SUPPLEMENTAL MATERIAL

Supplementary Methods

Temporal evolution of myocardial hemorrhage and edema in patients after acute ST-elevation myocardial infarction: pathophysiologic insights and clinical implications.

ClinicalTrials.gov registration NCT02072850

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Setting and study populations

STEMI patients

Screening, enrolment, and data collection were prospectively performed by cardiologists in the cardiac catheterization laboratories of the Golden Jubilee National Hospital, Glasgow, United Kingdom. This hospital is a regional referral centre for primary and rescue percutaneous coronary intervention (PCI). The hospital provides clinical services for a population of 2.2 million. A screening log was recorded, including patients who did not participate in the cohort study.

Healthy volunteers

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

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

The rationale for including healthy volunteers in this study is as follows. First, native T2/ T2* values may vary between CMR scanners and so a local reference range for native T2/ T2* is recommended in CMR guidelines [1]. Second, native T2/ T2* may vary spatially in the heart and therefore. Myocardial native T2/ T2* values were regionally segmented in regions-of-interest and summarised according to the AHA model [2].

Coronary angiogram acquisition and analyses

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

Percutaneous coronary intervention

Consecutive admissions with acute ST-elevation myocardial infarction (STEM) referred for emergency percutaneous coronary intervention (PCI) were screened for the inclusion and exclusion criteria. During ambulance transfer to the hospital, the patients received 300 mg of aspirin, 600 mg of clopidogrel and 5000 IU of unfractionated heparin [3, 4]. The initial primary PCI procedure was performed using radial artery access. A conventional approach to primary PCI was adopted in line with usual care in our hospital [3, 4]. Conventional bare metal and drug eluting stents were used in line with guideline recommendations and clinical judgement. The standard transcatheter approach for reperfusion involves minimal intervention with aspiration thrombectomy only or minimal balloon angioplasty (e.g. a compliant balloon sized according to the reference vessel diameter and inflated at 4-6 atmospheres 1-2 times). During PCI, glycoprotein IIbIIIa inhibitor therapy was initiated with high dose tirofiban (25 μg/kg/bolus)

followed by an intravenous infusion of 0.15 μg/kg/min for 12 hours, according to clinical judgement and indications for bail-out therapy [3, 4]. No reflow was treated according to contemporary standards of care with intra-coronary nitrate (i.e. 200 μg) and adenosine (i.e. 30 – 60 μg) [3, 4], as clinically appropriate. In patients with multivessel coronary disease, multivessel PCI was not recommended, in line with clinical guidelines [3, 4]. The subsequent management of these patients was symptom-guided.

Angiographic analysis

The coronary anatomy and disease characteristics of study participants were described based on the clinical reports of the attending cardiologist.

Outcome definitions

Coronary blood flow can be described based on the visual assessment of coronary blood flow revealed by contrast injection into the coronary arteries [3, 4].

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

CMR acquisition and analyses

CMR acquisition

CMR was performed on a Siemens MAGNETOM Avanto (Erlangen, Germany) 1.5-Tesla scanner with a 12-element phased array cardiac surface coil.

The cine-CMR for LV mass and function was collected before contrast administration. Cine-CMR involved a short axis LV stack and at least one long axis view (vertical long axis, horizontal long axis and / or 3 chamber view).

Steady-state free precession (SSFP) cine breath-hold sequences (with parallel imaging acceleration) was used. The heart was imaged in multiple parallel short-axis (SAX) planes 7-mm thick separated by 3-mm gaps, equating to approximately 10 slices and 30 cardiac phases with the following imaging parameters**:** Voxel size 2.0x2.0x7.0mm, TR/TE 39.6/1.12 ms, flip angle 55^{o}, matrix 192x192, slice thickness 7 mm, 3 mm gap.

T2 maps were acquired in contiguous short axis slices covering the whole ventricle, using an investigational prototype T2-prepared (T2P) TrueFisp sequence [5, 6]. Typical imaging parameters were: bandwidth ~947 Hz/pixel; flip angle 70°; T2 preparations: 0 ms, 24 ms, and 55 ms respectively; matrix 160 x 105 pixels; spatial resolution 2.6 x 2.1 x 8.0 mm; slice thickness 8 mm.

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

The MOLLI T1 cardiac-gated acquisition involved three inversion-recovery prepared look locker experiments combined within one protocol (3 (3) 3 (3) 5) [28].. The CMR parameters

were: bandwidth ~1090 Hz/pixel; flip angle 35°; echo time (TE) 1.1 ms; T1 of first experiment 100 ms; TI increment 80 ms; matrix 192 x 124 pixels; spatial resolution 2.2 x 1.8 x 8.0 mm; slice thickness 8 mm; scan time 17 heartbeats.

Late gadolinium enhancement images covering the entire LV were acquired 10-15 minutes after intravenous injection of 0.15 mmol/kg of gadoterate meglumine (Gd^{2+}-DOTA, Dotarem, Guebert S.A.) using segmented phase-sensitive inversion recovery (PSIR) turbo fast low-angle shot [7]. Microvascular obstruction was defined as a dark zone on early delayed enhancement imaging 1, 3, 5 and 7 minutes post-contrast injection and within an area of late gadolinium enhancement. Typical imaging parameters were: matrix = 192 x 256, flip angle = 25°, TE = 3.36 ms, bandwidth = 130 Hz/pixel, echo spacing = 8.7ms and trigger pulse = 2. The voxel size was 1.8 x 1.3 x 8 mm^3. Inversion times were individually adjusted to optimize nulling of apparently normal myocardium (typical values, 200 to 300 ms).

