Evaluation of myocardial mechanics with three-dimensional speckle tracking echocardiography in heart transplant recipients: comparison with two-dimensional speckle tracking and relationship with clinical variables

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Received 2 February 2013; accepted after revision 27 March 2013; online publish-ahead-of-print 23 April 2013

Aims
Two-dimensional speckle-tracking echocardiography (2D-STE) is limited by its inability to track tissue motion in three dimensions. This is particularly relevant in heart transplant recipients, in whom marked translational motion of the transplanted heart is present. We aimed to compare 3-dimensional (3D)- and 2D-STE-derived strain parameters, and to identify clinical features associated with myocardial mechanics in transplant recipients.

Methods and results
In 36 heart transplant recipients, global and regional left-ventricular (LV) longitudinal and circumferential strain (LSt and CSt), and radial displacement (RDisp) were obtained by 3D- and 2D-STE, and their results were compared. 3D-STE deformation from a subset of transplant recipients with preserved ejection fraction was compared with a control group of 25 subjects matched by gender, age, history of hypertension, and ejection fraction. Associations between global LSt and CSt and clinical, echocardiographic, and haemodynamic parameters in transplant recipients were investigated. 3D-STE yielded lower magnitude of global LSt compared with 2D-STE (−13 ± 3 vs. −16 ± 3%, \( P < 0.001 \)). The inferolateral wall was a source of variation between 3D- and 2D-STE both for LSt and CSt. Inferolateral wall 3D-STE-derived RDisp was greater than that observed in control subjects (7.4 ± 1.2 vs. 6.5 ± 1.7 mm, \( P = 0.03 \)), while anteroseptal RDisp was lower than controls (4.2 ± 1.0 vs. 7.3 ± 1.6 mm, \( P < 0.001 \)). Multiple regression analysis demonstrated that 3D-STE-derived LSt was independently associated with NYHA class (\( P < 0.001 \)), while 2D-STE-derived LSt was not.

Conclusion
Examination of LV mechanics by 3D- and 2D-STE deformation parameters in heart transplant recipients yields significantly discordant results. 3D-STE-derived LSt is independently associated with NYHA class, suggesting a clinically important relationship between functional status and myocardial mechanics.

Keywords
Speckle-tracking echocardiography • Three-dimensional echocardiography • Cardiac mechanics • Heart transplant
Introduction

Speckle-tracking echocardiography (STE) technology has emerged as a promising technique for evaluation of cardiac function due to its capability to examine altered myocardial mechanics in cardiovascular disease, even when standard echocardiographic measurements, such as left-ventricular (LV) volumes and ejection fraction, remain within the normal range.1-3 Both 3- and 2-dimensional (3D and 2D) algorithms from different vendors are commercially available for speckle-tracking analysis and have been applied to a variety of cardiovascular conditions.4,5

Although the experience with 3D-STE is less extensive than that with 2D-STE, it has been suggested that the former may be a more accurate tool for global and segmental assessment of LV function.6 This concept is based on the fact that 2D algorithms only track speckles in 2D planes, while speckles move in 3-dimensions, and so only a portion of the real motion can be detected. This limitation is particularly relevant in heart transplant recipients, in whom the typically greater size of the mediastinal cavity when compared with the donor heart, and the loss of support provided by the pericardial sac, results in a marked translational motion of the transplanted heart inside the chest during the cardiac cycle. This prominent translational motion is likely to present a significant limitation when using 2D-STE in these patients, since a large amount of speckles is likely to move in and out the 2D imaging plane.

Examination of myocardial mechanics is of clinical interest in heart-transplant recipients, since it has been observed that these patients often have subnormal functional capacity, even in the absence of significant allograft rejection or overt LV systolic dysfunction.7,8 While previous investigations have addressed myocardial mechanics after heart transplantation, focusing either on the diagnosis of rejection or its role against conventional parameters of LV function, they have all used tissue Doppler methods, such as left-ventricular (LV) volumes and ejection fraction,1-3,11-14 In view of the potential technical advantages of 3D-STE, the aim of this study was to compare 3D-STE-derived deformation parameters with both 2D-STE-derived deformation parameters and matched controls, and to identify clinical features that might be associated with myocardial mechanics in heart transplant recipients.

