Dynamic Edematous Response of the Human Heart to Myocardial Infarction:
Implications for Assessing Myocardial Area at Risk and Salvage

Running Title: Fernández-Jiménez et al.; Bimodal Post-STEMI Edema in Humans

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Abstract

**Background**—Clinical protocols aimed to characterize the post-myocardial infarction (MI) heart by cardiac magnetic resonance (CMR) need to be standardized to take account of dynamic biological phenomena evolving early after the index ischemic event. Here we evaluated the time-course of edema reaction in ST-segment elevation MI (STEMI) patients by CMR and assessed its implications for myocardium-at-risk (MaR) quantification both in patients and in a large animal model.

**Methods**—A total of 16 anterior STEMI patients successfully treated by primary angioplasty and 16 matched controls were prospectively recruited. In total, 94 clinical CMR exams were performed: STEMI patients were serially scanned (within the first 3 hours after reperfusion and at 1, 4, 7, and 40 days), and controls only once. T2 relaxation time in the myocardium (T2-Mapping) and the extent of edema on T2W-STIR (i.e. CMR-MaR) were evaluated at all timepoints. In the experimental study, 20 pigs underwent 40-min-ischemia/reperfusion followed by serial CMR exams at 120-min and 1, 4, and 7 days after reperfusion. Reference MaR was assessed by contrast-multidetector computed tomography (MDCT) during the index coronary occlusion. Generalized linear mixed models were used to take account of repeated measurements.

**Results**—In humans, T2 relaxation time in the ischemic myocardium declines significantly from early after reperfusion to 24 hours, and then increases up to day 4, reaching a plateau from which it decreases from day 7. Consequently, edema extent measured by T2W-STIR (“CMR-MaR”) varied with the timing of the CMR exam. These findings were confirmed in the experimental model by showing that only CMR-MaR values for day 4 and day 7 post-reperfusion, coinciding with the deferred edema wave, were similar to values measured by reference MDCT.

**Conclusions**—Post-MI edema in patients follows a bimodal pattern, which affects CMR estimates of MaR. Dynamic changes in post-STEMI edema highlight the need for standardization of CMR timing to retrospectively delineate MaR and quantify myocardial salvage. According to the present clinical and experimental data, a time window between day 4 and 7 post-MI seems a good compromise solution for standardization. Further studies are needed to study the effect of other factors on these variables.

**Key Words:** magnetic resonance imaging; edema; area at risk; ischemia reperfusion injury; model
Clinical Perspective

What is new?
- This work shows for the first time that myocardial edema in the week after STEMI in humans is a bimodal phenomenon.
- An initial wave of edema appears abruptly at reperfusion but it is significantly attenuated by 24 hours.
- The initial wave of edema is followed by a second (deferred) healing-related wave of edema several days after reperfusion reaching a plateau around 4 to 7 days after myocardial infarction.
- This bimodal edematous response has a major impact on retrospective myocardial area at risk and salvage quantification by cardiac magnetic resonance (CMR) given that measures of edema are greatly influenced by the timing of imaging.

What are the clinical implications?
- Both CMR imaging techniques and timing of post-infarction imaging for assessing myocardial area at risk and myocardial salvage should be standardized to take account of the pathophysiology of the bimodal edematous phenomenon.
- The timeframe between day 4 and 7 post-infarction seems a good compromise solution according to clinical and experimental data here presented.
- Our results have important implications for the design and interpretation of clinical trials using edema-sensitive CMR protocols to quantify myocardium at risk and/or myocardial salvage as an endpoint.
Noninvasive tissue characterization by cardiac magnetic resonance (CMR) after myocardial infarction (MI) offers the possibility to evaluate the impact of interventions designed to preserve cardiac function and predict long-term remodeling.\textsuperscript{1} It has been postulated that an intense edematous reaction confined to the post-ischemic region appears early after MI and persists in stable form for at least 1 week.\textsuperscript{2, 3} On the basis of this assumption, the use of edema-sensitive T2-CMR sequences to delineate the spatial extent of post-MI edema was rapidly incorporated as an index of the original occluded coronary artery perfusion territory (myocardium at risk, MaR).\textsuperscript{4, 5} Quantification of late gadolinium enhancement (LGE) and edema extent (assumed to delineate MaR) in the same imaging session has been extensively used to quantify the amount of salvaged myocardium, a theoretical surrogate of the effect of cardioprotective therapies,\textsuperscript{6, 7} thus reducing the required sample size in trials.\textsuperscript{8} Consequently, CMR-based myocardial salvage has been and continues to be used as an endpoint in multiple clinical and experimental studies.\textsuperscript{9}

Based on the assumed stable unimodal edematous reaction during the first week after MI, the timing of the endpoint imaging session in these studies varies considerably. However, recent work in the pig model showed that the post-MI edematous reaction is not stable, and instead follows a bimodal pattern.\textsuperscript{10} An initial reperfusion-related wave of edema appears abruptly upon reperfusion and dissipates at 24 hours. This is followed by a healing-related deferred wave of edema appearing several days after MI, peaking around post-reperfusion day 7.\textsuperscript{11} This coordinated bimodal edema pattern suggests that CMR-quantified MaR may vary according to the day of imaging, but to date this has not been tested in a controlled manner. Some recent studies evaluated MaR extent in patients according to the timing of post-MI imaging, but these were either retrospective analyses\textsuperscript{12} or did not systematically scan patients at the same time points.\textsuperscript{13} Consequently, whether this phenomenon occurs in MI patients is unclear.
This study was designed to address these specific 2 questions: 1) is post-MI edematous reaction bimodal in humans?, and 2) does the bimodal edematous reaction affect the CMR-based quantification of MaR and myocardial salvage? We designed a longitudinal clinical study in which ST-segment elevation MI (STEMI) patients successfully treated by primary angioplasty were prospectively recruited and CMR performed within the first 3 hours post-reperfusion and at 24 hours, 4 days, 7 days, and 40 days. The impact of the dynamic edematous response on post-MI CMR measures of MaR, infarct size (IS) and salvaged myocardium was evaluated in the pig model of reperfused MI by performing reference measures of MaR and a comprehensive serial CMR imaging study.

