ESC European Society of Cardiology

European Heart Journal - Cardiovascular Imaging (2023) **24**, 364–372
https://doi.org/10.1093/ehjci/jeac107

ORIGINAL PAPER

Histopathological validation of semi-automated myocardial scar quantification techniques for dark-blood late gadolinium enhancement magnetic resonance imaging

Hedwig M.J.M. Nies [1,2]*, Suzanne Gommers[2], Geertruida P. Bijvoet[1,3], Luuk I.B. Heckman[4], Frits W. Prinzen [1,4], Gaston Vogel[5], Caroline M. Van De Heyning[6], Amedeo Chiribiri[7], Joachim E. Wildberger[1,2], Casper Mihl[1,2], and Robert J. Holtackers [1,2,7]

[1]Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Maastricht, The Netherlands; [2]Department of Radiology & Nuclear Medicine, Maastricht University Medical Centre, PO Box 5800, AZ 6202, Maastricht, The Netherlands; [3]Department of Cardiology, Maastricht University Medical Centre, Maastricht, The Netherlands; [4]Department of Physiology, Maastricht University, Maastricht, The Netherlands; [5]Pie Medical Imaging, Maastricht, The Netherlands; [6]Department of Cardiology, Antwerp University Hospital and GENCOR, University of Antwerp, Antwerp, Belgium; and [7]School of Biomedical Engineering & Imaging Sciences, King's College London, London, UK

Received 23 February 2022; revised 6 May 2022; accepted 10 May 2022; online publish-ahead-of-print 20 June 2022

Aims To evaluate the performance of various semi-automated techniques for quantification of myocardial infarct size on both conventional bright-blood and novel dark-blood late gadolinium enhancement (LGE) images using histopathology as reference standard.

Methods and results In 13 Yorkshire pigs, reperfused myocardial infarction was experimentally induced. At 7 weeks post-infarction, both bright-blood and dark-blood LGE imaging were performed on a 1.5 T magnetic resonance scanner. Following magnetic resonance imaging (MRI), the animals were sacrificed, and histopathology was obtained. The percentage of infarcted myocardium was assessed per slice using various semi-automated scar quantification techniques, including the signal threshold vs. reference mean (STRM, using 3 to 8 SDs as threshold) and full-width at half-maximum (FWHM) methods, as well as manual contouring, for both LGE methods. Infarct size obtained by histopathology was used as reference. In total, 24 paired LGE MRI slices and histopathology samples were available for analysis. For both bright-blood and dark-blood LGE, the STRM method with a threshold of 5 SDs led to the best agreement to histopathology without significant bias (−0.23%, 95% CI [−2.99, 2.52%], $P = 0.862$ and −0.20%, 95% CI [−2.12, 1.72%], $P = 0.831$, respectively). Manual contouring significantly underestimated infarct size on bright-blood LGE (−1.57%, 95% CI [−2.96, −0.18%], $P = 0.029$), while manual contouring on dark-blood LGE outperformed semi-automated quantification and demonstrated the most accurate quantification in this study (−0.03%, 95% CI [−0.22, 0.16%], $P = 0.760$).

Conclusion The signal threshold vs. reference mean method with a threshold of 5 SDs demonstrated the most accurate semi-automated quantification of infarcted myocardium, without significant bias compared to histopathology, for both conventional bright-blood and novel dark-blood LGE.

* Corresponding author. Tel: +31 (0)43 387 4912, E-mail hedwig.nies@mumc.nl

Keywords late gadolinium enhancement • magnetic resonance imaging • myocardial infarction • semi-automated scar quantification • dark-blood late gadolinium enhancement

Introduction

Late gadolinium enhancement magnetic resonance imaging (LGE MRI) is a well-established imaging technique to distinguish infarcted from normal myocardium and forms the basis of clinical routine cardiac MRI protocols worldwide.[1–3] In patients with ischaemic heart disease, the presence and extent of myocardial infarction (MI) is a strong predictor of major adverse cardiovascular events (MACEs), superior to and independent of left ventricular (LV) function.[4] The assessment of the extent of LGE in MI therefore has significant prognostic value and is crucial to guide patient management.[5–7]

Hyperenhanced regions of MI can be quantified by manual contouring. This visual analysis, however, is time consuming and highly influenced by the observer which may lead to poor reproducibility.[8,9] Both significant under- and overestimation of manually defined infarct size have been reported when compared to histopathological findings.[8,10–12] In clinical practice, the observer therefore often reverts to qualitative evaluation, reporting the amount and location of hyperenhanced segments, as well as the estimated percentage of transmurality.[13,14] As an alternative to manual contouring, semi-automated intensity thresholding techniques were developed as a more standardized, objective quantification method capable to reduce intra- and interobserver variability. Such methods include the signal threshold vs. reference mean (STRM) method and the full-width at half-maximum (FWHM) method.

For the STRM method, a signal intensity (SI) threshold based on a number of standard deviations (SDs) above the mean signal of remote normal myocardium is used to identify infarcted myocardium. The FWHM method, however, starts from the highest SI in the infarcted myocardium and identifies a myocardial area as enhanced when its intensity exceeds half of the SI between that maximum SI and the minimum value in the myocardium.[8] Both methods have been extensively validated using conventional bright-blood LGE MRI, with and without histopathology as reference standard.[8–10,15–18]

When using conventional bright-blood LGE, however, the blood pool often has an almost equally high SI as the enhanced scar tissue, thereby leading to poor scar-to-blood contrast and hindering the assessment of small subendocardial scar tissue.[19] Various novel dark-blood LGE methods that lower the blood pool SI have been proposed to overcome this limitation and increase scar-to-blood contrast for improved detection of subendocardial scar patterns.[20] Among these is the readily available blood-nulled phase-sensitive inversion-recovery (PSIR) LGE method, which has recently been clinically validated in a cohort of 300 patients,[19] and histopathologically validated in an animal model of MI.[21] This novel dark-blood LGE method demonstrated superior visualization and quantification of ischaemic scar tissue compared to the current in-vivo reference standard (i.e. conventional bright-blood LGE).

