ORIGINAL RESEARCH

Effects of Intracoronary Alteplase on Microvascular Function in Acute Myocardial Infarction

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Background—Impaired microcirculatory reperfusion worsens prognosis following acute ST-segment–elevation myocardial infarction. In the T-TIME (A Trial of Low-Dose Adjunctive Alteplase During Primary PCI) trial, microvascular obstruction on cardiovascular magnetic resonance imaging did not differ with adjunctive, low-dose, intracoronary alteplase (10 or 20 mg) versus placebo during primary percutaneous coronary intervention. We evaluated the effects of intracoronary alteplase, during primary percutaneous coronary intervention, on the index of microcirculatory resistance, coronary flow reserve, and resistive reserve ratio.

Methods and Results—A prespecified physiology substudy of the T-TIME trial. From 2016 to 2017, patients with ST-segment–elevation myocardial infarction ≤6 hours from symptom onset were randomized in a double-blind study to receive alteplase 20 mg, alteplase 10 mg, or placebo infused into the culprit artery postreperfusion, but prestenting. Index of microcirculatory resistance, coronary flow reserve, and resistive reserve ratio were measured after percutaneous coronary intervention. Cardiovascular magnetic resonance was performed at 2 to 7 days and 3 months. Analyses in relation to ischemic time (<2, 2–4, and ≥4 hours) were prespecified. One hundred forty-four patients (mean age, 59±11 years; 80% male) were prospectively enrolled, representing 33% of the overall population (n=440). Overall, index of microcirculatory resistance (median, 29.5; interquartile range, 17.0–55.0), coronary flow reserve(1.4 [1.1–2.0]), and resistive reserve ratio (1.7 [1.3–2.3]) at the end of percutaneous coronary intervention did not differ between treatment groups. Interactions were observed between ischemic time and alteplase for coronary flow reserve (P=0.013), resistive reserve ratio (P=0.026), and microvascular obstruction (P=0.022), but not index of microcirculatory resistance.

Conclusions—In ST-segment–elevation myocardial infarction with ischemic time ≤6 hours, there was overall no difference in microvascular function with alteplase versus placebo.

Clinical Trial Registration—URL: https://www.clinicaltrials.gov. Unique identifier: NCT02257294. **(*J Am Heart Assoc*. 2020;9: e014066. DOI: 10.1161/JAHA.119.014066.)**

Key Words: cardiovascular magnetic resonance • fibrinolysis • microvascular obstruction • primary PCI • ST-segment–elevation myocardial infarction

Failed myocardial reperfusion affects approximately half of patients with acute ST-segment–elevation myocardial infarction (STEMI) following primary percutaneous coronary intervention (PCI),[1] and microvascular obstruction confers a worse prognosis.[2–4] Microvascular obstruction is an acute, but potentially reversible, pathology. The pathophysiology

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Accompanying Data S1 and Tables S1 through S4 are available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.119.014066

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Received August 22, 2019; accepted December 12, 2019.

Clinical Perspective

What Is New?

- Overall, there was no difference in index of microcirculatory resistance, coronary flow reserve, or resistive reserve ratio with alteplase versus placebo.
- Patients presenting with a limited ischemic time (<2 hours) had dose-related improvements in microvascular function (coronary flow reserve and resistive reserve ratio) with alteplase versus placebo.
- Patients presenting with longer ischemic times (≥4 hours) had increased microvascular obstruction extent with alteplase versus placebo.

What Are the Clinical Implications?

- The findings are relevant to ongoing trials of intracoronary fibrinolytics during primary percutaneous coronary intervention, and to clinicians considering bail-out lytic therapy in acute ST-segment–elevation myocardial infarction patients, with high thrombus burden and no reflow.

includes microvascular thrombi, endothelial disruption, and, if reperfusion does not occur, then irreversible hemorrhagic transformation occurs within the infarct core.[5]

Facilitated PCI with full- or half-dose fibrinolytic therapy given intravenously pre-PCI improves initial coronary flow, but promotes paradoxical thrombus formation, which counteracts fibrinolysis and is associated with higher residual thrombus burden, thrombotic complications, bleeding, and mortality.[6–8] In the T-TIME (A Trial of Low-Dose Adjunctive Alteplase During Primary PCI) trial (NCT02257294), we hypothesized that low-dose, intracoronary fibrinolysis with adequate anticoagulation would reduce intracoronary and microvascular thrombosis and distal embolization without activating thrombus formation, thereby reducing microvascular obstruction. However, as assessed by contrast-enhanced cardiovascular magnetic resonance (CMR) imaging, microvascular obstruction did not differ with low-dose intracoronary alteplase versus placebo.[9]

In contrast with CMR, the index of microcirculatory resistance (IMR) quantifies immediate efficacy of microcirculatory reperfusion.[10,11] Elevated IMR is quantitatively associated with microvascular obstruction,[12,13] myocardial hemorrhage,[12] worse recovery of infarct size,[14] and adverse left ventricular (LV) remodeling and function.[15] In acute STEMI, an IMR ≤32 post-PCI predicts recovery of LV function[16] whereas an IMR ≥32 predicts all-cause death or rehospitalization for heart failure.[13] An IMR >40 after primary PCI has been associated with all-cause death, heart failure readmissions, and major adverse cardiac events.[13–15]

Resistive reserve ratio (RRR) measures the vasodilatory capacity of the coronary microcirculation. It is the ratio between basal resting tone and resistance at maximal hyperemia and is lower when microvasodilatation is impaired. Coronary flow reserve (CFR) reflects epicardial and microcirculatory vasodilator capacity, as well as residual epicardial stenosis. CFR is the ratio of hyperemic to resting coronary blood flow. In acute STEMI, a lower RRR and CFR predict microvascular obstruction[12,17] and larger infarction.[17,18]

We prospectively investigated the effects of intracoronary alteplase during primary PCI on acute invasive parameters of microcirculatory function. We hypothesized that intracoronary alteplase would be associated with lower IMR, higher CFR, and higher RRR. Second, we hypothesized that the effects of alteplase on IMR, CFR, and RRR may vary with ischemic time, TIMI (Thrombolysis in Myocardial Infarction) thrombus grade, and TIMI coronary flow grade at the time of drug delivery (prespecified interaction analyses).

Methods

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study Design and Patient Selection

We performed a predefined, prospective, nested substudy within the main T-TIME trial. From August 2016 to December 2017, patients with acute STEMI from 3 hospitals in the United Kingdom were randomized in a 1:1:1 dose-ranging, double-blind study. The protocol is summarized in Figure 1. Participants were treated according to contemporary practice guideline recommendations.[4]

Patients were eligible for study participation if they presented with persistent ST-segment elevation or recent left bundle branch block within 6 hours of symptom onset and either an occluded culprit artery (TIMI coronary flow grade ≤1) or reduced flow (TIMI flow grade 2, slow but complete filling) in the presence of angiographic evidence of thrombus (TIMI thrombus grade ≥2). Eligibility required radial artery access, and the occlusion had to be in the proximal or mid segment of a major coronary artery. Exclusion criteria included a functional coronary collateral supply (Rentrop grade ≥2) to the culprit artery, cardiogenic shock, comorbidities with expected survival <1 year and contraindications to fibrinolysis, or CMR. The full list of eligibility criteria is provided in Data S1.

Enrollment into the physiology study within the 3 designated sites was based on prospective assessment of eligibility criteria, operator experience, and logistical considerations at the point of care. A screening register was prospectively completed to document the reasons for participation or not. Witnessed verbal assent to participate in the study was obtained in the catheterization laboratory, and written informed consent was subsequently obtained on the ward.

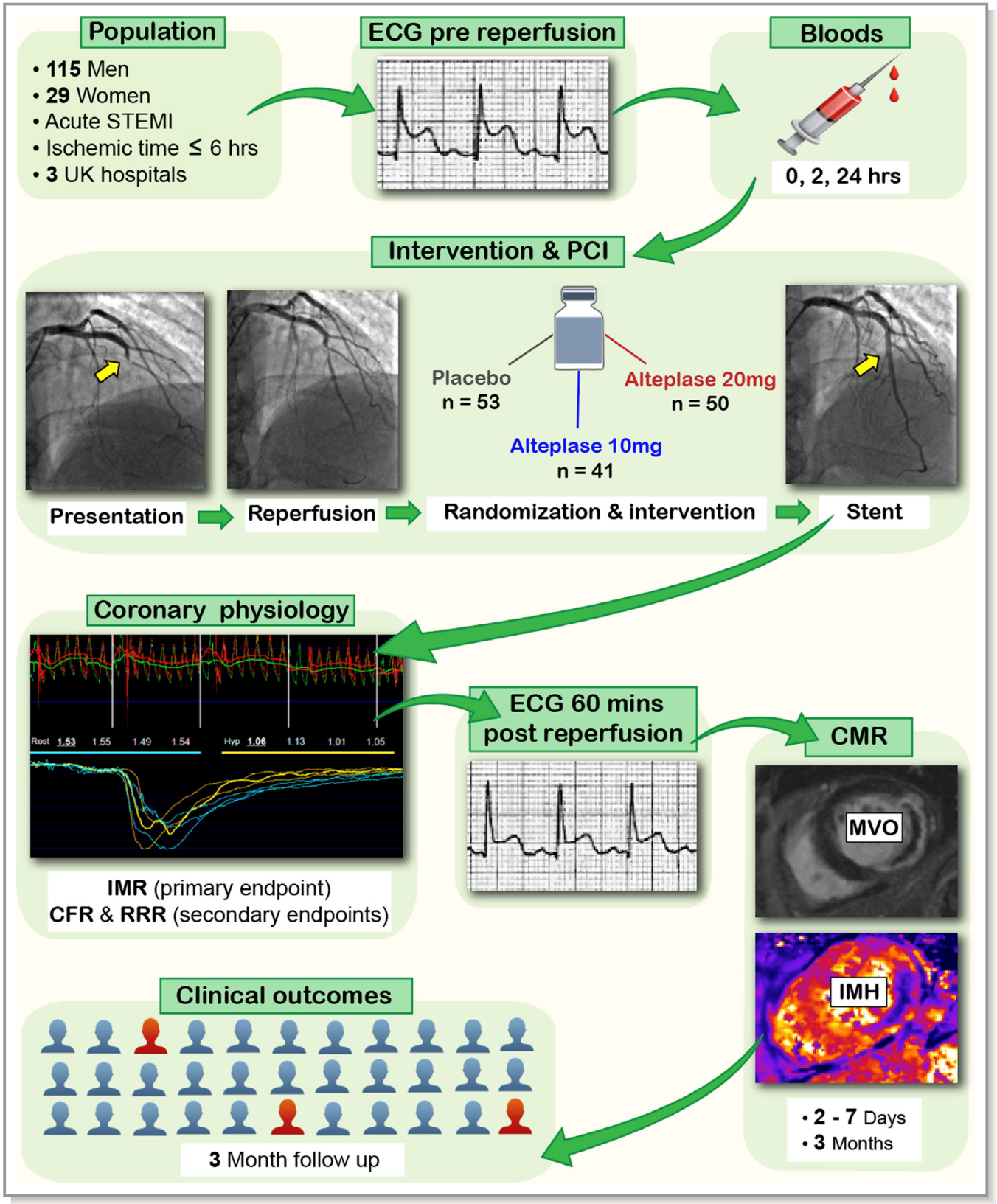

Figure 1. Illustration of the study design. Acute STEMI patients meeting the eligibility criteria were enrolled in the catheterization laboratory and randomized to placebo, alteplase 10 mg, or alteplase 20 mg, in a 1:1:1 dose-ranging, double-blind design. CFR indicates coronary flow reserve; IMH, intramyocardial hemorrhage; IMR, index of microcirculatory resistance; MVO, microvascular obstruction; PCI, percutaneous coronary intervention; RRR, resistive reserve ratio; STEMI, ST-segment–elevation myocardial infarction.

The study complied with the Declaration of Helsinki[19] and was approved by the West of Scotland Research Ethics Committee (reference 13-WS-0119).

Randomization and Intervention

Patients were randomized using an interactive voice-response–based system. The randomization sequence was computer generated, using the method of randomized permuted blocks of length 6, with stratification by location of STEMI (anterior versus nonanterior). The allocation sequence was on a 1:1:1 basis between placebo and the reduced dose alteplase groups (10, 20 mg; ie, one-tenth or one-fifth of the standard dose). Patients, staff, and researchers were blinded to treatment group allocation.

After successful reperfusion (TIMI flow grade ≥2) was achieved, using balloon angioplasty and/or aspiration thrombectomy, patients received the allocated intervention immediately in the catheterization laboratory. The 20-mL volume of study drug was manually infused into the culprit coronary artery over 5 to 10 minutes proximal to the culprit lesion, using either an intracoronary catheter or the guiding catheter if selectively engaged.

