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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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have detectable, pretransplant HLA antibodies; have received platelets or cryoprecipitate during the first 30 days after transplant; be discharged on mycophenolate mofetil; and to have had a retrospective positive crossmatch at the time of transplant (Table 1). Of the 139 recipients with positive HLA antibodies posttransplant, 108 (78%) had their first detectable HLA antibody within the first year after transplant, and 54 (39%) developed DSA. The median time to DSA development was 52 days posttransplant (interquartile range [IQR], 19-769).

Posttransplant HLA Antibodies Are Associated With BOS

Of the 441 subjects in the cohort, 415 were eligible for BOS assessment. Of these, 181 (44%) developed BOS during the analysis study period (median follow-up time, 4.68 years). The detection of HLA antibodies was a risk factor for BOS in univariate analysis (hazard ratio [HR], 1.57; 95% CI, 1.13-2.18). The detection of DSA was not a significant predictor for BOS (HR, 1.45; 95% CI, 0.84-2.55; $P = .19$). In the univariate analysis, pretransplant antibodies, type of transplant, PGD grade 3, and ARR were significantly associated with the development of BOS (Table 2). In our cohort, community-acquired viral infections, pathogenic *Aspergillus* pulmonary infections, and *Pseudomonas aeruginosa* pulmonary infection or colonization prior to the development of BOS were not different between those with and without BOS, and these infections were not associated with BOS in our cohort. In a multivariable regression model, the detection of HLA antibodies remained a significant predictor for BOS when controlling for pretransplant antibodies, type of transplant, PGD grade 3, and ARR (HR, 1.54; 95% CI, 1.03-2.29; $P = .04$) (Table 3).

Posttransplant HLA Antibodies Are Associated With Worse Survival

During the study period, 187 of the 441 subjects (42%) died (median survival, 4.36 years; IQR, 2.97-6.69). Detection of HLA antibodies posttransplant was associated with worse survival in univariate analysis (HR, 2.43; 95% CI, 1.81-3.27; $P < .0001$). Several variables were considered in separate univariate survival analyses (Table 4). Older age, native lung disease (cystic or restrictive), positive crossmatch, pretransplant HLA-antibody status, PGD grade 3, CMV pneumonitis, and BOS were significantly associated with worse survival in the univariate analysis and therefore were included in the multivariable analysis (Table 5). The detection of HLA antibodies posttransplant remained a significant predictor of worse survival in the multivariate survival model (HR, 1.53; 95% CI, 1.07-2.21; $P = .02$).

Table 1—*Demographics of 441 Lung Transplant Recipients*

Characteristics	Not HLA Positive Posttransplant (n = 302)	HLA Positive Posttransplant (n = 139)	*P* Value
Male sex	183 (61)	67 (48)	.01
Race			.01
White	278 (92)	114 (82)	
Black	24 (7)	22 (16)	
Other	2 (1)	3 (2)	
HLA-positive antibody pretransplant	22 (7)	63 (45)	<.0001
Bilateral transplant	291 (96)	138 (99)	.11
Native disease			.08
Cystic	60 (20)	14 (10)	
Obstructive	120 (40)	65 (47)	
Restrictive	114 (38)	56 (40)	
Vascular	8 (3)	4 (3)	
Age at transplant, median (IQR), y	57 (44-63)	53 (47-61)	.76
PGD grade 3 at 72 h after transplant	34 (11)	19 (14)	.48
Positive crossmatch	0 (0)	3 (2)	.01
Recipients receiving blood products in first 30 d after transplant			
RBCs	282 (93)	134 (96)	.20
Platelets	104 (34)	71 (51)	.0009
Plasma	196 (65)	94 (68)	.58
Cryoprecipitate	32 (11)	32 (23)	.0006
Study days, median (IQR)	1,752 (1,140-2,558)	1,447 (861-19,52)	.003
Died during study period	114 (38)	73 (53)	.004
Reached BOS status during study period[a]	117 (41)	64 (50)	.06
Posttransplant HLA tests per patient, median (IQR), No.	6 (4-9)	11 (8-16)	<.0001

Data given as No. (%) unless otherwise indicated. BOS = bronchiolitis obliterans syndrome; HLA = human leukocyte antigen; IQR = interquartile range; PGD = primary graft dysfunction.
[a]Based on 415 eligible recipients.

Table 2—*Univariate Analysis of Risk Factors for BOS*

Risk Factors	HR (95% CI)	*P* Value
Age > 56 y	1.06 (0.79-1.42)	.72
Pretransplant HLA-antibody positive	1.59 (1.11-2.26)	.01
Bilateral transplant	0.40 (0.21-0.76)	.005
Positive crossmatch	3.59 (0.50-26.1)	.21
PGD grade 3 at 72 h[a]	1.54 (1.02-2.34)	.04
CMV pneumonitis[b]	1.21 (0.85-1.72)	.30
ARR[b]	1.80 (1.13-2.87)	.014
Detection of HLA antibodies[b]	1.57 (1.13-2.18)	.007
Detection of DSA[b]	1.45 (0.84-2.552)	.19

ARR = acute rejection ratio; CMV = cytomegalovirus; DSA = donor human leukocyte antigen-specific antibodies; HR = hazard ratio. See Table 1 legend for expansion of other abbreviations.

[a]Limited to 356 recipients with repeat arterial blood gas values in first 72 h.

[b]Events considered before the development of BOS and in a time-dependent manner.

