Novel Insights into the Assessment and Therapeutics of Microcirculatory Injury in Acute Myocardial Infarction

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Abstract

Introduction:

Microvascular injury in acute ST-segment elevation myocardial infarction (STEMI) is an important predictor of adverse prognosis. However, persistent microvascular injury typically passes undetected after primary percutaneous coronary intervention (PCI), and is a problem of unmet therapeutic need. The aim of this work was to gain greater insight into the invasive assessment of acute microvascular injury following primary PCI, for risk-stratification, and to provide mechanistic insights into the effects of intracoronary alteplase on the microcirculation.

Methods:

The first part of the thesis sought to establish the influence of coronary flow on the effects of adjunctive intracoronary alteplase, and to investigate the effects of intracoronary alteplase on invasive physiology measures of microvascular function. Through a multi-centre, prospective, randomised controlled trial (T-TIME, NCT02257294), alteplase 10mg, and alteplase 20mg were compared to placebo, in patients undergoing primary PCI. Eligible participants presented within 6 hours from STEMI onset, and the study drug was administered before stent implantation. In 421 patients, TIMI (thrombolysis in myocardial infarction) flow grade was determined in the infarct-related artery immediately before study drug administration. In a subset of 144 patients, invasive physiology parameters were measured in the infarct-related artery at the end of the primary PCI procedure (the prespecified T-TIME physiology sub-study). These invasive physiology parameters included index of microcirculatory resistance (IMR), coronary flow reserve (CFR) and resistive reserve ratio (RRR). Microvascular obstruction (MVO) and myocardial haemorrhage were assessed on cardiovascular magnetic resonance (CMR) imaging at 2 to 7 days post-STEMI, and CMR imaging was repeated at 3 months.

The second part of the thesis sought to prospectively compare IMR, CFR, RRR, myocardial perfusion grade (MPG) and TIMI frame count (TFC), for predicting MVO and myocardial haemorrhage, and clinical outcomes at 1 year, in the T-TIME physiology sub-study. The following adjudicated clinical outcomes were assessed: major adverse cardiac events, heart failure hospitalisations, and all-cause death/ heart failure hospitalisations. Furthermore, a retrospective analysis was performed in 271 acute STEMI patients from a single-centre observational study (MR-MI, NCT02072850), for the derivation of a newly

conceived invasive physiology parameter termed temperature recovery time (TRT). The associations between TRT and MVO (on 2 to 7 day CMR imaging) and clinical outcomes, were assessed in the MR-MI cohort, and were prospectively validated in the T-TIME physiology sub-study population.

Results:

The main findings are summarised as follows:

- Low-dose intracoronary alteplase given early during primary PCI, was associated with increased occurrence of MVO and myocardial haemorrhage in participants who had TIMI flow ≤2 immediately preceding drug administration.
- In participants with TIMI 3 flow immediately preceding drug administration, there was no difference in MVO or myocardial haemorrhage with intracoronary alteplase compared to placebo.
- There was overall no difference in microvascular function, measured by IMR, CFR and RRR, between intracoronary alteplase and placebo groups.
- In patients with ischaemic time <2 hours, CFR and RRR were higher with alteplase 20mg vs. placebo, whereas in patients with ischaemic times ≥4 hours, MVO extent was higher with alteplase 20mg vs. placebo.
- In acute STEMI patients, lower RRR, IMR >40, and MPG ≤1 were associated with more MVO, myocardial haemorrhage presence and adverse clinical outcomes, whereas CFR ≤2 was not.
- In acute STEMI patients, TFC >27 was associated with adverse clinical outcomes, but was not associated with MVO or myocardial haemorrhage.
- Higher TRT independently predicted more MVO and adverse clinical outcomes, in two independent acute STEMI cohorts.

Conclusions:

The findings from this PhD are novel and clinically relevant. Invasive measures of microvascular injury during primary PCI allows potential for early administration of targeted adjunctive therapies to the highest risk patients. The data support IMR in conjunction with RRR instead of CFR, to select patients for adjunctive therapies. Moreover, TRT was found to detect failed microvascular perfusion and may have potential to refine risk stratification in acute STEMI. The findings raise a question as to the safety of intracoronary administration of alteplase in the context of STEMI when there is <TIMI 3 flow. Finally, the data suggest that future studies evaluating the effects of intracoronary fibrinolysis should limit recruitment to patients with short ischaemic time.

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Professor Colin Berry was chief investigator for the T-TIME trial (trial of low-dose adjunctive alteplase during primary PCI), and the MR-MI study (detection and significance of heart injury in ST-elevation myocardial infarction).

The Robertson Centre for Biostatistics validated the statistics in chapters 3 and 4.

Dr Peter McCartney analysed the cardiovascular magnetic resonance images from the T-TIME trial. Dr Campbell Tait led the coagulation analysis and Dr Peter Macfarlane led the electrocardiography analysis for the T-TIME trial. Dr Robin Weir, Dr Aengus Murphy and Dr Colin Petrie adjudicated clinical events, as part of the clinical event committee for the T-TIME trial. Dr David Carrick coordinated the MR-MI study, which was used as the retrospective derivation cohort for chapter 6.

I would like to thank the patients who participated, and the staff who recruited patients in the T-TIME trial and the MR-MI study.

Lastly, I am thankful for a few individuals who encouraged me to take an independent lead on designing the PhD thesis and publications, from the analyses I had available to lead on.

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.

My specific contribution to the T-TIME trial is as follows:

I contributed to writing the British Heart Foundation clinical PhD fellowship, entitled “the T-TIME coronary physiology study”, which was awarded to fund my PhD studies. I screened some of the patients for eligibility, assented patients into the study and obtained consent for some of the patients. In the catheterisation laboratory, I randomised some of the patients who met the eligibility criteria and reconstituted the study drug for some of the patients included in the trial. I regularly attended and actively participated in management meetings regarding the T-TIME trial. I performed site visits, and I gave feedback to sites on the quality of the angiographic and coronary physiology data obtained. I developed the angiogram and the coronary physiology standard operating procedures. I transferred data from coronary physiology and optical coherence tomography recordings, and angiograms, for analysis in the core laboratory. Furthermore, I coordinated transfer of angiographic and coronary physiology data from external sites to the central core laboratory in Glasgow. I was a point of contact for research staff at other sites. I analysed all the angiograms in the T-TIME trial, as well as all the coronary physiology data. I supervised some of the cardiovascular magnetic resonance imaging scans. I screened serious adverse events that had been reported to the Pharmacovigilance Unit by site research staff, and I forwarded relevant serious adverse events to the clinical events committee for review. I wrote the statistical analysis plan for the T-TIME coronary physiology sub-study, and I performed the statistical analyses for my PhD results chapters, and for publications that I am first author for. I contributed to conceiving the ideas for analyses reported in my PhD thesis, and I designed the structure of my PhD thesis, so that it forms a cohesive report of the subject and of the work I did. Last but not least, I interpreted data and I wrote the publications that I am first author for.

Annette Maznyczka

List of publications, abstracts and awards

Research Publications

Maznyczka A, McCartney P, Duklas P, McEntegart M, Oldroyd KG, Greenwood JP, Muir DF, Chowdhury S, Gershlick AH, Appleby C, Eteiba H, Cotton JM, Wragg A, Curzen N, Tait RC, Macfarlane PW, Welsh P, Sattar N, Petrie M, Ford I, Fox K.A.A, McConnachie A, Berry C. Effect of coronary flow on intracoronary alteplase: a pre-specified analysis from the T-TIME trial. 2020 (submitted).

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Awards

British Heart Foundation clinical research training fellowship (FS/16/74/32573): award total £263,332, for the T-TIME trial coronary physiology study

British Cardiovascular Interventions Society young investigator finalist prize, London, 2019

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Abbreviations

A

Activated clotting time (ACT)

Area under the curve (AUC)

C

Circumflex artery (Cx)

Cardiovascular magnetic resonance (CMR)

Confidence interval (CI)

Coronary flow reserve (CFR)

E

Enzyme-linked immunosorbent assay (ELISA)

H

Hyperaemic microvascular resistance (HMR)

Hazard ratio (HR)

I

Index of microcirculatory resistance (IMR)

Interquartile range (IQR)

L

Late gadolinium enhancement (LGE)

Left anterior descending artery (LAD)

Left ventricular (LV)

Left ventricular end diastolic pressure (LVEDP)

Left ventricular end diastolic volume (LVEDV)

Left ventricular end systolic volume (LVESV)

M

Major adverse cardiac events (MACE)

Microvascular obstruction (MVO)

Myocardial perfusion grade (MPG)

O

Odds ratio (OR)

P

Aortic pressure (Pa)

Plasminogen activator inhibitor -1 (PAI-1)

Distal coronary pressure (Pd)

Percutaneous coronary intervention (PCI)

Coronary wedge pressure (Pw)

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Q

Quantitative coronary angiography (QCA)

R

Receiver operating characteristic (ROC)

Reactive oxygen species (ROS)

Resistive reserve ratio (RRR)

Right coronary artery (RCA)

Recombinant tissue plasminogen activator (r-tPA)

S

Single-photon emission computed tomography (SPECT)

Standard deviation (SD)

ST-segment elevation myocardial infarction (STEMI)

T

Temperature recovery time (TRT)

Thrombolysis in myocardial infarction (TIMI)

TIMI frame count (TFC)

Mean transit time (Tmn)

V

Versus (vs)

Chapter 1: Introduction and literature review

1.1 Scale of the problem and the wider context of myocardial infarction research

ST-segment elevation myocardial infarction (STEMI) is a leading cause of premature morbidity and mortality worldwide[1] and it is relatively more common in men[2]. The incidence of STEMI in Europe ranges from 43 to 144 per 100,000 per year[3], and the incidence has been falling[4]. The mortality from contemporary STEMI management has significantly improved following the implementation of rapid reperfusion, with primary percutaneous coronary intervention (PCI), potent antithrombotic therapy, lifestyle modifications and secondary prevention[5-7]. Specifically, in contemporary practice the 30-day mortality rate following primary PCI is approximately 4%[6] and is even less in patients presenting without cardiogenic shock or cardiac arrest (approximately 2%)[8]. The 1-year mortality rate, following primary PCI, is approximately 4 to 7%[9-11]. The rate of heart failure hospitalisation at 1-year following primary PCI is approximately 5%[12 13].

As a result of the improvement in outcomes following contemporary STEMI management, current and future clinical trials now may need many thousands of patients to achieve adequate power to ascertain reductions in clinical outcome endpoints, including mortality, reinfarction and heart failure[14]. Therefore, clinical trials often use surrogate endpoints that are associated with clinical outcomes, to show differences between treatment groups. Cardiovascular magnetic resonance (CMR) imaging is useful in this regard, because continuous parameters, such as infarct size, can be measured that are associated with clinical outcomes[15]. Increasingly, assessment of the coronary microcirculation acutely in the catheterisation laboratory is being used in clinical trials, in a stratified therapy approach, to target adjunctive treatments to STEMI patients most likely to benefit[16]. Thereby, potentially reducing the number of patients needed to achieve adequate power to ascertain improvements in trial endpoints.

1.2 The coronary microcirculation and the “no-reflow” phenomenon

The coronary circulation consists of epicardial arteries, followed by small arteries called pre-arterioles (200 to 300μm in diameter), which taper into arterioles (10 to 200μm in diameter), and then capillaries (<10μm in diameter). Epicardial arteries make a negligible

contribution to flow resistance in the absence of significant stenosis[17]. On the other hand, pre-arterioles and arterioles contribute to flow resistance by changing their diameter, in order to maintain constant coronary perfusion over a range of perfusion pressures (Figure 1.1). Arterioles make the largest contribution to total coronary resistance[18]. The diameter of larger arterioles (100 to 200µm) is regulated by blood flow related stimuli (endothelial cell mediated vasodilatation)[19]. The diameter of intermediate arterioles (40 to100µm) is largely regulated by intravascular pressure changes, detected by vascular smooth muscle stretch receptors[20], however endothelial dependent flow induced mechanisms also have a role[21]. The diameter of the smaller arterioles (10 to 40µm) is regulated by tissue metabolites[22]. The diameter of capillaries is fixed, as their purpose is exchange of nutrients and oxygen[23]. Capillaries do not have a layer of vascular smooth muscle cells, which is found in arterioles. Therefore, capillaries consist only of an endothelial layer over a basal lamina, and are potentially susceptible to compression by intramyocardial or intraventricular pressures[18]. The microcirculation, i.e. vascular elements <300µm in diameter[24] that contribute to myocardial perfusion[18], is too small to be visualised with the spatial resolution available at angiography.

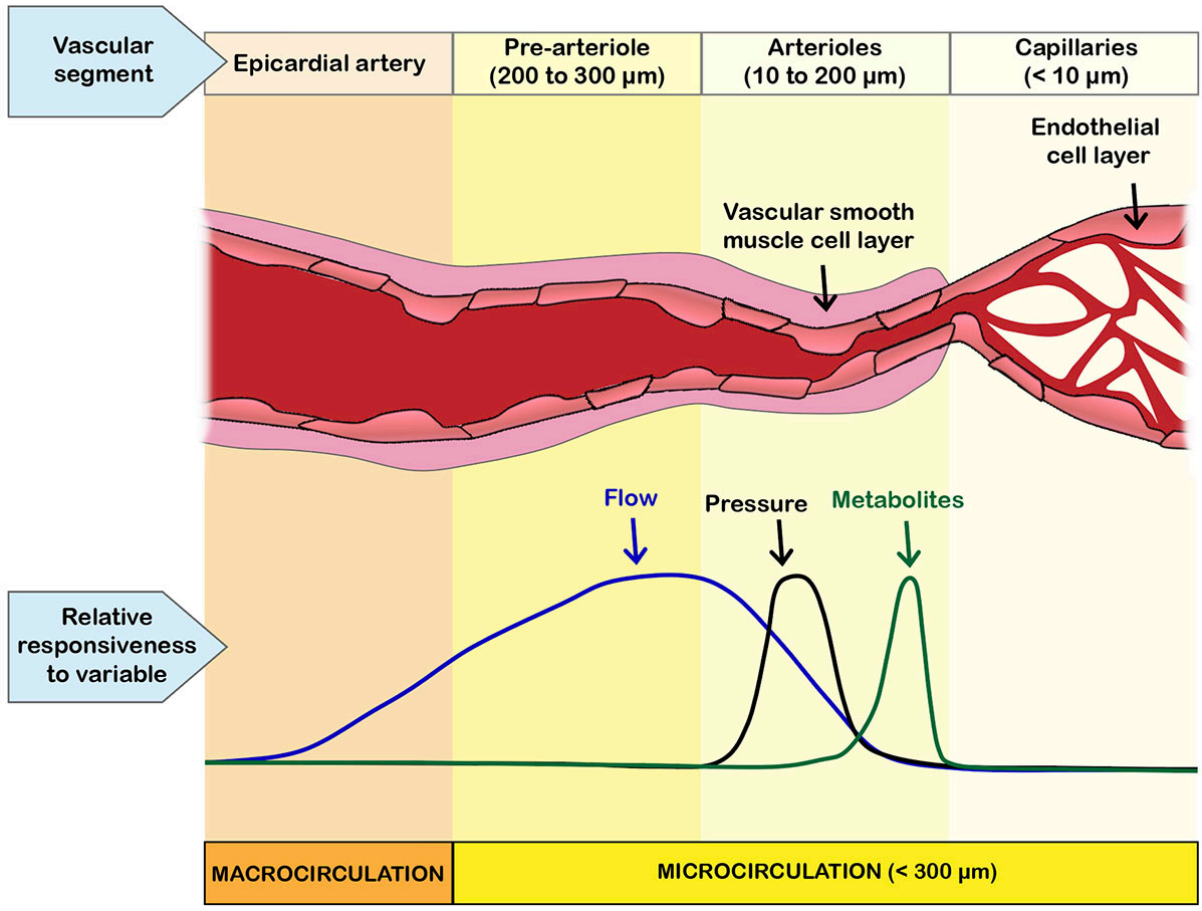

Figure 1.1 *Schematic diagram illustrating the anatomy of the coronary microcirculation and the relative responsiveness of vascular elements to blood flow, pressure and metabolites.*

Even though primary PCI is a life-saving treatment[25], and normal flow in the epicardial coronary artery is typically achieved, approximately half of STEMI patients sustain persistent impairment of microcirculatory blood flow[26 27]. When coronary flow is impaired, despite relief of the epicardial obstruction, this is described as "no reflow". Kloner *et al*[28] first reported coronary "no reflow" in the 1970s, in a canine model. Kloner *et al*[28] observed poor or absent perfusion in areas of the myocardium, despite complete epicardial coronary artery patency, and linked it to microvascular injury. At the time of epicardial coronary artery reopening following acute occlusion, myocardial reperfusion is achieved only in areas with anatomically preserved microvasculature, whereas reflow does not occur in myocardium with extensive microvascular damage. This is observed clinically as microvascular obstruction (MVO) on CMR imaging, and confers an adverse prognosis[27 29]. However, MVO usually passes undetected in daily practice, presenting an unmet therapeutic need.

1.3 Pathophysiology of microvascular injury

1.3.1 Distal embolization

Embolization of atherothrombotic debris from the infarct-related artery during primary PCI is one of the key contributors to MVO[30-33]. Distal embolization can cause mechanical obstruction, as well as activate local inflammatory pathways, in situ thrombosis[30] and release of vasoconstrictors, such as endothelin-1[34], from hypoxic cells. These processes in turn result in microvascular vasospasm, interstitial oedema and further cellular injury. The effects of distal embolization on myocardial damage are greater in patients who have large thrombus burden in the infarct-related artery[35].

1.3.2 Ischaemic injury

In a canine model, involving 90 minutes of coronary artery occlusion followed by reperfusion, Kloner *et al*[28] demonstrated histologically severe capillary damage, with endothelial protrusions that obstructed the capillary lumen, endothelial gaps (from destabilisation of cellular junctions[36]) and extravascular erythrocytes[28]. The erythrocyte extravasation leads to interstitial oedema, which compresses the microvascular capillaries[37].

On a molecular level, during ischaemia, glycogen is initially broken down by mitochondrial anaerobic glycolysis to produce adenosine triphosphate along with lactic acid[38]. This results in a decrease in pH, which then acts by negative feedback to inhibit further adenosine triphosphate production. A lack of adenosine triphosphate causes ionic pumps to fail, leading to increased cytosolic sodium concentrations[39], causing water to move into cells in an attempt to maintain the osmotic equilibrium, resulting swelling of cells[40]. Furthermore, calcium is released from the mitochondria into the cytoplasm and extracellular spaces, thereby activating calcium-dependent proteases[41].

1.3.3 Reperfusion injury

Reperfusion can paradoxically worsen myocardial injury. This was demonstrated in a rabbit model of 30 minutes of coronary occlusion, which showed that the extent of poorly or non-perfused myocardial regions increased following reperfusion[42]. Furthermore, in a rat model, 30 minutes of ischaemia without in vivo reperfusion showed only mild morphological changes to the coronary microvascular endothelium, whereas 30 minutes of ischaemia combined with 60 minutes of in vivo reperfusion resulted in visible microvascular damage[43].

A characteristic feature of reperfusion injury is intense oedema of myocytes, endothelial cells and the interstitium, which occludes the microvasculature due to external compression[34]. There is a reactive hyperaemia, due to the abrupt increase in capillary hydrostatic pressure, and subsequently a second wave of oedema is triggered by an influx of inflammatory cells, which increase microvascular permeability[44 45]. Carrick *et al*[46] reported that oedema follows a bimodal time course only in patients with accompanying myocardial haemorrhage, whereas in patients without myocardial haemorrhage, oedema seemed to evolve on a unimodal time course.

On a molecular level, once ischaemic tissue is reperfused, an influx of molecular oxygen catalyses enzyme reactions that produce reactive oxygen species (ROS). For example, xanthine oxidase degrades hypoxanthine to uric acid, thereby releasing superoxide[47 48]. Superoxide is converted to hydrogen peroxide and the hydroxyl radical. Hydroxyl radical production consequently leads to peroxidation of the lipid in cell membranes, which disrupts cell permeability, resulting in cell death. Lipid peroxidation of cellular membranes releases arachidonic acid, which is the substrate for production of prostaglandins,

thromboxanes and leukotrienes. Prostaglandins are vasodilators, but they are short-lived and rapidly depleted, resulting in uninhibited vasoconstriction and exacerbation of ischaemia. In particular, thromboxane A_2 promotes vasoconstriction and platelet aggregation. Moreover, leukotrienes activate inflammatory pathways, activate neutrophils, and result in accumulation of inflammatory cells[49]. Neutrophils secrete matrix metalloproteinases[50 51], which degrade basement membranes contributing to tissue destruction.

Platelets have emerged as a major contributing factor to ischaemia reperfusion injury[52 53]. First, activated platelets infiltrate the reperfused myocardium and contribute to injury by forming microthrombi, which cause microvascular plugging. This is mediated by platelets adhering to capillary endothelium, or to attached leukocytes. Platelet glycoproteins, such as IIbIIIa have a key role in platelet adhesion and aggregation. Possible mediators of platelet activation include the exposed extracellular matrices[52], and release of platelet-activating cytokines, such as stromal cell-derived factor 1 alpha[54]. Second, the formation of platelet-neutrophil aggregates[55 56] promotes additional pro-inflammatory leukocyte infiltration and vasoconstriction[57 58]. In particular, thromboxane A2 is a potent vasoconstrictor released by activated platelets. Third, activated platelets shed microparticles, which have the potential to increase inflammation[52]. Last, platelets activate cardiac-sympathetic afferent nerves during ischaemia[59], with associated tachyarrhythmia[59], leading to further myocardial damage by increasing the cardiac oxygen demand.

Overall, the mechanisms underlying myocardial reperfusion injury are complex, and the key pathways include the acute inflammatory response[60], endothelial dysfunction with impaired endothelium-dependent vasodilatation[61], intracellular calcium overload[34 62], opening of the mitochondrial permeability transition pore[34 63], increased generation of ROS[47 64], platelet aggregation and microthrombi formation[65].

1.3.4 Individual susceptibility to acute microvascular injury

In STEMI patients, hyperglycaemia rather than diabetes per se is associated with MVO on CMR imaging[66-69]. It has been postulated that endothelial dysfunction may increase the risk of reperfusion injury to the microcirculation. Indeed, in a hyperglycaemia canine model, endothelial-dependent coronary microvascular dilatation was impaired[70]. On the other hand, pre-existing hypertension in acute STEMI patients has not been associated with

CMR-defined MVO[71 72]. Current smoking has not been associated with MVO on CMR imaging in acute STEMI patients, but has been associated with myocardial haemorrhage when corrected for infarct size in some studies[73 74].

In STEMI patients, longer symptom onset to reperfusion time, i.e. ischaemic time >6 hours, is an independent predictor of angiographic no reflow[75-77] and CMR-defined MVO[78]. Advanced age is also an independent predictor of angiographic no-reflow[77 79], and this association may be related to increased vascular oxidative stress with advanced age. Other independent predictors of angiographic no-reflow are initial TIMI (thrombolysis in myocardial infarction) flow grade ≤1 and a high thrombus burden[77]. Furthermore, anterior location of MI is associated with larger infarct size and more MVO on CMR imaging, than non-anterior MI[80].

1.4 Diagnosis of coronary microvascular dysfunction and prognostic significance

1.4.1 Non-invasive assessment by CMR

A reference non-invasive technique for detection of microvascular pathology is CMR. However, CMR has limitations when considered as an imaging test of the efficacy of reperfusion therapy in acute STEMI patients. First, CMR is not feasible acutely, it is typically performed 2 to 7 days after primary PCI, so it is not suitable for early risk stratification and is not widely available[81]. Second, CMR is only possible in medically stable patients, so the sickest patients with the most severe forms of MI may be excluded. Furthermore, gadolinium contrast agents used in CMR imaging are contraindicated in patients with severe renal dysfunction, and some patients may not be able to undergo CMR imaging due to claustrophobia, cardiac devices, or indwelling metallic objects.

Microvascular obstruction

Late gadolinium enhancement (LGE) CMR imaging is used to evaluate MVO. After intravenous administration of gadolinium, the extracellular contrast agent perfuses through myocardial capillaries and diffuses within the extracellular compartment. The rupture of cardiac cells in the early post-infarcted tissue, increases the interstitial volume, which reduces the washout rate of the gadolinium contrast from the extracellular space. This results in an increased concentration of contrast, compared to healthy myocardium. The persistence of gadolinium in the infarct zone for a longer time than in healthy tissue, leads

to a regional hyperintense (bright) area on T1-weighted imaging. A hypointense core (black area, due to absence of contrast in the obstructed capillaries) represents MVO within the hyperintense infarct zone in the area of LGE.

The temporal change of MVO is very dynamic. By 3.5 hours following reperfusion, the zone of impaired perfusion extends from the sub-endocardium to the mid-myocardium[82]. During the first 24 hours following reperfusion the zone of impaired perfusion increases[42]. Imaging by CMR earlier than 2 days post-STEMI can over-estimate MVO. MVO is reasonably stable between days 2 to 7 post-STEMI. However, from 10 to 30 days post-STEMI, MVO diminishes markedly, and in a variable proportion of patients MVO persists (linked to myocardial haemorrhage)[26]. By 7 to 8 months post-reperfusion, MVO on CMR imaging has usually resolved[83].

Previous studies have shown that MVO predicts heart failure[27], adverse left ventricular (LV) remodelling, ventricular arrhythmia[84], major adverse cardiac events (MACE) and death[27 85 86]. There is a weak correlation between MVO and infarct size ($r=0.21$, $p<0.001$)[85] and MVO predicts adverse clinical outcomes independent of infarct size[86]. The pathophysiological mechanisms through which MVO adversely impacts prognosis may be due to MVO limiting delivery and transit of promoters involved in post-infarction remodelling, as well as macrophages needed for phagocytosis of cellular debris, to contribute to optimal infarct healing[87 88].

Myocardial haemorrhage

The most sensitive and established method for imaging myocardial haemorrhage is T2* based imaging[89 90]. However, a limitation of T2* imaging is that it requires relatively long breath holds to minimise respiratory movements, which is not possible in some patients. Therefore, there is difficulty in obtaining reliable diagnostic quality T2* images, in a proportion of patients[91]. On T2* maps, accumulation of paramagnetic haemoglobin degradation products, leads to a shortening of T2* relaxation times, resulting in reduced signal intensity (hypointense zone) on imaging, which represents tissue haemorrhage[92 93]. Previous validation in swine established that the hypointense zone on T2* maps anatomically correlates with severe capillary loss and destruction, resulting in tissue haemorrhage[92].

Myocardial haemorrhage is a pathological subset of MVO, and is an irreversible consequence of severe microvascular injury[94]. Myocardial haemorrhage occurs when endothelial cell injury compromises capillary integrity, leading to blood extravasation into the extracellular space. The external compression of capillaries by extravasated blood in the interstitial space potentiates progression of microvascular damage. In the longer term, haemoglobin degradation leads to deposition of cytotoxic levels of iron in the myocardium, which triggers inflammation and fibrosis[95 96]. Myocardial haemorrhage is associated with larger infarct size[93], heart failure, adverse LV remodelling[97 98], late arrhythmia risk[99], and is an even stronger predictor of MACE and death than CMR-defined MVO[26 100 101].

1.4.2 Non-invasive assessment by electrocardiography

Less ST-segment resolution 90 minutes post-reperfusion compared to baseline (i.e. 90 minutes after the initial restoration of flow in the infarct-related artery, compared to the electrocardiogram [ECG] on admission) is associated with MVO on CMR imaging (area under the curve [AUC]: 0.68, $p<0.05$)[102]. Incomplete ST-segment resolution (<70%) 60 minutes post-reperfusion relative to baseline, appears to be a better predictor of MVO on CMR imaging than angiographically-derived TIMI flow grade, or TIMI myocardial perfusion grade (MPG)[103]. However, studies reporting the relationship between ST-segment resolution and clinical outcomes have shown conflicting results[104 105]. A limitation of incomplete ST-segment resolution for predicting microvascular injury is that it requires calculation at least 60 minutes after the baseline ECG, and therefore is not immediately available during the primary PCI procedure. Furthermore, the optimal timing of ECG analysis remains uncertain[106].

1.4.3 Invasive assessment by angiography

The therapeutic window for treating microvascular injury after the onset of STEMI, occurs immediately after reperfusion has been established[43]. CMR-defined MVO is unsuitable for early risk stratification in STEMI patients, because it is only feasible in medically stabilised patients and is not available at the time of emergency PCI. By the time CMR is performed the therapeutic window for minimising microvascular injury has lapsed. On the other hand, angiographic and invasive coronary physiology surrogates of failed myocardial perfusion can be measured immediately after epicardial patency has been restored, to inform on microvascular dysfunction, or can be used as an endpoint to evaluate immediate therapeutic efficacy.

TIMI flow grade and TIMI frame count

The TIMI flow grade is a categorical grading system for epicardial antegrade flow, ranging from 0 (no flow) to 3 (complete antegrade flow). When assessed at the end of primary PCI, TIMI flow <3 is associated with larger infarct size, worse LV ejection fraction, and increased incidence of death, heart failure and MACE[107-111]. Furthermore, TIMI flow <3 early during the primary PCI procedure (i.e. pre-stenting) is associated with higher rates of death, MACE and lower LV ejection fraction, than TIMI flow <3 at the end of primary PCI, i.e. post-stenting[112]. Nonetheless, TIMI flow grade is a relatively insensitive surrogate of microvascular perfusion, so TIMI 3 flow does not guarantee adequate microvascular perfusion[26 103 113]. Furthermore, TIMI flow <3 at the end of primary PCI does not necessarily guarantee impaired microvascular perfusion[114].

Because of a relatively high inter-rater variability in grading TIMI flow, the corrected TIMI frame count (TFC) was introduced, which provides a quantitative assessment of coronary flow. TFC has relatively good inter-rater and intra-observer reproducibility[115-117]. However, nitrate use, dye injection at the beginning of diastole and slower heart rate have been shown to affect TFC[118 119]. A TFC ≥28 in the infarct-related artery is considered abnormal[120-123]. The prognostic significance of TFC is uncertain. Some studies showed that increased TFC is independently associated with increased mortality[121 124], whereas other studies showed no association between TFC and mortality[125 126].

Conversely, a very fast TFC at the end of PCI (TFC <14), which represents hyperaemic flow, has been associated with higher incidence of a composite of death/ MI ($p<0.001$) compared to normal flow (TFC 14 to 28), in patients with MPG ≤1 at the end of PCI[127]. This may be explained by hyperaemic arterial flow after reperfusion therapy for STEMI being caused by microembolization of platelet aggregates and atherothrombotic debris, with subsequent release of endogenous adenosine, a vasodilator, from surrounding territories[127-129].

Importantly, though TFC and TIMI flow grade at the end of primary PCI have been associated with MVO on CMR imaging, the associations are relatively weak (TFC: AUC=0.53, $p<0.05$; TIMI flow grade: AUC=0.54, $p<0.05$)[102].

TIMI Myocardial perfusion grade

Filling and clearing of contrast from a coronary angiogram is characterised by TIMI MPG, which is graded from 0 (no myocardial perfusion) to 3 (normal myocardial perfusion). MPG ≤1 at the end of primary PCI is associated with less myocardial salvage[130] and a higher incidence of MVO on CMR imaging[131], than MPG ≥2. Moreover, MPG ≤1 predicts mortality after STEMI, independent of TIMI flow grade[132].

However, MPG ≥2 is not synonymous with normal myocardial perfusion, and some of these patients have MVO on CMR imaging[103 131]. Another drawback of MPG is that visual assessment requires an experienced operator, and is limited by inter-rater variability[116]. Automated, off-line, computer-assisted quantification of myocardial blush is available, which has improved intra- and inter-observer variability[133] and has been shown to predict clinical outcomes[134].

1.4.4 Invasive assessment by coronary physiology

Angiographic parameters can lack sensitivity in clinical practice, with considerable observer disagreement[135], and are best assessed in a core laboratory. Invasive physiology measures of microvascular dysfunction in the infarct-related artery have potential for early risk stratification of patients, and to guide the selection of patients for adjunctive therapies during primary PCI[16]. Figure 1.2 depicts invasive physiology parameters for measuring microvascular function in acute STEMI, organised into flow- or resistance-based, and thermodilution- or Doppler-derived parameters. In acute STEMI, the use of fractional flow reserve (distal coronary pressure [Pd] divided by aortic pressure [Pa] during hyperaemia) in the infarct-related artery is not recommended[136], because the dynamic changes in the infarcting myocardial bed lead to variable trans-stenotic pressure gradients[137].

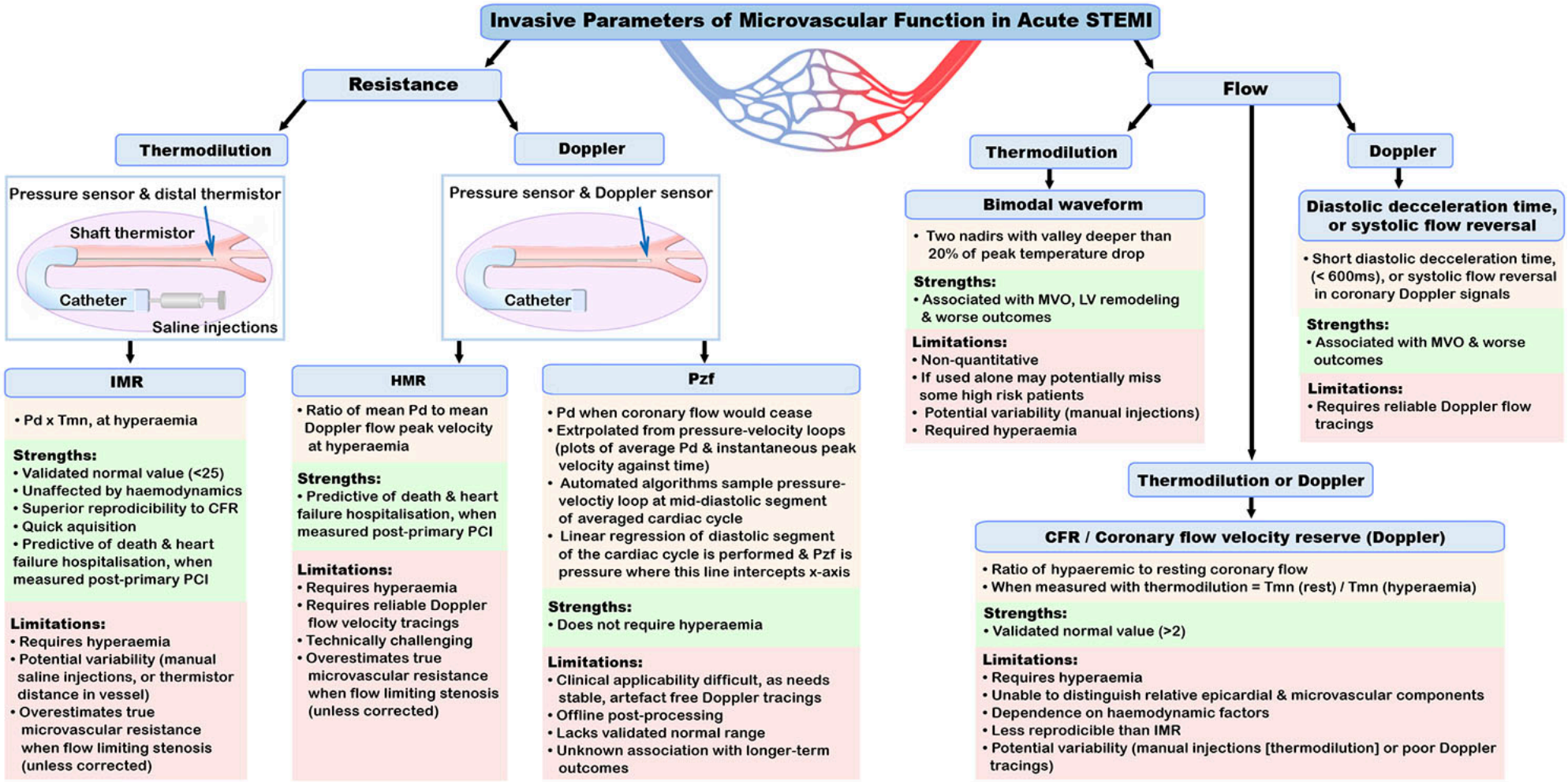

Figure 1.2 *Summary of invasive coronary physiology parameters to measure microvascular function in acute STEMI. Reproduced with permission from: Maznyczka A et al. The potential use of index of microcirculatory resistance (IMR) to guide stratification of patients for adjunctive therapy in acute myocardial infarction. JACC Cardiovasc Interv. 2019. 12(10): 951-966. Doi: 10.1016/j.jcin.2019.01.246*[16].

Index of microcirculatory resistance

Microvascular resistance is the force that the coronary microcirculation exerts against coronary flow. When maximal hyperaemia is induced, for example pharmacologically with adenosine, auto-regulation by the microcirculation is exhausted, resistance vessels are maximally dilated, and blood flow varies linearly with perfusion pressure[138], thereby the minimal microvascular resistance can be calculated. Ohm's law is applied for calculating minimal microvascular resistance.

Ohm's law states that current equals the voltage difference divided by resistance. When Ohm's law is applied to flow in blood vessels, the voltage difference is the pressure difference, the current is the blood flow, and the resistance is resistance to flow. Thereby, the resistance to blood flow is inversely related to absolute flow:

$$\textit{Resistance} = \frac{\textit{Pressure gradient}}{\textit{Absolute flow}}$$

Several assumptions are made when Ohm's law is applied to describe the relationship between blood flow and vascular resistance, therefore it is an imperfect approximation of resistance. The equation assumes that blood flow is constant and linear. However, in reality blood flow is laminar, the size and shape of vessels varies, and in large arteries the propagation of the pressure pulse depends on the elastic property of the arterial wall. Additionally, the non-homogenous nature of blood makes it difficult to ascertain a single value for viscosity, and in the microcirculation the fact that blood is a suspension of cells influences its flow properties[139]. Furthermore, when considering the coronary anatomy it is important to acknowledge that collaterals provide anastamotic connections between arteries without an intervening capillary bed. Collateral flow can affect pressure measurements, but is not accounted for when Ohm's law is applied.

For calculating the pressure gradient across the microcirculation, the venous pressure is assumed to be negligible, during maximal hyperaemia. Therefore, minimal microvascular resistance can be simplified as the ratio of Pd to flow, at maximal hyperaemia.

Thermodilution methodology can be used to obtain the mean transit time (Tmn) of manually injected normal saline, which is used as a surrogate of coronary flow. For this purpose, a coronary guidewire is used, which combines a distal pressure sensor and temperature sensor (Abbott Vascular, California). Using thermodilution to quantify coronary blood flow and volume is based on the following relationship:

$$\textit{Flow} = \frac{\textit{volume}}{\textit{Tmn (at maximal hyperaemia)}}$$

Because the vascular volume can be assumed to remain constant at maximal hyperaemia, the two above equations can be combined, so that microvascular resistance is proportional to the product of Pd and Tmn:

$$\textit{Microvascular resistance} = \textit{Pd (at maximal hyperaemia)} \div \frac{1}{\textit{Tmn (at maximal hyperaemia)}}$$

$$\textit{Microvascular resistance} = \textit{Pd x Tmn (at maximal hyperaemia)}$$

The index of microcirculatory resistance (IMR) is defined by the above equation, i.e. Pd x Tmn at maximal hyperaemia[140]. IMR was initially validated in animals[140 141]. In a swine model, IMR was compared with true microvascular resistance, defined as Pd divided by absolute coronary flow measured using ultrasonic flow probes[140]. Microvascular dysfunction was created artificially using microspheres injected into the coronary arteries. There was a modest correlation between IMR and total microvascular resistance ($r=0.54$, $p<0.001$)[140]. The findings were validated in an in vitro model, which demonstrated excellent correlation between IMR and true microvascular resistance ($r^2=0.94$)[142].

When severe stenosis is present, the contribution of collateral flow may lead to overestimation of IMR[143 144]. In these circumstances IMR requires correction using either coronary wedge pressure (Pw), i.e. corrected IMR = Pa x Tmn x ([Pd – Pw] / [Pa – Pw])[143], or Yong's formula[145] (corrected IMR = Pa x Tmn x [(1.35 x Pd / Pa) - 0.32]), with Pa, Tmn and Pd measured during hyperaemia. In the setting of stable ischaemic heart disease, i.e. not in the setting of acute STEMI, IMR has been shown to be influenced to a small extent by the amount of myocardium subtended by the epicardial artery under study[146].

In humans, IMR has been validated against positron emission tomography[147], CMR[148-156], single photon emission computed tomography (SPECT)[157] and echocardiographic[158 159] imaging. Stable patients without microvascular disease generally have IMR values <25[160-162]. In STEMI, IMR correlates with the adequacy of microvascular perfusion, evidenced by the association of elevated IMR with MVO[148-150 152 153 155 156] and myocardial haemorrhage on CMR imaging[150 156]. Moreover, Cuculi *et al*[163] showed that IMR is modifiable when assessed repeatedly within 24 hours of reperfusion.

Carrick *et al*[156] performed the largest study in STEMI patients (n=288) comparing IMR measured at the end of emergency PCI with MVO on CMR imaging (MR-MI study [detection and significance of heart injury in STEMI]). Carrick *et al*[156] found that IMR >27 was most closely associated with MVO and myocardial haemorrhage on CMR imaging. However, this IMR threshold differs from a meta-analysis of 6 studies by Bulluck *et al*[152], which suggested an IMR cut-off >41 at the end of primary PCI for predicting MVO on CMR imaging.

In acute STEMI, an elevated IMR at the end of PCI also correlates with worse recovery of infarct size[147 149 150 153 155 158], adverse LV remodeling and worse LV function[149 153 154 158 159]. Fearon *et al*[158] reported that an IMR ≤32 (median) at the end of primary PCI was associated with recovery of LV function (assessed echocardiographically). An IMR ≤32 correlated with improvement in wall motion score index at 3 months relative to baseline, whereas patients with IMR >32 had no change in wall motion score index[158]. The findings were validated by Faustino *et al*[159] and Lim *et al*[147] who reported improvements in

echocardiographic wall motion score index with IMR <26 and ≤33 respectively at the end of primary PCI.

Interestingly, De Maria *et al*[155] found that patients with CMR-defined MVO and IMR ≤40 had regression of infarct size, whereas those with IMR >40 did not. In patients without MVO on CMR imaging, those with IMR ≤40 had regression of infarct size, whereas those with IMR >40 did not[155]. One interpretation of these findings[155] is that patients with the highest IMRs might have sustained irreversible microvascular damage, that may potentially be less modifiable by adjunctive therapies.

Perhaps most clinically relevant, an IMR >40 after primary PCI predicts all-cause death, heart failure readmissions, and MACE[149 164 165]. In STEMI patients (n=253), Fearon *et al*[164] reported that IMR >40 (mean) at the end of primary PCI was independently associated with all-cause death (hazard ratio [HR]: 4.3 [95% confidence interval (CI): 1.3, 15.0] p=0.02), or all-cause death/ rehospitalisation for heart failure at 1 year (HR: 2.2 [95% CI: 1.1, 4.5] p=0.03). An IMR >31 (median) was independently associated with all-cause death/ rehospitalisation for heart failure at 1 year (HR: 3.1 [95% CI: 1.4, 6.6] p=0.004), but not with all-cause death alone[164]. Carrick *et al*[149] reported that IMR >40 at the end of emergency PCI was independently associated with all-cause death/ heart failure hospitalisations at 2 years, in 283 acute STEMI patients (odds ratio [OR]: 4.36 [95% CI: 2.10, 9.06] p<0.001). Fahrni *et al*[165] reported that IMR >40 at the end of primary PCI predicted major cardiac complications 30-days post-STEMI (16.7% vs. 0%, p<0.001) (n=261).

Advantages of IMR are as follows: (i) it can be performed at the time of primary PCI; (ii) from a technical perspective it is relatively straightforward to obtain the measurements; (iii) it is reproducible[166]; (iv) it has been robustly validated in animals and humans[140 141 147-162], and; (v) studies have shown that it can reliably predict clinical outcomes[149 164 165]. However, IMR has some potential limitations. First, large side branches might potentially result in overestimation of flow, and thereby underestimation of IMR, due to loss of saline down the side branches. Second, vessel compression during systole might potentially affect the result if saline is injected mainly during systole especially in bradycardic patients[167],

therefore Tmn is measured in triplicate and the mean value is recorded. Third, manual saline injections can potentially be a source of variability[168 169].

Hyperaemic microvascular resistance

Hyperaemic microvascular resistance (HMR) is measured with a combined Doppler-pressure wire (Phillips Volcano Corporation, California), in the infarct-related artery. It is the ratio of mean Pd to mean Doppler flow peak velocity. The mean Doppler peak flow velocity is used as a surrogate of coronary flow. In the setting of a flow limiting stenosis, HMR requires correction for the contribution of collateral flow to myocardial flow, however, when measured post-primary PCI in patients with no residual epicardial stenosis, corrections for the impact of collaterals are not required[170]. HMR correlates with MVO on CMR imaging (optimal cut-off >2.5mmHg/cm per second)[171 172], regional wall motion abnormalities[173], infarct size[174 175], and adverse LV remodeling[176]. In a comparative study (n=33 STEMI patients), Williams *et al*[172] found no significant difference between HMR and IMR for predicting the presence of extensive MVO (defined as >3ml MVO present) on CMR imaging (AUC: 0.83 vs. 0.72, p=0.22). In STEMI patients (n=145), an elevated HMR (>2.82 mmHg/cm per second) independently predicted long-term MACE (mean follow up 7 years) (HR: 1.74 [95% CI: 1.35, 2.26] p<0.001)[177]. In another study (n=130 STEMI patients), an elevated HMR (≥3.0 mmHg/cm per second) predicted all-cause death and hospitalisation for heart failure in the longer term (median follow up 3.2 years), HR: 7.0 (95% CI: 1.5, 33.7)[178].

The limitations of HMR relate to the technical challenges associated with obtaining adequate quality Doppler flow velocity tracings, resulting in higher failure rates than when measuring IMR using thermodilution methodology[169 178]. Another limitation is the lack of a consistent optimal threshold for predicting clinical outcomes.

Thermodilution-based absolute flow and resistance

The measurement of thermodilution-based absolute flow and resistance requires a pressure wire with a temperature sensor and a dedicated coronary catheter, with multiple side holes[179], for continuous saline infusion. The advantage of using continuous thermodilution to assess absolute coronary flow, is that it permits an invasive evaluation of microvascular resistance based on actual coronary flow measurement, and not a surrogate[180]. The method relies on some important observations. First, constant infusion of room temperature saline at 20ml/min through the dedicated catheter with multiple side holes produces steady-state

maximal hyperaemia equivalent to that obtained with adenosine[181]. Second, coronary flow can be calculated from the rate and temperature of infused saline and the temperature of mixed blood with the infused saline. The technique has been validated in vitro[179 182], in a canine model[183] and in humans[183-185], and there is significant agreement between thermodilution-based absolute flow and positron emission tomography derived myocardial blood flow (the gold standard for quantification of myocardial perfusion[186]) ($r=0.91$, $p<0.001$)[180].

The thermodilution-based absolute flow and resistance technique has potential to reduce variability from manual saline injections, and thereby has potential to provide a more accurate and reproducible assessment of coronary microvascular status[187]. Studies have shown that this approach is feasible in acute STEMI[184 185]. However, current limitations are: (i) there is no established "normal range" of values; (ii) it can be technically challenging, taking longer to perform than IMR measurements; (iii) data on association with clinical outcomes is lacking, and: (iv) there are no direct comparison studies with IMR. Moreover, because the technique requires complete mixing of blood and saline[183 185], the pressure wire tip should be ≥3cm distal to the infusion catheter tip, which precludes flow measurements in short vessels and may affect measurements in coronary segments proximal to large side branches[180]. Lastly, because continuous saline infusion elicits hyperaemia, the technique does not permit resting flow to be determined, therefore the technique does not allow assessment of coronary flow reserve (CFR).

Coronary flow reserve

CFR was first described by Gould *et al* in 1974[188], and it represents the property of coronary artery blood flow to increase in response to ischaemia[189], i.e. vasodilatory reserve. CFR is defined as the ratio of hyperaemic flow to resting coronary flow:

$$CFR = \frac{Hyperaemic\ flow}{Resting\ flow}$$

Because flow is the ratio of volume to Tmn, the CFR equation can be expressed as follows:

$$CFR = \left(\frac{Volume}{Tmn}\right) at\ hyperaemia \div \left(\frac{Volume}{Tmn}\right) at\ rest$$

Assuming the epicardial volume remains unchanged, thermodilution-derived CFR can be calculated as follows:

$$CFR = \frac{1}{Tmn\ at\ hyperaemia} \div \frac{1}{Tmn\ at\ rest}$$

$$CFR = \frac{Tmn\ at\ rest}{Tmn\ at\ hyperaemia}$$

Doppler-derived CFR, termed coronary flow velocity reserve, is defined as the ratio of average peak velocity during hyperaemia and at rest. A head-to-head comparison, in 40 patients with stable coronary artery disease, found that thermodilution-derived CFR had inferior agreement with positron emission tomography when compared to Doppler-derived CFR (r: 0.55 vs. 0.82, $p<0.001$)[169]. Moreover, higher intra-observer variability of thermodilution-, compared to Doppler-derived CFR measurements was demonstrated[169].

However, the quality of data was poorer with Doppler-derived CFR, than with thermodilution methodology[169]. The thermodilution technique for assessing CFR assumes a constant coronary vessel diameter[167], however in reality vessel diameter changes with endothelial-mediated vasodilatation[190].

In acute STEMI, CFR predicts MVO on CMR imaging, and larger infarct size, and the optimal threshold for abnormal CFR is ≤2.0[149 191]. However, previous studies have shown that CFR is inferior to IMR or HMR for predicting MVO, and does not add to the predictive utility to IMR or HMR[149 178].

An important limitation of CFR is that it is sensitive to both epicardial and microvascular disease[192 193]. The presence of residual epicardial coronary narrowing, as well as the presence of microvascular dysfunction, both lower CFR. Thus, CFR reflects epicardial and microcirculatory vasodilator capacity, as well as residual epicardial stenosis. Another limitation of CFR is that it is affected by determinants of resting coronary blood flow, i.e. heart rate and blood pressure, which diminishes the reproducibility of CFR[166]. Specifically, CFR decreases during pacing induced tachycardia or dobutamine infusion[166]. In contrast, IMR and HMR are independent of haemodynamic variation[166]. A further limitation of CFR is it's inferior reproducibility compared to IMR, with a mean coefficient of variation of 6.9 ± 6.5% for IMR compared to 18.6 ± 9.6% for CFR[166].

Resistive reserve ratio

The resistive reserve ratio (RRR) is a newer, less well-studied parameter, which is derived as the ratio between basal resting tone in the microcirculation (i.e. basal resistance index) and microcirculatory resistance at maximal hyperaemia (i.e. IMR)[194]:

$$RRR = \frac{\text{Basal resting tone in coronary microvasculature}}{\text{Microvascular resistance at maximal hyperaemia}}$$

$$RRR = \frac{Pd \text{ at baseline} \times Tmn \text{ at baseline}}{Pd \text{ at maximal hyperaemia} \times Tmn \text{ at maximal hyperaemia}}$$

RRR represents the ability of the coronary microcirculation to vary its resistance in response to a hyperaemic stimulus, such as adenosine[194]. Higher RRR values indicate greater vasodilatation of the microcirculation in response to hyperaemia. On the other hand, lower RRR values indicate poor vasodilator capacity of the coronary microcirculation. RRR is theoretically distinct from CFR, since RRR is an integrated index of both pressure and flow, whereas CFR is a surrogate marker of flow velocity[195].

Layland *et al*[194] found that RRR was lower in patients with STEMI (n=40) compared to patients with non-STEMI (n=50) or stable angina (n=50). In another study of 73 STEMI patients[196], RRR measured in non-culprit vessels was lower acutely vs. 1 month later, which indicates a blunted hyperaemic vasodilatory response acutely. Scarsini *et al*[197] investigated 24 STEMI patients, and found that RRR ≤1.98 (median for the cohort) at the end of primary PCI was associated with the extent of MVO on CMR imaging (2 days post-PCI) and infarct size assessed at 6 months. Scarsini *et al*[197] reported that RRR performed better compared to IMR and CFR in predicting 6-month infarct size (AUCs: 0.85, 0.70 and 0.67 respectively). However, these findings need to be verified in a larger cohort.

Lee *et al*[195] reported that lower RRR measured in non-culprit intermediate stenoses, in patients with stable ischaemic heart disease from 3 pooled registries (n=1245 patients), has utility incremental to CFR and FFR for predicting adverse clinical outcomes. Specifically, among patients with CFR >2 and deferred revascularisation, RRR <3.5 (median value for the cohort) was associated with increased risk of adverse clinical outcomes (a combination of all-cause mortality, MI, or any revascularisation events during 5-year follow up)[195]. Similarly, among patients with FFR >0.8 and deferred revascularisation, RRR<3.5 was associated with increased risk of adverse clinical outcomes[195]. The association between RRR measured in the infarct-related artery at the end of primary PCI and clinical outcomes is yet to be determined.

Thermodilution waveforms

Because downstream microvascular resistance influences coronary blood flow, the shape of the thermodilution waveform in the infarct-related artery may reflect microvascular dysfunction. In a study of 88 acute STEMI patients, Fukunaga *et al*[198] showed that a bimodal thermodilution waveform is associated with MVO on CMR imaging (within 2 weeks), adverse LV remodeling[199] (assessed by echocardiography at 6 months) and cardiac death at 6 months[198]. In an analysis of 278 acute STEMI patients from the MR-MI study, the association between bimodal thermodilution waveform and CMR-defined MVO or

myocardial haemorrhage became insignificant when the multivariable model included IMR >40[200]. However, bimodal waveform predicted all-cause death and heart failure hospitalisation at 4 years, independent of IMR >40[200].

The thermodilution bimodal waveform has limitations for translation into clinical practice. First, it is not quantitative, and so has limitations for detecting responses to adjunctive treatments in clinical trials. Second, the bimodal thermodilution waveform appears to underestimate microvascular dysfunction in STEMI, because the reported incidence of bimodal waveform ranges from 13%[200] to 17%[198], whereas MVO on CMR imaging occurs in about 50% of patients treated with primary PCI[26 103]. Third, off-line post-processing is required to analyse thermodilution waveforms.

Coronary zero flow pressure

Coronary zero flow pressure (Pzf) is another parameter that can be used to assess microvascular resistance and is measured with a combined Doppler-pressure wire (Phillips Volcano Corporation, California) in the infarct-related artery[175]. Pzf is measured from hyperaemic diastolic coronary pressure-flow velocity loops (i.e. plots of average Pd and instantaneous peak velocity against time). Automated algorithms sample the pressure-velocity loop at the mid-diastolic segment of the averaged cardiac cycle. A linear relationship between pressure and flow values exists during the mid-late phases of diastole. The slope of the regression line obtained over this period expresses microcirculatory conductance. Pzf is calculated by extrapolation from the regression line described above, and Pzf is the pressure where this line intercepts the x-axis. Specifically, Pzf is the Pd when theoretically, coronary flow would cease and mainly informs on the external pressure on the coronary microcirculation[201]. Theoretically, Pzf should reflect the intraluminal pressure required to maintain patent compressible elements against extravascular compression[202].

Pzf measured at the end of primary PCI predicts early infarct size[174], microvascular perfusion defects[171], adverse LV remodelling[203], myocardial salvage and LV function[175]. The advantage of Pzf over the other described invasive physiology parameters is that it permits the assessment of coronary flow over a pressure range without the interference of cardiac contraction, and Pzf does not require hyperaemia. However, Pzf appears less transferable to clinical practice, for the following reasons: (i) difficulties acquiring high

quality, artefact-free, Doppler tracings; (ii) a requirement for offline post-processing; and (iii) lack of a validated normal range, or longer-term outcome data[16].

Summary of invasive physiology testing for microvascular injury

In summary, invasive coronary physiology measurements are useful for early identification of microvascular dysfunction in acute STEMI. Compared to other invasive indices, IMR has more validation data suggesting a stronger potential for implementation into clinical practice. However, IMR is an imperfect predictor of MVO and prognosis, and it does not measure microvascular vasodilator capacity, whereas RRR does. If a novel invasive parameter were easy to obtain, reproducible and accurately predicted MVO and clinical outcomes, with incremental utility over IMR, this might represent an advance for clinical practice.

1.4.5 LV haemodynamics

The LV end diastolic pressure (LVEDP) is a simple haemodynamic measurement that is readily measured during cardiac catheterisation. Because of the proximity of the vascular and myocardial compartments, intramyocardial vessels may be susceptible to changes in LV cavity pressure[30]. An increased pressure in the LV cavity may result in transmitted external compressive forces on the microcirculation, thereby increasing endocardial capillary pressure, which may in turn lead to decreased perfusion pressure, with subsequent impairment of microvascular perfusion in the territory of the infarct-related artery[30 204]. This concept is supported by correlations between LVEDP with Pzf[201].

In a pooled analysis of TIMI trials, Kirtane *et al*[204] observed that LVEDP associates with angiographic measures of microvascular function in the territory of the infarct-related artery, i.e. LVEDP was higher in patients with MPG ≤1 vs. ≥2. Higher LVEDP was also associated with larger infarct size (assessed by nuclear scintigraphy)[205]. The relationships between LVEDP and IMR, CFR, RRR, or MVO, have not been previously described in STEMI. In contrast to IMR, which is a specific measure of microvascular resistance, multiple overlapping factors may affect LVEDP in acute STEMI, therefore LVEDP is not a specific measure of microvascular function. LVEDP measured during primary PCI reflects acute LV pump function, including the extent of ischaemic injury, filling, contractility and compliance (which can be reduced due to oedema in the myocardium). Other factors that may potentially affect LVEDP in acute STEMI, include intravascular

volume status[206 207], and vasodilatory drugs, e.g. nitrate, which may transiently reduce LVEDP.

LVEDP has prognostic value in patients treated by primary PCI[205 208-210]. Specifically, elevated LVEDP (>18mmHg [median] in one study[208], >22mmHg [median] in another study[209]) correlates with increased MACE, including heart failure and death, by 30 days[208], 90 days[209] and 2 years[208]. LVEDP at the end of primary PCI also predicts mortality in the long-term, i.e. at 8 years follow up (HR: 1.18 [95% CI: 1.02, 1.36] p=0.022, for 5mmHg incremental increase in LVEDP values)[205]. However, there is no validated, accepted, LVEDP threshold for predicting adverse clinical outcomes.

1.5 Therapeutic approaches for addressing microvascular injury

To date, there are no effective evidence-based therapeutic strategies for preventing microvascular injury in acute STEMI patients with "no reflow". Intracoronary isosorbide dinitrate is used on an ad hoc basis to treat patients with "no reflow" during PCI, and in small studies intracoronary isosorbide dinitrate was associated with improvements in TFC[211]. However, larger trials are needed to verify practice guideline recommendations on intracoronary nitrates for "no reflow".

Recent clinical trials of treatments designed to reduce microvascular injury, have failed to provide benefit, including: intravenous beta blockers[212]; intravenous morphine[213]; deferred stenting[214-218]; thrombus aspiration[219 220]; intracoronary adenosine[91]; sodium nitroprusside[91]; ticagrelor[221]; glycoprotein IIbIIIa inhibitors[222]; statins[223-225]; calcium channel blockers[226]; cyclosporine[227 228], and; ischaemic conditioning[229]. Evidence from key clinical trials is summarised in the following section.

1.5.1 Thrombus aspiration

Because the effects of distal embolization on myocardial damage are greatest in patients who have large thrombus burden in the infarct-related artery[35], clinical trials of thrombus aspiration during primary PCI were performed[219 220 230]. Following the TASTE trial[219] (thrombus aspiration during STEMI) (n=7244) and TOTAL trial[220] (trial of routine aspiration thrombectomy with PCI vs. PCI alone in patients with STEMI) (n=10,732), routine aspiration thrombectomy in all-comers is not recommended. However, a meta-

analysis of the 3 main trials of thrombus aspiration in STEMI, found that the sub-group with high thrombus burden had non-statistically significant trends towards reduced cardiovascular death, and increased stroke[231]. A selective strategy of bail-out aspiration thrombectomy in patients with high thrombus burden might be recommended, however, there is insufficient data to establish effectiveness[25].

1.5.2 Glycoprotein IIbIIIa inhibitors

Glycoprotein IIbIIIa inhibitors, for example abciximab, prevent platelet aggregation. In the CMR sub-study (n=795) of the AIDA STEMI trial (Abciximab intravenous vs intracoronary in STEMI) intracoronary administration of glycoprotein IIbIIIa inhibitors during PCI for STEMI did not improve MVO or infarct size, when compared with intravenous administration[222]. Intracoronary glycoprotein IIbIIIa inhibitors are recommended as a bailout strategy during primary PCI when there is "no reflow" and large thrombus burden[1], however, evidence for this recommendation is lacking.

1.5.3 Deferred stenting

Deferred stenting is a strategy whereby TIMI 3 flow is restored to the infarct-related artery with balloon angioplasty (without stenting), during the primary PCI procedure. The patient subsequently receives an infusion of heparin or glycoprotein IIbIIIa inhibitor on the ward to reduce thrombus burden, before returning to the catheterisation laboratory for stenting. In the DEFER-STEMI trial (deferred stenting vs. immediate stenting to prevent no- or slow-reflow in acute STEMI), 101 STEMI patients with ≥1 risk factor for no-reflow were randomised to deferred stenting (4 to 16 hours later) vs. standard primary PCI[214]. Deferred stenting was associated with fewer angiographic intraprocedural thrombotic events, lower incidence of final TIMI flow grade ≤2 and improved 6-month myocardial salvage index, however there was no difference in CMR-defined MVO at 2 to 7 days, or infarct size at 6 months with deferred stenting vs. standard primary PCI[214].

The DANAMI-DEFER 3 trial (deferred vs. conventional stent implantation in patients with STEMI) compared deferred stenting at 48 hours (n=603) with standard primary PCI (n=612) in STEMI patients <12 hours from symptom onset[215]. The primary endpoint (all-cause mortality, heart failure hospitalisation, recurrent MI, or unplanned revascularisation of the infarct-related artery) did not differ between patients randomised to deferred stenting vs. standard primary PCI (HR: 0.99 [95% CI: 0.76, 1.29] p=0.92)[215]. The CMR sub-study

(n=510) of the DANAMI-DEFER 3 trial demonstrated no difference in MVO or infarct size with deferred stenting vs. standard primary PCI[216].

The INNOVATION trial (impact of immediate stent implantation versus deferred stent implantation on infarct size and microvascular perfusion in patients with STEMI) (n=114) randomised patients within 12 hours of STEMI onset to deferred stenting (72 hours later) or standard primary PCI[217]. Overall, there was no difference in the primary endpoint (infarct size at 3 to 7 days post-STEMI), or CMR-defined MVO, between the 2 groups[217]. However, in the subset of patients with anterior STEMI (n=69), deferred stenting was associated with reduced infarct size (16.7% vs. 22.7%, p=0.017), and less MVO (43.8% vs.70.3%, p=0.047)[217]. An adequately powered randomised clinical trial may potentially be indicated, to assess whether deferred stenting may improve outcomes in patients with high thrombus burden[218].

1.5.4 Adenosine and sodium nitroprusside

Adenosine is a coronary vasodilator, which additionally inhibits adhesion/ migration of neutrophils, which may contribute to ischaemia-reperfusion injury[232]. Sodium nitroprusside is a direct nitric oxide donor that mediates potent arteriolar vasodilatation[233]. The open-label REOPEN-AMI trial (intracoronary nitroprusside vs. adenosine in acute MI)[234] found that the primary endpoint of incidence of complete (>70%) ST-segment resolution on the ECG at 90 minutes post-reperfusion was higher with intracoronary adenosine (2mg) (n=80) compared to saline control (n=80) (71% vs. 51%, p=0.009). However, a limitation of the REOPEN-AMI trial is that non-invasive imaging was not performed to assess MVO or infarct characteristics[234].

In the open-label REFLOW-STEMI trial (reperfusion facilitated by local adjunctive therapy in STEMI), vasodilator therapy with high-dose intracoronary adenosine (2 to 3mg) (n=82) and intracoronary sodium nitroprusside (n=79) during primary PCI did not reduce CMR-derived infarct size or MVO, when compared to standard PCI (n=86)[91]. In fact, high-dose intracoronary adenosine was associated with larger infarct size (12,0% vs. 8.3%, p=0.031), worse LV ejection fraction (42.5% vs. 45.7%, p=0.027) and an increase in MACE at 6 months (HR: 5.39 [95% CI: 1.18, 24.60] p=0.04)[91]. The findings suggest a safety concern with high dose intracoronary adenosine in STEMI patients with “no reflow”[91]. Adenosine may reduce coronary flow in STEMI when the infarct-related artery

has large collaterals and a profound fixed microvascular injury, which is unresponsive to adenosine, resulting in inter-coronary steal phenomenon, i.e. where blood is diverted away from the myocardium supplied by the infarct-related artery, to adjacent vascular beds, which dilated with adenosine[235].

1.5.5 Antiplatelet therapy

Ticagrelor has been reported to increase plasma adenosine levels, due to off-target properties[236]. The REDUCE-MVI trial (reducing microvascular dysfunction in acute MI by ticagrelor) evaluated 110 patients undergoing primary PCI, and found no differences in invasive measures of microvascular injury measured at 1-month, between patients randomised to ticagrelor vs. placebo[221].

1.5.6 Beta blockers

Recent STEMI guidelines suggest that intravenous beta blocker should be considered at presentation in patients undergoing primary PCI, without contraindications, i.e. acute heart failure or systolic blood pressure ≤120mmHg[1]. Intravenous administration of the β1 selective blocker metoprolol, before reperfusion, may impair neutrophil migration, by preventing changes to neutrophil structure, which are needed to initiate intracellular interactions and tissue infiltration, in the early phases of neutrophil recruitment[237].

The EARLY-BAMI trial (early-beta blocker administration before reperfusion primary PCI in patients with STEMI) was a randomised, double-blind trial, which compared intravenous metoprolol (2 x 5mg bolus, administered immediately before PCI) to placebo, in all-comer STEMI patients undergoing primary PCI, <12 hours from symptom onset (n=683)[212]. The primary endpoint (infarct size at 30 days, assessed by CMR imaging) did not differ between the metoprolol vs. placebo group (15.3% vs. 14.9%, p=0.616)[212]. MVO was not reported in the EARLY-BAMI trial[212]. In contrast, the METOCARD-CNIC trial (effect of metoprolol in cardioprotection during an acute MI) compared intravenous metoprolol (3 x 5mg bolus, administered in the ambulance before PCI) to standard care in patients with anterior STEMI undergoing primary PCI <6 hours from symptom onset (n=270)[238]. METOCARD-CNIC was a single-blind trial, i.e. the outcome assessors were blinded[239]. The primary endpoint (infarct size 5 to 7 days post-STEMI, assessed by CMR imaging) was smaller with intravenous metoprolol vs. control (25.6g vs. 32.0g, adjusted difference: -6.53 [95% CI: -11.39, -1.78] p=0.012). Moreover, there was a 40% reduction in CMR-defined MVO with intravenous metoprolol vs. control[237]. LV ejection fraction at 6

months post-STEMI was higher with intravenous metoprolol vs. control (48.7% vs. 45.0%, adjusted treatment effect: 3.49% [95% CI: 0.44, 6.55] p=0.025)[240]. At 2 years follow up, there was no difference in the prespecified composite outcome of death, heart failure admission, reinfarction and malignant arrhythmias between the intravenous metoprolol group and controls (10.8% vs. 18.3%, adjusted HR: 0.55 [95% CI: 0.26, 1.04] p=0.065)[240]. The contrasting findings of the EARLY-BAMI and METOCARD-CNIC trials may relate to the different dosing of metoprolol (10mg vs. 15mg) and the different trial populations (all-comers vs. anterior STEMI, and ischaemic time <12 hours vs. <6 hours). An adequately powered, double-blind, randomised clinical trial of intravenous metoprolol (15mg) in STEMI patients <6 hours from symptom onset vs. placebo may be warranted.

1.5.7 Statins

High-dose statin therapy has been postulated to prevent "no reflow", due to pleiotropic effects, including activation of mitochondrial potassium channels following statin pre-treatment[241], anti-inflammatory effects[242] and improvement of endothelial function. However, the effects of pre-treatment with statins on microvascular injury in STEMI patients is conflicting[243-245], and randomised clinical trials, which used CMR to assess infarct characteristics, have shown that statins prior to primary PCI did not reduce MVO[223 224], or infarct size[223 225].

1.5.8 Ischaemic conditioning

Ischaemic conditioning has been postulated to reduce reperfusion injury, and is defined as: (i) repeated brief episodes of mechanical ischaemic reperfusion (either at the site of the target organ or at a remote site), or (ii) pharmacological prevention of the opening of the mitochondrial permeability transition pore at the onset of reperfusion[202]. In randomised clinical trials, cyclopsorine, which inhibits the mitochondrial permeability transition pore, did not improve clinical outcomes, when administered intravenously during primary PCI[227 228]. The CONDI-2/ERICPPCI trial (effect of remote ischaemic conditioning on clinical outcomes in STEMI patients undergoing primary PCI) was a large multicentre, single-blind, randomised controlled trial (n=5401 primary PCI patients), which compared remote ischaemic conditioning to standard care[229]. The primary endpoint of the CONDI-2/ERICPPCI trial (12-month cardiac death or hospitalisation for heart failure) did not differ between the remote ischaemic conditioning group vs. standard care[229]. Infarct size (evaluated by troponin T levels) also did not differ between the remote ischaemic conditioning group vs. standard care[229].

1.5.9 Intracoronary hypothermia

In animal models of MI, hypothermia has been shown to reduce infarct size[246]. The underlying mechanism of this protective effect may be that intracoronary hypothermia reduces metabolic demand, which in turn reduces oxidative stress on the ischaemic myocardium, thereby attenuating myocardial reperfusion injury[247]. However, systemic hypothermia in humans has failed to demonstrate a reduction in infarct size, perhaps due to inability of the myocardium to reach the target temperature before reperfusion[248]. Furthermore, systemic cooling can cause adverse effects, such as volume overload and severe shivering[249]. Selective intracoronary hypothermia to the infarcted myocardium overcomes the limitations of systemic cooling, and has been shown to be feasible and safe[250]. In a pilot study of 60 patients undergoing primary PCI, intracoronary hypothermia before stent implantation was associated with smaller infarct size (2 to 7 day) assessed by CMR imaging, compared to standard care[251]. The ongoing EURO-ICE trial (European intracoronary cooling evaluation in patients with STEMI) is a randomised controlled trial, which is investigating localised intracoronary hypothermia administered to the infarct-related artery (pre-stent implantation), in anterior STEMI patients with symptom onset ≤6 hours (NCT03447834)[252 253]. The target sample size for EURO-ICE is 200 patients, the primary endpoint is 3-month infarct size (assessed by CMR imaging), and the estimated study completion date is in 2022[253].

1.6 Coronary physiology to select patients for treatments and to assess treatment efficacy

Because microvascular dysfunction does not occur in all STEMI patients, clinical trial design could potentially be improved by the initial selection of patients at increased risk, in whom adjunctive treatment might be of benefit. Some studies have used IMR as a tool for acute risk stratification, to select patients at high risk of "no reflow" for novel therapies during primary PCI[254 255], and to assess response to treatment[221 254-268]. Figure 1.3 depicts the potential use of IMR for prognostication and hypothetical future directions to guide stratification of STEMI patients for adjunctive therapeutic strategies.

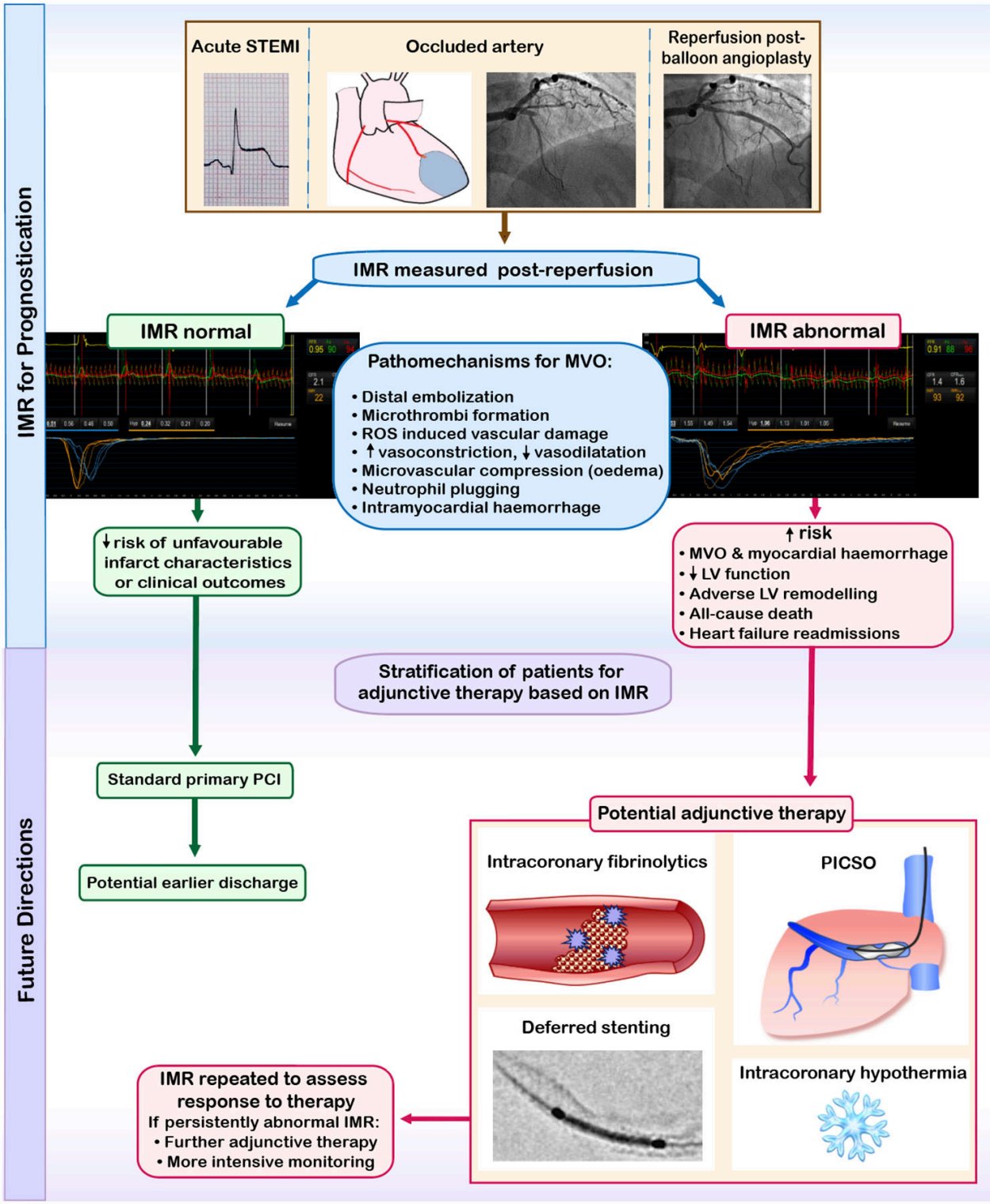

Figure 1.3 *Illustration of the potential value of IMR as a prognostic biomarker, and hypothetical scenarios using IMR to guide stratification of acute STEMI patients for adjunctive therapies in randomised trials. Abbreviation: PICSO = pressure-controlled intermittent coronary sinus occlusion. Adapted from: Maznyczka A et al. The potential use of index of microcirculatory resistance (IMR) to guide stratification of patients for adjunctive therapy in acute myocardial infarction. JACC Cardiovasc Interv. 2019. 12(10): 951-966. Doi: 10.1016/j.jcin.2019.01.246*[16].

1.6.1 Studies using IMR as a theragnostic biomarker

Stratified medicine is the identification of patient sub-groups (or endotypes) within a heterogeneous population, these being distinguishable by disease severity and potential for response to therapy[16 269]. A theragnostic biomarker is a metric that predicts therapeutic response[16]. In a small observational study with no control group (n=18), Morimoto *et al*[254] used IMR ≥30 immediately post-primary PCI, as a theragnostic biomarker to select patients for adjunctive intracoronary sodium nitroprusside (Table 1.1).

In the OxAMI-PICSO non-randomised pilot study (Oxford acute MI – pressure-controlled intermittent coronary sinus occlusion), De Maria *et al*[255] used IMR >40 pre-stenting to risk stratify anterior STEMI patients for treatment with PICSO (pressure-controlled intermittent coronary sinus occlusion) during primary PCI (Table 1.1). The postulated therapeutic action of PICSO is that cyclic balloon inflations in the coronary sinus for ≥30 minutes may redistribute blood from the remote non-ischaemic myocardium to under perfused areas, i.e. to the border zone of the ischaemic myocardium. The redistribution of blood when the balloon is inflated may be due to retrograde perfusion from the distal infarct-related artery, as well as augmented collateral inflow, from adjacent non-infarct-related arteries supplying the border zone of the myocardium. In addition, arterial pressure decreases during deflation of the coronary sinus balloon, which may create a suction effect, which enhances washout of embolic material. In OxAMI-PICSO, there was no difference in IMR at the end of primary PCI between PICSO and historical controls[255]. However, at 24 to 48 hours following primary PCI, IMR was lower in the PICSO group (24.8 vs. 45.0, $p<0.001$)[255]. There were no between-group differences in acute infarct size, or MVO, but 6-month infarct size (assessed by CMR imaging) was lower with PICSO (26.0% vs. 33.0%, $p=0.006$)[255]. The PiCSO-AMI-I trial (pressure-controlled intermittent coronary sinus occlusion in acute MI) is an ongoing, randomised (1:1) clinical trial, using pre-stenting IMR >40 to select acute anterior STEMI patients for intervention with PICSO (NCT03625869)[270]. The target enrolment for the PiCSO-AMI-I trial is 144 patients, and the estimated completion date is 2023[270].