Intracoronary Physiology Measurements and Analysis

IMR, CFR, and RRR were measured at the end of primary PCI, using a pressure- and temperature-sensing guidewire (Abbott Vascular, Santa Clara, CA). All patients received 200 μg of intracoronary nitroglycerin in the culprit artery. The calibrated wire was equalized to guide catheter pressure, then advanced to the distal third of the culprit artery. Using standard thermodilution methodology, mean transit time (Tmn) of a hand-injected 3-mL bolus of room-temperature saline was measured in triplicate at rest and during steady-state maximal hyperemia induced by intravenous adenosine (140 μg/kg/min). In order to mitigate the possibility of bias through disclosure of the IMR, CFR, and RRR results, the operators were blinded. The blinding process involved obscuring the display of the RadiAnalyzer Xpress monitor by turning it away from the clinician and other clinical staff. Experienced physiology technicians recorded the thermodilution data and quality assured the acquisition.

IMR was quantified by distal coronary pressure × Tmn during hyperemia.[20] At the end of primary PCI when IMR was measured after stenting, there was no residual epicardial stenosis, and therefore IMR correction with coronary wedge pressure[21] or Yong's formula[22] was not required. CFR was quantified by dividing resting Tmn by hyperemic Tmn.[23,24] RRR was quantified by dividing the baseline resistance index (distal coronary pressure × Tmn under resting conditions) by IMR.[25] The shape of the hyperemic thermodilution curves was assessed as narrow unimodal, wide unimodal, or bimodal.[26] A narrow unimodal waveform was defined as an acute temperature reduction (duration of <0.42 seconds from the beginning of the temperature reduction to nadir temperature), followed by rapid return to resting temperature. A wide unimodal waveform was defined as a temperature decrease to nadir >0.42 seconds, followed by a gradual return to baseline temperature. A bimodal waveform was defined as having 2 distinct nadirs, with a valley deeper than 20% of peak temperature drop.

All data were extracted from the RadiAnalyzer Xpress instrument and then analyzed offline using Coroflow software (Coroventis Research AB, Uppsala, Sweden) by an investigator blinded to treatment allocation and blinded to CMR data. The coronary physiology data were subject to a second read, and final data were established by consensus agreement.

Angiographic, ECG, and Troponin Analyses

ECG and angiographic end points were determined by blinded core laboratory analysis, using standard operating procedures.

Angiograms were analyzed by A.M.M. and then subject to a second read by 1 of 2 interventional cardiologists, both with >10 years of experience. Discrepancies were resolved by consensus agreement between the first and second reviewers, or where discrepancies remained consensus was reached after discussion with a third reviewer. The following were assessed in the culprit artery: TIMI coronary flow grade, TIMI frame count, myocardial perfusion grade, and TIMI thrombus grade. 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 when used to deliver study drug. Angiographic methods are described in detail in Data S1.

The absolute percentage ST-segment resolution on ECGs obtained 60 minutes after reperfusion compared with pre-reperfusion was calculated. Troponin T area under the curve was measured from blood samples obtained immediately prereperfusion (0 hours) and then again 2 and 24 hours later.

Cardiovascular Magnetic Resonance

CMR imaging was performed at 1.5 Tesla. The standard operating procedure for CMR included: (1) microvascular obstruction presence and extent (% LV mass) demonstrated by late gadolinium enhancement images; (2) myocardial hemorrhage presence and extent (% LV mass) demonstrated by T_2* mapped images; (3) infarct size (% LV mass) demonstrated by late gadolinium enhancement images; and (4) LV ejection fraction. Microvascular obstruction and

myocardial hemorrhage were reported 2 to 7 days post-STEMI; the other CMR parameters were reported at 2 to 7 days and 3 months post-STEMI. A detailed description of the CMR acquisition and analysis techniques are in Data S1.

Coagulation

Coagulation and hemostasis parameters were measured in peripheral blood samples taken prereperfusion, then 2 and 24 hours postreperfusion. The parameters included fibrinogen and plasminogen (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 activation).

Clinical Outcomes

Clinical outcomes were prospectively collected between the index event and 3-month follow-up. Major adverse cardiac events was defined as cardiovascular death, nonfatal myocardial infarction, or unplanned hospitalization for heart failure. All-cause death and heart failure hospitalization were also reported. All events were adjudicated by a clinical event committee who were independent of the trial and blinded to the treatment allocation.

Sample Size

Sample-size calculation was based on data from the MR-MI (Magnetic Resonance Imaging in Acute ST-Segment Elevation Myocardial Infarction) cohort study[12,15] in patients who fulfilled the eligibility criteria for T-TIME. For a comparison of IMR between 3 groups (placebo versus alteplase 10 mg versus alteplase 20 mg), assuming a mean IMR of 33.9 and an SD of 25.2, and assuming mean differences in IMR between the 10 and 20 mg alteplase groups versus placebo of 10 and 20, respectively, then 108 subjects (36/group) were needed for 85% power and a significance level of 0.05. For a comparison of CFR between 3 groups (placebo versus alteplase 10 mg versus alteplase 20 mg), assuming a mean CFR of 1.65 and an SD of 0.8, and assuming mean differences in CFR between the 10 and 20 mg alteplase groups versus placebo were 0.4 and 0.8, respectively, then 69 subjects (n=23/group) were needed with 85% power (α=0.05).

Statistical Analysis

Analyses were performed according to treatment received (alteplase 10 mg, 20 mg, or placebo). Primary and secondary outcomes were assessed using linear regression (continuous outcomes), logistic regression (binary outcomes), or proportional odds logistic regression (ordinal outcomes) to make treatment effect estimates. In linear regression models, logarithmic or square root transformations were used, where necessary, to improve model residual distributions. We performed post hoc analyses in prespecified subgroups. We prespecified subgroups of interest according to patient characteristics: (1) ischemic time (<2, 2–4, and ≥4 hours); (2) TIMI thrombus grade immediately prestudy drug (≤2 and ≥3); and (3) TIMI coronary flow grade immediately prestudy drug (≤2, and 3). These subgroups were based on an a priori concern that they were clinically relevant patient characteristics that could potentially impact on associations of alteplase with IMR, CFR, and RRR. Regression models were used to assess treatment effects within prespecified subgroups through use of treatment-by-subgroup interactions. Interaction test *P* values, reported from regression models, included treatment group as a 3-level categorical variable or as a 2-level categorical variable (active versus placebo) and treatment modeled as a linear trend across dose groups (0, 10, and 20 mg). Regression analyses were adjusted for location of the myocardial infarction. All tests were 2-tailed and assessed at the 5% significance level. There was no imputation for missing values, and no adjustments for multiple statistical comparisons were made. Data were analyzed using R software (R Development Core Team, Los Angeles, CA), according to a statistical analysis plan that was finalized before data lock.

Results

Study Population Characteristics

Participants' characteristics are shown in Tables 1 and 2. The flow of subjects through the study is summarized in Figure 2. The sample size (n=144) represented 33% of the overall study population, and their characteristics were broadly similar. Mean age was 59.4±10.5 years, and 80% were male. Median ischemic time was 2.5 hours (interquartile range [IQR], 2.0, 3.5), and 31 (22%) had an ischemic time <2 hours. The culprit coronary artery was the left anterior descending in 38% (n=54), circumflex in 17% (n=24), and right in 46% (n=66) of patients.

At initial coronary angiography, TIMI flow grade was ≤1 in 124 (88%) patients and 2 in 20 (12%) patients. The thrombus grade at presentation was 5 (occluded vessel) in 116 (81%) patients, 4 (thrombus dimension >2 vessel diameters) in 25 (17%) patients, and 3 (thrombus dimension >1/2 and <2 vessel diameters) in 3 (2%) patients. The residual thrombus grade postreperfusion immediately before study drug delivery was 4 in 46 (33%) patients, 3 in 62 (44%) patients, 2 in 21 (15%) patients, and 1 in 12 (9%) patients. All but one of the patients received the study intervention according to the protocol.

Table 1. Population Characteristics

	All [n=144]	Placebo [n=53]	Alteplase 10 mg [n=41]	Alteplase 20 mg [n=50]
Demographics				
Age, y	59.4±10.5	56.8±11.3	61.2±9.4	60.6±10.3
Male	115 (80%)	45 (85%)	31 (76%)	39 (78%)
BMI, kg/m^2	28.4±5.1	28.8±5.3	29.0±5.2	27.4±4.6
Ischemic time, h:mm median (IQR)	2:47 (2:03, 3:50)	2:40 (2:03, 3:52)	2:43 (1:53, 4:10)	2:54 (2:10, 3:36)
Medical history				
Hypertension	41 (28%)	14 (26%)	11 (27%)	16 (32%)
Hypercholesterolemia	21 (15%)	11 (21%)	6 (15%)	4 (8%)
Diabetes mellitus*	16 (11%)	6 (11%)	6 (15%)	4 (8%)
Smoking				
Current	68 (47%)	25 (47%)	17 (41%)	26 (52%)
Former	27 (19%)	13 (25%)	7 (17%)	7 (14%)
Never	49 (34%)	15 (28%)	17 (41%)	17 (34%)
Previous PCI	9 (6%)	3 (6%)	1 (2%)	5 (10%)
Previous MI	8 (6%)	2 (4%)	1 (2%)	5 (10%)
Angina	4 (3%)	2 (4%)	0 (0%)	2 (4%)
Stroke/TIA	3 (2%)	2 (4%)	0 (0%)	1 (2%)
Pre-existing maintenance medication				
Aspirin	20 (14%)	8 (15%)	4 (10%)	8 (16%)
$P2Y_{12}$ inhibitor				
Clopidogrel	1 (1%)	1 (2%)	0 (0%)	0 (0%)
Ticagrelor/prasugrel	2 (1%)	0 (0%)	1 (2%)	1 (2%)
Statin	25 (17%)	13 (25%)	6 (15%)	6 (12%)
Beta-blocker	14 (10%)	4 (8%)	4 (10%)	6 (12%)
ACEi or ARB	18 (12%)	7 (13%)	4 (10%)	7 (14%)
MRA	3 (2%)	1 (2%)	2 (5%)	0 (0%)
Hemodynamic measures and initial blood results on admission				
Heart rate, bpm	73.0±15.1	74.4±16.1	71.5±13.0	72.7±15.8
Systolic BP, mm Hg	140±26	140±28	141±23	138±27
Diastolic BP, mm Hg	82±15	84±17	82±13	81±16
Creatinine, μmol/L†	79±16	79±16	80±14	79±17
eGFR, mL/min/1.73 m^{2}†	91±21	94±22	88±18	92±22
Hemoglobin, g/L†	145.8±13.7	145.4±13.8	146.2±12.6	146.0±14.6
Platelet count, 10^9/L†	264.2±62.1	252.4±53.6	280.9±73.5	262.9±58.1

Data are mean±SD, or n (%), unless otherwise stated. ACEi indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; IQR, interquartile range; MI, myocardial infarction; MRA, mineralocorticoid receptor antagonist; PCI, percutaneous coronary intervention; TIA, transient ischemic attack.

*Diabetes mellitus was defined as a history of diet-controlled or treated diabetes mellitus.

†Missing data: creatinine, eGFR, hemoglobin, and platelets, 1 subject (alteplase 20 mg group).

Primary Physiology End Point

Median IMR for the entire population was 29.5 (IQR, 17.0, 55.0). Forty-eight percent (n=69) had an IMR >32 at the end of the procedure, and 40% (n=57) had an IMR >40. On logistic regression analysis, older age was the only baseline characteristic that independently predicted an IMR >32 (mean age was 57.2±9.5 in those with IMR ≤32 and mean age was 61.7±11.2 in those with IMR >32). Overall, IMR did not differ between the alteplase groups (10, 20 mg) and placebo (alteplase 10 mg: median 22.0 [IQR, 17.0, 42.0]

Table 2. Procedure Characteristics

	All [n=144]	Placebo [n=53]	Alteplase 10 mg [n=41]	Alteplase 20 mg [n=50]
Culprit artery				
LAD	54 (38%)	19 (36%)	17 (41%)	18 (36%)
Circumflex	24 (17%)	9 (17%)	9 (22%)	6 (12%)
RCA	66 (46%)	25 (47%)	15 (37%)	26 (52%)
Culprit artery diameter, mm	3.2 ±0.4	3.2±0.5	3.2±0.5	3.2±0.4
Balloon angioplasty prestent	141 (98%)	51 (96%)	41 (100%)	49 (98%)
Initial angiography				
TIMI coronary flow grade				
0	114 (79%)	47 (89%)	34 (83%)	33 (66%)
1	14 (10%)	2 (4%)	3 (7%)	9 (18%)
2	16 (11%)	4 (8%)	4 (10%)	8 (16%)
3	0 (0%)	0 (0%)	0 (0%)	0 (0%)
TIMI thrombus grade				
3	3 (2%)	0 (0%)	0 (0%)	3 (6%)
4	25 (17%)	6 (11%)	5 (12%)	14 (28%)
5	116 (81%)	47 (89%)	36 (88%)	33 (66%)
Immediately prestudy drug				
TIMI coronary flow grade*				
1	5 (4%)	2 (4%)	2 (5%)	1 (2%)
2	41 (29%)	14 (27%)	10 (24%)	17 (35%)
3	95 (67%)	35 (69%)	29 (71%)	31 (63%)
TIMI thrombus grade*				
1	12 (9%)	5 (10%)	2 (5%)	5 (10%)
2	21 (15%)	10 (20%)	7 (17%)	4 (8%)
3	62 (44%)	21 (41%)	21 (51%)	20 (41%)
4	46 (33%)	15 (29%)	11 (27%)	20 (41%)
Study drug administration				
Thrombectomy catheter	106 (74%)	39 (74%)	29 (71%)	38 (76%)
Guide catheter	35 (24%)	13 (25%)	11 (27%)	11 (22%)
Other	3 (2%)	1 (2%)	1 (2%)	1 (2%)
Poststudy drug				
PCI with stent implant	144 (100%)	53 (100%)	41 (100%)	50 (100%)
Total stent length, mm	35.6±13.2	33.6±12.2	38.1±14.6	35.8±13.0
Poststent dilatation	133 (92%)	46 (87%)	41 (100%)	46 (92%)
Acute therapy following first medical contact†				
Aspirin loading dose				
300 mg	142 (99%)	53 (100%)	40 (98%)	49 (98%)
None	2 (1%)	0 (0%)	1 (2%)	1 (2%)
Additional antiplatelet medication				
None	2 (1%)	0 (0%)	1 (2%)	1 (2%)
Clopidogrel	87 (60%)	27 (51%)	28 (68%)	32 (64%)