Methods

Clinical study

Design
Hemodynamically stable consecutive patients with a first anterior STEMI and undergoing primary percutaneous coronary intervention (PCI) were prospectively recruited between February 2015 and November 2015 ad hoc for this study. 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 from symptom onset to reperfusion of ≤8 hours. Additional mandatory inclusion criteria were evidence of complete occlusion in the proximal or mid portion of the left anterior descending (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.14, 15

CMR exams were performed within 3 hours of reperfusion (hyperacute reperfusion) and at 24 hours, 4 days, 7 days, and 40 days after reperfusion (Figure 1A). Normal T2 relaxation times (“baseline”) were obtained in 16 healthy age- and sex-matched volunteers. The study was approved by the hospital Ethics Committee, and all 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.16 On day-7 and day-40 CMR, LGE imaging was performed to assess infarct size 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.


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 analyzed by placing region of interest (ROI) at the transmural ischemic, infarcted (with or without including areas suggestive of IMH), salvage, and transmural remote areas 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.\textsuperscript{16, 17} The extent of edema, expressed as a percentage of LV mass (CMR-MaR), was initially identified using the full-width at half-maximum (FWHM) with subsequent manual correction and visual border delineation after tracing the endocardial and epicardial contours of T2W-STIR short-axis images.\textsuperscript{18} Hypointense areas within the edematous zone, corresponding to IMH, were included within the edematous region.\textsuperscript{19, 20} Additionally, IMH area was calculated by manual delineation of the hypointense areas on T2W-images\textsuperscript{19} and expressed as a percentage of LV mass.

IS, expressed as a percentage of LV mass, was defined according the extent of LGE 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.\textsuperscript{19, 20} Additionally, the size of the MVO area was calculated by manual delineation of the hypointense areas on LGE images\textsuperscript{19} and expressed as a percentage of LV mass. Detailed information about CMR imaging protocol and parameters, and imaging analysis is presented in Supplemental Methods.
Experimental study

Design and myocardial infarction procedure

The study was approved by Institutional and Regional Animal Research Committees.

To study the impact of the dynamic edematous response on post-MI CMR time profile measures of MaR, IS and salvaged myocardium, a group of 20 pigs underwent closed-chest reperfused MI by the percutaneous catheter-based technique, with 40 minute angioplasty-balloon occlusion of the mid-left LAD coronary artery, followed by balloon deflation and reestablishment of blood flow\textsuperscript{10} (Figure 1B). These pigs were sacrificed at 120 minutes (n=5), 24 hours (n=5), 4 days (n=5), or 7 days (n=5) after ischemia/reperfusion (I/R). 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).\textsuperscript{21} Comprehensive CMR scans were performed at every follow-up stage until sacrifice (i.e. animals sacrificed on day 7 underwent baseline, 120min, 24h, day4, and day7 CMR exams).

Full methods can be found in the supplementary appendix.

Arterial enhanced MDCT protocol and analysis

All MDCT studies were carried out on a 64-slice CT-scanner (Brilliance CT 64, Philips Healthcare, Cleveland, Ohio) after intravenous administration of 60 ml 400 mg/l/ml iomeprol (Iomeron 400, Bracco Imaging, Milano, Italy).\textsuperscript{21} MDCT images were analyzed using dedicated software (MR Extended Work Space 2.6, Philips Healthcare, Best, The Netherlands). MaR and remote areas were visually identified based on contrast enhancement differences, manually delineated, and expressed as a percentage of LV area.
CMR protocol and analysis

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 an 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 map sequence;10, 16 and a T1-IR-TFE sequence to assess IS and MVO. CMR images were similarly 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 group allocation. Detailed information about MDCT and CMR imaging protocol and parameters, and imaging analysis, can be found in the Supplementary Methods.

Statistical analysis

In the clinical study, the sample size calculation to detect a difference in T2 relaxation time in the ischemic myocardium between examination time-points after STEMI was pre-specified by using the user-written command \textit{nsize} (Stata 12.0). A sample size of 16 patients was determined on the basis of our previous experimental results,10 a 95% confidence level, a statistical power of 80%, a conservative significant mean difference to detect of 15 milliseconds in T2, a standard deviation of 12, and multiple pairwise comparisons between time-points.

Normal distribution of each data subset was checked using graphical methods and a Shapiro–Wilk test. Leven’s test was performed to check homogeneity of variances. For quantitative variables, data are expressed as mean $\pm$ standard deviation. For categorical variables, data are expressed as frequencies and percentages. To take account of repeated measures, generalized linear mixed models were conducted to analyze the time course of T2 relaxation time, CMR-
MaR, IMH, MVO, IS, and salvaged myocardium. Models evaluating the time course of T2 or CMR-MaR were further adjusted by extent of hemorrhage, including the amount of IMH expressed as a percentage of the LV as a covariate, given that this parameter is known to affect T2. Given the hypothesis-driven nature of the study, comparisons among different time-points were planned in advance. Nonetheless, p-value was adjusted for multiple comparisons using the Hochberg method.

All statistical analyses were performed with Stata v12.0 (StataCorp, College Station, Texas).

Results

Clinical study

General characteristics of the population

Clinical characteristics of the study population are summarized in Table 1. Serial CMR was performed with informed consent in 16 consecutive anterior STEMI patients fulfilling the inclusion criteria (mean age 58.8±14.5 years, 14 [87.5%] male) and successfully treated by primary PCI. A total of 94 CMR exams were performed: the 16 healthy volunteers were scanned once, and the 16 STEMI patients were scanned at 2.2±0.5 hours, 24.8±1.8 hours, 3.8±0.4 days, 6.8±0.6 days, and 41.7±4.3 days after reperfusion. In all patients, the first CMR scan was performed within the first 3 hours (90 to 180 minutes) after primary PCI. The timing for the initial CMR scan (around the peak of reperfusion-related wave of edema) was identified before in a dedicated separate group of 5 pigs undergoing serial CMR scans every 20 min during the 6 hours following reperfusion (see Supplemental Methods and Results, and Supplemental Figures 1 and 2). Evaluable T2-mapping and T2W-STIR data were available in 100% of CMR scans performed. Information on vital status was available for all participants.
Edema time course in STEMI patients

