The potential clinical role of ultrasonic strain and strain rate imaging in diagnosing acute rejection after heart transplantation

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KEYWORDS
Rejection; Heart transplantation; Strain and strain rate imaging

Abstract
Background: There has been a continued search for a more sensitive noninvasive technique for detecting sub-clinical acute rejection in heart transplant recipients. Ultrasonic deformation imaging (strain/strain rate) is sensitive in detecting sub-clinical abnormalities in regional systolic function and could potentially be sufficiently sensitive to detect changes in deformation induced by graft rejection.

Aim: To assess the use of strain (S) and strain rate (SR) imaging as a noninvasive method for monitoring and diagnosing acute rejection in heart transplant recipients.

Methods and results: A prospective preliminary study was carried out involving 31 consecutive heart transplant patients who underwent a total of 106 routine follow up endomyocardial biopsy with correlative cardiac ultrasound data. To assess regional longitudinal deformation, ultrasonic S and SR data were acquired from the intraventricular septum, left ventricular (LV) lateral and right ventricular free walls (RVFW). For radial deformation, data were obtained from the LV posterior wall (LVPW).

According to the International Society of Heart and Lung Transplantation criteria, 88 biopsies (Group 1) had grade 0 or IA rejection, and 18 biopsies (Group 2) had...
Introduction

Detecting acute sub-clinical allograft rejection is imperative after heart transplantation. Right ventricular endomyocardial biopsy currently remains the gold standard for diagnosing rejection but carries all the potential risk and drawbacks of an invasive procedure.

In the search for an alternative sensitive noninvasive method with which to diagnose sub-clinical rejection, techniques such as cardiac ultrasound, gamma scintigraphy, phosphorus-31 nuclear magnetic resonance spectroscopy, serological and immunological testing have all been tried but have had limited success.

The first attempts to diagnose acute rejection by cardiac ultrasound were based on attempts to detect abnormalities in filling of the heart. However, the process of rejection with cellular infiltrate and oedema is equally (or more likely) to cause abnormalities in the contractile properties of the myocardium. Thus, a sensitive technique which can measure abnormalities in systolic function may be more appropriate. Regional myocardial peak systolic velocity imaging (MVI) has been proposed as a sensitive method of detecting rejection – however, this was sensitive in detecting only >IIIA grade rejection. This lack of sensitivity/specificity for mild rejection is likely to be explained by the fact that regional velocity data do not solely represent contractile function but are also altered by cardiac motion.

Regional strain (S) and strain rate (SR) imaging is a new cardiac ultrasound modality (based on MVI) which allows the detection of abnormalities in regional contractile function. It is an accurate method for the noninvasive quantification of systolic deformation, and has been shown to detect changes in regional systolic function at an earlier sub-clinical stage than either conventional echocardiography or myocardial velocity imaging.

Thus, the purpose of this preliminary study was to assess the potential role of strain and strain rate imaging in the detection of acute allograft rejection proven by endomyocardial biopsy.

Methods

Patients

The study population consisted of 31 consecutive patients (23 men and 8 women) who underwent heart transplantation and had 106 biopsies carried out in the Cardiology Department of the University Hospital Gasthuisberg as part of their routine postoperative management. Physical examination and standard echocardiography including MVI (for the calculation of S and SR) were performed within 2–3 h after a right heart catheterization (which included the endomyocardial biopsy).

Endomyocardial biopsy (EMB)

All endomyocardial biopsies were performed according to the standard procedure used in post-transplant patients in our institution, i.e. a jugular approach, a Scholten bioprome and a minimum of three samples per biopsy session.

As per institution policy, acute rejection is monitored by serial myocardial biopsies performed weekly during the first 5 weeks post-transplantation then every 2 weeks in the second and third months, and then every 3 weeks till the 17th week, every 4 weeks till 29th week and next two times every 5 weeks and every 6 weeks till the end of the first year. After this, a biopsy is either taken 1 year after heart transplantation or when patients develop abnormal clinical findings. All the biopsies were read by an experienced pathologist who was blinded to the cardiac ultrasound findings.

Cellular rejection was graded using the International Society of Heart and Lung Transplantation (ISHLT) criteria: grade 0, no rejection; grade IA, focal (perivascular or interstitial) infiltrate without myocyte damage; grade IB, diffuse but sparse
infiltrate without myocyte damage; grade II (not used anymore in current clinical practice), one focuses on aggressive infiltrates and/or myocyte damage; grade IIIA, multifocal aggressive infiltrates and/or myocyte damage; grade IIIB, diffuse inflammatory process with myocyte necrosis; and grade IV, diffuse aggressive polymorphous infiltrate with haemorrhage and myocyte necrosis.

