Three-layer ultrasonic tissue characterization of the ventricular septum is predictive of prognosis in patients with non-obstructive hypertrophic cardiomyopathy

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Aims
A necropsy study of patients with hypertrophic cardiomyopathy (HCM) who died at a young age exhibited marked disarray and fibrosis in the mid-wall layer of the left ventricular (LV) myocardium. We assessed ultrasonic tissue characteristics in the three layers of the ventricular septum (VS), and correlated the result with long-term prognosis in HCM.

Methods and results
The magnitude of cyclic variation of integrated backscatter (CV-IB) was calculated in the three layers of the VS and the whole aspect of the LV posterior wall in 58 non-obstructive HCM patients and 20 healthy controls. All HCM patients were prospectively followed for an average period of 7.1 years for the occurrence of cardiac death or hospitalization due to heart failure. Each CV-IB of four regions was lower in HCM patients than in controls (all \( P < 0.01 \)). CV-IB of the VS mid-wall layer was lower in 14 HCM patients with cardiac events than in patients without (5.4 ± 0.6 vs. 7.4 ± 0.5 dB, \( P = 0.033 \)) although CV-IB of three other regions did not differ between the two groups. The optical cut-off point of \%CV-IB < 90\%, i.e. the ratio of CV-IB in the VS mid-wall layer to the mean value in the layers on both sides, was an independent predictor of cardiac events (hazard ratio, 6.12; 95% confidence interval, 1.62–66.6; \( P = 0.013 \)), with a positive predictive value of 44% and particularly with a high negative predictive value of 91%.

Conclusion
Patients with non-obstructive HCM are not likely to undergo cardiac events in the near future, when the CV-IB value is not significantly lower in the VS mid-wall layer than in the layers on both sides.

Keywords
Hypertrophic cardiomyopathy • Ultrasound tissue characterization • Integrated backscatter • Prognosis • Mid-wall layer • Ventricular septum

Introduction
Hypertrophic cardiomyopathy (HCM) is characterized by unexplained left ventricular (LV) hypertrophy associated with myocardial disarray and fibrosis.1–3 Conventional echocardiography is an established technique to assess cardiac morphology and function in patients with HCM. However, this current modality is not powerful enough in the evaluation of histological features or tissue characteristics of the myocardium because signals obtained by conventional echocardiography are diversely processed, depending on the property of the apparatus.4,5

Integrated backscatter (IB), an ultrasonic imaging technique, has been developed to provide useful information on histology. This technique, requiring little post-processing of the acoustic data, enables us to non-invasively characterize myocardial tissue.4,5

Whole aspect analysis of the ventricular wall by this technique has been applied to a variety of cardiac diseases. Its prognostic value has been reported in patients with acute myocardial
infarction,\textsuperscript{6} dilated cardiomyopathy,\textsuperscript{7} aortic stenosis,\textsuperscript{8} and cardiac amyloidosis.\textsuperscript{9} The IB technique has also been applied to HCM to characterize the histological features of the myocardium\textsuperscript{10,11} but at present, few data are available to predict the prognosis.

The normal muscle fibre orientation through the LV wall changes gradually from the endocardium to epicardium or to the right-sided endocardium of the ventricular septum (VS). Midway between the base and apex, latitudinal muscle fibres run circularly and continuously in the mid-wall layer through the VS and LV free wall and longitudinal or oblique fibres predominate in the layers on both sides.\textsuperscript{12–15} Necropsy\textsuperscript{16,17} and magnetic resonance imaging\textsuperscript{18} studies on patients with HCM clarified that myocardial disarray and fibrosis were present most prevalently in the mid-wall circular layer, particularly in and near the area of VS-free wall junctions. HCM patients who died at a young age exhibited destruction of the mid-wall circular layer with severe disarray and fibrosis.\textsuperscript{19} Thus, using the IB technique, we assessed ultrasonic myocardial tissue characteristics of the VS, paying attention to its three-layer architecture, and correlated the results with long-term prognosis in HCM patients.

