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ORIGINAL PAPER

Validation of contrast enhanced cine steady-state free precession and T2-weighted CMR for assessment of ischemic myocardial area-at-risk in the presence of reperfusion injury

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Abstract

The purpose of the study was to validate by histopathology, contrast enhanced cine steady-state free precession and T2-weighted CMR for the assessment of ischemic myocardial area-at-risk (AAR) in the presence of microvascular obstruction (MVO). Eleven anesthetized pigs underwent CMR 7 to 10 days post infarction. The area-at-risk was measured from T2-weighted fast spin echo (T2-STIR) and contrast-enhanced steady-state free precession magnetic resonance imaging (CE-SSFP) images using semi-automated algorithms based on a priori knowledge of perfusion territory. Also, late gadolinium enhancement (LGE) was performed to measure final infarct size (FIS). Histopathological comparison with Evans blue dye to define AAR and triphenyltetrazolium chloride to define FIS served as the reference. All infarcts demonstrated MVO on LGE images. Bland–Altman analysis showed no significant bias in AAR or myocardial salvage between T2-STIR and CE-SSFP or between CMR and histopathology. The mean differences ± 2SD from Bland–Altman analysis were: AAR: Evans Blue vs. T2-STIR [0.7%; + 13.5%; − 12.1%]; AAR: Evans Blue vs. CE-SSFP [0.1%; + 13.8%; − 13.7%]; AAR: T2-STIR vs. CE-SSFP [0.7%; + 6.2%; − 4.9%]; Salvage: Evans Blue − TTC vs. T2-STIR-LGE [0.8%; + 11.1%; − 9.6%]; Salvage: Evans Blue − TTC vs. CE-SSFP-LGE [0.1%; + 9.9%; − 9.6%]; Salvage: CE-SSFP-LGE vs. T2-STIR-LGE [0.7%; + 6.2%; − 4.9%]. Both T2-STIR and CE-SSFP sequences allow for unbiased quantification of AAR in the presence of ischemia/reperfusion injury when analysed by semi-automated algorithms. These experimental data, which was validated by histopathology, supports the use of CMR for the assessment of myocardial salvage during the subacute phase.

Keywords Magnetic resonance imaging · Myocardium at risk · Edema · Salvage · Final infarct size

Abbreviations

AAR	Area-at-risk
FIS	Final infarct size
CMR	Cardiovascular magnetic resonance
CE-SSFP	Contrast-enhanced steady-state free precession magnetic resonance imaging
ECG	Electrocardiogram
LV	Left ventricular
B-SSFP	Balanced-steady-state-free-precession
FOV	Field of view
TR	Repetition time
TE	Echo time
LGE	Late gadolinium enhancement
MVO	Microvascular obstruction
TTC	Triphenyltetrazolium chloride

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Introduction

Measuring myocardial salvage is essential to quantify the potential cardioprotective efficacy of the adjunctive cardioprotective intervention in patients with myocardial infarction undergoing primary percutaneous intervention [1]. Myocardial salvage is defined as the difference between the myocardial area-at-risk (AAR) and final infarct size (FIS). Measuring myocardial AAR by cardiovascular magnetic resonance (CMR) using T2-weighted imaging for detection of myocardial edema has been challenging and data regarding the validity of T2-weighted CMR for quantifying AAR are conflicting [2]. Thus, recent data from an experimental study and patients suggest that the bright signal on T2-weighted imaging reflects myocardial necrosis rather than AAR [3]. Paradoxically, in infarcts with reperfusion injury accompanied by microvascular obstruction with and without intramyocardial hemorrhage, delineation of AAR based on myocardial edema from T2-weighted imaging is problematic. This is because the actual T2-values within the core of an infarct with MVO is not different from the remote myocardium implying that algorithms based on T2-values will tend to underestimate the AAR [4]. In addition, a bimodal pattern of myocardial edema during the first week after ischemia/reperfusion has been demonstrated both in an experimental study [5] and in humans [6] indicating that edema appears abruptly upon reperfusion dissipates at 24 h and reappears with maximum day 7 after reperfusion. These conflicting data may very well reflect differences in species and different infarct morphologies as well as different time points in regard to the CMR scans. More recently, contrast-enhanced steady-state free precession magnetic resonance imaging (CE-SSFP) has been used to quantify AAR and validated against myocardial perfusion imaging using single-photon emission computed tomography (SPECT) in humans [7, 8]. Furthermore, early gadolinium enhancement has been validated in two experimental studies showing good agreement with AAR as defined from microsphere blood flow analysis after 48 h of reperfusion [9, 10]. The CMR assessment of AAR was based on either manual delineation [7, 11] or by counting pixels with signal intensity > 2SD from remote myocardium with inclusion of hypoenhanced pixels (interpreted as MVO) within an area of hyperenhancement [8, 9]. We sought to determine whether T2-STIR and CE-SSFP accurately depict AAR in an experimental porcine model of myocardial ischemia–reperfusion injury using histopathology as the reference for both AAR and FIS. Furthermore, we hypothesized that AAR could be defined with good intra- and inter-observer variability using semi-automated algorithms based on a priori knowledge of perfusion territory even in the presence of MVO [12, 13]. According to the bimodal pattern of myocardial edema during the first week, the CMR was performed in the subacute phase.

