Longitudinal Changes in Myocardial T-1 and T-2 Relaxation Times Related to Diffuse Myocardial Fibrosis in Aortic Stenosis; Before and After Aortic Valve Replacement

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Longitudinal changes in myocardial T1 and T2 relaxation times related to diffuse myocardial fibrosis in aortic stenosis; before and after aortic valve replacement

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Running title

Myocardial relaxation times after AVR
Longitudinal changes in myocardial T1 and T2 relaxation times related to diffuse myocardial fibrosis in aortic stenosis; before and after aortic valve replacement

Abstract

Background
Diffuse myocardial fibrosis is connected to adverse outcome in several cardiac diseases, but detection and quantification is challenging. Cardiovascular magnetic resonance (MR) relaxation times mapping represent promising imaging biomarkers for diffuse myocardial fibrosis.

Purpose/Hypothesis
The objective of this study was to investigate whether myocardial relaxation times can detect longitudinal changes in myocardial tissue composition associated with diffuse fibrosis in patients with severe aortic stenosis (AS) before and after aortic valve replacement (AVR).

Study type
Prospective longitudinal study.

Population/Subjects/Phantom/Specimen/Animal model
Fifteen patients with severe AS.

Field strength/Sequence
3 Tesla / 3(3)3(3)-MOLLI, T2-GraSE and 3D-QALAS

Assessment
Fifteen patients with severe AS accepted for AVR underwent MR examinations at three time points: before AVR, as well as 3 and 12 months after AVR. The MR examinations included a multiple-slice cine acquisition, 2D-MOLLI acquisitions for T1-mapping, 2D-GraSE acquisitions for T2-mapping and a 3D-QALAS acquisition for combined T1- and T2-mapping. Data from each patient were analyzed in 16 myocardial segments.
Statistical tests

The segment-wise T1 and T2 data were analyzed over time after surgery using linear mixed models with repeated measures.

Results

The results showed that T1 relaxation times were significantly (p<0.05) shorter 3 and 12 months postoperative than pre-operative and that the T2 relaxation times were significantly (p<0.05) longer 3 and 12 months postoperative than pre-operative for both 3D- and 2D-mapping methods. No significant changes were seen between 3 and 12 months postoperative.

Data conclusion

Our findings demonstrate that changes in myocardial relaxation times and thus tissue characteristics can be observed already early after AVR surgery, within the first 3 months. The significant changes in relaxation times from pre-operative examinations to the follow up may be interpreted as a reduction of interstitial fibrosis in the left ventricular wall.

Keywords

Relaxation times mapping, Aortic valve replacement, Longitudinal study, Aortic stenosis, Remodeling, Fibrosis
Introduction

Diffuse fibrosis is connected to an adverse outcome in several cardiac diseases including aortic stenosis (AS), but detection and quantification has shown to be challenging(1). In AS the left ventricular outflow is obstructed, which results in a pressure gradient across the aortic valve. In patients with severe AS, this often leads to concentric left ventricular hypertrophy to maintain adequate cardiac output. Left ventricular hypertrophy due to chronic pressure overload is associated with normal intra-cavity size, but increased wall thickness. An increased amount of interstitial fibrosis is present in concentric hypertrophy due to AS (2). The increased amount of interstitial fibrosis is assumed to be diffusely distributed in the myocardium. Myocardial fibrosis may be reversible(3), but among AS patients, presence of myocardial fibrosis has shown to dramatically increase the all-cause mortality risk(4,5), even after aortic valve replacement(6). The possibility to detect and quantify diffuse myocardial fibrosis at an early stage of the disease is therefore of clinical importance, both to quantify myocardial disease burden and to optimize timing of intervention.

Non-invasive methods to quantify diffuse myocardial fibrosis are lacking. Myocardial biopsies allow for analysis of myocardial fibrosis, but this approach is invasive and provides only regional information. Cardiovascular magnetic resonance allows, by the use of delayed contrast enhancement, for detection of focal myocardial fibrosis, but falls short in detection of diffuse fibrosis since normal unaffected myocardium is missing. Quantitative MR imaging and MR relaxation times mapping have shown to represent promising imaging biomarkers for diffuse myocardial fibrosis(7-9), but longitudinal studies using these methods are missing.