MR image analyses

The images were analysed on a Siemens work-station by observers with at least 3 years CMR experience (N.A., D.C., I.M, S.R.). All of the images were reviewed by experienced CMR cardiologists (C.B., N.T.). LV dimensions, volumes and ejection fraction were quantified using computer assisted planimetry (syngo MR®, Siemens Healthcare, Erlangen, Germany). All scan acquisitions were spatially co-registered.

T2 and T2 – standardized measurements in myocardial regions of interest*

LV contours were delineated with computer assisted planimetry on the raw T2* image and the last corresponding T2 raw image, with echo time of 55 ms [8]. Contours were then copied onto the color-encoded spatially co-registered maps and corrected when necessary by consulting the SSFP cine images. Apical segments were not included because of partial volume effects. Particular care was taken to delineate regions of interest with adequate

margins of separation from tissue interfaces prone to partial volume averaging such as between myocardium and blood. Each T2/ T2* map image was visually assessed for the presence of artifacts relating to susceptibility effects or cardio-respiratory motion. Each map was evaluated against the original images. When artifacts occurred, the affected segments were not included in the analysis.

T2/ T2* values were segmented spatially and regions of interest were defined as (1) remote myocardium, (2) injured myocardium and (3) infarct core. The regions-of-interest were planimetered to include the entire area of interest with distinct margins of separation from tissue interfaces to exclude partial volume averaging. The remote myocardial region-of-interest was defined as myocardium 180º from the affected zone with no visible evidence of infarction, edema or wall motion abnormalities (assessed by inspecting corresponding contrast enhanced T1-weighted, T2-weighted and cine images, respectively). The infarct zone region-of-interest was defined as myocardium with pixel values (T2) >2 SD from remote myocardium on T2-weighted CMR [5, 6]. The infarct core was defined as an area in the center of the infarct territory having a mean T2/ T2* value of at least 2 standard deviations (SDs) below the T2/ T2* value of the periphery of the area-at-risk.

In healthy volunteers, the mid-ventricular T2/T2* map was segmented into 6 equal segments, using the anterior right ventricular-LV insertion point as the reference point [2]. T2/T2* was measured in each of these segments, and regions-of-interest were planimetered distinct and separate from blood-pool and tissue interfaces. These segmental values were also averaged to provide one value per subject. Results are presented as average values for segments and slices.

Infarct definition and size

The presence of acute infarction was established based on abnormalities in cine wall motion, rest first-pass myocardial perfusion, and delayed-enhancement imaging. In addition,

supporting changes on the ECG and coronary angiogram were also required. Acute infarction was considered present only if late gadolinium enhancement was confirmed on both the axial and long axis acquisitions. The myocardial mass of late gadolinium (grams) was quantified using computer assisted planimetry and the territory of infarction was delineated using a signal intensity threshold of >5 standard deviations above a remote reference region and expressed as a percentage of total LV mass [9]. Infarct regions with evidence of microvascular obstruction were included within the infarct area and the area of microvascular obstruction was assessed separately and also expressed as a percentage of total LV mass.

Myocardial haemorrhage

Myocardial haemorrhage was scored visually. On the T2* maps, a region of reduced signal intensity within the infarcted area, with a T2* value of <20 ms [10-13], was considered to confirm the presence of myocardial haemorrhage.

Area-at-risk

Area-at-risk was defined as LV myocardium with pixel values (T1/T2) >2 standard deviations from remote myocardium [5, 6, 14-17]. In order to assess the area-at-risk the epicardial and endocardial contours on the last corresponding T2-weighted raw image with an echo time of 55 ms were planimetered [8]. Contours were then copied to the map and corrected when necessary by consulting the SSFP cine images.

Myocardial salvage

Myocardial salvage was calculated by subtraction of percent infarct size from percent area-at-risk [14, 17, 18]. The myocardial salvage index was calculated by dividing the myocardial salvage area by the initial area-at-risk.

Adverse remodelling

Adverse remodelling was defined as an increase in LV end-diastolic volume ≥ 20% at 6 months from baseline [19].

Reference ranges

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

Electrocardiogram

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

The extent of ST-segment resolution on the ECG assessed 60 minutes after reperfusion compared to the baseline ECG before reperfusion [3] was expressed as complete (≥70%), incomplete (30% to < 70%) or none (≤30%).

