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ORIGINAL ARTICLE

The long-term influence of repetitive cellular cardiac rejections on left ventricular longitudinal myocardial deformation in heart transplant recipients

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Summary

The aim of the study was to evaluate the long-term influence of repeated acute cellular rejections on left ventricular longitudinal deformation in heart transplantation (HTX) patients. One hundred and seventy-eight HTX patients were included in the study. Rejections were classified according to the International Society of Heart and Lung Transplantation (ISHLT) classification (0R–3R). Patients were divided into three groups according to rejection scores (RSs). Group 1: <50% of biopsies with 1R rejection and no ≥2R rejections; Group 2: ≥50% of biopsies with 1R rejection or one biopsy with ≥2R rejection; Group 3: ≥Two biopsies with ≥2R rejections. All patients had a comprehensive echocardiographic examination and coronary angiography. We found significantly decreasing global longitudinal strain (GLS) comparing to rejection groups (GLS group 1: -16.8 ± 2.4 (%); GLS group 2: -15.9 ± 3.3 (%); GLS group 3: -14.5 ± 2.9 (%), $P = 0.0003$). After excluding patients with LVEF < 50% or vasculopathy, GLS was still significantly reduced according to RS groups ($P = 0.0096$). Total number of 1R and 2R rejections correlated significant to GLS in a linear regression model. In contrast, we found fractional shortening and LVEF to be unaffected by repeated rejections. In conclusion, repeated cardiac rejections lead to impaired graft function as detected by decreasing magnitude of GLS. In contrast, traditional systolic graft function surveillance by LVEF did not correlate to rejection burden.

Introduction

Acute cellular rejection is an inflammatory response predominantly induced by T-lymphocytes. It can occur any time after heart transplantation (HTX), but most frequently in the first 3–6 months. Approximately one-third of the patients remain free of rejection episodes after 1 year [1,2]. It is well known, that rejections are associated with poor long-term outcome, including increased risk of developing cardiac allograft vasculopathy (CAV) [1,2]. However, long-term influence of repeated and severe cellular rejections on myocardial deformation has not been fully investigated. In various cardiac diseases, ischemia, edema, and fibrosis often predominantly affect the longitudinally oriented endocardial muscle fibers. The conventional estimation of left ventricular (LV) function is often assessed by LV ejection fraction (EF). 2D speckle tracking echocardiography (2D-STE) is a new echocardiographic modality for evaluation of myocardial longitudinal deformation. Global longitudinal strain (GLS) by 2D-STE has several methodological advantages, such as low interobserver variability and heart rate, angle, and load independence as compared to more traditional measures of LV function [3]. Longitudinal deformation by GLS has previously been shown to be impaired in HTX patients [4–6]. Furthermore, Doppler strain and GLS has been correlated to acute rejection [7–9], however, the long-term impact of previous cellular rejections on GLS remains unclear.

Thus, the aim of this study was to evaluate the impact of repeated and severe cellular rejections on LV function measured by 2D speckle tracking echocardiography.

Methods

Study population

The study population consisted of the HTX patients followed at Aarhus University Hospital, Skejby in the period 2011–2013 (n = 199) (Fig. 1). We excluded patients with unknown vasculopathy status, incomplete information of previous rejections, lack of echocardiography, and HTX <1 year ago. The most recent echocardiography within follow-up was used for graft function evaluation.

Echocardiography

We used a commercially available ultrasound system (Vivid 9; GE Healthcare, Horten, Norway) with a 3.5 MHz phased array transducer (M5S).

From a parasternal view, M-mode measurement included septal and posterior wall thickness, end-diastolic and end-systolic diameter of LV and right ventricle (RV).

From an apical view, two-dimensional LV-EF measurements were based on end-systolic/diastolic LV volumes, using the biplane method of disks [10].

Peak systolic mitral annular velocities (S') and tissue tracking (TT) were estimated in EchoPAC from tissue velocity image as an average of septal, lateral, anterior, and posterior mitral annulus velocity. GLS was obtained from frame-by-frame tracking of speckle patterns throughout the left-sided myocardium in standard 2D cine loops. The speckle area of interest was manually adjusted for optimal tracking results. We excluded segments with unacceptable low tracking quality, due to poor image acquisition or artifacts. GLS [11] was calculated by the software as the average longitudinal systolic strain of 17 myocardial segments [12] at peak value in systole. EchoPAC only allowed calculation of GLS when tracking quality was adequate in at least five of six segments in each view. In case of no GLS reported, due to exclusion of only one projection (two or more

