Quantitative Differentiation of LV Myocardium with and without Layer-Specific Fibrosis Using MRI in Hypertrophic Cardiomyopathy and Layer-Specific Strain TTE Analysis

Nobusada Funabashi, MD, Hiroyuki Takaoka, MD, Koya Ozawa, MD, Tomoko Kamata, MD, Masae Uehara, MD, Issei Komuro, MD and Yoshio Kobayashi, MD

Summary
To achieve further risk stratification in hypertrophic cardiomyopathy (HCM) patients, we localized and quantified layer-specific LV myocardial fibrosis on MRI in HCM patients using regional layer-specific peak longitudinal strain (PLS) and peak circumferential strain (PCS) in LV myocardium (LVM) on speckle tracking transthoracic echocardiography (TTE). A total of 18 HCM patients (14 males; 58 ± 17 years) underwent 1.5T-MRI and TTE. PLS and PCS in each layer of the LVM (endocardium, epicardium, and whole-layer myocardium) were calculated for 17 AHA-defined lesions. MRI assessment showed that fibrosis was classified as endocardial, epicardial, or whole-layer (= either or both of these). Regional PLS was smaller in fibrotic endocardial lesions than in non-fibrotic endocardial lesions (P = 0.004). To detect LV endocardial lesions with fibrosis, ROC curves of regional PLS revealed an area under the curve (AUC) of 0.609 and a best cut-off point of 13.5%, with sensitivity of 65.3% and specificity of 54.3%. Regional PLS was also smaller in fibrotic epicardial lesions than in non-fibrotic epicardial lesions (P < 0.001). To detect LV epicardial lesions with fibrosis, ROC curves of PLS revealed an AUC of 0.684 and a best cut-off point of 9.5%, with sensitivity of 73.5% and specificity of 55.5%. Using whole-layer myocardium analysis, PLS was smaller in fibrotic lesions than in non-fibrotic lesions (P < 0.001). To detect whole-layer LV lesions with fibrosis, ROC curves of regional PLS revealed an AUC of 0.674 and a best cut-off point of 12.5%, with sensitivity of 79.0% and specificity of 50.7%. There were no significant differences in PCS of LV myocardium (endocardium, epicardium, and whole-layer) between fibrotic and non-fibrotic lesions. Quantitative regional PLS but not PCS in LV endocardium, epicardium, and whole-layer myocardium provides useful non-invasive information for layer-specific localization of fibrosis in HCM patients.

Key words: Left ventricular myocardium, Layer-specific peak longitudinal strain, Speckle tracking transthoracic echocardiography, Magnetic resonance imaging

Risk stratification for major adverse cardiac events (MACE) including sudden death is important for the management of patients with hypertrophic cardiomyopathy (HCM) because it has been established that implantable cardioverter defibrillators for preventing sudden cardiac death are effective. Occurrence of non-sustained ventricular tachycardia (VT) and detection of late enhancement (LE) in left ventricular (LV) myocardium suggesting the presence of myocardial fibrosis, by T1 weighted cardiac magnetic resonance imaging (MRI), are very useful predictors for MACE in HCM patients as well as non-dilated LV size and mitral regurgitation and dilated phase HCM. Furthermore, the genetic variation in the SCN10A gene might be associated with cardiac conduction abnormalities in HCM patients. However, current stratification algorithms remain incomplete and novel non-invasive strategies are needed in order to effectively identify high-risk HCM patients.

We previously compared the clinical significance of occurrence of non-sustained VT and that of the presence of LE in LV myocardium, which suggested the presence of myocardial fibrosis, as detected by computed tomography (CT) during risk stratification for MACE in HCM patients without obstructed coronary arteries. We concluded that the presence of myocardial fibrosis in LV myocardium on CT is a stronger predictor of MACE compared with the presence of non-sustained VT in such patients.

