SUPPLEMENTAL MATERIAL

SUPPLEMENTAL METHODS

Clinical study

Design

Hemodynamically stable consecutive patients with a first anterior ST-segment-elevation acute myocardial infarction (STEMI) and undergoing primary percutaneous coronary intervention (PCI) were prospectively recruited between February 2015 and November 2015. Patients eligible for enrollment were aged 18 years or older, and showed symptoms consistent with STEMI for >90 minutes and ST-segment elevation ≥2 mm in ≥2 contiguous leads in V1 through V5, with an anticipated time of symptom onset to reperfusion of ≤8 hours. Additional compulsory inclusion criteria were evidence of complete occlusion in the proximal or mid portion of the LAD coronary artery (TIMI 0-1 initial flow) and successful primary angioplasty evidenced by appropriate reestablishment of coronary flow in the culprit artery (TIMI-3 flow after angioplasty). Exclusion criteria were Killip class III to IV, persistent systolic blood pressure <100 mmHg, persistent heart rate <50 bpm or >110 bpm, presence of bifascicular or trifascicular block, evidence of second- or third-degree atrioventricular block, atrial fibrillation, known history of previous MI, pregnancy, active breastfeeding, and the presence of metallic objects or devices incompatible with MR imaging. Patients were managed according to current clinical guidelines.[1, 2]

CMR exams were performed within 3 hours of reperfusion (hyperacute reperfusion) and at 24 hours, 4 days, 7 days, and 40 days after reperfusion. Baseline myocardial T2 relaxation time was measured in 16 healthy age- and sex-matched volunteers. The study was approved by the hospital Ethics Committee, and patients and volunteers gave written informed consent.

CMR protocol

CMR examinations were conducted with a Philips 1.5-Tesla Achieva whole-body scanner (Philips Healthcare, Best, the Netherlands) equipped with a 16-element phased-array cardiac coil. At all time-points, the imaging protocol included a standard segmented cine steady-state free-precession (SSFP) sequence to provide high-quality anatomical references; a T2-weighted short-tau triple inversion-recovery (T2W-STIR) sequence to assess the extent of edema and intramyocardial hemorrhage (IMH); and a T2-gradient-spin-echo mapping (T2-GraSE map) sequence to provide precise myocardial T2 relaxation time properties.[3] On day-7 and day-40 CMR, late gadolinium enhancement (LGE) imaging was performed to assess infarct size (IS) and microvascular obstruction (MVO), using a T1-weighted inversion recovery turbo field echo (T1-IR-TFE) sequence acquired 10 to 15 minutes after intravenous administration of 0.20 mmol gadobutrol contrast agent per kg body weight (Gadovist, Bayer HealthCare Pharmaceuticals). All sequences were acquired during expiration breath-hold mode.

The imaging parameters for the SSFP sequence were a FOV of 342 x 342 mm, a slice thickness of 8 mm with no gap, TR 3.0 ms, TE 1.5 ms, flip angle 60°, cardiac phases 30, voxel size 2.0 x 2.0 mm^2, and 1 NEX. The imaging parameters for the T2W-STIR sequence were FOV 320 x 320, slice thickness 10 mm, TR 2 heartbeats, TE 85 ms, voxel size 1.9 x 2.4 mm^2, delay 160 ms, end-diastolic acquisition, echo-train length 28, and 2 NEX. The imaging parameters for the T2-GraSE mapping were FOV 320 x 320 with an acquisition voxel size of 2.0 x 2.5 mm^2 and slice thickness 8 mm, TR 2 heartbeats, and eight echo times ranging from 23 to 194 ms, EPI factor 7. No registration algorithm was used before T2 maps estimation; however the presence of motion artifacts between different TE for every analyzed T2 map was specifically checked. To minimize motion artefact, the breath-hold per slice in the T2-GraSE sequence was less than 10 seconds to enable proper patient breath-hold during the acquisition at every time point, including the within 3 hours post-reperfusion exam. The imaging parameters for T1-IR-TFE were as follows: FOV 265 x 265, slice thickness 10 mm with no gap, TR 8.1 ms, TE 4.0 ms, flip angle 20°, voxel size 1.8 x 2.1 mm^2, inversion time 250 to 350 (optimized to null normal myocardium), TFE factor 18, averages 1.

SSFP, T2W-STIR and T1-IR-TFE sequences were performed to acquire 8-11 contiguous short axis slices covering the heart from the base to the apex, whereas T2-maps were analyzed in a mid-apical ventricular short axis slice corresponding to the same anatomical level in all acquisitions, in order to track T2 relaxation time changes over time.

CMR analysis

CMR images were analyzed using dedicated softwares (MR Extended Work Space 2.6, Philips Healthcare, The Netherlands; and QMassMR 7.6, Medis, Leiden, The Netherlands) by two observers experienced in CMR analysis and blinded to time-point allocation and patient identification.

T2-maps were automatically generated on the acquisition scanner by fitting the signal intensity of all echo times to a monoexponential decay curve at each pixel with a maximum likelihood expectation maximization algorithm. The different myocardial states were initially defined by the localization relative to LGE defined infarction.[4] Regions of interest (ROI) were manually drawn at the transmural ischemic, infarcted (with or without including areas suggestive of IMH), salvage and transmural remote areas; and then copied to the corresponding areas of the individual T2 maps. Care was taken to include the entire wall thickness and were individually adjusted by hand to avoid the ventricular cavities or image artefacts.

