Clin Transplant 2010: 24: 321–327 DOI: 10.1111/j.1399-0012.2009.01063.x

Clinical Transplantation

Relationship between a validated molecular cardiac transplant rejection classifier and routine organ function parameters

Cadeiras M, Shahzad K, John MM, Gruber D, von Bayern M, Auerbach S, Sinha A, Latif F, Unniachan S, Memon S, Mital S, Restaino S, Marboe CC, Addonizio LJ, Deng MC. Relationship between a validated molecular cardiac transplant rejection classifier and routine organ function parameters.
Clin Transplant 2010: 24: 321–327. © 2009 John Wiley & Sons A/S.

Abstract: Background: As acute cellular cardiac allograft rejection is a systemic process affecting the entire organism, we hypothesized that scores of a peripheral blood mononuclear cell gene expression profiling (GEP) test developed and validated to rule out International Society of Heart and Lung Transplantation (ISHLT) grade ≥3A/2R acute cellular cardiac allograft rejection also reflects biologically plausible changes of the routinely assessed clinical parameters.
Methods: We retrospectively analyzed 76 patients who underwent GEP testing, at the time of their routine clinical follow-up in our Institution between February 1, 2006 and January 31, 2007. Data were analyzed with *t*-test, nonparametric tests, bivariate Spearman's correlation, and multivariate linear regression modeling.
Results: More activated GEP-score correlated with longer corrected QT (QTc)-interval ($r = 0.377$, $p = 0.001$, $n = 63$), longer QRS duration ($r = 0.231$, $p = 0.03$, $n = 66$), higher heart rate ($r = 0.221$, $p = 0.037$, $n = 66$), higher serum creatinine ($r = 0.26$, $p = 0.01$, $n = 75$), higher gamma-glutamyl transferase (GGT) GGT ($r = 0.266$, $p = 0.037$, $n = 46$), lower pulmonary artery oxygen saturation ($r = -0.313$, $p = 0.003$, $n = 76$), lower platelet count ($r = -0.372$, $p = 0.001$, $n = 74$), lower monocyte count ($r = -0.208$, $p = 0.040$, $n = 72$), and lower high-density lipoprotein (HDL) HDL level ($r = -0.242$, $p = 0.041$, $n = 53$). Multivariate analysis showed a significant amount of variance in the GEP score independently explained by the variability of QTc-interval ($\beta = 1.998$, $p = 0.001$) and platelet count ($\beta = -1.540$, $p = 0.017$). *Post hoc* analysis of the 11 individual GEP-classifier genes showed WDRA40 ($p = 0.02$) and ras homolog gene family, member U (RHOU) RHOU ($p = 0.01$) independently related to mixed venous O_2Sat%.
Conclusion: A GEP test developed and validated to detect the absence of cardiac rejection correlates with electrocardiographic and hemodynamic cardiac parameters as well as renal, hepatic, bone marrow, and lipid metabolism parameters suggesting a complex relationship between rejection, leukocytes, and organ function within the continuum between alloimmunological quiescence and rejection.

Martin Cadeiras[a]*, Khurram Shahzad[a]*, Manju M. John[a]*, Dorota Gruber[a], Manuel von Bayern[a], Scott Auerbach[b], Anshu Sinha[c], Farhana Latif[a], Sreevalsa Unniachan[a], Sarfaraz Memon[a], Seema Mital[b], Susan Restaino[a], Charles C. Marboe[d], Linda J. Addonizio[b] and Mario C. Deng[a]

[a]Department of Medicine, Division of Cardiology, College of Physicians & Surgeons, [b]Department of Pediatrics, Division of Pediatric Cardiology, College of Physicians & Surgeons,[c]Department of Biomedical Informatics and [d]Department of Pathology, College of Physicians & Surgeons, Columbia University, New York, NY, USA

Key words: electrocardiography – gene expression – heart transplantation – hemodynamics – molecular testing

Abbreviations: CARGO, Cardiac Allograft Rejection Gene Expression Observational Study; CI, cardiac index; CO, cardiac output; CUMC, Columbia University Medical Center; ECG, electrocardiogram; EMB, endomyocardial biopsy; GEP, gene expression profiling; ISHLT, International Society of Heart and Lung Transplantation; MVO_2Sat, mixed venous oxygen saturation; NPV, negative predictive value; PBMC, peripheral blood mononuclear cells; QTc, corrected QT; RT-PCR, real time-polymerase chain reaction.