A precision medicine approach using pre-stenting IMR is being used to evaluate deferred stenting in a large ongoing clinical trial, with a target sample size of 880 acute STEMI patients) (NCT03581513)[271]. The comparator groups are IMR ≤40 randomised to either

deferred stenting or standard primary PCI, and IMR >40 randomised to either deferred stenting or standard primary PCI. The primary outcome is the prevalence of heart failure, repeat MI, or target vessel revascularisation at 1 year. Two more ongoing, randomised clinical trials, are using post-stent IMR (>30, or >32) as a theragnostic biomarker to guide stratified therapy with adjunctive intracoronary fibrinolytics during primary PCI, and are described in section 1.7.9 ([NCT02894138][272] and [ACTRN12618000778280][273]).

1.6.2 Studies using IMR to assess treatment efficacy

Non-randomised[254-257 274] and randomised[221 258 261-268] studies that measured IMR in STEMI patients, to assess the immediate efficacy of adjunctive treatments, which were designed to reduce microvascular injury, are presented in Tables 1.1 and 1.2. The quality of studies is variable, with some non-randomised[254-257 252], some without a control group[254 257 261], some without blinding[254 255 257 261 264-268], some without non-invasive imaging[254 256 258] and some with small sample sizes[254 256 257 259 264 266].

The timing for the IMR measurement to assess therapeutic response in different studies is not consistent. The majority of studies measured IMR post-stenting, at the end of the same PCI procedure[254 256 257 262-265 268]. However, some studies made another IMR measurement 2 days later[255], and some only measured IMR during an additional catheterisation laboratory procedure, performed 2 days post-PCI[259 260], or 4 to 5 days post-PCI[266]. The timing for IMR measurement is relevant, because there are dynamic changes in microvascular dysfunction following acute STEMI, related to ischaemic time[16]. IMR and CFR undergo partial recovery in the first 24 to 48 hours following reperfusion, with further recovery to 6 months[157 163 275 276]. It may be that in patients with a short duration of ischaemia a decrease in IMR over time post-PCI reflects a microvascular recovery response[16]. However, repeated invasive procedures to measure IMR 24 to 48 hours after primary PCI would create additional costs, logistical issues and potential safety risks, therefore information from invasive coronary physiology is most practically useful at the time of the primary PCI procedure.

Non-randomised study	Sample size	Intervention	Timing of IMR measurement	IMR findings	Infarct characteristics on non-invasive imaging
Vasodilatory therapy:					
Ito *et al*[256] 2010	40	Intracoronary nicorandil post-PCI vs. placebo control	IMR immediately after PCI, pre-study drug and post-study drug	• IMR was lower post- vs. pre-nicorandil (median values: 18.7 vs. 27.7, p<0.001). • No difference in IMR post vs. pre-control (23.8 vs. 24.3, p=0.819). • In the sub-group with baseline IMR ≥21, IMR was lower with nicorandil vs. control.	Not reported
Kostic *et al*[257] 2015	32	Intracoronary nicorandil post-PCI (no control)	IMR immediately post-PCI and 10 minutes post-nicorandil	IMR was lower post- vs. pre-nicorandil (mean values: 9.9 vs. 14.1, p<0.001)	Improvement in wall motion score index on echocardiography at 3 months post-PCI vs. 24 hours post-PCI (1.07 vs. 1.14, p=0.004)
Morimoto *et al*[254] 2012	18 patients with IMR ≥30 post-PCI	Intracoronary sodium nitroprusside (no control)	IMR immediately post-PCI pre-study drug and post-study drug	IMR was lower post- vs. pre-study drug (45 vs. 76, p=0.0006).	Not reported
Pressure-controlled intermittent coronary sinus occlusion:					
De Maria *et al*[255] 2018 **OxAMI-PICSO**	75 patients with IMR >40 (25 patients treated with PICSO and 50 historical controls)	PICSO vs. standard primary PCI	• IMR immediately post-PCI in all patients. • IMR 24 to 48 hours post-PCI in 20 PICSO patients and in 31 controls with IMR >40	• IMR was lower 24 - 48 hours post-PCI in the PICSO group vs. control group (median values: 24.8 vs. 45.0, p<0.001) • No difference between groups for IMR measured immediately post-PCI (p=0.40)	• Infarct size on CMR 6-months post-PCI was lower with PICSO vs. controls (26% vs. 33%, p=0.006). • No difference between PICSO vs. controls for infarct size (39% vs. 41%, p=0.42) or MVO occurrence (64.7% vs. 86.2%, p=0.08) at 2 days.
Aspiration thrombectomy:					
Firman et al[274] 2020	45 patients with high thrombus burden	Thrombus aspiration vs. standard primary PCI	IMR immediately post-PCI	No difference in IMR in patients who received thrombus aspiration vs. standard primary PCI (51.9 vs. 47.1, p=0.723)	No difference in global longitudinal strain on echocardiography at 6 months with thrombus aspiration vs. standard primary PCI (-13.0 vs. -12.8, p=0.912).

Table 1.1 *Non-randomised clinical studies, using IMR to measure the efficacy of interventions designed to reduce microvascular injury in STEMI patients.*

Randomised study	Sample size	Intervention	Primary endpoint	Timing of IMR	Difference in IMR +/ - CFR	Infarct characteristics on non-invasive imaging
Vasodilatory therapy:						
Ito *et al*[258] 2013	60 (crossover trial)	Intracoronary nicorandil vs. intracoronary nitroglycerin post-PCI	IMR	IMR immediately post-PCI (pre- and post-study drug)	• As a first administration nicorandil reduced IMR more than nitroglycerin (median values: 10.8 vs. 2.1, p=0.0002). • As a second administration, nicorandil further reduced IMR, whereas nitroglycerin did not (p<0.001).	Not reported
Thrombolytic therapy:						
Sezer *et al*[259] 2007	41	Intracoronary streptokinase vs. standard primary PCI	IMR and CFR at 2 days and infarct size at 6 months	IMR and CFR 2 days post-PCI	• IMR was lower with streptokinase (mean values: 16.3 vs. 32.5, p<0.001). • CFR was higher with streptokinase (mean values: 2.0 vs. 1.4, p=0.002)	No difference in infarct size by SEPCT at 6 months with streptokinase vs. controls (27.8% vs. 37.3%, p=0.17)
Sezer *et al*[260] 2009	95 in main study 85 had IMR	Intracoronary streptokinase vs. standard primary PCI	Infarct size at 6 months	IMR and CFR 2 days post-PCI	• IMR was lower with streptokinase vs. controls (mean values: 20.2 vs. 34.2, p<0.001). • CFR was higher with streptokinase vs. controls (mean values: 2.5 vs. 1.7, p<0.001)	Infarct size by SPECT 6 months post-primary-PCI was smaller with streptokinase vs. controls (22.7% vs. 32.9%, p=0.003)
Xiao et al[261] 2019	71 patients with high thrombus burden	Intracoronary prourokinase vs. thrombus aspiration	Combination of IMR and MPG	IMR immediately post-PCI	IMR was lower with prourokinase vs. thrombus aspiration (mean values: 28.2 vs. 33.6, p=0.005).	• LV ejection fraction by echocardiography (12 months post STEMI) was higher with prourokinase vs. thrombus aspiration (58.1% vs. 55.2%, p=0.043). • Infarct size by SPECT at 12 months was higher with prourokinase vs. thrombus aspiration (18.6% vs. 22.7%, p=0.046)

Table 1.2 *(page 1 of 3) Randomised studies, using IMR to measure the efficacy of interventions designed to reduce microvascular injury in STEMI patients*

Randomised studies	Sample size	Intervention	Primary endpoint	Timing of IMR	Difference in IMR +/ - CFR	Infarct characteristics on non-invasive imaging
Aspiration thrombectomy:						
Woo *et al*[262] 2014	63	Thrombus aspiration vs. standard primary PCI	IMR	IMR immediately post-PCI	IMR was lower with thrombus aspiration vs. controls (mean values: 23.5 vs. 34.2, p=0.018)	Reduction in wall motion score index on echocardiography during first admission for STEMI and 6 months later was greater with thrombus aspiration vs. controls (-0.12 vs. -0.004, p=0.001).
Hoole *et al*[263] 2015 **IMPACT trial**	41	Thrombus aspiration vs. balloon angioplasty	IMR	IMR immediately post-PCI	No difference in IMR between aspiration thrombectomy vs. balloon angioplasty (mean values: 43.3 vs. 44.6, p=0.90).	No difference in MVO occurrence, or infarct size, on CMR 1-day post-PCI with aspiration thrombectomy vs. balloon angioplasty.
Ahn *et al*[264] 2014 **ICAT trial**	40	Intracoronary bolus GPIIbIIIa inhibitor (abxicimab) vs. thrombus aspiration vs. both.	IMR	IMR immediately post-PCI	• IMR was lower in the combination group vs. abciximab only group (mean values: 23.5 vs. 66.9, p=0.001). • No difference in IMR between the combination group vs. aspiration thrombectomy only group (mean values: 23.5 vs. 37.2, p=0.07)	• MVO occurrence on CMR 5 days post-STEMI was lower in the combination group vs. abciximab only group (18.8% vs. 88.9%, p=0.002). • No difference in MVO occurrence in the combination vs. aspiration thrombectomy only group (18.8% vs. 66.7%, p=0.054)
Direct thrombin inhibition:						
van Geuns *et al*[265] 2017 **BIVAL trial**	64 in main study 52 had IMR	Bivalirudin vs. unfractionated heparin	Infarct size by CMR, 5 days post-PCI	IMR immediately post-PCI	IMR was lower with bivalirudin vs. unfractionated heparin (mean values: 43.5 vs. 68.7, p=0.014)	• No difference in mean infarct size on CMR with bivalirudin vs. heparin (25.0% vs. 27.1%, p=0.75). • No difference in MVO extent with bivalirudin vs. heparin (5.3g vs. 7.7g, p=0.17)

Table 1.2 *(page 2 of 3) Randomised studies, using IMR to measure the efficacy of interventions designed to reduce microvascular injury in STEMI patients*

Randomised studies	Sample size	Intervention	Primary endpoint	Timing of IMR	Difference in IMR +/ - CFR	Infarct characteristics on non-invasive imaging
Anti-platelet therapy:						
van Leeuwen et al[221] 2019 **REDUCE-MVI**	110	Ticagrelor vs. prasugrel	IMR 1-month post-PCI	IMR and CFR immediately after and 1-month post-PCI	• No difference in IMR at 1 month with ticagrelor vs. prasugrel (median values: 21 vs. 18, p=0.08). • No difference in CFR at 1 month with ticagrelor vs. prasugrel (mean values: 3.7 vs. 3.9, p=0.66).	• No difference in MVO at 2 to 7 days with ticagrelor vs. prasugrel (mean values: 11 vs. 17, p=0.35). • Less myocardial haemorrhage at 2 to 7 days with ticagrelor vs. prasugrel (8 vs. 18, p=0.04). • No difference in infarct size at 1 month with ticagrelor vs. prasugrel (7.6% vs. 8.7%, p=0.14).
Kirma *et al*[266] 2012	49	Intracoronary vs. intravenous GPIIbIIIa inhibitor	IMR and CFR	IMR and invasive CFR 4 to 5 days post-PCI	• IMR did not differ with intracoronary vs. intravenous tirofiban (mean values: 27 vs. 35, p=0.08). • CFR did not differ with intracoronary vs. intravenous tirofiban (mean values: 2.2 vs. 1.9, p=0.25)	No difference in infarct size on SPECT at 6 months with intracoronary vs. intravenous tirofiban (18% vs. 12%, p=0.26).
Ubaid *et al*[267] 2019	100	Intravenous cangrelor then oral ticagrelor vs. oral ticagrelor only	Platelet inhibition	IMR immediately post-PCI	No difference in IMR between cangrelor and ticagrelor groups	No difference in infarct size on CMR 3 months post-PCI between cangrelor and ticagrelor groups.
Park *et al*[268] 2016 **CV-TIME trial**	76	Ticagrelor vs. clopidogrel	IMR	IMR immediately post-PCI	IMR was lower with ticagrelor vs. clopidogrel (mean values: 22.2 vs. 34.4, p=0.005).	No difference in estimated infarct size by wall motion score index on echocardiography with ticagrelor vs. clopidogrel at 24 hours (1.55 vs. 1.61, p=0.41), or 3 months (1.42 vs. 1.47, p=0.57).

Table 1.2 *(page 3 of 3) Randomised studies, using IMR to measure the efficacy of interventions designed to reduce microvascular injury in STEMI patients*

1.7 Intracoronary fibrinolysis as a potential adjunctive treatment for acute microvascular dysfunction

There has been recent interest in the potential efficacy of intracoronary low-dose fibrinolytic therapy, to minimise microvascular damage, during primary PCI. The following section is an overview of intracoronary fibrinolytic therapy, which critically appraises the evidence from randomised clinical studies and describes the ongoing trials.

1.7.1 Mechanism of action of fibrinolytic drugs

Thrombolytic drugs activate endogenous fibrinolysis[130], by catalysing the formation of plasmin from plasminogen (Figure 1.4). Plasmin then breaks down the cross-linked fibrin matrix in thrombus[277], releasing soluble fibrin degradation products. Plasmin also has paradoxical procoagulant effects, due to the activation of factor V to Va. The factor X – Va complex converts prothrombin to thrombin, releasing prothrombin fragments F_{1+2}. Thrombin then converts fibrinogen to fibrin, forming thrombus. Thrombin also activates platelets, which are a source of coagulation proteins and release plasminogen activator inhibitor-1 (PAI-1) (a plasmin antagonist that can inhibit fibrinolysis).

The oldest thrombolytic drug is streptokinase, which is a protein produced by group B haemolytic streptococci bacteria[278 279] and purified for clinical use. Streptokinase (plasma half-life: 12 to 18 minutes)[280] is a first-generation, non-fibrin specific, allergenic, thrombolytic drug. Another first-generation, non-fibrin specific thrombolytic agent is urokinase. Urokinase is an enzyme cultured from human kidney cells and has a plasma half-life of 15 to 20 minutes[280].

Newer thrombolytic agents were developed to enable fibrin specificity. Prourokinase (plasma half-life: 9 minutes)[281] is a fibrin-specific, second-generation fibrinolytic agent. Prourokinase is a proenzyme precursor of urokinase. When two single chain prourokinase units are activated by fibrin-associated plasmin, they bind at the thrombus surface to form an active two chain urokinase molecule[282]. Alteplase (plasma half-life: 4 to 8 minutes)[281] is another second-generation fibrin-specific fibrinolytic drug. Alteplase is a serine protease found on endothelial cells and is manufactured from recombinant biotechnology, being referred to as recombinant tissue plasminogen activator (r-TPA).

Third-generation thrombolytic agents have been developed with focus on (i) longer half-lives (to enable intravenous bolus injections, instead of the continuous infusions required with the earlier-generation thrombolytics)[283], (ii) higher fibrin-specificity[284 285] (to increase potency and potentially decrease undesirable bleeding events), and (iii) greater resistance to plasminogen activator inhibitor-1 (to increase potential potency especially for platelet rich clots)[286 287]. Tenecteplase (plasma half-life: 20 minutes)[281] is a third-generation fibrin-specific r-TPA. Specifically, tenecteplase is a glycoprotein, developed by introducing modifications to the complementary deoxyribonucleic acid for natural TPA[283].

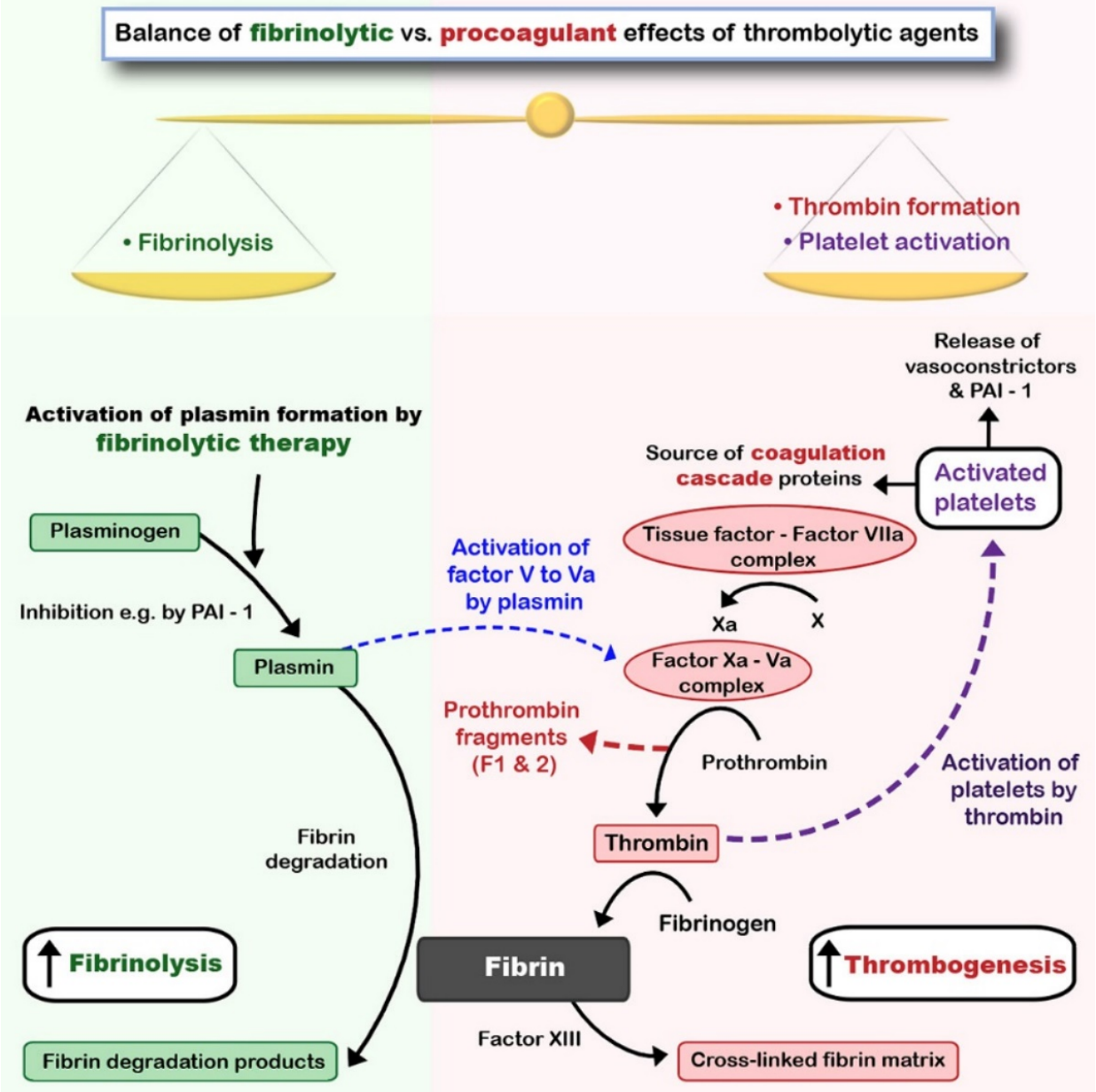

Figure 1.4 *Competing processes involved in local fibrinolytic therapy. Adapted and reproduced with permission from: Berry C, Maznyczka A, McCartney P. Failed myocardial reperfusion during primary PCI: an unmet therapeutic need. EuroIntervention. 2019. 14:1628-1630. Doi: 10.4244/EIJV14I16A279.*[288]

1.7.2 Insights from systemic fibrinolysis combined with PCI

The STREAM trial (strategic reperfusion early after MI) (n=939) explored a pharmaco-invasive strategy consisting of early fibrinolysis (within 3 hours of symptom onset) for patients unable to undergo primary PCI within 1 hour, followed by either rescue PCI (for failed thrombolysis), or non-urgent angiography within 24 hours[289]. When compared to primary PCI without fibrinolysis, the pharmaco-invasive strategy had more open vessels at the start of angiography[289]. There were no differences between the 2 groups for death, shock, heart failure, or reinfarction at 30-days, however the fibrinolysis arm was associated with an increased risk of intracranial haemorrhage (1.0% vs. 0.2%, p=0.04)[289].

These data should be put into context with the findings from studies of facilitated primary PCI, i.e. where thrombolysis (full- or half-dose) is followed by immediate pre-planned PCI, to mitigate the delay associated with PCI. Fibrinolytic therapy before immediate planned PCI improved initial vessel patency[290 291]. However, the ASSENT-4 trial[290] (assessment of the safety and efficacy of a new treatment strategy with PCI 4) found that primary PCI with prior full-dose tenecteplase (n=823) was associated with higher mortality, reinfarction, congestive heart failure and more ischaemic strokes than standard primary PCI without fibrinolysis (n=831). The FINESSE trial[291] (facilitated intervention with enhanced reperfusion speed to stop events) (n=2452) reported that facilitated primary PCI with combinations of reteplase plus abxicimab, or abxicimab alone did not improve clinical outcomes compared to standard primary PCI.

The results from the studies of facilitated primary PCI[290 291], may be partly explained by the fact that clopidogrel was not given at that time. Furthermore, the results of the ASSENT-4 trial[290] could potentially be explained by relatively inadequate anticoagulation and potentially paradoxical activation of thrombin, with formation of fibrin and thrombus in the group that received tenecteplase[292].

1.7.3 Intracoronary fibrinolysis

Intracoronary administration of fibrinolytic drugs allows lower systemic and higher local concentrations of the drug at the site of the thrombus. Registries have demonstrated that intracoronary fibrinolysis can be an effective treatment for acute coronary thrombosis[293-295]. Randomised clinical studies of adjunctive low-dose intracoronary fibrinolytic therapy during primary PCI are summarised in Table 1.3. These studies[259-261 292 296-303] did not show any significant bleeding events with intracoronary low-dose fibrinolytics.

A recent meta-analysis of 6 randomised controlled trials, found no difference in the primary endpoint, i.e.TIMI flow ≥2 in the infarct-related artery, with intracoronary fibrinolysis vs. standard care/ placebo[304]. Nonetheless, there was a higher incidence of complete ST-segment resolution with intracoronary fibrinolysis vs. placebo, and a trend favouring fewer in-hospital MACE with intracoronary fibrinolysis, which led the authors to conclude that intracoronary fibrinolysis is safe and potentially effective during primary PCI[304]. However, the primary endpoint of TIMI flow ≥2 is not synonymous with normalised myocardial perfusion, and the meta-analysis[304] did not include a subsequent sub-group analysis from T-TIME (trial of low-dose adjunctive alteplase during primary PCI), which raises a safety concern with intracoronary alteplase[303].

1.7.4 Intracoronary streptokinase

One of the earliest reports of intracoronary streptokinase administration in the management of STEMI patients (n=5) was by Rentrop in the 1970s[305]. The feasibility of intracoronary streptokinase was further demonstrated in an observational study of STEMI patients from the 1980s, which reported successful reperfusion in 38 of 46 single vessel occlusions associated with a first MI[306]. Moreover, it has been demonstrated histopathologically in a canine model of LAD occlusion, that intracoronary streptokinase administered over 30 minutes, after 2 hours of LAD occlusion, improved microvascular reperfusion, when compared to control[307].

The first randomised clinical trial of intracoronary streptokinase in STEMI patients was reported by Sezer *et al* in 2007[259]. Forty-one patients undergoing primary PCI <12 hours from symptom onset, were randomised to adjunctive intracoronary streptokinase (250 kU) infused over 3 minutes immediately post-stent implantation (n=21), or no additional

therapy (n=20). They reported improvements in mean CFR and IMR when catheterisation was repeated 2 days later, in the streptokinase group vs. standard care group (CFR: 2.0 vs. 1.4, p=0.002; IMR: 16.3 vs. 32.5, p<0.001)[259]. The findings suggest that streptokinase might reduce microvascular damage and improve myocardial reperfusion. When enrolment was increased to 95 patients, in a second report from the same group, mean TFC was lower and MPG ≥2 occurred more frequently in the streptokinase group (n=51) than with standard care (n=44) (TFC: 20.7 vs. 29.2, p<0.001; MPG ≥2: 86% vs. 36%, p<0.001)[260]. However, there was no difference in % ST-segment resolution between streptokinase and standard care groups[260]. At 6 months, Sezer *et al*[260] also recorded improvements in mean infarct size, LV volumes and LV ejection fraction (assessed by SPECT) in the streptokinase group vs. standard care group (infarct size: 22.7% vs. 32.9%, p=0.003; LV ejection fraction: 57.2% vs. 51.8%, p=0.018). There was no difference in MACE at 6 months, however the study was underpowered for clinical outcomes.

The findings reported by Sezer *et al*[259 260] should be interpreted with some caution. The intervention was open-label, with no placebo, and the acquisition and analysis of endpoints was mainly performed by unblinded investigators. Furthermore, the size of the cohort was relatively small, therefore the potential risk of type 1 statistical error (false positive) is increased.

Randomised Study	Number of patients	Thrombolytic drug	Comparator group	Blinded drug administration	Timing of study drug delivery	Thrombus aspiration	Main findings
McCartney *et al*[292] Maznyczka *et al*[302] 2019 and 2020 **T-TIME trial**	440 (main trial) 144 (physiology sub-study)	Alteplase 10mg or 20mg	Volume - matched saline (20ml)	Yes	Pre-stent	Used in 27% of patients	• Overall, no difference with alteplase vs. placebo for MVO assessed by CMR imaging (at 2 to 7 days), or 1-year clinical outcomes.
Gibson *et al*[296] 2020 **ICE T-TIMI 49 trial**	40	Tenecteplase 4mg x 2	Volume - matched saline (2ml x 2)	No	4mg pre-stent, followed by 4mg post-stent	Not reported	• More patients in the tenecteplase group had reduction in TIMI thrombus grade after the first bolus of study drug than controls. • Fewer patients in the Tenecteplase group had TFC <14 than in the placebo group. • No difference in MPG, or % diameter stenosis of the infarct-related lesion with tenecteplase vs. control.
Geng *et al*[297] 2018	230	Prourokinase 10mg	Volume - matched saline (10ml)	No	Pre-stent	Not reported	• Lower peak troponin I, higher rate of ST-segment resolution >70% and better myocardial perfusion assessed by echocardiography (at 7 days) with prourokinase vs. controls. • No difference in 6-month MACE.
Wang *et al*[298] 2020	182	Prourokinase 20mg	Volume - matched saline (10ml)	No	Pre-stent	Used in all patients	• Lower TFC, lower peak troponin, greater rates of ST-segment resolution >70% and improved 6-month LV ejection fraction with prourokinase vs. control. • Lower 6-month MACE rates with prourokinase vs. control.
Xiao et al[261] 2019	71	Prourokinase 10 – 20mg	Thrombus aspiration	No	Pre-stent	Only as comparator	IMR, MPG, TFC, 12-month infarct size, LV ejection fraction and MACE rates improved with prourokinase vs. thrombus aspiration.

Table 1.3 *(page 1 of 2) Summary of randomised clinical studies of adjunctive intracoronary fibrinolysis, during primary PCI. All studies reported that intracoronary low-dose fibrinolysis was not associated with significant bleeds.*

Randomised Study	Number of patients	Thrombolytic drug	Comparator group	Blinded drug administration	Timing of study drug delivery	Thrombus aspiration	Main findings
Greco *et al*[299] 2013 **DISSOLUTION trial**	102	Urokinase 200,000 IU	Volume - matched saline (10ml)	No	Pre-stent	Used in all patients	• Greater volume of thrombus aspirated with urokinase vs. control. • Higher rates of final TIMI 3 flow, MPG ≥2, and ST-segment resolution >70% with urokinase vs. control. • Lower 6-month MACE rates with urokinase vs. control
Sezer *et al*[259 260] 2007 and 2009	41 (2007) 95 (2009)	Streptokinase 250 kU (20ml)	Standard care	No	Post-stent	Not reported	• Better CFR, IMR, TFC, MPG ≥2 rates, and better 6-month infarct size, LV volumes and ejection fraction (by SPECT) with streptokinase vs. standard care. • No difference in 6-month MACE
Morales-Ponce *et al*[300] 2019	76	Tenecteplase 1/5th systemic dose (20ml)	Intracoronary glycoprotein IIbIIIa inhibitor (abciximab)	No	Pre-stent	Used in 34% of patients	• Higher TFC and lower rates of MPG ≥2 with tenecteplase vs. abciximab. • No difference in 4-month infarct size (by CMR).
Zhu *et al*[301] 2013 **ICTUS – AMI trial**	490	Urokinase 250 kU (20ml)	Intracoronary glycoprotein IIbIIIa inhibitor (tirofiban)	No	Pre-stent	Not reported	• No difference in rates of ST-segment resolution ≥70%. • Absolute % ST-segment resolution worse with urokinase vs. tirofiban

Table 1.3 *(part 2 of 2) Summary of randomised clinical studies of adjunctive intracoronary fibrinolysis, during primary. All studies reported that intracoronary low-dose fibrinolysis was not associated with significant bleeds.*

1.7.5 Intracoronary urokinase and up-front fibrinolysis before manual thrombectomy

The DISSOLUTION randomised clinical trial (delivery of thrombolytics before thrombectomy in patients with STEMI undergoing primary PCI) explored the effects of intracoronary urokinase prior to aspiration thrombectomy (n=102)[299]. Acute STEMI patients <12 hours from symptom onset, with TIMI 0 flow in the infarct-related artery on initial angiography were included. Up-front intracoronary urokinase (administered over 5 minutes) was associated with increased volume of thrombus aspirated with manual thrombectomy, compared to saline control. Up-front urokinase was also associated with a higher incidence of TIMI 3 flow at the end of PCI, increased rate of MPG ≥2 at the end of PCI, and increased rate of ST-segment resolution >70%[299]. At 6 months, MACE was less frequent in the urokinase group, driven by a reduction in rehospitalisation for heart failure (6% vs. 21%, log-rank p=0.044). The DISSOLUTION trial[299] had some limitations. First, it was an open-label study, so there was potential bias from cardiologists knowing whether the participants were receiving urokinase or saline. Second, non-invasive imaging to assess infarct size, or myocardial perfusion was not performed. To date, there are no other randomised trials of up-front intracoronary fibrinolysis, prior to manual thrombectomy, in STEMI patients with large thrombus burden.

The ICTUS-AMI trial (intracoronary tirofiban vs. urokinase as an adjunct to primary PCI in patients with acute STEMI)[301] compared adjunctive intracoronary urokinase to intracoronary glycoprotein IIbIIIa inhibitor (n=490). The adjunctive intervention was administered within 1 minute, immediately after restoration of infarct-related artery patency, pre-stent implantation. Even though there was no difference in the primary endpoint, (ST-segment resolution ≥70%), absolute % ST-segment resolution was worse in the urokinase group compared to the glycoprotein IIbIIIa inhibitor group[301].

1.7.6 Intracoronary prourokinase

The open-label randomised clinical studies of intracoronary prourokinase compared to volume-matched saline control[297 298], or compared to thrombus aspiration[261], during primary PCI, should be interpreted with some caution, because the administration of the intervention and analyses were performed by unblinded investigators.

Xiao *et al*[261] compared intracoronary prourokinase (10 to 20mg), which was administered distal to the lesion pre-stent implantation (n=38), to thrombus aspiration pre-stent implantation (n=33). STEMI patients <12 hours from symptom onset were included. IMR measured at the end of the PCI procedure was lower with prourokinase vs. thrombus aspiration (mean values: 28.2 vs. 33.6, p=0.005)[261]. At 12 months, LV ejection fraction assessed echocardiographically was higher with prourokinase vs. thrombus aspiration (58.1% vs. 55.2%, p=0.043) and infarct size assessed by SPECT was lower with prourokinase (18.6% vs. 22.7%, p=0.046)[261]. Moreover, 12-month MACE rates were lower with prourokinase vs. thrombus aspiration (45% vs. 70%, p=0.034)[261].

Geng *et al*[297] included STEMI patients <12 hours of symptom onset. Intracoronary prourokinase (10mg) was administered distal to the lesion over 3 minutes, pre-stent implantation (n=118), or for the control group 10ml of saline was administered in the same way (n=112). Peak levels of troponin I (surrogate marker of infarct size) were lower in the prourokinase group (mean: 21.5ng/L vs. 23.1ng/L, p=0.017)[297]. More patients in the prourokinase group had ST-segment resolution >70% than in the control group (89% vs. 79%, p=0.047)[297]. Myocardial perfusion, evaluated by contrast echocardiography 7-days after primary PCI, revealed higher myocardial blood flow with prourokinase compared with controls[297]. At 6 months, MACE rates did not differ between prourokinase vs. control groups (9% vs. 13%, p=0.434)[297].

External validation for the findings[297] was provided by Wang et al[298]. Acute STEMI patients <12 hours from symptom onset, were randomised to intracoronary prourokinase (20mg) administered proximal to the lesion over 3 minutes (n=92), or control, i.e.10ml saline administered in the same way (n=90)[298]. There were improvements in numerous surrogates of myocardial perfusion in the prourokinase group vs. control group, including: (i) mean TFC at the end of PCI (29.6 vs, 22.9, p=0.020), (ii) peak troponin I (mean: 52.2ng/L vs. 60.9ng/L, p=0.029), (iii) ST-segment resolution >70% (57% vs. 39%, p=0.017), and (iv) 6-month LV ejection fraction assessed by echocardiography (55.2 vs. 52.2, p=0.041)[298]. Interestingly, 6-month MACE rates were lower in the urokinase group compared to controls (11% vs. 22%, p=0.039)[298].

1.7.7 Intracoronary tenecteplase

Numerous case reports, or case series, have reported feasibility and efficacy with intracoronary tenecteplase or alteplase, during primary PCI, usually as a bail-out agent in STEMI patients with large thrombus burden and no-reflow[308-312], but also as an adjunct to PCI in cases of thrombotic complications and no-reflow[313 314].

Morales-Ponce *et al*[300] explored associations between adjunctive intracoronary tenecteplase (one fifth of systemic dose, infused over 3 minutes, pre-stent implantation) vs. intracoronary glycoprotein IIbIIIa inhibitor therapy, during primary PCI (n=76). Angiography was repeated 48 hours later to assess TFC and MPG. There was no difference in the primary endpoint (infarct size assessed by CMR imaging at 4 months) between tenecteplase vs. glycoprotein IIbIIIa inhibitor groups (17.0g vs. 21.1g, p=0.22)[300]. However, final TFC was higher in the tenecteplase group (18.2 vs. 14.2, p=0.02) and the proportion of participants with final MPG ≥2 was lower with tenecteplase (68% vs. 90%, p=0.03)[300]. The study was limited by the open-label design, lack of a volume-matched placebo control group, and CMR imaging was not performed within the first week from admission to allow quantification of MVO[300]. However, the findings are consistent with the ICTUS–AMI trial[301] (section 1.7.5). A potential explanation for the findings[300 301] may be the competing fibrinolytic and procoagulant effects of thrombolytic drugs[288] (Figure 1.4).

The first randomised study of adjunctive intracoronary tenecteplase compared to volume-matched placebo during primary PCI was ICE T-TIMI 49 (intracoronary tenecteplase during balloon angioplasty to treat heart attacks)[296]. ICE T-TIMI 49 was an open-label, randomised controlled pilot trial, of intracoronary tenecteplase (4mg, over 2 minutes) administered before and after stent implantation (n=20) during primary PCI, compared to volume matched control (n=20). All patients received intravenous glycoprotein IIbIIIa inhibitors. Patients were eligible for inclusion if they were within 6 hours of symptom onset, and had TIMI flow ≤1 on the initial angiogram. The median ischaemic time for the population was relatively short (approximately 2 hours). The primary endpoint (percent diameter stenosis of the infarct-related lesion after the administration of study drug) did not differ between treatment groups, neither did MPG, or 30-day mortality[296]. In ICE T-TIMI 49, there was a greater incidence of hyperaemic flow measured using TFC <14 (a marker

of distal embolization)[127] with placebo vs. intracoronary tenecteplase, and participants were more likely to have reduction in TIMI thrombus grade after the first bolus of intracoronary tenecteplase compared to placebo[296]. The ICE T-TIMI 49 study was not powered for clinical outcomes and cardiologists were not blinded during study drug administration[296]. Furthermore, the optimal dose of intracoronary tenecteplase is unknown, and non-invasive imaging to measure myocardial perfusion was not performed.

1.7.8 Intracoronary alteplase and the T-TIME trial

To date, the largest randomised controlled trial of intracoronary thrombolysis is T-TIME[292]. In T-TIME, STEMI patients ≤6 hours from symptom onset, with an occluded artery, or thrombus-laden patent artery, on initial angiography, were randomised to receive intracoronary alteplase or placebo, pre-stenting. The rationale for administering the drug before stent implantation, was based on an analysis from the INFUSE-AMI trial[315]. The INFUSE-AMI trial compared intracoronary glycoprotein IIbIIIa inhibitor pre-stent implantation vs. no glycoprotein IIbIIIa inhibitor therapy, and manual thrombectomy vs. no thrombectomy, during primary PCI for anterior STEMI, in a 2 x 2 factorial design[316]. Patients with pre-stent TIMI 3 flow (n=175) had lower 30-day infarct size (assessed by CMR imaging), compared to patients with TIMI 2 flow pre-stent (n=47), or TIMI flow ≤1 pre-stent (n=68) (15.5% vs. 22.6% vs. 19.5%, $p<0.001$)[315]. The findings suggest that optimisation of microvascular perfusion pre-stent implantation may reduce infarct size in patients undergoing primary PCI[315].

T-TIME intended to randomise 558 patients to alteplase 20mg, alteplase 10mg, or volume matched placebo. However, following a prespecified futility analysis, the Sponsor discontinued enrolment on December 21st 2017 based on a recommendation from the Data and Safety Monitoring Committee.

The futility analysis was performed when 40% of patients had been randomised and followed-up to 3 months. Considering MVO extent on CMR imaging, each active treatment arm was compared to the placebo arm and if the conditional power for showing a benefit over placebo was less than 30%, the recommendation was to stop recruitment. The sample size calculation for the T-TIME trial was based on data from the British Heart Foundation funded MR-MI study (detection and significance of heart injury in STEMI) [149] [156]. For 80% power at a 5% level of significance to detect between-group mean differences

in MVO extent of 1.49%, assuming mean (± standard deviation [SD]) of 3.2 (±5.1%) for MVO extent in the placebo group, a minimum sample size of 186 per group was needed (558 patients randomised in total).

Of the 440 patients randomised, the primary endpoint (mean amount of MVO on CMR imaging performed 2 to 7 days post-STEMI) did not differ between 20mg alteplase (3.5%) vs. placebo (2.3%) groups (mean difference: 1.16% [95% CI: -0.08, 2.41%] p=0.32)[292]. There was also no difference in mean amount of MVO between alteplase 10mg (2.6%) vs. placebo (mean difference: 0.29% [95% CI: -0.76, 1.35%] p=0.74)[292]. There was no difference in clinical outcomes at 1-year between treatment groups (MACE rates with alteplase 20mg [10%] vs. placebo [11%], OR: 0.96 [95% CI: 0.45, 2.04]; MACE rates with alteplase 10mg [15%] vs. placebo, OR: 1.52 [95% CI: 0.76, 3.05])[302]. However, even though T-TIME was designed to study 1-year outcomes it was not powered to do so.

In the T-TIME trial[292], intracoronary alteplase was administered after low-pressure balloon inflation, or thrombus aspiration, but before stent implantation. Hypothetically, persisting impaired antegrade flow in the infarct-related artery might influence the effect of intracoronary alteplase. One might speculate that impaired antegrade flow may lead to inadequate drug delivery to the microcirculation.

1.7.9 Ongoing trials of intracoronary fibrinolysis

Three other randomised controlled trials of low-dose intracoronary lytic therapy are ongoing (Table 1.4). In the STRIVE trial[317] (adjunctive low-dose tPA in primary PCI for STEMI) (NCT03335839), intracoronary alteplase is being administered via a microcatheter routinely to enhance local drug deliver. Intracoronary alteplase is also being investigated in the OPTIMAL trial[272] (optimal coronary flow after PCI for MI) (NCT02894138). Intracoronary tenecteplase is being investigated in the RESTORE-MI trial[273] (restoring microcirculatory perfusion in STEMI) (ACTRN12618000778280). In all three trials the comparator intervention is volume matched saline or water, and patients with ischaemic time up to 12 hours are included. In RESTORE-MI[273] and OPTIMAL[272], the study intervention is administered at the end of PCI after stent implantation, and patients are eligible for inclusion if they have a post-stent IMR in the infarct-related artery >32 or >30 respectively. However, it is unclear if this risk-based selection strategy, which is targeting patients with invasive evidence of microcirculatory damage, could be selecting some

patients with irreversible microvascular damage, in whom intracoronary fibrinolytics might exacerbate haemorrhagic transformation of the infarct core.

1.7.10 Summary of evidence for intracoronary fibrinolysis during primary PCI

The quality of evidence supporting intracoronary low-dose fibrinolysis during primary PCI is variable. The findings reported by different studies are inconsistent. Differences might be related to variability in timing of fibrinolytic drug administration, i.e. pre- or post-stent implantation, and different fibrinolytic agents and doses used. However, all trials so far were negative on their primary endpoints.

The following uncertainties remain: (i) whether low-dose intracoronary tenecteplase (which is less procoagulant than alteplase[318]) administered at the end of PCI to patients with large thrombus burden and short ischaemic time (<4 hours) might be beneficial; (ii) in which patient sub-groups might intracoronary fibrinolysis potentially be harmful, for example those with more aggressive antithrombotic therapy, or those who already have extensive (possibly irreversible) microvascular damage. The findings from further analyses from the T-TIME trial[292] should help towards unravelling uncertainties.

Trial and registration number	Estimated enrolment	Fibrinolytic agent	Risk stratified enrolment based on IMR	Primary endpoint	Estimated completion
RESTORE-MI[273] ACTRN12618000778280	n=800 Patients within 12 hours of symptom onset	Tenecteplase 1/3 of weight based systemic dose	Yes IMR >32	Cardiovascular mortality and rehospitalisation for heart failure at 2 years	December 2023
STRIVE[317] NCT03335839	n=200 Patients within 6 to 12 hours of symptom onset	Alteplase 10mg or 20mg	No	Composite of post-procedural MPG ≤1 and distal embolization, 30 days post-STEMI	December 2020
OPTIMAL[272] NCT02894138	n=80 Patients within 12 hours of symptom onset	Alteplase 20mg	Yes IMR >30	Infarct size assessed 2 to 6 days, and 3 months post-STEMI.	April 2021

Table 1.4 *Ongoing randomised clinical trials of adjunctive intracoronary fibrinolysis during primary PCI.*

1.8 Hypotheses and project aims

The principle aim of this work is to gain greater insight into the invasive assessment of acute microvascular injury following primary PCI, and to provide mechanistic insights to better understand the effects of intracoronary alteplase on the microcirculation. The first part of the thesis aims to determine whether adjunctive intracoronary alteplase during primary PCI has any effect on acute microvascular function, assessed by thermodilution-derived invasive physiology parameters, and to evaluate whether the effect of intracoronary alteplase is related to TIMI flow grade immediately preceding study-drug administration. The second part of the thesis aims to investigate the utility of novel physiology parameters, namely RRR and the newly conceived temperature recovery time (TRT), for predicting MVO on CMR imaging. In addition, the utility of IMR, CFR, RRR, MPG and TFC will be compared for predicting MVO, myocardial haemorrhage and prognosis following acute STEMI. The utility of TRT for predicting prognosis will also be investigated. The body of work incorporated in this thesis principally involves prospective analyses from the T-TIME randomised clinical trial[292], as well as a retrospective analysis from the MR-MI observational study[149].

The specific aims of this thesis are as follows:

1) To evaluate the effects of intracoronary alteplase, according to sub-groups of TIMI coronary flow grade immediately preceding study drug administration, during primary PCI (Chapter 3).

2) To evaluate the effects of intracoronary alteplase during primary PCI on IMR, CFR and RRR (Chapter 4).

3) To compare IMR, CFR, RRR, MPG and TFC, measured at the end of primary PCI, for predicting MVO, myocardial haemorrhage and clinical outcomes (Chapter 5).

4) To investigate whether a newly conceived parameter (TRT) is associated with MVO and clinical outcomes following acute STEMI (Chapter 6)

The specific hypotheses investigated in this thesis are stated below:

1) In acute STEMI patients with TIMI 3 flow immediately preceding study drug administration, intracoronary alteplase is associated with less MVO and less myocardial haemorrhage on CMR imaging, compared to placebo (Chapter 3).

2) In acute STEMI patients with TIMI flow ≤2 immediately before study drug administration, there is no difference in MVO or myocardial haemorrhage on CMR imaging with intracoronary alteplase vs. placebo (Chapter 3).

3) Intracoronary alteplase administered pre-stenting, is associated with lower IMR, higher CFR and higher RRR at the end of primary PCI, compared to placebo (Chapter 4).

4) In the sub-group of patients with shorter ischaemic time (≤2 hours), intracoronary alteplase administered pre-stenting is associated with lower IMR, higher CFR and higher RRR at the end of primary PCI, compared to placebo (Chapter 4).

5) In the sub-group of patients with TIMI 3 flow immediately preceding study drug administration, intracoronary alteplase is associated with lower IMR, higher CFR and higher RRR at the end of primary PCI, compared to placebo (Chapter 4).

6) In the sub-group of patients with TIMI thrombus grade ≥3 immediately pre-study drug administration, intracoronary alteplase is associated with lower IMR, higher CFR and higher RRR at the end of primary PCI, compared to placebo (Chapter 4).

7) Higher IMR and TFC, and lower CFR, RRR and MPG at the end of primary PCI are associated with MVO and myocardial haemorrhage on CMR imaging, and are associated with increased rates of MACE, heart failure hospitalisation, and the combination of all-cause death/ heart failure hospitalisation (Chapter 5).

8) Lower RRR at the end of primary PCI is more closely associated with MVO and myocardial haemorrhage on CMR imaging, than lower CFR (Chapter 5).

9) Higher TRT at the end of emergency PCI is associated with MVO on CMR imaging and is associated with increased rates of MACE, and the combination of all-cause death/ heart failure hospitalisation (Chapter 6).

Chapter 2: Methods

2.1 The T-TIME angiographic study

2.1.1 Trial design and population

T-TIME was an investigator-initiated, double-blind, parallel group, dose-ranging, phase 2 randomised clinical trial of low-dose adjunctive intracoronary alteplase during primary PCI (Clinical trial Identifier: NCT02257294). It was funded by the Efficacy and Mechanism Evaluation program of the National Institute for Health Research. The trial design is summarised in Figure 2.1.

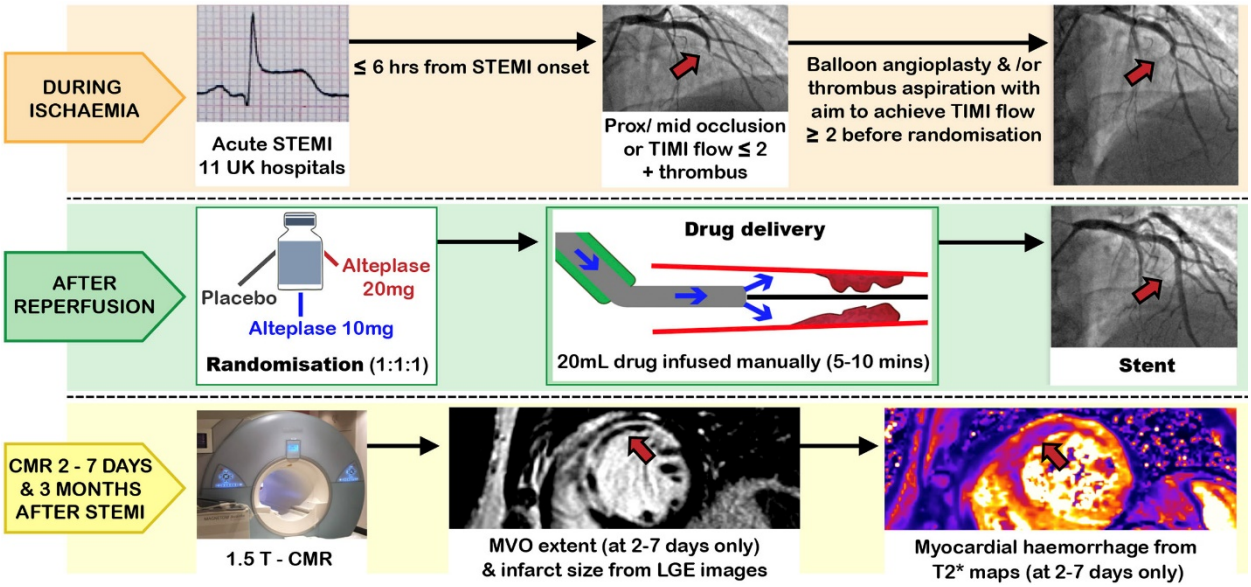

Figure 2.1 *Overview of the T-TIME trial design.*

From 17th March 2016 to 21st December 2017, patients with acute STEMI were enrolled by 11 U.K. hospitals. Screening and study drug administration occurred during standard care primary PCI. Enrolment was based on prospective assessments of eligibility criteria, operator experience and logistical considerations.

Patients were eligible for participation in the T-TIME trial if they presented with persistent ST-segment elevation or recent left bundle branch block, ≤6 hours from symptom onset and with an occluded infarct-related artery, or TIMI coronary flow grade 1 or 2, with TIMI thrombus grade ≥2. The rationale for limiting the ischaemic time to ≤6 hours was to

include patients with salvageable myocardium[319]. Other key inclusion criteria included radial artery access, and an occlusion of the proximal, or mid-segment of a major coronary artery, i.e. right coronary artery (RCA), left anterior descending (LAD), or circumflex (Cx). The rationale for including patients with impaired antegrade flow at initial angiography, in the presence of at least TIMI grade 2 thrombus, was to select patients at increased risk of distal embolization[320] and no-reflow.

An additional inclusion criteria for the angiographic study analysis of the T-TIME trial was the availability of a fluorostore or cine image, to allow TIMI coronary flow grade assessment immediately preceding study drug delivery.

Key exclusion criteria included: a functional collateral supply to the infarct-related artery (Rentrop grade[321] ≥2, i.e. at least partial filling of the infarct-related artery by the collateral vessel) and cardiogenic shock. The other exclusion criteria are shown in Figure 2.2. A screening log was prospectively completed to document the reasons for participation or not.

Seven sites recruited patients outwith, in addition to within “normal working hours” (09:00 to 17:00 on a weekday), i.e. Glasgow, Southampton, Leeds, Manchester, London, Middlesbrough and Wolverhampton. The other 4 sites recruited patients during “normal working hours” only (Leicester, Liverpool, Newcastle and Edinburgh).

- Normal flow in the infarct-related coronary artery at initial angiography (TIMI flow grade 3).
- Functional coronary collateral supply (Rentrop grade 2/3) to the infarct-related artery.
- Previous infarction in the infarct-related artery (known or suspected clinically, e.g. wall motion abnormality revealed by echocardiography).
- Cardiogenic shock (Killip Class IV), or multivessel PCI intended before the day 2-7 CMR scan.
- Estimated body weight <60kg, or comorbidity with expected survival <1 year.
- Pacemaker, implantable defibrillator, or contra-indication to contrast-enhance CMR imaging.
- Known impaired renal function (estimated glomerular filtration rate <30ml/min).
- Significant bleeding disorder either presently or within the past 6 months.
- Patient with current concomitant oral anticoagulation therapy (international normalised ratio >1.3), including apixaban, dabigatran and rivaroxaban.
- Any history of central nervous system damage (i.e. neoplasm, aneurysm, intracranial or spinal surgery).
- Severe hypertension (blood pressure >180/110 mmHg) not controlled by medical therapy.
- Major surgery, biopsy of a parenchymal organ, or significant trauma within the past 3 months (this includes any trauma associated with the current acute MI).
- Recent head trauma (<2 months), or prolonged cardiopulmonary resuscitation (>2 mins) in the past 2 weeks.
- Acute pericarditis and/ or subacute bacterial endocarditis.
- Acute pancreatitis, or active peptic ulceration.
- Severe hepatic dysfunction, including hepatic failure, cirrhosis, portal hypertension (oesophageal varices) and active hepatitis.
- Arterial aneurysm and known arterial/ venous malformation.
- Neoplasm with increased bleeding risk, or any known history of haemorrhagic stroke.
- Known history of ischaemic stroke, or transient ischaemic attack in the preceding 6 months.
- Dementia, or incapacity/ inability to provide informed consent.
- Hypersensitivity to gentamicin, or natural rubber.
- Previous randomisation to this study, or participation in a study with an investigational drug, or medical device within 90 days prior to randomisation.
- Women of child bearing potential (i.e. pre-menopausal), or breast feeding.
- Immunosuppressive therapy at any time in the preceding 3 months. This would include corticosteroids (but not inhaled or topical), drugs used following transplantation (e.g tacrolimus, cyclosporine), anti-metabolite therapies (e.g. mycophenolic acid, azathioprine, leflunomide and immunomodulators including biologics (e.g. adalimumab, or etanercept) and disease modifying anti-rheumatic drugs. This list is not exhaustive.
- Active/ prophylactic treatment with oral/ parenteral antibiotic, antifungal, or antiviral therapy.
- Any anti-cancer treatment (excluding surgery as this is covered above) at any time during the preceding 3 months, including chemotherapy, radiotherapy, and treatment with biologics, such as Vascular Endothelial Growth Factor Receptor inhibitors (e.g. bevacizumab, pazopanib). This list is not exhaustive.
- Any significant concurrent, or recent condition(s) not listed above that in the opinion of the treating clinician would pose an additional risk to the patient.

Figure 2.2 *Exclusion criteria for the T-TIME trial*

2.1.2 Consent process

Screening and study drug administration occurred during standard care primary PCI. Only patients who were sufficiently well to understand the information about the study, were eligible to participate. Patients who met the eligibility criteria (section 2.1.1) underwent verbal witnessed assent to participate, in the catheterisation laboratory. The investigator who obtained verbal assent signed the short consent form (Appendix 2). Since these were all emergency patients, it would have been inappropriate to delay treatment in order to obtain full written informed consent in the catheterisation laboratory. Written informed consent was obtained on the ward, within 24 hours of the hospital admission. The patient information sheet and a blank consent form, for patients to sign, is shown in Appendix 3.

Patients were informed that they could withdraw from the study at any time. Clinicians could withdraw patients if knowledge of the treatment was absolutely necessary for further management of the patient. Ethical approval for the study (reference: 13-WS-0119) was obtained from the West of Scotland Research Ethics Service (Appendix 1). The trial was conducted in accordance with the Declaration of Helsinki[322].

2.1.3 Standard of care for primary PCI

Standard care for initial coronary reperfusion was with balloon angioplasty, or aspiration thrombectomy for thrombus-containing lesions, according to the operator's discretion. If balloon angioplasty was performed the balloon was sized according to the expected lumen diameter, and inflated to a relatively low pressure (e.g. 4 to 8 atmospheres), to minimise thrombus embolization. Use of an under-sized balloon was not recommended, as this could leave an obstructive (thrombotic) stenosis, which is a substrate for re-occlusion during study drug preparation and administration. Post-dilatation of implanted stents was routine. Anti-thrombotic therapy included oral anti-platelet drugs, i.e. 300mg aspirin, and either 180mg ticagrelor, 60mg prasugrel, or 600mg clopidogrel, and intravenous heparin (5000 IU, or as per standard practice) at the first medical contact.

The target activated clotting time (ACT) was 250 to 300s, which was to be achieved prior to study drug administration. In line with optimal standard care, the ACT was checked every 20 minutes, to ensure therapeutic anticoagulation. As per clinical guidelines[1], bail out glycoprotein IIb/IIIa inhibitors could be used in the event of angiographic evidence of

large thrombus, slow- or no-reflow, or a thrombotic complication, although there is no evidence from randomised clinical trials for this strategy. In line with clinical guidelines, multivessel PCI in the acute setting was not recommended[1].

2.1.4 Randomisation and blinding

Patients were randomised using an interactive voice response-based system. The randomisation sequence was computer generated, using the method of randomised permuted blocks of length 6. Randomisation was stratified by the location of MI (anterior vs. non-anterior). The allocation sequence was on a 1:1:1 basis, between placebo and the alteplase groups. The researchers, clinical staff and patients were blinded to the treatment group allocation. Boehringer-Ingelheim U.K Ltd. Provided the study drugs (alteplase 10mg and 20mg), matched placebo, and sterile water for injection, but had no other involvement in the conduct of the study.

2.1.5 Intervention and protocol for study drug administration

Two doses of alteplase (10mg [one tenth of standard systemic dose] and 20mg [one fifth of standard systemic dose]) were compared to placebo. At the time of the study design, there were no other published randomised clinical trials comparing intracoronary alteplase to placebo, to help inform the lowest effective intracoronary dose of alteplase. In a previous open-label study, that compared intracoronary streptokinase to standard care, there was an improvement in IMR in the group that received one sixth of the standard systemic dose of intracoronary streptokinase, administered at the end of the primary PCI procedure[260]. Given that streptokinase is less effective than alteplase, a 10-fold lower dose (relative to standard systemic dose) was adopted as the lowest dose of alteplase in the T-TIME trial.

The reason for selecting alteplase in preference to streptokinase is because alteplase is fibrin specific. Streptokinase has a higher bleeding risk than fibrin-specific fibrinolytics, such as alteplase and tenecteplase[323].

Alteplase (a second generation lytic) was selected in preference to tenecteplase (a third generation lytic), because alteplase has a shorter plasma half-life, compared to tenecteplase[324 325]. Therefore, there was potentially less possibility of harmful remote bleeds with alteplase, and if a bleeding serious adverse event did occur this could theoretically have been worse with tenecteplase than with alteplase. Furthermore,

tenecteplase is more expensive than alteplase, so is less transferable to the National Health Service[324 325]. The indicative price of one vial of tenecteplase 10000 units is £602.70[325]. The indicative price of one vial of 10mg alteplase is £172.80, and that of one vial of 20mg alteplase is £259.20[324].

The reason for adopting intracoronary rather than systemic administration of the study drug, was to minimise the possibility of harmful remote bleeds. Systemic administration of reduced dose alteplase would have diluted the drug to ineffective concentrations at the site of the coronary lesion.

The protocol for study drug administration is provided in Appendix 4. The trial protocol encouraged achieving TIMI flow grade ≥2, using balloon angioplasty and/ or aspiration thrombectomy, prior to randomisation of participants. After randomisation, the allocated intervention was prepared, during which TIMI flow grade in the infarct-related artery deteriorated in a minority of patients, prior to administration of the study drug. The allocated intervention (alteplase 20mg, alteplase 10mg, or placebo) was administered before stent implantation. The rationale for administering the study drug before stent implantation was to potentially reduce the amount of thrombus present that could embolize distally during stent implantation, and to treat distal microvascular thrombosis, with the aim of achieving reperfusion to the microcirculation as early as possible.

First, the study drug powder needed to be reconstituted in solvent (sterile water), made up to a volume of 20ml. Second, using either a flushed intracoronary catheter, or a selectively engaged guide catheter, the 20ml volume of study drug was infused into the infarct-related artery, over 5 to 10 minutes, proximal to the lesion. Finally, the catheter was flushed with normal saline to ensure that all of the study drug had been administered.

The infusion period of up to 10 minutes was adopted based on pragmatic grounds. Bolus administration may have potentially limited first pass binding of alteplase with fibrin molecules, however, during slow manual infusion real-time thrombolysis had potential to further expose fibrin molecules within the thrombus as lysis progresses. Furthermore, the ‘deep-tissue’ beta half-life of alteplase is approximately 40 minutes[326], therefore persistent

local lysis could potentially occur within the distribution of the infarct-related artery after study-drug administration.

2.1.6 Angiogram acquisition and analysis

The angiogram acquisition protocol (Appendix 4) required stored fluoroscopy of study drug administration, to enable verification by the core laboratory that the guide catheter was selectively engaged in the infarct-related artery during study drug delivery. This also enabled core laboratory evaluation of TIMI coronary flow grade immediately preceding, and immediately after, study drug delivery. Participants were grouped according to TIMI flow grade (≤2 vs. 3) in the infarct-related artery immediately preceding study drug administration.

A deidentified copy of each angiogram was transferred to the Glasgow core laboratory by secure web-upload, or exceptionally by compact disc via courier. All the angiogram parameters were analysed prospectively using QAngio® XA Medis Suite software (Medis, Leiden, Netherlands). The angiograms were prospectively analysed by one researcher, who was blind to treatment allocation (i.e. alteplase 10mg, alteplase 20mg, or placebo) and blind to CMR, ECG, or coagulation data. A second read was performed by an experienced interventional cardiologist, involving combined review with the first investigator. Discrepancies were resolved by consensus agreement between the first and second reviewers, or where discrepancies remained consensus was reached after discussion with a third reviewer. The final angiography data, from the Glasgow core laboratory were submitted to the data coordination centre prior to database lock, after which no changes to the data were possible.

Inter-rater variability for TIMI coronary flow grade, TFC, and MPG at the end of the PCI procedure were evaluated in 440 patients. Inter-rater variability for TIMI coronary flow grade immediately preceding study drug delivery was evaluated in 65 consecutive patients. Inter-rater variability for TIMI thrombus grade immediately preceding study drug administration was evaluated in 90 patients. Intra-observer variability was evaluated after a 3-month interval, in 50 patients.

Identification of the cine/ fluorostore showing study drug delivery

When a fluorostore or cine image was available showing a thrombectomy catheter positioned proximal to the infarct-related lesion, or a selectively engaged guide catheter without contrast injection, this was taken to be study-drug administration. Confirmation was sought by establishing that the time gap between the adjacent fluorostore/ cine images was sufficiently long for study drug administration. Further confirmation was sought by verifying that the fluorostore/ cine image identified was consistent with information documented on the electronic case report form, regarding the method of study drug delivery and duration over which the study drug was infused.

When a fluorostore or cine image was not available during study drug administration, consecutive fluorostore/ cine images separated by a time gap sufficient for study-drug delivery, prior to stent deployment were identified. These were taken as the fluorostore/ cine images corresponding to immediately preceding and immediately after study drug delivery. Confirmation was sought by checking for consistency with information documented on the electronic case report form.

Multivessel disease

For the assessment of multivessel disease, a diseased non-culprit vessel was defined as having >50% stenosis in a major coronary artery, by angiographic visual assessment.

TIMI coronary flow grade

The TIMI coronary flow grade[327] (Figure 2.3) was assessed in the infarct-related artery at 4 time points: (i) on the initial image pre-reperfusion; (ii) immediately preceding study drug administration; (iii) immediately after study drug administration; and (iv) at the end of the PCI procedure after stent optimisation.

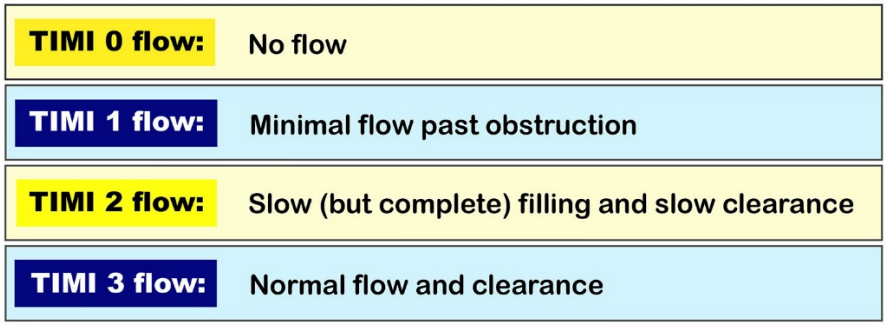

Figure 2.3 *Definitions for TIMI coronary flow grades*

TFC

The corrected TFC was assessed in the infarct-related artery at the end of the PCI procedure after stent optimisation. The first frame used for TFC was the frame when contrast entered the origin of the artery, and when the following criteria were met: (i) column of fully concentrated contrast extending across the entire width of the origin of the artery; (ii) contrast touching both borders of the artery's origin, and; (iii) antegrade motion of contrast. The last frame used for TFC was when contrast entered the end-point branch of the infarct-related artery, as specified in the original TFC description[120]. For the RCA the distal landmark was the first branch arising from the posterior lateral extension of the RCA after the origin of the posterior descending artery. For the Cx artery, the distal landmark was the endpoint of the marginal branch with the longest total distance along which contrast travels in the Cx system and yet passes through the infarct-related lesion. For the LAD artery, the distal landmark was the distal-most branch of the LAD, conventionally at the apex of the heart. The frame count for the LAD was divided by 1.7 to correct for the relatively longer vessel length.

MPG

MPG was evaluated for the infarct-related artery, at the end of the PCI procedure after stent optimisation. The definitions used to evaluate MPG[328] are shown in Figure 2.4.

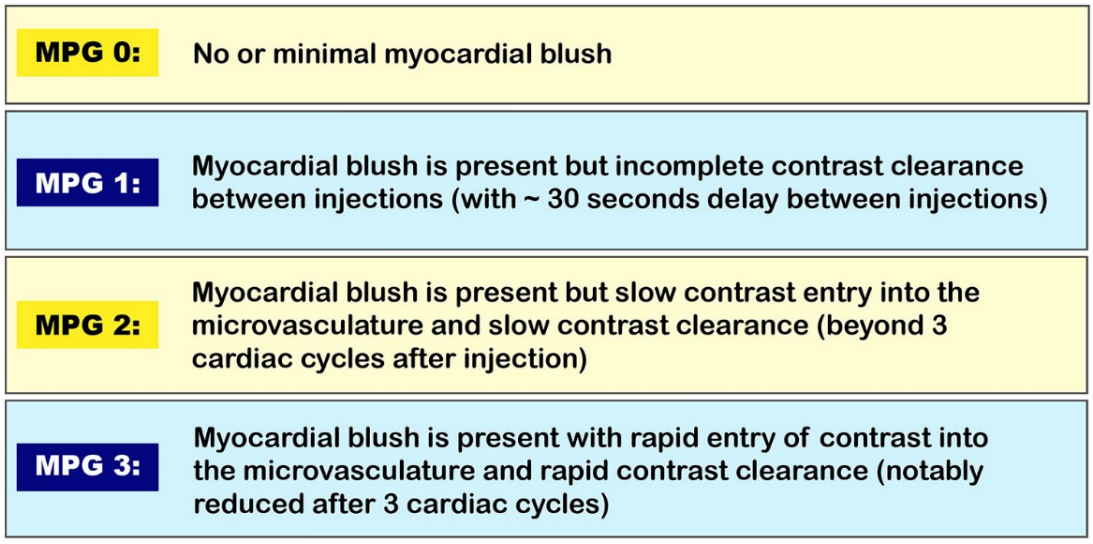

Figure 2.4 *Definitions for myocardial perfusion grades (MPG)*

TIMI thrombus grade

The TIMI classification of thrombus grade is the most widely used angiographic classification of coronary thrombus[329]. TIMI thrombus grade[330] (Figure 2.5) was evaluated

in the infarct-related artery on the initial image pre-reperfusion, immediately preceding study drug administration and at the end of the PCI procedure after stent optimisation.

A limitation of the TIMI thrombus grade classification is that the highest score, i.e. grade 5, is a totally occluded vessel, consequently the actual amount of thrombus relative to vessel diameter is unknown, yet the assumption of the TIMI scale is that grade 5 represents the highest thrombus burden. With the intent of overcoming this limitation, a modification was introduced in 2007 that incorporated insertion of a guidewire or 1.5mm balloon for crossing and recanalising the occlusive grade 5 thrombus, to improve determination of the amount of thrombus[331]. On balance the TIMI thrombus grade classification shown in Figure 2.5 was used, because it is universally accepted[329] and was user-friendly for the interventional cardiologists making decisions about whether or not a patient met the eligibility criteria for the T-TIME trial, which was important for facilitating recruitment.

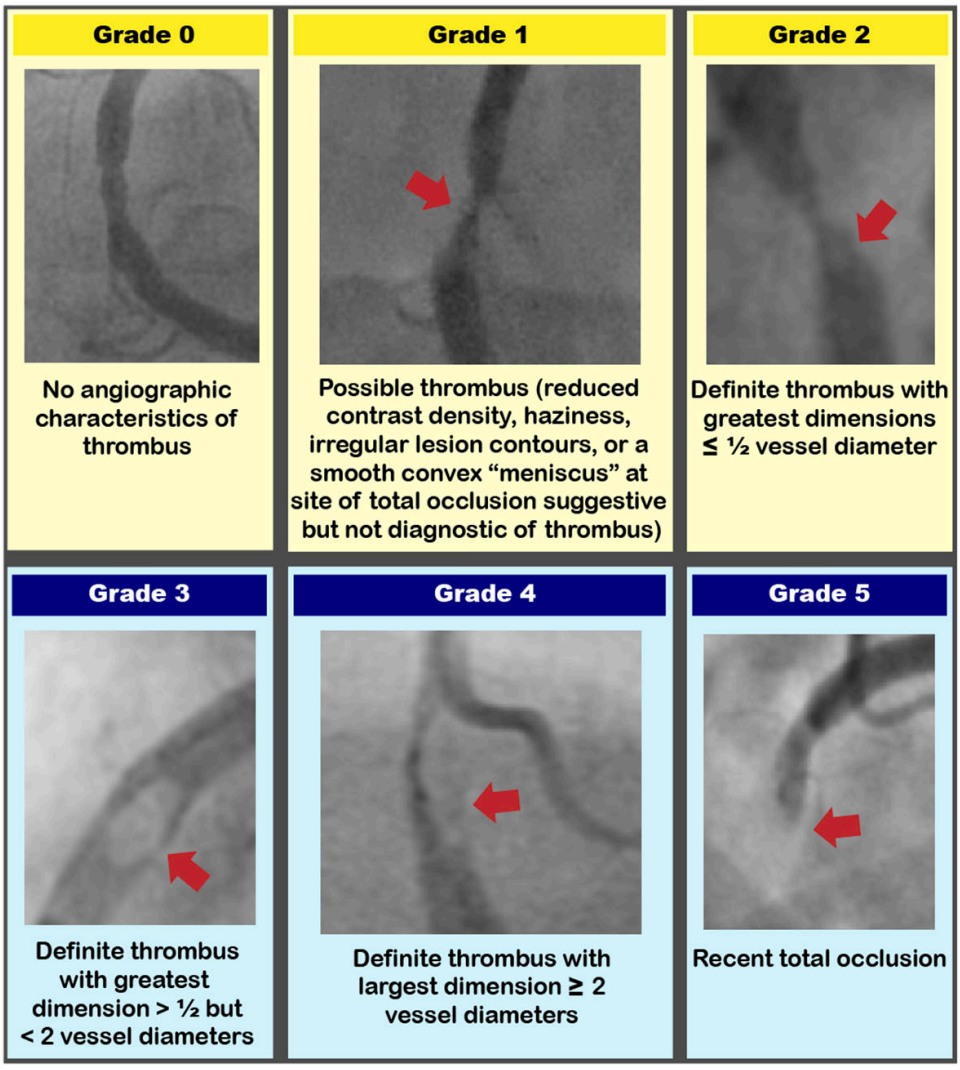

Figure 2.5 *Definitions for TIMI thrombus grades*

Quantitative coronary angiography

For quantitative coronary angiography (QCA), catheter calibration was performed using QAngio® XA Medis Suite software (Medis, Leiden, Netherlands), using information on the catheter size (6 or 5 French) documented in the electronic case report form. An angiographic projection perpendicular to the long axis of the vessel was used to avoid foreshortening. After selection of the end-diastolic frame, where the coronary segment was fully opacified with contrast, the QCA software measured different parameters, by automatic vessel edge detection algorithms. The parameters recorded included reference vessel diameter (average diameter of the coronary vessel assumed without atherosclerotic disease) and stent length.

2.1.7 LVEDP

Measurement of LVEDP was recommended in the T-TIME protocol, but was not mandatory, so it was measured at the discretion of the interventional cardiologist. The measurement of LVEDP occurred at the end of the cardiac catheterisation procedure, by positioning the right coronary catheter in the LV. LVEDP was then obtained from the LV pressure waveform at the point when the slope of the ventricular pressure upstroke changes, coinciding with the R wave on the ECG (Figure 2.6). The average LVEDP from five LV pressure waveforms was recorded.

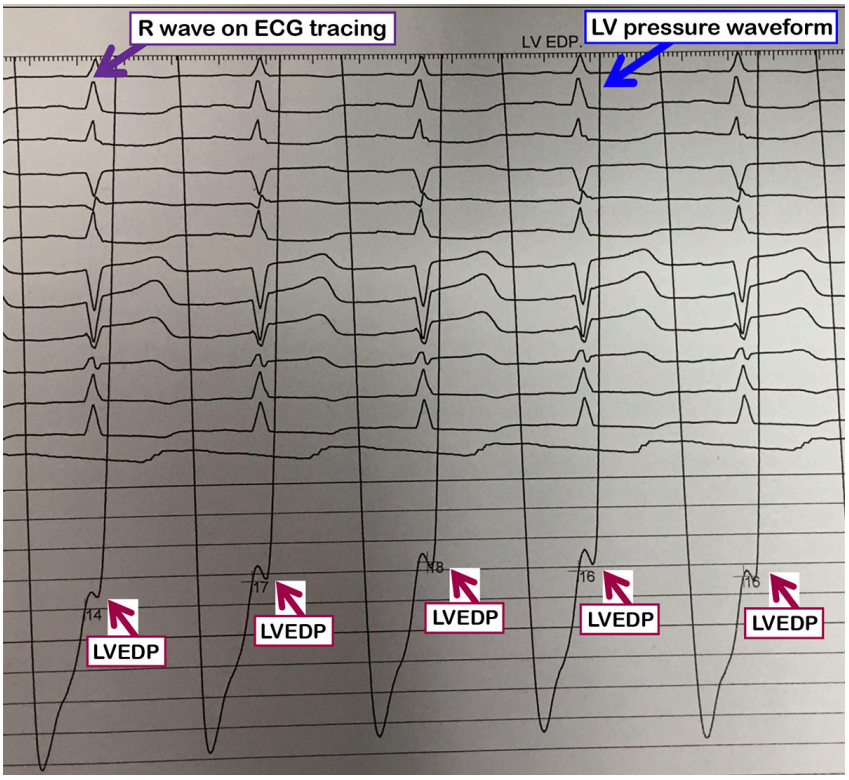

Figure 2.6 *LVEDP measurement*

2.1.8 ECG acquisition and analysis

The absolute percentage ST-segment resolution on 12-lead ECGs obtained 60 minutes after reperfusion, compared to pre-reperfusion were calculate centrally by the University of Glasgow ECG core laboratory, which is certified to ISO 9001: 2008 standards as a UKAS accredited organisation. The results from the ECG analyses, were submitted to the data coordination centre prior to database lock.

2.1.9 CMR acquisition and analysis

CMR was performed at 1.5 Tesla. CMR scans were acquired during the index hospitalisation (2 to 7 days post-STEMI), and 3 months later. MVO and myocardial haemorrhage were reported from the 2 to 7 day CMR scans. The other CMR parameters were reported from the 2 to 7 day and 3-month CMR scans. Deidentified copies of each CMR scan was transferred to the Glasgow core laboratory by secure web-upload, or when that was not feasible, then by compact disc via courier. The CMR scans were analysed using QMass Medis Suite MR software (Medis, Leiden, Netherlands). After the CMR scans underwent initial analysis by one investigator who, was blind to the angiographic findings and treatment allocation, there was a second read by a cardiologist with level 3 CMR certification (who was also blind to treatment allocation), involving combined review with the first investigator. Discrepancies were resolved by consensus agreement. The final CMR data, from the Glasgow core laboratory were submitted to the data coordination centre prior to database lock.

The CMR imaging protocol followed a standard operating procedure (Appendix 5) that included cine CMR imaging with steady-state free precession, T1-mapping, T2-mapping, T2*-maps and LGE imaging. The scan acquisitions were spatially co-registered and included different slice orientations to enhance diagnostic confidence.

Microvascular obstruction

To identify MVO, LGE CMR imaging 2 to 7 days post-STEMI was performed, and MVO was defined as a dark zone on early gadolinium enhancement imaging 1, 3, and 7 minutes after gadolinium-based contrast injection that persisted within an area of LGE at 10 to 15 minutes[89 332]. A difference in signal intensity of at least x 2 SD between the hypointense core of MVO and surrounding hyperintense zone of infarction without MVO was required. The myocardial mass of the dark zone was quantified by manual delineation and expressed

as % of LV mass. The presence or absence of MVO was also recorded. The appearance of cine and LGE images in orthogonal planes (long axis and short axis images), thinning of the myocardium, as well as oedema and T2*-weighted imaging helped discriminate MVO from other pathological processes which may mimic MVO post-infarction.

Myocardial haemorrhage

T2*-weighted CMR imaging was used to asses infarct zone haemorrhage, 2 to 7 days post-STEMI. A region of reduced signal intensity within the infarcted area, with a T2* value of <20ms was considered to confirm the presence of myocardial haemorrhage[15 26 333 334]. This area was manually delineated and expressed as a percentage of the total LV mass. The contours of the LV were delineated with computer assisted planimetry on the raw T2* image and the last corresponding T2 raw image, with echo time of 55ms[335]. Contours were then copied onto the colour-encoded spatially co-registered maps and corrected where necessary by consulting the steady-state free precession cine images. The presence or absence of myocardial haemorrhage was also recorded.

Infarct size

Infarct size was assessed at 3 months post-STEMI, since infarct remodelling is substantially complete by 3 months post-STEMI[336]. Infarct size was also assessed at 2 to 7 days post-STEMI. The presence of an acute infarction was established based on abnormalities in cine wall motion, rest first pass myocardial perfusion, and LGE imaging. Acute infarction was considered present only if LGE was confirmed in 2 imaging planes, i.e. on both axial and long axis acquisitions. The myocardial mass of late gadolinium was quantified using computer assisted planimetry. The territory of infarction was delineated using a signal intensity threshold of >5 SD above a remote reference region[337 338] and expressed as a percentage of the total LV myocardial mass.