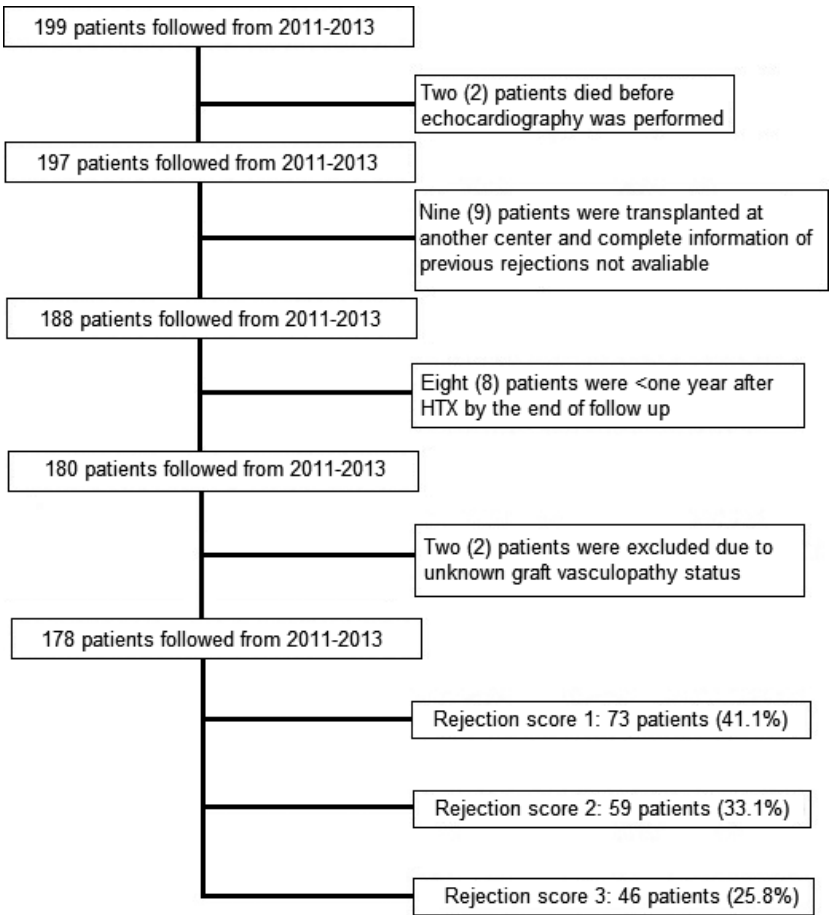

Figure 1 Consort diagram.

segments with inadequate tracking), we calculated the average of the remaining segments as measure for the projection. Subsequently, GLS was manually calculated as the average of all three projections.

A single investigator (TSC), blinded to clinical data, analyzed data offline, using dedicated software (EchoPAC PC SW-Only, 112; GE Healthcare, Milwaukee, WI, USA) and stored them digitally.

Intra-observer repeatability has previously been evaluated in our echocardiographic core laboratory. Analysis showed mean differences of 0.3% points for GLS (95% CI: −0.2;0.7) and −2% points for LV-EF (95% CI: −4;1). Coefficients of repeatability (1.96 SD on differences) on a relative scale were 11% for GLS (95% CI: 8;15) and 23% for LV-EF (95% CI: 18;32) [13].

Endomyocardial biopsy

Biopsies were performed by standard local hospital procedure using the internal jugular or femoral vein. Patients underwent routine biopsies the first 2 years after HTX. Biopsies were scheduled weekly during the first 6 weeks, every 2 weeks up to 3 months, then monthly up to 6 month, and every 2 months for the rest of the first postoperative year. Between years one and two, biopsies were taken every 3 months. Afterward, biopsies were only performed, when rejection was clinically suspected. All rejections classified as ≥2R were treated with intravenous methylprednisolone, 1 g for 3 days and basal oral immunosuppression was adjusted if necessary.

An experienced cardiac pathologist analyzed all biopsies, blinded to echocardiography, and coronary angiography (CAG). Acute cellular rejections were histopathologically graded according to guidelines of ISHLT (1R–3R) [14]. Rejection score (RS) was, prior to the study, divided in three groups:

RS group 1: no rejections higher than 1R and <50% of biopsies with 1R rejections.

RS group 2: either one episode of rejection ≥2R or >50% of biopsies with 1R rejections.

RS group 3: more than one episode of rejection ≥2R.

In addition, we calculated an alternative rejection score as previously described by Raichlin *et al.* [2]: Alternative rejection score = (total number of 1R or resolving rejections) × 1 + (total number of 2R rejections) × 2 + (total number of 3R rejections) × 3.

Angiography and cardiac allograft vasculopathy

To detect donor-transmitted coronary atherosclerosis, a baseline CAG was performed within the first 3 months after transplantation. Afterward CAG was performed annually to detect CAV. We used the latest CAG to determine vasculopathy status. An experienced cardiologist reviewed all CAG's and compared them to previous CAG, blinded to clinical status, echocardiography, and biopsies. In our study, we defined CAV as coronary lesions developed after baseline CAG (3 months). CAV was classified using guidelines of ISHLT [15]. Patients with previous percutaneous coronary intervention (PCI) were classified as 'severe vasculopathy', even if no present severe lesions were seen. We have previously published the correlation between CAV and myocardial graft function in the present HTX population [16].