Continued

Table 2. Continued

	All [n=144]	Placebo [n=53]	Alteplase 10 mg [n=41]	Alteplase 20 mg [n=50]
Ticagrelor	55 (38%)	26 (49%)	12 (29%)	17 (34%)
Prasugrel	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Unfractionated heparin, U median (IQR)	11 500 (10 000, 15 000)	10 000 (10 000, 14 000)	12 000 (10 000, 15 000)	12 000 (10 000, 15 375)
Intravenous morphine	134 (93%)	48 (91%)	38 (93%)	48 (96%)
Inhaled oxygen	20 (14%)	8 (15%)	7 (17%)	5 (10%)
Glycoprotein IIb/IIIa antagonist	8 (6%)	1 (2%)	5 (12%)	2 (4%)
Aspiration thrombectomy	23 (16%)	8 (15%)	8 (20%)	7 (14%)

Data are mean±SD, or n (%), unless otherwise stated. IQR indicates interquartile range; LAD, left anterior descending artery; PCI, percutaneous coronary intervention; RCA, right coronary artery; TIMI, Thrombolysis in Myocardial Infarction.

*Missing data: TIMI coronary flow grade and TIMI thrombus grade immediately prestudy drug, 3 subjects (2 placebo, 1 alteplase 20 mg group).

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

versus placebo, 33.0 [17.0, 57.0]; relative difference, 0.79 [95% CI, 0.58, 1.07]; *P*=0.125; alteplase 20 mg: 37.0 [20.0–57.8] versus placebo, 33.0; relative difference, 1.04 [95% CI, 0.78–1.38]; *P*=0.801; Table 3).

The intraclass correlation coefficient (ICC) for IMR, assessed from 30 consecutive patients, showed excellent intrarater reliability (ICC, 0.998 [95% CI, 0.997, 0.999]) and inter-rater reliability (ICC, 0.999 [95% CI, 0.998, 0.999]). The mean difference between repeated IMR measurements from 12 patients was 6.33 (*P*=0.076).

Secondary Physiology End Points

Median CFR for the population was 1.4 (IQR, 1.1, 2.0). At the end of the procedure, 115 (88%) patients had a CFR ≤2.0. The median RRR for the entire population was 1.7 (IQR, 1.3, 2.3). Overall, neither CFR nor RRR differed with alteplase versus placebo (Table 3). The mean difference between repeated CFR measurements from 9 patients was 0.07 (*P*=0.659). The mean difference between repeated RRR measurements from 9 patients was −0.04 (*P*=0.860). The ICC for RRR, assessed from 30 consecutive patients, showed excellent intrarater reliability (ICC, 0.988 [95% CI, 0.974, 0.994]) and inter-rater reliability (ICC, 0.988 [95% CI, 0.975, 0.994]).

Thermodilution waveforms or LV end-diastolic pressure did not differ with alteplase versus placebo.

Angiographic, ECG, and Troponin Results

As in the main trial, there were no differences in final TIMI coronary flow grade or TIMI frame count between treatment groups (Table 4). TIMI myocardial perfusion grade was higher in the alteplase 20 mg group compared with the placebo group (odds ratio, 2.16 [95% CI, 1.04, 4.49]; *P*=0.039). There was no difference in TIMI myocardial perfusion grade between the alteplase 10 mg versus placebo group (odds ratio, 1.32 [95% CI, 0.60, 2.92]; *P*=0.496). Percent ST-segment resolution 60 minutes postreperfusion did not differ with alteplase versus placebo (Table 4). Troponin T under the curve (0–24 hours) did not differ with alteplase versus placebo (Table 4).

Cardiovascular Magnetic Resonance Imaging Results

CMR was performed in 140 patients (97%) from 2 to 7 days after enrollment and in 135 patients (94%) at 3 months. Overall, there was no difference in microvascular obstruction or myocardial hemorrhage presence or extent, infarct size, myocardial salvage, LV ejection fraction, or volumes with alteplase versus placebo (Table S1).

Coagulation and Hematological Variables

There was an alteplase dose-related increase in systemic concentrations of fibrin D-dimer, reflecting fibrinoylsis, and prothrombin fragment F_{1+2}, reflecting activation of the clotting system, and a reduction in plasminogen, reflecting the intended effect of alteplase (Table S2). Systemic concentrations of fibrinogen and hemoglobin were similar between treatment groups (Table S2), indicating that effects of alteplase were localized to the heart.

Clinical Outcomes

Follow-up information was available for all patients at 3 months post-STEMI. Major adverse cardiac events occurred in 20 patients by 3 months, of whom 7 received placebo, 7

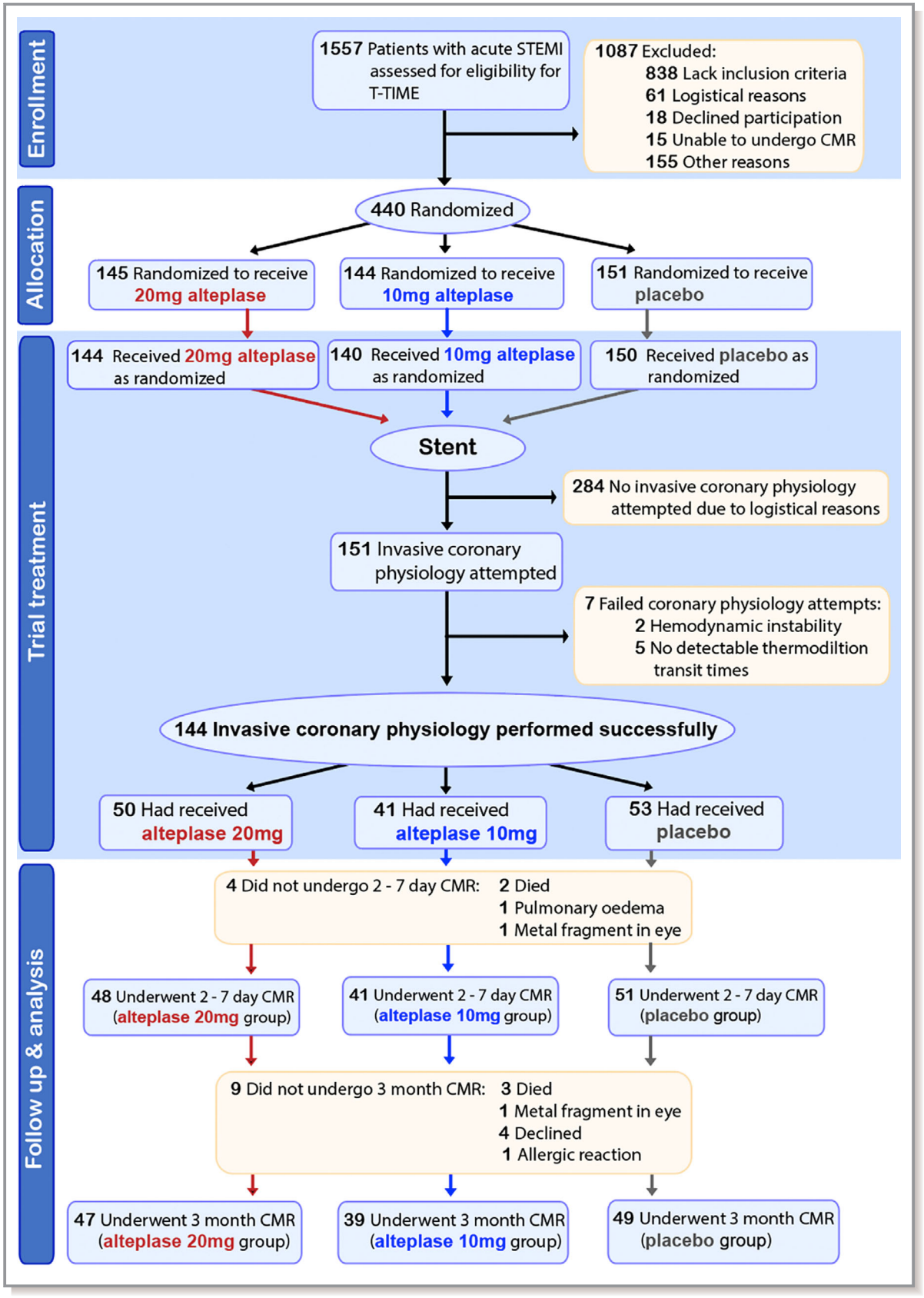

Figure 2. Diagram showing patient recruitment, randomization, and flow through the T-TIME physiology substudy. CMR indicates cardiovascular magnetic resonance; STEMI, ST-segment–elevation myocardial infarction; T-TIME, A Trial of Low-Dose Adjunctive Alteplase During Primary PCI.

Table 3. Coronary Physiology End Points

	Treatment Group				Treatment Effect			
					20 mg vs Placebo	10 mg vs Placebo	10 or 20 mg vs Placebo	Trend With Dose
	All (n=144)	Placebo (n=53)	Alteplase 10 mg (n=41)	Alteplase 20 mg (n=50)	Estimate (95% CI) *P* Value	Estimate (95% CI) *P* Value	Estimate (95% CI) *P* Value	Estimate (95% CI) *P* Value
IMR*	29.5 (17.0, 55.0)	33.0 (17.0, 57.0)	22.0 (17.0, 42.0)	37.0 (20.0, 57.8)	1.04 (0.78, 1.38) *P*=0.801	0.79 (0.58, 1.07) *P*=0.125	0.92 (0.71, 1.18) *P*=0.505	1.02 (0.88, 1.17) *P*=0.824
IMR >40†	57 (40%)	24 (45%)	11 (27%)	22 (44%)	0.93 (0.42, 2.05) *P*=0.864	0.42 (0.17, 1.02) *P*=0.054	0.66 (0.33, 1.34) *P*=0.251	0.96 (0.64, 1.43) *P*=0.840
IMR >32†	69 (48%)	27 (51%)	15 (37%)	27 (54%)	1.12 (0.51, 2.44) *P*=0.774	0.54 (0.23, 1.24) *P*=0.147	0.81 (0.41, 1.60) *P*=0.546	1.05 (0.71, 1.55) *P*=0.794
CFR*	1.4 (1.1, 2.0)	1.3 (1.1, 1.8)	1.4 (1.1, 1.9)	1.4 (1.1, 2.0)	1.03 (0.88, 1.20) *P*=0.732	1.01 (0.86, 1.19) *P*=0.900	1.02 (0.89, 1.17) *P*=0.777	1.01 (0.94, 1.09) *P*=0.732
CFR ≤2†	115 (80%)	44 (83%)	31 (76%)	40 (80%)	1.23 (0.45, 3.36) *P*=0.680	1.62 (0.59, 3.36) *P*=0.680	1.40 (0.58, 3.36) *P*=0.451	1.11 (0.68, 1.79) *P*=0.681
RRR*	1.6 (1.3, 2.3)	1.6 (1.3, 2.2)	1.6 (1.4, 2.6)	1.8 (1.3, 2.4)	1.02 (0.87, 1.20) *P*=0.795	1.04 (0.88, 1.23) p=0.658	1.03 (0.90, 1.18) =0.685	1.01 (0.93, 1.09) 0.790
Waveform‡								
Unimodal (narrow)	75 (52%)	26 (49%)	22 (54%)	27 (54%)	0.98 (0.46, 2.06) *P*=0.956	1.00 (0.45, 2.20) *P*=0.999	0.99 (0.52, 1.89) *P*=0.972	0.99 (0.68, 1.44) *P*=0.957
Unimodal (wide)	56 (39%)	25 (47%)	14 (34%)	17 (34%)				
Bimodal	13 (9%)	2 (4%)	5 (12%)	6 (12%)				
LVEDP, mm Hg§	17.0 (12.0, 20.8)	16.5 (13.2, 19.0)	19.0 (13.2, 22.8)	15.0 (12.0, 18.8)	0.89 (0.75, 1.05) *P*=0.172	1.05 (0.88, 1.25) *P*=0.579	0.96 (0.83, 1.12) *P*=0.609	0.94 (0.87, 1.03) *P*=0.184

Data are median (IQR) or n (%). Between-group comparisons derived from linear, logistic, or ordinal logistic regression models, adjusted for location of MI (see footnotes). CFR indicates coronary flow reserve; IMR, index of microcirculatory resistance; IQR, interquartile range; LVEDP, left ventricular end-diastolic pressure; MI, myocardial infarction; RRR, resistive reserve ratio.