LV ejection fraction and volumes

LV end-diastolic or systolic volumes and LV ejection fraction were quantified using automated planimetry, at 2 to 7 days post-STEMI and 3 months post-STEMI. The borders were assessed visually. Papillary muscles were not included. The LV apex was included.

2.1.10 Coagulation blood sampling and analysis

Peripheral intravenous blood samples were measured when site logistics permitted. The sampling-time points were 0, 2 and 24 hours post-PCI. Blood samples were centrifuged locally, with plasma separated and frozen within 2 hours of sampling. Frozen samples were

then transported on dry ice for central laboratory analysis, at the department of Haematology at the Glasgow Royal Infirmary. Plasma samples were stored at -80ºC until analysis. The final coagulation data, were submitted to the data coordination centre prior to database lock. Plasminogen was measured, because it's depletion correlates with systemic fibrinolysis[339]. Fibrin D-dimer was measured, as it is a product of fibrin lysis[340]. Prothrombin fragment F_{1+2} was measured, as it indicates thrombin generation and thrombosis[341] and correlates with the procoagulant effects of fibrinolytics[342]. Standard laboratory assays: high sensitivity Fibrin D-dimer by latex immunoassay[340], and plasminogen activity by chromogenic assay[339], were performed on an IL TOP700 analyser using HemosIL® reagents (Instrumentation Laboratory Company, Bedford, U.S). The prothrombin fragment F_{1+2} antigen level ELISA (enzyme-linked immunosorbent assay) was performed on a TECAN sunrise spectrophotometer (Labtech International Ltd. U.K.), using the Enzygnost F1 + 2 Mono (Siemens, Marburg, Germany) commercially available kit.

2.1.11 Trial management

The Robertson Centre for biostatistics within the Glasgow Clinical Trials Unit provided the trial-specific electronic data collection system, and functioned as an independent coordination centre for randomisation and data management. The trial was co-sponsored by the Glasgow and Clyde Health Board and the University of Glasgow. The sponsor undertook feasibility assessments at each site. The independent Data and Safety Monitoring Committee, had responsibility for potentially recommending early discontinuation of the trial due to a safety concern or futility, and met before the enrolment began, and twice again during the active phase of the trial. The funder required an interim analysis for futility and also specified the criteria.

2.2 The T-TIME physiology sub-study

2.2.1 Study design, population and ethical approval

The T-TIME physiology sub-study was a pre-defined, prospective, sub-study, within the main trial, and it was funded by a British Heart Foundation clinical PhD fellowship. It was approved by the West of Scotland Research Ethics Service (reference: 13-WS-0119, Appendix 1) and complied with the Declaration of Helsinki[322].

Patients with STEMI were eligible for inclusion in the physiology sub-study if they fulfilled the eligibility criteria for the main T-TIME trial (section 2.1.1 and Figure 2.2). Patients were recruited to the physiology sub-study at the same time, and in the same manner as to the main T-TIME trial (section 2.1.2). Every centre that recruited patients into the main T-TIME trial was invited to participate in the physiology sub-study. Three sites agreed to participate (Glasgow, Leeds and Wolverhampton) and all 3 had prior experience of clinical research using thermodilution-derived coronary physiology measurements. Enrolment into the physiology sub-study within the 3 designated sites was at the discretion of the operating cardiologists, based on operator experience and logistical considerations. Two of the sites (Glasgow and Leeds) also enrolled patients into the physiology sub-study outwith "normal working hours", i.e. outside 09:00 to 17:00 on a weekday. The dates of enrolment into the physiology sub-study were from 27th August 2016 to 17th December 2017.

2.2.2 Thermodilution data acquisition and analysis

The steps for thermodilution data acquisition are detailed in the standard operating procedure (Appendix 6). The coronary physiology data were acquired at the end of the PCI procedure, i.e. after stent implantation and post-stent optimisation.

Pressure wire preparation

A PressureWire™ Certus™ pressure- and temperature-sensitive guidewire (Abbott, Vascular, California, U.S.A) was calibrated outside the body. Two hundred micrograms of intracoronary nitroglycerin was administered to the infarct-related artery, to minimise effects of potential arterial spasm on the readings. The pressure- and temperature-sensitive guidewire was equalised to Pa, with the pressure sensor at the ostium of the guide catheter. The wire was advanced down the infarct-related artery, so that the distal sensor (3cm from

the wire tip) was in the distal third of the artery. Care was taken to ensure that all radiographic contrast was flushed out of the guide catheter with saline, because radiographic contrast can induce submaximal hyperaemia[343], which would have affected physiology measurements intended to be made during resting conditions. Meticulous attention was taken to ensure the guide catheter was appropriately engaged, and only guide catheters without side holes were used.

Thermodilution and hyperaemia

Using thermodilution, the Tmn of 3ml bolus of room temperature saline, injected briskly into a flushed catheter, was measured in triplicate at rest and during steady state hyperaemia. Caution was taken to ensure that the PressureWire^{TM} Certus^{TM} (Abbott, Vascular, California, U.S.A) was positioned similarly in the coronary artery on repeated measurements. In a previous study, the distance between the wire temperature-sensor and the guide tip was not significantly correlated with the mean hyperaemic Tmn[166]. Simultaneous measurements of mean Pa and Pd were made. Hyperaemia was induced by infusion of intravenous adenosine (140µg/kg/min), which is an extracellular signalling molecule that causes endothelium-independent coronary vasodilatation [344 345], primarily through activation of A_{2A} receptors[346]. When the response to adenosine was inadequate (i.e. fall in blood pressure <20% of resting value, rise in heart rate <10% from baseline, or no widening of pulse pressure), the adenosine infusion was increased to 210 µg/kg/min. Reproducibility of IMR was assessed in 13 patients, and reproducibility of CFR and RRR was assessed in 9 patients. Duplicate measurements were made by either continuing the adenosine infusion and repeating 3 additional hyperaemic Tmn measurements, or stopping the adenosine infusion to repeat 3 resting Tmn measurements then restarting the adenosine infusion to repeat 3 hyperaemic Tmn measurements.

Blinding

To mitigate the possibility of bias through disclosure of coronary physiology results, the operators were blinded by obscuring the display of the RadiAnalyzer^{TM}Xpress screen, i.e. turning it 180^{o}. The research fellow, or physiology technician quality assured the data acquisition, and documented the site reported IMR measurements, from the RadiAnalyzer^{TM}Xpress console, on a catheter laboratory worksheet (Appendix 7).

Analysis and Coroventis software

De-identified study number labelled files, containing the temperature- and pressure-wire recordings from Leeds, or Wolverhampton, were saved locally and then sent to the

Glasgow core laboratory for analysis. The coronary physiology data was analysed offline, using Coroventis software (Coroventis Research AB, Uppsala, Sweden). An example of coronary physiology data displayed on Coroview (Coroventis Research AB, Uppsala, Sweden) is provided in Figure 2.7.

Evaluation of the coronary physiology data was performed prospectively, by an investigator blind to treatment group allocation (i.e. placebo, alteplase 10mg, or alteplase 20mg), and blind to CMR, ECG, or coagulation data. The coronary physiology data were subject to a second read by an investigator, experienced in coronary physiology, involving combined review with the first investigator. Final data were established by consensus agreement between the two investigators. The final coronary physiology data, from the Glasgow core laboratory were submitted to the data coordination centre prior to database lock. Inter-rater variability for IMR, RRR and thermodilution waveforms was assessed in 30 consecutive patients, by a third investigator, who was blinded to all other data, and treatment group allocation. Intra-observer variability was assessed from 30 consecutive patients after a 3-month interval.

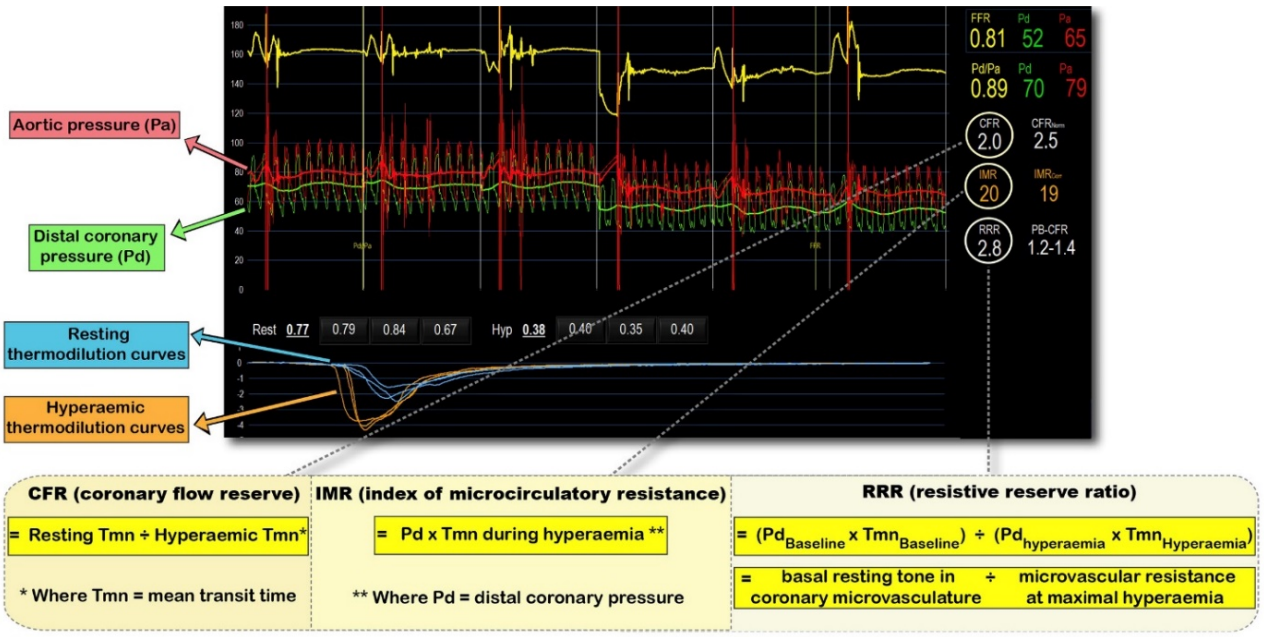

Figure 2.7 *An example of physiology data, displayed on Coroview (Coroventis Research AB, Uppsala, Sweden). Thermodilution curves are obtained in triplicate at rest (blue) and at hyperaemia (orange).*

2.2.3 CFR analysis

To quantify CFR, resting Tmn was divided by hyperaemic Tmn[167]. A threshold of 2.0 was used to dichotomise CFR in regression analyses, because CFR ≤2.0 is considered abnormal, based on published literature[149].

2.2.4 IMR analysis

The definition of IMR was as follows: Pd x Tmn during hyperaemia[140]. At the end of primary PCI, when IMR was measured, there was no residual epicardial stenosis in the infarct-related artery, therefore IMR correction with wedge pressure[143], or Yong's formula[145] was not required. Because IMR is a measure of the minimal achievable microvascular resistance[140], the Pd value used in the IMR equation should be taken when there is stable maximal hyperaemia. When analysing thermodilution data, the IMR value can change, by varying the position of the Pd marker. Therefore, care was taken to position the Pd marker at the lowest stable Pd/Pa, for core lab evaluation of IMR. An IMR >40, or IMR >32, was used to dichotomise IMR in multivariable analyses. This is because an IMR >40, or ≥32 predicts all-case death or heart failure hospitalisation[16], and is being used to select patients for clinical trials[255] (Deferred stenting in STEMI patients [NCT03581513][271], RESTORE-MI [ACTRN12618000778280][273]). Moreover, an IMR >32 predicts worse recovery of LV function after acute STEMI[158].

2.2.5 RRR analysis

The RRR measures the ability of the coronary microcirculation to change from baseline to minimum resistance, in response to a pharmacological hyperaemic stimulus. i.e. the vasodilatory capacity. Therefore, RRR reflects the ability to achieve maximal hyperaemia.

Baseline resistance index (BRI) is used in the equation for calculating RRR. The BRI is a measure of resting tone in the coronary microcirculation and was calculated using the following previously validated equation[194]:

$$\mathbf{BRI = Pd_{Baseline} \times Tmn_{Baseline}}$$

To measure the ability of the coronary microcirculation to undergo vasodilatation in response to adenosine, the RRR was calculated as previously described:

$$\mathbf{RRR = BRI \div IMR}$$

Because there are no established thresholds for abnormal, or normal RRR, RRR was dichotomised by the median in multivariable regression analyses, which is the conventional approach taken by previous studies[197].

2.2.6 Thermodilution waveform analysis

A narrow unimodal waveform was defined as an acute reduction in temperature (duration <0.42s from the beginning of the temperature reduction to nadir temperature) followed by a rapid return to resting temperature[198]. A wide unimodal waveform was defined as a temperature drop to nadir >0.42s, followed by a gradual return to baseline temperature. A bimodal waveform was defined as having 2 distinct nadirs, with a valley deeper than 20% of peak temperature drop[198].

Chapter 3: Implications of impaired coronary flow on the effects of adjunctive intracoronary alteplase during primary PCI: the T-TIME angiographic study

3.1 Abstract

Background: Following STEMI, MVO confers an adverse prognosis. Persistently impaired flow (<TIMI 3) in the infarct-related artery is a surrogate for failed myocardial perfusion. The aim of this chapter was to evaluate the effects of intracoronary alteplase, according to sub-groups of TIMI coronary flow grade immediately preceding study drug administration, during primary PCI.

Methods: Patients with STEMI from 11 U.K. hospitals, presenting ≤6 hours from symptom onset, were randomised to alteplase 20mg, alteplase 10mg, or placebo, during primary PCI. The intervention was administered by infusion into the infarct-related artery, after initial flow restoration (by balloon angioplasty and/or thrombus aspiration), before stent implantation. Core laboratory evaluation of TIMI flow grade in the infarct-related artery was performed immediately pre- and post-study drug administration, and at the start and end of the procedure. MVO, myocardial haemorrhage, and infarct size were assessed by CMR imaging at 2 to7 days after STEMI. Follow up CMR occurred at 3 months.

Results: TIMI flow was assessed after first treatment (balloon angioplasty and/ or aspiration thrombectomy) immediately before study drug administration, in 421 participants (mean age 61 ± 10 years, 85% male), and was 3, 2, or 1, in 267, 134 and 19 participants, respectively. Patients with TIMI flow ≤2 pre-drug had a higher incidence of MVO with alteplase (alteplase 20mg [53.1%] and 10mg [59.5%] combined vs. placebo [34.1%]; OR: 2.47 [95% CI: 1.16, 5.22] p=0.018 [interaction: p=0.005]) and increased incidence of myocardial haemorrhage with alteplase (alteplase 20mg [53.1%] and alteplase 10mg [57.9%] combined vs. placebo [27.5%]; OR: 3.26 [95% CI: 1.44, 7.36] p=0.004 [interaction: p=0.001]). Patients with TIMI flow ≤2 pre-drug had a trend towards larger infarct size with alteplase (alteplase 20mg [32.3 ± 13.1%] and alteplase 10mg [32.0 ± 12.7%] combined vs. placebo [28.1 ± 15.5%]; estimated mean difference: 3.87 [95% CI: -0.34, 8.07] p=0.072 [interaction: p=0.026]). In participants with TIMI 3 flow pre-study drug, there were no associations between alteplase and MVO, myocardial haemorrhage or infarct size. There were no interactions between TIMI flow grade pre-drug, alteplase and CMR findings at 3 months.

Conclusion: Intracoronary alteplase was associated with increased presence of MVO and myocardial haemorrhage, in STEMI patients with impaired flow (<TIMI 3) in the infarct-related artery immediately before study drug administration.

3.2 Introduction

Despite routinely restoring epicardial coronary patency with primary PCI, which is the standard of care for STEMI[1], MVO affects about half of patients[26] and confers an adverse prognosis[27 86]. The pathophysiology of MVO is multifactorial, however a key component is distal embolization and microvascular thrombi[31-33], especially fibrin rich thrombi[32]. There are no evidence-based treatments for MVO[91 212 347].

Facilitated PCI, using full-dose, or half-dose adjunctive therapy before PCI with stenting improves epicardial coronary flow acutely[259 306]. However, facilitated PCI may cause paradoxical thrombus formation and bleeding[290 291].

The T-TIME trial tested the hypothesis that low-dose intracoronary alteplase, administered shortly after initial treatment (i.e. balloon angioplasty and/ or thrombus aspiration) before stent implantation, would reduce intracoronary and microvascular thrombosis and distal embolization, thereby reducing MVO. However, as assessed by contrast enhanced CMR imaging, MVO did not differ with intracoronary alteplase vs. placebo[292].

Persistently reduced flow in the infarct-related artery (TIMI flow ≤2) after first treatment, is termed "angiographic no-reflow"[28 348], and is a surrogate for myocardial perfusion defects (assessed by SPECT[110] or by myocardial contrast echocardiography[349]). TIMI flow ≤2 in the infarct-related artery at the end of PCI in STEMI patients is associated with heart failure[350], larger infarct size[110], and mortality[107 350-352]. TIMI flow ≤2 early during primary PCI (pre-stenting) may be even more closely associated with mortality[112 315] and larger infarct size[315] than TIMI flow ≤2 post-stenting. In contrast, maintained TIMI 3 flow in the infarct-related artery after initial treatment may help restore microvascular function[349].

Persisting impaired flow in the infarct-related artery may influence the effect of intracoronary alteplase. Hypothetically, impaired antegrade coronary flow could potentially reduce the effective intracoronary delivery of alteplase to the microcirculation, thereby limiting lysis of microthrombi.

The following hypotheses were evaluated:

1) In acute STEMI patients with TIMI 3 flow immediately preceding study drug administration, intracoronary alteplase is associated with less MVO and less myocardial haemorrhage on CMR imaging compared to placebo.

2) In acute STEMI patients with TIMI flow ≤2 immediately before study drug administration, there is no difference in MVO or myocardial haemorrhage on CMR imaging with intracoronary alteplase vs. placebo.

3.3 Methods

The methods for the T-TIME angiographic study were described in detail in section 2.1.

3.3.1 Statistical analysis

The analysis according to TIMI flow grade immediately preceding study drug administration (≤2 vs. 3), was prespecified prior to database lock. Patients in whom TIMI flow grade was not evaluable immediately pre-drug administration, were excluded from this analysis. Patients were also excluded from the analysis if the study drug was not administered, or was administered post-stent implantation, or distal to the culprit lesion. The analyses were performed according to treatment received (alteplase 20mg, alteplase 10mg, or placebo).

Continuous data were generally described as mean ± SD if normally distributed, or median and interquartile range (IQR) if skewed. The trial endpoints were assessed using linear regression (continuous parameters), or logistic regression (binary parameters), to make treatment effect estimates. In linear regression models, logarithmic, or square root transformations were used where necessary to improve model residual distributions. Regression models were used to assess treatment effects through interactions, with treatment as 3-level and 2-level categorical variables. The regression analyses were adjusted for MI location (anterior vs. non-anterior). All tests were 2-tailed and assessed at the 5% significance level. Imputation for missing values was not performed, and there was no adjustment for multiple statistical comparisons. Data were analysed with SPSS (version 25.0, SPSS, IBM, Armonk, NY, USA).

3.4 Results

3.4.1 Population characteristics

One thousand five hundred and twenty-seven STEMI patients were screened (Figure 3.1). Four hundred and forty patients were randomised to alteplase 20mg, alteplase 10mg, or placebo. Nineteen patients were excluded from the analysis (Figure 3.1). Out of these 19 excluded patients, there were 7 in whom TIMI flow grade was unevaluable immediately before study drug administration, 5 in whom the study drug was not administered, 3 in whom the study drug was administered after stent implantation, and 4 in whom the study drug was administered distal to the lesion.

Four hundred and twenty-one patients were included in the analysis (mean age 60 ± 10 years, 85% male). Out of the 421 participants who were included, 1 participant who was randomised to 10mg of alteplase received 20mg of alteplase and 1 participant randomised to placebo received alteplase 20mg, because of handling errors.

Out of the patients with TIMI flow ≤2 pre-drug, the majority (87%) had TIMI 2 flow immediately preceding drug administration. The distribution of TIMI coronary flow grades immediately preceding drug administration were as follows: TIMI grade 0 in 1 patient (0.2%) who received alteplase 10mg; TIMI grade 1 in 19 patients (5%) of whom 8 received placebo, 4 received alteplase 10mg and 7 received alteplase 20mg; TIMI grade 2 in 134 patients (32%) of whom 42 received placebo, 44 received alteplase 10mg and 48 received alteplase 20mg, and; TIMI grade 3 flow in 268 patients (64%) of whom 93 received placebo, 87 received alteplase 10mg and 88 received alteplase 20mg.

The population characteristics for patients with TIMI flow ≤2 (n=154) or TIMI 3 flow immediately before drug administration were broadly similar. There was no difference in glycoprotein IIbIIIa inhibitor use between patients who had TIMI flow ≤2 vs. 3 immediately before study drug administration (p=0.476). However, LAD infarcts were relatively more frequent in patients with TIMI flow ≤2 pre-study drug (Tables 3.1 and 3.2). Anterior location of MI was the only population characteristic associated with TIMI flow ≤2 pre-study drug, on multivariable logistic regression analysis (OR: 1.61 [95% CI: 1.07, 2.43] p=0.023). Ischaemic time was not associated with TIMI flow ≤2 immediately pre-

study drug administration (OR: 1.05 [95% CI: 0.91, 1.22] p=0.499). Infarct-related artery occlusion after study drug administration occurred in 44 out of 334 patients (13%).

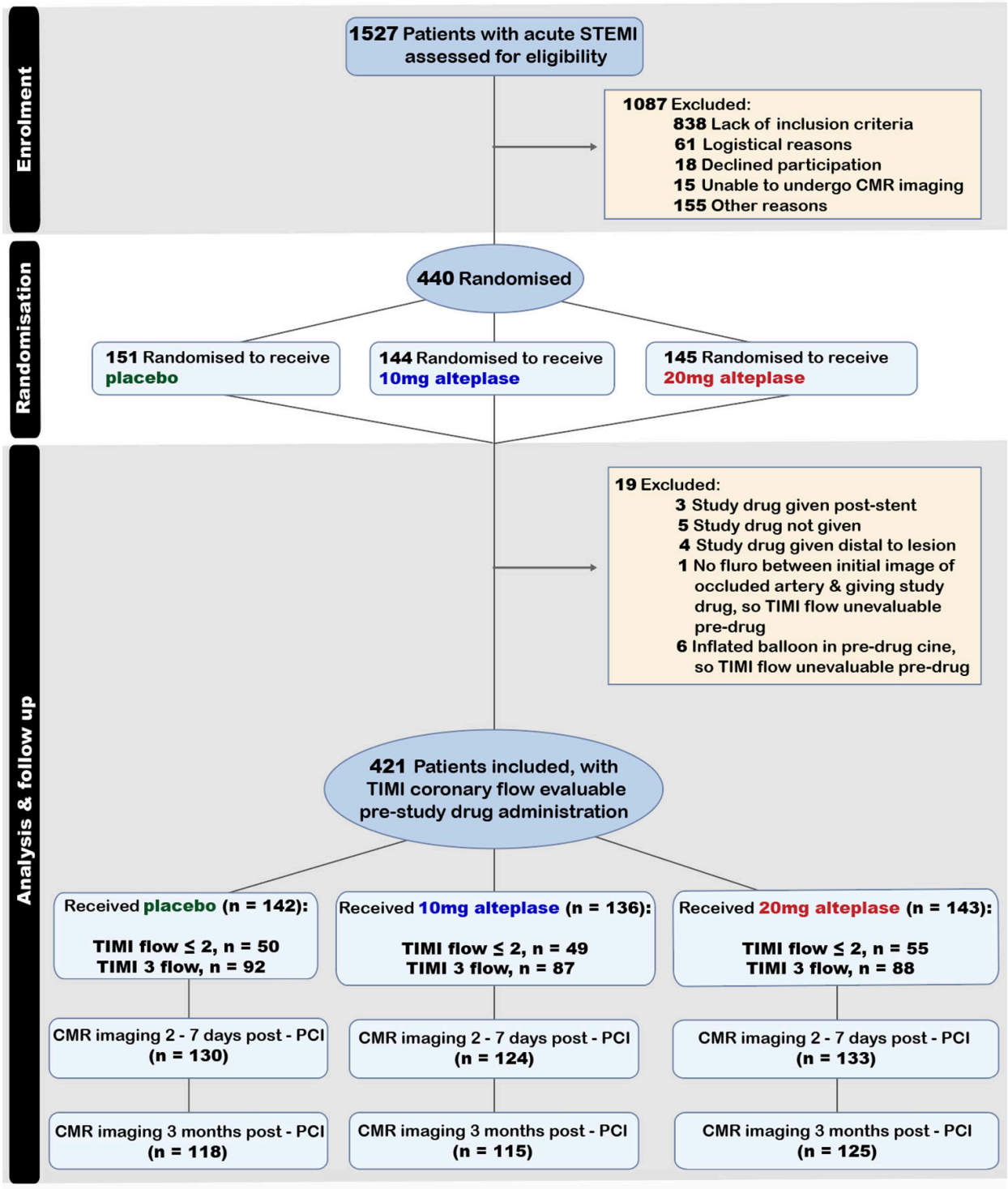

Figure 3.1 *Flow of subjects through the T-TIME angiographic study. CMR follow up is reported according to treatment received.*

	Impaired coronary flow immediately pre-drug (TIMI flow ≤ 2)				Normal coronary flow immediately pre-drug (TIMI 3 flow)			
	All (n=154)	**Placebo (n=50)**	**Alteplase 10mg (n=49)**	**Alteplase 20mg (n=55)**	**All (n=267)**	**Placebo (n=92)**	**Alteplase 10mg (n=87)**	**Alteplase 20mg (n=88)**
Age	59.5 ± 10.7	59.5 ± 11.3	58.7 ± 11.3	60.3 ± 9.7	61.2 ± 10.0	61.8 ± 10.7	60.1 ± 9.9	61.8 ± 9.5
Male	134 (87%)	44 (88%)	43 (88%)	47 (86%)	224 (84%)	76 (83%)	74 (85%)	74 (84%)
White	143 (93%)	46 (92%)	47 (96%)	50 (91%)	253 (95%)	89 (97%)	81 (93%)	83 (94%)
Asian	9 (6%)	3 (6%)	1 (2%)	5 (9%)	14 (5%)	3 (3%)	6 (7%)	5 (6%)
Body mass index (kg/m^2)	28.4 ± 4.8	29.1 ± 5.6	28.1 ± 4.2	27.9 ± 4.4	28.1 ± 5.0	28.1 ± 5.1	28.7 ± 5.2	27.6 ± 4.5
Heart rate at presentation, beats/ min	73.7 ± 17.2	72.1 ± 16.2	70.6 ± 15.3	78.0 ± 18.9	72.1 ± 20.1	73.5 ± 25.6	72.3 ± 16.6	70.3 ± 16.4
Systolic blood pressure at presentation, mmHg	132.4 ± 22.9	128.8 ± 21.5	135.8 ± 23.8	132.7 ± 23.3	134.9 ± 26.6	134.8 ± 28.4	134.3± 25.6	135.5 ± 25.8
Diastolic blood pressure at presentation, mmHg	81.1 ± 14.7	77.8 ± 15.1	81.1 ± 14.3	83.4 ± 14.5	80.0 ± 16.0	80.0 ± 17.2	80.6 ± 15.6	79.4 ± 15.1
Infarct location:								
Anterior	81 (53%)	26 (52%)	26 (53%)	29 (53%)	104 (39%)	38 (41%)	33 (38%)	33 (38%)
Non-anterior	73 (47%)	24 (48%)	23 (47%)	26 (47%)	163 (61%)	54 (59%)	54 (62%)	55 (63%)
Hypertension	53 (34%)	18 (36%)	15 (31%)	20 (36%)	82 (31%)	27 (29%)	28 (32%)	27 (31%)
Renal impairment *	1 (1%)	1 (2%)	0	0	5 (2%)	1 (1%)	3 (3%)	1 (1%)
Hypercholesterolaemia	40 (26%)	15 (30%)	13 (27%)	12 (22%)	56 (21%)	25 (27%)	14 (16%)	17 (19%)
Diabetes mellitus #	20 (13%)	5 (10%)	9 (18%)	6 (11%)	33 (12%)	13 (14%)	8 (9%)	12 (14%)
Smoking:								
Current	75 (49%)	28 (56%)	20 (41%)	27 (49%)	122 (46%)	42 (46%)	45 (52%)	35 (40%)
Former (stopped >3 months)	32 (21%)	9 (18%)	13 (27%)	10 (18%)	49 (18%)	17 (19%)	9 (10%)	23 (26%)
Never	47 (31%)	13 (26%)	16 (33%)	18 (33%)	96 (36%)	33 (36%)	33 (38%)	30 (34%)
Previous PCI	4 (3%)	1 (2%)	2 (4%)	1 (2%)	14 (5%)	6 (7%)	3 (3%)	5 (6%)
Angina	2 (1%)	1 (2%)	1 (2%)	0	13 (5%)	4 (4%)	4 (5%)	5 (6%)
Previous MI	2 (1%)	0	2 (4%)	0	15 (6%)	5 (5%)	3 (3%)	7 (8%)

Table 3.1 *(page 1 of 2) Baseline characteristics from the T-TIME angiographic study, by sub-groups of TIMI flow (≤2 vs. 3) immediately before study drug administration. Data are reported according to treatment received (n=421). Data are mean ± SD, or n (%) unless otherwise stated. *Renal impairment was defined as an estimated glomerular filtration rate <59 mL/min/1.73m^2. # Diabetes mellitus was defined as a history of diet-controlled or treated diabetes. Missing: body mass index (calculated as weight in kg divided by height in meters squared) n=2, creatinine or estimated glomerular filtration rate n=68, haemoglobin n=16, platelets n=30.*

	Impaired coronary flow immediately pre-drug (TIMI flow ≤ 2)				Normal coronary flow immediately pre-drug (TIMI 3 flow)			
	All (n=154)	**Placebo (n=50)**	**Alteplase 10mg (n=49)**	**Alteplase 20mg (n=55)**	**All (n=267)**	**Placebo (n=92)**	**Alteplase 10mg (n=87)**	**Alteplase 20mg (n=88)**
Stroke/ Transient Ischaemic Attack	0	0	0	0	5 (2%)	2 (2%)	1 (1%)	2 (2%)
Peripheral vascular disease	3 (2%)	2 (4%)	1 (2%)	0	9 (3%)	1 (1%)	2 (2%)	6 (7%)
Pre-existing maintenance medication:								
Aspirin	16 (10%)	6 (12%)	5 (10%)	5 (9%)	47 (18%)	20 (22%)	11 (13%)	16 (18%)
P2Y12 inhibitor								
Clopidogrel	1 (1%)	0	0	1 (2%)	1 (0.4%)	1 (1%)	0	0
Ticagrelor or prasugrel	4 (3%)	1 (2%)	0	3 (6%)	16 (6%)	8 (9%)	4 (5%)	4 (5%)
Statin	31 (20%)	11 (22%)	11 (22%)	9 (16%)	60 (23%)	27 (29%)	17 (20%)	16 (18%)
Beta blocker	14 (9%)	4 (8%)	6 (12%)	4 (7%)	26 (10%)	12 (13%)	8 (9%)	6 (7%)
ACE inhibitor or ARB	30 (20%)	8 (16%)	10 (20%)	12 (22%)	43 (16%)	13 (14%)	16 (18%)	14 (16%)
Mineralocorticoid receptor antagonist	2 (1%)	0	2 (4%)	0	2 (1%)	1 (1%)	0	1 (1%)
Symptom onset to reperfusion, median (IQR) hrs	2.7 (2.1, 3.8)	2.7 (2.1, 3.5)	2.7 (1.9, 4.2)	2.9 (2.1, 3.8)	2.6 (2.0, 3.8)	2.6 (2.0, 3.7)	2.8 (1.9, 4.0)	2.7 (2.0, 3.8)
Initial blood results on admission:								
Haemoglobin, g/dL	147.3 ± 13.2	144.9 ± 15.1	145.7 ± 11.0	151.0 ± 12.5	144.6 ± 13.3	144.0 ± 13.5	145.9 ± 13.6	143.8 ± 12.8
Platelet count, $10^3/\mu L$	259.7 ± 61.2	248.7 ± 61.2	273.3 ± 64.6	257.7 ± 56.8	262.6 ± 63.6	254.2 ± 61.0	269.7 ± 75.8	263.9 ± 50.5
Creatinine, µmol/L	80.5 ± 17.3	83.6 ± 19.2	74.8 ± 12.3	82.7 ± 18.2	80.9 ± 18.1	78.0 ± 17.3	83.4 ± 18.7	81.2 ± 18.2
eGFR (ml/min/1.73m^2)	92.7 ± 21.4	91.1 ± 21.6	96.4 ± 18.4	91.0 ± 23.4	88.9 ± 20.7	90.4 ± 20.9	86.9 ± 20.0	89.3 ± 21.3

Table 3.1 *(page 2 of 2) Baseline characteristics from the T-TIME angiographic study, by sub-groups of TIMI flow (≤2 vs. 3) immediately before study drug administration. Data are reported according to treatment received (n=421). Data are mean ± SD, or n (%) unless otherwise stated. *Renal impairment was defined as an estimated glomerular filtration rate <59 mL/min/1.73m^2. # Diabetes mellitus was defined as a history of diet-controlled or treated diabetes. Missing: body mass index (calculated as weight in kg divided by height in meters squared) n=2, creatinine or estimated glomerular filtration rate n=68, haemoglo0bin n=16, platelets n=30.*

	Impaired coronary flow immediately pre-drug (TIMI flow ≤ 2)				Normal coronary flow immediately pre-drug (TIMI 3 flow)			
	All (n=154)	**Placebo (n=50)**	**Alteplase 10mg (n=49)**	**Alteplase 20mg (n=55)**	**All (n=267)**	**Placebo (n=92)**	**Alteplase 10mg (n=87)**	**Alteplase 20mg (n=88)**
Culprit artery: LAD	82 (53%)	26 (52%)	27 (55%)	29 (53%)	109 (41%)	40 (44%)	35 (40%)	34 (39%)
Cx	12 (8%)	3 (6%)	3 (6%)	6 (11%)	41 (15%)	16 (17%)	14 (16%)	11 (13%)
RCA	60 (39%)	21 (42%)	19 (39%)	20 (36%)	117 (44%)	36 (39%)	38 (44%)	43 (49%)
Multivessel disease: 1	112 (73%)	34 (68%)	34 (69%)	44 (80%)	165 (62%)	62 (67%)	52 (60%)	51 (58%)
2	37 (24%)	13 (26%)	14 (29%)	10 (18%)	80 (30%)	25 (27%)	27 (31%)	28 (32%)
3	5 (3%)	3 (6%)	1 (2%)	1 (2%)	22 (8%)	5 (5%)	8 (9%)	9 (10%)
Initial TIMI coronary flow grade:								
0 (no flow)	119 (77%)	41 (82%)	38 (78%)	40 (73%)	216 (81%)	81 (88%)	67 (77%)	68 (77%)
1 (minimal flow)	9 (6%)	2 (4%)	2 (4%)	5 (9%)	23 (9%)	1 (1%)	12 (14%)	10 (11%)
2 (slow but complete flow)	25 (16%)	7 (14%)	8 (16%)	10 (18%)	23 (9%)	8 (9%)	6 (7%)	9 (10%)
3 (normal flow)	1 (1%)	0	1 (2%)	0	5 (2%)	2 (2%)	2 (2%)	1 (1%)
Initial TIMI thrombus grade: 0 - 2	0	0	0	0	0	0	0	0
3	3 (2%)	1 (2%)	1 (2%)	1 (2%)	8 (3%)	2 (2%)	1 (1%)	5 (6%)
4	32 (21%)	9 (18%)	9 (18%)	14 (26%)	43 (16%)	9 (10%)	19 (22%)	15 (17%)
5	119 (77%)	40 (80%)	39 (80%)	40 (73%)	216 (81%)	81 (88%)	67 (77%)	68 (77%)
QCA lesion length pre-drug (mm)	25.5 ± 11.2	26.7 ± 11.6	27.4 ± 12.4	22.7 ± 9.3	27.2 ± 11.3	26.7 ± 10.6	27.6 ± 11.6	27.5 ± 11.8
Mode of reperfusion:								
Aspiration thrombectomy	45 (29%)	12 (24%)	14 (29%)	19 (35%)	74 (28%)	23 (25%)	28 (32%)	23 (26%)
Balloon angioplasty	109 (71%)	38 (76%)	35 (71%)	36 (66%)	192 (72%)	69 (75%)	59 (68%)	64 (73%)
Primary stent	0	0	0	0	1 (0.4%)	0	0	1 (1%)
Balloon angioplasty pre-stent	144 (94%)	48 (96%)	46 (94%)	50 (91%)	244 (91%)	83 (90%)	82 (94%)	79 (90%)

Table 3.2 *(page 1 of 3) Procedural characteristics from the T-TIME angiographic study, by sub-groups of TIMI flow (≤2 vs. 3) immediately preceding study drug administration. Data are reported according to treatment received (n=421). Data are mean ± SD, or n (%), unless otherwise stated. None of the participants received intravenous or intracoronary treatment with bivalirudin, metoprolol, nicorandil, or sodium nitroprusside. Missing: ACT (n=96), aspirin loading dose (n=56), duration of study drug infusion (n=24), glycoprotein IIbIIIa inhibitor (n=11), inhaled oxygen (n=11), infarct related artery occlusion after study drug administration (n=87), CMR 2 to 7 days post-PCI (n=38), CMR 3 months post-PCI (n=66).*

	Impaired coronary flow immediately pre-drug (TIMI flow ≤ 2)				Normal coronary flow immediately pre-drug (TIMI 3 flow)			
	All (n=154)	**Placebo (n=50)**	**Alteplase 10mg (n=49)**	**Alteplase 20mg (n=55)**	**All (n=267)**	**Placebo (n=92)**	**Alteplase 10mg (n=87)**	**Alteplase 20mg (n=88)**
Method of study drug delivery:								
Thrombectomy catheter	112 (73%)	38 (76%)	36 (74%)	38 (69%)	188 (70%)	65 (71%)	58 (67%)	65 (74%)
Guide catheter	35 (23%)	10 (20%)	9 (18%)	16 (29%)	65 (24%)	21 (23%)	26 (30%)	18 (21%)
Other	7 (5%)	2 (4%)	4 (8%)	1 (2%)	14 (5%)	6 (7%)	3 (3%)	5 (6%)
PCI with stent implantation	152 (99%)	50 (100%)	48 (98%)	54 (98%)	266 (100%)	91 (99%)	87 (100%)	88 (100%)
Total number of stents deployed:								
0	2 (1%)	0	1 (2%)	1 (2%)	1 (0.0%)	1 (1%)	0	0
1	104 (68%)	35 (70%)	29 (59%)	40 (73%)	188 (70%)	59 (64%)	65 (75%)	64 (73%)
2	40 (26%)	13 (26%)	14 (29%)	13 (24%)	64 (24%)	30 (3%)	14 (16%)	20 (23%)
≥3	8 (5%)	2 (4%)	5 (10%)	1 (2%)	14 (5%)	2 (2%)	8 (9%)	4 (5%)
Post stent dilatation	133 (86%)	48 (96%)	42 (86%)	43 (78%)	233 (87%)	76 (83%)	76 (87%)	81 (92%)
Total length of stents deployed from QCA, mm	32.7 ± 14.4	32.9 ± 13.8	35.2 ± 16.2	30.2 ± 13.1	34.5 ± 14.4	34.9 ± 13.3	34.8 ± 14.2	33.9 ± 14.7
QCA reference vessel diameter post-stent, mm	3.3 ± 0.5	3.2 ± 0.5	3.3 ± 0.6	3.2 ± 0.4	3.2 ± 0.4	3.1 ± 0.4	3.2 ± 0.5	3.2 ± 0.4
Loading with aspirin at first medical contact	135 (88%)	44 (88%)	43 (88%)	48 (87%)	230 (86%)	78 (85%)	77 (89%)	75 (85%)
Aspirin loading dose, mg, n/ total (%):								
300	133/135 (99%)	44/44 (100%)	42/43 (98%)	47/48 (98%)	220/230 (96%)	73/78 (94%)	74/77 (96%)	73/75 (97%)
>300	2/135 (2%)	0	1/43 (2%)	1/48 (2%)	10/230 (4%)	5/78 (6%)	3/77 (4%)	2/75 (3%)

Table 3.2 *(page 2 of 3) Procedural characteristics from the T-TIME angiographic study, by sub-groups of TIMI flow (≤2 vs. 3) immediately preceding study drug administration. Data are reported according to treatment received (n=421). Data are mean ± SD, or n (%), unless otherwise stated. None of the participants received intravenous or intracoronary treatment with bivalirudin, metoprolol, nicorandil, or sodium nitroprusside. Missing: ACT (n=96), aspirin loading dose (n=56), duration of study drug infusion (n=24), glycoprotein IIbIIIa inhibitor (n=11), inhaled oxygen (n=11), infarct related artery occlusion after study drug administration (n=87), CMR 2 to 7 days post-PCI (n=38), CMR 3 months post-PCI (n=66).*

	Impaired coronary flow immediately pre-drug (TIMI flow ≤ 2)				Normal coronary flow immediately pre-drug (TIMI 3 flow)			
	All (n=154)	**Placebo (n=50)**	**Alteplase 10mg (n=49)**	**Alteplase 20mg (n=55)**	**All (n=267)**	**Placebo (n=92)**	**Alteplase 10mg (n=87)**	**Alteplase 20mg (n=88)**
Additional antiplatelet medication at first medical contact:								
None	18 (12%)	5 (10%)	5 (10%)	8 (15%)	30 (11%)	12 (13%)	8 (9%)	10 (11%)
Clopidogrel	55 (36%)	20 (40%)	20 (41%)	15 (27%)	90 (34%)	26 (28%)	29 (33%)	35 (40%)
Ticagrelor	75 (49%)	24 (48%)	22 (45%)	29 (53%)	142 (53%)	53 (58%)	49 (56%)	40 (46%)
Prasugrel	6 (4%)	1 (2%)	2 (4%)	3 (6%)	5 (2%)	1 (1%)	1 (1%)	3 (3%)
Unfractionated heparin, median (IQR), U	10000.0 (8000.0, 13000.0)	10000.0 (8000.0, 15000.0)	10000.0 (8000.0, 13000.0)	10000.0 (8000.0, 12000.0)	10000.0 (7000.0, 12000.0)	9000.0 (7000.0, 12000.0)	10000.0 (7500.0, 13000.0)	10000.0 (7000.0, 13000.0)
ACT (s)	276.3 ± 89.8	264.3 ± 89.8	303.4 ± 97.6	263.0 ± 78.3	284.0 ± 87.4	280.8 ± 88.5	294.9 ± 88.5	276.1 ± 85.2
Intravenous morphine	114 (74%)	37 (74%)	38 (78%)	39 (71%)	197 (74%)	62 (67%)	64 (74%)	71 (81%)
Inhaled oxygen, n/ total (%)	28/151 (19%)	8/49 (16%)	14/48 (29%)	6/54 (11%)	32/259 (12%)	14/90 (16%)	10/85 (12%)	8/84 (10%)
Glycoprotein IIb/IIIa inhibitor, n/ total (%)	28/151 (19%)	6/49 (12%)	11/48 (23%)	11/54 (20%)	38/259 (15%)	8/90 (9%)	17/85 (20%)	13/84 (16%)
Duration of study drug infusion, min	6.6 ± 2.0	6.9 ± 2.1	6.5 ± 2.0	6.5 ± 1.9	6.4 ± 1.9	6.2 ± 1.9	6.4 ± 1.9	6.7 ± 2.0
Infarct-related artery occlusion after study drug infusion, No/total (%)	21/121 (17%)	11/37 (30%)	5/40 (13%)	5/44 (11%)	23/213 (11%)	8/73 (11%)	6/71 (8%)	9/69 (13%)
Days from PCI to CMR imaging 2-7 days post-primary PCI, median (IQR)	4.0 (3.0, 6.0)	4.0 (3.0, 5.0)	4.0 (3.0, 6.0)	4.0 (2.8, 6.0)	4.0 (3.0, 6.0)	4.0 (2.8, 5.0)	5.0 (3.0, 6.0)	4.0 (4.0, 6.0)
Days from PCI to CMR imaging 3 months post-primary PCI, median (IQR)	91.0 (85.0, 98.8)	91.0 (85.0, 97.0)	92.0 (86.0, 99.5)	90.0 (85.0, 99.0)	90.0 (86.0, 95.3)	90.0 (85.8, 94.0)	90.0 (86.0, 96.0)	91.0 (86.0, 97.0)

Table 3.2 *(page 3 of 3) Procedural characteristics from the T-TIME angiographic study, by sub-groups of TIMI flow (≤2 vs. 3) immediately preceding study drug administration. Data are reported according to treatment received (n=421). Data are mean ± SD, or n (%), unless otherwise stated. None of the participants received intravenous or intracoronary treatment with bivalirudin, metoprolol, nicorandil, or sodium nitroprusside. Missing: ACT (n=96), aspirin loading dose (n=56), duration of study drug infusion (n=24), glycoprotein IIbIIIa inhibitor (n=11), inhaled oxygen (n=11), infarct related artery occlusion after study drug administration (n=87), CMR imaging 2 to 7 days post-PCI (n=38), CMR imaging 3 months post-PCI (n=66).*

One hundred and seventy-one patients (41%) were recruited on a weekend or outside 9am to 5pm, i.e. outwith "normal working hours". A breakdown of the number patients enrolled in the angiographic study by day of the week and by time to the nearest hour is shown (Figure 3.2).

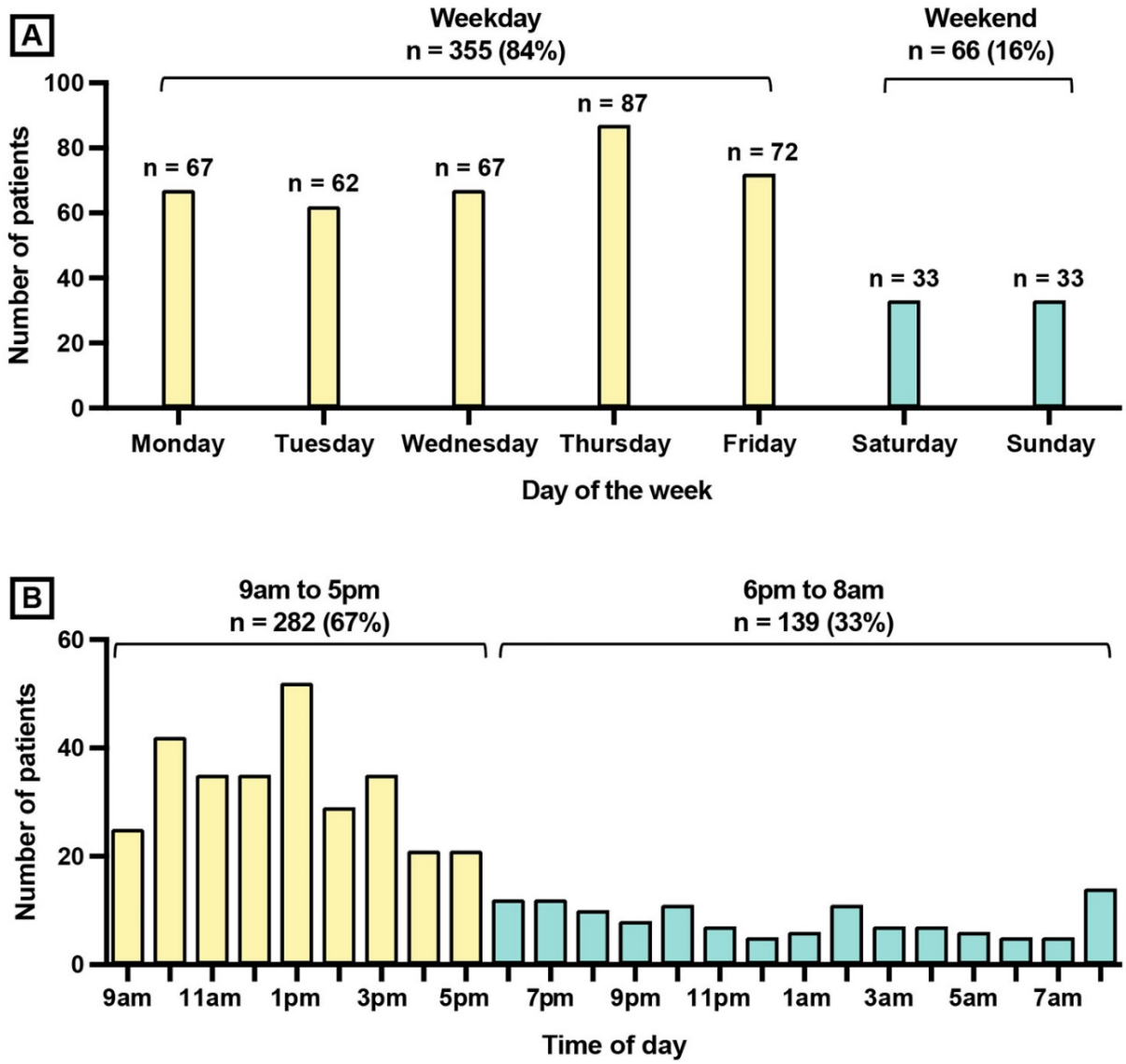

Figure 3.2 *Breakdown of the number of patients enrolled in the T-TIME angiographic study by day of the week (A) and by time of day to the nearest hour (B).*

3.4.2 Intra-observer and inter-rater reliability for angiographic parameters

Inter-rater variability was assessed for MPG and TIMI flow grade at the end of the PCI procedure, in the entire T-TIME trial population (n=440). Inter-rater variability was assessed for TFC at the end of the procedure, in 438 patients from the T-TIME trial population. There was excellent inter-rater reliability for TIMI flow grade at the end of the PCI procedure (kappa=0.94). The inter-rater reliability for TIMI flow grade immediately

pre-study drug, assessed in 65 consecutive participants, was also excellent (kappa=0.94). There was excellent inter-rater reliability for MPG (kappa=0.88) and for TFC (intra-class correlation coefficient: 0.996 [95% CI: 0.995, 0.996]), at the end of the PCI procedure.

There was excellent intra-observer reliability for TFC, MPG and TIMI flow grade, at the end of PCI in 50 consecutive participants (intra-class correlation coefficient for TFC: 0.999 [95% CI: 0.999, 1.000], kappa for MPG=0.85, and kappa for TIMI flow grade=0.90).

3.4.3 CMR endpoints

Three hundred and eighty-seven patients (92%) had CMR imaging performed 2 to 7 days post-primary PCI, and MVO presence/ absence was evaluable in 383 patients (91%). At 3 months post-primary PCI, CMR imaging was available in 358 participants (85%). The number of days from PCI to CMR imaging is shown in Table 3.2. The CMR results at 2 to 7 days post-primary PCI are shown in Table 3.3, and the results stratified by location of MI are shown in Table 3.4. In participants with TIMI 3 flow pre-study drug, there were no associations between alteplase and CMR parameters, apart from an increase in LV end diastolic volume with alteplase 10mg vs. placebo (Tables 3.3 to 3.5).

Due to the high proportion of participants with a 0 value for MVO extent (56% of participants) and myocardial haemorrhage extent (57% of participants), the median values for MVO and myocardial haemorrhage were 0 for all groups. Therefore, for these parameters the mean ± SD was reported despite not being an ideal summary for these data. Overall, the mean MVO extent was higher in patients who had TIMI flow ≤2 compared to TIMI 3 flow pre-study drug (3.7 ± 6.0% vs. 2.3 ± 4.2%; coefficient = 0.33 [95% CI: 0.05, 0.60] p=0.022 [from linear regression using square root transformed MVO]). Overall, the mean myocardial haemorrhage extent was also higher in patients who had TIMI flow ≤2 compared to TIMI 3 flow pre-study drug (2.7 ± 5.1% vs. 1.6 ± 3.4%; coefficient = 1.14 [95% CI: 0.24, 2.03] p=0.014, from linear regression).

MVO

Participants with TIMI flow ≤2 pre-study drug had MVO present more often with alteplase (placebo: 34% [n=15/44], alteplase 10mg: 60% [n=25/42], alteplase 20mg: 53% [n=26/49]; OR for alteplase 10mg and 20mg combined vs. placebo, 2.47 [95% CI: 1.16, 5.22] p=0.018) (Figure 3.3). Interactions were observed with MVO presence, between

TIMI flow pre-drug, and treatment analysed as 3-, or 2-level categorical variables (p=0.013 and p=0.005 respectively) (Table 3.3). When the 19 patients with TIMI 1 flow and the one patient with TIMI 0 flow immediately pre-drug were excluded, significant interactions remained between alteplase, TIMI flow pre-drug (2 vs. 3) and the presence of MVO (p=0.022) (Table 3.6).

Participants with TIMI flow ≤2 pre-study drug had increased extent of MVO (% LV mass) with alteplase 20mg compared to placebo (placebo: 2.6 ± 5.7%, alteplase 10mg: 2.7 ± 3.9%, alteplase 20mg: 5.4 ± 7.4%; estimated mean difference alteplase 20mg and 10mg combined vs. placebo 0.53 [95% CI: 0.06, 1.00] p=0.027) (Table 3.3). In participants with TIMI 3 flow pre-study drug, there was no association between alteplase and MVO extent or presence (Table 3.3). There was an interaction between MVO extent, TIMI flow pre-drug and treatment, when alteplase 10mg and 20mg were combined and compared with placebo (p=0.041), but not for treatment as a 3-level categorical variable (p=0.070) (Table 3.3).

Myocardial haemorrhage

Myocardial haemorrhage presence/ absence was evaluable in 366 of the patients (87%), 2 to 7 days post-PCI. Myocardial haemorrhage extent was evaluable in 348 of the patients (83%).

In patients who had TIMI flow ≤2 pre-study drug, myocardial haemorrhage occurred more often with alteplase than placebo (placebo: 28% [n=11/40], alteplase 10mg: 58% [n=22/38], alteplase 20mg: 53% [n=26/49]; OR for alteplase 10mg and 20mg combined vs. placebo = 3.26 [95% CI: 1.44, 7.36] p=0.004) (Figure 3.3). There were interactions between myocardial haemorrhage presence, TIMI flow pre-drug and treatment, analysed as 3- and 2-level categorical variables (p=0.004 and p=0.001 respectively) (Table 3.3). When the 19 patients with TIMI 1 flow pre-drug and the one patients with TIMI 0 flow immediately pre-drug were excluded, significant interactions remained between alteplase, TIMI flow pre-drug (2 vs. 3) and the presence of myocardial haemorrhage (p=0.009) (Table 3.6).

In patients with TIMI flow ≤2 pre-study drug there was a trend towards increased extent of myocardial haemorrhage (% LV mass) with alteplase (placebo: 1.7 ± 5.2%, alteplase 10mg: 2.2 ± 3.4%, alteplase 20mg: 3.8 ± 5.8%). In patients with TIMI flow ≤2 pre-study drug, the estimated mean difference for myocardial haemorrhage extent for alteplase 20mg vs. placebo was 2.15 (95% CI: 0.45, 3.85) p=0.014, however the estimated mean difference for myocardial haemorrhage extent for alteplase 10mg and 20mg combined vs. placebo was not statistically significant (Table 3.3). In participants with TIMI 3 flow pre-study drug, there was no association between alteplase and myocardial haemorrhage extent or presence (Table 3.3). There were no interactions between myocardial haemorrhage extent, TIMI flow pre-drug and treatment, analysed as 3-, or 2-level categorical variables (p=0.120 and p=0.179 respectively) (Table 3.3).

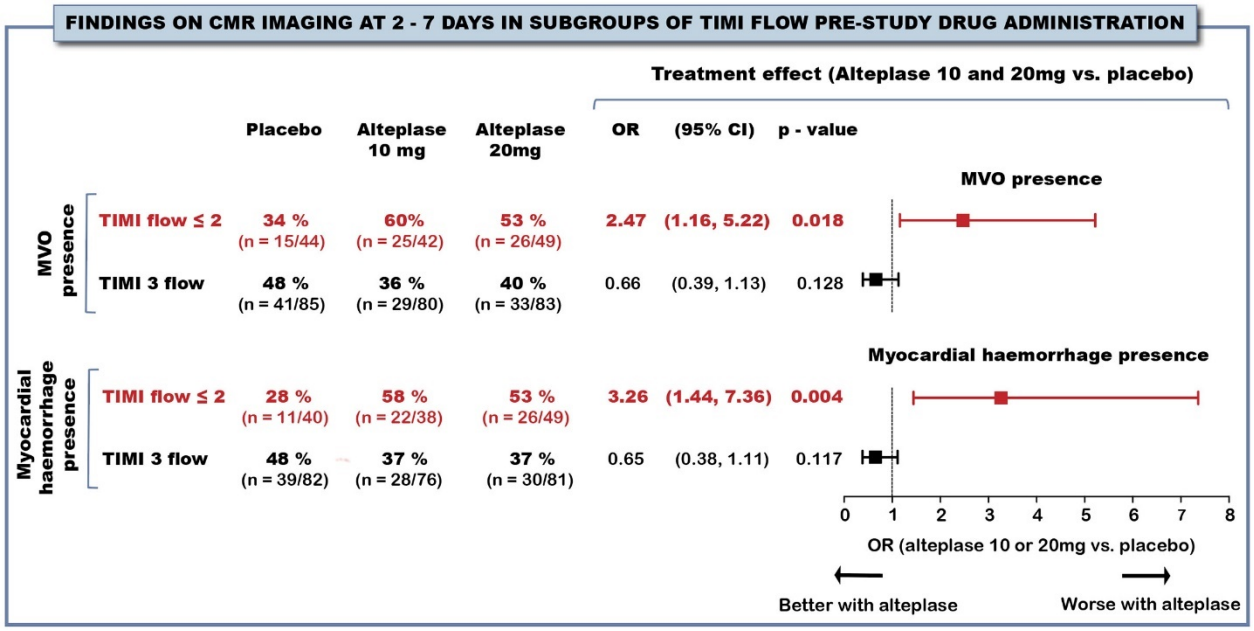

Figure 3.3 *Forrest plots showing increased MVO and myocardial haemorrhage presence associated with alteplase vs. placebo in patients with TIMI flow ≤2 immediately before study drug administration*

Infarct size

In patients with TIMI 3 flow pre-study drug, there were no associations between alteplase and infarct size (Tables 3.3 and 3.4). In patients with TIMI flow ≤2 pre-study drug, there was a trend towards larger infarct size (2 to 7 day) with alteplase than placebo (placebo: 28.1 ± 15.5, alteplase 10mg: 32.0 ± 12.7, alteplase 20mg: 32.3 ± 13.1). Though no differences in treatment effects were detected on linear regression (Table 3.3), an interaction was observed for association between infarct size (2 to 7 day), TIMI flow and

treatment as a 2-level categorical variable (p=0.026), but not for treatment as a 3-level categorical variable (p=0.076) (Table 3.3). There were no associations or interactions between TIMI flow sub-groups, alteplase, and infarct size at 3 months post-primary PCI (Table 3.5).

LV ejection fraction and volumes

There were no interactions between sub-groups of TIMI flow pre-study drug, alteplase, and LV ejection fraction, or volumes, at 2 to 7 days or 3 months post-PCI (Tables 3.3 and 3.5).

	Treatment group			Treatment Effect		Interaction p-value (treatment as a 3-level categorical variable)	Treatment Effect	Interaction p-value (treatment as a 2-level categorical variable)
	Placebo (n=142)	Alteplase 10mg (n=136)	Alteplase 20mg (n=143)	Alteplase 10mg vs. placebo	Alteplase 20mg vs. placebo		Alteplase (10mg or 20mg) vs. placebo	
				Estimate (95% CI) p-value	Estimate (95% CI) p-value		Estimate (95% CI), p-value	
MVO present (n/ total) (a)								
TIMI flow ≤2	15/44 (34.1%)	25/42 (59.5%)	26/49 (53.1%)	2.86 (1.19, 6.88 **p=0.019**	2.18 (0.94, 5.04) p=0.069	**0.013**	2.47 (1.16, 5.22) **p=0.018**	**0.005**
TIMI 3 flow	41/85 (48.2%)	29/80 (36.3%)	33/83 (39.8%)	0.61 (0.33, 1.14) p=0.119	0.72 (0.39, 1.34) p=0.298		0.66 (0.39, 1.13) p=0.128	
MVO extent (% of LV mass) (b)								
TIMI flow ≤2	2.6 ± 5.7	2.7 ± 3.9	5.4 ± 7.4	0.31 (-0.24, 0.86) p=0.269	0.72 (0.19, 1.25) **p=0.008**	0.070	0.53 (0.06, 1.00) **p=0.027**	**0.041**
TIMI 3 flow	2.2 ± 3.4	2.4 ± 4.8	2.3 ± 4.3	-0.09 (-0.49, 0.31) p=0.661	-0.06 (-0.45, 0.34) p=0.777		-0.07 (-0.42, 0.27) p=0.677	
Myocardial haemorrhage present (n/ total) (a)								
TIMI flow ≤2	11/40 (27.5%)	22/38 (57.9%)	26/49 (53.1%)	3.67 (1.42, 9.49) **p=0.007**	2.97 (1.22, 7.27) **p=0.017**	**0.004**	3.26 (1.44, 7.36) **p=0.004**	**0.001**
TIMI 3 flow	39/82 (47.6%)	28/76 (36.8%)	30/81 (37.0%)	0.64 (0.34, 1.21) p=0.168	0.66 (0.35, 1.23) p=0.188		0.65 (0.38, 1.11) p=0.117	

Table 3.3 *(page 1 of 3) Analysis of CMR parameters 2 to 7 days after primary PCI, in the T-TIME angiographic study, by sub-groups of TIMI flow (≤2 vs. 3) immediately before study drug administration (adjusted for MI location). Data are mean ± SD, median [IQR], or n (%), unless otherwise stated. Missing data: MVO extent or presence/ absence (n=38), myocardial haemorrhage extent (n=73), myocardial haemorrhage presence/ absence (n=55), infarct size (n=38), LV ejection fraction or volumes (n=34). Treatment effect estimates and interaction with treatment received are shown. (a) Treatment effect estimates reported as ORs between groups from a logistic regression model. (b) Treatment effect estimates reported as mean differences in square root transformed MVO extent between groups, from a linear regression model. (c) Treatment effect estimates reported as mean differences between groups, from linear regression. (d) Data analysed on a logarithmic scale, with treatment effect estimates reported as relative differences between groups.*

	Treatment group			Treatment Effect			Treatment Effect	
	Placebo (n=142)	Alteplase 10mg (n=136)	Alteplase 20mg (n=143)	Alteplase 10mg vs. placebo	Alteplase 20mg vs. placebo	Interaction p-value (treatment as a 3-level categorical variable)	Alteplase (10mg or 20mg) vs. placebo	Interaction p-value (treatment as a 2-level categorical variable)
				Estimate (95% CI) p-value	Estimate (95% CI) p-value		Estimate (95% CI) p-value	
Myocardial haemorrhage extent (% LV mass) (c)								
TIMI flow ≤2	1.7 ± 5.2	2.2 ± 3.4	3.8 ± 5.8	0.55 (-0.31, 2.42) p=0.562	2.15 (0.45, 3.85) **p=0.014**	0.120	0.50 (-0.04, 3.04) p=0.057	0.179
TIMI 3 flow	1.4 ± 2.8	1.8 ± 3.6	1.5 ± 3.8	0.29 (-1.00, 1.58) p=0.656	0.11 (-1.16, 1.38) p=0.867		0.20 (-0.91, 1.31) p=0.726	
Infarct size (% LV mass) (c)								
TIMI flow ≤2	28.1 ± 15.5	32.0 ± 12.7	32.3 ± 13.1	3.87 (-1.08, 8.81) p=0.126	3.87 (-0.90, 8.63) p=0.112	0.076	3.87 (-0.34, 8.07) p=0.072	**0.026**
TIMI 3 flow	26.0 ± 12.6	24.7 ± 11.7	22.8 ± 12.3	-1.35 (-4.92, 2.22) p=0.460	-2.68 (-6.22, 0.86) p=0.138		-2.03 (-5.09, 1.03) p=0.195	
LV ejection fraction (%) (c)								
TIMI flow ≤2	43.4 ± 10.5	41.5 ± 8.8	42.6 ± 8.8	-1.85 (-5.18, 1.48) p=0.276	-0.67 (-3.88, 2.53) p=0.681	0.681	-1.22 (-4.05, 1.62) p=0.400	0.431
TIMI 3 flow	44.8 ± 7.9	44.7 ± 7.4	45.4 ± 7.9	-0.03 (-2.43, 2.37) p=0.982	0.40 (-1.98, 2.79), p=0.740		0.19 (-1.87, 2.25) p=0.856	

Table 3.3 *(page 2 of 3) Analysis of CMR parameters 2 to 7 days after primary PCI, in the T-TIME angiographic study, by sub-groups of TIMI flow (≤2 vs. 3) immediately before study drug administration (adjusted for MI location). Data are mean ± SD, median [IQR], or n (%), unless otherwise stated. Missing data: MVO extent or presence/ absence (n=38), myocardial haemorrhage extent (n=73), myocardial haemorrhage presence/ absence (n=55), infarct size (n=38), LV ejection fraction or volumes (n=34). Treatment effect estimates & interaction with treatment received are shown. (a) Treatment effect estimates reported as ORs between groups from a logistic regression model. (b) Treatment effect estimates reported as mean differences in square root transformed MVO extent between groups, from a linear regression model. (c) Treatment effect estimates reported as mean differences between groups, from linear regression. (d) Data analysed on a logarithmic scale, with treatment effect estimates reported as relative differences between groups.*

	Treatment group			Treatment Effect		Interaction p-value (treatment as a 3-level categorical variable)	Treatment Effect	Interaction p-value (treatment as a 2-level categorical variable)
	Placebo (n=142)	Alteplase 10mg (n=136)	Alteplase 20mg (n=143)	Alteplase 10mg vs. placebo	Alteplase 20mg vs. placebo		Alteplase (10mg or 20mg) vs. placebo	
				Estimate (95% CI) p-value	Estimate (95% CI) p-value		Estimate (95% CI) p-value	
LV end-systolic volume (ml) (d)								
TIMI flow ≤2	96.2 [80.2, 118.9]	105.3 [85.6, 124.3]	95.5 [80.8, 113.6]	1.05 (0.93, 1.19) p=0.392	0.95 (0.85, 1.07) p=0.425	0.739	1.00 (0.90, 1.11) p=0.984	0.480
TIMI 3 flow	90.2 [75.9, 108.0]	92.9 [79.0, 113.4]	92.5 [72.3, 109.0]	1.08 (0.99, 1.18) p=0.070	1.01 (0.93, 1.10) p=0.819		1.05 (0.97, 1.13) p=0.242	
LV end-diastolic volume (ml) (d)								
TIMI flow ≤2	174.2 [153.9, 214.1]	177.3 [163.9, 212.3]	161.6 [142.6, 200.0]	1.02 (0.93, 1.12) p=0.721	0.94 (0.86, 1.03) p=0.171	0.340	0.97 (0.90, 1.06) p=0.525	0.141
TIMI 3 flow	162.2 [141.8, 190.1]	176.5 [155.5, 205.8]	170.5 [136.6, 194.3]	1.08 (1.01, 1.16) **p=0.021**	1.02 (0.95, 1.09) p=0.601		1.05 (0.99, 1.11) p=0.105	

Table 3.3 *(page 3 of 3) Analysis of CMR parameters 2 to 7 days after primary PCI, in the T-TIME angiographic study, by sub-groups of TIMI flow (≤2 vs. 3) immediately before study drug administration (adjusted for MI location). Data are mean ± SD, median [IQR], or n (%), unless otherwise stated. Missing data: MVO extent or presence/ absence (n=38), myocardial haemorrhage extent (n=73), myocardial haemorrhage presence/ absence (n=55), infarct size (n=38), LV ejection fraction or volumes (n=34). Treatment effect estimates & interaction with treatment received are shown. (a) Treatment effect estimates reported as ORs between groups from a logistic regression model. (b) Treatment effect estimates reported as mean differences in square root transformed MVO extent between groups, from a linear regression model. (c) Treatment effect estimates reported as mean differences between groups, from linear regression. (d) Data analysed on a logarithmic scale, with treatment effect estimates reported as relative differences between groups.*

		Treatment Group			Treatment Effect			Treatment Effect	
		Placebo (n=142)	Alteplase 10mg (n=136)	Alteplase 20mg (n=143)	Alteplase 10mg vs placebo	Alteplase 20mg vs. placebo	Interaction p-value (treatment as a 3-level categorical variable)	Alteplase (10mg or 20mg) vs. placebo	Interaction p-value (treatment as a 2-level categorical variable)
					Estimate (95% CI) p-value	Estimate (95% CI) p-value		Estimate (95% CI), p-value	
MVO present (n/ total) (a)									
Anterior MI:	TIMI flow ≤2	8/23 (34.8%)	11/22 (50.0%)	15/27 (55.6%)	1.88 (0.57, 6.21) p=0.304	2.34 (0.75, 7.37) p=0.145	0.354	2.12 (0.77, 6.13) p=0.151	0.150
	TIMI 3 flow	19/36 (52.8%)	16/34 (47.1%)	15/31 (48.4%)	0.80 (0.31, 2.03) p=0.633	0.84 (0.32, 2.19) p=0.720		0.82 (0.36, 1.84) p=0.625	
Non-anterior MI:	TIMI flow ≤2	7/21 (33.3%)	14/20 (70.0%)	11/22 (50.0%)	4.67 (1.30, 18.65) **p=0.022**	2.00 (0.58, 6.87) p=0.271	**0.014**	2.94 (0.98, 8.81) p=0.054	**0.012**
	TIMI 3 flow	22/49 (44.9%)	13/46 (28.3%)	18/52 (34.6%)	0.48 (0.21, 1.14) p=0.095	0.65 (0.29, 1.45) p=0.292		0.57 (0.28, 1.15) p=0.116	
MVO extent (% LV mass) (b)									
Anterior MI:	TIMI flow ≤2	3.7 ± 7.4	2.5 ± 3.4	6.3 ± 8.0	0.00 (-0.85, 0.86) p=0.993	0.73 (-0.09, 1.54) p=0.080	0.261	0.40 (-0.32, 1.13) p=0.277	0.284
	TIMI 3 flow	3.0 ± 4.1	2.9 ± 4.6	3.1 ± 5.6	-0.12 (-0.80, 0.57) p=0.738	-0.10 (-0.80, 0.60) p=0.778		-0.11 (-0.70, 0.49) p=0.072	
Non-anterior MI:	TIMI flow ≤2	1.4 ± 2.9	3.1 ± 4.5	4.2 ± 6.5	0.65 (-0.07, 1.37) p=0.079	0.71 (0.00, 1.41) p=0.050	0.156	0.68 (0.07, 1.29) **p=0.031**	0.053
	TIMI 3 flow	1.5 ± 2.6	2.1 ± 4.9	1.8 ± 3.3	-0.07 (-0.54, 0.40) p=0.775	-0.02 (-0.49, 0.44) p=0.922		-0.04 (-0.45, 0.36) p=0.828	

Table 3.4 *(page 1 of 3) Analysis of CMR parameters 2 to 7 days after primary PCI, in the T-TIME angiographic study, by sub-groups of TIMI flow (≤2 vs. 3) immediately before study drug administration, and by sub-groups of MI location (anterior [n=187], non-anterior [n=234]). Data are mean ± SD, or n (%), unless otherwise stated. Missing data: infarct size (n=66), LV ejection fraction or volumes (n=63). Treatment effect estimates and interaction with treatment received are shown. (a) Treatment effect estimates reported as ORs between groups from a logistic regression model. (b) Treatment effect estimates reported as mean differences in square root transformed MVO extent between groups, from a linear regression model. (c) Treatment effect estimates reported as mean differences between groups, from linear regression.*

		Treatment Group			Treatment Effect		Interaction p-value (treatment as a 3-level categorical variable)	Treatment Effect	Interaction p-value (treatment as a 2-level categorical variable)
		Placebo (n=142)	Alteplase 10mg (n=136)	Alteplase 20mg (n=143)	Alteplase 10mg vs. placebo	Alteplase 20mg vs. placebo		Alteplase (10mg or 20mg) vs. placebo	
					Estimate (95% CI) p-value	Estimate (95% CI) p-value		Estimate (95% CI) p-value	
Myocardial haemorrhage present (n/ total) (a)									
Anterior MI:	TIMI flow ≤2	7/21 (33.3%)	10/19 (52.6%)	15/27 (55.6%)	2.22 (0.62, 7.98) p=0.221	2.50 (0.77, 8.16) p=0.129	0.245	2.38 (0.83, 7.32) p=0.114	0.102
	TIMI 3 flow	17/34 (50.0%)	15/33 (45.5%)	12/29 (41.4%)	0.83 (0.32, 2.18) p=0.710	0.71 (0.26, 1.92) p=0.494		0.77 (0.33, 1.79) p=0.544	
Non-anterior MI:	TIMI flow ≤2	4/19 (21.0%)	12/19 (63.0%)	11/22 (50.0%)	6.43 (1.62, 30.35) **p=0.012**	3.75 (0.99, 16.56) p=0.061	**0.007**	4.79 (1.45, 19.13) **p=0.015**	**0.003**
	TIMI 3 flow	22/48 (45.8%)	13/43 (30.2%)	18/52 (34.6%)	0.51 (0.21, 1.20) p=0.129	0.63 (0.28, 1.40) p=0.254		0.57 (0.28, 1.17) p=0.124	
Myocardial haemorrhage extent (% LV mass) (c)									
Anterior MI:	TIMI flow ≤2	2.9 ± 7.1	2.8 ± 4.0	4.6 ± 6.4	-0.08 (-3.22, 3.05) p=0.959	1.77 (-1.10, 4.64) p=0.230	0.671	1.01 (-1.59, 3.61) p=0.447	0.752
	TIMI 3 flow	1.6 ± 3.0	2.0 ± 3.4	2.2 ± 5.2	0.39 (-2.08, 2.87) p=0.757	0.55 (-2.01, 3.10) p=0.676		0.46 (-1.71, 2.64) p=0.677	
Non-anterior MI:	TIMI flow ≤2	0.4 ± 1.1	1.6 ± 2.5	2.9 ± 4.9	1.21 (-0.94, 3.36) p=0.272	2.52 (0.56, 4.47) **p=0.010**	0.072	1.99 (0.23, 3.75) **p=0.028**	0.067
	TIMI 3 flow	1.3 ± 2.7	1.6 ± 3.8	1.1 ± 2.8	0.28 (-1.04, 1.60) p=0.679	-0.16 (-1.43, 1.11) p=0.804		0.04 (-1.08, 1.16) p=0.944	

Table 3.4 *(page 2 of 3) Analysis of CMR parameters 2 to 7 days after primary PCI, in the T-TIME angiographic study, by sub-groups of TIMI flow (≤2 vs. 3) immediately before study drug administration, and by sub-groups of MI location (anterior [n=187], non-anterior [n=234]). Data are mean ± SD, or n (%), unless otherwise stated. Missing data: infarct size (n=66), LV ejection fraction or volumes (n=63). Treatment effect estimates and interaction with treatment received are shown. (a) Treatment effect estimates reported as ORs between groups from a logistic regression model. (b) Treatment effect estimates reported as mean differences in square root transformed MVO extent between groups, from a linear regression model. (c) Treatment effect estimates reported as mean differences between groups, from linear regression.*

		Treatment Group			Treatment Effect		Interaction p-value (treatment as a 3-level categorical variable)	Treatment Effect	Interaction p-value (treatment as a 2-level categorical variable)
		Placebo (n=142)	Alteplase 10mg (n=136)	Alteplase 20mg (n=143)	Alteplase 10mg vs. placebo	Alteplase 20mg vs. placebo		Alteplase (10mg or 20mg) vs. placebo	
					Estimate (95% CI) p-value	Estimate (95% CI) p-value		Estimate (95% CI) p-value	
Infarct size (% LV mass) (c)									
Anterior MI:	TIMI flow ≤2	33.4 ± 17.0	37.6 ± 11.5	35.3 ± 15.4	4.13 (-3.97, 12.23) p=0.319	1.91 (-5.80, 9.61) p=0.628	0.382	2.91 (-3.94, 9.75) p=0.407	0.180
	TIMI 3 flow	33.1 ± 12.7	31.3 ± 12.0	28.4 ± 14.5	-1.75 (-8.24, 4.75) p=0.598	-4.71 (-11.36, 1.95) p=0.167		-3.16 (-8.79, 2.47) p=0.272	
Non-anterior MI:	TIMI flow ≤2	22.3 ± 11.4	25.8 ± 11.3	28.5 ± 8.3	3.57 (-2.35, 9.49) p=0.238	6.23 (0.45, 12.01) **p=0.036**	0.097	4.96 (-0.08, 10.01) p=0.055	**0.045**
	TIMI 3 flow	20.8 ± 9.8	19.8 ± 8.8	19.5 ± 9.4	-1.06 (-4.95, 2.83) p=0.595	-1.34 (-5.11, 2.43) p=0.488		-1.21 (-4.51, 2.10) p=0.475	

Table 3.4 *(page 3 of 3) Analysis of CMR parameters 2 to 7 days after primary PCI, in the T-TIME angiographic study, by sub-groups of TIMI flow (≤2 vs. 3) immediately before study drug administration, and by sub-groups of MI location (anterior [n=187], non-anterior [n=234]). Data are mean ± SD, or n (%), unless otherwise stated. Missing data: infarct size (n=66), LV ejection fraction or volumes (n=63). Treatment effect estimates and interaction with treatment received are shown. (a) Treatment effect estimates reported as ORs between groups from a logistic regression model. (b) Treatment effect estimates reported as mean differences in square root transformed MVO extent between groups, from a linear regression model. (c) Treatment effect estimates reported as mean differences between groups, from linear regression.*

	Treatment Group			Treatment Effect		Interaction p-value (treatment as a 3-level categorical variable)	Treatment Effect	Interaction p-value (treatment as a 2-level categorical variable)
	Placebo (n=142)	Alteplase 10mg (n=136)	Alteplase 20mg (n=143)	Alteplase 10mg vs placebo	Alteplase 20mg vs. placebo		Alteplase (10mg or 20mg) vs. placebo	
				Estimate (95% CI) p-value	Estimate (95% CI) p-value		Estimate (95% CI), p-value	
Infarct size (% LV mass) (a)								
TIMI flow ≤2	21.3 ± 14.7	22.1± 11.3	23.9 ± 13.0	1.11 (-3.61, 5.83) p=0.645	2.73 (-1.84, 7.30) p=0.242	0.488	1.97 (2.07, 6.01 p=0.339	0.261
TIMI 3 flow	17.5 ±11.2	16.3±10.3	16.2±10.3	-1.09 (-4.57, 2.39) p=0.539	-0.74 (-4.17, 2.70) p=0.675		-0.91 (-3.90, 2.08) p=0.552	
LV ejection fraction (%) (a)								
TIMI flow ≤2	47.4 ± 11.9	47.3 ± 8.7	46.8 ± 9.2	1.03 (0.88, 1.20) p=0.729	0.99 (0.85, 1.15) p=0.878	0.762	1.01 (0.88, 1.15) p=0.923	0.508
TIMI 3 flow	50.8 ± 6.8	49.1 ± 7.3	49.9 ± 7.9	1.10 (0.99, 1.23) p=0.085	1.03 (0.92, 1.15) p=0.640		1.06 (0.97, 1.17) p=0.210	
LV end-systolic volume (ml) (b)								
TIMI flow ≤2	81.6 [72.8, 114.7]	88.9 [71.4, 116.5]	92.1 [71.5, 110.1]	1.03 (0.88, 1.20) p=0.729	0.99 (0.85, 1.15) p=0.878	0.762	1.01 (0.88, 1.15) p=0.923	0.508
TIMI 3 flow	77.5 [60.7, 99.5]	85.9 [71.7, 103.3]	78.5 [65.8, 102.1]	1.10 (0.99, 1.23) p=0.085	1.03 (0.92, 1.15) p=0.640		1.06 (0.97, 1.17) p=0.210	

Table 3.5 *(page 1 of 2) Analysis of CMR parameters 3 months after primary PCI, in the T-TIME angiographic study, by sub-groups of TIMI flow (≤2 vs. 3) immediately before study drug administration (adjusted for MI location). Data are mean ± SD, median [IQR], or n (%), unless otherwise stated. Missing data: infarct size (n=66), LV ejection fraction or volumes (n=63). Treatment effect estimates and interaction with treatment received are shown. (a) Treatment effect estimates reported as mean differences between groups. (b) Data analysed on a logarithmic scale, with treatment effect estimates reported as relative differences between groups.*

	Treatment Group			Treatment Effect		Interaction p-value (treatment as a 3-level categorical variable)	Treatment Effect	Interaction p-value (treatment as a 2-level categorical variable)
	Placebo (n=142)	Alteplase 10mg (n=136)	Alteplase 20mg (n=143)	Alteplase 10mg vs. placebo	Alteplase 20mg vs. placebo		Alteplase (10mg or 20mg) vs. placebo	
				Estimate (95% CI), p-value	Estimate (95% CI), p-value		Estimate (95% CI), p-value	
LV end-diastolic volume (ml) (b)								
TIMI flow ≤2	170.2 [158.8, 207.1]	170.0 [152.9, 206.4]	174.0 [150.5, 195.1]	1.01 (0.91, 1.12) p=0.796	0.95 (0.86, 1.05) p=0.349	0.567	0.98 (0.90, 1.07) p=0.673	0.281
TIMI 3 flow	157.9 [138.9, 188.5]	173.6 [153.7, 205.6]	162.9 [140.4, 194.3]	1.08 (1.00, 1.16) **p=0.045**	1.01 (0.94, 1.08) p=0.847		1.04 (0.98, 1.11) p=0.213	

Table 3.5 *(page 2 of 2) Analysis of CMR parameters 3 months after primary PCI, in the T-TIME angiographic study, by sub-groups of TIMI flow (≤2 vs. 3) immediately before study drug administration (adjusted for MI location). Data are mean ± SD, median [IQR], or n (%), unless otherwise stated. Missing data: infarct size (n=66), LV ejection fraction or volumes (n=63). Treatment effect estimates and interaction with treatment received are shown. (a) Treatment effect estimates reported as mean differences between groups. (b) Data analysed on a logarithmic scale, with treatment effect estimates reported as mean differences between groups.*

	Treatment group			Treatment Effect		Interaction p-value (treatment as a 3-level categorical variable)	Treatment Effect	Interaction p-value (treatment as a 2-level categorical variable)
	Placebo (n=134)	Alteplase 10mg (n=131)	Alteplase 20mg (n=136)	Alteplase 10mg vs. placebo	Alteplase 20mg vs. placebo		Alteplase (10mg or 20mg) vs. placebo	
				Estimate (95% CI) p-value	Estimate (95% CI) p-value		Estimate (95% CI), p-value	
MVO present (n/ total) (a)								
TIMI 2 flow	13/36 (36.1%)	22/37 (59.5%)	21/43 (48.8%)	2.58 (1.00, 6.65) p=0.051	1.66 (0.67, 4.13) p=0.257	0.153	2.06 (0.92, 4.62) p=0.081	**0.022**
TIMI 3 flow	41/85 (48.2%)	29/80 (36.3%)	33/83 (39.8%)	0.61 (0.33, 1.14) p=0.119	0.72 (0.39, 1.34) p=0.298		0.66 (0.39, 1.13) p=0.128	
MVO extent (% of LV mass) (b)								
TIMI 2 flow	3.0 ± 6.2	2.8 ± 3.9	5.2 ± 7.2	0.26 (-0.36, 0.89) p=0.405	0.55 (0.18, 1.27) p=0.136	0.243	0.43 (0.17, 1.02) p=0.158	0.107
TIMI 3 flow	2.2 ± 3.4	2.4 ± 4.8	2.3 ± 4.3	-0.09 (-0.49, 0.31) p=0.661	-0.06 (-0.45, 0.34) p=0.777		-0.07 (-0.42, 0.27) p=0.677	
Myocardial haemorrhage present (n/ total) (a)								
TIMI 2 flow	10/33 (30.0%)	20/35 (57.1%)	21/43 (48.8%)	3.05 (1.12, 8.31) **p=0.029**	2.14 (0.82, 5.62) p=0.121	0.054	2.55 (1.07, 6.06) **p=0.034**	**0.009**
TIMI 3 flow	39/82 (47.6%)	28/76 (36.8%)	30/81 (37.0%)	0.64 (0.34, 1.21) p=0.168	0.66 (0.35, 1.23) p=0.188		0.65 (0.38, 1.11) p=0.117	

Table 3.6 *(page 1 of 2) Analysis of CMR parameters 2 to 7 days after primary PCI, in the T-TIME angiographic study, by sub-groups of TIMI flow (2 vs. 3) immediately before study drug administration (adjusted for MI location). Data are mean ± SD, or n (%), unless otherwise stated. Missing data: MVO extent or presence/ absence (n=78), myocardial haemorrhage extent (n=68), myocardial haemorrhage presence/ absence (n=51), infarct size (n=37). Treatment effect estimates and interaction with treatment received are shown. (a) Treatment effect estimates reported as ORs between groups from a logistic regression model. (b) Treatment effect estimates reported as mean differences in square root transformed MVO extent between groups, from a linear regression model. (c) Treatment effect estimates reported as mean differences between groups, from linear regression.*

	Treatment group			Treatment Effect			Treatment Effect	
	Placebo (n=134)	Alteplase 10mg (n=131)	Alteplase 20mg (n=136)	Alteplase 10mg vs. placebo Estimate (95% CI) p-value	Alteplase 20mg vs. placebo Estimate (95% CI) p-value	Interaction p-value (treatment as a 3-level categorical variable)	Alteplase (10mg or 20mg) vs. placebo Estimate (95% CI) p-value	Interaction p-value (treatment as a 2-level categorical variable)
Myocardial haemorrhage extent (% LV mass) (c)								
TIMI 2 flow	2.0 ± 5.7	2.0 ± 2.9	4.2 ± 6.0	1.95 (-0.33, 4.24) p=0.093	1.98 (-0.74, 4.69) p=0.151	0.132	0.50 (-0.04, 3.04) p=0.287	0.362
TIMI 3 flow	1.4 ± 2.8	1.8 ± 3.6	1.5 ± 3.8	0.29 (-1.00, 1.58) p=0.656	0.11 (-1.16, 1.38) p=0.867		0.20 (-0.91, 1.31) p=0.726	
Infarct size (% LV mass) (c)								
TIMI 2 flow	28.5 ± 16.4	30.5 ± 12.7	31.9 ± 13.5	2.46 (-3.91, 8.82) p=0.445	3.00 (-3.48, 9.48) p=0.359	0.158	2.69 (-2.67, 8.04) p=0.322	0.085
TIMI 3 flow	26.0 ± 12.6	24.7 ± 11.7	22.8 ± 12.3	-1.35 (-4.92, 2.22) p=0.460	-2.68 (-6.22, 0.86) p=0.138		-2.03 (-5.09, 1.03) p=0.195	

Table 3.6 *(page 2 of 2) Analysis of CMR parameters 2 to 7 days after primary PCI, in the T-TIME angiographic study, by sub-groups of TIMI flow (2 vs. 3) immediately before study drug administration (adjusted for MI location). Data are mean ± SD, or n (%), unless otherwise stated. Missing data: MVO extent or presence/ absence (n=78), myocardial haemorrhage extent (n=68), myocardial haemorrhage presence/ absence (n=51), infarct size (n=37). Treatment effect estimates and interaction with treatment received are shown. (a) Treatment effect estimates reported as ORs between groups from a logistic regression model. (b) Treatment effect estimates reported as mean differences in square root transformed MVO extent between groups, from a linear regression model. (c) Treatment effect estimates reported as mean differences between groups, from linear regression.*

3.4.4 LVEDP, angiographic and ECG endpoints

LVEDP was measured in 272 of the 421 patients (65%). In patients with TIMI flow ≤2 pre-study drug, there were no associations between alteplase and the following: LVEDP, TFC, MPG ≤1 and TIMI flow grade ≤2 at the end of PCI, and % ST-segment resolution (Table 3.7). In patients with TIMI 3 flow pre-study drug, alteplase was not associated with any of these parameters, apart from TFC and LVEDP (Table 3.7). In patients with TIMI 3 flow immediately pre-drug, LVEDP was lower with alteplase 20mg vs. placebo (mean difference: -0.16 (95% CI: -0.30, -0.02) p=0.025) (Table 3.7).