Statistics

Continuous data conforming to a normal distribution are presented as mean ± standard deviation (SD), and categorical data are presented as absolute values with percentages. We used ANOVA analysis when comparing continuous variables between groups and histograms and Q-Q-plots to check continuous values for normal distribution. We used linear regression model when comparing continuous variables and predicted value and residual to check the regression models. We used ROC analysis to identify the predictive ability of the variable on the end point. $P < 0.05$ was considered statistically significant. We used a standard statistical software package (STATA/IC 12; StataCorp LP, College Station, TX, USA).

Results

We included 178 HTX patients (78.7% men) from the 1st of January 2011 until the 1st of July 2013. Sixty-nine (38.8%) patients had some degree of angiographic CAV. Table 1 displays demographics of the three RS groups. We found no difference between the groups regarding recipient or donor age, time since transplantation, blood pressure, heart rate, and comorbidity (diabetes, hypertension, hypercholesterolemia, stroke/claudication). Significantly more patients in RS group 3 received oral prednisolone (47.8% in group 3 vs. 16.4% in group 1, $P = 0.0007$). Mean time between CAG and echocardiography was 75.8 ± 229.3 days and did not differ comparing the groups ($P = 0.91$). In 28 (15.7%) cases, CAG was performed >28 days after echocardiography but showed no sign of CAV in 23 cases and unchanged CAV in five cases. We found a nonsignificant trend toward higher degree of vasculopathy between the groups (RS group 1: 31.5%; RS group 2: 40.7%; RS group 3: 47.7%, $P = 0.194$).

Table 2 shows various echocardiographic parameters of the RS groups. The longitudinal myocardial function was impaired in patients with severe or repeated rejections. We observed a significant reduction in GLS between the groups (RS group 1 = −16.8 ± 2.4%; RS group 2 = −15.9 ± 3.3%;

Table 1. Patient characteristics by rejection score group.

	Rejection score 1 (n = 73)	Rejection score 2 (n = 59)	Rejection score 3 (n = 46)	ANOVA P
Men (%)	55 (75.3)	47 (79.7)	38 (82.6)	0.63
Age (years)	53.4 ± 16.8	55.3 ± 15.5	55.0 ± 12.5	0.76
Donor age (years)	40.4 ± 13.4	37.9 ± 13.3	36.5 ± 16.1	0.32
Time since transplantation (years)	8.4 ± 5.4	10.1 ± 5.7	9.6 ± 5.7	0.22
Reason for transplantation				
Ischemic heart disease (%)	28 (38.4)	20 (33.9)	16 (34.8)	0.85
Cardiomyopathy (%)	35 (47.9)	32 (50.8)	27 (58.7)	0.52
Congenital heart disease (%)	5 (6.8)	3 (5.1)	3 (6.5)	0.91
Other (%)	5 (6.8)	4 (6.8)	0 (0)	0.19
Weight (kg)	79.1 ± 15.8	82.4 ± 16.3	82.6 ± 16.2	0.41
Systolic blood pressure (mmHg)	140.1 ± 14.9	139.3 ± 17.5	134.3 ± 17.8	0.18
Diastolic blood pressure (mmHg)	86.2 ± 10.9	86.9 ± 10.5	83.9 ± 10.6	0.35
Heart rate (beats/min)	84.0 ± 11.4	83.0 ± 15.1	87.2 ± 14.8	0.28
Diabetes (%)	12 (16.4)	13 (22.0)	6 (13.0)	0.47
Hypertension (%)	66 (90.4)	48 (82.8)	40 (87.0)	0.44
Hypercholesterolemia (%)	67 (91.7)	58 (98.3)	44 (97.8)	0.14
Medication				
Prednisolone (%)	12 (16.4)	15 (25.4)	22 (47.8)	0.0007*
Ciclosporine (%)	28 (38.4)	30 (50.8)	21 (45.7)	0.35
Tacrolimus (%)	43 (58.9)	29 (49.2)	25 (54.3)	0.54
Mycophenolate (%)	49 (67.1)	39 (66.1)	30 (66.7)	0.99
Everolimus (%)	16 (21.9)	18 (30.5)	13 (28.3)	0.51
ACE or ATII inhibitor (%)	54 (74.0)	37 (62.7)	33 (71.7)	0.36
Statins (%)	62 (84.9)	53 (89.8)	39 (84.8)	0.66
Furosemide or bumetanide (%)	13 (17.8)	12 (20.3)	13 (28.3)	0.39
Thiazid (%)	18 (24.7)	9 (15.3)	9 (19.6)	0.41
Calcium channel blocker (%)	37 (50.7)	27 (45.8)	13 (28.3)	0.0496*
Aspirin (%)	40 (54.8)	28 (47.5)	21 (45.7)	0.56
Biochemistry				
Creatinine (μmol/l)	122.9 ± 60.5	140.5 ± 138.7	171.7 ± 145.5	0.09
Hemoglobin (mmol/l)	8.6 ± 1.2	8.7 ± 1.1	8.2 ± 1.1	0.09
Total cholesterol (mmol/l)	4.5 ± 1.1	4.5 ± 1.1	4.6 ± 1.2	0.92
Total number of EMBs	18.3 ± 4.4	21.0 ± 5.0	26.6 ± 7.1	<0.0001*
Number of EMBs showing 0R	13.3 ± 4.3	11.6 ± 4.5	11.9 ± 4.7	0.07
Number of EMBs showing 1R	4.9 ± 3.1	7.7 ± 3.8	10.3 ± 4.7	<0.0001*
Number of EMBs showing 2R	0	0.9 ± 0.5	2.9 ± 1.6	<0.0001*
Number of EMBs showing 3R	0	0.02 ± 0.1	0.07 ± 0.3	0.06
Number of EMBs with resolving	0.1 ± 0.3	0.9 ± 1.6	1.6 ± 2.1	<0.0001*
Alternative rejection score	5.0 ± 3.1	10.4 ± 4.0	17.7 ± 7.4	<0.0001*
Vasculopathy (%)	23 (31.5)	24 (40.7)	22 (47.8)	0.19