The extent of edema, expressed as a percentage of LV mass (CMR-MaR), was defined after manually tracing the endocardial and epicardial contours of T2W-STIR short-axis images. Abnormal areas were initially identified using the full-width at half-maximum (FWHM) method.[5, 6] Given that the solely use of FWHM may be prompt to patchy inaccurate estimations,[7, 8] extensive manual correction and visual border delineation were performed. Extreme care was taken to avoid including any artificially high signal intensity due to inadequately suppressed slow flow within the cavity space. Hypointense areas within the edematous zone, corresponding to IMH, were included within the edematous region.[9, 10] Additionally, the size of IMH area was calculated by manual delineation of the hypointense areas on T2W-images[9] and expressed as a percentage of

LV mass. Manual delineation of clear hypointense areas was permitted in the absence of discernible hyperintense myocardium.

IS, expressed as a percentage of LV mass, was defined according the extent of late gadolinium enhancement after manually tracing the endocardial and epicardial contours on T1-IR-TFE short axis images. Abnormal areas were defined using the FWHM, with manual correction if needed. Hypointense black areas within the necrotic zone, corresponding to MVO, were included within the necrotic area.[9, 10] Additionally, the size of the MVO area was calculated by manual delineation of the hypointense areas on LGE images[9] and expressed as a percentage of LV mass.

Experimental study

Design

The study was approved by Institutional and Regional Animal Research Committees. Myocardial infarction was induced in 5 castrated male Large-White pigs weighing 30 to 40 kg to identify the optimal time-window for the first post-reperfusion CMR scan in the clinical study. Reperfused MI was generated by the percutaneous catheter-based technique, with 40min angioplasty-balloon occlusion of the mid-LAD coronary followed by balloon deflation and reestablishment of blood flow.[11] CMR exams including CINE, T2W-STIR and T2-mapping were performed immediately before MI induction and at 20 minute intervals post-reperfusion to 6 hours, when LGE sequence was performed. Immediately after, animals were sacrificed and myocardial tissue samples from ischemic and remote areas were rapidly collected for histology and evaluation of water content.

In a second set of experiments, a total of 20 pigs underwent reperfused acute myocardial infarction induced experimentally by closed-chest 40-minute left anterior descending coronary artery ischemia/reperfusion (I/R). These pigs were sacrificed at 120 minutes (n=5), 24 hours (n=5), 4 days (n=5), and 7 days (n=5) after reperfusion. CMR scans including CINE, T2W-STIR, T2-mapping, and LGE sequences were performed at every follow-up stage until sacrifice. Thus, animals sacrificed on day 7 underwent baseline, 120 min, 24 hours, day 4, and day 7 CMR exams. In all pigs, arterial enhanced multidetector computed tomography (MDCT) was performed during

the index coronary occlusion, between minute 10 and minute 20 of ischemia, to delineate the reference MaR (hypoperfused region during coronary occlusion).[12]

Myocardial infarction procedure

The MI protocol has been detailed elsewhere.[11] Anesthesia was induced by intramuscular injection of ketamine (20 mg/kg), xylazine (2 mg/kg), and midazolam (0.5 mg/kg), and maintained by continuous intravenous infusion of ketamine (2 mg/kg/h), xylazine (0.2 mg/kg/h) and midazolam (0.2 mg/kg/h). Animals were intubated and mechanically ventilated with oxygen (fraction of inspired O_2: 28%). Central venous and arterial lines were inserted and a single bolus of unfractioned heparin (300 IU/kg) was administered at the onset of instrumentation. The LAD coronary artery, immediately distal to the origin of the first diagonal branch, was occluded for 40 minutes with an angioplasty balloon introduced via the percutaneous femoral route using the Seldinger technique. Balloon location and maintenance of inflation were monitored angiographically. After balloon deflation, a coronary angiogram was recorded to confirm patency of the coronary artery. Continuous infusion of amiodarone (300 mg/h) was maintained during the procedure in all pigs to prevent malignant ventricular arrhythmias. In cases of ventricular fibrillation, a biphasic defibrillator was used to deliver non-synchronized shocks.

Arterial enhanced MDCT protocol

Arterial enhanced multidetector computed tomography (MDCT) was performed during coronary occlusion in all pigs, between minute 10 and minute 20 of ischemia, to delineate the reference MaR (hypoperfused region during coronary occlusion).[12] All MDCT studies were performed on a 64-slice CT-scanner (Brilliance CT 64, Philips Healthcare, Cleveland, Ohio). The pigs were positioned supine, and all scans were performed in the cranio-caudal direction during free-breathing. Arterial phase MDCT was performed after intravenous administration of 60 ml iomeprol 400 mgI/ml (Iomeron 400, Bracco Imaging, Milano, Italy) at a flow rate of 3 ml/s followed by a 20-ml saline chaser bolus at the same flow rate. The scan delay was determined

using a bolus tracking technique. Data acquisition started 15 seconds after a threshold of 180 Hounsfield Units was reached in a region of interest placed in the descending aorta.[12]

MDCT examinations were acquired using retrospective cardiac triggered at the 75% of the cardiac cycle with 64 x 0.625 mm collimation and a pitch of 0.2, 120 kV tube voltage, 800 mA tube current and tube rotation time of 400 ms. Image reconstruction was performed with a 512x512 matrix size over a 273x273mm^2 FOV and 0.45mm slice thickness by using high resolution filter (Xres Sharp).