Methods

Forty-one non-selected heart-transplant recipients were recruited from the Heart Failure and Cardiac Transplant Center at Tufts Medical Center, Boston, MA, USA. Study patients provided informed consent. Only cardiac transplant recipients who were in a stable outpatient cardiovascular setting were included. NYHA class was determined from the clinical evaluation on the day of the echocardiogram, performed by a physician who was blinded to the echocardiographic data. A control group of 25 subjects matched by gender, age, history of hypertension, and ejection fraction was used for comparisons; control subjects were matched with transplant recipients for history of hypertension in order to control for the demonstrated effect of hypertension on myocardial mechanics. The control group had no evidence of left- or right-bundle branch block on electrocardiogram, and had normal 2D and Doppler echocardiograms. The Institutional Review Board approved the study protocol.

Echocardiography

The echocardiographic study was performed with a Toshiba Artida 4D System (Toshiba America Medical Systems, Tustin, CA, USA). Standard 2D and Doppler images were acquired15 with a PST-30SBT transducer and 3D data sets were acquired with a matrix array PST-25SX transducer. Two-dimensional data sets for speckle-tracking analysis included three short-axis views (basal, mid-ventricular, apical), and three apical longitudinal views (4-, 2-, and 3-chamber). Special care was taken to assure the acquisition of optimal circumferential cross-sectional slabs at the basal (mitral leaflets visible without the presence of papillary muscles), mid-ventricular (papillary muscles visible without the presence of mitral leaflets), and apical (towards the LV apex, absence of papillary muscles) levels. Adjustments in sector depth and angle yielded a temporal resolution of 80–100 frames per second.

Three-dimensional data sets consisted of apical full volumes created by the combination of six ECG-gated, wedge-shaped sub-volumes during a single breath hold. Optimization of 3D images and adjustments in depth and full-volume sector angles yielded a temporal resolution of 20–30 volumes per second. Exclusion criteria included significant (moderate or severe) valve dysfunction in the standard 2D and Doppler study, and irregular cardiac rhythm that precluded an adequate construction of the 3D full volume focused on the left ventricle.

Off-line processing of the 3D and 2D data sets for STE analysis was performed using the Wall Motion Tracking software (Toshiba Medical Systems) by an expert in the interpretation of echocardiographic images. For analysis of 3D data sets, the axis orientation was first optimized (Figure 1A and B). Semi-automated tracing of the endocardial and epicardial borders was rendered by placing, at end-diastole, six reference points (Figure 1C and D). Thickness of the region of interest was adjusted until a best match with the actual endocardial and epicardial surfaces was found. The automated tracking of borders throughout the cardiac cycle was then started, and the 3D images of the LV wall were automatically divided into a 16-segment model. The resulting tracings were manually modified only in those areas where the true endocardial and epicardial borders were not correctly tracked. If it was not feasible to either automatically or manually track one or more segments, the case was excluded; thus all 16 segments from included cases were considered for results.

The analysis of 2D data required processing of six data sets individually (three short-axis views and three apical long-axis views). Semi-automated tracing of the endocardial and epicardial borders was rendered by placing reference points on the endocardial border at end-systole. Both adjustment of thickness of the region of interest before the automated tracking of borders and manual modification of the resulting tracings were managed in the same way as in the 3D-STE analysis.

This study’s examination of myocardial mechanics focused on the analysis of myocardial longitudinal and circumferential strain (LSt and CSt), and radial displacement (RDisp). Longitudinal and circumferential strains refer to myocardial deformation (measured in %) in the direction tangential and circumferential, respectively, to the endocardial contour (a negative value indicates shortening). Radial displacement refers to myocardial excursion (measured in millimetres) in the direction normal to the endocardial surface (a positive value indicates displacement towards the centre of the cavity). All of these variables were derived from both 3D- and 2D-STE analysis. Three-dimensional-STE-derived global values for LSt, CSt, and RDisp were obtained from the rendered 16-segment model, while regional values were obtained by averaging the segmental values corresponding to the inferolateral and anteroseptal walls. Two-dimensional STE-derived global values for CSt and RDisp were obtained from the 16...
segments in the three LV short-axis views, and for LS from the 18 segments in the three LV apical longitudinal axis views; in order to have the best match with the 3D 16-segment model, the 4- and 3-chamber view apical septal segments, and the 4- and 3-chamber view apical lateral segments were averaged to render a single septal and lateral segment, respectively. Regional values were obtained by averaging the segmental values corresponding to the inferolateral and anteroseptal walls (septal and lateral apical segments from 4- and 3-chamber views were averaged as for global values).

