Strain rate imaging would predict sub-clinical acute rejection in heart transplant recipients

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Abstract

Objective: Non-invasive diagnosis of rejection is a major objective in the management of heart transplant recipients. The ability of strain rate (SR) imaging on echocardiograms to detect rejection in heart transplant recipients was investigated. Methods: A total of 396 endomyocardial biopsies, right-heart catheterisation and echocardiograms were performed in 35 heart transplant recipients. Mean values of systolic strain (e sys), peak systolic SR (SR sys), and peak early diastolic SR (SR dia) obtained from eight left ventricular segments were calculated. Results: According to the conventional International Society for Heart and Lung Transplantation criteria, 351 biopsies showed a rejection grade of 0 or 1a (group AR) whereas 45 biopsies showed a grade of 1b or higher (group AR+). The e sys, SR sys and SR dia were significantly different between group AR+ and group AR (e sys: 7.4±3.0 vs 9.4±4.4 mm/7, p<0.001, 2.5±1.8 vs 3.6±1.1/s, p<0.0001, and −1.9±1.6 vs −3.5±1.3/s, p<0.001, respectively). Multivariate analysis identified e sys (p<0.0001) as a strong predictor for group AR+, and e sys cut-off value of −27.4% was associated with a predictive accuracy of 82.3%. The combination of e sys and SR dia discriminated group AR+ from group AR- with a predictive accuracy of 84.8%. The pulmonary artery wedge pressure was higher in group AR+ than that in group AR (8.0±6.0 vs 6.3±2.6 mmHg, p<0.05). Conclusion: SR imaging is of potential clinical value for monitoring acute rejection in heart transplant recipients.

Keywords: Heart transplant; Rejection; Echocardiography; Diagnosis

1. Introduction

Non-invasive diagnosis of rejection is a major objective in the management of heart transplant recipients. Endomyocardial biopsy remains the gold standard of rejection surveillance in cardiac transplantation. However, this procedure is invasive, expensive and subject to sampling error and inter-observer variability [1], and it results in morbidity in 0.5—1.5% of heart transplant patients. Many non-invasive techniques have been investigated for their potential to diagnose rejection, including echocardiography, ultrasonic myocardial backscatter, radionuclide imaging, magnetic resonance imaging, intramyocardial electrogram recording and multiparametric immune monitoring [2—9]. However, none of these non-invasive imaging approaches was found sufficiently reliable to replace endomyocardial biopsy [2—8,10]. Gene expression profiling used for this purpose in the multicentre Cardiac Allograft Rejection Gene Expression Observation (CARGO) study [9] has several limitations including cost and general applicability, particularly for institutions that handle only a small number of heart transplant recipients, as well as an inability to detect mild rejection.

Strain rate (SR) imaging based on tissue Doppler imaging (TDI) allows the quantitative assessment of regional myocardial wall motion [11] reflecting both systolic and diastolic left ventricular (LV) functions [12,13]. SR imaging might be expected to have the potential to detect even mild rejection not associated with haemodynamic changes in heart transplant patients. We have therefore assessed the utility of SR imaging as a non-invasive technique for the diagnosis of sub-clinical acute cardiac rejection in the follow-up of heart transplant recipients.
2. Materials and methods

2.1. Patients and study design

Thirty-five adult heart transplant recipients (27 men, eight women, transplanted at 33.6 ± 13.1 years of age, transplanted between July 1993 and December 2006) underwent scheduled cardiac catheterisation and endomyocardial biopsy at 1 week, 2 weeks, 7 weeks, 3 months, 4.5 months, 6 months, 9 months, 1 year, 1.5 years and then every 6 months or 1 year after transplantation surgery, or when rejection was suspected on the basis of clinical symptoms. Conventional echocardiography and TDI were performed within 12 h after right-heart catheterisation. A total of 396 consecutive endomyocardial biopsy procedures accompanied by right-heart catheterisation and echocardiography were evaluated. A subset of 127 biopsies was also accompanied by left heart catheterisation to obtain LV end-diastolic pressure as well as by coronary angiography with intracoronary ultrasound.

