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Effect of coronary flow on intracoronary alteplase, a prespecified analysis from a randomised trial

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Abstract:	Objectives: Persistently impaired culprit artery flow (<TIMI 3) during primary percutaneous coronary intervention is a surrogate for failed myocardial perfusion. We evaluated the effects of intracoronary alteplase according to TIMI flow grade immediately preceding drug administration. Methods: In T-TIME, patients ≤6 hours from onset of ST-elevation myocardial infarction (STEMI), were randomised to placebo, alteplase 10mg, or alteplase 20mg, administered by infusion into the culprit artery, pre-stenting. In this pre-specified, secondary analysis, coronary flow was assessed angiographically, at the point immediately before drug administration. Microvascular obstruction, myocardial haemorrhage and infarct size were assessed by cardiovascular magnetic resonance (CMR), at 2-7 days and 3 months. Results: TIMI flow was assessed after first treatment (balloon angioplasty/ aspiration thrombectomy), immediately pre-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. In patients with TIMI flow≤2 pre-drug there was higher incidence of microvascular obstruction 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 higher incidence of myocardial haemorrhage (alteplase 20mg [53.1%] and 10mg [57.9%] combined vs. placebo [27.5%]; OR=3.26 [95% CI: 1.44-7.36, p=0.004] interaction p=0.001). These effects were not observed in participants with TIMI 3 flow pre-drug. There were no interactions between TIMI flow pre-drug, alteplase and 3-month CMR findings. Conclusion: In patients with impaired culprit artery flow (<TIMI 3) after initial balloon angioplasty/ thrombus aspiration, intracoronary alteplase was associated with increased presence of microvascular obstruction and myocardial haemorrhage.

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Effect of coronary flow on intracoronary alteplase, a pre-specified analysis from a randomised trial

Short title: Coronary flow and alteplase during primary PCI

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Abstract

Objectives: Persistently impaired culprit artery flow (<TIMI 3) during primary percutaneous coronary intervention is a surrogate for failed myocardial perfusion. We evaluated the effects of intracoronary alteplase according to TIMI flow grade immediately preceding drug administration.

Methods: In T-TIME, patients ≤6 hours from onset of ST-elevation myocardial infarction (STEMI), were randomised to placebo, alteplase 10mg, or alteplase 20mg, administered by infusion into the culprit artery, pre-stenting. In this pre-specified, secondary analysis, coronary flow was assessed angiographically, at the point immediately before drug administration. Microvascular obstruction, myocardial haemorrhage and infarct size were assessed by cardiovascular magnetic resonance (CMR), at 2-7 days and 3 months.

Results: TIMI flow was assessed after first treatment (balloon angioplasty/ aspiration thrombectomy), immediately pre-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. In patients with TIMI flow ≤2 pre-drug there was higher incidence of microvascular obstruction 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 higher incidence of myocardial haemorrhage (alteplase 20mg [53.1%] and 10mg [57.9%] combined vs. placebo [27.5%]; OR=3.26 [95% CI: 1.44-7.36, p=0.004] interaction p=0.001). These effects were not observed in participants with TIMI 3 flow pre-drug. There were no interactions between TIMI flow pre-drug, alteplase and 3-month CMR findings.

Conclusion: In patients with impaired culprit artery flow (<TIMI 3) after initial balloon angioplasty/ thrombus aspiration, intracoronary alteplase was associated with increased presence of microvascular obstruction and myocardial haemorrhage.

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Key Questions

What is already known?

- Microvascular obstruction following ST-segment elevation myocardial infarction (STEMI) confers a worse prognosis.
- There are no evidence-based treatments for microvascular obstruction.

What does this study add?

- Adjunctive intracoronary alteplase, during primary percutaneous coronary intervention (PCI), was associated with increased presence of microvascular obstruction and myocardial haemorrhage, in participants with impaired culprit artery flow (TIMI <3) at the time of study drug administration.
- Low-dose intracoronary lytic therapy in patients with STEMI, who have impaired coronary flow may be harmful.

How might this impact on clinical practice?

- The findings are relevant to trials of adjunctive intracoronary fibrinolytic therapy during primary PCI, and as a disincentive to clinicians when considering bail-out lytic therapy for angiographic “no reflow”.
- 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.

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Key Words

ST-segment elevation myocardial infarction

TIMI coronary flow grade

Fibrinolytics

Microvascular obstruction

Myocardial haemorrhage

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Abbreviations

AUC	Area-under-the-curve
CI	Confidence interval
CMR	Cardiovascular magnetic resonance
eGFR	Estimated glomerular filtration rate
IQR	Interquartile range
LGE	Late gadolinium enhancement
LV	Left ventricular
MVO	Microvascular obstruction
OR	Odds ratio
PCI	Percutaneous coronary intervention
QCA	Quantitative coronary angiography
SD	Standard deviation
STEMI	ST-segment elevation myocardial infarction
TIMI	Thrombolysis in Myocardial Infarction

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Introduction

Despite routinely restoring epicardial coronary patency with primary percutaneous coronary intervention (PCI), microvascular obstruction (MVO) affects about half of patients(1) and confers an adverse prognosis(2, 3). A key component of MVO is distal embolization and microvascular thrombi(4-6).

In the T-TIME trial (NCT02257294), we hypothesised that low-dose intracoronary alteplase, administered shortly after balloon angioplasty or aspiration thrombectomy, before stenting, would reduce intracoronary and microvascular thrombosis, and distal embolization, thereby reducing MVO. However, as assessed by contrast-enhanced cardiovascular magnetic resonance (CMR), MVO did not differ with intracoronary alteplase vs. placebo(7). Interestingly, in a T-TIME subgroup analysis, participants presenting ≥4 hours after symptom onset, had a dose dependent increase in mean amount of MVO and myocardial haemorrhage with alteplase vs. placebo(8). Invasively measured index of microcirculatory resistance did not differ with intracoronary alteplase vs. placebo(9), and there was no difference in clinical outcomes at 1-year between treatment groups(10).

Coronary angiography allows a semi-quantitative grading of coronary flow, according to the Thrombolysis in Myocardial Infarction (TIMI) flow grades(11). Persistently reduced flow in the culprit coronary artery (TIMI flow <3) after first treatment, is termed "no-reflow"(12, 13). TIMI flow <3 is a surrogate for impaired myocardial perfusion(14, 15) and predicts heart failure(16), larger infarct size(14) and mortality(16, 17). TIMI flow <3 early during the primary PCI procedure (pre-stenting) may be even more closely associated with mortality(18, 19) and larger infarct size(19), than TIMI flow <3 post-stenting. In contrast, recovery of TIMI 3 flow in the culprit artery after first treatment (balloon angioplasty/ thrombus aspiration) may help restore microvascular function(15).

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Persisting impairment of antegrade flow in the culprit artery may influence the effect of intracoronary alteplase. We hypothesised that impaired coronary flow reduces the effective delivery of alteplase to the microcirculation. The primary aim of this pre-specified secondary analysis was to assess the associations between TIMI flow grade, treatment group (placebo, alteplase 10mg, alteplase 20mg), and MVO. We also investigated associations between TIMI flow grade, treatment group and the secondary endpoints from the T-TIME trial(10).

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Methods

Trial Design

T-TIME was a randomised, double-blind, parallel group, phase 2 clinical trial of low-dose adjunctive intracoronary alteplase during primary PCI(7, 10). Patients were enrolled by 11 U.K. hospitals, from March 2016 to December 2017. The methodology has been described previously in detail(7) (Figure 1).

Consent

Screening and study drug administration occurred during standard care primary PCI. Witnessed verbal assent to participate was obtained in the catheterisation laboratory. Written informed consent was subsequently obtained on the ward. The study was approved by the West of Scotland Research Ethics Committee (reference 13-WS-0119).

Eligibility

Patients were eligible to participate if they presented with persistent ST-segment elevation or recent left bundle branch block, ≤6 hours from symptom onset, and with an occluded culprit artery (TIMI 0 flow), TIMI 1 flow (contrast passes beyond the obstruction, but fails to opacify the entire distal coronary bed), or reduced coronary flow (TIMI 2 flow, slow but complete filling), in the presence of TIMI thrombus grade ≥2.

Key exclusion criteria (Supplemental Methods) included a functional coronary collateral supply (Rentrop grade ≥2) to the culprit artery and cardiogenic shock.

Randomisation and Blinding

Participants 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, with stratification by location of MI (anterior vs. non-anterior). The

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allocation sequence was on a 1:1:1 basis, between placebo and the reduced dose of alteplase groups (10mg and 20mg), i.e. one tenth, or one fifth, of standard dose. The participants, staff and researchers were blinded to the treatment group allocation.

Interventions

The trial protocol encouraged achieving TIMI flow grade ≥2, using balloon angioplasty/ aspiration thrombectomy, prior to randomisation. After randomisation, the allocated intervention was prepared, during which TIMI flow grade deteriorated in a minority of patients prior to study drug administration, before stent deployment. The 20ml volume of study drug was manually infused into the culprit artery, over 5-10 minutes, proximal to the culprit lesion, using either an intracoronary catheter, or the guiding catheter if selectively engaged.

CMR

CMR (1.5 Tesla) was analysed by an investigator who was blind to the angiographic findings and treatment allocation. A second read was undertaken by a cardiologist with level 3 CMR certification. MVO presence and extent (% left ventricular [LV] mass) was revealed by late gadolinium enhancement (LGE), 10-15 minutes after administration of gadolinium-based contrast media. MVO was defined as a dark zone on early gadolinium enhanced imaging 1, 3, 5 and 7 minutes post-contrast injection that persisted within an area of LGE at 15 minutes. The myocardial mass of the dark zone was quantified by manual delineation and expressed as % of LV mass.

Myocardial haemorrhage presence and extent (% LV mass) was revealed by T2* mapping. A region of reduced signal intensity within the infarcted area, with a T2* value <20ms was considered to confirm the presence of myocardial haemorrhage. This area was manually delineated and expressed as % LV mass.

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The presence of acute infarction was established based on abnormalities in cine wall motion, rest first-pass myocardial perfusion, and LGE imaging, in 2 imaging planes. The myocardial mass of late gadolinium was quantified using a 5-standard deviation (SD) semi-automated method and expressed as % of total LV mass. Myocardial salvage was calculated by subtraction of percent infarct size from percent area-at-risk (as reflected by the extent of oedema) and the myocardial salvage index was calculated by dividing the myocardial salvage area by the initial area-at-risk.

MVO and myocardial haemorrhage were reported on CMR scans acquired 2–7 days post-STEMI. The other CMR parameters were reported from the 2–7 day and 3-month scans (Supplement).

Angiography, ECGs and Troponin

The ECG and angiographic parameters were determined by blinded core laboratory analysis (blinded to CMR data and treatment allocation).

The angiograms were analysed prospectively by one researcher, and then a second read was undertaken by an experienced interventional cardiologist. Discrepancies were resolved by consensus agreement. The following were assessed in the culprit artery: TIMI flow grade, TIMI frame count, myocardial perfusion grade, TIMI thrombus grade, and plaque characteristics (Supplement).

The angiogram acquisition protocol required stored fluoroscopy of study drug administration, to enable verification by the core laboratory that the guide catheter was selectively engaged in the culprit artery during drug delivery. This also enabled core laboratory evaluation of TIMI flow grade immediately before study drug administration, which was submitted to the data coordination centre prior to database lock. Participants were

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grouped according to TIMI flow grade (≤2 vs. 3) in the culprit artery immediately preceding study drug administration.