Right heart catheterization
A subset of 25 subjects had a clinically indicated right heart catheterization for surveillance endomyocardial biopsy, which also included, according to the regular practice in our institution, measurement of the following pulmonary artery catheter resting haemodynamic data: right atrial pressure, systolic, diastolic, and mean pulmonary artery pressure, pulmonary capillary wedge pressure, mixed venous oxygen saturation, cardiac output, and cardiac index. Heart transplant rejection was graded based on the 1990 International Society for Heart and Lung Transplantation grading system.16

Statistical analysis
Continuous variables were tested for normality using Kolmogorov–Smirnov and Shapiro–Wilk tests, and are shown as mean ± standard deviation or median [interquartile range (IQR)], as appropriate. Categorical variables are expressed as frequencies and percentages. Comparisons between 3D-STE- and 2D-STE-derived global and regional strain results, and between heart transplant recipient and control groups were performed using paired and unpaired Student’s t-tests, respectively. After construction of scatter plots for pairs of continuous variables, correlations between global strain parameters and LV volumes, stroke volume, ejection fraction, mass, and right heart catheterization-derived data were tested by Pearson correlation coefficient. Differences in strain variables for both NYHA functional class (Class I–II vs. Class III–IV) and grade of rejection on endomyocardial biopsy were tested by unpaired Student’s t-test and one-way analysis of variance, respectively. Linear regression (simple analysis followed by simultaneous multiple analysis for those variables with significance level at <0.1) was conducted to determine possible independent associations of several predefined clinical factors (patient age, donor age, cold ischaemic time, time since transplant, and NYHA functional class) with myocardial mechanics (global LS and CS). Prior to performing the multiple regression analysis, multicollinearity problems among the set of clinical variables was ruled out; it was confirmed both that the relationship between each of the possible predictor variables and the dependent variable was linear, and that the residual was normally distributed and uncorrelated to the predictors. Intra- and interobserver variability (reading by a second expert in the interpretation of echocardiographic images) was calculated as the absolute difference of the corresponding pair of repeated myocardial mechanics measurements, expressed as percentage of their mean. A two-tailed P-value <0.05 was considered statistically significant. Statistical analysis was performed using IBM SPSS Statistics 20.0 (IBM Corp., Armonk, NY, USA).

Results
Three-dimensional STE analysis was possible in 36 out of 41 transplant recipients, yielding feasibility for the technique of 88%. Demographic, clinical, and transplant-related characteristics and

Figure 1 Off-line processing of three-dimensional data sets for speckle-tracking analysis. (A) Five-plane display with default axis orientation on two orthogonally oriented LV longitudinal views, and three LV cross-sectional views. (B) Same five-plane display after manual rotation, tilt, and displacement of axes to ensure the exposure of the true endocardial border both in the 4-, 2-chamber views and in the apical, mid-, and basal cross-sectional views. (C) Following counterclockwise direction, six reference points are marked at the blood–tissue interface, first on the 4-, and then on the 2-chamber view. (D) Rendered 3D endocardial and epicardial borders, whose automated tracking throughout the cardiac cycle subsequently generates a 16-segment model.
Data derived from speckle-tracking analysis are shown in Table 1. Resting haemodynamic data obtained by right heart catheterization were available from 22 patients of the 36 with adequate 3D-STE analysis (Table 2).