*Data analyzed on logarithmic scale. Treatment effect estimates reported as relative differences between groups, with 95% CI and *P* value, from linear regression model adjusted for location of MI.

†Treatment effect estimates reported as odds ratios between groups, with 95% CI and *P* value, from logistic regression model adjusted for location of MI.

‡Treatment effect estimates reported as odds ratio between groups, with 95% CI and *P* value, from ordinal logistic regression model adjusted for location of MI.

§Missing data: LVEDP, 18 subjects (7 placebo, 3 alteplase 10 mg, and 8 alteplase 20 mg group).

received alteplase 10 mg, and 6 received alteplase 20 mg. Three patients died during the 3-month follow-up period, of whom 1 received alteplase 10 mg and 2 received alteplase 20 mg. There were 17 unplanned hospitalizations for heart failure by 3 months (7 in the placebo group, 6 in the alteplase 10 mg group, and 4 in the alteplase 20 mg group).

Subgroup Analyses

Treatment effect estimates for IMR, CFR, and RRR measured in the culprit coronary artery at the end of PCI in the prespecified subgroups, based on postulated pathophysiological mechanisms, are shown in Figure 3, Table 5, and Tables S3 through S4.

Ischemic time

There was no interaction between ischemic time and alteplase with IMR (Table 5). In patients with an ischemic time <2 hours, median IMR was 45.0 (23.0, 53.2) with placebo, 29.0 (17.0, 36.0) with alteplase 10 mg, and 19.5 (15.0, 22.8) with alteplase 20 mg. In patients with an ischemic time ≥4 hours, median IMR was 28.0 (19.0, 60.0) with placebo,

Table 4. ECG, Angiographic, and Troponin End Points

	Treatment Group				Treatment Effect			
	All (n=144)	Placebo (n=53)	Alteplase 10 mg (n=41)	Alteplase 20 mg (n=50)	20 mg vs Placebo Estimate (95% CI) *P* Value	10 mg vs Placebo Estimate (95% CI) *P* Value	10 or 20 mg vs Placebo Estimate (95% CI) *P* Value	Trend With Dose Estimate (95% CI) *P* Value
Absolute % ST-segment resolution 60 min*,†	46.6 (40.9)	45.1 (37.8)	45.7 (43.8)	48.8 (42.4)	4.15 (−11.71, 20.02) *P*=0.608	1.32 (−15.53, 18.16) *P*=0.878	2.89 (−11.04, 16.83) *P*=0.684	2.08 (−5.83, 9.98) *P*=0.607
TIMI flow grade post-PCI‡					1.70 (0.51, 5.69) *P*=0.391	1.43 (0.42, 4.84) *P*=0.565	1.57 (0.57, 4.32) *P*=0.387	1.31 (0.71, 2.41) *P*=0.383
1	3 (2%)	2 (4%)	0	1 (2%)				
2	15 (10%)	6 (11%)	5 (12%)	4 (8%)				
3	126 (88%)	45 (85%)	36 (88%)	45 (90%)				
TIMI MPG post-PCI‡								
0	42 (29)	18 (34)	15 (37)	9 (18)	2.16 (1.04, 4.49) *P*=0.039§	1.32 (0.60, 2.92) *P*=0.496	1.75 (0.91, 3.37) *P*=0.091	1.47 (1.02, 2.21) *P*=0.039§
1	3 (2)	3 (6)	0	0				
2	60 (42)	19 (36)	15 (37)	26 (52)				
3	39 (27)	13 (25)	11 (27)	15 (30)				
TFC post-PCI, median (IQR)‖	18.0 (14.0, 26.0)	18.0 (14.0, 26.0)	16.5 (14.0, 22.4)	22.0 (14.0, 24.5)	1.03 (0.84, 1.27) *P*=0.774	0.89 (0.72, 1.11) *P*=0.311	0.97 (0.80, 1.16) *P*=0.713	1.01 (0.91, 1.13) *P*=0.789
Troponin T AUC 0 to 24 h (mg/L)‡,‖	125.6 (143.2)	115.6 (139.5)	130.8 (142.4)	131.7 (150.5)	1.39 (0.83, 2.34) *P*=0.213	1.56 (0.91, 2.67) *P*=0.110	1.46 (0.93, 2.30) *P*=0.098	1.18 (0.91, 1.53) *P*=0.206

Data are mean±SD, or n (%), unless otherwise stated. Between-group comparisons derived from linear, logistic, or ordinal logistic regression models, adjusted for location of MI (see footnotes). AUC indicates area under the curve; IQR, interquartile range; MI, myocardial infarction; MPG, myocardial perfusion grade; PCI, percutaneous coronary intervention; TFC, TIMI frame count; TIMI, Thrombolysis in Myocardial Infarction.

*Treatment effect estimates reported as mean differences between groups, with 95% CI and *P* value, from linear regression model adjusted for MI location.

†Missing data: ST-segment resolution 60 min, 3 subjects (2 placebo, 1 alteplase 10 mg group). Troponin T AUC, 21 subjects (8 placebo, 5 alteplase 10 mg, and 8 alteplase 20 mg group).

‡Treatment effect estimates reported as odds ratio between groups, with 95% CI and *P* value, from a proportional odds logistic regression model, adjusting for MI location.

‖Data analyzed on a logarithmic scale. Treatment effect estimates reported as relative difference between groups, with 95% CI and *P* value, from linear regression model adjusted for MI location.

19.0 (17.0, 34.0) with alteplase 10 mg, and 51.0 (26.8, 59.0) with alteplase 20 mg.

Interactions were observed between ischemic time and alteplase for CFR (*P*=0.013) and RRR (*P*=0.026; Figure 3; Table 5). In patients with ischemic times <2 hours, median CFR was higher with alteplase (placebo, 1.2 [1.1, 1.7]; alteplase 10 mg, 1.4 [1.0, 1.8]; alteplase 20 mg, 2.0 [1.8, 2.3]). RRR was also higher with alteplase in patients with an ischemic time <2 hours (placebo, 1.5 [1.3, 1.9]; alteplase 10 mg, 1.6 [1.1, 2.2]; alteplase 20 mg, 2.2 [2.0, 2.6]). In patients with an ischemic time ≥4 hours, RRR was 2.0 (1.4, 2.7) with placebo, 2.3 (1.6, 2.8) with alteplase 10 mg, and 1.4 (1.0, 2.5) with alteplase 20 mg. In those with an ischemic time ≥4 hours, CFR was 1.7 (1.4, 2.0) with placebo, 1.8 (1.3, 2.6) with alteplase 10 mg, and 1.2 (1.0, 1.6) with alteplase 20 mg.

An interaction occurred between ischemic time and alteplase for amount of microvascular obstruction (*P*=0.022). In patients with an ischemic time ≥4 hours, alteplase increased the mean extent of microvascular obstruction: placebo (0.89 ± 1.65%), alteplase 10 mg (2.77 ± 4.54%), and alteplase 20 mg (5.97 ± 6.58%). In those with an ischemic time <2 hours, mean amount of microvascular obstruction was: 2.06 ± 3.93% with placebo, 2.22 ± 3.98% with alteplase 10 mg, and 0.00 ± 0.00% with alteplase 20 mg.

TIMI flow and thrombus grades prestudy drug

There was no interaction between treatment group and IMR, CFR, or RRR in the following subgroups: (1) TIMI coronary flow grade immediately prestudy drug

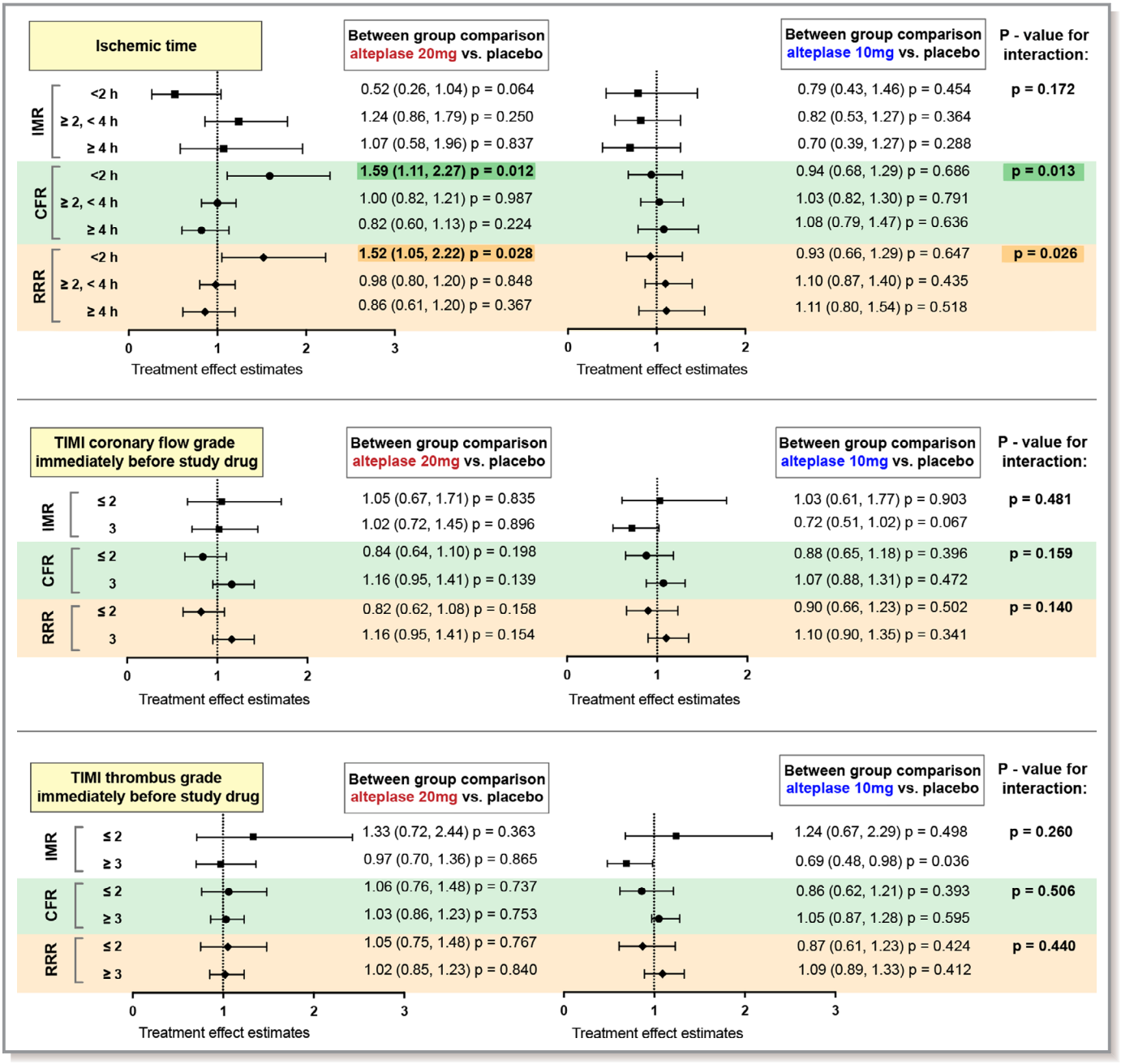

Figure 3. Forest plots showing treatment effect estimates and interaction *P* values for IMR (index of microcirculatory resistance), CFR (coronary flow reserve), and RRR (resistive reserve ratio) in subgroups for ischemic time and TIMI (Thrombolysis in Myocardial Infarction) coronary flow grade and thrombus grade immediately before study drug delivery.

dichotomized by ≤2 or 3; (2) TIMI thrombus grade immediately prestudy drug dichotomized by ≤2 or ≥3 (Figure 3 and Tables S3 and S4).

Discussion

The main finding is that overall microvascular function, assessed by IMR, CFR, and RRR, did not differ between alteplase and placebo groups.