Despite the superior performance of this dark-blood LGE method in both preclinical and clinical studies, the effect of the improved contrast level on semi-automated scar quantification techniques has not been evaluated yet. In this study, we therefore sought to evaluate the performance of various semi-automated quantification techniques, as well as manual contouring, to assess infarct size on both conventional bright-blood LGE and novel dark-blood LGE images, using histopathology as reference standard.

Methods

In 13 female Yorkshire pigs, reperfused MI was experimentally induced using coronary artery balloon catheter occlusion of part of the left circumflex artery. Occlusion lasted 90 min, followed by 180 min of controlled reperfusion under general anaesthesia. Further details on animal preparation have been described in previous work.[21] This study was approved by the Experimental Animal Committee of the Maastricht University (DEC2016-002) and animal handling complied with the Dutch Law on Animal Experimentation and the European Directive on the Protection of Animals used for Scientific Purposes (2010/63/EU).

MRI

At 7 weeks after MI induction, MRI was performed under general anaesthesia using a clinical 1.5 T MRI scanner (Ingenia, Philips Healthcare, Best, The Netherlands). After an intravenous double dose (0.2 mmol/kg) injection of gadobutrol (Gadovist; Bayer Pharmaceuticals, Berlin, Germany), both conventional bright-blood and novel dark-blood LGE images were acquired in randomized order (first method at 10 min, second method at 20 min after injection) to obtain a stack of short-axis and three long-axis views for each method. For both LGE methods, a standard electrocardiogram-triggered PSIR LGE sequence with a radiofrequency spoiled turbo field-echo readout was used. Typical sequence parameters were: echo time 3.0 ms, repetition time 6.1 ms, flip angle 25°, PSIR reference flip angle 5°, shot duration 135 ms, slice thickness 8 mm, acquired resolution 1.6×1.6 mm^2, reconstructed resolution 0.8×0.8 mm^2, parallel imaging acceleration factor 1.5, and two signal averages. The inversion time (TI) was set to null the signal of remote myocardium for conventional bright-blood LGE, while the TI was set for LV blood pool nulling for dark-blood LGE. Note that all other settings and parameters remained identical for both methods. The mechanism of the used dark-blood LGE method (blood-nulled PSIR LGE) has been described in detail before.[22] All LGE images were acquired in mid-diastole during end-expiratory ventilator stops. The given contrast dose reflects local protocol and current international guidelines.[1]

Histopathology

Directly following MRI examination, the animals were sacrificed, and their hearts removed for further analysis. Using a 3D printed mould with equally spaced cutting slots, the *ex vivo* hearts were sectioned into 8 mm thick axial slices. Each slice was then stained using 1% triphenyltetrazolium chloride (TTC) and fixated in 4% formalin. Each

slice was photographed digitally from both the apical and basal side while on graph paper. Before further analysis, matching LGE slices and pathology samples were visually selected based on anatomical landmarks, such as the papillary muscles and right ventricular insertion points.

Image analysis

All matching LGE slices were transferred to a remote workstation for analysis using a commercially available software package (CAAS MR Solutions v5.2.1; Pie Medical Imaging, Maastricht, The Netherlands). For each subject, the conventional bright-blood and novel dark-blood LGE images were separated, anonymized, and presented to an expert observer (S.G.) with >10 years of experience in cardiac MRI, who was blinded to histopathology analysis. Bright-blood and dark-blood LGE images of the same subject were never presented consecutively. Endo- and epicardial contours were manually drawn by the observer to define the myocardium. Papillary muscles and LV trabeculations were excluded from myocardial contours. The expert observer then assessed the hyperenhanced myocardium using manual contouring.

Subsequently, semi-automated quantification of hyperenhanced myocardium was performed using both the STRM and FWHM methods. For both methods, the earlier defined endo- and epicardial contours were used to minimize the influence of manual myocardial contouring on the semi-automated quantification results. For the STRM method, a region of interest (ROI) in the remote normal myocardium was manually selected as reference region for each slice. This ROI was consistently placed in the anterior septum. Thresholds varying from 3 to 8 SDs above the mean signal of the reference region were used to quantify the infarcted myocardium. For the FWHM method, the voxel with the highest SI was manually selected within the infarcted area for each slice. A threshold of 50% between that maximum SI and the minimum value in the myocardium was used to quantify the infarcted myocardium. For each LGE image, the percentage of infarcted LV myocardium was calculated by dividing the infarcted area by the total LV myocardium.

The digital photographs of the matching histopathology samples were also transferred to a remote workstation and analyzed using a freely available software package (ImageJ v1.53; U.S. National Institutes of Health, Bethesda, MD, USA). Another independent observer (L.I.B.H.), who was blinded to MRI analysis, then manually delineated the endo- and epicardial borders of the myocardium. Papillary muscles and LV trabeculations were excluded from these contours. Finally, the infarcted myocardium was assessed using manual contouring. For each histopathology sample, the percentage of infarcted LV myocardium was calculated by dividing the infarcted area by the total LV myocardium.

Statistical analysis

For both conventional bright-blood LGE and novel dark-blood LGE, the results of the semi-automated scar quantification techniques and manual contouring were compared against histopathology using the paired-sample *t*-test. Correlations between the quantification techniques and histopathology were determined using the Pearson's correlation test. Data are expressed as mean ± SD unless otherwise specified. Statistical tests were performed using a commercially available statistical software package (SPSS v26; IBM, Armonk, NY, USA) with statistical significance set at $P < 0.05$.

Results

Study population

MRI examination 7 weeks post-MI induction was successfully performed in 5 of 13 pigs. Reasons for dropouts include fatal arrhythmias within 4 h after MI induction ($n = 5$), death in a later stage but prior to MRI examination due to heart failure ($n = 2$), and cancelation of the MRI examination due to COVID-19 pandemic restrictions ($n = 1$). Animal weight of the surviving pigs at 7 weeks post-MI MRI examination was 87.2 ± 8.4 kg. The TIs for conventional bright-blood LGE were 307 ± 26 and 326 ± 20 ms when performed at 10 and 20 min after contrast administration, respectively. The TIs for dark-blood LGE were 189 ± 25 and 224 ± 13 ms, respectively. In total 24 MRI slices—acquired using both conventional bright-blood and novel dark-blood LGE—with 24 matching histopathology samples were available for analysis. *Figure 1* shows the results of the various quantification techniques on a short-axis MRI slice acquired using both conventional bright-blood and novel dark-blood LGE, with its matching histopathology sample as reference standard.

