Quantitative assessment of left ventricular volume and ejection fraction using two-dimensional speckle tracking echocardiography

Tomoko Nishikage1, Hiromi Nakai2, Victor Mor-Avi3, Roberto M. Lang3, Ivan S. Salgo4, Scott H. Settlemier4, Stephane Husson4, and Masaaki Takeuchi2*

1Echocardiographic Laboratory, Tane General Hospital, Osaka, Japan; 2Second Department of Internal Medicine, University of Occupational and Environmental Health, School of Medicine, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu 807-8555, Japan; 3Cardiac Imaging Center, University of Chicago, Chicago, IL, USA and 4Philips Medical Systems, Andover, MA, USA

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Aims Two-dimensional speckle tracking echocardiography (2DSTE) allows measurements of left ventricular (LV) volumes and LV ejection fraction (LVEF) without manual tracings. Our goal was to determine the accuracy of 2DSTE against real-time 3D echocardiography (RT3DE) and against cardiac magnetic resonance (CMR) imaging.

Methods and results In Protocol 1, 2DSTE data in the apical four-chamber view (iE33, Philips) and CMR images (Philips 1.5T scanner) were obtained in 20 patients. The 2DSTE data were analysed using custom software, which automatically performed speckle tracking analysis throughout the cardiac cycle. LV volume curves were generated using the single-plane Simpson’s formula, from which end-diastolic volume (LVEDV), end-systolic volume (LVESV), and LVEF were calculated. In Protocol 2, the 2DSTE and RT3DE data were acquired in 181 subjects. RT3DE data sets were acquired, and LV volumes and LVEF were measured using QLab software (Philips). In Protocol 1, excellent correlations were noted between the methods for LVEDV ($r = 0.95$), ESV ($r = 0.95$), and LVEF ($r = 0.88$). In Protocol 2, LV volume waveforms suitable for analysis were obtained from 2DSTE images in all subjects. The time required for analysis was 2 min per patient. Excellent correlations were noted between the methods for LVEDV ($r = 0.95$), ESV ($r = 0.97$), and LVEF ($r = 0.92$). However, 2DSTE significantly underestimated LVEDV, resulting in a mean of 8% underestimation in LVEF. Intra- and inter-observer variabilities of 2DSTE were 7 and 9% in LV volume and 6 and 8% in LVEF, respectively.

Conclusions Two-dimensional speckle tracking echocardiography measurements resulted in a small but significant underestimation of LVEDV and EF compared with RT3DE. However, the accuracy, low intra- and inter-observer variabilities and speed of analysis make 2DSTE a potentially useful modality for LV functional assessment in the routine clinical setting.

KEYWORDS
2-Dimensional speckle tracking echocardiography; Left ventricular function

Introduction
Accurate estimation of left ventricular (LV) volumes and systolic function using cardiac ultrasound is essential for the routine management of patients in clinical practice. Although several previous studies have demonstrated that real-time three-dimensional echocardiography (RT3DE) is more accurate for evaluating LV volumes and LV ejection fraction (LVEF) compared with M-mode and two-dimensional (2D) echocardiography,1–12 Two-dimensional echocardiography remains the most widely utilized technique in routine clinical practice. The biplane Simpson’s formula is recommended by the guidelines13 as the preferred method for the calculation of LV volumes and LVEF. However, this method requires manual tracing of the endocardial borders in the apical four- and two-chamber views, which is tedious and time consuming, and also dependent on the reader’s experience. Moreover, accurate endocardial border tracing is difficult in still end-diastolic and end-systolic frames, particularly in the apical lateral segments.

The recent development of 2D speckle tracking echocardiography (2DSTE) has allowed automatic measurements of regional displacement, tissue velocity, strain, and rotation to be performed without the need for manual tracing.

* Corresponding author. Tel: +81 93 603 1611; fax: +81 93 691 6913.
E-mail address: masaaki_takeuchi@hotmail.com

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tracings.14–19 We hypothesized that application of this methodology to the LV endocardial border would allow automatic measurements of LV volume and LVEF. Accordingly, the aims of this study were (i) to test the feasibility of 2DSTE for LV volume and LVEF measurements in a large group of patients, (ii) to validate these measurements against cardiac magnetic resonance (CMR) reference, and (iii) to determine the accuracy of speckle tracking using RT3DE-derived LV volumes and LVEF, as a reference technique.

