Supplementary Online Content

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Supplementary Material

Effect of low-dose intracoronary alteplase during primary percutaneous coronary intervention on microvascular obstruction in patients with acute myocardial infarction: a randomized clinical trial.

ClinicalTrials.gov: NCT02257294.

Introduction

Patients with acute ST-segment elevation myocardial infarction (STEMI) and a large thrombus burden evident at initial coronary angiography were enrolled. A three-arm dose-ranging design was adopted where either 10 mg or 20 mg of alteplase (representing one tenth and one fifth of the standard dose, respectively), or placebo. In contrast to giving adjunctive fibrinolytic therapy at the end of percutaneous coronary intervention (PCI),[1] the intervention was scheduled to occur early during the primary PCI procedure when residual thrombus burden is greatest and fibrinolytic therapy might be most effective. The study therapy was given after reperfusion of the infarct-related coronary artery, proximal to the culprit lesion and before stent implantation. We aimed to determine the lowest effective dose of alteplase in reducing microvascular obstruction, and other surrogate outcomes for efficacy, bleeding and mechanisms.

Supplementary Methods

Participants and eligibility criteria

Patients with a clinical diagnosis of acute STEMI were eligible for randomization according to the following eligibility criteria

Inclusion

- Acute myocardial infarction (symptom onset ≤ 6 hours) with persistent ST segment elevation or recent left bundle branch block
- Coronary artery occlusion (TIMI coronary flow grade 0 or 1) or, impaired coronary flow (TIMI flow grade 2, slow but complete filling) in the presence of definite angiographic evidence of thrombus (TIMI grade 2+).

- Proximal-mid culprit lesion location in a major coronary artery (i.e. the right, left anterior descending, intermediate or circumflex coronary artery)
- Radial artery access

Exclusion

Clinical criteria that would exclude the patient from the trial were evaluated by medical, research and nursing staff when the patient arrives in the catheter laboratory.

Coronary

- 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)

Clinical

- Cardiogenic shock (Killip Class IV)
- Multivessel PCI intended before cardiac magnetic resonance imaging (MRI) intended for day 2 – 7
- Previous infarction in the culprit artery (known or suspected clinically)
- Body weight estimated to be <60 kg
- Non-cardiac co-morbidity with expected survival <1 year
- Contra-indication to contrast-enhanced MRI

  Pacemaker
- Implantable defibrillator
- Known impaired renal function (eGFR<30ml/min)
- Significant bleeding disorder either at present or within the past 6 months

- Patients with current concomitant oral anticoagulant therapy (INR > 1.3), including apixaban, dabigatran, and rivaroxaban
- Any history of central nervous system damage (i.e. neoplasm, aneurysm, intracranial or spinal surgery)
- Known hemorrhagic diathesis
- Severe hypertension (BP >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 AMI)
- Recent trauma to the head or cranium (<2 months)
- Prolonged cardiopulmonary resuscitation (>2 minutes) within the past 2 weeks
- Acute pericarditis and/or subacute bacterial endocarditis e.g. valve mass or vegetation revealed by echocardiography
- 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-menopause) or breast feeding.
- Requirement for immunosuppressive drug therapy at any time during the past 3 months; whether administered orally, subcutaneously, intramuscularly or intravenously. This would include corticosteroids (but not inhaled or topical), drugs used following transplantation (e.g. tacrolimus, cyclosporine), anti-metabolite therapies (e.g. mycophenolic acid (Myfortic), azathioprine, leflunomide (Arava)), and immunomodulators including biologics (e.g. adalimumab (HUMIRA), etanercept (Enbrel), aldesleukin), and DMARDS (cyclophosphamide, methotrexate, etc.). Please note this list is not exhaustive and a requirement for other immunosuppressive drugs not listed would also exclude the patient.
- 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 past 3 months including chemotherapy, radiotherapy and treatment with biologics such as Vascular Endothelial Growth Factor Receptor (VEGFR) inhibitors (e.g. bevacizumab, pazopanib).
- Any significant concurrent or recent condition(s) not listed above that in the opinion of the treating clinician would pose an additional risk for the patient.

Informed consent

Screening, witnessed verbal informed consent, study drug administration and acute assessments of efficacy took place during the standard of care emergency primary PCI procedure in the cardiac catheterization laboratory. A screening log was prospectively

completed. Only patients who were sufficiently well to understand the information about the study, as described by the attending cardiologist, were eligible to participate. The decision as to whether a patient is eligible to be included was made and documented by the cardiologist. The study information sheet that had been approved by the research ethics committee was subsequently provided to each participant on the ward where written informed consent was obtained. The participants were followed-up unless consent was withdrawn.

Standard care

Standard care for coronary reperfusion was recommended according to contemporary practice guidelines[2] using either 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 minimize thrombus embolization. The balloon angioplasty was intended to stabilize the thrombotic lesion and prevent vessel reocclusion 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.

Secondary Outcomes

Central laboratory analyses

The central laboratory analyses of the primary and secondary outcomes were determined blind to treatment allocation.

Cardiac magnetic resonance acquisition and analysis

We used cardiac MRI to assess left ventricular dimensions, function and pathology 2 – 7 days and 3 months post-MI. MRI 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 localizers, T1-mapping, T2*-mapping, cine MRI with steady-state free precession (SSFP), and late gadolinium enhancement imaging 10 – 15 minutes after administration of contrast media.[3] The scan acquisitions were spatially co-registered and also included different slice orientations to enhance diagnostic confidence.

Cardiac MRI protocol

	Stage	Guidance	Time
			Min
Pre-contrast	Patient preparation	Patient instructions, scanner set-up, load the pump injector, check IV access is functional, acquire the ECG	5 / 5
	Localisers and planning	X3 orthogonal bright-blood, 2Ch, 3Ch, 4Ch	5 / 10
	T2* (pre-contrast)	SA full LV stack (8 - 10 slices) T2* multi-echo GRE	7 / 17
	T1 map (pre-contrast)	SA Basal/mid/apical slices MOLLI	2 / 19
Post-contrast	First pass perfusion	**Inject Gadovist, Dose 1** – 0.05 mmol/kg FLASH sequence 3 SAX per R-R interval over 1.5 min	4 / 23
		Inject Gadovist Dose 2 – 0.10 mmol/kg	1 / 24
		Wait 3 minutes for contrast equilibration	3 / 27
	Cine-SSFP	SA full stack 8 – 10 slices aligned to the Use the T2* SAX slice positions; single LAX	8 / 35
	Late enhancement	Start at t = 10 min post Dose 2 SA full stack aligned to the T2* positions	8 / 43
	T1 (post-contrast)	SA Basal/mid/apical slices MOLLI	2 / 45
	End of study	Patient briefing	5 / 50

Typical T2 imaging parameters*

T2* multi-echo GRE (preferred) or T2*-map Bandwidth ~814 (x8) Hz/pixel; flip angle 18°; matrix 256x115 pixels; spatial resolution 2.6 x 1.6 x 10 mm; slice thickness 8 mm with 2 mm gap.

Contrast media administration

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 in a weight-adjusted contrast volume this was in order to obtain information on myocardial perfusion.

First dose injection (D1 = 0.05 mmol/kg) was given to initiate the first-pass of contrast and the second dose, D2 = 0.1 mmol/kg 'top-up' injection, was given immediately after the first-pass. Therefore, the total dose of gadobutrol was 0.15 mmol/kg.

An automated pump injector was used for intravenous injection of gadolinium. The injection rate was 4 ml/sec. A 3-of-5 MRI acquisition protocol for short axis imaging of the first pass of gadolinium contrast perfusion was acquired at the same slice positions as the T2* scans. First-pass perfusion at rest for 'wash-in' microvascular obstruction quantification was performed with a fast low-angle shot (FLASH) sequence run simultaneously with the contrast injection. 'Normal' standard sequence, i.e. non work-in-progress implementation, for 3 short axis (SAX) per R-R interval over 1.5 min is used.