The diagnosis of cellular acute rejection was based on the conventional International Society for Heart and Lung Transplantation (ISHLT) criteria [14]. Given that none of the study subjects showed antibody-mediated rejection alone (without associated cellular rejection), only the grade of cellular rejection was used for classification of acute rejection in this study. Biopsy specimens showing an ISHLT grade 0 or 1a were classified as the acute rejection—negative (AR−) group, and those of grade 1b or higher were classified as the AR+ group.

Endomyocardial biopsy and haemodynamic testing are accepted and financially covered by the National Health Insurance System of Japan for the diagnosis of rejection and for obtaining information on the clinical condition of heart transplant recipients. The present study, including the biopsy procedure, was approved by the Institutional Review Board and Institutional Ethical Committee for Human Research of the National Cardiovascular Center, and was executed in accordance with the Declaration of Helsinki. All study subjects provided written informed consent with regard to the study procedures.

2.2. Echocardiography

Echocardiographic images were obtained in the parasternal long- and short-axis views and apical two- and fourchamber views with standard transducer positions and with the use of a Vivid Seven digital ultrasound system (GE VingMed Ultrasound, Horten, Norway). The LV ejection fraction was calculated by the Teichholz method. Continuous-wave Doppler echocardiography was performed, and peak early (E) and late (A) transmitral filling velocities and their ratio (E/A), deceleration time of E, and isovolumic relaxation time were measured from mitral inflow velocities.

On completion of the standard echocardiographic measurements, colour TDI was performed. Digital data were transferred for off-line analysis with the software incorporated into the Vivid Seven system. Scanning was performed longitudinally from the apex to acquire apical four- and two-chamber views with a 5.0-MHz phased-array transducer and a frame rate of 100 ± 20 frames s−1, depending on the heart rate, to minimise the noise level. Early diastolic annular velocity (Ea) was obtained by placing a tissue Doppler sample volume at the septal mitral annulus in the apical four-chamber view, and the E/Ea mitral flow ratio was calculated. Longitudinal strain and SR in the basal and apical segments of each (i.e., anterior, inferior, septal and lateral) wall were estimated by measuring the spatial velocity gradient over a computation area of 8 mm × 10 mm. The region of interest was continuously positioned within the interrogated segment with the use of a semiautomatic tracking algorithm and was analysed as described previously [15]. Systolic strain (E)t, peak systolic SR (Sr) and peak early diastolic SR (Srdia) were calculated from the averaged SR profiles.

Two examiners, who were unaware of the clinical status of the subject, performed echocardiographic analyses independently of each other. The reproducibility of each measure and SR determinations were assessed in nine subjects randomly allocated from the comparative study groups. Intra-observer reproducibility was assessed by a single observer (S.H.) on two separate occasions, whereas inter-observer reproducibility was assessed by two independent observers (S.H. and T.S.K.).

2.3. Statistical analysis

Data are presented as means ± standard deviation (SD). Normality was evaluated for each variable on the basis of normal distribution plots and histograms and by the Kolmogorov–Smirnov test. Clinical characteristics, echocardiographic indices and haemodynamic variables were compared between groups by Student’s unpaired two-tailed t-test or chi-square analysis. A p-value of <0.05 was considered statistically significant. Individual regression analysis was used to select potential independent predictors from echocardiographic indices for discriminating group AR+ from group AR−. Covariables examined included LV end-diastolic internal dimension, LV ejection fraction, E, the E/A ratio, isovolumic relaxation time, deceleration time of E, Emsys, Srsys, Srdia, Ems and the E/Ems ratio. Individual predictors of group AR+ selected on the basis of a p value of <0.05 were entered into a multivariate discriminant analysis. The discriminant score and discriminant probability were calculated using a discriminant function test. The optimal cut-off values of individual parameters for differentiation between group AR+ and group AR− were determined with a receiver-operating characteristic curve. The sensitivity, specificity and predictive accuracy were determined and expressed as percentages. The relations between haemodynamic variables and indices identified to discriminate independently group AR+ from group AR− were analysed with Pearson’s correlation coefficient. Inter- and intra-observer reproducibility were evaluated by means of the interclass correlation coefficient (ICC). All statistical analyses were performed with SPSS version 15 software (SPSS, Chicago, IL, USA).