Study design
This study included two separate protocols. In Protocol 1, 2DSTE measurements of LV volumes and LVEF were compared against CMR reference, which is currently the accepted standard reference method for LV volume measurements. Once validated against this rather expensive and time-consuming reference in a relatively limited number of patients, 2DSTE measurements of LV volumes and EF were obtained in a considerably larger number of patients and validated against RT3DE estimates, which were recently shown to compare favourably with CMR measurements.2,3,6,7,12 Of note, the latter data were obtained quickly and easily in the same setting and using the same equipment as 2DSTE data.

Methods
Protocol 1
Twenty patients with a wide range of LV volume [mean LV end-diastolic volume (LVEDV), 153 ± 80 ml; range, 34-402 ml] and LVEF (mean, 46 ± 16%; range, 9-72%) referred for CMR imaging were enrolled in this protocol (nine patients with dilated cardiomyopathy, six with LV hypertrophy, and five normal volunteers). Exclusion criteria were pacemaker or defibrillator implantation, claustrophobia, and other well-known contraindications to CMR. Patients who had no knowledge of the echocardiographic measurements.14–19 We hypothesized that application of this methodology to the LV endocardial border would allow automatic measurements of LV volume and LVEF. Accordingly, the aims of this study were (i) to test the feasibility of 2DSTE for LV volume and LVEF measurements in a large group of patients, (ii) to validate these measurements against cardiac magnetic resonance (CMR) reference, and (iii) to determine the accuracy of speckle tracking using RT3DE-derived LV volumes and LVEF, as a reference technique.

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Cardiac magnetic resonance assessment of left ventricular volume and ejection fraction
Cardiac magnetic resonance images were obtained in each patient using a 1.5 T scanner (Philips Medical Systems) with a phased-array cardiac coil. ECG-gated localized spin-echo sequences were used to identify the LV long axis. Steady-state free-precession dynamic gradient-echo cine loops were obtained during 10-15 s breath-holds. Data were reconstructed to obtain 30 frames per cardiac cycle. In all patients, 8-13 short-axis cine loops were obtained from just above the mitral annulus to just below the LV apex (9 mm slice thickness, no gaps). CMR cine loops were analysed offline with commercial software (ViewForum, Philips). In every short-axis slice, endocardial contour was manually traced at end-diastole and end-systole, while including the papillary muscles and the endocardial trabeculae in the LV cavity. LVEF was determined by disk-area summation method. All tracings were performed by an investigator experienced in the interpretation of CMR images, who had no knowledge of the echocardiographic measurements.

Protocol 2
A total of 196 subjects with a wide range of LVEF were screened to perform 2D and RT3DE echocardiography. Patients were selected based on image quality. Patients in whom the endocardium was not adequately visualized in two contiguous segments or three or more non-contiguous segments were excluded from the study. Patients were also excluded if they had rhythm disturbances that precluded the acquisition of adequate RT3DE data sets. A total of 15 patients were excluded using these criteria. Thus, the final group consisted of 181 patients. Informed consent was obtained from all patients.

Two-dimensional speckle tracking echocardiography assessment of left ventricular volume and left ventricular ejection fraction
In all subjects, apical four-chamber views were acquired during three consecutive cardiac cycles using high frame rate harmonic imaging (IE33, Philips system with a 5-5-1 transducer), while taking care to maximize the LV long-axis dimension. Data were subsequently transferred to a personal computer for off-line analysis using newly developed 2D speckle tracking software (TMQ, Qlab, Philips). One cardiac cycle was analysed in each patient. In the end-diastolic frame, three anatomic landmarks, including septal and lateral points on the mitral annulus and the apical endocardium, were manually initialized. Following initialization, the software automatically placed eight region of interests (ROIs) equidistantly on the endocardial LV cavity surface. Manual adjustment of ROI was performed when necessary. Subsequently, the software performed speckle tracking analysis using a block matching algorithm in the eight ROIs throughout the cardiac cycle. Further, manual adjustments of the position of ROI in the end-diastolic frame were performed as necessary followed by recalculation of speckle tracking algorithm throughout the cardiac cycle. LV volume vs. time curves were generated by calculating LV volume at each phase of the cardiac cycle using the single-plane Simpson’s formula, from which LVEDV, LV end-systolic volume (LVEV), and LVEF were automatically calculated (Figure 1).

Two-dimensional echocardiographic assessment of left ventricular volume and left ventricular ejection fraction
In each subject, 2D echocardiographic assessment of LV volumes and LVEF was performed in the identical apical four-chamber image in which 2DSTE was applied. The endocardial border at both end-diastolic and end-systolic frames was manually traced, and LVEDV, LVEV, and LVEF were calculated using the single-plane Simpson’s formula.