Typical first-pass imaging parameters: Saturation recovery with inversion pulse. T1 101 ms; TR/TE 194/0.98 ms; acquisition window 1000 ms. 1 concatenation; 3 SAX slices. If three slices could not be acquired within the R-R cycle, then 2 concatenations were used.

Cardiac mass & function incorporating area-at-risk imaging with contrast cine-SSFP was acquired 2 – 3 minutes following the second dose of contrast media to enable contrast equilibration. The cine-MRI for LV mass and function was collected during the interval

between the 3 min scan and late enhancement imaging. Cine-MRI was acquired with a short axis LV stack, slices aligned to T2* maps, from the mitral valve to the LV apex (usually 10 slices in total). Extra slices were acquired basally to the mitral valve that incorporate left ventricular outflow tract and also potentially to the apex.

Steady-state free precession (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 2 mm gaps, equating to approximately 10 slices and 30 cardiac phases.

Typical SSFP imaging parameters: Voxel size 2.0 x 2.0 x 8.0 mm; TR/TE 39.6/1.12 ms; flip angle 55°, matrix 192 x 192 pixels; slice thickness 8 mm, with 2 mm gap.

Late enhancement

Late microvascular obstruction and scar 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 three long axis views (vertical long axis, horizontal long axis and 3 chamber view) were acquired. Phase-sensitive inversion recovery MRI techniques reduce variability relating to myocardial nulling which is required for late gadolinium enhancement imaging of infarct vs. unaffected myocardium. If a phase-sensitive protocol was not used, a modified Look-Locker inversion time scout was performed prior to using an inversion recovery turbo gradient echo sequence. Phase swops were performed where appropriate to rule out artefact.

Poor breath-holding: A single shot technique or navigated late gadolinium enhancement imaging was used as an option for poor breath holders.

Typical late gadolinium enhancement and microvascular obstruction imaging parameters with phase-sensitive inversion recovery: matrix 192 x 256 pixels; flip angle 25°; 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 300 ms).

Post-contrast T1 mapping

Three short axis T1-maps (basal, mid and apical positions) that were spatially matched to the same slice positions as the pre-contrast T1-map scans >15 minutes after the top-up injection of contrast media.

Cardiac magnetic resonance imaging at 3 months

Cardiac magnetic resonance imaging (MRI) at 3 months follow-up involved the same scan protocol as was used in the baseline MRI scan. Renal function was known or checked. A full blood count was checked to provide hematocrit for the extracellular volume analysis from the post-contrast T1-map.

MRI analysis

The MRI analyses were undertaken using Medis® Suite MR (Medis, Leiden, NL) which is a vendor-independent post-processing software. P.McC. undertook the primary analyses of the scans and related analyses were reviewed by C.B. (second observer). The research staff were blinded to treatment allocation.

Infarct definition and 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 left ventricular mass.

Microvascular obstruction

Microvascular obstruction 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 myocardial mass (grams) of the dark zone was quantified by manual delineation and expressed as a percentage of left ventricular mass.

Myocardial edema

The presence of myocardial edema was established based on an area of increased signal intensity on the steady-state free precession 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 left ventricular mass mass.[3]

Myocardial salvage

Myocardial salvage was calculated by subtraction of percent infarct size from percent area-at-risk, as reflected by the extent of edema. 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 [4,5] was considered to confirm the presence of myocardial hemorrhage. The area was manually delineated and expressed as % left ventricular mass.

Coronary angiogram acquisition and analyses

The acquisition and analyses of the coronary angiograms were performed according to Standard Operating Procedures. Coronary angiograms were acquired during emergency care with cardiac catheter laboratory X-ray and information technology equipment. Feedback was provided to sites on the quality and completeness of the angiograms.

The angiograms were analyzed by trained observers (A.M, C.B., M.McE.) using imaging post-processing software (QAngio® XA Medis, Leiden, NL.). The observers were blinded to treatment assignment. 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. Therefore, the single plane projection showing the best opacified and most severe lesion with minimal foreshortening and minimal branch overlap was selected.

The Thrombolysis In Myocardial Infarction (TIMI) coronary flow grade[6] and thrombus grade[7] were assessed following initial angiography and at the end of the procedure. TIMI myocardial perfusion grade[8] and TIMI frame count[9] was assessed at the end of the procedure. The TIMI frame count and perfusion grade are angiographic measures of microvascular function.

TIMI Coronary Flow Grade

TIMI coronary flow grade of the infarct-related artery is independently predictive of prognosis.[6]

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 contrast entering the microvasculature and contrast washout, it is predictive of prognosis.[9]

TIMI myocardial perfusion grade	Definition
0	Minimal or no myocardial blush in the distribution of the infarct related artery.
1	Myocardial blush is present in the distribution of the infarct related 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 infarct related 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 infarct related 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.

Corrected TIMI Frame Count (TFC)

The TIMI frame count is an objective continuous variable index of coronary blood flow, representing the amount of time (in frames) for contrast dye to reach a standardized distal landmark, corrected for vessel length **(**corrected TFC, normal < 27 frames).[9]

Method: The corrected TFC is the number of cine frames required for contrast to first reach standardized distal coronary landmarks in the culprit artery and is measured with a frame counter on a cine viewer. A frame rate of 30 frames/second was used in the original description of the method. The common use of slower frame rates will require the count to be adjusted (e.g. 15 frames/second will require the frame count to be doubled). If the culprit vessel is the left anterior descending artery (LAD) the frame count then requires to be corrected by dividing by 1.7 (correcting for longer vessel length).

To calculate transit time (seconds) for dye to traverse the length of the artery to the distal landmark, the corrected TFC is divided by 30 and multiplied by 1000 to convert the time to milliseconds. To calculate the fraction of a cardiac cycle required for dye to traverse the artery (normalizing the corrected TFC for heart rate): Fraction of cardiac cycle = (corrected TFC/30 seconds)/ (60/heart rate). A frame count of 100 (a value that is the 99^{th} percentile of patent vessels) was imputed to an occluded vessel.

TIMI Coronary Thrombus Grade

Thrombus burden revealed during coronary angiography can be classified according to the TIMI thrombus grade.[7]

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 ≤ 1/2 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

Electrocardiogram acquisition and analysis

A 12-lead electrocardiogram (ECG) was obtained before coronary reperfusion and 60 minutes afterwards and again at 3 months. The electrocardiograms were analyzed in the University of Glasgow ECG core laboratory.

ECGs

A 12 lead ECG was performed according to standard methods. The ECG was acquired by trained cardiology staff using standard ECG recorders available in the cardiology department. ECG records were anonymized by inserting the patient ID number.

Anonymized ECGs were prospectively collected and uploaded to the e-CRF portal for electronic transfer to the University of Glasgow core laboratory during the course of the study. The ECG Core Lab is certified to ISO 9001: 2015 standards by a UKAS Accredited

Organization. The intention was that ECGs would be analyzed using the University of Glasgow ECG Analysis Program,[10] but this approach proved not to be possible in most participating centers, and so copies of all ECGs were uploaded to the e-CRF portal in pdf format. ECGs were checked for completeness and quality and feedback was provided to local sites.

The ECG outcomes include the change in summative ST elevation score on the 60 min ECG post-MI vs. pre-reperfusion, and the Selvester QRS score was taken as a surrogate ECG measure of infarct size at 3 months.[11] The Selvester score translates subtle changes in ventricular depolarization on the electrocardiogram to a surrogate measure of infarct size. There are a maximum of 32 points, with one point corresponding to 3% of the left ventricle.

