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Contrast-Enhanced CMR Overestimates Early Myocardial Infarct Size

Mechanistic Insights Using ECV Measurements on Day 1 and Day 7

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ABSTRACT

OBJECTIVES This study aimed to investigate whether an overestimation of infarct size on cardiac magnetic resonance (CMR) versus triphenyltetrazolium chloride (TTC) exists acutely and whether it remains after 7 days in an experimental pig model and to elucidate possible mechanisms.

BACKGROUND Overestimation of infarct size (IS) on late gadolinium enhancement CMR early after acute myocardial infarction has been debated.

METHODS Pigs were subjected to 40 min of left anterior descending artery occlusion and 6 h (n = 9) or 7 days (n = 9) reperfusion. IS by in vivo and ex vivo CMR was compared with TTC staining. Extracellular volume (ECV) was obtained from biopsies using technetium 99m diethylenetriamine pentaacetic acid (99mTc-DTPA) and light microscopy. TTC slices were rescanned on CMR enabling slice-by-slice comparison.

RESULTS IS did not differ between in vivo and ex vivo CMR ($p = 0.77$). IS was overestimated by 27.3% with ex vivo CMR compared with TTC ($p = 0.008$) acutely with no significant difference at 7 days ($p = 0.39$). Slice-by-slice comparison showed similar results. A significant decrease in ECV was seen in biopsies of myocardium at risk (MaR) close to the infarct (sometimes referred to as the peri-infarction zone) over 7 days (48.3 ± 4.4% vs. 29.2 ± 2.4%; $p = 0.0025$). The ECV differed between biopsies of MaR close to the infarct and the rest of the salvaged MaR acutely (48.3 ± 4.4% vs. 32.4 ± 3.2%; $p = 0.013$) but not at 7 days (29.2 ± 2.4% vs 25.7 ± 1.4%; $p = 0.23$).

CONCLUSIONS CMR overestimates IS compared with TTC acutely but not at 7 days. This difference may be explained by higher ECV in MaR closest to the infarct acutely that decreases during 7 days to the same level as the rest of the salvaged MaR. The increased ECV in the MaR closest to the infarct day 1 could be due to severe edema or an admixture of infarcted and salvaged myocardium (partial volume) or both. Nonetheless, this could not be reproduced at 7 days. These results have implications for timing of magnetic resonance infarct imaging early after acute myocardial infarction. (J Am Coll Cardiol Img 2015;8:1379–89) © 2015 by the American College of Cardiology Foundation.

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ABBREVIATIONS AND ACRONYMS

AMI = acute myocardial infarction

CMR = cardiac magnetic resonance

ECV = extracellular volume

Gd-DOTA = gadolinium-tetraazacyclododecane-tetraacetic-acid

IS = infarct size

LGE = late gadolinium enhancement

MaR = myocardium at risk

MI = myocardial infarction

TTC = triphenyltetrazolium chloride

^{99m}TC-DTPA = technetium 99m diethylenetriamine pentaacetic acid

Cardiac magnetic resonance (CMR) is superior to other imaging modalities for determining infarct size (IS) (1) and late gadolinium enhancement (LGE) CMR is considered the gold standard for quantifying acute and chronic myocardial infarction (MI) (2,3). The pathophysiological foundation for hyperenhancement on LGE-CMR is based on an increased distribution volume of an extracellular contrast agent within scarred tissue in chronic MI and in cells that have lost their cellular integrity due to necrosis in acute myocardial infarction (AMI) (4,5). Reimer and Jennings (6) described an ischemic zone histopathologically in AMI containing viable myocytes adjacent to the infarction as the border zone, i.e., salvaged myocardium. Arheden et al. (4,5) showed that salvaged myocardium (myocardium at risk [MaR] – infarction) had an increased extracellular volume (ECV) compared with normal myocardium measured by CMR and validated this by radioisotopes in rats subjected to ischemia. Experimental studies with LGE-CMR and histochemical staining acutely,

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however, have shown conflicting results regarding the presence and nature of a hyperenhanced zone of myocardium located immediately adjacent to and around the infarction that hyperenhances on CMR but is triphenyltetrazolium chloride (TTC) negative. Saeed et al. (7,8) characterized this as a "peri-infarction zone" in AMI by using a double-contrast-agent approach and found that the hyperenhanced area on CMR overestimates IS compared with TTC. Other studies have shown similar findings (9–11), but also good agreement between CMR and histopathology (12). Studies in humans have shown a decrease of hyperenhancement in the infarcted region during the first week after infarction (13,14). Resorption of edema has been proposed as a possible explanation for the reduction of hyperenhancement in the ischemically injured zone over time (7,13).

The biological mechanism behind early reduction of hyperenhancement in ischemically injured myocardium is, however, yet to be determined. Therefore, the purpose of this study was to: 1) investigate if the reported overestimation by CMR versus TTC remains after 7 days in an experimental pig model; and 2) elucidate possible mechanisms behind this phenomenon.

METHODS

EXPERIMENTAL PROTOCOL. The study conforms to the Guide for the Care and Use of Laboratory animals U.S. National Institutes of Health (NIH Publication No.85-23, revised 1996) and was approved by the local ethics committee. This experimental pig study consisted of 2 models, 1 of AMI and 1 of chronic MI. The study design is shown in **Figure 1**. See Online Appendix 1:1 for details on anesthesia.

Pigs were subjected to 40 min of left anterior descending artery occlusion and all pigs surviving infarction were imaged 6 h ($n = 9$) or 7 days ($n = 9$) after reperfusion. The balloon occlusion was placed randomly either after the first or second diagonal branch of the left anterior descending artery to obtain a wide range of infarct sizes. During in vivo imaging 0.2 mmol/kg gadolinium-tetraazacyclododecane-tetraacetic-acid (Gd-DOTA) (Dotarem, Guerbet, Roissy, France) was administrated intravenously 19 ± 3 min before LGE-CMR. An additional 0.2 mmol/kg of Gd-DOTA was administered 41 ± 7 min after the first contrast injection as well as 1,000 megabecquerel of technetium 99m diethylenetriamine pentaacetic acid (^{99m}Tc-DTPA) 15 min before heart explantation. The timing of imaging ensured both agents to be in equilibrium in normal and infarcted myocardium (4). For ex vivo imaging, the explanted hearts were suspended in plastic containers with deuterated water-filled balloons in the ventricles.