Bright-blood LGE

For bright-blood LGE, semi-automated quantification using the 5 SDs threshold method led to the best agreement with histopathology (−0.23%, 95% CI [−2.99, 2.52%], $P = 0.862$) (*Figure 2A*). The neighbouring threshold levels of 4 and 6 SDs led to a non-significant overestimation of 2.46% (95% CI [−0.37, 5.29%], $P = 0.086$) and underestimation of −2.19% (95% CI [−5.02, 0.63%], $P = 0.122$) in the percentage of infarcted myocardium, respectively. Other thresholds for the STRM method, as well as the FWHM method, led to a significant over- (3 SDs) and underestimation (7, 8 SDs, and FWHM) compared to histopathology. Manual contouring also led to a significant underestimation of −1.57% (95% CI [−2.96, −0.18%], $P = 0.029$) in the percentage of infarcted myocardium.

Dark-blood LGE

Of the evaluated semi-automated quantification techniques, the STRM method using the 5 SDs threshold led to the best agreement with histopathology (−0.20%, 95% CI [−2.12, 1.72%], $P = 0.831$) (*Figure 2B*). Other threshold levels for the STRM method showed significant over- (3 and 4 SDs) and underestimation (6, 7, and 8 SDs) of the percentage infarcted myocardium compared to histopathology. The FWHM method led to a non-significant underestimation of −1.18% (95% CI [−3.58, 1.22%], $P = 0.318$). Manual contouring on dark-blood LGE demonstrated the best agreement with histopathology, showing almost no bias (−0.03%, 95% CI [−0.22, 0.16%], $P = 0.760$).

Correlation plots and Bland–Altman plots for all quantification techniques, for both conventional bright-blood and dark-blood LGE with histopathology as reference standard, are shown in *Figure 3*. When comparing the best performing semi-automated scar quantification technique for each LGE method (which is 5 SDs for both LGE methods), dark-blood LGE shows less bias (−0.20

vs. −0.24%) and superior correlation ($r = 0.794$ vs. $r = 0.659$) than conventional bright-blood LGE when compared to histopathology. When using a manual assessment, dark-blood LGE also shows a significantly lower bias (−0.03 vs. −1.57%) and superior correlation ($r = 0.998$ vs. $r = 0.892$) than conventional bright-blood LGE when compared to histopathology.

Figure 4 shows an imaging example where local SI variations in the myocardium can be observed that affected the performance of the STRM quantification method, in particular for conventional bright-blood LGE when using thresholds >5 SDs.

Discussion

In this study, multiple scar quantification techniques were evaluated using conventional bright-blood LGE as well as novel dark-blood LGE, using histopathology in a porcine animal model with induced MI as reference standard. From the evaluated semi-automated quantification techniques, the STRM method with a threshold of 5 SDs showed the best agreement in scar size with histopathology, for both LGE methods. For dark-blood LGE, manual contouring showed an excellent agreement with histopathology, with no significant bias, closely followed by the STRM method using a threshold of 5 SDs. For bright-blood LGE, however, manual contouring showed a significant underestimation of −2% compared to histopathology, thereby being outperformed by semi-automated quantification using a threshold of 5 SDs. Such differences in infarct size are clinically relevant since a >7 fold increased risk for MACE has been described in patients with a mean relative MI size of only 1.4%.[23,24]

Previous studies have shown that the presence and extent of scar tissue in patients with MI has significant prognostic value, making evaluation of the infarcted areas using LGE MRI a crucial assessment.[5,6] In contrast to manual contouring, which is usually adequate for daily clinical routine, semi-automated techniques are increasingly used for quantitative and objective assessment of infarct size in research studies using LGE MRI as end point. While numerous studies evaluated different (semi-)automated scar quantification techniques for the conventional bright-blood LGE method, with both histopathology and manual contouring as reference standard, there is hardly any literature available on the performance of such techniques for the increasingly used novel dark-blood LGE methods.

In a recent abstract by Kotecha *et al.*,[17] semi-automated quantification of acute infarct size was performed for a