The lack of an overall treatment effect on microvascular function in the culprit artery contrasts with the findings of Sezer et al.[27] In their proof-of-concept study, low-dose intracoronary thrombolysis (streptokinase, 250 kU; n=51) was infused over 3 minutes through a guide catheter at the end of primary PCI and, when compared with standard care (n=44), resulted in a significant increase in CFR and decrease in IMR (CFR, 2.5 versus 1.7; $P<0.001$; IMR, 20.2 versus 34.2; $P<0.001$).[27]

There are important differences between our study and that of Sezer et al.[27] First, the study by Sezer et al[27] was not

Table 5. IMR, CFR, and RRR, Microvascular Obstruction and Myocardial Hemorrhage in Subgroups of Ischemic Time

Ischemic Time	Placebo [n=53]	Alteplase 10 mg [n=41]	Alteplase 20 mg [n=50]	Treatment Effect		Interaction *P* Value (treatment as 3-level categorical variable)	Treatment Effect	Interaction *P* Value (treatment as 2-level categorical variable)	Treatment Effect	Interaction *P* Value (treatment as per 10 mg increase in dose)
				20 mg vs Placebo	10 mg vs Placebo		10 or 20 mg vs Placebo		Trend With Dose	
IMR										
<2 h [n]	45.0 (23.0, 53.2) [10]	29.0 (17.0, 36.0) [13]	19.5 (15.0, 22.8) [8]	0.52 (0.26, 1.04) *P*=0.064	0.79 (0.43, 1.46) *P*=0.454	0.172	0.68 (0.39, 1.19) *P*=0.173	0.367	0.73 (0.52, 1.02) *P*=0.068	0.097
≥2, <4 h [n]	31.5 (15.5, 51.0) [30]	24.0 (15.0, 42.0) [17]	39.5 (20.0, 61.2) [32]	1.24 (0.86, 1.79) *P*=0.250	0.82 (0.53, 1.27) *P*=0.364		1.07 (0.76, 1.51) *P*=0.685		1.12 (0.93, 1.34) *P*=0.243	
≥4 h [n]	28.0 (19.0, 60.0) [13]	19.0 (17.0, 34.0) [11]	51.0 (26.8, 59.0) [10]	1.07 (0.58, 1.96) *P*=0.837	0.70 (0.39, 1.27) *P*=0.238		0.86 (0.51, 1.44) *P*=0.556		1.02 (0.75, 1.38) *P*=0.918	
CFR										
<2 h [n]	1.2 (1.1, 1.7) [10]	1.4 (1.0, 1.8) [13]	2.0 (1.8, 2.3) [8]	1.59 (1.11, 2.27) *P*=0.012*	0.94 (0.68, 1.29) *P*=0.686	0.013*	1.14(0.85, 1.55) *P*=0.379	0.652	1.24 (1.04, 1.49) *P*=0.019*	0.038*
≥2, <4 h [n]	1.3 (1.1, 1.8) [30]	1.3 (1.2, 1.8) [17]	1.4 (1.2, 2.0) [32]	1.00 (0.82, 1.21) *P*=0.987	1.03 (0.82, 1.30) *P*=0.791		1.01 (0.84, 1.21) *P* =0.915		1.00 (0.91, 1.10) 0.984	
≥4 h [n]	1.7 (1.4, 2.0) [13]	1.8 (1.3, 2.6) [11]	1.2 (1.0, 1.6) [10]	0.82 (0.60, 1.13) *P*=0.224	1.08 (0.79, 1.47) *P*=0.636		0.95 (0.72, 1.25) *P*=0.697		0.91 (0.78, 1.07) *P*=0.264	
RRR										
<2 h [n]	1.5 (1.3, 1.9) [10]	1.6 (1.1, 2.2) [13]	2.2 (2.0, 2.6) [8]	1.52 (1.05, 2.22) *P*=0.028*	0.93 (0.66, 1.29) *P*=0.647	0.026*	1.12 (0.82, 1.53) *P*=0.481	0.827	1.22 (1.01, 1.47) *P*=0.041*	0.093
≥2, <4 h [n]	1.6 (1.3, 2.2) [30]	1.6 (1.5, 2.6) [17]	1.6 (1.3, 2.2) [32]	0.98 (0.80, 1.20) *P*=0.848	1.10 (0.87, 1.40) *P*=0.435		1.02 (0.85, 1.23) *P*=0.833		0.99 (0.89, 1.1.0) *P*=0.838	
≥4 h [n]	1.9 (1.6, 2.3) [13]	2.3 (1.6, 2.8) [11]	1.4 (1.0, 2.5) [10]	0.86 (0.61, 1.20) *P*=0.367	1.11 (0.80, 1.54) *P*=0.518		0.98 (0.74, 1.31) *P*=0.907		0.93 (0.79, 1.1.0) *P*=0.420	
<2 hours [n]	2.1±3.9 [9]	2.2±4.0 [13]	0.0±0.0 [8]	−0.87 (−2.07, 0.33) *P*=0.155	−0.03 (−1.10, 1.03) *P*=0.950	0.039*	−0.35 (−1.34, 0.64) *P*=0.484	0.089	−0.42(−1.02, 0.17) *P*=0.161	0.010*
≥2, <4 h [n]	2.4±3.3 [29]	2.6±4.4 [17]	2.8±5.9 [31]	−0.14 (−0.77, 0.50) *P*=0.676	−0.14 (−0.90, 0.61) *P*=0.715		−0.14 (−0.72, 0.45) *P*=0.643		−0.07 (−0.38, 0.25) *P*=0.676	
≥4 h [n]	0.9±1.6 [13]	2.8±4.5 [11]	6.0 ±6.6 [9]	1.47 (0.40, 0.54) *P*=0.007*	0.49 (−0.52, 1.50) *P*=0.337		0.93 (0.05, 1.82) 0.039*		0.72 (0.19, 1.25) *P*=0.007*	
<2 h [n]	0.5±0.9 [7]	1.7±3.8 [11]	0.0±0.0 [8]	−0.51 (−4.44, 3.42) *P*=0.800	1.20 (−2.48, 4.87) *P*=0.523	0.153	0.48 (−2.91, 3.86) *P* =0.783	0.392	−0.30 (−2.25, 1.66) *P*=0.766	0.080

Continued

DOI: 10.1161/JAHA.119.014066

Table 5. Continued

Ischemic Time	Placebo [n=53]	Alteplase 10 mg [n=41]	Alteplase 20 mg [n=50]	Treatment Effect		Interaction *P* Value (treatment as 3-level categorical variable)	Treatment Effect	Interaction *P* Value (treatment as 2-level categorical variable)	Treatment Effect	Interaction *P* Value (treatment as per 10 mg increase in dose)
				20 mg vs Placebo	10 mg vs Placebo		10 or 20 mg vs Placebo		Trend With Dose	
≥2, <4 h [n]	2.2±3.3 [28]	2.7±4.4 [15]	2.2±4.9 [31]	−0.06 (−2.05, 1.92) *P*=0.950	0.41 (−2.04, 2.86) *P*=0.741		0.09 (−1.75, 1.93) *P*=0.926		−0.04 (−1.02, 0.95) *P*=0.941	
≥4 h [n]	0.8±1.7 [12]	1.6±3.1 [10]	5.1±5.9 [9]	4.25 (0.88, 7.61) *P*=0.013*	0.80 (−2.46, 4.06) *P*=0.632		2.42 (−0.41, 5.25) *P*=0.094		2.06 (0.39, 3.73) *P*=0.016	

Data are median (IQR) or mean±SD. Coronary physiology data were analyzed on a logarithmic scale, with treatment effect estimates reported as relative differences and 95% CIs with *P* values, derived from linear regression, adjusted for location of MI. Microvascular obstruction (% LV) was analyzed on a square root scale, with treatment effect estimates reported as mean differences and 95% CIs with *P* values, derived from linear regression, adjusted for location of MI. CFR indicates coronary flow reserve; IMR, index of microcirculatory resistance; IQR, interquartile range; LV, left ventricular; MI, myocardial infarction; RRR, resistive reserve ratio.

double blinded, whereas our study was. Second, streptokinase is not fibrin specific, whereas alteplase is. Third, all of the patients in Sezer et al's study[27] received a bolus of tirofiban glycoprotein IIbIIIa inhibitor therapy at the start of the procedure followed by tirofiban infusion for 12 hours, whereas only 6% of patients in our study received a glycoprotein IIbIIIa inhibitor, in line with current practice guideline recommendations.[28] Fourth, streptokinase was delivered poststent when 89% of the cohort had TIMI 3 coronary flow, whereas we administered alteplase prestent. In our study, 46 patients (32%) had TIMI coronary flow ≤2 immediately prestudy drug, which may have limited alteplase reaching the downstream microcirculation, and prothrombotic effects of fibrinolytics might be enhanced in conditions of slow flow.[29,30] Fifth, we measured coronary physiology immediately after the primary PCI procedure, whereas Sezer et al[27] measured IMR and CFR 48 hours after primary PCI, when IMR and CFR may have undergone partial recovery.[31–33]

In exploratory prespecified subgroup analyses, which were intended to provide mechanistic insights and should be interpreted as hypothesis generating, interactions were observed between ischemic time and alteplase with CFR and RRR, but not IMR. The improvement in microvascular vasodilator function with alteplase, as reflected by higher CFR and RRR, in patients with ischemic time <2 hours may be explained by those patients presenting with a brief ischemic time having intact microcirculation, which was modifiable by therapy, whereas those with a longer ischemic time may have had irreversible microvascular injury. While these observations could be attributed to type 1 statistical error, our findings are supported by consistent effects of ischemic time on extent of microvascular obstruction, for which the *P* value for interaction was significant.

In our study, alteplase was associated with more microvascular obstruction in patients with an ischemic time ≥4 hours. The findings suggest the possibility of alteplase having a detrimental effect on myocardial reperfusion in patients with longer ischemic time. The mechanism may involve alteplase promoting myocardial hemorrhage in circumstances of prolonged ischemia, characterized by capillary degradation[34] and myocyte necrosis. An increase in extravasation of blood into the interstitial space of the infarct core results in external compression of capillaries, with an associated increase in microvascular resistance. This leads to more microvascular obstruction and potentiates the progression of myocardial hemorrhage. The findings support the rationale to limit eligibility to a short ischemic time (eg, <4 hours).

A plausible explanation for the lack of interaction between ischemic time and alteplase with IMR could be because IMR measures microvascular resistance during maximal hyperemia, which might be less modifiable by intracoronary alteplase than microvascular vasodilator function (measured by RRR and CFR).

Two other ongoing trials of low-dose intracoronary fibrinolysis are using IMR as an eligibility criterion and to measure acute microvascular function after intervention. The RESTORE-MI (Restoring Microcirculatory Perfusion in STEMI) trial (ACTRN12618000778280) will randomize STEMI patients with IMR >32 (n=800) to intracoronary tenecteplase (one-third of weight-based systemic dose) or placebo, in a double-blind design, and those with IMR ≤32 will continue in a followup registry. Recently, a pilot trial in 36 patients with acute STEMI with symptoms ≤ 6 hours and TIMI 0/1 flow in the culprit artery demonstrated adjunctive low-dose (4 mg) of intracoronary tenecteplase given twice (post-reperfusion and at the end of PCI, 8 mg total) compared with placebo (saline) as an adjunct to primary PCI was feasible and safe but did not improve percent stenosis of the culprit lesion (primary outcome).[35] The smaller OPTIMAL (Optimal Coronary Flow After PCI for Myocardial Infarction) trial (NCT02894138) will randomize 80 STEMI patients with a poststenting IMR >30 to intracoronary alteplase (20mg), or placebo, in an open-label design. Both studies are including patients with ischemic times up to 12 hours. However, our findings suggest that therapeutic benefit with alteplase might be restricted to patients with a shorter ischemic time.

Limitations and Strengths

Because of the potential for type 1 error in the subgroup analyses, these should be interpreted as exploratory. Although we observed a higher TIMI myocardial perfusion grade post-PCI in the alteplase 20 mg group compared with the placebo group, this difference was not observed in the main trial,[9] and the significant *P* value may have occurred because of chance (type 1 statistical error).

Strengths of our study include the randomized, double-blind design, blinding of IMR, CFR, and RRR measurements to minimize bias, and their excellent inter- and intrarater reliability. Retention with CMR was high (94% at 3 months).

Conclusions

In acute STEMI with ischemic time ≤6 hours, there was, overall, no difference in culprit artery microvascular function (IMR, CFR, or RRR) at the end of PCI with alteplase versus placebo. Interactions were observed between ischemic time and alteplase on CFR, RRR, and microvascular obstruction, implying therapeutic benefit in patients presenting with a shorter ischemic time and a detrimental effect in patients with a longer ischemic time. Further research seems warranted.

Acknowledgments

We thank the patients who participated in this study and the T-TIME investigators and staff who supported it.

Sources of Funding

Dr Maznyczka is funded by a fellowship from the British Heart Foundation (FS/16/74/32573). Dr Berry is supported by grant RE/18/6/34217 from the British Heart Foundation. T-TIME was supported by grant 12/170/4 from the Efficacy and Mechanism Evaluation (EME) program of the National Institute for Health Research (NIHR-EME). Boehringer Ingelheim UK Ltd provided the study drugs (alteplase 10, 20 mg), matched placebo, and sterile water for injection. These organizations had no other involvement in the conduct of the study or in any aspect of the manuscript.

Disclosures

Dr Berry is employed by the University of Glasgow, which holds research and/or consultancy agreements with AstraZeneca, Abbott Vascular, Boehringer Ingelheim, GSK, HeartFlow, Opsens, and Novartis. Dr Oldroyd has received speaker fees and research support from Abbott Vascular and Boston Scientific. Dr Tait reported receiving grants from the National Institute for Health Research (NIHR) during the conduct of the study, consultancy fees and honoraria from Boehringer Ingelheim and Sobi, consultancy fees and nonfinancial support from Bayer Healthcare, NovoNordisk, and Shire, consultancy fees from Pfizer, and nonfinancial support from CSL Behring. Dr Cotton reported research support and speaker fees from Abbott Vascular. Dr Watkins reports speaker fees from Biosensors International, GE Healthcare, Abbott, Sanofi, and AstraZeneca. Welsh reported receiving grants from the Chief Scientist Office, Boehringer Ingelheim, and Roche. Sattar reported receiving grants and personal fees from Boehringer Ingelheim and personal fees from Amgen, Eli Lilly, Janssen, and AstraZeneca outside the submitted work. Dr Robertson reported receiving speaker fees and educational support from AstraZeneca and educational support from Abbott Vascular.