Safety

Parameters of hemostasis and coagulation, including fibrinogen concentration, plasminogen activity, Fibrin D-dimer, and Prothrombin F1+2 served as a surrogate measure of bleeding risk and safety.[12,13] TPA antigen levels were assessed as a potential measure of the systemic overflow of the alteplase administered into the culprit coronary artery. Hemostasis and coagulation parameters were measured in blood samples, when site logistics permitted. The sampling time-points were 0 hours, 2, and 24 hours post-reperfusion.

A depletion of fibrinogen and plasminogen following thrombolysis correlates with systemic fibrinolysis and may correlate with bleeding risk. Fibrin D-dimer concentration represents a specific measure of fibrinolysis. Fibrin D-dimer concentrations may correlate with the amount of clot lysis and may therefore represent a measure of residual clot burden. Fibrin D-dimer concentrations have the potential to correlate with efficacy and outcome. Prothrombin F1+2 is a measure of thrombin activation and correlates with the (undesired) procoagulant effect of thrombolysis. F1+2 are depressed by anti-coagulants administered before and during

PCI. Prothrombin F1+2 concentrations may associate with the dose of alteplase and placebo and potentially could correlate with adverse thrombotic events.

Local hospital blood sample handling

Blood samples collected into 0.109M sodium citrate (for hemostasis assays) or EDTA (for Troponin and NT-proBNP) were handled according to a sample handling manual which was provided to all sites. Research blood tests at T 0, 2 hrs. and 24 hrs. were preferred but not compulsory. 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 analyses

Blood samples

Research blood tests at T 0, 2 hrs. and 24 hrs. were preferred but not compulsory. In this way blood sample handling out-of-hours did not become a barrier to enrolment.

Troponin and NT-proBNP

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.

EDTA plasma samples were stored to analyze high-sensitivity cardiac troponin T and NT-proBNP 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).[14,15] Troponin T (ng/ml) was measured in blood samples collected at baseline (before study drug administration), and 2hr ± 1, 24 hr. ±12, 2-7 days, and 12 weeks (± 2 weeks) post-MI. NT-pro BNP (pg/ml) was measured in blood samples collected at baseline, 2-7 days and 12 weeks ± 2 in order to provide a biochemical measurement of left ventricular remodeling (within-subject change in NT-proBNP at follow-up from baseline).[16]

For measurement of both NT-proBNP and high sensitivity cardiac troponin T, we used an automated method (e411, Roche Diagnostics, Burgess Hill, United Kingdom) 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 0.0027 ng/ml, respectively.

For NT-proBNP, the coefficient of variation was 2.6% and 2.4% for control materials with mean NT-proBNP level of 4418 pg/ml and 142 pg/ml, respectively. The troponin T and NT-proBNP results were provided to the Robertson Centre for Biostatistics, University of Glasgow.

Hemostasis and coagulation laboratory methods

Fibrinogen and other hemostasis parameters served as a surrogate measure of bleeding and safety.[12,13] Hemostasis and coagulation parameters were measured in blood samples when site logistics permitted. The sampling time-points were 0 hours, and 2, and 24 hours post-reperfusion. The parameters included fibrinogen, fibrin D-Dimer and plasminogen activity, tPA antigen and prothrombin F1+2.

Sample Handling

All plasma samples were processed in a non-standard manner using anonymized bar coded samples by a trained member of staff.

Assays

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 analyzer using HemosIL® reagents (Instrumentation Laboratory Company, Bedford, US). The fibrinogen Clauss assay had a normal reference range 170 – 400 mg/dL (internally derived) and, an inter-assay coefficient of variation of 5.8% and 7.7% for low control samples with mean concentrations of 292 mg/dL and 222 mg/dL 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 range 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 F1+2 antigen levels) were performed on a TECAN Sunrise spectrophotometer (Labtech International Ltd, United Kingdom) 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 range 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.

Health outcomes

1. Major Adverse Cardiovascular and Cardiac Events (MACCE): cardiovascular death, non-fatal MI, unplanned hospitalization for TIA or stroke;
2. Major Adverse Cardiac Events (MACE): cardiac death, non-fatal MI, unplanned hospitalization for heart failure;
3. Spontaneous MACE: MACE, excluding MI associated with revascularization procedures (Type 4 or 5 MI);
4. MI associated with revascularization procedures (Type 4 or 5 MI);
5. All-cause mortality;
6. Unplanned hospitalization for heart failure;
7. All-cause death and unplanned hospitalization for heart failure;
8. BARC Type 3, Type 4, and Type 5 bleeding events.

Acute cerebrovascular and systemic bleeds were defined using the Bleeding Academic Research Consortium (BARC) standardized definitions for clinical trials. [17]

All of these events were adjudicated by a Clinical Event Committee (CEC) comprised of 3 cardiologists who were independent of the trial and blinded to the treatment allocation. The CEC charter was established before enrolment began. Coronary revascularization, including PCI and coronary artery bypass grafting (CABG), were prospectively recorded in the clinical report form. Information on serious adverse events during follow-up was obtained by contacting the patients by telephone and reviewing their medical records. Complications that were potentially related to the study procedure were prospectively recorded.

Trial management

The trial was conducted in line with Guidelines for Good Clinical Practice in Clinical Trials[18] and the study complies with the Declaration of Helsinki.[19] There was a Trial Management Group for operational activity, an independent Clinical Event Committee to adjudicate on serious adverse events for safety and efficacy outcomes, 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 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. This analysis was scheduled for when approximately 40% of patients had been randomized and followed-up to 3 months. Considering the primary outcome, each active treatment arm was compared to the placebo arm and if the conditional power for showing a benefit over placebo based on the current trend was less than 30%, then a recommendation would be made to halt that arm.

The Robertson Centre for Biostatistics within the Glasgow Clinical Trials Unit provided the trial-specific electronic data collection system, acted as an independent coordinating center for randomization and data management, and conducted the statistical analyses. 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. The sponsor prospectively monitored the study

for safety and monitoring, visits were undertaken in all of the sites. All serious adverse events were prospectively reported to the Pharmacovigilance Unit.

Statistical methods

Secondary outcomes were summarized in the final analysis set as a whole and by treatment group. All outcomes were compared between treatment groups using linear, binary logistic or proportional odds logistic regression models, for continuous, binary, and ordinal outcomes respectively, with adjustment for location of the MI. Treatment was initially included as a 3-level categorical variable, and each treatment group was compared with placebo; in a second model, treatment was included as a binary variable, to compare both alteplase groups combined with placebo. For continuous outcome measures, model residual distributions were examined and outcomes were transformed for modelling where necessary to improve model fit.

Regression models were extended to examine treatment effect differences between subgroups, by inclusion of interaction terms. Pre-specified subgroup analyses for the primary outcome were in relation to time from symptom onset to reperfusion (<2, ≥2 - <4, ≥4 hours), sex, age (<55, ≥55 - <65, ≥65 years), location of the MI (anterior, non-anterior), smoking status (never, former, current), initial TIMI Coronary Flow Grade (0, 1, ≥2), and use of antiplatelet medication prior to the MI.

