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Gene expression profiling scores in dual organ transplant patients are similar to those in heart-only recipients

Sandra A. Carey[a,b,1], Kristen M. Tecson[c], Aayla K. Jamil[b], Joost Felius[b], Theresa K. Wolf-Doty[d], Shelley A. Hall[a,b,*]

[a] Center for Advanced Heart and Lung Disease, Baylor University Medical Center, 3410 Worth St., Suite 250, Dallas, TX 75246, USA
[b] Annette C. and Harold C. Simmons Transplant Institute, Baylor Scott & White Research Institute, 3410 Worth St., Suite 560, Dallas, TX 75246, USA
[c] Baylor Heart and Vascular Institute, Baylor Scott & White Research Institute, 621 Hall St., Dallas, TX 75246, USA
[d] CareDx, 3260 Bayshore Blvd., Brisbane, CA 94005, USA

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ABSTRACT

Background: Serial gene expression profiling (GEP) may reduce the need for endomyocardial biopsies for detecting acute cellular rejection (ACR) after transplantation, but its performance in dual organ transplant recipients is currently unknown.

Methods: We analyzed 18 months of follow-up in a national cohort of 27 dual organ recipients (18 heart-kidney, 8 heart-liver, 1 heart-lung) matched to 54 heart-only recipients for gender, age, and time to first GEP (AlloMap®) test. ACR, antibody-mediated rejection (AMR), cytomegalovirus infections, biopsies, and longitudinal GEP scores were evaluated.

Results: During the first 90 days post-transplant, the mean GEP score for dual organ recipients was 25.2 ± 9.1, vs. 23.5 ± 7.7 for heart-only recipients (P = 0.48), with final GEP scores being 29.1 ± 6.1 and 32.3 ± 3.4, respectively (P = 0.34). GEP scores increased over time (P < 0.001) at a similar rate (P = 0.33) for both groups. One heart-only recipient had treated ACR (GEP score = 17). Fourteen subjects had cytomegalovirus infection, 8 of whom were dual-organ. During follow-up, mean GEP score among patients with cytomegalovirus infection was 32.3, compared to 26.7 (p < 0.001) in patients without cytomegalovirus. Only 4 (2%) of 233 biopsies were positive for mild AMR; all occurring in 2 heart-only recipients (GEP scores = 18–33).

Conclusions: This largest cohort to date suggests that dual organ transplantation alone should not be reason to omit GEP testing from post-transplant medical management, as the two groups' scores did not differ significantly. Confirming that GEP scores increase over time for heart-only and dual organ recipients and in the presence of cytomegalovirus infection, our work shows promise for the use of serial GEP testing in dual organ recipients.

1. Introduction

Despite advances in modern transplantation procedures and medical management, acute cellular rejection (ACR) remains a significant concern for organ recipients. Traditionally, cellular rejection could only be identified via invasive endomyocardial biopsy. In 2010, however, a non-invasive gene expression profiling (GEP) test from CareDx, AlloMap®, was proven to be non-inferior to routine endomyocardial biopsy for identifying the absence of cellular rejection during post-heart transplantation surveillance in patients with stable allograft function [1,2]. Since this finding, serial GEP testing has been widely utilized across multiple cardiac transplant centers internationally to aid clinicians in discerning whether or not heart transplant recipients are at risk for ACR [3–7]. Ultimately, the validation and acceptance of GEP testing will lower the need for biopsies. While accepted in the heart-only transplant community, the role of GEP testing in dual organ transplant recipients has yet to be established. Not only may the true rate of ACR be different in dual organ transplant recipients compared to heart-only transplantation [8], the magnitude of the GEP scores [9] and their rate of increase over time may also be different in this population.