3. Results

Among the 396 endomyocardial biopsy procedures performed throughout this study (ranging from 1 to 20 biopsies per patient), 210 biopsies showed conventional ISHLT grade 0
and 140 biopsies showed grade 1a, six biopsy specimens showed grade 1b, 16 biopsies showed grade 2 and 24 biopsies showed grade 3a. Twenty-seven patients experienced rejection between 1 and 6 times and were categorised as group AR+(grade 1b or higher rejection), and among those, 10 patients experienced grade 3a rejection. Thus, a total of 350 examinations were classified as group AR− and 45 examinations were classified as group AR+.

The clinical characteristics of patients classified as group AR− and group AR+ are summarised in Table 1. The reason for heart transplantation was non-ischaemic cardiomyopathy for all patients. All patients were in the NYHA (New York Heart Association) class 1 at the time of examination. The age at the time of transplantation and examination were not significantly different between the groups. Sex distribution and distribution of immunosuppressive regimen were not significantly different between the groups. The modified bicaval anastomosis technique [16] was applied to 56% and 61% of the subjects classified as group AR− and group AR+, respectively. None of the patients showed angiographically apparent coronary stenosis. The proportion of those showing an intimal thickness of Stanford Class IV on intracoronary ultrasound.

### 3.2. Discrimination of group AR+ from group AR−

Individual analysis revealed that the $e_{sys}$, $SR_{sys}$ and $SR_{dia}$ were significantly associated with sub-clinical rejection as defined by ISHLT grade 1b or higher (group AR+). These parameters were therefore used as dependent variables for multivariate analysis of discrimination between group AR− and group AR+. Only the $e_{sys}$ and $SR_{dia}$ were found to be independent predictors for discrimination of group AR+ from group AR (Table 3).

Receiver operator characteristic curve analysis identified the optimal cut-off value of $e_{sys}$ for discrimination between group AR− and group AR+ as $-27.4\%$; this value was associated with sensitivity, specificity and predictive accuracy of $82.2\%, 82.3\%$ and $82.3\%$, respectively. Similarly, a $SR_{dia}$ of $-2.8\,s^{-1}$ was associated with a sensitivity of $75.6\%$, specificity of $74.9\%$ and predictive accuracy of $75.0\%$. Relative cumulative frequency distribution plots of $e_{sys}$ and $SR_{dia}$ are shown in Fig. 2.

### 3.1. Echocardiographic parameters

Neither conventional echocardiographic indexes nor $E/E_{m}$ or the $E/E_{m}$ mitral flow ratio differed significantly between group AR− and group AR+ (Table 2). In contrast, $e_{sys}$, $SR_{sys}$ and $SR_{dia}$ differed significantly between the two groups (Table 2). Representative cross sections of myocardial biopsy specimens from patients showing no rejection (ISHLT grade 0) and ISHLT grade 3a rejection with SR imaging analysis are shown in Fig. 1.

Intra-observer reproducibility for strain analysis was excellent, with the ICC being 0.92, 0.94 and 0.94 for $e_{sys}$, $SR_{dia}$ and $SR_{sys}$, respectively. Similar results were obtained for inter-observer reproducibility, with ICC values of 0.90, 0.93 and 0.92 for $e_{sys}$, $SR_{dia}$ and $SR_{sys}$, respectively.

