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The Journal of Heart and Lung Transplantation

http://www.jhltonline.org

Gene expression profiling to study racial differences after heart transplantation

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KEYWORDS:
heart transplantation;
race;
acute rejection;
mortality;
calcineurin inhibitor;
gene expression profile score

BACKGROUND: The basis for increased mortality after heart transplantation in African Americans and other non-Caucasian racial groups is poorly defined. We hypothesized that increased risk of adverse events is driven by biologic factors. To test this hypothesis in the Invasive Monitoring Attenuation through Gene Expression (IMAGE) study, we determined whether the event rate of the primary outcome of acute rejection, graft dysfunction, death, or retransplantation varied by race as a function of calcineurin inhibitor (CNI) levels and gene expression profile (GEP) scores.

METHODS: We determined the event rate of the primary outcome, comparing racial groups, stratified by time after transplant. Logistic regression was used to compute the relative risk across racial groups, and linear modeling was used to measure the dependence of CNI levels and GEP score on race.

RESULTS: In 580 patients monitored for a median of 19 months, the incidence of the primary end point was 18.3% in African Americans, 22.2% in other non-Caucasians, and 8.5% in Caucasians ($p < 0.001$). There were small but significant correlations of race and tacrolimus trough levels to the GEP score. Tacrolimus levels were similar among the races. Of patients receiving tacrolimus, other non-Caucasians had higher GEP scores than the other racial groups. African American recipients demonstrated a unique decrease in expression of the *FLT3* gene in response to higher tacrolimus levels.

CONCLUSIONS: African Americans and other non-Caucasian heart transplant recipients were 2.5-times to 3-times more likely than Caucasians to experience outcome events in the Invasive Monitoring Attenuation through Gene Expression study. The increased risk of adverse outcomes may be partly due to the biology of the alloimmune response, which is less effectively inhibited at similar tacrolimus levels in minority racial groups.

J Heart Lung Transplant 2015;34:970–977

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Racial disparities in survival after solid organ transplantation were first recognized by Opelz and Terasaki[1] in 1977 when large differences between African American and Caucasian recipients were identified after kidney transplantation. Multiple reports since then have demonstrated

1053-2498/$ - see front matter
http://dx.doi.org/10.1016/j.healun.2015.01.987

worse survival after heart transplantation in African American recipients compared with other racial groups.[2–7] Many reasons have been suggested to account for racial disparities in post-transplant outcomes. These include socioeconomic and educational factors,[2] access to high-quality medical care,[4] compliance, a higher prevalence of comorbidities, such as hypertension, in African American recipients,[8] and fundamental immunologic differences.[9,10]

The Invasive Monitoring Attenuation through Gene Expression (IMAGE) study,[11] which examined the clinical utility of monitoring for acute rejection after heart transplantation using peripheral blood gene expression profiling, provided a unique opportunity to study the biology of racial differences in heart transplant outcomes. We sought to determine whether the incidence of acute rejection, graft dysfunction, death, or retransplantation varied according to race and to elucidate observed racial disparities in outcomes on the basis of immunosuppressive (calcineurin-inhibitor [CNI]) drug levels and peripheral blood gene expression patterns.

Methods

Study design, patients, and procedures

The IMAGE study was a randomized trial conducted at 13 United States heart transplant centers between January 2005 and October 2009. The study design and procedures have been described previously.[11,12] Adult heart transplant recipients, between 6 months and 5 years after transplant, were eligible for enrollment and were randomly assigned to undergo monitoring for rejection by means of gene expression profiling (GEP) or specimens obtained from routine endomyocardial biopsies (EMBs). Blood samples were also taken for GEP testing from patients assigned to the EMB group.

This study population of 602 patients consisted of 12% African Americans and 6% other non-Caucasian participants. Compliance with medical therapies and rejection surveillance was closely monitored throughout the study period by trial coordinators. Data on medications and laboratory results enabled us to examine differences in immunosuppressive drug doses and CNI trough blood levels among racial groups. Finally, data on composite gene expression (AlloMap) scores and expression of each of the 11 genes comprising this test were available. These individual genes were originally selected to distinguish immune activation associated with acute rejection from a quiescent state. Analysis of IMAGE study data thereby enabled us to correlate clinical outcomes in different races with individual gene expression, which may provide insight into the biology of racial differences in transplant outcomes.

Gene expression testing was performed with the use of the AlloMap test (CareDx), which evaluates expression levels of 11 informative genes that were shown by previous studies to distinguish between rejection and the absence of rejection.[13] Possible scores range from 0 to 40, with higher scores indicating a higher likelihood of histologic rejection. Patients were monitored for a maximum of 24 months, until they died, or until the study completion date, whichever occurred first.

Outcomes

The primary IMAGE trial outcome was the first occurrence of rejection with hemodynamic compromise, graft dysfunction due to other causes, retransplantation, or death. Rejection with hemodynamic compromise was defined as one or more of the following criteria: absolute drop in left ventricular ejection fraction $\leq 30\%$, proportional decrease in left ventricular ejection fraction $\geq 25\%$ compared with the first study visit, cardiac index <2 liters/min/m^2, and/or use of inotropic drugs to support circulation at the time of the rejection episode. Death from any cause was considered a secondary outcome.

Statistical analysis

For the current analysis, 580 patients who had at least one AlloMap score were studied. The patients were assigned to 1 of 3 groups by self-identified race as Caucasian, African American, and other non-Caucasian, where the other non-Caucasian category includes predominantly Hispanic, Asian, and Pacific Islander populations. Within each group, study visits were classified by the time period after transplant: 7 to 12 months, 13 to 36 months, and $>$ 36 months. Demographics (age, racial group, cytomegalovirus [CMV] status, and number of rejections in the first year) of each racial group were assessed for the sub-set of patients with visits in each time period. CMV status was coded as a binary variable: 1 for donor positive/recipient negative and 0 otherwise. For demographic variables, each of 3 pairwise comparisons between the racial groups were performed using the Tukey test for continuous variables and Fisher's test with correction for multiple hypothesis testing by the Bonferroni method for discrete variables.

Racial differences in event rates

Event-free progression after study entry, within each racial group, was assessed using Cox modeling. Event rates and death rates for each racial group in each time period were calculated by dividing the total number of events observed during that time by the total length of time patients were monitored.

Racial differences in CNI levels

Only visits at which patients were taking a CNI, either tacrolimus or cyclosporine, were included. CNI trough levels for each patient's visits falling within a particular time period were averaged to generate a summary for that patient and time period. Then, the patient averages were summarized across each racial group. When drug levels were compared between racial groups, pairwise comparisons, overall and within the 1-year to 3-year time period, were performed using the Tukey test. We chose to focus on Years 1 to 3 after transplant because most study visits occurred during this time period.

Doses of prednisone $>$ 20 mg/day and those administered for treatment of acute rejection were excluded from analyses of maintenance corticosteroid dosing.

Racial differences in GEP

GEP scores for patient visits falling within a particular time period were averaged to generate a summary for that patient and time period; patient averages were then summarized across each racial group. To compare GEP scores between racial groups, pairwise comparisons, overall and within the 1-year to 3-year time period, were performed using the Tukey test.

To study the determinants of GEP score, a multivariate model was fit on 435 patients treated with tacrolimus, with tacrolimus trough level, prednisone dose, racial group, time post-transplant, age, sex, and CMV status as predictors. Univariate models were