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A non-contrast CMR index for assessing myocardial fibrosis

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Abstract

Purpose: Safe, sensitive, and non-invasive imaging methods to assess the presence, extent, and turnover of myocardial fibrosis are needed for early stratification of risk in patients who might develop heart failure after myocardial infarction. We describe a non-contrast cardiac magnetic resonance (CMR) approach for sensitive detection of myocardial fibrosis using a canine model of myocardial infarction and reperfusion.

Methods: Seven dogs had coronary thrombotic occlusion of the left anterior descending coronary arteries followed by fibrinolytic reperfusion. CMR studies were performed at 7 days after reperfusion. A CMR spin-locking T₁ρ mapping sequence was used to acquire T₁ρ dispersion data with spin-lock frequencies of 0 and 511 Hz. A fibrosis index map was derived on a pixel-by-pixel basis. CMR native T₁ mapping, first-pass myocardial perfusion imaging, and post-contrast late gadolinium enhancement imaging were also performed for assessing myocardial ischemia and fibrosis. Hearts were dissected after CMR for histopathological staining and two myocardial tissue segments from the septal regions of adjacent left ventricular slices were qualitatively assessed to grade the extent of myocardial fibrosis.

Results: Histopathology of 14 myocardial tissue segments from septal regions was graded as grade 1 (fibrosis area, <20% of a low power field, n = 9), grade 2 (fibrosis area, 20–50% of field, n = 4), or grade 3 (fibrosis area, >50% of field, n = 1). A dramatic difference in fibrosis index (183%, $P < 0.001$) was observed by CMR from grade 1 to 2, whereas differences were much smaller for T₁ρ (9%, $P = 0.14$), native T₁ (5.5%, $P = 0.12$), and perfusion (–21%, $P = 0.05$).

Conclusion: A non-contrast CMR index based on T₁ρ dispersion contrast was shown in preliminary studies to detect and correlate with the extent of myocardial fibrosis identified

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histopathologically. A non-contrast approach may have important implications for managing cardiac patients with heart failure, particularly in the presence of impaired renal function.

Keywords

CMR; Myocardial diffuse fibrosis; Spin-lock $T_{1\rho}$; Myocardial infarction

1. Background

Cardiac fibrosis is one of the hallmarks of pathological left ventricular (LV) remodeling [1], which plays a significant role in myocardial response to injury. Excessive cardiac fibrosis leads to progression of heart failure [2]. Therefore, non-invasive imaging methods to quantitatively and reproducibly assess at an early stage the presence, extent, and turnover of myocardial fibrosis are needed for risk stratification of heart failure, and also to monitor the effects of treatment in patients after acute myocardial infarction (MI).

Historically, the most convenient and cost-effective imaging tool for fibrosis has been ultrasonic calibrated integrated backscatter [3]. Developed in the 1980s [4], it detects the decreased tissue elasticity and thus changes in acoustic impedance and reflectivity of the myocardium as collagen increases. However, this imaging method has low reproducibility and poor image quality due to certain technical limitations [5]. CMR T_1 mapping is a technique that enables quantification of extracellular volume fraction (ECV), a surrogate marker for myocardial fibrosis that has been extensively validated [6,7]. However, T_1 mapping requires the administration of gadolinium contrast agent to obtain pre- and post-contrast T_1 maps to calculate ECV, which may be contraindicated in some patients, especially those with impaired renal function (e.g., in heart failure, diabetes, and end-stage renal dysfunction) [8].

Spin-lock $T_{1\rho}$ contrast in MRI has been used extensively for the detection of collagen and proteoglycan contents in knee cartilage in patients with osteoarthritis [9] and in skeletal muscle [10]. This contrast approach measures spin-lattice relaxation time in the rotating frame at a spin-lock amplitude B_1 [11]. In myocardial muscle, $T_{1\rho}$ increased up to 2-fold in regions of chronic, non-reperfused MI (primarily replacement fibrosis or scar), in comparison with remote normal myocardial tissue [12,13]. A recent report by van Oorschot et al. demonstrated a good correlation of myocardial $T_{1\rho}$ and ECV values in 20 patients with dilated cardiomyopathy (Pearson $r = 0.66$) [14]. However, the sensitivity of $T_{1\rho}$ contrast to diffuse types of fibrosis, e.g. at the border zone between infarcted and non-infarcted myocardium after MI, appeared relatively low in other patients assessed previously by these investigators [15]. Several studies have adopted a $T_{1\rho}$ dispersion contrast, rather than a $T_{1\rho}$ contrast, to improve the sensitivity for assessing early cartilage degeneration [16], to investigate aging difference in skeletal muscle [17], and to explore metabolite with chemical exchange sensitive MRI [18]. The fundamental mechanism is likely to be the chemical exchange contribution, between free water protons and protons bound with macromolecules, on a time scale of the spin-locking field [19]. In this study, we hypothesized that a $T_{1\rho}$ dispersion contrast would provide another imaging approach with improved sensitivity for the assessment of diffuse myocardial fibrosis [20]. Based on a cardiac $T_{1\rho}$ technique

reported previously [21], we report initial evaluation for detection of myocardial fibrosis in a canine model of MI after coronary reperfusion.