Real-time three-dimensional echocardiography assessment of left ventricular volume and left ventricular ejection fraction
Harmonic real-time 3D imaging was performed using the same ultrasound system and a matrix-array transducer (X3-1, 1.9/3.8 MHz) to obtain a pyramidal volume data set. Gain and compression controls, as well as time-gain compensation settings, were optimized to enhance image quality. Care was taken to include the entire LV cavity within the pyramidal scan volume. RT3DE data sets were acquired using a wide-angle acquisition (93° x 80°) mode in which four wedge-shaped subvolumes (93° x 20° each) were obtained from four consecutive cardiac cycles. Data were acquired from the apical four-chamber position during held end-expiration. Acquisition was triggered to the ECG R-wave of each cardiac cycle.

Data sets were subsequently analysed off-line using commercial software (3DQ ADV, QLab, Philips) as described previously.15,16 Briefly, five anatomic landmarks were manually initialized on the end-diastolic frame in the non-foreshortened, apical four- and two-chamber views obtained by cropping the pyramidal data set. The 3D endocardial surface was automatically detected using a deformable shell model. Thereafter, the end-systolic frame was selected by identifying the frame with the smallest LV cavity cross-sectional area in both apical views. Following initialization, surface detection

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was then repeated on this frame to obtain end-systolic volumes. Finally, the computer algorithm automatically defined and traced the endocardial border in all frames of the cardiac cycle. ‘Casts’ of the LV endocardium were then automatically obtained from which LV volumes vs. time curves were derived. From these curves, LVEDV, LVESV, and LVEF were computed.

Inter- and intra-observer variabilities
Intra-observer variability was determined by having one observer repeating the measurements of 2DSTE- and RT3DE-derived LVEDV, LVESV, and LVEF in 35 randomly selected subjects 1 month later. Inter-observer measurement variability was determined by having a second observer measuring these variables in the same 35 subjects. Intra- and inter-observer variability values were then calculated as the absolute difference between the corresponding two measurements in per cent of their mean.

Statistical analysis
All values were expressed as mean ± SD. Agreement between 2DSTE and other modalities was evaluated by linear regression analysis with Pearson’s correlation coefficient. In addition, Bland-Altman analysis was used to determine the bias and limits of agreement between the corresponding measurements. The significance of inter-technique biases was tested using paired t-tests. A value of \( P < 0.05 \) was considered significant.

Results

Protocol 1

2DSTE images obtained in all study subjects were suitable for analysis and allowed the measurements of LV volumes and LVEF. Significant correlation was noted between 2DSTE and CMR for LV volumes (Figure 2), with no statistically significant difference between them. Although good correlation for LVEF was also noted, 2DSTE significantly underestimated LVEF with a mean difference of 5.2% \( (P < 0.005) \) with 95% limits of agreement at ± 15%.

Protocol 2

Both 2DSTE in the apical four-chamber view and RT3DE data sets were successfully acquired in all subjects. Patient characteristics are shown in Table 1. The frame rate in the 2D apical four-chamber views was 70 ± 10 frames/s (range, 39–98) in contrast to 18 ± 2 frames/s (range, 13–27) in the full-volume RT3DE data sets. Two-dimensional speckle tracking analysis algorithm resulted in an average LVEDV of 101 ± 48 mL (range, 48–295 mL), LVESV of 59 ± 45 mL (range, 19–238 mL), and LVEF of 46 ± 13% (range, 10–66%). Using the speckle tracking algorithm, poor tracking of the apical ROI, lateral wall ROI, and ROI at multiple locations were observed in four, two and two cases, respectively. LV volume vs. time curves showed drift in the LV volume curves in an additional three cases. The time
required for analysis, including endocardial initialization, adjustments of ROI, and computation of LV volume and LVEF, was usually <2 min. Manual tracing of standard 2D apical four-chamber view resulted in LVEDV of 100 ± 48 mL (range, 44–304 mL), LVESV of 52 ± 47 mL (range, 12–260 mL), and LVEF of 54 ± 17% (range, 2–79%). RT3DE surface detection algorithm yielded LVEDV of 109 ± 47 mL (range, 46–293 mL), LVESV of 56 ± 46 mL (range, 13–237 mL), and LVEF of 54 ± 17% (range, 9–78%).