Materials and methods

Animal model

Eleven female Danish domestic pigs weighing 40 kg were used for the experiments and the details of the experimental closed-chest porcine animal model of ischemia–reperfusion has been presented previously [4, 11]. The pigs were treated by the Danish law on animal experiments. After endotracheal intubation, anesthesia was maintained with isoflurane (2.5%) in oxygen and continuous rate infusion of fentanyl (3 mg/kg/h). The pigs were mechanically ventilated with a tidal volume of 425 mL (respiratory rate 12/min). Coronary occlusion was induced by placing a 2.5 mm angioplasty balloon in the left anterior descending artery (LAD) distal to the second diagonal branch artery and inflating it to 10 atm. The balloon occluded the LAD for 65 min and was then deflated and removed. At the end of the experiment the pigs were awakened and returned to their stables where they stayed for 7 to 10 days (average 8.5 days) before CMR imaging, harvesting, and subsequent histopathology were performed.

CMR protocol

All 11 pigs underwent CMR imaging before euthanasia. The sedation protocol was as described above except that continuous propofol infusion (12 mL/h) was used instead of isoflurane. CMR was performed using a 1.5 T Philips Achieva dStream whole body scanner with a dStream Torso 32-channel coil setup (Philips Medical Systems, Best, The Netherlands). All pigs were imaged in the supine position. First, a survey scan was performed to localise the heart and the diaphragm, and then left ventricular (LV) function was assessed using a retrospective, ECG-triggered balanced-steady-state-free-precession (B-SSFP) breath-hold cine sequence in the cardiac short-axis, vertical long axis and horizontal long axis planes. In the cardiac short-axis, the LV volume was completely encompassed by contiguous 8 mm slices with a spatial resolution of 1.22 mm × 1.22 mm and a field of view (FOV) of 288 mm × 288 mm. The following imaging parameters were used: repetition time (TR) 3.0 ms; echo time (TE) 1.5 ms; flip angle 60°; 30 heart phases. To ensure a strong T2-weigthing a T2-STIR fast spin echo sequence with a long echo time was obtained in the previously mentioned short-axis orientation to assess AAR. The sequence was navigator-gated and cardiac-triggered. The following imaging parameters were used: TR 2400 ms; TE

100 ms; echo train length 20; fat inversion time 180 ms; flip angle 90°; spatial resolution 0.54 mm × 0.54 mm in-plane; number of averages 2; slice thickness 8 mm; FOV 320 mm × 320 mm; 14 slices.

After gadolinium injection early CE-SSFP and late gadolinium enhancement (LGE) was performed to identify areas of microvascular obstruction (MVO), AAR and myocardial infarction, respectively. The intravenous bolus dose of 0.2 mmol/kg Gd-DTPA (Gadobutrol, Gadovist, Bayer Schering Pharma, Berlin) was administered manually. CE-SSFP was performed immediately after bolus injection of gadolinium in the cardiac short-axis with the same parameters as pre-contrast SSFP.

