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Echo rejection score: new echocardiographic approach to diagnosis of heart transplant rejection☆

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Abstract

Objective: The gold standard test in the diagnosis of heart transplant rejection is right ventricular (RV) myocardial biopsy, which is an invasive, time-consuming, expensive method. In an effort to find a reliable method to minimise the sequential use of myocardial biopsy, we assessed the main echocardiographic indices for the detection of allograft rejection. **Materials and methods:** Fifty myocardial specimens were examined in this prospective study, which assessed the prominent echocardiographic parameters propounded by previous studies as indicators of rejection. Prior to biopsy, all the patients underwent preoperative transthoracic echocardiography. The accuracy of the echocardiographic indices was compared with that of myocardial biopsy indices as the gold standard. At three myocardial segments, namely, RV base, interventricular septal (Sep) base and lateral left ventricular (Lat) base, peak systolic strain (RV-S, Lat-S and Sep-S) was measured. In addition, time to systole (TS) was measured at the same three segments, yielding the three variables of RV-TS, Sep-TS and Lat-TS. **Results:** Our logistic regression model revealed that the four factors of Lat-S (%), Sep-TS (ms), posterior wall thickness (PWT; mm) and left ventricular mass index (LVMI; g m^{-2}) could denote heart transplant rejection. We devised a new index, the echo rejection score, using the following formula: [(PWT + LVMI) − (Lat-S + Sep-TS)]. This new formula has an area under a curve of 0.932 and a cut-off point of 0; it yields a sensitivity of 100.0%, specificity of 71.0%, positive predictive value of 67.9% and a negative predictive value of 100.0%. If the echo rejection score is >0, there is a 67.9% possibility that a cardiac transplant patient is presenting with allograft rejection, while a score ≤0 denotes a 100% improbability of rejection. **Conclusion:** Our proposed method for screening patients at risk of acute cardiac rejection with echo rejection score showed a good sensitivity in detection of graft rejection. However, further study is required to determine if it can be used as an adjunct to the myocardial biopsy.

Keywords: Heart transplant; Echocardiography; Rejection

1. Introduction

Right ventricular (RV) myocardial biopsy and histological evaluation of obtained samples constitute the gold standard for the detection of acute rejection in patients who have undergone heart transplantation [1,2]. Nevertheless, this expensive procedure gives rise to various complications such as carotid artery puncture, prolonged bleeding, arrhythmias, coronary artery fistula and tricuspid regurgitation [2—6]. Attempts at developing a non-invasive test to screen patients at risk of acute allograft rejection have so far failed to gain wide acceptability [7—10]. We, therefore, sought a novel, accurate, non-invasive method to screen this group of patients.

2. Materials and methods

This prospective study, where 38 patients were included, was approved by the institutional ethics committee, and written informed consent was obtained from all the patients before inclusion into the study. All the heart transplantations were performed by a single team, with one surgeon, using only one surgical technique. Two pathologists and two echocardiographers, who were totally blinded to the parallel results of the other procedures to minimise inter-observer bias, performed the pathological and echocardiographic evaluations, respectively.

The accuracy of the echocardiographic indices was compared with that of RV myocardial biopsy as the gold standard.

2.1. Patients

The inclusion criteria were orthotopic cardiac transplantation at our centre between 2003 and 2006; and that

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myocardial biopsies were performed 1 month after surgery, 2 months after the first biopsy and at 3-month intervals subsequently for 1 year; or clinical suspicion of transplant rejection, tissue Doppler echocardiography was performed within 4 h of each biopsy. Patients who had post-transplant valvular prostheses, re-transplantation or insufficient imaging quality for analysis were excluded.

2.2. Cardiac catheterisation and myocardial biopsy

Myocardial biopsy was assumed as the gold standard test in our study.

Initially, a 7–9F sheath was inserted into the right internal jugular vein; the bioptome was guided through the tricuspid valve into the RV. Four to six samples were taken at each catheterisation from both RV wall and interventricular septum. Then, the samples were sent to the pathology laboratory.

2.3. Pathological evaluations

Haematoxylin–eosin dye was used to examine the tissue samples. Lymphocytic infiltration and myocardial cell necrosis were the most common pathological presentations after cardiac transplantation.

Two pathologists, blind to each other's results, reviewed the specimens. The standard grading system of the International Society of Heart and Lung Transplant (ISHLT) was employed in our study. Pathological grades that were more advanced than 3A were considered as rejection.