Data are presented as absolute number and present or mean ± standard deviation. EMB, endomyocardial biopsy.
*$P < 0.05$.

RS group 3 = −14.5 ± 2.9%, $P = 0.0003$) (Fig. 2). GLS was significantly reduced with higher degrees of RS, even in patients with LV-EF > 50% ($n = 168$, $P = 0.0024$) or patients without CAV ($n = 109$, $P = 0.026$). Tissue tracking values also decreased significantly between the groups ($P = 0.0398$), whereas S' did not ($P = 0.30$). We found no differences in fractional shortening (FS) ($P = 0.26$) and LV-EF ($P = 0.29$) between the groups. The diastolic parameters tended to show higher degree of restrictive filling in patients with repeated or severe rejections with higher E/A and E/e′ ratios, shorter IVRT and E-dec. However, only IVRT was significantly different in the ANOVA analysis ($P = 0.0014$). Figure 3 shows Box Plot diagrams of GLS, S', TT, and EF in relation to vasculopathy status.

We found a significant correlation between GLS and total numbers of 1R rejections in a linear regression analysis with $r = -0.28$, $\beta_1 = -0.19$ (95% CI: −0.29; −0.09), $P < 0.0001$. Total number of 1R rejections remained significantly correlated to GLS after adjustment for numbers of 2R rejections [$r = -0.32$,

Table 2. Echocardiographic parameters by rejection score group.

	Rejection score 1 (n = 73)	Rejection score 2 (n = 59)	Rejection score 3 (n = 46)	ANOVA P
Parasternal M-mode				
LA (cm)	4.5 ± 0.8	4.6 ± 0.9	4.9 ± 0.8	0.0221*
RV (cm)	3.0 ± 0.5	3.0 ± 0.5	3.1 ± 0.5	0.32
IVS (cm)	1.0 ± 0.2	1.1 ± 0.2	1.1 ± 0.2	0.19
PW (cm)	1.0 ± 0.1	1.1 ± 0.2	1.1 ± 0.1	0.10
LV EDD (cm)	4.7 ± 0.5	4.8 ± 0.7	4.8 ± 0.5	0.52
LV ESD (cm)	3.0 ± 0.5	3.2 ± 0.6	3.1 ± 0.5	0.22
FS (%)	36.2 ± 5.8	34.5 ± 5.4	35.0 ± 7.6	0.26
Mass (g)	174.1 ± 48.9	196.7 ± 70.2	189.9 ± 51.7	0.07
Apical				
EF Simpson Biplane (%)	64.4 ± 5.9	62.6 ± 8.5	62.4 ± 9.1	0.29
EDV (ml)	97.4 ± 25.2	101.0 ± 32.9	103.5 ± 22.8	0.49
ESV (ml)	35.0 ± 12.1	39.7 ± 24.3	39.3 ± 15.0	0.26
LA vol (ml)	78.3 ± 28.9	87.6 ± 40.7	95.1 ± 50.1	0.09
TAPSE (cm)	1.7 ± 0.3	1.6 ± 0.3	1.5 ± 0.4	0.0059*
Diastole				
E/A ratio	2.0 ± 0.8	2.3 ± 1.0	2.4 ± 1.2	0.10
E-dec (msec)	169.1 ± 32.5	163.9 ± 30.1	155.4 ± 34.2	0.08
IVRT (msec)	74.3 ± 11.7	74.7 ± 11.0	66.8 ± 14.0	0.0014*
E/E′ ratio	8.8 ± 3.6	10.2 ± 5.8	10.3 ± 4.3	0.16
Tissue Doppler				
S' mean (cm/s)	6.1 ± 1.3	6.2 ± 1.6	5.8 ± 1.5	0.30
TT mean (mm)	10.4 ± 2.0	10.2 ± 2.5	9.3 ± 2.3	0.0398*
2D-STE				
GLS (%)	16.8 ± 2.4	15.9 ± 3.3	14.5 ± 2.9	0.0003*
GLS without vasculopathy (%)	17.3 ± 1.9	16.8 ± 2.8	15.3 ± 2.3	0.0026*
GLS with vasculopathy (%)	15.6 ± 2.9	14.7 ± 3.7	13.7 ± 3.3	0.17
GLS if EF > 50%	17.0 ± 2.1	16.3 ± 3.0	15.2 ± 2.3	0.0024*