In the field of myocardial relaxation times mapping in relation to diffuse fibrosis, focus has mostly been on the longitudinal T1 relaxation. The longitudinal spin-lattice relaxation time,
T1, can be described as a loss of energy among the spins and as the recovery of the magnetization vector in z-direction, i.e. parallel to the static magnetic field. The relaxation is caused by dipole-dipole interactions, which are induced by fluctuations in the local magnetic field. Thermal molecular motions, such as rotations and vibrations, cause the fluctuations in the local magnetic field. Composition and solidity of a tissue determine how fast, and with what frequency, a dipole-dipole interaction can occur and thus how fast the T1 relaxation is. The fastest, i.e. shortest T1 relaxation time is found in tissue where fluctuations in the local magnetic field occur with a frequency close to the Larmor frequency. Studies have demonstrated a correlation between diffuse myocardial fibrosis and long native T1 relaxation time. A short T1 relaxation time in contrast enhanced myocardial tissue has also been correlated to increased amount of diffuse fibrosis.

The relationship between the transverse relaxation time, T2, and myocardial fibrosis is not studied to the same extent. The transverse spin-spin relaxation time can be described as loss of phase coherence among the spins in the transverse plane, resulting in an overall loss of signal. The loss of phase coherence is mostly due to magnetic interactions and magnetic disturbances with the surrounding molecules. How fast the spins tumble through space is a key aspect in the transverse relaxation. The faster they tumble, the longer the transverse relaxation time becomes. Larger water content enables spins to tumble faster and imply longer T2 relaxation time. In animal studies, correlation between myocardial fibrosis and T2 relaxation time has shown contradictory results. In mice studies, myocardial fibrosis has been correlated to both prolonged T2 relaxation time (17) and shortened T2 relaxation time (18,19), but there is a lack of studies in human myocardial tissue.
The aim of this study was to investigate whether myocardial T1 and T2 relaxation times can detect longitudinal changes in myocardial tissue composition associated with diffuse myocardial fibrosis in patients with severe AS prior to, three and 12 months after aortic valve replacement (AVR).

Materials and methods

All subjects gave written informed consent to participate in the study and approval was granted from the Regional Ethical Review Board.

Study population and MR examination

This study included patients with high gradient severe AS accepted for AVR between April 2014 and December 2015. Patients were accepted for surgical intervention according to current guidelines(20). Patients with a history of any other concomitant cardiac disease, or suffering from arrhythmias or claustrophobia were excluded. Fifteen patients (8 male, 7 female) accepted to participate in the study (Table 1).

The patients underwent MR examinations at three different time points: before AVR, as well as 3 and 12 months after AVR. The MR examinations included a multiple-slice short-axis cine balanced steady-state free precession (SSFP) acquisition, native 2D MOLLI (21) T1 acquisitions and 2D T2-GraSE acquisitions, at apical, mid-ventricular and basal slice positions. The patients also underwent a native 3D-QALAS (22) acquisition. All scans were performed using a Philips Ingenia 3T system.

The MOLLI acquisitions were performed using 3(3)3(3)5 acquisitions. The resolution was 1.22 mm x 1.22 mm in plane and with a slice thickness of 10 mm. A flip angle of 35° was
used together with a repetition time of 2.4 ms and an echo time of 1.13 ms. The MOLLI
acquisition was performed during a breath hold of 17 heartbeats and provided one 2D slice of
the left ventricular myocardium.

The T2-GraSE acquisitions, also providing one 2D slice, had the same resolution as the
MOLLI acquisitions and were performed during a breath hold of 15 heartbeats. Nine different
echo times, range between 9 ms and 84 ms, were used for the calculation of the T2-map and
the repetition time was 1000 ms.

