Reprinted from
THE JOURNAL OF
HEART AND LUNG TRANSPLANTATION

A Forum That Includes Basic Science, The Failing Heart, And Advanced Lung Disease

Volume 26 Number 4 **April 2007**

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Transplant Vasculopathy Is Associated With Increased AlloMap Gene Expression Score

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The AlloMap gene expression test is used for the non-invasive detection of rejection in heart transplant recipients. We evaluated the impact of transplant vasculopathy on AlloMap gene expression analysis. A total of 69 heart transplant recipients, mean age 53 years, were evaluated at a mean 35 months post-transplant. AlloMap score was determined on the same day of the endomyocardial biopsies. Twenty patients had evidence of vasculopathy by coronary angiography (vasculopathy group). These were compared to the remaining 49 patients (control group). The vasculopathy group had a longer mean follow-up duration (48.7 vs 28.8 months, $p < 0.01$), lower left ventricular ejection fraction (51% vs 60%, $p < 0.01$) and increased use of sirolimus (40% vs 16%, $p = 0.034$) compared with controls. Using the logistic regression model and bagging bootstrap approach to adjust for the time factor and potential confounders, the vasculopathy group had a significantly higher AlloMap score than the control group (32.2 ± 3.9 vs 26.1 ± 6.5, $p < 0.001$). There was a correlation of AlloMap score with time after transplantation ($r = 0.31$, $p = 0.01$). We found transplant vasculopathy to be associated with increased AlloMap score. J Heart Lung Transplant 2007;26:403-6. Copyright © 2007 by the International Society for Heart and Lung Transplantation.

The AlloMap gene expression test is used for the non-invasive detection of rejection and quiescence in heart transplant recipients.[1] Several factors influence AlloMap score, including time post-transplant, peripheral alloimmune activity, corticosteroid dose and cytomegalovirus infection.[2,3] However, the impact of coronary allograft vasculopathy (CAV) on AlloMap gene expression analysis has not been evaluated until now. We hypothesized that CAV would be associated with increased AlloMap score in the absence of significant rejection.

METHODS

Patient Population

A total of 69 heart transplant recipients, mean age 52 ± 12 years, were evaluated at a mean 35 ± 21 months post-transplant. The AlloMap test (XDx, Inc., South San Francisco, CA) was performed on the same day of the endomyocardial biopsies, which were graded by a blinded cardiac pathologist using revised criteria from the International Society for Heart and Lung Transplantation (ISHLT).[4] Heart biopsies had either no rejection ($n = 27$) or mild rejection (Revised Grade 1, $n = 42$) at the time of AlloMap analysis. Patients with Revised Grade ≥2 rejection on their biopsy were excluded from the analysis to avoid any bias related to rejection. Twenty patients had evidence of CAV by coronary angiography, performed within 4.3 ± 3 months of the AlloMap test. These patients were compared with the remaining 49 patients (the control group). Coronary artery occlusion of ≥50%, after exclusion of donor-transmitted disease, was required for a diagnosis of CAV. The angiographer was blinded to the AlloMap results.

Statistical Analysis

Data are presented as mean ± SD. Categoric variables were compared by chi-square or Fisher's exact test as appropriate. Continuous variables were compared using Student's *t*-test. Differences were considered significant at $p < 0.05$.

AlloMap scores were analyzed using the logistic regression model and bagging bootstrap approach to adjust for the time factor and account for other potential confounders, such as age, gender, rejection episodes, cytomegalovirus (CMV) infection, white blood cell count, creatinine, time post-transplant and immunosuppression (particularly corticosteroid dose). After re-sampling in the study group 200 times, vasculopathy entered into the model 53.5% of the time.

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Submitted June 16, 2006; revised October 10, 2006; accepted December 12, 2006.

