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Diffusion biomarkers in chronic myocardial infarction

Tanjib Rahman¹, Kévin Moulin², Daniel B. Ennis², Luigi E. Perotti¹

¹Department of Mechanical and Aerospace Engineering, University of Central Florida, Orlando, FL 32816, USA

²Department of Radiology, Stanford University, Stanford, CA 94305, USA

Abstract

Cardiac diffusion tensor magnetic resonance imaging (cDTI) allows estimating the aggregate cardiomyocyte architecture in healthy subjects and its remodeling as a result of cardiac disease. In this study, cDTI was used to quantify microstructural changes occurring in swine (N=7) six to ten weeks after myocardial infarction. Each heart was extracted and imaged *ex vivo* with 1mm isotropic spatial resolution. Microstructural changes were quantified in the border zone and infarct region by comparing diffusion tensor invariants – fractional anisotropy (FA), mode, and mean diffusivity (MD) – radial diffusivity, and diffusion tensor eigenvalues with the corresponding values in the remote myocardium. MD and radial diffusivity increased in the infarct and border regions with respect to the remote myocardium ($p < 0.01$). In contrast, FA and mode decreased in the infarct and border regions ($p < 0.01$). Diffusion tensor eigenvalues also increased in the infarct and border regions, with a larger increase in the secondary and tertiary eigenvalues.

Keywords

Cardiac Diffusion Tensor Imaging; Myocardial Infarction; Cardiac Microstructure; Radial Diffusivity

1 Introduction

Amongst existing imaging modalities, MRI and in particular T1 mapping and late gadolinium enhancement (LGE) are the most commonly used imaging techniques to detect and quantify infarct extension and location. Although recent studies have proposed the use of native T1 mapping to image myocardial infarction (MI), commonly used LGE and T1 mapping techniques rely on the use of contrast agents, which are not recommended for many patients with renal insufficiency. Furthermore, LGE and T1 mapping techniques do not provide direct information regarding the microstructural reorganization occurring in the infarcted myocardium.

Using cardiac diffusion tensor magnetic resonance imaging (cDTI), a non-contrast imaging technique, the myocardium microstructural organization can be inferred from the self-diffusion of water molecules. In particular, diffusion tensor invariants can be used to

quantify tissue changes occurring as a result of MI. For example, mean diffusivity (MD) represents the overall magnitude of diffusivity within the tissue and it has been shown to increase in infarcted myocardium [4,14,13]. Similarly, the fractional anisotropy (FA) represents the magnitude of anisotropy of the myocardial microenvironment and it decreases in infarcted myocardium due to cellular death and the loss of cellular structure.

The objective of this study was to further investigate changes in diffusion tensor invariants in remote, border, and infarcted myocardium, focusing on the analysis of primary, secondary, and tertiary eigenvalues and radial diffusivity.

2 Methods

2.1 Experimental and imaging procedures

Female Yorkshire swine (N=7) were considered in this study. Animal care and handling during the experimental procedures followed protocol # 2015–124 approved by the UCLA Institutional Animal Care and Use Committee.

As described in [5], under general anesthesia, a standard Seldinger technique was adopted to access the femoral artery. Subsequently, under X-ray fluoroscopy, a 7 French (Fr) balloon wedge-pressure catheter (110cm, Teleflex Arrow) was advanced over a metal guidewire to the left aortic sinus. At this location, a micro guidewire (Hi-Torque, Balance Middleweight Universal Guide Wire, 0.014 inches, Abbot Vascular) was used to sub-select a branch of the left anterior descending (LAD) or left circumflex (LCx) artery. Specifically, referring to the subjects number used in Figures 4 and 5, the LAD was sub-selected in subjects 3, 5, and 7, while the LCx was sub-selected in subjects 1, 2, 4, and 6. Subsequently, a balloon catheter (MINI TREK Coronary Dilatation Catheter, 1.5 mm diameter, Abbot Vascular) was inflated in the selected coronary branch and 2.5–3.0ml of microspheres (Polybead, Polystyrene 90 micron from Polysciences Inc) were injected. The balloon catheter was left inflated for one minute past microspheres' injection to avoid microspheres backflow and subsequently deflated and removed. In one subject, myocardial infarction was caused by a thrombus formed in the proximal LAD and microspheres were not injected. A comprehensive MRI exam was conducted six to ten weeks after infarct induction to allow enough time for scar formation.