Data are presented as mean ± standard deviation. 2D-STE, 2D-speckle tracking echocardiography; LA, left atrium; RV, right ventricle; IVS, interventricular septum; PW, posterior wall; LV EDD, left ventricle end-diastolic diameter; LV ESD, left ventricle end-systolic diameter; FS, fractional shortening; EF, ejection fraction; LV EDV, left ventricle end-diastolic volume; LV ESV, left ventricle end-systolic volume; LA vol, left atrium volume; TAPSE, tricuspid annular plane systolic excursion; IVRT, isovolumetric relaxation time; S', *Peak systolic* mitral annular velocities; TT, tissue tracking; GLS, global longitudinal strain.
*$P < 0.05$.

$\beta_1 = -0.14$ (95% CI: −0.25; −0.03), $P = 0.016$]. In addition, number of 1R rejections still remained significantly correlated to GLS after excluding patients with number of 1R ≥ 15 [$r = -0.25$, $\beta_1 = -0.21$ (95% CI: −0.33; −0.08), $P = 0.001$]. Likewise, we found a significant correlation between GLS and total number of 2R rejections with $r = -0.25$, $\beta_1 = -0.52$ [(95% CI: −0.82; −0.21), $P = 0.001$]. Total number of 2R rejections remained correlated to GLS after adjustment for numbers of 1R rejections [$r = -0.32$, $\beta_1 = -0.37$ (95% CI: −0.71; −0.02), $P = 0.040$]. Furthermore, number of 2R rejections remained significantly correlated to GLS after excluding patients with number of 2R ≥ 4 episodes [$r = -0.73$, $\beta_1 = -0.22$ (95% CI: −1.23; −0.23), $P = 0.004$]. We found no correlation between numbers of biopsies without rejection (0R) and GLS (Fig. 4). Finally, we found a significant linear correlation between the alternative rejection score and GLS [$r = -0.31$, $\beta_1 = -0.13$ (95% CI −0.20; −0.07), $P < 0.0001$] (Fig. 4). Alternative rejection score and GLS remained significantly correlated after excluding patients with alternative rejection score >25 [$r = -21$, $\beta_1 = -0.11$ (95% CI −0.20; −0.03), $P < 0.0001$]. A linear regression analysis showed no correlation between LV-EF and total numbers of 1R ($P = 0.293$) or 2R rejections ($P = 0.142$) or the alternative rejection score ($P = 0.073$) (Fig. 5). We found significant correlation between all measures of diastolic function and total number of 2R rejections in a linear regression analysis, with: IVRT: $r = -0.21$, $\beta_1 = -1.87$ (95% CI −3.14; −0.59), $P = 0.004$; E/A ratio: $r = 0.25$, $\beta_1 = 0.17$ (95% CI 0.07; 0.27), $P = 0.001$; E-dec: $r = -0.16$, $\beta_1 = -3.62$ (95% CI −6.95; −0.29), $P = 0.033$. E/A ratio was the only diastolic parameter significantly correlated to total