**Methods**

**Study population**

Consecutive patients referred to Department of Echocardiography in Matsushita Memorial Hospital from July 2000 to July 2001 and from April 2006 to June 2007 were enrolled in the present study. Of all, 91 patients were diagnosed as having non-obstructive HCM on the basis of conventional echocardiographic demonstration of an LV end-diastolic thickness \( \geq 15 \) mm and LV end-diastolic diameter \( \leq 55 \) mm in the absence of any cardiac or systemic disorder that could cause hypertrophy, such as severe hypertension defined as a systolic blood pressure \( \geq 160 \) mmHg and/or diastolic blood pressure \( \geq 100 \) mmHg or aortic stenosis defined as a peak jet velocity \( >3.0 \) m/s and/or an aortic valve area <1.5 cm\(^2\). LV outflow obstruction was defined as the peak jet velocity \( >3.0 \) m/s by the continuous wave Doppler method under resting conditions in the supine position. Furthermore, we selected HCM patients with asymmetric septal hypertrophy defined as the end-diastolic VS to LV posterior wall thickness ratio \( \geq 1.5 \); thus, nine patients with apical hypertrophy, five with diffuse hypertrophy, and three with lateral hypertrophy were excluded from the enrolment. We also excluded patients having any heart disease other than HCM which could possibly confound the IB analysis: five with atrial fibrillation, one with Wolff–Parkinson–White syndrome, five with moderate or severe mitral regurgitation, one with severe tricuspid regurgitation, one with a diameter reduction of \( \geq 50\% \) on coronary angiograms, one with a history of coronary artery bypass surgery, and two with a poor acoustic echo window were excluded from the enrolment.

Finally, 58 patients with non-obstructive HCM (44 men and 14 women; 27–80 years of age, mean 59 years) were enrolled in this follow-up study. The New York Heart Association class was I in 33 patients, II in 19, and III in 6 at the time when the echocardiogram was acquired. Episodic of syncope were observed in seven patients and ventricular tachycardia defined as a short run of three or more premature ventricular beats, in six patients. Survey of the first- or second-degree relatives of all patients revealed a family history of sudden cardiac death in 11 patients and family history of HCM in 24 patients. Of all, 25 patients underwent coronary angiography and 17 underwent exercise testing with scintigraphy; all of them were diagnosed as not having coronary heart disease. Coronary artery disease was not assessed in the remaining 16 patients with HCM because of the low probability of coronary stenosis; none of them had symptoms, hypertension, dyslipidaemia, or diabetes mellitus. There were no patients treated with permanent mechanical devices, e.g. pacemaker or implantable cardioverter-defibrillators. Genetic analysis was not performed in these patients.

We studied 20 age- and sex-matched healthy subjects as normal control (15 men and 5 women; 29–76 years of age, mean 58 years) to obtain normal IB values. All of them had normal findings on physical examination and electrocardiography, and had normal ventricular dimensions and function on echocardiography. Informed consent for this study was obtained from all patients with HCM and control subjects.

**Conventional echocardiography**

The echocardiographic examination was performed in control subjects and non-obstructive HCM patients using a SONOS 5500 (Philips Medical Systems, Best, Netherlands) with a wide-band sector transducer (S4). Conventional indexes were measured as: the end-diastolic diameter and fractional shortening of the LV, VS and LV posterior wall thickness at the level of the chordae, left atrial end-systolic diameter, E-wave velocity, E-wave to A-wave ratio, and E-wave deceleration time of the mitral valve.

**Integrated backscatter analysis**

Ultrasonic tissue characterization was performed by IB analysis in non-obstructive HCM patients and control subjects using the online acoustodensitometry software as previously described.\textsuperscript{6,9,19} In brief, IB images of the VS magnified ~2 diameters and the LV posterior wall without magnification were obtained from the parasternal long-axis view which included the anterior portion of the VS near the VS-free wall junction,\textsuperscript{20} and stored on a magneto-optical disc with maximum dynamic range of 60 dB and 60 image frames corresponding to almost two cardiac cycles.

