T2 values, with a nadir in both on day 3, (4) during the first 10 days post-reperfusion, T2 values within the infarct zone and hypointense core had a bimodal distribution in patients with myocardial haemorrhage whereas T2 values had a unimodal progressive increase in patients without myocardial haemorrhage, (5) changes in T2 values were inversely related to the occurrence and extent of haemorrhage, (6) the extent of oedema (area at risk, % LV mass) stayed fairly constant in size in both groups for the first 3 days and by day 10 was slightly less, (7) myocardial haemorrhage was associated with sustained reductions in LV ejection fraction and adverse LV remodelling from baseline through to 7 months.

Based on these observations, we conclude that myocardial haemorrhage increases progressively after reperfusion with a primary hyperacute phase < 12 hours post-MI

culminating in a peak 3 days later. The temporal changes in T2 relaxation times are inversely associated with myocardial haemorrhage. Our results provide further evidence that myocardial haemorrhage is an adverse prognostic complication post-MI, but the secondary phase between days 1 and 3 suggests there may be a therapeutic window to prevent haemorrhage should targeted therapies become available in the future.

Our data complement the myocardial oedema time-course study by Fernández-Jiménez *et al* (Fernandez-Jimenez et al., 2015). They described a bimodal pattern of myocardial oedema with peaks of percentage myocardial water content and T2 values acutely at 2 hours post-reperfusion and 7 days later associated with an intervening decrease in myocardial water content at 24 hours. They concluded that myocardial oedema occurred in a two "*waves*", one occurring abruptly after reperfusion and a second "*deferred wave of oedema*" appearing progressively and becoming maximal around 7 days. There could be different explanations for the *"second wavefront of oedema"* including, first, an increase in the absolute amount of water or, second, a reduction in infarct tissue mass and a *relative* increase in percentage water, or finally, an increase in the wet weight of tissue due progressive myocardial hemorrhage or hemorrhagic transformation (Fernandez-Jimenez et al., 2015). Our analysis supports this possibility. Oxidative denaturation of haemoglobin evolves over 1 – 3 days (Anzalone et al., 2004) and the product, deoxyhemoglobin, has paramagnetic effects that destroy T2 signal. Our results are consistent with concomitant oxidative denaturation and paramagnetic destruction of T2 signal within the infarct core consistent with earlier pre-clinical (Ghugre et al., 2011) and clinical (Zia et al., 2012) observations. Therefore, the peak in myocardial haemorrhage that we observed 3 days post-MI likely explains the reductions in co-localized T2 values at this time-point, in turn explaining the bimodal distribution in T2 values that was observed by Fernández-Jiménez *et al* (Fernandez-Jimenez et al., 2015).

The increase in the incidence of hemorrhage over time in some individuals is consistent with haemorrhagic transformation reflecting the natural history of wound-healing after tissue infarction, especially in reperfused patients treated with anti-thrombotic therapies (Anzalone et al., 2004, Fishbein et al., 1980, Alvarez-Sabin et al., 2013). On day 3 CMR, 5 patients without evidence of haemorrhage on T2* imaging had a hypointense cores on T2 maps. The mean T2 core value for these patients was greater than for patients with haemorrhage (54.5±4.5 ms vs. 51.8±4.6 ms; p=0.268). The hypointense core on T2 maps in the absence of haemorrhage likely represents a reduction in the amount of tissue water

within the infarct core due to cellular debris and obstructed capillary flow (Verhaert et al., 2011). The observation that the mean T2 core value is lower in patients with haemorrhage is consistent with the additional effect of paramagnetic depletion of T2 signal.

Our results have important clinical implications. First, the results translate experimental concepts proposed by Fernández-Jiménez *et al* (Fernandez-Jimenez et al., 2015) into clinical observations in patients. Second, our results should be helpful to plan the timing of CMR imaging post-MI for clinical and research purposes and indicate that the extent of edema reduces after 3 days post-MI. Third, our results provide further information on the adverse prognostic associations between myocardial haemorrhage and reductions in LV systolic function and adverse LV remodelling, consistent with previous studies (Beek et al., 2010, Eitel et al., 2011, Ganame et al., 2009, Kidambi et al., 2013). Finally, our results confirm that infarct pathologies evolve progressively post-MI and therefore, potentially, may be amenable to targeted preventative therapeutic interventions. Robbers *et al* (Robbers et al., 2013) proposed that myocardial haemorrhage was the final consequence of severe microvascular thrombosis and that therapeutic interventions that restored microvascular perfusion might in turn prevent myocardial haemorrhage. Conceivably, intra-coronary thrombolysis administered early after reperfusion and before stent implantation might reduce coronary thrombus burden and distal clot embolization, lyse microvascular thrombi and restore microvascular perfusion early post-MI. We are currently examining this hypothesis in a randomized, double-blind, placebo-controlled, parallel group trial of low-dose adjunctive alteplase during primary PCI (T-TIME; NCT02257294).

5.5.1 Limitations

We do not have pathological validation of our imaging results. Although pre-clinical studies enable pathological validation (Fernandez-Jimenez et al., 2015, Ghugre et al., 2011), the corollary is a stepped reduction in sample size and statistical power (n=20 at 2 hours post-MI vs. n=5 at 7 days post-MI (Fernandez-Jimenez et al., 2015)). The sample size in our cohort was preserved during follow-up. Although CMR was not possible before STEMI, we think it is reasonable to believe that there was no haemorrhage present in the STEMI patients before the event, implying a 'zero baseline', since remote T2 and T2* values in STEMI patients were similar to those measured in healthy individuals. We acknowledge that the differences in T2 (ms) and T2* are within the inter-observer range of values and that our findings do not confirm causality.

5.6 Conclusion

We have performed a comprehensive longitudinal clinical study of myocardial haemorrhage and oedema in a cohort of reperfused STEMI survivors. Myocardial haemorrhage peaked at day 3 post-MI in reperfused STEMI patients, and the temporal changes in oedema may be a secondary process.

6 Chapter 6: Prognostic significance of infarct core pathology in ST-elevation myocardial infarction survivors revealed by quantitative T2-mapping cardiac magnetic resonance

6.1 Introduction

Cardiac magnetic resonance (CMR) with T2-mapping is a recent advance for quantifying the extent and nature of ischaemic myocardial injury (Hammer-Hansen et al., 2014, Ugander et al., 2012, Verhaert et al., 2011, Giri et al., 2009) that has potential to extend what is known based on qualitative T2-weighted CMR (Garcia-Dorado et al., 1993, Higgins et al., 1983, McNamara et al., 1985). T2-weighted CMR enables detection of acute myocardial infarction (MI) and discrimination of acute from chronic MI (Abdel-Aty et al., 2004, Cury et al., 2008), and qualitative T2-weighted CMR delineates the ischaemic area-at-risk (Berry et al., 2010, Payne et al., 2011b, Aletras et al., 2006, Garcia-Dorado et al., 1993) and myocardial salvage, which is marker for the efficacy of reperfusion (Friedrich et al., 2008, Eitel et al., 2010).

Although qualitative T2-weighted CMR with dark blood turbo spin echo techniques has been the standard method for imaging myocardial oedema this method is hampered by image artefacts that limit quantitative assessment of heart injury (Wince and Kim, 2010, Kellman et al., 2007). Since the signal intensity is not linearly related to pathology, only the extent of oedema can be measured. Quantitative T2 mapping, which allows direct determination of T2 relaxation times, overcomes many of the inherent limitations associated with dark blood T2-weighted CMR and may allow for a more objective assessment of the infarct core (Giri et al., 2009, Verhaert et al., 2011, Ghugre et al., 2011, Zia et al., 2012, Ugander et al., 2012, Nassenstein et al., 2014, Park et al., 2013).

A hypointense core within the hyperintense infarct zone revealed by T2-weighted CMR is a common observation that in some (Basso et al., 2007, Payne et al., 2011a), but not all (Cannan et al., 2010, Jackowski et al., 2006), studies corresponds with histology evidence of myocardial haemorrhage. Some studies have shown that T2 hypointense infarct cores are associated with adverse remodelling (Ganame et al., 2009, Husser et al., 2013) and adverse clinical outcome (Amabile et al., 2012, Eitel et al., 2011), whereas others have shown that there is no prognostic significance beyond microvascular obstruction (Beek et al., 2010, Bekkers et al., 2010a).

In order to resolve this uncertainty, we studied the clinical associates and prognostic significance of a T2 hypointense core, revealed by quantitative T2 mapping.

6.2 Methods

6.2.1 Study population and STEMI management

We performed a prospective CMR cohort study in a single regional cardiac centre between 11 May 2011 and 22 November 2012. Three hundred and forty three STEMI patients provided written informed consent to undergo CMR 2 days and 6 months post-MI. The eligibility criteria and acute STEMI management are as described in detail in chapter 2.

6.2.2 CMR acquisition

CMR was performed as described in detail in chapter 2. The imaging protocol included cine MRI with steady-state free precession (SSFP), T2-mapping with full LV coverage (Giri et al., 2009, Verhaert et al., 2011), T2*-mapping (3 short-axis slices: base, mid and apex, incorporating infarct zone), and delayed-enhancement phase-sensitive inversion-recovery pulse sequences (Kellman et al., 2002). Patients and healthy volunteers underwent the same imaging protocol except that healthy volunteers <45 years did not receive gadolinium.

6.2.3 CMR analyses

T2 values were measured in myocardial regions of interest defined as: (1) remote myocardium, (2) injured myocardium and (3) infarct core, as previously described in detail in chapter 2.

The infarct zone region-of-interest was defined as myocardium with pixel values (T2) >2 SD from remote myocardium on T2-weighted CMR (Giri et al., 2009, Verhaert et al., 2011). The infarct core was defined as an area in the centre of the infarct territory having a mean T2 value of at least 2 standard deviations (SDs) below the T2 value of the periphery of the area-at-risk. The rest of the analyses are described in detail in chapter 2.

6.2.4 Health outcomes

We pre-specified adverse health outcomes that are pathophysiologically linked with STEMI. The primary composite outcome was all-cause death or heart failure hospitalisation (chapter 2).

Research staff screened for events from enrolment by checking the medical records and by contacting patients and their primary and secondary care physicians, as appropriate. Each event was reviewed by a cardiologist who was independent of the research team and blinded to all of the clinical and CMR data. The adverse events were defined according to standard guidelines (Thygesen et al., 2012) and categorised as having occurred during the index admission or post-discharge. All study participants were followed-up for a minimum of 18 months after discharge.

6.2.5 Statistical analyses

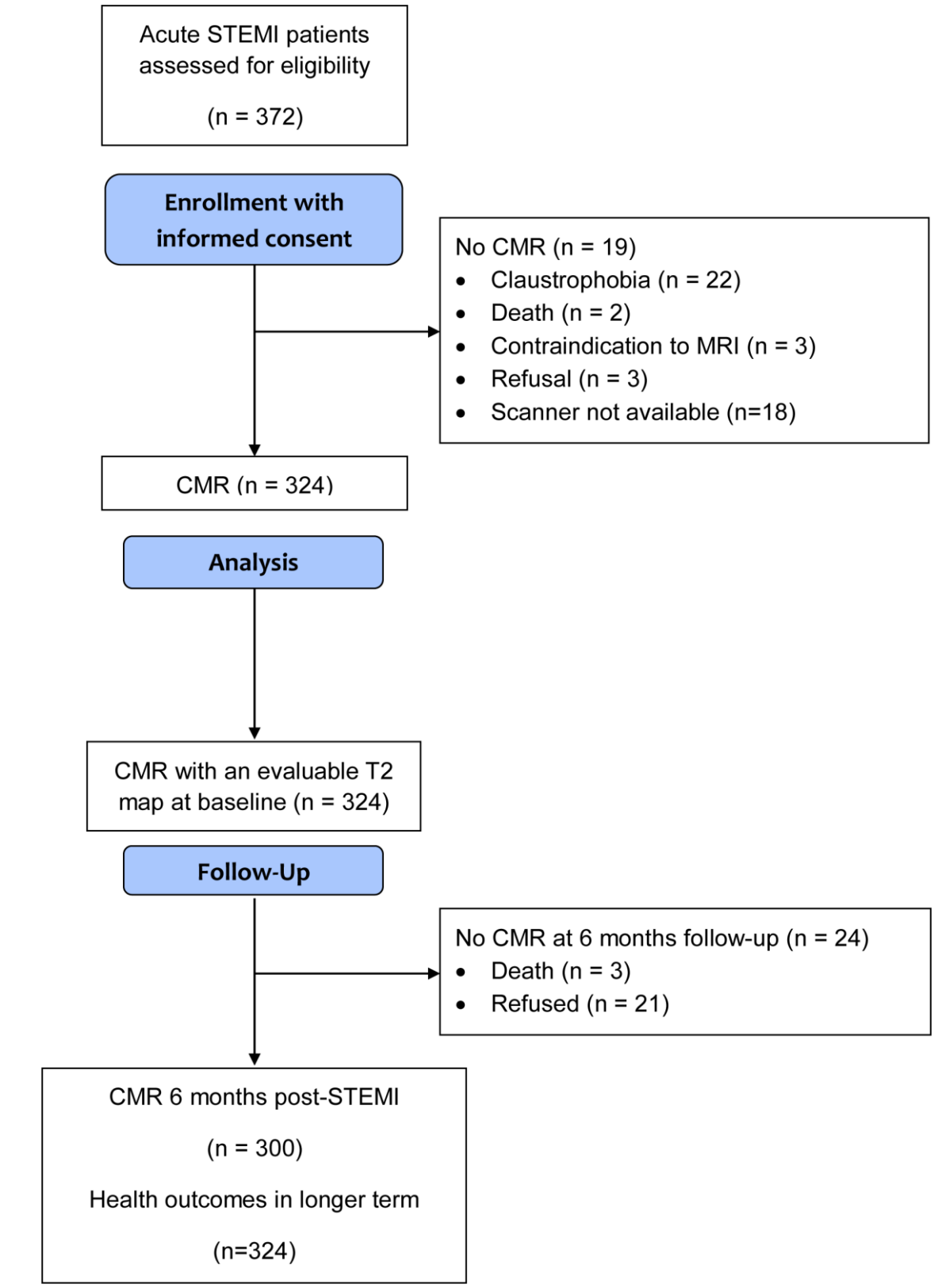
Categorical variables are expressed as number and percentage of patients. Most continuous variables followed a normal distribution and are therefore presented as means together with standard deviation. Those variables that did not follow a normal distribution are presented as medians with interquartile range. Differences between groups were assessed using one-way ANOVA, Kruskal-Wallis test or Fisher's where appropriate. Univariable and multivariable logistic regression analyses were performed to identify predictors of T2 hypointense core.

Kaplan-Meier and Cox proportional hazards methods were used to identify potential clinical predictors of all-cause death/heart failure events, including patient characteristics and CMR findings. A p-value > 0.05 indicates the absence of a statistically significant effect.

6.3 Results

Of 372 STEMI patients referred for emergency reperfusion therapy, 324 underwent CMR at 1.5 Tesla 2.2±1.9 days and all (100%) of these patients had evaluable T2-maps. 300 patients (93%) had repeat CMR 6 months later (figure 6-1). All patients (n=324) with CMR had vital status assessed at least 18 months after enrolment (figure 6-1).

Figure 6-1 Study flow diagram



6.3.1 Patient characteristics

The characteristics of the patients (n=324) are shown in table 6-1, including the patients with T2 hypointense infarct cores. The mean (standard deviation) age was 59 (12) years and 74% were male. 236 (73%) patients had an occluded culprit artery (TIMI coronary flow grades 0/1) at initial angiography.

Table 6-1 Baseline clinical and angiographic characteristics of patients with acute STEMI and a CMR, with evaluable T2 map, at baseline.

Characteristics*		All STEMI patients n=324
Clinical		
Age, years		59.30 (11.49)
Male sex, n (%)		237 (73.1%)
BMI, (kg/m^2)		28.79 (4.76)
History		
Hypertension, n (%)		105 (32.4%)
Current smoking, n (%)		196 (60.5%)
Hypercholesterolemia, n (%)		94 (29.0%)
Diabetes mellitus‡, n (%)		34 (10.5%)
Previous angina, n (%)		40 (12.3%)
Previous myocardial infarction, n (%)		25 (7.7%)
Previous PCI, n (%)		18 (5.6%)
Presenting characteristics		
Heart rate, bpm		78 (17)
Systolic blood pressure, mmHg		132 (25)
Diastolic blood pressure, mmHg		79 (14)
Time from symptom onset to reperfusion, min		253 (212)
Ventricular fibrillation†, n (%)		21 (6.5%)
Heart failure, Killip class at presentation, n (%)	I	233 (71.9%)
	II	68 (21%)
	III	17 (5.2%)
	IV	6 (9.1%)
ECG		

ST segment elevation resolution post PCI, n (%)		
Complete, ≥70 %		148 (45.8%)
Partial, 30% to < 70%		127 (39.3%)
None, ≤30%		48 (14.9%)
Coronary angiography		
Reperfusion strategy, n (%)		
Primary PCI		302 (93.2%)
Rescue PCI (failed thrombolysis)		14 (4.3%)
Successful thrombolysis		8 (2.5%)
Number of diseased arteries¥, n (%)	1	174 (53.7%)
	2	105 (32.4%)
	3	45 (13.9%)
Culprit artery, n (%)	Left anterior descending	121 (37.3%)
	Left circumflex	59 (18.2%)
	Right coronary	144 (44.4%)
TIMI coronary flow grade pre-PCI, n (%)	0/1	236 (72.8%)
	2	58 (17.9%)
	3	30 (9.3%)
TIMI coronary flow grade post-PCI, n (%)	0/1	4 (1.2%)
	2	15 (4.6%)
	3	305 (94.1%)

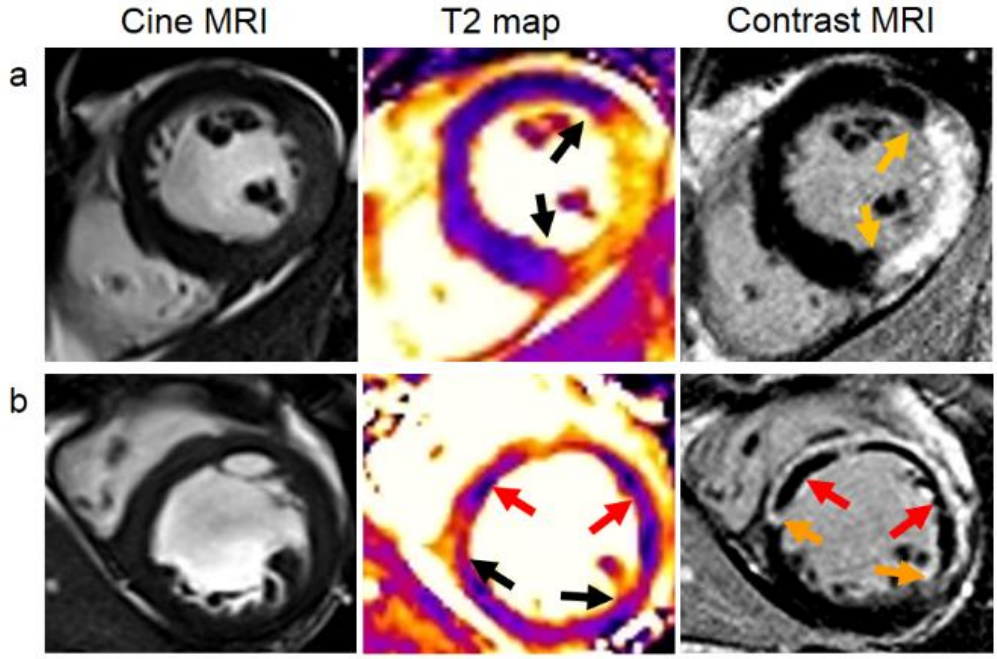
*Footnote: TIMI = Thrombolysis in Myocardial Infarction grade, PCI = percutaneous coronary intervention. Killip classification of heart failure after acute myocardial infarction: class I - no heart failure, class II - pulmonary rales or crepitations, a third heart sound, and elevated jugular venous pressure, class III - acute pulmonary edema, class IV - cardiogenic shock. * Data are given as n (%) or mean (SD). ‡ Diabetes mellitus was defined as a history of diet-controlled or treated diabetes. † Successfully electrically cardioverted ventricular fibrillation at presentation or during emergency PCI procedure. ¥ Multivessel coronary artery disease was defined according to the number of stenoses of at least 50% of the reference vessel diameter, by visual assessment and whether or not there was left main stem involvement.*

6.3.2 CMR findings

Initial CMR findings following hospital admission

The CMR findings and clinical cases are shown in table 6-2 and figure 6-2, respectively. At baseline, the mean myocardial infarct size was 18 ±14% of LV mass. Native T2 within the infarct core (53.9±4.8 ms) was higher than in the remote zone (49.7±2.1 ms; p<0.01) but lower than in the area-at-risk (62.9±5.1 ms) (p<0.01).

Figure 6-2 Acute STEMI cases, with and without T2 hypointense infarct core, revealed by CMR 2 days post-MI



(a) Patient with no T2 hypointense infarct core and no microvascular obstruction. (b) *Patient with both T2 hypointense infarct core (middle image, red arrows) and microvascular obstruction (right image, red arrows)*

Table 6-2 Comparison of CMR findings at baseline in STEMI patients and healthy volunteers and 6-month CMR findings in STEMI patients.

Characteristics*	STEMI patients n=324	Healthy volunteers n=50	p value
CMR findings 2 days post-MI			
LV ejection fraction, %	55.0 (9.6)	67.2 (4.5)	<0.0001
LV end-diastolic volume, ml			
Men	161.3 (33.3)	167.8 (31.6)	0.329
Women	125.0 (25.4)	134.1 (23.0)	0.104

LV end-systolic volume, ml			
Men	75.3 (26.6)	56.8 (14.9)	<0.0001
Women	55.1 (18.0)	43.6 (12.3)	<0.001
LV mass, g			
Men	144.54 (32.7)	124.5 (22.7)	<0.001
Women	99.1 (23.3)	92.0 (20.4)	0.151
Oedema and infarct characteristics			
Area at risk, % LV mass	31.9 (11.9)	-	
Infarct size, % LV mass	18.0 (13.5)	-	
Myocardial salvage, % of LV mass	13.9 (8.8)		
Myocardial salvage index, % of LV mass	49 (30)		
Early microvascular obstruction present, n (%)			
Late microvascular obstruction present, n (%)	164 (50.6)	-	
Late microvascular obstruction, % LV mass	2.9 (5.0)	-	
Myocardial haemorrhage, n (%)			
Myocardial native T2 values			
T2 remote myocardium (all subjects), ms	49.7 (2.1)	49.5 (2.5)	0.511
Men, ms	49.6 (2.0)	48.5 (2.1)	0.014
Women, ms	50.1 (2.1)	50.5 (2.5)	0.390
T2 area-at-risk, ms	62.9 (5.1)	-	-
T2 hypointense core present, n (%)	197 (61)	-	-
T2 hypointense infarct core, ms	53.90(4.8)	-	-
T2 of infarct tissue surrounding core, ms	68.5 (6.3)		
CMR findings 6 months post-MI (n = 300)			
LV ejection fraction, %	61.9 (9.4)		
LV end-diastolic volume, ml			
Men	168.6 (42.0)		
Women	127.3 (28.6)		
LV end-systolic volume, ml			
Men	68.0 (34.2)		
Women	46.3 (17.5)		
LV mass, g			
Men	127.5 (26.5)		
Women	92.0 (19.6)		

*Footnote: * Data are given as n (%) or mean (SD). Abbreviations: LV = left ventricle*

T2 maps were acquired with full LV coverage. In one patient, area at risk could not be measured due to SSFP off-resonance artefact. All other T2 maps were suitable for analysis. Infarct zone T2 values were higher in infarct tissue than infarct core ($p<0.001$) and remote myocardium ($p<0.001$).

6.3.3 Comparison of T2 hypointense core and microvascular obstruction

197 (61%) STEMI patients had a T2 hypointense core. Microvascular obstruction with early gadolinium- and late gadolinium enhancement CMR was revealed in 186 (57%) and 164 (51%) patients, respectively. All patients with late microvascular obstruction had evidence of a hypointense core on T2 imaging. 33 (10%) patients had a T2 hypointense core in the absence of late microvascular obstruction. 185 (99%) patients with early microvascular obstruction had a T2 hypointense core. Only 12 (4%) patients had a T2 hypointense core without evidence of early microvascular obstruction. The negative- and positive predictive values for T2 hypointense core and microvascular obstruction are summarised in table 6-3. In patients with both late microvascular obstruction and a T2 hypointense core (n = 164), the median (IQR) amount of T2 core (%LV mass) was greater than the median amount of microvascular obstruction (%LV mass) (5.2 (2.9, 9.2) vs. 3.5 (1.7, 8.4); p=0.006).

Table 6-3 Negative- and positive predictive values of T2 infarct core for microvascular obstruction and myocardial haemorrhage disclosed by a T2* core.

	Native T2 infarct core absent	Native T2 infarct core present	
Myocardial haemorrhage			
Myocardial haemorrhage absent	84	60	Specificity 58.3% 95% CI (49.8, 66.5)
Myocardial haemorrhage present	0	101	Sensitivity 100% 95% CI (96.4, 100)
	NPV 100% 95% CI (95.7, 100)	PPV 63.7% 95% CI (54.8, 70.2)	
Early MVO			
Early MVO absent	126	12	Specificity 91.3% 95% CI (89.6, 96.8)
Early MVO present	1	185	Sensitivity 99.5% 95% CI (97.0, 99.9)
	NPV 99.2% 95% CI (95.7, 99.9)	PPV 93.9% 95% CI (89.6, 96.8)	

Late MVO			
Late MVO absent	127	33	Specificity 79.4% 95% CI (72.3, 85.4)
Late MVO present	0	164	Sensitivity 100% 95% CI (97.8, 100)
	NPV 100% 95% CI (97.1, 100)	PPV 83.3% 95% CI (77.3, 88.2)	

95% CI – 95% confidence interval; MVO – microvascular obstruction; NPV – negative predictive value; PPV – positive predictive value. The CMR approaches for delineation of early MVO, late MVO and myocardial haemorrhage are described in the Methods (chapter 2).

6.3.4 Comparison of T2 hypointense core and myocardial haemorrhage

T2*-maps were available in 245 patients at baseline. Myocardial haemorrhage was revealed in 101 (41%) patients, all of whom had a corresponding T2 hypointense core (table 6-3). However, 64 (26%) patients had a T2 hypointense core in the absence of myocardial haemorrhage.

6.3.5 T2 values in STEMI patients vs. healthy controls

Fifty aged-matched healthy volunteers (52% male, 54±13 years) were included (table 3-7, chapter 3). The mean remote zone native T2 at the mid-ventricular level was higher in male STEMI patients than in male volunteers. The myocardial remote zone T2 values were similar in female patients and volunteers (section 3.6.1, chapter 3).

6.3.6 Intra- and inter-observer agreement of T2 measurements

The results for intra-class correlation coefficient for reliability of T2 measurements and Bland-Altman plots are shown in chapter 3, section 3.5.2.

6.3.7 Infarct core native T2: associations with clinical characteristics and inflammation

T2 core (ms) was univariably associated with LVEF at baseline (0.31 (0.04, 0.58); p=0.023) but not at 6 months. T2 core was not associated with LV end-diastolic volume at baseline or at 6 months (table 6-4).

In multivariable linear regression, native T2 in the infarct core was negatively associated with heart rate, Killip class and peak neutrophil count at presentation (all p<0.05) (table 6-4).

Table 6-4 Predictors of native T2 (ms) in the infarct core (n=197 subjects) in univariable and multivariable stepwise regression analyses.