There were no interactions between sub-groups of TIMI flow pre-drug, alteplase, and LVEDP, TFC, MPG or TIMI flow grade at the end of the PCI procedure (Table 3.7). There were no interactions between sub-groups of TIMI flow pre-study drug, alteplase, and absolute % ST-segment resolution at 60 minutes post-reperfusion compared to baseline (Table 3.6).

3.4.5 Coagulation variables

There were no interactions between sub-groups of TIMI flow pre-study drug, alteplase, and fibrin D-dimers, prothrombin fragment $F_{1+2,}$ or plasminogen (Table 3.8). There was an increase in systemic fibrin D-dimers (a product of fibrin lysis) and decrease in plasminogen 2 hours post-primary PCI relative to baseline, in patients with TIMI flow ≤2 or 3, pre-study drug. This is consistent with what is expected following intra-arterial fibrinolysis.

There was a significant increase in systemic prothrombin fragment F_{1+2} (a measure of thrombin activation) two hours post-primary PCI relative to baseline, with alteplase compared to placebo, in patients with TIMI flow ≤2 or 3 pre-drug (Table 3.8)

	Treatment Group			Treatment Effect		Interaction p-value (treatment as a 3-level categorical variable)	Treatment Effect	Interaction p-value (treatment as a 2-level categorical variable)
	Placebo (n=142)	Alteplase 10mg (n=136)	Alteplase 20mg (n=143)	Alteplase 10mg vs. placebo Estimate (95% CI) p-value	Alteplase 20mg vs. placebo Estimate (95% CI) p-value		Alteplase (10mg or 20mg) vs. placebo Estimate (95% CI), p-value	
Absolute % ST-segment resolution 60 min post-reperfusion relative to baseline(a)								
TIMI flow ≤2	45.0 ± 44.3	40.6 ± 52.1	37.7 ± 43.3	-4.43 (-21.96, 13.10) p=0.621	-7.37 (-24.21, 9.47) p=0.392	0.671	-6.02 (-20.91, 8.87) p=0.429	0.789
TIMI 3 flow	50.7± 36.4	44.4 ± 41.8	50.5 ± 46.0	-6.89 (-20.50, 6.73) p=0.322	0.34 (-13.59, 12.91) p=0.960		-3.44 (-14.99, 8.11) p=0.560	
TIMI flow grade post-PCI ≤2 (b)								
TIMI flow ≤2	19 (38.0)	15 (30.6)	22 (40.0)	0.72 (0.31, 1.66) p=0.432	1.09 (0.50, 2.39) p=0.838	0.282	0.90 (0.45, 1.81) p=0.762	0.071
TIMI 3 flow	5 (5.4)	12 (13.8)	11 (12.5)	2.80 (0.95, 8.34) p=0.064	2.53 (0.84, 7.61) p=0.099		2.66 (0.98, 7.27) p=0.056	
TFC (c):								
TIMI flow ≤2	26.5 [17.4, 39.4]	22.4 [15.5, 35.9]	28.0 [21.8, 40.5]	0.91 (0.73, 1.13) p=0.372	1.07 (0.87, 1.32) p=0.534	0.095	0.99 (0.82, 1.19) p=0.902	0.276
TIMI 3 flow	17.7 [12.0, 24.0]	20.0 [14.0, 26.0]	17.4 [12.9, 24.0]	1.18 (1.00, 1.39) **p=0.049**	1.08 (0.92, 1.26) p=0.377		1.12 (0.98, 1.29) p=0.099	

Table 3.7 *(page 1 of 2) Analysis of ECG and angiographic parameters at the end of primary PCI, in the T-TIME angiographic study, by sub-groups of TIMI flow (≤2 vs. 3) immediately before study drug administration (adjusted for location of MI). Data are mean ± SD, median [IQR], or n (%), unless otherwise stated. Missing data: % ST-segment resolution (n=43), TFC (n=2), LVEDP (n=154). Treatment effect estimates and interaction with treatment received are shown. (a) Treatment effect estimates reported as mean differences between groups. (b) Treatment effect estimates reported as ORs between groups from a logistic regression model. (c) Data analysed on a logarithmic scale, treatment effect estimates reported as relative differences between groups. (d) Data analysed on a logarithmic scale, with treatment effects reported as mean differences between groups.*

	Treatment Group			Treatment Effect			Treatment Effect	
	Placebo (n=142)	Alteplase 10mg (n=136)	Alteplase 20mg (n=143)	Alteplase 10mg vs. placebo Estimate (95% CI) p-value	Alteplase 20mg vs. placebo Estimate (95% CI) p-value	Interaction p-value (treatment as a 3-level categorical variable)	Alteplase (10mg or 20mg) vs. placebo Estimate (95% CI) p-value	Interaction p-value (treatment as a 2-level categorical variable)
MPG ≤1 (b):								
TIMI flow ≤2	24 (48.0)	23 (46.9)	33 (60.0)	0.95 (0.42, 2.13) p=0.895	1.65 (0.75, 3.66) p=0.214	0.050	1.27 (0.63, 2.54) p=0.501	0.634
TIMI 3 flow	31 (33.7)	36 (41.4)	23 (26.1)	1.43 (0.77, 2.65) p=0.264	0.71 (0.37, 1.37) p=0.308		1.02 (0.59, 1.77) p=0.932	
LVEDP mmHg (d):								
TIMI flow ≤2	16.5 (12.0-23.0)	20.0 (15.8-23.0)	17.5 (12.8-24.3)	0.14 (-0.09, 0.38) p=0.220	0.04 (-0.20, 0.28) p=0.740	0.221	0.09 (-0.11, 0.29) p=0.384	0.118
TIMI 3 flow	18.0 (14.0-25.0)	19.0 (13.8-23.0)	15.0 (12.0-20.0)	-0.02 (-0.16, 0.13) p=0.841	-0.16 (-0.30, -0.02) **p=0.025**		-0.09 (-0.21, 0.04) p=0.173	

Table 3.7 *(page 2 of 2) Analysis of ECG and angiographic parameters at the end of primary PCI, in the T-TIME angiographic study, by sub-groups of TIMI flow (≤2 vs. 3) immediately before study drug administration (adjusted for location of MI). Data are mean ± SD, median [IQR], or n (%), unless otherwise stated. Missing data: % ST-segment resolution (n=43), TFC (n=2), LVEDP (n=149). Treatment effect estimates and interaction with treatment received are shown. (a) Treatment effect estimates reported as mean differences between groups. (b) Treatment effect estimates reported as ORs between groups from a logistic regression model. (c) Data analysed on a logarithmic scale, treatment effects reported as relative differences between groups. (d) Data analysed on a logarithmic scale, with treatment effects reported as mean differences between groups.*

	Treatment Group			Treatment Effect		Interaction p-value (treatment as a 3-level categorical variable)	Treatment Effect	Interaction p-value (treatment as a 2-level categorical variable)
	Placebo (n=142)	Alteplase 10mg (n=136)	Alteplase 20mg (n=143)	Alteplase 10mg vs. placebo	Alteplase 20mg vs. placebo		Alteplase (10mg or 20mg) vs. placebo	
				Estimate (95% CI) p-value	Estimate (95% CI) p-value		Estimate (95% CI), p-value	
Plasminogen (U/dL) 2 hours post-PCI (a)								
TIMI flow ≤2	95.0 [88.3, 101.0]	91.0 [81.5, 100.8]	83.5 [74.8, 92.0]	-2.66 (-8.56, 3.25) p=0.378	-10.88 (-16.52, -5.25) **p<0.001**	0.378	-7.16 (-12.23, -2.09) **p=0.006**	0.623
TIMI 3 flow	96.0 [87.0, 104.5]	88.0 [80.0, 98.0]	84.0 [77.0, 92.0]	-6.90 (-11.20, -2.66) **p=0.002**	-10.49 (-14.73, -6.25) **p<0.001**		-8.74 (-12.45, -5.03) **p<0.001**	
Fibrin D-dimer (ng/mL) 2 hours post-PCI (b)								
TIMI flow ≤2	101.0 [69.5, 138.3]	319.5 [215.5, 633.0]	513.5 [266.8, 831.5]	3.64 (2.53, 5.22) **p<0.001**	4.91 (3.48, 6.93) **p<0.001**	0.563	4.29 (3.15, 5.83) **p<0.001**	0.299
TIMI 3 flow	117.0 [74.8, 169.0]	354.0 [224.0, 593.0]	421.0 [275.5, 641.5]	3.15 (2.43, 4.09) **p<0.001**	3.88 (3.00, 5.03) **p<0.001**		3.50 (2.80, 4.39) **p<0.001**	
Prothrombin fragment F_{1+2} (pmol/L) 2 hours post-PCI (b)								
TIMI flow ≤2	165.0 [134.0, 220.8]	161.1 [124.9, 260.8]	201.5 [147.4, 303.0]	1.20 (0.92, 1.57) p=0.183	1.22 (0.94, 1.57) p=0.136	0.909	1.21 (0.96, 1.52) p=0.103	0.925
TIMI 3 flow	155.5 [124.1, 267.0]	200.3 [144.0, 328.2]	199.1 [153.2, 303.0]	1.26 (1.04, 1.53) **p=0.019**	1.19 (0.98, 1.44) p=0.078		1.23 (1.04, 1.45) **p=0.017**	

Table 3.8 *(page 1 of 2) Analysis of coagulation variables after primary PCI, in the T-TIME angiographic study, by sub-groups of TIMI flow (≤2 vs. 3) immediately before study drug administration (adjusted for MI location). Data are mean ± SD, median [IQR], or n (%), unless otherwise stated. Missing data: coagulation variables 2 hours post-PCI (n=75), change in coagulation parameters at 2 hours relative to baseline (n=80). Treatment effect estimates and interaction with treatment received are shown. (a) Treatment effect estimates reported as mean differences between groups. (b) Data analysed on a logarithmic scale, with treatment effect estimates reported as relative differences between groups.*

	Treatment Group			Treatment Effect		Interaction p-value (treatment as a 3-level categorical variable)	Treatment Effect	Interaction p-value (treatment as a 2-level categorical variable)
	Placebo (n=142)	Alteplase 10mg (n=136)	Alteplase 20mg (n=143)	Alteplase 10mg vs. placebo	Alteplase 20mg vs. placebo		Alteplase (10mg or 20mg) vs. placebo	
				Estimate (95% CI) p-value	Estimate (95% CI) p-value		Estimate (95% CI) p-value	
Change in plasminogen (U/dL) at 2 hours relative to baseline (a)								
TIMI flow ≤2	1.0 [-2.0, 3.0]	-3.0 [-9.5, 4.5]	-10.0 [-15.0, -6.0]	-4.30 (-8.20, -0.40) **p=0.034**	-13.40 (-17.10, -9.60) **p<0.001**	0.110	-9.30 (-12.80, -5.80) **p<0.001**	0.609
TIMI 3 flow	1.0 [-3.3, 5.0]	-5.0 [-11.0, -0.8]	-9.5 [-16.0, -4.0]	-6.10 (-8.90, -3.30) **p<0.001**	-10.20 (-13.00, -7.40) **p<0.001**		-8.20 (-10.07, -5.60) **p<0.001**	
Ratio of fibrin D-dimer at 2 hours relative to baseline (b)								
TIMI flow ≤2	1.1 [1.0, 1.3]	3.2 [2.2, 6.0]	3.8 [2.0, 6.2]	3.27 (2.47, 4.32) **p<0.001**	3.52 (2.70, 4.59) **p<0.001**	0.213	3.40 (2.67, 4.32) **p<0.001**	0.907
TIMI 3 flow	1.1 [0.9, 1.5]	3.4 [2.2, 4.6]	4.9 [3.2, 7.4]	2.86 (2.35, 3.50) **p<0.001**	4.16 (3.41, 5.08) **p<0.001**		3.46 (2.91, 4.12) **p<0.001**	
Ratio of prothrombin fragment F_{1+2} at 2 hours relative to baseline (b)								
TIMI flow ≤2	1.1 [0.9, 1.3]	1.3 [1.1, 1.6]	1.2 [1.0, 1.6]	1.46 (1.16, 1.85) **p=0.002**	1.20 (0.96, 1.51) p=0.104	0.242	1.31 (1.08, 1.60) **p=0.008**	0.432
TIMI 3 flow	1.1 [0.9, 1.4]	1.2 [0.9, 1.5]	1.3 [1.1, 1.6]	1.18 (1.00, 1.39) p=0.057	1.20 (1.02, 1.42) **p=0.030**		1.19 (1.03, 1.38) **p=0.018**	

Table 3.8 *(page 2 of 2) Analysis of coagulation variables after primary PCI, in the T-TIME angiographic study, by sub-groups of TIMI flow (≤2 vs. 3) immediately before study drug administration (adjusted for MI location). Data are mean ± SD, median [IQR], or n (%), unless otherwise stated. Missing data: coagulation variables 2 hours post-PCI (n=75), change in coagulation parameters at 2 hours relative to baseline (n=80). Treatment effect estimates and interaction with treatment received are shown. (a) Treatment effect estimates reported as mean differences between groups. (b) Data analysed on a logarithmic scale, with treatment effect estimates reported as relative differences between groups.*

3.5 Discussion

In this prespecified analysis, low-dose intracoronary alteplase given early during primary PCI, was associated with increased presence of MVO and myocardial haemorrhage in participants who had TIMI flow ≤2 pre-drug administration (Figure 3.3). On the other hand, patients with TIMI 3 flow immediately pre-study drug administration had no difference in MVO or myocardial haemorrhage with alteplase compared to placebo. Furthermore, the relationship of intracoronary alteplase with infarct size 2 to 7 days following primary PCI depended on TIMI flow grade pre-drug administration.

The findings contrast with a previous study, which reported smaller 6-month infarct size (assessed by SPECT) in patients given adjunctive intracoronary streptokinase at the end of primary PCI (n=51) compared to standard primary PCI without streptokinase (n=44)[260]. In the previous study, intracoronary streptokinase was delivered post-stent, at the end of the PCI procedure, when 89% of the population had TIMI 3 flow[260], whereas in the T-TIME trial intracoronary alteplase was administered early after re-establishment of flow in the infarct-related artery pre-stenting, when only 63% of the population had TIMI 3 flow. The previous study was not double-blind, so it had an open design with unmasked interventions[260], and it is plausible that those findings might have been a consequence of type 1 statistical error, i.e. a false positive result.

The speculation that TIMI flow ≤2 at the time of study drug administration would in turn lead to inadequate drug delivery to the microcirculation, does not appear to be supported by the data. In fact, increased fibrin D-dimer, and lower plasminogen concentrations, were observed with alteplase in patients with TIMI flow ≤2 pre-drug, which indicates that fibrinolysis did indeed occur in this group of participants (Table 3.7).

An explanation for the findings presented in this chapter may be that in circumstances of slow microvascular flow, the undesired procoagulant effects of fibrinolytic therapy[288 353-358] were potentially augmented, thereby promoting microvascular thrombosis and worsening MVO (Figure 3.4). Indeed, increased prothrombin F_{1+2} concentrations were observed with alteplase (Table 3.7), despite therapeutic anticoagulation with heparin, which indicates thrombin generation and thrombosis[341]. Slow blood flow is one of the factors postulated by

Rudolph Virchow to propagate thrombus formation, the other 2 factors being vascular wall injury and hypercoagulable state due to abnormalities in coagulation, or fibrinolytic pathways, or platelet function[359]. The mechanism of the paradoxical procoagulant effect of alteplase, is that alteplase actives plasmin formation, and plasmin paradoxically increases thrombogenesis, due to the activation of factor V to Va[288].

Another explanation for the findings presented in this chapter may be that is circumstances of reduced antegrade flow myocardial perfusion is reduced, leading to higher local concentrations of alteplase, due to reduced "washout" of alteplase from the microcirculation. Alteplase activates plasmin formation, which degrades fibrin, and therefore increases bleeding propensity. Patients with TIMI flow ≤2 immediately pre-drug may have had move extensive microvascular injury, as evidenced by these patients having significantly more MVO and myocardial haemorrhage than the patients with TIMI 3 flow pre-drug. Myocardium with extensive microvascular damage from coronary occlusion is characterised by endothelial degradation, loss of capillary integrity and myocyte necrosis. In these circumstances, intracoronary alteplase appears to worsen extravasation of erythrocytes, resulting in myocardial haemorrhage in the infarct core (the secondary and irreversible manifestation of persistent MVO). An increase in extravasation of blood into the interstitial space of the infarct core results in external compression of capillaries, with an associated increase in microvascular resistance, thereby worsening MVO (Figure 3.4).

Insights from previous studies of glycoprotein IIbIIIa inhibitors are consistent with the findings reported in this chapter[155 360]. In a porcine model, with MI induced by balloon occlusion of the LAD (n=23), there was an increased incidence of myocardial haemorrhage when intracoronary glycoprotein IIbIIIa inhibitors were added to intravenous bivalirudin therapy[361]. In humans, peri-procedural glycoprotein IIbIIIa inhibitor use during STEMI (n=410) was an independent predictor myocardial haemorrhage, as assessed on T2-weighted CMR imaging[360]. Although a causal relationship cannot be assumed, because of the observational nature of that study, it is conceivable that more aggressive anti-thrombotic treatment may promote myocardial haemorrhage, especially in the context of extensive microvascular injury. Further support for this theory is from another study in 49 STEMI patients, which showed that more potent inhibition of platelet aggregation following glycoprotein IIbIIIa inhibitors was related to the occurrence of myocardial haemorrhage[362].

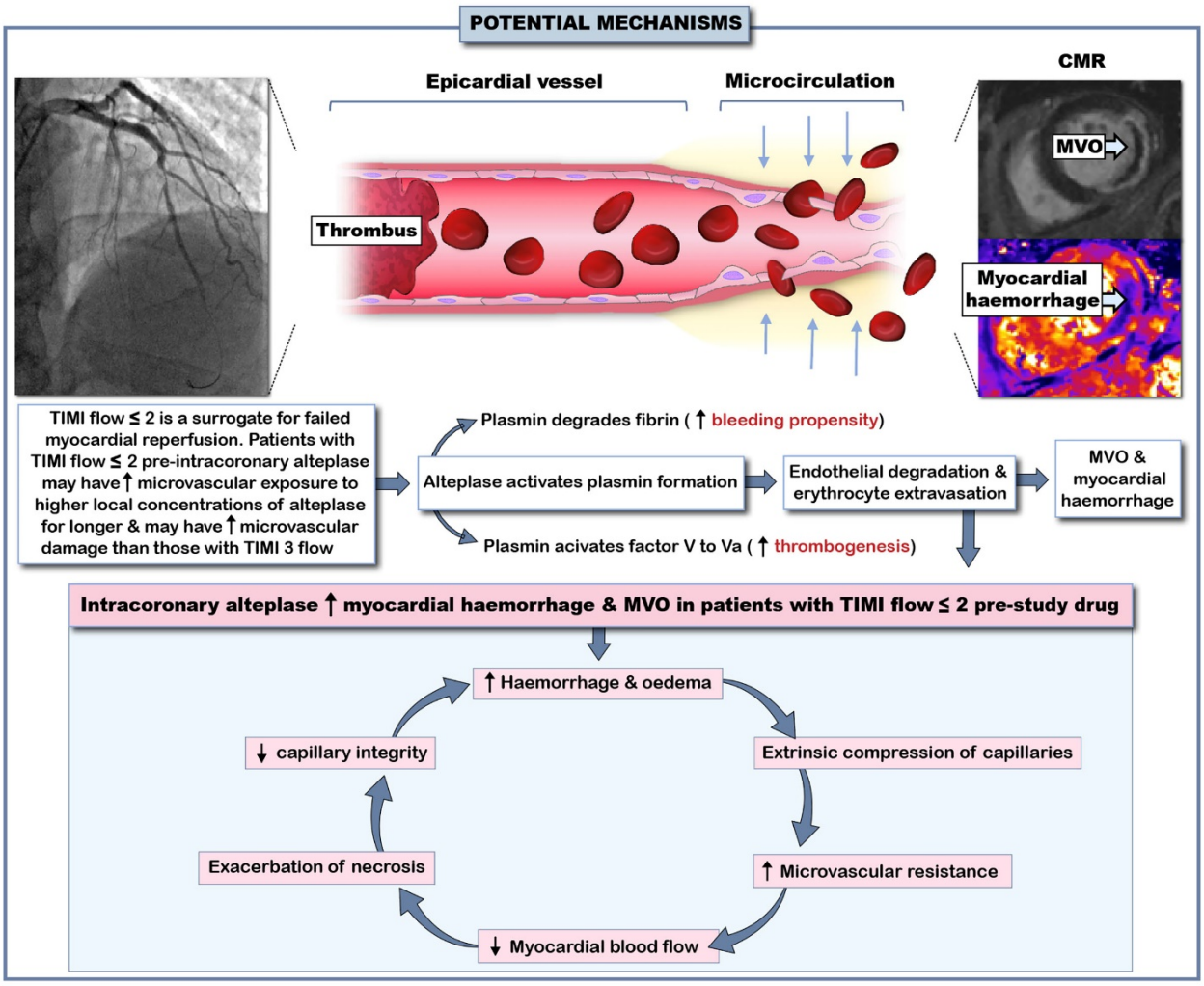

Figure 3.4 *Potential mechanisms for the association of intracoronary alteplase with increased MVO and myocardial haemorrhage presence, in patients with TIMI flow ≤2 immediately preceding study drug administration.*

In patients with reduced antegrade flow in the infarct-related artery, there may have been increased microvascular exposure to higher local concentrations of alteplase for longer. In patients with slow microvascular flow, intracoronary alteplase potentially promoted the undesired procoagulant effects of fibrinolytic therapy. TIMI flow ≤2 may indicate ongoing impaired myocardial reperfusion, due to extensive microvascular damage. In these circumstance, intracoronary fibrinolysis appears to worsen MVO and extravasation of erythrocytes, resulting in myocardial haemorrhage in the infarct core and potentially promotes microvascular thrombosis. An increased in extravasation of blood into the interstitial space results in external compression of capillaries with an associated increase in microvascular resistance. This leads to a further reduction in myocardial blood flow, and exacerbates myocardial necrosis and capillary destruction, which promotes further myocardial haemorrhage.

The observation that participants with TIMI flow ≤2 early after balloon angioplasty were more likely to have infarct-related lesions in the LAD is consistent with previous reports in the medical literature[363]. A potential explanation for this finding is that the LAD artery supplies a relatively larger territory of myocardium, thus LAD occlusion would likely have

resulted in relatively more extensive myocardial necrosis, making persistent TIMI flow ≤2 more likely during PCI. Analyses for interactions between TIMI flow pre-study drug, alteplase, and CMR parameters were performed separately for patients who had anterior MI and patients who had non-anterior MI (Table 3.4). In the group that had non-anterior MI (n=234) there were interactions between TIMI flow, alteplase and MVO presence, myocardial haemorrhage presence, or infarct size, consistent with the findings in the entire population studied (n=421). In the group that had anterior MI (n=187) these interactions were not observed, likely because the smaller number of patients limited statistical power to detect interactions.

The findings presented in this chapter are relevant as a disincentive to clinicians when considering bail-out lytic therapy for acute STEMI patients with high thrombus burden and angiographic "no reflow". The findings are also very relevant to ongoing clinical trials. Notably, the RESTORE-MI trial [ACTRN12618000778280][273] is randomising STEMI patients undergoing primary PCI (n=800) to adjunctive intracoronary tenecteplase or placebo, in a double-blind design, and a key inclusion criteria for that trial is a post-stent IMR >32 in the infarct-related artery, which signifies incomplete microvascular perfusion and microvascular dysfunction[156]. Importantly, the findings presented in this chapter suggest that low-dose intracoronary lytic therapy in STEMI patients who have incomplete perfusion after initial balloon angioplasty may be harmful. Therefore, the risk-based selection strategy used in the RESTORE-MI trial [ACTRN12618000778280][273], which targets patients with microvascular dysfunction evidenced by abnormal invasive coronary physiology, may enrol patients at risk of myocardial haemorrhage, which may be exacerbated by lytic therapy. Nonetheless, there are important differences in the design of T-TIME as compared to RESTORE-MI, notably, in relation to the timing of study drug administration (before, or after stent implantation, respectively) and the lytic agent (alteplase, or tenecteplase), which is relevant since tenecteplase is less procoagulant than alteplase[318].

The findings suggest that normal antegrade coronary flow is important for fibrinolytic therapy to be safe and effective. Therefore, future studies evaluating the effects of intracoronary lytic therapy should limit recruitment to patients with TIMI 3 flow at the time of study drug administration, which would be post-stent implantation for most patients, rather than pre-stent implantation.

3.6 Limitations

The strengths of this study are: (i) the analyses of source data were conducted in a double-blind manner to minimise the potential risk of bias, (ii) core-lab analyses were performed, (iii) the randomised design and (iv) CMR was available in almost all of the participants. Even though randomisation was not stratified according to TIMI flow grade post-reperfusion, the randomisation was stratified according to MI location (anterior vs. non-anterior), which was the only independent associate of TIMI flow pre-study drug. The regression analyses were adjusted for MI location, to limit the potential influence of confounding.

Because of the potential for type 1 statistical error (false positive) the findings should be interpreted as exploratory/ hypothesis generating, and causality should not be inferred. Nonetheless, whilst the results may be due to play of chance, the mechanism is plausible and type 1 statistical error appears less likely given that the findings for MVO and myocardial haemorrhage were consistent with infarct size at 2 to 7 days post-PCI. A potential limitation of not adjusting for multiple statistical comparisons is that the probability of a false positive result is increased[364]. Adjusting for multiple statistical comparisons, for example with the Bonferroni method, generally requires more stringent significance thresholds for p-values[365]. There is a danger that by correcting for multiplicity the probability of type 2 statistical error (false negative) is increased. The results from the analyses presented in this chapter include some very low interaction p-values, for example the interaction p-value for alteplase, coronary flow and myocardial haemorrhage presence was 0.001 (Table 3.3), which would likely remain significant even after adjustment for multiple statistical comparisons.

Lastly, TIMI 3 flow is not synonymous with optimal tissue perfusion, because MVO can be demonstrated in a substantial number of acute STEMI patients with TIMI 3 flow at the end of PCI[113]. Approximately 50% of acute STEMI patients with TIMI 3 flow at the end of PCI have MVO on CMR imaging[26 103]. In the present study, 42% of participants with TIMI 3 flow immediately pre-study drug administration had MVO present, and 40% of participants with TIMI 3 flow at the end of the PCI procedure had MVO present.

3.7 Future work

Future research is of interest to determine whether low-dose intracoronary tenecteplase (which has less unwanted procoagulant effects than alteplase[318]) administered after stent implantation, at the end of the primary PCI procedure (when TIMI 3 flow is routinely achieved), might have beneficial effects on the coronary microcirculation.

3.8 Conclusions

In conclusion, low-dose intracoronary alteplase administered pre-stent implantation was associated with an increased incidence of MVO and myocardial haemorrhage, in STEMI patients with impaired coronary flow (<TIMI 3) immediately pre-drug administration. Potential explanations include increased local exposure to alteplase enhancing endothelial damage and haemorrhagic transformation in the infarct core, and augmented procoagulant effects of alteplase promoting microvascular thrombosis. In patients with TIMI 3 flow in the infarct-related artery, pre-drug administration, alteplase was not associated with MVO or myocardial haemorrhage.

Chapter 4: Effects of intracoronary alteplase during primary PCI on IMR, CFR and RRR: the T-TIME physiology sub-study

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4.1 Abstract

Background: Impaired microcirculatory perfusion worsens prognosis following acute STEMI. The aim of the study presented in this chapter was to evaluate the effects of intracoronary alteplase, during primary PCI, on IMR, CFR and RRR.

Methods: The T-TIME coronary physiology study was a pre-defined, prospective, double-blind, sub-study, within the main T-TIME trial. From 2016 to 2017 patients with STEMI ≤6 hours from symptom onset were randomised, to receive alteplase 20mg, alteplase 10mg, or placebo infused into the infarct-related artery, after initial balloon angioplasty and/ or thrombus aspiration, but pre-stenting. IMR, CFR and RRR were measured at the end of the PCI procedure. CMR was performed at 2 to 7 days and 3 months post-PCI. Sub-group analyses in relation to ischaemic time ([i] <2 hours, [ii] ≥2, <4 hours and [iii] ≥4 hours), TIMI coronary flow grade immediately preceding study drug administration (≤2, and 3), and TIMI thrombus grade immediately before study drug administration (≤2 and ≥3) were prespecified.

Results: 144 patients (mean age: 59 ± 11 years, 80% male) were prospectively enrolled into the physiology sub-study, representing 33% of the overall population (n=440). The median IMR was 29.5 (IQR: 17.0 - 55.0), median CFR was 1.4 (IQR: 1.1 - 2.0) and median RRR was 1.7 (IQR: 1.3 - 2.3). Overall, IMR, CFR or RRR did not differ between alteplase and placebo groups. Interactions were observed between ischaemic time and alteplase for CFR (p=0.013), RRR (p=0.026) and MVO (p=0.022), but not for IMR. In patients with ischaemic time <2 hours, CFR was higher with alteplase 20mg vs. placebo (relative difference: 1.59 [95% CI: 1.11, 2.27] p=0.012), and RRR was also higher with alteplase 20mg vs. placebo (relative difference 1.52 [95% CI: 1.05, 2.22] p=0.028). In patients with ischaemic times ≥4 hours, MVO extent was higher with alteplase 20mg vs. placebo (mean difference: 1.47 [95% CI: 0.40, 0.54] p=0.007). There was no interaction

between TIMI flow, or thrombus grade, immediately preceding study drug administration, with alteplase, and IMR, CFR or RRR.

Conclusion: There was overall no difference in IMR, CFR or RRR with adjunctive intracoronary alteplase vs. placebo, during primary PCI. The results from sub-group analyses suggest that CFR and RRR may be improved with alteplase in patients presenting with an ischaemic time ≤2 hours, and that MVO extent may be worse with alteplase in patients presenting with an ischaemic time ≥4 hours.

4.2 Introduction

Failed myocardial perfusion following STEMI, revealed as MVO on CMR imaging, confers a worse prognosis[27]. There are no evidence-based treatments for MVO, so it is a clinical problem of unmet need. A key component for the pathophysiology of MVO is distal embolization and microvascular thrombi[31-33].

In contrast to CMR imaging, which is not readily available at the time of primary PCI, invasive coronary physiology parameters can quantify the immediate efficacy of microcirculatory reperfusion[16]. Among these parameters, IMR measures the minimal achievable microvascular resistance during maximal hyperaemia[140]. RRR reflects the microvascular vasodilator capacity, i.e. the ability of the coronary microcirculation to change from baseline to hyperaemia, reflecting the ability to achieve maximal hyperaemia[194 197]. RRR is the ratio between basal resting tone and resistance at maximal hyperaemia, and is lower when microvasodilatory capacity is impaired.

The aim of the present study was to evaluate the effects of low-dose intracoronary alteplase during primary PCI, on acute invasive coronary physiology measurements. The effects of ischaemic time on any associations between alteplase and IMR, CFR, or RRR was also assessed. The rationale for investigating this sub-group is that longer ischaemic time diminishes the efficacy of primary reperfusion therapies, including systemic fibrinolysis[367] and primary PCI[319]. The effects of TIMI coronary flow grade immediately before study drug administration, on any associations between alteplase and IMR, CFR, or RRR was also evaluated. The rationale for investigating this sub-group was the speculation that impaired antegrade flow in the infarct-related artery could influence the effects of intracoronary alteplase on the microcirculation. Lastly, the effects of TIMI thrombus grade immediately before study drug administration, on any associations between alteplase IMR, CFR or RRR was evaluated, to assess whether intracoronary alteplase might be effective compared to placebo, when administered to the most thrombus-laden lesions.

The following hypotheses were evaluated:

1) Intracoronary alteplase administered pre-stenting, is associated with lower IMR, higher CFR and higher RRR at the end of primary PCI, compared to placebo.

2) In the sub-group of patients with shorter ischaemic time (≤2 hours), intracoronary alteplase administered pre-stenting is associated with lower IMR, higher CFR and higher RRR at the end of primary PCI, compared to placebo.

3) In the sub-group of patients with TIMI 3 flow immediately preceding study drug administration, intracoronary alteplase is associated with lower IMR, higher CFR and higher RRR at the end of primary PCI, compared to placebo.

4) In the sub-group of patients with TIMI thrombus grade ≥3 immediately pre-study drug administration, intracoronary alteplase is associated with lower IMR, higher CFR and higher RRR at the end of primary PCI, compared to placebo.

4.3 Methods

Details of the specific methods used in the T-TIME physiology sub-study are described in section 2.2. The T-TIME physiology sub-study design is depicted in Figure 4.1.

4.3.1 Sample size calculation

The sample size calculation for the T-TIME physiology sub-study was based on data from patients in the British Heart Foundation MR-MI study[149 156] who fulfilled the eligibility criteria for T-TIME. For a comparison of IMR between the 3 treatment groups (placebo vs. alteplase 10mg vs. alteplase 20mg), assuming a mean IMR of 33.9 and SD of 25.2, and assuming mean differences in IMR for the10mg and 20mg alteplase groups vs. placebo of 10 and 20 respectively, then 108 subjects were needed for 85% power and a significance level of 0.05. For a comparison of CFR between the 3 treatment groups (placebo vs. alteplase 10mg vs. alteplase 20mg), assuming a mean CFR of 1.65 and SD of 0.8, and assuming mean differences in CFR between the 10mg and 20mg alteplase groups vs. placebo of 0.4 and 0.8 respectively, then 69 subjects were needed with 85% power (α = 0.05).

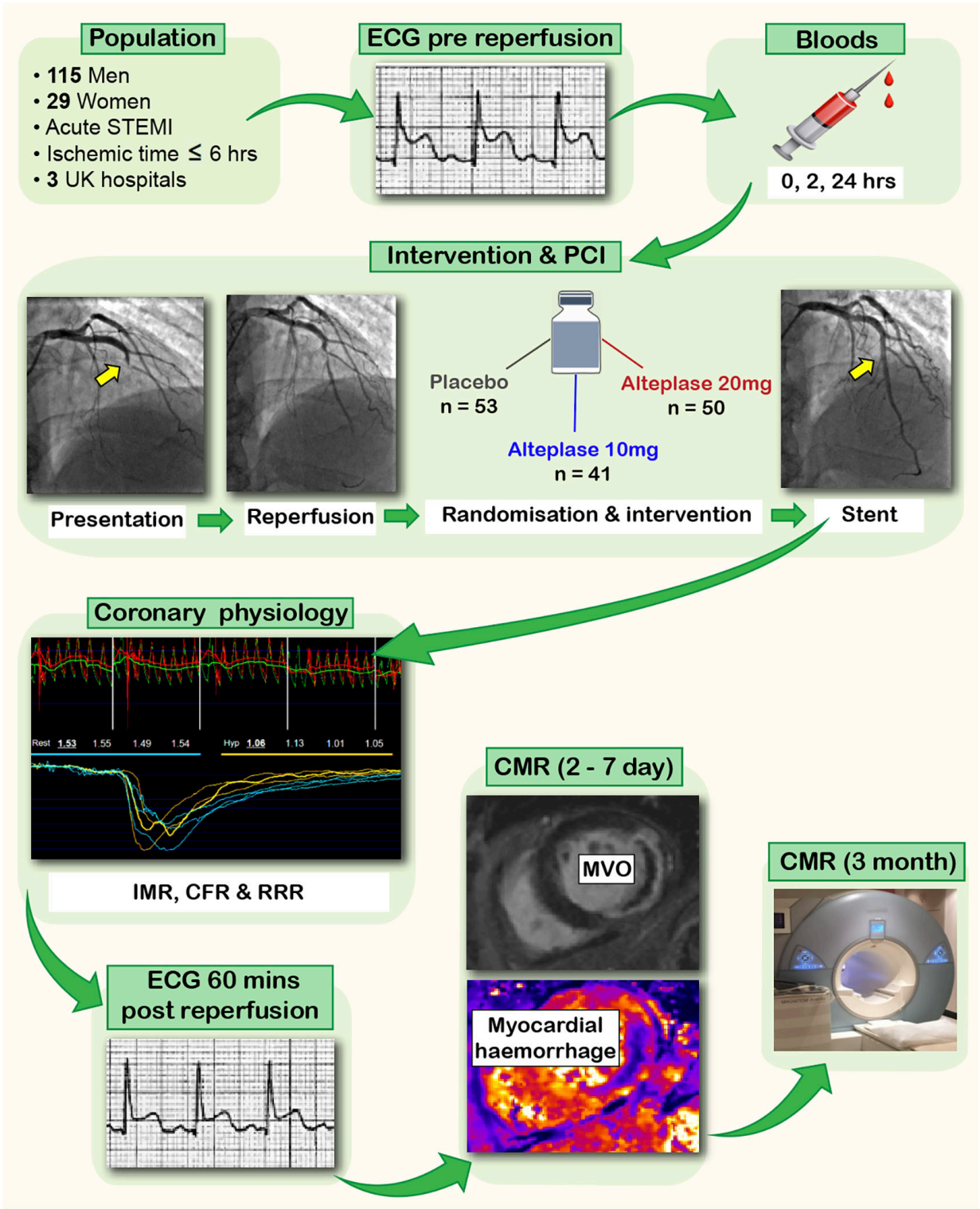

Figure 4.1 *Summary of study design for the T-TIME physiology sub-study. Adapted and reproduced with permission from: Maznyczka A et al. Effects of intracoronary alteplase on microvascular function in acute myocardial infarction. JAHA. 2020. 9(3): e014066. Doi: 10.1161/JAHA.119.014066*[366].

4.3.2 Statistical analyses

There was a prespecified statistical analysis plan (Appendix 8), which was finalised prior to database lock. Continuous data were summarised using mean ± SD, or median (IQR). MVO and myocardial haemorrhage extent were presented as mean ± SD, because the high proportion of patients with a 0 value for MVO or myocardial haemorrhage extent resulted in median values of 0 in each treatment group for MVO or myocardial haemorrhage. Categorical variables were reported as frequency and percentages. All the analyses were performed according to treatment received (alteplase 10mg, alteplase 20mg, or placebo).

Association analyses were performed using linear regression for continuous outcomes, logistic regression for binary outcomes, or proportional odds logistic regression for ordinal outcomes. Logarithmic or square root transformations were used where necessary, in linear regression models, to improve model residual distributions. The regression analyses were adjusted for the location of MI (anterior vs. non-anterior). Regression models were used to assess treatment effects with treatments as a 3-level categorical variable, and treatment as a 2-level categorical variable (alteplase 10mg and 20mg combined vs. placebo).

Post-hoc analyses were performed in prespecified sub-groups of interest, according to patient characteristics, i.e. ischaemic time ([i] <2 hours, [ii] > 2, ≤ 4 hours and [iii] ≥ 4 hours), TIMI coronary flow grade immediately pre-study drug (≤2, and 3) and TIMI thrombus grade immediately pre-study drug (≤2, and ≥3). The sub-groups were based on *a priori* concern that they are clinically relevant patient characteristics, which potentially could impact on associations of alteplase with IMR, CFR and RRR. Regression models were used to assess treatment effects within prespecified sub-groups through the use of treatment-by-sub-group interactions.

There was no imputation for missing values and no adjustments for multiple statistical comparisons were made. All tests were 2-tailed and assessed at the 5% significance level. Data were analysed using R (version 3.4.3, R Development Core Team, California, U.S.A) and SPSS (version 25.0, SPSS, IBM, Armonk, NY, USA).

4.4 Result

4.4.1 Population characteristics

The flow of subjects through the study is shown in Figure 4.2. One hundred and forty-four patients were enrolled into the physiology sub-study, which represented one third of the overall T-TIME population (n = 440) and their characteristics were broadly similar. The characteristics of the population are shown in Table 4.1 and procedure characteristics are shown in Table 4.2. The mean age was 59.4 ± 10.5 years, 80% were male, and the median ischaemic time was 2.5 hours (IQR: 2.0 – 3.5). The infarct-related artery was the RCA in 66 patients (46%), LAD in 54 patients (38%) and Cx in 24 patients (17%).

At the start of the coronary angiogram, TIMI coronary flow grade was 0 in 114 patients (79%), 1 in 14 patients (10%) and 2 in 16 patients (11%). The thrombus grade at the start of the angiogram was 5 in 116 patients (81%), 4 in 25 patients (17%) and 3 in 3 patients (2%) (Table 4.2).

All of the patients included in the physiology sub-study analysis, except for one, received the study drug according to the protocol. The one patient who did not receive the study drug according to protocol had the study drug delivered distal to the infarct-related lesion, and was in the alteplase 20mg group. That patient had no CMR performed, or coagulation parameters measured.

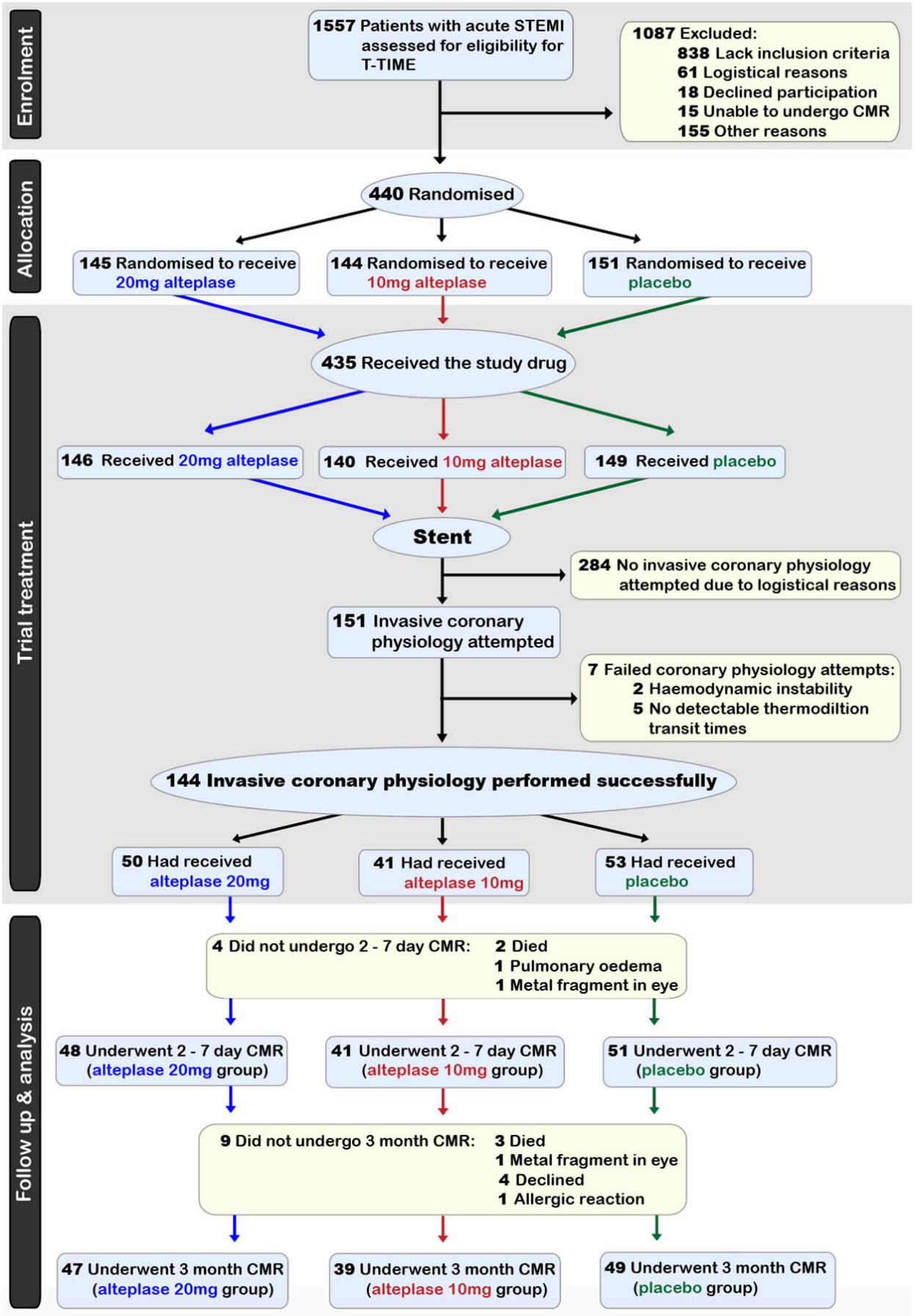

Figure 4.2 *Flow of subjects through the T-TIME physiology sub-study*[366].

	All [n=144]	Placebo [n=53]	Alteplase 10mg [n=41]	Alteplase 20mg [n=50]
Demographics				
Age, years	59.4 ± 10.5	56.8 ± 11.3	61.2 ± 9.4	60.6 ± 10.3
Male	115 (80%)	45 (85%)	31 (76%)	39 (78%)
Body mass index, kg/m^2	28.4 ± 5.1	28.8 ± 5.3	29.0 ± 5.2	27.4 ± 4.6
Ischaemic time, h:mm, median (IQR)	2:47 (2:03, 3:50)	2:40 (2:03, 3:52)	2:43 (1:53, 4:10)	2:54 (2:10, 3:36)
Medical history				
Hypertension	41 (28%)	14 (26%)	11 (27%)	16 (32%)
Hypercholesterolaemia	21 (15%)	11 (21%)	6 (15%)	4 (8%)
Diabetes mellitus*	16 (11%)	6 (11%)	6 (15%)	4 (8%)
Smoking:				
Current	68 (47%)	25 (47%)	17 (41%)	26 (52%)
Former	27 (19%)	13 (25%)	7 (17%)	7 (14%)
Never	49 (34%)	15 (28%)	17 (41%)	17 (34%)
Previous PCI	9 (6%)	3 (6%)	1 (2%)	5 (10%)
Previous MI	8 (6%)	2 (4%)	1 (2%)	5 (10%)
Angina	4 (3%)	2 (4%)	0 (0%)	2 (4%)
Stroke/ Transient Ischaemic Attack	3 (2%)	2 (4%)	0 (0%)	1 (2%)
Pre-existing maintenance medication				
Aspirin	20 (14%)	8 (15%)	4 (10%)	8 (16%)
$P2Y_{12}$ inhibitor:				
Clopidogrel	1 (1%)	1 (2%)	0 (0%)	0 (0%)
Ticagrelor/ Prasugrel	2 (1%)	0 (0%)	1 (2%)	1 (2%)
Statin	25 (17%)	13 (25%)	6 (15%)	6 (12%)
Beta-blocker	14 (10%)	4 (8%)	4 (10%)	6 (12%)
Angiotensin converting enzyme inhibitor/ angiotensin receptor blocker	18 (12%)	7 (13%)	4 (10%)	7 (14%)
Mineralocorticoid receptor antagonist	3 (2%)	1 (2%)	2 (5%)	0 (0%)
Haemodynamic measures and baseline blood results on admission				
Heart rate, beats per minute	73.0 ± 15.1	74.4 ± 16.1	71.5 ± 13.0	72.7 ± 15.8
Systolic blood pressure, mmHg	140 ± 26	140 ± 28	141 ± 23	138 ± 27
Diastolic blood pressure, mmHg	82 ± 15	84 ± 17	82 ± 13	81 ± 16
Creatinine†, µmol/L	79 ± 16	79 ± 16	80 ± 14	79 ± 17
Estimated glomerular filtration rate†	91 ± 21	94 ± 22	88 ± 18	92 ± 22
Haemoglobin†, g/L	145.8 ± 13.7	145.4 ± 13.8	146.2 ± 12.6	146.0 ± 14.6
Platelet count†, $10^9/L$	264.2 ± 62.1	252.4 ± 53.6	280.9 ± 73.5	262.9 ± 58.1

Table 4.1 *Population characteristics, from the T-TIME physiology sub-study[366]. Data are reported according to treatment received (n=144). Data are mean ± SD, or n (%), unless otherwise stated. † Missing data: Creatinine, estimated glomerular filtration rate, haemoglobin and platelets, one subject (alteplase 20mg group) * Diabetes mellitus was defined as a history of diet-controlled or treated diabetes.*

	All	Placebo	Alteplase 10mg	Alteplase 20mg
	[n=144]	[n=53]	[n=41]	[n=50]
Infarct-related artery: LAD	54 (38%)	19 (36%)	17 (41%)	18 (36%)
Cx	24 (17%)	9 (17%)	9 (22%)	6 (12%)
RCA	66 (46%)	25 (47%)	15 (37%)	26 (52%)
Number of vessels diseased: 1	83 (58%)	33 (62%)	22 (54%)	28 (56%)
2	49 (34%)	16 (30%)	16 (39%)	17(34%)
3	12 (8%)	4 (8%)	3 (7%)	5 (10%)
Balloon angioplasty pre-stent	141 (98%)	51 (96%)	41 (100%)	49 (98%)
Initial angiography pre-reperfusion				
TIMI coronary flow grade: 0	114 (79%)	47 (89%)	34 (83%)	33 (66%)
1	14 (10%)	2 (4%)	3 (7%)	9 (18%)
2	16 (11%)	4 (8%)	4 (10%)	8 (16%)
3	0 (0%)	0 (0%)	0 (0%)	0 (0%)
TIMI thrombus grade: 3	3 (2%)	0 (0%)	0 (0%)	3 (6%)
4	25 (17%)	6 (11%)	5 (12%)	14 (28%)
5	116 (81%)	47 (89%)	36 (88%)	33 (66%)
Immediately pre-study drug administration				
TIMI coronary flow grade: 1	5 (4%)	2 (4%)	2 (5%)	1 (2%)
2	41 (29%)	14 (27%)	10 (24%)	17 (35%)
3	95 (67%)	35 (69%)	29 (71%)	31 (63%)
Study drug administration				
Thrombectomy catheter	106 (74%)	39 (74%)	29 (71%)	38 (76%)
Guide catheter	35 (24%)	13 (25%)	11 (27%)	11 (22%)
Other	3 (2%)	1 (2%)	1 (2%)	1 (2%)
Post- study drug				
PCI with stent implant	144 (100%)	53 (100%)	41 (100%)	50 (100%)
QCA total stent length, mm	35.6 ± 13.2	33.6 ± 12.2	38.1 ± 14.6	35.8 ± 13.0
QCA post-stent infarct-related artery diameter, mm	3.2 ± 0.4	3.2 ± 0.5	3.2 ± 0.5	3.2 ± 0.4
Post stent dilatation	133 (92%)	46 (87%)	41 (100%)	46 (92%)
Acute therapy following first medical contact†				
Aspirin loading dose: 300mg	142 (99%)	53 (100%)	40 (98%)	49 (98%)
None	2 (1%)	0 (0%)	1 (2%)	1 (2%)
Additional antiplatelet medication:				
None	2 (1%)	0 (0%)	1 (2%)	1 (2%)
Clopidogrel	87 (60%)	27 (51%)	28 (68%)	32 (64%)
Ticagrelor	55 (38%)	26 (49%)	12 (29%)	17 (34%)
Prasugrel	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Unfractionated heparin, U median (IQR)	11500 (10000, 15000)	10000 (10000, 14000)	12000 (10000, 15000)	12000 (10000, 15375)
Intravenous morphine	134 (93%)	48 (91%)	38 (93%)	48 (96%)
Inhaled oxygen	20 (14%)	8 (15%)	7 (17%)	5 (10%)
Glycoprotein IIb/IIIa inhibitor	8 (6%)	1 (2%)	5 (12%)	2 (4%)
Aspiration thrombectomy	23 (16%)	8 (15%)	8 (20%)	7 (14%)

Table 4.2 *Procedural characteristics, from the T-TIME physiology sub-study[366]. Data are reported according to treatment received (n=421). Data are mean ± SD, or n (%), unless otherwise stated. Missing data: TIMI coronary flow grade immediately pre-study drug administration (3 subjects [2 placebo, 1 alteplase 20mg group]).* † *None of the patients received intravenous or intracoronary treatment with bivalirudin, metoprolol, nicorandil, or sodium nitroprusside.*

Sixty-seven patients (47%) were recruited on a weekend or outside 9am to 5pm, i.e. outwith "normal working hours". A breakdown of the number patients enrolled in the physiology sub-study by day of the week and by time to the nearest hour is shown (Figure 4.3).

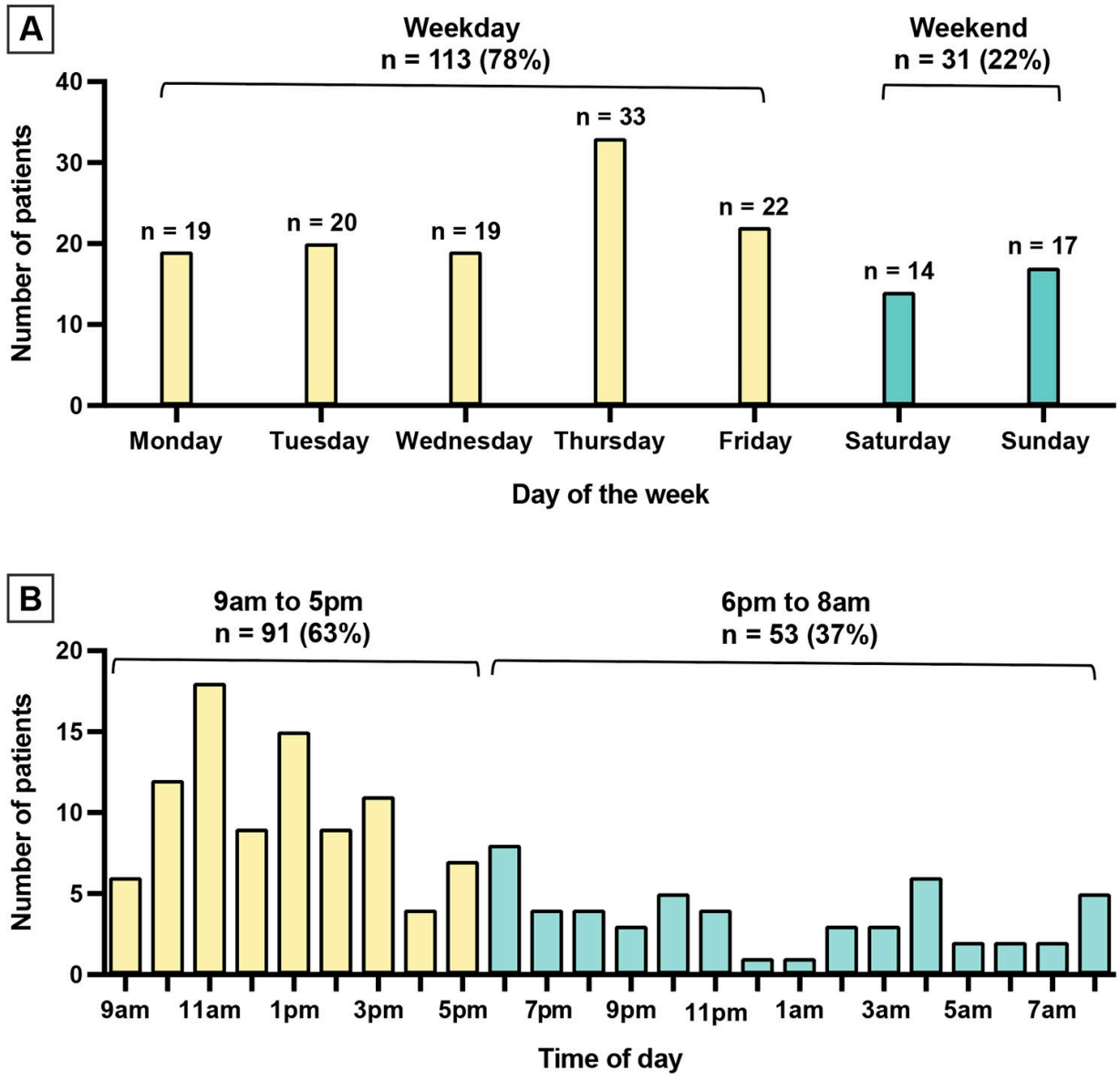

Figure 4.3 *Breakdown of the number patients enrolled in the T-TIME physiology sub-study by day of the week (A) and by time of day to the nearest hour (B).*

4.4.2 Correlations between site reported and core laboratory IMR data

There was excellent correlation between the 144 site-reported IMR values and IMR values reported by the core-laboratory ($r=0.99$, $p<0.0001$). The intra-class correlation coefficient was 0.993 (95% CI: 0.990, 0.995).

4.4.3 Intra-observer and inter-rater reliability for IMR, RRR, CFR thermodilution waveforms and TIMI thrombus grade

The intra-observer reliability for IMR assessed from 30 consecutive patients was excellent, with an intra-class correlation coefficient of 0.998 (95% CI: 0.997, 0.999). The inter-rater reliability for IMR assessed from 30 consecutive patients was also excellent (intra-class correlation coefficient: 0.999 [95% CI: 0.998, 0.999]).

For RRR there was excellent intra-observer reliability from 30 consecutive patients (intra-class correlation coefficient: 0.988 [95% CI: 0.974, 0.994]). The inter-rater reliability for RRR assessed from 30 consecutive patients was also excellent, with an intra-class correlation coefficient of 0.988 (95% CI: 0.975, 0.994). For CFR, the intraclass correlation coefficient was 1.0 for intra- and inter-rater reliability (assessed from 30 consecutive patients).

From 30 consecutive patients there was concordance in intra-observer reporting of thermodilution waveforms in 97% of patients (n=29), with a kappa value of 0.95 consistent with excellent intra-observer agreement[368]. There was concordance in inter-rater reporting of thermodilution waveforms in 26 out of 30 patients (87%) (kappa=0.79).

From 90 patients there was substantial, but imperfect, inter-rater reliability for TIMI thrombus grade assessed immediately before study drug administration (kappa=0.75). The intra-rater reliability for TIMI thrombus grade assessed in 50 patients immediately before study drug administration, was also substantial (kappa=0.72).

4.4.4 Repeatability of IMR, CFR and RRR

Paired IMR measurements, obtained 0.5 to 8 minutes apart, post-primary PCI by 4 cardiologists in 13 patients were correlated (r=0.80, p=0.001), with a mean difference between duplicate IMR measurements of 2.90 ± 8.69 (p=0.252). The coefficient of variation from duplicate IMR measurements was 15.5%. The Bland-Altman plot is shown in Figure 4.4. The mean difference between duplicate CFR measurements from 9 patients was 0.07 ± 0.44 (p=0.659) and the coefficient of variation was 24.1%. The mean difference between duplicate RRR measurements from 9 patients was -0.04 ± 0.73 (p=0.860) and the coefficient of variation was 24.1% The Bland-Altman plots are shown in Figure 4.4.

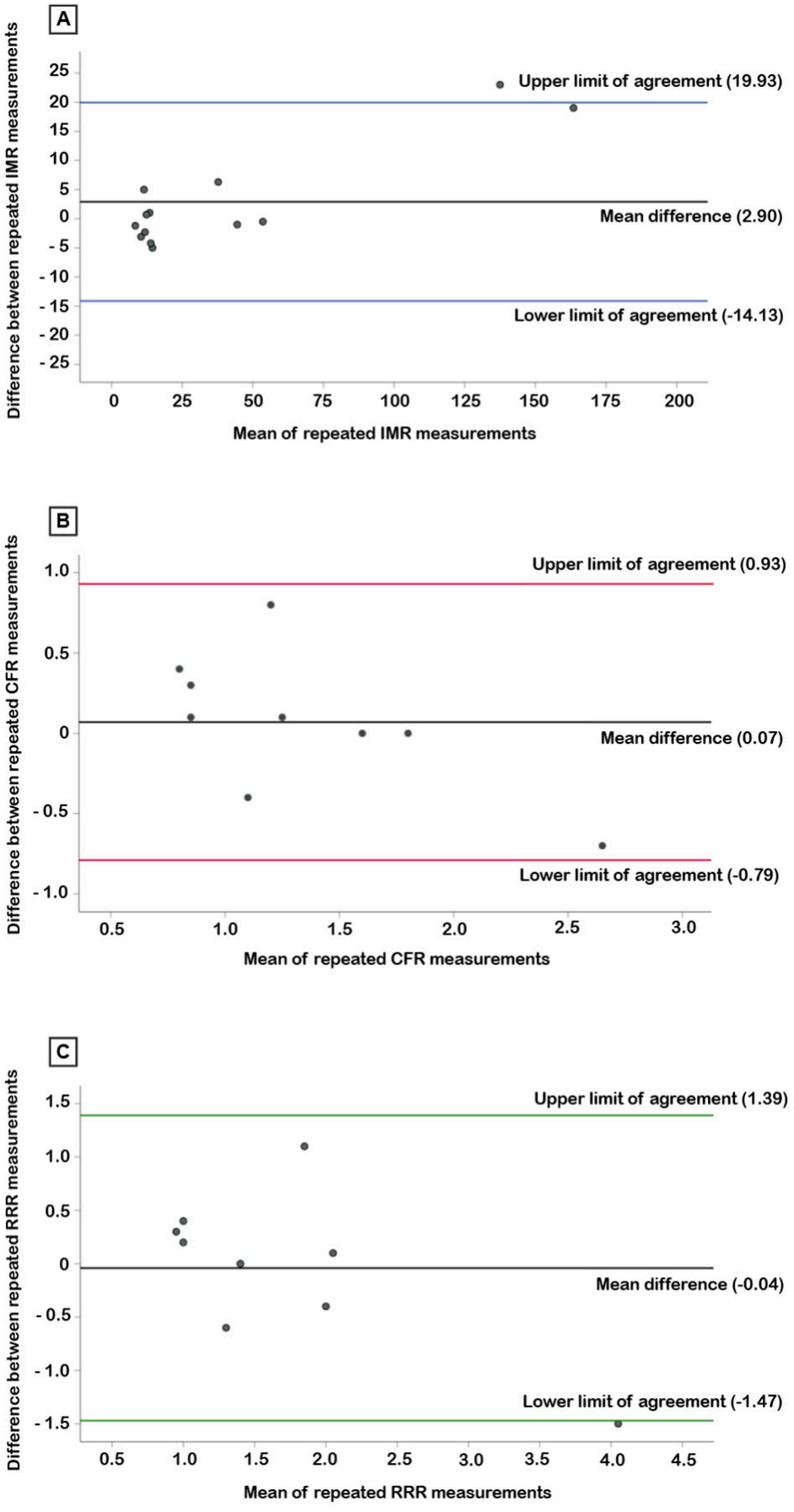

Figure 4.4 *Bland-Altman plots for duplicate IMR (A), CFR (B) and RRR (C) measurements. Horizontal lines are drawn at the mean difference and at the limits of agreement.*

4.4.5 Physiology endpoint: IMR

The median IMR for the entire population was 29.5 (IQR: 17.0 – 55.0). IMR >40 occurred in 57 patients (40%), and IMR >32 occurred in 69 patients (48%). Overall, IMR did not differ between the alteplase 20mg group (median IMR: 37.0 [IQR: 20.0 – 57.8]), alteplase 10mg group (median IMR: 22.0 [IQR: 17.0 – 42.0]) and placebo group (median IMR: 33.0 [IQR: 20.0 – 57.8]). The relative difference in IMR between patients who received alteplase 20mg vs. placebo was 1.04 (95% CI: 0.78, 1.38) p=0.801. The relative difference in IMR between patients who received alteplase 10mg vs. placebo was 0.79 (95% CI: 0.58, 1.07) p=0.125. Table 4.3 shows the treatment effects on IMR for alteplase 10mg and 20mg combined vs. placebo.

4.4.6 Physiology endpoints: CFR and RRR

The median CFR for the whole population was 1.4 (IQR: 1.1 – 2.0). CFR ≤2.0 occurred in 115 patients (88%). Overall, CFR did not differ between the alteplase 20mg group (median CFR: 1.4 [IQR: 1.1 – 2.0]), alteplase 10mg group (median CFR: 1.4 [IQR: 1.1 - 1.9]) and placebo group (median CFR: 1.3 [IQR: 1.1 – 1.8]). The relative difference in CFR between patients who received alteplase 20mg vs. placebo was 1.03 (95% CI: 0.88, 1.20) p=0.732. Treatment effects on CFR did not differ for alteplase 10mg vs. placebo, or for alteplase 10mg and 20mg combined vs. placebo (Table 4.3).

The median RRR for the population was 1.7 (IQR: 1.2 – 2.3). Overall, RRR did not differ between treatment groups (median for the alteplase 20mg group: 1.8 [IQR: 1.3 – 2.4], alteplase 10mg group: 1.6 [IQR: 1.4 – 2.6]), placebo group: 1.6 [IQR: 1.3 - 2.2]). The relative difference in RRR between patients who received alteplase 20mg vs. placebo was 1.02 (95% CI: 0.87, 1.20) p=0.795. Treatment effects on RRR did not differ for alteplase 10mg vs. placebo, or for alteplase 10mg and 20mg combined vs. placebo (Table 4.3).

4.4.7 Physiology endpoint: thermodilution waveforms

Thermodilution waveforms did not differ between treatment groups (Table 4.3).

	Treatment Group				Treatment Effect		
	All	**Placebo**	**Alteplase 10mg**	**Alteplase 20mg**	**10mg vs. placebo**	**20mg vs. placebo**	**10 or 20mg vs. placebo**
	[n = 144]	**[n = 53]**	**[n = 41]**	**[n = 50]**	**Estimate (95% CI) p-value**	**Estimate (95% CI) p-value**	**Estimate (95% CI) p-value**
IMR	29.5 (17.0, 55.0)	33.0 (17.0, 57.0)	22.0 (17.0, 42.0)	37.0 (20.0, 57.8)	0.79 (0.58, 1.07) p=0.125	1.04 (0.78, 1.38) p=0.801	0.92 (0.71, 1.18) p=0.505
IMR >40	57 (40%)	24 (45%)	11 (27%)	22 (44%)	0.42 (0.17, 1.02) p=0.054	0.93 (0.42, 2.05) p=0.864	0.66 (0.33, 1.34) p=0.251
IMR >32	69 (48%)	27 (51%)	15 (37%)	27 (54%)	0.54 (0.23, 1.24) p=0.147	1.12 (0.51, 2.44) p=0.774	0.81 (0.41, 1.60) p=0.546
CFR	1.4 (1.1, 2.0)	1.3 (1.1, 1.8)	1.4 (1.1, 1.9)	1.4 (1.1, 2.0)	1.01 (0.86, 1.19) p=0.900	1.03 (0.88, 1.20) p=0.732	1.02 (0.89, 1.17) p=0.777
CFR ≤2.0	115 (80%)	44 (83%)	31 (76%)	40 (80%)	1.62 (0.59, 3.36) p=0.680	1.23 (0.45, 3.36) p=0.680	1.40 (0.58, 3.36) p=0.451
RRR	1.6 (1.3, 2.3)	1.6 (1.3, 2.2)	1.6 (1.4, 2.6)	1.8 (1.3, 2.4)	1.04 (0.88, 1.23) p=0.658	1.02 (0.87, 1.20) p=0.795	1.03 (0.90, 1.18) p=0.685
Waveform:							
Unimodal (narrow)	75 (52%)	26 (49%)	22 (54%)	27 (54%)	1.00 (0.45, 2.20) p=0.999	0.98 (0.46, 2.06) p=0.956	0.99 (0.52, 1.89) p=0.972
Unimodal (wide)	56 (39%)	25 (47%)	14 (34%)	17 (34%)			
Bimodal	13 (9%)	2 (4%)	5 (12%)	6 (12%)			

Table 4.3 *Coronary physiology endpoints, from the T-TIME physiology sub-study, according to treatment received[366]. Data are median (IQR), or n (%). IMR, CFR and RRR were analysed on a logarithmic scale. Treatment effect estimates for IMR, CFR and RRR were derived from linear regression, with treatment effects reported as relative differences between groups, with 95% CIs and p-values (adjusted for location of MI). Treatment effect estimates for IMR >40, IMR >32 and CFR ≤2.0 were reported as odds ratios between groups, with 95% CIs and p-values, from logistic regression models (adjusted for location of MI). Treatment effect estimates for thermodilution waveforms were reported as odds ratio between groups, with 95% CIs and p-values, from a proportional odds logistic regression model (adjusted for location of MI).*

4.4.8 LVEDP, angiographic and ECG endpoints

One hundred and thirty-one patients had both LVEDP and IMR measured. There were no statistically significant correlations between LVEDP and IMR (r=0.2, p=0.087), CFR (r= -0.1, p=0.485), RRR (r= -0.1, p=0.475), or MVO (r=0.1, p=0.284). LVEDP did not differ with alteplase vs. placebo (Table 4.4).