At the end of the MRI exam, a double dose of a gadolinium-based contrast agent was injected (0.6ml/kg gadopentetate dimeglumine diluted in 10ml of saline). Ten minutes past the injection of the gadolinium-based contrast agent, the subjects were euthanized under general anesthesia by injecting intravenously a veterinary grade euthanasia solution at 0.1ml/lb of body weight (Euthasol®, Virbac). Post euthanasia, the heart was extracted, rinsed, and prepared for *ex vivo* imaging, including insertion in a 3D-printed mold to preserve the left and right ventricular anatomy as described in [3]. The 3D printed molds were based on the mid-diastasis ventricular configuration. A knee coil was used for imaging and the hearts were submerged in a perfluoropolyether solution (Fomblin, Solvay).

Ex vivo imaging was conducted in a 3T MRI scanner (Prisma, Siemens). Imaging protocols included: T1-weighted GRE (TE/TR=3.15ms/12ms; flip angle= 25°; field of

view = 160mm×160mm; spatial resolution = 1.0×1.0×1.0mm³, $N_{\text{avg}} = 6$), T2-weighted SE (TE/TR=89ms/15460ms; flip angle= 180°; field of view = 192mm × 192mm; spatial resolution = 1.0 × 1.0 × 1.0mm³, $N_{\text{avg}} = 8$), and cDTI (TE/TR=62ms/15560ms; field of view = 150mm×150mm; spatial resolution = 1.0 × 1.0 × 1.0mm³; b-values=0s/mm² and 1000s/mm²; $N_{\text{dir}} = 30$; $N_{\text{avg}} = 5$). In average, *ex-vivo* MRI exams started 2.5 hours post euthanasia. The cDTI sequence was a readout segmented sequence [10] with a twice refocused spin-echo encoding for correction of eddy currents [11].

2.2 Diffusion tensor reconstruction and image labeling

Diffusion tensors were reconstructed from the diffusion weighted images using an in-house Matlab [6] code (github.com/KMoulin/DiffusionRecon). Subsequently, tensor invariants and eigenvalues were calculated at each voxel location.

T1-weighted images were used to segment the myocardium into remote, border, and infarct zones. For this purpose, five short axis slices were selected from apex to base in each subject. Per each subject, small regions of interest (ROIs) were outlined on the remote myocardium of each slice. The mean signal intensity (μ_{remote}) and standard deviation (σ_{remote}) of the selected remote myocardium across slices were computed for each heart. All voxels with a signal intensity SI less than two standard deviations above the mean signal intensity of the remote myocardium (i.e., $SI < \mu_{\text{remote}} + 2\sigma_{\text{remote}}$) were labeled as remote zone. Small ROIs were then drawn in the brightest regions of the otherwise unlabeled myocardium to determine the mean signal intensity of the infarcted myocardium (μ_{infarct}). Following the segmentation method used by Schelbert et al. [12], a halfway value was calculated by averaging the mean of the remote myocardium and the mean of the infarcted myocardium. Voxels with signal intensity greater than two standard deviations above the mean of the remote myocardium, but less than the halfway value (i.e., $\mu_{\text{remote}} + 2\sigma_{\text{remote}} < SI < \frac{1}{2}(\mu_{\text{remote}} + \mu_{\text{infarct}})$) were labeled as border zone. The remaining voxels with signal intensity above the halfway value ($SI \geq \frac{1}{2}(\mu_{\text{infarct}} + \mu_{\text{remote}})$) were labeled as infarct.

