Ultrasonic Assessment of Myocardial Microstructure in Hypertrophic Cardiomyopathy Sarcomere Mutation Carriers With and Without Left Ventricular Hypertrophy

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Background—The noninvasive assessment of altered myocardium in patients with genetic mutations that are associated with hypertrophic cardiomyopathy (HCM) remains challenging. In this pilot study, we evaluated whether a novel echocardiography-based assessment of myocardial microstructure, the signal intensity coefficient (SIC), could detect tissue-level alterations in HCM sarcomere mutation carriers with and without left ventricular hypertrophy.

Methods and Results—We studied 3 groups of genotyped individuals: sarcomere mutation carriers with left ventricular hypertrophy (clinical HCM; n=36), mutation carriers with normal left ventricular wall thickness (subclinical HCM; n=28), and healthy controls (n=10). We compared measurements of echocardiographic SIC with validated assessments of cardiac microstructural alteration, including cardiac magnetic resonance measures of interstitial fibrosis (extracellular volume fraction), as well as serum biomarkers (NTproBNP, hs-cTnI, and PICP). In age-, sex-, and familial relation–adjusted analyses, the SIC was quantitatively different across subjects with overt HCM, subclinical HCM, and healthy controls (P<0.001). Compared with controls, the SIC was 61% higher in overt HCM and 47% higher in subclinical HCM (P<0.001 for both). The SIC was significantly correlated with extracellular volume (r=0.72; P<0.01), with left ventricular mass and E′ velocity (r=0.50, -0.60, respectively; P<0.01 for both), and with serum NTproBNP levels (r=0.36; P<0.01).

Conclusions—Our findings suggest that the SIC could serve as a noninvasive quantitative tool for assessing altered myocardial tissue characteristics in patients with genetic mutations associated with HCM. Further studies are needed to determine whether the SIC could be used to identify subclinical changes in patients at risk for HCM and to evaluate the effects of interventions. (Circ Heart Fail. 2016;9:e003026. DOI: 10.1161/CIRCHEARTFAILURE.116.003026.)

Key Words: echocardiography ■ hypertrophic cardiomyopathy ■ left ventricular hypertrophy ■ myocardium ■ sacromeres

Hypertrophic cardiomyopathy (HCM) is the most common genetic cardiomyopathy and is often caused by sarcomere gene mutations. Characteristic histopathologic features include myocyte hypertrophy, disarray, and fibrosis.1–5 However, there are presently limited noninvasive methods for assessing these changes in patients with HCM. Cardiac magnetic resonance imaging (CMR) is the current standard for noninvasive assessment of altered myocardial tissue composition. Late gadolinium enhancement (LGE) and quantification of extracellular volume (ECV) by T1 mapping techniques are believed to reflect dense and interstitial fibrosis, respectively.6–8 Although a mainstay in the clinical care of patients with HCM, echocardiography has traditionally been limited to the assessment of gross morphological features, such as left ventricular (LV) wall thickness, and functional features, including LV ejection fraction and outflow tract obstruction.4,7 Advances in echocardiographic image analysis now offer the ability to assess for the presence of microstructural tissue level changes.

See Clinical Perspective

Interactions between the ultrasound signal and myocardial tissue (eg, absorption and refraction) influence the grayscale signal intensity values produced at the myocardial–pericardial interface. Detectable variations in the resulting signal distribution can yield quantifiable differences between diseased versus healthy myocardium.9,10 We and others have shown that ultrasound-based tissue analyses can reveal alterations in myocardial microstructure that correlate with the histological presence...
of myocardial fibrosis (including both interstitial and dense replacement fibrosis) in the setting of a variety of myopathic disease processes, such as hypertensive heart disease, dilated cardiomyopathy, and postinfarct ischemic injury.\textsuperscript{10-14} Therefore, in this pilot study, we evaluated how ultrasound-based analytic techniques compare with CMR for assessing myocardial tissue-level alterations in HCM. We hypothesized that the echocardiographic signal intensity coefficient (SIC), an echocardiography-based measure of myocardial microstructure, can effectively detect tissue-level alterations in HCM sarcomere mutation carriers with and without LV hypertrophy (LVH).