One-layer (whole aspect), two-layer, and three-layer analyses of the VS were performed in patients with HCM and control subjects (Figure 1). We put the maximum circle as the region of interest across the anterior portion of the VS in one-layer analysis and across the right and left halves in two-layer analysis midway between the base and apex. In three-layer analysis, one circle with 21 × 21 pixels and a diameter of ~6 mm was put as the region of interest on each of the three layers in the anterior portion of the VS, with the centre at the middle portion of each layer. The non-magnified records of the LV posterior wall could not accommodate three circles of the same size in series across the wall, and hence it underwent one-layer analysis. This may be acceptable since the entire myocardium of the posterior segment has been reported to be almost intact even in the heart with marked disarray and fibrosis in other typical segments in the necropsy and magnetic resonance imaging studies.\textsuperscript{17,18}

Each region of interest was manually adjusted on a frame-by-frame basis to keep it inside each region throughout cardiac cycles, excluding endocardial and epicardial reflectors. The time-gain compensation levels were all equal and the lateral-gain compensation was not used. The pre-processing, focus position, persistence, compression, frame rate, and post-processing were maintained constant in all patients, avoiding signal saturation which may possibly cause estimation errors. After retrieving IB images, the time-intensity curves were calculated in the three layers of the VS (Figure 1) and the whole aspect of
the LV posterior wall. We measured the magnitude of cyclic variation of IB (CV-IB) defined as the difference between the minimum and maximum values during cardiac cycles. An experienced investigator blinded to the patients’ clinical information except IB images on a magneto-optical disc conducted IB analysis soon after the enrolment of patients into the study protocol and before the occurrence of cardiac events. After the manual setting of the region of interest, CV-IB was automatically calculated by the apparatus without being influenced by the analysers’ recognition of the VS thickness.

Follow-up

After echocardiographic examination, all the enrolled patients with non-obstructive HCM were prospectively followed for the occurrence of the combined endpoint of cardiac death or hospitalization due to heart failure. Cardiac death was defined as death from arrhythmia, progressive heart failure, or sudden death, i.e. a witnessed death within 1 h after the onset of symptoms or an unwitnessed death in a patient known to be alive and functioning normally 24 h before. Cardiac death was also based on the history of successfully resuscitated life-threatening arrhythmia. Heart failure was defined as developing pulmonary oedema and requiring intravenous treatments (inotropic agents, vasodilators, and diuretics), mechanical ventilation, or circulatory supports. During the follow-up period, the patients underwent standard medical treatment to control symptoms or arrhythmia, etc. Patient information was obtained from available medical records and interviews with patients or their physicians.

Statistical analysis

Categorical variables were compared by χ² test or Fisher’s exact test as appropriate. Continuous variables were expressed as mean ± standard deviation and compared using Student’s t-test. CV-IB indexes were expressed as mean ± standard error and compared using the Mann—Whitney U test or the Kruskal—Wallis test, followed by Bonferroni correction for a multiple comparison test. Wilcoxon matched-pairs signed-ranks test was used to analyse CV-IB indexes within each group. Receiver operator characteristics analysis was performed to determine the optimal cut-off point. Cox proportional hazards regression models were used to calculate hazard ratios and the 95% confidence interval for the comparison of cardiac events. The event-free curves were compared by Kaplan—Meier analysis with the log-rank test statistic. Intra-observer and inter-observer reproducibility for CV-IB were evaluated in three-layer VS analysis using mean absolute differences in 18 HCM patients enrolled in the present study. Intra-observer variability was assessed by one observer twice, and
inter-observer variability by two independent observers. A P-value of <0.05 was considered statistically significant.

Results

Follow-up data were obtained from all of the enrolled patients with non-obstructive HCM. During a follow-up period of 7.1 ± 2.9 years (range: 0.1–9.7), 44 patients remained clinically stable and 14 experienced a cardiac event: 1 patient died suddenly, 2 died of ventricular fibrillation or ventricular tachycardia, and 1 died of progressive heart failure; the remaining 10 required hospitalization because of heart failure.

The above two groups with or without cardiac events were well matched with respect to baseline characteristics and conventional echocardiographic measurements at the time of enrolment (Table 1) although the HCM patients with cardiac events tended to be older without statistical significance. The New York Heart Association functional class was similar in the two groups at the time of enrolment (Table 1), but was higher in patients with cardiac events (class I in 2 patients, II in 9, and III in 3) than in patients without cardiac events (I in 24, II in 14, and III in 6; P = 0.029) at the time when the patients finally consulted us under a stable condition after an average follow-up period of 6.8 years. Neither co-morbidities nor medications significantly differed between the two groups: hypertension (29 vs. 18%, P = 0.31), dyslipidaemia (21 vs. 11%, P = 0.29), and diabetes mellitus (14 vs. 7%, P = 0.35); beta-blockers (14 vs. 16%, P = 0.35), calcium antagonist (43 vs. 32%, P = 0.45), angiotensin-converting enzyme inhibitors/angiotensin receptor blockers (29 vs. 23%, P = 0.45), and amiodarone (14 vs. 2%, P = 0.14). Medications at the final follow-up did not differ between the two groups: beta-blockers (29 vs. 18%, P = 0.31), calcium antagonist (43 vs. 45%, P = 0.86), angiotensin-converting enzyme inhibitors/angiotensin receptor blockers (36 vs. 45%, P = 0.37), and amiodarone (14 vs. 7%, P = 0.35). Heart rate during the acquisition of IB images did not differ between patients with cardiac events and patients without events (61 ± 8 vs. 63 ± 10 beats/min, P = 0.71).