Univariable associations	coefficient (95% CI)	p value
Hypertension	-1.70 (-3.14, -0.27)	0.020
Heart rate, min	-0.04 (-0.09, -0.00)	0.048
Killip class 4	-5.53 (-9.43, -1.63)	0.006
Maximum log CRP	-0.96 (-1.53, -0.40)	<0.001
Maximum leucocyte count, ($x10^9L$)	-0.22 (-0.42, -0.02)	0.028
Maximum neutrophil count, ($x10^9L$)	-0.26 (-0.47, -0.04)	0.018
Maximum monocyte count, ($x10^9L$)	-0.26 (-0.47, -0.04)	0.018
Change in log CRP from baseline*	-0.21 (-0.53, -0.07)	0.003

Change in monocyte count, ($x10^9$L)	-0.26 (-0.47, -0.04)	0.018
Multiple stepwise regression	coefficient (95% CI)	p value
A. Including patient characteristics and angiographic data		
Hypertension	-1.49 (-2.92, -0.06)	0.041
Heart rate, beats per min	-0.04 (-0.08, -0.00)	0.056
Killip class 4	-5.01 (-8.92, -1.10)	0.012
*B. Including patient characteristics, angiographic data, and change in log CRP**		
Change in log CRP from baseline*	-0.17 (-0.34, 0.00)	0.050
Killip class 3	-3.30 (-6.40, -0.20)	0.037
*C. Including patient characteristics, angiographic data, and maximum leucocyte count**		
Heart rate, beats per min	-0.05 (-0.10, -0.00)	0.037
Killip class 3	-3.08 (-5.96, -0.20)	0.036
Killip class 4	-5.67 (-10.34, -0.99)	0.018
Maximum leucocyte count, ($x10^9$L)	-0.27 (-0.51, -0.03)	0.027
D. Including patient characteristics, angiographic data, and maximum		

*neutrophil count**		
Heart rate, beats per min	-0.05 (-0.01, -0.00)	0.048
Killip class 3	-3.14 (-6.02, -0.26)	0.033
Killip class 4	-5.66 (-10.33, -0.98)	0.018
Maximum neutrophil count, ($x10^9$L)	-0.28 (-0.53, -0.03)	0.028
*E. Including patient characteristics, angiographic data, and maximum monocyte count**		
Heart rate, beats per min	-0.05 (-0.01, -0.00)	0.058
Killip class 3	-2.78 (-5.69, 0.12)	0.060
Killip class 4	-4.88 (-9.66, -0.09)	0.046
Maximum monocyte count, ($x10^9$L)	-2.51 (-4.67, -0.35)	0.023

Footnote: The coefficient (95% confidence intervals) indicates the magnitude and direction of the difference in infarct core T2 (ms) for the patient characteristic (binary or continuous). For example, on average, infarct core native T2 (ms) is lower (-0.04 (-0.09, -0.00) for each 1 beat per min increase in heart rate.

For univariable analyses, all variables in Table 1 were tested and also the following baseline CMR parameters: area-at-risk, LV ejection fraction, LV end-diastolic volume, LV end-systolic volume and infarct size. Selected patient characteristics are shown. Separate multivariable analyses were performed for patient characteristics, angiographic data and CMR data. CMR parameters, which were all highly correlated with one another, were included separately in multiple stepwise regression models with patient characteristics and angiographic data to reduce multicollinearity.

6.3.8 Infarct core tissue characteristics and left ventricular outcomes

At 6 months, LV end-diastolic volume increased on average (SD) by 5 (25) ml in 295 patients with evaluable data (table 6-2). Adverse remodelling, defined as an increased LV end-diastolic volume by ≥20% at 6-months from baseline, occurred in 34 (11%) patients and 23 (68%) of these patients had both microvascular obstruction and T2 hypointense core at baseline. Native T2 in the infarct core was not associated with adverse remodelling.

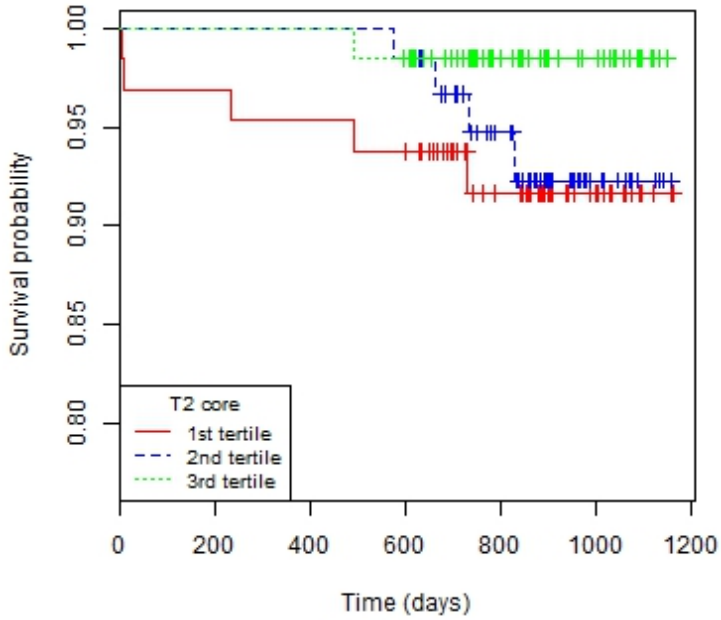
The area-at-risk revealed by T2-mapping CMR was associated with LVEF at follow-up (-0.16 (-0.27, -0.04); p=0.07) and with LV end-diastolic volume at follow-up (0.83 (0.46, 1.19); p<0.001), independent of LVEF (p<0.01) and LV end-diastolic volume at baseline (p<0.001).

6.3.9 Infarct core tissue characteristics and longer term health outcomes

324 (100%) patients had longer term follow-up information. The median duration of follow-up was of 860 days (minimum - maximum post-discharge censor duration 597 - 1162 days). Thirty four (10.5%) patients died or experienced a heart failure event. These events included 6 cardiovascular deaths, 4 non-cardiovascular deaths and 25 episodes of heart failure (Killip Class 3 or 4 heart failure (n=23) or defibrillator implantation n=2). Fourteen (4.3%) patients died or experienced a heart failure hospitalisation post-discharge.

T2-core (1 ms change) was associated with a reduced risk of all-cause death or heart failure hospitalisation (hazard ratio 0.786, 95% CI 0.658, 0.939; p=0.008) including after adjustment for LVEF at baseline (p=0.017) or LV end-diastolic volume at baseline (p=0.009) (figure 6-3; table 6-5).

Figure 6-3 Kaplan-Meier survival plot for T2 core; patients grouped as thirds



Kaplan-Meier survival curves for 197 STEMI patients grouped according to the native T2 value in the infarct core with patients grouped by thirds and all-cause death or first heart failure hospitalisation (n=14) after discharge from hospital to the end of follow-up (censor time 860 (597 to 1162) days). Infarct core native T2 values in the lowest tertile were associated with all-cause death or heart failure hospitalization; p=0.047.

Table 6-5 Relationships for infarct core T2 relaxation time (ms) revealed by CMR at baseline in 197 STEMI patients with an infarct core and all-cause death or first hospitalisation for heart failure post-discharge.

Associations	Hazard ratio (95% CI)	p value
Univariable associations		
Infarct core native T2, (for a 1 ms difference)	**0.786 (0.658, 0.939)**	**0.008**
LVEF at baseline, (for a 1% difference)	0.938 (0.890, 0.989)	0.017
Model A		
Infarct core native T2, (for a 1 ms difference)	**0.799 (0.664, 0.961)**	**0.017**
LVEF at baseline, (1% difference)	0.945 (0.882, 1.012)	0.107

14 (4.3%) patients experienced all-cause death or heart failure hospitalisation post-discharge discharge (median (range) follow-up duration of 860 days (597 to 1162) days). Given the limited number of adverse events, the models were specified to assess the prognostic relationships of infarct core native T2 with LV function, LV volume and infarct characteristics that were measured at approximately the same time 2 days after hospital admission.

6.4 Discussion

We have presented the largest ever single centre CMR study in acute STEMI survivors. The main findings of our study are: 1) T2-maps that were of diagnostic quality were obtained in all (100%) of the STEMI survivors early post-MI, and the T2 measurements were reliable; 2) Infarct core pathology delineated by a central zone of reduced T2 was associated with heart rate, acute systemic inflammation, as revealed by log CRP and the circulating concentrations of neutrophils and monocytes, and heart failure; 3) Myocardial haemorrhage always occurred in the presence of a T2 hypointense core, however 60 (37%) patients had a T2 hypointense core in the absence of haemorrhage 4) Infarct core native T2 was associated with LVEF early post-MI; 5) Infarct core pathology revealed by native T2 was independently associated with all-cause death or heart failure hospitalisation during longer term follow-up; 6) Microvascular obstruction always occurred in the presence of a T2 hypointense core.

Previous studies using dark blood T2-weighted imaging to evaluate the infarct core showed that microvascular obstruction occurred commonly in the absence of a T2 hypointense core (Amabile et al., 2012, Eitel et al., 2011, Ganame et al., 2009). In contrast, we have observed that all patients with microvascular obstruction had a hypointense core on T2-mapping. This disparity may be explained by differences in measurement sensitivity between quantitative T2-mapping and qualitative T2-weighted CMR methods (Giri et al., 2009, Nassenstein et al., 2014, Park et al., 2013, Verhaert et al., 2011). Our results are consistent with post-mortem histology (Jackowski et al., 2006) that found a T2 hypointense core always represented microvascular obstruction, with or without haemorrhage.

We also observed that a T2 hypointense core was more closely related to early microvascular obstruction (sensitivity 99.5% and specificity 91.3%) than late microvascular obstruction (sensitivity 100% and specificity 79.4%). This observation is consistent with previous studies that found significant correlation between the extent of hypointense core on T2-weighted imaging and the extent of microvascular obstruction by early gadolinium enhancement (Ganame et al., 2009, Mather et al., 2011b, O'Regan et al., 2009). A study by O'Regan et al (O'Regan et al., 2010), using T2-weighted imaging and a haemorrhage sensitive T2* technique, showed that late microvascular obstruction was highly associated with the extent of haemorrhage, defined by T2* imaging (r^2=0.87,

$p<0.001$), but the correspondence with early microvascular obstruction was weaker ($r^2=0.3$, $p<0.003$). This result supports the notion that the occurrence of a T2 hypointense core revealed by T2 mapping likely represents, at one end of the spectrum, intra-myocardial haemorrhage in patients with severe reperfusion injury and microvascular destruction, and at the other end of the spectrum, functional microvascular obstruction, with preserved endothelial integrity.

The paramagnetic effects of myocardial haemorrhage resulting in shortening of T2-relaxation times and thus a haemorrhagic infarct core will cause a hypointense zone on T2-weighted CMR (Basso et al., 2007, Bradley, 1993). In addition, functional microvascular obstruction (e.g. cellular debris and extrinsic oedema) may reduce the capillary flow and the effective tissue water content within the infarct core thereby reducing the supply of protons and subsequent reduction in T2 signal.

Consistent with other studies, we found that a T2 values within the infarct core were associated with adverse clinical outcome (Amabile et al., 2012, Eitel et al., 2011, Husser et al., 2013). Since signal intensity values in qualitative T2-weighted CMR are not clinically meaningful, the quantitative nature of T2 mapping adds incremental prognostic value over and above the binary classification of oedema by qualitative T2-weighted imaging.

There are few clinical studies using T2 mapping to assess ischaemic-reperfusion injury. A small study by Park et al. (Park et al., 2013), including 20 STEMI patients, found that the mean T2 value of remote myocardium was 50.3 (3.2) ms, which is in accordance with our result of 49.7 (2.1) ms. They excluded areas of microvascular obstruction when measuring T2 values in the infarct zone, which may explain why they found a T2 value of 67.9 (9.3) ms, compared to our T2 infarct zone value of 62.9 (5.1) ms, which encompassed the entire area-at-risk, including microvascular obstruction. Our findings are also in line with other small studies including Giri et al. (Giri et al., 2009) (remote myocardium = 50.5 (3.5) ms and infarct zone = 66.7 (1.9) ms), although there is no mention of whether microvascular obstruction was included in the infarct zone measurement. Verhaert et al. (Verhaert et al., 2011) noted that the T2 value within the infarct core, corresponding with the area of microvascular obstruction, was lower than the T2 value of surrounding infarcted tissue and measured 58.7 (6) ms, which was higher than our value of 53.9 (3.8) ms. However, their

study included 27 acute MI patients, 6 of whom were non-STEMIs and 7 of whom who were not revascularised.

T2 mapping resulted in evaluable data in all patients, owing to the relative insensitivity to motion artefacts. This is in part due to the integrated motion correction algorithm, which makes it particularly useful in this patient cohort, in whom poor breath holding and arrhythmia are prevalent. Since native T2 core can be determined without contrast, this prognostic parameter may be especially useful in patients with relative contraindications to intravenous gadolinium contrast.

6.4.1 Limitations

We lack pathological correlation of our imaging results and T2* values. The number of adverse events limited the number of variables that could be included in the multivariable models.

6.5 Conclusion

A hypointense infarct core revealed by T2-mapping was common and independently associated with all-cause death or heart failure hospitalisation post-discharge. Quantitative T2-mapping is a robust, reliable and prognostically informative imaging biomarker in STEMI patients undergoing CMR.

7 Chapter 7: Prognostic significance of infarct core pathology revealed by quantitative non-contrast T1-mapping, in comparison to contrast cardiac magnetic resonance imaging in reperfused ST-elevation myocardial infarction survivors

7.1 Introduction

Myocardial infarct size [(Holman et al., 1978, Pfeffer and Braunwald, 1990)] and microvascular obstruction [(van Kranenburg et al., 2014, Eitel et al., 2010, Eitel et al., 2011, Wu et al., 1998b, Hombach et al., 2005)] revealed by contrast-enhanced cardiac magnetic resonance (CMR) reflect the efficacy of reperfusion therapy and are prognostically important findings in survivors of ST-elevation myocardial infarction (STEMI).

Human tissue has fundamental magnetic properties, including the longitudinal (spin-lattice) relaxation time (native T1 in milliseconds). Native T1 is influenced by water content, binding with macromolecules (water mobility), and cell content [(Mathur-De Vre, 1984, Cameron et al., 1984)]. Native T1 CMR does not involve an intravenous contrast agent. Tissue water content increases as a result of ischemia, resulting in longer T1 times being a biomarker of more severe myocardial injury in localized myocardial regions [(Williams et al., 1980, Been et al., 1988, Higgins et al., 1983, Yang et al., 2007, Dall'Armellina et al., 2013, Dall'Armellina et al., 2012, Messroghli et al., 2003, Messroghli et al., 2007b, Ugander et al., 2012)].

The clinical significance of tissue changes within the infarct core in patients with acute reperfused STEMI has not been directly assessed. We hypothesised that baseline native T1 values would be 1) inversely associated with the severity of MI, including microvascular obstruction, 2) independently associated with left ventricular (LV) remodelling, and 3) independently associated with pre-defined health outcomes. Should these hypotheses be confirmed then infarct core native T1 mapping without an intravenous contrast agent might have potential as an alternative biomarker to microvascular obstruction revealed by contrast-enhanced CMR.

To investigate these hypotheses we measured native T1 in myocardial regions of interest in STEMI patients undergoing serial cardiac magnetic resonance (CMR) imaging 2 days and 6 months post-MI. We assessed the clinical determinants of native T1 within the hypointense infarct core and subsequent LV remodelling and examined its association with all-cause death and first hospitalisation for heart failure.

7.2 Methods

7.2.1 Study population and STEMI management

We performed a prospective CMR cohort study in a single regional cardiac centre between 14 July 2011 and 22 November 2012. Three hundred and forty three STEMI patients provided written informed consent to undergo CMR 2 days and 6 months post-MI. The eligibility criteria and acute STEMI management are described in detail in chapter 2.

7.2.2 CMR acquisition

CMR was performed, as described in detail in chapter 2. In brief, the imaging protocol included cine MRI with steady-state free precession (SSFP), native T1 mapping [(Messroghli et al., 2004, Messroghli et al., 2007b)], T2 mapping [(Giri et al., 2009, Verhaert et al., 2011)] (full LV coverage), T2*-mapping (3 short-axis slices), and delayed-enhancement phase-sensitive inversion-recovery pulse sequences [(Kellman et al., 2002)].

Native T1 maps were acquired in 3 short-axial slices (basal, mid and apical), using an optimised modified look-locker inversion-recovery (MOLLI) T1-mapping investigational prototype sequence [(Messroghli et al., 2004, Messroghli et al., 2007b)] before contrast administration. The prototype sequence did not involve motion correction.

CMR was also performed in 50 healthy volunteers of similar age and gender in order to obtain local reference values for myocardial native T1 (chapter 2).

7.2.3 CMR analyses

Approach to analyses is described in detail in chapter 2.

Each T1 map image was assessed for the presence of artefacts relating to susceptibility effects, or cardio-respiratory motion. Each colour map was evaluated against the original images. When artefacts occurred the affected segments were not included in the analysis.

Native myocardial T1 was measured in regions of interest defined as (1) remote myocardium, (2) injured myocardium and (3) infarct core. The hypointense infarct core was defined as an area in the centre of the infarct territory having a mean T1 value of at

least 2 standard deviations (SDs) below the T1 value of the periphery of the area-at-risk [(Giri et al., 2009, Verhaert et al., 2011)]. The assessment of T1 maps and adjudication (present/absent) of a hypointense core was performed independently by D.C.

7.2.4 Pre-specified health outcome

Described in detail in chapter 2, including independent adjudication of SAEs by cardiologists blinded to all other clinical and CMR data.

7.2.5 Statistical analyses

As described in chapter 2, categorical variables are expressed as number and percentage of patients. Most continuous variables followed a normal distribution and are therefore presented as means together with standard deviation. Those variables that did not follow a normal distribution are presented as medians with interquartile range. Differences in continuous variables between groups were assessed by the Student's t-test or analysis of variance (ANOVA) for continuous data with normal distribution, otherwise the nonparametric Wilcoxon rank sum test or Kruskal-Wallis test. Differences in categorical variables between groups were assessed using a Chi-square test or Fisher's test, as appropriate. Correlation analyses were Pearson or Spearman tests, as indicated. Random effects models were used to compute inter-and intra- rater reliability measures (intra-class correlation coefficient (ICC)) for the reliability of infarct core native T1 values measured independently by 2 observers in 12 randomly selected patients from the cohort.

Univariable and multivariable linear regression analyses were performed to identify associates of T1 values for (1) remote myocardium, (2) injured myocardium within the area-at-risk and (3) infarct core in all patients and (4) in patients without late microvascular obstruction. In backward stepwise linear regressions, the Akaike information criteria (AIC) was used as a measure of the relative quality of the models for this dataset, and the model with the minimum AIC value was reported. The CMR parameters that were all highly correlated with one another were included in multiple stepwise regression models with patient characteristics, angiographic data and blood results separately in order to reduce multi-collinearity. Where standardised regression coefficients are reported, these are calculated by multiplying the unstandardised coefficient by the standard deviation of the predictor, then dividing by the standard deviation of the response. Potential non-linear

relationships between T1 values in regions of interest and LV ejection fraction and end-diastolic volume were explored with restricted cubic splines and Loess plots. The relationships between the presence or absence of an infarct core revealed by native T1 CMR compared with early MVO, late MVO, presence of T2 core, and myocardial haemorrhage were explored in sensitivity analyses.

Receiver operating curve (ROC), Kaplan-Meier and Cox proportional hazards methods were used to identify potential clinical predictors of all-cause death/heart failure events and MACE, including patient characteristics, CMR findings and native T1. The net reclassification improvement (NRI) was calculated as described by Pencina et al [(Pencina et al., 2011)].

All p-values are 2-sided, and a p-value > 0.05 indicates the absence of a statistically significant effect. Statistical analyses were performed using R version 2.15.1 or SAS v 9.3, or higher versions of these programs.

7.3 Results

Of 343 STEMI patients referred for emergency reperfusion therapy, 300 underwent serial CMR at 1.5 Tesla 2.2±1.9 days and 6 months after hospital admission (figure 7-1). 292 STEMI patients had a T1-map acquisition and 288 (99%) had evaluable T1 data (figure 7-1). CMR follow-up at 6 months was achieved in 267 (93%) of the patients and the reasons for non-attendance are summarised in figure 7-1. Information on vital status and SAEs were available in all (100%) of the 288 participants.

7.3.1 Patient characteristics

Table 7-1 shows the characteristics of the patients, including the patients with a hypointense infarct core revealed by native T1 mapping (n=160 (56%), grouped by thirds of native T1).

7.3.2 Intra- and inter-observer agreement of T1 measurements

The results for intra-class correlation coefficient for reliability of T1 measurements and Bland-Altman plots are shown in chapter 3, section 3.5.2.

Figure 7-1 Study flow diagram

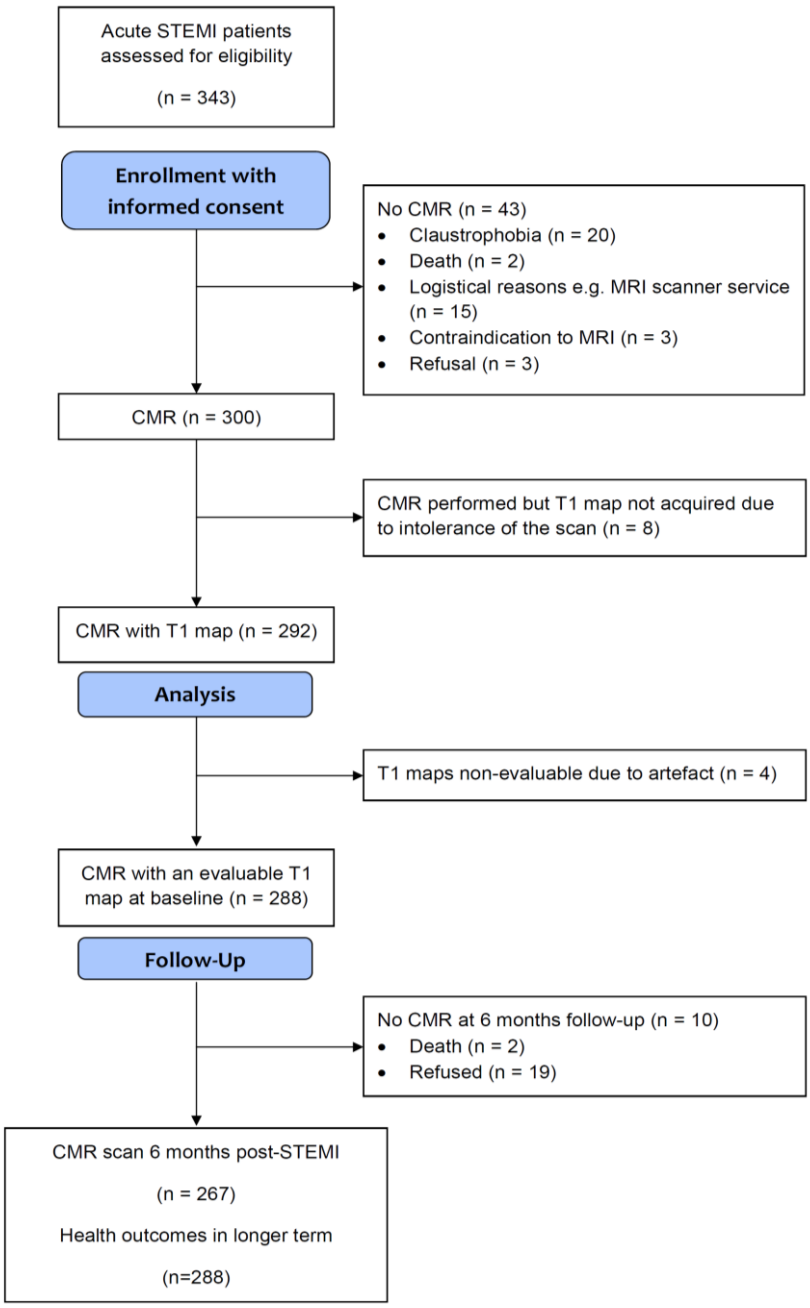


Table 7-1 Clinical and angiographic characteristics of 288 STEMI patients who had CMR with evaluable maps for myocardial native T1 magnetisation, including the subset of patients with an infarct core revealed by native T1 (all and categorized by tertiles of native T1).