POSTMORTEM PROTOCOL. After ex vivo whole heart imaging, hearts were sliced into 5-mm slices and incubated in TTC for 5 min. The slices were photographed (**Figure 2**, bottom row) on both apical and basal sides for infarct analysis. TTC analysis was performed blinded to the other techniques. See Online Appendix 1:2 for details.

CARDIAC MAGNETIC RESONANCE. All imaging was performed using a 1.5-T scanner (Philips Achieva, Best, the Netherlands). In vivo CMR was performed using a 32-channel cardiac coil. LGE-CMR was acquired with a 2-dimensional phase-sensitive inversion recovery and 3-dimensional inversion recovery sequence. A T2-weighted short tau inversion recovery sequence was used to assess MaR in vivo (15).

Ex vivo imaging of whole hearts were performed using a head coil. Images were acquired covering the entire left ventricle using a high resolution (0.5 × 0.5 × 0.5 mm) T1-weighted sequence (12) and a T2-weighted turbo spin echo sequence to assess MaR (16).

Ex vivo imaging of TTC-stained slices with apparent necrosis was performed with the same T1-weighted sequence as described above (**Figure 2**). See Online Appendix 1:3 for CMR parameters.

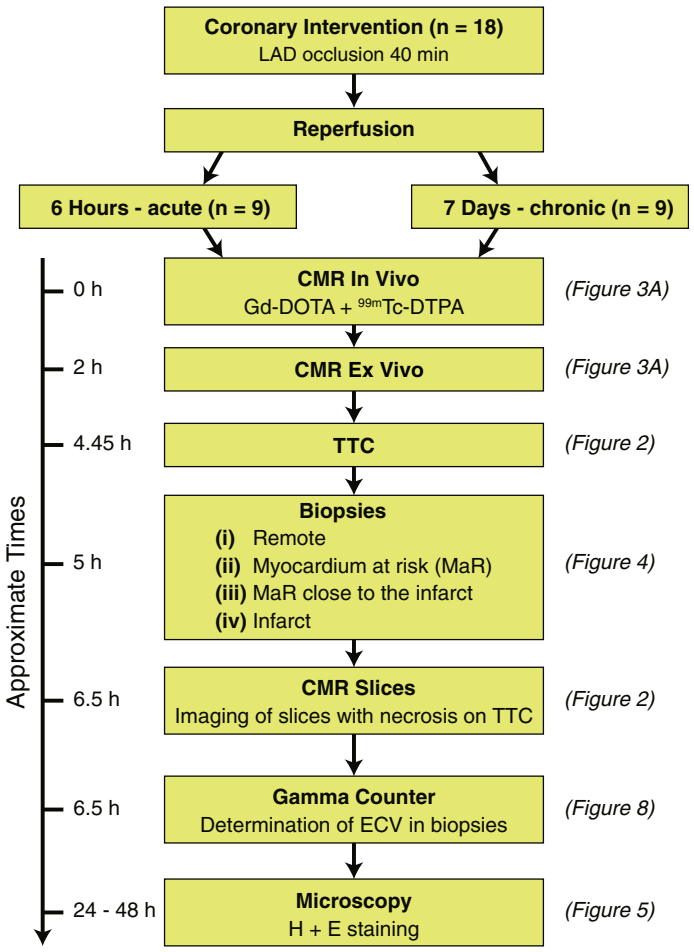
IMAGE ANALYSIS. The software Segment version 1.9 (Medviso AB, Lund, Sweden) was used for all image analysis. Analysis of in vivo LGE-CMR images (**Figure 3A**) was performed by manually delineating endocardium and epicardium followed by using a semiautomatic algorithm for infarct quantification (17). In vivo T2-weighted short tau inversion recovery images were delineated manually. Maximal wall thickness in infarcted and remote myocardium was measured on in vivo LGE-CMR in short axis slices.

For ex vivo whole heart contrast-enhanced images (**Figure 3A**), a threshold of 8 SD was used to quantify IS (17). T2-weighted images were delineated manually (16). Myocardial salvage index, defined as: 1 - (IS/MaR), was calculated both acutely and at 7 days using IS from both ex vivo CMR and TTC. Ex vivo TTC-stained slices (**Figure 2**) were analyzed by manually delineating the endocardium, epicardium, and infarction. Ex vivo magnetic resonance images of TTC-stained slices were analyzed on both basal and apical sides using the same threshold methodology as for ex vivo whole heart analysis. The outermost layer of the ex vivo CMR slices was identified using a novel off-line post-processing method, enabling direct comparison with photographed TTC slices. See Online Appendix 1:4 for details.

EXTRACELLULAR VOLUME ANALYSIS. For each animal, 4 consecutive slices with infarct by TTC were chosen, and 2-mm cylindrical biopsies were taken from 4 regions approximately 3.5 h after euthanization. One biopsy per slice (**Figure 4**) was taken in remote myocardium, 3 to 4 biopsies in MaR defined as part of the salvaged myocardium (i.e., with 4 to 6 mm proximity to the infarct), 2 to 3 biopsies in the MaR closest to the infarct (sometimes referred to as the peri-infarction zone) within the salvaged myocardium, that is, immediately adjacent to the solid infarct (approximately 2 mm from the TTC-positive tissue) and 1 biopsy in the infarction. Biopsies were taken from areas without microvascular obstruction/hemorrhage and biopsies in the salvaged MaR were taken epicardial/lateral to the biopsies of MaR closest to the infarct. Ex vivo T1-weighted imaging of the TTC-positive slices with biopsies and ex vivo T2-weighted imaging was performed to independently confirm the location of biopsies in order to ensure that biopsies were taken in areas with abnormal T2 signal. ECV was calculated using Equation 1, since ^{99m}Tc-DTPA distributes in the extracellular space similar to Gd-DOTA (4).

$$\text{ECV} = (\text{counts}_{\text{biopsy}}/\text{weight}_{\text{biopsy}})/(\text{counts}_{\text{plasma}}/\text{weight}_{\text{plasma}}) \quad \text{[Equation 1]}$$