### Three-dimensional- and 2D-STE analyses

Data derived from speckle-tracking analysis are shown in Table 3. Three-dimensional STE yielded lower magnitude of global LSt when compared with results derived from the 2D modality ($-13 \pm 3$ vs. $-16 \pm 3\%$, $P < 0.001$). No inter-technique differences were detected for global CSt ($-25 \pm 4$ vs. $-27 \pm 6\%$, $P = 0.1$). Regional analysis of the anteroseptal wall demonstrated higher LSt from 2D-STE compared with 3D-STE, but no other differences between the techniques were observed within the anteroseptal region. However, regional analysis of the inferolateral wall showed that 2D-STE-derived LSt, CSt, and RDisp were all higher than the corresponding 3D-STE parameters in that region (Table 3). Of note, the inferolateral wall RDisp was greater than that of the anteroseptal wall in transplant recipients, regardless of the technique used. In order to investigate if the inferolateral wall RDisp is particularly enhanced in transplant recipients, global and regional mechanics were compared between a subset of transplant recipients with preserved ejection fraction ($n = 25$) and controls ($n = 25$) that were matched by age, gender, and the presence of hypertension. This analysis showed a greater RDisp of the inferolateral wall in transplant recipients ($7.4 \pm 1.2$ vs. $6.5 \pm 1.7$ mm, $P = 0.03$), and a significantly attenuated anteroseptal RDisp ($4.2 \pm 1.0$ vs. $7.3 \pm 1.6$ mm, $P < 0.001$), compared with controls (Table 4).

### Associations and correlates of longitudinal and circumferential strain

Transplant recipient groups according to NYHA functional class differed in 3D-STE-derived LSt ($I–II$, $-13 \pm 2\%$ vs. III–IV, $-9 \pm 2\%$, $P = 0.001$), while this was not seen for LSt obtained by 2D-STE ($I–II$, $-16 \pm 3\%$ vs. III–IV, $-13 \pm 2\%$, $P = 0.2$). The same analysis
employing 3D- and 2D-STE-derived CSt again only showed differences for the 3D-STE-derived variable (3D-STE: I–II, −25 ± 4% vs. III–IV, −21 ± 6%, P = 0.03; 2D-STE: I–II, −27 ± 6% vs. III–IV, −30 ± 8%, P = 0.6). Regarding grade of rejection on endomyocardial biopsy, no differences between transplant recipient groups were detected both for 3D- and 2D-STE-derived LSt, or 3D- and 2D-STE-derived CSt.

Significant correlations were seen between 3D-STE-derived LSt and LV end-systolic volume (r = 0.40, P = 0.02) and ejection fraction (r = −0.72, P < 0.001). For 3D-STE-CSt, correlations were found with LV end-diastolic volume (r = 0.36, P = 0.03), end-systolic volume (r = 0.61, P < 0.001), ejection fraction (r = −0.83, P < 0.001), and LV mass (r = 0.40, P = 0.02). When 2D-STE-derived strain parameters were tested, only stroke volume and ejection fraction showed a significant correlation with LSt (−0.39, P = 0.04, and −0.47, P = 0.01, respectively). No correlations were found between any of the right heart catheterization-derived resting haemodynamics and 3D- or 2D-STE-derived LSt or CSt.

Table 4 Three-dimensional speckle-tracking analysis in transplant patients with ejection fraction ≥50% and matched controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>3D-STE Transplant patients (n = 25)</th>
<th>Controls* (n = 25)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSt (%)</td>
<td>−14 ± 2</td>
<td>−16 ± 3</td>
<td>0.004</td>
</tr>
<tr>
<td>CSt (%)</td>
<td>−27 ± 3</td>
<td>−30 ± 4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Anteroseptal LSt (%)</td>
<td>−12 ± 2</td>
<td>−15 ± 3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Inferolateral LSt (%)</td>
<td>−16 ± 3</td>
<td>−17 ± 3</td>
<td>0.3</td>
</tr>
<tr>
<td>Anteroseptal CSt (%)</td>
<td>−25 ± 3</td>
<td>−30 ± 5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Inferolateral CSt (%)</td>
<td>−28 ± 4</td>
<td>−31 ± 4</td>
<td>0.02</td>
</tr>
<tr>
<td>Wall radial displacement (mm)</td>
<td>4.2 ± 1.0</td>
<td>7.3 ± 1.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Anteroseptal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inferolateral</td>
<td>7.4 ± 1.2b</td>
<td>6.5 ± 1.7c</td>
<td>0.03</td>
</tr>
</tbody>
</table>

3D, three-dimensional; STE, speckle-tracking echocardiography; LSt, longitudinal strain; CSt, circumferential strain.