Characteristics*	All patients	Patients with a native T1 infarct core	Patients with a native T1 infarct core grouped by tertile of infarct core zone native T1 (ms) at baseline			P-value
			T1 core ≤ 973 ms	974 < T1 core ≤ 1010 ms	T1 core > 1010 ms	
	n = 288	n = 160 (56%)	n = 54 (33%)	n = 53 (33%)	n = 53 (33%)	
Age, years	59 (11)	59 (11)	59 (11)	57 (11)	61 (11)	0.238
Male sex, n (%)	211 (73)	123 (77)	46 (85)	37 (70)	40 (76)	0.144
BMI, (kg/m^2)	29 (5)	29 (5)	29 (4)	29 (5)	28 (5)	0.674
Medical history						
Hypertension, n (%)	93 (32)	57 (36)	17 (32)	21 (40)	19 (36)	0.684
Current smoking, n (%)	177 (62)	100 (62)	32 (59)	34 (64)	34 (64)	0.858
Hypercholesterolaemia, n (%)	82 (28)	44 (28)	12 (22)	17 (32)	15 (28)	0.527
Diabetes mellitus‡, n (%)	32 (11)	20 (12)	7 (13)	7 (13)	6 (11)	1.000
Previous angina, n (%)	34 (12)	21 (13)	8 (15)	4 (8)	9 (17)	0.304
Previous myocardial infarction, n (%)	23 (8)	15 (9)	5 (9)	3 (6)	7 (13)	0.415
Previous PCI, n (%)	16 (6)	14 (9)	4 (7)	3 (6)	7 (13)	0.414

Presenting characteristics							
Heart rate, bpm		78 (17)	78 (16)	80 (16)	79 (16)	76 (17)	0.401
Systolic blood pressure, mmHg		136 (24)	136 (22)	137 (24)	140 (23)	131 (19)	0.095
Diastolic blood pressure, mmHg		79 (14)	80 (14)	82 (14)	83 (13)	76 (13)	0.010
Time from symptom onset to reperfusion, min		174 (120, 311)*	188 (125, 388)	223 (145, 406)	163 (113, 313)	198 (128, 257)	0.268
Ventricular fibrillation†, n (%)		20 (7)	10 (6)	3 (6)	2 (4)	5 (9)	0.518
Heart failure, Killip class at presentation, n (%)	I	205 (71%)	101 (63)	29 (54%)	38 (72%)	34 (64%)	
	II	64 (22%)	43 (27)	15 (28%)	14 (26%)	14 (26%)	0.059
	III / IV	19 (7)	16 (10)	10 (18)	1 (2)	5 (9)	
ECG							
ST segment elevation resolution post PCI, n (%)							
Complete, ≥70 %		129 (45)	55 (35)	15 (28)	21 (40)	19 (36)	
Incomplete, 30% to < 70%		115 (40)	74 (46)	27 (50)	23 (44)	24 (45)	0.715
None, ≤30%		43 (15)	30 (19)	12 (22)	8 (15)	10 (19)	
Reperfusion strategy, n (%)							
Primary PCI		268 (93)	148 (92)	49 (91)	49 (92)	50 (94)	
Rescue PCI (failed thrombolysis)		13 (4)	10 (6)	4 (7)	3 (6)	3 (6)	1.000

Successful thrombolysis		7 (2)	2 (1)	1 (2)	1 (2)	0 (0)	
Coronary angiography							
Number of diseased arteries¥, n (%)	1	156 (54)	89 (56)	156 (54)	156 (54)	156 (54)	
	2	89 (29)	44 (28)	90 (31)	90 (31)	90 (31)	0.436
	3	42 (15)	24 (15)	42 (15)	42 (15)	42 (15)	
	LM	6 (2)	3 (2)	0 (0)	2 (4)	1 (2)	
Culprit artery, n (%)	LAD	108 (38)	60 (38)	22 (41)	19 (36)	19 (36)	
	LCX	51 (18)	31 (19)	10 (18)	12 (23)	9 (17)	0.915
	RCA	129 (45)	69 (34)	22 (41)	22 (42)	25 (47)	
TIMI coronary flow grade pre-PCI, n (%)	0/1	208 (72)	135 (84)	49 (91)	39 (74)	47 (89)	
	2	52 (18)	27 (13)	5 (9)	11 (21)	5 (9)	0.085
	3	28 (10)	4 (2)	0 (0)	3 (6)	1 (2)	
TIMI coronary flow grade post-PCI, n (%)	0/1	3 (1)	2 (1)	0 (0)	1 (2)	1 (2)	
	2	13 (4)	8 (5)	3 (6)	3 (6)	2 (4)	0.959
	3	272 (94)	150 (94)	51 (94)	49 (92)	50 (94)	
Medical therapy							
ACE-I or ARB		285 (99)	159 (>99)	54 (100)	53 (100)	52 (98)	0.663
Beta-blocker		278 (96)	158 (99)	53 (98)	52 (98)	53 (100)	1.000

Initial blood results on admission							
C-reactive protein, (mg/L)	median (IQR) range	3.0 (2.0 - 7.0) 0 - 265.0	4.0 (2.0, 8.0) 1.0 - 265	3.5 (2.0 - 11.0) 1.0 - 125.0	3.0 (1.0 – 6.2) 1.0 - 92.0	4.0 (2.0 - 7.0) 1.0 - 265.0	0.696
Leucocyte cell count ($x10^9L$)		12.4 (3.5)	12.8 (3.6)	12.9 (3.5)	13.3 (3.5)	12.3 (3.6)	0.310
Neutrophil count ($x10^9L$)		9.6 (3.2)	10.1 (3.3)	10.0 (3.4)	10.6 (3.4)	9.6 (3.0)	0.244
Monocytes ($x10^9L$)		0.4 (0.4)	0.9 (0.4)	1.0 (0.4)	0.9 (0.3)	0.9 (0.5)	0.485
NT-proBNP, pg/mL		824 (350, 1642)	1103 (628, 1849)	1456 (702, 2455)	980 (565, 1637)	1021 (529, 1436)	0.354

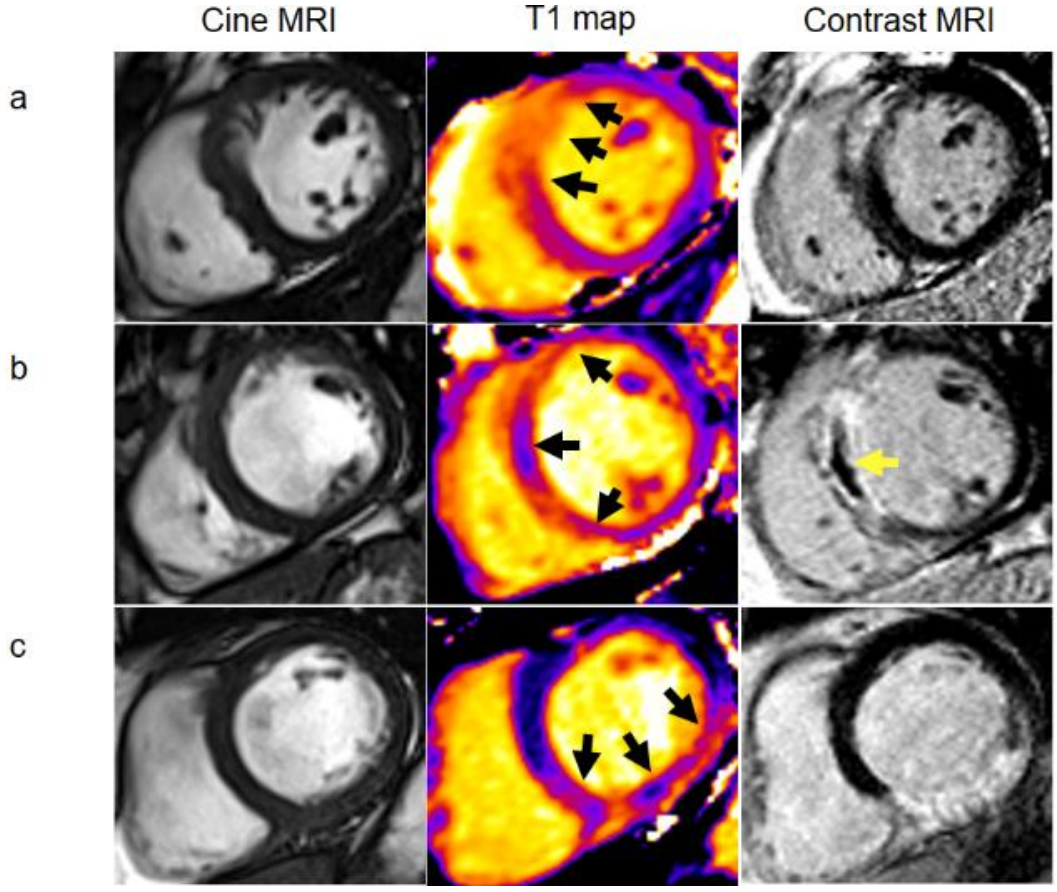
*Footnote: ACE-I or ARB = angiotensin converting enzyme inhibitor or angiotensin receptor blocker; LAD = Left anterior descending coronary artery; LCX = Left circumflex coronary artery; LM = left main coronary artery; RCA = right coronary artery; TIMI = Thrombolysis in Myocardial Infarction grade, PCI = percutaneous coronary intervention. Killip classification of heart failure after acute myocardial infarction: class I - no heart failure, class II - pulmonary rales or crepitations, a third heart sound, and elevated jugular venous pressure, class III - acute pulmonary edema, class IV - cardiogenic shock. * Data are reported as mean (SD), median (IQR), or N (%) as appropriate. P-values have been obtained from a one-way ANOVA or Fisher test. TIMI flow grades pre- and post-PCI were grouped 0/1 vs. 2/3 for this analysis. ‡ Diabetes mellitus was defined as a history of diet-controlled or treated diabetes. † Successfully electrically cardioverted ventricular fibrillation at presentation or during emergency PCI procedure. ¥ Multivessel coronary artery disease was defined according to the number of stenoses of at least 50% of the reference vessel diameter, by visual assessment and whether or not there was left main stem involvement. The blood results on admission and their changes during the first two days after admission are described in Supplementary Table 1. Missing data: Heart rate, n=1; Time from symptom onset to reperfusion, n=20; ST-segment resolution, n=1; CRP, n=7; leucocyte count, n=1. The patients are grouped according to tertile of T1 in hypo-intense core at baseline.*

7.3.3 Left ventricular function and pathology

Initial CMR findings following hospital admission

The CMR findings are summarised in table 7-2 and case examples are shown in figure 7-2. At baseline, the mean (SD) myocardial infarct size was 18 (14) % of LV mass. The average infarct core native T1 (996.9 (57.3)) was higher than native T1 in the remote myocardium (961 (25) ms; p<0.01) but lower than native T1 in the area-at-risk (1097 (52) ms; p<0.01).

Figure 7-2 Acute STEMI cases with different infarct core T1 results revealed by CMR 2 days post-MI and divergent longer term clinical outcomes



(a) Patient with no T1 hypointense infarct core and no microvascular obstruction. *Native T1 within the injury zone (middle) measured 1211 ms. Acute infarct size revealed by late gadolinium enhancement (right) was 22.2%. The LVEF and LV end-diastolic volume were 55.2% and 143.1 ml, respectively. Analysis of the repeat MRI scan after 6 months follow-up indicated that the final infarct size was 15.6% of LV mass and the LV end-diastolic volume had reduced to 103.0 ml. This patient had an uncomplicated clinical course.*

(b) Patient with both T1 hypointense infarct core and microvascular obstruction. *T1 mapping (middle) revealed a hypointense region within the infarct core, corresponding to the area of microvascular obstruction on contrast-enhanced MRI (right). Native T1 within the infarct core measured 1036 ms, which was substantially lower than the T1 value measured at the periphery of the infarct zone (1193 ms). Acute infarct size revealed by late gadolinium enhancement (right) was 33.0%. Microvascular obstruction depicted as the central dark zone within the infarct territory was 3.6% of LV mass. The LVEF and end-diastolic volume were 45.8% and 199.3 ml, respectively. The final infarct size at 6 months was 22.6% of LV mass and the LV end-diastolic volume had increased to 221.8 ml. This patient was re-hospitalized for new onset heart failure during follow-up.* ***(c) Patient with T1 hypointense infarct core, but no microvascular obstruction.*** *T1 mapping (middle) revealed a hypointense region within the infarct core, with a T1 value of 998 ms, which was substantially lower than the T1 value measured at the periphery of the infarct zone (1113 ms). Acute infarct size revealed by late gadolinium enhancement (right) was 30.7%. The left ventricular ejection fraction and end-diastolic volume were 50.2% and 152.6 ml, respectively. Six month follow-up MRI revealed final infarct size was 22.1% of left ventricular mass and there was a significant increase in left ventricular end-diastolic volume to 182.4 ml. This patient had no adverse events during follow-up.*

Table 7-2 Comparison of CM findings at baseline in 288 STEMI survivors and 6-month CMR findings in 267 STEMI patients.

Characteristics*	All patients	Patients with a native T1 infarct core	Patients with a native T1 infarct core grouped by tertile of infarct core zone native T1 (ms) at baseline			P-value
	All patients	Hypointense core	≤ 973 ms	974 < T1 core ≤ 1014 ms	> 1014 ms	
	n = 288	n = 160	n = 54	n = 53	n = 53	
CMR findings 2 days post-MI						
LV ejection fraction, %	55 (10)	52 (9)	52 (10)	51 (8)	53 (10)	0.418
LV end-diastolic volume, ml						
Men	162 (33)	168 (147, 187)	168 (22)	169 (36)	166 (30)	0.900
Women	124 (25)	125 (113, 145)	122 (30)	134 (26)	126 (21)	0.497
LV end-systolic volume, ml						
Men	73 (55, 94)	79 (64, 98)	75 (64, 94)	81 (74, 103)	76 (60, 100)	0.496
Women	53 (41, 66)	64 (50, 69)	64 (57, 71)	66 (57, 70)	56 (45, 65)	0.383
LV mass, g						
Men	142 (124, 159)	145 (130, 166)	149 (135, 170)	143 (126, 159)	141 (130, 160)	0.526

Women	97 (84, 108)	101 (89, 124)	103 (92, 113)	109 (93, 132)	97 (83, 101)	0.113
Edema and infarct characteristics						
Area at risk, % LV mass	32 (12)	40 (11)	37 (11)	35 (10)	36 (11)	0.482
Infarct size, % LV mass	16 (7, 27)	25 (16, 32)	25 (18, 34)	27 (18, 32)	22 (16, 32)	0.386
Myocardial salvage, % of LV mass	18 (12, 24)	17 (12, 23)	18 (12, 24)	17 (10, 22)	16 (13, 22)	0.546
Myocardial salvage index, % of LV mass	62 (44, 84)	49 (36, 62)	50 (40, 62)	46 (30, 62)	50 (40, 63)	0.590
Late microvascular obstruction present, n (%)	145 (50)	23 (14)	49 (91)	45 (85)	43 (81)	0.356
Late microvascular obstruction, % LV mass	0.1 (0.0, 3.5)	2.7 (0.8, 7.5)	5.2 (1.7, 10.5)	2.7 (0.9, 7.1)	1.7 (0.3, 4.7)	0.005
Myocardial haemorrhage, n (%)*	96 (40)	94 (67)	34 (76)	35 (70)	25 (54)	0.086
Myocardial native T1 values						
T1 remote myocardium (all subjects), ms	961 (25)	964 (26)	958 (28)	962 (20)	972 (28)	0.014
Men, ms	959 (25)	962 (26)	955 (29)	959 (19)	973 (26)	0.004
Women, ms	968 (25)	969 (26)	969 (22)	969 (22)	968 (36)	0.992
T1 infarct zone, ms	1097 (52)	1093 (52)	1052 (37)	1088 (33)	1140 (22)	<0.001
T1 hypointense infarct core, ms	997 (57)	997 (57)	938 (30)	995 (12)	1060 (37)	<0.001
Myocardial native T2 values						
T2 infarct core (n=171, ms)	54 (5)	54 (5)	52 (4)	53 (4)	56 (5)	<0.001

CMR findings 6 months post-MI (n=267)						
LV ejection fraction at 6 months, %	63 (57, 69)	60 (53, 65)	59 (53, 65)	59 (54, 64)	61 (54, 68)	0.542
LV end-diastolic volume at 6 months, ml						
Men	165 (140, 193)	176 (155, 204)	188 (160, 209)	169 (153, 197)	171 (156, 196)	0.367
Women	124 (110, 136)	120 (96, 139)	120 (96, 139)	130 (122, 153)	127 (118, 142)	0.338
LV end-systolic volume at 6 months, ml						
Men	61 (43, 78)	69 (56, 95)	73 (58, 98)	69 (62, 84)	63 (53, 96)	0.667
Women	43 (34, 58)	55 (44, 61)	45 (41, 56)	60 (50, 65)	53 (40, 57)	0.213

*Footnote: Abbreviations: LV = left ventricle, T1 = myocardial longitudinal relaxation time. Area-at-risk was measured with T2-mapping. Data are given as n (%) or mean (SD). P-values were obtained from one-way ANOVA, Kruskal-Wallis test, or a Fisher test. * Data are reported as mean (SD), median (IQR), or n (%) as appropriate. Data on T2*-CMR for myocardial haemorrhage were not available in 48 patients.*

Three T1 maps (basal-, mid-, and distal-ventricular levels) were measured in each patient (n=876 T1-maps overall) and 93% of these maps were suitable for analysis. Overall, 20 (6.8%) patients had poor quality T1 maps and 4 (1.3%) patients had no evaluable T1 maps (Figure 2). In all, 42 (4.8%) T1 maps were unsuitable for analysis because of SSFP off-resonance artefacts and 19 (2.2%) T1 maps were affected by motion artefacts. T1 values were higher in infarct tissue surrounding the infarct core than within the infarct core ($p<0.001$) and remote myocardium ($p<0.001$).

7.3.4 Baseline associates of infarct core native T1 (hypothesis 1)

The clinical characteristics that were univariably associated with infarct core native T1 time (ms) and were included in the multivariable models were systolic blood pressure at initial angiography, mmHg (-0.45 (-0.85, -0.05); p = 0.026), LV ejection fraction (%) (0.94 (-0.03, 1.91); p=0.057), infarct size (% LV mass) (-0.93 (-1.68, -0.18); p=0.016), minimum leucocyte count (x109L) (-3.97 (-6.93, -1.01); p=0.009), minimum neutrophil count (x109L) (-4.99 (-8.41, -1.56); p=0.005), maximum log CRP (-7.35 (-15.00, 0.31); p=0.060), maximum leucocyte count (x109L) (-2.53 (-5.09, 0.02); p=0.052), and maximum monocyte count (x109L) (-21.83 (-42.15, -1.51); p=0.035).

In multivariable regression analysis, native T1 in the infarct core was inversely associated with TIMI coronary flow grades at the end of emergency PCI, Killip class and neutrophil count at initial presentation (all p<0.04), independent of LVEF, LV end-diastolic volume or infarct size (table 7-3).

Infarct core native T1 (ms) was univariably associated with infarct core T2 (ms) (r=0.42; p<0.001) and infarct core T2* (ms) (r=0.36; p<0.0001).

Table 7-3 Associates of infarct core native T1 time (for a 10 ms difference) in 160 STEMI survivors with infarct core pathology revealed by native T1 mapping with CMR 2 days post-MI.

Multiple stepwise regression (for a 10 ms difference in infarct core T1)	coefficient (95% CI)	p value
*A. Including patient characteristics and angiographic data**		
Systolic blood pressure at initial angiography, mmHg	-0.05 (-0.09, -0.01)	0.007
Killip class 3 or 4	-3.84 (-6.87, -0.80)	0.014
TIMI flow grade 2 or 3 post-PCI	-7.51 (-15.42, 0.40)	0.063
*B. Including patient characteristics, angiographic data, and minimum neutrophil count**		
Systolic blood pressure at initial	-0.05 (-0.09, -0.01)	0.015

angiography, mmHg		
Killip class 3 or 4	-3.39 (-6.45, -0.33)	0.030
TIMI flow grade 2 or 3 at the end of PCI	-9.77 (-17.67, -1.87)	0.005
Minimum neutrophil count, ($x10^9$L)	-0.50 (-0.86, -0.15)	0.005
C. *Including patient characteristics, angiographic data, minimum neutrophil count*and T2 core (1 ms)*		
T2 core (1 ms)	0.50 (0.32, 0.67)	<0.001
Neutrophils	-0.39 (-0.71, 0.07)	0.016
Gender (male)	-2.32 (-4.25, 0.39)	0.019
SBP	-0.03 (-0.07, 0.00)	0.059
TIMI 2/3 post-PCI	-5.46 (-12.62, 1.70)	0.134

Footnote: The coefficient (95% confidence intervals (CI)) indicates the magnitude and direction of the effect of the patient characteristic (binary or continuous) on the infarct core T1 (ms). For example, in models A and B, on average, infarct core native T1 (10 ms difference) is 0.50 lower for each 1 mmHg increase in SBP.

** The clinical and angiographic characteristics that were assessed are listed in Table 1. The univariable associates with native T1 in the infarct core are described in the text. Separate multivariable analyses were performed for (A) patient characteristics and angiographic data and (B) CMR data. CMR parameters, which were all highly correlated with one another, were included separately in multiple stepwise regression models with patient characteristics and angiographic data to reduce multicollinearity.*

Similar results were obtained when area-at-risk, LV ejection fraction, LV end-systolic volume, and infarct size were included. Maximum leucocyte count (p=0.053) and maximum monocyte count (p=0.034) remained associates of infarct core native T1 after adjustment for LV end-diastolic volume. Similar results were also obtained in the multivariable model with LV end-diastolic volume for minimum leucocyte count (p=0.011).

7.3.5 Relationships for native T1 infarct core versus infarct pathology, including microvascular obstruction, infarct core T2 and myocardial haemorrhage

137 (86%) STEMI patients with a hypointense native T1 infarct core also had microvascular obstruction. In contrast, only 6.3% of those without hypointense infarct core had late microvascular obstruction. The negative- and positive predictive values of native

T1 infarct core for T2 core, early gadolinium enhancement, microvascular obstruction and myocardial haemorrhage disclosed by a T2* core are summarised in table 7-4.

Table 7-4 Negative- and positive predictive values of T1 infarct core for microvascular obstruction, T2 core and myocardial haemorrhage disclosed by a T2* core.

	Native T1 infarct core absent	Native T1 infarct core present	
T2 core			
T2 core absent	111	2	Specificity 98.2 % 95% CI (95.8, 100.0)
T2 core present	17	158	Sensitivity 90.3% 95% CI (85.9, 94.7)
	NPV 86.7% 95% CI (80.8, 92.9)	PPV 98.8% 95% CI (97.0, 100.0)	
Myocardial haemorrhage			
Myocardial haemorrhage absent	97	47	Specificity 67.4% 95% CI (60.5, 74.8)
Myocardial haemorrhage present	2	94	Sensitivity 97.9% 95% CI (95.2, 100.0)

	NPV 97.9% 95% CI (95.3, 100.0)	PPV 66.7% 95% CI (59.4, 74.2)	
Early MVO			
Early MVO absent	114	10	Specificity 91.9% 95% CI (87.1, 97.1)
Early MVO present	14	150	Sensitivity 91.5% 95% CI (87.3, 95.7)
	NPV 89.1% 95% CI (83.8, 94.6)	PPV 93.8% 95% CI (90.1, 97.7)	
Late MVO			
Late MVO absent	120	23	Specificity 83.9% 95% CI (78.4, 89.7)
Late MVO present	8	137	Sensitivity 94.5% 95% CI (90.8, 98.3)
	NPV 93.8% 95% CI (89.6, 98.1)	PPV 85.6% 95% CI (0.80, 0.91)	

Footnote: 95% CI – 95% confidence interval; MVO – microvascular obstruction; NPV – negative predictive value; PPV – positive predictive value. The CMR approaches for delineation of early MVO, late MVO and myocardial haemorrhage are described in the Methods.

7.3.6 Infarct core tissue characteristics as a marker of subsequent left ventricular remodelling (hypothesis 2)

At 6 months, LV end-diastolic volume increased on average (SD) by 5 (25) ml in 262 patients with evaluable data (table 7-2). Adverse remodelling occurred in 30 (12%) patients and 23 (77%) of these patients had a hypointense native T1 core at baseline. Infarct core native T1 (ms) was not associated with change in LV end-diastolic volume at follow-up (p=0.531).

There were 20 clinical characteristics that were univariable associates of adverse LV remodelling, defined as an increase in LV end-diastolic volume ≥ 20% at 6 months from baseline. 244 STEMI participants had complete data for these clinical characteristics and paired CMR scans at baseline and follow-up, and 136 of these patients had a hypointense native T1 core. The univariable characteristics and their p-values that were included in the multivariable model were: **infarct core native T1 (p=0.052),** age (p=0.909), male sex (p=0.847), body mass index (p=0.366), previous myocardial infarction (p=0.364), diabetes mellitus (p=0.491), previous percutaneous coronary intervention (p=0.639), **cigarette smoking (p=0.036),** history of hypertension (p=0.463), hypercholesterolaemia (p=0.912), history of angina (p=0.972), heart rate (p=0.569), systolic blood pressure at initial angiography (p=0.718), Killip class II vs. Killip class I (reference category) (p=0.437), Killip class III/IV vs. Killip class I (reference category) (p=0.574), **sustained ventricular arrhythmia (p=0.015),** symptom onset to reperfusion time (p=0.793), TIMI flow grade 2/3 vs. grade 1 (reference category) at initial angiography (p=0.648), ST segment resolution (none vs. complete (reference category), and p=0.966; incomplete vs. complete (reference category), p=0.089).

In multivariable regression, native T1 (ms, continuous) within the hypointense core was inversely associated with adverse remodelling (table 7-5).

In a sensitivity analysis, the occurrence of a hypointense core within the infarct zone on T1 mapping was associated with the odds ratio for being in the top quarter of an increase in LV end-diastolic volume at 6 months (native T1 core to predict Q4 (n=66) vs. Q1-3 (n=196) (n=26 missing); odds ratio 0.994 (0.987, 0.999); p=0.048).

Table 7-5 Multivariable associates of adverse LV remodelling revealed by CMR in STEMI survivors* after 6 months follow-up.

Multivariable associations	Odds ratio (95% CI)	p value
A Patient and angiographic characteristics		
Native T1 infarct core, per 10 ms	**0.91 (0.82, 1.00)**	**0.061**
Current smoking	5.27 (1.07, 26.00)	0.041
Sustained ventricular arrhythmia	16.06 (1.67, 154.43)	0.016
Incomplete ST-segment resolution	3.29 (0.85, 12.78)	0.085
B Patient and angiographic characteristics and infarct core native T2		
Native T2 infarct core, per 10 ms	1.01 (0.28, 3.67)	0.987
Native T1 infarct core, per 10 ms	0.91 (0.81, 1.01)	0.073
Current smoking	4.99 (0.99, 25.06)	0.051
Sustained ventricular arrhythmia	15.26 (1.57, 148.71)	0.019
Incomplete ST-segment resolution	3.18 (0.81, 12.43)	0.097
C Patient and angiographic characteristics and myocardial haemorrhage		
Myocardial haemorrhage	0.57 (0.14, 2.41)	0.449

Native T1 infarct core, per 10 ms	0.90 (0.81, 1.01)	0.070
Current smoking	4.78 (0.83, 27.52)	0.080
Sustained ventricular arrhythmia	11.70 (0.94, 144.88)	0.055
Incomplete ST-segment resolution	3.68 (0.90, 15.02)	0.069

Footnote: The odds ratio (95% confidence intervals) indicates the magnitude and direction for adverse LV remodelling. For a 10 ms increase in native T1 the odds ratio for adverse LV remodelling reduced (0.91 (0.82, 1.00); p=0.061). For a 1 ms increase in native T1 the odds ratio for adverse LV remodelling reduced (0.99 (0.98, 1.00); p=0.061).

** Twenty clinical characteristics at baseline that were univariable associates of adverse LV remodelling at 6 months post-MI were included in the multivariable model and these univariable associates are described in the text. 267 STEMI patients had CMR at 6 months and baseline and 23 of these patients had missing data of at least one of the univariable characteristics that were included in this multivariable model. C-statistic (area-under-the-curve (AUC)) for the multivariable model in 244 subjects but not including native T1 core: 0.95; C-statistic (AUC) for the model (above) including infarct core native T1 (n=136): 0.81; net reclassification index for incremental addition of T1 core to the model: 0.31, p = 0.184.*

When the multivariable model for adverse remodelling included infarct size, the area-under-the curve (AUC) without native T1 core (continuous, ms) was 0.823 and the AUC with T1 core values included was 0.857. Inclusion of native T1 core values neither increased nor reduced the predictive value of this model (net reclassification index p=0.16).There was no threshold for native T1 core value in the infarct core in relation to its association with LV outcomes at baseline or during follow-up.

7.3.7 Infarct core native T1 early post-MI and NT-proBNP, a biochemical measure of adverse outcome, at 6 months

Biomarker blood samples were collected in the STEMI patients who had been enrolled during office hours and NT-proBNP results were available in 151 (52%) of 288 STEMI

patients overall. 81 of these STEMI patients had evaluable T1 CMR maps at baseline and an NT-proBNP result at 6 months, and 50 (62%) of these patients had a hypointense core disclosed by T1 mapping. The characteristics of these patients were similar to those of the whole cohort (tables not included).

Native T1 within the infarct core and NT-proBNP were not associated at baseline. T1 values within the infarct core at baseline were associated with log NT-proBNP (per 1 pg/mL change) at 6 months (per 1 ms reduction in native T1: coefficient (95% CI) 0.01 (0.01, 0.00); p=0.015) (n=50), independent of LV end-diastolic volume and NT-proBNP at baseline.

7.3.8 Native T1 infarct core, microvascular obstruction, T2 core, myocardial haemorrhage and left ventricular outcomes at 6 months

The relationships for infarct core native T1 (binary and continuous), T2 core (binary and continuous), microvascular obstruction (binary, % LV mass) and myocardial haemorrhage (binary and continuous) for LV outcomes, including LV end-diastolic volume and LV ejection fraction, are shown in table 7-6. Native T1 (ms) was not associated with LV volumes at follow-up. The presence of a hypointense infarct core disclosed by native T1 and the presence and amount of microvascular obstruction were consistently and similarly associated with LV outcomes. Overall, there was no evidence of non-linearity between infarct core T1 (ms) and LV outcomes. Myocardial haemorrhage defined by T2* hypointense core was strongly associated with LV outcomes, including LVEDV and LVEF at 6-months. However, in multivariable regression, T2* (ms, continuous) was not associated with adverse remodelling or health outcome (all cause death or hospitalisation for heart failure).

In multivariable regression, native T1 (ms, continuous) within the hypointense core was inversely associated with adverse remodelling.

Table 7-6 The univariable relationships for infarct core characteristics revealed by native T1, T2, T2* and microvascular obstruction for LV outcomes at baseline and during follow-up in 288 STEMI patients.