FIGURE 1 Study Design



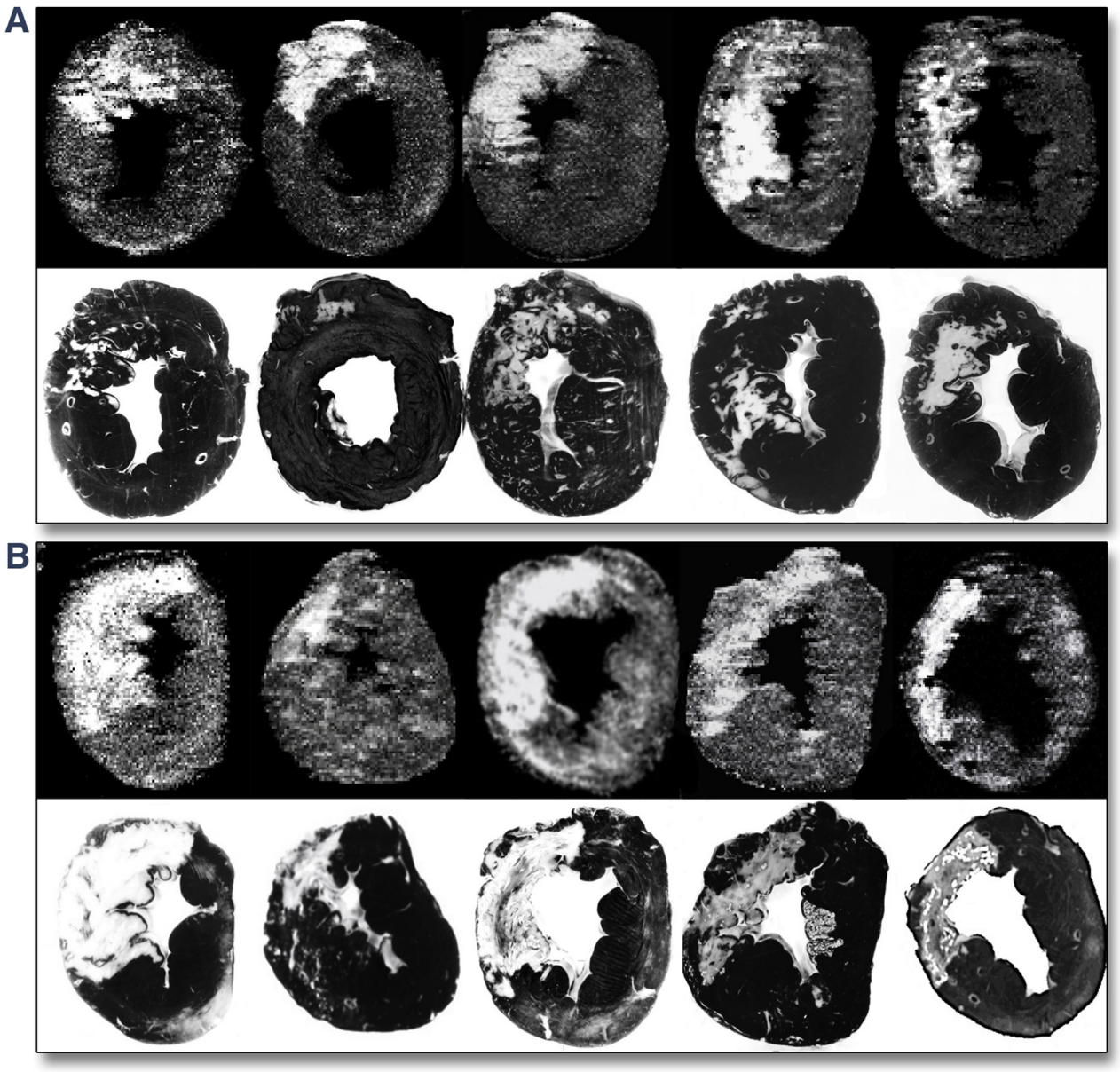
The flow chart shows the different steps from coronary artery occlusion to light microscopy. CMR = cardiac magnetic resonance; ECV = extracellular volume; H+E = hematoxylin-eosin; LAD = left anterior descending; TTC = triphenyltetrazolium chloride.

The average ECV for each region was calculated in each animal and subsequently used in the analysis. See Online Appendix 1.5 for details.

MICROSCOPY. Representative biopsies from all 4 regions were stained with hematoxylin-eosin and examined under a light microscope (**Figure 5**, Online Appendix 1:6).

STATISTICAL ANALYSIS. Results are expressed as mean ± standard error of the mean if not stated otherwise and Pearson correlation, Student paired and unpaired *t* tests, and bias according to Bland-Altman were used for comparison of IS, MaR, differences in ECV by microscopy and ^{99m}Tc-DTPA, and wall thickness. However, when using TTC as reference standard for CMR infarct size, bias was calculated as: (CMR-TTC)/TTC × 100. A random