The absolute percentage ST-segment resolution on ECGs obtained 60 minutes after reperfusion (i.e. after initial restoration of flow in the culprit artery), compared to pre-reperfusion was calculated. Troponin T area-under-the-curve (AUC) was measured in blood samples obtained immediately pre-reperfusion (0 hours), then at 2- and 24-hours post-reperfusion.

Coagulation

Coagulation and haemostasis parameters were measured in peripheral blood samples taken pre-reperfusion, then 2, and 24 hours post-reperfusion. The parameters included fibrinogen and plasminogen (both measures of systemic fibrinolysis), fibrin D-dimer (a measure of fibrin lysis), tissue plasminogen activator (a measure of endogenous fibrinolytic system activation and circulating alteplase) and prothrombin fragment F_{1+2} (a measure of thrombin generation).

Statistical Analysis

This study was a pre-specified secondary analysis in the T-TIME trial population. The analyses were performed according to treatment received (alteplase 20mg, 10mg, or placebo). The trial endpoints were assessed using linear regression (continuous variables), or logistic regression (binary variables), to make treatment effect estimates. In regression models, logarithmic, or square root transformations were used where necessary to improve model residual distributions. As MVO extent was not normally distributed, we adopted square root transformation for MVO extent, in keeping with the analysis plan for the main T-TIME trial(7). However, as 56% of patients had zero values for MVO extent we also performed a sensitivity analysis using bootstrapping confidence intervals (CIs) to model this endpoint.

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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 the location of MI (anterior vs. non-anterior). All tests were 2-tailed and assessed at the 5% significance level. There was no imputation for missing values and there were no adjustments for multiple statistical comparisons. Given the high proportion of participants with a 0 value for MVO extent and myocardial haemorrhage extent, the median values for MVO and myocardial haemorrhage were 0 for all groups, therefore the mean (SD) values were reported, despite not being ideal summaries for these data. Data were analysed using R (version 3.6.1, R Development Core Team, Auckland, New Zealand) and SPSS (version 25.0, SPSS, IBM, Armonk, NY, USA).

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Results

Population

Four hundred and forty participants were randomised to placebo, alteplase 10mg, or alteplase 20mg. Nineteen patients were excluded from the analysis (Figure 2): in 7 patients TIMI flow grade was unevaluable immediately before study drug administration; in 5 participants study drug was not given; in 3 participants study drug was administered post-stent implantation, and; in 4 participants study drug was administered distal to the lesion.

The analysis therefore included 421 participants (mean age 61±10 years, 85% male). Out of the 421 participants who were included, 1 participant who was randomised to 10mg of alteplase received 20mg, and 1 participant randomised to placebo received 20mg of alteplase, because of handling errors.

The baseline and procedural characteristics for participants with TIMI flow ≤2 (n=154) or TIMI 3 flow (n=267) pre-study drug were broadly similar (Tables 1, 2 and Supplemental Table 1).

The distribution of TIMI flow grades immediately before study drug administration was as follows: TIMI grade 0 in 1 participant (0.2%), who received alteplase 10mg; TIMI grade 1 in 19 participants (4.5%), of whom 8 received placebo, 4 received alteplase 10mg, and 7 received alteplase 20mg; TIMI grade 2 in 134 participants (31.8%), of whom 42 received placebo, 44 received alteplase 10mg and 48 received alteplase 20mg, and; TIMI grade 3 in 267 participants (63.4%), of whom 92 received placebo, 88 received alteplase 10mg and 87 received alteplase 20mg.

In multivariable logistic regression analysis, only anterior MI was associated with TIMI flow ≤2 pre-study drug (odds ratio [OR] 1.61 [95% CI 1.07-2.43] p=0.023). Ischaemic

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time (symptom onset to reperfusion time) was not associated with TIMI flow ≤2 immediately pre-study drug (OR 1.05 [95% CI 0.91-1.22] p=0.499).

CMR Parameters

CMR was performed in 387 participants (92%) at 2–7 days (Table 3 and Supplemental Table 2), and in 358 participants (85%) at 3-months post-STEMI (Table 4 and Supplemental Table 2). The CMR results (2-7 day) stratified by location of MI are shown in Supplemental Table 3. Baseline/ procedure characteristics were similar for patients who had MVO data available (n=383) vs. those with missing MVO data (n=38) (Supplemental Tables 4 and 5). This suggests that data was missing at random, and the impact of missing data should be affecting each treatment group in a similar way.

Mean MVO extent was higher in patients who had TIMI flow ≤2 (3.7 ± 6.0%) vs. TIMI 3 flow (2.3 ± 4.2%) immediately pre-drug (coefficient: 0.33 [95% CI: 0.05-0.60] p=0.022 [derived from linear regression, using square root transformed MVO]).

In participants with TIMI 3 flow pre-study drug, there were no associations between alteplase and infarct characteristics, apart from an increase in LV end-diastolic volume with alteplase 10mg vs. placebo (Tables 3 and 4, and Supplemental Table 2).

MVO

Participants with TIMI flow ≤2 pre-study drug, had MVO present more often with alteplase (placebo, 34.1% [n=15/44]; alteplase 10mg, 59.5% [n=25/42]; alteplase 20mg, 53.1% [n=26/49]; OR for alteplase 10mg and 20mg combined vs. placebo 2.47 [95% CI: 1.16-5.22] p=0.018) (Table 3 and Figure 3A). Interactions were observed for association 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). When the 19 patients

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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) (Supplemental Table 6).

Participants with TIMI flow ≤2 pre-drug, had increased extent of MVO (% LV mass) with alteplase (placebo 2.6 ± 5.7%, alteplase 10mg 2.7 ± 3.9%, alteplase 20mg 5.4 ± 7.4%, estimated mean difference [for MVO analysed on a square root scale] alteplase 20mg and 10mg combined vs. placebo 0.53 [95% CI: 0.06-1.00] p=0.027) (Table 3). There was an interaction between MVO extent (% LV mass), TIMI flow pre-drug and treatment, when alteplase 10mg and 20mg were combined vs. placebo (p=0.041), but not for treatment as a 3-level categorical variable (p=0.070) (Table 3). On bootstrap linear regression analysis, 20mg alteplase was associated with MVO extent when compared to placebo in patients with TIMI flow ≤2 pre-drug (mean difference: 3.37 [95% CI: 0.77-6.89] p=0.016), but not in patients with TIMI 3 flow pre-drug (mean difference: 1.91 [95% CI: -0.74, 3.01] p=0.287) (Supplemental Table 7).

Myocardial Haemorrhage

Myocardial haemorrhage presence/ absence was evaluable in 366 participants at 2-7 days, and myocardial haemorrhage extent was evaluable in 348 participants.

In participants with TIMI flow ≤2 pre-study drug, myocardial haemorrhage occurred more often with alteplase than placebo (alteplase 20mg, 53.1% [26/49], alteplase 10mg, 57.9% [n=22/38] vs. placebo, 27.5% [n=11/40]; OR for alteplase 10mg and 20mg combined vs. placebo: 3.26 [95% CI: 1.44-7.36] p=0.004) (Figure 3A). Interactions were observed between myocardial haemorrhage presence, TIMI flow pre-drug and treatment, analysed as 3-level, or 2-level categorical variables (p=0.004 and p=0.001 respectively) (Table 3). When the 19 patients with TIMI 1 flow and the one patient with TIMI 0 flow immediately pre-drug

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were excluded, significant interactions remained between alteplase, TIMI flow pre-drug (2 vs. 3) and the presence of myocardial haemorrhage (p=0.009) (Supplemental Table 6).

Infarct Size, Myocardial Salvage Index, LV Ejection Fraction and LV Volumes at 2-7 days and 3-months

An interaction was observed for association with infarct size (2-7 day), between TIMI flow and treatment analysed as a 2-level categorical variable (p=0.026). Similar findings were observed for myocardial salvage index at 2–7 days post-STEMI (Table 3).

In participants with TIMI flow ≤2 pre-study drug, alteplase was not associated with LV ejection fraction, or LV volumes 2-7 days post-STEMI (Table 3, Supplemental Table 2), or at 3-months (Table 4, Supplemental Table 2).

Angiographic and ECG Parameters

The inter-observer reliability for TIMI flow grade pre-study drug, assessed in 65 consecutive participants, was excellent (kappa=0.94). Occlusion of the culprit coronary artery after study drug administration occurred in 44 out of 334 patients (13%). There were no interactions between TIMI flow pre-drug, alteplase and angiographic or ECG surrogates of failed microvascular reperfusion (Supplemental Table 8).

Blood Chemistry

There were no interactions between TIMI flow pre-drug, alteplase and troponin T measured in 306 participants (Supplemental Table 8).

Coagulation

Regarding coagulation data (Table 5, Supplemental Table 9), there was an increase in fibrin D-dimers (a product of fibrin lysis), and decrease in plasminogen and fibrinogen, 2 hours post-primary PCI relative to baseline, with alteplase vs. placebo, regardless of TIMI

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flow grade pre-drug. This is consistent with what is expected following intra-arterial fibrinolysis. There was an increase in prothrombin fragment F_{1+2} (a measure of thrombin activation) two hours post-primary PCI relative to baseline, with alteplase vs. placebo, regardless of TIMI flow grade pre-drug (Table 5).

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Discussion

Low-dose adjunctive intracoronary alteplase administered early during primary PCI, was associated with increased MVO and myocardial haemorrhage in participants who had TIMI flow ≤2 pre-drug administration (Figure 3A). These effects were not observed in participants with normalised TIMI 3 flow.

Our findings contrast with a previous study (n=95), which reported smaller infarct size at 6 months in patients given intracoronary streptokinase immediately post-primary PCI(20). Differences between the previous study(20) and ours include the previous study was not double-blinded, streptokinase is not fibrin specific, whereas alteplase is, and streptokinase was delivered post-stent insertion in the previous study (when 89% of the cohort had TIMI 3 flow), whereas we administered alteplase pre-stent implantation.

We might speculate that impaired antegrade coronary flow would in turn lead to inadequate drug delivery to the microcirculation, resulting in less effective microvascular reperfusion. In fact, increased fibrin D-dimer and lower plasminogen concentrations were observed with alteplase in patients with TIMI flow ≤2 pre-drug (Table 5, and Supplemental Table 9), which indicates that fibrinolysis did indeed occur in this group of participants.

Our findings may be related to the undesired procoagulant effects of fibrinolytic therapy (Figure 3B). In circumstances of slow microvascular flow, intracoronary alteplase potentially promotes the procoagulant effects of alteplase(21), thereby promoting microvascular thrombosis and worsening MVO. Indeed, increased prothrombin fragment F_{1+2} concentrations were observed with intracoronary alteplase (Table 5), despite therapeutic anticoagulation with heparin, indicating thrombin generation and increased risk of thrombosis(22).

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Our findings may suggest that in circumstances of reduced antegrade flow, myocardial perfusion is reduced leading to prolonged, higher local concentrations of alteplase, due to reduced washout of alteplase from the microcirculation. TIMI 3 flow is not synonymous with normal myocardial perfusion, for example in our population 42% of patients with TIMI 3 flow pre-study drug administration had MVO present. However, participants with TIMI flow ≤2 immediately pre-study drug had more extensive microvascular injury, evidenced by these patients having significantly more MVO than the patients with TIMI 3 flow pre-drug. Myocardium with extensive microvascular damage from coronary occlusion, is characterised by loss of capillary integrity. In these circumstances, intracoronary fibrinolysis appears to worsen extravasation of erythrocytes, resulting in myocardial haemorrhage in the infarct core (an 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, which worsens MVO (Figure 3B).

Insights from previous studies of glycoprotein IIbIIIa inhibitors, are consistent with our findings(23, 24). An animal study demonstrated an increased incidence of myocardial haemorrhage with the addition of intracoronary glycoprotein IIbIIIa inhibitors(24), and in humans peri-procedural glycoprotein IIbIIIa inhibitors have also been associated with myocardial haemorrhage(24).

