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Predictive value of gene expression profiling for long-term survival after heart transplantation

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ABSTRACT

Objectives: Identifying patients at risk for impaired long-term survival after heart transplantation (HT) remains a clinical challenge. The aim of this analysis was to investigate whether the gene expression profiling test AlloMap® is related to long-term survival after HT.

Methods: 46 patients who underwent HT between 2006 and 2007 who were originally included into the CARGO II trial at our institution were investigated. Patients were divided in two groups according to an increase or decrease of the AlloMap® score between 6 and 9 months after HT. The primary endpoint of this study was long-term all-cause mortality.

Results: 23 patients showed an increase of the AlloMap® score between 6 and 9 months after HT whereas the remaining 23 patients presented with a decrease of the score. After a median follow-up time of 8.1 years (interquartile range 7.6–8.6), all-cause mortality was significantly elevated in patients with an AlloMap® increase compared with patients who showed a decrease of the score (log-rank $p = 0.005$). A ratio of the AlloMap® at 9 months to 6 months of 1.02 or less was associated with a negative predictive value for all-cause mortality of 100%.

Conclusions: Dynamic changes of the AlloMap® score between 6 and 9 months after HT were strongly related to all-cause long-term survival after HT. These results suggest that AlloMap® potentially displays a useful tool to estimate the patients' risk for long-term mortality.

1. Introduction

Cardiovascular disease is a major epidemic health burden accounting for almost 5 million deaths per year in Europe and the United States [1,2]. For patients with end stage heart failure refractory to optimal medical therapy (OMT), orthotopic heart transplantation (HT) remains the only definitive treatment option [3,4]. Results after HT have improved over time, largely due to improved peri- and postoperative management including more effective immunosuppressive protocols. This has led to a substantially reduced amount of acute allograft rejection episodes. However, chronic allograft rejection of heart transplants with its hallmark feature cardiac allograft vasculopathy (CAV) - a progressive luminal narrowing of the entire coronary vascular tree - still remains a substantial concern accounting for >13% of deaths >5 years after HT [5].

The gold standard for surveillance of graft rejection after HT is the endomyocardial biopsy (EMB). However, obtaining an EMB sample is invasive, uncomfortable and constitutes a potential risk for the patient [6]. Complications during or following EMB are relatively rare but can be associated with significant consequences for the patient. In addition, Crespo-Leiro et al. found a considerable variance among interpretations of biopsy samples by independent pathologists [7]. Furthermore, EMB does not allow for a definite exclusion of rejection. These findings emphasize the need for additional, reliable and less invasive surveillance tools for cardiac allograft rejection.

AlloMap® is a gene expression profiling test that quantifies the expression of selected genes in circulating leukocytes utilizing a single venous blood sample [8]. It was introduced to address above mentioned limitations associated with rejection surveillance through EMB. AlloMap® translates the gene expression of 20 genes into a score ranging from 0 to 40 with low scores reflecting a low risk for rejection [8]. In the initial *Cardiac Allograft Rejection Gene Expression Observational* (CARGO) trial, the AlloMap® was shown to adequately predict the absence from severe graft rejection [8]. Moreover, in the time period >-6 months after HT, an AlloMap® score < 27 was associated with a negative predictive value of 100%. In this population, this affected more than a quarter of all patients therefore representing a substantial amount of patients in whom EMB could potentially be spared. In

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addition, 499 patients confirmed the results of the initial CARGO study similarly revealing a high negative predictive value (95.5%) for graft rejection in patients >6 months after HT [9]. The *Invasive Monitoring Attenuation through Gene Expression* (IMAGE) study showed that monitoring for graft rejection primarily based on AlloMap® was not inferior to the standard surveillance protocol with regular EMBs regarding survival and the occurrence of adverse events [10]. As a consequence of these findings, utilization of AlloMap® was included in the most recent *International Society of Heart and Lung Transplantation* (ISHLT) Guidelines for the Care of Heart Transplant Recipients [11].

So far, studies have focused on investigating the usefulness of AlloMap® to predict the absence from graft rejection at the time of AlloMap® scoring. Little is known about the potential of the AlloMap® score to predict the future clinical course of the patient. We hypothesized that AlloMap® scores correlate with long-term outcomes of patients after HT and aimed to investigate this hypothesis within the current study.

2. Materials and methods

2.1. Study population

124 adult end stage heart failure patients who consecutively underwent HT between February 2006 and November 2007 at our institution were identified. Of those, patients who were originally included in the CARGO II trial were identified. For the present analysis we included patients who survived at least one year after HT and in whom the AlloMap® score was available at 6, 9, 12 and 18 months after HT. The final study population comprised $n = 46$ patients.