In standard clinical practice, rejection grades 0 and IA never received treatment. Grade IB is treated only when present on at least three consecutive biopsies and/or when accompanied by hemodynamic compromise. Grade IIIA always triggers anti-rejection treatment. On the basis of clinical relevance, patients for this study were divided into Group 1 with grade 0 or IA rejection and Group 2 with grade ≥IB rejection.

Right heart catheterization

Hemodynamic data were obtained at the time of the endomyocardial biopsy using a Swan–Ganz thermodilution catheter. The following parameters were measured or derived: right atrial pressure, pulmonary artery pressure, right ventricular systolic and diastolic pressure, pulmonary wedge pressure, cardiac output and pulmonary vascular resistance.

Standard echocardiography

Patients underwent a conventional transthoracic cardiac ultrasound examination together with the acquisition of myocardial velocity imaging data. Left ventricular M-mode measurements included septal and posterior wall thickness at end diastole, ring displacement of the lateral wall of left ventricle (LV), the interventricular septum and the right ventricular free wall (RVFW) all measured from an apical four-chamber view. Ejection fraction was measured using the Simpson method. Left ventricular filling was assessed by measuring inflow at the tips of the leaflets of the mitral valve using pulsed Doppler. The following parameters were measured: early diastolic peak flow velocity (E, cm/s), late diastolic velocity (A, cm/s), deceleration time of early filling (E-dec, ms), the isovolumetric relaxation time (IVRT, ms) and the duration of the late diastolic flow velocity (A-dur, ms).

Mitral and tricuspid annular velocities were measured by pulsed Doppler from an apical four-chamber view and were sampled in the lateral wall of the LV, the interventricular septum and the RVFW. A 2 mm sample volume was placed at the lateral and medial corner of the mitral annulus and in the lateral corner of the tricuspid annulus. Mitral and tricuspid myocardial velocity profiles were recorded for subsequent off-line analysis. The echocardiographic studies were performed using a Vivid 7 ultrasound scanner (GE Vingmed). The images were acquired from standard parasternal and apical views. Three heart cycles were stored in a cine-loop format and sent to an external workstation for post-processing.

Color Doppler myocardial imaging

Data acquisition

For each patient, a parasternal long axis and apical four-chamber view were acquired. For longitudinal deformation, real time two-dimensional MVI data were recorded from the septum, lateral LV wall and RVFW. For radial deformation, data were recorded from the LV posterior wall (LVPW).

A frame rate of 200–300 frames/s was used to acquire the data. An image sector angle of 15° and the optimal depth of imaging were used to increase temporal resolution. Special attention was paid to the color Doppler velocity range setting in order to avoid any aliasing within the image. Velocity aliasing was eliminated using appropriate PRF values.

Data analysis

All data were analyzed off-line. Myocardial velocity data were analyzed using a software package developed in our laboratory (Speqle 4, Software Package for Echocardiographic Quantification LEuven, Catholic University of Leuven, Belgium).9 For the evaluation of longitudinal function, the basal, mid and apical segments were analyzed for the LV lateral wall and septum and for the RVFW function the basal and apical segments were interrogated. For LV radial function, mid posterior wall data were analyzed.

Strain (S) defines the amount of local deformation expressed as a percentage and is derived by integrating the strain rate (SR) over time. SR measures the rate of segmental deformation and correspond to the local spatial velocity gradient, expressed in s⁻¹.7 By convention, SR values are positive when a myocardial segment thickens/lengthens and negative when a segment thins/shortens. Computation areas of 10 and 5 mm were used for the longitudinal and radial SR estimation, respectively. A semi-automatic M-mode based tracking algorithm was applied to maintain the sample volume within the region of interest throughout the cardiac cycle. To reduce the noise, SR profiles were averaged with a mask of 5 × 1
pixels over three consecutive cycles. The regional SR profiles were integrated over time to obtain the natural S profiles. Aortic valve opening and closure clips were introduced from the Pulsed wave blood pool Doppler tracings acquired with a similar R–R interval in order to determine the duration of ejection. Peak systolic strain and strain rate during the ejection period were assessed for each segment analyzed. For each patient nine segments were analyzed during one study.