References

1. Hombach V, Grebe O, Merkle N, Waldenmaier S, Hoher M, Kochs M, Wohrle J, Kestler HA. Sequelae of acute myocardial infarction regarding cardiac structure and function and their prognostic significance as assessed by magnetic resonance imaging. *Eur Heart J*. 2005;26:549–557.
2. de Waha S, Patel MR, Granger CB, Ohman EM, Maehara A, Eitel I, Ben-Yehuda O, Jenkins P, Thiele H, Stone GW. Relationship between microvascular obstruction and adverse events following primary percutaneous coronary intervention for ST-segment elevation myocardial infarction: an individual patient data pooled analysis from seven randomized trials. *Eur Heart J*. 2017;38:3502–3510.
3. Carrick D, Haig C, Ahmed N, McEntegart M, Petrie MC, Eteiba H, Hood S, Watkins S, Lindsay MM, Davie A, Mahrous A, Mordi I, Rauhalammi S, Sattar N, Welsh P, Radjenovic A, Ford I, Oldroyd KG, Berry C. Myocardial hemorrhage after acute reperfused st-segment-elevation myocardial infarction: relation to microvascular obstruction and prognostic significance. *Circ Cardiovasc Imaging*. 2016;9:e004148.
4. Levine GN, Bates ER, Bittl JA, Brindis RG, Fihn SD, Fleisher LA, Granger CB, Lange RA, Mack MJ, Mauri L, Mehran R, Mukherjee D, Newby LK, O'Gara PT,

Sabatine MS, Smith PK, Smith SC Jr. 2016 ACC/AHA guideline focused update on duration of dual antiplatelet therapy in patients with coronary artery disease: a report of the American College of Cardiology/American Heart Association Task Force On Clinical Practice Guidelines: an update of the 2011 ACCF/AHA/SCAI Guideline for Percutaneous Coronary Intervention, 2011 ACCF/AHA Guideline for coronary artery bypass graft surgery, 2012 ACC/AHA/ACP/AATS/PCNA/SCAI/STS Guideline for the diagnosis and management of patients with stable ischemic heart disease, 2013 ACCF/AHA Guideline for the management of ST-elevation myocardial infarction, 2014 AHA/ACC Guideline for the management of patients with non-ST-elevation acute coronary syndromes, and 2014 ACC/AHA Guideline on perioperative cardiovascular evaluation and management of patients undergoing noncardiac surgery. *Circulation*. 2016;134:e123–e155.

5. Robbers LF, Eerenberg ES, Teunissen PF, Jansen MF, Hollander MR, Horrevoets AJ, Knaapen P, Nijveldt R, Heymans NW, Levi MM, van Rossum AC, Niessen HW, Marcu CB, Beek AM, van Royen N. Magnetic resonance imaging-defined areas of microvascular obstruction after acute myocardial infarction represent microvascular destruction and haemorrhage. *Eur Heart J*. 2013;34:2346–2353.
6. Zalewski J, Bogaerts K, Desmet W, Sinnaeve P, Berger P, Grines C, Danays T, Armstrong P, Van de Werf F. Intraluminal thrombus in facilitated versus primary percutaneous coronary intervention: an angiographic substudy of the ASSENT-4 PCI (Assessment of the Safety and Efficacy of a New Treatment Strategy with Percutaneous Coronary Intervention) trial. *J Am Coll Cardiol*. 2011;57:1867–1873.
7. Assessment of the Safety and Efficacy of a New Treatment Strategy with Percutaneous Coronary Intervention (ASSENT-4 PCI) investigators. Primary versus tenecteplase-facilitated percutaneous coronary intervention in patients with ST-segment elevation acute myocardial infarction (ASSENT-4 PCI): randomised trial. *Lancet*. 2006;367:569–578.
8. Ellis SG, Tendera M, de Belder MA, van Boven AJ, Widimsky P, Janssens L, Andersen HR, Betriu A, Savonitto S, Adamus J, Peruga JZ, Kosmider M, Katz O, Neunteufl T, Jorgova J, Dorobantu M, Grinfeld L, Armstrong P, Brodie BR, Hermsnn HC, Montalescot G, Neumann FJ, Effron MB, Barathan ES, Topol EF; FINESSE Investigators. Facilitated PCI in patients with ST-elevation myocardial infarction. *N Eng J Med*. 2008;358:2205–2217.
9. McCartney PJ, Eteiba H, Maznyczka AM, McEntegart M, Greenwood JP, Muir DF, Chowdhury S, Gershlick AH, Appleby C, Cottin JM, Wragg A, Curzen N, Oldroyd KG, Lindsay M, Rocchiccioli JP, Shaukat A, Good R, Watkins S, Robertson K, Malkin C, Martin L, Gillespie L, Ford T, Petrie MC, Macfarlane PW, Tait RC, Welsh P, Sattar N, Weir RA, Fox KA, Ford I, McConnachie A, Berry C. Effect of low-dose intracoronary alteplase during primary percutaneous coronary intervention on microvascular obstruction in patients with acute myocardial infarction: a randomized clinical trial. *JAMA*. 2019;321:56–68.
10. Maznyczka AM, Oldroyd KG, McCartney P, McEntegart M, Berry C. The use of index of microcirculatory resistance (IMR) to guide stratification of patients for adjunctive therapy in acute myocardial infarction. *JACC Cardiovasc Interv*. 2019;12:951–966.
11. van Leeuwen MAH, van der Hoeven NW, Janssens GN, Everaars H, Nap A, Lemkes JS, de Waard GA, van de Ven PM, van Rossum AC, Ten Cate TJF, Piek JJ, von Birgelen C, Escaned J, Valgimigli M, Diletti R, Riksen NP, van Mieghem NM, Nijveldt R. Evaluation of microvascular injury in revascularized patients with ST-segment-elevation myocardial infarction treated with ticagrelor versus prasugrel. *Circulation*. 2019;139:636–646.
12. Carrick D, Haig C, Carberry J, May VTY, McCartney P, Welsh P, Ahmed N, McEntegart M, Petrie MC, Eteiba H, Lindsay M, Hood S, Watkins S, Mahrous A, Rauhalammi SM, Mordi I, Ford I, Radjenovic A, Sattar N, Oldroyd KG, Berry C. Microvascular resistance of the culprit coronary artery in acute ST-elevation myocardial infarction. *JCI Insight*. 2016;1:e85768.
13. Fearon WF, Low AF, Yong AS, McGeoch R, Berry C, Shah MG, Ho MY, Kim HS, Loh JP, Oldroyd KG. Prognostic value of the Index of Microcirculatory Resistance measured after primary percutaneous coronary intervention. *Circulation*. 2013;127:2436–2441.
14. De Maria GL, Alkhalil M, Wolfrum M, Fahrni G, Borlotti A, Gaughran L, Dawkins S, Langrish JP, Lucking AJ, Choudhury RP, Porto I, Crea F, Dall'Armellina E, Channon KM, Kharbanda RK, Banning AP. Index of microcirculatory resistance as a tool to characterize microvascular obstruction and to predict infarct size regression in patients with stemi undergoing primary PCI. *JACC Cardiovasc Imaging*. 2019;12:837–848.
15. Carrick D, Haig C, Ahmed N, Carberry J, Yue May VT, McEntegart M, Petrie MC, Eteiba H, Lindsay M, Hood S, Watkins S, Davie A, Mahrous A, Mordi I, Ford I, Radjenovic A, Oldroyd KG, Berry C. Comparative prognostic utility of indexes of microvascular function alone or in combination in patients with an acute ST-segment-elevation myocardial infarction. *Circulation*. 2016;134:1833–1847.
16. Fearon WF, Shah M, Ng M, Brinton T, Wilson A, Tremmel JA, Schnittger I, Lee DP, Vagelos RH, Fitzgerald PJ, Yock PG, Yeung AC. Predictive value of the index of microcirculatory resistance in patients with ST-segment elevation myocardial infarction. *J Am Coll Cardiol*. 2008;51:560–565.
17. Scarsini R, De Maria GL, Borlotti A, Kotronias RA, Langrish JP, Lucking AJ, Choudhury RP, Ferreira VM, Ribichini F, Channon KM, Kharbanda RK, Banning AP. Incremental value of coronary microcirculation resistive reserve ratio in predicting the extent of myocardial infarction in patients with STEMI. Insights from the Oxford Acute Myocardial Infarction (OxAMI) study. *Cardiovasc Revasc Med*. 2019;S1553–8389:30091.
18. Van Herck PL, Paelinck BP, Haine SE, Claeys MJ, Miljoen H, Bosmans JM, Parizel PM, Vrints CJ. Impaired coronary flow reserve after a recent myocardial infarction: correlation with infarct size and extent of microvascular obstruction. *Int J Cardiol*. 2013;167:351–356.
19. General Assembly of the World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA*. 2013;310:2191–2194.
20. Fearon WF, Balsam LB, Farouque HM, Caffarelli AD, Robbins RC, Fitzgerald PJ, Yock PG, Yeung AC. Novel index for invasively assessing the coronary microcirculation. *Circulation*. 2003;107:3129–3132.
21. Aarnoudse W, Fearon WF, Manoharan G, Geven M, van de Vosse F, Rutten M, De Bruyne B, Pijls NH. Epicardial stenosis severity does not affect minimal microcirculatory resistance. *Circulation*. 2004;110:2137–2142.
22. Yong AS, Layland J, Fearon WF, Ho M, Shah MG, Daniels D, Whitbourn R, Macisaac A, Kritharides L, Wilson A, Ng MK. Calculation of the index of microcirculatory resistance without coronary wedge pressure measurement in the presence of epicardial stenosis. *JACC Cardiovasc Interv*. 2013;6:53–58.
23. Knaapen P, Camici PG, Marques KM, Nijveldt R, Bax JJ, Westerhof N, Gotte MJ, Jerosch-Herold M, Schelbert HR, Lammertsma AA, van Rossum AC. Coronary microvascular resistance: methods for its quantification in humans. *Basic Res Cardiol*. 2009;104:485–498.
24. De Bruyne B, Pijls NH, Smith L, Aarnoudse W, Barbato E, Bartunek J, Bech GJ, Van De Vosse F. Coronary thermodilution to assess flow reserve: experimental validation. *Circulation*. 2001;104:2003–2006.
25. Layland J, Carrick D, McEntegart M, Ahmed N, Payne A, McClure J, Sood A, McGeoch R, MacIsaac A, Whitbourn R, Wilson A, Oldroyd K, Berry C. Vasodilatory capacity of the coronary microcirculation is preserved in selected patients with non-ST-segment-elevation myocardial infarction. *Circ Cardiovasc Interv*. 2013;6:231–236.
26. Fukunaga M, Fujii K, Kawasaki D, Sawada H, Miki K, Tamaru H, Imanaka T, Iwasaku T, Nakata T, Chibuya M, Akahori H, Masutani M, Kobayashi K, Ohyanagi M, Masuyama T. Thermodilution-derived coronary blood flow pattern immediately after coronary intervention as a predictor of microcirculatory damage and midterm clinical outcomes in patients with ST-segment-elevation myocardial infarction. *Circ Cardiovasc Interv*. 2014;7:149–155.
27. Sezer M, Cimen A, Aslanger E, Elitok A, Umman B, Burga Z, Yormaz E, Turkmen C, Adalet IS, Nisanci Y, Umman S. Effect of intracoronary streptokinase administered immediately after primary percutaneous coronary intervention on long-term left ventricular infarct size, volumes, and function. *J Am Coll Cardiol*. 2009;54:1065–1071.
28. O'Gara PT, Kushner FG, Ascheim DD, Casey DE Jr, Chung MK, de Lemos JA, Ettinger SM, Fang JC, Fesmire FM, Franklin BA, Granger CB, Krumholz HM, Linderbaum JA, Morrow DA, Newby LK, Ornato JP, Ou N, Radford MJ, Tamis-Holland JE, Tomasso CL, Tracy CM, Woo YJ, Zhao DX, Anderson JL, Jacobs AK, Halperin JL, Albert NM, Brindis RG, Creager MA, DeMets D, Guyton RA, Hochman JS, Kovacs RJ, Kushner FG, Ohman EM, Stevenson WG, Yancy CW; American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. 2013 ACCF/AHA guideline for the management of ST-elevation myocardial infarction: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *Circulation*. 2013;127:e362–e425.
29. Berry C, Maznyczka AM, McCartney P. Failed myocardial reperfusion during primary PCI: an unmet therapeutic need. *EuroIntervention*. 2019;14:1628–1630.
30. Morales-Ponce FJ, Lozano-Cid FJ, Martinez-Romero P, Gonzalez-Perez P, Sanchez-Brotons JA, Diaz-Torres I, Rodriguez-Yanez JC, Caro-Mateo P, Serrador-Frutos AM. Intracoronary tenecteplase versus abciximab as adjunctive treatment during primary percutaneous coronary intervention in patients with anterior myocardial infarction. *EuroIntervention*. 2019;14:1668–1675.
31. Cuculi F, Dall'Armellina E, Manlhiot C, De Caterina AR, Colyer S, Ferreira V, Morovat A, Prendergast BD, Forfar JC, Alp NJ, Choudhury RP, Neuauer S, Channon KM, Banning AP, Kharbanda RK. Early change in invasive measures of microvascular function can predict myocardial recovery following PCI for ST-elevation myocardial infarction. *Eur Heart J*. 2014;35:1971–1980.
32. Neumann FJ, Kosa I, Dickfeld T, Blasini R, Gawaz M, Hausleiter J, Schwaiger M, Schomig A. Recovery of myocardial perfusion in acute myocardial infarction after successful balloon angioplasty and stent placement in the infarct-related coronary artery. *J Am Coll Cardiol*. 1997;30:1270–1276.