### Table 1

**Clinical characteristics.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group AR− (n = 350)</th>
<th>Group AR+ (n = 45)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at transplant (years)</td>
<td>35.2 ± 2.7</td>
<td>34.1 ± 12.6</td>
<td>0.16</td>
</tr>
<tr>
<td>Age at examination (years)</td>
<td>37.1 ± 13.1</td>
<td>35.7 ± 12.6</td>
<td>0.50</td>
</tr>
<tr>
<td>Post-transplant duration (years)</td>
<td>1.8 ± 2.0</td>
<td>1.6 ± 13.1</td>
<td>0.79</td>
</tr>
<tr>
<td>Male (%)</td>
<td>76.3%</td>
<td>75.6%</td>
<td>0.94</td>
</tr>
<tr>
<td>Surgical technique</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modified bicaval anastomosis</td>
<td>56.5%</td>
<td>61.2%</td>
<td>0.38</td>
</tr>
<tr>
<td>Imunosuppression</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSA-based regimen</td>
<td>54.1%</td>
<td>44.4%</td>
<td>0.29</td>
</tr>
<tr>
<td>Tac-based regimen</td>
<td>45.9%</td>
<td>55.5%</td>
<td>0.29</td>
</tr>
<tr>
<td>MMF administration</td>
<td>100%</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Steroid withdrawal</td>
<td>41.1%</td>
<td>44.4%</td>
<td>0.79</td>
</tr>
<tr>
<td>Transplant coronary artery disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stanford class-IV IMT</td>
<td>39.1% (n = 115)</td>
<td>41.7% (n = 12)</td>
<td>0.89</td>
</tr>
</tbody>
</table>

### Table 2

**Echocardiographic parameters.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group AR− (n = 350)</th>
<th>Group AR+ (n = 45)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVDD (mm)</td>
<td>41.8 ± 4.6</td>
<td>43.2 ± 4.7</td>
<td>0.056</td>
</tr>
<tr>
<td>LVDs (mm)</td>
<td>26.2 ± 3.9</td>
<td>26.8 ± 4.1</td>
<td>0.33</td>
</tr>
<tr>
<td>IVST (mm)</td>
<td>9.4 ± 1.3</td>
<td>9.4 ± 1.5</td>
<td>1.0</td>
</tr>
<tr>
<td>PWT (mm)</td>
<td>9.5 ± 1.3</td>
<td>9.3 ± 1.5</td>
<td>0.34</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>61.9 ± 7.2</td>
<td>62.2 ± 8.5</td>
<td>0.80</td>
</tr>
<tr>
<td>TMF E (cm/s)</td>
<td>78.5 ± 19.2</td>
<td>80.9 ± 21.7</td>
<td>0.44</td>
</tr>
<tr>
<td>TMF E/a</td>
<td>2.4 ± 4.8</td>
<td>2.1 ± 0.7</td>
<td>0.68</td>
</tr>
<tr>
<td>TMF DcT (ms)</td>
<td>159.3 ± 30.4</td>
<td>163.0 ± 37.3</td>
<td>0.46</td>
</tr>
<tr>
<td>IRT (ms)</td>
<td>63.7 ± 17.5</td>
<td>61.8 ± 14.5</td>
<td>0.49</td>
</tr>
<tr>
<td>$E_{m}$ (cm/s)</td>
<td>10.1 ± 3.7</td>
<td>11.2 ± 3.4</td>
<td>0.058</td>
</tr>
<tr>
<td>$E/E_{m}$</td>
<td>7.4 ± 3.4</td>
<td>8.0 ± 6.2</td>
<td>0.32</td>
</tr>
<tr>
<td>$e_{sys}$ (%)</td>
<td>-32.6 ± 6.3</td>
<td>-20.7 ± 8.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$SR_{sys}$ (/s)</td>
<td>3.6 ± 1.1</td>
<td>2.5 ± 1.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$SR_{dia}$ (/s)</td>
<td>-3.5 ± 1.3</td>
<td>-1.9 ± 1.6</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are means ± SD. Abbreviations not defined in text: LVDD, LV end-diastolic internal dimension; LVDs, LV end-systolic internal dimension; IVST, thickness of the interventricular septum; PWT, thickness of the LV posterior wall; LVEF, LV ejection fraction; FS, fraction shortening; TMF, transmitial flow; DcT, deceleration time of $E$; IRT, isovolumic relaxation time.

Fig. 1. Representative pathological findings for endomyocardial biopsy specimens (a and b) and SR imaging recordings with strain analysis (c and d) of heart transplant recipients with ISHLT grade 0 (a and c) or 3a (b and d) rejection. Sections in (a) and (b) were stained with hematoxylin-eosin; scale bars, 100 μm.
respectively.

Data are means ± SD. Abbreviations not defined in text: CI, cardiac index; PAWP, pulmonary arterial wedge pressure; PA, pulmonary artery pressure; RA, right atrial pressure; LVEDP, LV end-diastolic pressure. The LVEDP was obtained in a subset of 127 examinations.