Biochemical and hematologic measurement of inflammation

Serial systemic blood sample were obtained immediately after reperfusion in the cardiac catheterization laboratory, and subsequently between 0600 - 0700 hrs each day during the

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

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

Hematologic measurement of inflammation

Leucocyte count and leucocyte sub-populations were measured as a hematologic measure of inflammation using sheath flow technology incorporating semi-conductor laser beam, forward and side scattered light (Sysmex XT200i and XT1800i for white blood cell and differential white blood cell counts, respectively). The linearity ranges for white blood cells was 0.00-440.0 x10(9) /L. The following are the normal ranges for full blood count parameters:

	MALE	**FEMALE**
WBC x 10^9/L	4.0 - 11.0	4.0 - 11.0

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

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

Statistics

Sample size calculation for serial imaging longitudinal study

The sample size of 30 was predetermined based on the incidence of infarct pathology (e.g. myocardial haemorrhage or microvascular obstruction) affecting at least one third of the cohort and quantitative data (e.g. T2 ms, edema area (%)) on 4 occasions in all subjects. In order to detect a minimal clinically significant correlation (r) of 0.6 between two continuous

variables then a sample size of 25 would be associated with 90% power and 5% significance (null hypothesis, r=0).The sample size aligns with the only other longitudinal CMR study in STEMI survivors with CMR performed on at least 3 occasions [20], and based on what might be feasible within the 3-year duration of this project.

Sample size calculation for the whole cohort

With an estimated haemorrhage incidence of 33% at 48 h post-STEMI, 100 subjects would have evidence of myocardial haemorrhage and 200 subjects would not. The study would have 90% power at a 5% level of significance using a two sided two sample t-test to detect a between-group difference in mean LV end-systolic volume index of 4.65 ml/m2 equivalent to three eighths of a common standard deviation (or an effect size of 0.375). We predicted a between-group difference in mean LVESVI of 4.65 ml/m2 equivalent to three eighths of a common standard deviation (or an effect size of 0.375). We also estimated that at least 30 major adverse cardiac events (MACE) would occur based on a conservative estimate of the event rate (10-12%) at 18 months. The sample size calculation was performed using nQuery version 7.0.

Statistical analysis

Categorical variables are expressed as number and percentage of patients. Most continuous variables followed a normal distribution and are therefore presented as means together with standard deviation. Those variables that did not follow a normal distribution are presented as medians with interquartile range. Differences between independent groups were assessed using t-tests, Mann-Whitney tests, or Fisher's tests where appropriate. Changes over time were assessed using generalized linear mixed effects models (LMEs) with time and haemorrhage as fixed effects, and subject ID as the only random effect. These models were fitted as full factorial models, with the interaction being removed only when not significant. Post-hoc multiple comparisons were performed with Tukey adjustment. Random

effects models were used to compute inter-and intra- rater reliability measures (intra-class correlation coefficient (ICC)) for the reliability of remote zone, infarct zone and infarct core T2 and T2* values measured independently by 2 observers in 20 randomly selected patients from the cohort. All statistical analyses were carried out using R v 2.15.1 or SAS v9.3, or later versions of these programs. A p-value > 0.05 indicates the absence of evidence for a statistically significant effect.

Trial Management

The study was conducted in line with Guidelines for Good Clinical Practice (GCP) in Clinical Trials. http://www.mrc.ac.uk/documents/pdf/good-clinical-practice-in-clinical-trials/

Trial management included a Trial Management Group, and an independent Clinical Trials Unit. Day to day study activity was coordinated by the Trial Management Group who was responsible to the Sponsor which was responsible for overall governance and that the trial was conducted according to GCP standards.

Clinical events were assessed and validated by an independent cardiologist (A.M.) who had access to relevant source clinical data. This cardiologist followed an agreed charter and he was blinded to all of the other clinical data.

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Supplementary Results

Table 1. Temporal changes in infarct zone end-diastolic wall thickness following acute reperfused STEMI.

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

Footnote: Data given as mean (standard deviation). The P-value were obtained from a linear mixed effects model to test for interactions of infarct zone end-diastolic wall thickness with hemorrhage and imaging time-point. Both groups of patients had a decrease in infarct wall thickness over time. On average, compared to patients without hemorrhage, this decrease was larger for those patients with hemorrhage.

Table 2.

(a) T2* (ms) within the *infarct zone* measured in the 6 patients who had evidence of intra-myocardial hemorrhage at day 3 but not 4 – 12 hrs post-MI.

		T2* infarct zone			
Subject	MR-MI number	4 – 12 hrs	Day 3	Day 10	7 months
1	1	24.3	23.9	26.0	27.4
2	4	36.3	33.3	32.5	32.6
3	21	29.6	28.4	26.7	31.6
4	24	Artefact	24.9	30.8	28.0
5	27	26.1	29.4	28.4	31.7
6	28	24.5	21.2	25.6	N/A*

(b) T2* (ms) within the *infarct core* measured in the 6 patients who had evidence of intra-myocardial hemorrhage 3, but not before.

		T2* infarct core			
Subject	MR-MI number	4 – 12 hrs	Day 3	Day 10	7 months
1	1	-	17.8	-	-
2	4	-	19.2	23.0	-
3	21	-	12.8	17.6	-
4	24	Artefact	13.6	16.5	-
5	27	-	19.4	21.5	24.9
6	28	-	10.7	12.8	N/A*

* imaging artefact preluded reliable measurement of T2* in this patient at 7 months.

Five patients had no evidence of a T2* hypointense core at 4-12 hours and subsequently developed T2* core on day 3. One patient's scan was not evaluable at 4-12 hours due to motion artefact, but there was evidence of T2* core on day 3, this has been updated in the manuscript text."

