FEATURED ARTICLE

Benefit of immune monitoring in heart transplant patients using ATP production in activated lymphocytes

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BACKGROUND: Balancing immunosuppression to prevent rejection while minimizing infection or drug toxicity risk is a major challenge in heart transplantation. Therapeutic drug monitoring alone is inadequate to measure the immune response. An immune monitoring (IM) assay (ImmuKnow; Cylex, Columbia, MD) performed on peripheral blood measures adenosine triphosphatase (ATP) release from activated lymphocytes and may predict the immune state. Therefore, we sought to determine the utility of IM in heart transplant recipients.

METHODS: Between November 2005 and July 2008, 296 heart transplant recipients had a total of 864 IM assays performed at 2 weeks to 10 years post-transplant and were correlated with infection and rejection events that occurred within 1 month after IM testing. All patients received standard triple-drug immunosuppressive therapy with tacrolimus, mycophenolate mofetil and corticosteroids, without induction therapy.

RESULTS: There were 38 infectious episodes and 8 rejection episodes. The average IM score was significantly lower during infection than steady state (187 vs 280 ng ATP/ml, \( p \) \(<\) 0.001). The average IM score was not significantly different during rejection when compared with steady state (327 vs 280 ng ATP/ml, \( p \) = 0.35). Interestingly, 3 of 8 rejection episodes were antibody-mediated rejections and had hemodynamic compromise and, for these, the mean IM score was significantly higher than for steady-state patients (491 vs 280 ng ATP/ml, \( p \) < 0.001).

CONCLUSIONS: The non-invasive IM test appears to predict infectious risk in heart transplant patients. The association between high IM scores and rejection risk is inconclusive due to the small number of rejection episodes. Further studies with larger sample sizes for rejection episodes are required.

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Heart transplantation is the preferred treatment for select patients with end-stage heart disease.1 The greatest impediments to survival in heart transplant recipients in the first year post-transplant are infection and rejection, whereas, over time, transplant coronary artery disease (a form of chronic rejection) and malignancy (resulting from inadequate immune surveillance of neoplastic cells) emerge as threats to long-term survival.1 These problems highlight the challenge of balancing the immune response in transplant recipients.

The primary goal of immunosuppression in transplant recipients is to achieve a state of quiescence. However, over-immunosuppression will result in an increased risk of...
infection and malignancy, whereas under-immunosuppression will result in an increased risk of rejection and transplant coronary artery disease. One of the major challenges of transplant management is measuring the effect of immunosuppressive therapy on the transplant recipient’s immune response. Drug monitoring values are not enough, and their primary value is for preventing drug toxicity, not in determining the immune response state and risk for rejection or infection.\textsuperscript{2}

An immune monitoring (IM) assay (ImmuKnow; Cylex, Columbia, MD) has been approved by the U.S. Food and Drug Administration for the detection of cell-mediated immunity in an immunosuppressed population.\textsuperscript{3} IM measures adenosine triphosphatase (ATP) release from activated lymphocytes and therefore correlates with the level of immune responsiveness. It has shown promise in assessing infection and rejection risk in solid-organ transplants,\textsuperscript{4} including kidney,\textsuperscript{5,6} pancreas\textsuperscript{7} and small bowel.\textsuperscript{8} However, in a recent study by Gupta et al, involving 111 heart transplant patients, scores did not correlate with infection \((n = 7)\) or rejection \((n = 1)\).\textsuperscript{9} The purpose of the current study was to investigate the utility of this IM assay in a large number of heart transplant recipients at our institution.

Methods

Patient population

Between November 2005 and July 2008, 337 heart transplant patients had 1,187 IM assays performed between 2 weeks and 10 years post-transplant. Of these, 323 IM samples from 41 patients were excluded due to an episode of infection or rejection that occurred within 1 month prior to the IM assay. The remaining 864 IM assays from 296 patients were used for analysis. All patients were treated with triple-drug immunosuppression (tacrolimus, mycophenolate, and corticosteroids) without induction therapy. Institutional review board (IRB) approval was obtained for the study.