	LVEDV at baseline	LVEDV at 6 months	LVEF at baseline	LVEF at follow-up

T1 core	Standardised β	-0.042	-0.035	0.151	0.055
(per 10 ms)	P-value	0.596	0.520	0.057	0.485
T1 core	β	16.410	13.80	-6.642	-4.652
(binary)	P-value	<0.0001	<0.0001	<0.0001	<0.0001
T2 core	Standardised β	0.035	0.057	0.159	-0.033
(per 10 ms)	P-value	0.653	0.282	0.037	0.586
T2 core	β	15.538	12.875	-6.542	-4.494
(binary)	P-value	<0.001	<0.0001	<0.0001	<0.0001
Myocardial	Standardised β	-0.158	-0.140	0.144	0.170
haemorrhage	P-value	0.115	0.038	0.151	0.023
(T2* core, per 10ms)					
Myocardial	β	17.205	16.811	-6.374	-5.769
haemorrhage	P-value	<0.0001	<0.0001	<0.0001	<0.0001
(T2* core, binary)					
Microvascular	Standardised β	0.186	0.209	-0.443	-0.283
obstruction					
(% of LV mass)	P-value	0.002	<0.0001	<0.0001	0.004
Microvascular	β	15.853	12.454	-6.620	-4.464
obstruction					
(binary)	P-value	<0.001	<0.0001	<0.0001	<0.0001

The relationships for infarct core native T1 relaxation time (per 10 ms), native T1 infarct core (binary), T2 core relaxation time (per 10 ms), T2 infarct core (binary), T2 core relaxation time (per 10 ms), myocardial haemorrhage (binary) and the presence and the amount of microvascular obstruction (n=145 STEMI patients) with LV outcomes are summarised by p-values and, for continuous predictors, standardised regression coefficients or odds ratios per standard deviation increase in native T1 (ms) or extent of microvascular obstruction (% of LV mass). Models with follow-up are adjusted for baseline. Binary predictors are summarised by p-values and unstandardized regression coefficients or odds ratios. The odds ratio (p-values) for adverse remodelling and infarct core characteristics are: native T1 core (per 10 ms) are 0.939, p=0.122; native T1 core (present/absent): 2.692, p=0.016; T2 core (present/absent): 2.874, p=0.026; myocardial haemorrhage (present/absent): 2.556, p=0.025; microvascular obstruction (% LV mass): 1.112, p=0.004; microvascular obstruction (present/absent) 1.883, p=0.115.*

7.3.9 Infarct core tissue characteristics and health outcomes (hypothesis 3)

All 288 patients had long term follow-up data completed. Thirty (10.4%) patients died or experienced a heart failure event. These events included 5 cardiovascular deaths, 3 non-cardiovascular deaths and 22 episodes of heart failure (Killip Class 3 or 4 heart failure (n=20) or defibrillator implantation n=2). Thirteen (4.5%) patients died or experienced a first heart failure hospitalisation post-discharge, and 8 (61.5%) of these patients had a hypointense infarct core at baseline.

Native T1 values (ms) within the hypointense infarct core (n=160 STEMI patients) were inversely associated with the risk of all-cause death or first hospitalization for heart failure post-discharge (for a 10 ms increase in native T1: hazard ratio 0.730, 95% CI 0.617, 0.863; $p<0.001$) including after adjustment for LVEF at baseline, LV end-diastolic volume at baseline, infarct core T2 (10 ms difference), and myocardial haemorrhage (figure 7-3; table 7-7). Infarct core T1 retained its prognostic significance over and above infarct core T2 and myocardial haemorrhage (table 7-7, models C – F). The net reclassification index for the inclusion of infarct core native T1 (ms) in a multivariable prognostic model for all-cause death or heart failure post-discharge was 1.129 (95% CI 0.516, 1.742); $p<0.001$) (table 7-7). Using ROC analysis, the C-index for infarct core native T1 for all-cause death or heart failure was 0.806. The C-indexes for the prognostic model without and with infarct core native T1 (ms) were 0.715 and 0.931, respectively.

Figure 7-3 Kaplan-Meier survival curves for 160 STEMI patients grouped according to the native T1 value in the infarct core with patients grouped by thirds (lowest T1 tertile vs. tertiles 2 and 3) and all-cause death or first heart failure hospitalisation (n=13) after discharge from hospital to the end of follow-up (censor time 839 (598 to 1099) days). Infarct core native T1 values in the lowest tertile were associated with all-cause death or heart failure hospitalisation.

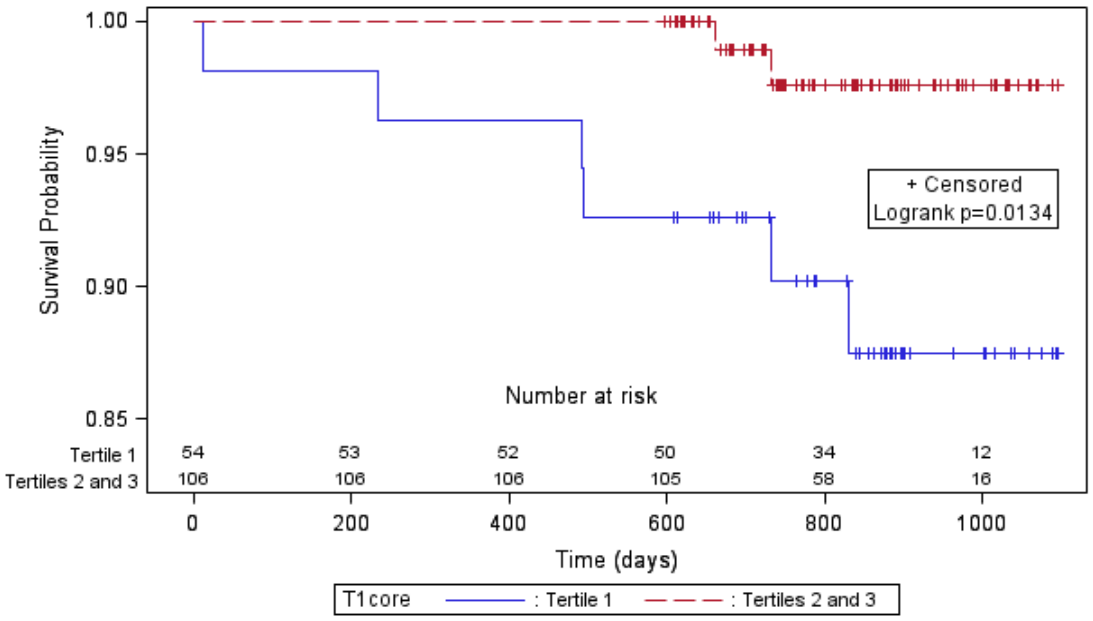


Table 7-7 . Relationships for infarct core T1 and T2 relaxation times (10 ms) revealed by CMR at baseline in 160 STEMI patients with an infarct core and all-cause death or first hospitalisation for heart failure post-discharge.

Associations	Hazard ratio (95% CI)	p value
Univariable associations		
Infarct core native T1, (for a 10 ms difference)	**0.730 (0.617, 0.863)**	**<0.001**
Myocardial haemorrhage	2.488 (0.814, 7.609)	0.110
LVEF at baseline, (for a 1% difference)	0.934 (0.885, 0.985)	0.013
Peak log eosinophil count, $x10^9$/L	0.617 (0.432, 0.881)	0.008
Model A		
Infarct core native T1, (for a 10 ms difference)	**0.744 (0.627, 0.883)**	**<0.001**
LVEF at baseline, (1% difference)	0.938 (0.883, 0.996)	0.036

Model B		
Infarct core native T1, (for 10 ms difference)	**0.737 (0.621, 0.875)**	**<0.001**
Peak log eosinophil count, (1 x10^9/L)	0.728 (0.476, 1.114)	0.144
Univariable associations		
Infarct core native T2, (for a 10 ms difference)	0.186 (0.032, 1.094)	0.063
Model C		
Infarct core native T2, (for a 10 ms difference)	**0.244 (0.039, 1.528)**	**0.132**
LVEF at baseline, (for a 1% difference)	0.932 (0.870, 0.998)	0.044
Model D		
Infarct core native T2, (for 10 ms difference)	**0.203 (0.034, 1.297)**	**0.093**
Peak log eosinophil count, x10^9/L	0.681 (0.460, 1.007)	0.054
Model E		
Infarct core T1, (for 10 ms difference)	**0.738 (0.624, 0.873)**	**<0.001**
Myocardial haemorrhage	1.965 (0.229, 16.864)	0.538
Model F		
Infarct core T1, (for 10 ms difference)	**0.752 (0.634, 0.893)**	**0.001**
Infarct core T2, (for a 10 ms difference)	0.428 (0.068, 2.683)	0.365
Myocardial haemorrhage	1.485 (0.159, 13.879)	0.729

Footnote: Thirteen (8.1%) patients experienced all-cause death or heart failure hospitalisation post-discharge discharge (median (range) follow-up duration of 841 (723 – 945) days). Given the limited number of adverse events, the models were specified to assess the prognostic relationships of infarct core native T1 versus circulating markers of systemic inflammation, LV function, LV volume and infarct characteristics that were measured at approximately the same time 2 days after hospital admission.

7.3.10 Prognostic importance of infarct core native T1: comparisons with microvascular obstruction and longer term health outcomes

In a univariate Cox model that included infarct core native T1 (ms), native T1 core (binary), T2 core (ms), T2 core (binary), myocardial haemorrhage and the presence (binary) and amount of microvascular obstruction (% LV mass), only infarct core native T1 (ms) ($p<0.001$) and the amount of microvascular obstruction (% LV mass) ($p<0.001$) were associated with all-cause death or first heart failure hospitalisation after discharge.

7.4 Discussion

The main findings of our study are: 1) native T1 mapping revealed without an intravenous contrast agent resulted in evaluable scans in a high percentage (93%) of STEMI survivors 2 days post-MI; 2) acute culprit coronary artery blood flow and circulating measures of systemic inflammation at the time of the hospital admission were multivariable associates of native T1 within the hypointense infarct core revealed by T1 mapping 2 days later; 3) native T1 values (ms) within the infarct core were clinically meaningful since they tended to be associated with adverse remodelling, NT-proBNP concentrations at 6 months, and all-cause death or heart failure hospitalisation post-discharge during longer term follow-up; 4) compared with infarct core T2 or myocardial haemorrhage revealed by T2* mapping, infarct core T1 was more consistently associated with LV surrogate outcomes and all-cause death or heart failure hospitalisation (table 7-7), implying T1 core is more closely linked with infarct pathology;; 5) compared with microvascular obstruction, a hypointense infarct core revealed by native T1 had similar prognostic significance for LV outcomes at 6 months and for post-discharge cardiac events, including all-cause mortality and heart failure hospitalisation, in the longer term (tables 7-6 and 7-7). Finally, this study adds to the emerging literature on the prognostic value of quantitative native T1 CMR [(Banypersad et al., 2015)] and reaffirms the prognostic importance of MVO post-STEMI [(van Kranenburg et al., 2014)].

The results of this study extend what is known about infarct core pathology, and also provide a potential mechanistic explanation. Infarct size [(Holman et al., 1978, Pfeffer and Braunwald, 1990)] and pathology, including microvascular obstruction [(van Kranenburg et al., 2014)], haemorrhage [(Eitel et al., 2011)], and salvage [(Eitel et al., 2010)], predict cardiac morbidity and mortality post-MI. These pathologies are revealed by contrast-enhanced CMR, and until recently, the assessment of infarct tissue without an intravenous contrast agent has been limited to T2-weighted and T2* imaging of myocardial haemorrhage [(Eitel et al., 2011, Yang et al., 2007, Payne et al., 2011b, Payne et al., 2011a, Robbers et al., 2013)]. T1-mapping methods, including MOLLI [(Messroghli et al., 2004, Messroghli et al., 2007b)] and shMOLLI [(Piechnik et al., 2010, Piechnik et al., 2013)], can now be integrated into clinical CMR protocols. Previous studies have assessed myocardial native T1 in experimental MI models *ex vivo* [(Higgins et al., 1983, Williams et al., 1980)], *in vivo* [(Yang et al., 2007)], or in proof-of-concept clinical studies involving

much smaller numbers of MI patients [(Dall'Armellina et al., 2013, Dall'Armellina et al., 2012, Messroghli et al., 2003, Messroghli et al., 2007b, Been et al., 1988)]. Our study extends these findings in a much larger STEMI cohort and provides new evidence that T1 core is more reflective of the severity of infarct injury and its prognostic importance than infarct core T2 and potentially also myocardial haemorrhage.

We found that a hypointense infarct core revealed by native T1 mapping and microvascular obstruction revealed by late gadolinium imaging, assessed independently by different observers, co-existed in 86% of patients implying a common pathological basis. The positive predictive value of early (dynamic) microvascular obstruction for native T1 infarct core was higher (93.8%) than that of late microvascular obstruction (85.6%). This difference can be explained by the occurrence of a hypointense T1 core in close association with microvascular obstruction in the early gadolinium enhancement imaging but a hypointense T1 core is less strongly associated with microvascular obstruction in the late gadolinium enhancement imaging. High negative predictive values were observed for both late microvascular obstruction and myocardial haemorrhage (T2* core) (93.8% and 97.9%, respectively) for a native T1 core. Early microvascular obstruction is to some extent a dynamic pathology since it may dissipate over time due to the contribution of reversible oedema and microvascular spasm. Native T1 is also affected by these pathologies, hence its closer association with early microvascular obstruction than with late microvascular obstruction which is a more persistent pathology because of its association with irreversible capillary destruction and intramyocardial haemorrhage (Robbers et al., 2013). This theory merits further assessment in pathology studies.

Our study builds on the results from previous studies of infarct core pathology (Wu et al., 1998b, van Kranenburg et al., 2014, Eitel et al., 2011). Dall'Armelina *et al.* studied 41 acute MI patients and found that native T1 values correlated with the segmental extent of MI and LV function acutely and with improvements in LV function at 6 months (Dall'Armellina et al., 2012). However, their study had some limitations. There were no age- or sex-matched controls, the sample size was limited (n=32 STEMI patients) so multivariable analyses were not performed, and 17% of the cohort did not have follow-up imaging. In some of their analyses, segments with microvascular obstruction were not included, Our study differed in a number of important ways from that of Dall'Armelina *et al.* (Dall'Armellina et al., 2012). First, our STEMI cohort was 10-fold larger in size, and

7% had primary reperfusion therapy with thrombolysis. We used a different T1-mapping method and CMR was performed at 1.5 Tesla rather than 3.0 Tesla (which is associated with higher T1 values). We assessed T1 values in all patients and specifically focused on patients with microvascular obstruction rather than excluding them. We also performed multivariable analyses to assess the prognostic significance of T1 values for LV outcomes, independent of clinical characteristics, including LV volume and the ischaemic area-at-risk.

We have compared infarct core pathology delineated by native T1 mapping with microvascular obstruction, which is an established prognostic CMR biomarker post-MI [(van Kranenburg et al., 2014)]. Native T1 mapping is obtained without the use of an intravenous gadolinium-based contrast agent whereas microvascular obstruction is revealed by serial CMR imaging of EGE and LGE after intravenous contrast administration. We observed a high degree of concordance between the occurrence of a hypointense infarct core depicted by native T1 CMR (56%) and late microvascular obstruction (50%) as revealed by contrast-enhanced CMR. Although both a native T1 core and microvascular obstruction are depicted as a hypointense core within the hyperintense infarct zone (figure 7-2), the physics of these CMR techniques is entirely different. On the one hand, a hypointense infarct core depicted by non-contrast native T1 mapping is due to local destruction of the T1 magnetisation signal. On the other hand, microvascular obstruction (figure 7-2) is due to a failure of gadolinium contrast to penetrate within the infarct core hence the dark zone where gadolinium is absent within the infarct zone. Both CMR methods are T1-weighted but contrast kinetics are not relevant for native T1 mapping since intravenous contrast is not administered. Accordingly, T1 mapping avoids the theoretical clinical risks and actual restrictions involved with gadolinium contrast-based imaging of microvascular obstruction.

Culprit artery coronary flow at the end of emergency PCI reflects the efficacy of coronary reperfusion, and reduced coronary flow initially independently predicted native T1 relaxation time within infarct core as assessed by CMR 2 days later. Similar associations also exist for microvascular obstruction [(Amabile et al., 2010, van der Laan et al., 2012)], and in our study, both infarct core native T1 and microvascular obstruction were independently associated with circulating biomarkers of acute systemic inflammation. The occurrence of an infarct core disclosed by native T1 mapping, and the nature of the core

(i.e. the native T1 value), were associated with the initial severity of MI (i.e. Killip heart failure class), systemic inflammation (i.e. leucocyte counts), and LV remodelling and health outcomes in the longer term. We think that the prognostic significance of native T1values within the hypointense core are a distinctive attribute compared with microvascular obstruction since signal intensity values within microvascular obstruction are not clinically meaningful beyond binary categorisation (i.e. present / absent). The clinical utility of native T1 as a novel non-contrast imaging biomarker for prognosis and risk stratification post-MI merits further prospective assessment.

The fact that infarct core pathology can be revealed without an intravenous contrast agent, and that this finding has similar prognostic significance with late microvascular obstruction, indicates that native T1 mapping could represent an alternative non-contrast CMR method for the assessment of infarct pathology in STEMI survivors. Intravenous gadolinium contrast represents a practical limitation for clinical CMR because of the risks associated with contrast allergy and advanced kidney disease. Since native T1 CMR mapping does not involve an intravenous contrast agent it is amenable to wider adoption. Furthermore, acquisition of the native T1 map does not prolong the CMR scan, in contrast to late gadolinium enhancement imaging for microvascular obstruction which is typically imaged 10 - 15 minutes after dosing [(Kramer et al., 2013)].

7.4.1 Limitations

We performed a single centre natural history study involving near-consecutive STEMI admissions. The STEMI patients in our natural-history study were recruited 24/7 therefore flow cytometry and routine NT-proBNP testing in all participants was not pragmatically possible.

T1 assessment is sensitive to motion artefacts and imperfect breath holding, which may reduce image quality. A shortened version of this sequence (ShMOLLI) involving only 9 heart beats has been developed, which shortens breath hold time and may help to account for these limitations [(Piechnik et al., 2010)]. Despite this, the MOLLI method has high precision reproducibility. Our T1 measurements are in good agreement with *in vivo* data published in the literature, including previous measurements using the ShMOLLI sequence [(Piechnik et al., 2010, Piechnik et al., 2013)].

The number of adverse events limited the number of variables that could be included in the multivariable models, however the associations between infarct core native T1 and a range of surrogate and clinical outcomes including adverse remodelling revealed by CMR, NT-proBNP and the primary health outcome (all cause death / heart failure), supports the adverse prognostic importance of infarct core native T1. Our study does not permit inference on causality, and other interpretations of our data are, of course, possible, and further studies are warranted.

7.5 Conclusions

We found that infarct core pathology revealed by native T1 maps had similar prognostic value compared with microvascular obstruction revealed by late gadolinium enhancement CMR. Native T1 mapping is potentially widely applicable in clinical practice, not limited by renal disease, and so potentially could represent an alternative non-contrast CMR option for the assessment of infarct pathology

8 Chapter 8: The index of microvascular resistance is an acute biomarker for myocardial haemorrhage and a clinical tool for risk stratification in reperfused survivors of acute-ST elevation myocardial infarction

Introduction

8.1 Introduction

Despite the success of primary percutaneous coronary intervention (PCI) in achieving coronary reperfusion in most patients with acute ST-elevation myocardial infarction (STEMI), failure of myocardial reperfusion affects almost half of STEMI patients (Ahmed et al., 2013, Carrick and Berry, 2013, Frohlich et al., 2013, Cochet et al., 2009, Hombach et al., 2005). The index of microvascular resistance (IMR) measured at the end of PCI predicts the subsequent occurrence of microvascular obstruction one week post-STEMI (McGeoch et al., 2010), and adverse LV remodelling, heart failure and all-cause mortality in the longer term (Layland et al., 2013, Payne et al., 2012, Fearon et al., 2013). Accordingly, IMR has potential to identify higher risk STEMI patients at a very early time-point for preventative therapies.

Experimental studies in a pig model of reperfused MI identified myocardial haemorrhage two days post-MI as an irreversible but potentially preventable outcome (Robbers et al., 2013). Using cardiac magnetic resonance (CMR) imaging, Robbers et al found that myocardial haemorrhage is preceded by microvascular obstruction which is characterised by fibrin-rich microvascular thrombi within intact capillaries. These results raised the question of whether IMR might be discriminative for microvascular obstruction (a therapeutic target) vs. myocardial haemorrhage (a manifestation of irreversible infarction). Accordingly, we aimed to assess whether IMR measured at the end of primary percutaneous coronary intervention (PPCI) might discriminate STEMI patients at risk of subsequent IMH.

We hypothesised that IMR would be 1) more strongly associated with myocardial haemorrhage, reflecting severe microvascular damage, than other pathologies with a reversible component, such as microvascular obstruction and 2) independently associated with adverse outcome post-STEMI including left ventricular (LV) remodelling and pre-defined health outcomes.

To investigate these hypotheses we measured IMR at the end of emergency PCI in acute STEMI patients undergoing serial cardiac magnetic resonance (CMR) imaging 2 days and 6 months post-MI. We assessed the clinical associates of IMR with infarct characteristics

and subsequent LV remodelling and examined its association with all-cause death and first hospitalisation for heart failure.

8.2 Methods

We performed a prospective single centre cohort study in 289 reperfused STEMI patients, as detailed in chapter 2. IMR measurement was not included in analyses if measured during the second procedure for the deferred patients in the deferred-stenting sub-study.

8.2.1 Index of microvascular resistance following coronary reperfusion

IMR and coronary flow reserve (CFR) were measured at the end of PPCI using guidewire based-thermodilution as described in detail in chapter 2. In brief, IMR is defined as the distal coronary pressure multiplied by the mean transit time of a 3 ml bolus of saline at room temperature during maximal coronary hyperemia, measured simultaneously (mmHg x s, or units) (Fearon et al., 2008, McGeoch et al., 2010, Payne et al., 2012). Coronary flow reserve is defined as the mean transit time at rest divided by the mean transit time during hyperaemia. Hyperemia was induced by 140 μ/kg/min of intravenous adenosine preceded by a 2 ml intracoronary bolus of 200 μg of nitrate. The mean aortic and distal coronary pressures were recorded during maximal hyperemia. In our study, the repeatability of IMR was assessed by duplicate measurements 5 minutes apart in a subset of 12 consecutive patients, in line with previous observations (Payne et al., 2012).

Cardiac magnetic resonance (CMR) was assessed 2 days and 6 months as described in detail in chapter 2. In brief, Cine-CMR was used to measure LV ejection fraction and volumes. IMH was defined as a hypointense infarct core with a T2* value <20 ms. Microvascular obstruction (MVO) was defined as a hypointense infarct core as revealed by late gadolinium contrast-enhanced CMR.

8.3 Results

289 STEMI patients had culprit coronary artery IMR measured acutely and patients underwent CMR at 2.1±1.8 days and 6 months later. The median IMR [interquartile range] was 24 [15–44]. 245 patients (86%) had evaluable T2*maps at baseline and 101 of these

patients (41%) had IMH. CMR follow-up at 6-months was achieved in 263 of the patients (91%) and all patients had longer-term health outcomes assessed.

8.3.1 Repeatability of IMR measurements

Repeated IMR measurements obtained by 4 different operators in 12 STEMI patients were highly correlated (r=0.99, P<0.001), with a mean difference between IMR measurements of 0.01 (mean standard error 1.59 [95% CI −3.52 to 3.54], p=0.48) (Payne et al., 2012).

8.3.2 Relationships for IMR with IMH and MVO

All of the patients with IMH had MVO, but 32 patients had MVO (13%) without IMH. IMR was higher in patients with IMH (37 [21 – 63]) than in patients without IMH (17 [12 – 33]), including those that had MVO in the absence of IMH (17 [13 – 39]; p<0.0001).

Using Receiver Operator Characteristic (ROC) analysis, the optimal cut-off for IMR in predicting IMH was 27 [area-under-the-curve (AUC) 0.73 (0.66, 0.79)]. The IMR cut-off for MVO was of 23.5 [AUC 0.68 (0.61, 0.75)]. IMR was more strongly associated with IMH (odds ratio 4.24 (2.38, 7.58); p<0.001) than for MVO (2.84 (1.70, 4.73); p<0.001).

8.3.3 IMR and adverse remodelling at 6-months

IMR measured acutely was independently associated with adverse remodelling at 6 months (1.01 (1.00, 1.03); p=0.006) and NT-proBNP at 6 months (4.64 (2.17, 7.12); p<0.001), including after adjustment for baseline LVEF and LVEDV (p=0.008).

8.3.4 IMR and LV function at 6 months

In multivariable regression IMR was inversely associated with LVEF at 6-months including after adjustment for baseline LVEF (regression coefficient -0.05 (95% CI -0.08, -0.01); p=0.02).

8.3.5 IMR and longer-term health outcomes

IMR was a weak multivariable associate of ACD/HF (n=30 events during admission and post-discharge (hazard ratio 1.016 (1.009, 1.023); p<0.001), whereas CFR was not (hazard ration 0.659 (0.382, 1.137); p=0.134). IMR values in the highest tertile were also associated with ACD/HF post-discharge (HR 3.30 (1.59, 6.86); p=0.001).

8.3.6 The comparative clinical utility of IMR versus CFR for acute risk assessment in reperfused STEMI patients

CFR was lower in patients with IMH (1.4 [1.0 – 1.8]) than in patients without IMH (1.7 [1.4 – 2.5]), including those that had MVO in the absence of IMH (1.5 [1.1 – 1.8]; p<0.001). Both IMR and CFR were associated with LVEF at 6-months, after adjustment for baseline LVEF (p=0.001 and p=0.029, respectively). In multivariable analyses including other clinical and angiographic characteristics, only IMR was associated with LVEF at 6-months (regression coefficient -0.05 (95% CI -0.08, -0.01); p=0.02). In contrast to IMR, CFR was not associated with adverse remodelling at 6 months (p=0.117).

8.4 Discussion

The main findings of our study are: 1) IMR measured in the culprit coronary artery after reperfusion is more strongly associated with myocardial haemorrhage than microvascular obstruction in STEMI survivors 2 days later and 2) compared with CFR, IMR has stronger prognostic importance and greater potential clinical utility for risk assessment post-STEMI.

Our paper adds to the emerging literature on the prognostic value of IMR (Fearon et al., 2013) and reaffirms the prognostic importance of myocardial haemorrhage post-STEMI (Eitel et al., 2011, Husser et al., 2013).

Our results have important clinical implications: IMR adds early prognostic information at the time of emergency reperfusion and so has potential to stratify patients at risk of myocardial haemorrhage for more intensive therapy.

8.5 Limitations

We performed a single centre natural history study involving near-consecutive STEMI admissions.

T2* CMR is sensitive to motion artefacts and imperfect breath holding, which and may reduce image quality. Our T2* measurements are in good agreement with *in vivo* data published in the literature (Kali et al., 2013b, O'Regan et al., 2010).

8.6 Conclusion

IMR is more strongly associated with myocardial haemorrhage than microvascular obstruction, and is independently associated with adverse remodelling and LV ejection fraction at 6 months. Compared with CFR, IMR has stronger prognostic importance and greater potential clinical utility for risk assessment post-STEMI. Since IMH is a secondary phenomenon post-MI, IMR measured at the end of PPCI has potential to risk stratify STEMI patients for targeted therapy for microvascular obstruction to achieve myocardial reperfusion and prevent myocardial haemorrhage.

9 Chapter 9: A Randomised Trial of Deferred Stenting versus Immediate Stenting to Prevent No-Reflow in Acute ST-Elevation Myocardial Infarction (DEFER STEMI)

9.1 Introduction

Primary percutaneous coronary intervention (PCI) with stenting immediately after coronary reperfusion is the guideline-recommended treatment for acute ST-elevation myocardial infarction (STEMI) and is effective at reducing mortality (Keeley et al., 2003, Steg et al., 2012). However, a substantial proportion of patients with STEMI develop chronic cardiac failure owing to poor restoration of microvascular function and myocardial perfusion. This occurrence is called the 'no-reflow' phenomenon. No-reflow is defined as an acute reduction in myocardial blood flow despite a patent epicardial coronary artery (Jaffe et al., 2008). Although substantial evidence supports the concept that the pathophysiology of no-reflow involves microvascular obstruction secondary to distal embolization of clot, microvascular spasm and thrombosis (Jaffe et al., 2008); irreversible microvascular injury and subsequent intramyocardial haemorrhage are now also thought to be important factors in the process (Robbers et al., 2013). It is likely that microvascular obstruction precedes myocardial haemorrhage, by causing hypoxic disruption of microvascular integrity in the core of the infarct (Robbers et al., 2013, Fishbein et al., 1980).