Our findings are relevant to clinicians when considering bail-out lytic therapy in acute STEMI patients with massive thrombus and angiographic “no reflow”. Our findings are also relevant to ongoing clinical trials. Notably, the RESTORE-MI trial (NCT03998319) is randomising patients with STEMI (n=800) to adjunctive intracoronary tenecteplase or placebo, in a double-blind design, during primary PCI. For the RESTORE-MI trial, a key inclusion criteria is a post-stent index of microcirculatory resistance >32 in the culprit artery, which signifies incomplete microvascular reperfusion and microvascular dysfunction(26).

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Our analyses raise the possibility that low-dose intracoronary lytic therapy in patients with STEMI, who have incomplete reperfusion at the end of PCI, may not reduce infarct pathology and, indeed, may be harmful. Nonetheless, there are important differences in the design of T-TIME as compared to RESTORE-MI, such as the timing of study drug administration (before or after stent implantation, respectively) and the lytic agent (alteplase vs. tenecteplase).

Strengths and Limitations

Strengths of our study include the double-blind design, high follow up rates with CMR, core-lab analyses, and the fact that TIMI flow grade immediately pre-study drug administration was prospectively analysed and was submitted to the data coordination centre prior to database lock. However, due to the potential for type 1 statistical error, the findings should be interpreted as exploratory/ hypothesis generating.

Although the randomisation was not stratified according to TIMI flow grade, the randomisation was stratified according to location of MI (anterior vs. non-anterior), which was the only independent associate of TIMI flow pre-drug. Regression analyses were adjusted for MI location, to limit the influence of confounding.

Conclusion

In STEMI patients with impaired coronary flow at the time of study drug administration, intracoronary alteplase was associated with increased incidence of MVO and myocardial haemorrhage.

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Contributorship Statement

A.M.M. wrote the manuscript. A.M.M. and C.B. conceived the idea for the manuscript. A.M.M., C.B., and M.McE. performed the angiogram analyses. A.M.M., P.D. and A.McC. performed the statistical analyses. P.J.M. and C.B. analysed the magnetic resonance images. P.W.M. analysed the ECGs. R.C.T. analysed the coagulation data. J.P.G., K.O., M.McE., C.B., D.F.M., S.C., A.H.G., C.A., H.E., J.M.C., A.W. and N.C. contributed to data acquisition. K.A.A., R.C.T., and N.C. contributed to interpreting the data and revising the work critically for intellectual content. All authors made the decision to submit. C.B. is guarantor.

Declaration

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Figure Titles and Legends

Figure 1. Graphical layout of trial protocol

Abbreviations: CMR, cardiovascular magnetic resonance; LGE, late gadolinium enhancement; MVO, microvascular obstruction; TIMI, Thrombolysis in Myocardial infarction; STEMI, ST-segment elevation myocardial infarction

Figure 2. Study flow diagram

CMR (cardiovascular magnetic resonance) follow up is reported according to treatment received. Abbreviations: MVO, microvascular obstruction; TIMI, Thrombolysis in Myocardial Infarction.

Study Flow Diagram.

Figure 3. Summary of main findings and potential mechanisms

A. Forrest plots showing increased MVO and myocardial haemorrhage presence associated with alteplase vs. placebo in participants with TIMI flow ≤2 at the time of study drug administration.

B. In participants with reduced antegrade flow in the culprit artery (TIMI flow ≤2), there may have been increased microvascular exposure to higher local concentrations of alteplase for longer. TIMI coronary flow ≤2 may indicate ongoing impaired myocardial reperfusion, due to extensive microvascular damage. In these circumstances, intracoronary fibrinolysis appears to worsen MVO and extravasation of erythrocytes, resulting in myocardial haemorrhage in the infarct core and potentially promotes microvascular thrombosis. An increase 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

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reduction in myocardial blood flow, and exacerbates myocardial necrosis and capillary destruction, which promotes further myocardial haemorrhage.

Abbreviations: CI, confidence interval; CMR, cardiovascular magnetic resonance; MVO, microvascular obstruction; TIMI, Thrombolysis in Myocardial Infarction.

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Table 1. Baseline characteristics, by subgroups of TIMI flow grade (≤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.

	Impaired coronary flow (TIMI flow ≤ 2)				Normal coronary flow (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 ± 10.6	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%)
Hypercholesterolemia	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 myocardial infarction	2 (1%)	0	2 (4%)	0	15 (6%)	5 (5%)	3 (3%)	7 (8%)
Stroke/ Transient Ischemic 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:								

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Aspirin	16 (10%)	6 (12%)	5 (10%)	5 (9%)	47 (18%)	20 (22%)	11 (13%)	16 (18%)
P2Y712 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 arrival at primary PCI centre, median (IQR) hrs	2.2 (1.6, 3.4)	2.2 (1.7, 3.1)	2.1 (1.5, 3.8)	2.5 (1.4, 3.4)	2.2 (1.5, 3.2)	2.0 (1.5, 3.1)	2.2 (1.5, 3.3)	2.2 (1.6, 3.2)
Arrival at primary PCI centre to reperfusion, median (IQR) hrs	0.4 (0.3, 0.6)	0.4 (0.3, 0.6)	0.4 (0.3, 0.6)	0.4 (0.3, 0.6)	0.4 (0.3, 0.6)	0.4 (0.3, 0.6)	0.4 (0.3, 0.6)	0.4 (0.3, 0.6)
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:								
Hemoglobin, 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

* Renal impairment was defined according to the estimated glomerular filtration rate (eGFR), with an eGFR <59 mL/min/1.73 m^2 fulfilling the criteria for renal impairment.

† 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), 2; creatinine, 68; eGFR, 68; haemoglobin, 16; platelets, 30.

Abbreviations: ACE, angiotensin-converting enzyme; ARB, angiotensin-receptor blocker; eGFR, estimated glomerular filtration rate; IQR, interquartile range; PCI, percutaneous coronary intervention; TIMI, Thrombolysis in Myocardial Infarction.

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Table 2. Procedure characteristics, by subgroups of TIMI flow grade (≤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.

	Impaired coronary flow (TIMI flow ≤ 2)				Normal coronary flow (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: *								
Left anterior descending	82 (53%)	26 (52%)	27 (55%)	29 (53%)	109 (41%)	40 (44%)	35 (40%)	34 (39%)
Circumflex	12 (8%)	3 (6%)	3 (6%)	6 (11%)	41 (15%)	16 (17%)	14 (16%)	11 (13%)
Right coronary artery	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 (5%)	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%)
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%)
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%)
Post stent dilatation	133 (86%)	48 (96%)	42 (86%)	43 (78%)	233 (87%)	76 (83%)	76 (87%)	81 (92%)

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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, No/ 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 (4.3)	5/78 (6%)	3/77 (4%)	2/75 (3%)
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)
Activated clotting time (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, No/ total (%)	28/151 (19%)	8/49 (16%)	14/48 (29%)	6/54 (11%)	32/259 (12%)	14/90 (15%)	10/85 (12%)	8/84 (10%)
Glycoprotein IIb/IIIa antagonist, No/ 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
Days from PCI to 2 – 7 days CMR, 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 3-month CMR, 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)

None of the participants received intravenous or intracoronary treatment with bivalirudin, metoprolol, nicorandil, or sodium nitroprusside.

* The angiographic parameters are based on central laboratory assessments.

† TIMI flow grade is a visual assessment of antegrade coronary artery flow at angiography, graded from 0 (no flow) to 3 (normal flow).

‡ TIMI thrombus grade allows the classification of thrombus burden (greatest dimension) revealed during coronary angiography. TIMI thrombus grade 0, no thrombus; 1, possible thrombus, with reduced contrast density, haziness, irregular lesion contour; 2, definite thrombus less than half

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the vessel diameter; 3, definite thrombus greater than half, but less than 2 vessel diameters; 4, definite thrombus greater than or equal to 2 vessel diameters; 5, total occlusion.

Missing: Activated clotting time, 96; aspirin loading dose, 56; duration of study drug infusion 24; glycoprotein IIb/IIIa antagonist, 11; inhaled oxygen, 11.

Abbreviations: CMR, cardiovascular magnetic resonance; IQR, interquartile range; PCI, percutaneous coronary intervention; QCA, quantitative coronary angiography; TIMI, Thrombolysis in Myocardial Infarction.

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Table 3. Analysis of CMR parameters 2–7 days after primary PCI, by subgroups of TIMI flow grade (≤2 vs. 3) immediately before study drug administration (adjusted for MI location [anterior vs. non-anterior]). Treatment effect estimates and interaction with treatment received are shown (see footnotes). Data are mean ± SD, median [IQR], or n (%), unless otherwise stated.

	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)
MVO presence (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 presence (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	

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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	
Myocardial salvage index (c)								
TIMI flow ≤2	0.4 ± 0.3	0.3 ± 0.2	0.3 ± 0.2	-0.06 (-0.16, 0.04) p=0.231	-0.09 (-0.18, 0.01) p=0.080	0.108	-0.07 (-0.16, 0.01) p=0.086	**0.049**
TIMI 3 flow	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	0.02 (-0.05, 0.09) p=0.593	0.04 (-0.03, 0.11) p=0.254		0.03 (-0.03, 0.09) p=0.329	
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	

(a) Treatment effect estimates reported as odds ratios 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 a linear regression model.
(d) Data analysed on a logarithmic scale. Treatment effect estimates reported as relative difference between groups.

The p values and 95% CI have not been adjusted for multiplicity, therefore these analyses should be interpreted as exploratory and not definitive.

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* Missing data: MVO extent, or presence/ absence (n=38); myocardial haemorrhage extent (n=73); myocardial haemorrhage presence/ absence (n=55); infarct size, or myocardial salvage index (n=38); LV ejection fraction (n=34)

† Given the high proportion of participants with a 0 value for MVO amount (56% of participants), and myocardial haemorrhage amount (57% of participants) the median value for MVO and myocardial haemorrhage was 0 for all groups, while the mean (SDs) are not ideal summaries for these data, it has been reported as such for this reason.

Abbreviations: CI, confidence interval; CMR, cardiovascular magnetic resonance; LV, left ventricular; MI, myocardial infarction; MVO, microvascular obstruction; TIMI, Thrombolysis in Myocardial Infarction.

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Table 4. Analysis of CMR parameters 3 months after primary PCI, by subgroups of TIMI flow grade (≤2 vs. 3) immediately before study drug administration (adjusted for MI location [anterior vs. non-anterior]). Treatment effect estimates and interaction with treatment received are shown (see footnotes). Data are mean ± SD, or median [IQR], unless otherwise stated.

	Treatment Group			Treatment Effect			Treatment Effect	
				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)
	Placebo (n=142)*	Alteplase 10mg (n=136)*	Alteplase 20mg (n=143)*	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, 7.3) 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, 2.7) p=0.675		-0.91 (-3.9, 2.08) p=0.552	
Myocardial salvage index (a)								
TIMI flow ≤2	0.5 ± 0.3	0.5 ± 0.2	0.5 ± 0.2	0.0 (-0.09, 0.10) p=0.929	-0.04 (-0.13, 0.06) p=0.437	0.844	-0.02 (-0.10, 0.07) p=0.671	0.623
TIMI 3 flow	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.02 (-0.05, 0.09) p=0.596	0.0 (-0.07, 0.07) p=0.940		0.01 (-0.05, 0.07) p=0.801	
LV ejection fraction (%) (a)								
TIMI flow ≤2	47.4 ± 11.9	47.3 ± 8.7	46.8 ± 9.2	-0.26 (-3.83, 3.32) p=0.889	-0.69 (-4.15, 2.77) p=0.696	0.805	-0.49 (-3.55, 2.57) p=0.754	0.632
TIMI 3 flow	50.8 ± 6.8	49.1 ± 7.3	49.9 ± 7.9	-1.7 (-4.31, 0.90) p=0.201	-1.13 (-3.71, 1.44) p=0.389		-1.41 (-3.65, 0.82) p=0.216	

(a) Treatment effect estimates reported as mean differences between groups.