FIGURE 2 Ex Vivo CMR and TTC of Short-Axis Slices



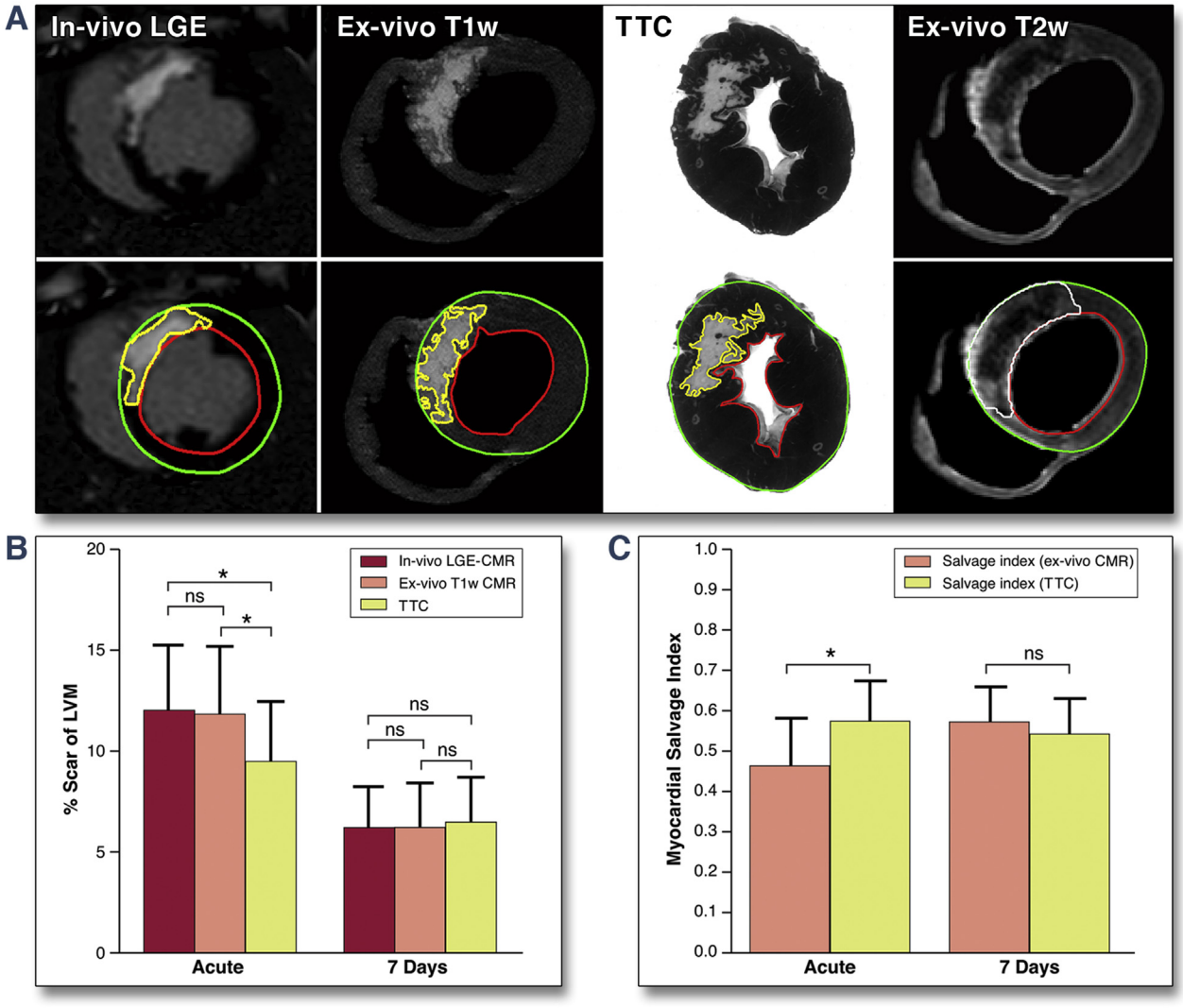
Corresponding CMR **(top row)** and TTC **(bottom row)** slices acutely **(A)** and at 7 days **(B)** after rescanning the photographed TTC stained slices. Note the hyperenhanced areas on CMR without apparent necrosis on the corresponding TTC slices acutely, not seen at 7 days. The method of off-line processing (Online Appendix 1:4) explains the lower signal-to-noise in the magnetic resonance images.

intercept mixed model was performed to correct for multiple observations within animals. Differences with $p < 0.05$ were considered statistically significant.

RESULTS

INFARCT SIZE IN AMI. Nine pigs were imaged acutely with in vivo and ex vivo CMR and analyzed with TTC staining (Figure 3A). Microvascular obstruction/hemorrhage was present in 3 animals. The IS (% scar of LVM) measured on CMR in vivo did not differ from ex vivo imaging ($p = 0.77$) (Figure 3B) and showed a high correlation ($r = 0.98$; $p < 0.0001$) and close agreement (bias: $0.2 \pm 2.1\%$). However, IS quantified on ex vivo CMR was larger compared with quantification of necrosis using TTC in whole hearts ($11.8 \pm 3.4\%$ vs.

FIGURE 3 Infarct Size and Myocardium at Risk

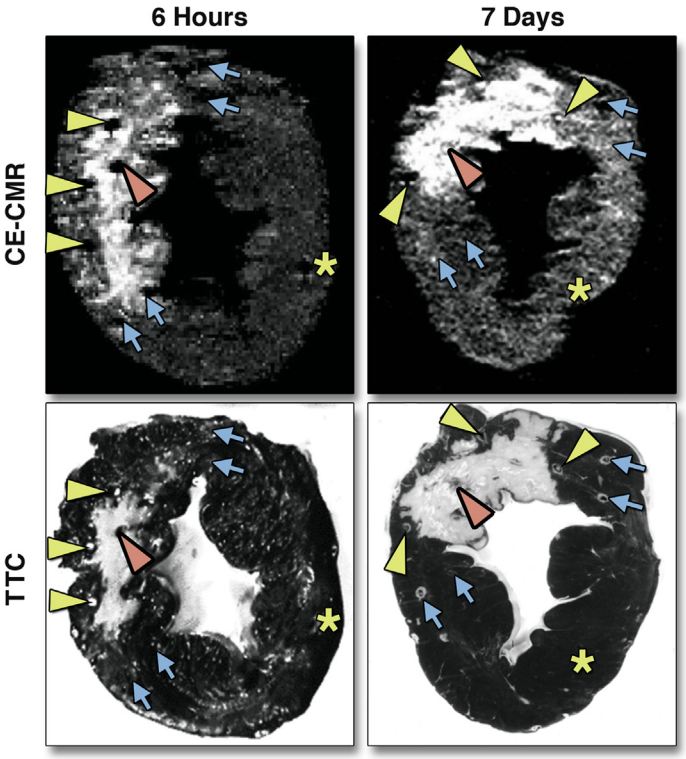


(A) Representative images of in vivo LGE-CMR, ex vivo T1w CMR, TTC-staining and ex vivo T2w CMR in one animal acutely. **(B)** Whole heart IS by in vivo CMR **(red)**, ex vivo CMR **(pink)**, TTC staining **(yellow)** acutely and at 7 days. **(C)** Myocardial salvage index with IS by CMR **(pink)** and TTC **(yellow)** respectively. IS was overestimated acutely with both in vivo and ex vivo CMR compared to TTC with no difference at 7 days. Hypoenhanced and hyperenhanced areas within the myocardium at risk **(white line)** on T2w images represent gadolinium within the infarcted myocardium and edema respectively. *$p < 0.05$. IS = infarct size; LGE = late gadolinium enhancement; other abbreviations as in Figure 1.