A significant correlation was found between 2DSTE and RT3DE measurements for LVEDV ($r = 0.95$), LVESV ($r = 0.97$), and LVEF ($r = 0.92$) (Figure 3). Compared with RT3DE, 2DSTE underestimated LVEDV, and slightly, but significantly overestimated LVESV, resulting in a significant underestimation of LVEF. Bland-Altman analysis of LVEF using both methods showed a mean difference of 8 with 95% limits of agreement at ±14%. The subgroup analysis showed that good correlation (LVEDV: $r = 0.93$, LVESV: $r = 0.90$, LVEF: $r = 0.80$) was still preserved in 42 patients who had globally reduced LVEF (28 ± 11% of mean LVEF by RT3DE, range, 9–44%) or in 29 patients with regional wall motion abnormalities (LVEDV: $r = 0.83$, LVESV: $r = 0.90$, LVEF: $r = 0.89$).

A significant correlation was also noted between the RT3DE and 2D manual tracing methods for LVEDV ($r = 0.93$), LVESV ($r = 0.96$), and LVEF ($r = 0.92$) (Figure 4). Although manual 2D tracing method significantly underestimated LV volume measurements, no differences of LVEF were noted between

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Clinical characteristic of study subjects ($n = 181$)</th>
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<tr>
<td>Age (years)</td>
<td>61 ± 14</td>
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<tr>
<td>Male (%)</td>
<td>143 (79%)</td>
</tr>
<tr>
<td>Height</td>
<td>163 ± 9 cm</td>
</tr>
<tr>
<td>Weight</td>
<td>61 ± 11 kg</td>
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<tr>
<td>Clinical diagnosis</td>
<td></td>
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<tr>
<td>CAD</td>
<td>57</td>
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<tr>
<td>HHD</td>
<td>21</td>
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<tr>
<td>LBBB</td>
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<tr>
<td>Others</td>
<td>33</td>
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<td>Normal volunteer</td>
<td>17</td>
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<tr>
<td>Risk factor</td>
<td></td>
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<tr>
<td>HT</td>
<td>98</td>
</tr>
<tr>
<td>DM</td>
<td>37</td>
</tr>
<tr>
<td>HL</td>
<td>59</td>
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<tr>
<td>Real time 3D echocardiographic data</td>
<td></td>
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<tr>
<td>LVEDV</td>
<td>108.8 ± 46.9 mL (range: 46 to 293 mL)</td>
</tr>
<tr>
<td>LVESV</td>
<td>56.3 ± 46.2 mL (range: 13 to 237 mL)</td>
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<tr>
<td>LVEF</td>
<td>53.6 ± 17.1% (range: 9 to 78%)</td>
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</tbody>
</table>

CAD, coronary artery disease; DM, diabetes mellitus; HHD, hypertensive heart disease; HL, hyperlipidemia; HT, hypertension; LBBB, left bundle branch block; LVEF, left ventricular ejection fraction; LVED(V)S(V), left ventricular end-diastolic (systolic) volume.
the two methods. Bland-Altman analysis of LVEF showed a mean difference of 0.1% with 95% limits of agreement at ±13%.

**Intra- and inter-observer variabilities**

Intra-observer variability in the measured LV volume and LVEF using 2DSTE was 6.7 ± 7.8 and 5.9 ± 5.8%, respectively. Inter-observer variability in the measured LV volume and LVEF using 2DSTE was 9.4 ± 8.0 and 7.7 ± 5.6%, respectively.

Intra-observer variability in the measured LV volume and LVEF using RT3DE was 7.7 ± 6.7 and 13.7 ± 12.3%. Inter-observer variability in the measured LV volume and LVEF using RT3DE was 8.3 ± 7.6 and 20.1 ± 14.0%, respectively.

**Discussion**

This study demonstrated that (i) 2DSTE evaluation of both LV volumes and LVEF correlated well with CMR, and (ii) although good correlation was noted between 2DSTE and RT3DE for LV volumes and LVEF, a small yet significant underestimation of LVEDV and LVEF was noted with 2D speckle tracking method. Despite these small differences, a shorter analysis time coupled with low observer variabilities makes this method potentially clinically useful.

Two-dimensional speckle tracking analysis was developed as a tool to measure regional LV function, using myocardial strain, strain rate, and torsion.14–19 The general principle of this technique relies on the tracking of natural acoustic markers (speckles) in the myocardium from frame to frame throughout the cardiac cycle using a sum of absolute differences algorithm.15,17 Tracking a specific speckle detail during serial frames allows measurements of temporal and spatial displacement of speckle targets, from which 2D strain and strain rate in the left ventricle can be calculated globally and regionally. The accuracy of 2D speckle tracking and its clinical utility for the assessment of regional LV function was demonstrated in several studies.14,20,21 Importantly, unlike tissue Doppler-derived strain and strain rate, speckle tracking is angle independent. Moreover, regional LV function can be assessed in all myocardial segments. The results of this study showed that this new method may also be useful for the evaluation of global LV function. Speckle tracking of the endocardial border is easier compared with tracking of the epicardium, because the acoustic mismatch between the endocardium and blood pool is large, resulting in more accurate tracking of the blood tissue interface.