Corresponding author: Mario C. Deng, MD, FACC, FESC, Associate Professor of Medicine, Director of Cardiac Transplantation Research, Department of Medicine, Division of Cardiology, Columbia University, 622 West 168th Street, PH12 STEM, Room 134, New York, 10032 NY, USA. Tel.: +1 212 305 0200/4600; fax +1 212 305 7439; e-mail md785@columbia.edu
*These authors contributed equally to this study.

Accepted for publication 7 July 2009

Endomyocardial biopsy (EMB) is the standard method used in heart transplantation rejection surveillance (1, 2) but has limitations (3–5). Recently, based on the rationale that gene expression profiling (GEP) of peripheral blood mononuclear cells (PBMC) reflects the activation of host responses by the cardiac allograft, the multicenter Cardiac Allograft Rejection Gene expression Observational (CARGO) study developed and validated a molecular classifier to distinguish International Society of Heart and Lung Transplantation (ISHLT) grade 0 rejection (i.e., quiescence) from moderate/severe (ISHLT ≥ 3A/2R) rejection (6). The CARGO study identified 252 candidate genes for which real-time qRT assays were developed. An 11 gene real time-polymerase chain reaction (RT-PCR) test was derived from a training set using linear discriminant analysis, converted into a scores (0–40), and validated prospectively in an independent set. The test distinguished biopsy-defined moderate/severe rejection from quiescence in the validation set, and had agreement of 84% (95% CI: 66–94) with ISHLT grade ≥3A/2R rejection. Patients >1 yr post-transplant with scores below 30 were very unlikely to have grade ≥3A rejection (negative predictive value [NPV] = 99.6%). Table 1 summarizes the genes selected for the final classifier. The post-CARGO initial clinical experience (7) reflects the high NPV as suggested by the original CARGO study (6). As the continuum between alloimmunologic quiescence and acute cellular cardiac allograft rejection is a systemic equilibrium process affecting the entire organism, we hypothesized that scores of a PBMC GEP test developed and validated to rule out ISHLT grade ≥3A/2R acute cellular cardiac allograft rejection are reflected in biologically plausible changes of the routinely assessed clinical parameters.

Table 1. Genes and pathways represented in GEP test

Genes in AlloMap test	Corresponding pathways
SEMA7A	Macrophage activation/PMNs
IL1R2, FLT3, ITGAM	Steroid responsiveness
PF4, G6B	Platelet production
MIR, WDR40A	RBC production (hematopoiesis)
PDCD1, ITGA4	T-cell activation and regulation
RHOU	Cell morphology

Methods

Study population

This study protocol was approved by the Institutional Review Board. De-identified data were retrospectively analyzed and a unique code number was used for each study subject. We analyzed all heart transplant patients who underwent GEP testing with AlloMap™ Molecular Test (XDx Inc., Brisbane, CA, USA) at the time of their protocol biopsy and routine follow-up encounters at our institution between February 1, 2006 and January 31, 2007. Orthotopic heart transplantation was performed by standard techniques (8–11). All transplant recipients received double or triple drug immunosuppressive therapy with the combination of the following drugs: prednisone, cyclosporine, tacrolimus or sirolimus, and azathioprine or mycophenolate mofetil. All patients were managed according to Columbia University Medical Center (CUMC) standard of care (12). Clinical Information of the patients was retrospectively collected from the medical records. Collected data consisted of demographics, medications, clinical status at the time of the encounter, immunosuppressive drug levels, and echocardiography, if available.