$9.4 \pm 3.0\%$; $p = 0.008$) (Figure 3B). This overestimation (bias: $27.3 \pm 22.2\%$) is demonstrated in Figures 6A and 6B. A comparison between ex vivo CMR and corresponding TTC slices in 5 animals after AMI is shown in Figure 2A. Furthermore, a slice-by-slice comparison between corresponding ex vivo CMR and TTC slices also showed an overestimation of IS (% scar per slice) acutely (bias: $25.5 \pm 26.9\%$; $p = 0.003$ on mixed model analysis) (Figures 7A and 7B). A larger area of hyperenhancement on CMR compared with the corresponding necrotic area on TTC indicates an increased distribution volume for Gd-DOTA in biopsies of MaR close to the infarct acutely.

INFARCT SIZE IN CHRONIC MI. Nine pigs were imaged after 7 days with in vivo and ex vivo CMR, and TTC staining. Microvascular obstruction/hemorrhage was found in 2 animals. The IS on CMR in vivo showed a high correlation ($r = 0.99$; $p < 0.0001$) and close agreement with ex vivo imaging (bias: $0.1 \pm 1.2\%$). In contrast to acutely, there was no significant

FIGURE 4 Biopsies on Ex Vivo CMR and TTC



Representative biopsies from AMI and at 7 days from 4 areas of the myocardium: remote myocardium **(yellow star)**, myocardium at risk (MaR) close to the infarct **(yellow arrowheads)**, salvaged MaR **(blue arrows)**, and infarction **(pink arrowhead)**.

difference in IS between in vivo and ex vivo CMR and TTC at 7 days (6.2 ± 2.0%, 6.1 ± 2.3% vs. 6.5 ± 2.2%; p = 0.36 and p = 0.39) (Figure 3B). Thus, there was no overestimation of IS by ex vivo CMR versus TTC at this time point (bias: -5.8 ± 15.0%) (Figures 6C and 6D). An example of corresponding ex vivo CMR and TTC slices in 5 different animals with chronic MI are shown in Figure 2B. A slice-by-slice comparison between IS on corresponding ex vivo CMR and TTC stained slices at 7 days showed a low bias (3.2 ± 26.4%; p = 0.87 on mixed model analysis) compared with acutely (Figures 7C and 7D).

MYOCARDIUM AT RISK, MYOCARDIAL SALVAGE INDEX, AND WALL THICKNESS. There was no statistically significant difference between total MaR on in vivo T2-weighted short tau inversion recovery compared with ex vivo T2-weighted turbo spin echo acutely (18.0 ± 3.4% LVM vs. 17.7 ± 3.5% LVM; p = 0.79; bias: 0.3 ± 3.7% LVM) or at 7 days (10.3 ± 2.6% LVM vs. 10.8 ± 2.8% LVM; p = 0.80; bias -0.4 ± 4.8% LVM). The myocardial salvage index with IS on ex vivo CMR was significantly smaller than with IS on TTC acutely (0.46 ± 0.12 vs. 0.57 ± 0.10; p = 0.007) (Figure 3C). At 7 days, there was no significant difference (0.58 ± 0.08 vs. 0.54 ± 0.09; p = 0.18) (Figure 3C). The ratio between IS on TTC and MaR was constant over 7 days (0.43 ± 0.10 vs. 0.46 ± 0.09; p = 0.81). The wall thickness was significantly larger acutely compared with 7 days in the infarcted region (9.5 ± 0.6 mm vs. 6.7 ± 0.3 mm; p = 0.0002) with no difference in remote myocardium (6.7 ± 0.3 mm vs. 6.3 ± 0.3 mm; p = 0.46).