Episodes of infection and rejection

IM scores were correlated with infection or rejection episodes that occurred within 1 month after IM testing. Infection episodes \((n = 38)\) were defined as those diagnosed by the treating physician and resulting in antibiotic therapy. Rejection \((n = 8)\) was defined as any treated episode of cellular or antibody-mediated rejection, with or without hemodynamic compromise (defined as left ventricular ejection fraction \(\leq 40\), cardiac index \(< 2.0\ \text{liters/min/m}^2\) and need for inotropic support). In our standard practice, only moderate or severe cellular rejection seen on endomyocardial biopsy (ISHLT Grade 2R or greater) and antibody-mediated rejection with left ventricular dysfunction merit treatment. Treatment ranges from an oral prednisone bolus and taper for asymptomatic rejection to cytolytic therapy with anti-thymocyte globulin for hemodynamically compromising rejection. There were 818 IM assays from patients without subsequent infection or rejection, which were used as controls.

Immune monitoring assay

Sodium heparin anti-coagulated whole blood samples were collected during clinic visits at the time of routine heart biopsies in the first year post-transplant. After 1 year, these samples were then obtained every 6 months. The IM assay was performed according to the manufacturer’s package insert. Briefly, 250 \(\mu\text{l}\) of anti-coagulated whole blood was diluted with sample diluent, added to wells of a 96-well microtiter plate, and incubated for 15 to 18 hours with phytohemagglutinin (PHA) in a 37°C, 5% CO\(_2\) incubator. The following day, CD4\(^+\) T cells were positively selected within the microwells using magnetic particles coated with anti-human CD4 monoclonal antibodies and a strong magnet, washed to remove residual cells, and lysed to release intracellular ATP. Released ATP was measured using luciferin/luciferase and a luminometer.

Data analysis

We performed a number of data analyses. First, we examined the relationship between IM levels and rejection or infection occurring within 1 month of the IM measurement. In this analysis, the primary exposure variable was the IM levels and the outcome variables were episodes of treated infection or rejection. Mean IM scores were compared using 2-tailed \(t\)-tests. \(p < 0.05\) was considered statistically significant. Because individuals contributed more than one observation (patients underwent serial IM measurements), we examined the relationship using generalized estimating equations and robust variance estimation assuming an independent working model for the within-cluster correlation.

We also examined the relationship between IM levels and the risk of rejection or infection within 1 month of the IM measurement using odds ratios. Odds ratios using logistic regression for infection and rejection in increments of 10 for IM scores from 50 to 600 were plotted to generate hazard curves for risks of infection and rejection in our heart transplant population. Analyses were performed using STATA, version 8.0 (StataCorp, College Station, TX).

Results

Patient population

The patient population consisted of 296 adult heart transplant recipients who underwent heart transplantation at our institution between November 2005 and July 2008. The majority of the patients were male (78%), with an average age of 54.6 \(\pm\) 12.8 years. The majority of patients were Caucasian (62%) followed by Asian (13%), Hispanic (10%)
and African American (7%). The main reasons for heart transplant were idiopathic (39%) or ischemic (36%). The 296 cardiac transplant patients had 864 immune monitoring assays obtained at 2 weeks to 10 years after heart transplant with most patients being <1 year post-transplant.

IM scores and infection

Within 1 month after an IM measurement, there were 38 episodes of infection: 20 bacterial; 11 viral; and 7 fungal. The average time from IM score to infectious episode was 11 ± 9 days. The average IM score was significantly lower in patients who developed an episode of infection within 1 month after the IM measurement compared with steady-state patients: 187 ± 126 ng ATP/ml vs 280 ± 126 ng ATP/ml, p < 0.001 (Figure 1).