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(b) Data analysed on a logarithmic scale. Treatment effect estimates reported as relative difference between groups.

The p values and 95% CI have not been adjusted for multiplicity, therefore these analyses should be interpreted as exploratory and not definitive.

*Missing data: infarct size (n=66); myocardial salvage index (n=72); LV ejection fraction (n=63).

Abbreviations: CI, confidence interval; CMR, cardiovascular magnetic resonance; IQR, inter quartile range; LV, left ventricular; SD, standard deviation; MI, myocardial infarction; TIMI, Thrombolysis in Myocardial Infarction.

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Supplemental Table 5. Analysis of coagulation variables at 2 hours compared to baseline, by subgroups of TIMI flow grade (≤2 vs. 3) immediately before study drug administration (adjusted for MI location [anterior vs. non-anterior]). Treatment effect estimates and interaction with treatment received are shown (see footnotes). Data are median [IQR], unless otherwise stated.

	Treatment Group			Treatment Effect			Treatment Effect	
				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)
	Placebo (n=142)*	Alteplase 10mg (n=136)*	Alteplase 20mg (n=143)*	Estimate (95% CI) p-value	Estimate (95% CI) p-value		Estimate (95% CI), p-value	
Ratio of fibrinogen at 2 hours relative to baseline (a)								
TIMI flow ≤2	1.00 [1.00, 1.14]	1.03 [0.90, 1.17]	0.97 [0.90, 1.05]	1.02 (0.95, 1.09) p=0.658	0.93 (0.87, 0.99) **p=0.021**	**0.032**	0.96 (0.91, 1.02) p=0.237	0.594
TIMI 3 flow	1.00 [0.90, 1.11]	1.00 [0.90, 1.10]	1.00 [0.90, 1.14]	0.98 (0.93, 1.03) p=0.336	0.99 (0.94, 1.04) p=0.761		0.98 (0.94, 1.03) p=0.467	
Change in plasminogen (U/dL) at 2 hours relative to baseline (b)								
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 (-10.70, -5.60) **p<0.001**	
Ratio of fibrin D-dimer at 2 hours relative to baseline (a)								
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**	

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Ratio of prothrombin fragment F_{1+2} at 2 hours relative to baseline (a)								
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**	
Ratio of tissue plasminogen activator at 2 hours relative to baseline (a)								
TIMI flow ≤2	1.1 [0.0, 3.0]	1.4 [1.2, 1.7]	1.5 [1.3, 2.0]	1.29 (0.95, 1.74) p=0.105	1.46 (1.09, 1.94) **p=0.011**	0.761	1.38 (1.06, 1.78) **p=0.015**	0.441
TIMI 3 flow	1.1 [-0.3, 2.0]	1.3 [1.1, 1.7]	1.6 [1.3, 1.9]	1.14 (0.92, 1.42) p=0.233	1.29 (1.04, 1.60) **p=0.021**		1.21 (1.01, 1.46) **p=0.041**	

(a) Data analysed on a logarithmic scale. Treatment effect estimates reported as relative difference between groups.

(b) Treatment effect estimates reported as mean differences between groups.

The p values and 95% CI have not been adjusted for multiplicity, therefore these analyses should be interpreted as exploratory and not definitive.

*Missing data: change in coagulation parameters at 2 hours relative to baseline (n=80)

Abbreviations: IQR, inter quartile range; MI, myocardial infarction; TIMI, Thrombolysis in Myocardial Infarction.

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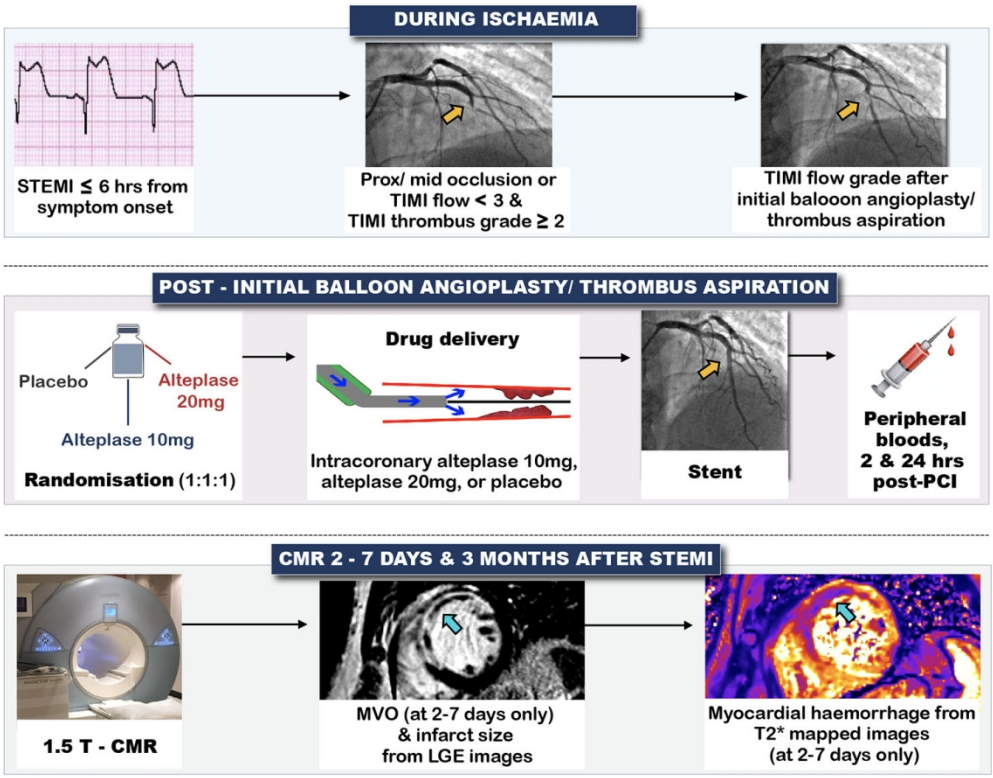

Figure 1. Graphical layout of trial protocol

127x98mm (300 x 300 DPI)

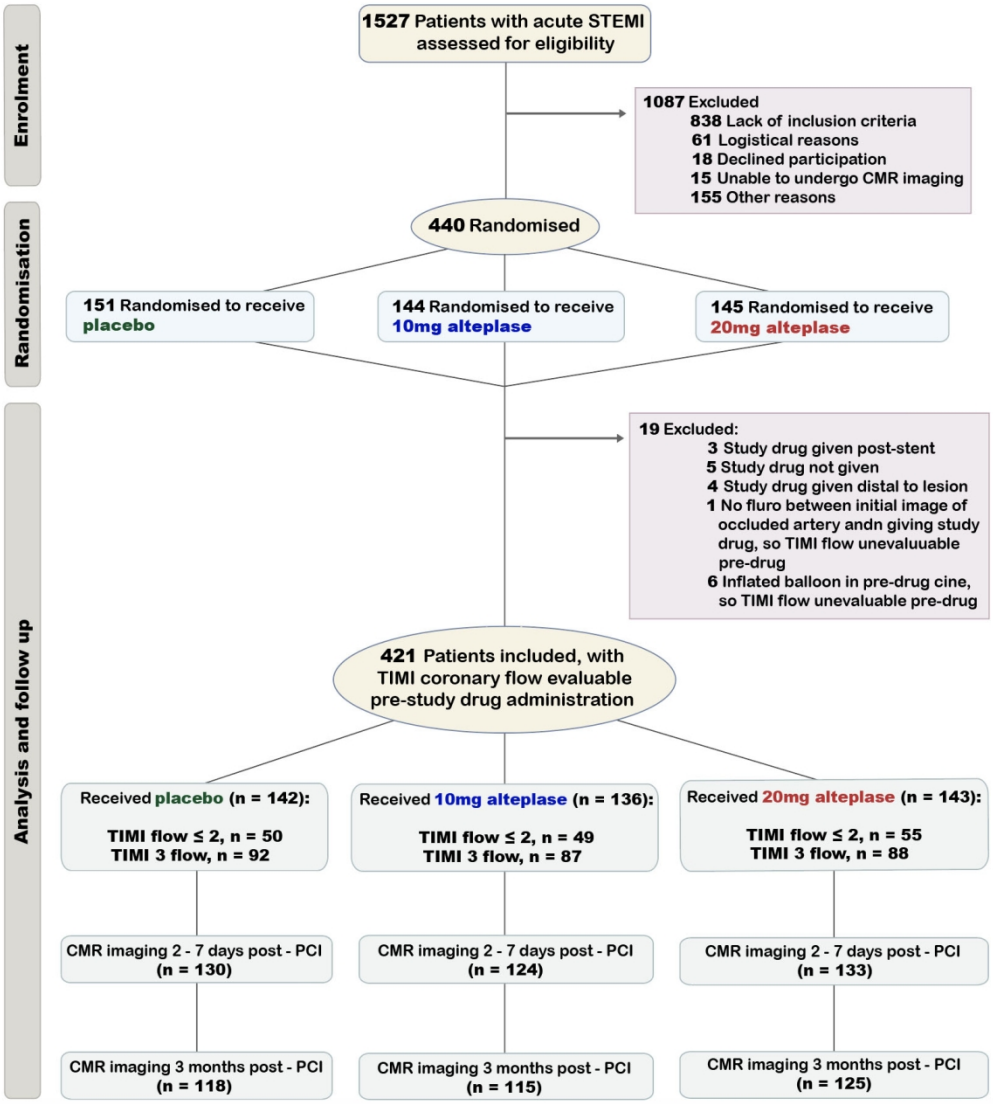

Figure 2. Study flow diagram

137x152mm (300 x 300 DPI)