The 3D-QALAS method provides full 3D coverage of the LV myocardium with simultaneous
mapping of T1- and T2 relaxation times in one breath hold. The 3D-QALAS acquisition had a
resolution of 2.0 mm x 2.0 mm in-plane and a slice thickness of 12.0 mm (reconstructed to
2.0 mm x 2.0 mm x 6.0 mm), flip angle of 5°, SENSE factor of 2 in phase direction and 1.2 in
slice direction, repetition time was 2.6 ms and echo time was 1.2 ms. The 3D-QALAS
acquisition was performed during a breath hold of 15 heartbeats and provided 13 short-axis
slices of the left ventricular myocardium.

Image analysis

Quantitative images from MOLLI and GraSE were generated immediately on the scanner
console. The 3D-QALAS data were exported from the scanner console and quantitative
images, i.e. T1- and T2 maps, were generated in a standalone version of SyMRI®
(SyntheticMR, Sweden). Analysis of the generated relaxation times maps were performed in
Segment v 1.9 R3644 (http://segment.heiberg.se). Epi- and endocardial borders were
manually contoured in the thirteen (representing the thirteen slices of the left ventricular
myocardium) T1- and T2 maps of each 3D-QALAS acquisition and in the three T1- and T2
maps from the 2D methods. Based on these manual contours, 16-segment bull’s eye plots were created automatically by the analysis software, excluding the apical cap that is not accessible from the short-axis acquisitions. The 16 segments were in accordance with the myocardial segment model recommended by the American Heart Association (AHA)(23). For each time point and each patient, an average value for T1- and T2 relaxation time were given for the area included in each segment of the 16-segment bull’s eye plot.

The balanced cine SSFP MR images were used to measure left ventricular wall mass, volumes and ejection fraction at the three different time points using a later version of Segment, v.2.1 R5726.

**Statistical analysis**
Segmental myocardial T1- and T2 relaxation times were compared between the three different time points. Data analysis was performed with IBM SPSS Statistics, Version 24.0 (Armonk, NY: IBM Corp.). All data were normally distributed according to the Kolmogorov-Smirnov test, thus parametric tests were used for the statistical analysis. In order to investigate differences over time, linear mixed models with repeated measures were used for segment-wise data. The different time points were defined as a fixed effect and the patients were defined as a random effect. Estimates of fixed effects will provide information about significant changes in relaxation times over the three time points. Statistical significance was set as p<0.05.

**Results**
Data were successfully acquired in all cases.
Patient characteristics

Heart rate, blood pressure and left ventricular ejection fraction were unaltered pre- versus postoperatively. A significant reduction in left ventricular wall mass (p<0.005) was seen from pre- to postoperatively. Characteristics of the patients, together with clinical parameters, can be seen in Table 1.

Cardiovascular magnetic resonance T1 and T2 relaxation times

To investigate changes in myocardial relaxation times longitudinally, the same myocardial segment from the same patient was followed over time. For both methods, the results showed that the T1 relaxation times were significantly shorter 3 and 12 months follow-up than pre-operatively (Figure 1) and that the T2 relaxation times were significantly longer 3 and 12 months follow-up than pre-operatively (Figure 2). No significant difference was seen in T1 or T2 relaxation times between 3 months and 12 months follow-up. A summary of the results is presented in Table 2.

For the 3D-method (3D-QALAS), the overall average native T1/T2 value from the 16 segments in all patients was 1169 ms/48 ms pre-operatively, 1142 ms/50 ms at the 3 months follow-up and 1126 ms/51 ms at the 12 months follow up (3.7% decrease in T1 and 4.6% increase in T2 values). For the 2D-methods (MOLLI and GraSE), the overall average T1/T2 value from the 16 segments in all patients was 1187 ms/50 ms pre-operatively, 1165 ms/52 ms at the 3 months follow-up and 1160 ms/53 ms at the 12 months follow up (2.3% decrease in T1 and 4.3% increase in T2).