*Matched by age, gender, history of hypertension, and ejection fraction.

Table 5 Simple linear regression analysis for 3D-STE-derived longitudinal strain

<table>
<thead>
<tr>
<th>Simple regression for 3D-STE-derived LSt</th>
<th>Standardized correlation coefficient</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient age</td>
<td>−0.26</td>
<td>0.1</td>
</tr>
<tr>
<td>Donor age</td>
<td>−0.37</td>
<td>0.03</td>
</tr>
<tr>
<td>Cold ischaemic time</td>
<td>−0.07</td>
<td>0.7</td>
</tr>
<tr>
<td>Elapsed time since transplant</td>
<td>−0.16</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Table 6 Multiple linear regression analysis for 3D-STE-derived longitudinal strain

<table>
<thead>
<tr>
<th>Multiple regression</th>
<th>Standardized correlation coefficient</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor age</td>
<td>−0.41</td>
<td>0.005</td>
</tr>
<tr>
<td>NYHA functional class</td>
<td>0.54</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Reproducibility

The intra- and interobserver variability obtained for 3D-STE-derived global strain parameters was as follows: LSt, 5 ± 4% and 6 ± 4%; CSt, 7 ± 5% and 9 ± 7%. The corresponding intra- and interobserver variability for 2D-STE-derived measurements was as follows: LSt, 9 ± 6% and 11 ± 6%; CSt, 11 ± 9% and 14 ± 11%.

Discussion

Cardiac transplant recipients have been the focus of attention in a number of recent studies investigating myocardial mechanics, mostly addressing the relationship of endomyocardial biopsy rejection grade with deformation parameters derived from 2D-STE. Although 2D-STE has inherent limitations that can be overcome by 3D-STE, the 3D approach to myocardial mechanics has not been employed to date in the evaluation of the transplanted heart. The major findings of this study reside in the different myocardial deformation results derived from 3D- and 2D-STE analyses in heart transplant recipients and, secondly, the possibility that clinical features of these patients might relate to specific strain values obtained in the 3D-STE analysis.

The enhanced translational displacement of the LV inferior and lateral walls can be frequently observed in the standard echocardiographic follow-up of transplanted patients. Since this...
exaggerated translational motion predominantly affects displacement of the free wall, our patients were studied investigating inferolateral vs. anteroseptal wall regional mechanics. Such enhanced translational motion is likely to be related both to some degree of disproportion between the size of the recipient mediastinal cavity and the size of the donor heart, and to the loss of support and hold given by the pericardial sac. In our study, when LSt and CSt derived from 3D- and 2D-STE were compared, significant differences were found between these techniques for the inferolateral wall LSt and CSt (Table 3). In addition, there was higher RDisp of the inferolateral wall by 2D-STE compared with 3D-STE. The finding of inter-technique differences for both LSt and CSt in the LV inferolateral wall suggests that the inferolateral wall represents a major source of discordance between 3D- and 2D-STE-derived deformation parameters, potentially due to enhanced translational motion of this wall; this concept is further supported by the higher RDisp of the inferolateral wall in transplant recipients compared with the control group. The observed differences between 2D-STE and 3D-STE are likely related to the inherent 2D limitation to tracking of the true endocardium due to noise and interference caused by speckles moving in and out of the image plane, which may thereby influence the evaluation of myocardial mechanics. Interestingly, the anteroseptal wall LSt also influenced the detected differences between both techniques despite the low RDisp of this wall in transplant recipients, indicating that, in addition to the inferolateral wall translational motion, anteroseptal mechanics might also be a source of discordant results between 3D- and 2D-STE.