T2 relaxation time

Mean myocardial T2 relaxation times in the 16 healthy volunteers (mean age 59.3±17.7 years, 12 [75%] male) were 53.1±4.1 ms and 51.1±4.5 ms for the mid-apical anteroseptal and posterolateral left ventricular walls, respectively. Compared with these values, hyperacute reperfusion in STEMI patients (≤3 hours) was associated with significantly longer T2 relaxation times in the ischemic area (Figure 2A-B). T2 relaxation time in STEMI patients showed a systematic and significant decrease at 24 hours post-MI. This was followed by a rebound increase, with T2 relaxation times on day 4 post-reperfusion reaching values similar to those observed during early reperfusion. Thereafter, T2 relaxation time progressively decreased, with values on day 40 similar to those observed at 24 hours. Similar results were obtained after adjusting T2 for the amount of IMH (Supplemental Table 1). During the first week after MI, T2 relaxation time in the remote myocardium showed a linear trend toward a progressive increase, albeit slight. T2 relaxation times in the ischemic and remote myocardium at different post-reperfusion time-points are summarized in Table 2. T2 relaxation time was longer in the transmural ischemic myocardium than in the remote myocardium at all time points evaluated. However, the differences observed at 24 hours and 40 days, albeit statistically significant, were of small magnitude and resulted in a wide overlapping of myocardial T2 values within ischemic and remote areas (Figure 2C). Individual trajectories for T2 relaxation time in the ischemic myocardium of STEMI patients are shown in Supplemental Figure 3.

Extent of edema (CMR-MaR)

The edematous area delineated by T2W-STIR sequences was similar in CMR scans performed at hyperacute reperfusion (≤3 hours) and on day 4 and day 7 (Figure 3). Conversely, the area of
edema was significantly smaller at 24 hours post-reperfusion. On day 40 post-MI, the area of edema was comparable to that seen at 24h. This time-course pattern for edema resembles that observed for T2 relaxation time, and similar results were obtained after adjusting the area of edema for the amount of IMH evaluated by T2W-STIR (Supplemental Table 2). Edematous area at different post-reperfusion time-points is summarized in Table 2. Individual patient trajectories for area of edema measurements are shown in Supplemental Figure 4.

Experimental study

Dynamics of CMR-MaR after reperfused MI as compared to the reference standard

CMR-measured MaR values at different times after reperfusion in pigs are summarized in Table 3 and Supplemental Table 3. Mean MaR as assessed by the MDCT reference method was 30.5±5.0 % of the LV. Due to initial swelling of the ischemic myocardium (Supplemental Table 4), CMR-measured MaR as delineated by T2W-STIR sequence was significantly higher than MaR measured by MDCT at early reperfusion (Figure 4A and 4B). Coinciding with the dissipation of the first edema wave,10 MaR was strikingly underestimated by CMR at 24 hours post-reperfusion. Conversely, CMR-estimated MaR values for day 4 and day 7 post-reperfusion, coinciding with the deferred edema wave,10 were similar to values measured by MDCT (i.e. no overestimation or underestimation). The dynamics of CMR-measured MaR resembled the time course for myocardial T2 relaxation time and water content in the ischemic area.10

Dynamics of CMR-measured IS, myocardial salvage, IMH and MVO after reperfused MI

CMR-measured IS and myocardial salvage in pigs are summarized in Table 3 and Supplemental Table 3. A progressive reduction of IS was observed during the first week after I/R (Figure 4C and 4D). Matching the temporal variations in CMR-MaR, CMR-estimated
myocardial salvage quantification [(MaR-IS)/MaR, %] also changed dynamically during the first week after I/R (Supplemental Figure 5).

CMR-estimated IMH and MVO are summarized in Supplemental Table 5. IMH was apparent at 24 h and peaked on day 4 post-I/R; in contrast, MVO was apparent at 120 min after reperfusion, peaking on day 1 post-I/R and progressively decreasing thereafter. The dynamics of CMR-estimated IMH are consistent with histologically evaluated IMH in the same model previously reported.11

Discussion

First demonstration of the post-infarction bimodal edema reaction in the human heart

This is the first comprehensive evaluation of STEMI patients by serial CMR to include the hyperacute post-reperfusion period (the first 3 hours). CMR scans timing was designed as per the protocol of our previous experimental studies, in which we demonstrated the existence of bimodal post-MI edema in pigs.10, 11

The main finding of the present clinical study is that, contrary to the accepted view, myocardial edema in the ischemic area after MI in humans is not stable, but rather follows a systematic bimodal pattern. An initial wave of edema appears abruptly very early after reperfusion but it is significantly attenuated by 24 hours. This is followed by a second (deferred) wave of edema several days after reperfusion reaching a plateau around 4 to 7 days after MI.

The initial wave of edema

To select the optimal timing for the first CMR scan in STEMI patients, we first analyzed the dynamics of the initial wave of edema in a series of 5 pigs; serial CMR scans were performed every 20 min until the reperfusion-related edema wave faded. Interestingly, this initial wave of
edema peaked very early, being significantly attenuated within a few hours after MI: at 180 minutes after reperfusion the edema had declined by approximately 50% from its maximum. In agreement with CMR data, quantification of myocardial water content and histological analysis at 6 hours after MI revealed partial resolution of the massive interstitial edema seen earlier after reperfusion (see Supplemental results). On the basis of these results in pigs, we decided to perform the first CMR scan in patients within a narrow 3-hour time-window after primary PCI to be able to detect the noon of the initial wave of edema.

Controversy on the bimodal post-infarction edema occurrence in humans

The recent demonstration of bimodal edema in the post-ischemic myocardium in pigs has generated intense discussion in the cardiac imaging field. Whether this phenomenon occurs in humans has been explored in 2 recent studies. Carrick and colleagues performed a longitudinal assessment of IMH and edema in 30 STEMI patients, concluding that “myocardial edema has a unimodal time course”. This population was more heterogeneous than the population examined here: 20% had an open artery on angiography (TIMI coronary flow grade 2-3) and only 30% had an anterior MI, whereas all patients in our study had an anterior infarction with an occluded artery on angiography. These factors might affect edema dynamics and visualization. In addition, patients in the Carrick, et al., study underwent 3 CMR exams within the first 10 days after MI, at 8.6±3.1 hours, 2.9±1.5 days, and 9.6±2.3 days. Importantly, the first of these examinations was performed between 4 and 12 hours after reperfusion, which according to the experimental data we present here is after the dissipation of the first edema wave. Indeed, the T2 values in the infarcted zone reported for the first CMR examination in Carrick, et al., are similar to those observed in the second scan in our clinical study, performed 24 hours after MI.
In the second report, Nordlund, et al., retrospectively analyzed pooled data from 3 studies assessing the MaR by qualitative CMR, concluding that no bimodal edema pattern was apparent. However, most patients in the evaluated studies underwent a single CMR scan at disparate times to from each other, and there were no systematic serial examinations. Importantly, no CMR scans were performed on day 0, and very few were performed on day 1 after MI. Moreover, no quantitative parametric T2-mapping was performed, despite this technique being demonstrated to improve detection and quantification of myocardial edema. Unlike these recent reports, our study was specifically designed to provide insight into the existence of bimodal edema in MI patients by mimicking time-points and CMR sequences performed in the previous experimental studies.