Arterial enhanced MDCT analysis

MDCT images were analyzed using dedicated software (MR Extended Work Space 2.6, Philips Healthcare, Best, The Netherlands). Short axes orientation were obtained from volumetric CT images by multi-planar reconstruction using equivalent anatomical coordinates used for T2W-STIR planning acquisition. In order to have equivalent LV sections, MDCT studies had to be reconstructed in slices equivalent in thickness and level to the CMR ones. Thus, T2W-STIR and multi-planar reconstructed (MPR) short axis CT images were co-registered in 13 to 15 short-axis LV slices by one observer. To ensure CT as independent reference for MaR, endocardial and epicardial borders from MPR CT short-axis images were manually traced by a different observer blinded to the co-registration information; and MaR and remote areas were visually identified based on contrast enhancement differences, manually delineated, and expressed as a percentage of LV area.

CMR protocol

Baseline CMR scans were performed immediately before myocardial infarction and scans were subsequently repeated at all post-infarction follow-up times until sacrifice. CMR examinations were conducted with a Philips 3-Tesla Achieva Tx whole body scanner (Philips Healthcare, Best, the Netherlands) equipped with a 32-element phased-array cardiac coil. The imaging protocol included a standard segmented cine SSFP sequence to provide high quality anatomical references,

and assessment of LV mass and wall thickness; a T2W-STIR sequence to assess the extent of edema and IMH; a T2-GraSE mapping sequence to provide precise myocardial T2 relaxation time properties;[3] and a LGE sequence to assess IS and MVO. To avoid interference with T2 measures at immediate reperfusion, gadolinium contrast was not administered at baseline CMR scans.

All sequences were acquired in free-breathing mode. The imaging parameters for the SSFP sequence were FOV 280 x 280 mm, slice thickness 6 mm with no gap, TR 2.8 ms, TE 1.4 ms, flip angle 45°, cardiac phases 30, voxel size 1.8 x 1.8 mm, and 3 NEX. The imaging parameters for the T2W-STIR sequence were FOV 300 x 300, slice thickness 6 mm, TR 2 heartbeats, TE 80 ms, voxel size 1.4 x 1.9 mm^2, delay 210 ms, end-diastolic acquisition, echo-train length 18, and 2 NEX. The imaging parameters for the T2-GraSE mapping were FOV 300 x 300 with an acquisition voxel size of 1.8 x 2.0 mm^2 and a slice thickness 8 mm, TR 2 heartbeats, and eight echo times ranging from 6.7 to 53.6ms, EPI factor 3. LGE imaging was performed 10 to 15 min after intravenous administration of 0.20 mmol of gadopentetate dimeglumine contrast agent per kg of body weight using a T1-IR-TFE sequence with the following parameters: FOV 280 × 280 mm, voxel size 1.6 × 1.6 mm, end-diastolic acquisition, thickness 6 mm with no gap, TR 5.6 ms, TE 2.8 ms, inversion delay time optimized to null normal myocardium, and 2 NEX.

SSFP, T2W-STIR, and T1-IR-TFE sequences were performed to acquire 13 to 15 contiguous short-axis slices covering the heart from the base to the apex, whereas T2-maps were analyzed in a mid-apical ventricular short axis slice corresponding to the same anatomical level in all acquisitions in order to track T2 relaxation time changes over time. In the experiments to identify the optimal time-window for the first post-reperfusion CMR scan in the clinical study, SSFP and T2W-STIR sequences were performed to acquire only 3 short axis slices (mid-basal, mid, and mid-apical) given that shorter time acquisitions were needed to image at 20 minute intervals.

CMR analysis

CMR images were analyzed using dedicated softwares (MR Extended Work Space 2.6, Philips Healthcare, The Netherlands; and QMassMR 7.6, Medis, Leiden, The Netherlands) by two

observers experienced in CMR analysis. LV mass, myocardial T2 relaxation time, and extent of edema, necrosis, IMH and MVO were determined.

LV endocardial borders were automatically traced with manual adjustment in each cine image. In the tracing convention used, the papillary muscles were included as part of the LV cavity volume. LV epicardial borders were also traced on the end-diastolic images to measure end-diastolic wall thickness, with LV mass computed as the end-diastolic myocardial volume (ie, the difference between the epicardial and endocardial volumes) multiplied by myocardial density (1.05 g/mL). Values of LV mass normalized to body surface area were calculated with the modified Brody's formula.[13]

T2-maps were automatically generated on the acquisition scanner by fitting the signal intensity of all echo times to a monoexponential decay curve at each pixel with a maximum likelihood expectation maximization algorithm. T2 relaxation maps were quantitatively analyzed by placing a wide transmural ROI at the ischemic and remote areas of the corresponding slice in all studies. Hypointense areas suggestive of IMH or MVO were included in the ROI for T2 quantification purposes.[3, 11, 14]

The extent of edema, expressed as a percentage of LV mass (CMR-MaR), was defined after manually tracing the endocardial and epicardial contours of T2W-STIR short-axis images. Abnormal areas were initially identified using the FWHM method.[5, 6] Given that the solely use of FWHM may be prompt to inaccurate patchy estimations,[7, 8] extensive manual correction and visual border delineation were performed. Areas corresponding to slow-flow artifacts were carefully excluded from edematous area. Hypointense areas within the edematous zone, corresponding to IMH, were included within the edematous region.[9, 10] Additionally, the size of the area of IMH was calculated by manual delineation of the hypointense areas on T2W-images,[9] and expressed as a percentage of LV mass. Manual delineation of clear hypointense areas was permitted in the absence of discernible hyperintense myocardium.