Treatment effect estimates were reported with 95% confidence intervals (CIs) and p-values. Where no suitable transformation was found, each active treatment group was compared to placebo using van Elteren tests, stratified by the location of the MI. Ordinal outcomes were compared between groups using proportional odds logistic regression models, adjusted for the location of the MI. Binary outcomes were compared between groups using logistic regression models, adjusted for the location of the MI. Logistic regression model results are

reported as odds ratios for each active treatment group vs. placebo, with 95% confidence intervals and p-values. For those outcomes measured at both 2 – 7 days and at 3 months, changes between the two time points were summarized, and regression models of 3 month outcomes were extended to include an adjustment for the day 2 – 7 measurement. The proportion of patients with major adverse cardiovascular events within 3 months and other binary outcomes were analyzed using the same methods, and time to events within 12 months was compared between groups using log rank tests. Health related quality of life was compared between groups using baseline-adjusted linear regression. All statistical analyses were carried out with or R v3.2.4 [R Development Core Team 2015].[20] The statistical analyses were conducted according to a pre-specified Statistical Analysis Plan (SAP), which was authored by the Trial Statistician and agreed by the Trial Steering Committee. The SAP was approved, and all statistical analysis programs were written and validated prior to database lock, at which point the randomized treatment allocations were released.

Post hoc analysis

A multiple imputation analysis was run as part of a post hoc analysis, using 10 imputed datasets. Two predictive models were used, one to predict the presence of MVO and one to predict the extent, if there was any. For the presence of MVO a logistic regression model was used with age, sex, location of MI, and time from symptom onset to reperfusion as predictors; for the extent of MVO (where present), a linear model for the log of MVO extent was used, using the same set of predictors. From these models, the presence of MVO and the extent (if present) was randomly imputed for patients with missing MVO data at 2-7 days, this was repeated 10 times. The primary analysis was then ran using the imputed datasets, and the results combined using Rubin’s rules.

Role of the funding source

The trial was funded by the Efficacy and Mechanism Evaluation (EME) program of the National Institute for Health Research (NIHR-EME). Boehringer Ingelheim U.K. Ltd. provided the study drugs including alteplase (10 mg, 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 this manuscript. The Chief Investigator had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Supplementary Results

Clinical events

Bleeding events on the day of the procedure

One patient who presented with acute inferior STEMI experienced recurrent intra-cranial bleeding and died 8 days after hospital admission. The final diagnosis was infective emboli to the right coronary artery and cerebral circulation secondary to gram-positive cocci endocarditis of the mitral valve. In addition to standard anti-thrombotic therapy with ticagrelor, this patient received 20 mg of intra-coronary alteplase. One other patient who received 10 mg of alteplase experienced a fatal myocardial rupture. This patient had Q-waves on the initial ECG and a history of chest infection treated with oral steroids.

Supplementary Discussion

Overview of current clinical trials

There is a widespread, growing interest in the potential efficacy of adjunctive intra-coronary fibrinolytic therapy during primary PCI. In our trial, the intervention was scheduled to occur at an early stage during primary PCI. The rationale was to infuse intra-coronary fibrinolytic therapy upstream of the culprit lesion when thrombus burden was greatest and fibrinolytic therapy might be most effective. Intra-coronary fibrinolytic therapy was infused before stent implantation in order to reduce mechanical distal embolization of thrombus. An alternative approach proposed by Sezer *et al* [1] was to administer adjunctive lytic therapy at the end of primary PCI. At this point, antegrade coronary flow is much improved compared to at the start of the procedure implying the potential for improved delivery of fibrinolytic therapy to the microcirculation. Based on the new knowledge from T-TIME, future trials should schedule adjunctive intra-coronary anti-thrombotic therapy for the end of the PCI.

Three trials are currently investigating this strategy (OPTIMAL NCT02894138; ACTRN12618000778280; STRIVE, NCT03335839). Two international, randomized, controlled phase 3 trials are investigating the efficacy of reduced doses of either alteplase or tenecteplase. The Adjunctive, Low-dose tPA in primary PCI for STEMI trial (STRIVE, NCT03335839) will enroll patients with acute STEMI that present <6 hours of symptom onset with a large thrombus burden at angiography (TIMI thrombus grade >3). The intervention involves open-label intra-coronary administration of alteplase (10 mg, 20 mg) or placebo during primary PCI, the primary composite outcome was major adverse cardiac events at 180 days although this was changed to a composite endpoint of post procedural myocardial blush grade 0/1 or distal embolization on the October 25, 2018. The Restoring

Microcirculatory Perfusion in STEMI (RESTORE-MI; ACTRN12618000778280) trial will enroll 1666 patients undergoing primary PCI <12 hours of symptom onset. In this double-blind trial, the efficacy and safety of intra-coronary administration of tenecteplase (one-third of the weight-adjusted systemic dose) will be compared with placebo in reperfused STEMI patients with evidence of microvascular dysfunction reflected by an index of micro-circulatory resistance (IMR) value > 32 at the end of PCI. The primary outcome is cardiovascular mortality at 24 months. Finally, the Optimal Coronary Flow After PCI for Myocardial Infarction (OPTIMAL) study is a single center pilot. The intervention involves selection of patients with an increased index of microvascular resistance (IMR >30) in the infarct-related artery at the end of PCI to receive an intra-coronary infusion (10 ml) of either 20 mg of alteplase or sodium chloride. The primary outcome is the ratio of myocardial infarct size to area at risk assessed by MRI early after enrolment (day 2-6) to assess the area at risk and again at 3 months to assess final infarct size. The overall sample size is 90 patients (n=40 per group), including 10 patients assigned to an observational group with no intervention.

The new knowledge from T-TIME is relevant to the design of these trials.

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eTable 1. Baseline Hematology and Coagulation Measured in 361 Patients

Baseline values	**Treatment received**		
	Placebo	10mg Alteplase	20mg Alteplase
Coagulation			
Fibrinogen (mg/dL) at baseline	339 (280, 390)	331 (260, 390)	327 (270, 380)
Plasminogen (U/dL) at baseline	93 (14)	93 (14)	94 (14)
Fibrin D-dimer (μg/mL) at baseline	0.09 (0.07, 0.16)	0.11 (0.07, 0.17)	0.10 (0.07, 0.16)
Prothrombin F1+2 (pmol/L) at baseline	164 (120, 220)	156 (122, 234)	156 (115, 242)
tPA (ng/ml) at baseline	10 (7.5, 12)	10 (8, 12)	9 (7.5, 12)

Footnote: All outcomes were pre-specified. tPA = Tissue plasminogen activator. Baseline Hematology and coagulation data presented based on treatment received.

325 **eTable 2. Procedure Characteristics and Outcomes (All Randomized Patients)**

	Randomly assigned, n (%)*		
Characteristics	Placebo (n = 151)**	Alteplase, 10 mg (n = 144)**	Alteplase, 20 mg (n = 145)**
Infarct-related artery			
Left anterior descending coronary	67 (44.4)	65 (45.1)	65 (44.8)
Circumflex	20 (13.2)	18 (12.5)	18(12.4)
Right	64 (42.4)	61 (42.4)	62 (42.8)
Infarct artery diameter, mm mean (SD)	3.2 (0.4)	3.3 (0.5)	3.2 (0.4)
Mode of reperfusion			
Aspiration thrombectomy	39/151 (25.8)	44/143 (30.8)	42/145 (29.0)
Balloon angioplasty	112/151 (74.2)	99/143 (69.2)	102/145 (70.3)
Primary stent	0/151 (0.0)	0/143 (0.0)	1/145 (0.7)
Balloon angioplasty pre-stent deployment	143 (94.7)	138 (95.8)	136 (93.8)
PCI with stent implantation	149/151 (98.7)	141/143 (98.6)	143/144 (99.3)
Total number of stents deployed			
0	2 (1.3)	2 (1.4)	1 (0.7)
1	102 (67.5)	98 (68.1)	106 (73.1)
2	43 (28.5)	30 (20.8)	33 (22.8)
≥3	4 (2.6)	14 (9.7)	5 (3.4)
Total length of stents deployed, mm mean (SD) [n=435]	33.4 (13.5)	35.0 (15.1)	32.3 (14.6)
Post-stent dilatation	129 (85.4)	124 (86.1)	125 (86.2)
TIMI flow grade at initial angiography‡			
0 (No flow)	130 (86.1)	113 (78.5)	111 (76.6)