Implications of the bimodal edema phenomenon for quantifying MaR and salvage

On the basis of an assumed stable edematous reaction lasting for several days after MI and despite recent controversy, T2-CMR sequences have been widely used to retrospectively quantify the MaR. In the present clinical study, we show that T2 relaxation time in the ischemic region changes systematically with the post-MI timing of the exam. In parallel, we confirmed significant variation in the extent of the MaR as measured by T2W-STIR. Consistent with the drop in T2 relaxation time at 1 day post-MI, T2W-estimated MaR at this time point was significantly lower than values obtained before and subsequently.

We experimentally confirmed clinical findings by accomplishing a comprehensive CMR serial imaging study in 20 pigs subjected to reperfused MI. Remarkably, we included pre-reperfusion MDCT imaging as a reference for the assessment of MaR which otherwise we considered unethical to perform in STEMI patients. In the experimental study, our results show that, due to the bimodal pattern of post-I/R edema formation, the extent of MaR delineated by
T2-CMR varies during the first week after I/R. Specifically, the edema-sensitive T2W-STIR CMR sequence overestimates MaR as compared to MDCT at early time points (120 min) after reperfusion which is in agreement with previous reports. This overestimate is mainly driven by swelling of the reperfused myocardium. By 24 hours, the scenario is completely altered, with a substantial resorption of edema and normalization of T2 relaxation time, resulting in systematic underestimation of MaR by CMR. This underestimation resulted in biologically implausible negative myocardial salvage data at 24 hours. This finding reinforces the idea that MaR (and consequently salvaged myocardium) cannot reliably be quantified by CMR around this time point. Conversely, on days 4 and 7, CMR-measured MaR was similar to MaR measured by MDCT.

More pronounced dynamic tissue changes were shown in the experimental model. This is a common phenomenon seen in the experimental setting in which many variables are controlled, as opposed to clinical studies. In addition, the more severe ischemic process in the porcine myocardium in the presence of poor collateral circulation, among other reasons, could influence the magnification of this phenomenon. However, the parallel courses of T2 and CMR-MaR fluctuations observed in the clinical and experimental settings strengthen the message of the present study. Thus, our data suggest that between day 4 and day 7 would be a good compromise solution for the delineation of theoretical MaR.

Nevertheless, our results highlight the need for caution in interpreting CMR of the post-MI heart. In the clinical study, three out the sixteen STEMI patients showed more limited changes in T2 and extent of edema (see Supplemental Figures 3 and 4). Remarkably, these three patients were older and showed significantly smaller infarcts and less extent of IMH and MVO, and greater myocardial salvage areas despite having longer intervals between symptom onset to
reperfusion (data not shown). We speculate that there might be several factors affecting the dynamics of the bimodal edematous reaction such as the existence of preformed collateral circulation, episodes of spontaneous reperfusion/re-occlusion during ischemia duration, or the presence of specific comorbidities. The impossibility of controlling these aspects in the clinical scenario, and the limited sample size preclude any definitive conclusion in this regard, but warrants further studies.

As compared to T2W-STIR, parametric T2-mapping might improve the detection and quantification of myocardial edema, but it is unlikely to alter the dynamic pattern of post-MI edema that is due to pathophysiological phenomena. The deferred edema wave is related to the post-MI healing process, and therefore interventions that protect the myocardium could affect the dynamics of edema, and thus bias MaR estimation. This idea is supported by recent suggestions that the extent of edema can be affected by the degree of damage or exposure to infarct-limiting interventions. However, patients in these studies received one CMR examination at a single time point, which was not the same for all. Therefore a dedicated study would be needed to provide evidence to support this hypothesis.

**Intramyocardial hemorrhage is not the main mechanism underlying bimodal post-MI edema**

In the clinical study, IMH, assessed by T2W-CMR, peaked around day 4 after reperfusion. This finding is in agreement with the present experimental CMR data and histologically validated data from our previous pig study. Given that T2 can be affected by hemorrhage, some authors have argued that the bimodal post-MI T2-CMR pattern could be explained entirely by the destructive paramagnetic effects of deoxyhemoglobin, rather than by a real fluctuation of tissue water content. However, if hemorrhage was the sole explanation for the bimodal T2 pattern, it
would be difficult to understand why T2 (both in the pig model and in the present clinical study) and water content (in the pig model) increased to day 4, coinciding with the maximum extent of hemorrhage. In fact, in the clinical study we observed no significant influence of IMH on T2 relaxation time or area of edema delineation. The finding that infarcted (either with or without IMH areas) and salvaged myocardium displayed the same bimodal pattern strengthen our results. In line with our data, Carrick, et al., found small differences in T2 (< 5 ms) between patients with and without hemorrhage, while Hammer-Hansen, et al., found that T2 relaxation time differed in the infarcted and salvaged myocardium, and both were significantly longer than remote in the post-reperfused dog heart. Interestingly, the later study followed animals at 4 and 48 hours after MI with results indicating a partial resolution of edema in the first 48 hours after reperfusion. Nevertheless, hemorrhage might exert some influence on T2 relaxation time, as we previously conceded.