**Previous studies**

As the quantitative assessment of LV function using manual tracing method is relatively tedious and observer dependent, automated approaches have attracted interest in the past. Acoustic quantification (AQ) is an automated border detection technique that has been used for the quantitative assessment of LV volume and function.22,23 However, AQ requires adequate visualization of the endocardial border to accomplish accurate tracking. Gain
setting heavily affects accurate tracking of the blood-tissue interface resulting in substantial measurement variability. These drawbacks have limited its routine use in clinical practice.

Current study

The results of Protocol 1 demonstrated that 2D speckle tracking analysis allows reliable assessment of LV volumes and LVEF when compared against CMR as a reference standard with relatively small bias and narrow limits of agreement. Although sample size was relatively small, our results contribute towards the validation of 2DSTE assessment of LV volume and LVEF.

In Protocol 2, all subjects who were enrolled on the basis of relatively good image quality were found suitable for 2DSTE analysis of LV volumes and LVEF. However, eight out of 181 subjects had suboptimal speckle tracking, especially in the apex and lateral wall. Additional three patients had a drift in the LV volume curve, which occurred as a result of obvious cardiac translation during data acquisition. When compared against RT3DE, 2D manual tracing method consistently underestimated both LVEDV and LVESV, a finding which is in agreement with previous studies. Although LVEDV by 2DSTE was also underestimated, in this study, compared with RT3DE, LVESV was slightly overestimated, resulting in an 8% underestimation of LVEF. In contrast to the 2D manual tracing and RT3DE methods, in which manual editing can be performed in both the end-diastolic as well as end-systolic frames, the speckle tracking software allows manual editing of the ROI position only in the end-diastolic frame, which might have contributed to this discrepancy.

In this study, LV volume and LVEF measurements obtained by 2D speckle tracking method were calculated using single-plane Simpson's role (apical four-chamber view) which necessitates mathematical geometric assumption. Previous studies have shown that a minimum of three to six long-axis cross sections is necessary for the accurate determination of LV function. However, our subgroup analysis still showed good correlation of LV volume and LVEF between 2DSTE and RT3DE measurements in patients with globally reduced LV systolic function and those with regional wall motion abnormalities. Thus, this method could be applicable in ischaemic patients with LV asynergy. Further studies are required to test this hypothesis.

Two-dimensional speckle tracking of the LV endocardial border provides LV volume vs. time curves throughout the cardiac cycle. Since the frame rates of 2DSTE are four to five times faster than those of full-volume RT3DE data sets, more detailed LV volume analysis could be potentially accomplished, including the analysis of diastolic function using parameters such as filling rates and per cent filling at specific time intervals of diastole.

Compared with previous studies, the inter-observer variability in LV volumes and LVEF using 2DSTE was smaller compared with those obtained with the 2D manual tracing and RT3DE. This finding is another advantage of 2DSTE compared with manual tracing for assessing global LV function.
Study limitations
We assessed only 2D speckle tracking in the apical four-chamber view and used single-plane Simpson’s formula for the calculation of LV volume and LVEF, because this software does not support the use of the bi-plane Simpson’s formula in its current stage of development. The study subjects did not constitute a consecutive series of subjects, and were selected according to image quality. In the future, the true feasibility of this method should be determined in a large series of consecutive patients. The accuracy of speckle tracking largely depends on image quality, and consequently poor endocardial delineation should result in inaccurate findings as a result of artificial error of endocardial tracking. However, the same situation occurs with manual tracing. Nevertheless, in this feasibility study, only 15 patients (<20%) were excluded because of inadequate image quality. We did not enrol patients with poor image quality, and therefore, subjects did not receive intravenous contrast agents to enhance endocardial border. Further studies are required to determine whether 2D speckle tracking would accurately track endocardial border on contrast-enhanced images.

Conclusions
2DSTE measurements of global LV function resulted in a small but significant underestimation of LVEDV and EF compared with RT3DE. However, its accuracy, low observer variability, and speed of analysis make 2DSTE an attractive alternative for the assessment of LV function in the clinical setting.

Conflict of interest: I.S.S., S.H.S. and S.H. are employees of Philips.

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