Overall, there were 261 IM scores that were considered to be in the over-immunosuppressive range (due to an IM score of <200 ng ATP/ml). Of these, 27 of 261 (10%) IM scores were associated with subsequent infectious episodes. This left 11 of 603 (2%) IM scores >200 ng ATP/ml that were associated with subsequent infectious episodes (p < 0.001, compared to the percentage of infections with an IM score of <200 ng ATP/ml).

There were no clinical parameters that would have led the clinicians to suspect the patients might be headed toward an infectious episode. Of these 38 infectious episodes, 14 (36%) required hospitalization or ICU treatment, but no patient died. All infectious complications were controlled. Anti-infective prophylaxis did not appear to influence IM values in patients on and off specific anti-infective antibiotics (data not shown).

IM scores and rejection

Within 1 month after an IM measurement, there were 8 episodes of treated rejection. The average IM score was not significantly different in patients who developed an episode of rejection compared with steady-state patients: 327 ± 175 ng ATP/ml vs 280 ± 126 ng ATP/ml, p = 0.35 (Figure 1). Interestingly, 3 of 8 rejection episodes were antibody-mediated and had hemodynamic compromise and, for these patients, the mean IM score was significantly higher than for steady-state patients: 491 ± 121 ng ATP/ml vs 280 ± 126 ng ATP/ml (p < 0.001).

IM-score hazard curves

We generated odds ratios for infection and rejection in increments of 10 ng ATP/ml for IM scores of 50 to 600. These scores were plotted to generate hazard curves for risks of infection and rejection in our heart transplant population (Figure 2). The optimal IM score range that minimizes infection and rejection risk occurs where the hazard curves for infection and rejection risk cross (black arrow). From our data, it would appear that an IM score in the range of 370 ± 150 ng ATP/ml would keep infection and rejection risk at <5%.

IM-score receiver operating characteristic curve for infection

We constructed a receiver operating characteristic (ROC) curve to test the performance of IM in predicting subsequent infections in heart transplant recipients (Figure 3). The ROC curve itself evaluates the performance of IM over a wide range of values, where the area under the ROC curve (AUC) quantifies the overall ability of the IM score in discriminating infection risk. The AUC of 72.8% indicates good test performance of IM in predicting subsequent infections. The cut-off that maximizes sensitivity and specificity, shown above, corresponds to an IM score of 200 ng ATP/ml, with a sensitivity of 71% and a specificity of 73%.
Steady-state IM scores

Steady-state IM scores were plotted based on time post-transplant, and no trend was observed ($R^2 = 0.0215$) (Figure 4).

Discussion

A major challenge in heart transplantation is balancing immunosuppression to achieve a state of quiescence in the graft while avoiding the toxicities and complications of over- and under-immunosuppression. A better understanding of the global immune response will facilitate individualization of immunosuppression to minimize the patient’s risk of infection, rejection, transplant coronary artery disease, malignancy and drug toxicities.

Our results are comparable to other studies in transplant patients. In a study of 504 solid-organ recipients, an IM score of $\leq 25$ ng ATP/ml was associated with a 12-fold increased risk of infection compared with a higher IM score, whereas an IM score of $\geq 700$ ng ATP/ml was associated with a 30-fold increased risk of rejection compared with a lower IM score. In pancreas transplantation, patients with an IM score of $< 100$ ng ATP/ml were 4 times more likely to develop infections. In pediatric liver transplant recipients, a low IM score was associated with a 100% probability of developing Epstein–Barr viremia, a marker for the development of post-transplant lymphoproliferative disorder in these patients. In small bowel transplant recipients undergoing tapering of immunosuppressive therapy, patients with a low IM score had no major adverse events during follow-up, whereas patients with high IM scores often progressed to rejection requiring steroids or OKT3.

These studies demonstrate the promise of IM in solid-organ transplant recipients, with the potential for tailoring immunosuppression based on the individual patient’s risk profiles for infection, rejection and malignancy.