TIMI flow grade or TFC at the end of PCI did not differ between treatment groups (Table 4.4). MPG was higher in the alteplase 20mg group vs. placebo (OR: 2.16 [95% CI: 1.04, 4.49] p=0.039). However, there was no difference in treatment effects on MPG for alteplase 10mg vs. placebo, or for alteplase 10mg and 20mg combined vs. placebo (Table 4.4).

As in the main T-TIME trial, there was no difference in absolute % ST-segment resolution 60 minutes post-reperfusion relative to baseline, between treatment groups (mean for the alteplase 20mg group: 48.8 ± 42.4%, alteplase 10mg group: 45.7 ± 43.8%, placebo group: 45.1 ± 37.8%) (Table 4.4).

	Treatment Group				Treatment Effect		
	All	**Placebo**	**Alteplase 10mg**	**Alteplase 20mg**	**10mg vs. placebo**	**20mg vs. placebo**	**10 or 20mg vs. placebo**
	[n=144]	**[n=53]**	**[n=41]**	**[n=50]**	**Estimate (95% CI) p-value**	**Estimate (95% CI) p-value**	**Estimate (95% CI) p-value**
LVEDP mmHg	17.0 (12.0-20.8)	16.5 (13.2-19.0)	19.0 (13.2-22.8)	15.0 (12.0-18.8)	1.05 (0.88, 1.25) p=0.579	0.89 (0.75, 1.05) p=0.172	0.96 (0.83, 1.12) p=0.609
TIMI flow grade post-PCI					1.43 (0.42, 4.84) p=0.565	1.70 (0.51, 5.69) p=0.391	1.57 (0.57, 4.32) p=0.387
1	3 (2%)	2 (4%)	0	1 (2%)			
2	15 (10%)	6 (11%)	5 (12%)	4 (8%)			
3	126 (88%)	45 (85%)	36 (88%)	45 (90%)			
MPG post-PCI					1.32 (0.60, 2.92) p=0.496	2.16 (1.04, 4.49) **p=0.039**	1.75 (0.91, 3.37) p=0.091
0	42 (29)	18 (34)	15 (37)	9 (18)			
1	3 (2)	3 (6)	0	0			
2	60 (42)	19 (36)	15 (37)	26 (52)			
3	39 (27)	13 (25)	11 (27)	15 (30)			
TFC post-PCI	18.0 (14.0-26.0)	18.0 (14.0-26.0)	16.5 (14.0-22.4)	22.0 (14.0-24.5)	0.89 (0.72, 1.11) p=0.311	1.03 (0.84, 1.27) p=0.774	0.97 (0.80, 1.16) p=0.713
Absolute % ST-segment resolution 60 min post-reperfusion relative to baseline	46.6 ± 40.9	45.1 ± 37.8	45.7 ± 43.8	48.8 ± 42.4	1.32 (-15.53, 18.16) p=0.878	4.15 (-11.71, 20.02) p=0.608	2.89 (-11.04, 16.83) p=0.684

Table 4.4 *LVEDP, angiographic and ECG endpoints, from the T-TIME physiology sub-study, according to treatment received[366]. Data are reported as mean ± SD, n (%), or median (IQR). Treatment effect estimates for TIMI flow grade, and MPG are reported as odds ratios, with 95% CIs and p-values, from proportional odds logistic regression (models adjusted for MI location). Treatment effects for TFC and LVEDP (analysed on a logarithmic scale) are reported as relative differences, with 95% CIs and p-values, from linear regression (models adjusted for MI location). Treatment effects for % ST-segment resolution are reported as mean differences, with 95% CIs and p-values, from linear regression (models adjusted for MI location). Missing data: LVEDP, 13 subjects (6 placebo, 1 alteplase 10mg, 6 alteplase 20mg); ST-segment resolution, 3 subjects (2 placebo, 1 alteplase 10mg group).*

4.4.9 CMR endpoints

Ninety seven percent of patients (n=140) had CMR imaging performed 2 to 7 days post-PCI, with MVO evaluable in all 140 patients. Ninety four percent of patients (n=135) had CMR imaging performed at 3 months post-PCI, with infarct size evaluable in 133 patients.

The number of days from PCI to CMR imaging is shown in Table 4.5. Given the high proportion of patients with a value of 0 for MVO (n=83 [59%]), the median MVO extent was 0.0 (IQR: 0.0 – 3.3). As in the main T-TIME trial, there was overall no difference between treatment groups for MVO, myocardial haemorrhage, infarct size, LV ejection fraction or LV volumes (Tables 4.6 and 4.7).

	All [n=144]	**Placebo [n=53]**	**Alteplase 10mg [n=41]**	**Alteplase 20mg [n=50]**
Days from PCI CMR imaging 2-7 days following primary PCI	4.0 (3.0 – 5.0)	4.0 (3.0 – 5.0)	5.0 (4.0 – 6.0)	4.0 (3.0 – 5.0)
Days from PCI to CMR imaging 3 months following primary PCI	90.0 (84.0 – 97.0)	89.0 (84.0 – 97.0)	90.5 (84.5 – 95.8)	90.0 (85.0 – 97.0)

Table 4.5 *Number of days from PCI to CMR imaging, in the T-TIME physiology sub-study[366]. Data are median (IQR). Missing data: 2 to 7 day CMR imaging (4 subjects [2 placebo, 2 alteplase 20mg group]); 3-month CMR imaging (9 subjects [5 placebo, 1 alteplase 10mg, 3 alteplase 20mg group]).*

4.4.10 Coagulation variables

The effects of alteplase on coagulation variables 2 hours post-PCI are shown in Table 4.8. Systemic concentrations of fibrin D-dimer were increased, and systemic concentrations of plasminogen were reduced in patients who received alteplase compared to placebo, reflecting fibrinolysis. Systemic concentrations of prothrombin fragment F_{1+2} were increased with alteplase compared to placebo, reflecting activation of the clotting system.

	Treatment Group				Treatment Effect		
	All	**Placebo**	**Alteplase 10mg**	**Alteplase 20mg**	**10mg vs. placebo**	**20mg vs. placebo**	**10 or 20mg vs. placebo**
	[n=144]	**[n=53]**	**[n=41]**	**[n=50]**	**Estimate (95% CI) p-value**	**Estimate (95% CI) p-value**	**Estimate (95% CI) p-value**
MVO extent (% LV)	2.5 ± 4.5	2.0 ± 3.1	2.5 ± 4.2	2.9 ± 5.7	0.03 (-0.50, 0.56) p=0.908	0.08 (-0.43, 0.59) p=0.766	0.06 (-0.50, 0.50) p=0.804
MVO present	57 (41%)	23 (45%)	16 (39%)	18 (38%)	0.78 (0.34, 1.81) p=0.566	0.73 (0.33, 1.64) p=0.449	0.76 (0.38, 1.52) p=0.432
Myocardial haemorrhage extent (% LV)	2.0 ± 3.9	1.6 ± 2.8	2.1 ± 3.8	2.4 ± 4.8	0.44 (-1.28, 2.16) p=0.619	0.72 (-0.87, 2.31) p=0.373	0.60 (-0.81, 2.01) p=0.403
Myocardial haemorrhage present	56 (41%)	22 (45%)	16 (41%)	18 (38%)	0.85 (0.36, 2.00) p=0.713	0.73 (0.33, 1.66) p=0.458	0.79 (0.38, 1.60) p=0.506
Acute infarct size (% LV)	24.1 ± 12.7	23.3 ± 12.9	26.6 ± 12.5	23.1 ± 12.8	2.57 (-2.29 7.42) p=0.300	-0.70 (-5.35, 3.94) p=0.767	0.80 (-3.27, 4.87) p=0.700
LV ejection fraction (%)	43.9 ± 8.3	44.3 ± 7.6	43.6 ± 7.6	43.7 ± 9.7	-0.25 (-3.55, 3.05) p=0.882	-0.32 (-3.48, 2.84) p=0.844	-0.29 (-3.04, 2.47) p=0.838
LV end systolic volume (ml), median (IQR)	90.5 (77.5, 108.3)	90.4 (80.9, 108.8)	92.9 (83.2, 106.8)	89.9 (66.1, 108.3)	1.00 (0.88, 1.12) p=0.946	0.94 (0.84, 1.06) p=0.306	0.97 (0.87, 1.07) p=0.503
LV end diastolic volume (ml), median (IQR)	166.0 (143.5, 188.4)	168.7 (151.3, 196.5)	73.5 (147.1, 187.9)	157.9 (131.6, 187.1)	0.99 (0.90, 1.09) p=0.850	0.94 (0.86, 1.03) p=0.189	0.96 (0.89, 1.04) p=0.360

Table 4.6 *CMR endpoints at 2 to 7 days post-PCI, from the T-TIME physiology sub-study, according to treatment received.[366] Data are mean ± SD, or n (%), or median (IQR). Treatment effect estimates for MVO extent (on square root transformed scale) were reported as mean difference in square root of MVO extent between groups, with 95% CIs and p-values from linear regression (adjusted for MI location).Treatment effect estimates for myocardial haemorrhage infarct size and LV ejection fraction, were reported as mean differences between groups, with 95% CIs and p-values, from linear regression (adjusted for MI location). Treatment effect estimates for MVO presence or myocardial haemorrhage presence were reported as odds ratios, with 95% CIs and p-values from logistic regression (adjusted for MI location). Treatment effect estimates for LV volumes (on a log-transformed scale) were reported as relative differences between groups, with 95% CIs and p-values from linear regression (adjusted for MI location). Missing data: MVO presence/ absence or extent, Infarct size, LV ejection fraction (4 subjects [2 placebo, 2 alteplase 20mg group]). LV end diastolic/ systolic volumes (4 subjects [2 placebo, 2 alteplase 20mg]). Myocardial haemorrhage extent (13 subjects [6 placebo [5 alteplase 10mg, 2 alteplase 20mg group]). Myocardial haemorrhage presence/ absence (8 subjects [8 placebo, 2 alteplase 10mg, 2 alteplase 20mg group]).*

	Treatment Group				Treatment Effect		
	All	**Placebo**	**Alteplase 10mg**	**Alteplase 20mg**	**10mg vs. placebo**	**20mg vs. placebo**	**10 or 20mg vs. placebo**
	[n=144]	**[n=53]**	**[n=41]**	**[n=50]**	**Estimate (95% CI) p-value**	**Estimate (95% CI) p-value**	**Estimate (95% CI) p-value**
Infarct size (% LV)	17.0 ± 11.5	17.0 ± 11.9	17.7 ± 11.0	16.5 ± 11.7	0.45 (-4.16, 5.06) p=0.848	-0.62 (-5.04, 3.80) p=0.782	-0.13 (-4.00, 3.74) p=0.947
LV ejection fraction	49.1 ± 8.4	49.5 ± 8.4	49.0 ± 6.5	48.9 ± 9.8	-0.32 (-3.79, 3.14) p=0.855	-0.45 (-3.77, 2.88) p=0.793	-0.39 (-3.29, 2.51) p=0.792
LV end systolic volume (ml), median (IQR)	81.1 (65.7, 102.2)	82.5 (69.0, 99.8)	81.8 (70.1, 92.9)	73.4 (61.6, 109.6)	0.97 (0.84, 1.12) p=0.687	0.95 (0.83, 1.09) p=0.484	0.96 (0.85, 1.08) p=0.513
LV end diastolic volume (ml), median (IQR)	162.8 (141.6, 186.2)	165.6 (149.2, 188.9)	164.6 (147.0, 176.9)	151.6 (132.7, 183.3)	0.96 (0.87, 1.06) p=0.403	0.93 (0.85, 1.02) p=0.140	0.95 (0.87, 1.02) p=0.170

Table 4.7 *CMR endpoints at 3 months post-PCI, from the T-TIME physiology sub-study, according to treatment received[366]. Data are mean ± SD, or median (IQR). Treatment effect estimates for infarct size and LV ejection fraction, were reported as mean differences between groups, with 95% CIs and p=values, from linear regression (adjusted for MI location). Treatment effect estimates for LV volumes (on a log-transformed scale) were reported as relative differences between groups, with 95% CIs and p-values from linear regression (adjusted for MI location). Missing data: LV ejection fraction (9 subjects [5 placebo, 1 alteplase 10mg, 3 alteplase 20mg group]). Infarct size (11 subjects [7 placebo, 1 alteplase 10mg, 3 alteplase 20mg group]). LV end diastolic/ systolic volume (9 subjects [5 placebo, 1 alteplase 10mg, 3 alteplase 20mg]).*

	Treatment Group				Treatment effect		
	All [n=144]	**Placebo [n=53]**	**Alteplase 10mg [n=41]**	**Alteplase 20mg [n=50]**	**10mg vs. placebo Estimate (95% CI) p-value**	**20mg vs. placebo Estimate (95% CI) p-value**	**10 or 20mg vs. placebo Estimate (95% CI) p-value**
ACT 2 hrs post-PCI (mean ± SD)	288.9 ± 92.0	302.3 ± 103.3	298.3 ± 83.1	266.9 ± 83.5	1.00 (0.89, 1.13) p=0.940	0.89 (0.80, 1.00) **p=0.046**	0.94 (0.85, 1.04) p=0.230
Plasminogen (U/dL) 2 hrs post-PCI	89.0 (78.2, 98.0)	96.0 (87.0, 106.0)	89.0 (82.5, 98.0)	81.5 (74.2, 91.8)	-5.20 (-11.00, 0.60) p=0.076	-12.9 (-18.4, -7.30) **p<0.001**	-9.40 (-14.30, -4.40) **p<0.001**
Change in plasminogen (U/dL) 2 hrs post-PCI relative to baseline	-6.0 (-11.0, 0.0)	0.0 (-5.0, 2.5)	-5.0 (-9.8, -2.0)	-11.0 (-15.2, -8.0)	-4.60 (-7.80, -1.30) **p=0.006**	-11.30 (-14.40, -8.10) **p<0.001**	-8.20 (-11.10, -5.30) **p<0.001**
D-dimers (ng/ml) 2 hrs post-PCI	245.5 (117.0, 455.8)	107.0 (66.0, 149.0)	287.0 (213.5, 587.5)	367.5 (228.8, 557.5)	3.23 (2.28, 4.58) **p<0.001**	3.49 (2.50, 4.87) **p<0.001**	3.37 (2.52, 4.50) **p<0.001**
Ratio of D-dimers 2 hrs post-PCI relative to baseline	2.11 (1.05, 3.72)	1.01 (0.95, 1.19)	2.96 (2.14, 4.06)	3.14 (2.16, 6.32)	2.78 (2.10, 3.68) **p<0.001**	3.24 (2.48,4.25) **p<0.001**	3.02 (2.39, 3.82) **p<0.001**
Prothrombin fragment F_{1+2} (pmol/L) 2 hrs post-PCI	178.8 (133.1, 244.2)	152.0 (118.6, 211.4)	183.0 (141.9, 291.9)	187.3 (150.5, 244.9)	1.27 (1.02, 1.58) **p=0.034**	1.24 (1.00, 1.52) **p=0.048**	1.25 (1.04, 1.50) **p=0.017**
Ratio of prothrombin fragment F_{1+2} 2hrs post-PCI relative to baseline	1.16 (1.02, 1.43)	1.07 (0.96, 1.44)	1.17 (1.03, 1.42)	1.18 (1.06, 1.40)	1.08 (0.87, 1.33) p=0.477	1. 08 (0.88, 1.32) p=0.466	1.08 (0.90 1.29) p=0.398

Table 4.8 *Coagulation and haematology variables, from the T-TIME physiology sub-study, according to treatment received[366]. Data are median (IQR) unless otherwise stated. Treatment effect estimates for ACT, plasminogen, and change in variables at 2 hours compared to baseline, are reported as mean differences between groups, with 95% CIs and p-values, from linear regression (adjusted for MI location). Treatment effects for D-dimers and prothrombin fragment F_{1+2} (analysed on logarithmic scales) reported as relative difference between groups, with 95% CIs and p-values, from linear regression (adjusted for MI location). Missing data: ACT (6 subjects [5 from placebo, 1 from alteplase 10mg group]), prothrombin fragment F_{1+2} two hours post-PCI (15 subjects [6 placebo, 3 alteplase 10mg, 4 alteplase 20mg group]). Plasminogen 2 hours post-PCI (10 subjects [4 placebo, 2 alteplase 10mg. 4 alteplase 20mg group]). Change in plasminogen, d-dimers, or prothrombin fragment F_{1+2} 2 hours post-PCI compared to baseline (15 subjects [6 placebo, 3 alteplase 10mg, 6 alteplase 20mg group]).*

4.4.11 Sub-group analyses

The results from the sub-group analyses are shown in Figures 4.5 to 4.8, and Tables 4.9 to 4.11. There was no interaction between TIMI flow grade immediately before drug administration, alteplase and IMR, CFR or RRR (Figure 4.5 and Table 4.9). Patients with TIMI 3 flow pre-study drug administration had numerically higher RRR and CFR with alteplase 20mg compared to placebo, and patients with TIMI flow ≤2 pre-study drug had numerically lower RRR and CFR with alteplase 20mg (Figure 4.5). However, there were no significant differences in treatment effects on linear regression (Table 4.9).

There was no interaction between ischaemic time, alteplase and IMR (Figure 4.6 and Table 4.10). There was an interaction between ischaemic time, alteplase and CFR, with treatment as a 3-level categorical variable (p=0.013) (Table 4.10). For patients with ischaemic times <2 hours, median CFR was higher with alteplase (alteplase 20mg: 2.0 [IQR:1.8 – 2.3], alteplase 10mg: 1.4 [IQR: 1.0 – 1.8], placebo: 1.2 [IQR: 1.1 – 1.7]) (Table 4.10). In patients with an ischaemic time ≥4 hours, median CFR was 1.2 (IQR: 1.0 - 1.6) for alteplase 20mg, 1.8 (IQR:1.3 – 2.6) for alteplase 10mg and 1.7 (IQR: 1.4 – 2.0) for placebo. An interaction occurred between ischaemic time, alteplase and RRR with treatment as a 3-level categorical variable (p=0.026) (Table 4.10). For patients with ischaemic times <2 hours, median RRR was higher with alteplase (alteplase 20mg: 2.2 [IQR:2.0 – 2.6], alteplase 10mg: 1.6 [IQR: 1.1 – 2.2], placebo: 1.5 [IQR: 1.3 – 1.9]). In patients with an ischemic time ≥4 hours median RRR was as follows: alteplase 20mg (1.4 [IQR: 1.0 – 2.5]), alteplase 10mg (2.3 [IQR:1.6 – 2.8]), placebo (2.0 [IQR: 1.4 – 2.7]).

Interaction occurred between ischaemic time, alteplase and MVO extent with treatment as a 3-level categorical variable (p=0.039). The interaction effect was driven by an increase in mean MVO extent with alteplase in patients with ischaemic times ≥4 hours (Figure 4.7 and Table 4.10): alteplase 20mg (5.97 ± 6.58%), alteplase 10mg (2.77 ± 4.54%), placebo (0.89 ± 1.65%).

There was no interaction between TIMI thrombus grade immediately before study drug administration, alteplase and IMR, CFR or RRR (Figure 4.8 and Table 4.11).

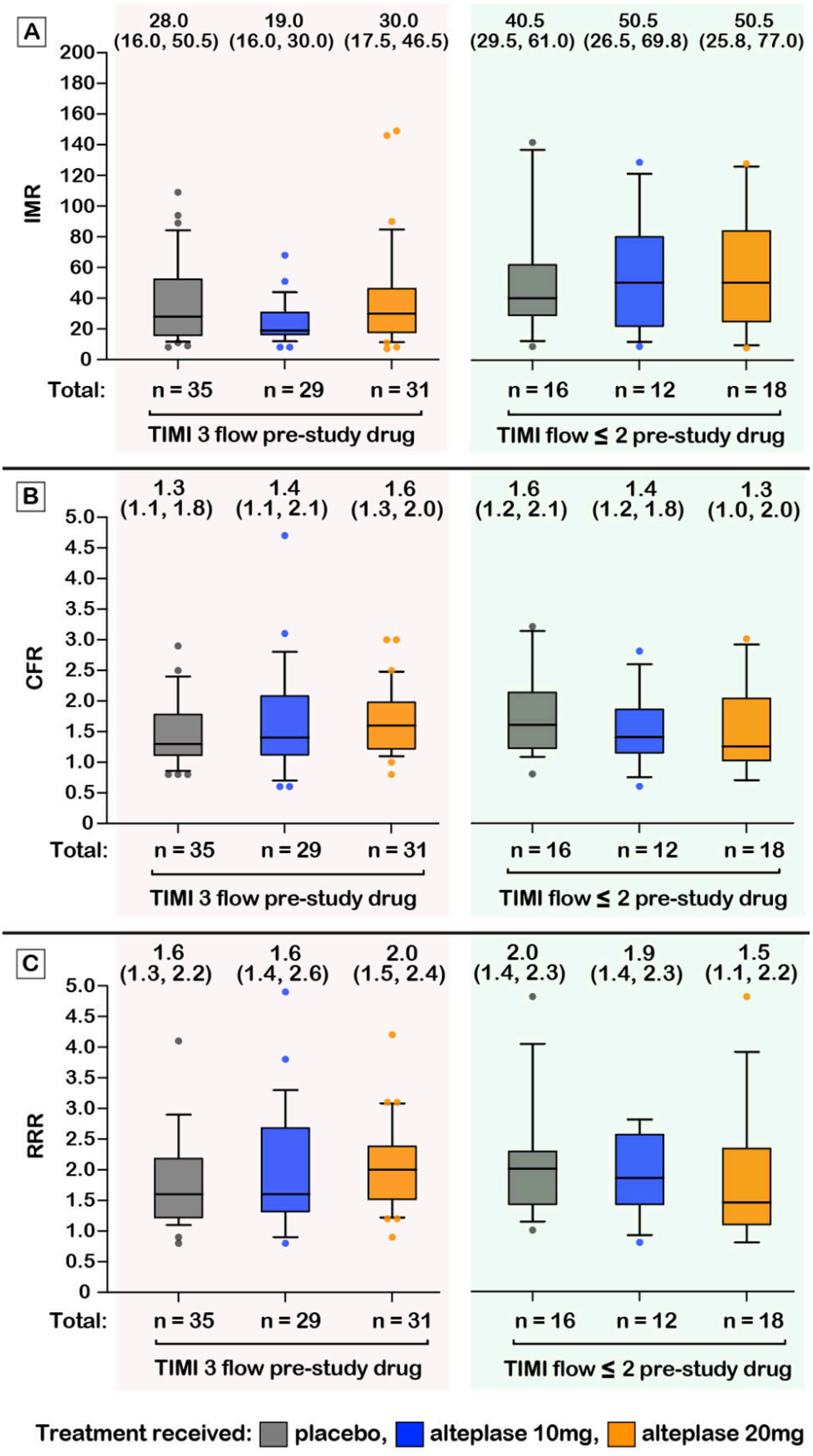

Figure 4.5 *IMR (A), CFR (B) and RRR (C), by treatment received, in sub-groups of TIMI coronary flow grade (≤2, 3) immediate before study drug administration. Boxes represent the median and IQR (values provided), with whiskers at the 10th and 90th percentiles. Values outside the 10th and 90th percentiles are presented as individual data points.*

TIMI flow pre-study drug	Treatment Effect		Interaction p-value (treatment as 3-level categorical variable)	Treatment Effect	Interaction p-value (treatment as 2-level categorical variable)
	10mg vs. placebo Estimate (95% CI) p-value	20mg vs. placebo Estimate (95% CI) p-value		10 or 20mg vs. placebo Estimate (95% CI) p-value	
IMR			0.481		0.473
≤ 2	1.03 (0.61, 1.77) p=0.903	1.05 (0.65, 1.71) p=0.835		1.05 (0.68, 1.62) p=0.834	
3	0.72 (0.51, 1.02) p=0.835	1.02 (0.72, 1.45) p=0.896		0.86 (0.64, 1.16) p=0.335	
CFR			0.159		0.074
≤ 2	0.88 (0.65, 1.18) p=0.396	0.84 (0.64, 1.10) p=0.198		0.85 (0.67, 1.09) p=0.201	
3	1.07 (0.88, 1.31) p=0.472	1.16 (0.95, 1.41) p=0.139		1.12 (0.95, 1.32) p=0.192	
RRR			0.140		0.065
≤ 2	0.90 (0.66, 1.23) p=0.502	0.82 (0.62, 1.08) p=0.158		0.85 (0.66, 1.09) p=0.203	
3	1.10 (0.90, 1.35) p=0.341	1.16 (0.95, 1.41) p=0.154		1.13 (0.95, 1.34) p=0.160	

Table 4.9 *Treatment effect estimates reported as relative differences and 95% CIs with p-values from linear regression and interactions with treatment received, for IMR, CFR and RRR, in sub-groups of TIMI coronary flow grade (≤2, 3) immediately before study drug administration.*[366] *Data analysed on logarithmic scale. The analyses were adjusted for MI location.*

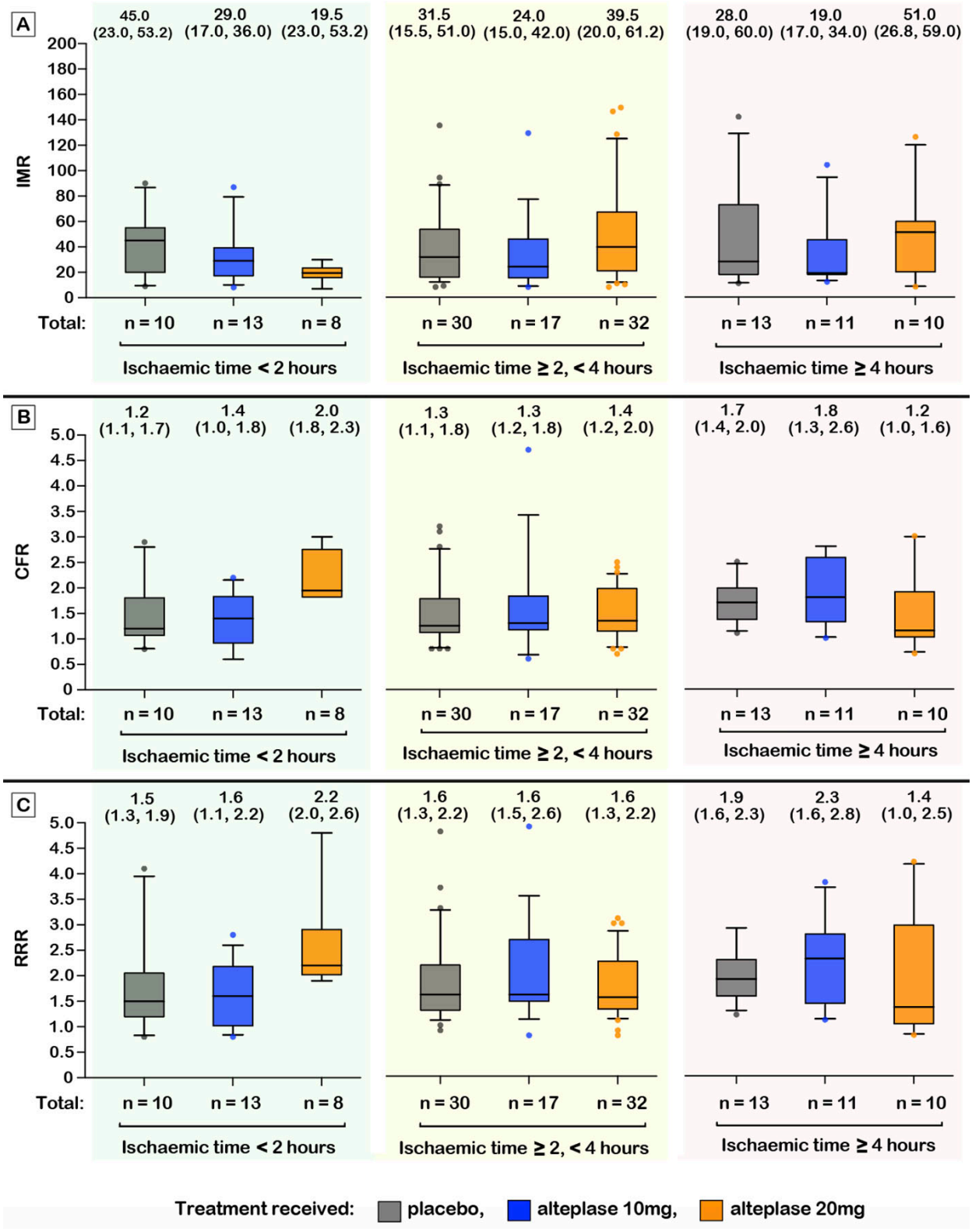

Figure 4.6 *IMR (A), CFR (B) and RRR (C), by treatment received, in sub-groups of ischaemic time (<2 hours, ≥ 2, < 4 hours, ≥4 hours). Boxes represent the median and IQR (values provided), with whiskers at the 10th and 90th percentiles. Values outside the 10th and 90thpercentiles are presented as individual data points.*

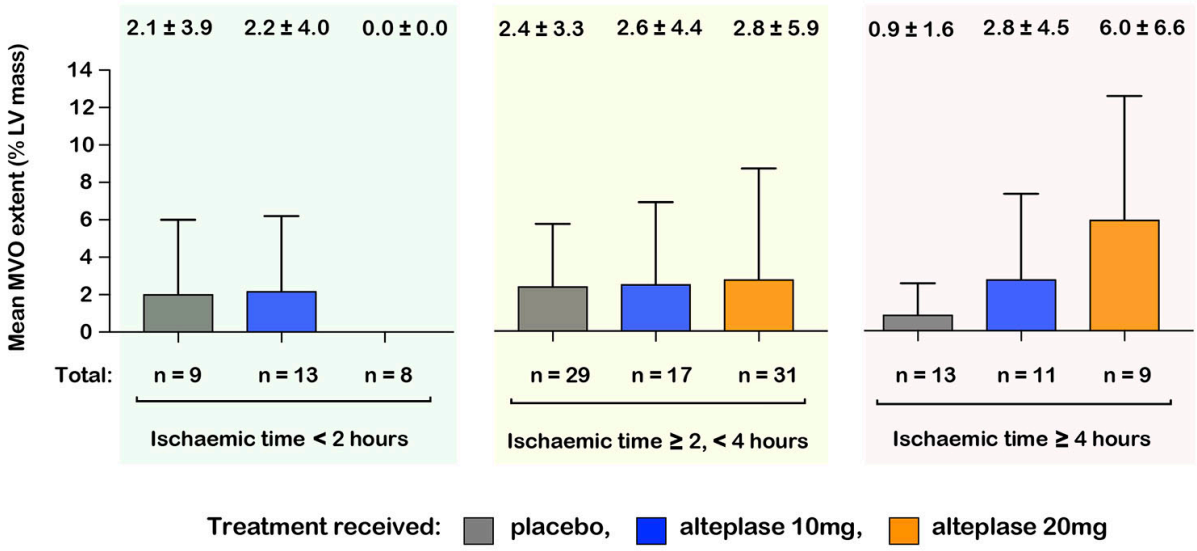

Figure 4.7 *Mean MVO extent (± SD), by treatment received, in sub-groups of ischaemic time (<2 hours, ≥2, < 4 hours, ≥4 hours).*

Ischaemic time	Treatment Effect		Interaction p-value (treatment as 3-level categorical variable)	Treatment Effect	Interaction p-value (treatment as 2-level categorical variable)
	10mg vs. placebo	20mg vs. placebo		10 or 20mg vs. placebo	
IMR			0.172		0.367
<2 hrs	0.79 (0.43, 1.46) p=0.454	0.52 (0.26, 1.04) p=0.064		0.68 (0.39, 1.19) p=0.173	
≥2, <4 hrs	0.82 (0.53, 1.27) p=0.364	1.24 (0.86 1.79) p=0.250		1.07 (0.76, 1.51) p=0.685	
≥4 hrs	0.70 (0.39, 1.27) p=0.238	1.07 (0.58, 1.96) p=0.837		0.86 (0.51, 1.44) p=0.556	
CFR			**0.013**		0.652
<2 hrs	0.94 (0.68, 1.29) p=0.686	1.59 (1.11, 2.27) **p=0.012**		1.14(0.85, 1.55) p=0.379	
≥2, <4 hrs	1.03 (0.82, 1.30) p=0.791	1.00 (0.82, 1.21) p=0.987		1.01 (0.84, 1.21) p=0.915	
≥4 hrs	1.08 (0.79, 1.47) p=0.636	0.82 (0.60, 1.13) p=0.224		0.95 (0.72, 1.25) p=0.697	
RRR			**0.026**		0.827
<2 hrs	0.93 (0.66, 1.29) p=0.647	1.52 (1.05, 2.22) **p=0.028**		1.12 (0.82, 1.53) p=0.481	
≥2, <4 hrs	1.10 (0.87, 1.40) p=0.435	0.98 (0.80, 1.20) p=0.848		1.02 (0.85, 1.23) p=0.833	
≥4 hrs	1.11 (0.80, 1.54) p=0.518	0.86 (0.61, 1.20) p=0.367		0.98 (0.74, 1.31) p=0.907	
MVO (% LV)			**0.039**		0.089
<2 hrs	-0.03 (-1.10, 1.03) p=0.950	-0.87 (-2.07, 0.33) p=0.155		-0.35 (-1.34, 0.64) p=0.484	
≥2, <4 hrs	-0.14 (-0.90, 0.61) p=0.715	-0.14 (-0.77, 0.50) p=0.676		-0.14 (-0.72, 0.45) p=0.643	
≥4 hrs	0.49 (-0.52, 1.50) p=0.337	1.47 (0.40, 0.54) **p=0.007**		0.93 (0.05, 1.82) **0.039**	
Myocardial haemorrhage (% LV)			0.153		0.392
<2 hrs	0.20 (-2.48, 4.87) p=0.523	-0.51 (-4.44, 3.42) p=0.800		0.48 (-2.91, 3.86) p=0.783	
≥2, <4 hrs	0.41 (-2.04, 2.86) p=0.741	-0.06 (-2.05, 1.92) p=0.950		0.09 (-1.75, 1.93) p=0.926	
≥4 hrs	0.80 (-2.46, 4.06) p=0.632	4.25 (0.88, 7.61) p=0.013		2.42 (-0.41, 5.25) p=0.094	

Table 4.10 *Treatment effect estimates and interactions with treatment received, for IMR, CFR, RRR, MVO extent and myocardial haemorrhage extent, in sub-groups of ischaemic time (<2 hours, ≥2 <4 hours, ≥4 hours). Coronary physiology data were analysed on a logarithmic scale, with treatment effect estimates reported as relative differences and 95% CIs, derived from linear regression.[366] MVO extent (% LV) were analysed on a square root scale, with treatment effect estimates reported as mean differences and 95%, derived from linear regression. For myocardial haemorrhage extent (% LV) treatment effect estimates were reported as mean differences between groups, with 95% CIs. The analyses were adjusted for location of MI.*

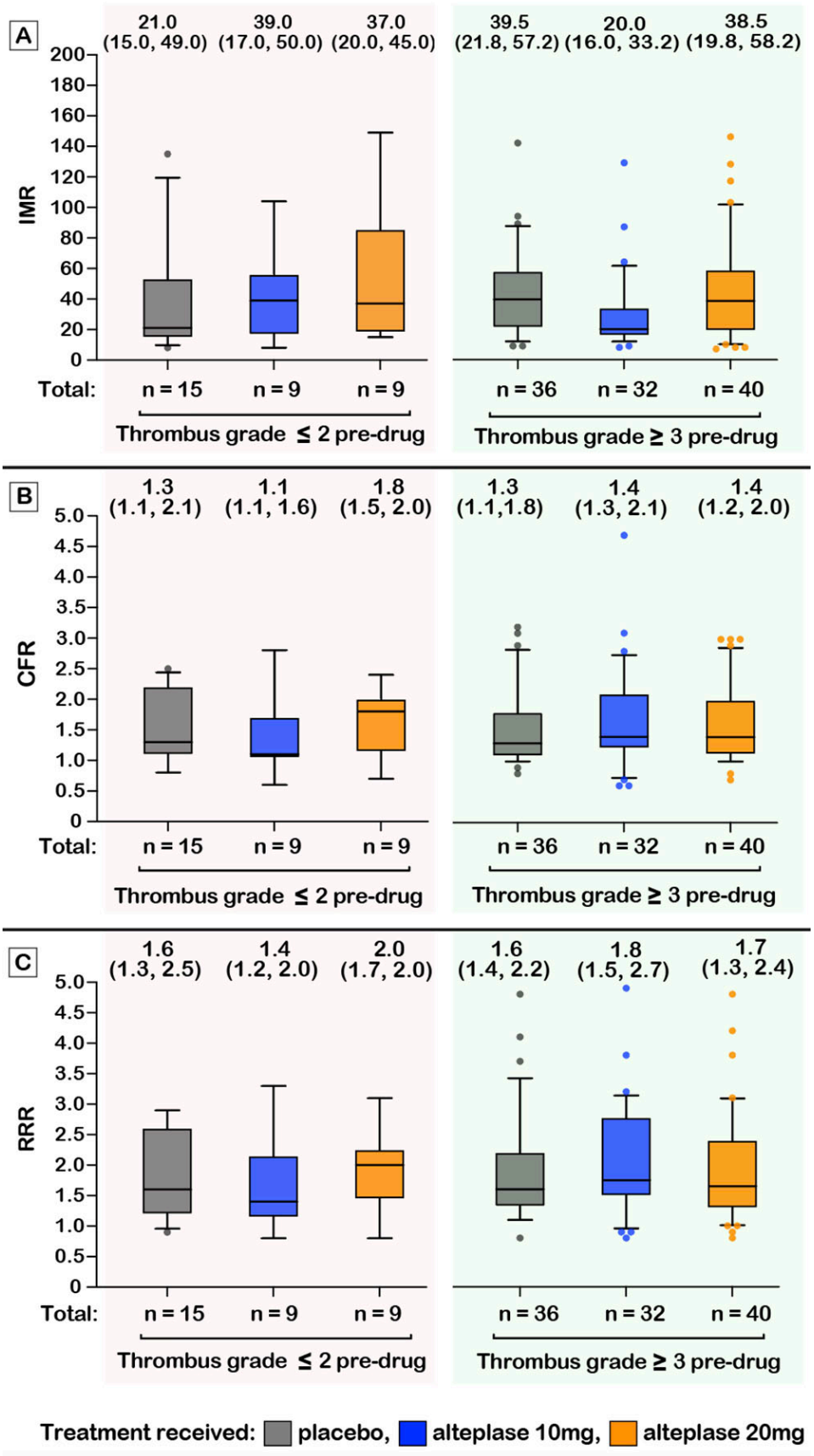

Figure 4.8 *IMR (A), CFR (B) and RRR (C), by treatment received, in sub-groups of TIMI thrombus grade (≤2, ≥3) immediate preceding study drug administration. Boxes represent the median and IQR (values provided), with whiskers at the 10th and 90th percentiles. Values outside the 10th and 90thpercentiles are presented as individual data points.*

TIMI thrombus grade pre-study drug	Treatment Effect		Interaction p-value (treatment as 3-level categorical variable)	Treatment Effect	Interaction p-value (treatment as 2-level categorical variable)
	10mg vs. placebo Estimate (95% CI) p-value	20mg vs. placebo Estimate (95% CI) p-value		10 or 20mg vs. placebo Estimate (95% CI) p-value	
IMR			0.260		0.158
≤ 2	1.24 (0.67, 2.29) p=0.498	1.33 (0.72, 2.44) p=0.363		1.28 (0.77, 2.13) p=0.348	
≥ 3	0.69 (0.48, 0.98) **p=0.036**	0.97 (0.70, 1.36) p=0.865		0.83 (0.62, 1.12) p=0.231	
CFR			0.506		0.616
≤ 2	0.86 (0.62, 1.21) p=0.393	1.06 (0.76, 1.48) p=0.737		0.96 (0.73, 1.26) p=0.759	
≥ 3	1.05 (0.87, 1.28) p=0.595	1.03 (0.86, 1.23) p=0.753		1.04 (0.89, 1.22) p=0.637	
RRR			0.440		0.595
≤ 2	0.87 (0.61, 1.23) p=0.424	1.05 (0.75, 1.48) p=0.767		0.96 (0.72, 1.27) p=0.768	
≥ 3	1.09 (0.89, 1.33) p=0.412	1.02 (0.85, 1.23) p=0.840		1.05 (0.89, 1.24) p=0.580	

Table 4.11 *Treatment effect estimates reported as relative differences and 95% CIs with p-values from linear regression and interactions with treatment received, for IMR, CFR and RRR, in sub-groups of TIMI thrombus grade (≤2, ≥3) immediately before study drug administration.[366] Data analysed on logarithmic scale. The analyses were adjusted for MI location.*

4.5 Discussion

In this prespecified, physiology sub-study of the T-TIME trial, there was overall no difference in microvascular function, measured by IMR, CFR and RRR, between alteplase and placebo groups.

The lack of an overall effect of adjunctive intracoronary alteplase on microvascular function in the infarct-related artery contrasts with the findings reported from a previous study, which used adjunctive intracoronary streptokinase during primary PCI[259 260]. In that study, low-dose intracoronary streptokinase (n=51) was infused into the infarct-related artery over 3 minutes through a guide catheter at the end of primary PCI, and when compared to standard care (n=44) the patients who received streptokinase had higher CFR (2.5 vs. 1.7, p<0.001) and lower IMR (20.2 vs. 34.3, p<0.001)[260].

There are differences between the study described in the above paragraph by Sezer *et al*[260] and the T-TIME physiology sub-study. First, the study by Sezer *et al*[260] was not double-blinded. Second, unlike alteplase, streptokinase is not fibrin specific. Third, in the study by Sezer *et al*[260] intracoronary streptokinase was administered after stent implantation, when 89% of the cohort had TIMI 3 flow in the infarct-related artery, whereas in the T-TIME physiology sub-study intracoronary alteplase was administered before stent implantation, when only 68% of the patients had TIMI 3 flow. Fourth, in the T-TIME physiology sub-study, IMR, CFR and RRR were measured immediately after stent optimisation at the end of the PCI procedure, whereas Sezer *et al*[260] measured IMR and CFR 48-hours after primary PCI, when IMR and CFR may have undergone partial recovery[163 275 276].

The prespecified sub-group analyses were exploratory and were intended to be interpreted as hypothesis generating, and to provide mechanistic insights. The reason for the absence of interaction between ischaemic time and alteplase with IMR, even though interactions were observed with CFR and RRR, could potentially be because IMR reflects different aspects of microvascular function than RRR or CFR, or due to type 2 statistical error (i.e. a false negative result), due to the relatively small number of patients in the sub-groups, which limited statistical power to detect differences. IMR measures the minimum achievable microvascular resistance with hyperaemia[140], but unlike RRR[194] and CFR[156 191],

IMR does not measure the "ability" of the microcirculation to vasodilate, i.e. vasodilator capacity, and therefore IMR might not reflect the full potential for the microcirculation to recover following reperfusion.

There are plausible potential mechanisms to explain the interactions that were observed between ischaemic time and alteplase, with CFR and RRR. The improvements in CFR and RRR with alteplase in patients with brief ischaemic times (<2 hours) may reflect those patients having an intact microcirculation, with preserved vasodilator capacity, which was modifiable by therapy. On the other hand, in patients with ischaemic times ≥4 hours, there may have been irreversible microvascular damage. Therefore, the microcirculation may have been resistant to modification by therapy in patients with longer ischaemic times. Indeed, patients with ischaemic times ≥4 hours had numerically lower CFR and RRR with alteplase 20mg, suggesting a trend to worse microvascular function with alteplase in patients with longer ischaemic times. This is consistent with the observation that alteplase was associated with more MVO in patients with ischaemic times ≥4 hours. The underlying mechanism is uncertain, but a potential explanation could be that in the setting of prolonged ischaemia with associated capillary degradation, alteplase may have promoted haemorrhage of erythrocytes into the interstitial space of the infarct core, with consequent external compression of capillaries, thereby worsening MVO. The findings suggest the possibility of alteplase having a detrimental effect on myocardial reperfusion in patients with ischaemic times ≥4. This is especially relevant to the ongoing RESTORE-MI trial (ACTRN12618000778280)[273], which is including STEMI patients with ischaemic times up to 12 hours, for adjunctive intracoronary tenecteplase or placebo during primary PCI.

Whilst the observed interactions between ischaemic time, alteplase and CFR, RRR and MVO could potentially be a consequence of type 1 statistical error (false positive result), the findings are supported by consistent effects of ischaemic time on the extent of MVO in the main T-TIME trial (n=440)[303]. In the main T-TIME trial population (n=440), patients with ischaemic times ≥4 had significantly more MVO and myocardial haemorrhage with alteplase compared to placebo, and there was a significant interaction between ischaemic time, alteplase and MVO (and myocardial haemorrhage) with treatment analysed as a linear trend (0mg, 10mg, 20mg), but not with treatment analysed as 2-level, or 3-level categorical variables[303].

With regards to the lack of interaction between TIMI flow grade immediately before drug administration, alteplase, and IMR, CFR or RRR, type 2 statistical error cannot be excluded (i.e. a false negative). The patient numbers in the treatment groups, for sub-groups of TIMI flow pre-drug, were relatively small, which limited power to detect significant interactions.

A possible explanation for the lack of interaction between TIMI thrombus grade immediately before study drug administration, alteplase and IMR, CFR or RRR, may be because distal embolization of thrombus to the microcirculation could have occurred at initial low-pressure balloon inflation, prior to the assessment of thrombus grade pre-study drug administration. Therefore, some of the patients assessed as having thrombus grade ≤2 immediately before study drug administration may have had very high thrombus burden that wasn't apparent angiographically at the time of study drug administration, because distal embolization of thrombus to the microcirculation had already occurred. A second consideration is that angiography is a relatively insensitive means of assessing thrombus burden, and intra-observer, and inter-rater variability was demonstrated in this study. Optical coherence tomography is a superior method, with high-resolution (10-20 μm)[369], for accurate determination of thrombus burden[369-372]. Furthermore, optical coherence tomography allows white thrombi (composed largely of platelets and leukocytes), which produce high signal intensity to be distinguished from red thrombi (composed mainly of fibrin and erythrocytes), which produce high backscattering protrusions in the artery lumen with signal attenuation[373]. It is not possible to distinguish red from white thrombus using the TIMI thrombus grade classification.

4.6 Limitations

The study presented in this chapter has many strengths, including: (i) multicentre enrolment, (ii) blinding of coronary physiology measurements during acquisition to minimise bias, (iii) double-blind, randomised design, (iv) excellent inter-rater and intra-observer reliability for physiology measurements, and (v) excellent follow up rates with CMR at 3 months (94%). However, the limitations need to be considered.

First, coronary physiology measurements were not performed consecutively in patients enrolled into the main T-TIME trial. Out of the 440 patients in the T-TIME trial, about two

thirds did not have coronary physiology measurements performed, due to logistical reasons, or operator inexperience with coronary physiology. Seven patients had failed attempts at coronary physiology measurements, due to haemodynamic instability or no detectable thermodilution transit times.

The most prevalent infarct-related artery in the T-TIME physiology study was the RCA (46%), followed by the LAD (38%), whereas for the main T-TIME trial the most prevalent infarct-related artery was the LAD (41%) and second most prevalent was the RCA (39%)[292]. In general, it may be easier and quicker to pass wires in the RCA than the LAD, which in circumstances of logistical pressures for catheterisation laboratory access may have led to slightly fewer pressure wire measurement being performed in the LAD.

Another limitation, is the potential for type 1 statistical error (false positive) in the sub-group analyses, therefore these should be interpreted as exploratory. Furthermore, duplicate IMR measurements were available in only 13 patients and duplicate CFR and RRR measurements were only available in 9 patients. Finally, there was some variability in angiographic thrombus assessment pre-study drug.

4.7 Future work

Further research is needed to validate the findings from the sub-group analyses, because of the potential for type 1 statistical error. In addition, analysis of the results from the T-TIME optical coherence tomography sub-study is of interest to determine whether low-dose intracoronary alteplase may have a differential effect in patients with the largest thrombus burden compared to lower thrombus burden pre-drug, assessed by optical coherence tomography. Lastly, it would be interesting to investigate whether intracoronary tenecteplase administered at the end of primary PCI, i.e. post-stent implantation, may result in improvement in invasive coronary physiology parameters measured before and after drug administration compared to placebo, in patients with short ischaemic times. The T-TIME physiology sub-study protocol did not include pressure wire studies pre-drug, because the drug was administered before stent implantation, and in that setting the infarct-related artery would have been at risk of re-occluding at the site of the unstented lesion.

4.8 Conclusions

In conclusion, acute STEMI patients with ischaemic times ≤6 hours had no overall difference in infarct-related artery microvascular function (IMR, CFR, or RRR) at the end of PCI with alteplase (administered following initial reperfusion and before stent implantation) compared to placebo. Interactions were observed between ischaemic time and alteplase on CFR, RRR and MVO, suggesting potential therapeutic benefit in patients presenting with a shorter ischaemic time, and a detrimental effect in patients with a longer ischaemic time.

Chapter 5: Comparison of acute invasive parameters for predicting microvascular injury and clinical outcomes after primary PCI

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5.1 Abstract

Background: Parameters that detect failed microvascular perfusion during primary PCI have potential for early risk stratification of patients, and to identify patients likely to benefit from adjunctive treatments. The aim of the research presented in this chapter was to compare IMR, CFR, RRR, MPG and TFC, for predicting MVO, myocardial haemorrhage and clinical outcomes.

Methods: In the T-TIME trial, 440 acute STEMI patients from 11 U.K. hospitals were prospectively enrolled. In a subset of 144 patients, IMR, CFR and RRR were measured at the end of primary PCI. In addition, MPG and TFC were measured at the end of the PCI procedure. MVO extent (% LV mass) was determined by CMR imaging performed 2 to 7 days after primary PCI. Myocardial haemorrhage was determined by T2* mapping. One year major adverse cardiac events (MACE), heart failure hospitalisations, and all-cause death/ heart failure hospitalisations were assessed.

Results: In these 144 patients (mean age 59 ± 11 years, 80% male), IMR >40 was multivariably associated with more MVO (coefficient: 0.54 [95% CI: 0.05, 1.03] p=0.030) and myocardial haemorrhage presence (OR: 2.67 [95% CI: 1.10, 6.51] p=0.031), independently of CFR ≤2.0, RRR ≤1.7, MPG ≤1 and TFC >27. Lower RRR was multivariably associated with more MVO (coefficient: -0.61 [95% CI: -0.98, -0.23] p=0.002) and myocardial haemorrhage presence (OR: 0.34 [95% CI: 0.15, 0.75] p=0.008). MPG ≤1 was also multivariably associated with more MVO (coefficient: 0.53 [95% CI: 0.07, 1.00] p=0.026) and myocardial haemorrhage presence (OR: 3.15 [95% CI: 1.35, 7.35] p=0.008), whereas CFR ≤2.0 and TFC >27 were not. IMR >40 was associated with heart failure hospitalisation (OR: 5.34 [95% CI: 1.80, 15.81] p=0.002), MACE (OR: 4.46 [95% CI: 1.70, 11.70] p=0.002), and all-cause death/ heart failure hospitalisation (OR: 4.08

[95% CI: 1.55, 10.79] p=0.005). Lower RRR was associated with heart failure hospitalisation (OR: 0.44 [95% CI:0.19, 0.99] p=0.047). TFC >27 and MPG ≤1 were also associated with clinical outcomes, whereas CFR ≤2.0 was not.

Conclusion: In acute STEMI, IMR >40, lower RRR and MPG ≤1, were associated with MVO, myocardial haemorrhage and clinical outcomes. TFC >27 was associated with clinical outcomes, but not MVO or myocardial haemorrhage. CFR ≤2.0 was not associated with MVO, myocardial haemorrhage, or clinical outcomes. Compared with CFR, RRR may have potential as a superior tool, to guide patient selection for adjunctive therapy.

5.2 Introduction

Invasive parameters, measured acutely in the infarct-related artery, during primary PCI can potentially be used to enable stratified medicine, which is the identification of patient sub-groups that are distinguishable from a heterogenous population by disease severity and potential response to treatments[269]. Early and accurate identification of acute microvascular injury in the catheterisation laboratory allows potential for early administration of targeted adjunctive therapies to the highest risk patients, before irreversible microvascular damage occurs. Furthermore, during primary PCI, invasive parameters measured acutely in the infarct-related artery, have potential to refine future clinical trial design, by enabling selection of higher risk STEMI patients with evidence of microvascular injury for adjunctive treatments[292].

In STEMI patients, MPG ≤1 at the end of PCI is associated with the presence of MVO on CMR imaging[131] and with mortality[125 132]. However, MPG as an angiographic surrogate measure of failed myocardial reperfusion has limitations: (i) it requires evaluation by an experienced observer for accurate interpretation[116], (ii) it has relatively high inter-rater variability[116], and (iii) it has limited specificity, since MPG ≥2 is not synonymous with adequate microvascular perfusion[103 131]. Increased TFC provides a quantitative, measure to assess coronary flow, and has relatively good inter-rater reproducibility[117 118 126]. A TFC ≤27 in the infarct-related artery is considered normal[120-123]. A limitation is that the prognostic value of TFC is unclear. A higher TFC following thrombolysis for STEMI independently predicted mortality in some studies[121 124], whereas in other studies higher TFC was not multivariably associated with mortality[125 126]. Moreover, TFC is increased (confounded) by the use of intracoronary nitrates[118 119].

Higher IMR values indicate greater degrees of microvascular dysfunction[156 164], and an IMR >40 predicts death, heart failure hospitalisations and MI[149 164]. CFR reflects epicardial and microcirculatory vasodilator capacity, as well as residual epicardial stenosis. A lower CFR predicts MVO, however, when compared to IMR >40, the combination of IMR>40 and CFR ≤2.0 did not add incremental prognostic value[149]. RRR is a newer invasive coronary physiology parameter, with no established cut-off value for normal/ abnormal. RRR evaluates the vasodilatory capacity of the coronary microcirculation and, therefore, the ability of the microcirculation to appropriately increase myocardial blood flow.

Specifically, RRR reflects the ability of the coronary microcirculation to vary its resistance in response to adenosine, i.e. a hyperaemic stimulus[194]. Higher RRR values signify greater vasodilatation of the microcirculation in response to hyperaemia, whereas lower RRR values signify poor vasodilator capacity of the coronary microcirculation. Lower RRR is associated with more MVO on CMR imaging[197]. Furthermore, lower RRR measured in patients with stable ischaemic heart disease is associated with a composite of all-cause mortality, MI, or any revascularisation events during 5-year follow up[195]. The prognostic significance of RRR in acute STEMI is unknown.

The aim of the present study was to compare the associations of IMR, CFR, RRR, MPG and TFC, with MVO (a reference surrogate measure of failed myocardial perfusion), myocardial haemorrhage (a secondary irreversible manifestation of persistent MVO) and clinical outcomes.

The following hypotheses were evaluated:

1) Higher IMR and TFC, and lower CFR, RRR and MPG at the end of primary PCI are associated with MVO and myocardial haemorrhage on CMR imaging.

2) Lower RRR at the end of primary PCI is more closely associated with MVO and myocardial haemorrhage on CMR imaging, than lower CFR.

3) Higher IMR and TFC, and lower CFR, RRR and MPG at the end of primary PCI are associated with increased rates of MACE, heart failure hospitalisation, and the combination of all-cause death/ heart failure hospitalisation

5.3 Methods

The design, population, data acquisition, and angiographic, ECG and CMR analyses for the T-TIME physiology sub-study was described in sections 2.1, 2.2, and Figure 2.2.

5.3.1 Clinical outcomes

All serious adverse events were prospectively reported to the Pharmacovigilance Unit, by site research staff. These events were initially screened by blinded investigators for forwarding to the clinical events committee. The events were then reviewed and

adjudicated by the clinical events committee, comprising 3 cardiologists who were blinded and independent of the trial. There was a pre-defined clinical events committee charter (Appendix 9). Clinical events were assessed at 1-year and clinical follow up was completed for all patients.

The prespecified clinical outcomes for this study were pathophysiologically linked with the natural history of STEMI. The clinical outcomes were: hospitalisation for heart failure; all-cause death/ hospitalisation for heart failure, and; MACE, defined as cardiac death, non-fatal MI, or hospitalisation for heart failure.

Hospitalisation for heart failure was defined as follows: (i) new/ worsening heart failure signs/ symptoms requiring initiation of, or increase in heart failure directed treatment, or occurring in a patient already receiving maximal heart failure therapy, or; (ii) confinement to bed predominantly due to heart failure symptoms, or; (iii) pulmonary oedema sufficient to cause increased respiratory rate and distress (not occurring concurrently with an acute MI, or worsening kidney function [that is not completely explained by worsening heart failure], or as the consequence of arrythmia without worsening heart failure), or; (iv) cardiogenic shock.

5.3.2 Statistical analysis

Categorical variables were reported as frequency and percentages. Continuous data were summarised using means if normally distributed, or medians if skewed. The associations between invasive parameters and MVO or myocardial haemorrhage extent were assessed by linear regression and were adjusted for the following covariates: CFR ≤2, RRR ≤1.7 (median value), IMR >40, TFC >27 and MPG ≤1 (all measured at the end of the primary PCI procedure). There was *a priori* concern that these covariates were clinically relevant confounders. The reason for not including continuous coronary physiology parameters as covariates together in the same model was due to collinearity. In linear regression models, square root transformation was applied to MVO to improve model residual distributions. The regression coefficients reported from linear regression represent mean change in the extent of the endpoint for a 1-unit increase in the predictor. The incremental predictive ability of RRR was assessed using the continuous net reclassification improvement[375]. Spearman rank coefficients were used to assess correlations.

Receiver operating characteristic (ROC) curve analysis was undertaken to investigate the relationships between IMR, CFR, RRR, or TFC, with MVO, and myocardial haemorrhage absence/ presence, and clinical outcomes. Optimal thresholds for predicting MVO, myocardial haemorrhage and clinical outcomes were derived from the ROC curves. In this, sensitivity and specificity were considered equally important, therefore the optimal cut-off was considered as the one giving the maximum Youden index. ROC comparisons were made using the DeLong method[376].

The associations with clinical outcomes were evaluated by calculating ORs, derived from logistic regression. A preponderance of heart failure episodes occurred during the index hospitalisation, therefore the assumptions for the Cox proportional hazards regression model for constant effects over time was not met.

All tests were 2-tailed and a p-value of <0.05 was considered statistically significant. There was no imputation for missing values and no adjustments for multiple statistical comparisons. Statistical analyses were performed in SPSS (version 25.0, SPSS IBM, Armonk, NY, USA), and MedCalc Statistical Software version 18 (MedCalc Software, Ostend, Belgium). The net reclassification improvement was calculated using the package PredictABEL in R (version 3.4.3, R Development Core Team, California, USA).

5.4 Results

The population and procedure characteristics for the T-TIME coronary physiology sub-study have been described in section 4.4.2, Tables 4.1 to 4.3 and Figure 4.2.

5.4.1 Associations with invasive parameters

Among the 144 patients, 57 (40%) had IMR>40, 115 (80%) had CFR ≤2, 77 (54%) had RRR ≤1.7, 29 (20%) had TFC >27 and 45 (31%) had MPG ≤1.

The characteristics that were associated with higher IMR, on multivariable linear regression, were: TIMI flow grade post-PCI ≤2 (p=0.026), MPG ≤1 post-PCI (p=0.016) and RRR ≤1.7 (p=0.009). The characteristics that were associated with lower RRR were:

CFR ≤2.0 ($p<0.001$) and IMR >40 ($p=0.034$). The only characteristic that was associated with lower CFR, on multivariable linear regression analysis, was RRR ≤1.7 ($p<0.001$).

The characteristics that were associated with higher TFC, on multivariable linear regression, were hypertension ($p=0.028$), lower estimated glomerular filtration rate ($p=0.011$), and TIMI flow grade post-PCI ≤2 ($p<0.001$). The characteristics that were associated with MPG ≤1 on multivariable logistic regression were lower estimated glomerular filtration rate ($p=0.019$), infarct-related artery (LAD vs. LCx vs. RCA) ($p<0.001$), TIMI flow grade post-PCI ≤2 ($p=0.043$) and IMR >40 ($p=0.023$).

IMR was correlated with RRR ($r= -0.32$, $p=0.0001$), CFR ($r= -0.30$, $p=0.0002$) and TFC ($r=0.59$, $p<0.0001$). CFR was correlated with RRR ($r=0.94$, $p<0.001$) (Figure 5.1), but not with TFC ($r= -0.05$, $p=0.549$). There was no statistically significant correlation between RRR and TFC ($r= -0.06$, $p=0.465$) (Figure 5.2).

When CFR was dichotomized by 2, RRR by 1.7 and IMR by 40, discordance between CFR and RRR occurred in 38 patients (26.4%), discordance between CFR and IMR occurred in 66 patients (45.9%), and discordance between RRR and IMR occurred in 50 patients (34.7%) (Figure 5.1). When RRR was dichotomised by 2 the results were broadly similar to when the 1.7 threshold was used (Figure 5.3).

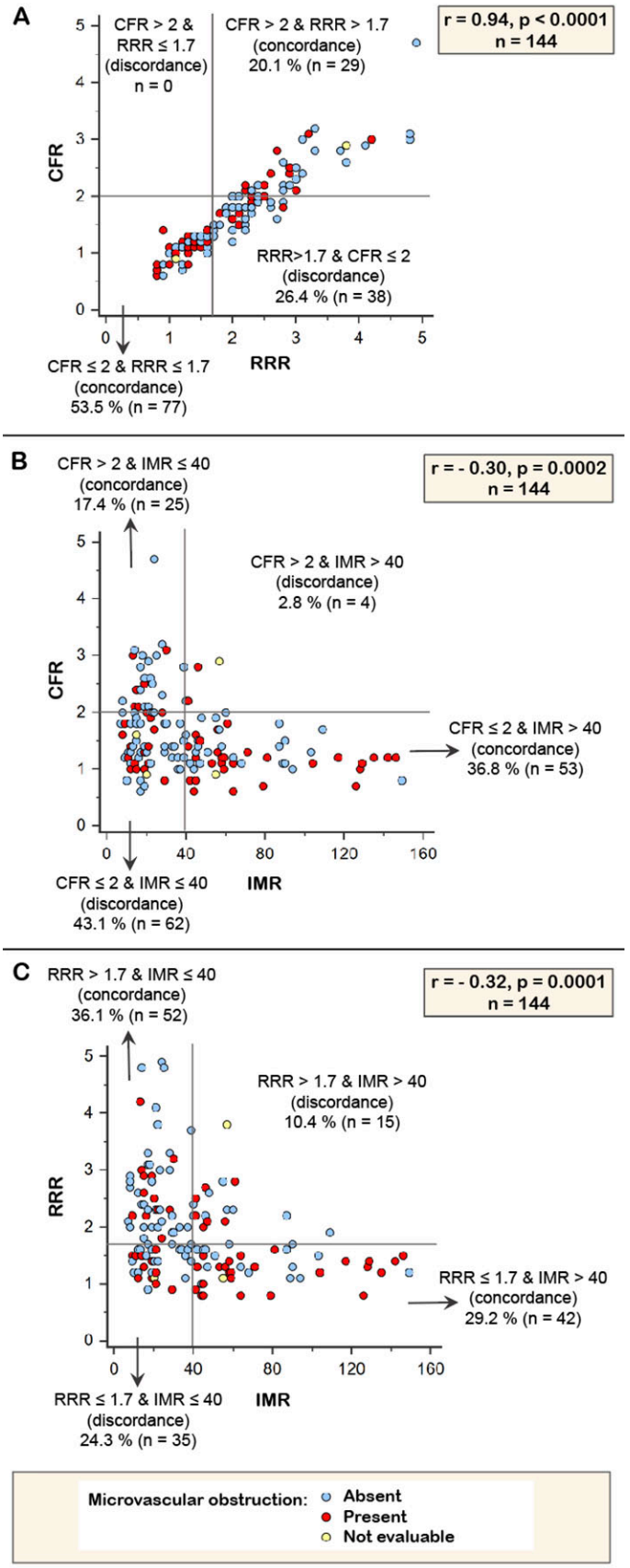

Figure 5.1 *Scatterplots showing correlations between: A, CFR and RRR; B, IMR and CFR; and C, IMR and RRR. Also shown is discordance between dichotomised coronary physiology parameters (CFR ≤2.0, RRR ≤1.7, IMR >40) and presence/ absence of MVO. Reproduced with permission from Maznyczka AM et al. Comparative significance of invasive measures of microvascular injury in acute myocardial infarction. Circ Cardiovasc Interv. 2020.13(5) [Epub ahead of print]*[374]*. Doi: 10.1161/CIRCINTERVENTIONS.119.008505*

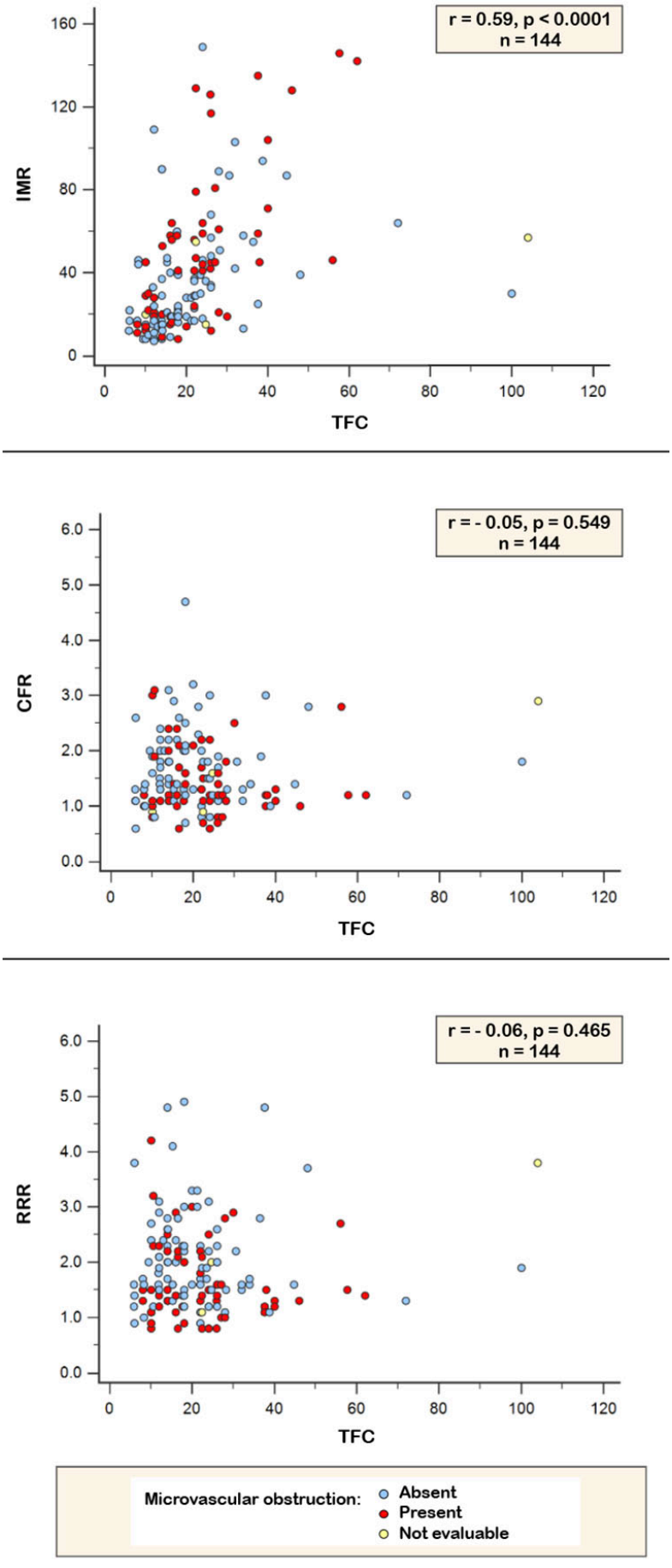

Figure 5.2 Scatterplots showing correlations between: (i) TFC and IMR, (ii) TFC and CFR, or (iii) TFC and RRR.

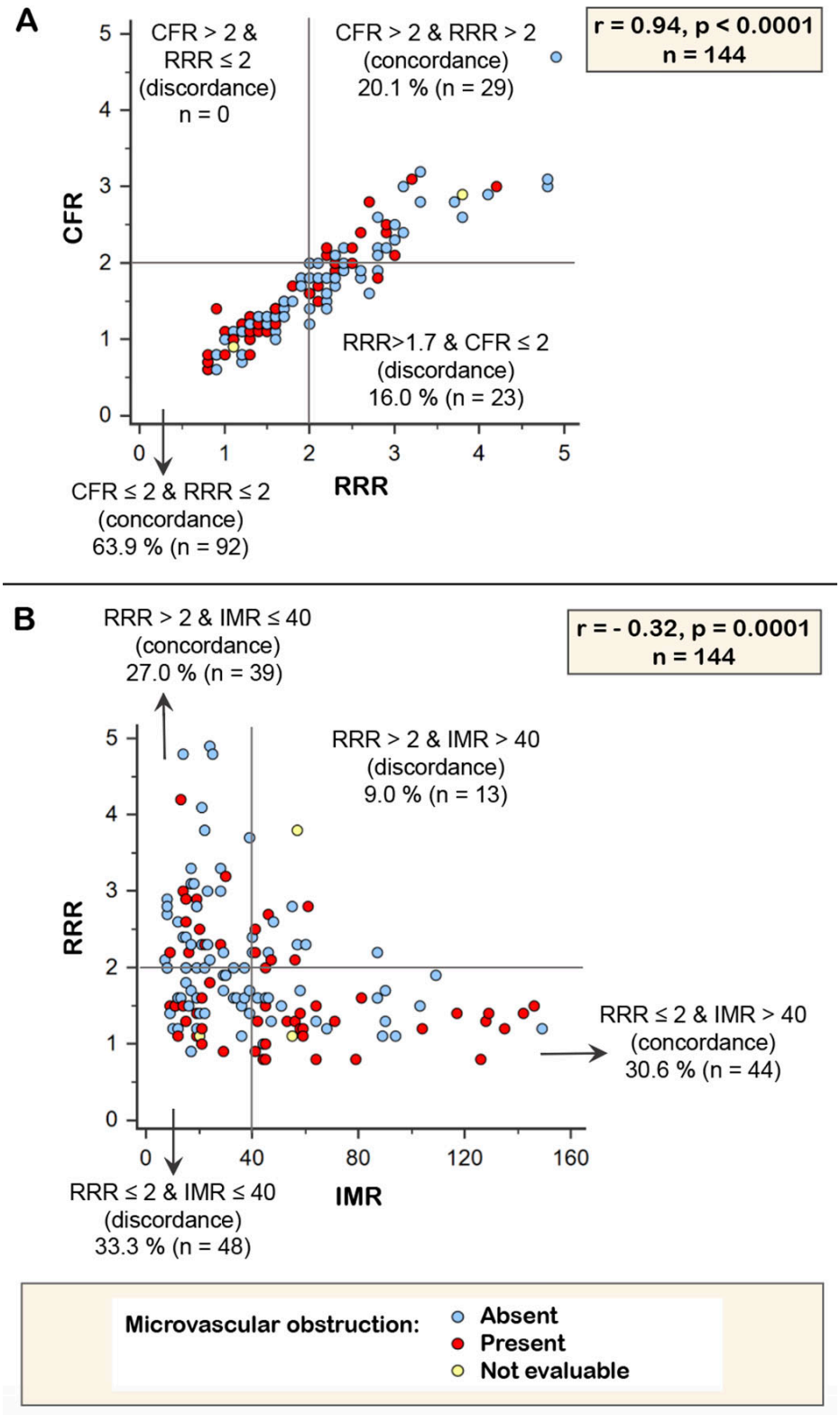

Figure 5.3 *Scatterplots showing correlations between: A, CFR and RRR; and B, IMR and RRR. Also shown is discordance between dichotomised coronary physiology parameters (CFR ≤2.0, RRR ≤2.0, IMR >40) and presence/ absence of MVO. Reproduced with permission from Maznyczka AM et al. Comparative significance of invasive measures of microvascular injury in acute myocardial infarction. Circ Cardiovasc Interv. 2020.13(5) [Epub ahead of print]*[374]*. Doi: 10.1161/CIRCINTERVENTIONS.119.008505*

5.4.2 Association of invasive parameters with MVO

IMR

Higher IMR measured acutely correlated with more MVO (Figure 5.4). Among the 140 patients who had CMR imaging 2 to 7 days post-PCI, IMR was >40 in 55, of whom 32 (58%) had MVO present, and IMR was ≤40 in 85, of whom 25 (29%) had MVO present.

The optimal IMR threshold from the AUC for predicting MVO presence was >40 (Figure 5.5). IMR >40 was multivariably associated with larger MVO extent (coefficient: 0.54 [95% CI: 0.05, 1.03]) p=0.030) and higher incidence of MVO (OR: 2.67 [95% CI: 1.11, 6.39] p=0.028), independently of CFR ≤2.0, RRR ≤1.7, TFC >27 and MPG ≤1 (Table 5.1). Continuous IMR, or IMR dichotomised by 30 (median value) were not multivariable predictors of MVO (Tables 5.1 and 5.2).

CFR

Lower CFR measured acutely correlated with more MVO (Figure 5.4). Among the patients who had CMR imaging 2 to 7 days post-PCI, CFR was ≤2.0 in 112, of whom 47 (42%) had MVO present, and CFR was >2.0 in 28, of whom 10 (36%) had MVO present.

Continuous CFR, CFR ≤2.0 or CFR dichotomised by median value (1.4) were not multivariable predictors of MVO (Tables 5.1 and 5.2). The optimal CFR threshold from the AUC for predicting MVO presence was ≤1.2 (Figure 5.5). CFR ≤1.2 was multivariably associated with larger MVO extent (coefficient: 0.60 [95% CI: 0.03, 1.17] p=0.039) and MVO presence (OR: 3.12 [95% CI: 1.06, 9.19) p=0.039) (Table 5.2).

RRR

Lower RRR measured acutely correlated with more MVO (Figure 5.4). Among the patients who had CMR imaging 2 to 7 days post-PCI, RRR was ≤1.7 (median value) in 75, of whom 37 (49%) had MVO present, and RRR was >1.7 in 65, of whom 20 (31%) had MVO present.

The optimal RRR threshold from the AUC for predicting MVO presence was ≤1.5 (Figure 5.5). RRR ≤1.5 was multivariably associated with larger MVO extent (coefficient: 0.91 [95% CI: 0.46, 1.35] p<0.001) and MVO presence (OR: 4.66 [95% CI: 1.89, 11.47)

p=0.001) (Table 5.2). Lower continuous RRR was also multivariably associated with larger MVO extent (coefficient: -0.61 [95% CI: -0.98, -0.23] p=0.002) and MVO presence (OR: 0.32 [95% CI: 0.14, 0.73) p=0.006) (Table 5.1). RRR ≤1.7 was a multivariable associate of larger MVO extent, but not MVO presence (Table 5.1).

The overall net reclassification improvement, reflecting the incremental predictive accuracy for detecting the presence of MVO, was 0.34 (95% CI: 0.06, 0.61, p=0.018) when RRR ≤1.7 was added to a baseline model containing CFR ≤2.0. When continuous RRR was added to a baseline model containing CFR ≤2.0 the net reclassification improvement for detecting MVO presence was 0.66 (95% CI: 0.36, 0.95, p<0.001). When the baseline model incorporated IMR >40, the overall net reclassification improvement for detecting the presence of MVO was 0.38 (95% CI: 0.05, 0.70, p=0.025) when RRR ≤1.7 was added, and was 0.29 (95% CI: -0.02, 0.59, p=0.068) when continuous RRR was added.

RRR and CFR in Combination

Compared with RRR >1.7 and CFR ≤2.0 combined (reference group), the group with the combination of RRR ≤1.7 and CFR ≤2.0 was associated with an increased odds of MVO presence (37/75 [40.3%] vs. 10/37 [27.0%]; OR, 2.63 [95% CI, 1.12, 6.18] p=0.027), and increased MVO extent (0.0 [IQR: 0.0 – 5.3] vs. 0.0 [IQR: 0.0-0.8]; coefficient, 0.74 [95% CI, 0.22, 1.25] p=0.006).

TFC

There was no statistically significant correlation between TFC and MVO (Figure 5.4). Among the patients who had CMR imaging 2 to 7 days post-PCI, TFC was >27 in 28, of whom 14 (50%) had MVO present. Among the patients who had CMR imaging 2 to 7 days post-PCI, TFC was ≤27 in 112, of whom 43 (38%) had MVO present.

The optimal TFC threshold from the AUC for predicting MVO presence was >22 (Figure 5.5). Continuous TFC, or TFC dichotomised by 22 (median value), 18, or 27 was not multivariably associated with MVO (Tables 5.1 and 5.2).

MPG

Among the patients who had CMR imaging 2 to 7 days post-PCI, MPG was ≤1 in 44 patients, of whom 28 (64%) had MVO present. Among the patients who had CMR imaging 2 to 7 days post-PCI, MPG was >1 in 96 patients, of whom 29 (30%) had MVO present.