2.4. Echocardiography

All the patients underwent preoperative transthoracic echocardiography (TTE) with a tissue Doppler imaging (TDI) analysis (GE Medical System, Vivid 7, Horton, Norway) during the day, leading up to biopsy (<4 h apart), with the recordings being taken by two experienced echocardiologists.

Left ventricular (LV) dimensions were measured using the two-dimensional guided M-mode method. LV volumes and ejection fraction (EF) were assessed using Simpson's equation through the apical two- and four-chamber views.

The mitral inflow parameters, including peak early diastolic wave velocity (*E*), late diastolic wave velocity (*A*), E-wave deceleration time and isovolumic relaxation (IVR) time, were measured. The pulmonary venous flow parameters measured include peak systolic wave (*S*), peak diastolic wave (*D*) and atrial reversal velocity peak.

The methodology of TDI has been previously described and validated [11,12].

At three myocardial segments, namely, RV base, interventricular septal (Sep) base and lateral LV (Lat) base, peak systolic strain (RV-S, Lat-S and Sep-S) was measured from the apical four-chamber view. In addition, time to systole (TS) was measured at the same three segments, yielding the three variables of RV-TS, Sep-TS and Lat-TS.

For TS, the beginning of the QRS complex was used as the reference point; and the end point was the beginning of the mechanical contraction in TDI tracing [8]. Myocardial strain was evaluated via the longitudinal axis of the apical four-chamber view in five consecutive heart beats with digitalised images.

Potential errors in the timing of the measurements were minimised by obtaining the highest possible temporal resolution of TDI images (2–8 ms). The averages of at least five consecutive beats were used for comparison. The continuous variables were considered and the percentage of changes in one person and between two persons was compared; 10 samples were measured by the two echocardiologists and each echocardiologist measured each sample twice. The new index was checked within this process.

2.5. Immunosuppressive regimen

Our protocol for transplant immunosuppressive therapy included the intravenous (IV) administration of 15 mg kg^{-1} methylprednisolone during operation before reperfusing the graft with recipient blood. That is, methylprednisolone (5 mg kg^{-1} day^{-1}) was administered intravenously in three divided doses after operation, followed by oral prednisolone for 4 weeks, and then the doses were tapered. Cyclosporine (1.5–2 mg $kg^{-1}day^{-1}$) was administered intravenously in three divided doses, followed by an oral intake of 5 mg kg^{-1} day^{-1} in three divided doses. Oral azathioprin (200 mg day^{-1}) was administered through nasogastric (NG) tube or orally for 2 weeks, which was changed to mycophenolate mofetil (CellCept®) thereafter.

2.6. Data analysis

All the results are expressed as mean ± standard deviation for the continuous variables and percent for the categorical variables. The correlation between rejection and the categorical variables and that between rejection and the continuous variables were defined with the chi-square test and Student's *t*-test, respectively. In addition, a logistic regression model was employed to calculate the odds ratio for the variables that influenced cardiac rejection in the patients. Receiver operating characteristic (ROC) curve analysis, positive predictive value and negative predictive value as well as specificity and sensitivity were used to assess the validity of the predictive cut-off point for the predictor. Using the binomial exact method, 95% confidence interval for (CI) sensitivity, specificity, positive predictive value and negative predictive value was calculated [13]. A *P*-value of <0.05 was considered statistically significant.

3. Results

In total, 50 specimens from 38 patients (M/F ratio = 1/1) were presented. The mean age of the patients and donors was 25.8 ± 9.6 and 23.0 ± 4.9, respectively. The specimens were collected over a period of 13.9 ± 20.3 months with a median of 7.0 (minimum: 1; and maximum: 63 months). Rejection was observed in 19 (38.0%) specimens in the pathological evaluations (grade ≥3A). The inter-observer and intra-observer variabilities, compared in 60 consecutive measurements, were 4.2% and 2.6%, respectively.

Table 1
Echocardiographic characteristics.