IS, expressed as a percentage of LV mass, was defined according the extent of late gadolinium enhancement after manually tracing the endocardial and epicardial contours on T1-IR-TFE short

axis images. Abnormal areas were defined using the FWHM, with manual correction if needed. Hypointense black areas within the necrotic zone, corresponding to MVO, were included within the necrotic area.[9, 10] Additionally, the size of the area MVO was calculated by manual delineation of the hypointense areas on LGE images,[9] and expressed as a percentage of LV mass.

Quantification of myocardial water content

Paired myocardial samples were collected within minutes of euthanasia from the ischemic myocardium of all pigs. Tissue samples were immediately blotted to remove surface moisture and introduced into laboratory crystal containers previously weighed on a high-precision scale. The containers were weighed before and after drying for 48 hours at 100°C in a desiccating oven. Tissue water content was calculated as follows: water content (%) = [(wet weight−dry weight)/wet weight] ×100. An empty container was weighed before and after desiccation as an additional calibration control.

Histological and immunnohistochemical analysis

Myocardial samples were collected within minutes of euthanasia from the ischemic (anteroseptal) and remote (posterolateral) mid-apical ventricular wall. Tissue samples were fixed in 10% neutral buffered formalin for 48 hours and processed by dehydrating the tissue in increasing concentrations of ethanol. Samples were then cleared in xylene, embedded in paraffin wax and cut into 4 micron sections.

For histopathological analysis, sections were stained with hematoxylin and eosin (H&E) and Masson's Trichrome. Necrotic tissue was identified by the presence of typical signs of coagulative necrosis, including marginal contraction bands, fading, and eventually loss of nuclei and striation in cardiomyocytes.

For immunohistochemical analysis, sections were deparaffinized and antigens were unmasked using heat induced epitope retrieval (HIER) with citrate buffer at pH6. Before incubation with

primary antibodies, endogenous peroxidase was blocked by incubation with H_2O_2 for 5 minutes, and endogenous antigens were blocked with fetal bovine serum (FBS) for 20 minutes.

Neutrophils were detected with mouse monoclonal anti-PM1 primary antibody (BMA biomedicals; T-3503) as previously described.[14] The secondary antibody was HRP-conjugated goat anti-mouse (Dako; P0447). Bound antibody was revealed by staining with diaminobenzidine, and nuclei were counterstained with hematoxylin. All immunohistochemical procedures were performed using an automated autostainer (Autostainer Plus®, Dako). For analysis, images were digitalized with a scanner (Nanozoomer-RS C110730®, Hamamatsu) and examined with image analysis software (Tissuemorph®, Visiopharm) by an experienced veterinary pathologist blinded to experimental procedure.

SUPPLEMENTAL RESULTS

Dynamics of the initial wave of edema

Five male pigs (mean body weight 36.4±2.9 kg) underwent CMR exam before MI induction (baseline) and at 20 min intervals after reperfusion for 6 hours (19 CMR exams per pig). Mean myocardial T2 relaxation time before MI induction was 44.3±1.6 ms and 43.0±1.3 ms for the mid-apical anteroseptal and posterolateral left ventricular walls, respectively. In the ischemic area, reperfusion was associated with an immediate sharp increase in T2 relaxation time above baseline values, reaching a peak at 40 minutes after reperfusion (**Supplemental Figure 1**). Thereafter, a progressive decrease in T2 was observed, with T2 relaxation time at 6 hours closer to the values obtained in baseline CMR scans. In the remote myocardium, T2 relaxation time showed no significant trend or differences at different times post reperfusion. Tissue water content in the formerly ischemic myocardium at 6 hours after reperfusion was 82.7 ± 1.0%. Histological analysis of such myocardial tissue at 6 hours after reperfusion revealed typical features of early acute transmural necrosis (**Supplemental Figure 2**).

SUPPLEMENTAL TABLES

Supplemental Table 1. Point estimates and differences in T2 relaxation time in the transmural ischemic myocardium relative to the value obtained in the hyperacute CMR exam (≤3 hour reperfusion), with adjustment for the extent of intramyocardial hemorrhage.

	Reperfusion time				
	≤ 3 hours	**24 hours**	**4 days**	**7 days**	**40 days**
T2 transmural ischemic, ms	80.8 (76.1, 85.4)	65.4 (60.8, 70.0)	80.5 (75.9, 85.1)	76.8 (72.1, 81.6)	65.4 (60.7, 70.2)
Δ T2 transmural ischemic, ms	-	-15.3 (-21.3, -9.4)	-0.3 (-6.2, 5.7)	-3.9 (-10.0, 2.2)	-15.3 (-21.3, -9.2)
Δ T2 transmural ischemic (*), ms	-	-14.7 (-20.7, -8.7)	1.0 (-5.3, 7.3)	-2.7 (-9.1, 3.7)	-15.1 (-21.1, -9.1)

To take account of repeated measures, a generalized linear mixed model was conducted to analyze the time course of T2 relaxation time. The model was further adjusted by extent of hemorrhage, including the amount of intramyocardial hemorrhage (IMH) expressed as a percentage of the left ventricle as a covariate.

Data are presented as point estimates (95% confidence interval), or mean difference (95% confidence interval) in T2 relaxation time (Δ T2) in the transmural ischemic myocardium relative to the first CMR examination, performed within 3 hours after reperfusion. The table shows nonadjusted differences and (*) differences adjusted for the amount of IMH (% of left ventricle). Globally, T2 relaxation time in the transmural ischemic area decreased 1.1 ms (95% CI, -3.1 to 1.0, p = 0.297) for every 1% absolute increase in IMH (expresses as a percentage of the left ventricle).