	Randomly assigned, n (%)*		
Characteristics	Placebo (n = 151)**	Alteplase, 10 mg (n = 144)**	Alteplase, 20 mg (n = 145)**
1 (Minimal flow)	3 (2.0)	14 (9.7)	15 (10.3)
2/3 (2= Slow but complete, 3=Normal flow)	18 (11.9)	17 (11.8)	19 (13.1)
TIMI thrombus grade at initial angiography§			
0-2 (0= No thrombus, 2= definite, <1/2 vessel diameter)	0 (0.0)	0 (0.0)	0 (0.0)
3 (definite, >1/2 but <2 diameters)	4 (2.6)	2 (1.4)	5 (3.4)
4 (Definite thrombus ≥ 2 vessel diameters)	18 (11.9)	28 (19.4)	29 (20.0)
5 (Total Occlusion)	129 (85.4)	114 (79.2)	111 (76.6)
Acute therapy following the first medical contact			
Aspirin	130 (86.1)	128 (88.9)	125 (86.2)
Loading dose of aspirin, mg			
300 mg	124/130 (95.4)	123/128 (96.1%)	121/125 (96.8)
>300 mg	6/130 (4.6)	5/128 (3.9)	4/125 (3.2)
Additional anti-platelet medication			
None	18 (11.9)	14 (9.7)	18 (12.4)
Clopidogrel	46 (30.5)	49 (34.0)	51 (35.2)
Ticagrelor	84 (55.6)	76 (52.8)	70 (48.3)
Prasugrel	3 (2.0)	5 (3.5)	6 (4.1)
Unfractionated heparin (U), median (IQR)	10,000 (7000, 12,250)	10,000 (7500, 13,000)	10,000 (7000, 13,000)
Inhaled oxygen	23/148 (15.5)	25/140 (17.9)	16/140 (11.4)
Intravenous morphine	105 (69.5)	109 (75.7)	112 (77.2)
Intravenous or intracoronary glycoprotein IIb/IIIa antagonist	17/148 (11.5)	31/140 (22.1)	25/140 (17.9)
Study drug treatment			

	Randomly assigned, n (%)*		
Characteristics	Placebo (n = 151)**	Alteplase, 10 mg (n = 144)**	Alteplase, 20 mg (n = 145)**
Drug administered	150 (99.3)	141 (97.9)	144 (99.3)
Study drug given according to protocol	149/150 (99.3)	139/141 (98.6)	141/144 (97.9)
Duration of study drug infusion, min, mean (SD)	6.4 (1.9)	6.6 (2.0)	6.6 (2.0)

Footnote: * Unless otherwise noted. **Unless otherwise stated. ‡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 revealed during coronary angiography. The angiographic parameters are based on central laboratory assessments. None of the patients received intravenous or intracoronary treatment with bivalirudin, metoprolol, nicorandil or sodium nitroprusside. IQR = interquartile range; PCI = percutaneous coronary intervention; TIMI = Thrombolysis in Myocardial Infarction grade.

eTable 3. Prespecified Analysis of Primary Outcome for Alteplase (10-mg and 20-mg Dose Combined) vs Placebo

Outcome	Alteplase vs Placebo p-value	Alteplase vs Placebo Effect estimates & 95% CI	Interaction p-value
Secondary analysis of primary outcome, microvascular obstruction, 2-7 days	0.28[a]	0.15 (-0.12, 0.42)	
Pre-specified analysis of primary outcome by characteristics at baseline			
Ischemic time			
<2 hours	0.59[a]	0.16 (-0.42, 0.74)	0.06[b]
2-4 hours	0.84[a]	-0.04 (-0.40, 0.32)	
≥ 4 hours	0.009[a]	0.81 (0.21, 1.42)	
Sex			
Male	0.13[a]	0.23 (-0.07, 0.52)	0.17[b]
Female	0.39[a]	-0.31 (-1.02, 0.40)	
Age			
<55 years	0.25[a]	0.29 (-0.21, 0.79)	0.65[b]
55-65 years	0.98[a]	-0.01 (-0.45, 0.44)	
≥ 65 years	0.40[a]	0.21 (-0.29, 0.71)	

Outcome	Alteplase vs Placebo p-value	Alteplase vs Placebo Effect estimates & 95% CI	Interaction p-value
MI location			
Anterior	0.66[a]	0.09 (-0.32, 0.50)	0.70[b]
Non-anterior	0.29[a]	0.20 (-0.17, 0.57)	
Smoking status			
Never	0.80[a]	0.06 (-0.41, 0.53)	0.57[b]
Former	0.15[a]	0.46 (-0.17, 1.10)	
Current	0.61[a]	0.10 (-0.29, 0.50)	
Initial TIMI Coronary flow grade			
0	0.37[a]	0.14 (-0.16, 0.43)	0.45[b]
1	0.15[a]	1.13 (-0.41, 2.67)	
2+	0.87[a]	0.06 (-0.71, 0.84)	
Pre-existing antiplatelet medication			
Yes	0.96[a]	-0.02 (-0.71, 0.68)	0.61[b]
No	0.24[a]	0.18 (-0.12, 0.48)	

Footnote:[a] between-group comparison p-values derived from linear regression model. [b] Interaction test p-values reported from regression models with treatment included as a 3-level categorical variable. All outcomes were pre-specified. MI = myocardial infarction; TIMI = Thrombolysis in Myocardial Infarction grade. Effect estimates reported as mean differences.

334 **eTable 4. Secondary Outcomes for Efficacy and Health Outcomes for Alteplase (10-mg or 20-mg Dose) vs Placebo**

Outcome	Alteplase v Placebo p-value	Alteplase v Placebo Effect estimates & 95% CI
Coronary angiogram		
TIMI flow grade after PCI		
0 - 3	0.32[a]	0.77 (0.47, 1.29)
TIMI myocardial perfusion grade after PCI‡		
0 - 3	0.26[a]	0.81 (0.57, 1.17)
Corrected TIMI frame count after PCI§	0.17[b]	1.08 (0.97, 1.22)
TIMI thrombus grade after PCI		
0 - 5	0.53[c]	1.45 (0.45, 4.63)
Electrocardiogram (acute)		
ST-segment resolution at 60 min, %	0.23[b]	-5.4 (-14.2, 3.5)
Biochemistry (acute)		
Troponin T area-under-the-curve	0.002[b]	1.53 (1.16, 2.01)
NT-proBNP, pg/ml, 2-7 days	0.59[b]	1.06 (0.86, 1.30)
Cardiac MRI (2-7 days)		
Microvascular obstruction, n (%)	0.77[c]	1.06 (0.70, 1.62)
Myocardial hemorrhage, n (%)	0.54[c]	1.15 (0.74, 1.77)
Myocardial hemorrhage, % left ventricular mass	0.18[b]	0.62 (-0.28, 1.52)
Infarct size, % left ventricular mass	0.68[b]	0.52 (-1.96, 3.00)
Extent of myocardial oedema, % left ventricular mass	0.40[b]	0.81 (-1.06, 2.67)
Myocardial salvage index	0.61[b]	-0.01 (-0.06, 0.04)