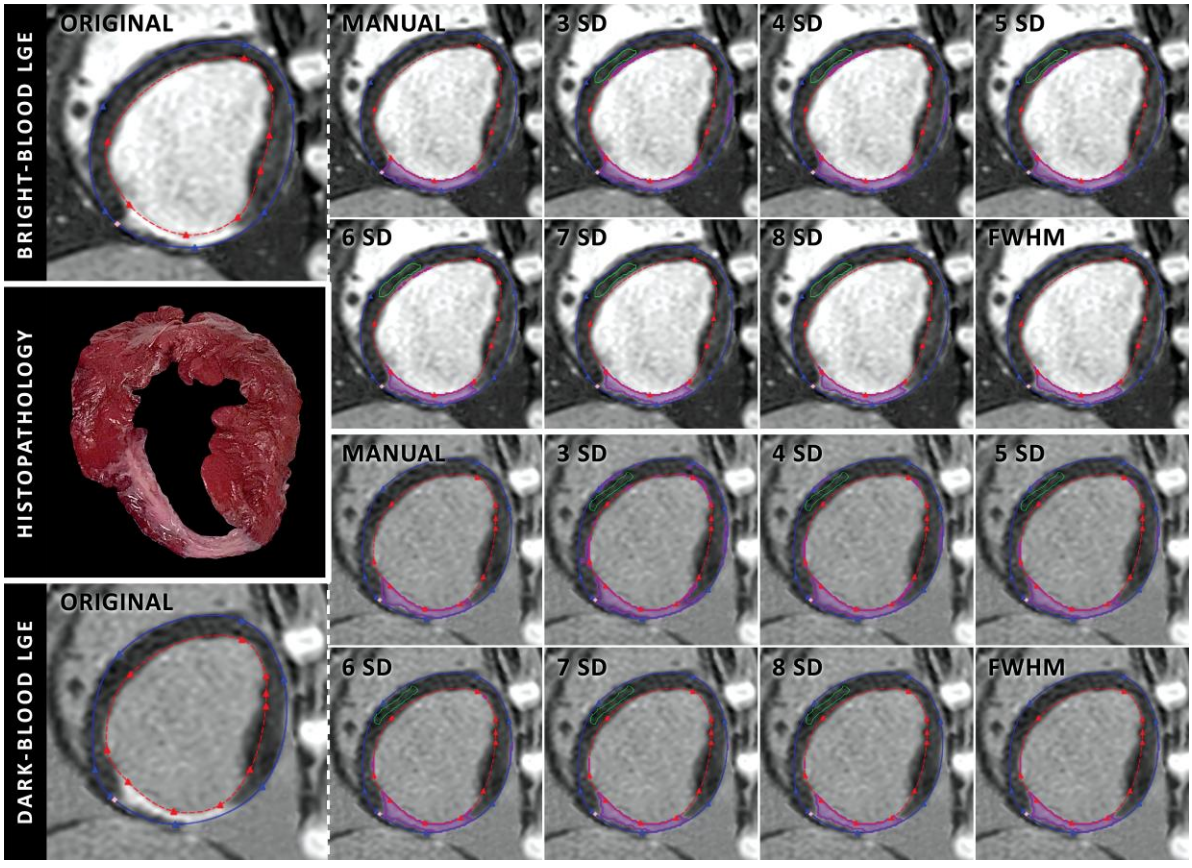

Figure 1 A short-axis MR image acquired using both conventional bright-blood LGE (top left) and novel dark-blood LGE (bottom left) with its corresponding histopathology slice (middle left). For each LGE method (upper and lower right), manual contouring as well as seven semi-automated scar quantification techniques (STRM method using thresholds ranging from 3 to 8 SDs and the FWHM method) were used to assess myocardial infarct size (purple). Note that identical endo- (red) and epicardial (blue) contours were used for all quantification techniques for each LGE method. The reference regions used for the STRM method are depicted in green.

T_2-preparation-based dark-blood LGE sequence and compared to manual contouring as reference. Accurate quantification of acute infarct size was achieved using the STRM method with the threshold set at 5 SDs. Both a threshold of 6 SDs, as well as the FWHM method, led to an underestimation of infarct size. Although no histopathology was available in that study and a different dark-blood LGE method was used, their results correspond with our findings. While an underestimation of approximately −5% was found for the FWHM method compared to manual contouring in their study, our results showed a much smaller underestimation of approximately −1%, partly explained due to the presence of two significant outliers of +12 and +18%. Without these, the FWHM method would have shown an underestimation of almost −3% compared to histopathology (and manual contouring).

Apart from evaluating semi-automated scar quantification techniques in novel dark-blood LGE only, conventional bright-blood LGE was performed to enable comparison with other literature. Early experimental studies using histopathology have used thresholds of 2 or 3 SDs for conventional bright-blood LGE.[2,3,8,25–27] An abundance of later studies, however, showed that such thresholds can lead to a significant overestimation of the infarcted myocardium, using manual contouring[9,15,28] or histopathology[10,12] as reference standard. Using manual contouring as reference standard, Bondarenko *et al.*[15] found thresholds of 5 and 6 SDs to agree best with manual contouring in a study of chronic MI. Flett *et al.*[9] found a threshold of 6 SDs to agree best to manual contouring in cases of acute MI, while thresholds of both 5 and 6 SDs agreed best in cases of chronic MI. These findings are in line with Vermes *et al.*,[28] who also found a threshold of 5 SDs to agree best in a study of acute MI using manual contouring as reference standard. With histopathology as reference standard, Gruszczynska *et al.*[10] reported that a threshold of 4 SDs correlated best in an animal model of acute MI. Since manual contouring was found to underestimate the percentage of infarcted myocardium by −2 to −3% in their study, manual contouring appeared to agree best when using a threshold of 5 or 6 SDs, which is in line with the findings of Flett *et al.*[9] and Vermes *et al.*[28] Also in our study, manual contouring in bright-blood LGE led to a significant underestimation of −2%, making manual contouring agree best when a threshold of 6 SDs was used. Compared to histopathology, however, a threshold of 5 SDs agreed best, whereas thresholds of 4 and 6 SDs showing a non-significant over- and underestimation, respectively.

With respect to the FWHM method, we only used, as per definition, thresholding at 50% between the maximum SI in the infarcted area and the minimum value in the myocardium, which showed a significant underestimation when using bright-blood LGE. Although these results differ from earlier findings that FWMH showed best agreement and even overestimated scar volume, it is noteworthy that these two studies investigated an acute MI setting, <24 h and within 5 days after MI induction, respectively, compared to a more chronic MI setting in our study.[8,10] Gruszczynska *et al.*[10] found that a threshold of 70% of the maximum SI, instead of the 50% used for the FWHM method, agreed best with histopathology.