**Methods**

**Study Sample**

We studied individuals who were originally referred to a specialty clinic at our institution for the evaluation of HCM, or enrollment in an HCM-related study protocol, as previously described.\textsuperscript{2} For this study, we included individuals with genotyping data available and who also underwent routine echocardiography (with a complete study performed and adequate-to-good quality images acquired for post hoc image analysis) in addition to newly concurrent CMR imaging (with sequences acquired that would allow for ECV quantification). All study participants were classified into one of the following groups: overt HCM patients (G+/LVH+), subclinical mutation carriers (G+/LVH−), and healthy controls (G−/LVH−). Criteria for inclusion in the overt HCM group included the presence of a sarcomere mutation and a maximal LV wall thickness of >12 mm. Exclusion criteria included contraindication to gadolinium administration, atrial fibrillation, infiltrative or storage disease, coronary heart disease, presence of a permanent pacemaker or implantable cardioverter-defibrillator, and systemic hypertension. All individuals undergoing research-based studies provided informed consent; the hospital institutional review board approved the study.

**CMR Imaging Measures**

As described previously,\textsuperscript{2} CMR image acquisition was performed in the standard supine position, using a 3.0 Tesla system (Tim Trio, Siemens, Erlangen, Germany) with cine imaging, steady-state–free precession imaging, and with the administration of gadolinium contrast. The LV endocardial and epicardial borders, with the exclusion of papillary muscles, were manually traced on short-axis consecutive cine images at end-diastole and at end-systole for the calculation of LV function and mass. LV mass was derived through the summation-of-discs method. In all participants, LV wall thickness was measured in the lateral, inferior, anterior, and posterior septal sections.

A standard LGE imaging protocol was performed to detect focal myocardial fibrosis, based on a segmental inversion-recovery pulse sequence. We used a semiautomated grayscale threshold technique to quantify LGE, expressed in grams and as a percentage of the total myocardial mass. All LGE analyses were performed previously using commercially available software (QMassMR, version 7.4, Medis, Leiden, The Netherlands). Additionally, T1 measurements were performed with a Look-Locker sequence and T1 relaxation rate was used to plot myocardial relaxation rate against blood pool relaxation rate with the slope calculated by linear regression.\textsuperscript{7} The myocardial ECV was calculated from this slope based on the methods detailed previously,\textsuperscript{19} and myocardial ECV fraction for each participant was determined as the average across multiple segmental views, as previously described.\textsuperscript{2} In study participants with LGE present, a second ECV was calculated after excluding regions that contained LGE.

**Echocardiographic Measures**

Standard echocardiograms and tissue Doppler interrogation were obtained using the Vivid-7 ultrasound system (GE Medical System, Milwaukee, WI). Measurements reflect the mean of 3 cardiac cycles. Tissue Doppler myocardial velocities were measured in early diastole (E′) at the inferior, lateral, septal, and anterior aspects of the mitral annulus in the apical 2-chamber and 4-chamber views. These measurements were averaged to calculate global values of E′ velocity. All echocardiographic images were acquired within 1 year of CMR imaging. Time between CMR imaging and echocardiographic acquisition was, on average, 18±60 days. All echocardiographic and CMR measurements were performed while blinded to genotype and clinical information.