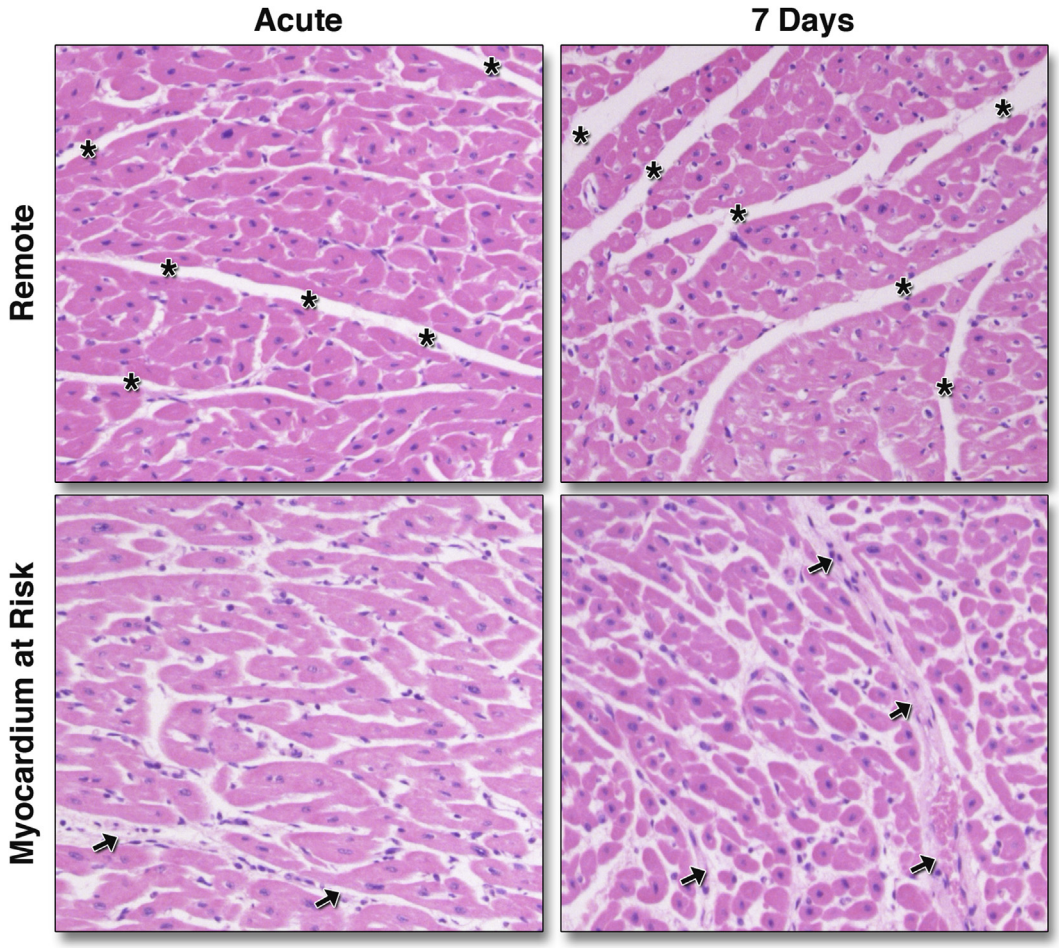
EXTRACELLULAR VOLUME. In total, 464 biopsies were taken from 7 animals in the acute and chronic cohort respectively (see Online Appendix 1:5 for details). Figure 4 shows where biopsies were taken on ex vivo CMR and corresponding TTC slice acutely and at 7 days. Figure 8 demonstrates differences in ECV between different regions at the 2 time points. There was no statistically significant difference in ECV acutely compared with 7 days in salvaged MaR (32.4 ± 3.2% vs. 25.7 ± 1.4%; p = 0.1). There was, however, a significant decrease in the biopsies of MaR close to the infarct (48.3 ± 4.4% vs. 29.2 ± 2.4%; p = 0.0025). A decrease of ECV in the infarction was also seen over 7 days (87.9 ± 7.1% vs. 61.6 ± 5.9%; p = 0.015). Furthermore, ECV in the biopsies of MaR close to the infarct was significantly higher than in salvaged MaR acutely (p = 0.013) but not at 7 days (p = 0.23). ECV of biopsies taken epicardially to the infarct in salvaged MaR was 36.1% acutely (n = 4 animals) and 23.9% (n = 1) at 7 days. In the biopsies of MaR closest to the infarct, ECV acutely was 54.5% epicardially (n = 5) and 48.6 % laterally (n = 7) of the infarction and at 7 days 27.0% epicardially (n = 5) and 29.7% laterally (n = 7).

MICROSCOPY. Microscopy confirmed findings on CMR and TTC with necrosis in the infarct acutely and at 7 days (see Online Appendix 1:6 for details). ECV by planimetry of microscopy images was higher in salvaged MaR compared with remote myocardium acute (34 ± 1% vs. 25 ± 1%; p < 0.0001) and at 7 days (33 ± 1% vs. 24 ± 1%; p < 0.0001) (Figure 5), demonstrating independently that the elevated ECV in salvaged myocardium remained at 7 days.

DISCUSSION

The present study has shown a systematic overestimation of IS early after MI with in vivo and ex vivo contrast-enhanced CMR compared with histochemical staining with TTC. This overestimation was attributed to an increased ECV, measured by radioisotopes in biopsies of MaR close to the infarct,

FIGURE 5 **ECV on Microscopy**



Microscopic sections (10×) from remote myocardium **(top row)** and salvaged myocardium at risk (MaR) **(bottom row)** in AMI **(left column)** and MI at 7 days **(right column)**. **Areas with a star** show rifts created during preparation and **arrows** denote blood vessels which both were excluded from analysis. This demonstrates that an increase in ECV in salvaged MaR, compared to remote myocardium, remains at 7 days following MI.

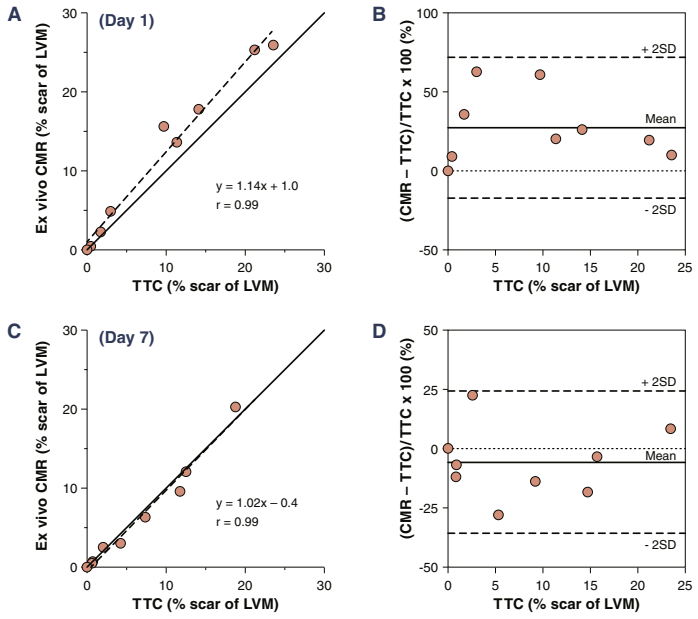
with corresponding contrast enhancement in the TTC-negative zone. However, no difference in IS was seen at 7 days between in vivo and ex vivo CMR compared with TTC. The better agreement between methods at 7 days compared with acutely is likely explained by a decrease in ECV in biopsies of MaR closest to the infarct. These results are valid both for whole heart comparison as well as for a direct slice-by-slice comparison between hyperenhanced areas on CMR and necrosis/scar on TTC using novel methodology.

HYPERENHANCEMENT EARLY AFTER INFARCTION. Our findings are in line with previous studies which found an average overestimation of IS in AMI with ex vivo CMR compared with TTC-staining, recalculated as: (CMR - TTC)/TTC × 100, by 14% to 50% (8,10,11). Several studies have described a reduction of IS in humans during the first week after AMI, hence 7 days was chosen as a time point for measuring IS (13,14).

Saeed et al. (8) showed an overestimation of IS in reperfused MI in rats using an extracellular contrast agent (Gd-DTPA) acutely (days 1 and 2) compared with TTC staining and a necrosis-specific contrast agent, attributing the overestimation to that Gd-DTPA "encompasses viable (peri-infarction zone) and nonviable portions" of the myocardium.