Supplemental Table 2. Point estimates and differences in edematous area relative to the value obtained in the hyperacute CMR exam (≤3 hour reperfusion), with adjustment for the extent intramyocardial hemorrhage.

	Reperfusion time				
	≤ 3 hours	**24 hours**	**4 days**	**7 days**	**40 days**
Area of edema, % of LV	39.9 (34.1, 45.7)	21.8 (16.1, 27.6)	42.8 (37.0, 48.6)	43.0 (37.1, 48.9)	20.3 (14.3, 26.2)
Δ Area of edema, % of LV	-	-18.0 (-24.2, -11.9)	2.9 (-3.2, 9.1)	3.1 (-3.2, 9.4)	-19.6 (-25.9, -13.3)
Δ Area of edema (*), % of LV	-	-19.0 (-25.3, -12.7)	1.0 (-5.7, 7.7)	1.2 (-5.6, 8.0)	-19.9 (-26.1, -13.6)

To take account of repeated measures, a generalized linear mixed model was conducted to analyze the time course of edematous area as measured by T2W-STIR. The model was further adjusted by extent of hemorrhage, including the amount of intramyocardial hemorrhage (IMH) expressed as a percentage of the left ventricle as a covariate.

Data are presented as point estimates (95% confidence interval), or mean difference (95% confidence interval) in edematous area (Δ Area of edema) relative to the first CMR examination, performed within 3 hours after reperfusion. The table shows nonadjusted differences and (*) differences adjusted for the amount of IMH (% of left ventricle). Globally, the area of edema increased 1.7% of the left ventricle (95% CI, -0.6 to 4.0, p = 0.154) for every 1% absolute increase in IMH (expresses as a percentage of the left ventricle).

Supplemental Table 3. Time course of myocardium at risk, infarct size, and myocardial salvage as assessed by cardiac magnetic resonance during the first week after reperfused myocardial infarction in pigs.

Endpoint	CMR measure	Follow up			
		R-120min	**R-24hours**	**R-Day4**	**R-Day7**
Sacrificed at 120 min	MaR, % of LV	51.6 (6.2)			
	Infarct size, % of LV	47.2 (2.9)			
	Myocardial salvage, %	3.7 (3.4)			
Sacrificed at 24 hours	MaR, % of LV	47.7 (5.7)	4.7 (3.7)		
	Infarct size, % of LV	46.8 (5.6)	40.0 (3.4)		
	Myocardial salvage, %	1.9 (1.7)	-1339 (1077)		
Sacrificed at 4 days	MaR, % of LV	50.0 (3.5)	3.8 (2.8)	33.4 (7.0)	
	Infarct size, % of LV	47.6 (3.6)	35.7 (3.7)	32.1 (3.3)	
	Myocardial salvage, %	4.6 (4.6)	-1209 (690)	-0.6 (26.7)	
Sacrificed at 7 days	MaR, % of LV	42.9 (5.7)	2.2 (1.7)	27.1 (3.4)	30.1 (2.3)
	Infarct size, % of LV	39.2 (3.8)	30.2 (3.1)	28.2 (4.6)	25.4 (4.0)
	Myocardial salvage, %	8.3 (6.4)	-1310 (996)	-4.4 (14.2)	15.7 (13.3)

Values are mean (standard deviation). Mean myocardium at risk assessed by MDCT reference standard for pigs was 33.7±5.8 % of the LV for those sacrificed at 120 minutes, 30.7±6.2 % for 24 hours, 29.8±3.9 % for day 4, and 28.3±4.3 % for day 7.

CMR: cardiac magnetic resonance; MaR: myocardium at risk; LV: left ventricle; I/R: ischemia/reperfusion; R: reperfusion.

Supplemental Table 4 Time profile of left ventricular mass and wall thickness ratio as assessed by cardiac magnetic resonance during the first week after reperfused myocardial infarction in pigs.

Endpoint	CMR measure	Follow up				
		Baseline	**R-120min**	**R-24hours**	**R-Day4**	**R-Day7**
Sacrificed at 120 min	LV mass, g/m^2	75.8 (7.9)	105.5 (11.1)			
	Wall thickness ratio, ischemic/remote	1.07 (0.04)	1.91 (0.23)			
Sacrificed at 24 hours	LV mass, g/m^2	79.6 (9.3)	103.8 (16.1)	81.5 (10.0)		
	Wall thickness ratio, ischemic/remote	1.08 (0.07)	2.43 (0.53)	1.25 (0.06)		
Sacrificed at 4 days	LV mass, g/m^2	72.5 (3.1)	108.6 (14.9)	81.3 (7.0)	81.7 (8.3)	
	Wall thickness ratio, ischemic/remote	1.07 (0.04)	1.88 (0.33)	1.16 (0.11)	1.15 (0.20)	
Sacrificed at 7 days	LV mass, g/m^2	64.0 (7.4)	92.6 (6.0)	71.1 (4.7)	73.6 (5.2)	74.3 (4.7)
	Wall thickness ratio, ischemic/remote	1.06 (0.18)	2.08 (0.36)	1.23 (0.13)	1.17 (0.15)	1.08 (0.06)
Pooled	**LV mass, g/m^2**	**73.0 (9.0)**	**102.6 (13.2)**	**78.0 (8.6)**	**77.6 (7.8)**	**74.3 (4.7)**
	Wall thickness ratio, ischemic/remote	**1.07 (0.09)**	**2.07 (0.41)**	**1.21 (0.11)**	**1.16 (0.17)**	**1.08 (0.06)**

Values are mean (standard deviation). No significant statistical differences were found between time-points except for R-120 min CMR, when LV mass and end-diastolic wall thickness ratio (MaR/remote) were significantly higher than at the other time-points due to the intense swelling of the ischemic myocardium at early reperfusion.