We also performed 2-dimensional (2D) speckle tracking transthoracic echocardiography (TTE) to compare the ability to predict the occurrence of MACE between global longitudinal strain (GLS) and global circumferential strain...
(GCS) in LV myocardium in HCM patients without obstructed coronary arteries. We concluded that both 2D LV GLS and GCS (2D LV GLS>GCS) on TTE can predict whether such patients will have a poor prognosis.9)

Furthermore, a recently developed multi-layer speckle tracking TTE technique has enabled the analysis of LV endocardium, epicardium, and whole layer myocardium strain separately.10-15) We speculated that using speckle tracking TTE for regional layer-specific peak longitudinal (PLS) and peak circumferential (PCS) strain in LV myocardium, we could localize and quantify layer-specific LV myocardial fibrosis confirmed by T1 weighted cardiac MRI in HCM patients.

Therefore, in this study, to achieve further risk stratification in HCM patients, we sought to ascertain the relationship between regional layer-specific peak strain (PLS and PCS) in LV myocardium and the presence of layer-specific LV myocardial fibrosis confirmed by T1 weighted cardiac MRI in HCM patients.

**Methods**

**Patient population:** A total of 18 HCM patients (14 males; mean age, 58 ± 17 years) underwent 1.5 Tesla (T) MRI (Achieva, Philips) and TTE (Vivid E9, GE Healthcare).

**Speckle tracking TTE:** Using TTE, PLS and PCS for each layer of the LV myocardium (endocardium, epicardium, and whole layer) among 17 lesions (PLS)16) and 12 lesions (mid and basal portions, PCS), as defined by the American Heart Association,17) were calculated using Echo PAC, version 113 (GE Healthcare) (Figure 1).

**MRI protocol:** Using MRI, the patients were placed supine in a clinical 1.5 T MRI with 5-channel cardiac coils around the chest. As previously reported, all MRIs were obtained using electrocardiogram (ECG) gating and during repeated episodes of breath-holding.18) Surface-coil intensity correction was performed for both cine MRI and MRI for LE. Cine MRIs were acquired using a steady-state free precession sequence. After acquiring cine MRIs on the 2- and 4-chamber long-axis projections, we obtained short axis cine MRIs encompassing the LV from base to apex. MRIs for LE were acquired 10 to 15 minutes after intravenous administration of 0.15 mmol/kg of gadopentetate dimeglumine (Magnevist; Schering AG). An inversion-recovery prepared, T1-weighted, three dimen-
sional gradient-echo sequence was used to obtain MRIs for LE in the same planes as the cine images. The inversion time was adjusted to minimize the signal from the normal myocardium in each patient by using a look-locker sequence to locate the null point of the normal myocardium. A typical inversion time for LE MRI ranged from 230 to 300 ms. Imaging data were analyzed on workstations (Ziostation 2, Ziosoft).

In the T1 weighted MRI acquired 10 minutes after contrast injection, we obtained images of the LV short axis view, the long axis 4 chamber views, and the long axis 2 chamber views. If there was an obvious high intensity area in the LV myocardium, we regarded this lesion as being LE positive.

The location of LE, suggesting the presence of myocardial fibrosis, was classified as endocardium, epicardium, or whole layer (= either or both the endocardium and epicardium) using T1-weighted MRI (Figure 2).

Figure 2. Typical late gadolinium enhancement image on T1 weighted cardiac magnetic resonance imaging suggesting the presence of myocardial fibrosis in a patient with hypertrophic cardiomyopathy. Myocardial fibrosis was observed in the whole-layer (mid layer dominant), in the whole range portion of the interventricular septum, and the LV anterior and inferior wall (arrows). LV indicates left ventricle; and RV, right ventricle.

Statistical analysis: For all analyses, $P < 0.05$ was considered to be statistically significant. Comparison of PLS and PCS (absolute values) on speckle tracking TTE between LV lesions (endocardium, epicardium, or whole layer) with and without fibrosis on MRI were performed using the t test (SPSS software, version 17.0, SPSS, Inc.).