33. Cuculi F, De Maria GL, Meier P, Dall'Armerllina E, de Caterina AR, Channon KM, Prendergast BD, Choudhury RP, Forfar JC, Kharbanda RK, Banning AP. Impact of microvascular obstruction on the assessment of coronary flow reserve, index of microcirculatory resistance, and fractional flow reserve after ST-segment elevation myocardial infarction. *J Am Coll Cardiol*. 2014;64:1894–1904.

34. Galaup A, Gomez E, Souktani R, Durand M, Cazes A, Monnot C, Teillon J, Le Jan S, Bouleti C, Briois G, Philippe J, Pons S, Martin V, Assaly R, Bonnin P, Ratajczak P, Janin A, Thurston G, Valenzuela DM, Murphy AJ, Yancopoulos GD, Tissier R, Berdeaux A, Ghaleh B, Germain S. Protection against myocardial infarction and no-reflow through preservation of vascular integrity by angiopoietin-like 4. *Circulation*. 2012;125:140–149.

35. Gibson CM, Kumar V, Gopalakrishnan L, Singh P, Guo J, Kazziha S, Devireddy C, Pinto D, Marshall JJ, Stouffer GA, Mavromatis K, Grip L, Bainey KR for the TIMI & PERFUSE Study Group. Feasibility and Safety of Low-Dose Intra-Coronary Tenecteplase During Primary Percutaneous Coronary Intervention for ST-Elevation Myocardial Infarction (ICE T-TIMI 49). *Am J Cardiol*. 2019; DOI: https://doi.org/10.1016/j.amjcard.2019.11.018.

SUPPLEMENTAL MATERIAL

Data S1.

Eligibility Criteria

Patients with a clinical diagnosis of acute ST-segment elevation myocardial infarction (STEMI) were eligible for randomization 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 randomization.
- 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
- 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 hemorrhagic diathesis
- Patient with current concomitant oral anticoagulation therapy (international normalized 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 trauma to the head (<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 (esophageal varices) and active hepatitis
- Active peptic ulceration
- Arterial aneurysm and known arterial/ venous malformation
- Neoplasm with increased bleeding risk

- Any known history of hemorrhagic stroke, or stroke of unknown origin
- Known history of ischemic stroke, or transient ischemic attack in the preceding 6 months
- Dementia
- Hypersensitivity to gentamicin, or natural rubber
- Incapacity, or inability to provide informed consent
- Previous randomization to this study, or participation in a study with an investigational drug, or medical device within 90 days prior to randomization
- 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.

Angiogram Acquisition & Analysis Methods

Coronary angiograms were acquired during emergency care with cardiac catheter laboratory X-ray and information technology equipment. The angiograms were analyzed 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.

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**.**

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

CMR Acquisition & 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[4]. 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. P.McC undertook the primary analysis of the scans and related analyses were reviewed by C.B. (second reviewer).

Late 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 Definition & 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 25^{o}; 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 Edema

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 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[4].

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 Hemorrhage

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[5,6] was considered to confirm the presence of myocardial hemorrhage. The area was manually delineated and expressed as % LV mass.

Table S1. CMR endpoints. Data are mean ± SD, or n (%), unless otherwise stated. Between-group comparisons derived from linear, logistic, or ordinal logistic regression models, adjusted for location of MI (see footnotes).

	Treatment Group				Treatment Effect			
	All	**Placebo**	**Alteplase 10mg**	**Alteplase 20mg**	**20mg vs. placebo**	**10mg vs. placebo**	**10 or 20mg vs. placebo**	**Trend with dose**
	[n=144]	**[n=53]**	**[n=41]**	**[n=50]**	**Estimate (95% CI) p-value**	**Estimate (95% CI) p-value**	**Estimate (95% CI) p-value**	**Estimate (95% CI) p-value**
CMR 2 – 7 day								
Microvascular obstruction extent (% LV) *† ‡	2.5 ± 4.5	2.0 ± 3.1	2.5 ± 4.2	2.9 ± 5.7	0.08 (-0.43, 0.59) p=0.766	0.03 (-0.50, 0.56) p=0.908	0.06 (-0.50, 0.50) p=0.804	0.04 (-0.21, 0.29) p=0.766
Microvascular obstruction presence * ‡ ‡	57 (41)	23 (45)	16 (39)	18 (38)	0.73 (0.33, 1.64) p=0.449	0.78 (0.34, 1.81) p=0.566	0.76 (0.38, 1.52) p=0.432	0.85 (0.57, 1.28) p=0.446
Myocardial haemorrhage extent (% LV) * ‡‡‡	2.0 ± 3.9	1.6 ± 2.8	2.1 ± 3.8	2.4 ± 4.8	0.72 (-0.87, 2.31) p=0.373	0.44 (-1.28, 2.16) p=0.619	0.60 (-0.81, 2.01) p=0.403	0.36 (-0.43, 1.15) p=0.372
Myocardial hemorrhage presence * ‡ ‡	56 (41)	22 (45)	16 (41)	18 (38)	0.73 (0.33, 1.66) p=0.458	0.85 (0.36, 2.00) p=0.713	0.79 (0.38, 1.60) p=0.506	0.86 (0.57, 1.29) p=0.458
Acute Infarct size (% LV) * ‡‡‡	24.1 ± 12.7	23.3 ± 12.9	26.6 ± 12.5	23.1 ± 12.8	-0.70 (-5.35, 3.94) p=0.767	2.57 (-2.29 7.42) p=0.300	0.80 (-3.27, 4.87) p=0.700	-0.33 (-2.66, 2.00) p=0.781
LV ejection fraction (%) * ‡‡‡	43.9 ± 8.3	44.3 ± 7.6	43.6 ± 7.6	43.7 ± 9.7	-0.32 (-3.48, 2.84) p=0.844	-0.25 (-3.55, 3.05) p=0.882	-0.29 (-3.04, 2.47) p=0.838	-0.16 (-1.74, 1.42) p=0.842
LV end systolic volume (ml), * median (IQR) ‡ ‡‡ ‡	90.5 (77.5, 108.3)	90.4 (80.9, 108.8)	92.9 (83.2, 106.8)	89.9 (66.1, 108.3)	0.94 (0.84, 1.06) p=0.306	1.00 (0.88, 1.12) p=0.946	0.97 (0.87, 1.07) p=0.503	0.97 (0.92, 1.03) p=0.308
LV end diastolic volume (ml), * median (IQR) ‡ ‡‡ ‡	166.0 (143.5, 188.4)	168.7 (151.3, 196.5)	73.5 (147.1, 187.9)	157.9 (131.6, 187.1)	0.94 (0.86, 1.03) p=0.189	0.99 (0.90, 1.09) p=0.850	0.96 (0.89, 1.04) p=0.360	0.97 (0.93, 1.01) p=0.190
CMR 3 months								
Infarct size (% LV) * ‡‡‡	17.0 ± 11.5	17.0 ± 11.9	17.7 ± 11.0	16.5 ± 11.7	-0.62 (-5.04, 3.80) p=0.782	0.45 (-4.16, 5.06) p=0.848	-0.13 (-4.00, 3.74) p=0.947	-0.31 (-2.52, 1.89) p=0.780

Myocardial salvage index * ‡‡‡	0.6 ± 0.2	0.6 ± 0.3	0.6 ± 0.2	0.6 ± 0.2	0.02 (-0.08, 0.12) p=0.707	0.02 (-0.08, 0.13) p=0.670	0.02 (-0.07, 0.11) p=0.642	0.01 (-0.04, 0.06) p=0.708
LV ejection fraction * ‡‡‡	49.1 ± 8.4	49.5 ± 8.4	49.0 ± 6.5	48.9 ± 9.8	-0.45 (-3.77, 2.88) p=0.793	-0.32 (-3.79, 3.14) p=0.855	-0.39 (-3.29, 2.51) p=0.792	-0.22 (-1.88, 1.43) p=0.792
LV end systolic volume (ml), * median (IQR) ‡ ‡‡ ‡	81.1 (65.7, 102.2)	82.5 (69.0, 99.8)	81.8 (70.1, 92.9)	73.4 (61.6, 109.6)	0.95 (0.83, 1.09) p=0.484	0.97 (0.84, 1.12) p=0.687	0.96 (0.85, 1.08) p=0.513	0.98 (0.91, 1.05) p=0.482
LV end diastolic volume (ml), * median (IQR) ‡ ‡‡ ‡	162.8 (141.6, 186.2)	165.6 (149.2, 188.9)	164.6 (147.0, 176.9)	151.6 (132.7, 183.3)	0.93 (0.85, 1.02) p=0.140	0.96 (0.87, 1.06) p=0.403	0.95 (0.87, 1.02) p=0.170	0.97 (0.92, 1.01) p=0.138
Change in LV end diastolic volume at 3 months from 2-7 days (ml), * median (IQR) ‡ ‡‡ ‡	-3.5 (-13.5, 10.0)	1.7 (-12.0, 14.6)	-4.7 (-13.1, 6.2)	-4.3 (-16.4, 12.8)	-7.80 (-17.82, 2.21) p=0.127	-9.53 (-19.98, 0.92) p=0.074	-8.60 (-17.34, 0.15) p=0.054	-3.92 (-8.93, 1.10) p=0.126

‡ Data analyzed on square root scale. Treatment effect estimates reported as mean differences in square root of MVO extent between groups, with 95% confidence interval and p-value, from linear regression model adjusted for MI location.

‡‡ Treatment effect estimates reported as odds ratios between groups, with 95% confidence interval and p-value, from a logistic regression model, adjusting for MI location.

‡‡‡ Treatment effect estimates reported as mean differences between groups, with 95% confidence interval and p-value, from linear regression model adjusted for MI location.

‡‡‡‡ Treatment effect estimates reported as relative differences between groups, on a log-transformed scale, with 95% confidence interval and p-value, from linear regression model adjusted for MI location.

*Missing data: Change in LV end diastolic volume at 3 months from 2 – 7 days, 9 subjects (5 placebo, 1 alteplase 10mg, 3 alteplase 20mg). Infarct size, LV ejection fraction, 2 – 7 days post-PCI, 4 subjects (2 placebo, 2 alteplase 20mg group). LV ejection fraction, 3 months post-PCI, 9 subjects (5 placebo, 1 alteplase 10mg, 3 alteplase 20mg group). Infarct size/ myocardial salvage index 3 months post-PCI, 11 subjects (7 placebo, 1 alteplase 10mg, 3 alteplase 20mg group). LV end diastolic/ systolic volume 2 – 7 days post-PCI, 4 subjects (2 placebo, 2 alteplase 20mg). LV end diastolic/ systolic volume 3 months post-PCI, 9 subjects (5 placebo, 1 alteplase 10mg, 3 alteplase 20mg). Microvascular obstruction extent/ presence, infarct size, LV ejection fraction, 4 subjects (2 placebo, 2 alteplase 20mg group). Myocardial hemorrhage extent 13 subjects (6 placebo, 5 alteplase 10mg, 2 alteplase 20mg group). Myocardial hemorrhage extent, 13 subjects (6 placebo, 5 alteplase 10mg, 2 alteplase 20mg). Myocardial hemorrhage presence/ absence, 8 subjects (8 placebo, 2 alteplase 10mg, 2 alteplase 20mg group †Microvascular obstruction was presented as mean ± SD, because the high proportion of patients with a 0 value for microvascular obstruction amount resulted in median values for microvascular obstruction of 0 in each group.

Table S2. Coagulation and hematological variables. Data are mean ± SD, or n (%), unless otherwise stated. Between-group comparisons derived from linear, logistic, or ordinal logistic regression models, adjusted for location of MI (see footnotes).