Outcome	Alteplase v Placebo p-value	Alteplase v Placebo Effect estimates & 95% CI
Left ventricular end-diastolic volume, ml	0.21[b]	1.03 (0.98, 1.08)
Left ventricular end-systolic volume, ml	0.20[b]	1.04 (0.98, 1.11)
Left ventricular ejection fraction, %	0.54[b]	-0.51(-2.15, 1.13)
Electrocardiogram (3months)		
Final infarct size (Selvester score)†	0.10[b]	1.69 (-0.33, 3.72)
Biochemistry (3 months)		
NT-proBNP, pg/ml	0.89[b]	0.98 (0.79, 1.23)
Change in NT-proBNP at 3 months from day 2 – 7, pg/ml	0.91[b]	16.30 (-263.50, 296.10)
Cardiac MRI (3months)		
Infarct size, % left ventricular mass	0.81[b]	0.30 (-2.13, 2.73)
Myocardial salvage index‡‡	0.89[b]	0.00 (-0.05, 0.05)
Left ventricular end-diastolic volume, ml	0.34[b]	1.03 (0.97, 1.08)
Left ventricular end-systolic volume, ml	0.20[b]	1.05 (0.97, 1.14)
Left ventricular ejection fraction, %	0.16[b]	-1.28 (-3.06, 0.51)
Health status and quality of life		
Health-related quality of life: EQ-5D†† health utility score	0.85[b]	0.003 (-0.03, 0.04)
Change from baseline health status at 3 months	0.83[b]	0.005 (-0.04, 0.05)
Health-related quality of life: EQ-5D†† visual analog score	0.09[b]	2.91 (-0.40, 6.22)
Change from baseline health status at 3 months	0.38[b]	1.73 (-2.12, 5.59)

Footnote: [a] between-group comparison p-values derived from proportional odds logistic regression model. ‡TIMI myocardial perfusion grade provides a score for ground-glass appearance "blush" of contrast entering the microvasculature and contrast washout. §Corrected TIMI frame count is an objective continuous variable index of coronary blood flow, representing the time (in cine frames) for contrast to reach a standardized landmark, corrected for vessel length (normal value = <27 frames).[b] between-group comparison p-values derived from linear regression model. [c] between-group comparison p-values derived from a logistic regression model. †Selvester score translates subtle

changes in ventricular depolarization on the electrocardiogram to a surrogate measure of infarct size, there is a maximum score of 32 points with one point corresponding to 3% of the left ventricle. ‡‡Myocardial salvage index is calculated by subtracting the infarct size from the extent of myocardial edema (represents jeopardized myocardium) and then indexing by dividing by the extent of myocardial edema, values range from 0 (no salvage) to 1 (complete salvage). ††EQ-5D is a standardized instrument used as a measure of health outcome, made up of two components 1) The health utility score, a descriptive system comprised of five dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression, scores for each are combined to give a maximum value of 1. 2)The visual analogue scale reports the patient's self-rated health on a visual analogue scale from 0 (worst imaginable) to 100 (best health imaginable). Baseline health status was assessed using the EQ-5D which was completed by patients at the time of their 2-7 day follow up visit.

eTable 5. Secondary Outcomes for Safety

Outcome	Treatment group[a]			Between-group comparison p-value[b]		
	Placebo (n=149)	Alteplase, 10 mg (n=140)	Alteplase, 20 mg (n=146)	20mg v Placebo	10mg v Placebo	Alteplase v Placebo
Coronary angiogram						
No-reflow, n (%)	26 (17.4)	19 (13.6)	18 (12.3)	0.88	0.48	0.63
Slow-reflow, n (%)	6 (4.0)	7 (5.0)	14 (9.6)			
Normal flow, n (%)	117 (78.5)	114 (81.4)	114 (78.1)			
Hematology and coagulation						
Activated clotting time (s) at 2 hours	256 (218, 300)	275 (229, 350)	250 (219, 310)	0.67	0.05	0.37
Ratio of fibrinogen (mg/dL) at 2 hours from baseline	1 (0.95, 1.1)	1 (0.92, 1.12)	1 (0.91, 1.08)	0.09	0.68	0.22
Change in plasminogen (U/dL) at 2 hours from baseline	1.7 (7.5)	-3.6 (8.9)	-9.7 (9.9)	<0.001	<0.001	<0.001
Ratio of fibrin D-dimer (ng/mL) at 2 hours from baseline	1.08 (0.95, 1.34)	3.37 (2.23, 4.79)	4.56 (2.91, 7.36)	<0.001	<0.001	<0.001
Ratio of prothrombin F1+2 (pmol/L) at 2 hours from baseline	1.05 (0.89, 1.34)	1.21 (1.03, 1.52)	1.27 (1.06, 1.57)	0.002	<0.001	<0.001
Ratio of tPA (ng/ml) at 2 hours from baseline	1.1 (1.0, 1.3)	1.3 (1.2, 1.7)	1.5 (1.3, 2.0)	<0.001	0.05	0.002
Hemoglobin (g/L), 24 hours	143.1 (11.3)	141.8 (12.9)	143.3 (13.5)	0.95	0.40	0.66
Change in hemoglobin (g/L) at 24 hours from baseline	-1.8 (9.5)	-3.9 (9.2)	-3.6 (10.0)	0.13	0.08	0.06

Footnote: All outcomes were pre-specified. Data summarized as mean±SD or median (interquartile range) for normal and non-normally distributed data respectively. [a]Treatment groups are as treated for safety measures. [b] between-group comparison p-values derived from linear, binary logistic, or proportional odds logistic regression model. Activated clotting time results were available in 364 patients. Repeated measurements were available in 351 patients. Fibrinogen, plasminogen, fibrin D-dimers, prothrombin F1+2, and tissue plasminogen activator were available in 385 patients at baseline and in 357 patients at 2 hours. Hemoglobin results were available in 422 patients at baseline and in 374 patients at 24 hours. tPA = Tissue plasminogen activator.

eTable 6. Secondary Outcomes for Safety With Effect Estimates and 95% Confidence Intervals

Outcome	**Effect Estimates and 95 % Confidence Intervals**		
	20mg v Placebo	10mg v Placebo	Alteplase v Placebo
Coronary angiogram			
No-reflow, slow-reflow, or normal flow			
No-reflow	1.04 (0.60, 1.81)	1.23 (0.69, 2.19)	1.13 (0.69, 1.83)
Slow-reflow			
Normal flow			
Hematology and Coagulation			
Activated clotting time (s) at 2 hours	1.0 (0.9, 1.1)	1.1 (1.0, 1.2)	1.0 (1.0, 1.1)
Ratio of fibrinogen (mg/dL) at 2 hours from baseline	0.97 (0.93, 1.01)	0.99 (0.95, 1.03)	0.98 (0.95, 1.01)
Change in plasminogen (U/dL) at 2 hours from baseline	-11.41 (-13.64, -9.19)	-5.34 (-7.60, -3.08)	-8.47 (-10.47, -6.47)
Ratio of fibrin D-dimer (ng/ml) at 2 hours from baseline	3.94 (3.36, 4.62)	3.11 (2.64, 3.65)	3.51 (3.06, 4.04)
Ratio of prothrombin F1+2 (pmol/L) at 2 hours from baseline	1.24 (1.08, 1.42)	1.29 (1.13, 1.48)	1.26 (1.13, 1.42)
Ratio of tPA (ng/ml) at 2 hours from baseline	1.35 (1.14, 1.60)	1.19 (1.00, 1.41)	1.27 (1.10, 1.47)
Hemoglobin (g/L), 24 hours	0.10 (-2.95, 3.15)	-1.31 (-4.38, 1.76)	-0.60 (-3.23, 2.04)
Change in hemoglobin (g/L) at 24 hours from baseline	-1.83 (-4.17, 0.51)	-2.11 (-4.45, 0.23)	-1.97 (-3.98, 0.04)

Footnote: All outcomes were pre-specified. tPA = Tissue plasminogen activator. Effect estimates reported as mean differences or relative differences.