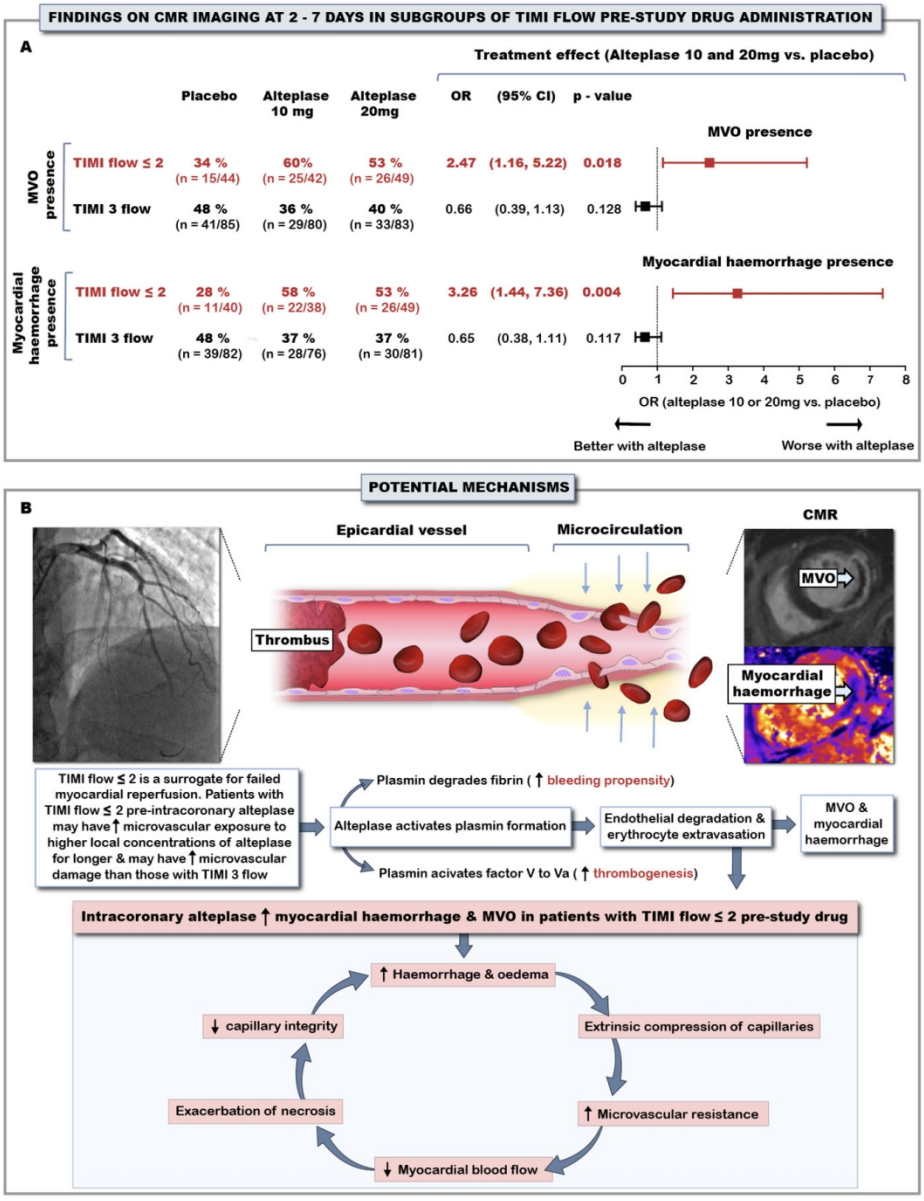

Figure 3. Summary of main findings and potential mechanisms

117x152mm (300 x 300 DPI)

SUPPLEMENTAL METHODS & RESULTS

Effect of coronary flow on intracoronary alteplase, a pre-specified analysis from a randomised trial

ClinicalTrials.gov: NCT02257294

Contents

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Eligibility Criteria

Patients with a clinical diagnosis of acute ST-segment elevation myocardial infarction (STEMI) were eligible for randomisation according to the following eligibility criteria:

Inclusion

- Acute MI (symptom onset ≤ 6 hours) with persistent ST-segment elevation or recent left bundle branch block
- Coronary artery occlusion (TIMI [Thrombolysis in Myocardial Infarction] coronary flow grade 0 or 1), or impaired coronary flow (TIMI coronary flow grade 2, slow but complete filling) in the presence of definite angiographic evidence of thrombus (TIMI grade 2 or more)
- Proximal-mid culprit lesion location in a major coronary artery (i.e. the right, left anterior descending, intermediate, or circumflex artery)
- Radial artery access
- Successful coronary reperfusion (TIMI coronary flow grade ≥2) pre-stent achieved prior to randomisation.
- Informed consent, i.e. only patients who were sufficiently well to understand the information about the study, as described by the attending cardiologist, were eligible to participate.

Exclusion

- Normal flow in the culprit coronary artery at initial angiography (TIMI grade 3)
- Functional coronary collateral supply (Rentrop grade 2/3) to the culprit artery
- Previous infarction in the culprit artery (known or suspected clinically, e.g. wall motion abnormality revealed by echocardiography)
- Cardiogenic shock (Killip Class IV)
- Multivessel percutaneous coronary intervention (PCI) intended before the day 2-7 cardiovascular magnetic resonance (CMR) scan
- Estimated body weight <60 kg

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- Non-cardiac co-morbidity with expected survival <1 year
- Contra-indication to contrast-enhance CMR imaging
- Pacemaker, or implantable defibrillator
- Known impaired renal function (estimated glomerular filtration rate <30ml/min)
- Significant bleeding disorder either at present or within the past 6 months
- Known haemorrhagic diathesis
- 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)
- Prolonged cardiopulmonary resuscitation (>2 minutes) within the past 2 weeks
- Acute pericarditis and/ or subacute bacterial endocarditis
- Acute pancreatitis
- Severe hepatic dysfunction, including hepatic failure, cirrhosis, portal hypertension (oesophageal varices) and active hepatitis
- Active peptic ulceration
- Arterial aneurysm and known arterial/ venous malformation
- Neoplasm with increased bleeding risk
- Any known history of haemorrhagic stroke, or stroke of unknown origin
- Known history of ischaemic stroke, or transient ischemic attack in the preceding 6 months
- Dementia

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- Hypersensitivity to gentamicin, or natural rubber
- Incapacity, or inability to provide informed consent
- 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
- Requirement for immunosuppressive therapy at any time during 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 or prophylactic treatment with oral, or parenteral antibiotic, antifungal, or antiviral therapy, to prevent or treat infection
- 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 (VEGFR) 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.

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Standard Care

Standard care for coronary reperfusion was with balloon angioplasty, or aspiration thrombectomy for thrombus-containing lesions. A coronary balloon diameter (mm) vs. lumen diameter (mm) relationship of <1:1 and a low inflation pressure were recommended to minimise thrombus embolization. The balloon angioplasty was intended to stabilise the thrombotic lesion and prevent vessel re-occlusion prior to stent implantation. Anti-thrombotic therapy included oral anti-platelet drugs and intravenous heparin (5000 IU, or as per standard practice) at the first medical contact. The target activated clotting time (ACT) was 250s.

Interventions

After initial balloon angioplasty/ thrombus aspiration, the participants were randomised using an interactive voice response-based system, and then received the allocated intervention. The study drug (placebo, alteplase 10mg, or alteplase 20mg) was manually infused before stent implantation. The drug was reconstituted by the clinical staff using 20ml of sterile water for injection. The cardiologist then infused the solubilised drug over 5-10 minutes directly into the culprit artery, proximal to the culprit lesions, using either an intracoronary catheter or the guiding catheter if selectively engaged.

Angiogram Acquisition & Analysis Methods

Coronary angiograms were acquired during emergency care with cardiac catheter laboratory X-ray and information technology equipment. The angiograms were analysed using post-processing software (QAngio® XA Medis, Leiden, NL.) by experienced investigators who were blinded to treatment allocation. Catheter calibration was performed using the catheter calibration function on MEDIS QAngio. For each lesion, a view perpendicular to the long axis of the vessel was used in order to avoid foreshortening and overlap of branches. The single plane projection showing the best opacified and most severe lesion with minimal foreshortening and minimal branch overlap was selected. Feedback was provided to sites on the quality and completeness of the angiograms.

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TIMI Coronary Flow Grade

The TIMI coronary flow grade was assessed using the following definitions(1):

TIMI coronary flow grade	Definition
0	No flow
1	Minimal flow past obstruction
2	Slow (but complete) filling and slow clearance
3	Normal flow and clearance

TIMI Myocardial Perfusion Grade

TIMI myocardial perfusion grade provides a score for ground-glass appearance ('blush') of the contrast entering the microvasculature and contrast washout. TIMI myocardial perfusion grade was assessed according to the following definitions(2):

TIMI myocardial perfusion grade	Definition
0	Minimal or no myocardial blush in the distribution of the culprit artery.
1	Myocardial blush is present in the distribution of the culprit artery. But there is incomplete clearance of dye between injections (with ~ 30 seconds between injections).
2	Myocardial blush is present in the distribution of the culprit artery. But there is slow contrast entry into the microvasculature and slow clearance of contrast. Specifically, blush is strongly persistent (i.e. either does not or only minimally diminishes in intensity) beyond 3 cardiac cycles after injection.
3	Myocardial blush is present in the distribution of the culprit artery, with normal entry and exit of dye (mild/ moderate persistence of dye beyond 3 cardiac cycles, but notably reduced after 3 cardiac cycles). Blush that is only mild intensity throughout 3 cardiac cycles after injection (washout phase), but fades minimally is also classified as grade 3.

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TIMI Frame Count

The TIMI frame count represents the amount of time (in frames) for contrast dye to reach a standardized distal landmark.(2) If the culprit vessel was the left anterior descending artery the frame count was divided by 1.7 (correcting for longer vessel length).

TIMI Coronary Thrombus Grade

Thrombus burden revealed during coronary angiography was classified according to the TIMI thrombus grade(3):

Thrombus grade	Definition
0	No angiographic characteristics of thrombus are present
1	Possible thrombus is present, with reduced contrast density, haziness, irregular lesion contour, or a smooth convex 'meniscus' at the site of total occlusion suggestive but not diagnostic of thrombus
2	Definite thrombus, with greatest dimensions ≤ half the vessel diameter
3	Definite thrombus but with greatest long axis dimension >1/2 but <2 vessel diameters
4	Definite thrombus, with the largest dimension ≥2 vessel diameters
5	Total occlusion

Lesion Characterisation

The culprit lesions were assessed for complexity using the modified American College of Cardiology/ American Heart Association score, which characterises coronary lesions as type A, B1 (one characteristic of a type B lesion), B2 (two or more characteristics of a type B lesion) and C.(4)

The culprit lesions were also assessed for complexity using a 6-point plaque characterisation score,(5) comprising:

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(i) Intraluminal filling defect consistent with thrombus

(ii) Ulcerated appearance, for example hazy contour, and/ or apple-core appearance

(iii) Irregularity of vessel borders

(iv) TIMI flow <3 beyond the lesion

(v) Moderate to severe calcification, i.e. calcification in more than one cine, outlining the full lumen

(vi) Lesion at a bifurcation point

CMR Acquisition and Analysis

CMR was performed using 1.5-T platforms (Siemens MAGNETOM Avanto, Erlangen, Germany and Philips Intera, Best, The Netherlands). The imaging protocol followed a standard operating procedure that included planning and localisers, T1-mapping, T2*-mapping, cine CMR with steady-state free precession (SSFP), and late gadolinium enhancement imaging 10 – 15 minutes after administration of contrast media.(6) The scan acquisitions were spatially co-registered and also included different slice orientations to enhance diagnostic confidence.

The intravenous contrast agent used in this study was gadobutrol (Gadovist®, Bayer: 1.5 mmol/ml solution for injection), which was administered in two doses. The first dose injection (0.05 mmol/kg) was given to initiate the first-pass of contrast. The second dose (0.1 mmol/kg) was given immediately after the first-pass. Therefore, the total dose of gadobutrol was 0.15 mmol/kg.

SSFP cine breath-hold sequences (with parallel imaging acceleration) were used. The heart was imaged in multiple parallel SAX planes 8-mm thick, separated by 2mm gaps, equating to approximately 10 slices and 30 cardiac phases. The CMR analyses were undertaken using Medis® Suite MR (Medis, Leiden, NL), by two trained investigators who were blinded to treatment allocation.

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Late Gadolinium Enhancement

Late microvascular obstruction (MVO) was imaged 10-15 minutes after intravenous Gadovist contrast administration, using in general a motion corrected T1-weighted phase-sensitive inversion recovery radiofrequency pulse sequence. A full stack, aligned to T2* scans (or cines) and 3 long axis views (vertical long axis, horizontal long axis and 3 chamber view) were acquired.

MVO was defined as a dark zone on early gadolinium enhancement imaging 1, 3, 5 and 7-minutes post-contrast injection that remained present within an area of late gadolinium enhancement at 15 minutes. The endocardial and epicardial borders were contoured. The myocardial mass (grams) of the dark zone was quantified by manual delineation and expressed as a percentage of total left ventricular (LV) mass.