Results

Patient background and TTE findings are presented in Table I and the MRI LV findings are presented in Table II.

Of a total of 306 lesions, fibrosis was detected in 72, 68, and 81 lesions in the LV endocardium, epicardium, and whole layer myocardium using T1 weighted MRI, respectively.

Endocardial analysis demonstrated that regional PLS was significantly smaller in fibrotic endocardial lesions (11.7 ± 6.8%) than in non-fibrotic endocardial lesions (15.0 ± 8.6%) on MRI ($P = 0.004$) (Figure 3). To detect LV endocardial lesions with fibrosis, receiver operating characteristic (ROC) curve analysis of regional PLS revealed an area under the curve (AUC) of 0.603 ($P = 0.008$) and a best cut-off point of 13.5%, with sensitivity of 63.9% and specificity of 53.8% (Figure 4).

Epicardial analysis demonstrated that regional PLS was significantly smaller in fibrotic epicardial lesions (6.8 ± 4.5%) than in non-fibrotic epicardial lesions (10.6 ± 6.3%) on MRI ($P < 0.001$) (Figure 5). To detect LV

Table II. Magnetic Resonance Imaging Left Ventricular (LV) Findings

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td>LV mass (g)</td>
<td>196 ± 93</td>
</tr>
<tr>
<td>LV end-diastolic volume (mL)</td>
<td>136 ± 34</td>
</tr>
<tr>
<td>LV end-systolic volume (mL)</td>
<td>60 ± 21</td>
</tr>
<tr>
<td>LV ejection fraction (%)</td>
<td>56 ± 9</td>
</tr>
<tr>
<td>Maximum LV wall thickness (mm)</td>
<td>20 ± 6</td>
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</tbody>
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At the examination, one patient had headache and cine magnetic resonance imaging could not be performed. These data were acquired from the remaining 17 patients.

Table I. Patient Backgrounds and TTE Findings

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>58 ± 17</td>
</tr>
<tr>
<td>Male</td>
<td>14 (78%)</td>
</tr>
<tr>
<td>Time interval between TTE and MRI</td>
<td>4 ± 5</td>
</tr>
<tr>
<td>Hypertrophic obstructive cardiomyopathy</td>
<td>4 (22%)</td>
</tr>
<tr>
<td>Dilated phase hypertrophic cardiomyopathy</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Left atrial dimension (mm)</td>
<td>44.1 ± 7.6</td>
</tr>
<tr>
<td>Left atrial volume index (mL/m²)</td>
<td>29.8 ± 11.0</td>
</tr>
<tr>
<td>Inter-ventricular septum thickness diameter on end diastole (mm)</td>
<td>16.9 ± 5.0</td>
</tr>
<tr>
<td>LV posterior wall thickness on end diastole (mm)</td>
<td>10.1 ± 1.9</td>
</tr>
<tr>
<td>LV end-diastolic dimension (mm)</td>
<td>48.1 ± 5.9</td>
</tr>
<tr>
<td>LV end-systolic dimension (mm)</td>
<td>29.8 ± 4.2</td>
</tr>
<tr>
<td>LV ejection fraction (%)</td>
<td>64.4 ± 6.5</td>
</tr>
</tbody>
</table>

TTE indicates transthoracic echocardiogram; MRI, magnetic resonance imaging; and LV, left ventricle.
epicardial lesions with fibrosis, ROC curve analysis of PLS revealed an AUC of 0.684 ($P < 0.001$) and a best cut-off point of 9.5%, with sensitivity of 73.5% and specificity of 55.5% (Figure 6).

For whole layer myocardial analysis, PLS was significantly smaller in fibrotic lesions (8.7 ± 4.9%) than in non-fibrotic lesions (12.5 ± 6.7%) on MRI ($P < 0.001$) (Figure 7). To detect whole layer (endocardial and/or epicardial) LV lesions with fibrosis, ROC curve analysis of regional myocardial PLS revealed an AUC of 0.674 ($P < 0.001$) and a best cut-off point of 12.5%, with sensitivity of 79.0% and specificity of 50.7% (Figure 8).