CMR: cardiac magnetic resonance; R: reperfusion; LV: left ventricle.

Supplementary Table 5. Time course of intramyocardial hemorrhage (IMH) and microvascular obstruction (MVO) assessed by cardiac magnetic resonance during the first week after reperfused myocardial infarction in pigs.

Endpoint	CMR measure	Follow up			
		R-120min	**R-24hours**	**R-Day4**	**R-Day7**
Sacrificed at 120 min	IMH, % of LV	0.0 (0.0)			
	MVO, % of LV	4.7 (4.8)			
Sacrificed at 24 hours	IMH, % of LV	0.5 (0.9)	2.2 (1.8)		
	MVO, % of LV	3.8 (3.6)	9.9 (5.4)		
Sacrificed at 4 days	IMH, % of LV	0.2 (0.5)	4.1 (1.3)	5.7 (2.5)	
	MVO, % of LV	1.5 (1.3)	7.5 (4.9)	4.7 (4.8)	
Sacrificed at 7 days	IMH, % of LV	0.2 (0.5)	4.3 (1.8)	4.4 (1.9)	1.4 (1.4)
	MVO, % of LV	5.1 (4.5)	6.5 (2.1)	2.3 (2.8)	1.8 (2.9)
Pooled	**IMH, % of LV**	**0.2 (0.5)**	**3.5 (1.8)**	**5.0 (2.2)**	**1.4 (1.4)**
	MVO, % of LV	**3.7 (3.7)**	**8.0 (4.3)**	**3.5 (3.9)**	**1.8 (2.9)**

Values are mean (standard deviation).

CMR: cardiac magnetic resonance; I/R: ischemia/reperfusion; IMH: intramyocardial hemorrhage; MVO: microvascular obstruction; LV: left ventricle.

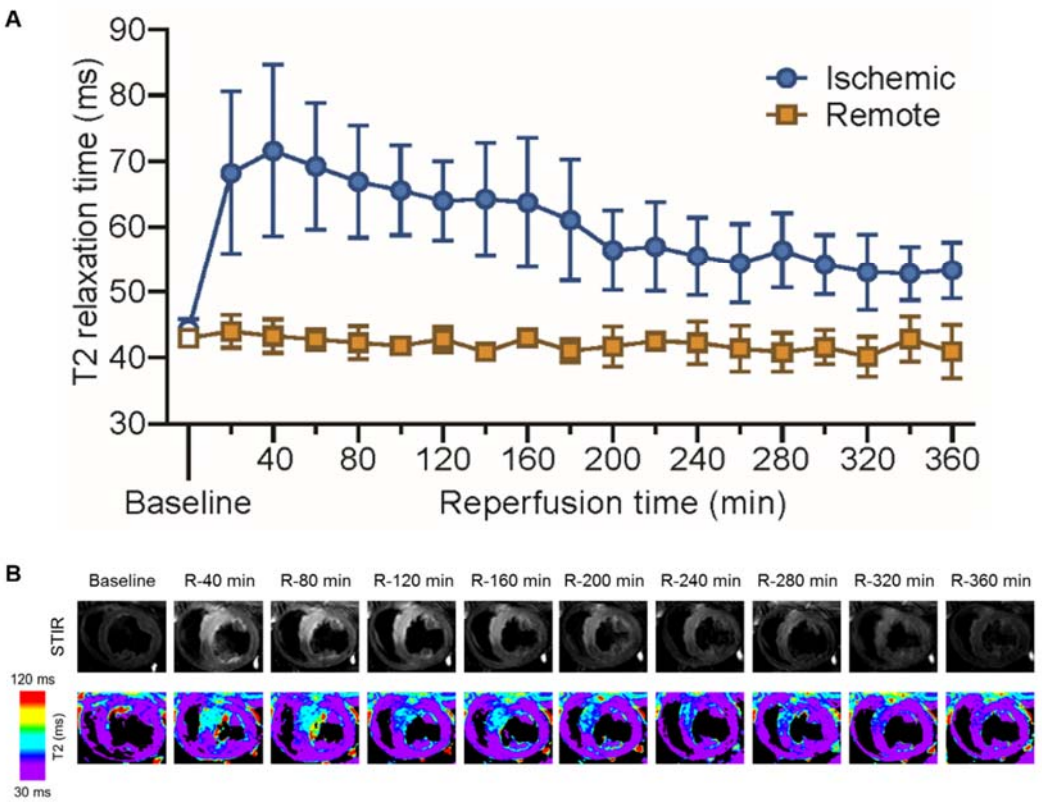
SUPPLEMENTAL FIGURES AND FIGURE LEGENDS

Supplemental Figure 1. Dynamics of the initial wave of edema

(A) Time course of T2 relaxation time (ms) in the ischemic and remote myocardium during the first 6 hours after ischemia/reperfusion in the pig model. Data are means and standard deviation. Cardiac magnetic resonance (CMR) scans were performed immediately before induction of myocardial infarction and at 20 minute intervals after reperfusion up to 6 hours, when pigs were sacrificed.