**Dynamics of infarct size over the first week after MI**

Consistent with previous observations, our experimental data show a progressive decrease of CMR-based IS. The substantial swelling of the early post-reperfused myocardium might explain the large IS detected in our experimental study 120 minutes after reperfusion. The early period after reperfusion is associated with significant transient expansion of extracellular volume (ECV); gadolinium gets trapped in this expanded ECV, but when ECV recedes, gadolinium no longer stays in this area. In this interpretation, acutely detected LGE does not necessarily equate to irreversible injury and may severely distort estimates of salvaged myocardium. These data highlight the importance of performing CMR infarct imaging within a consistently defined and narrow time frame, preferably at the end of the first week, when using IS as an endpoint in clinical trials during the acute post-MI period.
Chronotherapeutic approaches

New treatments demonstrating significant promise in preclinical experiments frequently produce no benefits in clinical trials,\textsuperscript{1, 39} and the present results hint that timing of intervention might be a key determinant of this mismatch.\textsuperscript{40-43} We believe the discovery of the bimodal nature of post-MI edema will help in the design and new therapies for reducing infarct size and post-MI left ventricular dysfunction.

In summary, we present the first demonstration that myocardial edema after MI is not stable in patients but instead follows a bimodal pattern, confirming recent experimental findings in pigs. The identification of such pattern has important biological, diagnostic, prognostic, and therapeutic implications, and opens a route to further exploration of factors influencing this phenomenon. Remarkably, this bimodal edematous response after MI has a major impact on CMR-MaR–and consequently–myocardial salvage quantification given that measures of edema are greatly influenced by the timing of post-MI imaging.

Limitations

Only anterior STEMI patients were recruited to the clinical study. The reasons for this choice include the avoidance of possible magnetic-field non-homogeneity related to the inferolateral wall.\textsuperscript{4, 29} These eligibility criteria closely resemble recommendations for patient selection in clinical trials of cardioprotective interventions.\textsuperscript{39, 44} The bimodal edema pattern may occur regardless of MI location; however, caution should be exercised when extrapolating results to other MI locations, especially regarding adequate visualization of the phenomenon by T2W-CMR in lateral MI where signal loss due to through-plane cardiac motion might occur.\textsuperscript{18} Given that patients were serially scanned, including one examination very early after reperfusion, we planned the shortest CMR protocol possible. For this reason we did not include T2* CMR as a
diagnostic method for quantifying IMH in vivo. Instead, we assessed both edema and hemorrhage by T2W-STIR imaging, a sequence validated and used for these purposes by many authors.

Extrapolation of the experimental results to the clinic should be done with caution. Nonetheless, the pig is one of the most clinically translatable large animal models for the study of reperfused MI. The similar edema and hemorrhage time-courses in the pig and the patient cohort highlight the great translational value of the pig model, especially considering the difficulty of performing a comprehensive CMR study that includes serial examinations within the first hours after reperfusion and reference techniques for the assessment of the MaR. The fact that myocardial edema and LGE follow a disparate dynamic pattern after ischemia/reperfusion highlights the complexity of measuring myocardial salvage in real practice. Thus, acutely detected LGE does not necessarily equate to irreversible injury and may contribute to severely distort estimates of salvaged myocardium when comparing against a pre-reperfusion standard to assess MaR. Other reasons might contribute to inaccurate estimations. Among them, it has been previously demonstrated that damage after ischemia/reperfusion may extend beyond the boundaries of the hypoperfused region during coronary occlusion; that MaR might slightly shrink in MDCT performed during coronary occlusion due to lack of perfusion in animal models with poor collateral circulation; and that residual edema in salvaged myocardium might contribute to overestimate of infarct size early after reperfusion.

Finally, it is fair to acknowledge that although previous studies have validated the use of MDCT to measure MaR, there is probably no perfect method for such purpose; as there is no consensus on a standardized method for the identification of MaR on T2W-CMR imaging. Underestimation of the maximum intensity at time points exhibiting shorter myocardial T2, i.e.
24 hour and day 40, could potentially bias results in the case of the FWHM method. However, we believe the ROI selection as initial thresholding for the FWHM method did not have a significant impact in our results for several reasons. First, the blinded analysis included manual correction and visual border delineation after initial thresholding. Second, the hemorrhagic area was larger at day 4 and day 7 coinciding with the largest edematous area delineated. Third, the demonstration of a similar bimodal edema pattern by the use of a quantitative and more objective method, i.e. T2-mapping, both in the human and pig myocardium strongly supports our findings here reported.

Conclusions

Contrary to the accepted view, the post-MI edematous reaction in patients is not stable, but follows a bimodal pattern. The initial edema wave appears early upon reperfusion and dissipates by 24 h. The deferred edema wave emerges thereafter and reaches a plateau lasting from approximately day 4 to day 7 post-reperfusion. Consequently, the MaR as measured by T2W-CMR changes dynamically according to timing of the CMR exam. Timing of CMR after MI for assessing MaR and salvaged myocardium needs to be standardized. According to the data presented, a timeframe between day 4 and day 7 after reperfusion seems a good compromise solution although some other factors might affect these variables.

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Disclosures

Javier Sánchez-González is a Philips Healthcare employee. The other authors declare no conflict of interest.

References


Table 1. Baseline patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>All patients (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>58.8±14.5</td>
</tr>
<tr>
<td>Male sex</td>
<td>14 (87.5)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27.1±2.9</td>
</tr>
<tr>
<td>Hypertension</td>
<td>9 (56.3)</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
</tr>
<tr>
<td>- Current smoker</td>
<td>9 (56.3)</td>
</tr>
<tr>
<td>- Ex-smoker (0-10 y before)</td>
<td>4 (25.0)</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>10 (62.5)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>3 (18.8)</td>
</tr>
<tr>
<td>Ischemia duration, min*</td>
<td>185±115</td>
</tr>
<tr>
<td>Killip class at recruitment</td>
<td></td>
</tr>
<tr>
<td>- I</td>
<td>11 (68.7)</td>
</tr>
<tr>
<td>- II</td>
<td>5 (31.3)</td>
</tr>
<tr>
<td>Infarct artery lesion location</td>
<td></td>
</tr>
<tr>
<td>- Proximal LAD</td>
<td>16 (100.0)</td>
</tr>
<tr>
<td>- Mid LAD</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Treatment at the time of PCI</td>
<td></td>
</tr>
<tr>
<td>- Heparin</td>
<td>16 (100.0)</td>
</tr>
<tr>
<td>- Oral antiplatelet</td>
<td></td>
</tr>
<tr>
<td>- Aspirin</td>
<td>14 (87.5)</td>
</tr>
<tr>
<td>- Clopidogrel</td>
<td>8 (50.0)</td>
</tr>
<tr>
<td>- Prasugrel</td>
<td>5 (31.3)</td>
</tr>
<tr>
<td>- Ticagrelor</td>
<td>4 (25.0)</td>
</tr>
<tr>
<td>- Thrombus aspiration</td>
<td>9 (56.3)</td>
</tr>
<tr>
<td>- GP IIb/IIIa during PCI</td>
<td>9 (56.3)</td>
</tr>
</tbody>
</table>