2.3 Data analysis

In order to compare tensor invariants and eigenvalues across remote, border, and infarct zones, 100 voxels per zone were randomly chosen from the previously labeled regions on the T1-weighted images for each subject. The labeled ROIs were then rigidly registered to the corresponding cDTI images, with the goal of computing FA, MD, mode, eigenvalues (e_1 , e_2 , and e_3) and radial diffusivity (Rd) – defined as the mean of e_2 and e_3 – associated with the remote, border, and infarct regions. Since a mold was inserted to preserve the heart configuration at mid-diastasis during the *ex vivo* image acquisition, a rigid registration was sufficient to register ROIs in the T1-weighted images to the corresponding cDTI regions.

Data was visualized using maps on representative slices as well as box and rain-cloud plots in order to compare the distributions of tensor invariants and eigenvalues across remote, border, and infarct regions. T1- and T2-weighted images were normalized by the standard deviation of the noise per slice.

All infarct, border, and remote data were screened using a non-parametric Kruskal-Wallis test with Bonferroni *ad hoc* adjustment. Pairwise non-parametric Mann-Whitney U test was also performed on the infarct-remote, border-remote, and infarct-border regions. $p < 0.01$ was considered significant.

3 Results

Figure 1 illustrates five representative T1-weighted and T2-weighted short axis slices from an infarcted heart along with their corresponding $b = 0\text{s/mm}^2$ and $b = 1000\text{s/mm}^2$ images.

Figure 2 illustrates five representative T1-weighted short-axis slices from the same infarcted heart, along with the corresponding FA, MD, e_1 , e_2 , e_3 , and Rd maps. The border and infarct regions are identifiable due to their hyperenhanced nature in the T1-weighted images. In the corresponding quantitative maps, the infarct and border regions are marked by a higher MD, e_1 , e_2 , e_3 , Rd and a lower FA compared to the remote myocardium.

Figure 3 quantitatively illustrates the differences in MD, FA, Mode, e_1 , e_2 , e_3 , Rd, T1, and T2-weighted normalized signals in the infarct, border, and remote regions. The upper and lower edges of each boxplot indicate the 25th (Q1) and 75th (Q3) percentiles and the top and bottom whiskers indicate 1.5 times the interquartile range measured from Q1 and Q3. Overall mean, median, and IQR across subjects are further summarized in Table 1.

Figure 4 illustrates the percent difference – per subject and overall – observed in median values for border and infarct regions with respect to the remote myocardium. Amongst diffusion tensor invariants, eigenvalues, and Rd the largest percent differences are recorded for e_2 (e_2 increases by 36% and 115% from remote to border and infarct zones, respectively), e_3 (e_3 increases by 40% and 155% from remote to border and infarct zones, respectively), and Rd (Rd increases by 37% and 128% from remote to border and infarct zones, respectively). Mean diffusivity (MD) and radial diffusivity (Rd) per subject are also reported in Fig. 5 (ref. Appendix).

In all statistical analysis (ref. Section 2.3), the computed p values were less than 0.01, rejecting the null hypothesis that observations were part of the same distributions.

4 Discussion

In this study, *ex vivo* cDTI data acquired six to ten weeks after myocardial infarction was used to quantify differences in tensor invariants and eigenvalues in infarct, border, and remote myocardial regions. Rd, e_2 , and e_3 present the largest increase in the infarct and border zones compared to the remote myocardium.

Across all subjects, FA and Mode decreased in the infarct and border regions, while MD, eigenvalues, and Rd increased. In all cases, the percent increase or decrease was higher in the infarct region than in the border zone. These trends concur with previous studies [7,4,2]. After chronic myocardial infarction, it has been histologically validated that the infarct region is largely comprised of replacement fibrosis [2] causing an overall increase in

the extracellular volume ratio [9]. This increase in extracellular volume ratio likely drives the observed increase in MD and eigenvalues and decrease in FA found in the infarct region.