Table 3
Multivariate discriminant analysis for differentiation of group AR⁺ and group AR⁻.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Discriminant coefficient</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>εsys</td>
<td>1.31</td>
<td>1.24—1.40</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SRsys</td>
<td>0.99</td>
<td>0.70—1.41</td>
<td>0.98</td>
</tr>
<tr>
<td>SRdia</td>
<td>1.62</td>
<td>1.19—2.20</td>
<td>0.0022</td>
</tr>
</tbody>
</table>

CI, confidence interval.

A discriminant function test revealed that a discriminant score (Z) defined using the following equation yielded the highest discriminant probability of 84.8%: Z = 8.58687 + (0.276782εsys) + (0.483395SRdia)

where Z > 0 indicates a diagnosis of ISHLT grade 1b or higher (group AR⁺) rejection and Z < 0 indicates a diagnosis of ISHLT grade 1a rejection or no rejection (group AR⁻).

3.3. Haemodynamic variables

Haemodynamic variables obtained by right-heart catheterisation were not significantly different between the groups, except for pulmonary arterial wedge pressure (PAWP). The PAWP was significantly higher in group AR⁺ than in group AR⁻. LV end-diastolic pressure was obtained in a subset of 127 examinations, which was not significantly different between the groups (Table 4). The εsys, SRsys, and SRdia were only weakly correlated with PAWP (r = 0.13, p = 0.0069, r = –0.16, p = 0.0016, r = 0.017, p = 0.0006, respectively).

Table 4
Haemodynamic parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group AR⁻ (n = 350)</th>
<th>Group AR⁺ (n = 45)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI (l min⁻¹ m⁻²)</td>
<td>3.6 ± 0.8</td>
<td>3.7 ± 0.7</td>
<td>0.42</td>
</tr>
<tr>
<td>PAWP (mmHg)</td>
<td>7.4 ± 3.0</td>
<td>9.4 ± 4.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean PA (mmHg)</td>
<td>13.8 ± 3.7</td>
<td>14.5 ± 3.4</td>
<td>0.22</td>
</tr>
<tr>
<td>Mean RA (mmHg)</td>
<td>3.3 ± 2.4</td>
<td>3.6 ± 2.9</td>
<td>0.44</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>8.3 ± 3.5 (n = 77)</td>
<td>9.7 ± 4.8 (n = 9)</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Data are means ± SD. Abbreviations not defined in text: CI, cardiac index; PAWP, pulmonary arterial wedge pressure; PA, pulmonary artery pressure; RA, right atrial pressure; LVEDP, LV end-diastolic pressure. The LVEDP was obtained in a subset of 77 examinations of grade 0 group, and 9 examinations of grade 3a groups, respectively.

3.4. Comparison of strain rate and haemodynamic parameters between those without rejection (ISHLT grade 0) and those with grade 3a

As a sub-analysis, the comparison of parameters derived from SR imaging and haemodynamic variables obtained from patients whose biopsies showed no evidence of rejection (ISHLT grade 0) and those that showed grade 3a, to verify the ability of SR imaging to detect treatment required rejection. The results were shown in Table 5. The εsys, SRsys, and SRdia were significantly different between the groups; however, haemodynamic variables except for cardiac index and PAWP were not significantly different. The cardiac index was rather higher in those with grade 3a rejection than that with grade 0.

4. Discussion

We have demonstrated the ability of SR imaging derived from TDI to discriminate ISHLT grade 1b or higher rejection from ISHLT grade 1a or no rejection in heart transplant recipients. A εsys cut-off value of –27.4% was associated with a sensitivity of 82.2%, specificity of 82.3% and predictive
Monitoring Attenuation through Gene Expression