Table 3. Amount of myocardial hemorrhage (%LV mass) for the patients that had hemorrhage (T2* core) evident at that point in time.

	4 - 12 hours n=7	3 days n=13	10 days n=11	P-value*
Myocardial hemorrhage, %LV mass	5.5 (4.3, 6.2)	7.0 (4.9, 7.5)	4.2 (3.0, 5.6)	0.009

Table 4.

Response: LVEF P-value for interaction: <0.001

		Comparisons	Estimate, 95% CI	p-value
Effect of time	Hemorrhage	Time 1, Time 2		0.98870
		Time 1, Time 3		0.10026
		Time 1, Time 4		0.19967
		Time 2, Time 3		0.52739
		Time 2, Time 4		0.72684
		Time 3, Time 4		0.99999
	No hemorrhage	Time 1, Time 2		0.13294
		Time 1, Time 3	7.00 (2.03, 11.97)	<0.001
		Time 1, Time 4	8.52 (3.55, 13.49)	<0.001
		Time 2, Time 3		0.73273
		Time 2, Time 4		0.17458
		Time 3, Time 4		0.98193
Effect of hemorrhage	Time 1	Hemorrhage, no hemorrhage		0.91821
	Time 2	Hemorrhage, no hemorrhage		0.37353
	Time 3	Hemorrhage, no hemorrhage		0.58632
	Time 4	Hemorrhage, no hemorrhage		0.18031

Interpretation: There were no significant differences over time in LVEF in those patients with hemorrhage. Those without hemorrhage have LVEF significantly higher on average 10 days and 7 months post-MI compared to at 4 – 12 hrs post-MI.

Time 1 = 4 – 12 hrs
Time 2 = day 1
Time 3 = day 3
Time 4 = 7 months

LVEDV: p-value for interaction = 0.0222

		Comparisons	Estimate, 95% CI	p-value
Effect of time	Hemorrhage	Time 1, Time 2		0.9988
		Time 1, Time 3		0.5533
		Time 1, Time 4	15.78 (1.96, 20.61)	0.0133
		Time 2, Time 3		0.9011
		Time 2, Time 4	13.05 (-0.77, 26.87)	0.0789
		Time 3, Time 4		0.7406
	No hemorrhage	Time 1, Time 2		1.0000
		Time 1, Time 3		1.0000
		Time 1, Time 4		0.6920
		Time 2, Time 3		1.0000
		Time 2, Time 4		0.6215
		Time 3, Time 4		0.7233
Effect of hemorrhage	Time 1	Hemorrhage, no hemorrhage		1.0000
	Time 2	Hemorrhage, no hemorrhage		1.0000
	Time 3	Hemorrhage, no hemorrhage		0.9951
	Time 4	Hemorrhage, no hemorrhage		0.5119

Interpretation: There was no significant change over time in LVEDV among patients without hemorrhage. Those with hemorrhage had, on average, significantly higher LVEDV 7 months vs. 4 – 12 hrs post-MI.

AAR: p-value for interaction = <0.001 NOTE: Scans 1 – 3 only

		Comparisons	Estimate, 95% CI	p-value
Effect of time	Hemorrhage	Time 1, Time 2		0.22334
		Time 1, Time 3		0.82219
		Time 1, Time 4		-
		Time 2, Time 3	-8.51 (-15.86, -1.17)	0.01288
		Time 2, Time 4		-
		Time 3, Time 4		-
	No hemorrhage	Time 1, Time 2		0.39096
		Time 1, Time 3		0.74301
		Time 1, Time 4		-
		Time 2, Time 3	-7.07 (-13.12, -1.02)	0.01185
		Time 2, Time 4		-
		Time 3, Time 4		-
Effect of hemorrhage	Time 1	Hemorrhage, no hemorrhage		0.23057
	Time 2	Hemorrhage, no hemorrhage		0.10828
	Time 3	Hemorrhage, no hemorrhage		0.27253
	Time 4	Hemorrhage, no hemorrhage		-

Interpretation: All patients had a significant decrease in AAR between CMR at 3 and 10 days post-MI. This decrease is larger for patients with hemorrhage compared to in patients without hemorrhage.

Infarct size: p-value for interaction = <0.001

		Comparisons	Estimate, 95% CI	p-value
Effect of time	Hemorrhage	Time 1, Time 2		0.83847
		Time 1, Time 3	-5.75 (-9.22, -2.27)	<0.001
		Time 1, Time 4	-7.29 (-10.68, -3.91)	<0.001
		Time 2, Time 3	-7.32 (-10.80, -3.85)	<0.001
		Time 2, Time 4	-8.87 (-12.26, -5.49)	<0.001
		Time 3, Time 4		0.86934
	No hemorrhage	Time 1, Time 2		1.00000
		Time 1, Time 3	-2.94 (-5.89, 0.02)	0.05332
		Time 1, Time 4	-3.86 (-6.88, -0.84)	0.00336
		Time 2, Time 3	-3.16 (-6.12, -0.21)	0.02719
		Time 2, Time 4	-4.09 (-7.10, -1.07)	0.00141
		Time 3, Time 4		0.98182
Effect of hemorrhage	Time 1	Hemorrhage, no hemorrhage	17.24 (8.12, 26.36)	<0.001
	Time 2	Hemorrhage, no hemorrhage	18.59 (9.47, 27.71)	<0.001
	Time 3	Hemorrhage, no hemorrhage	14.43 (5.28, 23.59)	<0.001
	Time 4	Hemorrhage, no hemorrhage	13.81 (4.67, 22.94)	<0.001

Interpretation: In both groups of patients, infarct size decreased over time. This decrease was smaller in the patients without hemorrhage patients compared to in those patients with hemorrhage. This may be explained in part due to hemorrhage patients having a significantly larger infarct size, on average, than non-hemorrhage patients at each scan. This difference is smaller by CMR at 7 months than at CMR done 4-12 hrs post-MI.