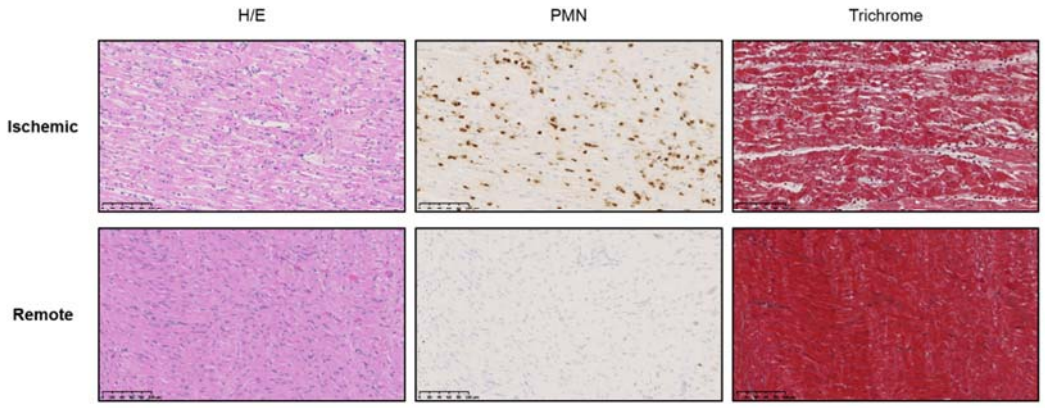
(B) Representative images from an animal that underwent 40min-I/R and serial CMR T2W-STIR and T2-mapping exams to study the precise dynamics of the first edema wave. Due to space restrictions, the representative images shown were taken at 40 minute intervals. All T2 maps were scaled between 30 and 120 ms.

CMR: cardiac magnetic resonance; R: reperfusion; STIR: Short-tau inversion recovery; ms: milliseconds.



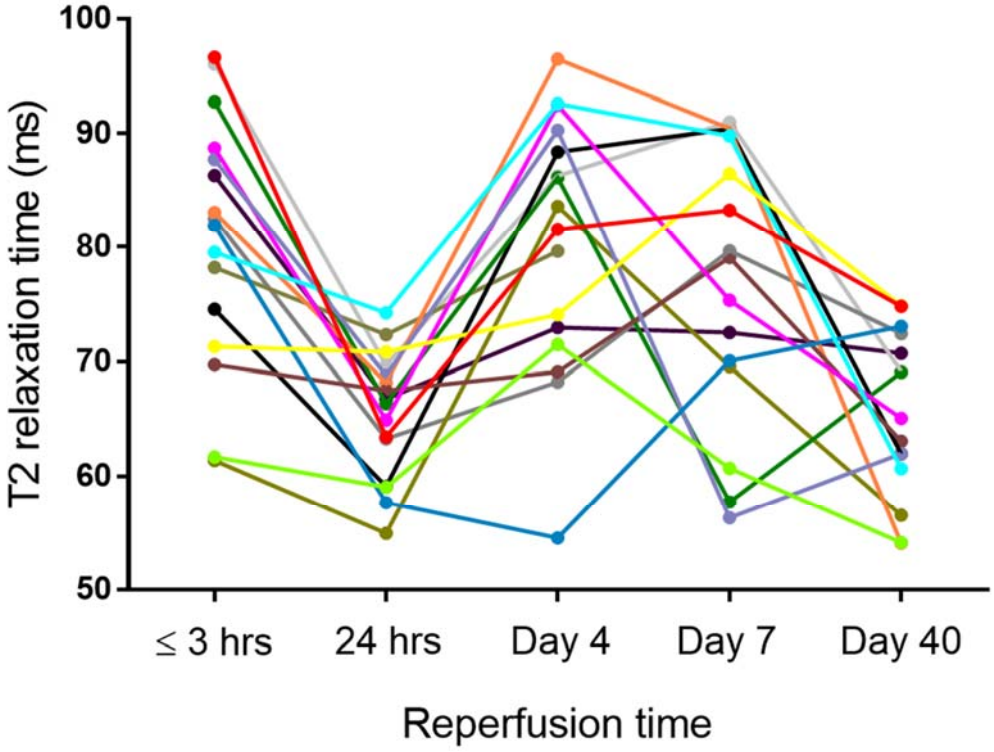
Supplemental Figure 2. Histological analysis of porcine myocardium 6 hours after ischemia/reperfusion

Representative histological images of ischemic myocardium (top) and remote myocardium (bottom) 6 hours after 40-minute ischemia and reperfusion (I/R) in the pig model. Images show staining with hematoxylin and eosin (H/E), anti-PM1 antibody (PMN), and Masson's trichrome. Neutrophils were quantified and interstitial hemorrhage was graded from 0 (absence) to 5 (very severe). The remote area showed no relevant pathological findings at this time-point. In contrast, ischemic myocardium exhibited typical features of acute transmural myocardial infarction, with extensive coagulative necrosis, contraction bands, loss of nuclei and striation in cardiomyocytes, wavy fibers, and cell edema. Interstitial edema in the ischemic area at 6 hours post-I/R was significantly lower than at 120 min after ischemia onset,[14] consistent with partial resolution of the initial wave of edema. Massive tissue infiltration by neutrophils (473±190 cells per mm^2 in the lesion area) was observed at 6 hours post-I/R, which was at least as high as that observed at 24 hours post-I/R.[14] Mild interstitial hemorrhage was detected (median score of 1; interquartile range, 0-1). Scale bars, 100μM.