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 1053-2498/07/$-see front matter. doi:10.1016/j.healun.2006.12.011

Table 1. Baseline Characteristics

	Control (n = 49)	Vasculopathy (n = 20)	p-value
Donor age (years)	33 ± 12	35 ± 12	NS
Recipient age (years)	52 ± 13	51 ± 12	NS
Male recipient gender	36 (73.4%)	14 (70%)	NS
Etiology of heart disease			
DCM/ICM/restrictive/hypertrophic/congenital	24/20/1/1/3	12/8/0/0/0	NS
LVAD as a bridge to transplant	14 (28%)	4 (20%)	NS
HLA mismatch	5.1 ± 1	5 ± 1	NS
PRA >20%	8 (16.1%)	4 (20%)	NS
PRA T cell	12 ± 20	14 ± 17	NS
PRA B cell	8 ± 14	7 ± 7	NS
Ischemia time (min)	198 ± 36	192 ± 32	NS
CMV status			
$D^+R^+/D^-R^+/D^+R^-/D^-R^-$	14/10/10/15	8/6/3/3	NS
CMV infection requiring treatment	7 (14%)	2 (10%)	NS
Follow-up duration (months)	28.8 ± 15.6	48.7 ± 30	0.0005
Biopsy at time of AlloMap			
Grade 0	20	7	NS
Grade 1R	29	13	NS
Average number of episodes of ACR per patient during the entire follow-up period	1.4 ± 1.5	2.6 ± 2.4	0.02
History of acute vascular rejection	2 (4.1%)	2 (10%)	NS
WBC	6.6 ± 2.4	7.1 ± 1.8	NS
Creatinine (mg/dl)	1.4 ± 0.4	1.6 ± 0.7	NS
Type of immunosuppression			
Prednisone	35 (71.4%)	10 (50%)	NS
Cyclosporine	15 (30.6%)	7 (35%)	NS
Tacrolimus	31 (63.2)	11 (55%)	NS
Mycophenolate mofetil	39 (79.6%)	11 (55%)	0.04
Azathioprine	1 (2%)	0	NS
Sirolimus	8 (16.3%)	8 (40%)	0.034
Everolimus	0	1 (5%)	NS
Dose of immunosuppression			
Prednisone (mg/d)	5.5 ± 3.8	6.0 ± 2.7	NS
Cyclosporine (mg/d)	215 ± 81	179 ± 62	NS
Tacrolimus (mg/d)	5.2 ± 2.8	3.4 ± 2.3	NS
Mycophenolate mofetil (mg/d)	1,942 ± 1017	1,796 ± 920	NS
Azathioprine (mg/d)	50	N/A	N/A
Sirolimus (mg/d)	2.4 ± 0.9	1.6 ± 0.7	NS
Everolimus (mg/d)	2	N/S	N/A
Cyclosporine blood level (ng/ml)	160 ± 79	186 ± 65	NS
Tacrolimus blood level (ng/ml)	8.7 ± 2.8	6.8 ± 3.2	NS
MPA blood level (mg/liter)	2 ± 1.3	1.9 ± 1.1	NS
Sirolimus blood level (ng/ml)	8.2 ± 4.2	8.6 ± 3.7	NS

DCM, dilated cardiomyopathy; ICM, ischemic cardiomyopathy; LVAD, left ventricular assist device; PRA, panel-reactive antibody; CMV, cytomegalovirus; D, donor; R, recipient; ACR, acute cellular rejection; WBC, white blood cell count; MPA, mycophenolic acid.

RESULTS

Both groups had similar baseline characteristics, except for a longer follow-up duration in the CAV group, and thus an increase number of episodes of acute rejection over the follow-up period (Table 1). In addition, because of CAV, an increased use of sirolimus was noted in this group. The different immunosuppressant agents are shown in Table 1. As expected, the CAV group had a lower left ventricular ejection fraction and a larger number of patients with graft dysfunction, compared with controls (Table 2). A higher percentage of patients in the CAV group had an AlloMap score of >30 when compared with controls (Table 2). There was a correlation (weak) of AlloMap score with time post-transplantation (Figure 1).