In both one-layer and two-layer analyses, CV-IB of every layer of the VS and the LV posterior wall was lower in non-obstructive HCM patients than in control subjects (each, P < 0.01) but did not differ between patients with and without cardiac events (Figure 2). In three-layer analysis, however, CV-IB of the VS mid-wall layer was lower in HCM patients with cardiac events than in those without (5.4 ± 0.6 vs. 7.4 ± 0.5 dB, P = 0.033) although CV-IB of the right- and left-sided layers did not differ between the two groups. In HCM patients with cardiac events, CV-IB was lower in the VS mid-wall layer than in the two layers on both sides (P = 0.014 and P = 0.038) although CV-IB was similar in the three VS layers in patients without cardiac events. We then calculated the ratio of CV-IB of the VS mid-wall layer to the mean value of CV-IB of the layers on both sides as CV-IB, which was lower in HCM patients with cardiac events (81.7 ± 5.3%) than in those without cardiac events (103.0 ± 5.1%) or control subjects (106.2 ± 5.5%, P < 0.001; Figure 3).

The receiver operator characteristics curve of CV-IB of the mid-wall layer and CV-IB for the detection of cardiac events

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Baseline characteristics at the time of enrolment</th>
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<tbody>
<tr>
<td></td>
<td>Control (n = 20)</td>
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<tr>
<td>Age (years)</td>
<td>58 ± 21</td>
</tr>
<tr>
<td>Men</td>
<td>15 (75%)</td>
</tr>
<tr>
<td>NYHA functional classification</td>
<td></td>
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<tr>
<td>I</td>
<td>20 (100%)</td>
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<tr>
<td>II</td>
<td>–</td>
</tr>
<tr>
<td>III</td>
<td>–</td>
</tr>
<tr>
<td>Syncope</td>
<td>–</td>
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<tr>
<td>Ventricular tachycardia</td>
<td>–</td>
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<tr>
<td>Family history of sudden death</td>
<td>–</td>
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<tr>
<td>Family history of HCM</td>
<td>–</td>
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<tr>
<td>Echocardiographic findings</td>
<td></td>
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<tr>
<td>LV end-diastolic diameter (mm)</td>
<td>45 ± 5</td>
</tr>
<tr>
<td>LV fractional shortening (%)</td>
<td>41 ± 7</td>
</tr>
<tr>
<td>Ventricular septum thickness (mm)</td>
<td>9 ± 1</td>
</tr>
<tr>
<td>LV posterior wall thickness (mm)</td>
<td>9 ± 1</td>
</tr>
<tr>
<td>Left atrial diameter (mm)</td>
<td>28 ± 3</td>
</tr>
<tr>
<td>E (cm/s)</td>
<td>74 ± 21</td>
</tr>
<tr>
<td>E/A</td>
<td>1.13 ± 0.43</td>
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<tr>
<td>Deceleration time (ms)</td>
<td>211 ± 40</td>
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Data are mean ± standard deviation or number (%). A P-value for HCM patients vs. without cardiac events. HCM, hypertrophic cardiomyopathy; LV, left ventricular; NYHA, New York Heart Association.
demonstrated that the optimal cut-off was 5.9 dB and 90%, respectively (Figure 4). Mid-wall CV-IB and CV-IB below the cut-off point was accompanied significantly by cardiac events with a hazard ratio of 3.18 (95% confidence interval, 1.06–9.52; \(P = 0.038\)) and 6.62 (95% confidence interval, 1.48–30.3; \(P = 0.014\)), respectively. Furthermore, these cut-off points remained a reasonable predictor of cardiac events after adjustment for age, gender, dyslipidaemia, diabetes mellitus, and medications (beta-blockers, calcium antagonist, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, and amiodarone) with a hazard ratio of 4.08 (95% confidence interval, 1.01–16.3; \(P = 0.048\)) and 6.12 (95% confidence interval, 1.62–66.6; \(P = 0.013\)), respectively.