2. Methods

2.1. Animal model and protocol

All animal protocols were approved by the Institutional Animal Care and Use Committee at our local institute. The animal preparation was a clinically relevant model of coronary thrombosis and fibrinolytic-induced reperfusion (with recombinant human tissue-type plasminogen activator, rt-PA) as described [22]. Briefly, seven mongrel dogs (21.3 ± 3.4 kg) underwent aseptic left thoracotomy surgery and exposure of the left anterior descending coronary artery (LAD). A stainless steel needle electrode and an MR-compatible electromagnetic flow probe (Transonic Systems) were placed on the artery below the first diagonal branch to monitor the change in coronary artery blood flow. The chest was then closed in layers and the dogs recovered from surgery.

Coronary thrombosis was induced by application of anodal current to the coronary electrode from a 9 V battery with the cathode attached to the skin. One hour after complete thrombotic occlusion, rt-PA (1 mg/kg) was given to induce lysis of the thrombus and coronary reperfusion. Coronary patency was confirmed and followed with use of the flow probe as described [22].

CMR scans were at 7 days after reperfusion. Anesthesia was induced with isoflurane (1–5%) delivered through a facemask followed by a cuffed endotracheal tube. The electrocardiogram (ECG) and arterial oxygen saturation were monitored continuously.

2.2. CMR imaging protocol

All Cardiac MR studies were performed on a Siemens 3T Trio System (Siemens Medical Solution, Malvern, PA) using an 8-element phased-array coil as the receiver. After scout imaging to localize the short-axial slices, the following scans were performed: (1) native T_1 mapping - measurements of myocardial T_1 were performed using a modified fast look-locker imaging sequence that was developed and validated [23]; (2) non-contrast $T_{1\rho}$ dispersion imaging at two spin-lock frequencies (SLFs), 0 Hz and 510 Hz; (3) first-pass perfusion imaging for myocardial blood flow (MBF) measurements, with a dual-bolus approach (10 and then 100 $\mu\text{mol/kg}$ of the MR contrast agent Multihance, Bracco Diagnostic, Princeton, NJ) [24]; and (4) late gadolinium enhancement (LGE) imaging - acquired after an additional injection of 50 $\mu\text{mol/kg}$ Multihance, using a clinical LGE protocol in the MRI system [25]. All imaging parameters are shown in Table 1.

The $T_{1\rho}$ sequence scanned each slice with two breath-holds to acquire $T_{1\rho}$ maps at two SLFs. This worked well in anesthetized, respiratory-controlled animals without any apparent registration motion artifacts. The $T_{1\rho}$ imaging sequence applied three 180° refocusing RF pulses and a rotary echo scheme for spin-lock pulses in the $T_{1\rho}$ -preparation module to reduce B_0 and B_1 inhomogeneity artifacts (Fig. 1) [21]. Triggered by every third ECG signal, the specific absorption rate was controlled under the safety limits imposed by the MRI system manufacturer. In our initial study with normal dogs (unpublished data), this

approach provided the best image quality, given the limited breath-hold time, restriction of specific absorption rate, and desired image resolution. The $T_{1\rho}$ (0 Hz) is essentially T_2 that can be also used to assess myocardial edema.

2.3. Histopathology

After the CMR, the hearts were perfused in situ with Evans blue dye, with the coronary artery reoccluded using a suture tie to estimate the previous non-perfused 'region-at-risk' in transverse slices of the left ventricle. The slices were then placed in triphyltetrazolium chloride to delineate the unstained (infarcted) muscle in each slice. The slices were then fixed in 4% paraformaldehyde solution for morphologic examination. Two adjacent ventricular slices were selected, based on approximately the same distance to the ventricular base as two MRI slices. Tissue specimens from the mid-septal zone bordering the non-perfused myocardium in these slices were embedded in paraffin, cut in 5 μ m sections, and stained with Masson's trichrome stain for morphologic identification of collagen [26].