Angiographic no-reflow (defined according to the TIMI coronary flow grade as 0 or 1) occurs in approximately 10% of cases of primary PCI and is a consequence of initial reperfusion or PCI procedures including stent deployment. An acute reduction in angiographic flow may be observed after stent deployment and expansion, suggesting that the negative effect on distal flow may be the consequence of increased atherosclerotic and thrombotic material embolization in the microvasculature. The risk factors associated with no-reflow include patient characteristics, such as increasing age and delayed presentation, and coronary characteristics such as a completely occluded culprit artery and heavy thrombus burden (Jaffe et al., 2008, Harrison et al., 2013, Morishima et al., 2000, Antoniucci et al., 2001, Ndrepepa et al., 2010).

No therapies have been shown to prevent no-reflow and when it occurs, treatment by administration of vasodilator drugs (Vijayalakshmi et al., 2006) and intra-aortic balloon counter-pulsation therapy is empirical (Vijayalakshmi et al., 2006, Jaffe et al., 2008, Steg et al., 2012, Windecker et al., 2014). In primary PCI, stenting occurs immediately after coronary reperfusion at a time when clot burden may be greatest and vascular spasm due to

acute reperfusion injury may be pronounced. Thus, the rationale behind a deferred stenting strategy is that a period of time spent on the coronary care unit, with anticoagulant and antiplatelet therapies, reduces vascular reactivity and thrombus burden, such that deferred stenting is safe and effective.

We hypothesised that after initial coronary reperfusion and normalisation of coronary blood flow, brief deferral of stenting might reduce the occurrence of angiographic no-reflow, MVO and IMH, compared to usual care with immediate stenting and increase myocardial salvage. We investigated this hypothesis in a real-life clinical setting involving STEMI patients treated with primary PCI.

9.2 Methods

9.2.1 Trial design

We performed a prospective randomised controlled parallel group trial in STEMI patients enrolled in a single centre between 11 March 2012 to 21 November 2012. The trial was a proof-of-concept trial nested in the larger prospective cohort study.

9.2.2 Participants and eligibility criteria

Patients at risk of no-reflow were selected if radial artery access was used and one or more of the following *inclusion criteria* were present:

1) *Clinical history (Jaffe et al., 2008, Morishima et al., 2000, Antoniucci et al., 2001, Ndrepepa et al., 2010, Vijayalakshmi et al., 2006)*: previous myocardial infarction, increased age (i.e. age ≥ 65 years), duration of symptoms > 6 hours;

2) *Culprit coronary artery abnormalities (Jaffe et al., 2008, Morishima et al., 2000, Antoniucci et al., 2001, Ndrepepa et al., 2010, Vijayalakshmi et al., 2006)*: an occluded artery (Thrombolysis in Myocardial Infarction (TIMI) grade 0/1 (1985)) at initial angiography, heavy thrombus burden (TIMI ≥ grade 2 (Gibson et al., 1996)), long lesion length (≥ 24 mm), small vessel diameter i.e. ≤ 2·5 mm;

3) *Clinical signs of acute microvascular injury after initial reperfusion (Jaffe et al., 2008, Morishima et al., 2000, Antoniucci et al., 2001, Ndrepepa et al., 2010, Vijayalakshmi et al., 2006)*: persistent ST-elevation > 50%.

The *exclusion criteria* were:

1) Absence of normal (TIMI grade 3) coronary blood flow after initial reperfusion with aspiration thrombectomy with or without balloon angioplasty. The residual severity of the culprit stenosis was not relevant to participation provided TIMI grade 3 flow was evident;

2) Cardiogenic shock;

3) A contra-indication to magnetic resonance imaging (e.g. permanent pacemaker);

4) Inability to give informed consent.

9.2.3 Setting and PCI procedure

Consecutive STEMI admissions were screened for these inclusion and exclusion criteria. During ambulance transfer to the hospital, the patients received 300 mg of aspirin, 600 mg of clopidogrel and 5000 IU of unfractionated heparin (Steg et al., 2012, Windecker et al., 2014). A conventional approach to primary PCI was adopted in line with usual care in our hospital (Steg et al., 2012, Windecker et al., 2014). Conventional bare metal and drug eluting stents were used. Covered stents or investigational stents designed to reduce thrombus embolisation were not used (Stone et al., 2012a). The guideline to cardiologists recommended minimal intervention for initial reperfusion with aspiration thrombectomy only or minimal balloon angioplasty (e.g. a compliant balloon sized according to the reference vessel diameter and inflated at 4-6 atmospheres 1-2 times). Bail-out PCI because of coronary dissection or repeated angioplasty to minimize stenosis severity were not permitted and patients treated in this way were not eligible to participate. Provided TIMI grade 3 flow had been achieved with initial reperfusion therapy then the residual stenosis severity had no influence on eligibility. During PCI, glycoprotein IIbIIIa inhibitor therapy was initiated with high dose tirofiban (25 μg/kg/bolus) followed by an intravenous infusion of 0.15 μg/kg/min for 12 hours (Steg et al., 2012, Windecker et al., 2014). No reflow was treated according to contemporary standards of care with intra-coronary nitrate (i.e. 200

μg) and adenosine (i.e. 30 – 60 μg) (Steg et al., 2012, Windecker et al., 2014), as clinically appropriate.

In patients with multivessel coronary disease, multivessel PCI was not recommended, in line with clinical guidelines (Steg et al., 2012, Windecker et al., 2014). The subsequent management of these patients was symptom-guided.

9.2.4 Informed consent

The amendment to the original study protocol to include the deferred stenting sub-study was approved by the West of Scotland Research Ethics Committee, reference 10-S0703-28 (appendix 5). Witnessed informed consent was verbally obtained after coronary reperfusion in eligible patients in the cardiac catheter laboratory. When the patient returned to the Coronary Care Unit an amended Patient Information Sheet approved by the local ethics committee was provided (appendix 6) and written informed consent was then obtained (appendix 7). The patients who were not randomised were included in a registry.

9.2.5 Randomisation, implementation and blinding

Randomisation took place immediately after obtaining verbal consent using a web-based computer tool with a concealed random allocation sequence provided by the independent clinical trials unit and implemented by the catheter laboratory physiologist. Randomisation was on a 1:1 basis between usual care with immediate stenting and deferred stenting.

9.2.6 Interventions

The deferred PCI strategy involved an intention-to-stent 4 to 16 hours after initial coronary reperfusion. This time interval was based on a balance between competing benefits and risks. A short minimum period (4 hours) was adopted given our concern about the theoretical time-related risk of coronary reocclusion. In practice, a guideline of at least 8 hours was recommended for the deferred PCI to permit the beneficial effects of reperfusion and anti-thrombotic therapies and in order that all patients could be treated between 0700 – 2300 hrs during the first 24 hours of admission to ensure that the second procedure occurred at a time which facilitated a rest period for the patient and the staff. Finally, an upper limit of 16 hours was set to minimise any prolongation of the hospital admission.

The treatment protocol for deferred patients included transfer to the Coronary Care Unit, continuous intravenous infusion of glycoprotein IIbIIIa inhibitor therapy (tirofiban, 0·15 μg/kg/min) and administration of subcutaneous low molecular weight heparin (enoxaparin, 1 mg/kg 12 hourly) for up to 16 hours (extended tirofiban infusion chart protocol included in appendix 8). The radial artery sheath used for PCI was retained or removed according to operator and patient preference. Arterial blood pressure and the radial sheath site were monitored in the Coronary Care Unit. All patients also had continuous ECG monitoring in the Coronary Care Unit.

Usual care included immediate stenting in the catheter laboratory and intravenous glycoprotein IIbIIIa inhibitor therapy for 12 hours (tirofiban, 0·15 μg/kg/min). After the PCI procedure was completed the patients returned to the Coronary Care Unit and were treated with optimal secondary prevention measures (Steg et al., 2012).

9.2.7 Primary outcome

The primary outcome was the incidence of no/slow-reflow (Steg et al., 2012), defined as absent flow (TIMI flow grade 0), incomplete filling (TIMI flow grade 1) or slow-reflow but complete filling (TIMI 2) of the culprit coronary artery during or at the end of PCI as revealed by the coronary angiogram during the first or second procedure. The definition of no-reflow also required the absence of coronary dissection or obstruction (e.g. due to thrombus) that could cause a decrease in coronary blood flow (Jaffe et al., 2008).

9.2.8 Secondary outcomes

The secondary outcomes included angiographic, ECG and MRI parameters.

9.2.9 Angiographic secondary outcomes

The angiographic secondary outcomes were no-reflow (TIMI flow grade 0/1), final TIMI flow grade (1985), corrected TIMI frame count (Gibson et al., 1996), TIMI myocardial blush grade (Gibson et al., 2000), the occurrence of intra-procedural thrombotic events (McEntegart et al., 2012), (defined as the development of new or increasing thrombus, abrupt vessel closure, or distal embolisation occurring at any time during the procedure in the culprit vessel or any significant side branch measuring ≥ 2 mm). Embolisation was

defined as a distal filling defect with an abrupt 'cut-off' in one of the peripheral coronary artery branches of the infarct-related vessel, distal to the site of angioplasty (Windecker et al., 2014).

The TIMI coronary flow grade (described in chapter 2) is straightforward to evaluate in the catheter laboratory hence TIMI flow grade was used as an eligibility criterion for participation in the study.

Tissue myocardial perfusion (blush) grade

Coronary angiography also provides other information on coronary blood flow and myocardial perfusion. The TIMI blush grade is an ordinal score for contrast washout at the end of the angiogram (Steg et al., 2012), and the TIMI blush grade is also predictive of prognosis (Gibson et al., 2000, van 't Hof et al., 1998).

Table 9-1 Definitions of TIMI myocardial blush grade.

TIMI Blush grade	
0	No myocardial blush
1	Minimal blush and very slow clearing (e.g. present at beginning of next cine)
2	Good blush with slow clearing of myocardial contrast (present at end of cine but gone at beginning of next)
3	Good blush and normal clearing (i.e. gone by end of cine)

TIMI frame count

The TIMI frame count is a simple objective continuous variable index of coronary blood flow, representing the amount of time (in frames) for contrast dye to reach a standardized

distal landmark, corrected for vessel length (Gibson et al., 1996). The corrected TIMI frame count (CTFC) is predictive of prognosis (Gibson et al., 1996, Gibson et al., 1999).

Method of CTFC

The CTFC is the number of cine frames required for contrast to first reach standardized distal coronary landmarks in the culprit artery and was measured with a frame counter on a cine viewer (normal < 27 frames). In the left anterior descending artery this figure is corrected to account for increased vessel length and the frame count is divided by 1.7. A frame count of 100, a value that is the 99th percentile of patent vessels, was imputed to an occluded artery. CTFC is a measure of time, and data were converted when necessary according to film speed (e.g. 30 frames/s). The CTFC was divided by 30 to calculate the transit time for dye to traverse the length of the artery to the landmark in seconds and multiplied by 1000 to calculate the time in milliseconds. This was used along with the heart rate to calculate the fraction of a cardiac cycle required for dye to traverse the artery: fraction of cardiac cycle (CTFC/30 seconds) / (60s/heart rate). Calculation of the fraction of a cardiac cycle required for dye to traverse the culprit artery normalises the CTFC for heart rate.

Intra-procedural thrombotic events

An intra-procedural thrombotic event was defined as the development of new or increasing thrombus, abrupt vessel closure, no reflow or slow reflow, or distal embolization occurring at any time during the procedure (McEntegart et al., 2012). Embolisation was defined as a distal filling defect with an abrupt "cut-off" in one of the peripheral coronary artery branches of the infarct-related vessel, distal to the site of angioplasty. Each complication was assessed relative to the status of the previous frames. Thus, if thrombus was present at baseline but then resolved only to recur later, this was coded as an intra-procedural thrombotic event. Similarly, thrombus at baseline that qualitatively "grew" in subsequent frames was considered an intra-procedural thrombotic event. Conversely, baseline thrombus that persisted in size without growing, diminished, or resolved was not considered an intra-procedural thrombotic event.

Comparison of stent strategy between procedures for the deferred group

In the deferred group, the intended stent strategy at the end of the first procedure was prospectively recorded and stent dimensions were compared to the actual stents used in the second procedure by the same operator. In addition, thrombus burden at the start of the second procedure was compared to the end of the first procedure. All of the angiographic outcomes were adjudicated blind to treatment allocation by an independent central core laboratory.

9.2.10 ECG secondary outcomes

The ECG secondary outcomes included the occurrence of complete (≥70), partial (30% to < 70%) or no (≤30%) ST-segment resolution on the electrocardiogram (ECG) assessed 60 minutes after reperfusion compared to the baseline ECG before reperfusion (Windecker et al., 2014, Steg et al., 2012). In addition, ST segment elevation was measured on the baseline ECG before reperfusion in order to estimate the extent of initial myocardial jeopardy with the Aldrich ST-elevation score (Aldrich et al., 1988).

9.2.11 MRI secondary outcomes

The MRI secondary outcomes included the occurrence of microvascular obstruction with late gadolinium enhancement on cardiac magnetic resonance imaging (MRI) 2 days after reperfusion (Kramer et al., 2013), final infarct size at 6 months (Kramer et al., 2013, Kellman et al., 2002), myocardial salvage (Berry et al., 2010, Payne et al., 2012, Giri et al., 2009), and myocardial salvage index (Berry et al., 2010, Payne et al., 2012, Giri et al., 2009) (both derived using final infarct size). Myocardial salvage (% left ventricular volume) was defined as the difference between the initial jeopardised area-at-risk revealed by T2-weighted MRI at baseline (Berry et al., 2010, Payne et al., 2012, Giri et al., 2009) and final infarct size revealed by contrast-enhanced MRI at 6 months (Berry et al., 2010). The myocardial salvage index was defined as infarct size at 6 months indexed to the initial area-at-risk (Payne et al., 2012).

The ECG and MRI outcomes were also adjudicated blind to treatment allocation.

9.2.12 Safety outcomes

The potential risks of the second catheterisation e.g. bleeding, contrast nephropathy, and procedure-related complications e.g. intra-procedural thrombotic events, and health outcomes were included as safety outcomes.

Clinical outcome measures included the occurrence of heart failure, re-infarction, bleeding and cardiac death during the index admission and after discharge as defined in the Clinical Event Committee Charter (appendix 4). All potential clinical events were adjudicated blinded to treatment allocation by an independent Clinical Event Committee comprised of 3 cardiologists from Ninewells Hospital, Dundee (J.I., A.D., S.H.T.)

All study participants were followed-up for a minimum of 8 months after discharge. Information on adverse events was obtained by clinical review of the patients and primary and secondary care records during follow-up.

9.2.13 Coronary angiogram acquisition and analyses

Coronary angiograms were acquired during usual care with cardiac catheter laboratory X-ray (Innova®, GE Healthcare) and information technology equipment (Centricity®, GE Healthcare). The angiograms underwent independent analysis in the Cardiovascular Research Foundation Angiographic Core Laboratory, New York, NY, USA by staff who were blinded to treatment assignment.

I coded and de-identified the angiograms, then transferred the CDs by courier to be analysed at an independent core laboratory (Cardiovascular Research Foundation, New York, New York) by technicians blinded to randomization and clinical outcomes. Quantitative analyses of coronary, stent and thrombus dimensions were performed with Medis QAngio XA v7.2.34 (Medis Medical Imaging Systems, Leiden, Netherlands) image analysis software. In addition to routine pre- and post-procedural quantitative and qualitative assessments, additional analyses of every cineangiographic frame were performed. Intra-procedural complications were independently assessed for each angiographic run.

Registry patients

The coronary angiograms of the non-randomised patients were analysed by 3 experienced interventional cardiologists (M.B., A.S., W.S.H.) who were independent of the lead site. All of the angiograms were independently and separately adjudicated by two cardiologists (M.B., A.S.) and disagreements were resolved by consensus established independently by a third cardiologist (W.S.H.).

9.2.14 ECG and MRI acquisition and analyses

A 12 lead electrocardiogram (ECG) was obtained before coronary reperfusion and 60 minutes afterwards with Mac-Lab® technology (GE Healthcare) in the catheter laboratory and a MAC 5500 HD recorder (GE Healthcare) in the Coronary Care Unit. The ECGs were acquired by trained cardiology staff. The ECGs were de-identified and transferred to the local ECG management system. The ECGs then underwent blinded analysis by R.W. who was trained by the University of Glasgow ECG Core Laboratory which is certified to ISO 9001: 2008 standards as a UKAS Accredited Organization.

Cardiac MRI was performed approximately 2 days after reperfusion and the sequence protocol is explained in detail in Chapter 2. In brief, the imaging protocol included cine MRI with steady state free precession, T2-weighted oedema imaging, T2* CMR and early and late gadolinium enhancement imaging. The initial area-at-risk was delineated with T2 mapping MRI (Giri et al., 2009). Myocardial haemorrhage was defined as a hypointense region within the infarct core, with a T2* value <20 ms. Microvascular obstruction was defined as a central dark zone on early contrast enhancement imaging 1, 3, 5 and 7 minutes post-contrast injection and present within an area of late gadolinium enhancement (Kramer et al., 2013). Myocardial infarction was imaged using a segmented phase-sensitive inversion recovery turbo fast low-angle shot (Kellman et al., 2002). The MRI scans were analysed by observers blinded to the treatment group allocation of the study participants.

9.2.15 Sample size

Based on a clinical audit in our hospital and a literature review (Morishima et al., 2000, Antoniucci et al., 2001, Ndrepepa et al., 2010), we estimated that the incidence of no/slow-reflow (TIMI ≤2) during primary PCI would be 40% in selected patients with one or more of our predefined risk factors and 10% in the deferred PCI group. A minimum of 84

patients (n=42 per group) would provide 85% power to reject the null hypothesis with a type 1 error of 0·05.

9.2.16 Statistical methods

Means and standard deviations were used to summarise approximately Normally distributed continuous data. Medians and interquartile ranges (IQR) or Geometric means and standard deviations were used to describe skewed continuous data. Counts and percentages were used to summarise categorical data. All tests were two-tailed and assessed at the 5% significance level. Comparisons of continuous variables used paired or unpaired t-tests for Normally distributed data, or Wilcoxon tests or t-tests after logarithmic transformation for skewed data. Differences in proportions were assessed using ordinal logistic regression with exact confidence intervals for odds ratios, or Mcnemar's tests for paired comparisons. Differences in ordinal data between groups were assessed using ordinal logistic regression with estimates of odds ratios and 95% confidence intervals. All statistical analyses were performed using R version 2.15.2 or SAS version 9.2 (or higher versions of these programs).

The Robertson Centre for Biostatistics acted as an independent coordinating centre for randomisation and its statisticians conducted the analyses. The study was monitored for safety by the sponsor. All serious adverse events were prospectively reported to the Pharmacovigilance Unit of the Clinical Trials Unit. The trial was approved by the National Research Ethics Service (reference 10/S0703-28). The registry was approved by the hospital's Caldicott Guardian and Clinical Governance office. The clinical trial registration number was NCT 01717573 and the trial sponsor was the National Waiting Times Centre Board, NHS Scotland.

9.3 Results

Four hundred and eleven patients were treated with primary PCI between 11 March 2012 and 21st November 2012 and all of these patients were included in a registry (figure 9-1; table 9-2). Of these, 101 patients (mean age 60 years, 69% male) were randomised (n=52 deferred group, n=49 immediate stenting; figure 9-1) by 8/13 (62%) cardiologists. The trial

stopped when all patients had a minimum follow-up period of six months and all randomised patients were included in the analysis.

Figure 9-1 Study flow diagram

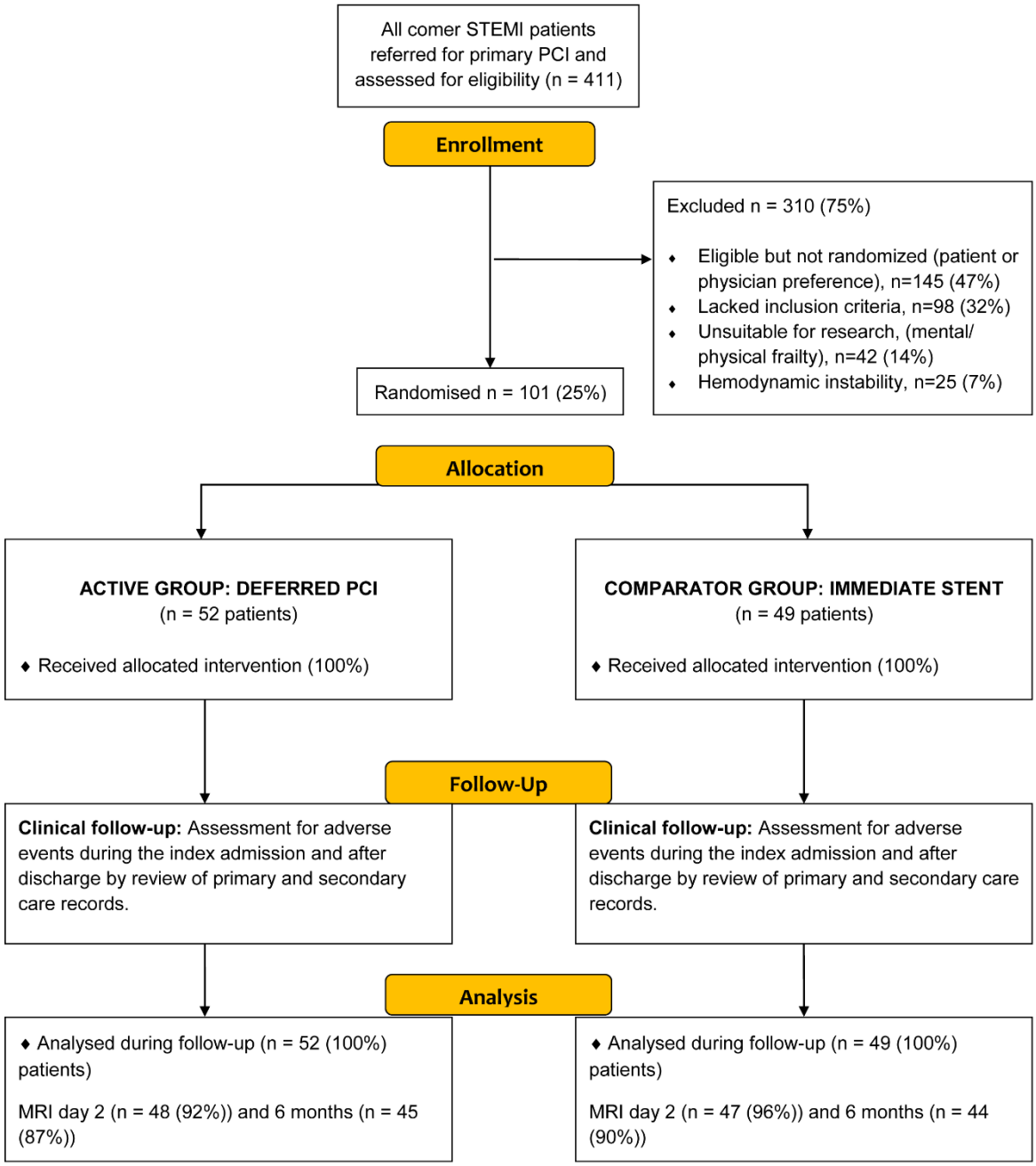


Table 9-2 Baseline clinical and angiographic characteristics of all-comers.

Characteristics*		Randomly assigned groups: Immediate Stenting n = 49	Randomly assigned groups: Deferred Stenting n = 52	Registry§ n = 310
Clinical				
Age, years		61·7 (12·2)	57·6 (10·9)	61·4 (12·9)
Male sex, n (%)		36 (73·5%)	34 (65·4%)	196 (63·2%)
Heart rate, bpm		83 (17)	77 (17)	83 (32)
Systolic blood pressure, mmHg		138 (27)	141 (24)	131 (28) §
Diastolic blood pressure, mmHg		79 (17)	83 (11)	77 (16) §
Diabetes mellitus‡, n (%)		6 (12·2%)	7 (13·5%)	30 (9·7%)
Previous myocardial infarction, n (%)		2 (4·1%)	5 (9·6%)	30 (9·7%)
Previous percutaneous coronary intervention, n (%)		2 (4·1%)	2 (3·8%)	21 (6·8%)
Heart failure, Killip class at presentation	I II III	35 (71·4%) 13 (26·6%) 1 (2·0%)	38 (73·1%) 12 (23·1%) 2 (3·8%)	-
Procedure				
Time from symptom onset to reperfusion (first balloon or aspiration thrombectomy), min		183 (131, 337)	166 (124, 276)	184 (124, 338)
Time from symptom onset to reperfusion >12 hours, n (%)		5 (10·2%)	1 (1·9%)	6 (5·9%)
Coronary angiography				

Characteristics*		Randomly assigned groups		Registry§
		Immediate Stenting n = 49	Deferred Stenting n = 52	n = 310
Number of diseased arteries, n (%)	1	26 (55·3%)	22 (45·8%)	
	2	15 (31·9%)	17 (35·4%)	-
	3	6 (12·8%)	9 (18·8%)	
Culprit artery, n (%)	LAD	18 (36·7%)	15 (28·8%)	131 (42·3%)§
	LCX	6 (12·2%)	11 (21·2%)	42 (13·5%)§
	RCA	25 (51·1%)	25 (48·1%)	132 (42·6%)
	VG	0 (0·0%)	1 (1·9%)	2 (0·6%)
	LM	0 (0·0%)	0 (0·0%)	3 (1·0%)
TIMI coronary flow grade pre-PCI¥, n (%)	0/1	39 (79·6%)	40 (76·9%)	200 (64·5%)§
	2	7 (14·3%)	6 (11·5%)	43 (13·9%)
	3	3 (6·1%)	6 (11·5%)	67 (21·6%)§
Lesion length§, mm		15·4 (11·2, 20·6)	13·5 (11·2, 17·8)	-
Coronary artery diameter at the start of the procedure§, mm				
- proximal to the culprit lesion		3·2 (0·7)	3·2 (0·6)	-
- distal to the culprit lesion		2·7 (0·6)	2·7 (0·6)	-
Thrombus present§, n (%)		47 (95·9%)	51 (98·1%)	284 (91·6%)
Thrombus area§, mm^2		13·0 (8·3, 20·2)	19·9 (12·0, 1·3)	-
TIMI thrombus grade, n (%)	0/1	21 (42·9%)	22 (42·3%)	151 (48·9%)
	2	6 (12·2%)	6 (11·5%)	62 (20·1%)
	3	10 (20·4%)	7 (13·5%)	60 (19·4%)
	4	12 (24·5%)	17 (32·7%)	36 (11·7%)

Characteristics*	Randomly assigned groups		Registry§
	Immediate Stenting	Deferred Stenting	
	n = 49	n = 52	n = 310
Jeopardised myocardium by the ECG Aldrich score (% left ventricle) †[14]	20 (17, 30)	19 (15, 26)	-
Procedure details			
Aspiration thrombectomy, n (%)	42 (85·7%)	46 (88·5%)	-
Glycoprotein IIbIIIa inhibitor therapy, n (%)	46 (98·9%)	51 (98·1%)	
Pre-dilatation, n (%)	36 (73·5%)	46 (88·5%)	-
Post-dilatation, n (%)	35 (71·4%)	30 (57·7%)	-
Final inflation pressure, kPa	17·4 (2·4)	16·4 (3·2)	-
Intra-coronary adenosine therapy, n (%)	4 (8·2%)	3 (5·8%)	
Number of stents: 0	0	3 (5·8%)	
1	39 (79·6%)	33 (63·5%)	
2	9 (18·4%)	16 (30·8%)	
3	1 (2·0%)	0	
Contrast volume, ml	205 (172, 250)	278 (238, 312)	

*Footnote: TIMI = Thrombolysis in Myocardial Infarction grade, ECG = electrocardiogram. * Means±SD or median (interquartile range) for normal and non-normally distributed data, respectively. ‡ Diabetes mellitus was defined as a history of diet-controlled or treated diabetes. Killip classification of heart failure after acute myocardial infarction: class I - no heart failure, class II - pulmonary rales or crepitations, a third heart sound, and elevated jugular venous pressure, class III - acute pulmonary oedema, class IV - cardiogenic shock. A diseased artery was defined as an epicardial*

artery (≥ 2 mm) with one or more lesions ≥ 50% of the reference vessel diameter. ¥ TIMI coronary flow grade pre-PCI was not evaluable in 1 patient in the immediate stenting group. Intra-coronary adenosine (10 – 30 μg) was administered as bolus therapy during primary PCI as clinically-indicated for reduced coronary flow. The clinical and treatment characteristics of the patients included in the immediately stented group and the deferred group were similar except for total volume of contrast which was greater in the deferred group (p<0·0001). Procedure details and outcomes include the first and second procedure in the deferred stent group. Two deferred patients experienced culprit artery reocclusion before the planned second procedure. The coronary flow grades at the end of the first procedure and at the start of the second procedure differed in three other deferred patients as follows: two patients changed from TIMI flow grade 3 to TIMI 2 and one patient changed from TIMI flow grade 2 to TIMI 3. None of the patients received bail-out or covered stents.
§ The following clinical characteristics differed between the registry patients and the randomly assigned patients who were enrolled in the trial: systolic blood pressure (p=0·003), diastolic blood pressure (p=0·022), TIMI thrombus grade 4 (p<0·0001) and TIMI flow grade pre-PCI (TIMI 0/1 p=0·015; TIMI 3 p=0·007). Quantitative coronary and ECG analyses were done in the randomised patients but not in the registry patients.