Indeed, a higher extracellular volume fraction accords with less hindered water self-diffusion owing to fewer cardiomyocyte diffusive barriers compared to healthy myocardium. In fact, water molecules in healthy myocardium are more constrained in the radial direction, therefore the increase in self-diffusion in infarcted myocardium is noticeably larger in the radial direction.

This agrees with the markedly higher e_2 (associated to the sheetlets' direction) and e_3 (associated with the direction normal to the sheetlets) values in the infarct region compared to e_1 values (associated with the aggregate cardiomyocyte preferential direction). Similarly, although less significant, an unequal increase in e_1 , e_2 , and e_3 with respect to the remote myocardium is visible in the border region, which is likely a mixture of scar tissue and viable myocardium.

Radial diffusivity is a concise measure representative of the changes in the sheetlet and cross fiber directions, where, as discussed above, the largest differences due to an increase in extracellular volume ratio are expected. Accordingly, together with e_2 and e_3 , Rd increased the most in the infarct and border regions compared to the remote myocardium.

The diffusion tensor eigenvalues and invariants reported in the current study are in good agreement with previously computed values. For example, the mean e_1 , e_2 , and e_3 values computed in Pashakhanloo et al. [8] compared to the ones computed in the current study (values computed in the current study are reported in parenthesis) were, respectively: $1.125(1.16)e-3\text{mm}^2/\text{s}$, $0.923(0.90)e-3\text{mm}^2/\text{s}$, and $0.725(0.72)e-3\text{mm}^2/\text{s}$ in the infarct region; $0.901(0.71)e-3\text{mm}^2/\text{s}$, $0.625(0.42)e-3\text{mm}^2/\text{s}$, and $0.495(0.29)e-3\text{mm}^2/\text{s}$ in the remote myocardium.

Pashakhanloo et al. [8] reported mean MD and FA in the infarcted tissue to be $0.924e-3\text{mm}^2/\text{s}$ and 0.23 respectively. These values are similar to mean MD and FA values computed in this study and equal to $0.91e-3\text{mm}^2/\text{s}$ and 0.27, respectively. However, in the non-infarct regions, Pashakhanloo et al. [8] have reported mean MD and FA to be $0.674e-3\text{mm}^2/\text{s}$ and 0.31, respectively, whereas mean MD and FA values computed in the current study were $0.47e-3\text{mm}^2/\text{s}$ and 0.44, respectively. This larger discrepancy may be due to the fact that in [8] the myocardium has been subdivided in remote and infarct regions only, as opposite to the current study where a border region has also been considered. A higher MD and lower FA in the remote myocardium may be a consequence of including part of the border region in the remote myocardium in studies considering only two regions. Differences in the precise tissue handling methods and duration from explant to imaging may also play a role.

Kung et al. [4] computed median FA values equal to 0.464, 0.417, and 0.330 in the remote, border, and infarct regions, respectively, whereas the corresponding values computed in the current study are 0.44, 0.40, and 0.24 respectively.

This study also presents limitations. First, only seven subjects were included. Although the data across all subjects is consistent, future studies should include a larger cohort. Moreover, all data were acquired *ex vivo*. This allowed acquiring high resolution and high SNR data without motion artifacts. Further studies using *in vivo* data at a lower resolution and SNR are necessary. Additional work is also needed to compare how border and infarct zones detected with cDTI data alone compare to LGE data. Owing to its relatively large changes, Rd is as a key candidate to determine if infarct and border zones are adequately detected from cDTI data. Finally, the size of the infarct varied significantly across subjects, likely leading to variations in the border zone and extent of the microstructural changes. Although controlling the infarct size is challenging, a larger sample size will enable better understanding the effect of infarct size on the measured cDTI quantities.