Random samples of 15 additional SR studies were read by two readers experienced in MVI to evaluate interobserver agreement and each reader reread a random sample of echocardiogram to evaluate intrareader agreement. Our intraobserver and interobserver variability for SR imaging was previously published.10

Statistical analysis

Statistical analysis was performed using STATISTICA (data analysis software system) version 6.0. Values are reported as mean ± SD (standard deviation). The comparison between the groups was performed using an ANOVA (analysis of variance) test. Plots of receiver operating characteristic (ROC) curves determined sensitivity and specificity.

Statistical significance was inferred for \( p < 0.05 \).

Results

Clinical data

A total of 106 myocardial biopsies were obtained (range 1–11 per patient) during the study period. Eighty-eight biopsies (40 grade 0 and 48 grade 1A) from 21 heart patients were graded <1B (Group 1) and 18 biopsies (16 grade 1B and 2 grade 3A) from 10 patients were graded ≥1B (Group 2). The characteristics of the two patient groups are summarized in Table 1. All patients were in NYHA class I at the time of biopsy. There was no significant difference between the groups for donor heart age, gender, reason for transplantation, systolic and diastolic blood pressure or heart rate.

Catheterization data

The results of pulmonary vascular resistance, mean pulmonary artery pressure (PAP), pulmonary capillary wedge pressure and right atrial pressure (RAP) were higher in Group 2. Only PAP and RAP were statistically different (\( p < 0.05 \)) (Table 2).

Traditional echocardiographic indices

There were no significant differences in M-mode measurements or in transmitral Doppler flow velocity data (Table 3) between Groups 1 and 2.

Mitral and tricuspid displacement and velocity data

Early diastolic mitral velocities were not significantly different between the two groups. Tricuspid annular displacement and velocity tended to be lower in Group 2 (Tables 3 and 4). There was also no significant change in mitral ring (lateral and septal) displacement between Groups 1 and 2 (Table 3). However, in both groups, mitral–septal ring displacement was lower compared to normal values of patients without heart transplantation.11

Table 1 Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group 1, ISHLT &lt;1B</th>
<th>Group 2, ISHLT ≥1B</th>
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<tbody>
<tr>
<td>Number of patients</td>
<td>21</td>
<td>10</td>
</tr>
<tr>
<td>No. of EMB</td>
<td>88</td>
<td>18</td>
</tr>
<tr>
<td>Male</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>Donor age (years)</td>
<td>32 ± 11</td>
<td>31 ± 12</td>
</tr>
<tr>
<td>Reason for heart transplantation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAD</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>DCMP</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>VHD</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Others</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>131 ± 9</td>
<td>130 ± 9.6</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>84 ± 8</td>
<td>85 ± 8</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>81 ± 11</td>
<td>85 ± 11</td>
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</tbody>
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Values are \( n \) or mean ± SD.

EMB, endomyocardial biopsy; CAD, coronary artery disease; DCMP, dilated cardiomyopathy; VHD, valvular heart disease.

Table 2 Right heart catheterization

<table>
<thead>
<tr>
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<th>Group 1, ISHLT &lt;1B</th>
<th>Group 2, ISHLT ≥1B</th>
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<tbody>
<tr>
<td>PVR [(dyne x s)/cm(^5)]</td>
<td>121 ± 40</td>
<td>147 ± 36</td>
</tr>
<tr>
<td>PAP (mmHg)</td>
<td>18 ± 4</td>
<td>22 ± 4.8*</td>
</tr>
<tr>
<td>RAP (mmHg)</td>
<td>5.9 ± 2.7</td>
<td>8.5 ± 2.5*</td>
</tr>
<tr>
<td>PCWP (mmHg)</td>
<td>11 ± 3</td>
<td>13.2 ± 5.0</td>
</tr>
<tr>
<td>CO (l/min)</td>
<td>4.8 ± 0.9</td>
<td>4.9 ± 1.4</td>
</tr>
</tbody>
</table>

Values are mean ± SD, *\( p < 0.05 \).

PVR, pulmonary vascular resistance; PAP, mean pulmonary artery pressure; RAP, right atrial pressure; PCWP, pulmonary capillary wedge pressure; CO, cardiac output.
Potential clinical role of ultrasonic S and SR imaging in diagnosing acute rejection

Statistical differences were found between Group 1 (with rejection < ISHLT IB) and the normal values of patients without heart transplantation.\textsuperscript{10}

Interventricular septum

S and SR measurements were decreased in both groups in all the segments of the interventricular septum compared to normal values (patients without heart transplantation)\textsuperscript{10,12} but no changes were found between the transplant groups.