It is also noteworthy that consistent with previous observations, transplant recipients in our study showed attenuated LSt and CSt when compared with controls (matched by gender, age, history of hypertension, and ejection fraction), mainly due to decreased values of these parameters in the anteroseptal wall. These findings, together with diminished anteroseptal wall RDisp in transplanted subjects (Table 4), might be explained by either reduced or paradoxical septal motion, both frequently observed after heart transplantation. Although conventional parameters of systolic thickening and curvature are typically preserved in patients with paradoxical septal motion, the observed decrease in anteroseptal LSt and CSt may indicate subtle impairments in septal wall motion.

The analysis of possible clinical features that might be related to 3D-STE-derived functional parameters yielded an independent association between LSt and both donor age and NYHA functional class. Interestingly, a negative correlation was observed between donor age and LSt. The specific relationship of donor age and LSt or CSt has not been evaluated in other studies performed on cardiac transplant recipients. Previously reported age-related events in terms of myocardial mechanics (i.e. decreasing LSt compensated by increasing CSt) have been thought to be related to progressive endocardial stiffening that favours the performance of epicardial muscle fibres. The net result is an increased LV wringing motion, where the shortening in the circumferential direction (mostly driven by epicardial fibres) overcompensates the diminished shortening in the longitudinal direction (mostly driven by endocardial fibres). The fact that relatively older transplant recipients (quartiles 1–3: 48–64 years) had relatively young donors (quartiles 1–3: 19–40 years) makes us hypothesize that endocardial stiffening in donor hearts may still be at early stages, which modifies the assumption that advancing age should associate with attenuated LSt in transplant recipients. This finding needs to be further evaluated, and elucidation of its clinical implications warrants specific investigations.

One of the key observations of this study was the correlation between LSt and NYHA functional class. When the multiple linear regression analysis was adjusted by LV ejection fraction in post-hoc analysis, NYHA functional class remained independently associated with LSt. Of note, myocardial mechanics examined by 2D technology did not demonstrate an association with functional status; the absence of such an association might be explained by the technical limitation of 2D imaging to tracking the enhanced myocardial translational motion in transplanted hearts. These observations suggest that the range of LSt values derived from 3D-STE provides useful information beyond conventional echocardiographic parameters (i.e. end-diastolic and end-systolic volumes), which could be of particular interest for those patients with preserved ejection fraction and heart-failure symptoms. In this regard, global LSt has been suggested as a superior predictor of outcome to ejection fraction in the non-transplanted population. Future follow-up studies examining functional status and cardiovascular events in transplant recipients and its possible relationship with myocardial mechanics, not only during systole but also at diastole, are needed to further evaluate these findings.

**Limitations**

The differences observed between 3D- and 2D-STE were not compared with an independent standard for myocardial mechanics, which precludes us from validating technique accuracy. Discordant results between different ultrasound vendor platforms have been reported, which should be taken into consideration when interpreting the strain values in the present work. Among the patients in our study, the majority had preserved or mildly reduced LV ejection fraction and no subjects had acute cellular rejection of greater than mild degree; thus, future studies that include a broader range of transplant recipients would be helpful to further understand the relationship between 3D-STE parameters, rejection status, and LV systolic function in these patients. Finally, our study was not designed to investigate the mechanisms that could participate in the observed correlates of 3D-STE-derived global LSt, and further extensive studies are warranted to elucidate those aspects.

**Conclusions**

Left-ventricular mechanics as assessed by 3D- and 2D-STE-derived deformation parameters, namely LSt and CSt, yield significantly discordant results between both techniques in cardiac transplant recipients. In addition, 3D-STE confirms significantly altered global and regional mechanics in transplant recipients compared with matched controls. The independent association seen between NYHA functional class and global LV function as assessed by 3D-STE-derived LSt suggests a clinically relevant relationship between functional status and myocardial mechanics in heart transplant recipients.
Funding
The Cardiovascular Imaging and Hemodynamic Laboratory at Tufts Medical Center has received an equipment grant from Toshiba America Medical Systems (Tustin, CA, USA). J.A.U.-M. has received a research grant from Fundación Alfonso Martín Escudero (Madrid, Spain).

Conflict of interest: The laboratory where this work was conducted received an equipment grant from Toshiba America Medical Systems (Tustin, CA, USA).

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