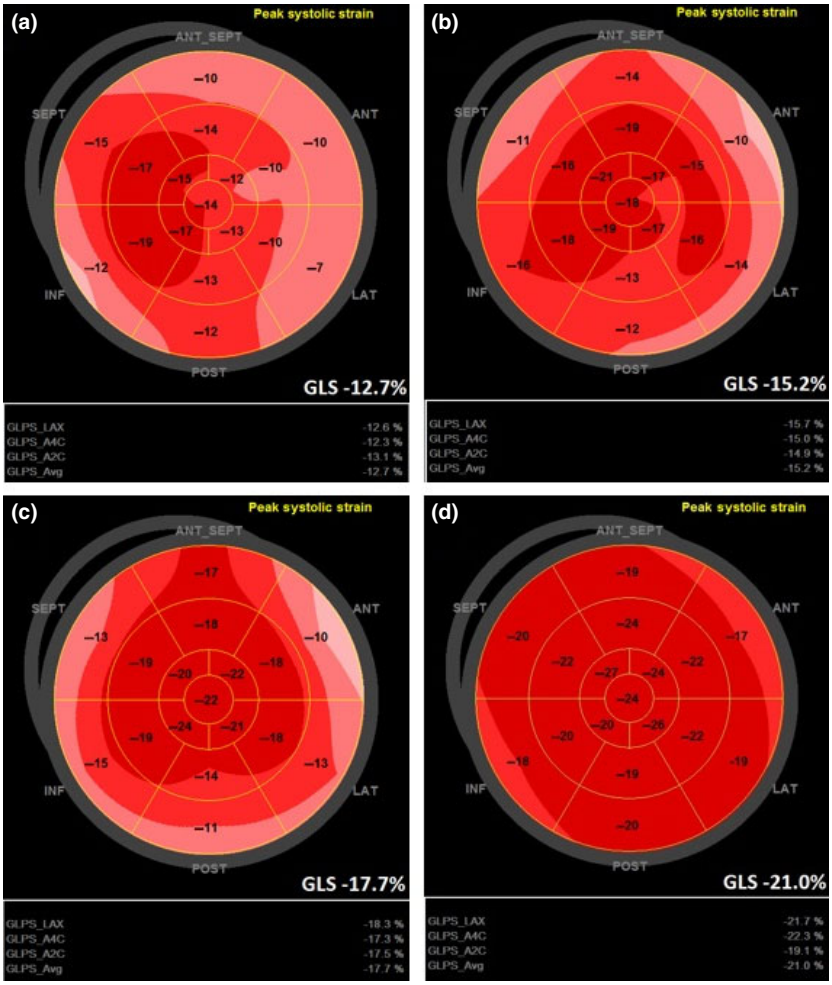

Figure 2 Bulls eye 17 segments model of global longitudinal strain. (a) Male, 63.7 years old heart transplantation (HTX) patient. Time since transplantation 8.7 years. Coronary angiography without vasculopathy. Previously 18 1R or resolving rejections and three 2R rejections. LV-EF 67.2%, GLS −12.7%. (b) Male, 46.6 years old HTX patient. Time since transplantation 8.8 years. Coronary angiography without vasculopathy. Previously eight 1R or resolving rejections, one 2R rejection. LV-EF 62.2%, GLS −15.2%. (c) Male, 49.4 years old HTX patient. Time since transplantation 13.4 years. Coronary angiography without vasculopathy. No previous 1R, 2R, or 3R rejections. LV-EF 69.2%, GLS −17.7%. (d) Male, 59.2 years old healthy control. LV-EF 65.3%, GLS −21.0%.

number of 1R rejections [$r = 0.18$, $\beta_1 = 0.04$ (95% CI 0.01; 0.07), $P = 0.017$].

In a multivariate analysis, GLS was significantly correlated to RS after adjustment for image quality, gender, donor age, graft age, blood pressure (systolic and diastolic), heart rate, serum creatinine, serum hemoglobin, serum cholesterol, weight, vasculopathy status, LV-EF, prednisolone treatment, and calcium inhibitor treatment [$r = -0.77$, $\beta 1 = -0.72$ (95% CI: −1.2; −0.3), $P = 0.002$]. In a ROC analysis, differentiating GLS in patients with highest rejection burden (RS group 3) from GLS in patients with lower rejection burden (RS group 1 and RS group 2), we found area under the curve of 0.70. Optimal cutoff was GLS = −14.7% leading to moderate sensitivity of 55.7% and high specificity of 80.6%.

The longitudinal function of the right ventricle, measured by tricuspid annular plane systolic excursion (TAPSE) decreased significantly with higher rejection score group ($P = 0.0059$). In a linear regression model, TAPSE was significantly correlated to number of 1R rejections [$r = -0.16$, $\beta 1 = -0.01$ (95% CI: −0.03; −0.001), $P = 0.039$], 2R rejections [$r = -0.24$, $\beta 1 = -0.06$ (95% CI: −0.10; −0.02), $P = 0.001$] and alternative rejection score [$r = -0.25$, $\beta 1 = -0.01$ (95% CI: −0.02; −0.01), $P = 0.001$].

Discussion

The main finding of this study is that repeated rejections leads to impaired longitudinal systolic LV function,