*Mean time from symptom onset to reperfusion; LAD: left anterior descending coronary artery; GP: glycoprotein; PCI: primary percutaneous intervention. Data are presented as mean ± standard deviation, or n (%).
Table 2. Cardiac magnetic resonance data of patients

<table>
<thead>
<tr>
<th></th>
<th>Reperfusion time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤ 3 hours</td>
</tr>
<tr>
<td>T2 transmural ischemic, ms</td>
<td>80.8 (10.9)</td>
</tr>
<tr>
<td>T2 transmural remote, ms</td>
<td>52.5 (6.7)</td>
</tr>
<tr>
<td>T2 infarct incl. hypointense core, ms</td>
<td>80.5 (16.4)</td>
</tr>
<tr>
<td>T2 infarct excl. hypointense core, ms</td>
<td>87.2 (15.1)</td>
</tr>
<tr>
<td>T2 salvaged, ms</td>
<td>70.2 (9.7)</td>
</tr>
<tr>
<td>MaR, % of LV</td>
<td>39.9 (13.0)</td>
</tr>
<tr>
<td>IMH, % of LV</td>
<td>0.6 (0.5)</td>
</tr>
<tr>
<td>Infarct size, % of LV</td>
<td>-</td>
</tr>
<tr>
<td>MVO, % of LV</td>
<td>-</td>
</tr>
</tbody>
</table>

Data are presented as mean (standard deviation). T2 maps were analyzed by placing ROIs at the transmural ischemic, infarcted (with or without including areas suggestive of IMH), salvaged, and transmural remote areas 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. The different myocardial states were initially defined by the localization relative to LGE. One patient died between day-4 and day-7 CMR, and one patient was unable to undergo LGE imaging due to severe renal impairment; therefore T2 information from the different myocardial states within the ischemic region was obtained from 14 out of 16 patients.

T2 relaxation time was longer in the transmural ischemic myocardium than in the remote myocardium at all time points evaluated (≤3 hours, day1, day4, day7, and day40 after reperfusion). At the 24 hour time-point, T2 values were 65.4±5.5 ms and 57.2±6.2 ms in the ischemic and remote myocardium respectively (p<0.01). Similar results (even of greater magnitude) were shown when comparing T2 in the ischemic and remote myocardium at day 40 (65.4±5.5 ms vs. 52.9±8.3 ms, p<0.01). On the other hand, T2 relaxation time in the ischemic myocardium showed significant variations across time: T2 at 24h was statistically shorter than at ≤3 hours (65.4±5.5 ms vs. 80.8±10.9 ms, p<0.01), than at day4 (65.4±5.5 ms vs. 80.5±11.3 ms, p<0.01), and than at day 7 (65.4±5.5 ms vs. 76.8±12.1 ms, p<0.01). In contrast, T2 in the ischemic myocardium at 24h did not differ from T2 at day 40 (65.4±5.5 ms vs. 54.7±7.2 ms, p=0.99).

CMR: cardiac magnetic resonance; MaR: myocardial area at risk; LV: left ventricle; ms: milliseconds; IMH: intramycardial hemorrhage; MVO: microvascular obstruction.
Table 3. Time profile of CMR-assessed myocardium at risk, infarct size, and myocardial salvage during the first week after reperfused myocardial infarction in pigs.

<table>
<thead>
<tr>
<th></th>
<th>Follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R-120min</td>
</tr>
<tr>
<td><strong>CMR</strong></td>
<td></td>
</tr>
<tr>
<td>MaR, % of LV</td>
<td>48.1 (6.0)</td>
</tr>
<tr>
<td>Infarct size, % of LV</td>
<td>45.1 (5.3)</td>
</tr>
<tr>
<td>Myocardial salvage, %</td>
<td>4.7 (4.7)</td>
</tr>
<tr>
<td>N= 20</td>
<td>N= 15</td>
</tr>
</tbody>
</table>

Values are mean (standard deviation). CMR data for each time point correspond to pooled data from all animals undergoing 40min-I/R. N values decrease over time because 5 pigs were sacrificed after each CMR exam for histological measurement of water content. The extent of myocardium at risk was assessed by MDCT reference during coronary occlusion in all animals (MDCT-MaR). MDCT-MaR as assessed during the index coronary occlusion was 30.5±5.0%, 29.6±4.7%, 29.1±3.9%, and 28.3±4.3% of LV; for pigs followed up to 120 minutes (n=20), 24 hours (n=15), 4 days (n=10) and 7 days (n=5) after reperfusion. Myocardial salvage as assessed by MDCT/CMR [(MDCT MaR – CMR infarct size) / MDCT MaR, %] in each of these groups was -50.6±24.4% at 120 minutes, -20.4±16.9% at 24 hours, -4.8±17.1% at 4 days, and 9.1±19.9% at 7 days after reperfusion, respectively. Note that MDCT was performed in all pigs only once (during the index ischemic event). Supplemental Table 3 shows all individual data from animals sacrificed at each time point.

CMR: cardiac magnetic resonance; MDCT: multidetector computed tomography; MaR: myocardium at risk; IS: infarct size; LV: left ventricle; I/R: ischemia/reperfusion; R: reperfusion.
Figure Legends

Figure 1. Study design

(A) Clinical study design. Twenty-two consecutive anterior STEMI patients fulfilling the inclusion criteria were assessed for eligibility: 3 patients refused to participate; 1 patient experienced anxiety and a claustrophobic reaction requiring premature termination of the first CMR; 1 patient felt sick with vomiting before the first CMR, which could not be performed; and 1 patient had a failed CMR study due to frequent episodes of premature ventricular contraction and non-sustained ventricular tachycardia during the scan. The clinical study population thus included 16 consecutive hemodynamically stable anterior STEMI patients reperfused by primary PCI. Cardiac magnetic resonance (CMR) examinations including T2W-STIR and T2-GraSE mapping sequences were per protocol scheduled at the following times after reperfusion: within the first 3 hours and at 24 hours, 4 days, 7 days, and 40 days. To take account of baseline values, myocardial T2 relaxation time was measured in 16 healthy age- and sex-matched volunteers.