eTable 7. Clinical Events

	Treatment group (as treated), n (%)			
Outcome	Placebo (n=149)	Alteplase 10mg (n=140)	Alteplase 20mg (n=146)	p-value
CV death, non-fatal MI, unplanned hospitalization for stroke or TIA (MACCE)	5 (3.4)	6 (4.3)	6 (4.1)	0.91
CV death, non-fatal MI or unplanned hospitalization for heart failure (MACE)	15 (10.1)	18 (12.9)	12 (8.2)	0.43
All-cause death	1 (0.7)	3 (2.1)	3 (2.1)	0.58
Cardiac death	1 (0.7)	3 (2.1)	2 (1.4)	0.45
Myocardial infarction associated with revascularization procedures (Type 4 or 5)	2 (1.3)	3 (2.1)	1 (0.7)	0.54
Fatal or non-fatal myocardial infarction	3 (2.0)	6 (4.3)	5 (3.4)	0.51
Stroke or transient ischemic attack	0 (0.0)	0 (0.0)	2 (1.4)	0.22
Heart failure requiring unplanned hospitalization	13 (8.7)	13 (9.3)	8 (5.5)	0.43
All-cause death or heart failure	14 (9.4)	15 (10.7)	11 (7.5)	0.64
All Bleeding				
Minor bleeds (BARC 1 – 2)	4 (2.6)	3 (2.1)	3 (2.1)	0.32
Major bleeds (BARC 3 – 5)	0 (0.0)	1 (0.7)	1 (0.7)	0.55
Bleeding on day of procedure				
Minor bleeds (BARC 1 – 2)	2 (1.3)	2 (1.4)	3 (2.1)	0.90
Major bleeds (BARC 3 – 5)	0 (0.0)	1 (0.7)	1 (0.7)	0.55

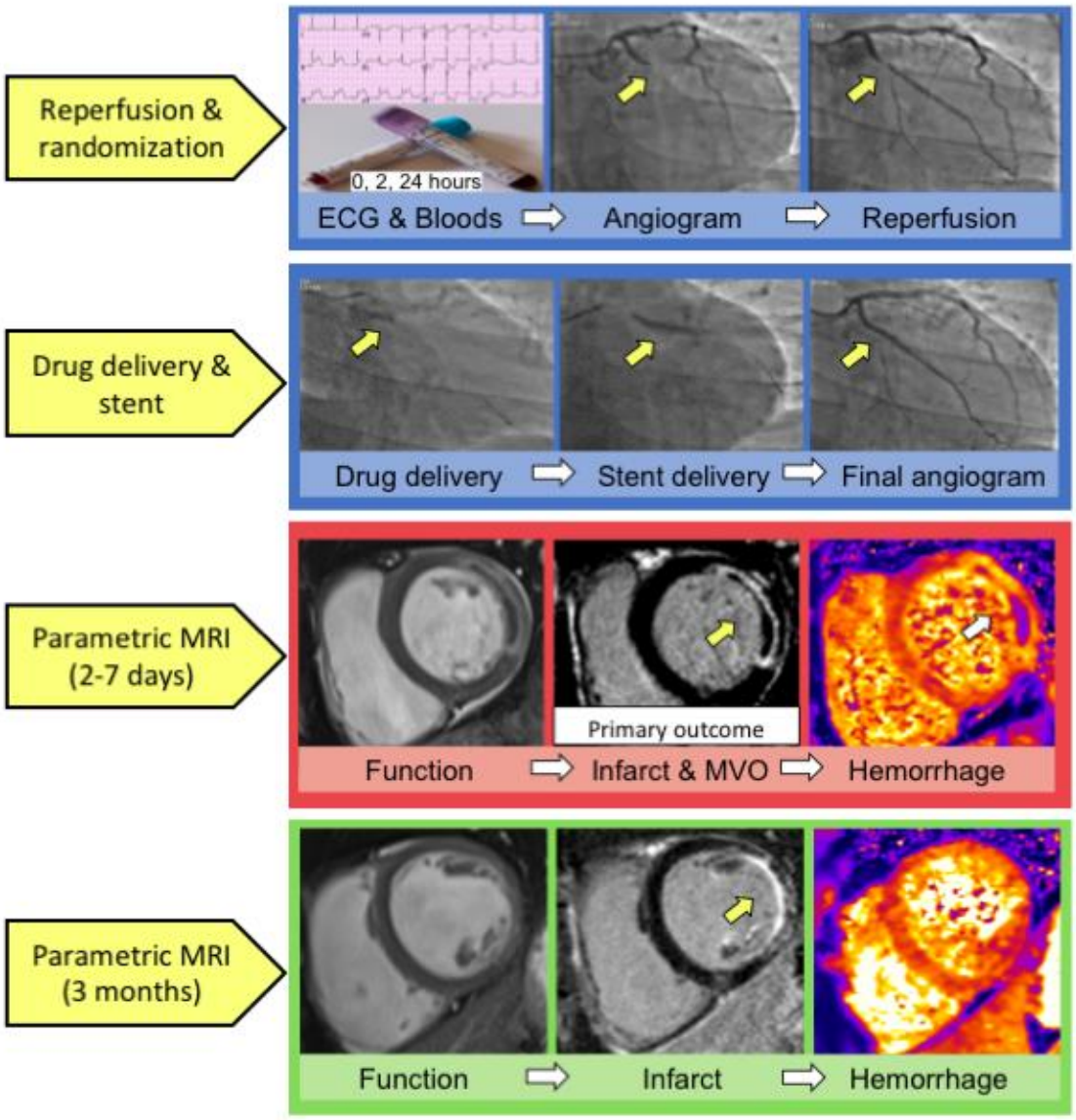
Footnote: Data reported as number (n) and percentage of treatment group (%). Treatment groups are as treated for clinical events. BARC = Bleeding Academic Research Consortium, CV = Cardiovascular, MACE = major adverse cardiac events, MACCE = Major adverse cardiac and cardiovascular events; MI = Myocardial infarction, TIA = Transient Ischemic attack.

eFigure 1. Graphical Layout of the Study Protocol

eFigure 2. Clinical Case Examples: Two patients with anterior STEMI. One patient had evidence of microvascular obstruction (red arrow) complicated by myocardial hemorrhage (yellow arrow) that persisted to 3 months. The other patient had neither of these pathologies and by 3 months, infarct size had reduced.