2. Objective

We hypothesized that serial GEP testing in dual organ recipients

* Corresponding author at: Center for Advanced Heart and Lung Disease, Baylor University Medical Center, 3410 Worth St., Suite 250, Dallas, TX 75246, USA.
E-mail addresses: Sandra.Carey@Abbott.com (S.A. Carey), Kristen.Tecson@BSWHealth.org (K.M. Tecson), Aayla.Jamil@BSWHealth.org (A.K. Jamil), Joost.Felius@BSWHealth.org (J. Felius), TWolf@CareDx.com (T.K. Wolf-Doty), Shelley.Hall@BSWHealth.org (S.A. Hall).
[1] Present address: Abbott, St. Jude Medical Dr., St. Paul, MN 55117, USA

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yields results comparable to those in heart-only transplant patients. Thus, we sought to investigate the pattern of dual organ recipients' serial GEP scores, determine its relationship to clinical endpoints, including treated ACR, cytomegalovirus (CMV) viremia, and antibody-mediated rejection (AMR), and compare the results to those from heart-only transplant recipients.

3. Materials and methods

This is a retrospective study approved by the Institutional Review Board of the Baylor University Medical Center. Approximately 18 months of follow-up data, including the results of GEP testing for patients transplanted between September 2007 and July 2016, were extracted from the Annette C. and Harold C. Simmons Transplant Institute's registry and from the national Outcomes AlloMap Registry (OAR) maintained by CareDx (Brisbane, CA). This multi-center, national cohort was assembled by matching heart-only recipients (controls) to dual organ recipients (cases) based on gender, age (± 5 years), and the time from transplantation to first GEP test (± 21 days) in a ratio of 2:1.

The process for AlloMap GEP testing has been described elsewhere [3]. Briefly, 1 cell preparation tube of blood is collected from the transplant recipient, centrifuged to isolate the peripheral blood mononuclear cells, processed to release RNA, and preserved at −15 °C. The preserved samples are sent to the CareDx laboratories via overnight shipment for analysis. There, after purification, RNA is reverse-transcribed into complementary DNA, which is added to each of 60 wells containing gene-specific primers and probes. The test uses real-time polymerase chain reaction (RT-PCR) to quantitate expression levels of a preselected panel of 20 genes: 11 genes informative about allograft rejection and 9 genes for normalization and quality control, used in the calculation of a logarithmic GEP score ranging from 0 to 39.

Due to varying standards with respect to time points of GEP testing across centers, a method to standardize testing time was essential. The 18 months of follow-up data were segmented into 6 quarters, each comprised of 90 days. For patients with more than one GEP score in a given quarter, the average score for that quarter was recorded. Only 25% of subjects had 2 or more scores in a given quarter, indicating that only a small amount of information was lost with this synchronization method. To ascertain the performance of GEP in the dual organ transplant community, we performed several analyses. Specifically, we developed linear mixed models to assess changes in GEP scores over time in order to compare serial GEP scores between dual and single organ recipients and to compare serial scores among those with and without CMV infections. CMV infection was treated as a time-varying covariate, as the presence or absence of CMV infection fluctuated over time. In the instance that a subject had conflicting CMV infection statuses per quarter, the data were treated as if there was an infection for that quarter and the single corresponding GEP score was used. AMR was monitored and graded following standard of care.

4. Results

There were 54 heart-only recipients matched to 27 dual organ recipients, yielding a total sample size of 81 (Table 1). Of the 27 dual organ recipients, 18 (67%) had heart-kidney transplants, 8 (30%) had heart-liver transplants, and 1 (4%) had a heart-lung transplant. Table 1 shows that the control group was well matched with the dual-organ sample, which consisted of 63% males and had a median age of 57 (25th percentile, 48; 75th percentile, 61) years and median time to first GEP test of 87 (74; 161) days.

In the 1st quarter following transplant, the average GEP score for dual organ recipients was 25.2 ± 9.1, compared to 23.5 ± 7.7 for heart-only recipients (P = 0.48). In the final (6th) quarter of the follow-up period, the average GEP score for dual organ recipients was 29.1 ± 6.1, compared to 32.3 ± 3.4 for heart-only recipients (P = 0.34). The linear mixed model assessing serial GEP scores between transplant groups yielded an intercept (first-quarter estimate) of 22.5 points for the heart-only recipients' and 24.6 points for the dual organ recipients (P = 0.32). The estimated rate of change in GEP scores for dual organ recipients was 1.0 (points per quarter) and 1.6 for heart-only recipients (P = 0.36). Hence, the initial GEP scores did not differ significantly between those who received 1 or 2 organs, nor did their rate of change (Fig. 1). However, the GEP scores significantly increased over time for both transplant groups ($P < 0.001$).