LGE was acquired 15 min after gadolinium injection using a 3D phase sensitive inversion recovery-prepared T1-weighted gradient echo sequence with the following parameters: TR 5.78 ms; TE 2.78 ms; echo train length 20; inversion time ~ 320 ms; flip angle 25°; spatial resolution 1.5 mm × 1.5 mm; slice thickness 8 mm; FOV 350 mm × 350 mm; 14 slices acquired in the LV short-axis with no interslice gap. Following CMR, the pigs were kept under anaesthesia and moved to the operating room for organ harvesting.

Histology

After midline sternotomy was performed, a snare was placed around the LAD distal to the second diagonal branch, at the same level as the previously performed balloon occlusion. Then 25 mL 10% Evans blue dye was injected into the left auricle to delineate the AAR. Subsequently, the animal was euthanized and the heart was excised. The heart was then cut into consecutive 8 mm-thick short-axis slices. The slices were then stained with 2% triphenyltetrazolium chloride (TTC) solution to delineate the infarct (infarcted, yellowish-white; non-infarcted, brick-red). Each slice was photographed with a digital camera (Nikon, Tokyo, Japan) to rmeasure myocardial AAR and infarction.

Data analysis

CMR

Two observers (WYK and ESH), blinded to the distribution of the groups, analysed all the CMR images using the semi-automatic, feely available software Segment version 1.9 R3697. First, LV volumes and function were calculated from the end-diastolic and end-systolic phases of the short-axis cine images by manual delineation of endocardial and epicardial borders. Second, myocardial infarct size was determined in LGE images by a semi-automated algorithm [14]. This algorithm accounts for partial volume effects and intermediate signal intensities by assigning a weighting to hyperenhanced voxels depending on the signal intensity, rather than classifying voxels as either 100% infarcted or normal.

The infarct size was expressed as a percentage of the LV myocardium (infarction volume/LV myocardium volume × 100%). The AAR was quantified from both T2-STIR and CE-SSFP images by semi-automated algorithms in percentage of the LV myocardium (AAR/LV myocardium volume × 100%). The algorithm for delineating AAR from T2-STIR images is based on intensity classification by an expectation maximization algorithm and utilization of a priori information on AAR. Thus, as input to the automatic algorithm, the manual delineation of endocardial and epicardial borders was used and the user defined the culprit artery as the left anterior descending artery, based on the overall appearance of the hyper enhanced region and also defined the right ventricular insertion points in T2-STIR or CE-SSFP images, to correctly rotate the maximal extent model [13]. Surface coil intensity correction and incorporation of the infarct region from LGE images was added in the algorithm delineating AAR from CE-SSFP [12]. Myocardial salvage was calculated as the difference between AAR and FIS.

All CMR images were matched and aligned.

SSFP sequences have a T2/T1 relationship that in a simplified form can be described by Formula 1 (SSFP signal with T1 and T2 relationship) [15]. In areas of myocardial edema, T2 relaxation time will increase and after gadolinium administration, the T1 relaxation time will decrease—which results in an overall increase in signal intensity in myocardial lesions with intact perfusion. Areas with microvascular obstruction will show decreased signal intensity compared to the remote myocardium.

$$Signal \approx \frac{T_2}{T_1} \frac{M_0 \sin\theta}{(1-\cos\theta)+(1+\cos\theta)} e^{-\frac{T_E}{T_2}}. \quad (1)$$

Histology

Two observers (SFP and ESH) analyzed by consensus and manual delineation all photographed images of the myocardial slices using the Adobe Photoshop software (Adobe Systems Inc., San Jose, CA, USA). First, myocardial volume was determined by manually tracing the epicardium and endocardium. Second, FIS defined as yellowish-white myocardium areas on TTC stained slices was manually delineated and expressed as a percentage of the LV myocardium (infarction area/left ventricle myocardium × 100%). Finally, the myocardial area stained with Evans blue was manually delineated, and the AAR was determined by the formula: (left ventricle wall area − Evans blue area/left ventricle myocardium × 100%).