A previous study using IM in heart transplant patients demonstrated no correlation between IM scores and infection or rejection. The major contention in this study was the very small number of infection ($n = 7$) and rejection ($n = 1$) episodes. With such small numbers, significant correlations would be difficult. Furthermore, the investigators included infection and rejection episodes that occurred within 6 months of the selected IM score. This long time period appears excessive because IM scores do change over this 6-month time period.

The IM test offers many advantages over other measures of immune function. Although IM scores can predict the risk for developing infection or rejection in transplant recipients, there was little correlation between IM scores and CD4 T-cell counts or immunosuppressive drug levels in a cross-section of heart transplant patients (data not shown). This is due to the fact that each heart transplant patient has their own unique response to immunosuppression. For example, the same tacrolimus levels of 8 ng/ml in 2 individual heart transplant recipients might reflect IM scores of 200 and 400 ng ATP/ml, respectively, illustrating the 2 patients’ differing immune response to immunosuppression. Another reason why the IM score is a better predictor than other measures such as CD4 T-cell count or immunosuppressive drug levels is that the IM test measures T-cell activation, which is important because the target of most immunosuppression is the T cell. Furthermore, by using a whole blood sample, the immune response is assessed in the continuous presence of immunosuppressive drugs during overnight incubation. By measuring global immunity, this assay allows for the assessment of the aggregate impact of multiple immunosuppressive medications together.

Other advantages to the IM test are that it can be performed by standard laboratories with results available within 24 hours, and it does not require specialized research equipment. In addition, immune activation to the allograft may be preceded by the presence of activated cells in the circulation, so the IM test may offer an earlier marker of infection or rejection risk. Over time, there does not appear to be a significant correlation with IM scores because most immunosuppression is rather stable at $> 1$ year post-transplant.

Although the results of this study offer useful insight into the role of IM testing in heart transplant recipients, some
limitations do bear mentioning. First, the study was retrospective and may have inherent biases. A randomized trial utilizing IM to guide immunosuppression in hopes of minimizing complications is warranted. Second, there were only a small number of rejection episodes in the study population, limiting the power to detect an association between IM scores and rejection. It would be of great interest to determine if there is a difference in IM scores for cellular vs antibody-mediated rejection episodes. A larger study with more rejection episodes will be needed to ascertain these potential differences. Third, changes to immunosuppression were performed for patients with extreme IM scores of $<100 \text{ ng ATP/ml}$ ($7\%$) and $>600 \text{ ng ATP/ml}$ ($1\%$), which was done for “learning curve” experience. For IM scores of $<100 \text{ ng ATP/ml}$, immunosuppression was reduced (decrease in tacrolimus or mycophenolate doses), resulting in an appropriate increase of IM scores in 49\% of these cases. Similarly, for IM scores of $>600 \text{ ng ATP/ml}$, immunosuppression was increased (increase in tacrolimus or mycophenolate doses), resulting in an appropriate decrease of IM scores in 64\% of these cases. It is likely that greater changes in immunosuppression will be needed to result in the appropriate change in IM scores. There was no impact on rejection/infection episodes in these patients with extreme IM scores because the IM scores did not change appreciably with immunosuppression dose changes. Therefore, treatment of extreme IM scores did not appear to have affected the outcome of this observational study. The hazard risk curves for infection and rejection risk were constructed to demonstrate how IM data could be used. Certainly, more data will help to refine the accuracy and validity of IM testing. In addition, it is not known whether IM is cost-effective. A randomized trial in which immunosuppression could be prospectively guided by IM is needed in order to evaluate cost-effectiveness.

In conclusion, this study has established that IM scores of $<200 \text{ ng ATP/ml}$ can predict infection risk in heart transplant recipients. The association between higher IM scores and rejection remains inconclusive due to the small number of rejection episodes assessed. This study offers promise for the utility of IM testing in the individualization of immunosuppression to balance the risks of infection and possibly rejection in heart transplant recipients. A study with a larger sample size of rejection episodes is needed.

**Disclosure statement**

J.K. received speaker honoraria from Cylex, Columbia, MD. The other authors have not conflicts of interest to disclose.

**References**