Table 1
Sample characteristics (n = 81).

Variable	Dual organ Transplant (n = 27)	Heart-only Transplant (n = 54)	P-value
Age (y)	57 [48, 61]	56.5 [48, 62]	0.10
Body mass index (kg/m^2)	26.6 ± 5.6 (n = 17)	28.7 ± 4.8 (n = 47)	0.06
Race/ethnicity			0.25
Caucasian	14 (52%)	36 (67%)	
Black	13 (48%)	14 (26%)	
Hispanic	0 (0%)	4 (7%)	
Male recipient	17 (63%)	34 (63%)	NA
Male donor	14 (58%) (n = 24)	25 (58%) (n = 43)	0.57
Positive recipient CMV serology	16 (64%) (n = 25)	32 (63%) (n = 51)	0.71
Mismatched CMV serology	6 (22%)	9 (18%) (n = 51)	0.70
Time to first GEP test (d)	87 [74, 161]	85 [75, 160]	0.60
First GEP score	28 [20, 34]	25.5 [19, 29]	0.51
1st quarter GEP score	25.2 ± 9.1 (n = 15)	23.5 ± 7.7 (n = 30)	0.48
2nd quarter GEP score	27.2 ± 7.8 (n = 20)	26.1 ± 5.1 (n = 38)	0.60
3rd quarter GEP score	27.4 ± 5.6 (n = 15)	28.2 ± 5.1 (n = 28)	1.00
4th quarter GEP score	30.3 ± 4.0 (n = 15)	30.5 [27.3, 32.5] (n = 25)	0.26
5th quarter GEP score	30.4 ± 6.4 (n = 12)	28.8 ± 5.5 (n = 17)	0.80
6th quarter GEP score	29.1 ± 6.1 (n = 11)	32.3 ± 3.4 (n = 13)	0.34
Organ transplanted:			NA
Heart-only	–	68 (100%)	
Heart + kidney	18 (67%)	–	
Heart + liver	8 (30%)	–	
Heart + lung	1 (4%)	–	

Comparing the largest subgroup of dual organ recipients (heart-kidney, n = 18) to their matched heart-only recipients (n = 36) yielded a first-quarter estimate of 25.0 points for heart-kidney recipients and 22.2 points for heart-only patients (P = 0.34). The slope estimate (change in GEP) was 1.3 points per quarter for heart-kidney recipients and 1.5 points per quarter for heart-only recipients (P = 0.76). Hence, neither the starting point nor the rate of change in GEP scores differed significantly between those who received a heart-only or heart-kidney transplant (Fig. 2).

Only 1 patient had treated ACR (grade 1R), which occurred in the second quarter (between 91 and 180 days post-transplant); this person was a heart-only recipient and had a GEP score of 17 at the nearest GEP test (measured following the treatment for rejection). One heart-only recipient died in the 2nd quarter, 112 days after transplant (1.2% overall, 1.9% of the heart-only group), with rejection of the transplanted heart provided as the cause of death. Her closest GEP score to death was 27 and her grade of rejection was 1R, both of which were determined 85 days following transplant (27 days prior to death).

During the 18 months following transplantation, 14 subjects developed a CMV infection, 8 (57%) of whom were dual-organ recipients. Throughout the follow-up, the overall median of GEP scores corresponding to CMV infections in any quarter was 34 (28; 35.5); the overall median corresponding to CMV-free GEP scores was 28.3 (24; 31.3). Of the 8 dual-organ recipients with CMV, 7 (88%) received a heart-kidney transplant, and the remaining subject received a heart-