Statistical analysis

Histograms and QQ-plots assessed data distribution. The significance of group differences was evaluated with Wilcoxon matched-pairs signed rank test. Data are presented as mean ± 95% confidence intervals. The Bland–Altman analysis was done for the comparison of between CMR and histology and limits of agreement are presented as mean difference ± 2SD. Interobserver and intraobserver agreement for CMR assessment of AAR, FIS and myocardial salvage was evaluated by Bland–Altman analysis. A value of $p < 0.05$ was considered statistically significant. All statistical analysis was performed using GrapPad Prism 7.02 (La Jolla, CA 92037, USA).

Results

Physiological values (mean/SD) obtained during CMR were: heart rate 57 ± 13 min^{-1}; LV end-diastolic volume 95 ± 21 mL; LV end-systolic volume 49 ± 18 mL; stroke volume 45 ± 10 mL, LV ejection fraction 49 ± 10%.

In Table 1, average values and statistical tests from histology, T2-STIR, CE-SSFP and LGE are presented. We observed no significant differences in AAR when comparing histology to T2-STIR and CE-SSFP. There were also no significant differences in myocardial salvage measured by CMR compared to histology. AAR measured by T2-STIR and CE-SSFP was significantly larger than FIS measured from TTC. FIS measured by TTC showed no significant difference to LGE. All infarcts demonstrated MVO on LGE images, and 3 out of 11 had intramyocardial hemorrhage verified from histopathology.

Bland–Altman analysis showed no significant bias in AAR, FIS or myocardial salvage between T2-STIR and CE-SSFP or between CMR and histopathology (Fig. 1). The mean differences ± 2SD from Bland–Altman analysis were: AAR: Evans Blue vs. T2-STIR [0.7%; + 13.5%; − 12.1%]; AAR: Evans Blue vs. CE-SSFP [0.06%; + 13.8%; − 13.7%]; AAR: T2-STIR vs. CE-SSFP [0.7%; + 6.2%1; 14.9%]; Salvage: Evans Blue − TTC vs. T2-STIR-LGE [0.8%; + 11.1%; − 9.6%]; Salvage: Evans Blue − TTC vs. CE-SSFP-LGE [0.1%; + 9.9%; − 9.6%]; Salvage: CE-SSFP-LGE vs. T2-STIR-LGE [0.7%; + 6.2%; − 4.9%].

In Fig. 2, the correlations between histology, T2-STIR and CE-SSFP are presented showing an overall significant correlation.

Representative matched cross-sectional images with the delineated infarct and AAR are shown in Fig. 3.

The interobserver and intraobserver agreement for CMR assessment of AAR, FIS and myocardial salvage is shown in Table 2. Overall both T2-STIR and CE-SSFP showed good agreement for assessment of AAR while the interobserver variability for assessment of myocardial salvage was poorer due to the combined variability in assessment of both AAR and FIS.

Discussion

Our results demonstrate that T2-STIR and CE-SSFP CMR sequences using semi-automated algorithms allow for unbiased assessment of AAR and myocardial salvage in a clinically relevant experimental porcine model of myocardial ischemia–reperfusion injury using histopathology as the reference for FIS and AAR. Previous experimental studies showed similar good agreement for AAR measured

Table 1 Area-at-risk (AAR), myocardial salvage and final infarct size (FIS) presented by average values ± 2 SD

	Average value (%)	Histology vs. MR	T2-STIR vs. CE-SSFP
AAR			
Histology (Evans Blue)	35.3 ± 9.7%		
T2-STIR	34.5 ± 5.5%	p = 0.52	
CE-SSFP	35.2 ± 6.3%	p = 0.64	p = 0.52
Salvage			
Histology (Evans Blue-TTC)	11.3 ± 6.7%		
T2-STIR-LGE	10.5 ± 5.0%	p = 0.97	
CE-SSFP-LGE	11.2 ± 4.3%	p = 0.76	p = 0.52
FIS			
Histology (TTC)	24.0 ± 6.4%		
LGE	24.0 ± 4.6%	p = 0.97	
T2-STIR		p = 0.001	
CE-SSFP		p = 0.001	