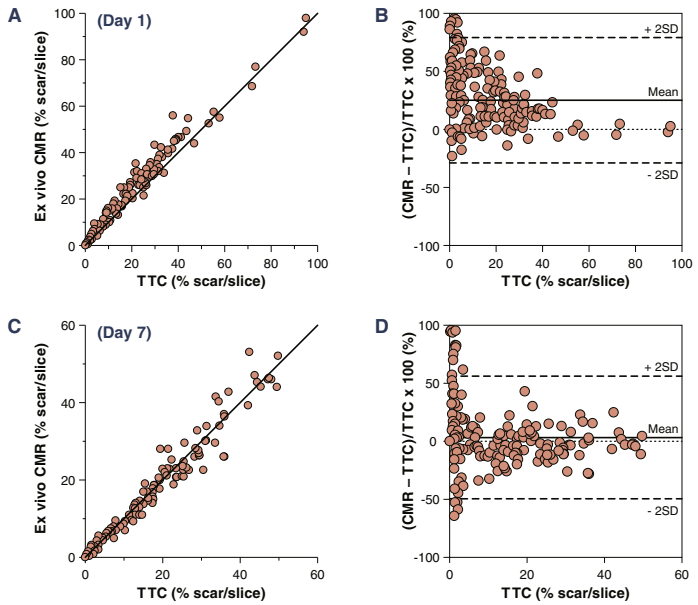
On the other hand, Kim et al. (12) showed a close agreement of both reperfused and nonreperfused IS on ex vivo CMR and TTC at 1 day, 3 days, and 8 weeks after infarction, concluding that "hyperenhancement does not occur in reversibly injured

FIGURE 6 Whole Heart Infarct Quantification With Ex Vivo CMR and TTC



(A) Ex vivo CMR versus TTC as reference standard in AMI. **(B)** Bias in AMI: 27.3 ± 22.2%. **(C)** Ex vivo CMR versus TTC at 7 days. **(D)** Bias at 7 days: −5.8 ± 15.0%. Thus, IS is overestimated by ex vivo CMR acutely but not at 7 days. **Left panels: solid lines** = line of identity, **dashed lines** = linear regression. LVM = left ventricular mass.

FIGURE 7 Slice-by-Slice Comparison of IS Between Ex Vivo CMR and TTC



(A) Ex vivo CMR versus TTC as reference standard in AMI. **(B)** Bias in AMI: 25.5 ± 26.9%. **(C)** Ex vivo CMR and TTC at 7 days. **(D)** Bias in MI at 7 days: 3.2 ± 26.4%. Ex vivo CMR overestimated IS compared to TTC acutely but not at 7 days. **Left panels: solid lines** = line of identity. Abbreviations as in Figure 1.

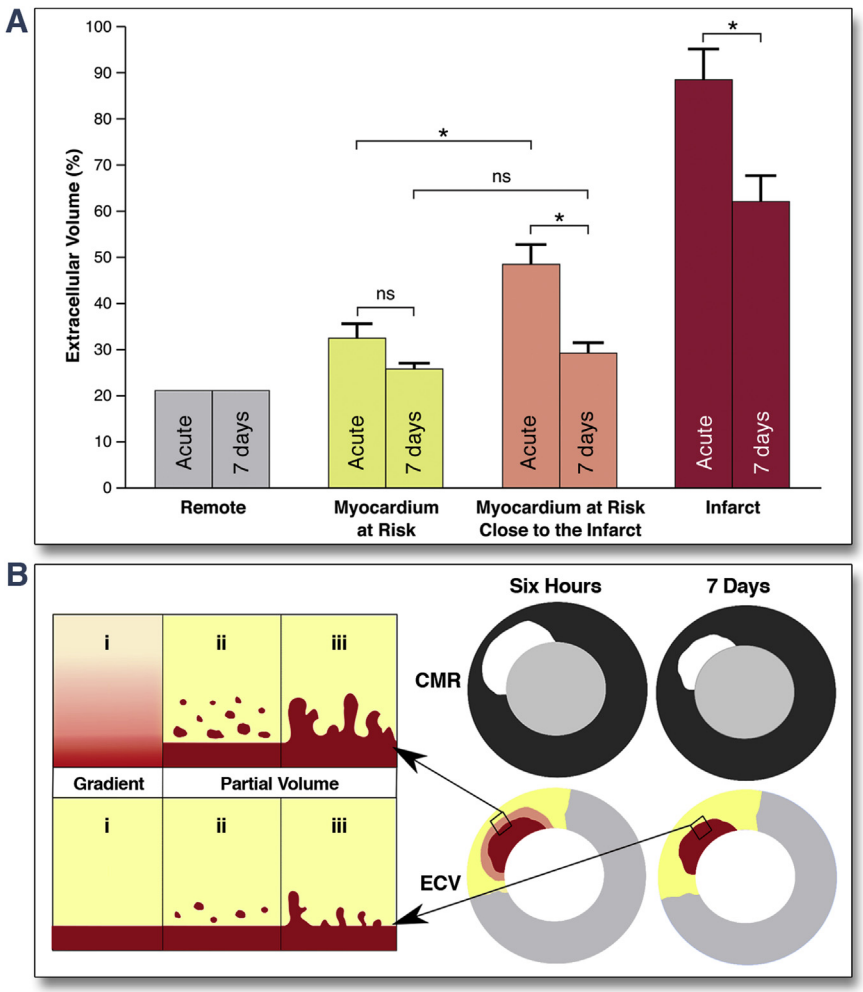
regions." However, these investigators did not "exclude the possibility of enhancement of a peripheral region surrounding the infarction that resolves by 24 hours."