MPG ≤1 was multivariably associated with larger MVO extent (coefficient: 0.53 [95% CI: 0.07, 1.00] p=0.026) and MVO presence (OR: 3.61 [95% CI: 1.56, 8.38] p=0.003).

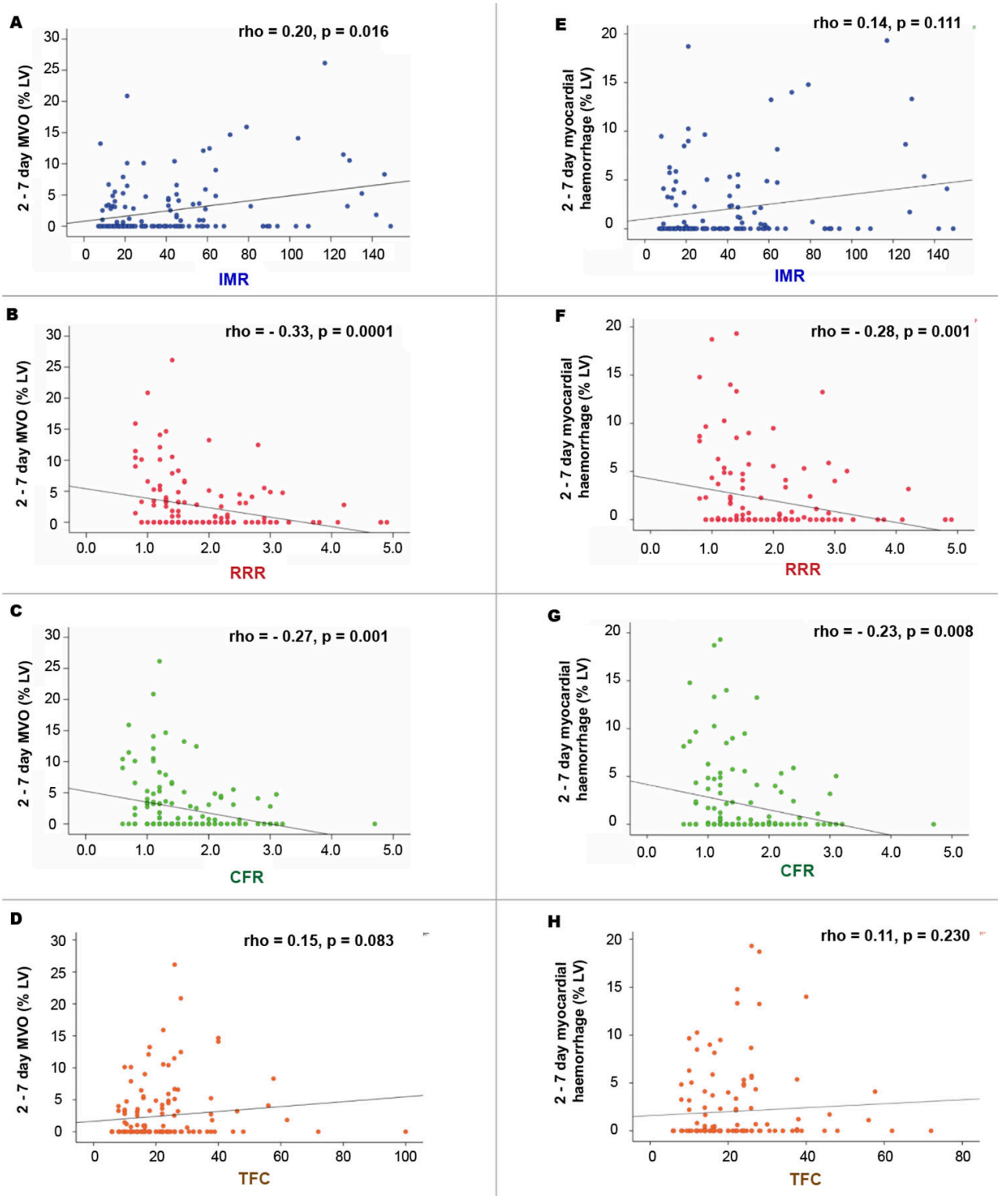

Figure 5.4 *Scatterplots showing correlations between IMR, RRR, CFR and TFC, with MVO (A, B, C, D) and myocardial haemorrhage (E, F, G, H).*

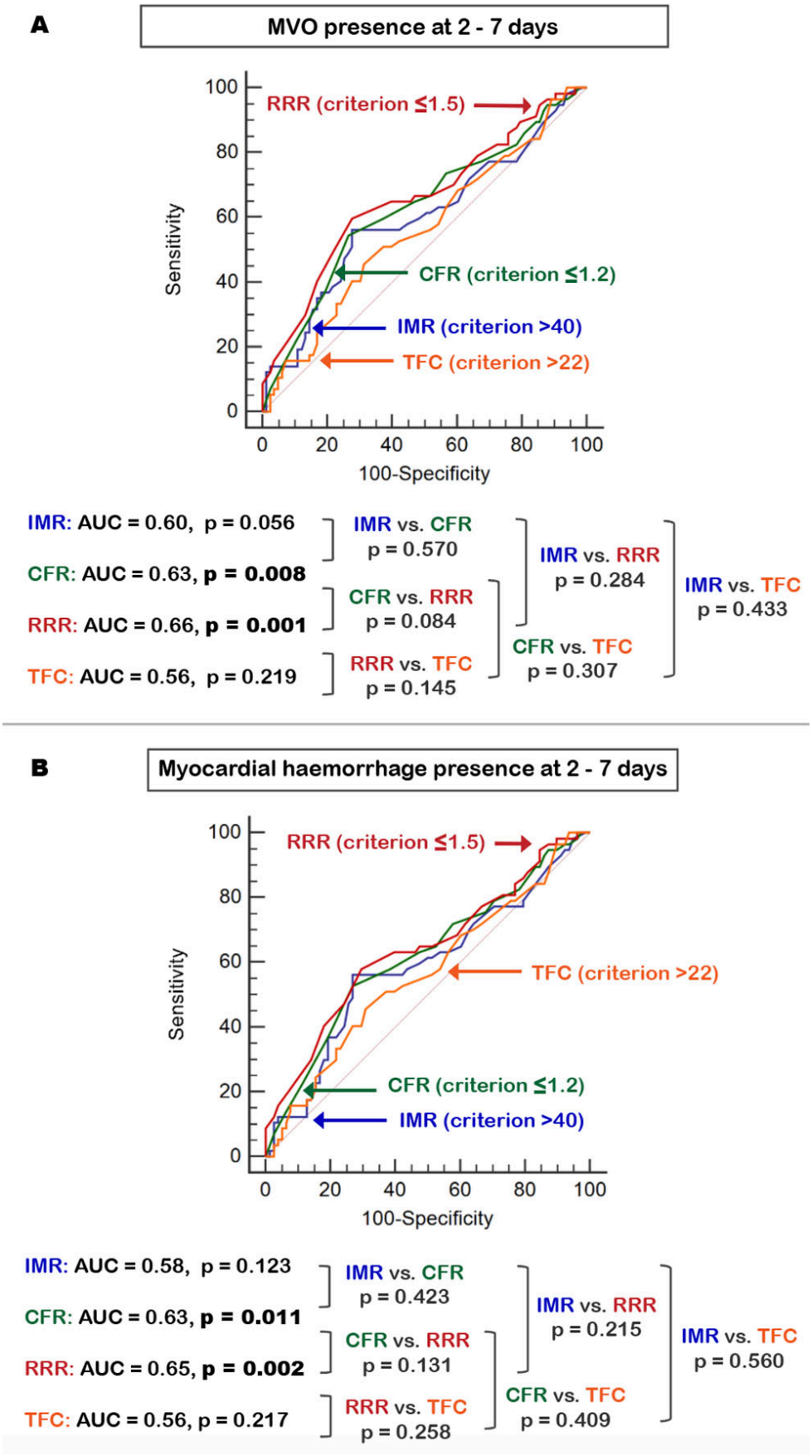

Figure 5.5 *ROC curves showing the performance of IMR, CFR, RRR and TFC for predicting MVO presence/ absence (A), and myocardial haemorrhage presence/ absence (B).*

	Association with MVO extent (% LV) (n=140)		Association with MVO presence (n=140)	
	Univariable	**Multivariable**	**Univariable**	**Multivariable**
Continuous IMR*	0.01 (0.01, 0.02) **p=0.001**	0.01 (-0.00, 0.01) p=0.077	1.01 (1.00, 1.02) **p=0.024**	1.01 (0.99, 1.02) p=0.460
IMR >40*	0.78 (0.36, 1.20) **p<0.001**	0.54 (0.05, 1.03) **p=0.030**	3.34 (1.64, 6.80) **p=0.001**	2.67 (1.11, 6.39) **p=0.028**
Continuous CFR†	-0.50 (-0.81, -0.18) **p=0.002**	-0.11 (-0.60, 0.38) p=0.654	0.52 (0.29, 0.93) **p=0.028**	0.92 (0.35, 2.40) p=0.856
CFR ≤2.0†	0.35 (-0.19, 0.88) p=0.200	-0.30 (-0.90, 0.30) p=0.325	1.30 (0.55, 3.07) p=0.548	0.45 (0.14, 1.42) p=0.173
Continuous RRR‡	-0.45 (-0.69, -0.20) **p<0.001**	-0.61 (-0.98, -0.23) **p=0.002**	0.51 (0.31, 0.83) **p=0.007**	0.32 (0.14, 0.73) **p=0.006**
RRR ≤1.7 (median)‡	0.67 (0.26, 1.09) **p=0.002**	0.60 (0.11, 1.09) **p=0.017**	2.19 (1.09, 4.39) **p=0.027**	2.35 (0.92, 5.97) p=0.074
Continuous TFC	0.01 (-0.01, 0.03) p=0.208	-0.01 (-0.02, 0.01) p=0.520	1.01 (0.99, 1.04) p=0.389	0.98 (0.95, 1.01) p=0.203
TFC >27 §	0.38 (-0.15, 0.91) p=0.162	-0.19 (-0.75, 0.37) p=0.493	1.61 (0.70, 3.69) p=0.266	0.56 (0.20, 1.58) p=0.275
MPG ≤1 ||	0.71 (0.27, 1.16) **p=0.002**	0.53 (0.07, 1.00) **p=0.026**	4.04 (1.90, 8.59) **p<0.001**	3.61 (1.56, 8.38) **p=0.003**

Table 5.1 *Associations of invasive parameters with the extent of MVO, from linear regression and with MVO presence from logistic regression. Results are reported as regression coefficient or OR (95% CI) and p-value. MVO extent (2 to 7 days post-PCI) was analysed on a square root scale.*
** Covariates in multivariable analyses for association of IMR with MVO: CFR ≤2.0, RRR ≤1.7, TFC >27 and MPG ≤1.*
† Covariates in multivariable analyses for association of CFR with MVO: IMR >40, RRR ≤1.7, TFC >27 and MPG ≤1.
‡ Covariates in multivariable analyses for association of RRR with MVO: IMR >40, CFR ≤2.0, TFC >27 and MPG≤1.
§ Covariates in multivariable analyses for association of TFC with MVO: IMR >40, CFR ≤2.0, RRR ≤1.7 and MPG ≤1.
|| Covariates in multivariable analyses for association of MPG ≤1 with MVO: IMR >40, CFR ≤2.0, RRR ≤1.7 and TFC >27.

	Association with MVO extent (% LV) (n=140)		Association with MVO presence (n=140)	
	Univariable	**Multivariable**	**Univariable**	**Multivariable**
IMR>30 (median)*	0.43 (0.01, 0.85) **p=0.045**	0.10 (-0.37, 0.56) p=0.687	1.76 (0.89, 3.47) p=0.105	1.11 (0.48, 2.53) p=0.814
CFR≤1.2 (optimal threshold from AUC)†	0.85 (0.44, 1.27) **p<0.001**	0.60 (0.03, 1.17) **p=0.039**	3.31 (1.62, 6.75) **p=0.001**	3.12 (1.06, 9.19) **p=0.039**
CFR≤1.4 (median)†	0.65 (0.23, 1.06) **p=0.002**	0.35 (-0.74, 1.43) p=0.528	2.09 (1.04, 4.18) **p=0.038**	1.65 (0.24, 11.15) p=0.610
RRR≤1.5 (optimal threshold from AUC)‡	0.98 (0.57, 1.38) **p<0.001**	0.91 (0.46, 1.35) **p<0.001**	3.86 (1.89, 7.88) **p<0.001**	4.66 (1.89, 11.47) **p=0.001**
TFC >22 (Optimal threshold from AUC) §	0.60 (0.17, 1.03) p=**0.007**	0.17 (-0.33, 0.66) p=0.508	1.84 (0.92, 3.69) p=0.087	0.77 (0.31, 1.92) p=0.772
TFC >18 (median) §	0.44 (0.02, 0.86) **p=0.040**	0.06 (-0.39, 0.51) p=0.797	1.52 (0.77, 3.00) p=0.224	2.25 (0.89, 5.68) p=0.451

Table 5.2 *Associations of invasive parameters with the extent of MVO, from linear regression and with MVO presence from logistic regression. Different dichotomisations for IMR, CFR, RRR and TFC are shown, including according to median values and according to the optimal thresholds from AUCs. Results are reported as regression coefficient or OR (95% CI) and p-value. MVO extent (2 to 7 days post-PCI) was analysed on square root scales.*
** Covariates in multivariable analyses for association of IMR with MVO: CFR ≤2.0, RRR ≤1.7, TFC >27 and MPG ≤1.*
† Covariates in multivariable analyses for association of CFR with MVO: IMR >40, RRR ≤1.7, TFC >27 and MPG ≤1.
‡ Covariates in multivariable analyses for association of RRR with MVO: IMR >40, CFR ≤2.0, TFC >27 and MPG ≤1.
§ Covariates in multivariable analyses for association of corrected TFC post-PCI with MVO: IMR >40, CFR ≤2.0, RRR ≤1.7 and MPG ≤1.

5.4.3 Association of invasive parameters with myocardial haemorrhage

IMR

There was no statistically significant correlation between IMR and myocardial haemorrhage extent (Figure 5.4). IMR >40 was multivariable associated with myocardial haemorrhage presence (OR: 2.67 [95% CI: 1.10, 6.51] p=0.031], but not myocardial haemorrhage extent (coefficient: 0.96 [95% CI: -0.67, 2.60] p=0.246) (Table 5.3). Continuous IMR, or IMR dichotomised by 30 (median value) were not multivariably associated with myocardial haemorrhage (Tables 5.3 and 5.4).

CFR

Lower CFR was correlated with more myocardial haemorrhage (Figure 5.4). However, CFR ≤2.0, CFR ≤1.2 (optimal threshold from AUC), CFR ≤1.4 (median value) and continuous CFR were not multivariably associated with myocardial haemorrhage (Tables 5.3and 5.4).

RRR

Lower RRR was correlated with more myocardial haemorrhage (Figure 5.4). RRR ≤1.5 (optimal threshold from AUC) was multivariably associated with larger myocardial haemorrhage extent (coefficient: 2.30 [95% CI: 0.77, 3.83] p=0.004) and myocardial haemorrhage presence (OR: 4.06 [95% CI: 1.65, 10.04] p=0.002).

Lower continuous RRR was also multivariably associated with larger myocardial haemorrhage extent (coefficient: -1.49 [95% CI: -2.71, -0.27] p=0.017) and myocardial haemorrhage presence (OR: 0.34 [95% CI: 0.15, 0.75] p=0.008). RRR ≤1.7 was multivariably associated with larger myocardial haemorrhage extent, but not myocardial haemorrhage presence (Table 5.3).

The overall net reclassification improvement, reflecting the incremental predictive accuracy for detecting the presence of myocardial haemorrhage was 0.34 (95% CI: 0.05, 0.52 [p=0.021]), when RRR ≤1.7 was added to a baseline model containing CFR ≤2.0. When continuous RRR was added to a baseline line incorporating CFR ≤2.0, the net reclassification improvement for detecting myocardial haemorrhage presence was 0.62 (95% CI: 0.32, 0.91 [p<0.001]).

When the baseline model incorporated IMR >40, the overall net reclassification improvement for detecting the presence of myocardial haemorrhage was 0.39 (95% CI: 0.04, 0.72 [p=0.026]) when RRR ≤1.7 was added, and was 0.25 (95% CI: -0.07,0.56 [p=0.131]) when continuous RRR was added.

CFR and RRR in combination

Compared with RRR >1.7 and CFR ≤2.0 combined (reference group), the group with the combination of RRR ≤1.7 and CFR ≤2.0 was associated with increased odds of myocardial haemorrhage presence (36/72 [50.0%] vs. 10/36 [27.8%]; OR, 2.60 [95% CI, 1.10, 6.17] p=0.030), and with increased myocardial haemorrhage extent (0.00 [IQR: 0.0 - 4.6] vs. 0.0 [IQR: 0.0 - 0.2]; coefficient, 1.85 [95% CI: 0.11, 3.58] p=0.037).

TFC

There was no statistically significant correlation between TFC and myocardial haemorrhage extent (Figure 5.4). Neither continuous TFC, TFC >27, TFC >22 (optimal from AUC), or TFC >18 (median value) were associated with myocardial haemorrhage (Tables 5.3 and 5.4).

MPG

MPG ≤1 was multivariably associated with the presence of myocardial haemorrhage (OR: 3.15 [95% CI: 1.35, 7.35] p=0.008), but not with myocardial haemorrhage extent (coefficient: 0.43 [95% CI: -1.16, 2.01] p=0.594) (Table 5.3).

	Association with myocardial haemorrhage extent (% LV) (n=131)		Association with myocardial haemorrhage presence (n=136)	
	Univariable	**Multivariable**	**Univariable**	**Multivariable**
Continuous IMR*	0.03 (0.01, 0.05) **p=0.014**	0.02 (-0.01, 0.04) p=0.132	1.01 (1.00, 1.02) p=0.090	1.00 (0.99, 1.01) p=0.901
IMR >40*	1.46 (0.09, 2.83) **p=0.037**	0.96 (-0.67, 2.60) p=0.246	3.27 (1.59, 6.72) **p=0.001**	2.67 (1.10, 6.51) **p=0.031**
Continuous CFR†	-1.32 (-2.32, -0.33) p**=0.009**	-0.50 (-2.09, 1.10) p=0.539	0.52 (0.29, 0.93) **p=0.028**	0.90 (0.34, 2.37) p=0.835
CFR ≤2.0†	1.16 (-0.48, 2.80) p=0.165	-0.30 (-2.26, 1.67) p=0.766	1.34 (0.56, 0.32) p=0.511	0.48 (0.15, 1.51) p=0.207
Continuous RRR‡	-1.13 (-1.90, -0.36) **p=0.004**	-1.49 (-2.71, -0.27) **p=0.017**	0.51 (0.31, 0.83) **p=0.007**	0.34 (0.15, 0.75) **p=0.008**
RRR ≤1.7 (median)‡	1.82 (0.50, 3.14) **p=0.007**	1.66 (0.30, 3.28) **p=0.046**	2.20 (1.09, 4.44) **p=0.028**	2.29 (0.90, 5.83) p=0.083
Continuous TFC	0.02 (-0.04, 0.08) p=0.491	-0.02 (-0.09, 0.05) p=0.589	1.01 (0.98, 1.04) p=0.397	0.97 (0.93, 1.01) p=0.091
TFC >27 §	0.73 (-1.05, 2.50) p=0.420	-0.32 (-2.24, 1.60) p=0.743	1.56 (0.66, 3.68) p=0.312	0.54 (0.18, 1.56) p=0.253
MPG ≤1 ||	0.90 (-0.58, 2.37) p=0.231	0.43 (-1.16, 2.01) p=0.594	3.72 (1.75, 7.95) **p=0.001**	3.15 (1.35, 7.35) **p=0.008**

Table 5.3 *Associations of invasive parameters with the extent of myocardial haemorrhage (2 to 7 days post-PCI), from linear regression and with myocardial haemorrhage presence from logistic regression. Results are reported as regression coefficient or OR (95% CI) and p-value.*

** Covariates in multivariable analyses for association of IMR with myocardial haemorrhage: CFR ≤2.0, RRR ≤1.7, TFC >27 and MPG ≤1.*
† Covariates in multivariable analyses for association of CFR with myocardial haemorrhage: IMR >40, RRR ≤1.7, TFC >27 and MPG ≤1.
‡ Covariates in multivariable analyses for association of RRR with myocardial haemorrhage: IMR >40, CFR ≤2.0, TFC >27 and MPG≤1.
§ Covariates in multivariable analyses for association of TFC with myocardial haemorrhage: IMR >40, CFR ≤2.0, RRR ≤1.7 and MPG ≤1.
|| Covariates in multivariable analyses for association of MPG ≤1 with myocardial haemorrhage: IMR >40, CFR ≤2.0, RRR ≤1.7 and TFC >27.

	Association with myocardial haemorrhage extent (% LV) (n=131)		Association with myocardial haemorrhage presence (n=136)	
	Univariable	**Multivariable**	**Univariable**	**Multivariable**
IMR>30 (median)*	0.64 (-0.72, 1.99) p=0.353	-0.15 (-1.70, 1.40) p=0.848	1.68 (0.84, 3.34) p=0.141	1.05 (0.45, 2.44) p=0.907
CFR≤1.2 (optimal threshold from AUC)†	2.00 (0.63, 3.37) **p=0.005**	1.16 (-0.76, 3.08) p=0.234	3.24 (1.57, 6.69) **p=0.001**	2.85 (0.96, 8.48) p=0.059
CFR≤1.4 (median)†	1.76 (0.44, 3.08) **p=0.010**	0.76 (-2.73, 4.25) p=0.667	2.09 (1.04, 4.22) **p=0.039**	1.58 (0.24, 10.59) p=0.638
RRR≤1.5 (optimal threshold from AUC)‡	2.44 (1.12 3.76) **p<0.001**	2.30 (0.77, 3.83) **p=0.004**	3.56 (1.73, 7.30) **p=0.001**	4.06 (1.65, 10.04) **p=0.002**
TFC >22 (optimal threshold from AUC) §	1.76 (0.39, 3.13) **p=0.012**	1.29 (-0.36, 2.93) p=0.124	1.77 (0.87, 3.60) p=0.112	0.74 (0.29, 1.88) p=0.530
TFC >18 (median) §	1.20 (-0.15, 2.54) p=0.080	0.62 (-0.87, 2.11) p=0.412	1.45 (0.73, 2.89) p=0.286	0.69 (0.30, 1.61) p=0.396

Table 5.4 *Associations of invasive parameters with the extent of myocardial haemorrhage (2 to 7 days post-PCI), from linear regression and with myocardial haemorrhage presence from logistic regression. Different dichotomisations for IMR, CFR, RRR and TFC are shown, including according to median values and according to the optimal thresholds from AUCs. Results are reported as regression coefficient or OR (95% CI) and p-value.*

** Covariates in multivariable analyses for association of IMR with myocardial haemorrhage: CFR ≤2.0, RRR ≤1.7, TFC >27 and MPG ≤1.*
† Covariates in multivariable analyses for association of CFR with myocardial haemorrhage: IMR >40, RRR ≤1.7, TFC >27 and MPG ≤1.
‡ Covariates in multivariable analyses for association of RRR with myocardial haemorrhage: IMR >40, CFR ≤2.0, TFC >27 and MPG ≤1.
§ Covariates in multivariable analyses for association of corrected TFC post-PCI with myocardial haemorrhage: IMR >40, CFR ≤2.0, RRR ≤1.7 and MPG ≤1.

5.4.4 Association of invasive parameters with clinical outcomes

At 1-year follow up, there were 19 adjudicated hospitalisations for heart failure, 22 for all-cause death/ heart failure hospitalisations, and 23 MACE events.

IMR

In patients with IMR >40, heart failure hospitalisations occurred in 14 patients (24.6%) at 1 year, death/ heart failure hospitalisation occurred in 15 patients (26.3%), and MACE occurred in 16 (28.1%) patients. The optimal IMR threshold from the AUC for predicting clinical outcomes was 44 (Figure 5.6). Higher continuous IMR, IMR >40, IMR >44, and IMR >30 (median value), were all associated with heart failure hospitalisations, all-cause death/ heart failure hospitalisation, and MACE (Table 5.5).

CFR

In patients with CFR ≤2.0, heart failure hospitalisations occurred in 18 patients (15.7%), death/ heart failure hospitalisation occurred in 20 patients (17.4%) and MACE occurred in 21 patients (18.3%). Neither continuous CFR, CFR ≤2.0, nor CFR ≤1.4 (median value) were associated with clinical outcomes. The optimal CFR threshold was ≤1.8 for predicting heart failure hospitalisation (OR: 8.47 [95% CI: 1.09, 65.70] p=0.041). The optimal CFR threshold was ≤1.3 for predicting all-cause death/ heart failure hospitalisation (OR: 2.89 [95% CI: 1.1.0, 7.58] p=0.032) (Figure 5.6). The optimal CFR threshold from the AUC for predicting MACE was ≤1.3 (Figure 5.6), however on logistic regression analysis CFR ≤1.3 was not associated with MACE (OR: 2.49 [95% CI: 0.98, 6.31] p=0.055).

RRR

In patients with RRR ≤1.7, heart failure hospitalisations occurred in 14 (18.2%) patients, death/ heart failure hospitalisation occurred in 16 (20.8%) patients, and MACE occurred in 16 (20.8%) patients. The optimal RRR threshold from the AUC for predicting heart failure hospitalisations was ≤1.6 (Figure5.5) (OR: 3.23 [95% CI: 1.10, 9.52] p=0.033). The optimal RRR threshold for predicting all-cause death/ heart failure hospitalisations was also ≤1.6 (OR: 3.14 [95% CI:1.15, 8.57] p=0.025). The optimal RRR threshold for predicting MACE was ≤2.2 (OR: 4.99 [95% CI: 1.12, 22.37] p=0.036). Lower continuous RRR was associated with heart failure hospitalisations, but was not associated with all-cause death/ heart failure hospitalisations, or MACE (Table 5.5). RRR ≤1.7 was not associated with clinical outcomes.

CFR and RRR in combination

RRR ≤1.7 and CFR ≤2.0 combined did not enhance the prognostic significance of RRR >1.7 and CFR ≤2.0 combined, for association with heart failure hospitalisations (14/77 [18.2%] vs. 4/38 [10.5%]; OR, 1.89 [95% CI: 0.58, 6.19] p=0.294), death/ heart failure hospitalisations (16/77 [20.8%] vs. 4/38 [10.5%]; OR, 2.23 [95% CI: 0.69, 7.21] p=0.180), or MACE (16/77 [20.8%] vs. 5/38 [13.2%]; OR, 1.73 [95% CI: 0.58, 5.15] p=0.324).

TFC

Higher continuous TFC, TFC >27, TFC >26 (optimal threshold from AUC), and TFC >18 (median value) were all associated with heart failure hospitalisations, all-cause death/ heart failure hospitalisation, and MACE (Table 5.5).

MPG

MPG <1 was associated with heart failure hospitalisations, all-cause death/ heart failure hospitalisation, and MACE (Table 5.5).

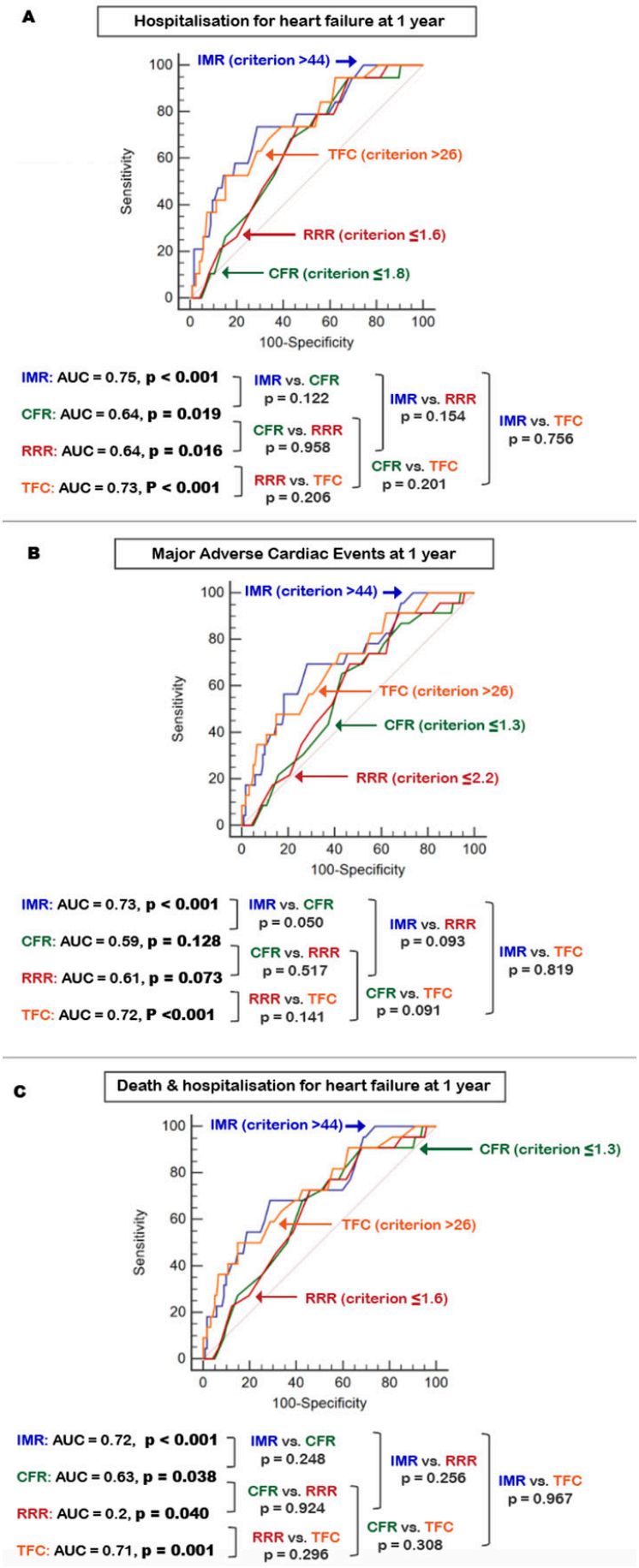

Figure 5.6 *ROC curves showing the performance of IMR, CFR, RRR and TFC for predicting heart failure hospitalisation (A), MACE (B), and all-cause death/ heart failure hospitalisation (C).*

	Heart failure hospitalisation (n=19 events)	All-cause death & heart failure hospitalisation combined (22 events)	Major adverse cardiac events (n=23 events)
	Crude OR (95% CI) p-value	**Crude OR (95% CI) p-value**	**Crude OR (95% CI) p-value**
Continuous IMR	1.02 (1.01, 1.04) **p<0.001**	1.02 (1.01, 1.03) **p=0.001**	1.02 (1.01, 1.03) **p=0.001**
IMR >40	5.34 (1.80, 15.81) **p=0.002**	4.08 (1.55, 10.79) **p=0.005**	4.46 (1.70, 11.70) **p=0.002**
IMR >44 (optimal threshold from AUC)	6.92 (2.32, 20.63) **p=0.001**	5.33 (2.00, 14.18) **p=0.001**	5.85 (2.21, 15.47) **p<0.001**
IMR >30 (median)	3.56 (1.21, 10.50) **p=0.021**	2.70 (1.03, 7.09) **p=0.044**	2.93 (1.13, 7.64) **p=0.028**
Continuous CFR	0.39 (0.15, 1.04) p=0.060	0.48 (0.20, 1.14) p=0.097	0.57 (0.26, 1.28) p=0.174
CFR ≤2.0	5.20 (0.66, 40.65) p=0.116	0.35 (0.08, 1.60) p=0.177	3.02 (0.67, 13.68) p=0.152
CFR≤ optimal threshold from AUC*	8.47 (1.09, 65.70) **p=0.041**	2.89 (1.10, 7.58) **p=0.032**	2.49 (0.98, 6.31) p=0.055
CFR ≤1.4 (median)	2.67 (0.91, 7.86) p=0.075	2.58 (0.95, 7.04) p=0.064	2.18 (0.84, 5.66) p=0.112
Continuous RRR	0.44 (0.19, 0.99) **p=0.047**	0.55 (0.28, 1.10) p=0.092	0.59 (0.31, 1.14) p=0.119
RRR ≤optimal threshold from AUC†	3.23 (1.10, 9.52) **p=0.033**	3.14 (1.15, 8.57) **p=0.025**	4.99 (1.12, 22.37) **p=0.036**
RRR ≤1.7 (median)	2.76 (0.94, 8.11) p=0.066	2.67 (0.98, 7.27) p=0.055	2.25 (0.86, 5.85) p=0.097
Continuous TFC	1.04 (1.01, 1.07) **p=0.007**	1.05 (1.02, 1.09) **p=0.002**	1.05 (1.02, 1.08) **p=0.002**
TFC >27	6.20 (2.23, 17.27) **p<0.001**	5.78 (2.18, 15.30) **p<0.001**	5.25 (2.01, 13.69) **p=0.001**
TFC >26 (optimal threshold from AUC)	6.20 (2.23, 17.27) **p<0.001**	5.78 (2.18, 15.30) **p<0.001**	5.25 (2.01, 13.69) **p=0.001**
TFC >18 (median)	3.68 (1.25, 10.85) **p=0.018**	1.05 (1.02, 1.09) **p=0.002**	3.89 (1.43, 10.55) **p=0.008**
MPG ≤1	4.78 (1.74, 13.17) **p=0.002**	3.24 (1.28, 8.20) **p=0.013**	2.91 (1.17, 7.23) **p=0.022**

Table 5.5 *Unadjusted odds ratios and 95% CI, from logistic regression, showing associations of invasive parameters with clinical outcomes at 1-year post-STEMI (n=144). Different dichotomisations for IMR, CFR, RRR and TFC are shown, including according to median values and according to the optimal thresholds from AUCs.*

**CFR ≤1.8 was the optimal threshold from AUC for predicting heart failure hospitalisation; CFR ≤1.3 was the optimal threshold from AUC for all-cause death and heart failure hospitalisation; CFR ≤1.3 was the optimal threshold from AUC for MACE.*

†RRR ≤1.6 was the optimal threshold from AUC for predicting heart failure hospitalisation; RRR ≤1.6 was optimal threshold from AUC for predicting all cause death of heart failure hospitalisation; RRR≤2.2 was the optimal threshold for predicting MACE.

5.5 Discussion

The findings presented in this chapter provide novel insights into the comparative clinical significance of invasive measures of microvascular function during primary PCI. Lower RRR was consistently associated with more MVO, even when different dichotomisations were used, and RRR ≤1.7 had incremental predictive utility compared to CFR ≤2.0 or IMR >40. When CFR or IMR were dichotomised by the optimal thresholds from the AUC (≤1.2 and >40 respectively), then these parameters were associated with more MVO. However, other thresholds, or continuous CFR and IMR, were not associated with MVO extent. MPG ≤1 predicted larger MVO extent, whereas TFC was not associated with MVO.

Lower RRR was consistently associated with more myocardial haemorrhage, even when different dichotomisations were used, and RRR ≤1.7 had incremental predictive utility compared to CFR ≤2.0 or IMR >40. A higher incidence of myocardial haemorrhage was associated with IMR >40 or MPG ≤1. Myocardial haemorrhage was not associated with CFR or TFC.

Lower IMR and higher TFC were consistently associated with all 3 types of clinical outcomes, even when different dichotomisations were tested. MPG ≤1 was also associated with all 3 types of clinical outcomes. Lower RRR (dichotomised by the optimal thresholds from the AUC) was associated with all 3 types of clinical outcomes. Lower continuous RRR was associated with heart failure hospitalisation, but not all-cause death/ heart failure hospitalisations or MACE. There was no association between CFR ≤2.0 and clinical outcomes. Lower CFR (dichotomised by the optimal threshold from the AUC) was associated with heart failure hospitalisations, and all-cause death/ heart failure hospitalisations, but was not associated with MACE. Other CFR thresholds, or continuous CFR was not associated with clinical outcomes.

Different aspects of microvascular function are reflected by IMR and RRR. Given that IMR does not reflect microvascular vasodilator capacity, it may not reflect the full potential for the microcirculation to recover following reperfusion. On the other hand, RRR reflects vasodilator capacity of the coronary microcirculation, i.e. it is a measure of the capacity of the coronary microcirculation to change from baseline to hyperaemia,

reflecting the ability to achieve maximal hyperaemia[194 197]. RRR may be superior to IMR in its ability to integrate a changing resistance from resting to hyperaemic conditions, as opposed to a fixed or absolute resistance assessed by IMR.

Compared with CFR, RRR was a better predictor of MVO and myocardial haemorrhage. Compared with CFR, RRR may better reflect the potential for the microcirculation to recover following reperfusion. RRR may provide additional information than what is obtained from currently available measures of microvascular function (CFR and IMR). The findings suggest that RRR may have potential as a superior tool compared with CFR to guide patient selection for adjunctive therapy.

Although RRR and CFR were correlated, discordance between high and low dichotomised CFR and RRR values occurred in 38 patients (26%), indicating that these parameters have overlapping and distinct behaviours. Furthermore, differences were observed in the associations of CFR and RRR, with MVO, myocardial haemorrhage and clinical outcomes, implying that CFR and RRR do not have equivalent clinical significance.

In a prior study consisting of 45 STEMI patients, RRR ≤1.98 (median for the cohort) at the end of primary PCI was associated with MVO extent 2 days following PCI[197]. The results presented in this chapter add to the findings from the previous study[197], by validating the findings in a larger cohort and showing that RRR is associated with clinical outcomes.

The reliable identification of patients with high probability of having microvascular damage has the potential to identify patients in the catheterisation laboratory for adjunctive therapies and inclusion in therapeutic trials. A test that has a binary cut-off, to identify normal and abnormal patients, is generally helpful for patient stratification in the clinic and in therapeutic trials. However, the optimal threshold may vary between different populations and different endpoints of interest. Therefore, IMR, RRR, CFR and TFC were dichotomised according to optimal thresholds from the AUC and median values, in addition to established thresholds, i.e. >40 for IMR and ≤2.0 for CFR[149].

Discordance was observed between dichotomised IMR >40 and MVO presence in just over one third of patients (42%), which is similar to prior literature[155]. Discordance between RRR ≤1.7 and MVO presence occurred in 51% of patients. Discordance between CFR ≤2.0 and MVO presence occurred in 58% of patients. There may be several explanations. First the extent of MVO varies in patients who have MVO present. The highest IMRs, lowest CFRs and lowest RRRs generally correspond to greater amounts of MVO, hence one expects discordance when binary thresholds are applied. Second, microvascular dysfunction is dynamic within minutes to the first few days following reperfusion. When microvascular function is measured immediately after primary PCI, reversible oedema and microvascular spasm may contribute more to microvascular dysfunction, than on CMR imaging 2 to 7 days later, where irreversible microvascular injury (including myocardial haemorrhage) may persist.

Although TFC was associated with clinical outcomes, it was not associated with MVO or myocardial haemorrhage. Therefore, the findings do not support the use of TFC as a theragnostic biomarker to select patients for adjunctive therapies aimed at limiting microvascular damage, or to evaluate the response of the microvasculature to therapies.

Although MPG ≤1 was multivariably associated with MVO, myocardial haemorrhage, and clinical outcomes, it has limitations for clinical translation as a theragnostic biomarker to select patients for adjunctive therapies in the catheterisation laboratory and to evaluate response to therapies. This is because MPG is non-quantitative and requires evaluation by an experienced observer for accurate interpretation[116].

5.6 Limitations

This study has many strengths, including: (i) independent adjudication of clinical events, (ii) core laboratory analyses, (iii) multicentre enrolment, and (iv) blinding of coronary physiology measurements to minimise bias. However, a limitation is that the T-TIME population was selected for participation in a clinical trial, and there was a relatively low number of clinical events, which limited power to detect statistically significant associations. Furthermore, these were all emergent PCI cases, hence the patients would not have withheld from caffeine, which could have affected response to adenosine[377]. Therefore, maximal hyperaemia could not be guaranteed in all of the patients. However,

these limitations apply to the previously published coronary physiology studies in the context of acute STEMI[140 148 155 156].

5.7 Future work

Further research is needed to determine whether the findings can be replicated in an independent population of acute STEMI patients. Another possibility for future research could be to investigate whether RRR may have utility in the assessment of the microcirculation in patients with ischaemia and no obstructive coronary disease.

5.8 Conclusions

In conclusion, in STEMI patients presenting within 6 hours of symptom onset, lower continuous RRR, IMR >40 and MPG ≤1 were associated with more MVO, myocardial haemorrhage presence and clinical outcomes, whereas CFR ≤2.0 was not. TFC >27 was associated with clinical outcomes, but not MVO or myocardial haemorrhage. Compared with CFR, RRR may have potential as a superior tool, to guide patient selection for adjunctive therapy.

Chapter 6: Thermodilution-derived temperature recovery time (TRT) a novel predictor of microvascular reperfusion and prognosis after acute STEMI

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6.1 Abstract

Background: Novel parameters that detect failed microvascular reperfusion might better identify the patients who are most likely to benefit from adjunctive treatments during primary PCI. The aim of this chapter was to investigate whether a newly conceived parameter (thermodilution-derived temperature recovery time [TRT]) would be associated with MVO and clinical outcomes, following acute STEMI.

Methods: TRT was derived retrospectively and validated prospectively in two distinct acute STEMI populations; from the MR-MI study and T-TIME trial, respectively. TRT was defined as the duration (in seconds) from the nadir of the hyperaemic thermodilution curve to 20% from return of the temperature to baseline. TRT was measured immediately post-PCI. The extent of MVO (% LV mass) was assessed by CMR, 2 to 7 days after emergency PCI. Participants were followed up for all-cause death/ heart failure hospitalisation, and MACE.

Results: In the derivation cohort (n=271, mean age 60 ± 12 years, 72% male), higher TRT was associated with more MVO (coefficient: 4.09 [95% CI: 2.70, 5.48] p<0.001), independent of IMR >32, CFR ≤2.0, hyperaemic Tmn >0.4 (median value), thermodilution waveform (bimodal or wide unimodal vs. narrow unimodal), age and ischaemic time. At 5-years, higher TRT was multivariably associated with all-cause death/ heart failure hospitalisation (OR: 4.41 [95% CI: 2.08, 8.25] p<0.001) and MACE (OR: 4.05 [95% CI: 2.00, 8.21] p<0.001), independent of IMR >32, CFR ≤2.0, hyperaemic Tmn >0.4 (median value) and thermodilution waveform. In the validation population (n=144, mean age 59 ± 11 years, 80% male) the findings were confirmed prospectively.

Conclusion: TRT is a novel invasive parameter for detecting MVO and predicting prognosis. TRT may represent a novel diagnostic advance, with potential to refine risk stratification in acute STEMI.

6.2 Introduction

MVO reflects failed microvascular reperfusion following STEMI. MVO affects about half of STEMI patients treated with standard primary PCI[26], and is associated with a worse prognosis[27 86], however there are no effective evidence-based therapies or interventions to treat MVO[91 212 347]. Novel parameters that accurately measure failed microvascular reperfusion, and provide prognostic information incremental to clinical indices, have potential to refine clinical trial design.

Invasive coronary physiology parameters provide an immediate assessment of post-PCI microvascular function. Among these parameters, IMR has been validated in animals[140 141] and humans[153 155 156 166]. Higher IMR values indicate greater degrees of microvascular dysfunction[156 164]. An IMR ≥32, or >40 predicts all-cause death or heart failure hospitalisations[164]. An IMR ≥32 also predicts worse recovery of LV function following acute STEMI[158]. IMR >32 is being used to select patients for clinical trials (RESTORE-MI [ACTRN12618000778280][273]). IMR >40 is also being used to select patients for clinical trials[255], [NCT03581513][271]. However, IMR has limitations for clinical translation. For example, manual saline injection can be a source of variability[16], and when calculating IMR the clinician should verify that the software has correctly positioned the Pd marker at the lowest stable Pd/Pa in hyperaemia.

CFR[191] and bimodal thermodilution waveforms[198] have been associated with MVO and clinical outcomes, following acute STEMI. However, due to variations in resting flow, CFR has inferior reproducibility, compared to IMR[166]. The potential of bimodal waveform for clinical translation is limited by requiring off-line processing for waveform classification. Furthermore, bimodal waveform is detected in only 9% to 17% of acute STEMI patients[198 200 366], thus if used alone bimodal waveform may miss some high-risk patients[16].

Theoretically, the later part of the thermodilution curve might be less susceptible to variability in the speed of manual intracoronary saline injections. This chapter details the conception and evaluation of a novel parameter, termed TRT, which is the time taken (in

seconds) for temperature recorded at the distal wire sensor to recover from the nadir value to baseline body temperature, during maximal hyperaemia.

The following hypotheses were evaluated:

1) Higher TRT at the end of emergency PCI is associated with MVO on CMR imaging.

2) Higher TRT at the end of emergency PCI is associated with increased rates of MACE, and the combination of all-cause death/ heart failure hospitalisation.

6.3 Methods

6.3.1 Summary of overall study design

The summary of the study design for this chapter is depicted in Figure 6.1. TRT was derived from a retrospective cohort of 288 all-comer acute STEMI patients, from a single centre in Glasgow, in the British Heart Foundation funded MR-MI study (NCT02072850)[149]. The patients included in the MR-MI study were recruited near consecutively, and all underwent emergency PCI. The derivation cohort population is described in more detail in section 6.3.2.

Validation of TRT was performed prospectively in patients from the physiology sub-study of the T-TIME trial[366]. The design, population, data acquisition and analysis for the T-TIME physiology sub-study was described in sections 2.1, 2.2, 5.4.1 and Figure 2.2.

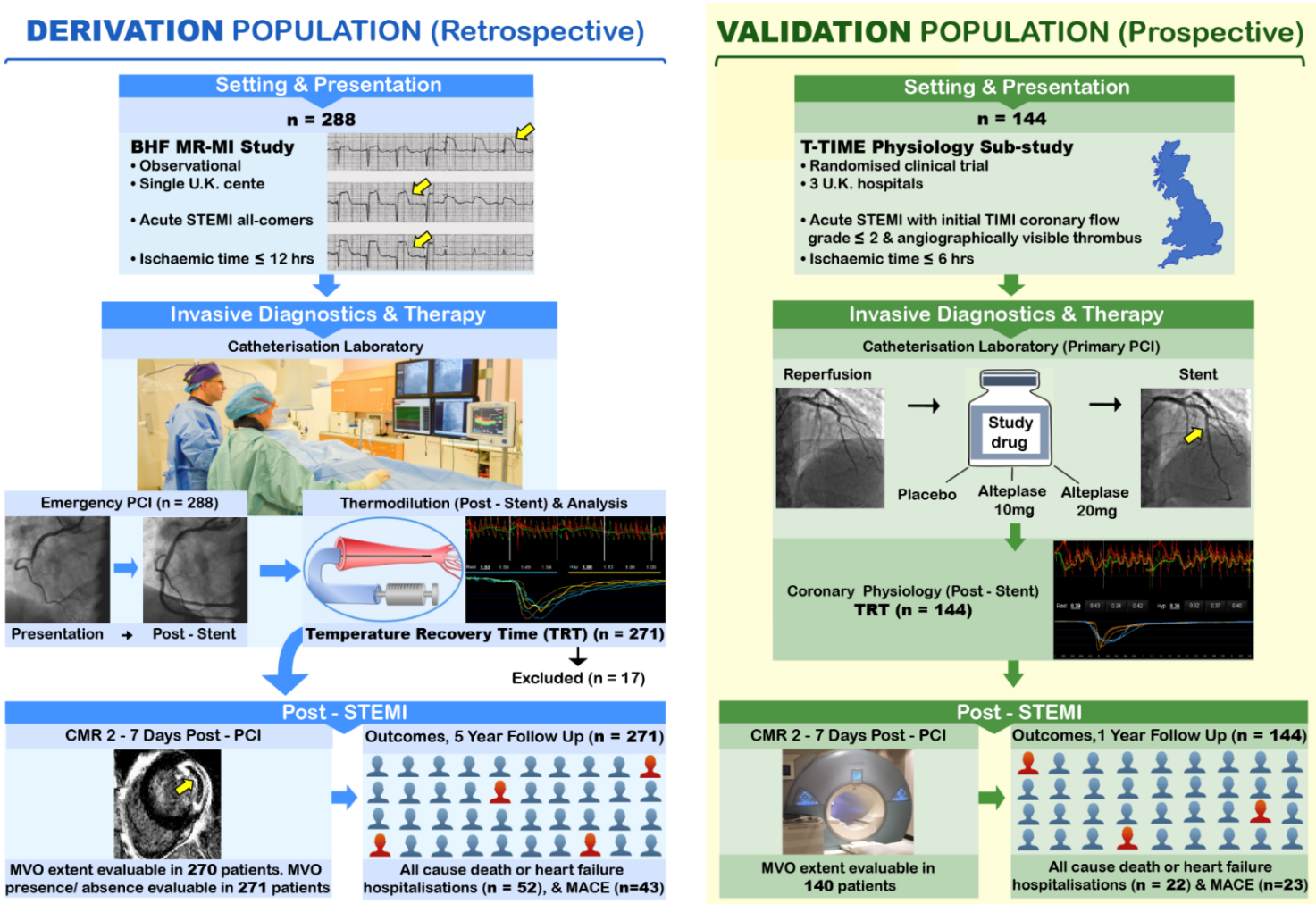

Figure 6.1 *Study design for the retrospective derivation and prospective validation of TRT. Abbreviation: BHF, British Heart Foundation. Reproduced with permission from Maznyczka AM et al. Thermodilution-derived temperature recovery time, a novel predictor of microvascular reperfusion and prognosis after myocardial infarction. EuroIntervention. 2020 [Epub ahead of print]. Doi: 10.4244/EIJ-D-19-00904.*

6.3.2 Derivation cohort (MR-MI) setting and population

Enrolment into the MR-MI cohort study was undertaken at the Golden Jubilee National Hospital in Glasgow, from 11th May 2011 to 22nd November 2012. Near consecutive patients were screened for inclusion in the study. Patients were included in the MR-MI study if they had acute STEMI within 12 hours of symptom onset, treated with primary PCI or emergency intravenous thrombolysis followed by PCI. Patients were excluded if they had contraindications to contrast enhanced CMR imaging. The MR-MI study was approved by the West of Scotland Research Ethics Service (10-S0703-28) (Appendix 10). Witnessed verbal assent to participate was obtained in the catheterisation laboratory and written informed consent was subsequently obtained on the ward.

6.3.3 Derivation cohort (MR-MI) standard of care for primary PCI

Patients in the MR-MI population received 300mg of aspirin, 600mg of clopidogrel and 5000 units of intravenous unfractionated heparin. The PCI procedures were performed via the radial artery. The standard of care at the time of enrolment into the MR-MI study was to initiate glycoprotein IIbIIIa inhibitor therapy in most cases during the primary PCI procedure[81 379], using initial bolus of tirofiban (25μg/kg) followed by intravenous infusion (0.15μg/kg/min), and to perform thrombus aspiration in the majority of patients. In line with clinical guidelines, multivessel PCI in the acute setting was not recommended[81 379].

6.3.4 Derivation cohort (MR-MI) physiology, angiogram, ECG and CMR acquisition and analyses

Angiogram acquisition in the MR-MI study was undertaken in the same way as was described for the T-TIME trial (section 2.1.6). TIMI coronary flow grade (Figure 2.3) was evaluated at the start and at the end of the PCI procedure, according to core laboratory standards. TFC and MPG (Figure 2.4) were evaluated at the end of the PCI procedure, according to core laboratory standards. The ECG and coronary physiology acquisition and analysis for the MR-MI study was undertaken as described for the T-TIME trial (sections 2.1.7 and 2.1.8). In the MR-MI study CMR imaging was obtained 2 to 7 days after PCI, and MVO was analysed in the same way as was described for the T-TIME trial (section 2.1.8).

6.3.5 Derivation cohort (MR-MI) clinical outcomes

For the MR-MI study serious adverse events were independently assessed by a cardiologist, who was blinded to all other clinical data. MACE was defined as cardiac

death, or unplanned hospitalisation for MI or heart failure. Five-year follow-up was completed for all the participants in the MR-MI study, initially via telephone contact and clinic visits, and later from review of electronic medical records.

6.3.6 TRT definition, acquisition and analysis

TRT was measured at the end of the PCI procedure, using a pressure- and temperature-sensing guidewire (Abbott Vascular, California, U.S.A), using standard thermodilution methodology, as described in section 2.2.2. TRT was defined as the duration in seconds, from the nadir of the hyperaemic thermodilution curve, to 20% from baseline temperature on the recovery part of the thermodilution curve (Figure 6.2).

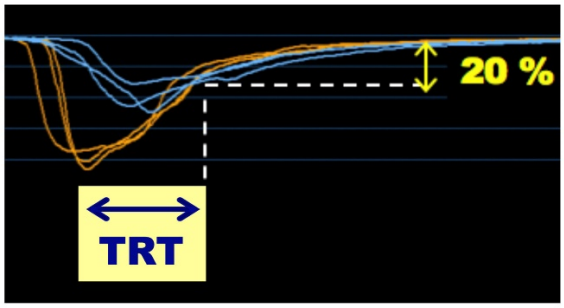

Figure 6.2 *Definition of TRT: duration (seconds) from the nadir of the hyperaemic thermodilution curve to 20% from return to baseline temperature.*

Time-temperature data was extracted into Excel files, from the distal thermistor, for the first 100 patients from the MR-MI cohort. The extracted time-temperature data showed the temperature recorded at the distal thermistor in the infarct-related coronary artery, in 0.01 second intervals from the start of the recording. The distal thermistor was sensitive to small errors in the measurements for the recordings made close to when the temperature returned exactly to baseline. To identify the endpoint for measuring TRT, that was best at predicting MVO, and all-cause death/ heart failure hospitalisations, five percentage cut-offs (7.5%, 10%, 20%, 30%, and 60%) from the end of the recordings were compared, from the first 100 consecutive patients in the derivation (MR-MI) cohort (Figure 6.3). The percentage cut-off that gave the highest AUC for predicting MVO, and all-cause heath/ heart failure hospitalisation on ROC analysis was used to define TRT (Figure 6.3). The 20% cut-off was found to have the best AUC for predicting MVO presence, and all-cause death/ heart failure hospitalisation (Figure 6.3). The mean value for TRT was obtained from triplicate measurements, and was calculated using an automated algorithm, using dedicated software (Coroventis^{TM}, Uppsala, Sweden). Coroventis software did not have automated algorithms for calculating the duration of the thermodilution recovery curve from peak temperature

drop to 7.5%, 10%, 30%, or 60% from baseline temperature. There was excellent correlation between manually calculated TRT duration from peak temperature drop to 20% from baseline, using the data extracted into Excel files, and the TRT measurement calculated by Coroventis using the automated algorithm (rho: 0.98, 95% CI: 0.97, 0.99), p<0.001).

In the derivation cohort, TRT was calculated retrospectively. In the validation cohort, TRT was calculated prospectively and was submitted to the clinical trial data coordination centre prior to database lock.

6.3.7 Statistical analyses

Between-group comparisons of continuous variables used the Student's t-test (if normally distributed data), or Mann-Whitney test (if skewed data). Differences in proportions were assessed with a Chi-squared test. Associations with MVO extent were assessed with linear regression, and the regression coefficients represented mean change in MVO extent for a 1-unit increase in TRT. Association with MVO presence, or clinical outcomes were evaluated using ORs, derived from logistic regression. A preponderance of heart failure episodes occurred during the index hospitalisation, therefore the assumption for the Cox proportional hazards regression model for constant effects over time was not met. The associations with MVO were adjusted for the following covariates: age, ischaemic time, CFR ≤2, IMR >32, hyperaemic Tmn >0.4 (median value) and thermodilution waveform (bimodal or wide unimodal vs. narrow unimodal). There was *a priori* concern that these covariates were clinically relevant confounders. Non-dichotomised continuous TRT, IMR, CFR, or hyperaemic Tmn were not included together in multivariable models due to collinearity. Stepwise regression was performed and the validity of regressions was verified by analysis of model residuals, and multicollinearity. The predictive value of TRT was evaluated using the McNemar test. ROC curve analysis was performed to detect the optimal thresholds for predicting MVO. In this, sensitivity and specificity were considered equally important, therefore the optimal cut-off was considered as the one giving the maximum Youden Index. ROC comparisons were made using the DeLong method[376]. All tests were 2-tailed and a p-value of <0.05 was considered statistically significant. There was no imputation for missing values. Statistical analyses were performed in SPSS (version 25.0, SPSS, IBM, Armonk, NY, USA), or MedCalc Statistical Software version 18 (MedCalc Software, Ostend, Belgium).

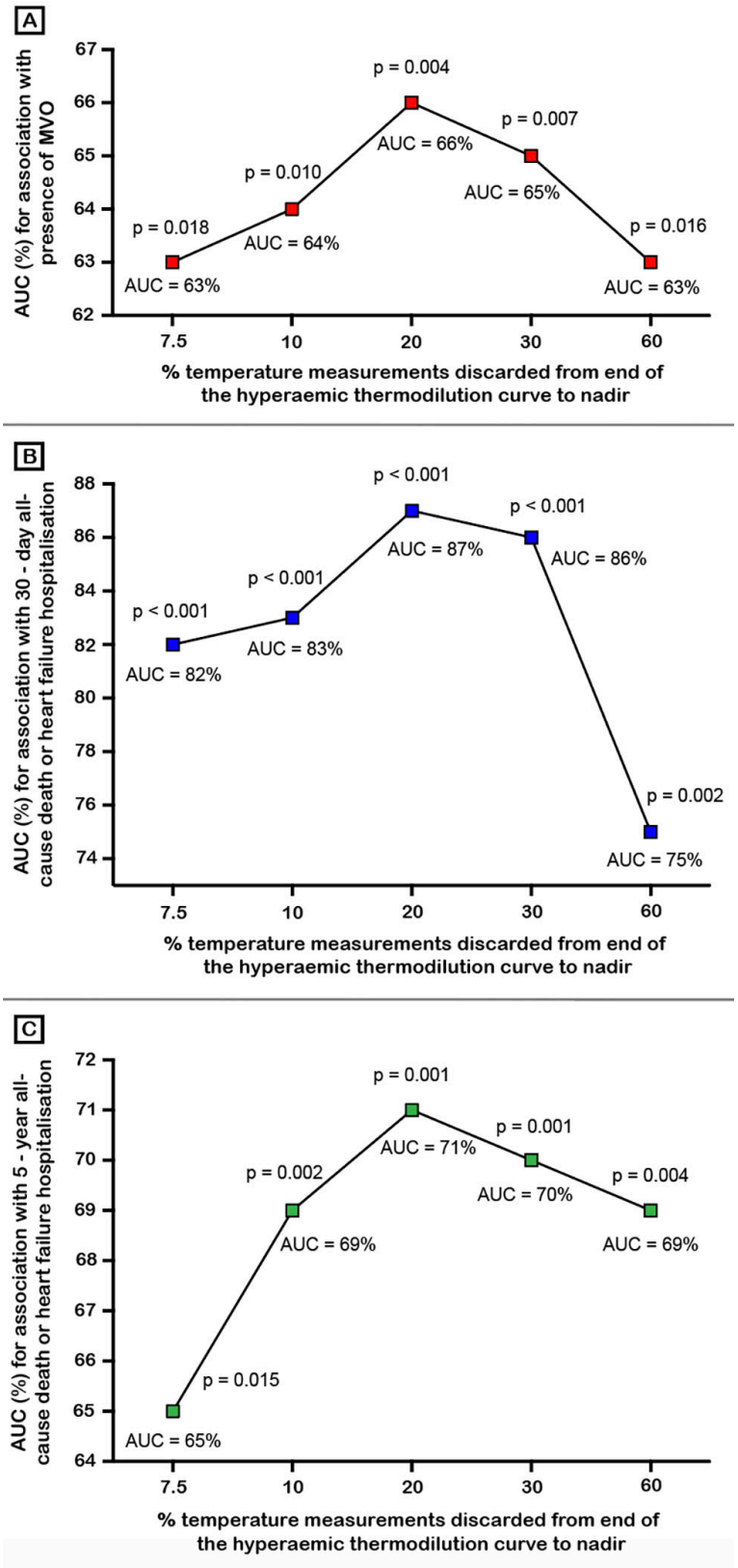

Figure 6.3 *Derivation of the optimal definition for TRT. Data are from the first 100 consecutive patients in the derivation (MR-MI) population. AUCs from ROC curve analyses, for predicting (A) MVO; (B) 30-day all-cause death/ heart failure hospitalisation, and; (C) 5-year all-cause death/ heart failure hospitalisation. Five different cut-offs from the end of the thermodilution curve (i.e. 7.5%, 10%, 20%, 30% and 60%), were tested. The dots represent AUCs. The mean was calculated from 3 hyperaemic thermodilution curves for each percentage cut-off. Reproduced with permission from Maznyczka AM et al. Thermodilution-derived temperature recovery time, a novel predictor of microvascular reperfusion and prognosis after myocardial infarction. EuroIntervention. 2020 [Epub ahead of print]. Doi: 10.4244/EIJ-D-19-00904.*

6.4 Results

6.4.1 Population characteristics

Derivation (MR-MI) population

Seventeen patients were excluded from the derivation (MR-MI) population, because coronary physiology recordings were not suitable for digital analysis. Among the 271 included patients (mean age 60 ± 12 years, 72% male), the majority (92%) underwent primary PCI, whilst 7 had PCI after successful thrombolysis, and 14 had PCI after failed thrombolysis. The population and procedure characteristics for the derivation population are shown in Tables 6.1 and 6.2.

Derivation Population Characteristics	**Overall (n=271)**	**TRT ≤0.5 (n=143)**	**TRT >0.5 (n=128)**	**p-value**
Age, years	59.5 ± 1.5	57.2 ± 11.1	62.0 ± 11.4	**0.001**†
Male	194 (72%)	98 (69%)	96 (75%)	0.239‡
Current smoker	170 (63%)	98 (69%)	72 (56%)	**0.037**‡
Body mass index, kg/m^2	28.7 ± 4.8	29.2 ± 4.9	28.0 ± 4.5	**0.040**†
Hypertension	85 (31%)	45 (32%)	40 (31%)	0.969‡
Diabetes mellitus	30 (11%)	15 (11%)	15 (12%)	0.747‡
Previous myocardial infarction	18 (7%)	9 (6%)	9 (7%)	0.808‡
Reperfusion strategy				0.344‡
Primary PCI	250 (92%)	134 (94%)	116 (91%)	
Thrombolysis followed by PCI	21 (8%)	9 (6%)	12 (9%)	
Presenting heart rate, beats per minute	78.1 ± 16.7	78.2 ± 16.9	78.0 ± 16.7	0.928†
Presenting systolic blood pressure, mmHg	135.2 ± 25.1	134.5 ± 24.8	133.2 ± 25.4	0.217†
Ischaemic time, hours, median (IQR)	2.92 (2.0 - 5.4)	2.8 (2.0 - 5.0)	3.4 (2.2 - 5.7)	0.101§
Baseline creatinine, μmol/L	76.9 ± 18.8	74.5 ± 19.1	79.7 ± 18.1	0.022‡
Procedural Characteristics				
Culprit artery: LAD/ left main stem	100 (37%)	50 (35%)	50 (39%)	0.364‡
Cx	52 (19%)	32 (22%)	20 (16%)	
RCA	119 (44%)	61 (43%)	58 (45%)	
Number of main vessels diseased: 1	148 (55%)	78 (55%)	70 (55%)	0.650‡
2	84 (31%)	42 (29%)	42 (33%)	
3	39 (14%)	23 (16%)	16 (13%)	
Thrombus aspiration	195 (72%)	102 (71%)	93 (73%)	0.808‡
Glycoprotein IIbIIIa inhibitor	249 (92%)	132 (92%)	117 (91%)	0.786‡

Table 6.1 *Population characteristics, grouped according to high and low TRT, from the derivation (MR-MI) cohort. Data are mean ± SD, or n (%) unless otherwise stated. †student's t-test. ‡χ^2 test. §Mann-Whitney test. Missing data: ischaemic time, n=18; creatinine n=1.*

Derivation Population Parameters	Overall (n=271)	TRT ≤0.5 (n=143)	TRT >0.5 (n=128)	p-value
ECG				
ST-segment resolution: Complete, ≥70%	123 (46%)	76 (54%)	47 (37%)	**0.020**‡
Incomplete <70%	147 (54%)	66 (47%)	81 (63%)	
Angiogram				
Initial TIMI coronary flow grade: 0	157 (58%)	73 (51%)	84 (66%)	**0.029**‡
1	34 (13%)	18 (13%)	16 (13%)	
2	43 (16%)	25 (18%)	18 (14%)	
3	37 (14%)	27 (19%)	10 (8%)	
Final TIMI coronary flow grade: 1	1 (0.4%)	1 (1%)	0	**0.003**‡
2	13 (5%)	1 (1%)	12 (9%)	
3	257 (95%)	141 (99%)	116 (91%)	
TIMI frame count post-PCI, median (IQR)	14.0 (10.0 - 24.0)	12.0 (8.2 - 20.0)	17.8 (10.6 - 25.4)	**0.004**§
Myocardial perfusion grade post-PCI: ≤1	76 (28%)	32 (22%)	44 (34%)	**0.028**‡
≥2	195 (72%)	111 (78%)	84 (66%)	
Coronary Physiology Post-PCI				
Hyperaemic Pa, mmHg, median (IQR)	76.0 (68.0 - 87.0)	76.0 (68.0 - 89.0)	76.0 (67.0 - 86.5)	0.515§
Hyperaemic Pd, mmHg, median (IQR)	70.0 (60.0 - 80.0)	69.0 (60.0 - 79.8)	71.0 (60.0 - 81.5)	0.983§
IMR, median (IQR)	24.0 (15.0 - 43.0)	15.4 (11.0 - 21.1)	44.1 (27.0 - 63.0)	**<0.001**§
IMR >32	100 (37%)	13 (9%)	87 (68%)	**<0.001**‡
IMR >40	75 (28%)	4 (3%)	71 (56%)	**<0.001**‡
CFR, median (IQR)	1.6 (1.1-2.1)	1.7 (1.3-2.4)	1.4 (1.0-1.8)	**<0.001**§
CFR ≤2	196 (72%)	93 (66%)	103 (83%)	**0.002**‡
Hyperaemic Tmn, (s) median (IQR)	0.4 (0.2-0.6)	0.2 (0.2-0.3)	0.6 (0.4-0.9)	**<0.001**§
Hyperaemic Tmn >median	132 (49%)	22 (15%)	110 (1%)	**<0.001**‡
Waveform: Bimodal	34 (13%)	5 (4%)	29 (23%)	**<0.001**‡
Wide unimodal	98 (36%)	38 (27%)	60 (47%)	
Narrow unimodal	139 (51%)	100 (70%)	39 (31%)	
CMR 2 to 7days after PCI				
Time from PCI to CMR imaging (days)	2.1 ± 1.9	2.2 ± 1.9	1.9 ± 1.9	0.174†
MVO extent (% LV mass)	0.2 (0.0 - 3.3)	0.0 (0.0 - 1.6)	2.0 (0.0 - 6.8)	**<0.001**§
MVO present	137 (50.6)	52 (36.4)	85 (66.4)	**<0.001**‡

Table 6.2 *From the derivation (MR-MI) cohort, ECG, angiographic, and coronary physiology parameters on admission and CMR findings at 2 to 7 days post-STEMI, grouped according to high and low TRT. Data are mean ± SD, or n (%) unless otherwise stated. ‡χ^2 test. §Mann-Whitney test. †student t-test. Missing: CFR, n=6; Pa post-PCI, n=57; ST-segment resolution, n=1, MVO extent, n=1.*

From the derivation population, TRT had a right-skewed distribution (Figure 6.4). The median TRT was 0.5 (range: 0.1 – 2.5).

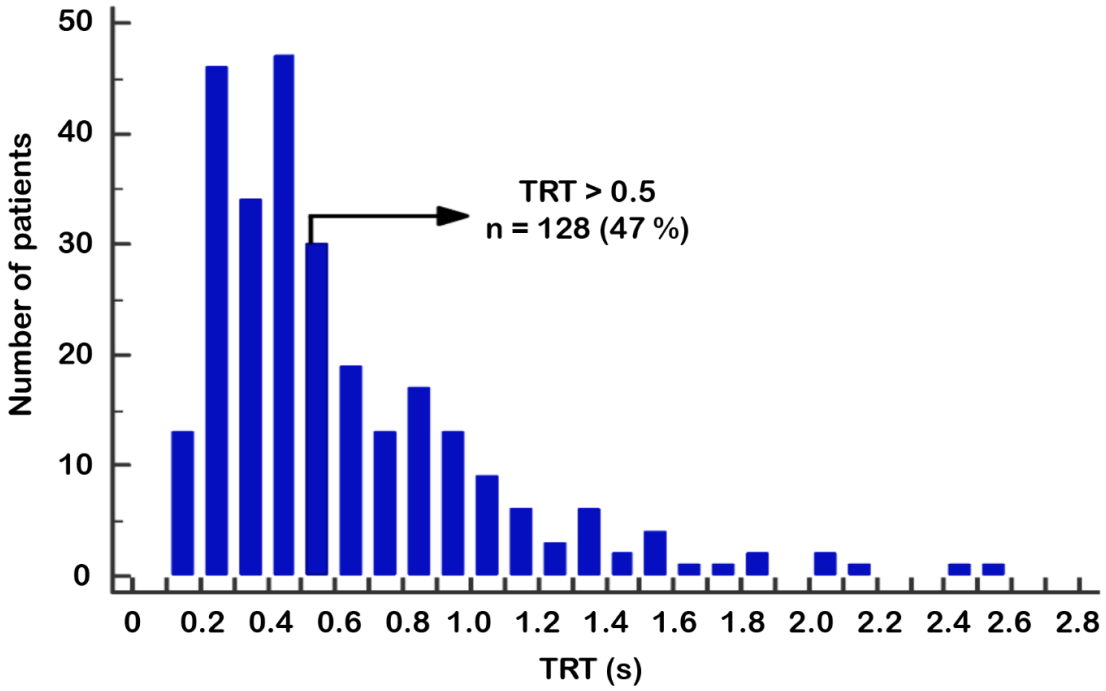

Figure 6.4 *From the derivation (MR-MI) population, distribution of TRT (n=271). Reproduced with permission from Maznyczka AM et al. Thermodilution-derived temperature recovery time, a novel predictor of microvascular reperfusion and prognosis after myocardial infarction. EuroIntervention. 2020 [Epub ahead of print]. Doi: 10.4244/EIJ-D-19-00904*

TRT was correlated with IMR (r=0.8, p<0.001), CFR (r= -0.3, p<0.001) and with hyperaemic Tmn (r=0.9, p<0.001). In the derivation (MR-MI) population, characteristics associated with higher TRT, in multivariable linear regression analysis, were as follows: IMR >32 (p<0.001), hyperaemic Tmn >0.4 (median value) (p<0.002), CFR ≤2.0 (p<0.001), bimodal or wide unimodal thermodilution waveform (p=0.018), TIMI coronary flow grade post-PCI ≤2 (p<0.001), and higher creatinine (p=0.044).

Validation (T-TIME) population

The population and procedure characteristics for the validation population, i.e. the T-TIME cohort, have been described in section 4.4.2, and Tables 4.1 to 4.3. The median TRT in the validation population (n=144) was 0.5 (range: 0.2 – 2.4). Characteristics associated with higher TRT in the validation population, in multivariable linear regression were: bimodal or wide unimodal thermodilution waveform, higher TFC and hyperaemic Tmn >0.4 (median value) (all p<0.001).

In the T-TIME population there was no overall significant treatment effect of intracoronary alteplase on TRT. The relative difference in TRT for 20mg alteplase (median: 0.6 [IQR: 0.4 – 1.1]) vs. placebo (median: 0.6 [IQR: 0.4 -1.0]) was 1.19 (95% CI: 0.93, 1.51) p=0.164. The relative difference in TRT for 10mg alteplase (median: 0.5 [IQR: 0.3 – 0.7]) vs. placebo was 0.90 (95% CI: 0.69, 1.15) p=0.394. The relative difference in TRT for 10mg and 20mg combined vs. placebo was 1.04 (95% CI: 0.84, 1.29) p=0.686.

6.4.2 Associations of TRT with MVO

Derivation (MR-MI) population

In the derivation population, CMR imaging with MVO presence/ absence evaluable was available in 271 participants, at 2 to 7 days post-PCI, and MVO extent was evaluable in 270 patients. MVO was present in 138 patients (51%). On ROC curve analysis there was no difference in the discriminatory ability of TRT compared to IMR for predicting MVO presence (Figure 6.5). The TRT threshold with the highest combination of sensitivity (61%) and specificity (68%) for predicting MVO was >0.5.

Figure 6.6 shows discordance between IMR and TRT. TRT dichotomised by 0.5 correctly predicted MVO presence or absence, in 65% (n=175/ 271) of patients. IMR dichotomised by 32 correctly predicted MVO presence or absence in 62% (n=167/271) of patients. There was no difference in the predictive accuracy for detecting MVO presence or absence when TRT dichotomised by 0.5, and IMR dichotomised by 32 were compared, using the McNemar test (difference: 2.95% [95% CI: -2.39%, 8.29%] p=0.341).

In the derivation population, higher continuous TRT was multivariably associated with larger MVO extent (coefficient: 4.24 [CI: 2.26, 6.22] p<0.001), independent of IMR >32, CFR ≤2.0, hyperaemic Tmn >0.4 (median value), thermodilution waveform, age and ischaemic time (Table 6.3). This means that MVO extent increased by 4.24 units for every 1-unit increase in TRT. TRT was also a multivariable predictor of MVO presence (OR: 3.01 [95% CI: 1.75, 5.16] p<0.001) (Table 6.3). The findings were similar when IMR >32 was substituted for IMR >40 in multivariable regression analyses (Table 6.4).