Mid-wall CV-IB < 5.9 dB yielded a reasonable predictive value for cardiac events with a sensitivity of 64%, a specificity of 68%, an accuracy of 67%, a positive predictive value of 39%, and a negative predictive value of 86%. The predictive value was ameliorated on the basis of CV-IB < 90% with a sensitivity of 79%, a specificity of 68%, an accuracy of 71%, a positive predictive value of 44%, and a negative predictive value of 91%. The combination of CV-IB < 5.9 dB and CV-IB < 90% yielded a sensitivity of 71%, a specificity

**Figure 2** Cyclic variation of integrated backscatter (CV-IB) in patients with hypertrophic cardiomyopathy (HCM) and control subjects. Left, one-layer analysis of the ventricular septum and the left ventricular posterior wall. Middle and Right, two-layer and three-layer analyses of the ventricular septum. *\(P < 0.01\) vs. control; †\(P < 0.05\) vs. HCM patients without cardiac events.

**Figure 3** Cyclic variation of integrated backscatter (CV-IB) in the ventricular septum of control subjects and patients with hypertrophic cardiomyopathy (HCM). Left, CV-IB of the mid-wall layer. Right, the ratio of CV-IB of the mid-wall layer to the mean value of layers on both sides.
of 82%, an accuracy of 79%, a positive predictive value of 56%, and a negative predictive value of 90%.

Of 19 non-obstructive HCM patients having both mid-wall CV-IB ≥5.9 dB and %CV-IB ≥90%, 7 (37%) experienced cardiac events: three of four dying of cardiac death had both CV-IB ≥5.9 dB and %CV-IB ≥90%. In contrast, no cardiac events occurred in 29 (83%) of 35 HCM patients having mid-wall CV-IB ≥5.9 dB or %CV-IB ≥90%.

Kaplan–Meier cumulative event-free curves (Figure 5) showed a 5-year event-free period in all of the HCM patients having mid-wall CV-IB ≥5.9 dB or %CV-IB ≥90%, but in 81% or 85% of HCM patients having mid-wall CV-IB <5.9 dB or %CV-IB <90%, respectively.

Episodes of syncope, ventricular tachycardia, or family history of sudden cardiac death was more frequent in patients with mid-wall CV-IB ≥5.9 dB (21 vs. 7%, P = 0.14; 50 vs. 9%, P = 0.002; 36 vs. 5%, P = 0.006) and patients with %CV-IB <90% (29 vs. 5%, P = 0.039; 36 vs. 14%, P = 0.066; 29 vs. 7%, P = 0.051). Of the two patients with marked VS thickening ≥30 mm, one with 3.0 dB and 46% died suddenly and the other with 6.0 dB and 103% remained clinically stable.

CV-IB was measured repeatedly in 8 HCM patients. During the average follow-up period of 5.8 (range: 5.3–6.8) years, mid-wall CV-IB and %CV-IB slightly decreased from 6.8 ± 0.7 to 6.3 ± 0.9 dB (P = 0.57) and from 97 ± 14 to 94 ± 6% (P = 0.78), with the rate of decline more marked in three patients having cardiac events (6.3 ± 0.4 to 4.6 ± 0.3 dB and 88 ± 6 to 82 ± 5%, respectively) than in patients having no cardiac events (7.2 ± 1.1 to 7.3 ± 1.2 dB and 103 ± 23 to 103 ± 9%, respectively).

Intra-observer and inter-observer variability of CV-IB showed a small difference in mean values in the right-sided layer of the VS (0.4 ± 0.3 and 0.6 ± 0.3 dB), in the mid-wall layer (0.5 ± 0.4 and 0.6 ± 0.3 dB), and in the left-sided layer (0.5 ± 0.3 and 0.7 ± 0.4 dB) in 18 HCM patients, of whom 5 showed mid-wall CV-IB ≥5.9 dB and %CV-IB ≥90%. Intra-observer and inter-observer variability did not lead to the change of classification of the 18 patients according to the cut-off point of CV-IB ≥5.9 dB and %CV-IB ≥90%.