Table 2. AlloMap Score and Left Ventricular Function

	Control (n = 49)	Vasculopathy (n = 20)	p-value
Mean AlloMap score	26.1 ± 6.5	>32.2 ± 3.9	<0.001
AlloMap score >30	15 (30%)	14 (70%)	0.0026
Mean LVEF (%)	60 ± 5	51 ± 10	<0.0001
Number of patients with EF <45%	0	4 (20%)	0.005

LVEF, left ventricular ejection fraction.

Using the logistic regression model and bagging bootstrap approach, CAV was still associated with a significantly increased AlloMap score (p = 0.0002) in the absence of significant rejection.

DISCUSSION

The main finding of this study is that patients with CAV have a significantly increased AlloMap gene expression score. CAV remains a major cause of morbidity and mortality in patients surviving beyond the first year after cardiac transplantation. Both immune and non-immune mechanisms have been implicated in the pathogenesis of CAV.[5] However, the exact molecular mechanisms underlying the progression of CAV are unknown. CAV affects 40% to 60% of transplant recipients by 5 years based on coronary angiography findings.[6] However, intravascular ultrasound (IVUS) detected an abnormal coronary intimal thickness in about 50% of patients as early as 1 year after cardiac transplantation.[7] Several studies have attempted to identify functional, molecular and cellular surrogate markers of vasculopathy to monitor disease activity. Some of these markers are related to the extracellular matrix, the renin-angiotensin system, the fibrinolytic system, adhesion receptors and markers of inflammation.[5] However, the clinical utility of these markers has to yet to be confirmed and validated in large clinical trials. The AlloMap test has been a helpful non-invasive diagnostic tool in the management of cardiac transplant recipients, including anticipation of future allograft rejection and dysfunction.[8]

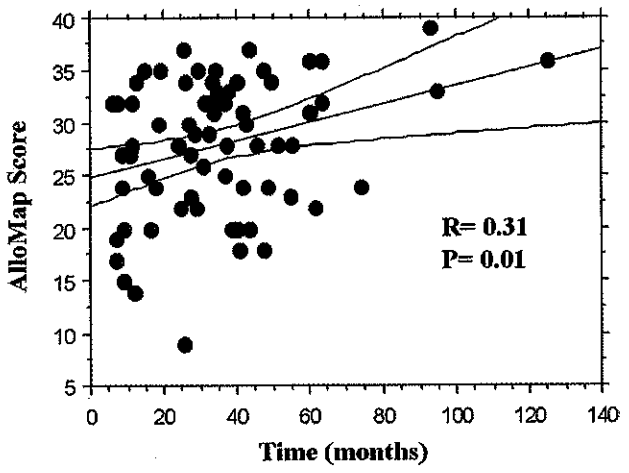

Figure 1. Correlation between AlloMap score and time post-transplant.

It is clear from the CARGO (Cardiac Allograft Rejection Gene Expression Observational) study that AlloMap scores increase with time post-transplant while the risk of rejection decreases.[1] A similar finding was seen in the present study, where we noted a correlation between AlloMap score and time post-transplant. Corticosteroid dose is also known to influence AlloMap score[3] and this variable was included in our statistical analysis. The AlloMap was done on the same day of the heart biopsy and we excluded patients who had significant rejection (Revised Grade ≥2) on their biopsies, thus eliminating any possible bias that could be influenced by acute rejection. Our findings unmask another important variable that may influence AlloMap gene expression analysis, specifically presence of CAV, and therefore may provide another explanation for a high score with a negative biopsy for rejection.

The main limitation of this study is its retrospective nature. Intravascular ultrasound was not performed in our analysis. CAV was diagnosed by angiography, which is the standard at most institutions. Another limitation is related to the inherent differences in baseline characteristics, but this was accounted for in our statistical analysis. Prospective studies will be required to determine the predictive capacity of the AlloMap test in identifying patients at high risk for CAV without concomitant allograft rejection.

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