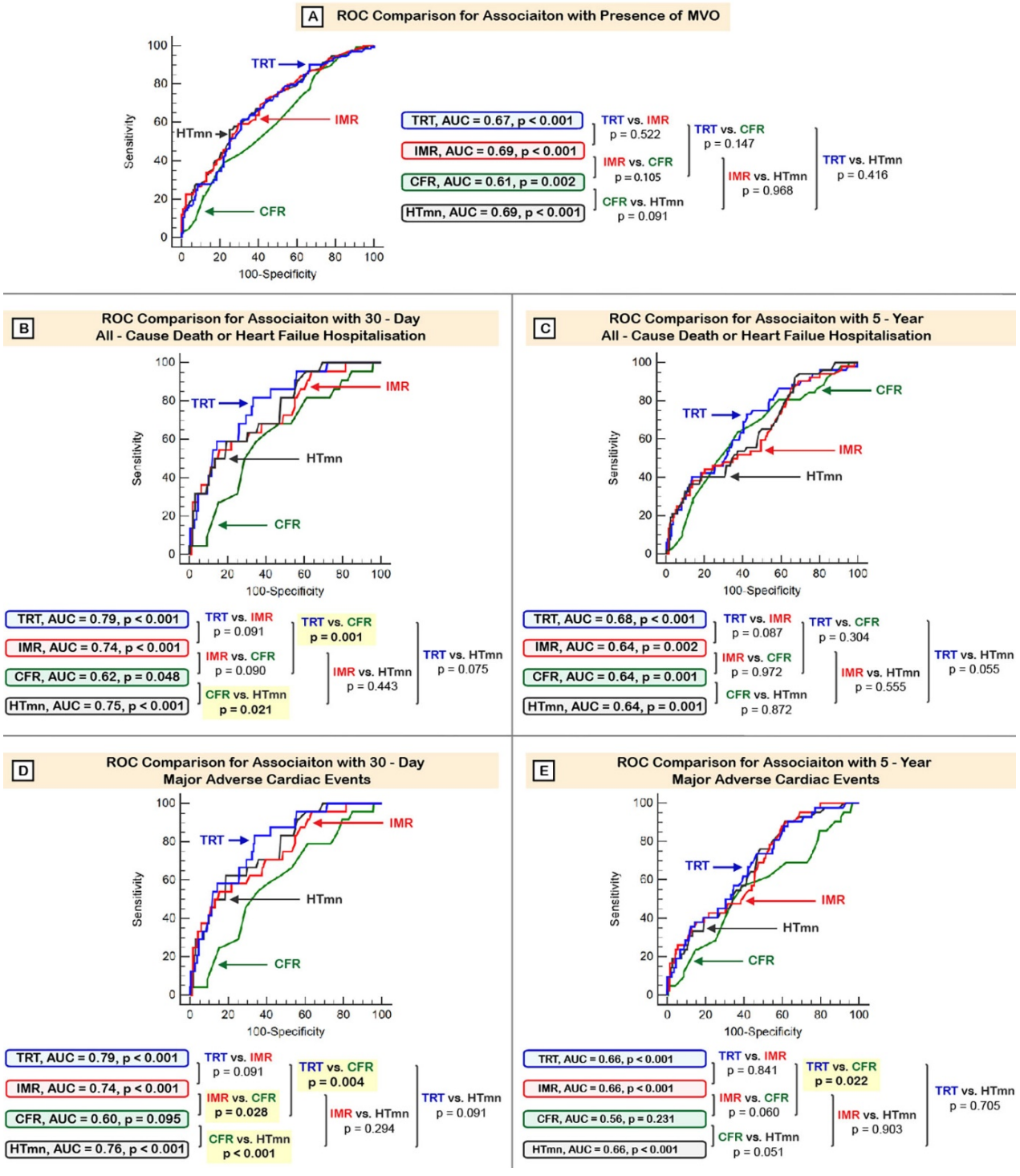

Figure 6.5 *ROC curves from the derivation (MR-MI) population, for association of TRT, IMR, CFR and hyperaemic Tmn with: (A) presence of MVO; (B & C) all-cause death/ heart failure hospitalisation; and (D & E) MACE.*

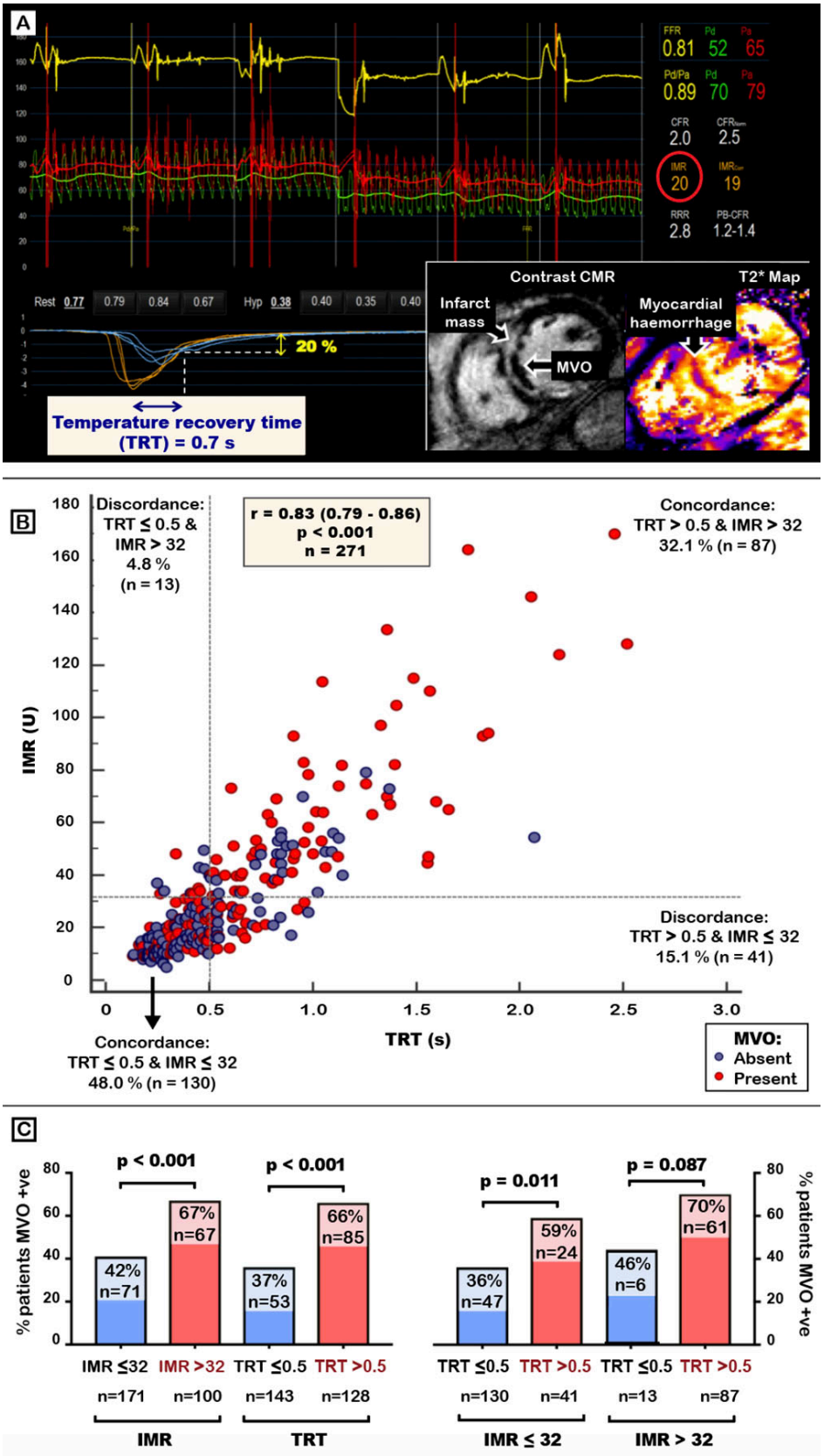

Figure 6.6 *From the derivation (MR-MI) population: (A) example of IMR – TRT discordance. The patient had high TRT and normal IMR at the end of primary PCI. CMR imaging revealed transmural antero-septal MI, complicated by MVO; (B) scatter graph showing IMR – TRT discordance, and IMR/ TRT – MVO discordance, and; (C) patients with MVO stratified by combinations of IMR ≤32, IMR >32, TRT ≤0.5, and TRT >0.5 (p-values from Chi-squared test). Reproduced with permission from Maznyczka AM et al. Thermodilution-derived temperature recovery time, a novel predictor of microvascular reperfusion and prognosis after myocardial infarction. EuroIntervention. 2020 [Epub ahead of print]. Doi: 10.4244/EIJ-D-19-00904.*

	Univariable analysis		Multivariable analysis	
	OR/ coefficient (95% CI)	**p-value**	**OR/ coefficient (95% CI)**	**p-value**
Associations with MVO extent (%LV mass) on CMR 2-7 days post-PCI*				
TRT continuous	4.38 (3.07, 5.68)	**<0.001**	4.09 (2.70, 5.48)	**<0.001**
TRT >0.5	2.84 (1.71, 3.97)	**<0.001**	2.69 (1.49, 3.89)	**<0.001**
TRT >0.5 (IMR >32, substituted by continuous IMR in model)			1.53 (-0.18, 3.23)	0.080
**Other variables in multivariable model: age, ischaemic time, IMR >32, CFR ≤2, hyperaemic Tmn >median, thermodilution waveform*				
Associations with MVO presence on CMR 2-7 days post-PCI†				
TRT continuous	5.52 (2.59, 11.77)	**<0.001**	5.26 (2.52, 11.74)	**<0.001**
TRT >0.5	3.36(2.04, 5.53)	**<0.001**	3.01 (1.75, 5.16)	**<0.001**
TRT >0.5 (IMR >32, substituted by continuous IMR in model)			1.72 (0.78, 3.77)	0.178
† Other variables in multivariable model: age, ischaemic time, IMR >32, CFR ≤2, hyperaemic Tmn >median, thermodilution waveform				
Associations with 30-day death/ heart failure hospitalisation‡				
TRT continuous	6.64 (2.90, 15.24)	**<0.001**	7.37 (2.37, 22.94)	**0.001**
TRT >0.5	8.14 (2.35, 28.20)	**0.001**	7.13 (1.78, 28.66)	**0.006**
TRT >0.5 (IMR >32, substituted by continuous IMR in model)			4.48 (1.14, 17.63)	**0.032**
‡Other variables in multivariable model: IMR >32				
Associations with death/ heart failure hospitalisation at median 5-years§				
TRT continuous	3.85 (1.97, 7.51)	**<0.001**	4.14 (2.08, 8.25)	**<0.001**
TRT >0.5	3.11 (1.63, 5.93)	**0.001**	6.44 (2.48, 16.71)	**<0.001**
TRT >0.5 (IMR >32, substituted by continuous IMR in model)			5.37 (2.00, 14.43)	**0.001**
§Other variables in multivariable model: IMR >32, CFR ≤2, hyperaemic Tmn>median, thermodilution waveform				
Associations with 30-day MACE‡				
TRT continuous	6.51 (2.89, 14.69)	**<0.001**	7.38 (2.41, 22.55)	**<0.001**
TRT >0.5	9.16 (2.66, 31.51)	**<0.001**	8.57 (2.18, 33.63)	**0.002**
TRT >0.5 (IMR >32, substituted by continuous IMR in model)			5.18 (1.34, 19.99)	**0.017**
‡Other variables in multivariable model: IMR >32				
Associations with MACE at median 5-years§				
TRT continuous	3.55 (1.79, 7.03)	**<0.001**	4.05 (2.00, 8.21)	**<0.001**
TRT >0.5	2.39 (1.21, 4.71)	**0.012**	2.65 (1.32, 5.30)	**0.006**
TRT >0.5 (IMR >32, substituted by continuous IMR in model)			2.19 (0.76, 6.29)	0.145
§Other variables in multivariable model: IMR >32, CFR ≤2, hyperaemic Tmn>median, thermodilution waveform				

Table 6.3 *From the derivation (MR-MI) cohort, logistic and linear regression analyses, showing associations between TRT and MVO, or TRT and clinical outcomes (with IMR >32 in multivariable models).*

	Univariable analysis		Multivariable analysis	
	OR/ coefficient (95% CI)	**p-value**	**OR /coefficient (95% CI)**	**p-value**
Associations with MVO extent (%LV mass) on CMR 2-7 days post-PCI*				
TRT continuous	4.38 (3.07, 5.68)	**<0.001**	4.09 (2.70, 5.48)	**<0.001**
TRT >0.5	2.84 (1.71, 3.97)	**<0.001**	1.70 (0.25, 3.15)	**0.022**
TRT >0.5 (IMR >40, substituted by continuous IMR in model)			1.53 (-0.18, 3.23)	0.080
***Other variables in multivariable model: age, ischaemic time, IMR >40, CFR ≤2, hyperaemic Tmn >median, thermodilution waveform*				
Associations with MVO presence on CMR 2-7 days post-PCI†				
TRT continuous	5.52 (2.59, 11.77)	**<0.001**	5.26 (2.36, 11.74)	**<0.001**
TRT >0.5	3.36 (2.04, 5.53)	**<0.001**	3.01 (1.75, 5.16)	**<0.001**
TRT >0.5 (IMR >40, substituted by continuous IMR in model)			1.72 (0.78, 3.77)	0.178
† Other variables in multivariable model: age, ischaemic time, IMR >40, CFR ≤2, hyperaemic Tmn >median, thermodilution waveform				
Associations with 30-day mortality or heart failure hospitalisation‡				
TRT continuous	6.64 (2.90, 15.24)	**<0.001**	6.27 (1.89, 20.78)	**0.003**
TRT >0.5	8.14 (2.35, 28.20)	**0.001**	5.77 (1.44, 23.14)	**0.013**
TRT >0.5 (IMR >40, substituted by continuous IMR in model)			4.48 (1.14, 17.63)	**0.032**
‡*Other variables in multivariable model: IMR >40*				
Associations with mortality or heart failure hospitalisation at median 5-years§				
TRT continuous	3.85 (1.97, 7.51)	**<0.001**	4.14 (2.08, 8.25)	**<0.001**
TRT >0.5	3.11 (1.63, 5.93)	**0.001**	5.81 (2.16, 15.63)	**<0.001**
TRT >0.5 (IMR >40, substituted by continuous IMR in model)			5.37 (2.00, 14.43)	**0.001**
§*Other variables in multivariable model: IMR >40, CFR ≤2, hyperaemic Tmn>median, thermodilution waveform*				
Associations with 30-day MACE‡				
TRT continuous	6.51 (2.89, 14.69)	**<0.001**	6.06 (1.88, 19.57)	**0.003**
TRT >0.5	9.16 (2.66, 31.51)	**<0.001**	6.81 (1.74, 26.62)	**0.006**
TRT >0.5 (IMR >40, substituted by continuous IMR in model)			5.18 (1.34, 19.99)	**0.017**
‡*Other variables in multivariable model: IMR >40*				
Associations with MACE at median 5-years§				
TRT continuous	3.55 (1.79, 7.03)	**<0.001**	4.05 (2.00, 8.21)	**<0.001**
TRT >0.5	2.39 (1.21, 4.71)	**0.012**	2.65 (1.32, 5.30)	**0.006**
TRT >0.5 (IMR >40, substituted by continuous IMR in model)			2.19 (0.76, 6.29)	0.145
§*Other variables in multivariable model: IMR >40, CFR ≤2, hyperaemic Tmn>median, thermodilution waveform*				

Table 6.4 *From the derivation (MR-MI) cohort, summary of logistic and linear regression analyses, showing associations between TRT and MVO, or TRT and clinical outcomes (with IMR >40 in multivariable models).*

Validation (T-TIME) population

In the validation population, higher continuous TRT was multivariably associated with MVO extent (coefficient: 2.86 [95% CI: 1.26, 4.46] p=0.001). Case examples are shown in Figure 6.7.

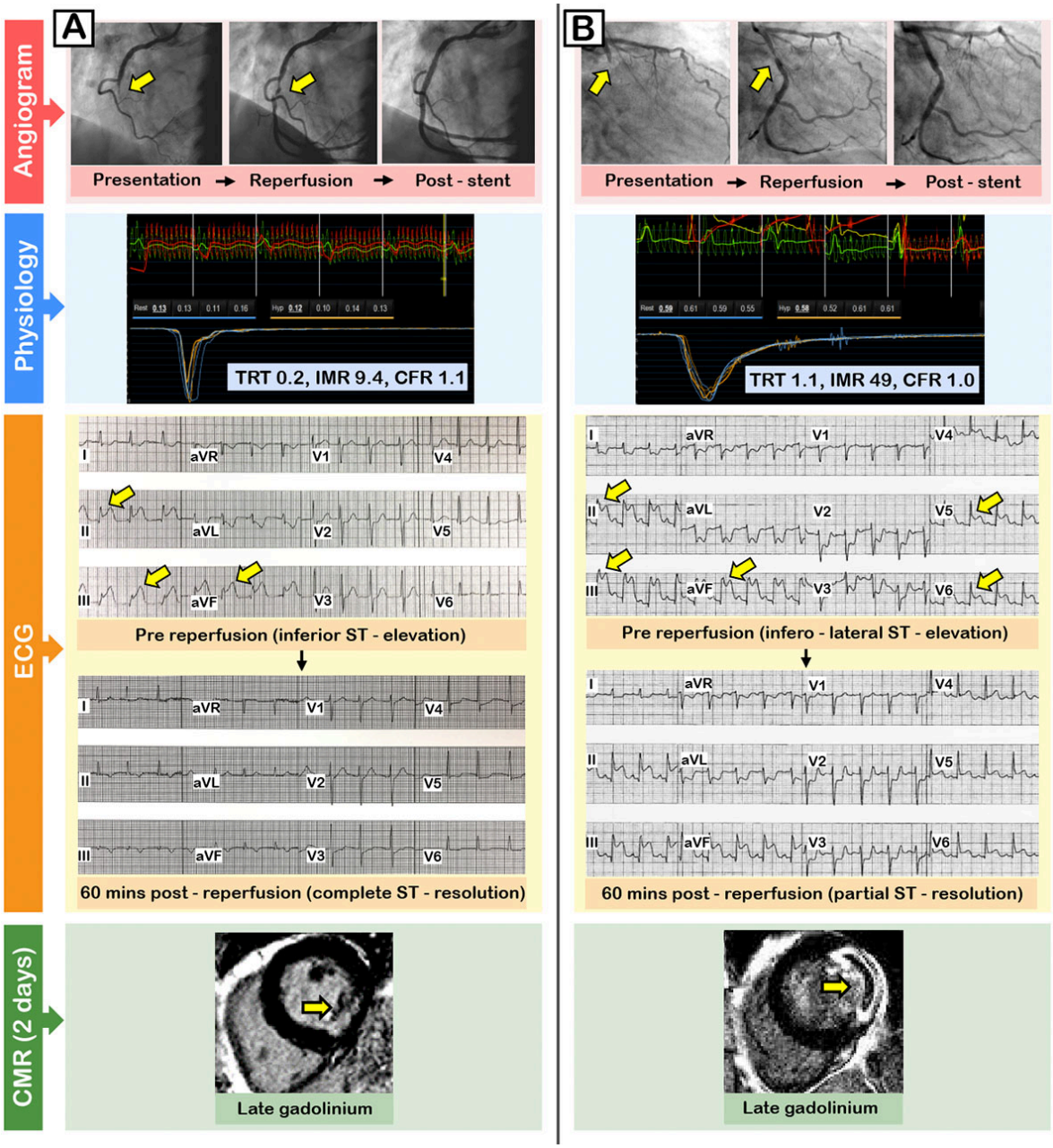

Figure 6.7 *Case examples showing TRT measured in 2 patients with acute STEMI treated by primary PCI. Both patients had TIMI 3 flow in the infarct-related artery, post-PCI.* ***A****, Normal TRT: The infarct-related artery was an occluded RCA. IMR and TRT were normal, indicating successful myocardial reperfusion. ECGs showed complete resolution of inferior ST-segment elevation. CMR imaging performed 2-days post STEMI revealed a small subendocardial infarct without MVO.* ***B****, Increased TRT: The infarct-related artery was an occluded Cx. Invasively measured microvascular function was abnormal (IMR 49, TRT 1.1), indicating impaired myocardial reperfusion. ECGs showed only partial resolution of infero-lateral ST-segment elevation. CMR imaging, 2-days later, revealed MVO. Reproduced with permission from Maznyczka AM et al. Thermodilution-derived temperature recovery time, a novel predictor of microvascular reperfusion and prognosis after myocardial infarction. EuroIntervention. 2020 [Epub ahead of print]. Doi: 10.4244/EIJ-D-19-00904*

6.4.3 Associations of TRT with clinical outcomes

Derivation (MR-MI) population

In the first 30 days following acute STEMI, all-cause death/ heart failure hospitalisation occurred in 22 patients (8%), and MACE occurred in 24 patients (9%). ROC curve analysis revealed that TRT had superior discriminatory ability compare to CFR for predicting 30-day clinical outcomes (Figure 6.5). In the derivation cohort, continuous TRT was a multivariable predictor of 30-day death/ heart failure hospitalisation (OR: 7.37 [95% CI: 2.36, 22.94] p<0.001), and 30-day MACE (OR: 7.38 [95% CI: 2.41, 22.55) p<0.001), with IMR >32 in the model (Table 6.3). TRT >0.5 was also a multivariable predictor of 30-day death/ heart failure hospitalisation (OR: 4.48 [95% CI: 1.14, 17.63] p=0.032) and 30-day MACE (OR: 5.18 [95% CI: 1.34, 19.99] p=0.017), with continuous IMR in the model (Table 6.3). The findings were similar when IMR >32 was substituted for IMR >40 in multivariable models (Table 6.4).

During a median follow-up of 5 years in the derivation cohort, 52 patients died or experienced heart failure hospitalisation, and 43 patients experienced MACE. ROC curve analysis showed that TRT had superior discriminatory ability compared to CFR for predicting 5-year MACE (Figure 6.5). Continuous TRT was a multivariable predictor of 5-year death/ heart failure hospitalisation (OR: 4.14 [95% CI: 2.08, 8.25] p<0.001), and 5-year MACE (OR: 4.05 [95% CI: 2.00, 8.21] p<0.001), with IMR >32 in the model (Table 6.3). TRT >0.5 was also a multivariable predictor of 5-year death/ heart failure hospitalisation, and MACE, with IMR >32 in the model (Table 6.3). The findings were similar when IMR >32 was substituted for IMR >40 in multivariable models (Table 6.4).

Validation (T-TIME) population

During 1-year follow up in the validation population, all-cause death/ heart failure hospitalisation occurred in 22 patients (15%), and MACE occurred in 23 patients (16%). Higher continuous TRT was associated with all-cause death/ heart failure hospitalisation (OR: 6.10 [95% CI: 2.42, 15.42] p<0.001) and MACE (OR: 7.65 [95% CI: 1.87, 31.29] p=0.005), independent of IMR >32, CFR ≤2.0, hyperaemic Tmn >median and thermodilution waveform. The results were similar when IMR >32 was substituted for IMR >40 in the multivariable model (all-cause death/ heart failure hospitalisation: OR, 5.85 [95% CI: 1.43, 23.94] p=0.014) and (MACE: OR, 5.84 [95% CI: 1.44, 23.76] p=0.014).

6.4.4 Repeatability of TRT

Paired TRT measurements, obtained 0.5 to 8 minutes apart, post-primary PCI by 4 cardiologists in 13 patients were correlated (r=0.93, p<0.001), with a mean difference between duplicate TRT measurements of 0.02 ± 0.08 (p=0.489). The coefficient of variation from duplicate TRT measurements was 12.7%. The coefficient of variation from duplicate IMR measurements was 15.5%. The Bland-Altman plot is shown in Figure 6.8.

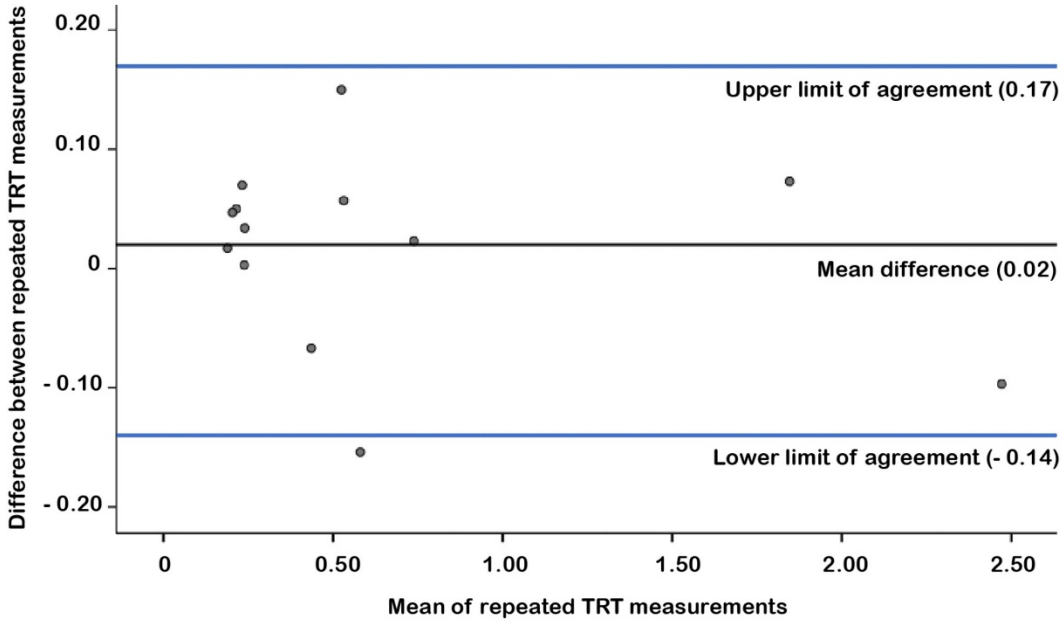

Figure 6.8 *Bland-Altman plot of differences plotted against the averages of paired TRT measurements. Horizontal lines are drawn at the mean difference and at the limits of agreement.*

6.4.5 Associations of TRT with systemic and coronary haemodynamics

The relationships between TRT, IMR, CFR or hyperaemic Tmn, with Pa, Pd and heart rate were examined in the derivation (MR-MI) cohort, because predictive performance may be impaired if influenced by haemodynamic conditions. Pd did not correlate with TRT (r= -0.04, p=0.543), or hyperaemic Tmn (r= -0.01, p=0.883). However, Pd and IMR were correlated (r=0.3, p<0.001), and Pd was correlated with CFR (r=0.2, p=0.014). Pa did not correlate with TRT (r= -0.1, p=0.311), or hyperaemic Tmn (r= -0.05, p=0.506). However, Pa was correlated with IMR (r=0.2, p=0.001) and CFR (r=0.2, p=0.011). TRT, IMR, or TRT were not correlated with heart rate (TRT [r= -0.1, p=0.399]; IMR [r=0.01, p=0.926]; hyperaemic Tmn [r= -0.03, p=0.615]). However, CFR was correlated with heart rate (r=0.1, p=0.030).

6.5 Discussion

In this chapter a novel parameter has been described and validated, for measuring the efficacy of microvascular reperfusion. Higher TRT was a multivariable predicted of more MVO and clinical outcomes, in two independent acute STEMI cohorts.

Though TRT and IMR are correlated, discordance occurred between high and low IMR (dichotomised by 40) and TRT (dichotomised by 0.5) in 20% of patients (n=54) from the derivation cohort, indicating that TRT and IMR are not one and the same. When an IMR threshold of 40 was used, discordance between high and low dichotomised IMR and TRT occurred in 23% of patients (n=61) from the derivation cohort. TRT measures different aspects of the thermodilution curve than hyperaemic Tmn, which is used to calculate IMR. For calculating IMR, the Tmn starts at the steepest downslope of the temperature-time curve (measured by the wire's shaft thermistor). The transit time measurement ends midway between the peak temperature drop (measured by the wire's distal thermistor) and 10% from return of the distal thermistor temperature to baseline. In contrast, the TRT measurement only incorporates the part of the temperature-time curve from peak temperature drop to 20% from return to baseline temperature. The independence of TRT, but not IMR, from coronary and systemic haemodynamics further distinguishes TRT from IMR.

With regards to translation to clinical practice, measuring TRT could potentially be more straightforward for use by clinicians who are less experienced with thermodilution methodology. This is because TRT is calculated precisely using an automated algorithm using dedicated software (Coroventis^{TM}, Uppsala, Sweden) in the catheterisation laboratory. On the other hand, for precise calculation of IMR the operator must verify that the position of the marker is at the lowest stable Pd/Pa during hyperaemia, which is not always positioned correctly by the automated software. The classification of bimodal waveforms is not automatically determined by the analysis software, and thus an experienced operator is required to interpret the thermodilution waveform. CFR, which reflects epicardial and microcirculatory vasodilator capacity, and residual epicardial stenosis, is calculated using an automated algorithm, however CFR does not improve the predictive ability of IMR alone[149].

The clinical relevance of these findings is that they support the potential clinical utility of TRT for refining risk stratification and selection of acute STEMI patients for adjunctive treatments during primary PCI. The reliable identification of patients with a high probability of having MVO may potentially improve research study design, by improving selection of the highest risk patients in the catheterisation laboratory for inclusion in therapeutic trials. Furthermore, accurate identification of acute STEMI patients who will not develop MVO, could potentially reduce healthcare costs through stratification and triage for early hospital discharge[165]. Though MVO revealed by CMR imaging can accurately detect failed myocardial reperfusion, CMR is not feasible in the acute setting, and therefore does not have potential clinical utility for selecting patients for adjunctive treatments during primary PCI.

6.6 Limitations

The strengths of this study include the high follow up rates for CMR, the prospective validation of TRT in a distinct population independent from the derivation population, and independent adjudication of clinical events, however there are some limitations.

The study presented in this chapter was not designed to comprehensively test the reproducibility of TRT. Paired TRT measurements were only available in 13 participants, which is insufficient to draw conclusions on the reproducibility of TRT compared to IMR.

Limitations of the bolus-derived thermodilution technique should be considered. First, manual bolus-injections of saline may be a source of variability[16]. Previous published literature has shown that the speed of injection is a source of variability in calculating cardiac output using thermodilution[380]. It is theoretically plausible that when injection speed is slower, it would take longer for the initial part of the intracoronary thermodilution curve to reach the peak temperature drop, than when injection speed is faster. It is potentially plausible that the recovery part of the thermodilution curve (nadir to return to baseline body temperature) may be less sensitive to variability in manual injection speed. However, there is no data available that evaluates this theory, and therefore future research is needed. Thermodilution-based absolute flow and microvascular resistance measurements, using continuous infusion of saline to induce steady-state maximal hyperaemia, has potential to have superior reproducibility than manual bolus saline

injections[185]. However, thermodilution-based absolute flow requires a dedicated infusion catheter with side holes[183], can be more technically challenging and although early studies have shown feasibility larger studies in STEMI are awaited[184].

A second limitation of the bolus-derived thermodilution technique is that higher intra-observer variability of thermodilution-, compared to Doppler-derived CFR measurements has been demonstrated[169]. It is important to consider that reliable Doppler flow velocity tracings are required for measurement, and Doppler flow velocity signals may be inconsistent and are particularly influenced by the wire tip position[135]. This weakness of Doppler derived parameters is exemplified by a study that evaluated HMR as a predictor of MVO after acute STEMI, and found that 8% of invasive measurements had to be excluded due to poor Doppler flow signals or iatrogenic coronary dissection in one patient[178].

Although TRT was derived and validated in distinct populations, the limited number of composite outcome events constrained the statistical power of multivariable models. A more robust way to validate TRT could be a comparison with positron emission tomography, or an experimental model with perivascular flow probes or microspheres, enabling calculation of coronary flow and myocardial resistance.

6.7 Future work

Future research is warranted to further validate the novel findings reported in this chapter and to study the reproducibility of TRT compared to IMR in a larger number of patients. Moreover, future research is of interest to determine whether this new metric (TRT) may have utility in the assessment of the microcirculation in patients with ischaemia and no obstructive coronary artery disease.

6.8 Conclusion

In conclusion, higher TRT predicts more MVO and clinical outcomes following acute STEMI, independently of established predictors. TRT represents a novel parameter for detecting failure of microvascular perfusion and may have potential to refine risk stratification after primary PCI. Further research is needed to evaluate the potential clinical utility of TRT-guided selection of patients for adjunctive treatments during primary PCI.

Chapter 7: Concluding remarks and future directions

The work presented in this thesis provides novel insights regarding the use of invasive quantitative parameters to measure impaired myocardial perfusion, and to predict 1-year clinical outcomes. The ideal test should be straightforward to perform during the primary PCI procedure, reliable, prognostically validated, and responsive to the effects of therapies on microvascular perfusion. A test that fulfils these criteria would potentially be a practice advance for the following areas of interest: (i) selection of the highest risk patients for adjunctive therapies designed to minimise microvascular injury; (ii) selection of the lowest risk patients for potential early discharge, and; (iii) serving as an immediate test of the efficacy of therapies. In current clinical practice, persistent microvascular injury typically passes undetected after primary PCI, and is a clinical problem of unmet therapeutic need.

The findings from the comparison of established and novel invasive measures of coronary microvascular function, for predicting MVO, myocardial haemorrhage and prognosis in acute STEMI patients, adds novel understanding on the potential role of RRR as a measure of microvascular function and as a prognostic biomarker for risk stratification. The findings in Chapter 5 showed for the first time, that lower RRR in acute STEMI patients was associated with adverse clinical outcomes. IMR and RRR reflect different aspects of microvascular function, i.e. RRR is a measure of the vasodilatory capacity of the microcirculation, whereas IMR is a measure of the minimum achievable microvascular resistance at peak hyperaemia. RRR and IMR are complimentary, and the findings presented in chapter 5 support IMR in conjunction with RRR, instead of CFR, as a tool to select patients for adjunctive therapy during primary PCI. Future studies are anticipated to determine the wider external validity of these findings.

Although IMR is a validated invasive coronary physiology test for evaluating the immediate efficacy of microcirculatory reperfusion, it has some limitations for clinical translation. In chapter 6, a newly conceived parameter, termed TRT, was described and validated for the first time, and was shown to be predictive of MVO and prognosis, independent of IMR >32, or IMR >40, and other established predictors. TRT is a novel diagnostic advance for detecting failed microcirculatory reperfusion, which may have potential to refine risk stratification and selection of patients for adjunctive treatments after

primary PCI. Further research is warranted to validate the findings in other cohorts and to examine the reproducibility of TRT, compared to IMR, in a larger number of patients.

This thesis also provides novel insights into the mechanistic effects of intracoronary alteplase, administered during primary PCI, on the coronary microcirculation. The notable finding of associations between intracoronary alteplase and MVO, and myocardial haemorrhage presence, in patients with TIMI flow ≤2 immediately preceding study drug administration, suggests that low-dose intracoronary alteplase may be harmful when administered to STEMI patients who have impaired coronary flow (chapter 3). The potential explanations include increased local exposure to alteplase, due to impaired washout of alteplase from the microcirculation, which appears to enhance haemorrhagic transformation in the infarct core and seems to augment the undesired procoagulant effects of alteplase, thereby promoting microvascular thrombosis. These interpretations were supported by results from the analyses of coagulation parameters. Nonetheless, no adjustment for multiplicity was made in this sub-group analysis, which should be interpreted as exploratory and not definitive. Further research is needed to validate the findings.

Although no overall differences in acute invasive measures of microvascular function (IMR, CFR, or RRR) were seen after intracoronary alteplase vs. placebo, the sub-group analyses in the T-TIME physiology sub-study provide further novel insights into the mechanistic effects of low-dose intracoronary alteplase (chapter 4). Notably, in the sub-group of patients with ischaemic time <2 hours, there were significant improvements in CFR and RRR (but not IMR) with alteplase vs. placebo, whereas in the sub-group of patients with ischaemic time ≥4 hours, MVO was worse with alteplase vs. placebo. These clinically relevant results, do not support administering intracoronary alteplase in patients with STEMI presenting with an ischemic time ≥4 hours. However, due to the potential for type 1 statistical error, the findings from the sub-group analyses, should be interpreted as exploratory/ hypothesis generating, rather than definitive.

In summary, the implications of the research presented in this thesis is that RRR and TRT may have clinical utility to risk stratify patients for adjunctive treatments, in the catheterisation laboratory. This targeted therapy approach has potential to reduce the

number of patients needed in clinical trials for STEMI research, to achieve adequate power to ascertain improvements in trial endpoints. Secondly, the finding of increased presence of MVO with alteplase vs. placebo in patients with TIMI flow <3 is clinically relevant, as a disincentive to interventional cardiologists when considering bail-out intracoronary alteplase for angiographic "no-reflow".

The weaknesses of the project should be considered. Importantly, the T-TIME population may be a relatively low risk population of STEMI patients, because the eligibility criteria excluded higher risk patients with ischaemic times >6 hours, or patients with cardiogenic shock. As a result, most of the patients (59%) included in the T-TIME physiology sub-study did not have MVO present. This may affect the generalisability of the findings to other populations of STEMI patients, and may have affected the magnitude of the differences in MVO between treatment groups. For example, in the T-TIME angiographic study the absolute difference in the extent of MVO with 20mg of alteplase vs. placebo in patients with TIMI flow ≤2 immediately before drug delivery was only 2.8% (% LV mass). Although this difference was found to be statistically significant (p-value <0.05), it is uncertain whether this difference is clinically meaningful, since it did not translate to a difference in infarct size at 3-months between the 20mg alteplase group vs. placebo group.

Another important consideration is whether the effect sizes that formed the foundation of the sample size calculation for the T-TIME physiology sub-study were realistic. This sample size calculation assumed a mean IMR of 33.9 and SD of 25.2. Whereas the actual mean and SD for IMR in the T-TIME physiology sub-study were 40.7 and 32.4 respectively. Therefore, the actual SD was larger than what was expected, leading to a decreased power. However, given that the result of the main T-TIME trial found overall no difference in MVO between alteplase vs. placebo, it is unlikely that having more patients in the T-TIME physiology sub-study would have resulted in an overall difference in IMR between treatment groups. Furthermore, the sample size calculation assumed a mean difference in IMR of 10 units between alteplase 10mg and placebo, and a mean difference in IMR of 20 units between alteplase 20mg and placebo. Expecting to detect a between-group difference in mean IMR of 20 units may be considered unrealistic, since the previous study by Sezer *et al* found a statistically significant difference in IMR of 13.6 units between streptokinase vs. placebo[260].

Another weakness of the project is that the trial was stopped early by the sponsor, for futility, following an interim analysis. When a clinical trial is stopped due to futility, this means that the interim data imply a very low likelihood of observing statistically significant superior efficacy if the trial continues to termination[381]. The potential disadvantage of stopping a clinical trial early, due to futility is that the premature termination of a trial could lead to equivocal results. However, exposing patients to potential side effects of a therapy in a clinical trial that has little chance of demonstrating efficacy may be unethical. As a result of T-TIME stopping early, the number of patients in each treatment arm for the sub-group analyses were relatively small, which may have resulted in type 2 statistical error, i.e false negative results. On the other hand, with multiple statistical testing in sub-group analyses there is also a risk of type 1 statistical error. Therefore, the findings from the sub-group analyses are exploratory, and should be interpreted with caution.

Several uncertainties remain, including whether intracoronary tenecteplase administered at the end of primary PCI (post-stenting), after normal antegrade flow has been established, might have a beneficial effect on minimising microvascular injury in patients with short ischaemic time (<4 hours). Tenecteplase has higher fibrin specificity than alteplase[382], and is also less procoagulant than alteplase[318]. One can therefore speculate, that intracoronary tenecteplase administered post-stenting, in acute STEMI patients with large thrombus burden and short ischaemic time might possibly have potential to be an effective treatment for microvascular thrombosis and distal embolization. Further research is needed, and the findings from the ongoing RESTORE-MI trial [ACTRN12618000778280][273], should help address uncertainties.

Recent randomised controlled trials of therapeutic interventions designed to reduce microvascular injury have failed to provide evidence of benefit. Therefore, MVO is a common adverse complication of acute STEMI, for which there is no evidence-based therapy. At present, the most effective strategy to limit microvascular injury in STEMI appears to be restoring coronary flow as quick as possible, to shorten the ischaemic time. Further research is warranted. Given the conflicting results of the EARLY BAMI[212], and METOCARD-CNIC trials[239], intravenous metoprolol may merit further investigation in an adequately powered randomised clinical trial. Furthermore, the results of the ongoing

randomised studies to evaluate whether PICSO[270] or intracoronary hypothermia[253] might reduce infarct size, in patients undergoing primary PCI are eagerly anticipated.

Regarding future work for risk stratification of patients in the catheterisation laboratory, during primary PCI, application of computational fluid dynamics to derive angiography-based pressure wire free parameters are emerging. Recently, IMRangio defined as Pa (measured during hyperaemia) multiplied by quantitative flow ratio (Pd/Pa derived from the angiogram) and multiplied by TFC in the infarct-related artery correlated with pressure wire derived IMR and MVO in 45 acute STEMI patients[383]. In another study, quantitative flow ratio was derived from 130 acute STEMI patients using contrast induced-hyperaemia, and was correlated with MVO on CMR imaging[384]. A limitation of current techniques is that calculation of quantitative flow ratio requires a stenosis, so it is being measured pre-stent implantation. A better time point to assess microvascular function in the infarct-related artery would be post-stenting. A prospective trial is ongoing to investigate whether quantitative flow ratio computed post-stenting in the infarct-related artery, using a partially inflated balloon, is associated with CMR defined MVO (NCT03910400)[385]. Looking forwards, measuring microvascular function in the infarct-related artery post-stenting, from the angiogram, without the need for a pressure wire has potential to advance clinical research.

Appendix 1: Ethical approval for the T-TIME trial and the physiology sub-study

NHS
Greater Glasgow and Clyde

WoSRES
West of Scotland Research Ethics Service

West of Scotland REC 1
Ground Floor, Tennent Building
Western Infirmary
38 Church Street
Glasgow
G11 6NT

Prof Colin Berry
Reader
University of Glasgow
126 University Place
Glasgow
G12 8TA

Date 8th August 2013
Direct line 0141-211-6270
Fax 0141-211-1847

ear Prof Berry

Study title: **A randomised, double blind, placebo-controlled, parallel group, Trial of low-dose adjunctive alTeplase during prIMary PCI (T-TIME)**
REC reference: **13/WS/0119**
EudraCT number:
IRAS project ID: **117753**

Thank you for your letter of 31 July 2013, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the NRES website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the Co-ordinator Miss Sharon Jenner, sharon.jenner@ggc.scot.nhs.uk.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites listed in the application, 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).

Non-NHS sites

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. We 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.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study 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 a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

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

Clinical trial authorisation must be obtained from the Medicines and Healthcare products Regulatory Agency (MHRA).

The sponsor is asked to provide the Committee with a copy of the notice from the MHRA, either confirming clinical trial authorisation or giving grounds for non-acceptance, as soon as this is available.

It is the 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 final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Advertisement		
Covering Letter		17 May 2013
Covering Letter		19 July 2013
Covering Letter		07 August 2013
GP/Consultant Information Sheets	1.0	17 May 2013
Investigator CV		16 May 2013
Other: Other changes to the short patient information sheet		19 July 2013
Other: From protocol page 78	1.0	29 April 2013
Other: Changes to the full patient information sheet		19 July 2013
Participant Consent Form: Short (tracked changes)	1.0	31 July 2013
Participant Consent Form: Main (tracked changes)	1.0	31 July 2013
Participant Information Sheet: Short (tracked changes)	1.0	31 July 2013
Participant Information Sheet: Main (tracked changes)	1.0	31 July 2013
Protocol	1.0	29 April 2013
REC application	1.0	16 May 2013
Response to Request for Further Information		19 July 2013
Response to Request for Further Information		31 July 2013

Statement of compliance

This Committee is recognised by the United Kingdom Ethics Committee Authority under the Medicines for Human Use (Clinical Trials) Regulations 2004, and is authorised to carry out the ethical review of clinical trials of investigational medicinal products.

The Committee is fully compliant with the Regulations as they relate to ethics committees and the conditions and principles of good clinical practice.

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.

After ethical review

Reporting requirements

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
- Notification of serious breaches of the protocol
- 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.

Feedback

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.

Further information is available at National Research Ethics Service website > After Review

13/WS/0119	**Please quote this number on all correspondence**

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

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

Yours sincerely

On behalf of
Dr John Hunter
Chair

Email:sharon.jenner@ggc.scot.nhs.uk

Enclosures: "After ethical review – guidance for researchers" *[SL-AR1]*

Copy to: *Dr Maureen Travers, NHS Greater Glasgow & Clyde*

WoSRES
West of Scotland Research Ethics Service

NHS
Greater Glasgow and Clyde

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Date 20 July 2016
Direct line 0141 232 1807
E-mail WoSREC1@ggc.scot.nhs.uk

Dear Dr Gillespie

Study title:	**A randomised, double blind, placebo-controlled, parallel group, Trial of low-dose adjunctive alTeplase during prIMary PCI (T-TIME)**
REC reference:	**13/WS/0119**
EudraCT number:	**2014-004405-32**
Amendment number:	**Substantial Amendment 5.0 4.0 2016/07/05 (REC Ref AM06)**
Amendment date:	**06 July 2016**
IRAS project ID:	**117753**

The above amendment was reviewed by the Sub-Committee in correspondence. The amendment relates to an updated protocol. Some of the changes to the protocol include:

1. To reflect an update in clinical guidelines for aspiration thrombectomy to Class IIB
2. Clarification on inclusion criterion: a requirement for reduced flow in the culprit artery (ie TIMI grade 2) in the presence of significant thrombus (Grade 2+), or, absent (TIMI grades 0 or 1) coronary flow at initial angiography. Proximal-mid culprit lesions.
3. Update on recent, relevant clinical trials
4. Addition of a coronary physiology sub-study
5. Minor typographical errors and clarification of the text, including re-numbering of the references as appropriate.

The amendment also includes the addition of a site and removal of 2 sites.

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*
Covering letter on headed paper		05 July 2016
Notice of Substantial Amendment (CTIMP)	Substantial Amendment 5.0 4.0 2016/07/05 (REC Ref AM06)	06 July 2016
Participant consent form [Short Consent includes PIS]	3.1	23 June 2016
Participant consent form [Full Short Consent includes PIS]	3.1	23 June 2016
Participant information sheet (PIS) [Short PIS includes consent]	3.1	23 June 2016
Participant information sheet (PIS) [Full PIS includes consent]	3.1	23 June 2016
Research protocol or project proposal [Summary of changes from version 3.3 to 4.0]	4.0	05 July 2016
Research protocol or project proposal [Clean]	4.0	05 July 2016
Research protocol or project proposal [Track Changes]	4.0	05 July 2016

New Sites

Research site	*Principal Investigator / Local Collaborator*
St Bartholomew's Hospital	Andrew Wragg

The amendment relates to the addition of new site(s) and/or investigator(s) within the National Health Service (NHS) or Health and Social Care (HSC) in Northern Ireland. The site-specific assessment for the site(s) will therefore form part of the research governance review. The Site-Specific Information (SSI) Form for the site should be included with the application for R&D approval.

On behalf of the Committee, I am pleased to confirm the extension of the favourable opinion to the new site(s) and/or investigator(s), subject to management permission being given by the relevant NHS/HSC R&D office(s) prior to the study starting at the site.

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

This Committee is recognised by the United Kingdom Ethics Committee Authority under the Medicines for Human Use (Clinical Trials) Regulations 2004, and is authorised to carry out the ethical review of clinical trials of investigational medicinal products.

The Committee is fully compliant with the Regulations as they relate to ethics committees and the conditions and principles of good clinical practice.

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.

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

13/WS/0119:	**Please quote this number on all correspondence**

Yours sincerely

Abibat Ackwumi

On behalf of
Dr Malcolm Booth
Chair

Enclosures: *List of names and professions of members who took part in the review*

Copy to: *Dr Maureen Travers, NHS Greater Glasgow and Clyde*
Professor Colin Berry, University of Glasgow and NHS Greater Glasgow & Clyde Health Board

Appendix 2: T-TIME trial short patient information sheet and consent form (for use in the catheterisation laboratory for witnessed assent to participate)

SHORT PATIENT INFORMATION SHEET AND CONSENT FORM

Research title: T-TIME Trial

PURPOSE

This is a guide to the information to be provided to the patient if they indicate they may be willing to take part in this research study in order that they may give verbal consent to take part.

The provision of the information and the agreement from the patient to take part must be witnessed by another member of staff in the cath lab.

If the patient consents to take part in the study this form should be signed by the person providing the information to the patient,

Invite the patient to take part in the research study investigating a potential new way to protect the heart after a heart attack.

Why are they being asked to take part?

- You are being asked to participate in this project because you have experienced a heart attack caused by a blood clot in one of the blood vessels in your heart.
- The decision to take part is voluntary. You do not have to be in this study to be treated for your heart attack. If you decide not to take part in this study then you will receive standard care.

What is the aim of the study?

- We are looking at ways of improving how the clot can be reduced in size to help protect the heart from further injury. Alteplase is a drug that breaks down blood clots and we would like to see if by giving a low dose of this directly into the clot in the heart artery might reduce heart injury and improve heart function, health and well being after a heart attack.

What will happen to me?

- This study has three groups of patients and you will be allocated to one of these at random:
    - Group 1: An inactive form of the drug (a placebo) will be injected into the blocked artery using an infusion catheter at the start of the angioplasty procedure which you will be undergoing.
    - Group 2: 10% of the standard dose of Alteplase will be injected in the blocked artery using an infusion catheter at the start of the angioplasty.
    - Group 3: 20% of the standard dose of Alteplase will be injected in the blocked artery using an infusion catheter at the start of the angioplasty.
    - You will have an equal chance of being treated with each of these groups and neither you nor your doctor will know which treatment you have been given.
- The medication in this study is approved for use in Europe, including the UK, however giving it directly into the clogged artery is considered investigational in this study.

- In this study, you will have the standard procedures given routinely for your condition, such as angiography (x-ray pictures of your heart blood vessels), balloon angioplasty, possible stenting and anticoagulation.
- You will have ECGs during your hospital stay and at your 3 month follow up visit, and blood samples collected during your hospital stay for routine tests and research purposes.
- You will have also have an MRI (magnetic resonance imaging) scan 2 days, and at 3 months after your treatment.
- For Sites participating in the OCT Substudy: You may also be asked to participate in an imaging substudy (OCT) which involves an additional two scans at the time of PCI.
- You may also be asked to participate in a coronary physiology substudy which involves additional measurements using a standard diagnostic guidewire after the PCI procedure.
- You will be in this study for up to 3 years with standard follow-ups and additional follow up by phone over this period. We would wish to obtain information on your health and wellbeing in the longer term from hospital and government records.

What are the risks?

- The risks of this clinical trial include many of the same risks that are associated with routine treatment of heart attack.
- Risks that could occur with the study treatment are:

  Death

  Heart: Reduced blood flow in the heart blood vessel, chest pain or heart attack, heart rhythm disturbance (irregular, fast, slow), heart blood vessel injury/trauma (spasm, dissection, rupture, tear, blood clot formation, recurrence of vessel narrowing, migration of air/tissue/blood clots, low blood pressure);

  Brain: Disturbance of blood flow to the brain, possible stroke;

  Bleeding: The most common, important side effect of treatment with alteplase is bleeding. This can become serious, and may, very rarely, be fatal, (fewer than 1 in every 10,000 patients treated). Bleeding is more likely to occur when alteplase is used in combination with other anticoagulant or antithrombotic medicines. This could result in complications such as anaemia (a low blood cell count), haematoma (bruising) or stroke.

  Other potential side effects are:

  - Pain, bleeding and bruising at the puncture site (after PCI treatment). These side effects are rare (fewer than 1 in every 100 patients treated).

If you are a woman of child-bearing age (i.e. before the menopause) you cannot take part in this study.

If you have had recent internal bleeding (< 6 months), a recent stroke (< 6 months), intra-cranial or intra-spinal surgery or trauma within 2 months, recent surgery (< 2 months), intra-cranial tumour or aneurysm, a known bleeding problem, pericarditis, pancreatitis, endocarditis, liver disease, peptic ulcer, varices or severe hypertension (systolic blood pressure >180 mmHg or diastolic blood pressure > 110 mm Hg), a

heart attack in the same part of your heart, allergy to gentamicin or your body weight is estimated to be < 60 kg, you should not take part in this study.

It is important that you let the study team know about any current or recent (within the last 3 months) medicines or other treatments you have received. You should not take part in this study if you meet any of the following criteria:

- **Treatment for an infection:** You are currently being treated for an infection (unless minor) or you routinely take medicines to prevent infection
- **Immunosuppressant treatment:** You are currently being treated with, or have received treatment with immunosuppressant drugs within the last 3 months. Immunosuppressent medicines include prednisolone , tacrolimus, ciclosporin., azathioprine, cyclophosphamide, and methotrexate. Other medicines, sometimes called 'biologics', also have an effect on the immune system and include adalimumab, rituximab and etanercept.
- **Anti-cancer treatment:** You have received chemotherapy or radiotherapy within the last 90 days.
- The infusion and thrombus aspiration catheters have not been previously studied in depth for treating heart attack patients. Therefore, unforeseeable risks may be involved.

Do I need to take part?

- No. You are free to withdraw your consent from the study at any time and your health care will not be affected in any way.

What are the benefits of taking part?

- Being in this study may be of no direct benefit to you but others may benefit from the results of this research in the future.

Will my taking part in this study be kept confidential?

- Yes. Your personal health information, including your medical data, will be collected by the study doctor and other site staff for this study's research purposes and every effort will be made to ensure it is kept confidential.

What if something goes wrong?

- The normal National Health Service complaints mechanisms will be available to you.
- There are no payments made to participants.
- There will be no compensation for you or your insurance company in the event of an injury.

More detailed information about this study will be provided in a separate information and consent form that you will be asked to read and sign after your condition is stabilised, if you agree to continue with the study.

Contact your study doctor for questions about the research or injuries from the research at the number on the front of this form.

SIGNATURES

I confirm that information about this study was explained to the patient, and any questions they had about this were answered. They agreed to take part in and consent to the procedures required by the above study, and were informed that relevant sections of their medical notes and data collected during the study, may be looked at by individuals from Greater Glasgow Health Board (Co-Sponsor), from regulatory authorities, or from the NHS hospital, where it is relevant to their taking part in this research. They gave permission for these individuals to have access to their records.

_____	_____
Patient Name (please print)	Date

_____	_____	_____
Name of person taking Consent (please print)	Signature	Date

Appendix 3: T-TIME trial patient information sheet and consent form (for use on the ward for signed consent to participate)

PATIENT INFORMATION SHEET

T-TIME: Evaluation of low dose intra-coronary alteplase during and after PCI

Invitation to take part in a research study

We would like to invite you to take part in a research study investigating a possible new way to protect the heart. You may recall that you were asked to participate in this research study when you were admitted to hospital with a heart attack. You underwent treatment for your heart attack, and at the same time were enrolled in the above study. We would now like to be able to take the time to provide you with all the information about the study. It is important that you read and understand the study information below before giving consent to continue to participate. Please ask any questions you may have about this study. One of our research team will go through the information sheet with you and answer any questions you may have.

you choose not to participate in the study you will be treated according to the hospital's normal standard-of-care.

What is the aim of the study?

Your heart attack was caused by a blockage in the artery that provides blood to the heart muscle. The blockage was caused by a blood clot. We are trying to find ways of improving how the clot can be reduced in size in order to protect the heart. Alteplase is a drug that breaks down blood clots. The study aims to show whether or not low or very low dose alteplase given directly into the heart artery might improve heart injury and function and your health and well being after a heart attack.

Why am I being asked to take part?

You are being asked to participate in this research study because you have experienced a heart attack. Up to 618 patients in 8 - 14 NHS hospitals are expected to take part in this study. Male and female patients who are experiencing a heart attack have been invited to take part.

What does the study involve?

During a heart attack an artery which provides oxygen to the heart muscle is blocked by a blood clot. Standard care involves opening the clogged artery with a procedure called percutaneous coronary intervention (PCI). PCI involves an X-ray of the heart arteries (an "angiogram"). This is performed by passing a small tube via a blood vessel in your arm (under local anaesthetic) to the site of the clot. The X-rays show where the clot is blocking your artery, and this is then sucked out with a very thin tube and/or a tiny balloon is inflated in your artery to widen it (angioplasty), and/or a stent (thin metal tube) is put in to hold the artery open (standard care).

Doctors may have used a drug called alteplase. Alteplase is not normally used in PCI, although alteplase can be given when PCI is not initially possible (e.g. journey to hospital will take some time e.g. longer than 2 hours). In this case, alteplase is normally given through a vein in your arm (intravenous, IV) to reduce the size of a blood clot. For this study, patients will be assessed based on the type of an additional treatment they will be given, once, at the start of their PCI procedure.

Group 1: Inactive drug (placebo) injected into the blocked artery using a standard catheter normally used during the procedure.

Group 2: Alteplase 10 mg (10% of the standard dose).

Group 3: Alteplase 20 mg (20% of the standard dose).

Alteplase is approved in the UK and Europe to be given at the standard dose into a vein; however the effect of giving it directly into the clogged artery in the heart at a low, or very low, dose is what is being evaluated in this study.

Do I have to take part?

Your participation in this research study is voluntary. If you do not wish to participate further this will not affect your future treatment. You are free to withdraw your participation in this research study at any time without fear of penalty or loss of medical care. Your doctor may stop your participation in this research study without your consent for medical or other reasons.

What will happen to me if I take part?

Once you agreed to take part, you received randomly (by chance) one of the three treatment groups described above. Neither you nor your doctor or nurses know which treatment you received. This is called blinding. To keep the blinding for this study, the nurse and/or doctor you will see during follow-up may not be the same as the one who carried out your procedure. Should it become medically necessary, your study doctor, or any other doctor treating you can obtain the information on the treatment you received to ensure that you are treated safely and appropriately. You will be provided with an alert card and this information sheet. You should carry the alert card with you at all times and show it to any health care professional that treats you.

You will be asked to have an ECG heart tracing, blood test, and heart MRI scan 2 days after, or as soon as possible within the next week, and again at 3 months after the procedure. You will be contacted by phone or seen at the clinic visit 1 year after the procedure and at 12 month intervals there after.

You may also have been invited to take part in one (or both) of two sub-studies.

The first is the heart artery imaging sub-study with optical coherence tomography (OCT), which is done at the time of the PCI.

OCT involves recording and looking at images of inside the heart artery with a thin tube and lens. OCT takes about 5 - 10 minutes and will be done during the PCI in order to assess the amount of clot in the artery.

The second is a coronary physiology substudy to record the index of microvascular resistance (IMR) and coronary flow reserve (CFR), both indicators of heart function, before administration of the study drug and again after the PCI procedure. This can be done in a few minutes using a standard diagnostic guidewire.

Blood thinning treatment is always given during PCI and in many hospitals heparin is the drug used. In this study, you will receive intravenous heparin, adjusted to body weight, and the heparin effect will be checked at the start and during the PCI. Sometimes another blood thinning treatment is needed and this is usually glycoprotein IIbIIIa inhibitor (gpIIbIIIa) therapy. If the doctor thinks additional treatment is needed then gpIIbIIIa therapy will be given as per local practice.

ECGs and routine blood tests (including for blood clotting (coagulation)) will be performed after the procedure and before you are released from the hospital. You will have the MRI scan for the study about

2 days after and will also be asked to complete a health questionnaire at this time. These tests are part of the research study. We would like your permission to store blood samples that are surplus to what is needed for routine tests for future research. Any future research with these samples would be subject to research ethical approval.

An MRI scanner takes detailed pictures of the heart. For this study, pictures of the heart will be taken to help your doctor evaluate the area of injury caused by your heart attack. To perform the MRI scan, you will lie inside a large tunnel-like tube for up to one hour. The MRI machine produces a loud thumping or humming noise. Contrast dye will be given through a drip in your arm to help improve the quality of the pictures.

The MRI scanner is very safe for people who do not have metal implants in their body. You will be asked questions to check this before being given the scan. The dye used during the cardiac MRI scans is called gadolinium. It is generally harmless and will be washed out through your kidneys. Severe impairment of kidney function would rule out giving the gadolinium but you would still be able to have the MRI scan (albeit without gadolinium). Side effects of the contrast 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. An MRI trained radiographer or doctor will be conducting your cardiac MRI scan. If your MRI scan results in a finding of something important or unexpected the result will be passed on to you and your General Practitioner. We would usually involve the opinion of a doctor who has specialist experience related to the finding.

We would like you to return to hospital 3 months later for a repeat MRI scan of your heart. There will be no increased risk for having an extra MRI scan. In addition, you will be asked about your health and any medicines you are taking at this 3 month visit and again at 12 monthly intervals from the start of the study (by clinic visit or telephone contact). If we cannot reach you by telephone we would like to be able to contact your General Practitioner to obtain information on your wellbeing and treatment. If you have other visits to a hospital or doctor, your study doctor or research staff will ask you for information about your health from these visits.

Your angiogram, OCT artery imaging (if taking part in the sub-study), ECG, blood samples, and MRI scans will be assessed and measurements made in a central laboratory.

Is there any long term follow up?

We would also like to ask you permission to obtain information on your future wellbeing to determine the longer term effects of this treatment. We can obtain this information by linking to records held by the Government (e.g. Registrar General) or NHS (e.g. health records). We would also like to obtain information on your drug therapy (medication) and any new medical diagnoses / hospital admissions or new medical conditions that you may have been diagnosed with in the future and again we can obtain this information through confidential electronic record linkage. This would not require us to contact you directly.

What are the potential risks or discomforts?

The risks that could occur are:

Bleeding: The most common, important side effect of treatment with alteplase is bleeding. This can become serious, and may, very rarely, be fatal, (fewer than 1 in every 10,000 patients treated). Bleeding is more likely to occur when alteplase is used in combination with other anticoagulant or blood thinning medicines. This could result in complications such as anaemia (a low blood cell count), haematoma (bruising) or stroke.

Alteplase may cause the following problems:

- Heart: Reduced blood flow in the heart blood vessel causing chest pain or heart attack, palpitations, heart blood vessel injury and low blood pressure;
- Brain: Disturbance of blood flow to the brain, possible stroke;

Other potential side effects are:

- Wrist and groin puncture site (radial / femoral pulse): Mild pain, bleeding and bruising at the puncture site (after PCI treatment) are common. More important pain, bleeding and swelling is less common (fewer than 1 in every 50 patients treated).
- If you are a woman of child-bearing age (i.e. before the menopause) you should not be taking part in this study.

  Please let us know if you have had recent internal bleeding (< 6 months), a recent stroke (< 6 months), surgery in your head or back or trauma within 2 months, recent surgery (< 2 months), a tumour or aneurysm in your head, a known bleeding problem, inflammation affecting the lining of your heart or your pancreas, heart valve infection, liver disease, stomach ulcer, varices or very high blood pressure (systolic blood pressure >180 mmHg or diastolic blood pressure > 110 mm Hg), allergy to the antibiotic gentamicin or your body weight is estimated to be < 60 kg.

  It is important that you let the study team know about any current or recent (within the last 3 months) medicines or other treatments you have received. You should not take part in this study if you meet any of the following criteria:

  - **Treatment for an infection:** You are currently being treated for an infection (unless minor) or you routinely take medicines to prevent infection
  - **Immunosuppressant treatment:** You are currently being treated with, or have received treatment with immunosuppressant drugs within the last 3 months. Immunosuppressent medicines include prednisolone , tacrolimus, ciclosporin., azathioprine, cyclophosphamide, and methotrextate. Other medicines, sometimes called 'biologics', also have an effect on the immune system and include adalimumab, rituximab and etanercept.
  - **Anti-cancer treatment:** You have received chemotherapy or radiotherapy within the last 90 days.

- The infusion and thrombus aspiration catheters have not been previously studied in depth for treating heart attack patients. Therefore, unforeseeable risks may be involved
- There may be other unforeseen risks which are not currently known.

What are the potential benefits from this research study?

There is no guarantee of improvement in your health or success of the procedure. Others who have a heart attack in the future, but who are not participating in this study, may benefit from the results of this study.

You will not receive any money for participating in this study. Money for lost wages or other direct/indirect costs is not available.

Who is funding the research?

The study is being funded by the National Institute of Health Research (NIHR) who will reimburse the hospital for the procedures over and above standard care necessary to undertake the study, and the pharmaceutical company, Boehringer Ingelheim, is donating the study drug. Your study doctor is not being paid for including you in the study.

What if I want to make a complaint?

If you have a concern about any aspect of the study then you should speak to the researchers in the first instance. If you remain unhappy and wish to complain formally you can do this though the NHS complaints procedure <add local hospital complaints office details>

What if something goes wrong?

In the unlikely event you are harmed from your participation in this study, medical treatment will be available to you. If this is due to someone's negligence then you may have grounds for a legal action for compensation against the study Sponsors, Greater Glasgow and Clyde Health Board and the University of Glasgow, but you may have to pay your legal costs. The Sponsors have taken out insurance to cover this eventuality, and the normal NHS complaints mechanisms will still be available to you.

There are no payments to patients participating in this study.

Confidentiality

Your identity will remain confidential at all times and all of your personal information will be processed in accordance with the Data Protection Act. You will be given a unique study number. We ask permission however to retain your contact details so as to phone you twice a year to ask about your general health and medication, as mentioned earlier. This information will only be available to the study team who are all bound by NHS rules on confidentiality.

Study data, images and results may be provided to experts out with your NHS Board/Trust, government agencies and published in medical literature or conferences; however no information will be released through which you could be identified. In addition, the clinical research team, the study sponsor, Greater Glasgow and Clyde Health Board, and regulatory authorities may request access to your medical record and the study data for monitoring or audit purposes. You have a right to privacy and the doctors involved in this research study will take all reasonable measures to protect the confidentiality of your records.

Your GP will be informed of your participation in this study. By signing the consent form you will be agreeing that your GP can be notified.

Questions?

If you need additional information regarding this research study, your rights as a research patient, or in the event you develop a research-related problem, contact **your local research nurse** at phone number **XXX XXX XXXX**.

Who has reviewed the study?

West of Scotland Research Ethics Committee 1.

Contact for further information

If you have any questions or concerns relating to the study please don't hesitate to get in touch with "INSERT PI CONTACT DETAILS", Department of Cardiology, INSERT PI ADDRESS. Telephone: INSERT TELEPHONE NUMBER

An independent contact for the study is: INSERT LOCAL INDPENDENT CONTACT, INSERT INDEPENDENT CONTACT ADDRESS, Telephone: INSERT INDEPENDENT CONTACT TELEPHONE NUMBER

PATIENT CONSENT FORM

Title of Protocol	T-TIME: Evaluation of low dose intra-coronary alteplase during and after PCI

Please initial box

1. I confirm that I have read and understood the patient information sheet version 5.0, dated 30/06/17 and that I have had any questions I have about the study answered. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without my medical care being affected. ☐
3. I understand that sections of any of my medical notes and imaging may be looked at by the research team/laboratories where this relates to this study, and for auditing and monitoring by the Sponsor or other regulatory authorities. I give permission for these individuals to have access to my records. ☐
4. I agree that my GP will be informed of my participation. ☐
5. I agree that my contact details can be retained by the study team for use in relation to study procedures. ☐
6. I agree to follow-up information being collected on my future wellbeing and treatment from NHS and Government health records. ☐
7. I understand that the hospital has no formal program for compensating patients for medical injuries arising from this research. Medical treatment will be provided for injuries as usual ☐
8. I agree that surplus blood samples may be retained for use in future research. ☐
9. I agree to take part in this study ☐

OPTIONAL SUB-STUDY

10. I agreed to take part in the IMR substudy Yes ☐ No ☐ N/A ☐

OPTIONAL SUB-STUDY

11. I agreed to take part in the OCT substudy and for the images and data obtained from my OCT scan to be used for this study Yes ☐ No ☐ N/A ☐

Patient Name (Print)	Patient Signature	DATE
Person obtaining Consent (Print)	Signature	DATE

3 copies- one for patient, one for notes, and one for study site file

Appendix 4: T-TIME trial standard operating procedures for angiogram acquisition and for study drug preparation and administration

T-TIME SOP for Coronary Angiography & Intervention

Index

Author & Contact details

Colin Berry: Email: colin.berry@glasgow.ac.uk;

Annette Maznyczka: Email: amaznyczka@nhs.net;

Phone: switchboard – 01419515000; Cell – 07831698479 & for the T-TIME Research Team

Please do not hesitate to make contact any time should any query arise.

Flow diagram of key points

1. Suggest obtain angiogram of non-infarct related artery (IRA)
2. Confirm eligibility and assent before reperfusion which must be achieved before randomisation
3. Obtain study bloods if not done already
4. Check ACT – give additional unfractionated heparin if ACT<250 s
5. Angiogram of infarct-related artery (IRA)
6. Decide on best orthogonal views
7. Wire the culprit artery
8. Reperfuse with balloon/thrombectomy
9. Nitrate (manifold flush)
10. Repeat the angiogram with the 'best views' (baseline angiogram post-reperfusion & prior to study therapy)
11. Non heparinised saline flush
12. Administer study drug (see guidance below)
13. Non-heparinised saline flush (to ensure residual volume in catheter is administered)
14. Check ACT – give additional unfractionated heparin if ACT<250 s
15. Repeat the angiogram post study drug & prior to stenting.
16. Stent & post-dilate, as appropriate
17. Nitrate (manifold flush)
18. Orthogonal views (same as in baseline angiogram post-reperfusion & prior to study therapy)
19. Measure LVEDP
20. Consider IMR measurement with PressureWire Certus (suggest use the initial wire as a buddy)

Diagnostic imaging with coronary angiography

Eligibility Criteria

The angiographic inclusion criteria (Appendix 1) must be present before informed assent and randomisation. The cardiologist must assess the initial angiogram to confirm eligibility.

The T-TIME team have provided posters for the catheter laboratory facilitate the study flow.

Intra-arterial nitrate therapy may be helpful in patients with hypertension.

Angiogram Acquisition

The coronary angiogram will be obtained during the emergency care primary percutaneous coronary intervention (PCI) procedure. Initial views of the culprit artery flow should be obtained at baseline in line with standard practice.

Please consider undertaking non-culprit artery angiography first in order to assess for prognostically important multivessel disease and to assess for functional collaterals to the culprit artery (an exclusion criterion)

Consider using a frame rate of up to 30 frames/second, in line with standard clinical practice for assessment of blush grade.

Magnification mode: 15 – 20 cm.

Record the French size of the catheter in the e-CRF (5 French or 6 French)

200 – 300 µg intra-coronary nitrate should be administered to attenuate epicardial artery spasm.

Coronary angiograms will be performed in the usual way before reperfusion to guide PCI, and then again after treatment to document the final results of the procedure.

Since the coronary guidewire will already be in place for the PCI, positioning of the aspiration catheter should be straightforward. No additional cine angiograms will be needed just fluoroscopy to guide and confirm the position of the aspiration catheter, which should be advanced 1 – 2 cm proximal to the lesion for study drug infusion.

Angiographic views: A comprehensive angiogram should be performed, in line with usual care, at the start and end of the PCI procedure. Orthogonal views of the culprit lesion should be obtained. For the purposes of the secondary angiographic outcomes, the EXACT SAME ANGLES of the x-ray image intensifier should be used at the beginning and end of the PCI in order that matched comparisons of the angiographic images can be made by the core laboratory.

Angiogram timing – key time points

1. **Standard of care prior to reperfusion**
2. **Post-reperfusion pre-study drug administration**
3. **Post-study drug administration**
4. **End-of-procedure (final angiographic views)**

Angiogram imaging: key points

1. **BASELINE ANGIOGRAM:** Cine-angiography orthogonal views should be obtained AFTER reperfusion but BEFORE study drug administration. This angiogram will represent the study baseline angiogram, and will set the projections for future acquisitions.

1) The angiogram should image the full distribution of the culprit artery. As a general rule

Culprit artery	Indicative angiographic projections (°)
Left anterior descending	RAO 10° CRANIAL 30° PA 0° CRANIAL 30°
Intermediate or Circumflex	RAO 10° CAUDAL 30°
Right	LAO 20° CRANIAL 20°

2) Centre the artery of interest, avoid overlap with side-branches and fore-shortening, so adjustments of the angiographic projections should be made to optimise the coronary imaging.
3) Timing and duration of the angiogram: The cine acquisition should start without contrast and then continue for at least 3 heart beats and finish after the blush. Imaging the 'wash-out' of contrast through to the venous phase is important to calculate the TIMI Blush Grade.
4) The projection (cranial, caudal, left, right) should be carefully noted since the exact same positions should be used for the final angiogram.
5) The French size of the catheter should be recorded in the e-CRF

2. **ANGIOGRAM OF STUDY DRUG ADMINISTRATION**: An angiogram recording by cine or fluoroscopy should be obtained to evidence the administration of the study drug and demonstrate that the study drug was administered proximal, and so upstream, of the culprit lesion using an intra-coronary catheter for infusion of the study drug.

3. **ANGIOGRAM POST-STUDY DRUG ADMINISTRATION**: A cine-angiogram should be obtained post-study drug administration to optimise stent placement (standard care).

4. **COMPLICATIONS DURING THE PROCEDURE**: A cine-angiogram should be obtained of any complication that occurs during the procedure e.g. thrombus embolisation, no-reflow, coronary dissection.

5. **FINAL ANGIOGRAM:** A final angiogram at the end of the procedure should be obtained in exactly the same projection as the baseline angiogram.

Angiogram annotation

All angiograms must be anonymised prior to upload with all patient identifiers removed.

The method used is the same as that for other data such as ECG and MRI, the required method is detailed below:

ID: TTIME_SiteID_ParticipatantNo_YearMMDD

Name: ANGIOGRAM_TIMEPOINT

Example:

ID: TTIME_01_054_20160912

NAME: ANGIOGRAM_BASELINE

Image resolution: 512 x 512

Angiogram export

We recommend that all of the angiogram acquisitions should be exported to the e-CRF for core laboratory analysis. At the very least, the subset of recordings with the key cine acquisitions should be uploaded. It is the responsibility of the Local Principal Investigator to ensure that the appropriate cine recordings are uploaded to the e-CRF. Since the upload will most likely be performed by a research nurse, the cardiologist must review the angiogram to denote which recordings should be exported.

Guidance on coronary intervention

Primary PCI

PCI should be performed in line with contemporary best practice for a thrombus-laden coronary artery.

The approach to reperfusion is not part of the study protocol and therefore use of balloon angioplasty or aspiration thrombectomy is left to the discretion of the operator. The CRF has mandatory fields for the reperfusion technique.

If balloon angioplasty is performed, it is suggested that a balloon sized to the expected lumen diameter may be most appropriate and inflated at comparatively low inflation pressure e.g. 4 – 8 atmospheres. An under-sized balloon may leave an obstructive (thrombotic) stenosis, which expectedly is a substrate for re-occlusion before stent implantation (including during study drug preparation and administration).

Although post-dilatation provokes distal embolisation, the risk of stent thrombosis in an undersized or malapposed stent may present greater harm to the patient, and post-dilatation is anticipated to be routine.

Anti-coagulation and anti-platelet therapy

Please see Appendix 2 for the protocol text on anticoagulation that is intended to reflect standard care.

Key points

Active management of therapeutic anticoagulation – please ensure that the ACT is therapeutic i.e. target 250 – 300 s before study drug is administered. Since the study drug preparation and infusion takes several minutes, sub-optimal anticoagulation could be associated with coronary re-occlusion.

LV end-diastolic pressure

Please measure and record LVEDP towards the end of the procedure.

IMR sub-study

Clinicians are encouraged to measure IMR at the end of the PCI procedure, when clinically appropriate. There is an SOP specifically for the IMR sub-study. A cath lab worksheet has been provided. This worksheet should be completed by the physiologist during the procedure. The Glasgow group offer to visit to provide on-site training for IMR and also invite clinicians to visit Glasgow.

OCT sub-study

OCT is designated for Glenfield Hospital, the Golden Jubilee, and Wythenshawe Hospital

Please see SOP for OCT.

Guidance on study drug administration

Rationale for slow manual infusion of the study drug rather than bolus administration:

The overall rationale of intra-coronary lysis after reperfusion and before stent implantation is to both prevent and treat microvascular obstruction due to thrombus within the culprit artery, including the culprit lesion and the distal branches.

An infusion period of up to ten minutes has been adopted on pragmatic grounds. Bolus administration may have limited first pass binding of alteplase with fibrin molecules and systemic release will dilute the drug to ineffective systemic concentrations. By slow infusion directly upstream of the thrombotic lesion, the rationale is to lyse thrombus both within the culprit lesion and distally in the microcirculation. Real-time lysis of clot may be achieved during the infusion, leading to further exposure of fibrin molecules within the thrombus as lysis progresses. The half-life of alteplase is 5 minutes, but the deep tissue half life is ~45 minutes, so persistent local lysis may occur within the distribution of the culprit artery following local drug administration.

The rationale for infusing the study drug before stent implantation is to lyse and degrade thrombus at the earliest opportunity after reperfusion in order to reduce the likelihood of thrombus embolisation following stent implantation and to restore microvascular perfusion by lysis of micro-thrombi within capillaries.

Study Drug Administration, c.t.d.

T0: Study drug administration (single infusion up to 10 min) in catheter laboratory at start of PCI (after reperfusion but before stenting)

Vital sign observations (heart rate and rhythm, blood pressure)

ACT check at 20 min intervals during the PCI and administration of unfractionated heparin as needed

See Study Flow Chart: *** *always aim to give the full volume of study drug****

- ⇒ Aspiration thrombectomy may be performed for thrombus-containing lesions on a selective basis, as per contemporary guidelines. Direct stenting is preferred rather than initial balloon angioplasty in order to minimise embolisation of thrombus. If balloon angioplasty is performed, then low balloon inflation pressures are recommended to minimise thrombus embolisation.
- ⇒ *The study pack is determined by the randomisation system with either the voice/telephone (IVRS) or web-based (IWRS) trials unit system*
- ⇒ *Dissolve study drugs* (1 vial per 10 ml diluent into one syringe e.g. 20 ml)
- ⇒ *Intra-coronary infusion catheter (e.g. aspiration thrombectomy catheter or perfusion catheter):* The catheter must be fully flushed with 0.9% sodium chloride immediately prior to administration of the study drug. The catheter should then be advanced on the coronary guidewire into the culprit coronary artery proximal to the lesion.
- ⇒ *In the case of a RCA or LMS lesion, the guide catheter may be used for drug infusion (assuming there is good engagement of the guide catheter with the coronary ostium and no proximal side branches).*
- ⇒ *Infuse study drug (20 ml)* – slow, manual infusion of up to 10 minutes but a minimum of 5 minutes (slow manual infusion rate = 2 ml / min). *If a thrombectomy catheter is used to infuse the study drug, the catheter may be intermittently withdrawn on 1 – 2 occasions (e.g. for a 1 minute period) to facilitate antegrade blood flow.*
- ⇒ *Finally, flush the catheter with normal (0.9%) saline to ensure that all of the study drug has been completely administered*
- ⇒ *Please record the duration of study drug administration in minutes*

Specific considerations for study drug administration

Steps to mitigate the risk of spontaneous reocclusion before stent implantation

- Suggest angioplasty with a balloon that is close to the expected lumen diameter (e.g. 0.9 – 1.0: 1), or at least a balloon that is not significantly under-sized (e.g. 2 mm balloon in a 3.5 – 4 mm vessel), in order to avoid leaving an obstructive, thrombotic lesion prior to stent implantation (e.g. during study drug administration)
- Study drug infusion is suggested up to 10 minutes but can be shorter in line with clinical judgement. Please document the infusion time.
- If an intra-coronary catheter or over-the-wire balloon is used to administer the study drug, avoid approximating the end of the catheter into the culprit lesion. The catheter should always remain proximal to the lesion and not be inserted into it.

Management of angiographic complications

- If there are clinical features of re-occlusion, e.g. recurrent ST-segment elevation, we suggest to withdraw the catheter (retain the study drug!), perform gentle balloon angioplasty, and then completely administer the remaining volume of the study drug.
- If coronary flow reduces to TIMI 0/1 from TIMI 2 or 3 flow grades, please note report this in the Cardiologist Questionnaire (Appendix 3) and have the research staff report an adverse event.

Appendix 1. Angiographic eligibility criteria

Angiographic Inclusion Criteria

(Angiographic criteria will be reviewed after initial coronary angiography)

- Coronary artery occlusion (TIMI coronary flow grade 0 or 1)
- or
- Impaired coronary flow (TIMI flow grade 2, slow but complete filling) in the presence of definite angiographic evidence of thrombus (TIMI grade 2+).
- Proximal-mid culprit lesion location in a major coronary artery (i.e. the right, left anterior descending, intermediate or circumflex coronary artery)
- Radial artery access

[TIMI thrombus grade definitions: grade 2 represents definite thrombus with greatest dimensions less than or equal to half the vessel diameter; grade 3 represents definite thrombus but with the greatest dimension more than half but less than two vessel diameters; grade 4: thrombus present—large size: as in grade 3 but with the largest dimension greater than or equal to two vessel diameters].

NB: When a 5 French guide catheter is used then an alternative intra-coronary infusion catheter may be used instead of an aspiration thrombectomy catheter (which may only be passed through a 6 French guide catheter).

Main Angiographic Exclusion Criteria

- Normal coronary flow grade (TIMI flow grade 3) at initial angiography
- Functional coronary collateral supply (Rentrop grade 2/3) to the culprit artery
- Multivessel PCI intended before the day 2 -7MRI scan

Appendix 2. Anticoagulation

Specific considerations on anticoagulation (from the protocol):

Anti-thrombotic therapy will be in accordance with optimal standard care before and during primary PCI. This is expected to include unfractionated heparin (minimum 5000 IU) at the first medical contact combined with dual anti-platelet therapy with aspirin and either 600 mg of clopidogrel, 60 mg of prasugrel or 180 mg of ticagrelor in line with standard care.

On arrival in the catheter laboratory the ACT should be checked to confirm prior anti-coagulation (target ACS 250 s) and supplementary heparin should be given, as appropriate, either if not given before arrival or the ACT is less than 250 s. During primary PCI, the ACT should be checked every 20 min to ensure therapeutic anti-coagulation (i.e. ACT 250 s) during the procedure in line with optimal standard care. Intravenous heparin should be re-administered as needed according to the ACT.

