

Tissue Doppler Echocardiography in the Diagnosis of Heart Transplantation Rejection

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Abstract

Background: Endomyocardial biopsy (EMB) is the gold standard method for the diagnosis of cellular rejection (CR) after heart transplantation (HT).

Objective: To test the hypothesis that tissue Doppler imaging (TDI) could detect CR \geq 3A and add diagnostic information compared to conventional Doppler.

Methods: Fifty-four HT patients underwent 129 EMB and a TDI echocardiographic study within 24 hours. We compared HT patients with CR \geq 3A versus HT patients with CR $<$ 3A, with a normal matched control group (13 patients). We measured TDI systolic (S), early diastolic (e'), late diastolic (a') velocities and e'/a' ratio in the left ventricular annulus, basal and medium (mid) segments of the septal (SEP), lateral (LAT), inferior (INF), anterior (ANT) walls; and in the right ventricular annulus.

Results: HT patients showed CR \geq 3A in 39/129 (30.2%) EMB. The best isolated predictor for CR diagnosis was a'LAT, with a sensitivity of 76.3%, specificity of 73.8% ($p = 0.001$). In the multivariate analysis, a'LAT ($p = 0.001$), a'SEP ($p = 0.002$), e'/a' LAT ratio ($p = 0.006$), e'Mitral/ e'LAT ratio ($p = 0.014$), SINF ($p = 0.009$) predicted CR \geq 3A. We obtained a score with a sensitivity of 88.2%, accuracy of 79.6% and negative predictive value of 92.9% to diagnose CR \geq 3A. Conventional Doppler (mitral and pulmonary venous flow) was not relevant to predict CR $>$ 3A.

Conclusion: TDI added diagnostic information to predict CR \geq 3A compared to conventional Doppler. A TDI-based model could become a potential method to detect CR \geq 3A after heart transplantation. (Arq Bras Cardiol. 2011; [online]. ahead print, PR.0-0)

Keywords: Echocardiography, Doppler; diagnosis; graft rejection; heart transplantation.

Introduction

Despite advances in immunosuppressive therapy over the past decade, acute cellular rejection (CR) of the donor heart remains an important factor related to long-term morbidity and mortality^{1,2}. Many noninvasive techniques for rejection diagnosis have been investigated, but none was found to be sufficiently reliable to replace endomyocardial biopsies (EMB) in clinical practice³.

However, routine biopsy is a major inconvenience to the patients and is also risky and costly. The strategy of performing biopsies after the first year post-transplant has been questioned and attempts to reduce their frequency have also been reported. Nevertheless, rejection surveillance is extensively based on EMB performed at predefined intervals⁴.

Tissue Doppler imaging (TDI) is an echocardiographic technique that enables the measurement of systolic and

diastolic velocities within the myocardium. TDI may increase the accuracy of conventional Doppler echocardiography because it integrates information on regional function to estimate global cardiac function and it does not seem to be strongly affected by varying preload conditions⁵. As left ventricular diastolic dysfunction is an early event during allograft rejection, this technique may be useful to detect rejection non-invasively⁶.

However, the role of TDI in the evaluation of subjects after heart transplantation (HT) and for the diagnosis of CR remains unclear⁷⁻⁹. Thus, the purpose of this study was to investigate TDI abnormalities in myocardial relaxation and contraction velocities in HT patients, and also to analyze the additional TDI diagnostic information compared to conventional Doppler concerning significant CR.

Methods

Study population

Between December 2001 and April 2004, 59 consecutive patients aged \geq four years old submitted to HT were referred to undergo EMB at The Heart Institute (InCor), University of São Paulo Medical School, São Paulo, Brazil, at least seven days

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after surgery. Patients had been submitted to orthotopic HT due to heart failure of any etiology. Exclusion criteria included patients with inadequate echocardiographic imaging, LV wall motion abnormalities, presence or suspicion of humoral rejection, graft vascular disease, EMB with evidence of Chagas' disease reactivation, EMB with inconclusive diagnosis and atrial fibrillation.

The final study population consisted of 54 patients (34.9 \pm 20.7), ranging from 4 to 70 years old, 39 males (72.2%), with an average of 354 (ranging from 7 to 3570) days post-HT when undergoing the EMB. The HT etiologies were dilated idiopathic cardiomyopathy, 19/54 (35.2%), Chagas' disease, 16/54 (29.6%), ischemic cardiomyopathy, 6/54 (11.1%). A bicaval surgical technique was performed in 42 patients (77.8%). The study protocol was approved by the Hospital scientific and ethical research committee and all participants signed an informed consent.

Study design

This was a cross-section study followed by a prospective phase involving HT patients who underwent EMB, matched with a group of normal volunteers. All patients underwent EMB and a TDI echocardiographic study, performed within 24 h after the EMB. The researchers involved in these two procedures were blinded to the results of the other technique. Cellular rejection was considered following the International Society of Heart and Lung Transplantation (ISHLT) criteria^{10,11}, as follows: 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 = one focus only with aggressive infiltrates and/or myocyte damage; grade IIIA = multifocal aggressive infiltrates and/or myocyte damage; grade IIIB = diffuse inflammatory process with myocyte necrosis; grade IV = diffuse aggressive polymorphous infiltrate with hemorrhage and myocyte necrosis. The findings of the echocardiographic study were correlated with the results of the EMB, taking into consideration the presence or absence of significant CR (CR \geq 3A)^{10,11}.