Salvage: p-value for interaction = <0.0001

		Comparisons	Estimate, 95% CI	p-value
Effect of time	Hemorrhage	Time 1, Time 2		0.69677
		Time 1, Time 3		0.91272
		Time 1, Time 4	12.68 (5.75, 19.61)	<0.001
		Time 2, Time 3		0.99998
		Time 2, Time 4	8.87 (1.94, 15.80)	0.00297
		Time 3, Time 4	9.67 (2.35, 17.00)	0.00179
	No hemorrhage	Time 1, Time 2		0.42992
		Time 1, Time 3		1.00000
		Time 1, Time 4	8.51 (2.21, 14.81)	0.00124
		Time 2, Time 3		0.50022
		Time 2, Time 4		0.39163
		Time 3, Time 4	8.22 (2.04, 14.40)	0.00171
Effect of hemorrhage	Time 1	Hemorrhage, no hemorrhage		0.35680
	Time 2	Hemorrhage, no hemorrhage		0.29278
	Time 3	Hemorrhage, no hemorrhage		0.81881
	Time 4	Hemorrhage, no hemorrhage		0.95104

Interpretation: In patients with hemorrhage, myocardial salvage consistently increased from CMR 3 days post-MI onwards. In patients without hemorrhage, salvage increased mainly between 10 days and 7 months, and this increase was, on average, smaller than that observed in patients with hemorrhage.

Myocardial hemorrhage (LV mass): Only effect of time

Comparisons	Estimate, 95% CI	Estimate, 95% CI
Time 1, Time 2	1.47 (0.36, 2.59)	0.00406
Time 1, Time 3		0.71110
Time 1, Time 4		0.42747
Time 2, Time 3	-1.01 (-2.13, 0.11)	0.09331
Time 2, Time 4	-2.13 (-3.25, -1.02)	<0.001
Time 3, Time 4	-1.12 (-2.24, -0.01)	0.04834

Interpretation: On average, hemorrhage increased between CMR at 4 -12 hrs vs 3 days, and then reduced progressively afterwards.

T2* infarct ms: p-value for interaction = <0.001

Effect of time	Hemorrhage	Time 1, Time 2		0.34738
		Time 1, Time 3		0.99990
		Time 1, Time 4		0.99959
		Time 2, Time 3		0.63120
		Time 2, Time 4		0.73585
		Time 3, Time 4		1.00000
	No hemorrhage	Time 1, Time 2		0.66928
		Time 1, Time 3		0.99686
		Time 1, Time 4	-5.06 (-8.62, -1.51)	<0.001
		Time 2, Time 3		0.21676
		Time 2, Time 4	-7.00 (-10.61, -3.40)	<0.001
		Time 3, Time 4	-4.27 (-7.88, -0.67)	0.00813
Effect of hemorrhage	Time 1	Hemorrhage, no hemorrhage	-8.44 (-13.08, -3.81)	<0.001
	Time 2	Hemorrhage, no hemorrhage	-12.97 (-17.54, -8.40)	<0.001
	Time 3	Hemorrhage, no hemorrhage	-8.16 (-12.79, -3.52)	<0.001
	Time 4	Hemorrhage, no hemorrhage		0.18641

Interpretation: T2* (ms) within the infarct zone did not change over time in patients with hemorrhage. When compared with day 3 vs. day 1 patients without hemorrhage had significantly higher T2* than those with hemorrhage. T2* (ms) decreased over time in patients without hemorrhage, such that at 7 months, their T2* values were not significantly different, on average, to those of patients with evidence of hemorrhage initially.

T2* core ms:

Time 1, Time 2	-5.76 (-9.42, -2.11)	<0.001
Time 1, Time 3		0.1659
Time 1, Time 4		0.8159
Time 2, Time 3		0.1357
Time 2, Time 4	-7.51 (2.69, 12.33)	<0.001*
Time 3, Time 4	4.79 (-0.07, 9.65)	0.0549*

Interpretation: On average, T2* core decreased over time, although a slight increase was observed between scans 3 and 4.