2.4. CMR data and histopathologic analysis

A myocardial fibrosis index (mFI) can be defined as follows:

$$\text{mFI}(\omega_1) = T1_{\rho}(\omega_1) - T1_{\rho}(0)$$

where mFI was calculated on a pixel-by-pixel basis, with units of msec. Two short axial images matching the locations of the tissue slices were analyzed for each imaging session, based on the distances from the tissue locations to the base of the hearts. The $T_{1\rho}$ (ω_1), $T_{1\rho}$ (0), mFI, and T_1 maps were all generated using custom-made software written in MATLAB (MathWorks, Natick, MA). MBF maps were obtained using another custom-made software (Perfusion Imaging Toolkit) that employed the Fermi function deconvolution to determine absolute MBF (ml/min/g) [27].

Due to severe artifacts in the anterior and lateral regions of the LV caused by the metal needle in the LAD, image analysis for this study was performed only in the mid-anteroseptal region. Regions-of-interest (ROIs) were drawn in this region that matched the location of the specimens in histopathology. Despite being comprised of both normal and infarcted myocardium, this region has been shown to develop fibrosis several days after MI with impairment of energy metabolism and myocardial function [28,29]. Non-contrast $T_{1\rho}$, native T_1 , mFI, and MBF in these ROIs were then measured on the corresponding maps. Using Masson's trichrome-stained slides of the septal tissue, a pathologist blinded to the MRI results visually estimated the percentage of fibrosis area compared to the total tissue area at 4 $\times$ (low power) and confirmed at 20 $\times$ (high power). The fibrosis extent was then scored as grade 1 (fibrosis area, <20% of a low power field, low), grade 2 (fibrosis area, 20–50% of field, moderate), or grade 3 (fibrosis area, > 50% of field, high) as described previously [30]. The differences in image parameters between grades were compared using unpaired two-tailed Student's *t*-tests. Significance was defined as $P < 0.05$.

3. Results

Because there were two mid-anteroseptal regions analyzed in each scan per dog, there were a total of 14 slides for analysis. Based on the microscopic examination, nine slides from five dogs were scored as grade 1, four slides from two dogs were scored as grade 2, and one slide was scored as grade 3. There was no edema evident in the septal regions in $T_{1\rho}$ (0) maps.

In Table 2, native T_1 , $T_{1\rho}$, and mFI increased with increasing grades, whereas MBF decreased as expected. Comparing mFI to $T_{1\rho}$ at SLF of 510 Hz and native T_1 , mFI demonstrated the largest difference from grade 1 to grade 2 fibrosis. This difference in mean mFI was 183% ($P < 0.001$), whereas the differences in other indices were 8.8% ($P = 0.14$) for $T_{1\rho}$ (510 Hz), 5.5% ($P = 0.12$) for native T_1 , and -21.1% ($P = 0.05$) for MBF. Only the mFI index showed a significant difference between the two grades. Statistical comparisons were not feasible for grade 3 because of the single sample. Fig. 2 shows image examples of grade 1, 2, and 3 fibrosis in the septal region of the ventricular slices from three different animals, as well as the corresponding histopathology.

4. Discussion and conclusion

This study reports a sensitive index for the detection of myocardial fibrosis without use of an exogenous contrast agent. Correlating with histopathologic results, the index demonstrated approximately a 2-fold difference between low and moderate fibrosis grades, whereas other standard MRI parameters showed only 5–21% differences (Table 2).

The animal model of MI and reperfusion was followed by structural remodeling with scar formation at the MI site (the anterior regions) and tissue changes in the septal myocardium that we imaged in this study, including formation of the diffuse interstitial and replacement fibrosis. After MI and reperfusion, during the early stages of myocardial healing (24 h – 2 weeks), several cytokines, including TGF- β 1, TNF- α , and IL-1 contribute to the conversion of cardiac fibroblasts into myofibroblasts [31]. The latter produce fibrillar type I and III collagens globally, i.e., increased collagen deposition in the interstitial compartment of non-infarcted myocardium [32]. Therefore, it is not unexpected to observe some infarction and diffuse fibrosis at the border zone of infarction in the septum at 7 days after a fairly large anterior MI and reperfusion in our canine model [33].