In patients who underwent more than one EMB, variations in myocardial TDI were analyzed in order to compare the different histopathological exams. The control group was comprised of 13 healthy volunteers matched by age and sex, without symptoms and previous history of cardiac diseases. Clinical examination, electrocardiogram at rest and the echocardiogram were considered to be normal.

Endomyocardial biopsy

The EMB were carried out in accordance with the protocol adopted in the institution, as follows: weekly during the first month of HT and afterwards at greater intervals according to clinical suspicion of CR. The analyses were carried out according to the criteria of the International Society for the Heart and Lung Transplantation (ISHLT) standardized cardiac biopsy grading¹¹.

Echocardiographic study

Examinations were recorded using a commercially available echocardiographic machine (Philips HDI 5000, Bothell, Washington, USA) equipped with a Tissue Doppler imaging

software. All studies were performed with patient on the left lateral decubitus in order to obtain the images of the parasternal and apical views. All echocardiographic measurements were carried out in accordance with the recommendations of the American Society of Echocardiography¹², and each value is represented as an average of 3 beats.

Conventional pulsed Doppler

Measurements of the peak velocities and diastolic profile parameters of mitral inflow and pulmonary venous flow signals were performed as previously described¹³.

Tissue Doppler echocardiography

TDI acquisitions were performed placing the sample volume in the central region of the myocardium, parallel to the direction of movement of the region of interest. The velocity spectral curve was obtained with a 5-mm sample volume; gain was minimized to optimize the tissue signal and to allow minimal background noise. The Nyquist limit was adjusted to a velocity range of 16 to 20 cm/s.

For the study of the myocardial velocities, we employed a model to analyze the different regions of the ventricles, in which the movements took place in radial (parasternal view) and longitudinal direction (apical 4-chamber and 2-chamber views). In all areas, we measured peak systolic (S), early (e') and late (a') diastolic velocities. In the posterior wall of the LV and in the regions of the LV and RV annulus, the e'/a' ratio was also obtained.

In the apical views, both the annulus, basal and mid segments were assessed at the following walls: septal, lateral, inferior and anterior of the left ventricle and the tricuspid annulus from the right ventricle. In addition, the short-axis circumferential fiber velocities were measured from the posterior mid segment. Short-axis circumferential fiber movement as determined by pulsed TDI have been employed to study some sorts of hypertrophic distribution that sometimes occurs after heart transplantation¹⁴. The index suggested by Nagueh et al¹⁵ was employed, which used the ratio between the early diastolic wave of the mitral flow (E mitral) and the early diastolic wave from the lateral wall measured by DTI (e'LAT). All the measurements were carried out in expiratory apnea.

Statistical analysis

All data are reported as mean \pm SD (95% confidence intervals). Unpaired Student's t-test was used for comparison of continuous variables and paired t-test for comparing follow-up patients. Linear and stepwise regression multivariate analyses were employed to look for relationships among continuous variables on univariate analyses. Receiver-operator curves were employed to evaluate the clinical and epidemiological ability of selected measurements and $p < 0.05$ was considered relevant.

Results

Cross-section study

Endomyocardial Biopsy

One hundred twenty nine EMB were carried out, 35/54 (64.8%) patients underwent more than one procedure,

ranging from one to eight, with an average of 2.4 biopsies per patient. Twenty-two out of 54 patients studied (40.7%) presented CR \geq 3A. CR \geq 3A was observed in 39/129 EMB (30.2%). Baseline characteristics of control and HT groups are summarized in Table 1.

Conventional echocardiography

Measurements by two-dimensional echocardiography and pulsed Doppler ($p < 0.05$) in control and HT groups with or without CR \geq 3A are summarized in Table 1. The univariate analysis of the pulsed wave Doppler parameters considering the ROC curve area for the diagnosis of CR \geq 3A, described mitral A wave velocity as the best parameter ($p = 0.013$). Considering a cut-off point of 35.2 cm/s, we found an accuracy of 59.8%, sensitivity of 59.5%, specificity of 60%, positive

predictive value of 40.7% and negative predictive value of 75%. Upper right pulmonary vein flow parameters did not add diagnostic information to CR \geq 3A. Also, mitral inflow velocities did not show statistical relevance in the multivariate analysis to predict CR \geq 3A.

Tissue Doppler imaging

The TDI study was carried out with success in all patients and regions studied. The velocities of the e' wave, a' wave and S wave, TDI e'/a' ratio and (e'Mitral / e'LAT ratio) in control group, RC \geq 3A and without RC \geq 3A in the LV and lateral tricuspid annulus are listed in Tables 2, 3 and 4.