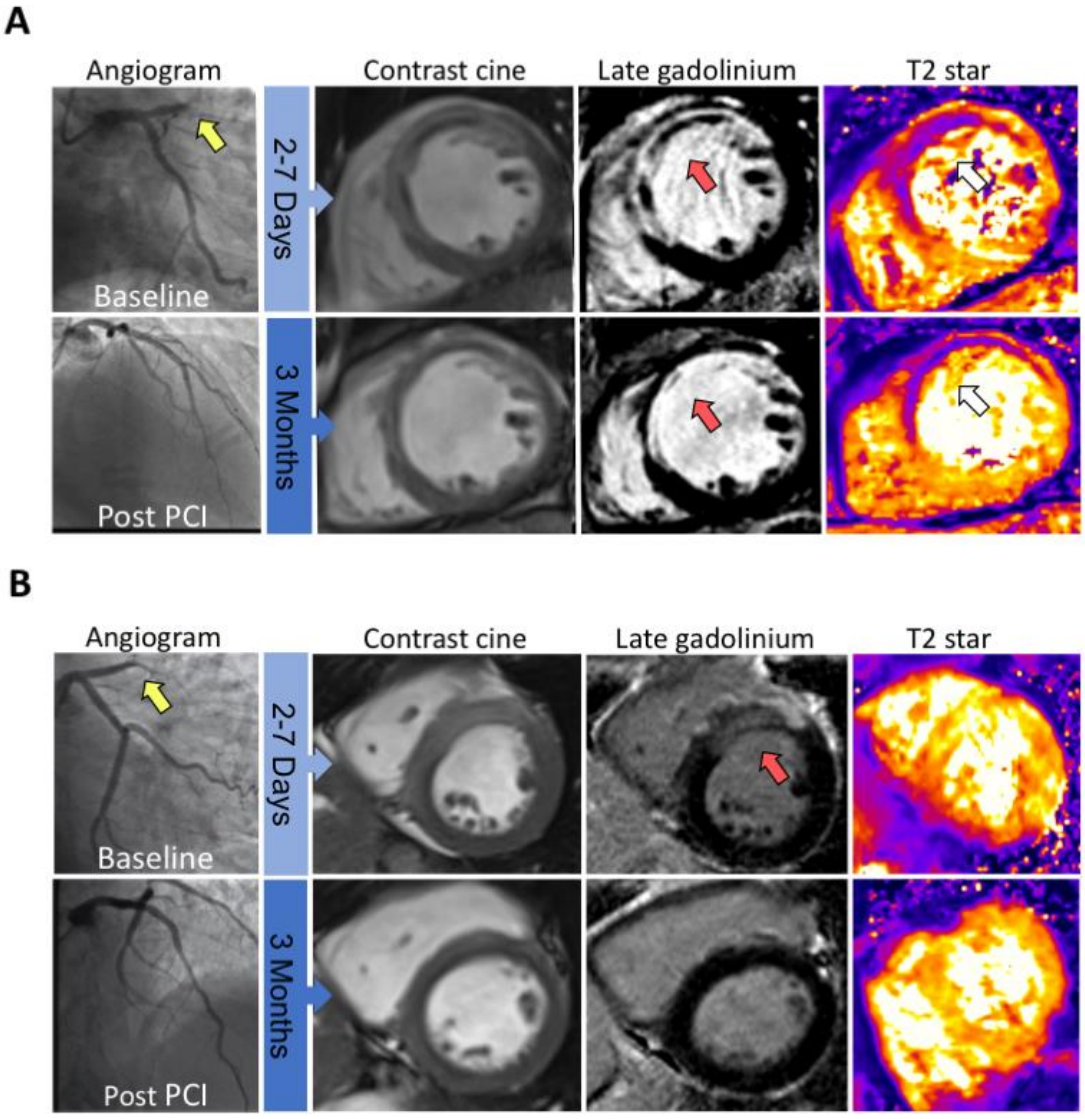
eFigure 3. Plot of troponin T AUC.

eFigure 1. Graphical layout of the trial protocol.



Patients presenting with acute ST-segment-elevation myocardial infarction meeting the study eligibility criteria were enrolled by research staff in the cardiac catheterization laboratory following reperfusion of the culprit artery. Blood tests were performed acutely (0, 2, 24 hours) and again at the time of multi-parametric Magnetic Resonance Imaging (MRI) at 2-7 days and 3 months. An Electrocardiogram was obtained prior to reperfusion (baseline), at 60 minutes and then again at 3 months. Parametric MRI, starting from left: cine imaging allows calculation of left ventricular function and volumes; Late gadolinium-enhanced MRI allows determination of infarct size (bright white area) and microvascular obstruction (hypointense, black core) within the infarct highlighted with the yellow arrow; T2*MRI, far right image revealed myocardial hemorrhage (white arrow) within the infarct core. MVO, microvascular obstruction.

eFigure 2. Clinical case examples.

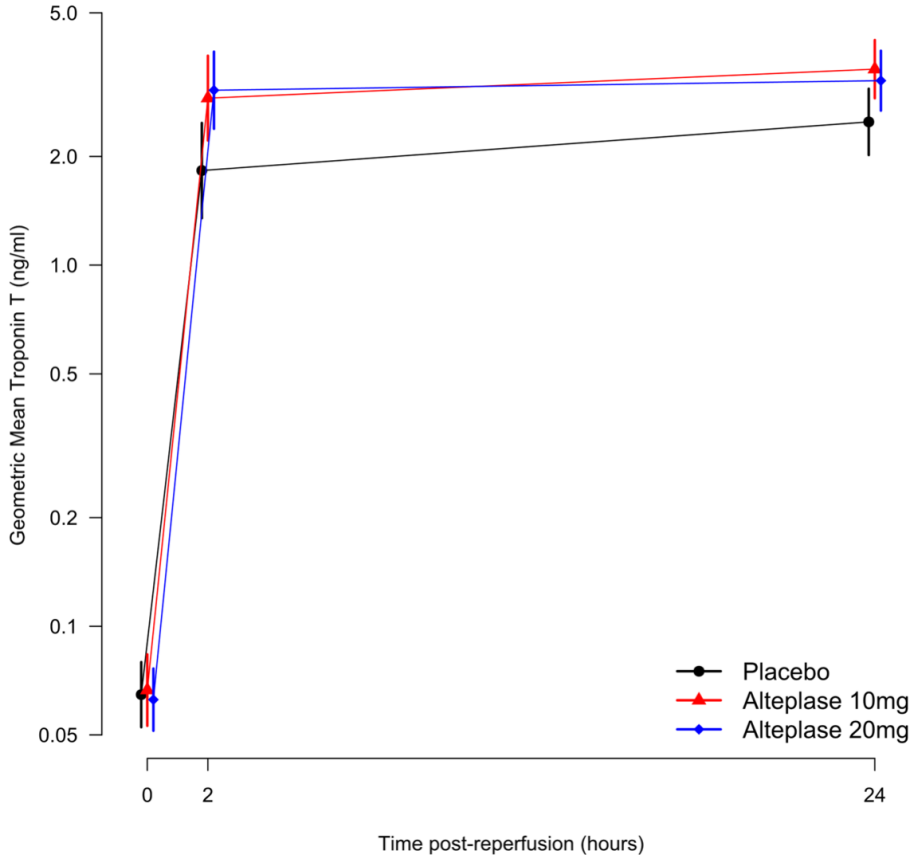


Two patients, both with acute anterior ST-segment-elevation myocardial infarction (STEMI) treated successfully with primary percutaneous coronary intervention (PCI) within 4 hours of symptom onset. Each patient had thrombolysis in myocardial infarction (TIMI) grade 3 flow at the end of PCI. Magnetic resonance imaging (MRI) was performed at 5, and 3 days post reperfusion respectively.

A: Patient with hemorrhagic infarction on MRI. Diagnostic angiogram (top far left image, yellow arrow) revealed an occluded left anterior descending (LAD) artery with TIMI 0 flow. T2*-MRI (top far right image) revealed myocardial hemorrhage (white arrow) within the infarct core. Late gadolinium-enhanced MRI revealed microvascular obstruction (top second from right image, red arrow) within the bright area of infarction. The microvascular obstruction within the infarct core spatially corresponded with the myocardial hemorrhage. There was evidence of persistent haemoglobin breakdown products (bottom far right image, white arrow), a reduction in ejection fraction, increased LV end-diastolic volumes and thinned myocardium in the infarct territory on follow up MRI at 3 months compared to baseline. This represents a case of failed myocardial reperfusion despite successful PCI.

B: Patient with an anterior infarct but no MRI evidence of reperfusion injury. Diagnostic angiography (top, far left image, yellow arrow) revealed an occluded LAD artery with TIMI 0 flow. Late gadolinium-enhanced MRI revealed an anterior infarct (top second from right image, red arrow) with no evidence of microvascular obstruction and no evidence of hemorrhagic transformation on T2*-MRI. There was an improved ejection fraction and a reduction in infarct mass on follow up MRI at 3 months. This represents a case of successful myocardial reperfusion.

eFigure 3. Plot of Troponin T AUC



Troponin T (ng/L) area-under-the-curve (AUC) was measured from blood samples obtained immediately before reperfusion (0 hours) and then again at 2 and 24 hours. Analyzed samples at all three time points were available in 317 patients.