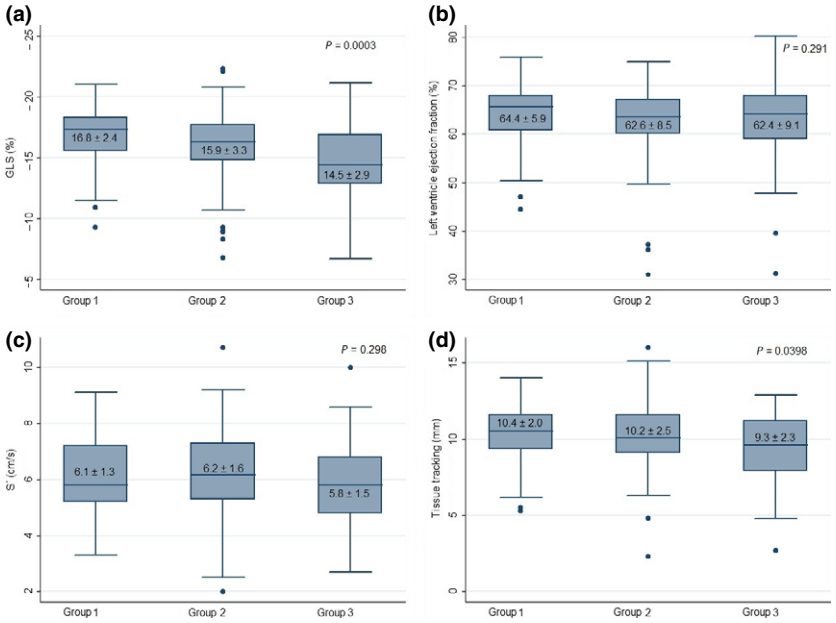

Figure 3 Box plots and ANOVA analysis. Each box plot shows the mean ± SD. (a) Global longitudinal strain. *T*-tests: Rejection score (RS) Group 1 versus Group 2: $P = 0.084$, Group 1 versus Group 3: $P < 0.0001$, Group 2 versus Group 3: $P = 0.0295$. (b) Left ventricle ejection fraction. *T*-tests: RS Group 1 versus Group 2: $P = 0.1634$, Group 1 versus Group 3: $P = 0.1562$, Group 2 versus Group 3: $P = 0.9122$. (c) Peak systolic mitral annular velocities (*S'*). *T*-tests: RS Group 1 versus Group 2: $P = 0.8599$, Group 1 versus Group 3: $P = 0.1527$, Group 2 versus Group 3: $P = 0.1890$. (d) Tissue tracking. *T*-tests: RS Group 1 versus Group 2: $P = 0.4691$, Group 1 versus Group 3: $P = 0.0084$, Group 2 versus Group 3: $P = 0.0948$.

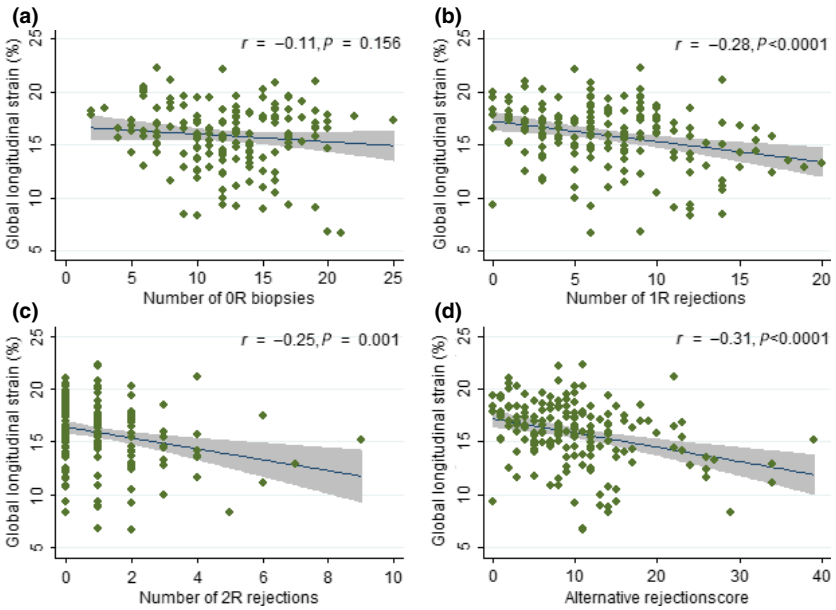

Figure 4 Scatter plots with regression lines and 95% CI for total number of biopsies showing no rejection (a), total number of 1R-rejections (b), total number of 2R-rejections (c), and alternative rejection score (d) in reference to global longitudinal strain.

measured by GLS, whereas traditional measures of systolic function, such as LV-EF, did not correlate to RS. We found an absolute difference of −2.3% in GLS (−16.8; −14.5%) comparing RS group 1 with RS group 3. The correlation between GLS and RS remained significant after excluding patients with known vasculopathy and LV-EF below 50%. The results remained significant after adjustment for potential confounders in a multivariate