Factor	Non-rejection		Rejection		PV
	Mean	SD	Mean	SD	
RV-S	16.45	4.71	13.95	4.78	0.076
Sep-S	15.90	4.35	11.84	4.83	0.003
Lat-S	15.16	4.69	12.47	2.97	0.030
RV-TS	87.74	12.85	76.11	19.12	0.013
Sep-TS	85.74	14.58	72.79	9.85	0.001
Lat-TS	76.10	18.88	72.89	12.97	0.519
LVEDd (cm)	44.61	3.50	43.68	2.87	0.336
LVESd (cm)	24.90	2.17	25.37	2.11	0.461
E Wave (cm s^{-1})	89.00	11.36	96.53	13.10	0.037
HR (beat min^{-1})	83.06	8.50	87.89	7.10	0.044
IVRT (ms)	71.71	9.72	69.63	9.42	0.462
PHT (ms)	55.81	4.71	55.00	4.88	0.565
E′ by TDI (cm s^{-1})	17.58	2.59	16.21	2.02	0.055
A′ by TDI (cm s^{-1})	7.65	1.36	7.47	1.61	0.688
LVEF (%)	60.32	3.86	57.37	3.86	0.012
PWT (mm)	9.61	0.99	10.74	1.52	0.008
S wave/*D* wave ratio	0.65	0.06	0.59	0.06	0.001
LVM (g m^{-2})	86.52	7.18	97.53	8.08	0.000
RVd	28.90	1.49	30.05	2.41	0.042

RV-S, right ventricular peak systolic strain; Sep-S, septal peak systolic strain; Lat-S, left ventricular lateral peak systolic strain; RV-TS, right ventricular time to systole; Sep-TS, septum time to systole; Lat-TS, lateral time to systole; LVESd, left ventricular end systolic dimension; LVEDd, left ventricular end diastolic dimension; HR, heart rate; *E* wave, mitral *E* wave peak velocity; IVRT, isovolumetric relaxation time; PHT, pressure half-time; *E′*, tissue Doppler *E′* wave peak velocity; *A′*, tissue Doppler *A′* wave peak velocity; PWT, LV posterior wall thickness; *S* wave, pulmonic vein *S* wave peak velocity; *D* wave, pulmonic vein *D* wave peak velocity; LVMI, LV mass index; RVd, right ventricular dimension.

Table 1 presents the result of the echocardiographic evaluations in the two groups of transplant rejection and non-rejection.

Our logistic regression model revealed that the four factors of Lat-S (%), Sep-TS (ms), posterior wall thickness (PWT; mm) and LV mass index (LVMI; g m^{-2}) could indicate heart transplant rejection (Table 2).

A new index, the echo rejection score, was devised using the following formula: [(PWT + LVMI) − (Lat-S + Sep-TS)]. This factor has an area under a curve of 0.932 (Fig. 1), a cut-off point of 0, sensitivity of 100.0% (95% CI: 82.4%, 100.0%), specificity of 71.0% (95% CI: 52.0%, 85.8%), positive predictive value of 67.9% (95% CI: 47.6%, 84.1%) and a negative predictive value of 100.0% (95% CI: 84.6%, 100.0%). Echo rejection score >0 is indicative of a 67.9% possibility of allograft rejection, and a score ≤ 0 stands for a 100% improbability of rejection.

Table 2
Model of logistic regression.

	B	SE	Wald	df	Sig.	Exp(*B*)	95% CI for Exp(*B*)	
							Lower	Upper
Lat-S	−0.478	0.219	4.742	1	0.029	0.620	0.403	0.953
Sep-TS	−0.175	0.074	5.588	1	0.018	0.839	0.726	0.970
PWT	1.226	0.578	4.498	1	0.034	3.408	1.098	10.581
LVM	0.258	0.107	5.845	1	0.016	1.294	1.050	1.595

Lat-S, left ventricular lateral peak systolic strain; Sep-TS, septum time to systole; PWT, LV posterior wall thickness; LVM, LV mass index.

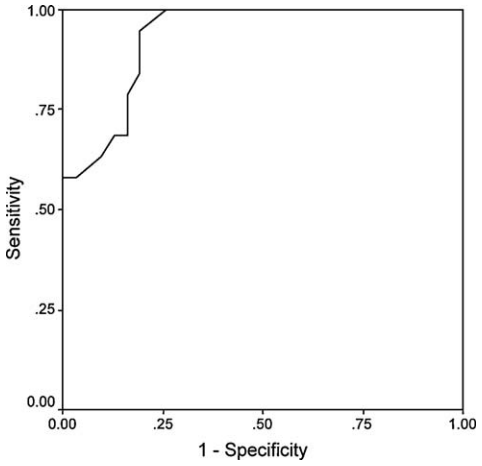

Fig. 1. ROC curve.

4. Discussion

Sequential endomyocardial biopsy is the gold standard test for the detection of allograft heart rejection; however, it is inconvenient and has potential risk for the patient. Over the years, different echocardiography modalities have become of interest as a non-invasive test for the detection of allograft heart rejection but they have several limitations. Although some studies have related E- and A-wave peak velocity as well as pressure half-time and deceleration time to allograft rejection, other reports have failed to demonstrate this relationship [2].