In figure 3, the average percentage difference between follow up and preoperatively, are presented in a segment-wise manner and color-coded to get an overview of the myocardial
relaxation times data. Figure 4 shows mid-ventricular T1- and T2-maps acquired with 3D-QALAS, MOLLI and T2-GraSE at the three different time points in one of the patients.

Discussion

In this study, we show that myocardial relaxation times, both T1 and T2 display changes in patients with severe AS from before AVR to 12 months after AVR that are statistically significant. Native myocardial T1 relaxation time was found to significantly decrease from before surgery to 12 months after surgery and myocardial T2 relaxation time was found to significantly increase from before surgery to 12 months after surgery. A significant decrease in T1 and increase in T2 could already be seen at the 3 months follow up. Furthermore, there was in general a good consistency in the results between the 2D-mapping methods and the 3D-mapping method used in this study.

Comparing these findings with T1 and T2 values in healthy volunteers is not obvious, as the normal range of relaxation times is large in a healthy cohort and even larger in cohorts of patients with myocardial pathologies. If such a comparison is made anyway, it is important to compare values acquired with the same mapping sequence and from the same specific site, as recommended by the T1 mapping consensus statement(24) and a T1 mapping review article by Puntmann et al (25). Previously, T1 and T2 relaxation times of healthy cohorts have been published from this site using the same mapping sequences as in this study (22,26). Pooling the 20 healthy volunteers from these two studies, the average T1 relaxation times were 1131 ms and 1117 ms for 3D-QALAS respectively MOLLI, and the average T2 relaxation times were 51.6 ms and 49.2 ms for 3D-QALAS respectively T2-GraSE. For all relaxation times we see the same trend over time after surgery, that the relaxation times for the aortic stenosis patients approach those of the healthy cohort, except for T2-GraSE. However, in this study
our main focus is not on differences between patients and healthy subjects. Instead, we have chosen to follow the same patient over time and thus focus on changes in myocardial relaxation times on a longitudinal basis.

Interstitial fibrosis in AS patients has in several studies been associated with increased native myocardial T1 relaxation time (12,13,27) but, to our knowledge, the association with the effect of reversed remodeling after surgical intervention has not been studied. Our findings, which focus on individual changes in relaxation times after AVR, demonstrate a decrease in native T1 relaxation time from before AVR to 12 months after AVR, which may be interpreted as a reduction of interstitial fibrosis. The largest decrease in T1 relaxation time occurs between pre AVR and 3 months post AVR, for both the MOLLI method and the 3D-QALAS method (Figure 1). Between 3 months and 12 months of follow up, only a slight non-significant additional decrease in T1 can be noted.

The transverse T2 relaxation time was also investigated with respect to changes over time after surgery. For both methods, a significant increase in T2 times was found at the 12 months follow up, compared with pre-operatively. Shorter T2 relaxation time before surgery and longer T2 relaxation time after surgery may indicate an increased tumbling speed of spins, following the Bloembergen-Purcell-Pound (BPP) theory (28), and thus increased relative amount of water content in the tissue after surgery. Assuming that the included patients have elevated amounts of interstitial fibrosis before the AVR, fibrotic myocardial tissue is thus stiffer with lower water content and spins tumble slower through space, implying more interactions with surrounding molecules and thereby shorter T2 relaxation time. However, contradicting results have been shown in animal studies, correlating collagen level or the amount of fibrotic tissue with both longer and shorter T2 relaxation times (17-19). The
association between myocardial interstitial fibrosis and T2 relaxation time in human myocardial tissue found in this study may provide important addition to our body of knowledge of myocardial tissue composition in the setting of fibrosis.

Myocardial edema, myocardial inflammation and iron content are also known to affect MR relaxation times, and can therefore potentially also contribute to the changes found in this study. Especially myocardial edema and inflammation related to surgery are possible confounders of changes in the MR relaxation times. However, a potential presence of myocardial edema or inflammation after surgery is expected to result in an increase in T1 as well as T2 after surgery(29), while we observed an increase in T2 in combination with a decrease in T1. Also the fact that T1 and T2 after surgery change towards normal values indicates that the amount of edema or inflammation is relatively small 3 and 12 months after surgery, which is in accordance with Dongaonkar et al(30). The observed decrease in left ventricular mass after surgery may also affect the relaxation times. A left ventricular mass reduction solely caused by a reduction of muscle tissue, would result in a relative increase in fibrotic tissue and consequently an increased T1. We observed a decreased T1, which indicates a larger reduction of fibrotic tissue than muscle tissue. The results therefore seem to be predominantly explained by a reduction of myocardial fibrosis over time after AVR.