Surveillance of transplant rejection

Patients were routinely seen at pre-specified time intervals based on CUMC rejection surveillance protocol for heart transplant recipients, i.e., every week during the first month, every two wk during the next 2 months, every four wk until month 6, every eight wk until first yr, during the second year every 3–6 months, and thereafter every 3–12 months during the rest of their follow-up. At the time of biopsy, patients undergo evaluation of the cardiac allograft with invasive hemodynamics and laboratory tests. For this analysis, patients required an evaluation with right heart catheterization, EMB, electrocardiogram (ECG), and routine blood sampling at the time of GEP testing (±7 d). Because some patients were tested multiple times, we selected for the single time point analysis only one sample per patient with complete hemodynamic, ECG, and laboratory information that was closest to one-yr post-heart transplantation.

Endomyocardial biopsy

Endomyocardial biopsies were performed with a Caves-Scholten bioptome, usually via a right internal jugular venous sheath. Three to six pieces of endomyocardium were obtained from the right ventricular septum and graded using the ISHLT 1990 and 2005 classifications (1, 2) by one of two senior pathologists.

Invasive hemodynamics

Invasive hemodynamics is routinely assessed at the time of protocol endomyocardial biopsies in most of the patients in our heart transplant program using a pulmonary catheter. Procedures are done in a dedicated biopsy suite under similar conditions for all patients through a right internal jugular vein access. Hemodynamic variables are recorded before obtaining the biopsy specimens with a 7.0 F Swan Ganz Catheter, which includes right atrial pressure, mixed venous oxygen saturation (MVO_2Sat), pulmonary capillary wedge pressure as well as cardiac output (CO), and cardiac index (CI) measured by thermodilution technique, as well as the Fick equation, because of the high incidence of significant tricuspid regurgitation in heart transplant patients as follows: $CO(L/min) = [(1.34 \times BSA \times 10)/(1.35 \times Hb\ (mg/dL) \times (97\% - O_2Sat\%)\ /100)]$ (average of two measurements).

QTc-interval measurement

QT-interval was measured on ECGs routinely recorded at the time of the patient's clinical visit for rejection surveillance. Resting 12-lead ECGs were recorded at a paper speed of 25 mm/s. QT-interval duration was determined automatically by using a computer program ECG recorder and one independent observer blinded to the molecular scores who manually determined the QT and RR interval duration on three different high-quality tracings defined as a standard limb or pre-cordial ECG lead in which the maximal T wave amplitude exceeded 0.25 mV. Using a 600% computer aided magnification of the ECG, each QT-interval was measured from the beginning of the QRS complex to the point at which the T wave returned visibly to the isoelectric line. When U waves exceeded 50% of the T wave, the duration of the U wave was included in QT measurement. When the T wave was interrupted by a U wave, the end of the T wave was defined as the nadir between the T and the U wave. Criteria for exclusion of the ECG from study were supraventricular or ventricular arrhythmias except for occasional PVC, pacemaker rhythms, readable recordings from <3 leads, and poor recording quality.

Routine laboratory testing

At the time of patient's clinical visit for rejection surveillance patients underwent normal laboratory testing including complete blood count, liver function tests, coagulation profile, and lipid profile, etc. For this analysis, we retrospectively collected the data by reviewing the medical records and the information obtained from the laboratory testing within ± 1 d of the GEP testing was included in the analysis.

Gene expression testing

Peripheral venous blood samples for GEP were drawn from each patient within six h before the initiation of the hemodynamic study and biopsy. Assessment of GEP with AlloMap molecular expression testing is described elsewhere (6, 7). Briefly, 8.5 cc of whole blood obtained from either a peripheral or central line was collected into a Cell Preparation Tube (CPT tube; Becton-Dickinson, Franklin Lakes, NJ, USA) followed by centrifugation, cell isolation, freezing at −20°C and overnight shipment in dry ice to the CLIA-certified laboratory (XDx Laboratory) for further processing. This gene expression test is a 20-gene (11 informative genes and nine control genes), quantitative RT-PCR assay run in triplicate that applies a proprietary mathematical algorithm that combines the gene expression values from genes associated with cardiac allograft rejection and generates an integer score ranging from 0 to 40. Molecular score and cycle thresholds for each individual gene component of the molecular test were requested from the manufacturer for an exploratory *post hoc* analysis.