To analyse the potential prognostic relevance of the AlloMap® score for long-term survival, we investigated two strategies: 1) The isolated AlloMap® score at 6, 9, 12 and 18 months post-HT was analyzed for correlations with long-term survival and 2) Dynamic changes of AlloMap® scores during these time intervals were investigated. A dynamic change of the AlloMap® score was defined as any increase or decrease of AlloMap® scores between the defined time intervals. The primary endpoint of this analysis was long-term all-cause mortality and the secondary endpoint was long-term rejection-related mortality. Survival data were gathered upon review of medical records and personal contact with the patients, their relatives or their general practitioners.

2.2. Sample collection and processing

Blood collection and processing was performed according to the CARGO II study protocol which was published previously [9,12]. Briefly, 8 mL of venous blood was withdrawn from the patient into a CPT tube (BD Vacutainer®, Becton Dickinson, NJ, USA) containing sodium citrate. Mononuclear cells were isolated using density gradient centrifugation (1750 g, 15 min at room temperature). The cells were lysed using RLT Buffer (Qiagen) and extracted RNA was snap frozen at −70 °C. After shipment to the CareDx reference laboratory, the lysate was further purified and the cDNA synthetized. The expression of the genes of interest was quantified using real time PCR. The detailed RT-PCR protocol was published previously [8].

2.3. Statistical analysis

All numerical data are given as median (25.-75- percentile) due to non-normal distribution. Differences between groups were compared using the Mann-Whitney-*U* test. Categorical variables are presented as the absolute number and percentages and differences between groups were compared with the Chi-square test or Fisher's exact test where appropiate. Long-term survival was estimated with the Kaplan-Meier method and compared between groups with the log-rank test. All statistical analyses were performed with IBM SPSS Statistics for Windows, Version 22.0., Armonk, NY: IBM Corp.

Table 1
Baseline characteristics of the study population.

	AlloMap® 6 M < 9 M ($n = 23$)	AlloMap® 6 M > 9 M ($n = 23$)	*p*-Value
Age (years)	48 (25–58)	53 (39–65)	0.18
Female (n/%)	5 (21.7%)	5 (21.7%)	1.0
Predominant cardiac diagnosis			
DCM (n/%)	15 (65.2%)	11 (47.8%)	0.23
ICM (n/%)	7 (30.4%)	12 (52.2%)	
other (n/%)	1 (4.3%)	0	
VAD before HT (n/%)	6 (26.1%)	8 (34.8%)	0.52
Gender mismatch (n/%)	12 (52.2%)	10 (43.5%)	0.56
CMV status			
Donor and recipient negative (n/%)	4 (17.4%)	8 (34.8%)	0.31
Donor and recipient positive (n/%)	5 (21.7%)	7 (30.4%)	0.50
Donor positive, recipient negative (n/%)	6 (26.1%)	6 (26.1%)	1.0
Donor negative, recipient positive (n/%)	8 (34.8%)	2 (8.7%)	0.04
CMV therapy (n/%)	3 (13%)	6 (26.1%)	0.46
Donor age (years)	35 (24–44)	43 (34–49)	0.09
Prednisolone before 6 months	22 (95.7%)	23 (100%)	1.0
Prednisolone between 6 and 9 months	22 (95.7%)	22 (95.7%)	1.0
Tacrolimus before 6 months	18 (78.3%)	20 (87.0%)	0.70
Tacrolimus between 6 and 9 months	18 (78.3%)	20 (87.0%)	0.70
Cyclosporine before 6 months	5 (21.7%)	2 (8.7%)	0.41
Cyclosporine between 6 and 9 months	5 (21.7%)	2 (8.7%)	0.41
Mycophenolate before 6 months	18 (78.3%)	15 (65.2%)	0.33
Mycophenolate between 6 and 9 months	18 (78.3%)	15 (65.2%)	0.33
Sirolimus before 6 months	1 (4.3%)	2 (8.7%)	1.0
Sirolimus between 6 and 9 months	1 (4.3%)	2 (8.7%)	1.0
Everolimus before 6 months	0	0	–
Everolimus between 6 and 9 months	0	0	–
ASS before 6 months	7 (30.4%)	4 (17.4%)	0.49
ASS between 6 and 9 months	7 (30.4%)	4 (17.4%)	0.49
Clopidogrel before 6 months	0	0	–
Clopidogrel between 6 and 9 months	0	0	–