**Echocardiographic Tissue Analysis**

We used a computational image analysis method developed on the ImageJ software platform v1.46 (National Institutes of Health, Bethesda, MD) to assess myocardial tissue characteristics for a user-selected region-of-interest.\textsuperscript{8,10} The image analysis method is applied to transthoracic echocardiographic images with the following parameters: B-mode imaging, parasternal long-axis view, end-diastolic frame, and adequate visualization of the mid-to-basal inferolateral endocardial, myocardial, and pericardial regions (Figure 1). The pericardial area directly adjacent to the mid-to-basal inferolateral myocardium (ie, at the myopericardial interface) was selected. The 25th percentile of pericardial signal intensity distribution (p) was obtained and the SIC was calculated as SIC=1-p/256 (Figure 1). The SIC, as a marker of myocardial microstructure, represents aggregate myocardial tissue changes following interaction of the ultrasound signal through maximum thickness of the myocardium, as previously described.\textsuperscript{8,10} In this study, we applied the ImageJ algorithm to quantify the SIC measure on echocardiographic images for all study participants while blinded to the genotype and clinical status. Previous studies have demonstrated the correlation coefficients for inter-reader and intrareader reproducibility of SIC at 0.89 and 0.90, respectively.\textsuperscript{10}

**Figure 1.** The signal intensity coefficient (SIC) measurement is based on the ultrasonic signal intensity distribution derived from a pericardial region of interest located at the myopericardial interface at the level of the mid-to-basal inferolateral wall, as shown in A. The SIC value is calculated from the distribution of signal intensity values captured from within the region of interest; the distribution signal intensity values, along a standard gray scale, from a sample region of interest is shown in B.
Biomarker Assessments

Serum biomarkers were assessed at the time of CMR imaging through protocols that included the collection of peripheral blood. Blood samples were processed within 60 minutes of phlebotomy, and were stored at −80°C before assays, which were performed in a blinded fashion using commercially available reagents. The assays included the following markers: carboxy-terminal propeptide of procollagen type I (PIPC; Quidel Corporation, San Diego, CA), amino terminal propeptide of B-type natriuretic peptide (NT-proBNP; Roche, Indianapolis, IN), and high-sensitivity cardiac troponin I (hs-cTnI; Singulex, Atlanta, GA).

Statistical Analyses

We assessed clinical, echocardiographic, and CMR characteristics across the 3 study participant groups using linear and logistic regression models assuming an exchangeable correlation structure within families. Robust standard errors were calculated in each model to account for any additional correlation within families and any possible deviation from the exchangeable correlation assumption. In the event that a model did not converge under the assumption of an exchangeable correlation structure, it was re-fitted assuming independence. Analyses were further adjusted by age and sex in addition to the measures taken into account for family relationship; for comparison of clinical and imaging traits between groups, trait values were expressed as mean±standard error, and observed differences in means between groups were assessed for statistical significance after controlling for age and sex. Associations between continuous imaging traits were evaluated using Pearson correlation. Based on multiple comparisons being made across 3 groups, we considered a 2-tailed P value of 0.05/3=0.017 as statistically significant. Because of the pilot nature of the study, we did not further adjust the P value threshold for the number of tests performed across multiple clinical characteristics. All analyses were performed using STATA v12.1 (StataCorp, College Station, TX).

Results

Clinical characteristics for the total study cohort, including adjusted mean values of imaging and biomarker measures, are shown in the Table. For reference, unadjusted imaging and biomarker measurement values are shown by subgroup in the Table. Participants with HCM had imaging performed for research purposes or for the following clinical indications: a positive family history (13/36 patients; 36.1%), symptoms (8/36; 22.2%), routine screening (11/36; 30.6%), after an acute cardiovascular event (1/36; 2.8%), or for a second opinion (2/36; 5.6%). Twelve (33.3%) of these patients had (or would subsequently receive after their index echocardiogram) a primary prevention ICD and 1 (2.8%) received a secondary prevention ICD. The mean (SD) NYHA functional class at the time of echocardiogram was 1.3 (0.5). Of patients with confirmed presence or absence of syncope, 5/35 (14.3%) patients had a history of syncope. Four (11.1%) were treated with surgical or catheter-based septal reductive therapy. Across genotype–phenotype groups, individuals with overt HCM (G+/LVH+) were older than both individuals with subclinical HCM (G+/LVH−) and healthy controls (G−/LVH−). Individuals with overt HCM were also more likely to be men than those with subclinical HCM. Consistent with previously reported results from this cohort,2 conventional echocardiographic measures distinguished between overt HCM and healthy controls, and between overt and subclinical HCM, but not between subclinical HCM and healthy controls (Table).