T2* remote ms: p-value for interaction = 0.6537
p-value for time: 0.4723
p-value for hemorrhage: 0.8056

T2 injured ms: p-value for interaction = <0.001

Effect of time	Hemorrhage	Time 1, Time 2		0.98732
		Time 1, Time 3	5.31 (0.10, 10.51)	0.04169
		Time 1, Time 4	-8.82 (-13.92, -3.73)	<0.001
		Time 2, Time 3	6.80 (1.59, 12.01)	0.00196
		Time 2, Time 4	-7.33 (-12.43, -2.24)	<0.001
		Time 3, Time 4	-14.13 (-19.34, -8.93)	<0.001
	No hemorrhage	Time 1, Time 2		0.78038
		Time 1, Time 3		0.17623
		Time 1, Time 4	-10.12 (-14.72, -5.52)	<0.001
		Time 2, Time 3		0.97261
		Time 2, Time 4	-12.44 (-16.97, -7.91)	<0.001
		Time 3, Time 4	-13.93 (-18.46, -9.40)	<0.001
Effect of hemorrhage	Time 1	Hemorrhage, no hemorrhage		0.99979
	Time 2	Hemorrhage, no hemorrhage		0.56607
	Time 3	Hemorrhage, no hemorrhage		0.87094
	Time 4	Hemorrhage, no hemorrhage		0.91392

Interpretation: Those patients without hemorrhage saw a decrease in T2 injured over time. The patients with hemorrhage had an increase in T2 injured between scans 1 and 3, and then a significant drop between scans 3 and 4. Overall, the decrease between scans 1 and 4 was slightly higher for those patients without hemorrhage, but this was not statistically significant.

T2 core ms: p-value for interaction = 0.0083

Effect of time	Hemorrhage	Time 1, Time 2		0.23994
		Time 1, Time 3		0.42151
		Time 2, Time 3	7.24 (1.93, 12.55)	0.00145
	No hemorrhage	Time 1, Time 2		1.00000
		Time 1, Time 3		0.24757
		Time 2, Time 3		0.49722
Effect of hemorrhage	Time 1	Hemorrhage, no hemorrhage		0.99255
	Time 2	Hemorrhage, no hemorrhage		0.88945
	Time 3	Hemorrhage, no hemorrhage		1.00000

Interpretation: The only significant change in T2 core was between scans 2 and 3, in the hemorrhage group.

T2 remote ms: p-value for interaction = <0.001

Effect of time	Hemorrhage	Time 1, Time 2		0.66211
		Time 1, Time 3	2.03 (0.50, 3.47)	0.00162
		Time 1, Time 4	1.87 (0.38, 3.36)	0.00413
		Time 2, Time 3		0.26208
		Time 2, Time 4		0.42299
		Time 3, Time 4		0.99998
	No hemorrhage	Time 1, Time 2		0.99851
		Time 1, Time 3		0.66401
		Time 1, Time 4	1.64 (0.28, 2.99)	0.00706
		Time 2, Time 3		0.94939
		Time 2, Time 4	1.36 (0.03, 2.70)	0.04083
		Time 3, Time 4		0.46811
Effect of hemorrhage	Time 1	Hemorrhage, no hemorrhage		1.00000
	Time 2	Hemorrhage, no hemorrhage		0.99055
	Time 3	Hemorrhage, no hemorrhage		0.46811
	Time 4	Hemorrhage, no hemorrhage		0.99996

Interpretation: The majority of the increase in T2 remote for those patients with hemorrhage happened between scans 1 and 3 (with a slight but non-significant decrease between scans 3 and 4). For those patients without hemorrhage, the increase occurred mainly between scans 2 and 4, without any decrease, on average.

Infarct wall thickness (cm) P-value for interaction: <0.001

Effect of time	Hemorrhage	Time 1, Time 2		0.53078
		Time 1, Time 3		0.92438
		Time 1, Time 4	-0.36 (-0.49, -0.22)	<0.001
		Time 2, Time 3	-0.14 (-0.27, -0.004)	0.03957
		Time 2, Time 4	-0.44 (-0.57, -0.31)	<0.001
		Time 3, Time 4	-0.30 (-0.44, -0.17)	<0.001
	No hemorrhage	Time 1, Time 2		0.52688
		Time 1, Time 3		0.99997
		Time 1, Time 4	-0.19 (-0.31, -0.07)	<0.001
		Time 2, Time 3		0.31148
		Time 2, Time 4	-0.26 (-0.38, -0.15)	<0.001
		Time 3, Time 4	-0.18 (-0.29, -0.06)	<0.001
Effect of hemorrhage	Time 1	Hemorrhage, no hemorrhage		0.99993
	Time 2	Hemorrhage, no hemorrhage		0.99960
	Time 3	Hemorrhage, no hemorrhage		1.00000
	Time 4	Hemorrhage, no hemorrhage		0.82363

Figure Legends for main text.

Figure 1. Study flow diagram

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

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

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

Figure 3. Time-course of CMR T2 relaxation times (% LV mass) during the first 10 days after ischemia/reperfusion. Patients without hemorrhage have a progressive rise in T2 relaxation times during the first 10 days post-reperfusion, whereas patients with hemorrhage

have a bimodal pattern. Myocardial hemorrhage peaks on day 3 post-reperfusion. Baseline T2 values are taken from age and sex-matched healthy volunteers (n = 50).

Supplementary Figure Legends

Figure 1. Myocardial edema (% LV mass) at 4 – 12 hrs, 3 days and 10 days post-MI according to the presence or absence of hemorrhage.

Figure 2. Bland-Altman plot for inter-observer agreement of myocardial remote zone T2* values.

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

Figure 4. Bland-Altman plot for inter-observer agreement of infarct core T2* values.