There were no significant differences in PCS of LV myocardium (endocardium, epicardium, and whole layer) between fibrotic lesions (30.6 ± 7.6%, 10.8 ± 5.5%, and 17.7 ± 6.4%, respectively) and non-fibrotic lesions (33.4 ± 9.5%, 10.8 ± 6.6%, and 19.3 ± 7.8%, respectively) using MRI (Figures 9-11).

**Discussion**

We sought to ascertain the relationship between regional layer-specific peak strain (PLS and PCS) in LV myocardium and the presence of layer-specific LV myo-
In this study, we conclude that quantitative regional PLS but not PCS in LV endocardium, epicardium, and whole layer myocardium provides useful non-invasive information for layer-specific localization of fibrosis confirmed on MRI in HCM patients.

Clinical significance, implications, and importance of this study: Until now, myocardial fibrosis on MRI on its own,5,4 or sole use of whole-layer myocardial strain on TTE,9,10 have been reported as candidates for predictors of HCM in patients.

Furthermore, fibrotic lesions on MRI or CT have been reported to have impaired (whole layer) strain values on TTE,1,20 and the relationship between the presence of LE on MRI and decrement of strain values in each myocardial lesion is estimated to strongly interact with each other.

Currently, due to developments in TTE technology, we can measure myocardial strain in endocardial and epicardial layers separately, while also being able to measure whole layer as well. We have already reported the utilities of these novel layer-specific strain measurements.10-15 The spatial resolution of TTE is superior to that of MRI, therefore, if accurate diagnosis of the presence of layer-specific LV myocardial fibrosis is confirmed by T1 weighted cardiac MRI, we speculate that the regional layer-specific peak strain values measured on TTE would be reduced in the corresponding layer-specific lesions with myocardial fibrosis using MRI. Therefore, in this study, we determined the detailed relationship between regional layer-specific peak strain (both PLS and PCS) in LV myocardium and the presence of layer-specific LV myocardial fibrosis confirmed by T1 weighted cardiac MRI in HCM patients. We believe both technologies would be novel and robust risk stratified predictors for HCM patients.

Secondly, we aimed to differentiate HCM from other myocardial diseases such as cardiac amyloidosis. We aimed to do this by identifying layer-specific strain on TTE and/or the presence of layer-specific LV myocardial fibrosis on MRI. Cummings and colleagues used a pattern-based approach to assess delayed enhancement in non-ischemic cardiomyopathy on MRI.21 Spatial resolution on MRI is superior to that of single photon emission CT and positron emission tomography. However, these techniques are inferior to that of CT and TTE, therefore, a partial volume effect may have influenced their results. To confirm the reliability of their hypothesis, we have to confirm that the regional layer-specific peak strain measure-
Utility of a multi-layer strain technique on 2D speckle tracking TTE: Using a multi-layer strain technique on 2D speckle tracking TTE, we previously evaluated compensatory mechanisms in HCM patients with preserved LV ejection fraction.\(^{14}\) In that study, we advocated a new myocardial characteristic indicator; this was the myocardial percentage endocardial strain dependency, as represented by 2D LV GLS and GCS. In such patients, we achieve this and confirm the aforementioned hypothesis, it would have been better to use a homogenous population and only include HCM patients to be evaluated in this study.

Figure 8. Longitudinal strain (PLS) (absolute values) to detect left ventricular whole layer lesions with fibrosis on cardiac magnetic resonance imaging in patients with hypertrophic cardiomyopathy. ROC curve of regional myocardial PLS to detect whole layer (endocardial and/or epicardial) LV lesions with fibrosis revealed an area under the curve (AUC) of 0.674 (\(P < 0.001\)) and a best cut-off point of 12.5%, with sensitivity of 79.0% and specificity of 49.3%.