Tissue Doppler and cellular cardiac rejection \geq 3A

No RV lateral annulus TDI parameter presented significant difference between the groups with and without CR \geq 3A. In LV posterior wall, a significant difference was found only in the late velocity (a' Mid post) between the groups with and without CR \geq 3A. In the walls of the LV analyzed on the apical views, the a' wave velocities were significantly lower in all regions, with the exception of the mid segment of the interventricular septum. The e' wave velocities were lower among patients with CR \geq 3A only in the septal annulus. The S wave velocities were significantly lower in patients with CR \geq 3A in the annulus and the basal region of the septum and the lateral wall of the LV. In the other segments, there were no significant differences in the systolic velocities.

In the univariate analysis by the ROC curve, the best area was obtained with the late diastolic velocities of the lateral annulus of the LV (a'LAT) (Figure 1). With a cutoff point of 5.6 cm/s, it was observed an accuracy of 74.6%, sensitivity of 76.3%, specificity of 73.8%, positive predictive value of 58% and negative predictive value of 86.8% for the diagnosis of CR \geq 3A.

Multivariate analysis

Using logistic regression, a model with 6 predictive parameters was obtained to predict CR \geq 3A: the late velocity of the lateral (ALATan) and septal LV annulus (a' SEP), the systolic velocity of the inferior annulus (SINF), the early diastolic velocity of the midlateral segment (e'LATmid),

Table 1 - Clinical and conventional Doppler echocardiographic features in control group and in HT group, with and without CR \geq 3A

Variable	Control	Without CR \geq 3A	With CR \geq 3A
Age (years)	35.6 \pm 4.3 (27.3 - 44)	36.6 \pm 2.0 (32.7 - 40.4)	43.4 \pm 2.9 (37.7 - 49.1)
Heart rate (bpm)	70.2 \pm 10.5 †‡ (64.5 - 75.9)	92.1 \pm 1.6 * (89 - 95.2)	85.6 \pm 1.8 (82 - 89)
Post HT time (days)	-	341.0 \pm 79.4 (185.4 - 496.7)	385.4 \pm 144.3 (102.5 - 668.2)
LVMI (g/m ²)	80.2 \pm 3.4 †‡ (73.6 - 86.8)	91.7 \pm 2.2 (87.5 - 95.9)	95.8 \pm 3.3 (89.3 - 102.3)
LVDd (mm)	47.6 \pm 1.3 † (45 - 50.3)	44.4 \pm 0.5 (43.4 - 45.5)	45.8 \pm 1.0 (44.5 - 47.1)
LVEF Simpson (%)	63.9 \pm 1.4 (61.2 - 66.6)	63.1 \pm 0.8 (61.5 - 64.6)	62.1 \pm 1.0 (60.1 - 64.1)
RVEF Simpson (%)	65.8 \pm 1.5 †‡ (62.9 - 68.6)	54.8 \pm 0.9 (53 - 56.6)	53.1 \pm 1.6 (50 - 56.3)
AMitral (cm/s)	52.5 \pm 3.9 †‡ (44.9 - 60.2)	40.5 \pm 1.5 * (37.5; 43.5)	35.2 \pm 1.8 (31.8 - 38.6)

AMitral - late diastolic velocity of mitral flow; CR - cellular cardiac rejection, LVMI - left ventricle mass index; LVDd - left ventricle diastolic diameter; LVEF - left ventricle ejection fraction; RVEF - right ventricle ejection fraction; HT - heart transplantation. $p < 0.05$: *, with rejection versus without rejection; † control versus without rejection; ‡ control versus with rejection.

Table 2 - TDI parameters of left ventricle posterior wall and tricuspid annulus in control group and in HT patients with and without CR \geq 3A

Parameter	Control	Without CR \geq 3A	With CR \geq 3A
e' mid posterior	11.3 \pm 0.7 (10 - 12.6)	12.3 \pm 0.4 (11.6 - 13)	12.3 \pm 0.3 (11.3 - 13.4)
a' mid posterior	5.1 \pm 0.6 (4.1 - 6.2) ‡	4.8 \pm 0.2 (4.4 - 5.3) *	4.0 \pm 0.2 (3.6 - 4.5)
e'/a' ratio mid posterior	2.5 \pm 0.2 (2 - 2.9)	2.9 \pm 0.2 (2.6 - 3.2)	3.1 \pm 0.2 (2.7 - 3.7)
S mid posterior	6.5 \pm 0.2 (5.9 - 7.1) †‡	8.3 \pm 0.2 (7.9 - 8.7)	8.1 \pm 0.3 (7.5 - 8.7)
e' tricuspid annulus	14.3 \pm 0.8 (12.8 - 15.7) †‡	8.2 \pm 0.3 (7.6 - 8.9)	7.4 \pm 0.4 (6.6 - 8.3)
a' tricuspid annulus	11.9 \pm 1.0 (9.9 - 13.9) †‡	8.3 \pm 0.4 (7.6 - 9.0)	7.7 \pm 0.5 (6.6 - 8.7)
e'/a' ratio tricuspid annulus	1.3 \pm 0.2 (1.0 - 1.6) †	1.0 \pm 0.1 (0.9 - 1.2)	1.1 \pm 0.1 (0.9 - 1.4)
S tricuspid annulus	12.9 \pm 0.4 (12.1 - 13.8) †‡	8.2 \pm 0.2 (7.8 - 8.8)	8.0 \pm 0.4 (7.2 - 8.7)

Velocity are expressed as mean cm/s \pm SE (95% CI); a' - late diastolic velocity; e' - early diastolic velocity; S - systolic peak velocity, mid - medium segment; CR - cellular cardiac rejection; HT - heart transplantation; $p < 0,05$: *, with rejection versus without rejection; † control versus without rejection; ‡ control versus with rejection.