Infarct Size

The presence of acute infarction was established based on abnormalities in cine wall motion, rest first-pass myocardial perfusion, and late gadolinium enhancement imaging in two imaging planes. The myocardial mass of late gadolinium (grams) was quantified using computer assisted planimetry and the territory of infarction was delineated using a 5 standard deviation method and expressed as a percentage of total LV mass. Typical late gadolinium enhancement and MVO imaging parameters with phase sensitive inversion recovery: matrix 192 x 256 pixels; flip angle 25º; TE 3.36 ms; bandwidth 130 Hz/pixel; echo spacing 8.7ms and trigger pulse 2. The voxel size is 1.8 x 1.3 x 8 mm. Inversion times individually adjusted to optimize nulling of apparently normal myocardium (typical values, 200 to 300ms).

Myocardial Oedema

The presence of myocardial oedema was established based on an area of increased signal intensity on the SSFP cine images (acquired two minutes after gadolinium contrast

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injection). The myocardial mass was calculated by manual delineation in end-diastole and end-systole. The values were averaged and expressed as a percentage of LV mass.(6)

Myocardial Salvage

Myocardial salvage was calculated by subtraction of percent infarct size from percent area-at risk, as reflected by the extent of oedema. The myocardial salvage index was calculated by dividing the myocardial salvage area by the initial area-at-risk.

Myocardial Haemorrhage

On the T2* parametric maps, a threshold of 20ms was applied. A region of reduced signal intensity within the infarcted area, with a T2* value of <20 ms(7)(8) was considered to confirm the presence of myocardial haemorrhage. The area was manually delineated and expressed as % LV mass.

Local Hospital Blood Sample Handling

Blood samples were measured when site logistics permitted. The sampling time-points were 0, 2 and 24 hours post-PCI. Blood samples were collected into 0.109M sodium citrate (for haemostasis assays), or EDTA (Troponin). The blood samples were centrifuged locally and plasma separated and frozen within 2 hours of sampling. Frozen plasma samples were subsequently transported on dry ice for central laboratory analysis in the department of Haematology, Macewan Building, 16 Alexandra Parade, Glasgow Royal Infirmary, G31 2ER. Plasma samples were stored at -80ºC until analysis, with residual samples being transferred to the Glasgow Biorepository for storage at the end of the study.

Central Laboratory Analysis for Troponin T

EDTA plasma samples were stored at -80ºC in the Glasgow Royal Infirmary until batch analysis at the end of the study. The biochemical analyses were performed in the British Heart Foundation Glasgow Cardiovascular Research Centre.

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EDTA plasma samples were stored to analyse high-sensitivity cardiac troponin T (ng/ml) on first thaw. Serial measurements of troponin T using the Roche high-sensitivity assay were used to provide a biochemical measurement of infarct size (area-under-the-curve).

For measurement of high sensitivity cardiac troponin T, we used an automated method (e411, Roche Diagnostic, Burgess Hill, U.K.) calibrated and quality controlled using the manufacturers reagents. We also participated in the National External Quality Assurance Scheme (NEQAS). The lower limit of detection of Troponin T is 0.003 ng/ml and the 99th percentile value in a healthy subpopulation is 0.0014 ng/ml (Roche Diagnostics, data on file). The between-assay coefficient of variations were 2.2% and 4.2% for control materials with mean Troponin T concentrations of 2.098 ng/ml and 0.00027 ng/ml, respectively.

Central Laboratory Analysis for Coagulation Parameters

The coagulation parameters measured in this study included fibrinogen and plasminogen (both measures of coagulation and systemic fibrinolysis), fibrin D-Dimer (a measure of fibrin lysis), tissue plasminogen activator (tPA) (a measure of endogenous tPA and any circulating alteplase) and prothrombin fragment F1+2 (a measure of thrombin activation). A depletion of fibrinogen and plasminogen following thrombolysis correlates with systemic fibrinolysis and may correlate with bleeding risk. Prothrombin fragment F1+2 is a measure of thrombin activation and correlate with the (undesired) procoagulant effect of thrombolysis. Prothrombin fragment F1+2 is depressed by anti-coagulants administered before and during PCI.

Standard laboratory assays (Fibrinogen by Clauss method; high sensitivity Fibrin D-Dimer by latex immunoassay; and Plasminogen Activity by chromogenic assay were performed on an IL TOP700 analyser using HemosIL® reagents (Instrumentation Laboratory Company, Bedford, U.S.). The fibrinogen Clauss assay had a normal reference rages 170 – 4.0 g/L (internally derived) and an inter-assay coefficient of variation of 5.8% and 7.7% for

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low control samples with mean concentrations of 2.92 g/L and 2.22 g/L respectively. The fibrin D-Dimer assay had a normal reference range <0.230 μg/ml (manufacturer derived), and an inter-assay coefficient of variation of 11.7% and 5.2% for control samples with mean concentrations of 0.343 μg/ml and 0.770 μg/ml respectively. The plasminogen activity assay had a normal reference rage 80 – 133 U/dL (manufacturer derived), and an inter-assay coefficient of variation of 2.1% and 1.8% for control samples with mean concentrations of 95.4 U/dL and 29.6 U/dL, respectively.

Non-standard laboratory ELISA assays (tissue plasminogen activator [tPA] and Prothrombin fragment F1+2 antigen levels) were performed on a TECAN Sunrise spectrophotometer (Labtech International Ltd, U.K.) using Zymutest tPA Antigen (Hyphen BioMed, Neuville-sur-oise France) and Enzygnost F1+2 Mono (Siemens, Marburg, Germany) commercial kits respectively. The tPA antigen assay had a normal reference range <10 ng/ml (manufacturer derived), and an inter-assay coefficient of variation of 4.7% and 11% for control samples with mean concentrations of 11.0 ng/ml and 3.1 ng/ml, respectively. The F1+2 assay had a normal reference rage 69 – 229 pmol/L (manufacturer derived) and an inter-assay coefficient of variation of 7.9% for a normal control sample with a mean concentration of 97.6 pmol/L.

Trial Management

There was a Trial Management Group for operational activity, an independent Data and Safety Monitoring Committee and a Trial Steering Committee to coordinate the trial and liaise with the Sponsor and Trials Unit. Each committee had a charter that was established before enrolment started.

The independent Data and Safety Monitoring Committee met before the enrolment began, and twice again during the active phase of the trial. This committee had responsibility for potentially recommending early discontinuation of the entire study or an individual arm,

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because of safety concerns or due to futility. The funder, the Efficacy and Mechanism Evaluation (EME) program of the National Institute for Health Research (NIHR) required an interim analysis for futility and also specified the criteria. Following a prespecified futility analysis, performed when 40% of the trial population had reached 3 months follow-up, the Data and Safety Monitoring Committee recommended that enrolment into the T-TIME trial should be discontinued on December 21 2017.

The Robertson Centre for biostatistics within the Glasgow Clinical Trials Unit provided the trial-specific electronic data collection system, acted as an independent coordination centre for randomisation and data management. The trial was approved by the National Research Ethics Service (reference 13/WS/0119). The clinical trial registration number is NCT02257294 and the trial was co-sponsored by the University of Glasgow and greater Glasgow and Clyde Health Board, NHS Scotland. The sponsor undertook feasibility assessments at each site, visits were undertaken in all of the sites. All serious adverse events were prospectively reported to the Pharmacovigilance Unit.

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Supplemental Table 1. Additional procedure characteristics, by subgroups of TIMI flow grade (≤2 vs. 3) immediately before study drug administration. Data are reported according to treatment received (n=421). Data are mean ± SD, or n (%).

	Impaired coronary flow (TIMI flow ≤ 2)				Normal coronary flow (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)**
American Heart Association culprit lesion type: *								
B2	42 (27%)	13 (26%)	15 (31%)	14 (26%)	62 (23%)	17 (19%)	20 (23%)	25 (28%)
C	112 (73%)	37 (74%)	34 (69%)	41 (75%)	205 (77%)	75 (82%)	67 (77%)	63 (72%)
Culprit lesion plaque characterisation score: † *								
2	1 (1%)	0	1 (2%)	0	3 (1%)	1 (1%)	2 (2%)	0
3	20 (13%)	5 (10%)	6 (12%)	9 (16%)	76 (29%)	27 (29%)	26 (30%)	23 (26%)
4	115 (75%)	40 (80%)	35 (71%)	40 (73%)	164 (61%)	54 (59%)	53 (61%)	57 (65%)
5	17 (11%)	4 (8%)	7 (14%)	6 (11%)	24 (9%)	10 (11%)	6 (7%)	8 (9%)
6	1 (1%)	1 (2%)	0	0	0	0	0	0
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
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%)

* The angiographic parameters are based on central laboratory assessments.

† The plaque characterisation score comprised one point for each of: intraluminal thrombus, ulceration, irregularity of vessel borders, TIMI flow <3 beyond the lesion, moderate-severe calcification and bifurcation.

Abbreviations: QCA, quantitative coronary angiography.

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Supplemental Table 2. Analysis of CMR derived LV end-diastolic and end-systolic volumes, by subgroups of TIMI flow grade (≤2 vs. 3) immediately before study drug administration (adjusted for MI location [anterior vs. non-anterior]). Data are median [IQR].

	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	
CMR parameters 2-7 days after primary PCI								
LV end-diastolic volume (ml)								
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	
LV end-systolic volume (ml)								
TIMI flow ≤2	96.2 [80.2, 118.9]	105.3 [85.6, 124.3]	95.5 [80.8, 113.6]	0.0 (-0.85, 0.86) p=0.993	0.73 (-0.08, 1.54) p=0.080	0.261	0.40 (-0.32, 1.13) p=0.277	0.284
TIMI 3 flow	90.2 [75.9, 108.0]	92.9 [79.0, 113.5]	92.5 [72.3, 109.0]	-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=0.72	
CMR parameters 3 months after primary PCI								
LV end-diastolic volume (ml)								
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	
LV end-systolic volume (ml)								
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	

Data analysed on a logarithmic scale. Treatment effect estimates reported as relative difference between groups.

The p values and 95% CI have not been adjusted for multiplicity, therefore these analyses should be interpreted as exploratory and not definitive.

* Missing data: LV volumes 2 – 7 days after primary PCI (n=34), LV volumes 3 months after primary PCI (n=63).

* Missing data: LV volumes 2 – 7 days after primary PCI (n=34), LV volumes 3 months after primary PCI (n=63).

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Supplemental Table 3. Analysis of selected CMR parameters 2-7 days after primary PCI, by subgroups of TIMI flow grade (≤2 vs. 3) immediately before study drug administration, and by subgroups of MI location (anterior [n=187], non-anterior [n=234]). Treatment effect estimates and interaction with treatment received are shown (see footnotes). Data are mean ± SD, or n (%), unless otherwise stated.