Glycoprotein IIbIIIa therapy

GpIIbIIIa inhibitor therapy should be administered for 'bail-out' as per clinical guidelines (1). The indications for bail-out GpIIbIIIa inihibitor therapy include angiographic evidence of massive thrombus, slow or no-reflow, or a thrombotic complication (1). The choice of gpIIbIIIa inhibitor is as per local practice. The recommended dose for abciximab (Reopro) is 0.25 mg/kg given as an intravenous bolus, followed by a continuous intravenous infusion of 0.125 microgram/kg per min (to a maximum of 10 microgram/min) for 12 hours. The recommended dose for tirofiban (Aggrastat) is 25 microgram/kg given as a bolus followed by an intravenous infusion of 0.15 microgram/kg/min for up to 24 hours. Eptifibitide may also be used in line with standard care.

Bivalirudin

If bivalirudin is used as the main anti-coagulant then intravenous heparin (70 – 100 U/kg) should still be administered initially to ensure immediate therapeutic anticoagulation and then the bivalirudin should be continued during and after the PCI at the standard dose until the patient has returned to the ward for up to 4 hours in line with contemporary practice.

Anti-platelet therapy

Following primary PCI, in line with optimal standard care in each site, dual anti-platelet therapy should be maintained for at least 3 months (6 months for a drug eluting stent) with clopidogrel, ticagrelor or prasugrel or as per standard practice at study site. Therefore, during the critical early treatment period, anti-thrombotic therapy will be optimal and standardised in all patients.

Appendix 3. Cardiologist questionnaire

Cardiologist feasibility questionnaire to capture information about study drug administration

Cardiologist	Response
1. Was the study drug easily prepared?	Y / N
If not, why?	
2. Did the patient receive all of the study drug?	Y / N
If not, why?	
3. Was slow manual infusion achieved through the intra-coronary catheter proximal to the culprit lesion?	Y / N
If not, why?	
4. Were there any problems with administering the study drug?	Y / N
If yes, what were the problems?	Placement of the infusion catheter Y/N Patient factors (e.g. cardio-respiratory instability) Y/N
5. Do you attribute any clinical problems to the study drug or treatment approach?	Y / N
If yes, what were the problems?	

Appendix 5: T-TIME trial standard operating procedure for CMR image acquisition and analysis

Cardiac Magnetic Resonance Analysis Standard Operating Procedure

Title: T-TIME Cardiovascular Magnetic Resonance (CMR) standard operating	Version: 2.0
Status: Draft	Revised by:
Version 1.0 date:	08/09/2015
Version 2.0 date:	19/07/2016
Public registration: https://clinicaltrials.gov/ct2/show/NCT02257294	NCT02257294

Purpose

1. To provide a comprehensive description of the approach to image analysis.
2. To optimise the analysis of the primary and secondary outcomes relating to cardiovascular magnetic resonance (CMR) in the T-TIME trial.

Scope

All individuals who analyse CMR data as a part of the T-TIME trial.

Key points

1. The primary outcome of the T-TIME trial is microvascular obstruction (% left ventricular (LV) mass) on the baseline CMR scan, as revealed by late gadolinium enhancement (LGE) imaging.
2. Core laboratory CMR analyses will be conducted blinded to the treatment group assignment and clinical outcomes. All of the data will be reviewed by at least 2 trained observers with expertise in CMR.
3. Customisation of image analysis programme. It is intended that the output of variables from the imaging analysis programme shall be matched to the list of variables designated to be provided to the clinical trials unit. By so-doing, the possibility of transcription errors is removed / minimsed.

Core Laboratory Staff

Name	Designation	Role in Image Analysis	Telephone	Email
Prof Colin Berry	Principal Investigator	Primary responsibility for reviewing and approving all of the MRI data	0141 330 1671 (office) 0141 951 5180 (hospital) 0783 169 8479 (mobile)	Colin.Berry@glasgow.ac.uk
Vanessa Orchard	Lead Radiographer, Imaging Analyst	Primary analysis - Analysis of T1 maps pre- and post-contrast, ECV calculation, image processing for perfusion analyses Second observer for all data	0141 951 5875	Vanessa.Orchard@gjnh.scot.nhs.uk
Peter McCartney	T-TIME Clinical Fellow, Imaging Analyst	Primary analysis – LV mass & function, area-at-risk (contrast-enhanced SSFP), microvascular obstruction and infarct size (contrast enhanced CMR), myocardial haemorrhage (T2* mapping) Second observer for all data	0141 951 5000	Peter.McCartney@glasgow.ac.uk
Dr Sasha Radjenovic	MR Physicist	T1-phantom – coordination and analysis; first pass perfusion analysis for myocardial blood flow at rest Second observer for all data	0141 330 3306 (office)	Aleksandra.Radjenovic@glasgow.ac.uk

CMR core laboratory image analysis

CMR data processing and analysis quality

Data handling

A de-identified CMR scans will be made available to the core laboratory in Glasgow using a secure web-upload or CD/DVD. The scan details will include the patient study code and date of the scan. If data must be transferred in a physical format it should be sent either by courier or registered post. A log of documents sent and received is maintained. The scan will include the patient study code and date of the scan.

Transfer of MRI scans from the sites

A de-identified electronic copy of each MRI scan will be made available by secure web-upload to the core laboratory in Glasgow, or when this is not feasible, then by CD/DVD. The CMR analyses will be conducted in line with local standard operating procedures.

CMR analysis – research data

The purpose of the core laboratory is research data analysis, and not to provide a clinical report. A clinical report should be issued for each CMR scan in line with local standards of clinical care. The scans will be prospectively analysed at the core laboratory on a dedicated workstations, blinded to treatment group assignment and clinical outcomes.

Main objectives

1. Quality assurance with site feedback
2. Provision of results for efficacy evaluation (primary and secondary outcomes)
3. Provision of results for mechanism evaluation
4. Generate a MRI database to support future education, training and research.

Imaging analysis equipment

VDU: Philips

Computers:

Software: QMass Medis Suite MR (Medis, Leiden, Netherlands). The 'industry-standard' software enables semi-automated standardised thresholding and border-delineation of areas-of-interest.

Environment: Core laboratory based in the Golden Jubilee National Hospital and the BHF Glasgow Cardiovascular Research Centre. The equipment in each site is identical. Access to imaging data is enabled by 'roving' licence log-in. De-identified imaging data are transferred between sites using secure, encrypted storage devices until such times as the University and NHS Golden Jubilee install network links.

Procedure for analysis of imaging datasets

General set-up

The scans from all of the centres will be coded, de-identified and analysed blind to treatment group assignment and clinical outcomes. CMR scans will be prospectively analysed by image analysts with expertise in cardiac CMR.

User-involvement will be retained to adjust endocardial / epicardial borders (e.g. exclude blood pool, exclude nonsense parameterization, etc.) and image artefacts. The imaging analysis software is an essential part of a core laboratory approach. The border settings for the contours applied to LV borders and infarct pathologies i.e. regions of interest (ROI), should be applied throughout the multimodality set of images in order to ensure co-registration of ROI between acquisitions. For paired measurements for secondary outcomes, the baseline and follow-up CMR scans will be analysed together (side-by-side). This approach will ensure standardised settings and measurements for the baseline and follow-up scans, including spatial matching of the infarct zone and relevant ROIs.

Workflow and organisation

The scans will be assessed by two trained observers at least one of whom should also have at least 5 years' experience in CMR imaging. The first analyses will be undertaken by Dr. Peter McCartney and Ms. Vanessa Orchard. The second analysis will involve a combined review with the first observer and second observer (Colin Berry, Sasha Radjenovic) of the initial images, borders and related results. The third step will involve a final check before sending to the CTU.

The workflow is dichotomized. Peter McCartney will lead on the analyses of LV borders, area-at-risk, infarct size, microvascular obstruction and myocardial haemorrhage. The second analysis will be primarily led by Colin Berry. Vanessa Orchard will lead on the analyses of pre- and post-contrast T1 maps and image processing of first pass perfusion for quantitative analysis of

myocardial blood flow. The second analysis of those data will be primarily led by Sasha Radjenovic.

Release of data to the CTU

The third step of review will involve a group-review of the results of the imaging analyses to assess for 'none-sense' results e.g. due to transcription error, or outlying datasets. The review should involve at least 3 individuals together. Individual images may be subject to further review. The overall aim is for results to be transferred to the CTU on a 3-monthly basis. In turn, feedback from the CTU Data Manager on those data is also anticipated.

1. MRI image quality

Each CMR scan will be logged and assessed for quality and feedback will be provided to the local site, as appropriate.

MRI image quality is influenced by technical and patient-related factors e.g. breathing artifact. Sometime, image quality is insufficient to be of diagnostic purposes. Image quality can be graded based on assessment by experienced observers and a Likert scale will be used:

1: 'excellent' defined as "no artifacts"

2: 'good' defined as "containing minor artifacts which do not adversely affect diagnostic value";

3: average' defined as "borderline clinical use due to the image quality";

4: 'poor defined as "major artifacts exist and clinical use is, therefore, not advised";

5: 'extremely poor' defined as "major artifacts exist and the images are not clinically useful";

The Likert scale will be used to record (A) Overall scan quality (for the whole study), (B) the quality of the key datasets, i.e. contrast-enhanced cine-MRI, T1 map, T2* map, first pass perfusion, late enhancement, post-contrast T1-maps.

Scans that are classified as Likert grade (4) or (5) will be designated in the database as unsuitable for analysis. The data on scan quality is considered essential in order to prospectively quality assure the imaging data, provide feedback to sites going forward, optimize the overall quality of the imaging data and so reduce measurement variability for the primary and secondary imaging outcomes.

Imaging analysis for the primary and secondary outcomes

The primary and secondary outcomes are listed in Appendix 1.

Methodology

There may be different analysis approaches for some of the CMR parameters. The methods proposed for each set of parameters reflect the optimal methods based on the current literature and best practice.

There are several options for the analysis of infarct size, and the consensus of expert opinion currently favours (1) full-width half maximum (FWHM) for infarct zone vs. remote zone signal intensity (S.I.) or (2) x5SD threshold difference between signal intensity in the infarct zone vs. remove zone. Potentially, the x5SD over-estimates infarct size as compared with the FWHM approach.

The approach to delineation of MVO is much less mature. The standard approach is to discriminate MVO from the surrounding territory of infarction by a difference of >x2SD, regardless of the method for determination of infarct size. To date, there are no publications that have described comparisons of the incidence and extent (% LV mass) of MVO, when assessed with different infarct sizing methods. Nor are there publications in which a manual approach has been compared with automated computerized approaches. In T-TIME, our plan had been to prospectively assess this question prior to the outset of the trial. However, the imaging analyst who had been initially employed and designated to undertake this work left his post without completing the project. Therefore, the MVO methodology project has been re-assigned to an imaging analysis (Aidan Morgan, post-BMedSci) who has spent one year undertaking MRI analysis in the BHF MR-MI study
https://clinicaltrials.gov/ct2/show/NCT02072850

The results of the MVO analysis will be used to inform the decision on which approach to finally use for the blinded analyses in T-TIME. Reflecting the need to maintain workflow within the study, the CMR scans will analysed using both x5SD and FWHM. Potentially, these analyses will support a future publication on methodological differences.

Primary and secondary outcomes and outcomes for mechanisms evaluation

CMR measurements, including LV dimensions, LVEF, infarct scar, haemorrhage, MVO, and LV remodelling index at follow-up (minimum infarct wall thickness / maximum remote zone

thickness in mid-diastole), will be prospectively analysed and recorded in a database. Contrast-enhanced SSFP will be used to assess area-at-risk, and T2* CMR will be used to assess infarct zone haemorrhage.

LV dimensions: LV dimensions, volumes and ejection fraction will be quantified using automated planimetry (QMass Medis Suite MR (Medis, Leiden, Netherlands). The borders will be assessed by visually and adjusted to improve border approximation, wherever necessary. Papillary muscles will not be included. The base of the LV stack will be exclude the atrio-ventricular plane with minimum involvement of the LV outflow tract. The LV apex will be included. The approach will be standardized with the same observer applying the LV settings for all of the scans at baseline and follow-up.

Adverse remodeling: Adverse remodeling was defined as an increase in LV end-diastolic volume ≥ 20% at 6 months from baseline.

Area-at-risk: Area-at-risk will be defined as LV myocardium with pixel values on the contrast-enhanced cine scan >2 SDs from remote myocardium. The myocardium in each LV short-axis slice will be manually analysed by delineating the LV epicardial and endocardial borders. The cine-set of images for each slice will be reviewed throughout the cardiac cycle (from end-diastole to end-systole) and the border of the hyperintense zone of edema will be defined by the user taking care to exclude artefact, blood pool and apparently normal tissue. The size of the AAR will be expressed as a percentage of the total LV myocardial volume.

Myocardial Salvage: Myocardial salvage will be calculated by subtraction of percent infarct size from percent area-at-risk. The myocardial salvage index will be calculated by dividing the myocardial salvage area by the initial area-at-risk.

Myocardial haemorrhage: The source imaging data may be a T2*-GRE method or a map from a T2*-map sequence. A hypointense core within the infarct zone on T2* CMR will be taken to represent myocardial haemorrhage.

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. 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. On the T2* maps, a region of reduced signal intensity within the infarcted area, with a

T2* value of <20 ms will be considered to confirm the presence of myocardial hemorrhage. T2* map image was assessed for the presence of artefacts relating to susceptibility effects or cardio-respiratory motion. Each motion-corrected series was evaluated for image alignment. Each map was evaluated against the original images. When artefacts occurred, the affected segments were not included in the analysis. The presence/absence and extent (% LV) of myocardial haemorrhage will be recorded, as well as Likert image quality and artefacts.

Infarct size: Quality assurance will be performed, including Likert rating and artefact assessments. The number of slices will be verified to ensure that duplicate acquisitions are not included in the analysis dataset. The myocardial mass of late gadolinium (grams) will be quantified using computer assisted planimetry. The presence of acute infarction will be established based on abnormalities in cine wall motion, rest first-pass myocardial perfusion, and delayed-enhancement imaging. Acute infarction will be considered present only if late gadolinium enhancement is confirmed on both the axial and long axis acquisitions. The myocardial mass of late gadolinium (grams) will be quantified using software programmes computer (QMass Medis Suite MR (Medis, Leiden, Netherlands)) with user-adjustment to exclude nonsense borders and artefacts. Two approaches will be invoked, namely x5SD and FWHM above a remote reference region and expressed as a percentage of total LV mass [12]. Infarct regions with evidence of microvascular obstruction will be included within the infarct zone.

Microvascular obstruction: The presence, extent (percentage of total LV mass) and distribution of microvascular obstruction (MVO) within the infarct zone will be assessed separately. MVO is defined as a dark zone on first pass imaging post-contrast injection and within an area of late gadolinium enhancement. Microvascular obstruction is classified as relevant (central dark zone with a sub-endocardial or intra-mural distribution) surrounded by hyperintense late enhancement and non-relevant (dots or nil). A difference in SI of at least x2SD difference between the hypointense core of MVO and surrounding hyperintense zone of infarction without MVO is required.

These analyses will be conducted by Peter McCartney and Colin Berry.

Native T1 maps pre- and post-contrast will be measured in regions of interest (infarct core, infarct zone, remote zone) and extracellular volume will provide data to help inform mechanistic evaluations. These analyses will be conducted by Vanessa Orchard with the support and supervision of Colin Berry and Sasha Radjenovic.

Myocardial native longitudinal relaxation time (T1) reflects tissue water content and cellularity T1-mapping analysis will make use of images obtained pre- and 15 minutes post-gadolinium

contrast administration. T1 maps are acquired in 3 short-axial slices (basal, mid and apical), using typically a modified look-locker inversion-recovery (MOLLI) investigational prototype sequence (Work-in-Progress (WIP) method 448, Siemens Healthcare) or ShMOLLI method.

Native T1-mapping

Native T1 mapping - standardised measurements in myocardial regions of interest

Native T1 mapping is a CMR method providing a parametric colour-encoded anatomical map in which the T1 value is encoded in each pixel. The native T1 map analyses are informed by contemporary CMR guidelines from SCMR. T1 maps will be assessed for image quality against a Likert scale and artefacts recorded. The type of map (MOLLI, ShMOLLI, other) will also be recorded.

The overall aim is to ensure spatial co-registration of image slices and ROI within-subject scans for T1 maps, pre- and post-contrast, as well as with other scan domains eg. T2* for haemorrhage. A team-based approach is envisaged to ensure like-for-like analysis.

LV contours will be delineated with computer assisted planimetry on the raw T1 image and copied onto the colour-encoded spatially co-registered map. Apical segments will not included because of partial volume effects. Particular care will be 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 pool. Each T1 map image will be assessed for the presence of artefacts relating to susceptibility effects, or cardio-respiratory motion. Each colour map will be evaluated against the original images. When artefacts occur the affected segments were not included in the analysis.

Myocardial T1 values will be segmented spatially and regions of interest were defined as (1) remote myocardium, (2) injured myocardium and (3) infarct core. The regions of interest will be planimetered to include the entire area of interest with distinct margins of separation from tissue interfaces to avoid partial volume averaging. The remote myocardium region of interest will be 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 cine images and late gadolinium enhanced images, respectively). The infarct zone region of interest will be defined as myocardium with pixel values (T1) >2 SD from remote myocardium. The hypointense infarct core will be 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. The assessment of T1 maps and adjudication (present/absent) of a hypointense core was performed independently by V.O., C.B, and P.M.

ECV measurement

LV contours were delineated with computer-assisted planimetry on the best spatially matched raw T1 image and copied onto color-coded spatially co-registered maps. Care was taken to have adequate margins of separation from tissue interfaces, such as myocardium and blood, to prevent partial volume averaging. Regions of interest will be drawn in 1) infarct zone, including the entire area of injury; 2) remote myocardium, placed 180° from infarct zone; 3) LV blood pool. Measurements will also performed in infarct zone excluding core. Regions of interest will be copied between the pre- and post-contrast T1 maps with manual correction to maintain margins of separation from tissue interfaces. ECV will be calculated as a ratio of corresponding T1 values measured pre- and post- contrast in each of the regions of interest. The use of imaging analysis software (Medis, Leiden) should enable automatic registration of ROIs. ECV will be calculated using Eq. (1), where Lambda (λ)=ΔR1myocardium/ΔR1blood, ΔR1=R1post-contrast-R1pre-contrast, R1=1/T1 [9]. Hematocrit (HCT) will be measured at the time of scanning.

$$ECV = (1\text{-}HCT) \times \lambda$$

Analysis of first pass contrast CMR: Using the modified ACC / AHA 16-segment nomenclature, segmental first pass will be interpreted as normal or abnormal. Each segmental abnormality will be scored on the basis of the transmural extent of the perfusion defect (absolute percentage). This value can then be described by the biostatistician in an ordinal system: 0=no defect, 1=1% to 50%, 2=51% to 100%). The apical cap (segment 17) will not be assessed because of the short-axis acquisition, so this segment will be treated as missing (i.e. 16 segment model). A perfusion defect will be deemed relevant only if it persists beyond peak myocardial enhancement. In an exploratory analysis, since non-transmural perfusion defects can be discriminated with high spatial resolution CMR, the myocardium will be divided into endocardial and epicardial segments, thus resulting in 32 segments in total. The quantitative analyses of myocardial perfusion will be led by Vanessa Orchard and Sasha Radjenovic.

Pilot data

Evidence of the reproducibility of MVO:

The day 2 CMR scans of 25 consecutive STEMI patients treated with primary PCI in the Golden Jubilee National Hospital (September-October 2012) were independently assessed by 2 experienced observers. The CMR scans were analysed for the presence and extent of late MVO. There was 100% agreement between the 2 observers for MVO (Cohen's Kappa statistic=1). Twenty patients had MVO and the mean±SD extent of MVO in these patients were 2.9±3.4% and 3.2±3.6%, respectively. The 95% confidence intervals for systematic bias between the observers was -0.64% to 0.18% which indicates that there was no evidence of bias (p=0.26). The 95% limits of agreement were -2.2% to 1.7%.

Non-standard scans and incidental findings

Not all datasets will always follow the standard format, due to unforeseen factors (e.g. repeated acquisitions, technical issues, patient compliance, etc.). In these cases, analysis procedure will be implemented where applicable, and more experienced imaging staff and PI consulted.

Occasionally, incidental findings (observations of potential clinical significance that are unexpectedly discovered and unrelated to the purpose or variables of the study) may appear. These are anticipated to be detected at the site by the attending clinical staff. but should prognostically relevant incidental findings be disclosed during the core laboratory analysis the finding(s) the local site will be informed as a duty of care.

Data storage

Imaging data (scans and their related analyses) will be stored on the workstations and also separately on data drives. It is anticipated that networked storage will be enabled during the lifetime of the project.

Quality assurance

Feedback to sites

There will be a continuous quality assurance process throughout the trial with feedback to sites on CMR scan quality. An assessment of CMR data acquisition and transfer quality will be

obtained from each site before enrolment of study participants, including of a T1 phantom for calibration, wherever possible. The feedback should include the Likert rating of overall scan quality and for components of each scan, and details of the nature of the imaging problems. Where there are no issues with data quality then no feedback is necessary.

Core laboratory approach

Core laboratory CMR analyses will be undertaken by a trained staff with expertise in cardiac CMR. All of the data will be reviewed and approved by the CMR cardiologist (Prof Berry) and quantitative analyses will be assured by Dr Radjenovic. Therefore, all scans will be reviewed by at least 2 trained observers.

Data reporting to the CTU

The MRI data will be routinely reported to the CTU. The core lab team will schedule a data review meeting prior to transfer of data to quality assure the results before release to the CTU. The frequency of each data export to the CTU should be no less frequently than once every 3 months. By so-doing, the data will be prospectively entered and managed by the CTU to avoid down-stream delays with CTU quality assurance checks, including of missing data.

Quality assurance within the core laboratory

Inter and intra observer variability will be assessed periodically e.g. annually. All analyses (contours, reports) will be archived, and extracted measurements recorded using single entry on a main Excel datasheet. Data entry accuracy is validated periodically by control checks and logical checks performed to ensure plausibility of results.

Unforeseen events

Data loss

At the end of an analysis session, all MR analyses conducted at the core laboratory (contour files and reports) are automatically backed up to a local picture archiving and communications system (PACS), with an incremental version number indicator to identify repeated analyses. MRI variables defined in Appendix 2 are recorded to an electronic Clinical Research Facility database (e-CRF) after each scan is analysed.

Once identified, a potential data loss is investigated following local operating procedures. Any incident of a potential data loss is reported to the Principal Investigator (PI).

Infrastructural loss

Critical assets for CMR analysis include: access to the de-identified CMR data, analysis workstation and PACS link, and access to e-CRF. A full or partial loss of any of these will present a significant challenge to MR analysis.

Analysis workstations are password protected and located at a secure University building. Access to a local PACS is limited to the dedicated workstations within analysis room, which can be accessed only by the staff working at the MR unit. The premises are secured with fire alarm, crime prevention systems and close-circuit television (CCTV) system. Electronic study portal (e-CRF) is password protected and regularly backed up.

Incident response follows dynamic risk assessment process, and reconstruction is aimed to maximise the recovery of the data and resources, as managed by the PI.

Appendix 1: Primary and secondary efficacy parameters

A final list of the MRI variables to be provided to the Clinical Trials Unit is established in line with the protocol and the customised read-out of the imaging analysis programme. The MRI variable list is pre-specified as a near automated output to be provided to the trials unit.

Primary efficacy parameter for the clinical trial

The amount of MVO (% of left ventricular mass) revealed by LGE 10 – 15 minutes after contrast administration on an MRI scan performed 2 days post-MI.

Secondary efficacy parameters

MRI at baseline

First pass MVO extent (% of LV); NB that FP will be acquired in 3 SAX levels to provide an index of %LV FP MVO

Early MVO extent (% of LV) on 1 min post-gadolinium contrast enhanced MRI, adjusted for area-at-risk at baseline

Late MVO (presence / absence) on LGE

Initial infarct size (LGE)

Initial myocardial salvage index (initial infarct size/area-at-risk)

LV end-diastolic volume index (LVEDVI)

LV end-systolic volume index (LVESVI)

LV remodelling index (minimum infarct wall thickness / maximum remote zone thickness mid-diastole)

LV ejection fraction (LVEF)

Systolic wall thickening in the culprit artery territory

Wall motion score index

Myocardial haemorrhage (presence/absence)

Myocardial haemorrhage extent (% of LV)

Follow-up MRI

Final infarct size

Final myocardial salvage index (final infarct size/initial area-at-risk)

Change in infarct size 3 months after procedure (2 day post PCI versus 3 month post-MI)

Final LV end-diastolic volume index (LVEDVI)

Final LV end-systolic volume index (LVESVI)

Final LV ejection fraction (LVEF)

Final systolic wall thickening in the culprit artery territory

Final wall motion score index

Change from baseline LV end-diastolic volume index (LVEDVI)

Change from baseline LV end-systolic volume index (LVESVI)

Change from baseline LV ejection fraction (LVEF)

Change from baseline in systolic wall thickening in the culprit artery territory

Change from baseline in wall motion score index

Primary efficacy variable

ALTERNATIVE HYPOTHESIS: A Chi squared test (continuity correction) will be used to compare the proportions with logistic regression being used to calculate confidence intervals. Since there are 3 groups (alteplase 20mg, alteplase 10mg and placebo), we will first compare alteplase 20 mg vs. placebo with $p<0.05$ required for significance. If this test is significant we will then test alteplase 10 mg vs. placebo. This hierarchical approach will conserve the overall type I error at 5%. Hence we will require a minimum of 558 patients with data for late MVO on the Day 2 MRI scan. To allow for deaths and intolerance of MRI (e.g. claustrophobia) we will recruit 618 patients (n=206/group). The sample size will be sufficiently large to address feasibility, safety and efficacy. However, since efficacy, safety and ease-of-use will all be important when considering the approach to alteplase administration, information on these parameters will be evaluated to form an overall strategic view to select one for the future Phase III trial.

Secondary efficacy analysis

Between group differences in infarct size & myocardial salvage as quantitative traits (% of LV) will be assessed using ANOVA adjusting for initial area-at-risk (% of LV). Other continuous outcomes will be analysed in a similar fashion where data are approximately normally distributed and where baseline levels are available for adjustment. Where this is not the case, two sample t-tests and corresponding confidence intervals will be used. Where data are clearly not normally distributed (e.g. laboratory variables) standard transformations will be applied to achieve approximate normality prior to analysis. CLINICAL OUTCOMES will be presented with Kaplan-Meier time-to-event curves & compared where appropriate using log rank tests. The angiographic parameters will be correlated with clinical outcomes.

Appendix 2: MRI variables recorded

Key points

1. CMR is performed twice – 2 days (baseline) and 3 months (follow-up) post-MI
2. The primary outcome of the T-TIME trial is microvascular obstruction (MVO) as revealed by late gadolinium enhancement 10-15 minutes after gadolinium contrast (0.15 mmol/L) administration.

Table 1: MRI variables recorded at the baseline and follow-up

Variable name	Parameter description	Unit
Scan image quality	Overall scan quality	High/adequate/poor/unusable
Late_MVO_presence	MVO presence/absence, on 15 post-gadolinium contrast enhanced MRI	Yes / no
Late_MVO_extent	Amount of microvascular obstruction (MVO)	% of left ventricular (LV) mass
Infarct_size	Infarct size	% of LV mass
Haemorrhage_presence	Myocardial haemorrhage presence/absence	Yes / no
Haemorrhage_extent	Myocardial haemorrhage extent	% of LV mass
T1_AAR	T1 relaxation time measured in region-of-interest (ROI) of injured area-at-risk (AAR)	ms
T1_core	T1 relaxation time measured in ROI at infarct core	ms
T1_peri	T1 relaxation time measured in ROI at peri-infarct tissue oedema zone	ms
T1_remote	T1 relaxation time measured in ROI at remote non-infarcted ('healthy') zone	ms

T2*_injured	T2* relaxation time measured in ROI of injured zone	ms
T2*_core	T2* relaxation time measured in ROI at infarct core	ms
T2*_peri	T2* relaxation time measured in ROI at peri-infarct tissue zone	ms
T2*_remote	T2* relaxation time measured in ROI at remote non-infarcted ('healthy') zone	ms
First pass MVO extent	First pass microvascular obstruction (MVO) extent	% of LV mass
MBF	Myocardial blood flow (perfusion) at rest Record three ROI values: 1) infarct, 2) infarct core, 3) remote NB: z-level will be determined by maximal extent of infarct size at scan 1 or scan2. If no lesion present, record mid-ventricular MBF for remote.	ml/g/min
Delta-T	Time delay between AIF and myocardial regions 1, 2 and 3 (defined as above). z-level defined in the same way as for MBF	ms
Early_MVO_presence	MVO presence/absence, on 1 min post-gadolinium contrast enhanced MRI	Yes / no
Early_MVO_extent	Early MVO extent, on 1 min post-gadolinium contrast enhanced MRI	% of LV
LVEDVI	LV end-diastolic volume index	ml/m^2
LVESVI	LV end-systolic volume index	ml/m^2

LVEF	LV ejection fraction	%
WMSI	Wall motion score index	
Remodelling_index	LV remodelling index (minimum infarct wall thickness / maximum remote zone thickness, at mid diastole)	
Culprit_wall_thickening	Systolic wall thickening in the culprit artery territory (with respect to end-diastolic)	%
Salvage	Myocardial salvage index (infarct size / area-at-risk)	

Appendix 6: T-TIME trial standard operating procedure for acquisition of coronary physiology measurements

T-TIME Standard Operating Procedure for Coronary Physiology

Protocol Version 4.0; Clinicaltrials.gov registration: NCT02257294

Contact details

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Introduction

The index of microvascular resistance (IMR) is a simple coronary guidewire-based method for assessing coronary microvascular function (1-3). IMR can be used to study the pathophysiology of microvascular function in the culprit coronary artery of patients with acute ST-segment elevation myocardial infarction (STEMI) (4-9).

T-TIME is a randomised placebo-controlled trial designed to determine the safety and efficacy of reduced doses of intra-coronary alteplase (a thrombolytic drug), as an adjunct to standard care (primary-PCI) in patients with acute STEMI (10). The primary outcome is the amount of severe persistent heart injury, as reflected by microvascular obstruction (MVO) revealed by cardiac MRI 2 – 7 days later.

T-TIME presents an opportunity to assess microvascular injury in the territory of the culprit coronary artery using guidewire-based coronary thermodilution. The objective of this sub-study is to elucidate whether IMR (a test of microvascular function), measured at the end of primary-PCI, differs between alteplase- and placebo- treated groups in T-TIME participants. The project has potential to validate IMR as a novel test for identifying patients who might benefit from this new intra-coronary alteplase treatment approach. In this case, IMR could be used to immediately identify patients at risk of MVO (i.e. IMR>40) and potentially be then used to identify patients who might benefit from targeted therapy with reduced dose-intra-coronary alteplase.

Safety

The Glasgow group has measured IMR studies in several hundred patients with acute STEMI their experience is that IMR measurement are routinely feasible, safe and informative (6-9).

Cost

The Certus pressure wire will be provided to sites at no cost by the University of Glasgow for use in the trial

Key objectives for the coronary physiology sub-study in T-TIME

1. Perform guidewire-based thermodilution to assess CFR and IMR.
2. Quality assurance including training and feedback to participating sites.

Coronary physiology parameters derived from thermodilution

Principles

Since coronary flow and resistance are inversely related, microvascular function can be measured by integrating pressure and temperature when measured simultaneously. The technique of measuring coronary flow based on transit time is called thermodilution. The

inverse of coronary transit time is representative of flow. This measurement can be achieved by injection of a bolus of saline at room temperature (e.g. 18◦C) which mixes with blood (body temperature 37◦C) and the change in temperature reflects coronary flow.

Practical considerations

The measurements can be made using a pressure- and temperature-sensitive coronary guidewire. The parameters can be easily determined using thermodilution at rest (3 saline injections) followed by thermodilution (3 further injections of saline) during hyperaemia induced by intravenous adenosine. Each injection involves manually administering 3 – 4 ml of saline (room temperature). The intra-coronary injection of saline should be given as a brisk bolus directly into a flushed guide catheter. The same volume should be given for all of the injections. The wire position should also remain constant.

Parameters

The parameters are 1) the index of microvascular resistance (IMR, measured during hyperemia), 2) coronary flow reserve (CFR) and 3) the resistance reserve ratio (RRR). The equations which explain these parameters are listed in Appendix 1.

Blinding

IMR is a prognostic marker, and so the result might influence clinician's behaviour. For example, a very high IMR result might stimulate a clinical decision to give additional therapy e.g. glycoprotein IIbIIIa inhibitor therapy (even if there is no clinical evidence to support such a plan). It is also plausible that the IMR result may be associated with the randomly assigned study therapy. Therefore, we propose that the IMR result should be obscured from the attending cardiologist. Blinding can be easily achieved by obscuring the haemodynamic console from the attending cardiologist, i.e. turning it 180◦. Blinding will help to minimise bias.

Methods

Timing

IMR is requested to be measured at the end of the procedure after stent implantation and post-stent optimisation. The IMR measurement would be the final action in the primary PCI procedure.

Practical considerations

- A PressureWire Certus (Abbot) should be used. This wire connects with a cable to the RADIAnalyzer console (not the Quantien Bluetooth wire).
- Use the RADIAnalyzer and access the CFR page view by clicking enter 'System', then click enter CFR page.

- Keep the standard wire in situ and use this wire as a 'buddy wire' in order to safely pass the diagnostic wire through the stent and into the distal half of the culprit artery.
- Staff should prepare adenosine for intravenous administration (infusion rate 140 µg/kg/min). In order to be time efficient, the IV adenosine could be prepared whilst the guidewire is being positioned.
- The Certus wire has a microsensor 3 cm from the distal tip. The guide-wire should be calibrated outside the body, equalized with aortic pressure at the ostium of the guide catheter and then advanced 6 - 9 cm into the distal third of the culprit artery as is normal practice for FFR measurement.
- FFR is not valid when measured in the culprit artery of a patient with acute STEMI, therefore, the focus of this study is on CFR, IMR and related parameters.

Measurement of microvascular parameters

IMR and CFR can be measured at the same time. The basal resistance index is defined as the distal coronary pressure multiplied by the mean transit time of a 3 ml bolus of saline at room temperature during resting conditions, measured simultaneously (mmHg x s, or units) (1-9). IMR is measured in the same way with resistance minimised with steady-state hyperemia induced by IV adenosine (at least 60 s before the first saline injection). The mean aortic (RED) and distal coronary (GREEN) pressures should be recorded during maximal hyperaemia.

Previous studies in patients with stable coronary disease have established that IMR is repeatable and independent of haemodynamic variations, including heart rate, blood pressure and myocardial contractility (1-5).

If the Certus wire is used during PCI, the distal coronary wedge pressure during balloon occlusion may be measured.

Recordings should be made on the CFR page (Figure 1).

Set-up

All recordings should be made on the 'CFR page' of the RADIXExpress console.

Practical steps for set-up and guidewire positioning

1) The pressure wire should be flushed in its package tubing
2) Connect the wire flat on the cath lab table to the RADIExpress console
3) Calibrated and zero the wire pressure recording to ensure standardised measurements.
4) Avoid handling the pressure sensor, 3 cm from the tip of the wire on the proximal part of the marker
5) Pass the wire through a needle introducer, advance through the guide catheter to is distal end
6) Withdraw the needle introducer and close the Tuohy-Borst Y connector (i.e. close on the wire).
7) Equalise aortic (red) and pressure wire (green) tracings.
8) Ensure the guide catheter is intubated, co-axial and not associated with a damped / ventricularised coronary pressure waveform.
9) Re-insert the Tuohy-Borst Y connector to advance the coronary wire into the coronary artery (ideally 60 mm (2 marker lengths) beyond the stenosis, where possible), and then remove the Tuohy-Borst to make the recordings.
10) Give 200 micrograms of intra-coronary nitrate.
11) Ensure that all radiographic contrast is flushed out of the guide catheter.
12) Record resting pressure (Pa, aortic and Pd, distal coronary) for at least 15 heart beats.

Administration of intra-venous adenosine

1) Administer intravenous adenosine (140 ug/kg/min) with a rise in heart rate (rise) and fall in blood pressure (including separation of systolic and diastolic recordings vs. baseline). Adenosine is contra-indicated in patients with significant asthma (e.g. routine use of bronchodilator therapy) and heart block).

2) Response to adenosine: typical changes in blood pressure, heart rate and symptoms should be recorded prospectively to confirm a haemodynamic response to adenosine. Following a 2 minute infusion period, typical haemodynamic changes indicative of a functional response to adenosine response are:

- symptoms of chest tightness, chest pain, wheeze
- fall in systolic blood pressure by 20% of the resting value

- fall in diastolic blood pressure by >20% of the resting value - widening of pulse pressure - rise in heart rate >10% from baseline 3) When the response to adenosine is inadequate the standard dose of adenosine (140 mcg/kg/min) should be increased up to 210 mcg/kg/min in order to best ensure maximal hyperaemia. 4) With steady state hyperaemia (typically after 60 s of adenosine infusion), record the lowest FFR value.
Administration of intra-coronary adenosine
• Not recommended for IMR measurement; intravenous adenosine is preferred. We have found this approach to be safe in several hundred patients with acute STEMI.
IMR quality assurance
• Consider obtaining a second IMR value during the same diagnostic procedure is good practice.

Recording the microvascular parameters – RADIAnalyzer

Practical considerations

1. Obscure the monitor from the attending cardiologist by turning it 180 degrees.

2. Create the patient record using the study number, not the patient name.

3. Access the CFR page by pressing enter 'System' and then enter 'CFR' on the RADIExpress console, then press "record"

4. Ensure steady resting conditions

5. Flush the guide catheter of all contrast. For resting and hyperaemic injections the guide catheter needs to be engaged to ensure saline delivery into the coronary artery

6. ***ENSURE THE AORTIC PRESSURE (Pa, RED) is recorded (i.e. pressure line 'open').***

7. ENGAGE THE GUIDE CATHETER so that it is sitting in the coronary artery to ensure that the saline passes directly into the coronary artery and not the aorta.

8. Resting injections x3 – each bolus is 3 ml of saline (room temperature); with each injection a thermodilution curve is displayed in blue and a transit time is displayed above. The mean transit time reflects the average of 3 measurements.

9. Please repeat any injections for any outlying/discordant transit times

10. Switch on the IV adenosine (140 µg/kg/min) and wait for 1 - 2 min (confirm clinical response to adenosine)

11. FLUSH THE GUIDE CATHETER of saline that may have warmed in the guide catheter inside the patient between saline injections since warming can increase variability. Therefore, each saline injection should be preceded by flushing the guide with saline so the volume of fluid injected has uniform room temperature

12. Perform 3 consecutive saline bolus injections during hyperaemia.

13. Please repeat any injections for any outlying/discordant transit times

14. Please record the data on the T-TIME catheter laboratory worksheet. A physiologist or trained nurse could do this on behalf of the team. The recordings should be written down at the time of the measurement acquisitions.

Training

Training for those not experienced in invasive measurements of coronary microvascular function will be provided by staff from Abbot, Annette Maznyczka and Prof Colin Berry, as appropriate. Please contact Prof Berry (details, page 1).

Data handling

For simplicity, it is proposed that the physiological recording for each patient is saved and exported to the Glasgow Core Laboratory for analysis. Since each console has space for 40 recordings only, ***export should be done once per week*** or immediately after the study recording has been obtained.

De-identified study number labeled pressure wire files .xml and .jpg should be saved locally and then emailed to: amaznyczka@nhs.net

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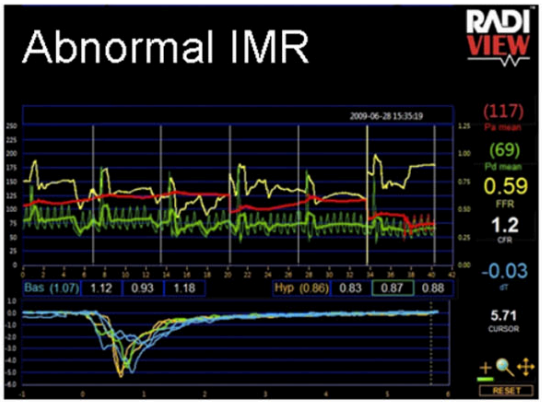
Appendix 1. Case examples

Index of microvascular resistance
IMR* = Pd x transit time



IMR = 59 x 0.19 = 12
Low / normal IMR

IMR = 69 x 0.86 = 59
Elevated IMR

* Measured during peak hyperaemia achieved by intravenous infusion of adenosine (140 μg/kg/min)

The recording on the left is an example of thermodilution curves (blue at rest, orange at hyperaemia) that are closely associated (high precision). On the right (abnormal IMR), the resting thermodilution curves (blue) have more variability but the hyperaemic recordings (orange) are closely matched.

Appendix 2. Equations

Pd/Pa = Resting pressure index

$$FFR = \frac{\text{Pd, distal coronary pressure during hyperaemia}}{\text{Pa, aortic presssure during hyperaemia}}$$

$$CFR = \frac{\text{Coronary flow at hyperaemia}}{\text{Coronary blood flow at rest}} \Rightarrow \frac{\text{Transit time (rest)}}{\text{Transit time (hyperaemia)}}$$

IMR = Hyperaemic transit time × Pd

(during adenosine hyperaemia supplemented by an intra-coronary bolus of nitrate (200μg) or adenosine)

Resting resistance index = Pd (rest) x mean transit time (rest)

RRR = Basal resistance index / IMR

Appendix 7: T-TIME catheterisation laboratory worksheet

T-TIME Catheter Laboratory Worksheet

Study Number: **Date:** **Time:** hrs

1. ECG Timepoints

Cath Lab arrival ☐
** 1st Intervention ECG (post reperfusion/pre drug delivery) ☐
Procedure end ☐

2. Enter patient CHI and initials in radi analyzer (IMR substudy)

3. Patient response to adenosine Enter patients

	HR, bpm	SBP, mmHg	DBP, mmHg
Rest, adenosine			
During peak hyperaemia (> 1 – 2 min			

3. Resting data (Blue)

Segment	Pa	Pd	Mean Transit (rest)	Pd/Pa	HR	Wedge	RI (PdxTrest)	RPP

4. Hyperaemic data (Orange)

Segment	Pa	Pd	FFR	Mean Transit (hyp)	CFR	IMR (PdxThyp	RRR	RPP

5. LV end-diastolic pressure - ..... mmHg

6. Please press 'Stop view' when exiting patient record on radi analyzer

Appendix 8: T-TIME trial statistical analysis plan for the physiology sub-study

The British Heart Foundation T-TIME Coronary Physiology Study

Statistical Analysis Plan – version 1.1

Authors:
Dr Annette Maznyczka
BHF clinical research training fellow

Professor Alex McConnachie
Assistant Director of Biostatistics, Robertson Centre for Biostatistics

Professor Colin Berry
Honorary Consultant Cardiologist and Professor of Cardiology

Title: The British Heart Foundation T-TIME Coronary Physiology Study Statistical Analysis Plan	Version: 1.1
Status: Draft	Revised by: Annette Maznyczka Signature: A. Maznyczka Date: 31.10.2018
Original date: 06/07/2018	
Revision date: 17/09/2018	Approved by: Colin Berry Signature: Colin Berry Date: 29.10.2018 Approved by: Alex McConnachie Signature: Date: 02/11/18
Public registration: https://clinicaltrials.gov/ct2/show/NCT02257294	NCT02257294

1. STUDY BACKGROUND

The T-TIME trial (Trial of low-dose adjunctive alTeplase during primary percutaneous coronary intervention (PCI)) is a randomised, placebo-controlled trial in patients with ST-segment elevation myocardial infarction (STEMI), to test the hypothesis that a therapeutic strategy involving intracoronary alteplase will reduce microvascular obstruction (MVO) and future risk of heart failure.

Standard care with primary PCI does not include alteplase, therefore the following three arm design of adjunctive treatment following reperfusion but before stenting was implemented in the TTIME trial protocol:

Control Arm: placebo
Arm A: alteplase 10mg
Arm B: alteplase 20 mg

In the T-TIME IMR sub-study IMR will be acquired at the end of the PCI procedure using the Certus diagnostic wire (Abbott, UK). Enrolment will be based on operator discretion (i.e. operator experience with IMR).

The index of microvascular resistance (IMR) is a prognostically validated invasive test of microvascular dysfunction in the territory of the culprit coronary artery in acute STEMI patients. IMR represents an invasive test of the efficacy of myocardial reperfusion. Further, IMR has potential value as a biomarker of the effect of treatment targeting microvascular injury in STEMI. The utility of IMR measured at the end of PCI to serve as an immediate test of the efficacy of local thrombolysis to attenuate microvascular injury will be validated with the results of the TTIME trial.

2. OBJECTIVES

Primary Objective:

- To assess whether IMR, measured at the end of primary PCI, differs between those treated with alteplase and placebo treated groups

Secondary Objectives:

1. To assess whether, in patients with ischaemic time >/= 4 hours, alteplase will be associated with higher IMR, reflecting potential more severe microvascular injury from myocardial haemorrhage.
2. To assess whether, in patients with better coronary flow prior to drug, alteplase will be associated with lower IMR
3. To assess whether, in patients with higher thrombus burden, alteplase will be associated with lower IMR
4. To assess whether, in patients with higher IMR at the end of primary PCI, alteplase will be associated with myocardial haemorrhage and other CMR parameters, troponin area-under-the curve and parameters of haemostasis and coagulation, reflecting a mechanism of treatment effect (alteplase vs. placebo).
5. To assess the utility of IMR for the prediction of MVO and/ or myocardial haemorrhage, measured 2-7 days and 3 months after PCI, in a selected high-risk population
6. To assess the utility of IMR for the prediction of troponin area-under-the-curve.
7. To assess the utility of IMR for the prediction of other surrogate measures post MI including left ventricular remodelling, function, infarct size and infarct size regression disclosed by CMR and NT-proBNP at 3 months.
8. To assess the utility of IMR for the prediction of clinical outcomes and quality of life, measured up to 12 months after PCI

9. To assess whether other coronary physiology parameters (including coronary flow reserve (CFR), resistive reserve ratio (RRR), and thermodilution waveforms e.g. temperature recovery time (TRT)) differ between those treated with alteplase and placebo treated groups
10. To assess the utility of other coronary physiology parameters (including CFR, RRR, and thermodilution waveforms) for the prediction of CMR parameters measured at 2 – 7 days and 3 months, and clinical and quality of life parameters up to 12 months.

3. SAMPLE SIZE

The target sample size for the physiology sub-study is 256 subjects and the minimum sample size to provide worthwhile data is 108 subjects. Based on pilot data from the BHF MR-MI study (ClinicalTrials.gov: NCT02072850) in patients who fulfilled the eligibility criteria for T-TIME, if the mean and SD of IMR are 33.9 and 25.2 respectively, there would be 85% power to detect a between-group difference of 10 IMR units (placebo vs. alteplase (10 mg & 20 mg groups combined) with an alpha of 0.05. Allowing for up to 10% of patients with incomplete data, the target sample size is 256. For a comparison of IMR between 3 groups (placebo vs. alteplase 10 mg vs. alteplase 20 mg) if the mean (SD) IMR in the placebo group is 33.9 (25.2), and there are mean differences in IMR between the 10 mg & 20 mg alteplase groups vs. placebo of 10 and 20 respectively, then 108 subjects (n=36/group) would be needed (85% power) (minimum sample size).

Considering CFR, if 170 patients have CFR measured at the end of PCI (~10% for incomplete data) and 150 subjects have evaluable data then for a comparison of CFR between two groups (placebo vs. alteplase (10 mg and 20 mg groups combined)), and the mean (SD) CFR is 1.65 (0.80), there would be 85% power to detect a difference of 0.4 units (half a SD) with an alpha of 0.05. For a comparison of CFR between 3 groups (placebo vs. alteplase 10 mg vs. alteplase 20 mg), and the mean differences in CFR between the 10 mg and 20 mg alteplase groups vs. placebo are 0.4 and 0.8 respectively, for the same CFR in the placebo group, then 69 subjects (n=23 / group) will be needed, with 85% power.

4. STUDY DESIGN

Details of the T-TIME trial design, randomization, inclusion and exclusion criteria are described in the T-TIME trial study protocol.

5. STATISTICAL ANALYSIS PLAN GENERAL PRINCIPLES

All analyses will be carried out in those who received study therapy, with "by-treatment" analyses carried out according to the therapy received.

Data will be summarised overall, and by treatment group. Continuous variables will be summarised as the number of observations, number of missing values, mean, standard

deviation, median, quartiles, and range. Categorical variables will be summarised as the number of observations, number of missing values, frequencies, and percentages. Linear, or logistic regression, or Cox regression analysis will be used as appropriate. A p value <0.05 will be considered significant.

Missing data will not be imputed. No adjustments will be made for multiple comparisons.

The association between IMR and alteplase will be investigated in subgroups to help provide mechanistic insights.

The secondary outcomes may be dichotomised to facilitate analyses and/or presentation. Additional secondary outcome variables may include the following.

6. BASELINE CHARACTERISTICS

Baseline characteristics will be summarised in the population as a whole, by treatment group (alteplase vs. placebo). The baseline characteristics reported may include:

- Age
- Sex
- Treatment times
- Heart rate
- Blood pressure
- Body mass index
- Serum creatinine and/ or estimated glomerular filtration rate
- Hypertension
- Renal impairment
- Diabetes mellitus
- Smoking (never, former, current – some days, current – every day)
- Previous MI
- Previous PCI
- CCS angina class
- NYHA functional class
- Killip class
- Medications
- Troponin
- Ischaemic time (time from symptom onset to first treatment)
- TIMI flow grade on initial angiogram
- TIMI thrombus grade on initial angiogram
- Infarct related artery
- Reference vessel diameter
- Number of main coronary vessels with stenoses > 50%
- Rentrop collateral grade to the culprit artery
- Dominance
- Total stent length
- Medications

7. SECONDARY OUTCOME VARIABLES FOR ACUTE INDICES OF MYOCARDIAL REPERFUSION FROM INVASIVE CORONARY PHYSIOLOGY, ANGIOGRAM AND ECG

The secondary outcome variables for acute indices of myocardial reperfusion obtained from the invasive coronary physiology, angiogram and ECG, may include the following:

Invasive coronary physiology and Left ventricular (LV) haemodynamics

- Invasive coronary flow reserve (CFR), IMR and fractional flow reserve (FFR) at the end of PCI. In addition, these parameters may be collected during the procedure, when logistics permit.
- Invasive temperature recovery time (TRT) at the end of PCI +/- temperature recovery constant at the end of PCI
- LV end-diastolic pressure

Coronary angiography

- TIMI myocardial blush grade at the end of the procedure
- TIMI frame count (TFC) at the end of the procedure
- TIMI coronary flow grade at the end of the procedure
- TIMI coronary flow grade immediately post study drug
- Intraprocedural change in TIMI myocardial blush grade (pre-study drug vs. at the end of PCI)
- Intraprocedural change in TFC (pre-study drug vs. at the end of PCI)
- Intraprocedural change in TIMI coronary flow grade (immediately pre- vs. post study drug)
- Intra-procedural thrombotic events

ECG

- % ST-segment resolution on the ECG (pre- vs. 60 min post-reperfusion with primary PCI)
- Surrogate ECG markers of infarct size (Selvester QRS score, Anderson ST Acuteness score)

8 CMR OUTCOMES

8.1 CMR INFARCT PATHOLOGY OUTCOMES

CMR performed 2-7 days post-MI:

- Extent of late MVO (% of left ventricular (LV) mass) revealed by late (10 – 15 minutes after contrast administration) gadolinium contrast-enhanced MRI.

- Presence/ absence of late MVO (% of LV mass), 10 – 15 minutes after contrast administration on MRI.
- Initial infarct size (% of LV)
- Myocardial haemorrhage (presence/ absence)
- Myocardial haemorrhage extent (% of LV)
- Early MVO extent (% of LV) on 1 minute post-gadolinium contrast enhanced MRI, adjusted for area at risk at baseline.
- Myocardial perfusion in the infarct zone
- Myocardial perfusion in the remote zone
- Infarct zone perfusion indexed to remote zone perfusion

CMR performed 3 months post MI:

- Final infarct size (% LV mass)
- Infarct size regression (Infarct size on MRI performed on day 2-7 minus infarct size performed 3 months post STEMI)
- Myocardial salvage index ([area at risk measured on 2 – 7 day CMR – infarct size on 3 month CMR] / area at risk on 2 – 7 day CMR)
- Change in infarct size from baseline.

8.2 CMR FUNCTION AND REMODELLING OUTCOMES

CMR performed 2-7 days post-MI:

- LV end-diastolic volume index (LVEDVi)
- LV end-systolic volume index (LVESVi)
- LV ejection fraction (LVEF)
- LV remodelling index (minimum infarct wall thickness/ maximum remote zone thickness mid-diastole)
- LV sphericity index at end-systole (maximum longitudinal LV diameter (i.e. tip mitral valve to LV apex)/ maximal short-axis diameter).
- LV sphericity index at end-diastole (maximum longitudinal LV diameter (i.e. tip mitral valve to LV apex)/ maximal short-axis diameter).
- Myocardial strain

CMR performed at 3 month follow up:

- LVEDVi
- LVESVi
- LVEF
- Change from baseline LVEDVi
- Change from baseline LVESVi
- Change from baseline LVEF

- LV remodelling index (minimum infarct wall thickness/ maximum remote zone thickness mid-diastole)
- LV sphericity index at end-systole (maximum longitudinal LV diameter (i.e. tip mitral valve to LV apex)/ maximal short-axis diameter).
- LV sphericity index at end-diastole (maximum longitudinal LV diameter (i.e. tip mitral valve to LV apex)/ maximal short-axis diameter).
- Myocardial strain
- LV diastolic myocardial wall thickness to volume

9. ADDITIONAL SECONDARY OUTCOME VARIABLES

Angiographic:

- TIMI thrombus grade at the end of PCI
- TIMI thrombus grade immediately post drug
- Intraprocedural change in TIMI thrombus grade (immediately pre-vs. post study drug)
- LV end diastolic pressure

Biochemical indices of infarct size and remodelling:

- Troponin T at 0, 2, 24 hours
- NT-proBNP 2-7 days and 3 months

Haemostasis and coagulation

- Plasminogen, tissue plasminogen activator, fibrinogen, D-dimers, Prothrombin F1+2, activated clotting factor (ACT)

The haemostasis and coagulation samples of systemic blood are collected at baseline, 2 hours and 24 hrs. These data will inform safety and mechanisms evaluations.

Quality of Life

- EQ5D-5L questionnaire 2-7 days post MI and 3 months post MI

10. CLINICAL OUTCOMES

Health outcomes are included in the 12 month follow-up of study particiants. These will be determined by independent, blinded adjudication of serious adver events (SAEs), and will be analysed as part of the 12 month analysis. After the main 12 month trial analyses have been completed, associations between coronary physiology parameters and clinical outcomes will be investiated as part of this analysis (Secondary Objective 10, above).

- Adjudicated health outcomes

- Major Adverse Cardiovascular and Cardiac Events (MACCE): cardiovascular death, non-fatal MI, hospitalisation for transient ischaemic attack or stroke
- Major Adverse Cardiac Events (MACE): cardiac death, non-fatal MI, hospitalisation for heart failure
- All cause mortality or hospitalisation for heart failure
- All cause mortality
- Unplanned hospitalisation for heart failure
- Cardiac death or non-fatal MI

11. DOCUMENT HISTORY

This Statistical Analaysis Plan is based on the peer-reviewed grant application approved by the British Heart Foundation (FS/16/74/32573; November 2016). v1_0 of the Statistical Anlysis Plan for the British Heart Foundation T-TIME Coronary Physiology Study analysis was drafted on 06/07/2018, before database lock for the main trial. The Statistical analysis plan was revised on 17/09/2018, prior to submitting the coronary physiology database to the Clinical Trials Unit for second data lock.

Appendix 9: T-TIME trial clinical event adjudication charter

Clinical Event Committee Charter

Version No: 2.0

Study Title:	A randomised, double blind, placebo-controlled, parallel group Trial of low-dose adjunctive alTeplase during prIMary PCI
Short Title:	T-TIME
Chief Investigator:	Professor Colin Berry
CEC Chairman	Dr Robin Weir
Co-Sponsors:	NHS Greater Glasgow & Clyde and The University of Glasgow
EudraCT No.:	2014-004405-32
Sponsor Ref:	GN12CA450
REC No.:	13/WS/0119
ClinicalTrials.gov identifier:	NCT02257294
Endpoint Office:	EndpointOffice@glasgowctu.org

Contents

1. Introduction

T-TIME is a double-blind, randomised, parallel group, placebo-controlled clinical trial designed to examine the efficacy and safety of reduced dose intra-coronary alteplase in STEMI patients receiving primary PCI. The primary objective is to determine the lowest effective dose of alteplase in reducing MVO. The results of T-TIME will inform the rationale and design of a potential larger Phase 3 trial.

Clinical events identified as potentially relevant to the designated secondary health outcomes will be assessed by a Clinical Event Committee (CEC). The CEC will be independent of both the investigators and the sponsor and will be blinded regarding any information relating to the randomisation group.

2. Purpose

The purpose of this charter is to delineate the roles, responsibilities and procedures for the adjudication of cardiovascular events occurring in T-Time.

3. Composition of the Clinical Event Committee (CEC)

The CEC consists of at least 3 cardiovascular physicians who have expertise in the diagnosis and treatment of cardiovascular disorders and in the medical aspects of clinical trials.

All members of the committee will have documented Good Clinical Practice and study specific training.

CEC member	Affiliation	Contact details
Dr Robin Weir (Chair)	Hairmyres Hospital, Lanarkshire	Robin.Weir@lanarkshire.scot.nhs.uk
Dr Colin Petrie	Monklands Hospital Airdrie ML6 0JS	Colin.Petrie@lanarkshire.scot.nhs.uk
Dr Aengus Murphy	Monklands Hospital Airdrie ML6 0JS	aengus.murphy@lanarkshire.scot.nhs.uk

In the event that a CEC member is unable to continue participation, the CEC Chairman will recommend a replacement to the Sponsor. The Sponsor has the final decision as to the replacement.

4. Roles and Responsibilities

The role of the CEC in T-Time is:

- To provide independent and unbiased review of clinical endpoint events which occur during the trial.
- To ensure unified and unambiguous events evaluation practices across the trial, through application of standardised event criteria, per protocol specifications.
- To compensate for regional diversity in medical practice in the area of event evaluation and classification, thereby reducing the impact of this diversity.

4.1. CEC Chairman

The CEC Chairman (Dr Robin Weir) will be responsible for:

- Acting as the primary liaison between the CEC and the Sponsor
- Proposal of CEC members
- The overall conduct of the CEC
- Developing the CEC Charter

4.2. CEC members

CEC members will be responsible for:

- Reading and understanding the content of the CEC charter
- Reviewing the relevant de-identified clinical data about a subject identified as having experienced a suspected event of interest requiring adjudication
- Adjudicating pre-specified clinical events of interest in keeping with the study definitions outlined in this charter
- Completion of adjudication forms
- Timely submission of adjudication decisions
- Communicating with the CEC Chairman about needs when necessary
- Attending scheduled CEC meetings throughout the study

4.3. Glasgow Clinical Trials Unit (GCTU) Endpoints Office

The CEC will be supported by the GCTU Endpoints (EP) Office. The EP Office will:

- Take receipt of EP information packs
- Review EP forms and source documents to ensure that the documents are fully redacted and that evidence required to support endpoint adjudication has been submitted
- Liaise with study sites regarding any queries resulting from the review
- Submit EP packs to the CEC for review
- Liaise with sponsor representatives , site staff , investigators and CEC

- Coordinate CEC meetings if required

5. Clinical Events to be reviewed

The T-Time study will use electronic data capture. The identification of potential endpoints, uploading of source documents, completion of endpoint forms, collation of endpoint packages and CEC review of potential endpoints will be facilitated by the T-Time study web portal.

6. Identification of potential endpoints

Potential endpoint events requiring review by the CEC will be identified following review of all SAEs by the Chief Investigator (CI) or a designated representative approved by the Sponsor.

SAE reports for each potential endpoint event will be reviewed by the CEC. Where the report contains sufficient information to allow adjudication of the event, the event will be classified. Where additional information is required before adjudication can take place this will be requested from the site.

Site teams will complete the required Case Report Forms for the event type and upload the required source data (detailed in Section 9) for these events.

The CEC will re-review the SAE report provided by the local investigator and relevant source clinical data provided to adjudicate on the cause of the event. The SAE record and source documents (detailed in Section 9) are expected to contain sufficient information to adjudicate on the cause(s) of the event.

The CEC will review and classify all reported instances of Major Adverse Cardiovascular and Cardiac Events (MACCE) and additional events to facilitate the assessment of efficacy and safety. This will include the review and classification of:

1. All causes of death
2. Stroke/ Transient Ischaemic Attack
3. Non-fatal Myocardial infarction (MI) (i.e. any recurrent MI after index hospitalisation)
4. Heart Failure requiring hospitalisation
5. **Heart failure complicating the index acute myocardial infarction**
6. Bleeding (BARC types 3-5-defined in section 7.2.3)

7. Endpoint Definitions

Endpoint definitions will align with the 2014 ACC/AHA Key Data Elements and Definitions for Cardiovascular Endpoint Events in Clinical Trials Hicks KA, et al. [1]and the "Third Universal Definition of Myocardial Infarction" (Thygesen et al Eur Heart J 2012)[2] for diagnosis of myocardial infarction.

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. The CEC will discuss such cases at a full CEC meeting and adjudicate them using their clinical expertise and the totality of the evidence before arriving at a classification decision that is based on full consensus.

7.1. 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.

7.1.1. 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:

7.1.1.1. 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.

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[1] Hicks KA, Tcheng JE, Bozkurt B, Chaitman BR, Cutlip DE, Farb A, Fonarow GC, Jacobs JP, Jaff MR, Lichtman JH, Limacher MC, Mahaffey KW, Mehran R, Nissen SE, Smith EE, Targum SL, 2014 ACC/AHA Key Data Elements and Definitions for Cardiovascular Endpoint Events in Clinical Trials, *Journal of the American College of Cardiology* (2015), doi: 10.1016/j.jacc.2014.12.018.

[2] Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD, et al. Third universal definition of myocardial infarction. Eur Heart J. 2012;33:2551–67

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 ischaemia, 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 CEC 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 ischaemia (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.

7.1.1.2. **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.

e. Type 3 MI ~ Cardiac death with symptoms suggestive of myocardial ischaemia and presumed new ischaemic ECG changes or new LBBB, but death occurring before blood samples could be obtained, before cardiac biomarker could rise, or in rare cases cardiac biomarkers were not collected.

f. 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)

7.1.1.3. 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 CEC will adjudicate on 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)

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.

7.1.1.4. **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 CEC 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.

7.1.1.5. **Death due to cardiovascular procedures**

Death due to cardiovascular procedures refers to death caused by the immediate complications of a cardiac procedure.

7.1.1.6. **Death due to Other Cardiovascular Causes** refers to a cardiovascular death not included in the above categories but with a specific known cause [e.g. pulmonary embolism or peripheral arterial disease)

7.1.2. Non-cardiovascular deaths

A non-cardiovascular death is defined as any death with a specific cause 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.

7.1.3. 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.

7.2. Non-fatal Cardiovascular Events

Date of onset: For purposes of classification, when classifying events that are a cause of hospitalisation, 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.

7.2.1. 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.

Diagnosis of spontaneous or PCI/CABG-related acute myocardial infarction:

Note: this applies to post randomisation acute myocardial infarction not the index myocardial infarction

A rise and/or fall of cardiac biomarkers (troponin or CK-MB) should usually be detected wherever possible with at least one value above the upper reference limit (URL) together with clinical evidence of new myocardial ischaemia with at least one of the following:

Clinical symptoms and/or signs consistent with new ischaemia

ECG evidence of acute myocardial ischaemia or new left bundle branch block (LBBB) (Table, below).
Development of new 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

Specific clinical classification of different types of myocardial infarction from Universal Definition of Myocardial Infarction (Thygesen et al Eur Heart J 2012)

Myocardial infarctions will be clinically classified as:

Type 1

Spontaneous myocardial infarction related to ischaemia due to a primary coronary event such as plaque erosion and/or rupture, fissuring, or dissection.

Type 2

Myocardial infarction secondary to ischaemia 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 ischaemia, 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 related to percutaneous coronary intervention (PCI)

Myocardial infarction associated with PCI is arbitrarily defined by elevation of cTn values >5 x 99th percentile URL in patients with normal baseline values (≤99th percentile URL) or a rise of cTn values >20% if the baseline values are elevated and are stable or falling. **In addition**, **either** (i) symptoms suggestive of myocardial ischaemia, or (ii) new ischaemic ECG changes or new LBBB, or (iii) angiographic loss of patency of a major coronary artery or a side branch or persistent slow or no-flow or embolisation, or (iv) imaging demonstration of new loss of viable myocardium or new regional wall motion abnormality is required.

Type 4b: Myocardial infarction related to stent thrombosis

Myocardial infarction associated with stent thrombosis is detected by coronary angiography or autopsy in the setting of myocardial ischaemia and with a rise and/or fall of cardiac biomarkers values with at least one value above the 99th percentile URL.

Type 5: Myocardial infarction related to coronary artery bypass grafting (CABG)

Myocardial infarction associated with CABG is arbitrarily defined by elevation of cardiac biomarker values >10 x 99th percentile URL in patients with normal baseline cTn values (≤99th percentile URL). In addition, either (i) new pathological Q waves or new LBBB, or (ii) angiographic documented new graft or new native coronary artery occlusion, or (iii) imaging evidence of new loss of viable myocardium or new regional wall motion abnormality.

ECG manifestations of acute myocardial ischaemia (in absence of left ventricular hypertrophy and left bundle branch block)

From Universal Definition of Myocardial Infarction (Thygesen et al Eur Heart J 2012)

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 ischaemia. 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.

ECG changes associated with prior myocardial infarction

From Universal Definition of Myocardial Infarction (Thygesen et al Eur Heart J 2012)

- 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]
- R wave ≥0.04 sec in V1–V2 and R/S ≥1 with a concordant positive T wave in absence of conduction defect.

[a]The same criteria are used for supplemental leads V7–V9.

7.2.2. Heart Failure

7.2.2(a) Heart failure complicating the index acute myocardial infarction

For the diagnosis of heart failure complicating the index acute MI, the following criteria must be fulfilled at a time-point between the completion of the primary percutaneous coronary interventional procedure used to treat the qualifying MI and discharge from hospital at the end of the index admission:

There should be:

1. 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

2. Investigative evidence of structural or functional heart disease (if available) 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 catheterisation).

And

3. Need for new/increased therapy* specifically for the treatment of heart failure

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 uptitration 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.

Note on oral therapy: In general, for an event to qualify as **heart failure complicating the index acute MI** on the basis of oral heart failure therapy (i.e. in cases where none of the non-pharmacological treatment modalities listed above have been utilised), 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:

a) 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 uptitration of heart failure therapy as part of the routine optimisation of medical therapy)
and
b) 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 CEC will adjudicate on clinical presentation and, if available, investigative evidence.

And

The CEC should be satisfied that heart failure was the primary disease process accounting for the clinical presentation.

7.2.2(b) Heart failure requiring hospitalisation

For the diagnosis of heart failure requiring hospitalisation, 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:

4. 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

5. Investigative evidence of structural or functional heart disease (if available) 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 catheterisation).

And

6. Need for new/increased therapy* specifically for the treatment of heart failure

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 uptitration 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.

Note on oral therapy: In general, for an event to qualify as **heart failure requiring hospitalisation** on the basis of oral heart failure therapy (i.e. in cases where none of the non-pharmacological treatment modalities listed above have been utilised), 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:

c) 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 uptitration of heart failure therapy as part of the routine optimisation of medical therapy)
and

d) 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 CEC will adjudicate on clinical presentation and, if available, investigative evidence.

And

The CEC should be satisfied that heart failure was the primary disease process accounting for the clinical presentation.

7.2.3. Bleeding

BARC bleeding is defined as:

Type 2 –not for CEC review

Any overt, actionable sign of haemorrhage (e.g., more bleeding than would be expected for a clinical circumstance, including bleeding found by imaging alone) that does not fit the criteria for type 3, 4, or 5 but does meet at least one of the following criteria: (1) requiring nonsurgical, medical intervention by a healthcare professional, (2) leading to hospitalization or increased level of care, or (3) prompting evaluation

Type 3

- **Type 3a**

Overt bleeding plus haemoglobin drop of 3 to <5 g/dL* (provided haemoglobin drop is related to bleed)
Any transfusion with overt bleeding

- **Type 3b**

Overt bleeding plus haemoglobin drop ≥5 g/dL* (provided haemoglobin drop is related to bleed)
Cardiac tamponade
Bleeding requiring surgical intervention for control (excluding dental/nasal/skin/haemorrhoid)
Bleeding requiring intravenous vasoactive agents

- **Type 3c**

Intracranial haemorrhage (does not include microbleeds or haemorrhagic transformation, does include intraspinal)

Subcategories confirmed by autopsy or imaging or lumbar puncture
Intraocular bleed compromising vision

Type 4: CABG-related bleeding

Perioperative intracranial bleeding within 48 h
Reoperation after closure of sternotomy for the purpose of controlling bleeding
Transfusion of ≥5 U whole blood or packed red blood cells within a 48-h period[‡]
Chest tube output ≥2L within a 24-h period

Type 5: Fatal bleeding

- **5a** Probable fatal bleeding: no autopsy or imaging confirmation but clinically suspicious
- **5b** Definite fatal bleeding: overt bleeding or autopsy or imaging confirmation.

7.2.4. 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 ≥ 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**:

a) neurology or neurosurgical specialist.

b) brain imaging procedure (at least one of the following):

(i) CT scan.

(ii) MRI scan.

(iii) cerebral vessel angiography.

c) lumbar puncture (i.e. spinal fluid analysis diagnostic of intracranial haemorrhage).

****If a stroke is reported but evidence of confirmation of the diagnosis by the methods outlined above is absent, the event will be discussed at a full CEC meeting. In such cases, the event may be adjudicated as a stroke on the basis of the clinical presentation alone but full CEC consensus will be mandatory.

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:

- **Ischaemic (non-hemorrhagic) stroke**

(i.e. caused by an infarction of central nervous system tissue)

or

- **Hemorrhagic stroke*****

(i.e. caused by non-traumatic intraparenchymal, intraventricular or subarachnoid hemorrhage)

or

- **Stroke type (i.e. hemorrhagic or ischaemic) 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 by the CEC

7.2.5. Transient Ischaemic Attack

- Transient ischaemic attack (TIA) is any focal neurological deficit consistent with a cerebrovascular event with sudden onset, as defined above, that resolves within 24 hours.

8. Adjudication process

8.1. Review of potential endpoints

CEC review of potential endpoints will be facilitated by the T-Time study web portal. Reviewers will be able to view documents relating to the event and complete an adjudication form. Reviewers will also be able to request additional information if required.

For the first 10 reported events requiring adjudication, the events will be reviewed at a CEC meeting with all 3 members present. The purpose of this initial committee review will be to ensure that all committee members are applying the endpoint definitions as described in this charter and that all members are aligned in their applications of the definitions to the classifications of events. In this review of the initial 10 events, full consensus will be required for each final classification decision.

8.1.1. Phase 1 CEC review

Each SAE report for a potential endpoint event will be reviewed independently by two CEC members. Reports will be allocated for review in a manner that ensures that events are distributed to the members on an even basis.

On confirmation in the electronic system that an SAE report ready for review, an email notification will be sent to 2 members of CEC indicating that an event available for adjudication. On receipt of the email

notification the reviewer will complete the review and adjudication in a timely manner. The CEC member will:

- Access the T-Time web portal (https://www.glasgowctu.org/TTIME/login) and select **Endpoints**
  - Identify the potential endpoint for review using the Participant No and SAE ID
  - Review the details of the event by accessing the SAE Report
  - Classify the event according to the EP definitions as detailed in this charter.
  - Complete the EP Adjudication Form.

If the reviewer unable to classify the event and considers that additional information is required before a classification decision can be made this option should be selected on the review form. An automatic email notification will be forwarded to the site (copied to the EP Office) advising that additional information is required. When new information becomes available, it will be re-submitted to the two adjudicators initially assigned the event. In instances where it is confirmed that efforts to obtain requested information have been unsuccessful (e.g. because the study site has indicated that the information is not available), classification of the event will be deferred pending review by the CEC Chairman or discussion at a scheduled CEC meeting.

If the reviewer is able to classify the event the adjudication form should be completed, saved and submitted. An automatic email notification will be forwarded to the EP Office advising that an event has been classified.

If the two reviewers are in agreement the adjudication decision will be accepted and the endpoint classified. If 2 different classifications are given, or if one or both of the reviewers are unable to reach a decision or request that the case is referred to the CEC Chairman, the case will be forwarded to the CEC Chairman for review and classification.

8.1.2. Phase 2 - review by CEC Chairman

If the CEC Chairman is able to classify the event the adjudication form should be completed with the Type of Event, saved and submitted. An automatic email notification will be forwarded to the EP Office advising that an event has been classified.

If the CEC Chairman is unable to arrive at a classification verdict for an event because of incomplete or inadequate information and it is felt that such information may be obtainable (i.e. the study site has not

indicated that the information required is unavailable), the Chairman will detail the precise information/documentation that is needed to achieve classification and this will be requested from the site by the EP Office.

If the CEC Chairman is unable to classify the event it will be referred to the full committee for review and classification

8.1.3. Phase 3- review by full CEC

The CEC will convene at regular intervals throughout the study as required. In general, these will be face- to- face meetings, however, if for some reason a face- to- face meeting is not possible, a meeting by teleconference may substitute.

The frequency of meetings depends on the quantity of clinical events received by the CEC. A meeting may be cancelled if there is no business for discussion or cases to be reviewed by full committee.

The primary objective of CEC meetings is the **Phase 3 review** and classification of those events for which a final classification decision has not been achieved by the Phase 1 or Phase 2 review process already outlined above. Phase 3 review of an event constitutes the discussion and adjudication of the event by the CEC as a group.

If the CEC are unable to arrive at a classification verdict for an event because of incomplete or inadequate information and it is felt that such information may be obtainable (i.e. the study site has not indicated that the information required is unavailable), the Chairman will detail the precise information/documentation that is needed to achieve classification and this will be requested from the site by the EP Office. Adjudication of the event will be deferred and reviewed subsequently at a CEC meeting when the information requested has been made available (or, when, despite best efforts, it is confirmed that the information will not be obtainable).

8.1.4. Adjudication timelines

The CEC members will make every effort to review events and to enter their classification decisions onto the electronic Adjudication Form within 2 to 4 weeks from the time that the event data is received. To facilitate the prompt adjudication of events, it is expected that adverse event data received by the CEC will be as clean and complete as possible and that any CEC data-queries are resolved in a timely fashion.

If necessary, the above timelines may be amended as the study progresses, if the CEC and the other relevant parties agree on a new schedule of event turn-around time.

9. Information to be provided to CEC

Information to be provided for event classification will include:

- Subject study identification number and event details
- Serious Adverse Event form
- Supportive source documentation where requested following initial Phase 1 SAE review

Source Documentation

The following de-identified source documents (if available) will be provided to the CEC to facilitate the review and adjudication of events if requested :

Death

- Hospital Discharge Summary/Death Summary *
- Autopsy Report
- Death Certificate

*Or the clinical equivalent if the above unavailable

Acute Myocardial Infarction

- Hospital Discharge Summary*
- ECGs
  - Pre-Randomisation/Screening- *Trial ECG available via electronic system*
  - Baseline (prior to event but post-randomization)- *Trial ECG available via electronic system*
  - 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 ischaemia

*Or the clinical equivalent if the above unavailable

Stroke/TIA

- Hospital Discharge Summary*
- Neurology Consultation Report(s)
- Reports for other investigations undertaken:
    - CT Brain Scan Report
    - MRI Brain Scan Report- *Trial scan available via electronic system if event during index admission*
    - Cerebral Angiography Report
    - Lumbar Puncture Report

*Or the clinical equivalent if the above unavailable

Heart failure complicating the index acute myocardial infarction

- Hospital Discharge Summary*
- Clinical note entry in the medical record
- Prescription of diuretic therapy
- Chest X-Ray Report
- 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 Catherization

*Or the clinical equivalent if the above unavailable

Heart Failure requiring hospitalisation

- Hospital Discharge Summary*
- Chest X-Ray Report
- 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 Catherization

*Or the clinical equivalent if the above unavailable

Bleeding (BARC types 3-5)

- Hospital discharge summary*
- Haemoglobin measurements throughout admission

* *Or the clinical equivalent if the above unavailable

10. Document History

Version	Date	Reason for change
1.0	19/12/2016	Initial creation
2.0	21/11/2017	Updated to reflect changes to CEC review process and the addition of a new endpoint - **Heart failure complicating the index acute myocardial infarction**

11. Approvals

The following CEC and Sponsor representatives have approved this Charter:

Name	Role	Signature	Date
Professor Colin Berry	Chief Investigator		
Dr Robin Weir	CEC Chairman		
Dr Colin Petrie	CEC Member		
Dr Aengus Murphy	CEC Member		
Dr Maureen Travers	Sponsor Representative		

Appendix 10. Ethical approval for the MR-MI study

Duplicate letter

WoSRES
West of Scotland Research Ethics Service

West of Scotland REC 1
Western Infirmary
West of Scotland Research Ethics Service
Ground Floor, Tennent Institute
38 Church Street
Glasgow
G11 6NT

Telephone: 0141-211-6238
Facsimile: 0141-211-1847
Ref AHT/SAJ

06 May 2010

Dr Colin Berry
Consultant Cardiologist
BHF Glasgow Cardiovascular Research Centre,
126 University Place
Glasgow
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.

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

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