Measures of Myocardial Microstructural Alterations

As previously described,2 CMR LGE was detectable only in subjects with clinically overt HCM. In contrast, ECV quantification was significantly different among the study cohorts, with the highest values in the overt HCM subgroup, intermediate values in subclinical HCM, and lowest values in controls (Table). In this study, echocardiographic SIC was measurable and also quantitatively different across all study groups. Compared with healthy controls, the SIC was on average 2.6-fold higher in those with overt HCM and 1.9-fold higher in those with subclinical HCM (P<0.0001 for both). As illustrated in Figure 2, both LV mass and global E′ velocity distinguished between overt and subclinical HCM, but not between subclinical HCM and healthy controls; by contrast, ECV and SIC measures were significantly different when comparing not only subjects with overt from subclinical disease, but also the subclinical HCM group from the healthy controls.

Correlations With Echocardiographic Signal Intensity Coefficient

In the total study sample, the SIC was significantly correlated with CMR-based quantification of ECV in addition to echocardiographic LV mass and global E′ velocity (Figure 3). In patients with measureable LGE (n=29), the correlation between SIC and LGE was r=0.45 (P<0.01). Pearson correlation between SIC and maximal wall thickness was 0.57 and between SIC and thickness of the inferolateral wall (where SIC is measured) was 0.49. With respect to serum biomarkers, the correlation between SIC and log NT-proBNP was r=0.59 (P<0.001; Figure 3), with high-sensitivity cTnI was r=0.14 (P=0.21) and with PICP was r=0.15 (P=0.31). Within 1 standard deviation, there was an agreement among ECV and SIC when a cut-off for abnormal was defined as that above 0.33 for both measures (Figure 4). Although we observed a range of values for both the SIC and the ECV in our cohort, there were no individuals who had values within the 2.5th and 97.5th percentiles for SIC with simultaneously high (>97.5th percentile) or low (<2.5th percentile) values for ECV (Figure 4).

Discussion

This pilot study was designed to evaluate whether a novel echocardiography-based metric could facilitate the detection of tissue-level alterations in cardiac structure in patients with sarcomere gene mutations associated with HCM. We found that, overall, echocardiographic SIC values differed among groups of patients with overt HCM compared with normal controls and subclinical mutation carriers. Differences in SIC were also observed between sarcomere mutation carriers with normal LV wall thickness compared to healthy controls. Echocardiographic SIC was tightly correlated with myocardial ECV by CMR, which is considered a robust measure of interstitial myocardial fibrosis. Furthermore, the SIC correlated significantly with echocardiographic LV mass and myocardial relaxation velocity as well as with serum NT-proBNP levels all with the expected directionality of associations. These results suggest that increased SIC has a physiological impact leading to myocardial relaxation and hemodynamic stress.
Thus, our results suggest that the echocardiographic SIC, like the CMR-based quantification of ECV, may provide useful insights regarding subtle abnormalities in myocardial tissue composition across a spectrum of disease states.

The SIC and similar ultrasound measures of myocardial microstructure, such as integrated backscatter, have been studied in the setting of multiple cardiac disease phenotypes including hypertensive heart disease, postinfarct ischemic injury, diabetic cardiomyopathy, and aortic stenosis.10,12,20–34 Most prior methods have been limited by reliance on the integrated or mean value of backscatter signal intensities,18 time delays for algorithms requiring cyclic variation assessment,35 or aliased data from low frame rates.36 By contrast, the SIC utilizes grayscale values from the complete ultrasonic signal distribution to yield information regarding tissue microstructure; the SIC also minimizes signal reconstruction artifacts by using end-diastolic B-mode frames.9 Shifts in the signal intensity distribution, as captured by the SIC measure, seem to represent tissue level changes that may include myocardial hypertrophy, increased myocyte density, and collagen deposition.37–41 Therefore, variation in the SIC, although measured in a prespecified region of interest of the LV, could represent the diffuse tissue-level alterations that are known to occur in HCM, such as myofibril disarray and interstitial fibrosis.