Table 3 - TDI parameters of septal and lateral annulus and segments of left ventricle walls in control group and in HT patients with and without CR \geq 3A

Parameter	Control	without CR \geq 3A	with CR \geq 3A
e' septal annulus	10.6 \pm 0.6 (9.5 - 11.6) †‡	9.1 \pm 0.2 (8.6 - 9.6)	7.9 \pm 0.3 (7.3 - 8.5) *
a' septal annulus	9.2 \pm 0.7 (7.8 - 10.6) †‡	7.1 \pm 0.2 (6.6 - 7.5)	5.2 \pm 0.2 (4.8 - 5.7) *
e'/a' ratio septal annulus	1.2 \pm 0.1 (1 - 1.5)	1.3 \pm 0.1 (1.2 - 1.4)	1.6 \pm 0.1 (1.4 - 1.7) *
A basal septal	7.4 \pm 0.6 (6.3 - 8.6) †‡	5.4 \pm 0.1 (5.0 - 5.8)	4.4 \pm 0.2 (3.9 - 4.9) *
S basal septal	7.2 \pm 0.2 (6.8 - 7.6) †‡	6.2 \pm 0.1 (6.0 - 6.5)	5.7 \pm 0.2 (5.3 - 6.1) *
e' Mid septal	9.5 \pm 0.6 (8.4 - 10.6) †‡	7.4 \pm 0.2 (6.9 - 7.9)	7.4 \pm 0.3 (6.7 - 8.2)
a' mid septal	6.2 \pm 0.5 (5.2 - 7.2) †‡	4.4 \pm 0.1 (4.0 - 4.7)	3.9 \pm 0.1 (3.6 - 4.3)
S mid septal annulus	6.1 \pm 0.3 (5.5; 6.8) †‡	5.3 \pm 0.1 (5.0; 5.6)	5.0 \pm 0.2 (4.5; 5.5)
e' lateral annulus	14.6 \pm 1.0 (12.6 - 16.6)	12.9 \pm 0.4 (12 - 13.6)	12.4 \pm 0.3 (11.3 - 13.7)
a' lateral annulus	7.9 \pm 0.7 (6.6 - 9.2) †‡	6.5 \pm 0.2 (6.2 - 6.9)	4.9 \pm 0.2 (4.6 - 5.3) *
e'/a' lateral annulus	1.8 \pm 0.2 (1.4 - 2.1) ‡	2.1 \pm 0.1 (1.9 - 2.3)	2.6 \pm 0.2 (2.3 - 3) *
e' mitral/e' lateral annulus	5.5 \pm 0.4 (4.8 - 6.1)	6.2 \pm 0.3 (5.8 - 6.8)	6.2 \pm 0.4 (5.4 - 7)
S lateral annulus	9.3 \pm 0.6 (8.2 - 10.4)	9.2 \pm 0.2 (8.8 - 9.7)	8.3 \pm 0.3 (7.9 - 8.9) *
e' basal lateral	12.2 \pm 0.9 (5.4 - 7.3)	13.4 \pm 0.5 (12.5 - 14.3)	12.8 \pm 0.7 (11.4 - 14.3)
a' basal lateral	6.4 \pm 0.5 (7.1 - 8.3) †‡	5.0 \pm 0.2 (4.7 - 5.3)	4.3 \pm 0.2 (4 - 4.7) *
S basal lateral	7.7 \pm 0.3 (7.1 - 8.3)	8.5 \pm 0.2 (8 - 8.9)	7.7 \pm 0.3 (7 - 8.4) *
e' mid lateral	9.8 \pm 0.8 (8.4 - 11.4)	11.6 \pm 0.4 (10.8 - 12.5)	10.5 \pm 0.5 (9.6 - 11.5)
a' mid lateral	5.6 \pm 0.4 (4.8 - 6.4) ‡	4.6 \pm 0.2 (4.3 - 5)	3.8 \pm 0.2 (3.5 - 4.2) *
S mid lateral	7.1 \pm 0.3 (6.4 - 7.8)	7.7 \pm 0.2 (7.4 - 8.2)	7.0 \pm 0.3 (6.5 - 7.5) *

Velocity are expressed as mean cm/s \pm SE (95% CI); a' - late diastolic velocity; e' - early diastolic velocity; S - systolic peak velocity; mid - medium segment; CR - cellular cardiac rejection; HT - heart transplantation; p < 0.05: *, with rejection versus without rejection; † control versus without rejection; ‡ control versus with rejection.