Left ventricular lateral wall

Peak systolic S values were decreased in the rejection group (ISHLT \geq IB). These were statistically significant for basal and mid wall segments (Fig. 1). Peak systolic SR values were decreased significantly in Group 2 in basal, mid and apical segments of the lateral LV wall (Fig. 2). There were no statistical changes found between Group 1 and normal values.\textsuperscript{10}

Radial deformation

A parasternal short axis was used to quantify regional radial systolic function of the left ventricle (106 segments were analyzed).

The maximal values of peak systolic S of the mid segment of LVPW were significantly lower in the group with a rejection score \geq IB compared to the non-rejection group < IB (\(p < 0.001\)). Similarly, a significant drop of SR values was noticed in Group 2 (\(p < 0.001\)) (Fig. 3). The scatterplots of the peak systolic S and SR values of the LVPW showing a significant decrease with grade \geq IB rejection are presented in Fig. 4.

According to ROC analysis, radial S for LVPW \leq 30% could predict rejection \geq IB with sensitivity of 85%, specificity of 90% and a negative predictive value of 93%. A cutoff value of SR < 3.0 s\textsuperscript{-1} had a sensitivity of 80%, a specificity of 86% and a predictive negative value for acute rejection 89%.

Discussion

Heart transplantation, where appropriate, is a standard therapy for end-stage of heart failure. However, detecting acute rejection of the transplanted organ is a major problem. This occurs most frequently during the first year after heart transplantation and should be diagnosed as soon as possible (preferably at a sub-clinical level). Currently, endomyocardial biopsy remains the gold standard in diagnosing acute rejection. However, this invasive method can, on occasion, give rise to serious complications. Because of this, considerable clinical research has been undertaken to find...
a reliable and sufficiently sensitive noninvasive way of detecting acute rejection.

The spectrum of histological changes in the myocardium which occur during acute rejection include myocardial oedema, lymphocyte infiltration, increased mass and myocardial necrosis. These may affect both myocardial contractile function and left ventricular filling due to both an increase in myocardial stiffness and abnormal relaxation.\textsuperscript{13} Thus, prior imaging approaches to identify acute rejection in the early phase have attempted to detect related abnormalities in either systolic or diastolic function.

A series of studies have shown standard gray scale echocardiography (either 2D or M-mode) to have a low sensitivity in detecting changes in either systolic or diastolic function due to acute rejection. This is probably because M-mode measurements of wall thickness and LV mass are often influenced by either technical or operator errors. Blood pool Doppler abnormalities in left ventricular filling have been reported to be the earliest manifestation of acute rejection detectable by cardiac ultrasound but changes in left ventricular filling patterns will only detect >IIIa rejection.\textsuperscript{14} However, the changes in transmitral Doppler flow indices are also rather non-specific in detecting rejection as they are markedly influenced by other variables such as heart rate, age and loading conditions.\textsuperscript{13} Furthermore, the heart of a transplanted

<table>
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<th>Table 5 Longitudinal and radial systolic function of the left and right ventricle estimated by systolic strain rate (SR) and strain (S)</th>
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<tr>
<td><strong>Apical four chamber</strong></td>
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<tr>
<td>Lateral LV wall</td>
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<td>Basal</td>
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<td>Mid</td>
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<td>Apical</td>
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<td>Septum</td>
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<td>Basal</td>
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<td>Mid</td>
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<td>Apical</td>
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<td>RV free wall</td>
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<tr>
<td>Basal</td>
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<tr>
<td>Apical</td>
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<td>SAX</td>
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<td>LVPW</td>
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Values are mean ± SD; *p < 0.05, **p < 0.001.

LAX, long axis; SAX, short axis; LVPW, left ventricular posterior wall; LV, left ventricle; RV, right ventricle; SR, strain rate; S, strain; ISHLT, International Society of Heart and Lung Transplantation.

Figure 1  Peak systolic strain values were significantly lower in Group 2 with rejection score >IB compared to Group 1 with rejection score <IB in RVFW and LV lateral wall.
patients is denervated. This usually results in a sinus tachycardia which can give rise to a pseudo-restrictive ventricular filling pattern similar to that which occurs during acute rejection.\textsuperscript{15} Our lack of any statistical difference between the groups in the standard cardiac ultrasound data sets measured in the current study (2D/gray scale blood pool Doppler data sets) would further confirm the relative shortcomings of the above methods.