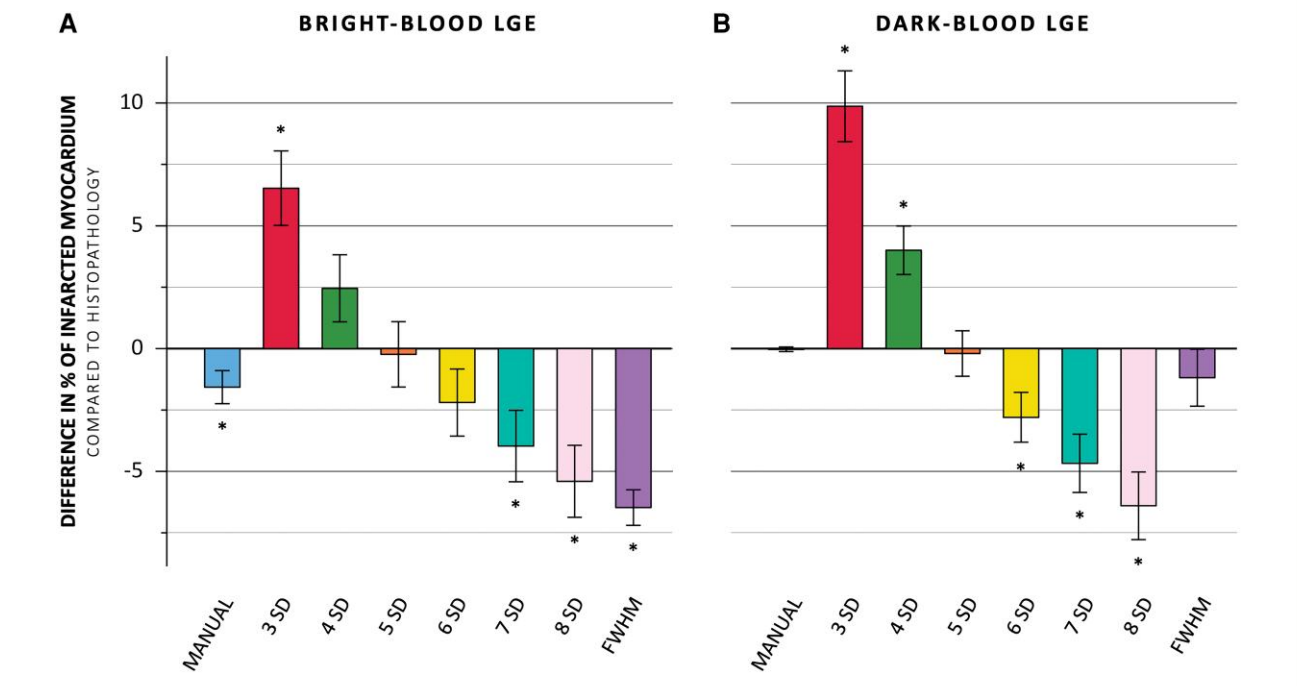

Figure 2 The performance of manual contouring and various semi-automated scar quantification techniques for both conventional bright-blood LGE (left panel, A) and novel dark-blood LGE (right panel, B), using histopathology as reference standard. The height of the bars indicates the mean difference in infarcted myocardium between the indicated method and histopathology, with the error bars indicating the standard error. Please note that for manual delineation on dark-blood LGE, only the error bars can be observed as the bias is close to zero. The asterisks indicate a significant difference ($P < 0.05$).

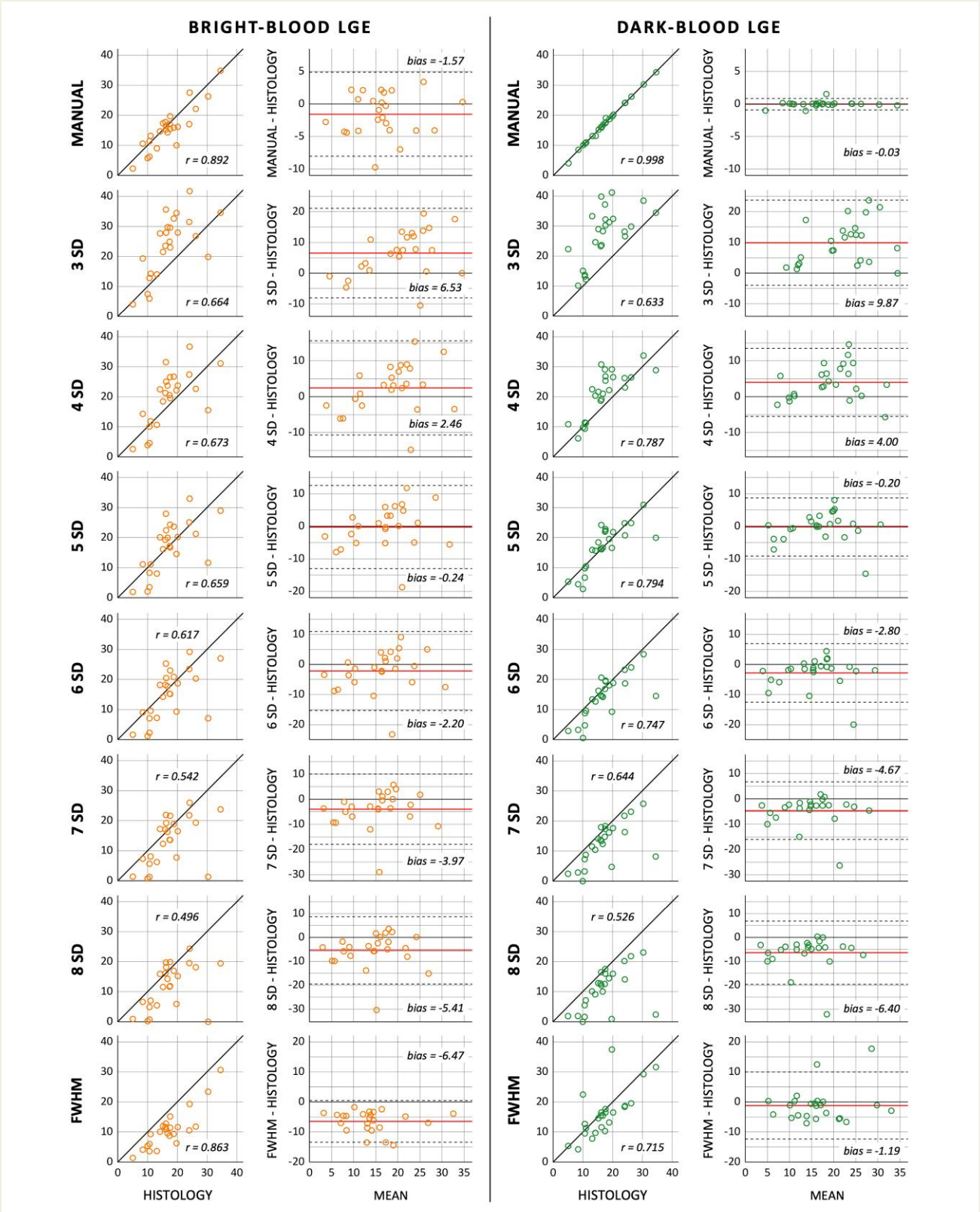

Figure 3 Correlation plots and Bland–Altman plots for manual contouring and all semi-automated quantification techniques for both conventional bright-blood LGE (orange) and novel dark-blood LGE (green) using histopathology as reference standard. All axes indicate the percentage of infarcted LV myocardium. For each quantification technique, the Pearson's correlation coefficient (correlation plot) and bias (Bland–Altman plot) are indicated.