(B) Experimental study design. The study population comprised 20 pigs weighing 30-40 kg which underwent closed-chest 40 min reperfused acute anterior myocardial infarction. These pigs were sacrificed at 120 minutes (n=5), 24 hours (n=5), 4 days (n=5), and 7 days (n=5) after MI. Arterial enhanced multidetector computed tomography was performed during coronary occlusion in all pigs as a reference standard for measuring the myocardial area at risk. CMR scans—including T2W-STIR, T2-mapping and LGE imaging—were performed at every follow-up stage until sacrifice. CMR: cardiac magnetic resonance; STEMI: ST-segment elevation myocardial infarction; PCI: percutaneous coronary intervention; I/R: ischemia/reperfusion. MDCT: multidetector computed tomography.
Figure 2. Temporal evolution of myocardial T2 relaxation time in STEMI patients

(A) Time course of absolute T2 relaxation time (ms) in the ischemic and remote myocardium in ST-segment elevation myocardial infarction (STEMI) patients. Data are means and standard deviation. For baseline values, myocardial T2 relaxation time was measured in 16 healthy age- and sex-matched volunteers. Dashed lines represent hypothetical mean trajectories for T2 from baseline to the hyperacute post reperfusion phase (≤ 3 hours). At all time-points after reperfusion, T2 relaxation time in the ischemic myocardium of patients differed significantly from baseline values in healthy volunteers. Notably, CMR T2-mapping revealed similar T2 values at ≤3 hours and on day 4 and day 7 post-reperfusion; in contrast, T2 relaxation time was significantly lower at 24 hours and on day 40 post-MI.

(B) Representative images from an anterior STEMI patient who underwent serial CMR T2-mapping examinations at 150 minutes, 26 hours, 4 days, 7 days and 44 days after reperfusion. For baseline CMR T2-mapping, an image from a healthy volunteer is shown. All T2 maps were scaled between 30 and 120 milliseconds. ms: milliseconds.

(C) T2 values distribution in the ischemic and remote myocardium in STEMI patients at different time points. Mean and standard deviation from all individual ROIs placed in these areas at all time points were analyzed. Blue and red colors represent distribution of T2 values in the remote and ischemic myocardium, respectively. Green color represents the overlapping of T2 values, i.e. pixels from both areas having the same T2. The percentages shown in each panel represent the % of the ischemic myocardium ROI with T2 above two standard deviations from mean T2 in the remote myocardium (pink). Despite mean T2 relaxation time in ischemic myocardium at all time points was longer than mean T2 in the remote, overlapping was patent and widest at 24h time point.
Figure 3. Time profile of edematous area in STEMI patients.

(A) Time profile of edematous area in STEMI patients, evaluated by T2W-STIR imaging. Data are means and standard deviation. CMR T2W-STIR scans at \( \leq 3 \) hours and on day 4 and day 7 revealed a similar edematous area (% LV); in contrast, edematous area was significantly smaller at 24 hours and on day 40 post-MI. Note the parallel courses of T2 relaxation time fluctuations and extent of edema by CMR.

(B) Representative contiguous short-axis images from an anterior STEMI patient who underwent serial CMR T2W-STIR examinations at 150 minutes, 26 hours, 4 days, 7 days and 44 days after reperfusion.

LV: left ventricle; T2W: T2-weighted; STIR: short-tau inversion recovery.

Figure 4. Temporal evolution of CMR-MaR and infarct size after reperfused myocardial infarction in the pig model.

Time profile of (A) MaR evaluated by T2W-STIR imaging and (C) infarct size evaluated by T1-IR-TFE in pigs subjected to 40min-I/R. Arterial enhanced MDCT was performed during coronary occlusion in all pigs as a reference standard measure of MaR. Data are shown as mean ± standard error of the mean. (B, D) Representative images from a pig that underwent MDCT during coronary occlusion followed by serial T2W-STIR (B) and LGE (D) examinations at 120 minutes, 24 hours, 4 days, and 7 days after reperfusion.

MaR: myocardium at risk; LV: left ventricle; T2W: T2-weighted; STIR: short-tau inversion recovery; IR: inversion-recovery; TFE: turbo-field echo; MDCT: multidetector computed tomography.
Dynamic Edematous Response of the Human Heart to Myocardial Infarction: Implications for Assessing Myocardial Area at Risk and Salvage

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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 atroventricular 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. 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 mm², 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 mm², 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 mm² 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 mm², 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. 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. Given that the solely use of FWHM may be prompt to patchy inaccurate estimations, 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. Additionally, the size of IMH area was calculated by manual delineation of the hypointense areas on T2W-images 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. Hypointense areas on LGE images were included within the necrotic area. Additionally, the size of the MVO area was calculated by manual delineation of the hypointense areas on LGE images 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. 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).  

**Myocardial infarction procedure**

The MI protocol has been detailed elsewhere. 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). 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.\textsuperscript{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\textsuperscript{2} FOV and 0.45mm slice thickness by using high resolution filter (Xres Sharp).

\textit{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.

\textit{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; 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 mm², 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 mm² and a slice thickness 8 mm, TR 2 heartbeats, and eight echo times ranging from 6.7 to 53.6 ms, 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 x 280 mm, voxel size 1.6 x 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.\textsuperscript{9, 10} Additionally, the size of the area MVO was calculated by manual delineation of the hypointense areas on LGE images,\textsuperscript{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 (\%) = \([\text{wet weight}−\text{dry weight}]/\text{wet weight}\] ×100. An empty container was weighed before and after desiccation as an additional calibration control.

*Histological and immunohistochemical 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 $\text{H}_2\text{O}_2$ 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. 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 (Nanoczhoumer-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 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.

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

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.

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

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.