Myocardial velocity imaging has recently been shown to be an adjunct to assess the filling of the transplanted heart.\textsuperscript{16} It is a robust technique for determining the velocity of regional myocardial motion in both the radial and longitudinal directions in non-transplant hearts but the values obtained post-transplantation are markedly influenced by the exaggerated overall motion of the transplanted heart. This is a major limitation when using velocities to describe regional function in such hearts. Despite this, Dandel et al.\textsuperscript{17} showed that early diastolic peak wall motion velocity and relaxation time measured by PW-TDI at the base of the left ventricular posterior wall appeared to have high sensitivity in diagnosing >II grade rejection. Stengel et al.\textsuperscript{18} also showed that severe rejection (ISHLT ≥III) could be excluded when there was a high late diastolic mitral annular velocity. On the other hand, Stengel et al. suggested that a reduced late diastolic mitral annular velocity was not predictive of rejection. In all these studies, acute rejection was diagnosed on the basis of abnormalities in left ventricular filling as there was no sufficiently sensitive tool to detect abnormal regional systolic function. As an alternative approach, global parameters of ventricular systolic function have been used to detect what are potentially a series of regional functional abnormalities. However, global parameters were also found to be insensitive.\textsuperscript{19} This may be explained by the fact that the regional changes induced by sub-clinical rejection may not be widespread enough to alter global function indices, as rejection can be non-uniform. This may explain the relative insensitivity in detecting <grade IIB rejection using global parameters.\textsuperscript{19}

Potentially, S/SR imaging indices are independent of overall cardiac motion\textsuperscript{7} and thus could be sensitive in detecting regional functional abnormalities induced by acute rejection.

A series of recent clinical studies have shown that in cardiac amyloidosis, Fabry’s disease, Friedrich’s
ataxia and diabetes, systolic myocardial contractile function is decreased and sub-clinical changes were earlier and better detected by S/SR imaging than by conventional echocardiographic indices. Indeed, in both amyloid and Friedrich’s abnormalities systolic function could be identified at a sub-clinical level. These studies have shown the sensitivity and accuracy of this new ultrasound modality in the early diagnosis of systolic dysfunction in these diseases. We postulated that the myocardial oedema which appears during acute rejection would result in decreased systolic deformation as such interstitial fluid would be incompressible. Thus SR imaging might be sufficiently sensitive to detect sub-clinical changes in regional systolic function. Our preliminary study showed that in a transplanted heart, a significant reduction in peak systolic S and SR in both radial and longitudinal deformation could be detected in early rejection grade.

However, we could detect regional abnormalities in deformation caused by rejection only by analyzing LV free wall data as deformation in the septum is consistently reduced in all patients who have undergone cardiopulmonary by-pass. Thus septal and RV free wall data sets should not be used to attempt to identify regional changes induced by acute rejection.

According to our data obtained from patients with grade IIIA rejection, decreased values of the S and SR were found to be non-uniform throughout the LV free walls. This would suggest that rejection can be a non-uniform process. Further investigation is needed to confirm these findings.

On the basis of this preliminary study, SR imaging could be potentially a good technique in detecting the changes in systolic deformation in LV free wall segments of the myocardium induced by acute sub-clinical rejection. However, further studies with increased number of patients are required to confirm these changes in this group of patients.

Limitations

A potential risk of mis-diagnosing acute rejection could be due to the coexistence of coronary artery disease in patients post-heart transplantation. This can also result in a decrease in regional myocardial deformation in the affected segments. However, ischaemic segments will have a different pattern of deformation either at rest or during dobutamine challenge with reduced end-systolic strain and ischaemic post-systolic thickening indicating the presence of coronary artery disease. This is a totally different pattern of deformation than that found in acute rejection.

The image artifacts, such as reverberations, can degrade the calculation of the regional deformation. Signal noise could be a potential problem in this group of patients as this is amplified during the strain rate calculation. To minimize this problem, all data were acquired at narrow sector angle with the wall placed in the center of the image. Finally, 954 segments were obtained and analyzed and none was excluded from the study on the basis of the curves.

Conclusions

S/SR imaging might be a good technique and additional tool for detecting ≥IB grade of acute rejection. The myocardial deformation, as assessed by S/SR imaging could be of clinical value in monitoring and diagnosing acute rejection in heart transplant recipients and could improve patients’ management by reducing the number of biopsies performed.
References