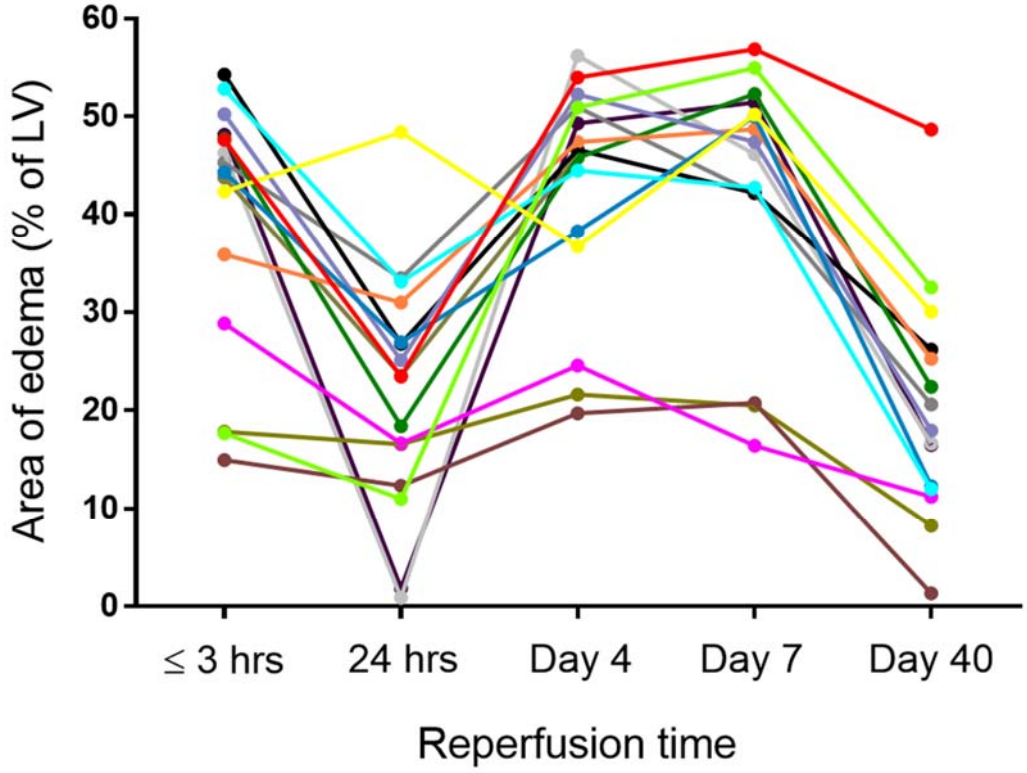
Supplemental Figure 3. Individual patient T2 relaxation time trajectories in the ischemic myocardium

Line-plots showing changes in individual T2 relaxation times in the ischemic myocardium of anterior STEMI patients after reperfusion by primary PCI. Cardiac magnetic resonance was scheduled within the first 3 hours and at 24 hours, 4 days, 7 days, and 40 days after reperfusion. T2 values at all time-points were obtained from T2-GraSE mapping sequences.[3]



Supplemental Figure 4. Individual patient trajectories for area of edema

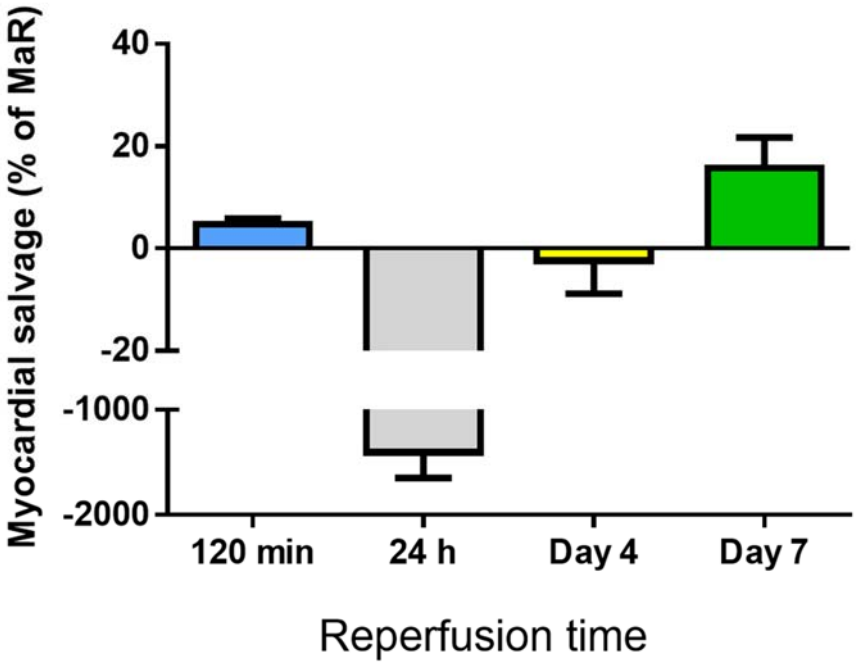
Line-plots showing changes in individual area of edema measurements of anterior STEMI patients after reperfusion by primary PCI. Colors identify the same individuals as in Supplemental Figure 3. Cardiac magnetic resonance was scheduled within the first 3 hours and at 24 hours, 4 days, 7 days, and 40 days after reperfusion. Edematous area at all time points was determined from T2W-STIR sequences.



Supplemental Figure 5. Temporal evolution of myocardial salvage assessed by cardiac magnetic resonance after reperfused myocardial infarction in pigs.

Data are means ±standard error of the mean.

MaR: myocardium at risk.



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