EXTRACELLULAR VOLUME. Arheden et al. (4,5) showed that ECV can be measured by CMR using T1 measurements before and after contrast injection. Furthermore, the authors also showed an increased ECV in the salvaged MaR compared with normal myocardium, similar to the present study. The difference seen between days 1 and 7 is that the biopsies of MaR closest to the infarct have an ECV in between that of the infarction and salvaged myocardium. Thus, there is an increased ECV in the biopsies of MaR closest to the infarct seen acutely but not at 7 days after MI (Figure 8). This could be explained by the following mechanisms that diminish at 7 days: 1) severe edema in MaR closest to the infarct; or 2) partial volume effect due to an admixture of viable and necrotic cells due to islands or peninsulas of necrotic tissue reaching into the still viable MaR or a combination of these (Figure 8B). However, if this increased ECV in MaR closest to the infarct would be solely explained by partial volume effect due to sampling of an admixture of viable and necrotic cells acutely, then this should be distinguishable also at 7 days. This was however not seen, possibly explained by resorption of edema and/or remodeling of the infarct border with reduced patchiness (Figure 8B).

ECV in the infarction and wall thickness of the infarcted myocardium was significantly decreased at 7 days compared to acutely which likely is attributed to resorption of edema and infiltration of inflammatory cells. Microscopy demonstrated an elevated ECV in salvaged MaR compared with remote myocardium and, more interestingly, that ECV in this region was constant over 1 week. This observation is in line with biopsy findings in the present study and previous work in humans (15) and provides independent pathophysiological evidence for the validity to use T2-weighted CMR (18) or T1 mapping (19) to quantify MaR during the first week after AMI.

MYOCARDIAL SALVAGE. Due to the rate of infarct evolution, 50% of MaR is irreversibly injured in pigs after 40 min of ischemia, therefore, this period of occlusion was chosen in the present study (20). In contrast to dogs, who experience a wavefront progression of infarction, pigs show an archipelago-like progression of infarction with more scattered islands of necrosis inside MaR, which yields a salvaged MaR not only epicardial to the infarction but also laterally (16). Lateral extension of MaR is also seen in humans

FIGURE 8 ECV in AMI and MI at 7 Days



(A) ECV acutely and at 7 days after MI are shown for biopsies from: remote myocardium, salvaged myocardium at risk (MaR), MaR close to the infarct and infarction. A significant decrease of ECV in biopsies of MaR close to the infarct was seen over 7 days ($p = 0.0025$). Furthermore, there was a significant difference in ECV between the salvaged MaR and biopsies of MaR close to the infarct acutely ($p = 0.013$), however no significant difference was seen at 7 days ($p = 0.23$). *$p < 0.05$. **(B)** Schematic drawing of a LV short-axis slice in AMI at 6 h and at 7 days. Acutely the hyperenhanced area on CMR **(white)** is larger compared to at 7 days. This is explained by an ECV gradient between salvaged MaR **(yellow)** and the biopsies of MaR close to the infarct **(pink)**. The ECV in the zone closest to the infarction, which hyperenhances on CMR acutely, decreases during 7 days to the same level as the rest of salvaged MaR. The blow up shows possible pathophysiological explanations to the findings of an increased ECV in the zone closest to the infarction acutely and at 7 days: 1) severe edema that regresses at 7 days or partial volume effect due to an admixture of viable and nonviable cells; 2) in an archipelago like pattern; and/or 3) peninsula like pattern which diminishes over 1 week.

(15,21). The current study was designed to get a range of infarct sizes, which resulted in different mean IS acutely and at 7 days. However, MaR depends on the location of the coronary occlusion and final IS depends on both duration of ischemia and size of the ischemic myocardium (22). Therefore, the myocardial salvage index was calculated for IS on both CMR and TTC and was significantly smaller acutely than at 7 days using IS on CMR, but with no difference with TTC as IS reference. Determination of accurate IS acutely has important clinical implications to determine myocardial salvage after revascularization therapy and for individual patient prognosis (23). This study shows the importance of performing CMR at a consistent narrow window after MI in cardioprotection studies. Variations in timing of CMR after infarction yield

differences in myocardial salvage index due to diminishing hyperenhancement toward the true infarct size during the first week after infarction.

STUDY LIMITATIONS. ECV of infarcted pigs was normalized against ECV from pigs without intervention, imaging, or TTC-staining for biopsies to be taken in desired regions. Before punching biopsies in the MaR closest to the infarct, both the apical and basal slices were checked for viability. The assumption was made that the apical and basal surface was representative of the whole biopsy tissue cylinder, with the possibility of partial volume effect due to sampling of both viable and nonviable myocardium in MaR close to the infarct. However, the procedure was identical acutely and at 7 days, and the observed difference between those time points is therefore likely not related to how biopsies were taken. Furthermore, no T1 mapping was performed, which could have had added value. The experimental setup did not allow for baseline examination and continuous follow-up with imaging of the same pig. Thus, 2 cohorts of pigs were imaged at 2 time points. Manual delineations were used for TTC analysis and MaR (16).

CONCLUSIONS

This study has shown that in vivo and ex vivo contrast-enhanced CMR overestimates IS acutely compared with histopathology with TTC. This overestimation acutely may be explained by increased ECV in MaR close to the infarct, which shows contrast enhancement on CMR but is negative on TTC. In the MaR close to the infarct, the ECV decreases over 7 days to the same level as the rest of the salvaged myocardium and at this time point no significant difference in IS was seen between in vivo and ex vivo CMR compared with TTC. The increased ECV seen acutely in the MaR close to the infarct, but not at 7 days, could be due to severe edema, or partial volume due to sampling of an admixture of viable and necrotic cells, or a combination thereof. These results highlight the importance of performing magnetic resonance infarct imaging acutely within a consistent narrow time period when using IS in clinical trials. Furthermore, the ECV was elevated constantly in salvaged MaR over 1 week, which renders edema imaging feasible during this time.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: When quantifying infarct size with contrast-enhanced CMR, time after infarction need to be taken into account as imaging early after infarction will overestimate infarct size due increased ECV of MaR close to the infarct.

TRANSLATIONAL OUTLOOK 1: This study shows the importance of performing CMR at a consistent narrow window after MI in cardioprotection studies. Systematic bias in the timing of CMR after infarction yields differences in myocardial salvage index due to diminishing hyperenhancement toward the true infarct size during the first week after infarction.

TRANSLATIONAL OUTLOOK 2: Accurate infarct size acutely has important clinical implications to determine myocardial salvage after revascularization therapy and for individual patient prognosis.

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KEY WORDS extracellular volume, magnetic resonance imaging, myocardial infarction

APPENDIX For an expanded Methods and Results section, please see the online version of this article.