According to studies based on endomyocardial biopsies (31) and late gadolinium enhancement (LGE) MR (32), fibrotic tissue and left ventricular hypertrophy often decreases after AVR. The decrease in left ventricular hypertrophy was in those studies found at an early stage, while the decrease in fibrotic tissue could be seen first after a longer period of time. In this study, changes in myocardial relaxation times, together with left ventricular mass, are seen already at an early stage (three months) after AVR. In contrast to data based on LGE and
biopsies, our findings propose that myocardial relaxation times, both T1 and T2, are early markers of changes in myocardial tissue characteristics and may reflect the amount of fibrotic tissue after AVR.

For each subject, myocardial relaxation times have been analyzed on a segment-wise manner over time. Differences between pre-operatively and follow up were calculated for each segment and are illustrated in figure 3. The figure demonstrates variations of the differences in relaxation times over the myocardial segments. In general, the variations between the segments seem to be a little larger for the 2D-methods than for the 3D-method. One factor may be the use of an iterative analysis approach instead of a conventional curve fitting approach for the 3D-QALAS method. This iterative approach handles several possibly confounding factors that could affect the estimate of T1 and T2, and makes the method more stable. The difference between the 2D methods and the 3D method may also be explained by issues arising in scan – rescan situations, such as positioning of the shim-box, the exact slice positioning and inflow effects, that seem to influence the 2D-methods more than the 3D-method (26). Comparing preoperative data with the 12 months follow up (right column in figure 3), there seems to be a larger change in relaxation times in the septal segments than in the lateral segments of the left ventricular myocardium. This observation is not statistically significant but is thought provoking and may indicate that septal segments are more likely to undergo a larger positive remodeling with respect to hypertrophic response than for example lateral segments, in accordance with Dweck et al (33).

Recently, studies have shown an inability for relaxation times mapping methods to separate healthy myocardium from pathological myocardium on an individual basis, due to the broad range of normal tissue relaxation times values(34,35). In our opinion, the strength and the
clinical potential of relaxation times mapping lies in longitudinal studies, i.e. following the
same subject over time. In such studies, the naturally occurring differences in relaxation times
between subjects are not of importance. So far, there is a lack of studies illustrating the
potential of using myocardial relaxation times in longitudinal studies, as well as using the
combination of T1- and T2 relaxation times in patients with severe AS. This study therefore
contributes with new applications in the field, combined myocardial T1- and T2 mapping in a
longitudinal manner.

Limitations
This study contains a limited number of included patients. However, despite the low number
of patients, we found significant changes in MR relaxation times over time after AVR.
Myocardial relaxation times representing normal tissue are well known to differ depending on
for example vendor and mapping method used (36), which is a limitation when generalizing
values from a single center. According to that, we have chosen to include both 3D and 2D
mapping methods in this study, which we see as a strength, as all methods show significant
results.

Conclusions
Our findings demonstrate that changes in myocardial relaxation times and thus tissue
characteristics can be observed already early after AVR surgery, within the first 3 months.
The significant changes in relaxation times from pre-operative examinations to the follow up
may be interpreted as a reduction of interstitial fibrosis in the left ventricular wall.
References


Table 1. Patient characteristics presented as mean ± one standard deviation.