Table 4 - TDI parameters of inferior and anterior annulus and segments of left ventricle walls in control group and in HT patients with and without CR \geq 3A

Parameter	Control	without CR \geq 3A	with CR \geq 3A
e' inferior annulus	12.0 \pm 0.7 (10.6 - 13.2)	11.3 \pm 0.3 (10.7 - 11.9)	10.5 \pm 0.4 (9.7 - 11.4)
a' inferior annulus	9.5 \pm 0.6 (8.4 - 10.6) †‡	7.6 \pm 0.2 (7.2 - 8.1)	5.9 \pm 0.2 (5.4 - 6.3) *
e'/a' ratio inferior annulus	1.3 \pm 0.1 (1.0 - 1.6)	1.5 \pm 0.1 (1.4 - 1.7)	1.9 \pm 0.1 (1.6 - 2.1) *
S inferior annulus	8.5 \pm 0.3 (8.0 - 9.0)	8.6 \pm 0.2 (8.2 - 9.0)	8.2 \pm 0.3 (7.7 - 8.7)
S basal inferior	7.5 \pm 0.2 (7.2 - 7.9)	7.8 \pm 0.2 (7.4 - 8.2)	7.3 \pm 0.3 (6.8 - 7.7)
e' mid inferior	9.3 \pm 0.6 (8.0 - 10.5)	9.1 \pm 0.4 (8.4 - 9.8)	9.1 \pm 0.5 (8.1 - 10.0)
a' mid inferior	6.0 \pm 0.4 (5.1 - 6.8) ‡	5.4 \pm 0.2 (5.1 - 5.8)	4.7 \pm 0.2 (4.2 - 5.1) *
S mid inferior	6.4 \pm 0.3 (5.9 - 6.8)	6.3 \pm 0.2 (6 - 6.6)	6.2 \pm 0.3 (5.7 - 6.7)
e' anterior annulus	12.1 \pm 0.7 (10.7 - 13.7) †‡	9.9 \pm 0.3 (9.4 - 10.4)	9.4 \pm 0.5 (9.4 - 10.4)
a' anterior annulus	8.3 \pm 0.6 (7.0 - 9.7) †‡	6.3 \pm 0.2 (6 - 6.7)	5.2 \pm 0.2 (4.8 - 5.7) *
e'/A ratio anterior annulus	1.6 \pm 0.2 (1.2 - 1.9)	1.6 \pm 0.1 (1.5 - 1.7)	1.9 \pm 0.1 (1.6 - 2.2) *
S anterior annulus	8.2 \pm 0.4 (7.4 - 9.0)	7.9 \pm 0.2 (7.6 - 8.3)	7.5 \pm 0.3 (6.9 - 8.0)
S basal anterior	6.9 \pm 0.1 (6.4 - 7.5)	6.9 \pm 0.2 (6.5 - 7.3)	6.4 \pm 0.2 (6 - 6.8)
e' mid anterior	8.1 \pm 0.6 (6.9 - 9.4)	8.4 \pm 0.3 (7.9 - 8.9)	8.0 \pm 0.4 (7.2 - 8.7)
a' mid anterior	5.1 \pm 0.3 (4.4 - 5.8) †‡	3.8 \pm 0.1 (3.6 - 4.1)	3.4 \pm 0.1 (3.2 - 3.7) *
S mid anterior	6.0 \pm 0.3 (5.4 - 6.6)	6.4 \pm 0.2 (6 - 6.8)	5.8 \pm 0.2 (5.4 - 6.2)

TDI - tissue Doppler imaging; Velocity are expressed mean cm/s \pm SE (95% CI); a' - late diastolic velocity; e' - early diastolic velocity; S - systolic peak velocity; mid - medium segment; CR - cellular cardiac rejection; HT - heart transplantation; p < 0.05: *, with rejection versus without rejection; † control versus without rejection; ‡ control versus with rejection.