Non-contrast mFI imaging may provide an alternative to the current approach for assessing myocardial fibrosis. The mFI imaging is based on $T_{1\rho}$ dispersion contrast, rather than the absolute $T_{1\rho}$ contrast. The latter has been reported by several groups to show up to a 1-fold higher $T_{1\rho}$ value for myocardial scar tissue induced by MI [12,13,20]. For other regions with less collagen content, such as the border zone of the infarct, the amount of increase in $T_{1\rho}$ is much less, making the distinction and quantification of differences in fibrosis more problematic. On the other hand, the $T_{1\rho}$ dispersion contrast produced a much greater difference in $T_{1\rho}$ between two SLFs, resulting in detection of more subtle differences in the extent of fibrosis, even within the border zone region [20]. For this reason, mFI is defined as the difference between $T_{1\rho}$ (0) and $T_{1\rho}$ (ω_1), rather than a composite metric $R_2 - R_{1\rho}$ ($R_{1\rho} = 1/T_{1\rho}$) [16] or the ratio of $T_{1\rho}$ (ω_1) and $T_{1\rho}$ (0) [34].

For normal myocardial tissue, the mFI contrast between 0 and 510 Hz of SLF should be relatively small [20]. For myocardial tissue with excess collagen, as with the diffuse type of fibrosis, $T_{1\rho}$ dispersion contrast is expected to increase and then reach much higher intensity in regions of densely packed collagen. The quantified mFI derived from the $T_{1\rho}$ dispersion contrast indeed showed approximately a 2-fold difference between fibrosis in grades 1 and 2 (Table 2). The moderate grade 2 fibrosis was composed of both diffuse and densely packed collagen (producing some LGE-enhanced areas). Although the low grade 1 fibrosis was defined as <20% by area, it was noted that the fibrosis content of grade 1 was negligible by visual inspection (Fig. 2). Although absolute $T_{1\rho}$ values also increased, the difference between two grades was not statistically significant, demonstrating greater sensitivity for the mFI contrast to detect myocardial diffuse types of fibrosis. To our knowledge, this is the first report for systematic investigation of $T_{1\rho}$ dispersion contrast in myocardial tissue with a diffuse type of fibrosis.

Despite a few studies in ex vivo tissue, in vivo animal, and human patients, the precise mechanisms of $T_{1\rho}$ to detect myocardial fibrosis remain unknown. Among possible contributors to alter $T_{1\rho}$ signals in myocardial tissue, chemical exchange on a time scale of γB_1 or intermediate exchange regimen are likely to play an important role in the modulation of $T_{1\rho}$ signals [35,36,37]. By subtracting $T_{1\rho}(0)$ from $T_{1\rho}(\omega_1)$ (to result in mFI), the intrinsic T_2 effect affecting both $T_{1\rho}(0)$ and $T_{1\rho}$ may be cancelled out or much reduced. This may “amplify” the chemical exchange effect in the relaxation times when water protons to exchange on the time scale of γB_1 are locked without dephasing [19,38].

Although increased $T_{1\rho}$ in myocardial scar tissue was thought to be associated with increased macromolecular content such as collagen ex vivo studies in agarose and other protein phantoms pointed in an opposite direction, i.e., an increase in agarose/protein concentration resulted in a decrease in phantom $T_{1\rho}$ [39,40]. In addition, loss of proteoglycan and glycosaminoglycan (GAG) in cartilage appeared to be associated with the elevation of $T_{1\rho}$. Nevertheless, based on chemical exchange theory [16,18], an increased concentration of water protons bound to macromolecules leads to increases in both $R_{1\rho}$ and mFI. The magnitude of the increase is modulated by the chemical shifts and exchange rates of macromolecules. After myocardial infarction, production of structural proteins such as collagens is elevated to facilitate in heart healing. Other nonstructural extracellular matrix proteins proteoglycans, and GAGs are all upregulated as orchestrators of myocardial extracellular matrix (ECM) remodeling contributing to inflammation, angiogenesis, and fibrosis [41]. These nonstructural proteins include matricellular proteins (tenascins, osteopontin, periostin) hyaluronan (the largest GAG in cardiac ECM), and small leucine-rich proteoglycans (biglycan, decorin, lumican) [42]. These upregulated proteins with varied size and weight would increase the exchange sites, resulting in increased mFI. However, the dynamic changes of all proteins would alter the mFI during myocardial remodeling. More studies are needed to determine the correlation of mFI with extracellular volume and whether mFI reflects the content of collagen or other macromolecules in myocardial ECM.