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

Figure 6. Bland-Altman plot for inter-observer agreement of myocardial injury zone T2values.

Figure 1.

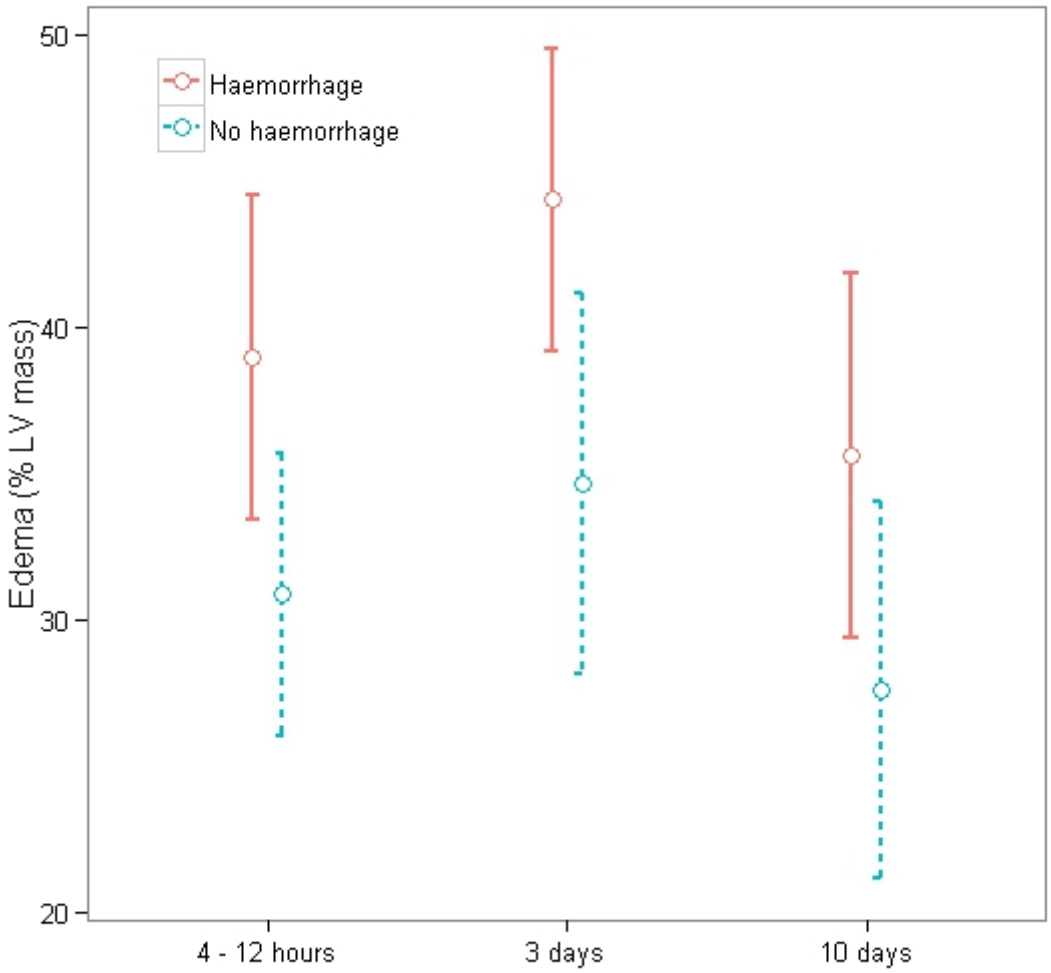

Haemorrhage
No haemorrhage
Edema (% LV mass)
50
40
30
20
4 - 12 hours
3 days
10 days

Figure 2.

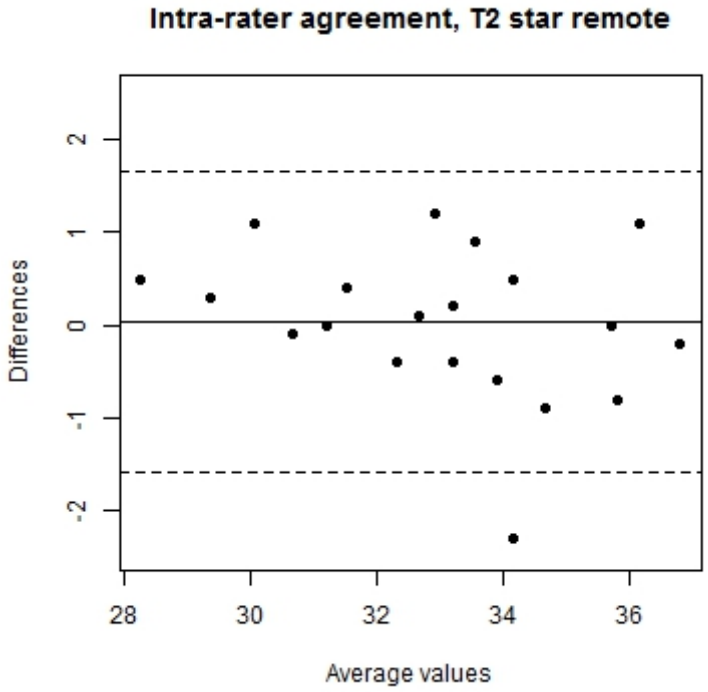

Intra-rater agreement, T2 star remote
Differences
2
1
0
-1
-2
28
30
32
34
36
Average values

Figure 3.