Immediate vs. deferred stenting groups

9.3.1 Angiographic findings

The incidence of no/slow-reflow post stenting (primary end-point) was significantly lower, in the deferred stenting group: [odds ratio 0.16 (0.04, 0.59), p=0.006 (table 9-3)]. Distal embolisation and intra-procedural thrombotic events were also less frequent in the deferred stenting group (table 9-3). Post stenting, TIMI grade 3 flow and myocardial blush grades were higher in the deferred stenting group (table 9-3). Within the deferred stenting group there was a significant reduction in the proportion of patients with angiographic evidence of thrombus at the start of the second vs. the first procedure (98.1% vs. 62.7%; p<0.0001). Coronary thrombus area reduced significantly between the end of the first and start of the

second angiograms [geometric mean for the ratio of the thrombus areas (95% CI) 0.67 (0.53, 0.85)].

Table 9-3 Primary and secondary angiographic and ECG outcomes.

Outcome*		Randomly assigned groups: Immediate Stenting n = 49	Randomly assigned groups: Deferred Stenting n = 52	Odds ratio (95% CI)	p value†	Registry, n = 310
Primary outcome						
No- or slow-reflow (TIMI 0 to 2), n (%)¢		14 (28·6%)	3 (5·8%)	0·16 (0·04, 0·59)	0·006	45 (14·5%)
Secondary angiographic outcomes						
No-reflow (TIMI 0 or 1), n (%)		7 (14·3%)	1 (2·0%)	0·12 (0·01, 1·04)	0·054	16 (5·2%)
Final TIMI coronary flow grade post-PCI, n (%)¥	3	39 (79·6%)	49 (98%)	0·08 (0·01, 0·67)	0·019	273 (88·6%)
	2	6 (12·2%)	0			25 (8·1%)
	0/1	4 (8·2%)	1 (2·0%)			10 (3·2%)
Final TIMI myocardial blush grade post-PCI, n (%)§	3	26 (53·1%)	39 (79·6%)	0·28 (0·12, 0·68)	0·005	-
	2	18 (36·7%)	9 (18·4%)			
	0/1	5 (10·2%)	1 (2·0%)			
No- or slow-reflow (TIMI 0 to 2), **with MBG ≤ 1**, n (%)		5 (10.2%)	1 (2.0%)	0.18 (0.02, 1.56)	0.119	
No- or slow-reflow (TIMI 0 to 2), **with MBG ≤ 2**, n (%)		12 (24.5%)	2 (3.9%)	0.13 (0.03, 0.60)	0.009	
All intra-procedural thrombotic events, n		28	9	-	-	68

Patients with at least one intra-procedural thrombotic event, n (%)	16 (32·7%)	5 (9·6%)	0·22 (0·07, 0·67)	0·008	63 (20·3%)
Distal embolisation, n (%)	10 (20·4%)	1 (1·9%)	0·08 (0·01, 0·65)	0·018	5 (1·3%)
Other secondary outcome					
ECG: Resolution of ST segment elevation 60 min post PCI, n (%)					-
Complete, ≥70 %	19 (38·8%)	26 (50·0%)			
Partial, 30% to < 70%	21 (42·9%)	15 (28·8%)	0·77 (0·37, 1·6)	0·484	
None, ≤30%	9 (18·4%)	11 (21·2%)			

9.3.2 Comparison of stent strategy between procedures in the deferred group

Compared with the intended stent strategy at the end of the first procedure, there was a 0.5 mm increase in maximum stent diameter (p<0.0001) and 3 mm increase in total length (p=0.002), evaluated by the same operator for both procedures (table 9-4 and 9-5). Three deferred patients did not receive a stent. In one patient, repeat arterial access was not possible because of peripheral arterial disease. In the other two patients, the culprit lesions had only minimal residual stenoses.

Table 9-4 Comparison of intended stenting strategy at the end of the first PCI procedure compared to the actual strategy during the second procedure in the deferred stent group.

	Deferred stenting group n = 49		
Characteristic	Procedure 1	Procedure 2	p value
Maximum stent diameter, mm	3.0 (3.0, 3.5)	3.5 (3.0, 4.0)	<0.0001
Total stent length, mm	28 (18, 32)	28 (20, 40)	0.002
Patients with an increase in maximum stent diameter for that procedure, n (%)	2 (4%)	36 (75%)	

Footnote: Three patients who did not receive a stent in the second procedure were excluded from the analysis.

Table 9-5 Median increase in stent diameter and length between procedures for the deferred stent group.

Characteristic		p value
Median increase in maximum stent diameter in procedure 2 versus procedure 1, mm	0.5	<0.0001
Median increase in total stent length in procedure 2 versus procedure 1, mm	3	0.002

9.3.3 MRI findings

The MRI results 2 days and 6 months post-MI are described in table 9-6 and case examples are shown in figure 9-2. Compared with immediate stenting, myocardial salvage (% left ventricular mass) (19·7 (13·8, 26·0) vs.14·7 (8·1, 23·2); p=0·027) and salvage index (%) (68 (54, 82) vs. 56 (31, 72); p=0·031) at 6 months were greater in the deferred group. The incidence of both microvascular obstruction and myocardial haemorrhage were lower in the deferred PCI group, although this was not statistically significant.

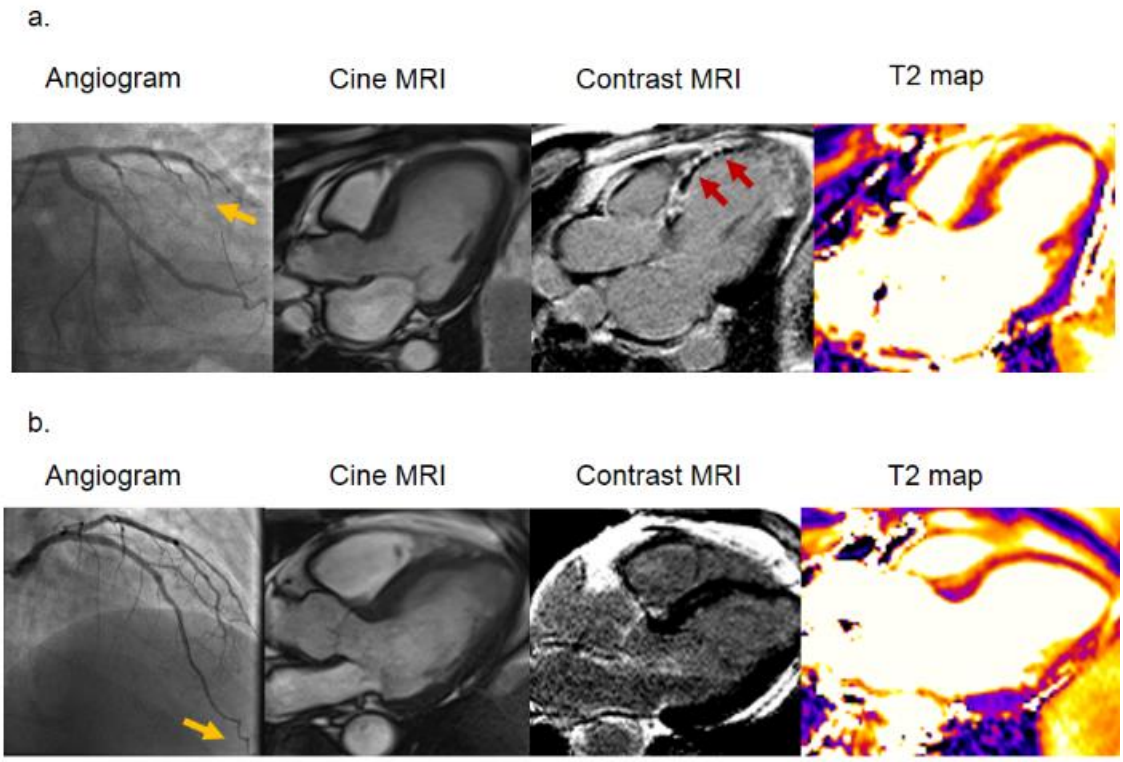
Table 9-6 Contrast-enhanced cardiac MRI findings during the index hospitalisation and after 6 months follow-up.

Characteristics*	Immediate stenting	Deferred PCI †	p value
MRI 2 days post-MI	*n = 47*	*n = 48*	
Microvascular obstruction, n (%)	29 (61·7)	23 (47·9)	0·155
Myocardial haemorrhage, n (%)¥	19 (46.1)	14 (33.3)	0.225
MRI 6 months post-MI	*n = 44*	*n = 45*	

Characteristics*	Immediate stenting	Deferred PCI †	p value
Myocardial salvage, % left ventricular volume	14·7 (8·1, 23·2)	19·7 (13·8, 26·0)	0·027
Myocardial salvage index, %	56 (31, 72)	68 (54, 82)	0·031
Infarct size, % of left ventricular volume	14·3 (6·3 , 20·3)	9·0 (4·3 , 16·0)	0·181

*Footnote: * Means±SD and median (interquartile range) are used for normal and non-normally distributed data. The initial area-at-risk (% of left ventricular volume) revealed by MRI 2 days post-MI was similar in patients randomised to immediate stenting (31·6 (20·8 , 37·4)) compared to in patients randomised to deferred PCI (28·4 (23·4 , 36·6); p=0.577). † Compared with the immediate stenting group, favorable directional changes were observed in the deferred PCI group for left ventricular end-systolic volume, left ventricular end-diastolic volume and ejection fraction, their changes at 6 months from baseline (data not shown). ¥Missing data: 6 patients in each group had non-evaluable T2* maps at baseline. The time from randomisation to MRI was 60 (18-97) hours and 55 (22-90) hours in the immediate stenting and deferred groups, respectively.*

Figure 9-2 Angiogram and MRI images from 2 patients with acute reperfused STEMI. One patient treated with a conventional primary PCI and the other with deferred PCI



(a) Usual care with immediate stenting. *The angiogram (left) revealed no-reflow (orange arrow, TIMI grade 1 flow) after stenting LAD. Cine MRI (middle left) revealed moderate left ventricular systolic dysfunction. Late gadolinium enhancement (LGE) imaging (middle right) revealed transmural infarction with microvascular obstruction (red arrows). There was minimal myocardial salvage because final infarct size nearly equalled area-at-risk (AAR) on T2 mapping (right).* ***(b) Deferred PCI.*** *The angiogram revealed brisk flow post-stent. Cine MRI (middle left) again revealed moderate left ventricular systolic dysfunction, however this is consistent with largely stunned but viable myocardium, since this patient had minimal evidence of infarction on LGE imaging (middle right) and no microvascular obstruction. The ischaemic AAR was far greater than the final infarct size and therefore myocardial salvage was substantial.*

9.3.4 Adverse events and safety

In hospital events after randomisation

In the deferred stent group recurrent ST elevation myocardial infarction prior to stenting occurred in two patients. One patient had a severe intra-mural dissection within the culprit

lesion in the left anterior descending coronary artery associated with absent flow in a large diagonal side-branch. Five hours after initial reperfusion the patient experienced recurrent chest pain associated with anterior ST re-elevation. Repeat coronary angiography was performed within 30 minutes and confirmed re-occlusion of the culprit artery. The patient received a stent and his subsequent clinical course was uncomplicated. A second patient who inadvertently had not received low molecular weight heparin therapy in the coronary care unit reinfarcted prior to stenting. This patient was treated with a stent within 30 minutes of symptom onset and had an uncomplicated clinical course. One further patient experienced an abrupt culprit artery closure and intra-procedural thrombotic event due to a guidewire-related dissection.

There were no bleeding events or in-hospital deaths. There was a greater volume of contrast used in the deferred group (278 ml (238, 312) vs. 205 ml (170, 250); $p<0{\cdot}0001$). No cases of contrast nephropathy occurred.

Post-discharge events

The mean (SD) duration of follow-up was 352 (79) days from randomisation. Three patients in the deferred group and one patient in the immediate stenting group experienced a non-ST segment elevation myocardial infarction. Two additional patients in the immediate stenting group were hospitalised with unstable angina, one of who was treated with PCI. There was one non-cardiovascular death due to small cell lung carcinoma in the deferred group.

9.4 Discussion

The main findings of our study are that compared with standard care with immediate stenting, brief deferral of stenting after initial reperfusion, reduced angiographic no-reflow, tended to reduce intramyocardial haemorrhage and microvascular obstruction, and increased myocardial salvage.

We implemented a novel strategy to prevent no-reflow in at-risk patients with STEMI undergoing primary PCI. A simple approach was adopted for treatment stratification and randomisation by the cardiologist. We identified patients with initial evidence of successful

reperfusion and with clinical risk factors for no-reflow, and from these patients the study participants were randomised to immediate stenting or to an intention-to-stent strategy within 4 - 16 hours including prolonged anti-thrombotic therapy. The strategy of deferred stenting in primary PCI represents a radical change from standard care.

We have observed that deferred completion of PCI in selected STEMI patients reduced no-reflow, distal embolisation and intra-procedural thrombotic complications compared to conventional treatment with immediate stenting. Final coronary flow grade and myocardial blush grade were also better in the deferred group. Two patients in the deferred group experienced early recurrent myocardial infarction before the second procedure. During longer term follow-up, myocardial salvage measured with cardiac MRI was significantly greater in the deferred group. The favourable effect on myocardial salvage is important. Salvage was objectively measured with MRI and was derived from results obtained after 6 months follow-up indicating a beneficial treatment effect that is sustained over time. Finally, myocardial salvage is a prognostically validated surrogate outcome that is a major therapeutic target in primary PCI (Steg et al., 2012, Windecker et al., 2014).

Our trial results reflect a balance of potential benefits and potential risks. The trial was conducted during usual care and our intervention was based on simple clinical eligibility criteria. The anti-thrombotic strategy involved a mechanical component (i.e. deferral of stent implantation to avoid/minimize thrombus embolisation) and a therapeutic component based on prolonged treatment with low molecular weight heparin (1 mg/kg) and glycoprotein IIb/IIIa inhibitor therapy during the interval between the first and second PCI procedure. Glycoprotein IIb/IIIa inhibitor therapy is an evidence-based anti-thrombotic treatment (Steg et al., 2012, Windecker et al., 2014) and was included in therapeutic strategy in order to reduce thrombus burden before stent implantation in the deferred group (Windecker et al., 2014). Although these treatments also increase the risk of bleeding, no bleeding problems occurred in the deferred group probably because radial artery access was used in all patients. Accordingly, our strategy has potential to be widely applicable.

Our strategy was based on selection of patients with at least one clinical and/or angiographic risk factor for no-reflow (Morishima et al., 2000, Ndrepepa et al., 2010, Antoniucci et al., 2001). We felt that the intervention would not be appropriate in 'all comers' for three reasons. Firstly, the efficacy of deferred stenting was likely to be greatest

in the patients at highest-risk of no/slow-reflow. Secondly, the risk of recurrent myocardial infarction could not be mitigated in patients who were at low risk of no-reflow on clinical grounds. Thirdly, a strategy which involved all-comers would be difficult to implement due to the large number of additional second procedures.

The clinical risk profiles of the randomised and registry patients differed. Compared with the registry patients, anterior myocardial infarction due to left anterior descending coronary artery thrombosis and an occluded culprit artery (TIMI 0/1) were much more common in the trial patients. Anterior myocardial infarction and an occluded culprit artery are both associated with large infarct size (Berry et al., 2010, Payne et al., 2012, Srinivasan et al., 2009) and an adverse prognosis (Steg et al., 2012, Windecker et al., 2014). These baseline differences between randomized and registry patients can be explained by appropriate risk stratification and patient selection by the cardiologists at the time of primary PCI.

In order to assess whether or not clinicians could stratify patients at risk of no-reflow, information on all-comers was collected and those not randomised were included in a registry. The incidence of no/slow-reflow in the registry patients was 14.5%, nearly half the incidence of this event observed in the immediately stented patients and over double the incidence of no- or slow-reflow in the deferred group. This indicates the patient selection approach correctly identified a sub-group of STEMI patients in whom the incidence of no/slow-reflow was lower (table 9-3).

Our study was performed during normal emergency care and all-comers were prospectively screened and documented. However, as might be expected with a new intervention which represents a radical change from standard care, patient enrolment was influenced by physician preference and in the absence of clinical evidence to support this strategy, 5/13 cardiologists in our primary PCI service did not randomise any patients.

Thrombus is mechanistically involved in no-reflow and stent implantation may cause distal embolisation of clot and microvascular thrombosis (Bekkers et al., 2010b, Niccoli et al., 2009). Based on the rationale for our intervention, we examined whether coronary thrombus burden might be lower at the start of the second PCI compared to the start of the first procedure (when stenting is normally performed) and this indeed was the case. Furthermore, thrombus in the culprit artery had dissipated during the intervening period.

Thus, coronary stent implantation in the deferred group of patients occurred when thrombus burden was less, and so the substrate for distal embolisation and microvascular thrombosis had diminished. This may explain the lower incidence of no-reflow in the deferred group.

Two patients in the deferred group had early recurrent myocardial infarction. One of these patients had a complex culprit lesion with an intra-mural dissection (Holmes et al., 1988) and persistently reduced side branch flow (TIMI grade 1). The other patient was a protocol violation as they had not received low molecular weight heparin after the initial procedure. Both patients were treated with PCI expeditiously and without complication. These events contain learning which should be used to optimize the design of a future clinical trial. For example, persistent flow reduction (TIMI 0/1) in the side branch of a culprit bifurcation lesion would be an exclusion criterion. Overall, the balance of the benefit of reduced no-reflow versus the risk of recurrent myocardial infarction needs to be tested in a large multicentre randomised controlled trial.

There were 5 patients in the usual care group compared with 1 in the deferred group that had a time from symptom onset to reperfusion greater than 12 hours. Given the small sample size, this may have affected the outcome, especially with regard to the difference in myocardial salvage at 6-months. In addition, 3 patients in the usual care group versus 1 in the deferred group never received Tirofiban, which may have confounded results.

Therapeutic strategies for the prevention and treatment of no-reflow have been intensively investigated in recent years (Stone et al., 2012b, Vlaar et al., 2008, Steg et al., 2012, Vijayalakshmi et al., 2006). However, none of the previously studied interventions have improved clinical outcomes in large multicentre randomised trials. Other clinical trials of deferred stenting in primary PCI are also underway including MIMI (NCT01360242), PRIMACY (NCT01542385), and DANAMI-3 (NCT01435408). The designs of these trials differ compared to DEFER-STEMI. For example, these trials involve a longer delay before stent implantation (i.e. at least 1 - 2 days), which theoretically increases the risk of recurrent myocardial infarction and bleeding, and prolongs hospital stay. Recent studies (Escaned et al., 2013, Kelbaek et al., 2013, Freixa et al., 2013, Isaaz et al., 2006), including three non-randomised case series (Escaned et al., 2013, Isaaz et al., 2006, Kelbaek et al., 2013) and a systematic review (Freixa et al., 2013), reported results which support the

notion that deferred stenting may be safe in appropriately selected patients. Taken together with the trials that are currently recruiting, these recent publications highlight the rapidly growing interest in this new therapeutic approach in primary PCI.

9.4.1 Implications for clinical practice

Our strategy of deferred stenting in selected STEMI patients with risk factors for no-reflow represents a potential new treatment paradigm. The strategy involves a balance between competing risks and benefits that merits prospective evaluation in a large clinical trial. On the one hand, we have shown that deferred stenting reduces no-reflow and increase myocardial salvage. On the other hand, there may be an increased risk of early recurrent STEMI. A deferred stent strategy involves a second procedure and so procedure-related costs may be higher. Our study design timed the second procedure 4 - 16 hours after the first in order to keep the second procedure within working hours and so optimise feasibility. On the other hand, the strategy has the potential to reduce healthcare costs overall by reducing the clinical consequences of no-reflow (e.g. heart failure and its related cost burden). Only a large clinical trial designed to assess patient experience, health outcomes, quality of life, and cost-effectiveness can address these uncertainties.

9.4.2 Limitations

Investigators and patients were unblinded in our study. For this reason, the primary and secondary outcomes underwent independent analysis blind to treatment group assignment in order to prevent ascertainment bias. Our estimates for the expected incidences of no-/slow-reflow were slightly higher than the observed rates. The reasons for this may be multifactorial and may reflect the effect of core laboratory adjudication over investigator reported events. Our study design did not include an angiographic control in the immediate stenting group, but we do not think this is relevant since the occurrence of no-reflow and other angiographic sequelae, such as intra-procedural thrombotic events, is due to the effect of PCI. Although two patients experienced recurrent STEMI and the outcome of these patients was favourable, and the learning from these experiences will inform the design of a future trial. Advanced peripheral vascular disease may limit vascular access for repeated procedures. Glycoprotein IIbIIIa inhibitor therapy and unfractionated heparin were used rather than bivalirudin (Steg et al., 2012), and the former anti-thrombotic

combination therapy remains widely used worldwide. Some of the registry patients were eligible for randomisation but were not included because of physician preference. We believe this behaviour is to be expected in a pragmatic trial with a disruptive intervention which conflicts with the standard of care, and demonstration of a treatment effect in the randomized patients arguably makes our trial results all the more striking.

Some of the MRI and ECG parameters were numerically but not statistically different between treatment groups. We think this is related to the sample size, especially since the longer term MRI results confirmed greater myocardial salvage in the deferred group.

9.5 Conclusions

For the first time, we have conducted a proof-of-concept trial and found that deferred stenting in primary PCI reduced angiographic no-reflow, tended to reduce IMH and MVO, and increased myocardial salvage compared to conventional primary PCI with immediate stenting. Two patients had recurrent myocardial infarction which represents important balancing information on potential risks. The strategy is simple, pragmatic and potentially widely applicable. Our results support the rationale for a substantive multicentre clinical trial to assess the cost-effectiveness of early deferred completion of PCI after reperfusion versus conventional treatment in STEMI patients at risk of no-reflow.

10 Chapter 10: Conclusions and future directions

In summary, I found that myocardial haemorrhage defined by T2* mapping is a biomarker for prognostication in STEMI survivors. For the first time we have performed a serial imaging analysis for the evolution and time-course of IMH and MVO in the early reperfusion period. Hemorrhage occurs in primary and secondary phases within the first 10 days post-MI and is a secondary phenomenon to the initial occurrence of microvascular obstruction. The dynamic evolution of haemorrhage suggests that microvascular damage may be modifiable. Therapeutic interventions designed to preserve microvascular integrity, given before or immediately after reperfusion, could prevent haemorrhage and this possibility merits prospective assessment in randomised controlled trials.

This thesis also adds to the growing body of evidence for the clinical utility of quantitative assessment of relaxation times, using parametric mapping techniques. T2 mapping proved to be a robust sequence with evaluable images in all patients. T2 core represents a novel biomarker with potential for infarct characterisation and prognostication. Using a comprehensive multi-parameter CMR protocol we showed that a hypointense T2 core was more closely related with MVO than IMH.

In chapter 7, I showed that infarct core pathology revealed by T1 mapping had superior prognostic value compared to infarct core T2 and myocardial haemorrhage, and similar prognostic value compared to MVO, an established prognostic CMR biomarker, revealed by contrast-enhanced CMR. T1 mapping is potentially widely applicable in this patient setting and avoids the theoretical risks and actual restrictions associated with contrast-enhanced CMR, so could represent an alternative non-contrast CMR option for the assessment of infarct pathology.

I have also demonstrated that IMR adds early prognostic information at the time of emergency reperfusion and has the potential to stratify patients at risk of IMH for more intensive therapy.

Finally, for the first time we have found that a strategy of deferred stenting in selected patients reduced angiographic no-reflow in primary PCI and tended to reduce MVO and IMH. This intervention is pragmatic and potentially widely applicable. Our results support the rationale for a multicentre trial to assess the safety and cost-effectiveness of deferred stenting in primary PCI.

In addition to the DEFER-STEMI programme (Carrick et al., 2014), this thesis has stimulated the MRC-EME funded T-TIME Phase II trial (Clinicaltrials.gov NCT02257294). Conceivably, intra-coronary thrombolysis administered early after reperfusion and before stent implantation might reduce coronary thrombus burden and distal clot embolisation, lyse microvascular thrombi and restore microvascular perfusion early post-MI. We are currently examining this hypothesis in a randomised, double-blind, placebo-controlled, parallel group trial of low-dose adjunctive alteplase during primary PCI (T-TIME).

Duplicate letter

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06 May 2010

Dr Colin Berry
Consultant Cardiologist
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G12 8TA

Dear Dr Berry

Study Title:	**Cardiac magnetic resonance imaging: new pathological insights and their functional and clinical significance in ST elevation acute myocardial infarction.**
REC reference number:	**10/S0703/28**
Protocol number:	**Version 1.1**

The Research Ethics Committee reviewed the above application at the meeting held on 04 May 2010. Thank you for attending to discuss the study.

Ethical opinion

The Committee had a few questions for Dr Berry which he answered to the Committee's satisfaction

a. What biomarkers do you intend to study? You explained that naturetic peptides and cpds related to bleeding into heart muscle will be studied
b. Patients who are admitted to the Golden Jubilee Hospital are normally discharged at approximately day 3 discharged to their local hospital. If patients are waiting for day 5 for a MRI will they be required to stay at the Golden Jubilee Hospital for longer? You advised the committee that the pressure on beds would not permit a long term stay than usual.
c. How will the 50 patients who receive the 5 MRI's be chosen? The patients who give consent and are willing to return for the extra scans will be invited.
d. Further research on samples taken will require approval from a REC committee. You acknowledged the need for further approval
e. The Committee wondered how researchers would get information on the drugs taken by patients during the study. You hoped to get the information at the 6 month scan or through ISD (possibly).

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Appendix 1 – Ethical approval

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

The Committee has not yet been notified of the outcome of any site-specific assessment (SSA) for the non-NHS research site(s) taking part in this study. The favourable opinion does not therefore apply to any non-NHS site at present. I will write to you again as soon as one Research Ethics Committee has notified the outcome of a SSA. In the meantime no study procedures should be initiated at non-NHS sites.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

For NHS research sites only, management permission for research ("R&D approval") should be obtained from the relevant care organisation(s) in accordance with NHS research governance arrangements. Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk. Where the only involvement of the NHS organisation is as a Participant Identification Centre, management permission for research is not required but the R&D office should be notified of the study. Guidance should be sought from the R&D office where necessary.

Sponsors are not required to notify the Committee of approvals from host organisations.