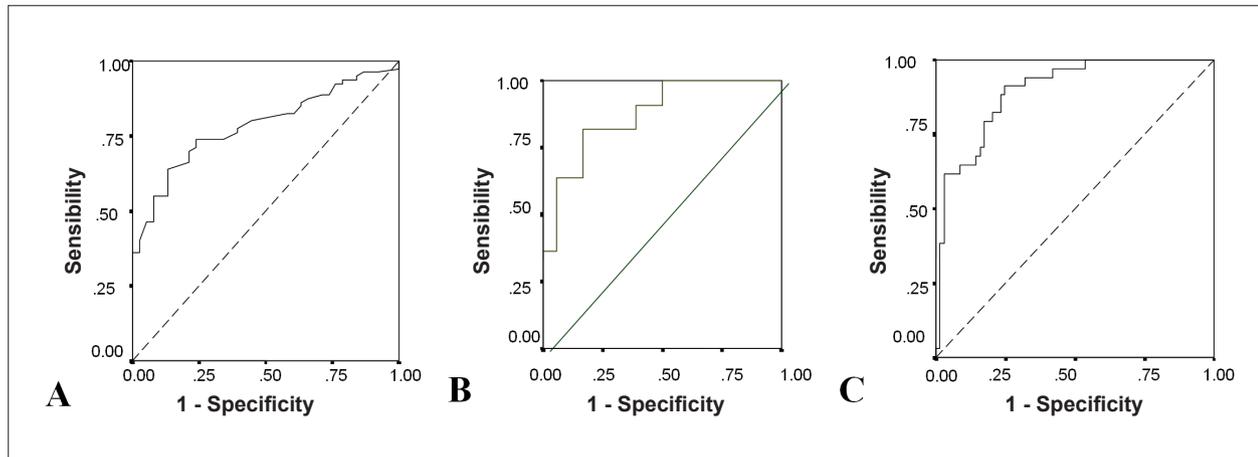


Figure 1 - Receiver operator curve (ROC) analysis for differentiation between the presence or absence of acute cellular rejection (CR) in heart transplanted patients. A) ROC for late diastolic velocity of lateral annulus of left ventricle (aLAT) diagnosis, B) ROC for the aLAT and fall of 7% in patients initially without CR and C) ROC for the multivariate model.

the e'/a' LAT ratio and the e'mitral/e'LAT ratio when all the variables were analyzed together (Table 5). The following predictive score was adopted:

Score = 16.923 - (1.068 x a'SEP - 1.118 x a'LAT - 1.508 x e'/a'LAT Ratio - 0.488 x e' Mitral/e'LAT Ratio + 0.570 x SINf - 0.264 x e' LAT mid).

The cut-off point obtained by the analysis of the ROC curve was equal to 0.619039.

The distribution of variables in the multivariate analysis demonstrated a sensitivity of 88.2%, specificity of 75.4%, accuracy of 79.6%, positive predictive value of 63.8% and a negative predictive value of 92.9% for the diagnosis of CR \geq 3A.

Prospective phase study

Considering the patients who were submitted to more than one EMB, we determined 2 groups: 1 - a group of 29 patients,

initially not presenting CR \geq 3A at the first EMB; 2 - a group of 25 patients, who presented CR \geq 3A at the first EMB.

We observed the behavior of (a'LAT) in these groups and found significant variations in the evolution of the variable in accordance to histological changes (Table 6), (Figure 2).

Taking into account all patients without CR \geq 3A at the time of the first EMB, we established, using the ROC curve, that a fall of 7% in the initial value of the a'LAT presented an accuracy of 79%, sensitivity of 81.8%, specificity of 77.8%, positive predictive value of 69.2% and negative predictive value of 87.5% for the diagnosis of CR \geq 3A (Figure 1).

Discussion

Our results showed the feasibility of TDI for early diagnosis of significant cellular rejection and that TDI added diagnostic information concerning conventional echocardiogram for such issue. We observed LV regional variations in patients presenting CR \geq 3A, which could suggest heterogeneous impairment of the inflammatory process in the graft. Besides, TDI serial

Table 5 - Tissue Doppler Imaging (TDI) parameters in the multivariate analysis

Parameter	Coefficient	Standard error	p	Odds ratio
Constant	16.923	4.194	<0.001	-
a' septal annulus	-1.068	0.3113	0.001	0.344
a' lateral annulus	-1.118	0.37	0.002	0.327
e'/a' ratio lateral annulus	-1.508	0.549	0.006	0.221
E mitral/ e'LAT annulus ratio	-0.488	0.199	0.014	0.614
S inferior annulus	0.57	0.218	0.009	1.768
E mid lateral wall	-0.264	0.13	0.042	0.768

TDI - tissue Doppler imaging; a' - late diastolic velocity; e' - early diastolic velocity; E mitral - early diastolic velocity of mitral flow; S - systolic peak velocity; LAT - left ventricular lateral wall.

Table 6 - Prospective phase study. Variation on late diastolic lateral annulus velocity (ALATan) according to EMB evolution. Groups 1 and 2, patients initially without RC \geq 3A. Groups 3 and 4 patients initially with RC \geq 3A

Group	Patients (n)	1 st Echo ALATan (cm/s)	2 nd Echo ALATan (cm/s)	p
1. Without CR \geq 3A	18	6.26 \pm 0.4	6.76 \pm 0.37	0.2
2. Development CR \geq 3A	11	6.83 \pm 0.38	5.23 \pm 0.39	0.001
3. CR \geq 3A with regression after treatment	19	4.94 \pm 0.26	6.59 \pm 0.27	<0.001
4. CR \geq 3A persistence	6	5 \pm 0.5	4.97 \pm 0.31	0.95

CR - cardiac rejection; ALATan - late diastolic velocity of left ventricle lateral annulus.