However, the diagnostic utility of Doppler echocardiographic haemodynamic compromise and all-cause mortality [19]. Thus, early detection, preferably at a sub-clinical level, and appropriate treatment for rejection is critical. We describe conventional ISHLT grade 1b or higher rejection as ‘sub-clinical’ acute rejection in the article. The identification of sub-clinical rejection and treatment for this in a clinical setting would be a distant and of unknown significance, and the separation for 1a to 1b would not have clinical relevance. However, we may reconsider or optimise an immunosuppressive regimen for recipients with recurrent 1b rejection, especially in their early phase after transplant; thus, a non-invasive investigation of grade 1b or higher rejection might be of clinical value. We believe that the close watching for signs of rejection, even if it would not be accompanied by haemodynamic changes, in other words, if it is ‘sub-clinical’, would be meaningful. This methodology described in the article can be easily repeated, is non-invasive and less costly compared with biopsy; therefore, we could in advance think about optimising immunosuppressive therapy or checking the patients’ compliance when this non-invasive method brings a suspicion of acute rejection.

Endomyocardial biopsy remains as the gold standard in rejection surveillance, although this procedure is invasive, expensive, subject to sampling error and inter-observer variability [1], and in some occasions, causes serious complications. Thus, considerable clinical research has been carried out to find a sensitive non-invasive technique to diagnose acute rejection; however, none of these non-invasive imaging approaches was found sufficiently reliable to replace endomyocardial biopsy [2–8,10]. The Invasive Monitoring Attenuation through Gene Expression (IMAGE) trial followed by the CARGO study [9] was designed to test the hypothesis that the gene expression analysis was not inferior to an endomyocardial biopsy-based strategy for detecting cardiac allograft dysfunction, rejection with haemodynamic compromise and all-cause mortality [19]. However, the IMAGE trial has not yet focussed on the sub-clinical stage of acute rejection, which we investigated in this study.

Acute rejection of heart transplants is characterised histologically by infiltration of inflammatory cells and interstitial oedema, which ultimately results in structural and functional abnormalities of the allograft. These changes during acute rejection affect both myocardial contractile function and left ventricular filling due to an increase in myocardial stiffness and abnormal relaxation [20]. Several studies have demonstrated a significant difference in Doppler filling parameters between rejected hearts and those without rejection in heart transplant recipients [6,21]. However, the diagnostic utility of Doppler echocardiographic parameters has been limited by low sensitivity as well as by inter- and intra-patient variability [6,20,21].

SR imaging based on tissue TDI allows the quantitative assessment of regional myocardial wall motion [11] reflecting both systolic and diastolic LV function [12,13], relatively independent of overall cardiac motion [15]. Therefore, SR imaging would have a potential of detecting regional functional abnormality induced by acute rejection. With the use of multivariate analysis, we have now shown that $i_{sys}$ and $SR_{dia}$ are powerful predictors for ISHLT grade 1b or higher rejection, with $i_{sys}$ being the most specific predictor of this condition. The ability of SR imaging indices to discriminate ISHLT grade 1b or higher rejection from ISHLT grade 1a or no rejection may be likely attributable to its relations to LV stiffness and/or filling abnormality, which leads to a weak correlation between SR imaging parameters and PAWP. The $i_{sys}$ is a systolic parameter; however, elastic recoil, which is determined by LV end-systolic volume, is an important determinant of relaxation rate. Therefore, an $i_{sys}$ would be expected to reflect LV diastolic performance and LV stiffness, which explains why $i_{sys}$ showed a weak correlation with PAWP.

We used the mean values of SR imaging indices obtained from eight LV segments for our analysis, which potentially reflect the abnormalities of rejected hearts induced by the heterogeneity of tissue texture of the myocardium due to eccentrically located infiltration of inflammatory cells and interstitial oedema during acute rejection. Conversely, our results may indicate that SR imaging may be used to detect heterogeneity of myocardial properties much earlier than visual assessment. Although TDI had been initially reported to be relatively insensitive to changes in preload [22], SR imaging is considered to be load dependency, as are myocardial Doppler velocities [11]. Filling pressures would thus be expected to affect SR imaging indexes [23]. This load dependency should be taken into account when deformation parameters are used to identify potential changes in contractility and lusitropic properties. In calculating the mean values of SR imaging indices in this study, we hypothesised that the heterogeneity of rejected hearts would outweigh the effect of load dependency.