		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 presence (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 (1.25, 17.44) **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	0.0 (-0.85, 0.86) p=0.993	0.73 (-0.08, 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=0.72	
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 (-0.48, 0.44) p=0.922		-0.04 (-0.45, 0.36) p=0.828	
Myocardial haemorrhage presence (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 (21.1)	12/19 (63.2)	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 (0.22, 1.22) 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	1.74 (-1.10, 4.64) p=0.230	0.472	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=0.012**	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	

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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	331. ± 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, 2.1) p=0.475	
Myocardial salvage index (c)									
Anterior-MI:	TIMI flow ≤2	0.4 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	-0.09 (-0.22, 0.04) p=0.197	-0.05 (-0.18, 0.08) p=0.434	0.217	-0.07 (-0.18, 0.04) p=0.241	0.085
	TIMI 3 flow	0.3 ± 0.2	0.4 ± 0.2	0.4 ± 0.3	0.05 (-0.06, 0.15) p=0.402	0.08 (-0.03, 0.18) p=0.173		0.06 (-0.03, 0.15) p=0.202	
Non-anterior MI:	TIMI flow ≤2	0.4 ± 0.3	0.3 ± 0.3	0.2 ± 0.2	-0.03 (-0.18, 0.12) p=0.676	-0.13 (-0.28, 0.01) p=0.077	0.213	-0.08 (-0.21, 0.04) p=0.196	0.231
	TIMI 3 flow	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	0.00 (-0.10, 0.10) p=0.990	0.02 (-0.08, 0.11) p=0.740		0.01 (-0.07, 0.09) p=0.836	
LV ejection fraction (%) (c)									
Anterior-MI:	TIMI flow ≤2	38.8 ± 12.6	39.8 ± 8.0	40.8 ± 8.8	0.96 (-4.10, 6.02) p=0.711	2.03 (-2.83, 6.9) p=0.414	0.969	1.54 (-2.77, 5.84) p=0.485	0.896
	TIMI 3 flow	41.0 ± 8.4	41.3 ± 7.7	43.2 ± 7.0	0.29 (-3.81, 4.39) p=0.890	2.12 (-2.08, 6.32) p=0.323		1.16 (-2.39, 4.71) p=0.522	
Non-anterior MI:	TIMI flow ≤2	48.4 ± 3.2	43.4 ± 9.5	44.7 ± 8.4	-4.97 (-9.35, -0.59) **p=0.027**	-3.69 (-7.92, 0.54) p=0.089	0.199	-4.29 (-8.00, -0.57) **p=0.025**	0.093
	TIMI 3 flow	47.4 ± 6.3	47.2 ± 6.1	46.8 ± 8.2	-0.25 (-3.10, 2.59) p=0.861	-0.69 (-3.47, 2.09) p=0.627		-0.48 (-2.91, 1.94) p=0.697	

(a) Treatment effect estimates reported as odds ratios 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.

The p values and 95% CI have not been adjusted for multiplicity, therefore these analyses should be interpreted as exploratory and not definitive.

* Missing data: MVO extent, or presence/ absence (n=38); myocardial haemorrhage extent (n=73); myocardial haemorrhage presence/ absence (n=55); infarct size, or myocardial salvage index (n=38); LV ejection fraction (n=34)

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† Given the high proportion of participants with a 0 value for MVO amount (56% of participants), and myocardial haemorrhage amount (57% of participants) the median value for MVO and myocardial haemorrhage was 0 for all groups, while the mean (SDs) are not ideal summaries for these data. It has been reported as such for this reason.

Abbreviations: CI, confidence interval; CMR, cardiovascular magnetic resonance; LV, left ventricular; MI, myocardial infarction; MVO, microvascular obstruction; TIMI, Thrombolysis in Myocardial Infarction.

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Supplemental Table 4. Baseline characteristics according to availability of MVO data (complete vs. missing). Data are mean ± SD, or n (%), unless otherwise stated.

	MVO data available (n=383)	MVO data missing (n=38)	P-value
Age	60.5 ± 10.0	61.9 ± 12.8	0.501
Male	327 (85%)	31 (82%)	0.482
White	359 (94%)	37 (97%)	0.715
Asian	22 (6%)	1 (3%)	0.710
Body mass index (kg/m^2)	28.1 ± 4.9	29.1 ± 4.9	0.235
Heart rate at presentation, beats/ min	72.4 ± 19.1	75.2 ± 18.6	0.396
Systolic blood pressure at presentation, mmHg	133.8 ± 25.1	135.4 ± 27.3	0.730
Diastolic blood pressure at presentation, mmHg	81.1 ± 14.7	80.0 ± 16.0	0.683
Anterior myocardial infarction	170 (44%)	15 (40%)	0.610
Hypertension	117 (31%)	18 (47%)	**0.044**
Hypercholesterolemia	83 (22%)	13 (34%)	0.103
Diabetes mellitus †	45 (12%)	8 (21%)	0.120
Smoking:			
Current	176 (46%)	21 (55%)	0.308
Former (stopped >3 months)	74 (19%)	7 (18%)	1.000
Never	133 (35%)	10 (26%)	0.370
Pre-existing maintenance medication:			
Aspirin	54 (14%)	9 (24%)	0.148
Statin	77 (20%)	14 (37%)	**0.023**
Beta blocker	33 (9%)	7 (18%)	0.074
ACE inhibitor or ARB	62 (16%)	11 (29%)	0.069
Symptom onset to arrival at primary PCI centre, median (IQR) hrs	2.2 (1.5, 3.2)	2.5 (1.7, 3.5)	0.354
Arrival at primary PCI centre to reperfusion, median (IQR) hrs	0.4 (0.3, 0.6)	0.6 (0.4, 0.7)	**0.002**

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Initial blood results on admission:			
Hemoglobin, g/dL	145.6 ± 13.6	145.8 ± 10.3	0.892
Platelet count, $10^3/\mu L$	260.6 ± 60.9	270.9 ± 80.4	0.486
Creatinine, µmol/L	80.9 ± 17.7	78.6 ± 18.6	0.546
eGFR (ml/min/1.73m^2)	90.1 ± 20.4	92.5 ± 28.5	0.679

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Supplemental Table 5. Procedure characteristics according to availability of MVO data (complete vs. missing). Data are mean ± SD, or n (%), unless otherwise stated.

	MVO data available (n=383)	MVO data missing (n=38)	P-value
Culprit artery:			
Left anterior descending	176 (46%)	15 (40%)	0.497
Circumflex	44 (12%)	9 (24%)	**0.040**
Right coronary artery	163 (43%)	14 (37%)	0.606
>50% stenosis in ≥2 major coronary arteries	134 (35%)	10 (26%)	0.370
Initial TIMI coronary flow grade:			
≤1	337 (88%)	30 (79%)	0.126
≥2	46 (12%)	8 (21%)	0.126
Initial TIMI thrombus grade:			
3/4	76 (20%)	10 (26%)	0.398
5	307 (80%)	28 (74%)	0.398
American Heart Association culprit lesion type A	287 (75%)	30 (79%)	0.695
Culprit lesion plaque characterisation score ≥4	292 (76%)	29 (76%)	0.100
QCA lesion length pre-drug (mm)	26.8 ± 11.4	25.0 ± 10.0	0.305
Reperfusion achieved with balloon angioplasty	269 (70%)	32 (84%)	0.089
Balloon angioplasty pre-stent	354 (92%)	34 (90%)	0.523
Study drug delivered with thrombectomy catheter	278 (73%)	22 (58%)	0.062
Total number of stents deployed ≥2	115 (30%)	11 (29%)	1.000
Post-stent dilatation	337 (88%)	29 (76%)	0.072
Total length of stents deployed from QCA (mm)	34.0 ± 14.4	32.8 ± 14.9	0.638
QCA reference vessel diameter post-stent (mm)	3.2 ± 0.5	3.2 ± 0.4	0.535
Unfractionated heparin, median (IQR), U	10000.0 (75000.0, 13000.0)	8750.0 (7125.0, 12000.0)	0.135

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Activated clotting time (s)	281.7 ± 88.0	273.0 ± 94.0	0.673
Intravenous morphine	284 (74%)	27 (71%)	0.700
Inhaled oxygen (%)	55 (15%)	5 (15%)	1.000
Glycoprotein IIb/IIIa antagonist (%)	57 (15%)	9 (27%)	0.091
Duration of study drug infusion (min)	6.5 (2%)	6.4 (2%)	0.679

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Supplemental Table 6. Analysis of CMR parameters 2–7 days after primary PCI, by subgroups of TIMI flow grade (2 vs. 3) immediately before study drug administration (adjusted for MI location [anterior vs. non-anterior]). Treatment effect estimates and interaction with treatment received are shown (see footnotes). Data are mean ± SD, median [IQR], or n (%), unless otherwise stated.

	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 presence (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 presence (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	
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	
Myocardial salvage index (c)								
TIMI 2 flow	0.4 ± 0.3	0.3 ± 0.2	0.3 ± 0.2	-0.05 (-0.17, 0.08) p=0.464	-0.07 (-0.18, 0.04) p=0.205	0.201	-0.06 (-0.15, 0.04) p=0.245	0.117
TIMI 3 flow	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	0.02 (-0.05, 0.09) p=0.593	0.04 (-0.03, 0.11) p=0.254		0.03 (-0.03, 0.09) p=0.329	

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Supplemental Table 7. Analysis of MVO extent (% LV mass) 2-7 days after primary PCI, by subgroups of TIMI flow grade (≤2 vs. 3) immediately before study drug administration, with treatment effects derived by bootstrapping (10,000 replicates, stratified by the location of myocardial infarction).

	Treatment Effect on MVO extent		Treatment Effect on MVO extent
	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
TIMI flow ≤2	2.19 (-1.40, 4.08) p=0.284	3.37 (0.77, 6.89) **p=0.016**	2.80 (-0.09, 5.51) p=0.057
TIMI 3 flow	1.96 (-0.65, 3.20) p=0.237	1.91 (-0.74, 3.01) p=0.287	1.99 (-0.57, 2.92) p=0.246

Missing data: MVO extent (n=38).

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Supplemental Table 8. Analysis of electrocardiographic, biochemical and angiographic parameters, by subgroups of TIMI flow grade (≤2 vs. 3) at the time of study drug administration (adjusted for MI location [anterior vs. non-anterior]). Treatment effect estimates and interaction with treatment received are shown (see footnotes). Data are mean ± SD, median [IQR], or n (%), unless otherwise stated.

	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 10 mg (n=136)*	Alteplase 20 mg (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	
Absolute % ST-segment resolution 60 min (a)								
TIMI flow ≤2	45.0 ± 44.3	40.6 ± 52.1	37.7 ± 43.3	-4.43 (-21.96, 13.1) 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	
Troponin T (ng/mL) AUC, 0–24 hours (b)								
TIMI flow ≤2	2.66 [1.10, 5.20]	2.94 [1.73, 6.86]	4.60 [1.20, 8.19]	1.67 (0.96, 2.89) p=0.071	1.83 (1.07, 3.12) **p=0.029**	0.662	1.75 (1.09, 2.80) **p=0.021**	0.402
TIMI 3 flow	3.16 [1.16, 5.76]	2.67 [1.53, 5.71]	3.47 [1.57, 6.30]	1.38 (0.92, 2.08) p=0.120	1.34 (0.90, 2.00) p=0.151		1.36 (0.96, 1.92) p=0.082	
TIMI coronary flow grade post-PCI ≤2 (c)								
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	0.134	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	
Corrected TIMI frame count post-PCI (b)								
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	
Myocardial perfusion grade post-PCI ≤1 (c)								
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	

(a) Treatment effect estimates reported as mean differences between groups.

(b) Data analysed on a logarithmic scale. Treatment effect estimates reported as relative difference between groups.

(c) Treatment effect estimates reported as odds ratios between groups, from a logistic regression model.

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The p values and 95% CI have not been adjusted for multiplicity, therefore these analyses should be interpreted as exploratory and not definitive.
*Missing data: % ST-segment resolution (n=43); Troponin AUC (n=115); corrected TIMI frame count post-PCI (n=2).

Abbreviations: AUC, area-under-the-curve; CI, confidence interval; IQR, interquartile range; MI, myocardial infarction; SD, standard deviation; PCI, percutaneous coronary intervention; TIMI, Thrombolysis in Myocardial Infarction.

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Supplemental Table 9. Analysis of coagulation variables, at 2 hours, at 24 hours, and at 24 hours compared to baseline, by subgroups of TIMI flow grade (≤2 vs. 3) immediately before study drug administration (adjusted for MI location [anterior vs. non-anterior]). Treatment effect estimates and interaction with treatment received are shown (see footnotes). Data are median [IQR], unless otherwise stated.