TDI indices, such as peak diastolic mitral velocity and diastolic mitral annular velocity, were found to be decreased in allograft rejection and showed more sensitivity than isovolumetric relaxation time [14,15]; however, reports of the relationship of TDI indices with graft rejection also have been inconsistent and it should be noted that these indices could be influenced by overall movements of the transplanted heart. Acute cellular rejection does not have a homogeneous pattern and as stated by Stengel and coworkers [16] and the reduced sensitivity of TDI in detecting low-grade rejection could be that the inflammation involves a certain region of the myocardium.

Strain pattern TDI is one of the newly developed methods in echocardiography, which demonstrates the percentage of change in the original dimension of a specific segment of the myocardium. Selected points of the myocardium are locally evaluated [17,18]; since the findings are free from the influence of respiration and systolic or diastolic variations, this method can quantitatively detect minor abnormal alterations of ventricular movement and ventricular deformities and timings very early in their courses [19]. Recent studies have demonstrated that these abnormal changes and deformities correlate with the indices of myocardial contractility, including stroke volume and EF [20,21]. This method has demonstrated promising results in patients with acute or chronic cardiac ischaemia and various types of cardiomyopathy [18,22—24]; nonetheless, the application of the results of this method in diagnosing the acute rejection of transplanted heart has not yet been tried extensively.

In the present study, we sought to assess the most favoured echocardiographic indices advocated by previous investigators as predictors of cardiac transplant rejection. Moreover, we made use of TDI to measure timings (electromechanical indices) and evaluated systolic strain pattern TDI. In our logistic regression model of the conventionally used indices, only an increase in the LVMI and the PWT correlated with rejection; and of our new indices, a decrease in the Lat-S and the Sep-TS correlated with rejection.

The decrease in strain indices can be observed in ischaemic heart disease and cardiomyopathy due to reduced contractility and elasticity of the myocardium [18,22–24]. The same mechanism is anticipated in patients experiencing rejection of heart transplant because of the presence of inflammation and, thus, an increase in the interstitial fluid and cell mediators. Nevertheless, the decrease in TS indices was not as predictable as that in strain indices. The conversion of electrical to mechanical function is expected to be delayed due to the inflammatory processes, leading to an increase in these indices. The decrease in these indices, however, can be justified by the hypothesis that advanced degrees of rejection can increase the sensitivity of myocardial cells to electrical impulses.

A thorough assessment of our four variables enabled us to devise a very practical and easy-to-calculate formula for screening patients at risk of heart transplant rejection.

We chose a 100% negative predictive value because it is crucial that we reliably predict via echocardiography that the patient is 100% unlikely to suffer cardiac transplant rejection and, therefore, there is no need for biopsy. However, if the score following echocardiography is >0, the cardiac transplant is likely to be rejected. It is worthy of note that although 32.1% of patients with a score >0 do not have their allograft rejected and their biopsy tends to be normal, it is vital that we do not miss applying the formula, [(PWT + LVMI) − (Lat-S + Sep-TS)], to any patient, so that we can reduce the number of biopsies performed for our patients. We believe that our echocardiographic approach to the diagnosis of cardiac transplant rejection is very practical inasmuch as not only is echocardiography a non-invasive and relatively inexpensive method that can be found in every centre but also the indices that are used are very easy to calculate.

The reproducibility of the parameters employed, use of only one surgical technique in all the patients and the assessment of the majority of the indices given preference to by previous studies are all the advantages of the present study. Our results, however, will gain wider acceptability if verified through more extensive studies with larger sample sizes, which would in turn render the ICs narrower.

Our echo rejection score did not show sufficient accuracy for the diagnosis of cardiac transplant rejection in all the patients; however, it did precisely rule out the possibility of rejection in about 71% of the patients. It can, therefore, have application in screening for those at risk of acute rejection of cardiac transplant as a complementary method to myocardial biopsy because it can be both easily added to the routine follow-up visits of patients and readily performed in a session of echocardiographic evaluations.

5. Limitations

Of the 50 samples taken from 38 patients, 19 developed rejections, which certainly necessitate a larger sample size to confirm our results. Conversely, the low incidence of rejection could have overestimated the sensitivity and the negative predictive value.

Another limitation of this study is the biopsy grading, which was based on the 1990 grading system for allograft heart rejection — this classification was revised in 2005. Since this study was started in 2003, we used the older ISHLT classification, which needs to be revised in future studies.

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