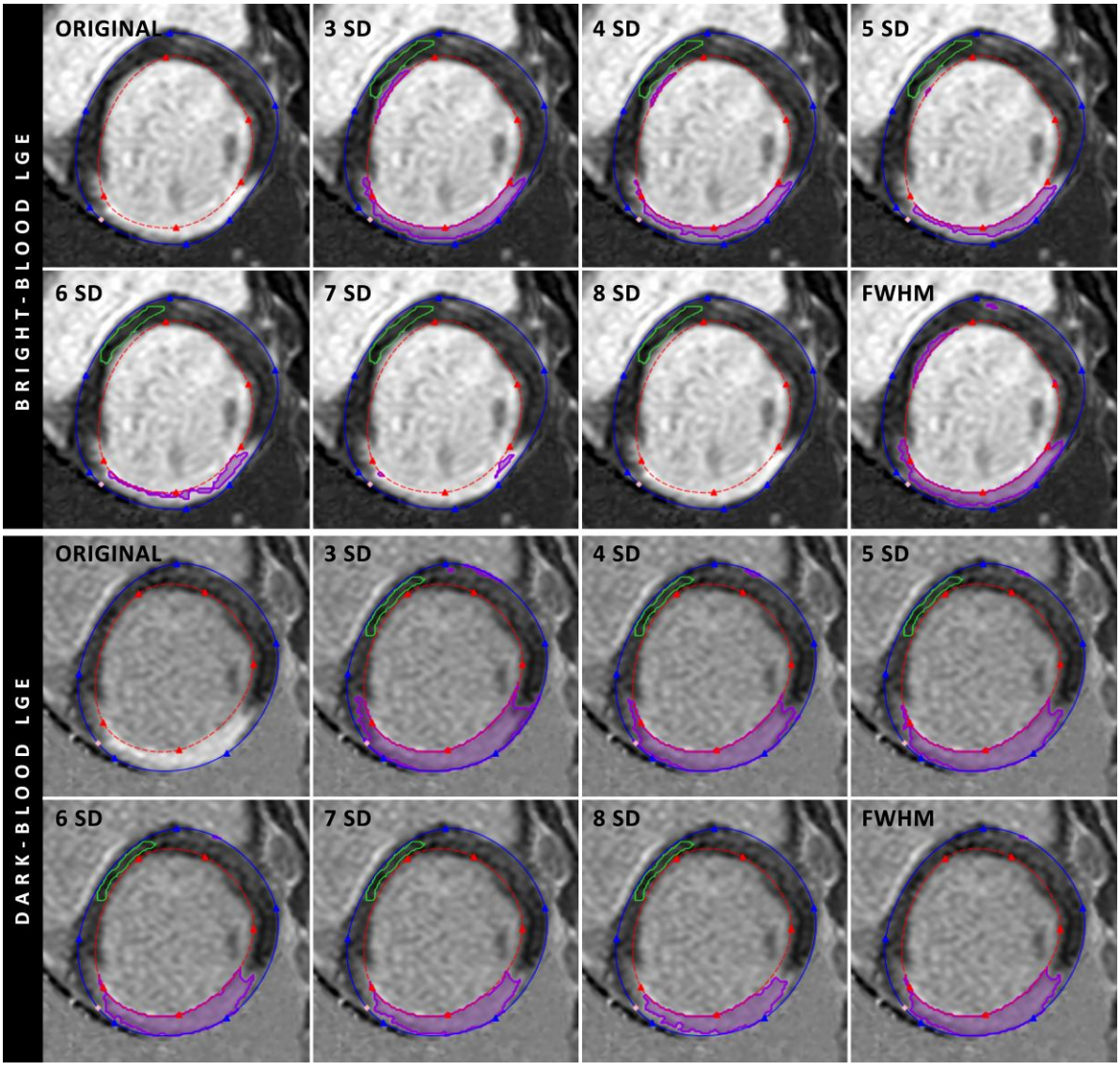

Figure 4 Imaging example of SI variation within the normal myocardium that affected the performance of the STRM quantification method, in particular for conventional bright-blood LGE when using thresholds >5 SDs. Although the reference region (green) is consistently positioned in the septum for both LGE methods, notable differences in infarct size (purple) are observed between both methods when using higher thresholds for the STRM method. Since the FWHM method uses the maximum SI instead of reference region in remote myocardium, its performance is less sensitive to local SI variations and therefore performs well in this case. Note that the same endo- (red) and epicardial (blue) contours have been used for all quantification techniques for each LGE method.

General limitations of semi-automated scar quantification techniques

While semi-automated scar quantification techniques can overcome some of the drawbacks of manual contouring, these techniques have their limitations as well. The presence of surface coil intensity variations may lead to local SI variations in the myocardium. The location of the remote region of reference myocardium, required for the STRM method, may therefore affect its performance.[10] Although the reference region was consistently positioned in representative remote myocardium in the septum, local variations in SI of this normal myocardium may result in a large SD in normal myocardial signal, which is used for the STRM method. Consequently, and in particular when using conventional bright-blood LGE imaging, hardly any hyperenhanced tissue is detected for thresholds >5 SDs while infarction is clearly present (*Figure 4*). Thresholding methods based on percentages of the maximum SI in the infarcted tissue have the advantage of being less dependent of signal variation in the reference region, as only the maximum SI in the infarcted area (single point) is selected.

Although semi-automated techniques are considered more standardized and objective than visual assessment, various steps in the

analysis are still observer dependent. Endo- and epicardial contours need to be drawn manually, and also the reference region and the pixel with highest SI, required for the STRM and FWHM methods, need to be selected manually. In addition, when utilizing semi-automated quantification techniques for the assessment of acute MI, the presence of areas of microvascular obstruction has to be considered. Since these regions are not detected as infarcted myocardium due to their dark appearance, manual tracing and thus additional observer interaction is required. Recently, however, fully automated scar quantification techniques that use machine learning to overcome the need for any observer interaction have been proposed as a promising alternative to semi-automated techniques.[29–31]

Specific study limitations

Despite the careful conduction of the present histopathological validation study, there are limitations that should be addressed. In our study, 5 of 13 animals completed the entire study protocol and were therefore available for histopathological validation. Although this number may seem limited, the goal of MI induction was to achieve a maximum amount of scar tissue and therefore the observed drop-out was as expected. The majority of animals died as a result of severe arrhythmias within 4 h post-MI. The high vulnerability for post-MI arrhythmias in these animals has also been described in earlier studies.[32] Despite the limited number of samples, the authors feel that additional animal experiments would not have altered the main study outcomes and additional animal experiments would have been unethical.