	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	
Fibrinogen (g/L) 2 hours post-PCI (a)								
TIMI flow ≤2	3.3 [2.7, 4.0]	3.2 [2.6, 3.6]	3.1 [2.7, 3.6]	0.98 (0.88, 1.1) p=0.784	0.94 (0.84, 1.05) p=0.250	0.684	0.96 (0.87, 1.05) p=0.391	0.955
TIMI 3 flow	3.3 [2.8, 3.9]	3.0 [2.6, 3.8]	3.3 [2.8, 3.7]	0.96 (0.88, 1.04) p=0.279	0.97 (0.89, 1.05) p=0.441		0.96 (0.90, 1.03) p=0.282	
Plasminogen (U/dL) 2 hours post-PCI (b)								
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 (a)								
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 (a)								
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**	
Tissue plasminogen activator (ng/mL) 2 hours post-PCI (a)								
TIMI flow ≤2	11.0 [8.3, 13.0]	14.0 [11.0, 16.0]	15.0 [12.0, 19.3]	1.26 (1.00, 1.59) p=0.056	1.45 (1.16, 1.82) **p=0.001**	0.977	1.36 (1.11, 1.66) **p=0.003**	0.869
TIMI 3 flow	11.0 [9.0, 13.0]	13.0 [11.0, 17.0]	14.0 [12.0, 16.5]	1.30 (1.10, 1.54) **p=0.003**	1.48 (1.25, 1.76) **p<0.001**		1.39 (1.20, 1.61) **p<0.001**	
Fibrinogen (g/L) 24 hours post-PCI (a)								
TIMI flow ≤2	3.6 [3.0, 4.5]	3.6 [3.1, 4.4]	3.6 [3.0, 4.4]	1.03 (0.92, 1.16) p=0.576	0.99 (0.89, 1.11) p=0.927	0.384	1.01 (0.92, 1.12) p=0.791	0.176
TIMI 3 flow	3.8 [3.3, 4.6]	3.5 [2.8, 4.3]	3.5 [3.0, 4.1]	0.94 (0.86, 1.02) p=0.143	0.92 (0.85, 1.00) p=0.063		0.93 (0.87, 1.00) p=0.052	
Plasminogen (U/dL) 24 hours post-PCI (b)								
TIMI flow ≤2	91.0 [86.0, 102.0]	91.6 [84.8, 99.3]	86.0 [77.0, 94.0]	0.14 (0.00, 45.15) p=0.506	0.00 (0.00, 0.34) **p=0.021**	0.230	0.01 (0.00, 1.85) p=0.086	0.519
TIMI 3 flow	96.0 [83.0, 107.0]	88.0 [77.0, 99.3]	90.0 [80.0, 96.0]	0.00 (0.00, 0.07) **p=0.002**	0.00 (0.00, 0.16) **p=0.005**		0.00 (0.00, 0.06) **p<0.001**	

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Fibrin D-dimer (ng/mL) 24 hours post-PCI (a)								
TIMI flow ≤2	103.0 [59.0, 150.0]	162.0 [112.0, 371.8]	224.0 [151.0, 344.0]	2.05 (1.46, 2.87) **p<0.001**	2.11 (1.51, 2.94) **p<0.001**	0.205	2.08 (1.55, 2.78) **p<0.001**	0.078
TIMI 3 flow	130.0 [80.5, 201.5]	190.0 [112.8, 379.0]	224.0 [133.0, 325.0]	1.44 (1.13, 1.85) **p=0.004**	1.56 (1.23, 1.99) **p<0.001**		1.50 (1.22, 1.85) **p<0.001**	
Prothrombin fragment F_{1+2} (pmol/L) 24 hours post-PCI (a)								
TIMI flow ≤2	197.0 [145.0, 262.2]	191.7 [129.7, 297.3]	204.0 [155.0, 321.0]	1.04 (0.80, 1.36) p=0.750	1.21 (0.93, 1.58) p=0.159	0.643	1.13 (0.89, 1.42) p=0.319	0.802
TIMI 3 flow	226.0 [153.6, 334.0]	226.8 [173.5, 324.6]	234.0 [166.4, 327.7]	1.09 (0.89, 1.32) p=0.404	1.08 (0.89, 1.31) p=0.416		1.08 (0.92, 1.28) p=0.336	
Tissue plasminogen activator (ng/mL) 24 hours post-PCI (a)								
TIMI flow ≤2	10.0 [8.0, 12.0]	11.0 [8.8 12.0]	10.0 [8.0, 13.0]	1.02 (0.84, 1.24) p=0.829	1.05 (0.87, 1.28) p=0.594	0.803	1.04 (0.88, 1.23) p=0.666	0.627
TIMI 3 flow	9.0 [7.0, 11.5]	10.0 [8.0 12.0]	10.0 [8.0, 12.0]	1.04 (0.91, 1.20) p=0.549	1.14 (0.99, 1.31) p=0.068		1.09 (0.97, 1.23) p=0.152	
Ratio of fibrinogen at 24 hours relative to baseline (a)								
TIMI flow ≤2	1.12 [1.00, 1.26]	1.17 [1.10, 1.35]	1.16 [1.00, 1.37]	1.08 (0.99, 1.17) p=0.077	1.05 (0.96, 1.14) p=0.296	**0.040**	1.06 (0.99, 1.14) p=0.107	**0.013**
TIMI 3 flow	1.20 [1.00, 1.33]	1.10 [0.90, 1.35]	1.11 [1.00, 1.25]	0.95 (0.89, 1.01) p=0.101	0.95 (0.89, 1.00) p=0.071		0.95 (0.90, 1.00) **p=0.044**	
Change in plasminogen (U/dL) at 24 hours relative to baseline (b)								
TIMI flow ≤2	1.0 [-4.0, 5.5]	-3.0 [-9.0, 4.0]	-6.0 [-11.5, -2.3]	-2.57 (-6.46, 1.32) p=0.197	-7.05 (-10.89, -3.21) **p<0.001**	0.074	-4.87 (-8.25, -1.49) **p=0.005**	0.239
TIMI 3 flow	2.0 [-3.0, 6.0]	-6.5 [-10.3, 0.0]	-7.0 [-11.8, -0.3]	-7.52 (-10.36, -4.67) **p<0.001**	-7.22 (-10.04, -4.40) **p<0.001**		-7.37 (-9.81, -4.93) **p<0.001**	
Ratio of fibrin D-dimer at 24 hours relative to baseline (a)								
TIMI flow ≤2	1.1 [0.8, 1.3]	1.8 [1.2, 3.3]	1.6 [1.0, 2.7]	2.01 (1.46, 2.77) **p<0.001**	1.67 (1.22, 2.30) **p=0.002**	**0.019**	1.83 (1.38, 2.42) **p<0.001**	0.249
TIMI 3 flow	1.3 [0.9, 1.7]	1.7 [1.0, 2.5]	2.2 [1.4, 3.4]	1.26 (1.00, 1.59) p=0.055	1.76 (1.40, 2.22) **p<0.001**		1.49 (1.22, 1.83) **p<0.001**	
Ratio of prothrombin fragment F_{1+2} at 24 hours relative to baseline (a)								
TIMI flow ≤2	1.2 [1.0, 1.6]	1.5 [13.9, 2.0]	1.3 [1.0, 1.9]	1.26 0.93, 1.71) p=0.134	1.23 (0.91, 1.66) p=0.173	0.520	1.25 (0.96, 1.62) p=0.103	0.200
TIMI 3 flow	1.4 [0.9, 1.9]	1.4 [1.0, 1.6]	1.4 [1.2, 2.1]	0.87 (0.70, 1.09) p=0.219	1.16 (0.93, 1.45) p=0.182		1.01 (0.83, 1.22) p=0.937	
Ratio of tissue plasminogen activator at 24 hours relative to baseline (a)								
TIMI flow ≤2	1.1 [0.9, 1.3]	1.2 [1.0, 1.3]	1.1 [0.9, 1.3]	1.05 (0.78, 1.41) p=0.761	0.98 (0.73, 1.32) p=0.894	0.454	1.01 (0.78, 1.31) p=0.926	0.444
TIMI 3 flow	0.9 [0.8, 1.2]	1.0 [0.8, 1.2]	1.1 [0.8, 1.4]	0.84 (0.68, 1.05) p=0.121	0.95 (0.76, 1.18) p=0.635		0.89 (0.74, 1.08) p=0.240	

(a) Data analysed on a logarithmic scale. Treatment effect estimates reported as relative difference between groups.

(b) Treatment effect estimates reported as mean differences between groups.

The p values and 95% CI have not been adjusted for multiplicity, therefore these analyses should be interpreted as exploratory and not definitive.

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*Missing data: coagulation parameters 2 hours post-PCI (n=75); coagulation parameters 24 hours post-PCI (n=71); change in coagulation parameters at 24 hours relative to baseline (n=97).

Abbreviations: IQR, inter quartile range; MI, myocardial infarction; TIMI, Thrombolysis in Myocardial Infarction.

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8. Carrick D, Haig C, Ahmed N, et al. Myocardial hemorrhage after acute reperfused ST-segment-elevation myocardial infarction: relation to microvascular obstruction and prognostic significance. Circ Cardiovasc Imaging 2016;9(1):e004148.

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CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	Randomised trial is not stated in title, because the submitted manuscript is a substudy of a randomised trial.
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	Page 4 main manuscript
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	Page 8 main manuscript
	2b	Specific objectives or hypotheses	Pages 8 & 9 main manuscript
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	Page 10 to 12 main manuscript, and page 15 supplement.
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	Page 14 supplement
Participants	4a	Eligibility criteria for participants	Pages 3 – 5 supplement
	4b	Settings and locations where the data were collected	Page 10 main manuscript
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	Page 11 main manuscript
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	Page 9 main manuscript
	6b	Any changes to trial outcomes after the trial commenced, with reasons	Page 10 main manuscript
Sample size	7a	How sample size was determined	Page 10 main manuscript
	7b	When applicable, explanation of any interim analyses and stopping guidelines	Page 14 supplement

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Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	Pages 10 and 11 main manuscript
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	Pages 10 and 11 main manuscript
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	Pages 10 and 11 main manuscript
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	Pages 10 and 11 main manuscript
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	Pages 10 and 11 main manuscript
	11b	If relevant, description of the similarity of interventions	n/a
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	Pages 13 and 14 main manuscript
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	Pages 13 and 14 main manuscript
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Page 37 main manuscript
	13b	For each group, losses and exclusions after randomisation, together with reasons	Page 37 main manuscript
Recruitment	14a	Dates defining the periods of recruitment and follow-up	Page 10 main manuscript
	14b	Why the trial ended or was stopped	Page 14 supplement
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Pages 27 to 28 main manuscript
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	Pages 32 to 36 main manuscript
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	Pages 32 to 36 main manuscript and pages 16 to 26 supplement
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	Pages 32 to 36 main manuscript and pages 16 to 26 supplement
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	Pages 32 to 36 main manuscript and pages 16 to 26 supplement
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	These were detailed

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			in the original publication of the T-TIME randomised trial. McCartney P et al. JAMA. 2019 321(1):56-68. doi: 10.1001/jama.2018.19802. The manuscript submitted to Heart is a substudy of the T-TIME trial.
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	Page 21 main manuscript
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	Page 21 main manuscript
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	Pages 19 to 21 main manuscript
Other information			
Registration	23	Registration number and name of trial registry	Page 8 main manuscript
Protocol	24	Where the full trial protocol can be accessed, if available	Page 10 main manuscript
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	Page 2

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

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