Figure 9. Comparison of peak circumferential strain (PCS) (absolute values) on speckle tracking transthoracic echocardiography between left ventricular (LV) endocardial lesions with and without fibrosis on cardiac magnetic resonance imaging (MRI) in patients with hypertrophic cardiomyopathy. There were no significant differences in the PCS of LV endocardium between fibrotic lesions (\(n = 39\)) (30.6 ± 7.6%) and non-fibrotic lesions (\(n = 177\)) (33.4 ± 9.5%) on MRI.

Figure 10. Comparison of peak circumferential strain (PCS) (absolute values) on speckle tracking transthoracic echocardiography between left ventricular (LV) epicardial lesions with and without fibrosis on cardiac magnetic resonance imaging (MRI) in patients with hypertrophic cardiomyopathy. There were no significant differences in the PCS of LV epicardium between fibrotic lesions (\(n = 34\)) (10.8 ± 5.5%) and non-fibrotic lesions (\(n = 182\)) (10.8 ± 6.6%) on MRI.

The third aim was to understand the mechanism of how myocardial fibrosis influences each strain value. To achieve this and confirm the aforementioned hypothesis, it would have been better to use a homogenous population and only include HCM patients to be evaluated in this study.
concluded that 2D LV GLS was lower than in controls but endocardial GCS was maintained in compensation for reduction in endocardial GLS. Consequently, the percentage endocardial GCS dependency may increase, and with a larger LV size, the compensatory effect will be smaller.

**Study limitations:** This was a non-randomized study in a single center with a small number of patients. We used a novel multilayer TTE technology, and given that the number of patients was small and there were only a few observation periods, we did not detect anyone with events during the observation periods. Therefore, we could not directly evaluate whether regional layer-specific peak strain in LV myocardium and/or the presence of layer-specific LV myocardial fibrosis confirmed by T1 weighted cardiac MRI were robust prognostic predictors in HCM patients.

In this study, we did not evaluate the inter-observer and intra-observer consistency of either layer-specific localization of fibrosis on MRI or layer-specific PLS and PCS in LV myocardium on speckle tracking TTE. However, we have previously evaluated inter- and intra-observer consistency in LV myocardial strain measurements using a novel multi-layer technique in patients with severe aortic stenosis and preserved LV ejection fraction.\(^4\)\(^5\) Furthermore, we also evaluated inter- and intra-observer correlation coefficients for a proportion of endocardial GLS and GCS dependency estimates which were defined as the ratio of endocardial strain to epicardial strain in HCM patients with preserved LV ejection fraction\(^4\)\(^6\) and we generated reliable results. The population in this report was comprised of patients with severe aortic stenosis but the measurement of layer-specific strain on TTE was automatically performed using TTE machines and if sufficient image data are acquired, inter- and intra-observer consistency would be less dependent on the different types of disease, and furthermore, both severe aortic stenosis and HCM have thickened LV walls. Therefore, we believe we can adapt the inter- and intra-observer consistency in the measurement of LV myocardial strain using a novel multilayer technique in patients with severe aortic stenosis to that compatible with HCM.

When using layer-specific analysis incorporating both TTE and MRI, both modalities are required for excellent spatial resolution and reduction of partial volume effects. Generally, the spatial resolution of MRI is inferior to that of TTE. Therefore, the accuracy of layer-specific localization of fibrosis on MRI may be poorer than that of layer-specific measurements of PLS and PCS in LV myocardium on speckle tracking TTE, which may have influenced the results presented in this study.

For analysis of MRI, it is generally preferable that it be performed not only by cardiologists but also by radiologists, or jointly by a radiologist and a cardiologist.

**Conclusion:** Quantitative regional PLS but not PCS in endocardium, epicardium, and whole myocardium provides useful non-invasive information for layer-specific localization of fibrosis on MRI in HCM patients.

**Disclosures**

**Conflicts of interest:** There is no conflict of interest to declare.

**References**

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