[Other conditions specified by the REC – optional. Indicate where final versions of documents should be provided to the committee for information, e.g. information sheet]

The Committee required the following amendments to the Patient Information Sheet as follows :

a. Page 2 2nd paragraph change "electric bed" to couch which slides into the machine
b. Page 2 3rd paragraph should read "two small plastic tubes or cannulas".
c. Page 2 3rd paragraph additional sentence to be added that further approval will be required by REC Committee
d. Page 2 paragraph 5 side effects of Gadolinium to be added
c. Page 2 paragraph 6 Participant to be advised that on their 2nd visit for a MRI they can bring along a CD of their own choice or they can ask a relative to bring one in
d. Page 3 Is there any long term follow up - 2nd sentence delete "any future hospitalisation and in the event that you pass away" insert "you future well-being".
e. Page 3 What are the risks? The impact of any incidental finding will be followed up by referral to the appropriate specialist if not dealt with by cardiology staff.
f. Page 3 What are the potential benefits of taking part? 1st sentence delete "may not" and insert "are unlikely to"
g. Page 3 What are the potential benefits of taking part? Delete 2nd sentence "you will be getting special scans etc"
h. Page 4 Will my GP be informed delete "If you agree" should read "We will inform your GP etc".
i. Page 4 Who has reviewed the study should read West Scotland of Research Committee (1)
The above amendments to come back to the Coordinator for checking and filing

It is responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Covering Letter		07 March 2010
REC application	Version 2.5	07 March 2010
Protocol	Version 1.1	07 March 2010
Investigator CV		07 March 2010
Participant Information Sheet	Version 1.1	07 March 2010
Participant Consent Form	Version 1.1	07 March 2010
GP/Consultant Information Sheets	Version 1.1	07 March 2010
Summary/Synopsis		07 March 2010
Summary CV for supervisor (student research)		07 March 2010
Summary CV for student		07 March 2010

Membership of the Committee

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Now that you have completed the application process please visit the National Research Ethics Service website > After Review

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nres.npsa.nhs.uk.

10/S0703/28	Please quote this number on all correspondence

With the Committee's best wishes for the success of this project

Yours sincerely

Dr John Hunter
Chair

Email: andrea.torrie@ggc.scot.nhs.uk

Enclosures: *List of names and professions of members who were present at the meeting and those who submitted written comments*
"After ethical review – guidance for researchers" [SL-AR1 for CTIMPs, SL-AR2 for other studies]

Copy to: *Dr Catherine Sinclair*

West of Scotland REC 1

Attendance at Committee meeting on 04 May 2010

Committee Members:

Name	*Profession*	*Present*	*Notes*
Dr D Attwood	Dentistry	Yes	
Dr Rosemarie Davidson	Consultant in Clinical Genetics	Yes	
Mr John Devitt	Printer (Retired)	Yes	
Dr K Duffy	Research	Yes	
Mr McKenzie Gibson	Manager - Optical Company/retired Physics Lecturer	Yes	
Dr A Heuchan	Consultant Neonatal Medicine	Yes	
Dr John Hunter	Chairman West of Scotland (1) Ethics	Yes	
Dr Peter Hutchison	GP/Vice Chair	Yes	
Mr Eoin MacGillivray	Lay Member	Yes	
Dr J D McClure	Statistician	Yes	
Mr Jim McHugh	Insurance	No	
Dr T Moores	Consultant Paediatric Anaesthetist	Yes	
Dr Audrey Morrison	Research Practitioner	Yes	
Dr G Robertson	Consultant Oncologist	Yes	
Mr C Rodden	Pharmacist	Yes	
Mr R Sim	Investments (Retired)	Yes	
Dr M Sproule	Consultant Radiologist	Yes	
Dr J Thorburn	Anaesthetist (Retired)	Yes	

Also in attendance:

Name	*Position (or reason for attending)*
Dr J Godden	Scientific Officer
Miss Sharon Jenner	Secretariat

Appendix 2 – Patient information sheet

Patient Information Sheet

<u>Project title</u>: Detection and significance of heart injury in ST elevation MI

You are being asked to take part in a clinical research study. Before you decide it is important for you to understand why the research is being done and what it will involve for you. Please take time to read the following carefully and discuss it with others if you wish. Please ask us if there is anything you are unclear about or if you would like more information. Take time to decide whether or not you wish to take part.

What does the title mean and what is the purpose of the study?

Treatment of heart attack (myocardial infarction) has traditionally concentrated on opening the large heart arteries, whether by "clot busting" medication or balloons and stents (angioplasty). We now know that damage to the heart's tiny blood vessels also occurs during heart attack and this can contribute to longer-term heart damage. We plan to take measurements, which represent damage to the heart's small blood vessels during treatment for heart attack with angioplasty. We will then perform a special heart scan, an MRI scan, which would allow us to look at the blood supply to the heart, to look at the amount of damage to the heart as a whole and at the amount of damage to the small blood vessels. We would also like to obtain a blood and urine sample at the time of your admission to hospital and with each MRI scan in order to study some circulating cells and chemicals that may be involved in heart muscle and blood vessel repair. Our aim is to identify patients with significant damage to the hearts small blood vessels at the time of angioplasty therefore allowing us to identify future patients with treatment to minimise damage at the earliest opportunity.

Why have I been chosen?

You have had a heart attack and you require an angiography procedure to look at the arteries that supply the heart.

Do I have to take part?

No, it is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and will be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?

During the angiography/angioplasty procedure we will make measurements that represent damage to the hearts small blood vessels. This involves injecting dye into the heart arteries under x-ray guidance allowing us to identify if any blockages are present. A tiny wire will be passed into the relevant heart artery allowing us to inflate balloons and deploy stents (like small scaffolds) over the blocked area. We will use a pressure and temperature sensitive guidewire during the procedure. This wire is routinely used in our clinical practice. The measurements will take an additional 10 minutes during the procedure and do not pose any additional risk to you. While these measurements are being taken a drug called adenosine is used to increase the blood flow through the heart arteries.

You will have two heart MRI scans. One will occur within 48 hours after the angiography/angioplasty procedure and the other will be at around six months after your heart attack at a time that is convenient for you. If you agree, we would also like you to have two other MRI scans after the angiography procedure in order to study how heart injury changes. These 'extra' MRI scans would take place on the day you are admitted to hospital, and after discharge day 7 to 10.

The MRI scans last approximately one hour each. The scanner is basically tunnel shaped, like large "polo" mint, which is open at both ends. You are slid into the centre of the "polo" on a couch and the scans are taken. Some people find it a little enclosing but you can come out at any time.

Before you go into the scanner, you will be invited to provide a urine sample. Following this, two small plastic tubes or cannulas (similar to that used when putting in a drip) will be

inserted into the veins in your arms by a doctor. We would like to draw about 40 millilitres (about 3 tablespoonfuls) of blood from one of the plastic cannulas, and also ask you to provide a urine sample. We will examine new cells, such as progenitor cells, that may be involved in heart blood vessel injury/repair. We will also measure some of the circulating growth factors (small chemicals in the blood) which stimulate the release of these cells. We will count the number of these cells in each blood sample, and also prepare DNA and RNA from these cells to examine whether the genetic make-up has any connection with heart muscle and blood vessel repair (as assessed by MRI). Small blood and urine samples will be stored in a freezer to be analysed at a later stage, particularly when new markers of disease will have been developed by us or by other scientists. Further approval will be required by the ethics committee for future studies with these samples.

Following this, the cannula will permit us to inject gadolinium dye during your MRI scan.

Gadolinium is a clear fluid like water. It is used in MRI scanning because it accumulates in abnormal tissue and "lights up" that area so the scanner can detect it. It is useful in telling us which parts of the heart are abnormal, if any. After a short while the gadolinium fades away and is removed from your body (within a few hours). There is a very small risk of kidney damage or allergy after gadolinium contrast administration.

When you are in the scanner you will need to wear a pair of headphones. These are necessary because of the loud knocking noise that occurs when the pictures are being taken. The headphones allow you to listen to music of your choice (you may bring your own CD) and allow us to communicate with you throughout the scan. Whilst in the scanner, you will be given an emergency buzzer and can very quickly be taken out should you feel uncomfortable. During the scan you will be asked to hold your breath at times to improve the quality of the pictures. During the 2nd MRI visit you may bring a CD of your own choice or you can ask a relative to bring one in.

Is there any long term follow up: There is no direct follow up once you have had a repeat scan at 6 months. However, in the future, we would like to obtain information on your future well-being from health records held by the National Health Service or Government (e.g. Registrar General). We would also like to obtain information on your drug therapy (medication). We can obtain this information through confidential electronic NHS and government records. This will not require us to contact you directly.

Women only: The effect of MRI scans on babies is unknown- for this reason, anyone who is pregnant or becomes pregnant during the study will be excluded. If you think you may be pregnant please inform the study doctor.

What are the risks?

There is no additional risk by taking these extra measurements during your angioplasty. The MRI scanner is very safe if you have not metal implants in your body.

The dye used during the cardiac MRI scans is called gadolinium. It is generally harmless and will be washed out of your system by your kidneys. Side effects include mild headache and nausea. Rarely (less than 1 % of the time) low blood pressure and light-headedness occurs. Very rarely (less than one in a thousand), patients are allergic to the contrast agent.

Senior doctors will be present during your angioplasty procedure and a senior doctor will be present during your cardiac MRI scans. The impact of any incidental finding will be followed up by referral to the appropriate specialist if not dealt with by cardiology staff.

The amount of blood and urine drawn does not place you at any risk.

We would like to involve medical and/or physics students in our research team in order that they learn about and engage in research in imaging and heart disease.

What are the potential benefits of taking part?

You are unlikely to benefit directly from taking part in the study but the information that we get may help to improve treatment of patients in the future. This will provide additional information about your health, which could influence your future treatment. While the blood and urine results may be useful for clinical research purposes, we do not anticipate these results to be useful for the treatment of your condition.

What if something goes wrong?

If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal NHS complaints mechanisms will be available to you.

Will my GP be informed?

We will inform your GP that you have agreed to take part in this study.

Will my taking part in this study be kept confidential?

All information that is collected about you during the course of the research will be kept strictly confidential. Any information about you that leaves the hospital will have your name and address removed so that you cannot be recognised from it. Your personal information will be kept on file and stored in a secure place at the BHF Glasgow Cardiovascular Research Centre and in the Department of Cardiology. All examinations (including urine and blood results and gene data) will be labelled with a code and not with any personal details so that all analyses will be carried out anonymously. All information which is collected about you during the course or the research will be kept strictly confidential. Any information about you which leaves the hospital or the Clinical Investigation Unit will have your name and address removed so that you cannot be recognised from it.

What will happen to the results of the research study?

When the results become available they will be submitted to medical journals where they will be considered for publication. The final results will also be submitted to national and international medical conferences where they will be considered for publication. At the BHF Glasgow Cardiovascular Research Centre we will have events to inform the public about our ongoing research and about results from this and other studies.

You will not be identified in any report or publication.
If you would like a copy of the results, please ask your study doctor.

Who is organising and funding the research?

This study is organised by doctors from the Department of Cardiology, Golden Jubilee National Hospital, and scientists from the BHF Glasgow Cardiovascular Research Centre at Glasgow University. The study is funded by charities and researchers will not receive any payment for conducting this study.

Who has reviewed the study?

The West of Scotland Research Ethics Committee and the National Waiting Times Board has reviewed this study.

Who can I contact for further information?

Study doctors: **Dr David Carrick**

Department of Cardiology
Golden Jubilee National Hospital
Telephone: 0141-951-5875 or 0141 951 5180

Supervisor: Dr Colin Berry

Thank you for taking the time to read this patient information sheet.

Appendix 3 – Patient consent form

CONSENT FORM

Title of project:

Detection and significance of heart injury in ST elevation MI

Name of researcher: Dr Colin Berry; Dr David Carrick **Please initial box**

1. I confirm that I have read and understand the information sheet for the above study and have had the opportunity to ask questions. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that sections of any of my medical notes may be looked at by responsible individuals from the research team or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐
4. I agree to take part in the above study. ☐

.		
Name of patient	Date	Signature
Name of Person taking consent (if different from researcher)	Date	Signature
Researcher	Date	Signature

1 for patient; 1 for researcher; 1 to be kept with hospital notes

Appendix 4 – Clinical event adjudication charter

Clinical Event Adjudication Charter

Detection and Significance of Heart Injury in ST Elevation Myocardial Infarction –

The BHF MR-MI study

NCT02072850

Rationale for the independent adjudication of clinical events

As a measure of enhanced Pharmacovigilance (PV) and Good Clinical Practice, a cardiologist who was independent of the clinical research team was designated to review deaths (due to any cause) and specifically cardiovascular events of interest. At a high level, such events of interest will include death of any cause, non-fatal acute myocardial infarction, non-fatal stroke, hospitalization due to unstable angina, hospitalization due to heart failure and coronary revascularization procedures (i.e. percutaneous coronary intervention, coronary artery bypass grafting). The revascularization procedures will not be considered to be major adverse events of interest but will be reviewed by the independent clinician to ensure that events of interest (e.g. acute myocardial infarction) have not been missed.

The clinician will review cases of interest to determine if they meet accepted diagnostic criteria. Causality assessments will not be made by the clinician, nor will the clinician possess governance authority. The cardiologist will be blinded regarding any information relating to the imaging measurements.

All deaths and pre-specified major adverse cardiovascular events (i.e. "MACE"-type events) will be prospectively collected by investigators and classified independently by the independent cardiologist. Details on these pre-specified events are listed in section 4.

As noted above, events of interest will be identified primarily by the investigator, who may use an eCRF checkbox to mark any event as a "CV event of interest". The study was under regulatory review by the National Research Ethics Service and the National Waiting Times Board (NWTB) which is the Sponsor.

Objective of the Event Adjudication Charter

The purpose of this document is to delineate the roles, responsibilities and procedures in regards to the adjudication of cardiovascular events occurring in the BHF MR-MI study.

Study Coordinator

The independent cardiologist is assisted by the study coordinator (Dr David Carrick, BHF Cardiovascular Research Centre, University of Glasgow; david.carrick@nhs.net) who is a registered physician based in the University of Glasgow and Golden Jubilee National Hospital and who has considerable previous experience in the conduct of clinical cardiology studies.

The coordinator will:

Assist with preparation of the source clinical data

Enter the classification verdicts of the independent cardiologist into the database

Events to be reviewed by the independent cardiologist

3.1 Deaths

The independent cardiologist will review all reported deaths and classify the cause of death according to the following schema:

Non-cardiovascular

A definite non-cardiovascular cause of death must be identified.

Cardiovascular (CV)

Death due to acute myocardial infarction

Death due to stroke

Sudden cardiac death

Other CV death (e.g. heart failure, pulmonary embolism, cardiovascular procedure-related)

Undetermined cause of death (i.e. cause of death unknown)

3.2 Non-fatal cardiovascular events

The independent cardiologist will review and adjudicate the following reported non-fatal cardiovascular events:

Acute myocardial infarction

Hospitalization for unstable angina/other angina*/chest pain*

Stroke/TIA/Other cerebrovascular events (i.e. subdural/extradural hemorrhage)**

Heart failure requiring hospitalization

Coronary revascularization procedures (i.e. percutaneous coronary intervention, coronary artery bypass grafting)***

Renal failure (>25% rise in creatinine from baseline or an absolute increase in serum creatinine of 0·.5 mg/dL (44 µmol/L) after a radiographic examination using a contrast agent (Barrett NEJM 2006;354:379-86)

Bleeding according to the ACUITY criteria (Stone Am Heart J 2004;148:764-75)

Note: Other non-fatal cardiovascular events will not routinely be reviewed by the independent cardiologist. These events will be reviewed by trained and qualified clinical research staff in the Golden Jubilee National Hospital to ensure that potential cardiovascular events requiring adjudication are not missed. If the review suggests that a potential cardiovascular event requiring adjudication may have been missed, further information will be requested, as required and, if necessary, the event will be allocated for adjudication.

*Hospitalization for other angina or for chest pain are not study events of interest but such events will be reviewed by the independent cardiologist to ensure that acute myocardial infarction or hospitalization for unstable angina events have not been missed.

**TIAs and other cerebrovascular events (subdural haemorrhage, extradural haemorrhage) will be reviewed to ensure that stroke events have not been missed.

***Coronary revascularization procedures (i.e. percutaneous coronary intervention, coronary artery bypass grafting) are not study events of interest but will be reviewed by the independent cardiologist to sure that study events of interest (e.g. acute myocardial infarction, hospitalization for unstable angina) have not been missed.

Adverse Event definitions

For those event-types requiring adjudication, each event will usually be adjudicated on the basis of strict application of the endpoint definitions below. However, the clinical likelihood that a suspected event has occurred will be individually assessed even in the absence of fulfilment of all of the criteria specified in the event-definition, recognizing that information may at times be difficult to interpret (e.g. the exact measurement of ECG changes may be imprecise) or unavailable.

Overall, event definitions should align with the "Standardized definitions for endpoint events in cardiovascular trials' Hicks KA et al May 2011 and the "Third Universal Definition of Myocardial Infarction" Thygesen et al Eur Heart J 2012.

4.1 Deaths

In cases where a patient experiences an event and later dies due to that event, the event causing death and the death will be considered as separate events *only* if they are separated

by a change in calendar day. If the event causing death and the death occur on the same calendar day, death will be the only event classified.

4.1.1 Cardiovascular deaths

Cardiovascular death includes death resulting from an acute myocardial infarction, sudden cardiac death, death due to heart failure, death due to stroke and death due to other cardiovascular causes as follows:

Death due to Acute Myocardial Infarction refers to a death usually occurring up to 30 days after a documented acute myocardial infarction (verified either by the diagnostic criteria outlined below for acute myocardial infarction, above, or by autopsy findings showing recent myocardial infarction or recent coronary thrombus) due to the myocardial infarction or its immediate consequences (e.g. progressive heart failure) and where there is no conclusive evidence of another cause of death.

If death occurs before biochemical confirmation of myocardial necrosis can be obtained, adjudication should be based on clinical presentation and other (e.g. ECG, angiographic, autopsy) evidence.

NOTE: This category will include sudden cardiac death, involving cardiac arrest, often with symptoms suggestive of myocardial ischemia, and accompanied by presumably new ST elevation*, or new left bundle branch block*, or evidence of fresh thrombus in a coronary artery by coronary angiography and/or at autopsy, but death occurring before blood samples could be obtained, or at a time before the appearance of cardiac biomarkers in the blood (i.e. myocardial infarction Type 3 – see section 4.2.1, below).

*If ECG tracings are not available for review, the independent cardiologist may adjudicate on the basis of reported new ECG changes that have been clearly documented in the case records or in the case report form.

Death resulting from a procedure to treat an acute myocardial infarction [percutaneous coronary intervention (PCI), coronary artery bypass graft surgery (CABG)], or to treat a complication resulting from acute myocardial infarction, should also be considered death due to acute myocardial infarction.

Death resulting from a procedure to treat myocardial ischemia (angina) or death due to an acute myocardial infarction that occurs as a direct consequence of a cardiovascular investigation/procedure/operation that was not undertaken to treat an acute myocardial

infarction or its complications should be considered as a death due to other cardiovascular causes.

Sudden Cardiac Death refers to a death that occurs unexpectedly in a previously stable patient. The cause of death should not be due to another adjudicated cause (e.g. acute myocardial infarction Type 3 – see section 4.2.1 below).

The following deaths should be included.

a. Death witnessed and instantaneous without new or worsening symptoms

b. Death witnessed within 60 minutes of the onset of new or worsening symptoms unless a cause other than cardiac is obvious.

c. Death witnessed and attributed to an identified arrhythmia (e.g., captured on an ECG recording, witnessed on a monitor), or unwitnessed but found on implantable cardioverter-defibrillator review.

d. Death in patients resuscitated from cardiac arrest in the absence of pre-existing circulatory failure or other causes of death, including acute myocardial infarction, and who die (without identification of a non-cardiac aetiology) within 72 hours or without gaining consciousness; similar patients who died during an attempted resuscitation.

Unwitnessed death without any other cause of death identified (information regarding the patient's clinical status in the 24 hours preceding death should be provided, if available)

Death due to Heart Failure refers to a death occurring in the context of clinically worsening symptoms and/or signs of heart failure without evidence of another cause of death (e.g. acute myocardial infarction).

Death due to heart failure should include sudden death occurring during an admission for worsening heart failure as well as death from progressive heart failure or cardiogenic shock following implantation of a mechanical assist device.

New or worsening signs and/or symptoms of heart failure include any of the following:

a. New or increasing symptoms and/or signs of heart failure requiring the initiation of, or an increase in, treatment directed at heart failure or occurring in a patient already receiving maximal therapy for heart failure

Note: If time does not allow for the initiation of, or an increase in, treatment directed at heart failure or if the circumstances were such that doing so would have been inappropriate

(e.g. patient refusal), the adjudication will be based on the clinical presentation and, if available, investigative evidence.

b. Heart failure symptoms or signs requiring continuous intravenous therapy (i.e. at least once daily bolus administration or continuous maintenance infusion) or chronic oxygen administration for hypoxia due to pulmonary oedema.

c. Confinement to bed predominantly due to heart failure symptoms.

d. Pulmonary oedema sufficient to cause tachypnoea and distress **not** occurring in the context of an acute myocardial infarction, worsening renal function (that is not wholly explained by worsening heart failure/cardiac function) or as the consequence of an arrhythmia occurring in the absence of worsening heart failure.

e. Cardiogenic shock **not** occurring in the context of an acute myocardial infarction or as the consequence of an arrhythmia occurring in the absence of worsening heart failure.

Cardiogenic shock is defined as systolic blood pressure (SBP) < 90 mm Hg for greater than 1 hour, not responsive to fluid resuscitation and/or heart rate correction, and felt to be secondary to cardiac dysfunction and associated with at least one of the following signs of hypoperfusion:

Cool, clammy skin ***or***

Oliguria (urine output < 30 mL/hour) ***or***

Altered sensorium ***or***

Cardiac index < 2·2 L/min/m^2

Cardiogenic shock can also be defined if SBP < 90 mm Hg and increases to ≥ 90 mm Hg in less than 1 hour with positive inotropic or vasopressor agents alone and/or with mechanical support.

Death due to Stroke refers to death after a documented stroke (verified by the diagnostic criteria outlined below for stroke or by typical post mortem findings) that is either a direct consequence of the stroke or a complication of the stroke and where there is no conclusive evidence of another cause of death.

NOTE: In cases of early death where confirmation of the diagnosis cannot be obtained, the independent may adjudicate based on clinical presentation alone.

Death due to a stroke reported to occur as a direct consequence of a cardiovascular investigation/procedure/operation will be classified as death due to other cardiovascular cause.

Death due to subdural or extradural haemorrhages will be adjudicated (based on clinical signs and symptoms as well as neuroimaging and/or autopsy) and classified separately by the CV-EAC.

Death due to Other Cardiovascular Causes refers to a cardiovascular death not included in the above categories [e.g. pulmonary embolism, cardiovascular intervention (other than one performed to treat an acute myocardial infarction or a complication of an acute myocardial infarction – see definition of death due to myocardial infarction, above), aortic aneurysm rupture, or peripheral arterial disease]. Mortal complications of cardiac surgery or non-surgical revascularization should be classified as cardiovascular deaths.

4.1.2 Non-cardiovascular deaths

A non-cardiovascular death is defined as any death that is not thought to be due to a cardiovascular cause. There should be unequivocal and documented evidence of a non-cardiovascular cause of death.

Further sub-classification of non-cardiovascular death will be as follows:

Pulmonary

Renal

Gastrointestinal

Infection (includes sepsis)

Non-infectious (e.g., systemic inflammatory response syndrome (SIRS))

Malignancy

Haemorrhage, not intracranial

Accidental/Trauma

Suicide

Non-cardiovascular surgery

Other non-cardiovascular, specify: _____

4.1.3 Undetermined cause of death

This refers to any death not attributable to one of the above categories of cardiovascular death or to a non-cardiovascular cause (e.g. due to lack of information such as a case where the only information available is "patient died"). It is expected that every effort will be made to provide the adjudicating committee with enough information to attribute deaths to either a cardiovascular or non-cardiovascular cause so that the use of this category is kept to a minimal number of patients.

4.1.4 Non-fatal Cardiovascular Events

Date of onset

For purposes of classification, when classifying events that are a cause of hospitalization, the date of admission will be used as the onset date. In cases where the stated date of admission differs from the date the patient first presented to hospital with the event (e.g. because of a period of observation in an emergency department, medical assessment unit or equivalent), the date of initial presentation to hospital will be used (provided that the patient had not been discharged from hospital in the interim).

For events where an admission date is not applicable (or not available), the date of onset as stated by the investigator will be used.

4.2.1 Acute myocardial infarction

Note on biomarker elevations:

For cardiac biomarkers, laboratories should report an upper reference limit (URL). If the 99th percentile of the upper reference limit (URL) from the respective laboratory performing the assay is not available, then the URL for myocardial necrosis from the laboratory should be used. If the 99th percentile of the URL or the URL for myocardial necrosis is not available, the MI decision limit for the particular laboratory should be used as the URL.

Spontaneous acute myocardial infarction:

A rise and/or fall of cardiac biomarkers (troponin or CK-MB) should usually be detected (see note below) with at least one value above the upper reference limit (URL) together with evidence of myocardial ischemia with at least one of the following:

Clinical presentation consistent with ischemia

ECG evidence of acute myocardial ischemia (as outlined in Table 1, below) or new left bundle branch block (LBBB).

Development of pathological Q waves on the ECG (see Table 2, below)

Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality

Autopsy evidence of acute myocardial infarction

If biomarkers are elevated from a prior infarction, then a spontaneous myocardial infarction is defined as:

a. One of the following:

o Clinical presentation consistent with ischemia

o ECG evidence of acute myocardial ischemia (as outlined in Table 1, below) or new left bundle branch block. [The events committee will adjudicate in the context of the sequential ECG changes that are commonly seen in acute ST elevation/acute non-ST elevation myocardial infarction.]

o New pathological Q waves (see Table 2, below). [The events committee will adjudicate in the context of the sequential ECG changes that are commonly seen in acute ST elevation/acute non-ST elevation myocardial infarction.]

o Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality

o Autopsy evidence of acute myocardial infarction

AND

b. Both of the following:

o Evidence that cardiac biomarker values were decreasing (e.g. two samples 3-6 hours apart) prior to the suspected acute myocardial infarction*

o ≥ 20% increase (and > URL) in troponin or CK-MB between a measurement made at the time of the initial presentation with the suspected recurrent myocardial infarction and a further sample taken 3-6 hours later

*If biomarkers are increasing or peak is not reached, then a definite diagnosis of recurrent myocardial infarction is generally not possible.

Percutaneous coronary intervention-related acute myocardial infarction

Peri-percutaneous coronary intervention (PCI) acute myocardial infarction is defined by any of the following criteria. Symptoms of cardiac ischemia are not required.

Biomarker elevations within 48 hours of PCI:

• Troponin or CK-MB (preferred) > 5 x URL ***and***

• No evidence that cardiac biomarkers were elevated prior to the procedure;

OR

• Both of the following must be true:

o ≥ 50% increase in the cardiac biomarker result

o Evidence that cardiac biomarker values were decreasing (e.g. two samples 3-6 hours apart) prior to the suspected acute myocardial infarction

New pathological Q waves or new left bundle branch block (LBBB).

[If the PCI was undertaken in the context of an acute myocardial infarction, the events committee will adjudicate in the context of the sequential ECG changes that are commonly seen in acute ST elevation/acute non-ST elevation myocardial infarction.]

Autopsy evidence of acute myocardial infarction

Coronary artery bypass grafting-related acute myocardial infarction

Peri-coronary artery bypass graft surgery (CABG) acute myocardial infarction is defined by the following criteria. Symptoms of cardiac ischemia are not required.

Biomarker elevations within 72 hours of CABG:

• Troponin or CK-MB (preferred) > 10 x URL ***and***

• No evidence that cardiac biomarkers were elevated prior to the procedure;

OR

• Both of the following must be true:

o ≥ 50% increase in the cardiac biomarker result

o Evidence that cardiac biomarker values were decreasing (e.g. two samples 3-6 hours apart) prior to the suspected acute myocardial infarction

AND

One of the following:

New pathological Q-waves (preferably with evidence of persistence)

[If the CABG was undertaken in the context of an acute myocardial infarction, the events committee will adjudicate in the context of the sequential ECG changes that are commonly seen in acute ST elevation/acute non-ST elevation myocardial infarction.]