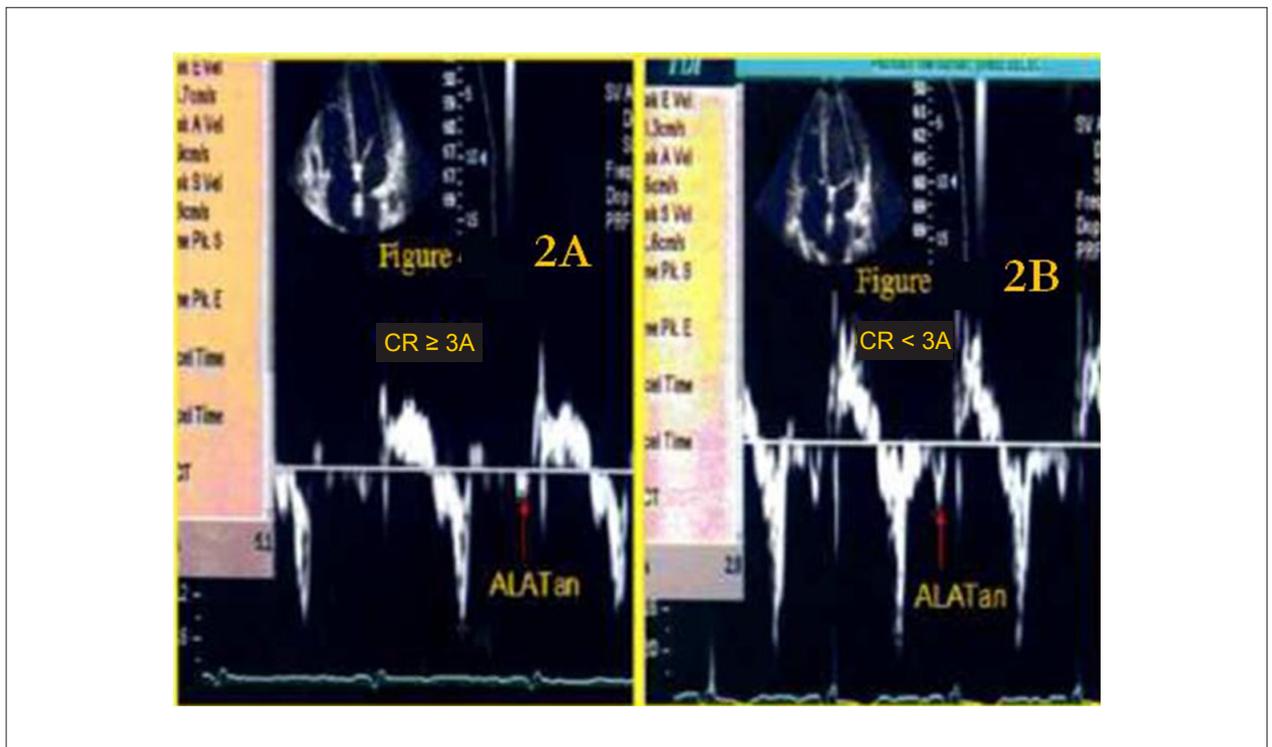


Fig. 2 - Consecutive TDI studies in a patient with acute cellular rejection ($CR \geq 3A$). Left (2A) - Small a'LAT annulus velocity (arrow) during $CR \geq 3A$ acute phase. Right (2B) - Significant increase in a'LAT annulus velocity (arrow) after $CR \geq 3A$ resolution.

analysis showed good diagnostic accuracy in detecting $CR \geq 3A$, demonstrating different behaviors of a'LAT velocities according to histological changes.

Heart transplant group versus normal group on tissue Doppler

To our knowledge, this is the first TDI report which quantitatively characterized various segments of the graft matched by a normal group. In relation to RV systolic function, our data suggest the presence of contractile alteration, which was discordant to a previous report¹⁶. The increase in the graft RV volume and thickness, shortly after HT, is an adaptation mechanism to pulmonary hypertension, generally present in the receptor. The regression of such changes during time is already known. However, RV systolic function seems to be preserved in stable patients without CR. The evidence of tricuspid insufficiency, common among HT patients, can overestimate the functional parameters on conventional echocardiography^{17,18}. The TDI study does not seem to be affected by this influence, as we may observe in our series, with very low systolic velocities of the tricuspid annulus among transplanted patients.

Taking into consideration RV diastolic function, we observed that the reduction in e' and a' wave velocities in the tricuspid annulus may suggest a more advanced degree of impairment of the RV diastolic function in the HT group. In concordance with our series, Valentine et al showed RV diastolic alterations in post-HT patients on conventional Doppler, with a reduction in a' wave velocities of the tricuspid flow among transplanted patients¹⁹.

In the LV systolic function study, our results indicated accentuated regional alterations in the interventricular septum relative to the control group, which was not found in previous conventional echocardiographic reported data²⁰. Left ventricular diastolic dysfunction could be confirmed by the reduction in the e' and a' wave velocities in relation to the control group, especially in the septal and anterior regions. These velocities are inversely proportional to the degree of diastolic dysfunction²¹. The alterations in the diastolic function are already known to occur among post-HT patients and our findings are in accordance with those demonstrated beforehand, in which the occurrence of a restrictive physiological pattern was observed early in the first weeks after the graft implant, with partial regression in the follow-up period²².

Pulsed Doppler and diagnosis of cellular rejection $\geq 3A$

The lack of statistical relevance of the conventional echocardiographic parameters studied in the multivariate analysis to predict $CR \geq 3A$ can be understood taking into account the hemodynamic influence on such issues. These findings are in agreement with previous studies^{22,23}.