<table>
<thead>
<tr>
<th>Parameter; average±sd</th>
<th>Before AVR</th>
<th>3 months after AVR</th>
<th>12 months after AVR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [years]</td>
<td>69 ± 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>8 Male, 7 Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure [mmHg]</td>
<td>142 ± 12</td>
<td>141 ± 13</td>
<td>143 ± 9</td>
</tr>
<tr>
<td>Diastolic blood pressure [mmHg]</td>
<td>78 ± 9</td>
<td>83 ± 8</td>
<td>83 ± 6</td>
</tr>
<tr>
<td>Heart rate [bpm]</td>
<td>63 ± 9</td>
<td>65 ± 9</td>
<td>65 ± 10</td>
</tr>
<tr>
<td>Left ventricular wall mass [g]</td>
<td>152 ± 38</td>
<td>127 ± 33</td>
<td>117 ± 28</td>
</tr>
<tr>
<td>Left ventricular ejection fraction [%]</td>
<td>63 ± 11</td>
<td>63 ± 6.1</td>
<td>66 ± 5.8</td>
</tr>
</tbody>
</table>
Table 2. Comparison of myocardial relaxation times by linear mixed model with repeated measures.

<table>
<thead>
<tr>
<th></th>
<th>Pre-operatively vs 3 months</th>
<th>Pre-operatively vs 12 months</th>
<th>3 months vs 12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Native T1 3D-QALAS</strong></td>
<td>Estimate: -40.2 p-value &lt;0.05</td>
<td>Estimate: -56.0 p-value &lt;0.05</td>
<td>Estimate: -15.9 p-value: not sig.</td>
</tr>
<tr>
<td><strong>Native T1 MOLLI</strong></td>
<td>Estimate: -18.4 p-value &lt;0.05</td>
<td>Estimate: -24.3 p-value &lt;0.05</td>
<td>Estimate: -5.93 p-value: not sig.</td>
</tr>
<tr>
<td><strong>T2 3D-QALAS</strong></td>
<td>Estimate: 1.58 p-value &lt;0.05</td>
<td>Estimate: 2.26 p-value &lt;0.05</td>
<td>Estimate: 0.677 p-value: not sig.</td>
</tr>
<tr>
<td><strong>T2 GraSE</strong></td>
<td>Estimate: 1.63 p-value &lt;0.05</td>
<td>Estimate: 2.14 p-value &lt;0.05</td>
<td>Estimate: 0.509 p-value: not sig.</td>
</tr>
</tbody>
</table>
**Figure legends**

Figure 1. Average myocardial T1 relaxation times at the three different time points. Overall average myocardial relaxation times followed over time; pre-surgery, 3- and 12-months post AVR for native T1 values with 3D-QALAS and MOLLI. The p-values are from the linear mixed model test and represent changes in each individual segment over the three time points.

Figure 2. Average myocardial T2 relaxation times at the three different time points. Overall average myocardial relaxation times followed over time; pre-surgery, 3- and 12-months post AVR for T2 values with 3D-QALAS and T2-GraSE. The p-values are from the linear mixed model test and represent changes in each individual segment over the three time points.

Figure 3. Average percentage differences in relaxation times for each myocardial segment. Blue indicates a decrease in relaxation time, while red indicates an increase between preoperative relaxation time and three respectively 12 months postoperative relaxation time.

Figure 4: Illustration of mid-ventricular maps with 3D-QALAS, MOLLI and GraSE in a patient with severe AS. The T1 and T2 relaxation times maps are acquired at three different time points: pre AVR, 3 months post AVR and 12 months post AVR.
Figures

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Figure 2. Average myocardial T2 relaxation times at the three different time points. Overall average myocardial relaxation times followed over time; pre-surgery, 3- and 12-months post AVR for T2 values with 3D-QALAS and T2-GraSE. The p-values are from the linear mixed model test and represent changes in each individual segment over the three time points.
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Severe AS patients (April 2014–Dec 2015) TAVI + open heart AVR n=274

TAVI n=139

Open heart AVR n=135

Declined to participate, pacemaker, atrial fibrillation, arrhythmia, concomitant cardiac disease, claustrophobia n=120

Included n=15
Pre operative

T1 3D-QALAS

3 months post

T1 MOLLI

12 months post

T2 3D-QALAS

T2 GraSE