New LBBB (preferably with evidence of persistence)

Angiographically documented new graft or native coronary artery occlusion

Imaging evidence of new loss of viable myocardium

OR

Autopsy evidence of acute myocardial infarction

Note: For a diagnosis of acute myocardial infarction, a rise and/or fall of cardiac biomarkers should usually be detected. However, myocardial infarction may be adjudicated for an event that has characteristics which are very suggestive of acute infarction but which does not meet the strict definition because biomarkers are not available (e.g. not measured) or are non-contributory (e.g. may have normalized).

Suggestive characteristics are:

Typical cardiac ischemic-type pain/discomfort
(except for suspected acute myocardial infarction occurring in the context of PCI or CABG where this requirement need not apply)

AND

New ECG changes* or other evidence to support a diagnosis of acute myocardial infarction (e.g. imaging evidence of new loss of viable myocardium/new regional wall motion abnormality or angiography demonstrating occlusive coronary thrombus)

*If ECG tracings are not available for review, the adjudication may be made on the basis of reported ECG changes that have been clearly documented in the case records or in the case report form.

Clinical classification of different types of myocardial infarction

Myocardial infarctions will be clinically classified as:

Type 1

Spontaneous myocardial infarction related to ischemia due to a primary coronary event such as plaque erosion and/or rupture, fissuring, or dissection.

Type 2

Myocardial infarction secondary to ischemia due to either increased oxygen demand or decreased supply, e.g. coronary artery spasm, coronary embolism, anaemia, arrhythmias, hypertension, or hypotension.

Type 3

Sudden unexpected cardiac death, including cardiac arrest, often with symptoms suggestive of myocardial ischemia, accompanied by presumably new ST elevation, or new LBBB, or evidence of fresh thrombus in a coronary artery by angiography and/or at autopsy, but death occurring before blood samples could be obtained, or at a time before the appearance of cardiac biomarkers in the blood.

Type 4a

Myocardial infarction associated with PCI.

Type 4b

Myocardial infarction associated with stent thrombosis as documented by angiography or at autopsy.

Type 5

Myocardial infarction associated with CABG.

Myocardial infarctions will be further sub-classified as:

ST segment elevation myocardial infarction (STEMI).

or

Non-ST segment elevation myocardial infarction (NSTEMI).

or

Myocardial infarction, type (i.e. STEMI or NSTEMI) unknown.

Table 1: ECG manifestations of acute myocardial ischemia (in absence of left ventricular hypertrophy and left bundle branch block)

ST elevation
New ST elevation at the J-point in two anatomically contiguous leads with the cut-off points: ≥ 0·2 mV in men (> 0·25 mV in men < 40 years) or ≥ 0·15 mV in women in leads V2-V3 and/or ≥ 0·1 mV in other leads.
ST depression and T wave changes
New horizontal or down-sloping ST depression ≥ 0·05 mV in two contiguous leads; and/or new T wave inversion ≥ 0·1 mV in two contiguous leads.
The above ECG criteria illustrate patterns consistent with myocardial ischemia. In patients with abnormal biomarkers, it is recognized that lesser ECG abnormalities may represent an ischemic response and may be accepted under the category of abnormal ECG findings.

Table 2: Pathological Q waves:

Any Q-wave in leads V2-V3 ≥ 0·02 seconds or QS complex in leads V2 and V3
Q-wave ≥ 0·03 seconds and ≥ 0·1 mV deep or QS complex in leads I, II, aVL, aVF, or V4-V6 in any two leads of a contiguous lead grouping (I, aVL, V6; V4-V6; II, III, and aVF) a
A The same criteria are used for supplemental leads V7-V9, and for the Cabrera frontal plane lead grouping.

4.2.2 Hospitalization for unstable angina

For the diagnosis of hospitalization due to unstable angina there should be emergency/unplanned admission to a hospital setting (emergency room, observation or inpatient unit) that results in at least one overnight stay (i.e. a date change) with fulfilment of the following criteria:

There should be:

1. Cardiac ischemic-type symptoms at rest (chest pain or equivalent) or an accelerating pattern of angina (e.g. exercise-related ischemic-type symptoms increasing in frequency and/or severity, decreasing threshold for onset of exercise related ischemic type symptoms) but without the fulfilment of the above diagnostic criteria for acute myocardial infarction.

and

2 The need for treatment with parenteral (intravenous, intra-arterial, buccal, transcutaneous or subcutaneous) anti-ischemic/antithrombotic therapy and/or coronary revascularization.

and

3a ECG manifestations of acute myocardial ischemia (New ST-T changes meeting the criteria for acute myocardial ischemia - as outlined in Table 1, section 5.2.1).

or

3b Angiographically significant coronary artery disease thought to be responsible for the patient's presentation. [If both invasive and CT angiographic imaging of the coronary arteries were performed, the results of the invasive coronary angiogram should take preference.]

and

4 The independent clinician should be satisfied that unstable angina was the primary reason for hospitalization.

4.2.3 Hospitalization for other angina*

For the diagnosis of hospitalization for other angina, there should be emergency/unplanned admission to a hospital setting (emergency room, observation or inpatient unit) that results in at least one overnight stay (i.e. a date change) with fulfilment of the following criteria:

There should be:

Typical cardiac ischemic-type symptoms but without the fulfilment of the above diagnostic criteria for acute myocardial infarction or unstable angina.

and

2 The need for treatment with new or increased anti-angina therapy (excluding sublingual nitrate therapy).

and

3a Investigations undertaken in view of the event (e.g. exercise ECG or stress myocardial perfusion scan) showing evidence of reversible myocardial ischemia.

or

3b Coronary angiography showing angiographically significant coronary disease thought to be responsible for the patient's presentation. [If both invasive and CT angiographic imaging of the coronary arteries were performed, the results of the invasive coronary angiogram should take preference.]

and

4 The independent clinician should be satisfied that angina was the primary reason for hospitalization.

4.2.4 Hospitalization for other chest pain*

There should be:

Emergency/unplanned admission to a hospital setting (emergency room, observation or inpatient unit) that results in at least one overnight stay i.e. a date change) due to chest pain but where the definitions (above) of acute myocardial infarction, hospitalization for unstable angina or hospitalization for other angina are not met.

The independent clinician should be satisfied that chest pain was the primary reason for hospitalization.

*These events are not study cardiovascular events of interest but the definitions provided for these events will be used by the independent clinician to categorise reported myocardial infarction, angina and chest pain events that do not meet the study definition of acute myocardial infarction or hospitalization for unstable angina.

4.2.5 Stroke

Stroke is defined as an acute episode of neurological dysfunction caused by focal or global brain, spinal cord, or retinal vascular injury.

A For the diagnosis of stroke, the following 4 criteria should usually be fulfilled:

1. Rapid onset* of a focal/global neurological deficit with at least one of the following:

Change in level of consciousness

Hemiplegia

Hemiparesis

Numbness or sensory loss affecting one side of the body

Dysphasia/aphasia

Hemianopia (loss of half of the field of vision of one or both eyes)

Complete/partial loss of vision of one eye

Other new neurological sign(s)/symptom(s) consistent with stroke

*If the mode of onset is uncertain, a diagnosis of stroke may be made provided that there is no plausible non-stroke cause for the clinical presentation.

2. Duration of a focal/global neurological deficit $\geq$ 24 hours

or

< 24 hours if

(i) this is because of at least one of the following therapeutic interventions:

(a) pharmacologic i.e. thrombolytic drug administration.

(b) non-pharmacologic i.e. neurointerventional procedure (e.g. intracranial angioplasty).

or

(ii) brain imaging available clearly documenting a new haemorrhage or infarct.

or

(iii) the neurological deficit results in death

3. No other readily identifiable non-stroke cause for the clinical presentation (e.g. brain tumour, hypoglycaemia, peripheral lesion).

4. Confirmation of the diagnosis by at least one of the following**:

neurology or neurosurgical specialist.

brain imaging procedure (at least one of the following):

CT scan.

MRI scan.

cerebral vessel angiography.

Lumbar puncture (i.e. spinal fluid analysis diagnostic of intracranial haemorrhage).

B If the acute neurological deficit represents a worsening of a previous deficit, this worsened deficit must have:

Persisted for more than one week

Or < one week if

(i) this is because of at least one of the following therapeutic interventions:

(a) pharmacologic i.e. thrombolytic drug administration.

(b) non-pharmacologic i.e. neurointerventional procedure (e.g. intracranial angioplasty).

or

(ii) brain imaging available clearly documenting an appropriate new CT/MRI finding.

or

(iii) the neurological deficit results in death

Strokes will be further sub-classified as:

Ischemic (non-haemorrhagic) stroke

(i.e. caused by an infarction of central nervous system tissue)

or

Haemorrhagic stroke***

(i.e. caused by non-traumatic intraparenchymal, intraventricular or subarachnoid haemorrhage)

or

Stroke type (i.e. haemorrhagic or ischemic) unknown (i.e. when imaging/other investigations are unavailable or inconclusive).

***Subdural and extradural haemorrhages will be adjudicated (based on clinical signs and symptoms as well as neuroimaging and/or autopsy) and classified separately.

4.2.6. Heart Failure requiring hospitalization

For the diagnosis of heart failure requiring hospitalization, there should be emergency/unplanned admission to a hospital setting (emergency room, observation or inpatient unit) that results in at least one overnight stay (i.e. a date change) with fulfilment of the following criteria:

There should be:

Clinical manifestations of new or worsening heart failure including at least one of the following:

New or worsening dyspnoea on exertion

New or worsening dyspnoea at rest

New or worsening fatigue/decreased exercise tolerance

New or worsening orthopnoea

New or worsening PND (paroxysmal nocturnal dyspnoea)

New or worsening lower limb or sacral oedema

New or worsening pulmonary crackles/crepitations

New or worsening elevation of JVP (jugular venous pressure)

New or worsening third heart sound or gallop rhythm

And

1 Investigative evidence of structural or functional heart disease (<u>if available</u>) with at least *one* of the following:

Radiological evidence of pulmonary oedema/congestion or cardiomegaly.

Imaging (e.g. echocardiography, cardiac magnetic resonance imaging, radionuclide ventriculography) evidence of an abnormality (e.g. left ventricular systolic dysfunction, significant valvular heart disease, left ventricular hypertrophy).

- Elevation of BNP or NT-proBNP levels.
- Other investigative evidence of structural or functional heart disease (e.g. evidence obtained from pulmonary artery catheterization).

And

3 Need for new/increased therapy***** specifically <u>for the treatment of heart failure</u>

including at least one of the following:

New or increased oral therapy for the treatment of heart failure

(See note on oral therapy, below)

Initiation of intravenous diuretic, inotrope, vasodilator or other recognised intravenous heart failure treatment or up-titration of such intravenous therapy if already receiving it

Mechanical or surgical intervention (e.g. mechanical or non-invasive ventilation, mechanical circulatory support, heart transplantation, ventricular pacing to improve cardiac function), or the use of ultrafiltration, hemofiltration, dialysis or other mechanical or surgical intervention that is specifically directed at treatment of heart failure.

<u>Note on oral therapy</u>: In general, for an event to qualify as *heart failure requiring hospitalization* on the basis of *oral* heart failure therapy (i.e. in cases where none of the non-pharmacological treatment modalities listed above have been utilized), the new or increased oral therapy should include oral diuretics. However, in special cases, other new or increased oral therapy (e.g. hydralazine/long acting nitrate, aldosterone antagonist) may be accepted provided that the adjudication committee is satisfied that:

the new or increased oral therapy was primarily directed at treating clinical manifestations of new or worsening heart failure (rather than, for example, initiation or up-titration of heart failure therapy as part of the routine optimization of medical therapy)

and

the totality of the evidence indicates that heart failure, rather than any other disease process, was the primary cause of the clinical presentation.

*****If time does not allow for the initiation of, or an increase in, treatment directed at heart failure or if the circumstances were such that doing so would have been inappropriate (e.g. patient refusal), the independent clinician will adjudicate on clinical presentation and, if available, investigative evidence.

and

4 The independent clinician should be satisfied that heart failure was the primary disease process accounting for the clinical presentation.

4.2.7. Renal Failure requiring hospitalisation

Contrast-induced nephropathy: is defined as either a greater than 25% increase of serum creatinine or an absolute increase in serum creatinine of 0·5 mg/dL after a radiographic examination using a contrast agent.

4.2.8. Bleeding requiring hospitalisation

Bleeding: is defined according to the ACUITY criteria: major bleed = intracranial or intraocular bleeding; bleeding at the site of angiography requiring intervention; a hematoma of 5 cm in diameter; a reduction in haemoglobin level of at least 4 g/dL in the absence of overt bleeding or 3 g/dL with a source of bleeding; or transfusion.

6.1 Event identification

The BHF MR-MI study will use paper-based and electronic data capture (EDC). Those events requiring independent validation (see section 4) will be reported by the Investigator via the EDC (electronic data capture) system.

6.2 Incomplete event data

If, having reviewed the event data pertaining to an event, the independent cardiologist deems that the information provided is insufficient for the purposes of event adjudication, an electronic request for further information detailing the information required will be made. The date of request will be recorded electronically and the event will be classified as not adjudicated/pending additional information.

Clinical data to be provided

The trial management team (including Prof Berry, Dr Carrick, Ms Joanne Kelly CRN) will provide event data for each potential cardiovascular event requiring adjudication to the independent cardiologist.

Data to be included for event classification will include:

Subject study identification number and event details

On request: Relevant de-identified CRF data (including any relevant event-specific CRFs e.g. the *myocardial infarction/hospitalization for unstable angina/other angina/*

chest pain event form).

Supportive source documentation as required

Baseline and subsequent scheduled ECGs obtained during study participation.

All clinical data would be de-identified.

De-identified Source Documentation

The following source documents (if available) will be provided to the independent cardiologist as part of the standard dossier contents for cardiovascular events requiring review/adjudication:

Death

Hospital Discharge Summary/Death Summary

Autopsy Report

Death Certificate

Admission History & Physical (if applicable)

Acute Myocardial Infarction/Hospitalization for Unstable Angina/Other Angina/Chest Pain

Hospital Discharge Summary

ECGs

Pre-Randomization/Screening

Baseline (prior to event but post-randomization)

During Event

Post-Event

Relevant Procedure/Operation Reports

Relevant Laboratory Reports (e.g. that document the cardiac enzyme/marker measurements provided – peak values and pre-procedure and post-procedure values, where applicable)

Reports for other investigations taken:

PCI Report

CABG Report

Coronary Angiography Report

Echocardiogram Report

Exercise ECG Report

Stress Myocardial Perfusion Scan Report

Other investigation report undertaken to test for presence of reversible myocardial ischemia

Admission History & Physical

Stroke/TIA/Other cerebrovascular events

Hospital Discharge Summary

Neurology Consultation Report(s)

Reports for other investigations undertaken:

CT Brain Scan Report

MRI Brain Scan Report

Cerebral Angiography Report

Lumbar Puncture Report

Admission History & Physical

Heart Failure requiring hospitalization

Hospital Discharge Summary

Chest X-Ray Report

Prescription Sheets/Medication Administration Records

Echocardiogram Report

Relevant Laboratory Reports (e.g. for peak BNP/NT-proBNP)

Reports for other investigations undertaken:

Cardiac Magnetic Resonance Imaging

Radionuclide Ventriculogram Scan

Pulmonary Artery Catherisation

Admission History & Physical

Coronary revascularization procedure

Hospital Discharge Summary

Relevant Procedure/Operation Reports

Bleeding

Hospital Discharge Summary

Relevant Procedure/Operation Reports

Hb

Blood transfusion results

Diagnostic and therapeutic procedures (e.g. gastroscopy).

CEC Quality assurance

For the purposes of quality assurance, 10 % of all events initially classified may be subject to review by the CEC again. If there are any discrepancies between the initial and the subsequent adjudication decisions, the Chairman and the Sponsor will discuss the steps necessary to ensure reconciliation and resolution of the issue.

NHS
Greater Glasgow and Clyde

WoSRES
West of Scotland Research Ethics Service

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BHF Glasgow Cardiovascular Research Centre,
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Date 23rd February 2012
Direct line 0141-211-6238
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Ref AHT/SAJ

Dear Prof Berry

Study title: **Cardiac magnetic resonance imaging: new pathological insights and their functional and clinical significance in ST elevation acute myocardial infarction.**
REC reference: **10/S0703/28**
Amendment number: **amendment 3 14 February 2012**
Amendment date: **14 February 2012**

The above amendment was reviewed on 21 February 2012 by the Sub-Committee in correspondence.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Participant Information Sheet: tracked changes	versiion 1.4	14 February 2012
Protocol	version 1.2	14 February 2012
Notice of Substantial Amendment (non-CTIMPs)	amendment 3 14 February 2012	14 February 2012
Covering Letter		14 February 2012

Appendix 5 – Study amendment, ethical approval

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

10/S0703/28:	**Please quote this number on all correspondence**

Yours sincerely

Andrea H Torrie

Dr John Hunter
Chair

E-mail: andrea.torrie@ggc.scot.nhs.uk

Enclosures: *List of names and professions of members who took part in the review*

Copy to: *Dr Catherine Sinclair, Golden Jubilee National Hospital / National Waiting Times Board*

West of Scotland REC 1

Attendance at Sub-Committee of the REC meeting on 21 February 2012

Name	*Profession*	*Capacity*
Dr John Hunter	Chairman West of Scotland (1) Ethics	Expert
Dr Peter Hutchison	Vice Chair/GP	Expert
Dr Audrey Morrison	Research Practitioner	Expert
Mr Robin Sim	Investments (Retired)	Lay

Also in attendance:

Name	*Position (or reason for attending)*
Miss Sharon Jenner	Secretariat
Mrs A Torrie	Senior/Lead Administrator

Appendix 6 – Amended patient information sheet

Version 1.4 February 2012 Patient Information Sheet

<u>Project title</u>: Detection and significance of heart injury in ST elevation MI

You are being asked to take part in a clinical research study. Before you decide it is important for you to understand why the research is being done and what it will involve for you. Please take time to read the following carefully and discuss it with others if you wish. Please ask us if there is anything you are unclear about or if you would like more information. Take time to decide whether or not you wish to take part.

What does the title mean and what is the purpose of the study?

Treatment of heart attack (myocardial infarction) has traditionally concentrated on opening the large heart arteries, whether by "clot busting" medication or balloons and stents (angioplasty). We now know that damage to the heart's tiny blood vessels also occurs during heart attack and this can contribute to longer-term heart damage. We plan to take measurements, which represent damage to the heart's small blood vessels during treatment for heart attack with angioplasty. We will then perform a special heart scan, an MRI scan, which would allow us to look at the blood supply to the heart, to look at the amount of damage to the heart as a whole and at the amount of damage to the small blood vessels. We would also like to obtain a blood and urine sample at the time of your admission to hospital and with each MRI scan in order to study some circulating cells and chemicals that may be involved in heart muscle and blood vessel repair. Our aim is to identify patients with significant damage to the hearts small blood vessels at the time of angioplasty therefore allowing us to identify future patients with treatment to minimise damage at the earliest opportunity.

During the angioplasty procedure, the cardiologist may feel that placing a stent in your heart artery might be harmful since blood flow might get worse, a situation known as 'no reflow'. When no-reflow happens patients usually feel more unwell. The delayed stenting approach should allow the heart artery to begin healing and so delayed stenting might be safer. The purpose of the study is to work out if waiting for a few hours before placing a

stent in your heart artery might reduce the risk of 'no-reflow', compared to usual care with stenting at the time of the initial procedure.

Why have I been chosen?

You have had a heart attack and you require an angiography procedure to look at the arteries that supply the heart. If you are asked to take part in the 'stent later' sub-study this is because your cardiologist feels you may be at risk of 'no reflow'.

Do I have to take part?

No, it is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and will be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at anytime, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?

During the angiography/angioplasty procedure we will make measurements that represent damage to the hearts small blood vessels. This involves injecting dye into the heart arteries under x-ray guidance allowing us to identify if any blockages are present. A tiny wire will be passed into the relevant heart artery allowing us to inflate balloons and deploy stents (like small scaffolds) over the blocked area. We will use a pressure and temperature sensitive guidewire during the procedure. This wire is routinely used in our clinical practice. The measurements will take an additional 10 minutes during the procedure and do not pose any additional risk to you. While these measurements are being taken a drug called adenosine is used to increase the blood flow through the heart arteries.

You will have an equal chance of being treated by stenting later (about 4 – 8 hrs) or usual care with stenting at the time of the initial angioplasty. Once the stent has been placed (either directly or after a few hours), you would then carry on with the study and usual care.

You will have two heart MRI scans. One will occur within 48 hours after the angiography/angioplasty procedure and the other will be at around six months after your heart attack at a time that is convenient for you. If you agree, we would also like you to

have three other MRI scans after the angiography procedure in order to study how heart injury changes. These 'extra' MRI scans would take place on the day you are admitted to hospital, on each of the first two days after admission and after discharge on day 5 - 7.

The MRI scans last approximately one hour each. The scanner is basically tunnel shaped, like large "polo" mint, which is open at both ends. You are slid into the centre of the "polo" on a couch and the scans are taken. Some people find it a little enclosing but you can come out at any time.

Before you go into the scanner, you will be invited to provide a urine sample. Following this, two small plastic tubes or cannulas (similar to that used when putting in a drip) will be inserted into the veins in your arms by a doctor. We would like to draw about 40 millilitres (about 3 tablespoonfuls) of blood from one of the plastic cannulas, and also ask you to provide a urine sample. We will examine new cells, such as progenitor cells, that may be involved in heart blood vessel injury/repair. We will also measure some of the circulating growth factors (small chemicals in the blood) which stimulate the release of these cells. We will count the number of these cells in each blood sample, and also prepare DNA and RNA from these cells to examine whether the genetic make-up has any connection with heart muscle and blood vessel repair (as assessed by MRI). Small blood and urine samples will be stored in a freezer to be analysed at a later stage, particularly when new markers of disease will have been developed by us or by other scientists. Further approval will be required by the ethics committee for future studies with these samples.

Following this, the cannula will permit us to inject gadolinium dye during your MRI scan.

Gadolinium is a clear fluid like water. It is used in MRI scanning because it accumulates in abnormal tissue and "lights up" that area so the scanner can detect it. It is useful in telling us which parts of the heart are abnormal, if any. After a short while the gadolinium fades away and is removed from your body (within a few hours). There is a very small risk of kidney damage or allergy after gadolinium contrast administration.

When you are in the scanner you will need to wear a pair of headphones. These are necessary because of the loud knocking noise that occurs when the pictures are being

taken. The headphones allow you to listen to music of your choice (you may bring your own CD) and allow us to communicate with you throughout the scan. Whilst in the scanner, you will be given an emergency buzzer and can very quickly be taken out should you feel uncomfortable. During the scan you will be asked to hold your breath at times to improve the quality of the pictures. During the 2nd MRI visit you may bring a CD of your own choice or you can ask a relative to bring one in.

Is there any long term follow up: There is no direct follow up once you have had a repeat scan at 6 months. However, in the future, we would like to obtain information on your future well being from health records held by the National Health Service or Government (e.g. Registrar General). We would also like to obtain information on your drug therapy (medication). We can obtain this information through confidential electronic NHS and government records. This will not require us to contact you directly.

Women only: The effect of MRI scans on babies is unknown - for this reason, anyone who is pregnant or becomes pregnant during the study will be excluded. If you think you may be pregnant please inform the study doctor.

What are the risks?

There is no additional risk by taking these extra measurements during your angioplasty. If you are in the stent-later group there is a very small chance (<1 in 100) that your artery may block when you are on the ward. If this happens you would experience some chest pain and you would be treated by going back to the cath lab earlier than planned.

The MRI scanner is very safe if you have not metal implants in your body.

The dye used during the cardiac MRI scans is called gadolinium. It is generally harmless and will be washed out of your system by your kidneys. Side effects include mild headache and nausea. Rarely (less than 1 % of the time) low blood pressure and light-headedness occurs. Very rarely (less than one in a thousand), patients are allergic to the contrast agent.

Senior doctors will be present during your angioplasty procedure and a senior doctor will be present during your cardiac MRI scans. The impact of any incidental finding will be followed up by referral to the appropriate specialist if not dealt with by cardiology staff.

The amount of blood and urine drawn does not place you at any risk.

We would like to involve medical and/or physics students in our research team in order that they learn about and engage in research in imaging and heart disease.

What are the potential benefits of taking part?

You are unlikely to benefit directly from taking part in the study but the information that we get may help to improve treatment of patients in the future. This will provide additional information about your health, which could influence your future treatment. While the blood and urine results may be useful for clinical research purposes, we do not anticipate these results to be useful for the treatment of your condition.

What if something goes wrong?

If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal NHS complaints mechanisms will be available to you.

Will my GP be informed?

We will inform your GP that you have agreed to take part in this study.

Will my taking part in this study be kept confidential?

All information that is collected about you during the course of the research will be kept strictly confidential. Any information about you that leaves the hospital will have your name and address removed so that you cannot be recognised from it. Your personal information will be kept on file and stored in a secure place at the BHF Glasgow Cardiovascular Research Centre and in the Department of Cardiology. All examinations (including urine and blood results and gene data) will be labelled with a code and not with any personal details so that all analyses will be carried out anonymously. All information which is collected about you

during the course or the research will be kept strictly confidential. Any information about you which leaves the hospital or the Clinical Investigation Unit will have your name and address removed so that you cannot be recognised from it.

What will happen to the results of the research study?

When the results become available they will be submitted to medical journals where they will be considered for publication. The final results will also be submitted to national and international medical conferences where they will be considered for publication. At the BHF Glasgow Cardiovascular Research Centre we will have events to inform the public about our ongoing research and about results from this and other studies.

You will not be identified in any report or publication.
If you would like a copy of the results, please ask your study doctor.

Who is organising and funding the research?

This study is organised by doctors from the Department of Cardiology, Golden Jubilee National Hospital, and scientists from the BHF Glasgow Cardiovascular Research Centre at Glasgow University. The study is funded by charities and researchers will not receive any payment for conducting this study.

Who has reviewed the study? The West of Scotland Research Ethics Committee and the National Waiting Times Board has reviewed this study.

Who can I contact for further information?

Study doctors: **Dr David Carrick**

Department of Cardiology
Golden Jubilee National Hospital
Telephone: 0141-951-5875 or 0141 951 5180

Supervisor: Professor Colin Berry

Thank you for taking the time to read this patient information sheet.

Appendix 7 – amended patient consent form

Version 1.2 April 2012

CONSENT FORM

Title of project:

Detection and significance of heart injury in ST elevation MI

Name of researcher: Professor Colin Berry

Please initial box

1. I confirm that I have read and understand the information sheet for the above study and have had the opportunity to ask questions. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that sections of any of my medical notes may be looked at by responsible individuals from the research team or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐
4. **I agree to take part in the 'delayed stent' sub-study.** ☐
5. **I agree to follow-up information being collected on my future well-being and treatment from NHS and Government health records.** ☐
6. I agree to take part in the above study. ☐

Name of patient	Date	Signature
Name of Person taking consent (if different from researcher)	Date	Signature
Researcher	Date	Signature

Appendix 8 – Tirofiban infusion protocol up to 16 hours

- Patient with body weight < 84 Kg – Standard infusion will last 16 hours. If the patient has not been taken back to the lab after 16 hours set up dilute tirofiban infusion.

- Patient with body weight 85-92 Kg – Standard infusion will run out between 12 and 16 hours. If a new infusion is required use a second vial for high dose tirofiban but seek advice from medics if patient has not been to the lab by 16 hours. May decide to reduce tirofiban dose to 0.01 microgrammes/Kg/minute.

- Patient with body weight >92Kg - Original infusion will run out before 12 hours. Use a second vial for high dose tirofiban but seek advice from medics if patient has not been to the lab by 16 hours. May decide to reduce dose to lower dose tirofiban at 0.01 microgrammes/Kg/minute.

- Remember to reduce infusion rate by 50% if CrCl is <30ml/minute

Ms Joanne Dunne, Cardiology Pharmacist, Golden Jubilee National Hospital

Telephone 0141 951 5805

3rd April 2012

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