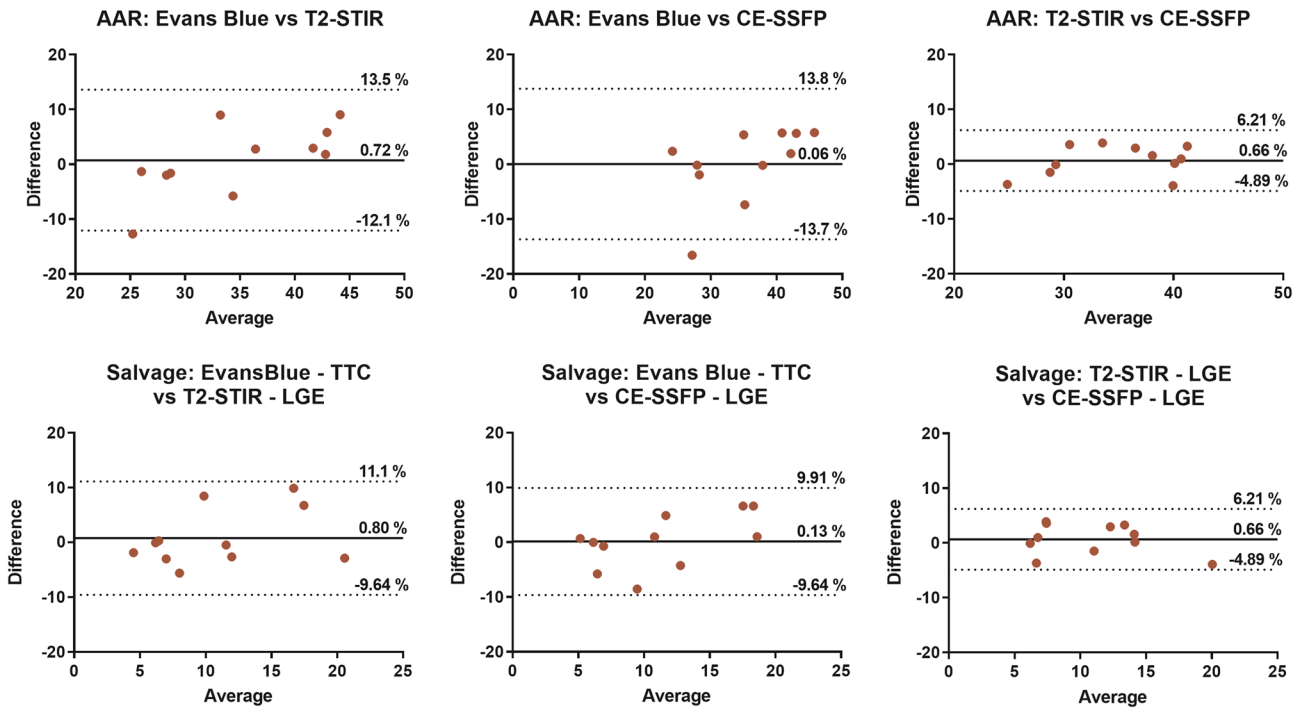

Fig. 1 Bland–Altman plots of AAR and Salvage. In the Bland–Altman plots, solid lines represent the mean and dashed lines represent the upper and lower limits of agreement