In the present study, we dichotomise the result of endomyocardial biopsy-proven rejection of conventional ISHLT grade 0 or 1a and grade 1b or higher grades of rejections. The reason we employed the conventional ISHLT grading system instead of the revised ISHLT grading system of 2005 [24] was that we still use a combination of conventional and revised versions of ISHLT rejection grading system for rejection diagnosis in our institution. The separation for conventional ISHLT grade 1a to grade 1b may not have clinical relevance. However, we might reconsider or optimise immunosuppressive regimens for transplant recipients who develop recurrent 1b rejections, particularly in their early phase after heart transplantation in a clinical setting. Therefore, we consider that a non-invasive investigation of detecting grade 1b or higher rejection might thus be of clinical value in considering further treatment or deciding the timing of endomyocardial biopsy, although further prospective studies are required to confirm that this methodology could optimise the timing of routine cardiac biopsy in heart transplant recipients. We speculate that the reason we failed to demonstrate the correlation between the strain rate parameters and haemodynamic parameters was the small number of acute rejections with any changes in...
haemodynamics. To put it the other way round, this study revealed that strain rate parameters such as $c_{sys}$ could have a potential of detecting sub-clinical acute rejection that is not accompanied by haemodynamic changes. Indeed, the biopsy-proven grade 3a rejection, which may require adequate treatment, were not associated with haemodynamic compromise, but associated with a reduction of absolute values of SR parameters in the present study (Table 5).

The major limitation of this study is a potential risk of misdiagnosing acute rejection because of the co-existence of transplant coronary artery disease. This can also result in an abnormal myocardial deformation in SR imaging in the affected segments. Another limitation of this study is we do not consider a possibility of co-existing antibody-mediated rejection. In our institution, we had performed immunofluorescence and/or immunoperoxidase such as C4d staining, only when patients were suspected to have antibody-mediated rejection or high Panel Reactive antibody. In addition, although we stated that endomyocardial biopsies would have drawbacks such as sampling errors and inter-observer variability, we adopted histological findings of cellular rejection standard as a benchmark because it still is a gold standard. We admit that such potential errors of biopsy findings of rejection may influence our results in the present study.

In addition, this study contains a statistical limitation. In the present study, we employed total number of examination instead of the number of the actual patients, leaving time-series processing out of consideration. We understand that mixed models (generalised linear model) or generalised estimating equation is the most appropriate methods to analyse our data. However, this study was not preliminarily designed for time-series processing; therefore, we could calculate neither the correlation coefficient of repeated measurements nor the impact of defective data on the results. Instead of mixed models, we performed sub-analysis using receiver operator characteristic curve analysis and discriminant analysis based on the data obtained from 27 patients who underwent examinations at three time points of the same interval (0, 1 and 2 years post-transplantation) extracted from total examinations, and the result of sub-analysis was similar to the results derived from the total number of examinations; therefore, we presume that none of examination obtained from a specific patient biased the analysis.

The result of our study is not sufficient enough to replace standard endomyocardial biopsy, because biopsy results could provide information of not only rejection diagnosis but also chronic rejection, infection or toxic causes. However, by means of using this non-invasive method, we could presume in advance whether a patient has a possibility of ongoing rejection, and might reduce the frequency of biopsy, especially for patients with inaccessible jugulars. Furthermore, we may avoid routine biopsy procedure for stable patients who have no history of rejection episodes for a long time after transplant. We could optimise medical therapy or pay more attention on the clinical state of patients who are suspected to have sub-clinical acute rejection on the basis of the results of this non-invasive method in advance, and then we could determine the necessity of biopsy procedure after the treatment. The appropriate use of this methodology as a supplement to invasive techniques, if not as a primary diagnostic technique, might play an important role in post-transplant patient care.

Further prospective studies are needed to confirm the superiority of this non-invasive method that could detect treatment-requiring rejection over endomyocardial biopsy-proven rejection.

5. Conclusions

SR imaging is relatively easy to perform and is not expensive or time-consuming because the data can be analysed off-line after routine echocardiographic analysis. SR imaging derived from TDI might thus be of clinical value in monitoring sub-clinical acute rejection in heart transplant recipients. Further prospective studies are warranted to determine an influence of transplant coronary artery disease and whether adoption of this non-invasive approach might allow a reduction in the frequency of endomyocardial biopsy in such patients.

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References