Figure 4 Receiver operator characteristics curve for detection of cardiac events and cyclic variation of integrated backscatter (CV-IB) of the ventricular septum in patients with hypertrophic cardiomyopathy. Left, based on CV-IB of the mid-wall layer. Right, based on the ratio of CV-IB of the mid-wall layer to the mean value of the layers on both sides.

Figure 5 Long-term prognosis and cyclic variation of integrated backscatter (CV-IB) of the ventricular septum in patients with hypertrophic cardiomyopathy. Left, based on CV-IB of the mid-wall layer. Right, based on the ratio of CV-IB of the mid-wall layer to the mean value of layers on both sides.
Discussion

During an average follow-up period of more than 7 years, 24% of our non-obstructive HCM patients died or required hospitalization because of heart failure. Cecchi et al.\textsuperscript{11} also reported that 16 of 51 (31%) patients with HCM died or severely deteriorated during an average follow-up of more than 8 years. Thus, the identification of HCM patients at risk is clinically important but risk stratification still remains to be a challenge in HCM because of marked heterogeneity in clinical expression, natural history, and prognosis.\textsuperscript{2,3,22} The present study suggested that ultrasonic myocardial tissue characterization separately in the three layers of the VS was predictive of cardiac events. In particular, the high negative predictive values, 86% and 91%, of mid-wall CV-IB and %CV-IB, respectively, strongly suggested that the patients with mid-wall CV-IB and %CV-IB in the patients without cardiac events did not likely to experience cardiac events in the near future. However, mid-wall CV-IB and %CV-IB in the patients without cardiac events ranged widely and overlapped largely with those values in the patients with events below the cut-off points (Figure 3), leading to relatively low positive predictive values.

CV-IB of every layer of the VS was lower in our non-obstructive HCM patients than in normal controls in concordance with previous studies by other researchers in which CV-IB obtained from the one-layer VS analysis was significantly smaller equally in patients with HCM\textsuperscript{10,11,23,24} than in normal subjects. An additional IB analysis\textsuperscript{11} dividing the LV wall into two layers showed that CV-IB did not differ with layers in both the VS and the LV posterior wall for these two diseases. In another two-layer analysis,\textsuperscript{24} CV-IB was also equal in the VS for the two diseases; in the LV posterior wall, however, CV-IB was lower in the endocardial half than in the epicardial half for pressure overload hypertrophy, although it was similar in the two layers for HCM. It may be difficult to make a reliable discrimination of HCM from pressure overload hypertrophy on the basis of these two-layer analyses. In addition, these studies\textsuperscript{10,11,23,24} did not present prognostic data in HCM unlike other studies on various cardiac diseases.\textsuperscript{6–9} In the present study, neither one-layer nor two-layer analysis of the VS revealed any correlation between CV-IB and cardiac events.

We measured CV-IB separately in the three layers of the VS in our patients with HCM, taking the normal\textsuperscript{12–15} and abnormal myocardial architecture\textsuperscript{17} into account. CV-IB was significantly lower in the mid-wall layer than in the layers on both sides in patients with cardiac events, whereas CV-IB in the three layers of VS was similar in patients without. Thus, CV-IB <5.9 dB or %CV-IB <90% of the VS mid-wall layer turned out to be a reasonable predictor of cardiac events. This result is consistent with the previous autopsy study on patients with HCM.\textsuperscript{17} Of 47 autopsied hearts with HCM, 36 (77%) exhibited that this mid-wall circular unit was destroyed by disarray and fibrosis, particularly in and near the area of the junction. Of all, 11 patients with an intact mid-wall circular unit died at a mean age of 60 years, whereas 26 patients with severe destruction of the mid-wall circular unit died at a mean age of 25 years. An unfavourable effect of the LV mid-wall layer destruction is also seen in the study by means of magnetic resonance imaging in which the extent of scar in the mid-wall layer was inversely correlated with systolic wall thickening and ejection fraction of the LV even in asymptomatic HCM patients.\textsuperscript{18}