Our results suggest that the SIC could be used as a non-invasive measure of tissue-level changes in the assessment of individuals with subclinical HCM and established disease. By definition, neither LV mass nor wall thickness differentiates subclinical HCM (G+/LVH−) from healthy controls (G−/LVH−) groups. However, the finding of increased SIC in individuals with subclinical (G+/LVH−) disease provides further corroboration that tissue-level pathology can exist in the absence of overt structural alterations in sarcomere mutation carriers. The SIC also demonstrated an inverse correlation

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Overt HCM (G+/LVH+)</th>
<th>Subclinical HCM (G+/LVH−)</th>
<th>Control (G−/LVH−)</th>
<th></th>
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<th></th>
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</thead>
<tbody>
<tr>
<td>n</td>
<td>36</td>
<td>28</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Age, y</td>
<td>42.3 (2.1)</td>
<td>29.7 (2.3)</td>
<td>25.9 (3.9)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.15</td>
</tr>
<tr>
<td>Women, n (%)</td>
<td>31 (7.8)</td>
<td>57 (9.5)</td>
<td>50 (17)</td>
<td>0.17</td>
<td>0.038</td>
<td>0.30</td>
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<tr>
<td>Causal gene, n (%)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>MYH7</td>
<td>8 (22)</td>
<td>12 (43)</td>
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<tr>
<td>MYBPC3</td>
<td>24 (67)</td>
<td>13 (46)</td>
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<td>TNNT2</td>
<td>2 (6)</td>
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<td>TNNI3</td>
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<td>1 (4)</td>
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<tr>
<td>MYL2</td>
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<tr>
<td>MYL3</td>
<td>1 (3)</td>
<td>0 (0)</td>
<td>...</td>
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<tr>
<td>Conventional echocardiographic measures</td>
<td></td>
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<tr>
<td>LV mass, g</td>
<td>159.0 (8.9)</td>
<td>96.4 (9.7)</td>
<td>109 (15.8)</td>
<td>0.005</td>
<td>&lt;0.001</td>
<td>0.09</td>
</tr>
<tr>
<td>Maximal LV wall thickness</td>
<td>17.7 (0.7)</td>
<td>8.4 (0.8)</td>
<td>8.6 (1.3)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.90</td>
</tr>
<tr>
<td>LV ejection fraction, %</td>
<td>61 (2)</td>
<td>61 (2)</td>
<td>59 (3)</td>
<td>0.11</td>
<td>0.99</td>
<td>0.08</td>
</tr>
<tr>
<td>Global E′ velocity, cm/s</td>
<td>10.0 (0.4)</td>
<td>13.9 (0.4)</td>
<td>15.4 (0.8)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.13</td>
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<td>Myocardial macro- and micro-structural measures</td>
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<tr>
<td>LGE, g</td>
<td>14.7 (3.4)</td>
<td>0</td>
<td>0</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>LGE, % LV mass</td>
<td>7.0 (1.4)</td>
<td>0</td>
<td>0</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>ECV (average)</td>
<td>0.37 (0.01)</td>
<td>0.33 (0.01)</td>
<td>0.26 (0.01)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ECV excluding LGE</td>
<td>0.36 (0.01)</td>
<td>0.33 (0.01)</td>
<td>0.26 (0.01)</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Signal intensity coefficient</td>
<td>0.42 (0.01)</td>
<td>0.30 (0.01)</td>
<td>0.16 (0.02)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<td>Serum biomarker levels</td>
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</tr>
<tr>
<td>PICP, μg/L</td>
<td>101.0 (7.0)</td>
<td>86.5 (5.8)</td>
<td>76.1 (9.9)</td>
<td>0.14</td>
<td>0.24</td>
<td>0.14</td>
</tr>
<tr>
<td>Troponin I, pg/mL</td>
<td>15.1 (4.9)</td>
<td>5.5 (3.8)</td>
<td>4.1 (6.5)</td>
<td>0.11</td>
<td>0.19</td>
<td>0.63</td>
</tr>
<tr>
<td>NT-proBNP, pg/mL</td>
<td>445 (92)</td>
<td>17 (73)</td>
<td>0</td>
<td>0.019</td>
<td>0.006</td>
<td>0.009</td>
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</table>