Calculations and statistical analysis

Data were analyzed using SPSS 15.0 statistical analysis software (SPSS Inc., Chicago, IL, USA). All variables were assessed for distribution characteristics. As GEP scores were not normally distributed, Spearman's correlation was used and coefficients and one-sided p-values for the correlation were calculated for the selected variables. Outliers, defined as >2 SD, were not considered in the analysis. Spearman's correlation coefficients were reported when appropriate. Variables which were found significant by calculating two sided p-value and those which meet the criteria of 80% complete data points were also analyzed in a multivariate approach to understand the extent of the variance in the molecular score that can be predicted using the combination of univariate significant parameters in a linear regression model. We used *t*-test for quantitative data analysis or nonparametric tests (Mann–Whitney *U*-test) when appropriate. Continuous variables are presented as mean $\pm$ SD, unless otherwise noted. A p-value of <0.05 was considered significant.

Results

Patients

A total of 218 blood samples were obtained for GEP testing from 115 patients. The routine parameters were available for 76 patients that were included in the analysis. In patients with multiple samples, we selected the sample closest to the first year post-transplant for the single time point analysis. The mean age of the patients was 42 ± 19.9 yr, 76% were males and most patients were Caucasian (67%). The immunosuppression regimen most commonly consisted of cyclosporine A, mycophenolate, and prednisone. No patient received a prednisone dose ≥20 mg/d. In addition to immunosuppressants, the other medications taken by these patients included diuretics (n = 31), calcium channel blockers (n = 26), angiotensin converting enzyme inhibitors (n = 23), angiotensin receptor blockers (n = 5), beta-blockers (n = 13), statins (n = 42), and aspirin (n = 49).

Sample result distribution

Median time (25th–75th percentile) from transplantation until the samples were obtained was 1471 (476–2634) d. Rejection grading was 0R in 50 samples and 1R in 24 cases. Two samples did not have a biopsy grade associated. Overall mean GEP score was 29.8 ± 5.4. The descriptive statistics are provided in Table 2.

Correlation between organ function parameters and GEP scores

Our results showed a positive correlation of GEP score with corrected QT (QTc)-interval ($r = 0.377$, $p = 0.001$, $n = 63$), QRS duration ($r = 0.231$, $p = 0.03$, $n = 66$), heart rate ($r = 0.221$, $p = 0.037$, $n = 66$), serum creatinine ($r = 0.26$, $p = 0.01$, $n = 75$), GGT ($r = 0.266$, $p = 0.037$, $n = 46$), and a negative correlation with pulmonary artery O_2Sat ($r = -0.313$, $p = 0.003$, $n = 76$), platelet count ($r = -0.372$, $p = 0.001$, $n = 74$), monocyte

Table 2. Baseline characteristics of the study population

Variable	Mean ± SD	Range	N	%
Age	41.8 ± 19.9	15.9–79.7	76	100.0
GEP score	29.4 ± 5.7	12–38	76	100.0
Time Tx-GEP score ([r])	1471 (476–2634)	145–2665	76	100.0
Race and GEP score				
Asian	29 ± 9.8	15–38	4	5.3
Black	26.7 ± 7.6	13–36	7	9.2
Hispanic	31.1 ± 5.1	19–38	14	18.4
Caucasian	29.4 ± 5.2	12–38	51	67.1
Gender and GEP score				
Female	30 ± 4.9	19–38	18	23.7
Male	29.3 ± 6.0	12–38	58	76.3
ISHLT 05 grade and GEP score				
0R	29.2 ± 5.7	13–38	51	67.1
1R	29.5 ± 5.9	12–36	22	28.9
2R	33.00	33–33	1	1.3
ND	33.0 ± 7.1	28–38	2	2.6
Prednisone dose (mg/d)	5.1 ± 2.3	1–10	59	77.6
Serum cyclosporine level (ng/mL)	184.9 ± 73.1	81–395	50	65.8
Serum tacrolimus level (ng/mL)	8.6 ± 3.5	1.5–15.2	20	26.3
MMF level (μg/mL)	1.7 ± 1.3	0.5–6.4	55	72.4
Sirolimus level (ng/mL)	11.6 ± 7.3	5.3–25.5	6	7.9
Hemoglobin (g/dL)	12.7 ± 1.6	8.9–17.9	74	97.4
White blood cells (10^9/L)	7.3 ± 2.5	2.5–15.6	74	97.4
Platelets (10^9/L)	227.4 ± 68.6	113–427	74	97.4
Right atrial pressure (mmHg)	6.2 ± 4.6	1–25	74	97.4
Wedge pressure (mmHg)	11.3 ± 6.0	3–32	76	100.0
Mixed venous O_2 saturation (%)	73.5 ± 6.3	50–83.5	76	100.0
Cardiac output (L/min)	6.0 ± 1.5	2.9–11	70	92.1
Cardiac index (L/min/m^2)	3.3 ± 0.8	1.5–5.2	66	86.8
Serum creatinine	1.68 ± 1.34	0.6–12.1	76	100.0
QTc (m/s)	444.2 ± 27.3	385.5–508.3	68	89.5