The myocardial collagen content or myocardial fibrosis may be a determinant of IB values in animal hearts\textsuperscript{25} or human transplanted hearts.\textsuperscript{26} In the study on 20 HCM patients by Mizuno et al.,\textsuperscript{27} there was a high correlation between IB values and the percentage of the fibrotic area in the endomyocardium. In the autopsy study by Shirani et al.\textsuperscript{28} on HCM patients who died suddenly, the calculated volume fraction of interstitial collagen was ~8 times greater than in control subjects. Furthermore, studies of one-layer VS analysis on patients with HCM showed that the septal IB value was significantly correlated with abnormal electrical properties including ventricular late potentials and ventricular tachycardia,\textsuperscript{19} or with the LV stiffness as assessed by Doppler echocardiography,\textsuperscript{29} which are possibly related to myocardial fibrosis and have been reported to be predictive of cardiac death or hospitalization due to heart failure in HCM.\textsuperscript{2,3,30,31} Myocardial fibrosis predominating in the LV mid-wall layer, ischaemic not necessarily because of coronary stenosis but rather secondary to myocardial disarray, may be a common factor leading to the unfavourable prognosis in the present and previous study.\textsuperscript{17}

The prognostic value in the present study was brought about by the IB analysis, an ultrasonic imaging technique, applied to the detection of the destroyed septal mid-wall layer, which is considered to be a part of the destroyed LV circular muscle layer running continuously through the mid-wall of the VS and LV free wall. The circular muscle fibres constitute an important functional unit for the generation of pressure in the LV. If we approximate the LV shape to a prolate spheroid, then the law of Laplace is expressed as $P = \frac{\mu}{2R_0} + \frac{\lambda}{R_1}$, where $P$ is intra-ventricular pressure; $\mu$ and $\lambda$ are latitudinal and longitudinal tensions, respectively; and $R_0$ and $R_1$ are latitudinal and longitudinal radii of curvature, respectively. As $R_0$ is smaller than $R_1$, the IB value in the vicinity of the equator or midway between the base and apex, $\phi$, contributes more to the generation of $P$ than does $\phi/2$. Thus, the destruction of the normal circular unit of muscle fibres of the LV mid-wall in HCM will naturally be associated with systolic and diastolic impairment of the LV function, leading to a poor prognosis. This destruction, a finding not yet well known, is likely to result from retention of an abnormal foetal myocardial architecture in which the septal latitudinal muscle bundle was continuous with the right ventricular free wall.\textsuperscript{17,33}

Study limitations

In order to put a circle with a diameter of ~6 mm as the region of interest within each of the three layers of the VS, the VS was recorded under 2 diameters of magnification. The effect of this magnification on the acquisition of IB values is not known. However, IB values were compared under the same grade of magnification, which condition may be safely acceptable. The optimal size of the region of interest as well as the lowest limit of thickness of each layer may remain to be determined to obtain more objective data. We were able to obtain meaningful results using IB values in the anterior portion of the VS near the VS-free wall junction, but the optimal position for IB analysis remains unclear; IB values of the right ventricle were not assessed in the present study.

We have not yet obtained either necropsy or magnetic resonance imaging data either from our own HCM patients or control subjects.
to compare with their IB findings; the data should confirm our present results concerning the prognosis in relation to myocardial fibrosis. CV-IB was correlated negatively with the percentage of fibrotic area in the endomyocardium in HCM patients, but there are no reports on the direct relation between CV-IB findings and magnetic resonance imaging of the myocardium. However, reduced CV-IB in hypertrophic myocardium was associated with a reduced ultrasonic strain rate and the strain rate has been proved to be a reliable tool to clinically diagnose regional fibrosis as assessed by late enhancement on magnetic resonance imaging.

The size of our study population may not be large enough to extrapolate the current result to the general population of patients with HCM. A larger study population may enable us to correlate more strictly the data of CV-IB with other clinical variables associated with an unfavourable prognosis in HCM, such as diastolic dysfunction, non-sustained ventricular tachycardia, extreme LV hypertrophy, left atrial size, atrial fibrillation, etc., reinforcing the function, non-sustained ventricular tachycardia, extreme LV hypertrophy, left atrial size, atrial fibrillation, etc. reinforcing the prognostic significance of the present result.

Conclusion
Ultrasonic myocardial tissue characterization separately in the three layers of the VS was predictive of cardiac events, with a good negative predictive value, which may support the practice of echocardiography as a new clinical tool for the purpose of risk stratification in patients with non-obstructive HCM.

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Conflict of interest: none declared.

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