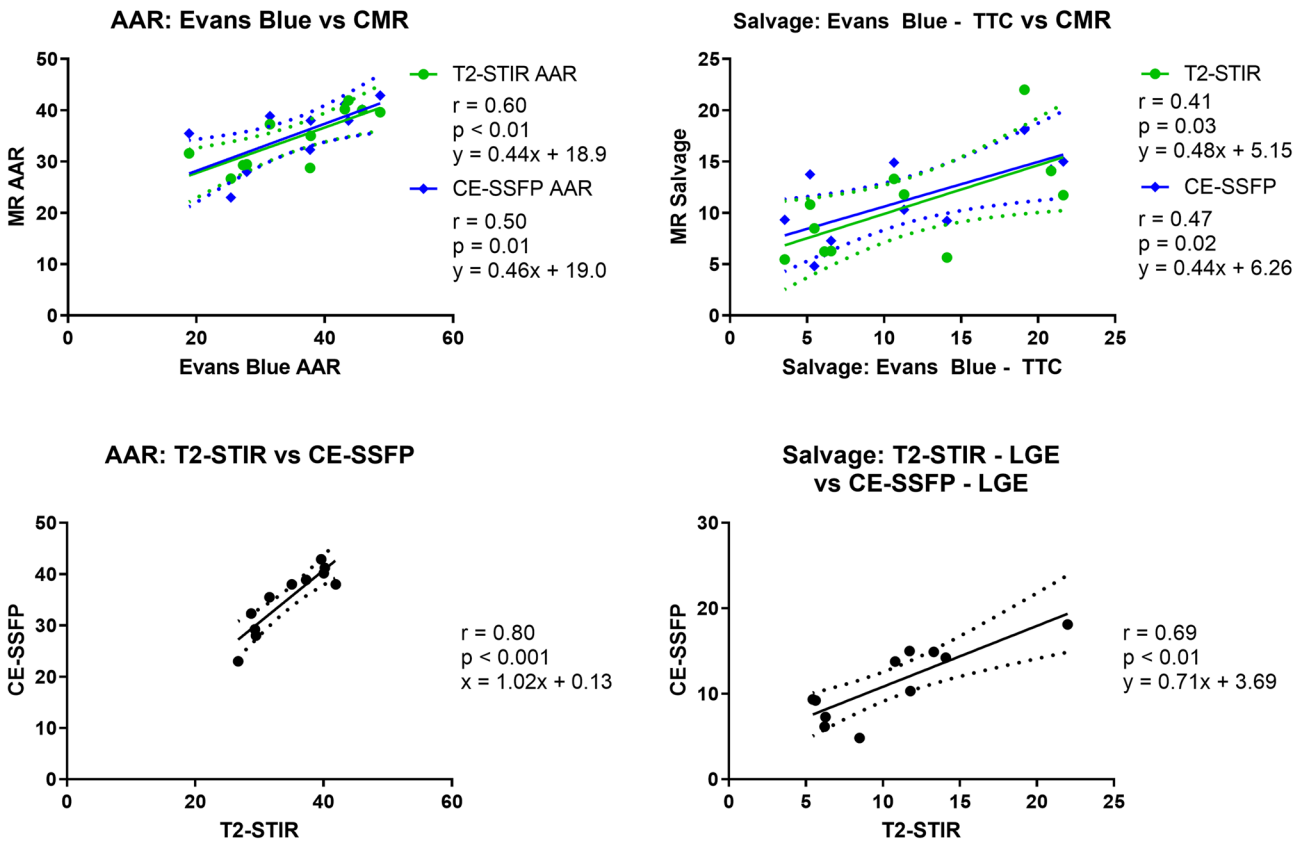

Fig. 2 Scatterplots for AAR and Salvage measurements. CMR sequences were compared to the reference histology and in between sequences

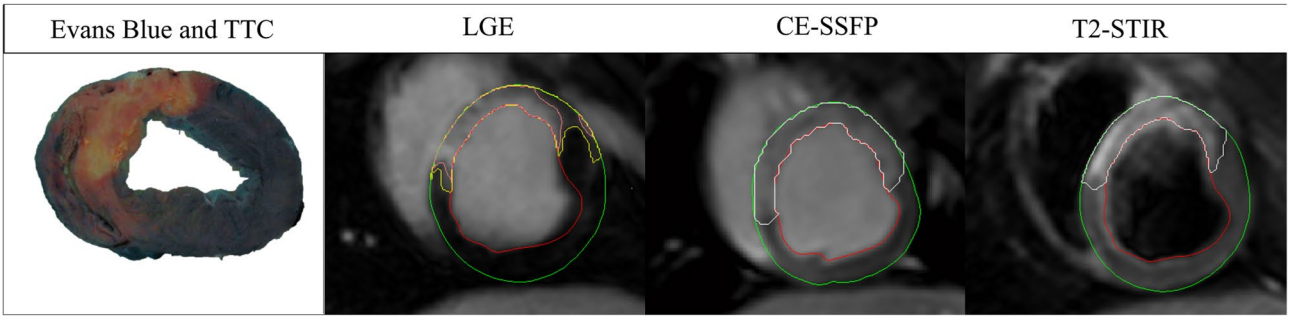

Fig. 3 Representative images showing histopathology, LGE, CE-SSFP and T2-STIR

Table 2 Inter- and intra-observer agreement for CMR assessment of area-at-risk (AAR), myocardial salvage and final infarct size (FIS) presented by average differences ± 2 SD of differences