All statistical comparisons are non-significant.
([r]): median, rank and 25% and 75% quartiles.

count ($r = -0.208$, p = 0.040, n = 72), and HDL ($r = -0.242$, p = 0.041, n = 53). The strongest positive correlation was observed with QTc-interval and GGT and strongest negative correlation with sirolimus level and platelet count (Table 3).

Multivariate analysis

To understand the contribution of each univariate cardiac allograft-related variables to the GEP score, we constructed a linear regression model. All univariately significant parameters (excluding those with <80% completed data points or only significant on one-sided test) were entered into the model. The parameters included into the multivariate linear regression model were serum creatinine, platelet count, O_2Sat, and QTc-interval. To eliminate the colinearity problem, the variables were standardized before entering into the model. The multiple regression coefficient R for the model was 0.582 (goodness of fit, R^2 0.339, adjusted R^2 0.293). The independent predictors were platelet count and QTc-interval (Table 4).

Table 3. Bivariate correlation analysis between the gene expression profiling score and hemodynamic, electrocardiographic, laboratory, pharmacological, and clinical variables

Variable	r	p-value	N
Right atrial pressure	0.119	0.157	74
Pulmonary capillary pressure	0.121	0.149	76
Mixed venous O_2 saturation*	−0.313	0.003[b]	76
Cardiac output	−0.155	0.12	66
Cardiac index	−0.159	0.10	66
ECG QTc*	0.377	0.001[b]	63
ECG PR	0.011	0.467	64
ECG QRS	0.231	0.03[a]	66
ECG heart rate	0.221	0.037[a]	66
Hemoglobin	−0.171	0.07	74
WBC count	−0.031	0.397	74
Monocyte count	−0.208	0.040[a]	72
Lymphocyte count	0.023	0.424	72
Basophil count	−0.048	0.346	72
Neutrophil count	0.035	0.385	72
Eosinophil count	0.099	0.205	72
Platelet count*	−0.372	0.001[b]	74
Serum creatinine*	0.260	0.01[a]	75
Total bilirubin	0.059	0.312	72
Direct bilirubin	0.170	0.077	72
AST	0.094	0.217	72
ALT	0.095	0.213	72
Alkaline phosphatase	−0.025	0.418	72
GGT	0.266	0.037[a]	46
APTT	−0.203	0.225	16
INR	−0.302	0.128	16
Triglycerides	0.02	0.443	53
Cholesterol	−0.002	0.494	54
HDL	−0.242	0.041[a]	53
LDL	0.038	0.397	50
Prednisone dose	−0.114	0.193	60
Mycophenolate mofetil level	−0.193	0.079	55
Cyclosporine level	−0.074	0.301	52
Tacrolimus level	−0.384	0.052	19
Sirolimus level	−0.639	0.044[a]	8

[a]p ≤ 0.05 (one-tailed); [b]p ≤ 0.01 (one-tailed); *p ≤ 0.05 (two-tailed).