In conclusion, diffusion tensor invariants, eigenvalues, and Rd are promising biomarkers that can be acquired without the use of a contrast agent and can help detecting and quantifying the microstructural changes occurring in infarct and border regions.

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Appendix

Mean diffusivity (MD) and Radial diffusivity (Rd) data are reported separately for each subject in Fig. 5. As also evident in Fig. 4, there is a significant variability across subjects, in part as a function of infarct size.

Fig. 5.

Grouped box plots overlaid on top of corresponding Mean diffusivity (MD) and Radial diffusivity (Rd) data points (cross and circular markers) in the infarct (left, purple), border (center, yellow), and remote (right, blue) myocardial regions for each subject. Cross markers represent data points 1.5 IQR above Q3 or below Q1.

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Fig. 1. T1-weighted, T2-weighted, $b = 0 \text{ s/mm}^2$, and $b = 1000 \text{ s/mm}^2$ images for representative short-axis slices in an infarcted subject (subject number 3 in the current study).

Fig. 2. T1-weighted, Fractional Anisotropy (FA), Mean Diffusivity (MD), primary (e_1), secondary (e_2), and tertiary (e_3) eigenvalue maps for representative short-axis slices. Infarct and border regions are visible from diffusion tensor invariants, eigenvalues, and Rd maps. Images are shown for subject 3 in the current study.

Fig. 3. Rain-cloud plots [1] overlaid to the corresponding box plots for FA, MD, Mode, eigenvalues e_1 , e_2 , and e_3 , radial diffusivity (Rd), normalized T1- and T2-weighted signal intensities in the infarct, border, and remote myocardial regions.

Fig.4.

Bar plots illustrating percent differences in median FA, MD, Mode, e_1 , e_2 , e_3 , Rd, T1-weighted, and T2-weighted normalized signal intensities in the border and infarct regions with respect to the remote myocardium. The dotted line indicates the median percentage change when data from all subjects are considered together. The percent differences reported directly on the plots are computed between remote-border and remote-infarct overall medians.

Table 1.

Overall mean, median, and Q1-Q3 computed over all subjects for FA, MD, Mode, eigenvalues, Rd, T1- and T2-weighted normalized signals.

	FA			MD (1e-3 mm ² /s)			Mode		
	Infarct	Border	Remote	Infarct	Border	Remote	Infarct	Border	Remote
Mean	0.27	0.40	0.44	0.91	0.65	0.47	0.14	0.40	0.54
Median	0.24	0.40	0.44	0.90	0.61	0.46	0.05	0.52	0.67
Q1, Q3	0.17, 0.34	0.29, 0.50	0.35, 0.53	0.65, 1.15	0.49, 0.76	0.39, 0.55	-0.24, 0.62	0.12, 0.78	0.34, 0.86
	e ₁ (1e-3 mm ² /s)			e ₂ (1e-3 mm ² /s)			e ₃ (1e-3 mm ² /s)		
	Infarct	Border	Remote	Infarct	Border	Remote	Infarct	Border	Remote
Mean	1.16	0.93	0.71	0.90	0.60	0.42	0.72	0.44	0.29
Median	1.16	0.91	0.70	0.88	0.55	0.41	0.72	0.39	0.28
Q1, Q3	0.91, 1.40	0.75, 1.10	0.61, 0.80	0.63, 1.14	0.43, 0.71	0.33, 0.50	0.48, 0.93	0.29, 0.53	0.20, 0.37
	Rd (1e-3 mm ² /s)			T1-weighted			T2-weighted		
	Infarct	Border	Remote	Infarct	Border	Remote	Infarct	Border	Remote
Mean	0.81	0.52	0.36	491	253	138	21.1	13.0	8.2
Median	0.79	0.47	0.35	374	235	112	13.1	9.4	5.6
Q1, Q3	0.55, 1.04	0.35, 0.62	0.27, 0.43	243, 678	117, 346	72, 191	8.4, 21.2	6.5, 15.6	4.6, 7.3