Since minimal intra- and interobserver bias has been reported for manual contouring and both STRM and FWHM methods when assessing ischaemic myocardial scar, intra- and interobserver variability has been omitted in the present study.[8,9,28,33]

Even though our study evaluated seven widely available semi-automated scar quantification techniques (STRM using various thresholds and FWHM), other quantification techniques have been proposed that were not included in our study due to their limited availability.[10–12,28]

Future outlook

As per study protocol, all animals underwent their final MRI examination at 7 weeks post-MI. Therefore, histopathological evaluation was only available in a more chronic infarct setting. Future research should also focus on the acute MI setting with the potential presence of areas of microvascular obstruction that change in size over time.

Although his study focused solely on induced MI, myocardial scar can also originate from a variety of different non-ischaemic aetiologies. Even though semi-automated scar quantification techniques have been evaluated in some of these non-ischaemic cardiomyopathies using conventional bright-blood LGE,[9,16,28,33,34] these techniques have not been investigated in non-ischaemic cardiomyopathies using dark-blood LGE methods yet. Future research should therefore focus on evaluating semi-automated scar quantification techniques for dark-blood LGE methods in cases with non-ischaemic cardiomyopathies.

Conclusion

For both conventional bright-blood LGE and novel readily available dark-blood LGE, accurate semi-automated quantification of infarcted myocardium can be obtained using the signal threshold vs. reference mean method with a threshold of 5 SDs, without significant bias compared to histopathology. Despite this method was found superior to manual contouring for conventional bright-blood LGE, manual contouring using dark-blood LGE outperformed semi-automated quantification and showed the most accurate quantification compared to histopathology in this study. Although semi-automated methods are increasingly used for quantitative and objective assessment of infarct size in research studies using LGE MRI as end point, manual contouring is usually adequate for daily clinical routine.

Acknowledgements

The authors would like to thank Frans van Nieuwenhoven and Marion Kuijper for their key roles in the design and initiation of the study, and conduction of the animal experiments.

Conflicts of interest: G.V. is an employee of Pie Medical Imaging. The other authors declare that they have no competing interest.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. Kramer CM, Barkhausen J, Bucciarelli-Ducci C, Flamm SD, Kim RJ, Nagel E. Standardized cardiovascular magnetic resonance imaging (CMR) protocols: 2020 update. *J Cardiovasc Magn Reson* 2020;**22**:17.
2. Fieno DS, Kim RJ, Chen EL, Lomasney JW, Klocke FJ, Judd RM. Contrast-enhanced magnetic resonance imaging of myocardium at risk: distinction between reversible and irreversible injury throughout infarct healing. *J Am Coll Cardiol* 2000;**36**:1985–1991.
3. Kim RJ, Fieno DS, Parrish TB, Harris K, Chen EL, Simonetti O, *et al.* Relationship of MRI delayed contrast enhancement to irreversible injury, infarct age, and contractile function. *Circulation* 1999;**100**:1992–2002.
4. Lee SA, Yoon YE, Kim JE, Park JJ, Oh IY, Yoon CH, *et al.* Long-term prognostic value of late gadolinium-enhanced magnetic resonance imaging in patients with and without left ventricular dysfunction undergoing coronary artery bypass grafting. *Am J Cardiol* 2016;**118**:1647–1654.
5. Vincenti G, Masci PG, Monney P, Rutz T, Hugelshofer S, Gaxherri M, *et al.* Stress perfusion CMR in patients with known and suspected CAD: prognostic value and optimal ischemic threshold for revascularization. *JACC Cardiovasc Imaging* 2017;**10**:526–537.
6. Garcia MJ, Kwong RY, Scherrer-Crosbie M, Taub CC, Blankstein R, Lima J, *et al.* State of the art: imaging for myocardial viability: a scientific statement from the American Heart Association. *Circ Cardiovasc Imaging* 2020;**13**:e000053.
7. Choi KM, Kim RJ, Gubernikoff G, Vargas JD, Parker M, Judd RM. Transmural extent of acute myocardial infarction predicts long-term improvement in contractile function. *Circulation* 2001;**104**:1101–1107.
8. Amado LC, Gerber BL, Gupta SN, Rettmann DW, Szarf G, Schock R, *et al.* Accurate and objective infarct sizing by contrast-enhanced magnetic resonance imaging in a canine myocardial infarction model. *J Am Coll Cardiol* 2004;**44**:2383–2389.
9. Flett AS, Hasleton J, Cook C, Hausenloy D, Quarta G, Ariti C, *et al.* Evaluation of techniques for the quantification of myocardial scar of differing etiology using cardiac magnetic resonance. *JACC Cardiovasc Imaging* 2011;**4**:150–156.
10. Gruszczynska K, Kirschbaum S, Baks T, Moelker A, Duncker DJ, Rossi A, *et al.* Different algorithms for quantitative analysis of myocardial infarction with DE MRI: comparison with autopsy specimen measurements. *Acad Radiol* 2011;**18**:1529–1536.
11. Hsu LY, Ingkanisorn WP, Kellman P, Aletras AH, Arai AE. Quantitative myocardial infarction on delayed enhancement MRI. Part II: clinical application of an automated feature analysis and combined thresholding infarct sizing algorithm. *J Magn Reson Imaging* 2006;**23**:309–314.
12. Hsu LY, Natanzon A, Kellman P, Hirsch GA, Aletras AH, Arai AE. Quantitative myocardial infarction on delayed enhancement MRI. Part I: animal validation of an automated feature analysis and combined thresholding infarct sizing algorithm. *J Magn Reson Imaging* 2006;**23**:298–308.