Table 4. Multivariate linear regression model including significant variables

Variable (n = 63)	β	SE	Std β	t	Sig. (p)	95% CI (β)
O_2sat	−0.944	0.591	−0.182	−1.596	0.116	−2.127 to 0.240)
Platelet count*	−1.540	0.627	−0.280	−2.455	0.017	−2.796 to −0.284
QTc*	1.998	0.566	0.380	3.532	0.001	0.866 to 3.130
Serum creatinine	1.065	1.343	0.088	0.793	0.431	−1.622 to 3.753

*p ≤ 0.05.

GEP score and QTc-interval

QTc-interval has been described as an important non-invasive tool for the detection of acute cellular cardiac allograft rejection (13). We identified four samples with 2R/3A rejection as a positive control and compared their mean GEP score and QTc-interval with our study cohort of quiescence samples. The mean GEP score in 2R/3A samples was 32 (n = 4) vs. 30 (n = 50) in 0R and mean QTc-interval was 451 (n = 4) vs. 433 (n = 45), respectively. To further understand the correlation between GEP and electrocardiographic parameter of QTc-interval, we looked into a case study displaying a longitudinal data in a 57-yr-old male with dilated non-ischemic cardiomyopathy, who underwent orthotopic heart transplantation in October 2005. The post-operative monitoring was performed using the GEP strategy. The patient had a stable course during the first two yr yet developed graft dysfunction in January 2008. The clinically indicated EMB demonstrated ISHLT 1R/1A mild rejection and absence of antibody-mediated rejection. The coronary angiogram demonstrated absence of coronary artery disease. Therefore, this clinical presentation was considered graft

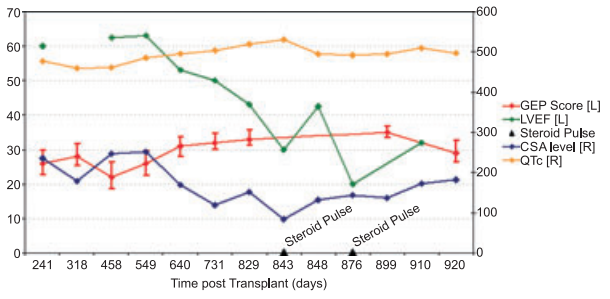

Fig. 1. Case study: post-transplant changes in GEP score, CsA level, QTc, and LVEF in a 57-yr-old male with dilated non-ischemic cardiomyopathy, who was monitored non-invasively using GEP-strategy (left axis scale [L], right axis scale [(R)].

dysfunction of unexplained mechanism and the patient listed for re-transplantation. During the follow-up monitoring, the GEP scores track closely with the QTc parameter on the routine ECG (Fig. 1). The trend of increasing GEP scores which is paralleled by increasing QTc scores is coincident with the drop in left ventricular ejection fraction. Neither cellular nor antibody-mediated rejection as defined by histology or invasive heart catheter results help explained the early deterioration.

Relationship between organ function parameters and individual gene components of the molecular test

In an exploratory *post hoc* analysis, we explored to which extent relationships found with the molecular score would be specifically explained by individual gene components. Among the 11 genes included in the GEP score MIR ($p = 0.009$), WDR40A ($p = 0.005$) and RHOU ($p = 0.021$) were correlated with $MVO_2Sat\%$, WDR40A ($p = 0.036$), and ITGAM ($p = 0.017$) with HR and ITGAM also with QRS duration ($p = 0.029$), PDCD1 with serum creatinine ($p = 0.034$), SEMA7A ($p = 0.037$) and PDCD1 ($p = 0.002$) with monocyte count, G6b ($p = 0.028$) and PF4 ($p = 0.008$) with GGT, RHOU with HDL ($p = 0.021$) and MIR ($p = 0.007$), and WDR40A ($p = 0.001$) to platelet count. Multivariate modeling, controlled for possible confounding variables showed WDR40A and RHOU independently related to $MVO_2Sat\%$, WDR40A, and ITGAM to heart rate, MIR, and WDR40A to platelet count, SEMA7A and PDCD1 to monocyte count, PDCD1 to serum creatinine, GGT to G6B and PF4, and HDL to RHOU. No individual component of the score was predictive of QTc or duration of the QRS interval.