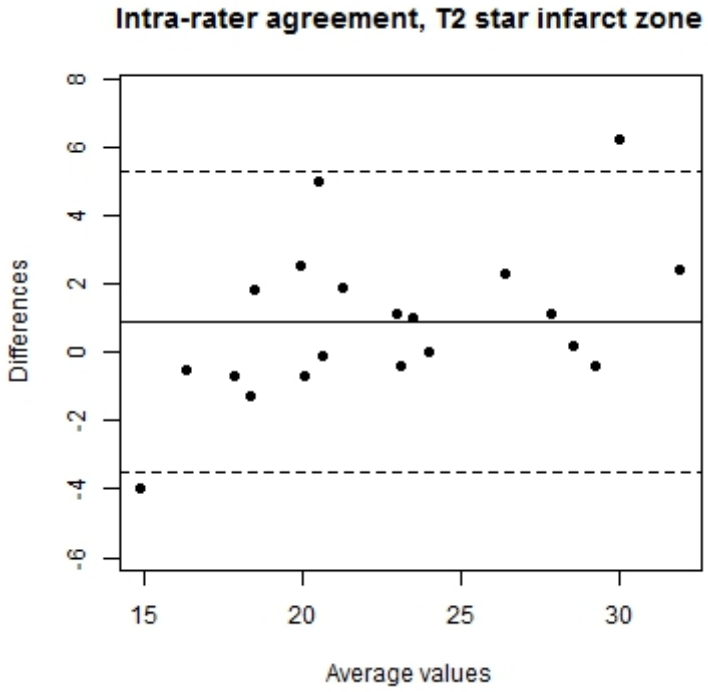
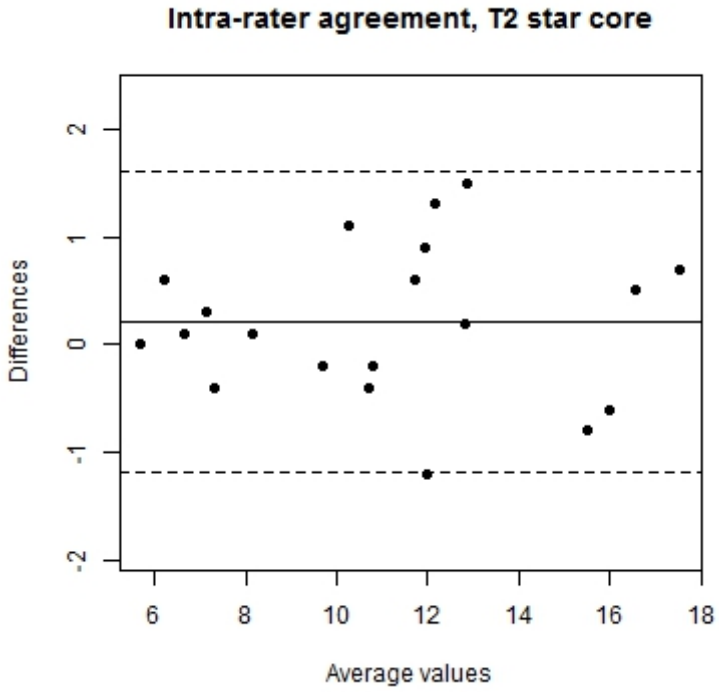

Intra-rater agreement, T2 star infarct zone
Differences
8
6
4
2
0
-2
-4
-6
15
20
25
30
Average values

Figure 4.


Intra-rater agreement, T2 star core
Differences
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1
0
-1
-2
6
8
10
12
14
16
18
Average values

Figure 5.

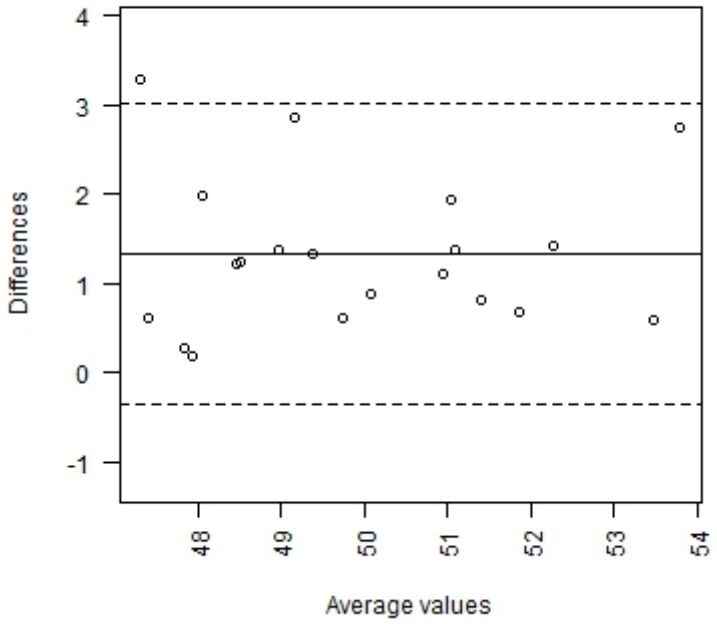

Figure 6.