Conventional echocardiographic measures, myocardial macrostructural measures, and serum biomarker levels are shown as mean (standard error) adjusted for age, sex, and family relation. ECV indicates extracellular volume; LGE, late gadolinium enhancement; LV, left ventricular; NT-proBNP, N-terminal of B-type natriuretic peptide; and PICP, carboxy-terminal propeptide of procollagen type I.

*A Bonferroni-corrected *P* value threshold of 0.017 (0.05/3) is considered significant.
with \( E' \) velocity and serum NT-proBNP levels, reinforcing the relationship between myocardial microstructure and subtle perturbations in diastolic function and hemodynamic stress in pathogenesis of HCM.

Importantly, the SIC was significantly correlated with CMR-based quantification of ECV, an established imaging-based marker of interstitial myocardial fibrosis.\(^1,6\) The SIC could reflect a variety of possible tissue-level changes, and we speculate that the SIC may predominantly represent interstitial myocardial fibrosis in the setting of HCM. Although CMR measures of ECV reflect alterations involving the extracellular components of the myocardial microstructure, the echocardiographic SIC provides an aggregate measure of intracellular and extracellular microstructural alterations. Thus, while acquired from different imaging modalities, the SIC and ECV could be used in a complementary manner to evaluate the progression of myocardial disease and provide valuable information regarding the phenotypic evolution of HCM. Further benefits of developing an ultrasound-based technique for assessing tissue characteristics include ease and safety of serial measurements and feasibility in patients with implanted devices.

Previous studies of echocardiography in HCM have investigated associations of strain-based measures of myocardial deformation with the degree of detectable fibrosis.\(^4,6,8\) The SIC now offers an echocardiographic method for assessing cardiac microstructure that can add to the information provided by strain-based measures of cardiac function in HCM, within the same imaging modality. It should be noted that the extent to

**Figure 2.** Comparison across genotype–phenotype groups using measures of left ventricular mass (A), global \( E' \) velocity (B), extracellular volume (C), and the signal intensity coefficient (D) are shown; a Bonferroni-corrected threshold of 0.017 (0.05/3) is considered statistically significant for the \( P \) values displayed (from multivariable-adjusted analyses).

**Figure 3.** Associations of the signal intensity coefficient with left ventricular mass (A), global \( E' \) velocity (B), extracellular volume (C), and log NT-proBNP (D).
which either the SIC or ECV, as measures of microstructural disease, may be useful for predicting adverse outcomes in HCM has yet to be determined.