Cardiac allograft vasculopathy and molecular score

Data on cardiac allograft vasculopathy (CAV) were available for 52 out of 76 patients (68.4%). Out of them 10 patients had evidence of CAV. Five patients had non-obstructive CAV and four patients had moderate or severe disease, and one patient had ectasia of the right coronary artery (RCA). The average AlloMap Score in patients with CAV was 29.4 ± 7.63, while in patients without CAV was 30.42 ± 4.93 (p-value 0.29, *t*-test, two-tailed).

Discussion

As the continuum between alloimmunological quiescence and acute cellular cardiac allograft rejection is a systemic process affecting the entire organism (4, 14–20), we hypothesized that scores of a PBMC GEP test developed and validated to rule out ISHLT grade ≥3A/2R acute cellular cardiac allograft rejection are reflected in biologically plausible changes of the routinely assessed clinical parameters. In this retrospective analysis, we found that a GEP score validated to detect the absence of moderate/severe acute cellular cardiac allograft rejection demonstrates a significant correlation with cardiac, renal, hepatic, bone marrow, and lipid metabolism parameters. The electrocardiographic parameter QTc-interval and platelet count were independent predictors of the GEP score. These are novel and interesting findings that have to be carefully interpreted.

To our knowledge, the pilot data presented here provide first evidence that such a link exists between the cardiac allograft and the recipient peripheral leukocytes. Both hemodynamics and QTc-interval have been previously evaluated to detect/predict acute cellular cardiac allograft rejection. Electrophysiologic changes such as QTc-interval may be more sensitive than hemodynamic changes in detecting altered myocardial properties related to myocardial edema. Early studies using intramyocardial electrography critieria of reduced voltage were shown to be sensitive indicators of allograft rejection (13, 19–21).

The relationship between the GEP score and kidney function (serum creatinine), liver function (GGT), bone marrow function (platelets), and lipid metabolism (HDL cholesterol) is complex. In this study, it is important to emphasize that the observed interaction between organ function parameters and GEP score is descriptive and does not, with further study, allow to infer causality in either direction, neither from organ dysfunction toward GEP nor from GEP toward organ dysfunction. A higher GEP score indicating a higher likelihood of rejection was associated with a downregulation of platelet membrane gene PF4 and G6B (22), yet the association with platelet count needs further explanation. It is tempting to speculate that the described association reflects the molecular impact of the rejection process on different system levels. The association of GEP score with HDL cholesterol may reflect a catabolic metabolism started with the proinflammatory mechanisms of allograft rejection.

Limitations of this study include the retrospective design, small number of patients, and modest correlation coefficients. However, we feel that statistically weak correlation may have biologic relevance. Although the existence of a correlation between organ dysfunction and GEP scores is interesting, a cause–effect relationship is difficult to establish. Because of these limitations, these findings need to be investigated further.

Conclusion

In summary, our results suggest that a GEP test developed and validated to detect the absence of cardiac allograft rejection weakly correlates with other biologic parameters, which may reflect an interaction between leukocytes and organ function. A recipient peripheral leukocyte GEP signature related to rejection correlates with organ function at different system levels, in a complex pattern that merits further study. If confirmed, GEP may provide information about the underlying graft physiology within the continuum between alloimmunological quiescence and rejection.