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>CMR measure</th>
<th>Follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>R-120min</td>
</tr>
<tr>
<td>Sacrificed at 120 min</td>
<td>MaR, % of LV</td>
<td>51.6 (6.2)</td>
</tr>
<tr>
<td></td>
<td>Infarct size, % of LV</td>
<td>47.2 (2.9)</td>
</tr>
<tr>
<td></td>
<td>Myocardial salvage, %</td>
<td>3.7 (3.4)</td>
</tr>
<tr>
<td>Sacrificed at 24 hours</td>
<td>MaR, % of LV</td>
<td>47.7 (5.7)</td>
</tr>
<tr>
<td></td>
<td>Infarct size, % of LV</td>
<td>46.8 (5.6)</td>
</tr>
<tr>
<td></td>
<td>Myocardial salvage, %</td>
<td>1.9 (1.7)</td>
</tr>
<tr>
<td>Sacrificed at 4 days</td>
<td>MaR, % of LV</td>
<td>50.0 (3.5)</td>
</tr>
<tr>
<td></td>
<td>Infarct size, % of LV</td>
<td>47.6 (3.6)</td>
</tr>
<tr>
<td></td>
<td>Myocardial salvage, %</td>
<td>4.6 (4.6)</td>
</tr>
<tr>
<td>Sacrificed at 7 days</td>
<td>MaR, % of LV</td>
<td>42.9 (5.7)</td>
</tr>
<tr>
<td></td>
<td>Infarct size, % of LV</td>
<td>39.2 (3.8)</td>
</tr>
<tr>
<td></td>
<td>Myocardial salvage, %</td>
<td>8.3 (6.4)</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>CMR measure</th>
<th>Baseline</th>
<th>R-120min</th>
<th>R-24hours</th>
<th>R-Day4</th>
<th>R-Day7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sacrificed at 120 min</td>
<td>LV mass, g/m²</td>
<td>75.8 (7.9)</td>
<td>105.5 (11.1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wall thickness ratio, ischemic/remote</td>
<td>1.07 (0.04)</td>
<td>1.91 (0.23)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sacrificed at 24 hours</td>
<td>LV mass, g/m²</td>
<td>79.6 (9.3)</td>
<td>103.8 (16.1)</td>
<td>81.5 (10.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wall thickness ratio, ischemic/remote</td>
<td>1.08 (0.07)</td>
<td>2.43 (0.53)</td>
<td>1.25 (0.06)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sacrificed at 4 days</td>
<td>LV mass, g/m²</td>
<td>72.5 (3.1)</td>
<td>108.6 (14.9)</td>
<td>81.3 (7.0)</td>
<td>81.7 (8.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wall thickness ratio, ischemic/remote</td>
<td>1.07 (0.04)</td>
<td>1.88 (0.33)</td>
<td>1.16 (0.11)</td>
<td>1.15 (0.20)</td>
<td></td>
</tr>
<tr>
<td>Sacrificed at 7 days</td>
<td>LV mass, g/m²</td>
<td>64.0 (7.4)</td>
<td>92.6 (6.0)</td>
<td>71.1 (4.7)</td>
<td>73.6 (5.2)</td>
<td>74.3 (4.7)</td>
</tr>
<tr>
<td></td>
<td>Wall thickness ratio, ischemic/remote</td>
<td>1.06 (0.18)</td>
<td>2.08 (0.36)</td>
<td>1.23 (0.13)</td>
<td>1.17 (0.15)</td>
<td>1.08 (0.06)</td>
</tr>
<tr>
<td>Pooled</td>
<td>LV mass, g/m²</td>
<td>73.0 (9.0)</td>
<td>102.6 (13.2)</td>
<td>78.0 (8.6)</td>
<td>77.6 (7.8)</td>
<td>74.3 (4.7)</td>
</tr>
<tr>
<td></td>
<td>Wall thickness ratio, ischemic/remote</td>
<td>1.07 (0.09)</td>
<td>2.07 (0.41)</td>
<td>1.21 (0.11)</td>
<td>1.16 (0.17)</td>
<td>1.08 (0.06)</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>CMR measure</th>
<th>Follow up</th>
<th></th>
<th>R-24hours</th>
<th>R-Day4</th>
<th>R-Day7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>R-120min</td>
<td>R-Day2</td>
<td>R-Day4</td>
<td>R-Day7</td>
<td></td>
</tr>
<tr>
<td>Sacrificed at 120 min</td>
<td>IMH, % of LV</td>
<td>0.0 (0.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MVO, % of LV</td>
<td>4.7 (4.8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sacrificed at 24 hours</td>
<td>IMH, % of LV</td>
<td>0.5 (0.9)</td>
<td>2.2 (1.8)</td>
<td>5.7 (2.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MVO, % of LV</td>
<td>3.8 (3.6)</td>
<td>9.9 (5.4)</td>
<td>4.7 (4.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sacrificed at 4 days</td>
<td>IMH, % of LV</td>
<td>0.2 (0.5)</td>
<td>4.1 (1.3)</td>
<td>5.7 (2.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MVO, % of LV</td>
<td>1.5 (1.3)</td>
<td>7.5 (4.9)</td>
<td>4.7 (4.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sacrificed at 7 days</td>
<td>IMH, % of LV</td>
<td>0.2 (0.5)</td>
<td>4.3 (1.8)</td>
<td>4.4 (1.9)</td>
<td>1.4 (1.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MVO, % of LV</td>
<td>5.1 (4.5)</td>
<td>6.5 (2.1)</td>
<td>2.3 (2.8)</td>
<td>1.8 (2.9)</td>
<td></td>
</tr>
<tr>
<td>Pooled</td>
<td>IMH, % of LV</td>
<td>0.2 (0.5)</td>
<td>3.5 (1.8)</td>
<td>5.0 (2.2)</td>
<td>1.4 (1.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MVO, % of LV</td>
<td>3.7 (3.7)</td>
<td>8.0 (4.3)</td>
<td>3.5 (3.9)</td>
<td>1.8 (2.9)</td>
<td></td>
</tr>
</tbody>
</table>

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, consistent with partial resolution of the initial wave of edema. Massive tissue infiltration by neutrophils (473±190 cells per mm² 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. 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.
SUPPLEMENTAL REFERENCES


edema after ischemia/reperfusion is not stable and follows a bimodal pattern: imaging and histological tissue characterization. J Am Coll Cardiol. 2015;65:315-23.