Several limitations of our study merit consideration. Our results are based on a cross-sectional study design involving a small cohort. Additional studies assessing the SIC in larger samples of individuals followed longitudinally are needed to validate our findings and to characterize how SIC may be used to track disease progression. In addition, myocardial tissue samples were not available for providing corroborative histological data regarding the presence and nature of fibrosis or other tissue-level changes. Importantly, it is not yet clear if the SIC distinguishes between replacement fibrosis and interstitial fibrosis. However, the lack of LGE in subclinical (G+/LVH-) individuals who had increased SIC measures (relative to controls), in addition to the observed stronger relation of SIC with ECV compared to with LGE, suggests that dense scar or replacement fibrosis may not be as important a contributor. Additional imaging and experimental studies could help to further clarify the underlying tissue pathology reflected by variation in the SIC. It is important to note that the median age of gene-positive but phenotype-negative individuals in our study is potentially older than the age at which many young individuals present for HCM screening. Furthermore, we could not prospectively assess the proportion of individuals with abnormal SIC who subsequently develop clinical HCM. Longitudinal studies in larger cohorts are needed to corroborate and extend our observations before any of our findings may be considered generalizable. Notwithstanding these limitations, our study had several strengths. Echocardiography and CMR were performed within a relatively short time period of each other. Rigorous statistical analyses accounted for genetic similarities within families, age, and sex. In addition, the study cohort was genotyped, allowing for the statistical analyses of subclinical disease in comparison to healthy controls and patients with overt disease.

Our findings indicate that, similar to the CMR-based quantification of ECV, measurement of the echocardiographic SIC is a noninvasive method that offers the ability to assess changes in myocardial tissue composition. The SIC seems to detect subtle changes that may occur early in the pathogenesis of HCM, as increased SIC was present in sarcomere mutation carriers with otherwise normal LV morphology and wall thickness. Thus, the SIC could serve as an effective as well as accessible imaging tool for assessing tissue-level alterations in the setting of HCM, offering additional and complementary information to conventional echocardiographic and existing CMR measures. Further studies are needed to elucidate the relationship between variation in the SIC and specific tissue-level alterations that manifest in HCM. Additional longitudinal investigations are also needed to determine whether the SIC could serve as a reliable marker of disease progression or response to therapies for HCM.

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**Disclosures**

None.

**References**


heartjnl-2013-304923.


5. Urban-Moral JA, Rowin EJ, Maron MS, Crean A, Pandian NG. Investigation of global and regional myocardial mechanics with 3-di-
mensional speckle tracking echocardiography and relations to hyper-


13. Holland MR, Gibson AA, Peterson LR, Areces M, Schaffer JE, Perez JE, Miller JG. Measurements of the cyclic variation of myocardial back-


15. Di Bello V, Talini E, Delle Donne MG, Aghini-Lombardi F, Monzani F, La Carrubba S, Antonini-Canterini F, Dani FL, Di Salvo G, Carej S, Marzilli M, Research Section of the Italian Society of Cardiovascular Echography (SIC). New echocardiographic techniques in the evaluation of left vent-


grated backscatter tissue characterization of remote myocardial infarc-

19. Finch-Johnston AE, Gussak HM, Mobley J, Holland MR, Petrovic O, Pérez JE, Miller JG. Cyclic variation of integrated backscatter: depen-
dence of time delay on the echocardiographic view used and the myocar-


22. Feinberg MS, Gussak HM, Dávila-Román VG, Baumann CM, Miller JG, Pérez JE. Dissociation between wall thickening of normal myocardium


**CLINICAL PERSPECTIVE**

There are currently limited tools available for noninvasively assessing myocardial tissue alterations that may be present in the setting of genetic mutations associated with hypertrophic cardiomyopathy. The ability to detect tissue-level changes in myocardial structure that are possible precursors to the development of overt left ventricular hypertrophy could lead to improved care for patients with potentially disease-causing mutations. This pilot study examined a novel echocardiographic measure, the signal intensity coefficient (SIC), as a method for noninvasively quantifying subclinical left ventricular structural alterations in people carrying genetic variants associated with hypertrophic cardiomyopathy. Overall, we observed that the SIC could differentiate between individuals with versus without overt left ventricular hypertrophy among people carrying hypertrophic cardiomyopathy-related mutations; furthermore, the SIC also differentiated between phenotypically silent mutation carriers and normal controls. In our study, the SIC measure performed comparably to a validated cardiac magnetic resonance imaging measure of cardiac fibrosis. More work is needed to prospectively investigate whether genotype-positive individuals with abnormal SIC will subsequently develop overt clinical hypertrophic cardiomyopathy, whether abnormal SIC can predict relevant outcomes, and whether this measure can track the myocardial response to emerging therapies.
Ultrasonic Assessment of Myocardial Microstructure in Hypertrophic Cardiomyopathy Sarcomere Mutation Carriers With and Without Left Ventricular Hypertrophy
Pranoti Hiremath, Patrick R. Lawler, Jennifer E. Ho, Andrew W. Correia, Siddique A. Abbasi, Raymond Y. Kwong, Michael Jerosch-Herold, Carolyn Y. Ho and Susan Cheng