References

1. Billingham ME, Cary NR, Hammond ME et al. A working formulation for the standardization of nomenclature in the diagnosis of heart and lung rejection: Heart Rejection Study Group. The International Society for Heart Transplantation. J Heart Transplant 1990: 9: 587.
2. Stewart S, Winters GL, Fishbein MC et al. Revision of the 1990 working formulation for the standardization of nomenclature in the diagnosis of heart rejection. J Heart Lung Transplant 2005: 24: 1710.
3. Baraldi-Junkins C, Levin HR, Kasper EK, Rayburn BK, Herskowitz A, Baughman KL. Complications of endomyocardial biopsy in heart transplant patients. J Heart Lung Transplant 1993: 12: 63.
4. Deng MC, Erren M, Roeder N et al. T-cell and monocyte subsets, inflammatory molecules, rejection, and hemodynamics early after cardiac transplantation. Transplantation 1998: 65: 1255.
5. Marboe CC, Billingham M, Eisen H et al. Nodular endocardial infiltrates (Quilty lesions) cause significant variability in diagnosis of ISHLT Grade 2 and 3A rejection in cardiac allograft recipients. J Heart Lung Transplant 2005: 24(7 Suppl.): S219.
6. Deng MC, Eisen HJ, Mehra MR et al. Noninvasive discrimination of rejection in cardiac allograft recipients using gene expression profiling. Am J Transplant 2006: 6: 150.
7. Starling RC, Pham M, Valantine H et al. Molecular testing in the management of cardiac transplant recipients: initial clinical experience. J Heart Lung Transplant 2006: 25: 1389.
8. Grande AM, Rinaldi M, D'Armini AM et al. Orthotopic heart transplantation: standard versus bicaval technique. Am J Cardiol 2000: 85: 1329.
9. Schnoor M, Schäfer T, Lühmann D, Sievers HH. Bicaval versus standard technique in orthotopic heart transplantation: a systematic review and meta-analysis. J Thorac Cardiovasc Surg 2007: 134: 1322.
10. Traversi E, Pozzoli M, Grande A, et al. The bicaval anastomosis technique for orthotopic heart transplantation yields better atrial function than the standard technique: an echocardiographic automatic boundary detection study. J Heart Lung Transplant 1998: 17: 1065.
11. Dreyfus G, Jebara V, Mihaileanu S, Carpentier AF. Total orthotopic heart transplantation: an alternative to the standard technique. Ann Thorac Surg 1991: 52: 1181.
12. Deng MC. Cardiac transplantation. Heart 2002: 87: 177.
13. Richartz BM, Radovancevic B, Bologna MT, Frazier OH. Usefulness of the QTc interval in predicting acute allograft rejection. Thorac Cardiovasc Surg 1998: 46: 217.
14. Almeida DR, Carvalho AC, Pessoa C et al. Hemodynamic study with Swan-Ganz catheterization, concomitant to endomyocardial biopsy in heart transplantation patients. Importance in the early diagnosis of rejection. Arq Bras Cardiol 1993: 61: 171.
15. Deng MC, Erren M, Kammerling L et al. The relation of interleukin-6, tumor necrosis factor-alpha, IL-2, and IL-2 receptor levels to cellular rejection, allograft dysfunction, and clinical events early after cardiac transplantation. Transplantation 1995: 60: 1118.
16. Mehra MR, Uber PA, Walther D et al. Gene expression profiles and B-type natriuretic peptide elevation in heart transplantation: more than a hemodynamic marker. Circulation 2006: 114(1 Suppl.): I21.
17. Mena C, Wencker D, Krumholz HM, McNamara RL. et al. Detection of heart transplant rejection in adults by echocardiographic diastolic indices: a systematic review of the literature. J Am Soc Echocardiogr 2006: 19: 1295.
18. Mills RM, Naftel DC, Kirklin JK et al. Heart transplant rejection with hemodynamic compromise: a multiinstitutional study of the role of endomyocardial cellular infiltrate. Cardiac Transplant Research Database. J Heart Lung Transplant 1997: 16: 813.
19. Tenderich G, Jahanyar J, Zittermann A, Schleithoff SS, Wlost S, Körfer R. Predictive value of ECG changes for acute cardiac rejections in heart transplant recipients. Med Klin (Munich) 2006: 101: 99.
20. Vrtovec B, Stojanovic I, Radovancevic R, Yazdanbakhsh AP, Thomas CD, Radovancevic B. Statin-associated QTc interval shortening as prognostic indicator in heart transplant recipients. J Heart Lung Transplant 2006: 25: 234.
21. Warnecke H, Müller J, Cohnert T et al. Clinical heart transplantation without routine endomyocardial biopsy. J Heart Lung Transplant 1992: 11: 1093.
22. Li J, Cadeiras M, Prinz von Bayern M et al. G6b-B cell surface inhibitory receptor expression is highly restricted to CD4+ T-cells and induced by interleukin-4-activated STAT6 pathway. Hum Immunol 2007: 68: 708.