	Treatment Group				Treatment effect			
	All	**Placebo**	**Alteplase 10mg**	**Alteplase 20mg**	**20mg vs. placebo**	**10mg vs. placebo**	**10 or 20mg vs. placebo**	**Trend with dos**
	[n=144]	**[n=53]**	**[n=41]**	**[n=50]**	**Estimate (95% CI) p-value**	**Estimate (95% CI) p-value**	**Estimate (95% CI) p-value**	**Estimate (95% C p-value**
Hemoglobin (g/L) at 24 hrs * ‡	142.1 ± 12.5	143.5 ± 10.8	141.1 ± 12.1	141.6 ± 14.5	-1.98 (-6.90, 2.94) p=0.431	-2.61 (-7.72, 2.49) p=0.316	-2.28 (-6.55, 2.00) p=0.297	-1.00 (-3.46, 1.4 p=0.423
Activated clotting time (s) at 2 hrs * ‡‡	288.9 ± 92.0	302.3 ± 103.3	298.3 ± 83.1	266.9 ± 83.5	0.89 (0.80, 1.00) **p=0.046**	1.00 (0.89, 1.13) p=0.940	0.94 (0.85, 1.04) p=0.230	0.94 (0.89, 1.00 **p=0.048**
Fibrinogen 2 hrs post-PCI * ‡‡‡	3.3 ± 0.9	3.4 ± 1.0	3.3 ± 1.1	3.3 ± 0.6	1.00 (0.90, 1.11) p=0.960	0.98 (0.88, 1.09) p=0.703	0.99 (0.90, 1.08) p=0.809	1.00 (0.95, 1.05 p=0.954
Plasminogen (U/dL) 2 hrs post-PCI *‡	89.4 ± 14.7	95.3 ± 14.3	90.1 ± 12.4	82.5 ± 14.2	-12.9 (-18.4, -7.30) **p<0.001**	-5.20 (-11.00, 0.60) p=0.076	-9.40 (-14.30, -4.40) **p=0.0002**	-6.40 (-9.20, -3.7 **p<0.001**
Change in plasminogen (U/dL) 2 hrs post-PCI compared to baseline *‡	-5.3 ± 9.0	-0.1 ± 7.8	-4.7 ± 8.0	-11.3 ± 7.3	-11.30 (-14.40, -8.10) **p<0.001**	-4.60 (-7.80, -1.30) **p=0.006**	-8.20 (-11.10, -5.30) **p<0.001**	-5.60 (-7.20, -4.1 **p<0.001**
D-dimers 2 hrs post-PCI * ‡‡‡	394.1 ± 543.3	142.4 ± 127.3	536.2 ± 695.8	541.7 ± 580.8	3.49 (2.50, 4.87) **p<0.001**	3.23 (2.28, 4.58) **p<0.001**	3.37 (2.52, 4.50) **p<0.001**	1.88 (1.58, 2.23 **p<0.001**
tPA 2 hrs post-PCI (ng/ml) * ‡‡‡	23.2 ± 54.4	17.4 ± 35.6	15.4 ± 8.0	36.1 ± 84.1	1.55 (1.22, 1.97) **p=0.0004**	1.16 (0.90, 1.49) p=0.244	1.36 (1.10, 1.68) **p=0.005**	1.24 (1.10, 1.40 **p=0.0004**

Ratio of tPA 2 hrs post-PCI compared to baseline * ‡‡‡	1.58 ± 1.34	1.16 ± 0.27	1.43 ± 0.59	2.17 ± 2.09	1.58 (1.36, 1.84) **p<0.001**	1.20 (1.03, 1.41) **p=0.022**	1.39 (1.21, 1.60) **p<0.001**	1.26 (1.16, 1.35 **p<0.001**
Prothrombin fragment F_{1+2} (pmol/L) 2 hrs post-PCI, median (IQR) * ‡‡‡	178.8 (133.1, 244.2)	152.0 (118.6, 211.4)	183.0 (141.9, 291.9)	187.3 (150.5, 244.9)	1.24 (1.00, 1.52) **p=0.048**	1.27 (1.02, 1.58) **p=0.034**	1.25 (1.04, 1.50) **p=0.017**	1.11 (1.00, 1.24 **p=0.046**

‡ Treatment effect estimates reported as mean differences between groups, with 95% confidence interval and p-value, from linear regression model adjusted for MI location.

‡‡ Treatment effect estimates reported as relative differences between groups, with 95% confidence interval and p-value, from linear regression model adjusted for MI location.

‡‡‡ Data analyzed on logarithmic scale. Treatment effect estimates reported as relative difference between groups, with 95% confidence interval and p-value, from linear regression model adjusted for MI location.

* Missing data: Hemoglobin at 24 hours, 6 subjects (3 placebo, 3 alteplase 20mg group). Activated clotting time, 6 subjects (5 from placebo, 1 from alteplase 10mg group). tPA/ fibrinogen/ d-dimer 2 hours post-PCI, 10 subjects (4 placebo, 2 alteplase 10mg, 4 alteplase 20mg group). tPA ratio 2 hours post-PCI compared to baseline, 15 subjects (6 placebo, 3 alteplase 10mg, 6 alteplase 20mg group). Prothrombin fragment F_{1+2} two hours post-PCI, 15 subjects (6 placebo, 3 alteplase 10mg, 4 alteplase 20mg group). Plasminogen 2 hours post-PCI, 10 subjects (4 placebo, 2 alteplase 10mg. 4 alteplase 20mg group). Change in plasminogen 2 hours post-PCI compared to baseline 15 subjects (6 placebo, 3 alteplase 10mg, 6 alteplase 20mg).

Table S3. IMR, CFR and RRR in sub-groups of TIMI coronary flow grade immediately before study drug delivery. Data are median (IQR). Data analyzed on logarithmic scale. Treatment effect estimates reported as relative differences, derived from linear regression models, adjusted for location of MI.

TIMI flow grade pre-study drug	Placebo [n=53]	Alteplase 10mg [n=41]	Alteplase 20mg [n=50]	Treatment Effect		Interaction p-value (treatment as 3-level categorical variable)	Treatment Effect	Interaction p-value (treatment as 2-level categorical variable)	Treatment Effect	Interaction p-value (treatment as per 10mg increase in dose)
				20mg vs. placebo	10mg vs. placebo		10 or 20mg vs. placebo		Trend with dose	
IMR ≤ 2 [n]	40.5 (29.5, 61.0) [16]	50.5 (26.5, 69.8) [12]	50.5 (25.8, 77.0) [18]	1.05 (0.65, 1.71) p=0.835	1.03 (0.61, 1.77) p=0.903	0.481	1.05 (0.68, 1.62) p=0.834	0.473	1.03 (0.81, 1.31) p=0.828	0.883
3 [n]	28.0 (16.0, 50.5) [35]	19.0 (16.0, 30.0) [29]	30.0 (17.5, 46.5) [31]	1.02 (0.72, 1.45) p=0.896	0.72 (0.51, 1.02) p=0.835		0.86 (0.64, 1.16) p=0.335		1.00 (0.84, 1.20) p=0.959	
CFR ≤ 2 [n]	1.6 (1.2, 2.1) [16]	1.4 (1.2, 1.8) [12]	1.3 (1.0, 2.0) [18]	0.84 (0.64, 1.10) p=0.198	0.88 (0.65, 1.18) p=0.396	0.159	0.85 (0.67, 1.09) p=0.201	0.074	0.92 (0.80, 1.05) p=0.198	0.056
3 [n]	1.3 (1.1, 1.8) [35]	1.4 (1.1, 2.1) [29]	1.6 (1.3, 2.0) [31]	1.16 (0.95, 1.41) p=0.139	1.07 (0.88, 1.31) p=0.472		1.12 (0.95, 1.32) p=0.192		1.08 (0.98, 1.18) p=0.136	
RRR ≤ 2 [n]	2.0 (1.4, 2.3) [16]	1.9 (1.4, 2.3) [12]	1.5 (1.1, 2.2) [18]	0.82 (0.62, 1.08) p=0.158	0.90 (0.66, 1.23) p=0.502	0.140	0.85 (0.66, 1.09) p=0.203	0.065	0.90 (0.79, 1.04) p=0.155	**0.046**
3 [n]	1.6 (1.3, 2.2) [35]	1.6 (1.4 2.6) [29]	2.0 (1.5, 2.4) [31]	1.16 (0.95, 1.41) p=0.154	1.10 (0.90, 1.35) p=0.341		1.13 (0.95, 1.34) p=0.160		1.08 (0.97, 1.19) p=0.148	

Table S4. IMR, CFR and RRR in sub-groups of TIMI thrombus grade immediately before study drug delivery. Data are median (IQR). Data analyzed on logarithmic scale. Treatment effect estimates reported as relative differences, derived from linear regression models, adjusted for location of MI.

Thrombus grade pre-study drug	Placebo [n=53]	Alteplase 10mg [n=41]	Alteplase 20mg [n=50]	Treatment Effect		Interaction p-value (treatment as 3-level categorical variable)	Treatment Effect	Interaction p-value (treatment as 2-level categorical variable)	Treatment Effect	Interaction p-value (treatment as per 10mg increase in dose)
				20mg vs. placebo	10mg vs. placebo		10 or 20mg vs. placebo		Trend with dose	
IMR ≤ 2 [n]	21.0 (15.0, 49.0) [15]	39.0 (17/0, 44.0) [9]	37.0 (20.0, 45.0) [9]	1.33 (0.72, 2.44) p=0.363	1.24 (0.67, 2.29) p=0.498	0.260	1.28 (0.77, 2.13) p=0.348	0.158	1.16 (0.85, 1.57) p=0.353	0.158
≥ 3 [n]	39.5 (21.8, 57.2) [36]	20.0 (16.0, 33.2) [32]	38.5 (19.8, 58.2) [40]	0.97 (0.70, 1.36) p=0.865	0.69 (0.48, 0.98) p=0.036		0.83 (0.62, 1.12) p=0.231		0.99 (0.84, 1.17) p=0.231	
CFR ≤ 2 [n]	1.3 (1.1, 2.1) [15]	1.1 (1.1, 1.6) [9]	1.8 (1.5, 2.0) [9]	1.06 (0.76, 1.48) p=0.737	0.86 (0.62, 1.21) p=0.393	0.506	0.96 (0.73, 1.26) p=0.759	0.616	1.02 (0.86, 1.20) p=0.843)	0.976
≥ 3 [n]	1.3 (1.1, 1.8) [36]	1.4 (1.3, 2.1) [32]	1.4 (1.2, 2.0) [40]	1.03 (0.86, 1.23) p=0.753	1.05 (0.87, 1.28) p=0.595		1.04 (0.89, 1.22) p=0.637		1.01 (0.93, 1.11) p=0.765	
RRR ≤ 2 [n]	1.6 (1.3, 2.5) [15]	1.4 (1.2, 2.0) [9]	2.0 (1.7, 2.0) [9]	1.05 (0.75, 1.48) p=0.767	0.87 (0.61, 1.23) p=0.424	0.440	0.96 (0.72, 1.27) p=0.768	0.595	1.01(0.86, 1.20) p=0.865	0.948
≥ 3 [n]	1.6 (1.4, 2.2) [36]	1.8 (1.5, 2.7) [32]	1.7 (1.3, 2.4) [40]	1.02 (0.85 1.23) p=0.840	1.09 (0.89, 1.33) p=0.412		1.05 (0.89, 1.24) p=0.580		1.01 (0.92, 1.11) P=0.863	

Supplemental References:

1. Group TS. The Thrombolysis in Myocardial Infarction (TIMI) trial. Phase I findings. The New England journal of medicine 1985;312:932-6.
2. Gibson CM, Murphy SA, Rizzo MJ, Ryan KA, Marble SJ, McCabe CH, Cannon CP, Van de Werf F, Braunwald E. Relationship between TIMI frame count and clinical outcomes after thrombolytic administration. Thrombolysis In Myocardial Infarction (TIMI) Study Group. Circulation 1999;99:1945-50.
3. Gibson CM, de Lemos JA, Murphy SA, Marble SJ, McCabe CH, Cannon CP, Antman EM, Braunwald E; TIMI Study Group. Combination therapy with abciximab reduces angiographically evident thrombus in acute myocardial infarction: a TIMI 14 substudy. Circulation 2001;103:2550-4.
4. Sorensson P, Heiberg E, Saleh N, Bouvier F, Caidahl K, Tornvall P, Rydén L, Pernow J, Arheden H. Assessment of myocardium at risk with contrast enhanced steady-state free precession cine cardiovascular magnetic resonance compared to single-photon emission computed tomography. Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance 2010;12:25.
5. Anderson LJ, Holden S, Davis B, Prescott E, Charrier CC, Bunce NH, Firmin DN, Wonke B, Porter J, Walker JM, Pennell DJ. Cardiovascular T2-star (T2*) magnetic resonance for the early diagnosis of myocardial iron overload. European heart journal 2001;22:2171-9.
6. Carrick D, Haig C, Ahmed N, Ahmed N, McEntegart M, Petrie MC, Eteiba H, Hood S, Watkins S, Lindsay MM, Davie A, Mahrous A, Mordi I, Rauhalammi S, Sattar N, Welsh P, Radjenovic A, Ford I, Oldroyd KG, Berry C. Myocardial Hemorrhage After Acute Reperfused ST-Segment-Elevation Myocardial Infarction: Relation to Microvascular Obstruction and Prognostic Significance. Circulation Cardiovascular imaging 2016;9:e004148.