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SUPPLEMENTAL MATERIAL

Supplemental Table. Unadjusted Imaging and Biomarker Characteristics By Genotype-Phenotype Groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overt HCM (G+/LVH+)</th>
<th>Subclinical HCM (G+/LVH-)</th>
<th>Control (G-/LVH-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>36</td>
<td>28</td>
<td>10</td>
</tr>
</tbody>
</table>

Conventional Echocardiographic Measures

<table>
<thead>
<tr>
<th></th>
<th>Overt HCM (G+/LVH+)</th>
<th>Subclinical HCM (G+/LVH-)</th>
<th>Control (G-/LVH-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV mass, g</td>
<td>171.0±70.1</td>
<td>84.9±24.2</td>
<td>97.3±28.6</td>
</tr>
<tr>
<td>Maximal LV wall thickness</td>
<td>18.4±5.4</td>
<td>7.7±1.2</td>
<td>7.8±1.8</td>
</tr>
<tr>
<td>LV ejection fraction, %</td>
<td>60±14</td>
<td>62±5</td>
<td>60±4</td>
</tr>
<tr>
<td>Global E’ velocity, cm/s</td>
<td>9.6±2.3</td>
<td>14.2±2.4</td>
<td>16.0±2.1</td>
</tr>
</tbody>
</table>

Myocardial Microstructural Measures

<table>
<thead>
<tr>
<th></th>
<th>Overt HCM (G+/LVH+)</th>
<th>Subclinical HCM (G+/LVH-)</th>
<th>Control (G-/LVH-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LGE, g</td>
<td>16.0±26.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LGE, % LV mass</td>
<td>7.5±10.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ECV (average)</td>
<td>0.37±0.04</td>
<td>0.33±0.04</td>
<td>0.25±0.02</td>
</tr>
<tr>
<td>ECV excluding LGE</td>
<td>0.37±0.04</td>
<td>0.33±0.04</td>
<td>0.25±0.02</td>
</tr>
<tr>
<td>Signal intensity coefficient</td>
<td>0.42±0.05</td>
<td>0.30±0.04</td>
<td>0.16±0.05</td>
</tr>
</tbody>
</table>

Serum Biomarker Levels

<table>
<thead>
<tr>
<th></th>
<th>Overt HCM (G+/LVH+)</th>
<th>Subclinical HCM (G+/LVH-)</th>
<th>Control (G-/LVH-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PICP, ug/L</td>
<td>99.8±40.0</td>
<td>86.3±24.2</td>
<td>78.9±32.3</td>
</tr>
<tr>
<td>Troponin I, pg/mL</td>
<td>13.6±17.5</td>
<td>6.3±19.3</td>
<td>4.9±4.8</td>
</tr>
<tr>
<td>NT-proBNP, pg/mL</td>
<td>389±538</td>
<td>41±32</td>
<td>29±28</td>
</tr>
</tbody>
</table>

Values are shown as mean ± standard deviation.
LV, left ventricular; LGE, late gadolinium enhancement; ECV, extracellular volume; PICP, carboxy-terminal propeptide of procollagen type I; NT-proBNP, N-terminal of B-type natriuretic peptide.