There are several limitations in this pilot study besides the small number of animals. The animal model is not specialized for creating myocardial fibrosis, but rather MI. Fortunately, both replacement and diffuse interstitial fibrosis can be observed in the septum adjacent to

the anterior MI region in some animals. Using this model is the first step to prove the concept of this non-contrast MRI approach. Although there were no artifacts observed in the septal regions, there were relatively large signal void artifacts in the anterior, lateral, and sometimes posterior regions due to the metal needle in the coronary artery (Fig. 2, dashed circles). Improvements in our animal model currently under development will eliminate the needle electrode increasing our ability to assess fibrosis in all regions of the heart. Another limitation is that two scans are needed for mFI measurements. Measurement errors could arise due to misregistration and cardiac motion. One solution is to use interleaved acquisition of k-space data of two $T_{1\rho}$ images in one scan. This CMR sequence is under development. Finally, whether this index can be used in the acute phase of MI, in the presence of interstitial edema is unknown. The current study only analyzed the septal region away from the acute infarct area. No edema was detected in this region. With future studies in a modified MI preparation, characteristics of mFI in the acute infarct area will be defined as well.

In summary, we have presented initial data using a non-contrast imaging index for the detection of myocardial fibrosis in a canine model of MI and reperfusion. Histopathological differences in fibrosis extent at 7 days appear to account for large differences in this index. While this index has the potential for quantification of fibrosis content, much more work is needed to characterize its sensitivity and specificity against the gold standards of molecular structure and histopathology.

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Fig. 1.

Schematic diagrams of the cardiac $T_{1\rho}$ -preparation sequence with three 180° refocusing pulses and rotary echo scheme for the reduction of B_0 and B_1 inhomogeneity. Three $T_{1\rho}$ -weighted images are acquired at three different TSL (10, 30, 50 ms) to form one $T_{1\rho}$ map. The sequence uses a segmented data acquisition scheme to acquire data and a trigger delay time is varied to ensure data acquisition time to occur at the mid-diastole for the reduction of cardiac motion.

Fig. 2.

Examples of various contrasts of images and maps for fibrosis grade 1 (top), grade 2 (middle), and grade 3 (bottom). From left to right, LGE images, zoomed LGE images on septal regions, $T_{1\rho}$ maps (510 Hz), $T_{1\rho}$ maps (0 Hz), and mFI maps (ms) in the same septal regions, as well as corresponding trichrome-stained histopathological images (blue = fibrosis). The green rectangles in mFI maps indicate approximate areas where the histopathological slides were located. The values in mFI maps indicated the mean mFI measured in the green rectangle covered regions. The dashed circles in the LGE images point to the artifacts induced by metal needles. The collagen deposit was shown in blue in histopathological slides. The units of color scale for $T_{1\rho}$ and mFI maps are ms. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1.

MRI sequences and parameters.

	T ₁ -mapping	T ₁ ρ mapping	First-pass perfusion	LGE
Pulse sequence	Modified single-shot look-locker	T ₁ ρ-prepared segmented gradient-echo	Saturation recovery turbo-FLASH	Phase-sensitive inversion recovery
TR/TE/flip angle	2.3 ms/1.1 ms/15°	3.4 ms/1.7 ms/15°	2.2 ms/1.1 ms/15°	4.4 ms/2.3 ms/20°
TI or time of spin-lock	TI = 100–7000 ms	TSL = 10, 30, 50 ms	TI = 100 ms	TI = 280–340 ms
B ₁	N/A	0 and 12 μT	N/A	N/A
Segment. #	Single-shot	31–41	Single-shot	23
FOV	220 × 170 × 8 mm ³	220 × 170 × 8 mm ³	220 × 170 × 8 mm ³	220 × 170 × 8 mm ³
Matrix	160 × 120 × 1	192 × 150 × 1	192 × 72 × 1	256 × 156 × 1
Acquisition Time/slice	30 cardiac cycles (~600 ms/cycle)	45 cardiac cycles (~600 ms/cycle)	70 cardiac cycle (~600 ms/cycle)	16 cardiac cycles (~600 ms/cycle)

MRI parameters of myocardial tissue at different fibrosis grades determined by histopathology (mean $\pm$ standard deviation).

Table 2.

	Native T ₁ (ms)	T ₁ ρ (0 Hz) (ms)	T ₁ ρ (510 Hz) (ms)	mFI (ms)	MBF (ml/min/g)	LGE?
Grade 1 (n = 9)	1218 $\pm$ 43	38.6 $\pm$ 4.0	42.5 $\pm$ 3.6	3.9 $\pm$ 1.5	0.85 $\pm$ 0.15	No
Grade 2 (n = 4)	1285 $\pm$ 93	34.3 $\pm$ 4.0	46.2 $\pm$ 4.8	10.9 $\pm$ 0.9 *	0.67 $\pm$ 0.07	Some
Grade 3 (n = 1)	1413	43.3	67	22.7	0.39	Yes

* $P < 0.001$, Grade 2 vs. Grade 1.