We believe that the lack of usefulness of the use of pulsed Doppler to predict $CR \geq 3A$ could be related to some reasons as previously stated by Stengel et al⁹: first of all, pulsed Doppler could be subject to certain important influences such as age, heart rate and loading conditions; second, denervation of cardiac transplanted subjects could lead to a restrictive filling pattern what could be related to diastolic dysfunction, without the evidence of cardiac rejection.

Tissue Doppler and diagnosis of cellular rejection $\geq 3A$

This is the first study, as far as we know, which has evaluated the diagnosis of CR $\geq 3A$ using a multi-regional TDI model including basal and medium segments of the LV walls. The importance of TDI to predict CR $\geq 3A$ is highlighted by the sudden reduction in a' wave velocity in 12 regions, as well as by the decrease in the S wave velocity in 5 regions, of the e' wave velocity in one region of the LV and by the definition of 6 independent predictors in the multivariate model.

In the LV, around 75% of the patients in the group with CR $\geq 3A$ presented velocities a' SEP and a' LAT below the level of the upper limit of the first quartile in the group of patients with CR. Our results are in accordance with a study published more recently⁹, which was the first one to show the benefit of the late diastolic wave velocity (a') for the diagnosis of CR $\geq 3A$. In another study, a' wave velocities in mitral annulus were significantly lower in the group of patients with CR, in agreement with our report²³. Thus, we believe that graft atrial dysfunction could be observed by studying the a' wave velocities on TDI and also that graft atrial dysfunction could be considered one of the main functional consequences of the myocardial aggression in significant CR.

In spite of previous reports we found lesser importance of the early diastolic wave velocity (e') for the diagnosis of CR $\geq 3A$, what could be explained by the emphasis on radial movement of the heart or uni-dimensional TDI technique used on such studies^{24,25}.

In relation to systolic function during CR $\geq 3A$, regional differences found in our series might suggest a heterogeneous form of muscular impairment in this type of cardiac lesion. No study published to date has shown greater importance of the systolic variables compared to diastolic parameters to predict CR. Our multi-regional model study came to a score involving systolic and diastolic features, what could lead to a better understanding of a complex situation such as CR.

Evolutionary phase study

This is the first study, as far as we know, with pulsed TDI and the use of the (a') wave for the diagnosis of CR $\geq 3A$, which sequentially evaluated HT patients according to histological evolution. Regarding our study, we supposed that the a' LAT variable was useful to establish the occurrence of CR $\geq 3A$ and this parameter does not appear to be under the influence of prior alterations in diastolic function. Other observational studies did not analyze a' waves and found significant variations in S and e' waves^{8,26}. Tissue Doppler parameters (mitral and tricuspid e'/e' ratios have been reported as non-invasive measures to rule out transplant rejection in pediatric heart transplant recipients²⁶. Eventually, similar echocardiographic analyses may be useful to rule out transplant rejection in the adult population.

Previous studies employing echocardiography have addressed acute allograft rejection²⁷. However, some bias may be troublesome concerning such data. For instance, some studies present small sample sizes, others define poorly demographic information or surgical techniques or demonstrate non-blinded comparisons²⁸. Other important point is the presentation of technical echocardiographic data in a clinical comprehensive fashion. In our study, concerning repetitive Tissue Doppler studies, a fall of 7% in the initial value of the a' LAT presented a high sensitivity (81.8%), as well as a high negative predictive value (87.5%) for the diagnosis of CR $\geq 3A$.

The echocardiographic score derived from this study, comprising information from different segments of the left ventricle by combining diastolic and systolic parameters may be a useful tool for the for the diagnosis of CR $\geq 3A$. Its use demonstrated very high values concerning sensitivity (88.2%) and negative predictive value (92.9%) to rule out severe cardiac rejection. Also, the use of such score added diagnostic information (higher sensitivity, specificity, accuracy, negative predictive value, positive predictive value) when compared to the best univariate TDI derived parameter (a' LAT) to detect CR $\geq 3A$. We are absolutely aware of the complexity of the format of such score, but perhaps it may just reflect the complex process of cardiac transplantation rejection.

Limitations

There are specific limitations regarding the TDI technique, such as those arising from the angle of inclination of the Doppler study and the complex movements of the heart, which could interfere with myocardial velocity registration. However, longitudinal movements as studied in our series may provide less interference. Another limitation of this investigation could be related to the presence of regional wall motion abnormalities in HT patients, which led to the exclusion of such kind of patients in our study. A new echocardiographic technique that could be used to overcome some of the TDI limitations, such as the angle dependence, could use the strain and strain rate, which could certainly add some information to cardiac rejection diagnosis.

Conclusion

This multiregional TDI-based study proved to be clinically feasible and added diagnostic information to predict CR $\geq 3A$, when compared to conventional Doppler. The multivariate model presented better accuracy than isolated TDI parameters for the diagnosis of CR $\geq 3A$. The use of the multiregional TDI score could be a new noninvasive diagnostic option for the screening of CR $\geq 3A$, which could lead to decreasing costs and risks related to EMB procedures.

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