	Inter-observer	Intra-observer
AAR		
T2-STIR	[− 0.38; + 3.10; − 3.9]	[− 0.30; + 1.2; − 1.8]
CE-SSFP	[2.6; + 6.9; − 1.8]	[0.4; + 2.5; − 1.7]
Salvage		
T2-STIR-LGE	[0.02; + 6.1; − 6.0]	[− 0.65; + 1.6; − 2.9]
CE-SSFP-LGE	[3.0; + 9.9; − 2.7]	[0.02; + 3.0; − 2.9]
FIS		
LGE	[− 0.4; + 3.5; − 4.3]	[− 0.3; + 2.2; − 1.5]

from CMR compared to SPECT after 6 h [7] and compared to microspheres after 48 h [8]. In the present study CMR was performed 7 to 10 days after infarction, which according to previous studies is a suitable time point to measure AAR since myocardial edema after ischemia–reperfusion is bimodal reaching a plateau at 4 to 7 days postinfarction [4, 5]. The pathophysiological basis for depicting the AAR by T2-STIR is the occurrence of myocardial edema as a consequence of myocardial ischemia followed by reperfusion. The AAR assessment by CE-SSFP CMR was in close agreement to T2-STIR indicating that early contrast enhancement represents similar pathology since in the presence of myocardial edema, the extracellular volume will increase [9]. Much of the debate regarding quantification of AAR by T2-weigthed CMR also involves the segmentation analysis and most often either manual tracings or predefined threshold based on signal intensities are used. Neither approach is ideal and both approaches will tend to underestimate the AAR in the presence of MVO due to the fact that T2-values within the core of the infarct are not different from the remote myocardium [4]. In the present study, semi-automated delineation of AAR [12, 13] and FIS [11] was done using the freely available software Segment version 1.9 R3697. The proposed algorithms are based on a priori knowledge of perfusion territory such that the operator needs to define the infarct location. The advantage of such a priori knowledge is the robustness since even in the presence of subtle signal changes the algorithms will depict an AAR representing the infarct location. In comparison algorithms which are strictly based on arbitrary signal intensities are very much dependent on good to excellent image quality. Intraobserver agreement for assessment of myocardial salvage was good while the interobserver agreement as expected was poorer using the semiautomated algoritms. The reason for this is largely due to the manual delineation of the left ventricular myocardium which was done since the automated contouring was not accurate in most cases. Therefore, when measuring myocardial salvage from CMR it is recommended that one experienced observer analyses the dataset. In the future multi-contrast CMR with fully automated and quantitative assessment of myocardial salvage may be possible provided that the different pathologies, which will occur in the presence of reperfusion injury, can be resolved and correctly quantitated.

Study limitations

Since only LAD infarcts were studied other infarct locations may affect the CMR quantification of AAR and FIS using algorithms that are based on a priori knowledge of perfusion territory. Future studies in patients will be required to test these algorithms in different infarct locations. The optimal timing of the CMR examination postinfarction for optimal assessment of AAR using STIR and CE-SSFP sequences was not studied. Therefore, the present results are only valid during the subacute phase, i.e. 7 to 10 days post infarction.

Conclusion

Both T2-STIR and CE-SSFP sequences allow for unbiased quantification of AAR in the presence of ischemia/reperfusion injury with microvascular obstruction when analysed by semi-automated algorithms based on a priori knowledge of perfusion territory. These experimental data, which was

validated by histopathology, supports the use of CMR for the assessment of myocardial salvage during the subacute phase.

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