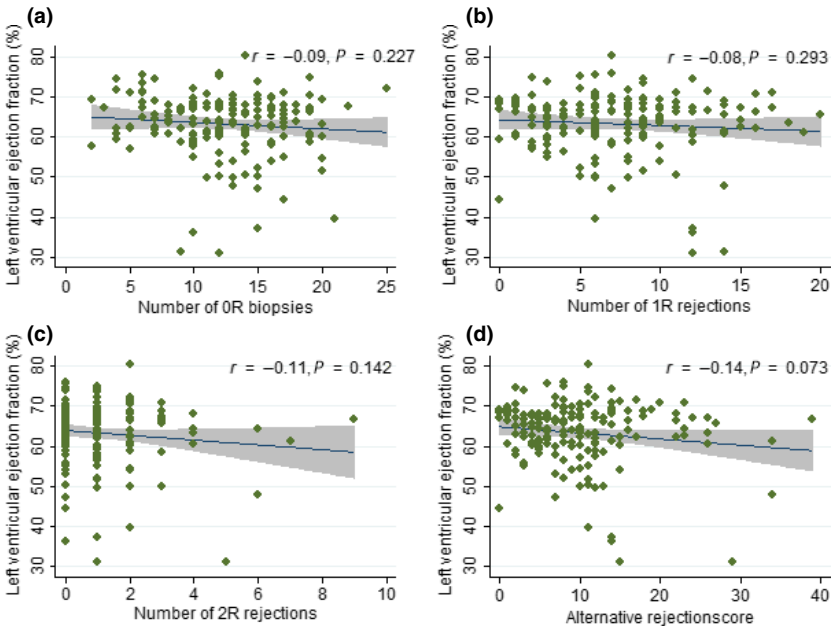

Figure 5 Scatter plots with regression lines and 95% CI for total number of biopsies showing no rejection (a), total number of 1R-rejections (b), total number of 2R-rejections (c), and alternative rejection score (d) in reference to left ventricular ejection fraction.

analysis and after excluding patients with highest rejection burden.

Acute cardiac rejections are associated with lymphocyte infiltration, myocardial necrosis, and local edema [1,17]. It is well known from several other cardiac diseases that early stages of fibrosis and edema often affect the subendocardial fibers of the myocardium, leading to impaired longitudinal myocardial function [3]. In accordance with our findings, other studies have demonstrated reduced longitudinal myocardial function, measured by GLS, in stable HTX patients [4–6], during acute rejections [9], and graft failure [18]. GLS has also proved to be an important prognostic marker in the early [19] and intermediate phase (within 24 months) after transplantation [20]. In addition, our group previously found significant correlation between CAV and GLS [16]. Several factors, such as the surgical procedure, ischemic transport damage, LV-remodeling, rejections, hypertension, fibrosis due to immunosuppressive treatment, and impaired micro- and macro-vascular perfusion, potentially affect the longitudinal function in HTX patients. Despite several possible confounders, our present study demonstrates that GLS is affected by repeated rejections. Importantly, the correlation between cardiac rejections and longitudinal myocardial deformation was noted for both repeated 1R and 2R rejections. It is noteworthy that even 1R rejections influence long-term graft function, as the common conception and recommendations state that 1R rejections are without clinical significance and should not be treated [21]. The present results challenge this assumption as we showed impaired LV longitudinal systolic function in patients with repeated 1R rejections.

We found unaffected LV-EF and FS after repeated cardiac rejections. This, combined with higher inter- and intraobserver variability compared to GLS, limits the use of LV-EF and FS in the overall monitoring of graft function with respect to rejections. GLS seems to possess incremental value to the estimation of LV function by LV-EF and diastolic Doppler measurements during the long-term follow-up and should therefore be considered for graft function monitoring and potential prognostication of HTX patients.

In our study, we found significantly correlation between RV TAPSE and previous rejection burden. The correlation was noted for both 1R and 2R rejections. Previous studies have described impaired longitudinal function of RV measured by TAPSE and tissue Doppler in stable HTX patients [22–24], but to the best of our knowledge, this is the first study to display the influence of previous rejection on RV function.

The demonstrated correlation between impaired longitudinal function and rejections in our study might have several explanations. It has previously been demonstrated that cardiac rejections do not lead to fibrosis [25–27]. In our study, we noted a nonsignificant relation between CAV and RS. Previous studies demonstrated increased risk of CAV development in patients with high rejection burden, suggesting an immune mediated rejection-related cause of

vasculopathy [2]. However, in our study, GLS was still significantly correlated to RS group after excluding patients with CAV. Longitudinal myocardial function is dependent of both the epicardial vessels and the microvascular system. Hiemann *et al.* [28] demonstrated that microvascular dysfunction is very common in HTX patients and associated with adverse prognosis. Inflammation-induced impaired microvascular perfusion might be triggered by severe or repeated rejections resulting in impaired longitudinal myocardial deformation. Finally, patients with severe or repeated rejection might have received higher dose of immunosuppressive treatment in periods after HTX, which is associated with myocardial fibrosis [25,26].

Limitations

We did not evaluate the microvascular perfusion or cardiac fibrosis, which could be relevant to assess the cause of impaired long axis function in this population.

Conclusion

Both repeated 1R and 2R rejections lead to long-term impaired longitudinal myocardial deformation, measured by GLS, in HTX patients independent of graft vasculopathy. In contrast, traditional systolic graft function surveillance by LV-EF did not correlate to rejection burden. GLS possess technical advantages compared to standard measures of LV systolic function and seems suitable for graft monitoring with respect to rejections. Further studies are needed to determine the overall prognostic value of GLS.

Authorship

TC: designed the study, performed the study, collected data and wrote the paper. BBL: designed the study and revised the paper. HE: designed the study and revised the paper. SH: pathological work. SHP: designed the study and revised the paper.

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