CHEST

Original Research

TRANSPLANTATION

Implications for Human Leukocyte Antigen Antibodies After Lung Transplantation

A 10-Year Experience in 441 Patients

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Background: **Long-term survival after lung transplant is limited by the development of chronic and progressive airflow obstruction, a condition known as bronchiolitis obliterans syndrome (BOS). While prior studies strongly implicate cellular rejection as a strong risk factor for BOS, less is known about the clinical significance of human leukocyte antigen (HLA) antibodies and donor HLA-specific antibodies in long-term outcomes.**

Methods: **A single-center cohort of 441 lung transplant recipients, spanning a 10-year period, was prospectively screened for HLA antibodies after transplant using flow cytometry-based methods. The prevalence of and predictors for HLA antibodies were determined. The impact of HLA antibodies on survival after transplant and the development of BOS were determined using Cox models.**

Results: **Of the 441 recipients, 139 (32%) had detectable antibodies to HLA. Of these 139, 54 (39%) developed antibodies specific to donor HLA. The detection of posttransplant HLA antibodies was associated with BOS (HR, 1.54; $P = .04$) and death (HR, 1.53; $P = .02$) in multivariable models. The detection of donor-specific HLA antibodies was associated with death (HR, 2.42; $P < .0001$). The detection of posttransplant HLA antibodies was associated with pretransplant HLA-antibody detection, platelet transfusions, and the development of BOS and cytomegalovirus pneumonitis.**

Conclusions: **Approximately one-third of lung transplant recipients have detectable HLA antibodies, which are associated with a worse prognosis regarding graft function and patient survival.**

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Abbreviations: ARR = acute rejection ratio; BOS = bronchiolitis obliterans syndrome; CMV = cytomegalovirus; DSA = donor human leukocyte antigen-specific antibodies; HLA = human leukocyte antigen; HR = hazard ratio; IQR = interquartile range; ISHLT = International Society for Heart and Lung Transplant; PGD = primary allograft dysfunction

Long-term outcomes after lung transplant are limited by the development of bronchiolitis obliterans syndrome (BOS), a condition of progressive airflow decline. One of the strongest risk factors for BOS is the number and severity of acute cellular rejection episodes marked by T-cell infiltrates around blood vessels and bronchioles in the allograft.[1] More recently, antibody-mediated, humoral or B-cell, rejection is being recognized as a possible risk factor for poor long-term outcomes in solid-organ transplantation. Initial reports from renal transplant recipients described endothelial injury that was distinctly different from cellular rejection and that corresponded to clinical decline.[2,3] In addition, complement split products in tissue samples and human leukocyte antigen (HLA) antibodies detected in serum corresponded to allograft dysfunction.[4-6] In lung transplant, centers have reported widely varying rates of antibody-mediated rejection based on a tissue diagnosis.[7-9] The difficulties of a tissue diagnosis in lung transplant antibody rejection are evidenced by the inability of two national conferences on allograft rejection to create a consensus definition.[10,11]

Rather than focus on tissue, many centers are using serum HLA antibodies to identify possible antibody-mediated rejection. Recent advances in the determination of HLA antibodies by solid-phase technologies have increased the sensitivity and specificity of HLA-antibody detection. While likely not the only antibodies produced in this type of rejection, HLA antibodies

provide a marker for B-cell activation. To our knowledge, our group was one of the first to report that lung transplant recipients who develop donor-specific HLA antibodies (DSA) have a higher risk of developing BOS and of worse posttransplant survival compared with individuals who did not develop DSA.[12] Subsequent studies have confirmed that pretransplant presence of HLA antibodies is associated with worse survival, and in small series, HLA antibodies detected posttransplant are associated with rejection and allograft dysfunction.[12-15] More recently, a prospective study at a single center noted that recipients with DSA who received treatment did not have an increased risk for acute cellular rejection, lymphocytic bronchiolitis, BOS, or worse survival.[16]

Given the diverse reports on the incidence of HLA antibodies and association with allograft dysfunction, we sought to review our large recipient cohort with extended longitudinal follow-up for HLA antibodies and to outline the risk factors for and incidence and implications of detection of HLA antibodies after lung transplant. Since 2000, we have used a prospective screening protocol for HLA antibodies. We specifically focused on HLA antibodies, given the lack of consensus regarding a histologic definition of antibody rejection.

Materials and Methods

Study Cohort

Adults (≥18 years old) receiving a first, cadaveric lung transplant at Duke University Medical Center between January 1, 2000, and October 1, 2008, with at least 30-day survival were eligible for this study. Multiorgan, living lobar, and retransplant recipients were excluded. All recipients received standardized immunosuppression, pulmonary function tests, and transbronchial biopsies as described in the supplemental material (e-Appendix 1).[17] The study was approved through the Duke University institutional review board (IRB#00007005).

HLA Antibody Determination and Screening Protocol

Prior to transplant and serially after transplant, all recipients are screened for the presence and specificity of HLA antibodies. Routine screening is done to coincide with surveillance bronchoscopies at 1, 3, 6, 9, and 12 months posttransplant. Additional HLA antibody screens are performed in the setting of clinical decline. Data collection for this analysis ended April 1, 2011.

Allograft Assessments

Acute rejection was defined as perivascular infiltrates detected on transbronchial biopsies as described by International Society for Heart and Lung Transplant (ISHLT) guidelines.[11] We used a time-dependent acute rejection ratio (ARR), where the sum of the ISHLT grade A scores was divided by the total number of transbronchial biopsies and considered as a time-dependent predictor. BOS was defined as progressive airflow obstruction according to the ISHLT guidelines.[18] In addition, to be eligible for our BOS analysis, recipients had posttransplant survival of ≥90 days and had undergone at least five pulmonary function tests. Primary allograft dysfunction (PGD) grade 3 was defined by a Pao_2/Fio_2 ratio <200 at 72 h posttransplant and the presence of radiographic infiltrates.[19] Recipients who were on extracorporeal membrane oxygenation at 72 h posttransplant were considered to have PGD grade 3.

Statistical Analysis

Demographic variables were compared between individuals with and without HLA antibodies using χ^2, Fisher, or Wilcoxon rank-sum tests as appropriate. Cox proportional hazards models were used to evaluate the impact of both time-dependent and time-independent covariates on allograft survival, BOS, and detection of posttransplant HLA antibodies and DSA. The first episodes of cytomegalovirus (CMV) pneumonitis, ARR, detection of HLA antibodies, and DSA were all limited to occurrence prior to BOS onset and were considered time-dependent covariates. The selection of predictor variables was based upon statistical differences between patients with and without HLA antibodies and prior published risk factors. In addition, the development of BOS and detection of HLA antibodies and DSA were also considered time-dependent covariates in the survival model; HLA antibodies and DSA also were considered time-dependent covariates in the BOS model. Each predictor variable was first entered into a univariate model; those meeting a significance level ≤ .05 were included in the multivariable regression model. Analyses were performed using SAS version 9.2 (SAS Institute Inc).

Results

Study Cohort Demographics and HLA Evaluation

There were 460 lung transplant recipients who met initial inclusion criteria and were eligible for analysis. A total of 5,813 individual serum samples were evaluated for HLA antibodies, including 2,119 pretransplant samples and 3,694 posttransplant samples. Of these 460 recipients, 19 were subsequently excluded from the analysis because they had no posttransplant HLA-antibody tests. Of the remaining 441 subjects, 139 (32%) had detectable HLA antibodies; these composed the positive HLA-antibody cohort. The remaining 302 (71%) subjects composed the negative HLA-antibody cohort. HLA antibody-positive recipients were more likely to be female; non-white;

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