13. Mahrholdt H, Wagner A, Holly TA, Elliott MD, Bonow RO, Kim RJ, *et al.* Reproducibility of chronic infarct size measurement by contrast-enhanced magnetic resonance imaging. *Circulation* 2002;**106**:2322–2327.
14. Kwon DH, Halley CM, Carrigan TP, Zysek V, Popovic ZB, Setser R, *et al.* Extent of left ventricular scar predicts outcomes in ischemic cardiomyopathy patients with significantly reduced systolic function: a delayed hyperenhancement cardiac magnetic resonance study. *JACC Cardiovasc Imaging* 2009;**2**:34–44.
15. Bondarenko O, Beek AM, Hofman MB, Kühl HP, Twisk JW, van Dockum WG, *et al.* Standardizing the definition of hyperenhancement in the quantitative assessment of infarct size and myocardial viability using delayed contrast-enhanced CMR. *J Cardiovasc Magn Reson* 2005;**7**:481–485.
16. Mikami Y, Kolman L, Joncas SX, Stirrat J, Scholl D, Rajchl M, *et al.* Accuracy and reproducibility of semi-automated late gadolinium enhancement quantification techniques in patients with hypertrophic cardiomyopathy. *J Cardiovasc Magn Reson* 2014;**16**:85.
17. Kotecha T, Martinez-Naharro A, Lambe T, Francis RD, Chacko LIZA, Brown JM, *et al.* Quantification of myocardial infarct size and microvascular obstruction using dark-blood late gadolinium enhancement. *Eur Heart J Cardiovasc Imaging* 2019;**20**:jez112.003.
18. Khan JN, Nazir SA, Horsfield MA, Singh A, Kanagala P, Greenwood JP, *et al.* Comparison of semi-automated methods to quantify infarct size and area at risk by cardiovascular magnetic resonance imaging at 1.5 T and 3.0 T field strengths. *BMC Res Notes* 2015;**8**:52.
19. Holtackers RJ, Van De Heyning CM, Nazir MS, Rashid I, Ntalas I, Rahman H, *et al.* Clinical value of dark-blood late gadolinium enhancement cardiovascular magnetic resonance without additional magnetization preparation. *J Cardiovasc Magn Reson* 2019;**21**:44.
20. Holtackers RJ, Van De Heyning CM, Chiribiri A, Wildberger JE, Botnar RM, Kooi ME. Dark-blood late gadolinium enhancement cardiovascular magnetic resonance for improved detection of subendocardial scar: a review of current techniques. *J Cardiovasc Magn Reson* 2021;**23**:96.
21. Holtackers RJ, Gommers S, Heckman LIB, Van De Heyning CM, Chiribiri A, Prinzen FW. Histopathological validation of dark-blood late gadolinium enhancement MRI without additional magnetization preparation. *J Magn Reson Imaging* 2022;**55**:190–197.
22. Holtackers RJ, Chiribiri A, Schneider T, Higgins DM, Botnar RM. Dark-blood late gadolinium enhancement without additional magnetization preparation. *J Cardiovasc Magn Reson* 2017;**19**:64.
23. Kwong RY, Chan AK, Brown KA, Chan CW, Reynolds HG, Tsang S, *et al.* Impact of unrecognized myocardial scar detected by cardiac magnetic resonance imaging on event-free survival in patients presenting with signs or symptoms of coronary artery disease. *Circulation* 2006;**113**:2733–2743.
24. Omori T, Kurita T, Dohi K, Takasaki A, Nakata T, Nakamori S, *et al.* Prognostic impact of unrecognized myocardial scar in the non-culprit territories by cardiac magnetic resonance imaging in patients with acute myocardial infarction. *Eur Heart J Cardiovasc Imaging* 2018;**19**:108–116.
25. Gerber BL, Rochitte CE, Bluemke DA, Melin JA, Crosille P, Becker LC, *et al.* Relation between Gd-DTPA contrast enhancement and regional inotropic response in the periphery and center of myocardial infarction. *Circulation* 2001;**104**:998–1004.
26. Hillenbrand HB, Kim RJ, Parker MA, Fieno DS, Judd RM. Early assessment of myocardial salvage by contrast-enhanced magnetic resonance imaging. *Circulation* 2000;**102**:1678–1683.
27. Oshinski JN, Yang Z, Jones JR, Mata JF, French BA. Imaging time after Gd-DTPA injection is critical in using delayed enhancement to determine infarct size accurately with magnetic resonance imaging. *Circulation* 2001;**104**:2838–2842.
28. Vermes E, Childs H, Carbone I, Barckow P, Friedrich MG. Auto-threshold quantification of late gadolinium enhancement in patients with acute heart disease. *J Magn Reson Imaging* 2013;**37**:382–390.
29. Ghanbari F, Joyce T, Kozerke S, Guaricci A, Masci P, Pavon A, *et al.* Performance of a machine-learning algorithm for fully automatic LGE scar quantification in the large multi-national derivate registry. *Eur Heart J Cardiovasc Imaging* 2021;**22**:jeab090.023.
30. Torlasco C, Papetti D, Mene R, Artico J, Seraphim A, Badano L, *et al.* Dark blood ischemic LGE segmentation using a deep learning approach. *Eur Heart J Cardiovasc Imaging* 2021;**22**:jeab090.020.
31. Lange T, Stiermaier T, Backhaus S, Boom P, Kowallick J, Lotz J, *et al.* Fully automated artificial intelligence-based myocardial scar quantification for diagnostic and prognostic stratification in patients following acute myocardial infarction. *Eur Heart J Cardiovasc Imaging* 2021;**22**:jeaa356.299.
32. Sattler SM, Skibsbye L, Linz D, Lubberding AF, Tfelt-Hansen J, Jespersen T. Ventricular arrhythmias in first acute myocardial infarction: epidemiology, mechanisms, and interventions in large animal models. *Front Cardiovasc Med* 2019;**6**:158.
33. Spiewak M, Malek LA, Misko J, Chojnowska L, Milosz B, Klopotowski M, *et al.* Comparison of different quantification methods of late gadolinium enhancement in patients with hypertrophic cardiomyopathy. *Eur J Radiol* 2010;**74**:e149–153.
34. Cochet H, Iriart X, Allain-Nicolaï A, Camaioni C, Sridi S, Nivet H, *et al.* Focal scar and diffuse myocardial fibrosis are independent imaging markers in repaired tetralogy of Fallot. *Eur Heart J Cardiovasc Imaging* 2019;**20**:990–1003.