Posttransplant DSA Is Associated With Worse Survival

In the univariate analysis, the first detection of DSA as a time-dependent predictor was associated with worse survival (HR, 3.43; 95% CI, 2.37-4.98; $P \leq .0001$). DSA class I or DSA class II modeled as time-dependent predictors in separate univariate analyses were also associated with worse survival (class I HR, 3.49; 95% CI, 2.17-5.62; $P \leq .0001$; class II HR, 4.23; 95% CI, 2.80-6.37; $P \leq .0001$). The development of any DSA, DSA class I, and DSA class II were considered in separate multivariable models that included age, pretransplant HLA-antibody status, native lung disease, PGD grade 3, CMV pneumonitis, and BOS. Controlling for these other variables, the detection of any DSA was a significant predictor for worse survival (HR, 2.39; 95% CI, 1.51-3.78; $P = .0002$) (Table 6). In a separate multivariable survival analysis, both the detection of DSA class I (HR, 2.43; 95% CI, 1.34-4.41; $P = .003$) and the detection of DSA class II (HR, 3.12; 95% CI, 1.96-4.97; $P \leq .0001$) also predicted worse survival after lung transplant (Table 7).

Table 3—*Multivariable Analysis of Risk Factors for BOS*

Risk Factors	HR (95% CI)	*P* Value
Pretransplant HLA-antibody positive	1.26 (0.82-1.94)	.30
Bilateral transplant	0.43 (0.22-0.81)	.01
PGD grade 3 at 72 h	1.49 (0.89-2.50)	.13
ARR[a]	1.92 (1.19-3.08)	.007
Detection of HLA antibodies[a]	1.54 (1.03-2.29)	.04

See Table 1 and 2 legends for expansion of abbreviations.

[a]Events considered before the development of BOS and in a time-dependent manner.

Table 4—*Univariate Analysis of Risk Factors for Death*

Risk Factors	HR (95% CI)	*P* Value
Age > 56 y at transplant	1.37 (1.03-1.83)	.03
Sex	0.99 (0.74-1.33)	.96
White race	0.79 (0.51-1.23)	.30
Pretransplant HLA-antibody positive	1.92 (1.38-2.66)	<.0001
Positive crossmatch	4.71 (1.15-19.3)	.03
Native lung disease (reference group: all others)		
Cystic	0.53 (0.34-0.82)	.005
Obstructive	1.03 (0.77-1.38)	.84
Vascular	0.96 (0.36-2.59)	.96
Restrictive	1.39 (1.04-1.86)	.03
Bilateral transplant	0.72 (0.37-1.41)	.34
PGD grade 3 at 72 h	1.56 (1.00-2.42)	.05
Development of CMV pneumonitis[a]	1.65 (1.19-2.28)	.003
Detection of HLA antibodies[a]	2.43 (1.81-3.27)	<.0001
Development of BOS[a]	12.9 (9.08-18.4)	<.0001
Development of DSA[a]	3.43 (2.37-4.98)	<.0001
Development of DSA class I[a]	3.49 (2.17-5.62)	<.0001
Development of DSA class II[a]	4.23 (2.80-6.37)	<.0001

See Table 1 and 2 legends for expansion of abbreviations.

[a]Time-dependent covariate.

Predictors for HLA Detection After Transplant

In univariate Cox regression analyses, sex, race, positive crossmatch, platelet transfusion, cryoprecipitate transfusion, pretransplant HLA antibodies, CMV pneumonitis (prior to HLA antibody detection), and the development of BOS (prior to HLA antibody detection) were associated with posttransplant HLA antibodies (Table 8) and, therefore, included in the multivariable model. Of note, all of the recipients had at least one posttransplant HLA-antibody test prior to the development of CMV or BOS. In the multivariable model, platelet transfusion (HR, 1.56; 95% CI, 1.05-2.33; $P = .03$), pretransplant HLA antibodies (HR, 6.19; 95% CI, 4.35-8.81; $P \leq .0001$), CMV pneumonitis (HR, 2.03; 95% CI, 1.08-3.79; $P = .03$), and the development of BOS (HR, 9.53; 95% CI, 5.40-16.8; $P \leq .0001$) remained significant predictors for HLA antibodies after transplant (Table 9).

Table 5—*Multivariable Model of HLA Antibodies and Risk Factors for Death*

Risk Factors	HR (95% CI)	*P* Value
Age > 56 y at transplant	1.24 (0.89-1.76)	.21
Cystic	1.01 (0.59-1.73)	.97
Restrictive	1.21 (0.86-1.70)	.27
Positive crossmatch	1.98 (0.44-8.94)	.37
Pretransplant HLA-antibody positive	1.26 (0.84-1.89)	.28
PGD grade 3 at 72 h	1.25 (0.73-2.14)	.41
Development of CMV pneumonitis[a]	1.38 (0.97-1.97)	.07
Development of BOS[a]	12.08 (8.46-17.2)	<.0001
Detection of HLA antibodies[a]	1.53 (1.07-2.21)	.02

See Table 1 and 2 legends for expansion of abbreviations.

[a]Time-dependent covariate.

Table 6—*Multivariable Model of DSA and Risk Factors for Death*

Risk Factors	HR (95% CI)	*P* Value
Age >56 y at transplant	1.28 (0.91-1.80)	.16
Cystic	1.10 (0.64-1.88)	.74
Restrictive	1.13 (0.80-1.60)	.48
Positive crossmatch	1.24 (0.27-5.69)	.79
Pretransplant HLA-antibody positive	1.31 (0.90-1.92)	.16
PGD grade 3 at 72 h	1.30 (0.76-2.22)	.34
Development of CMV pneumonitis[a]	1.45 (1.02-2.06)	.04
Development of BOS[a]	12.57 (8.80-17.98)	<.0001
Development of DSA[a]	2.39 (1.51-3.78)	.0002

See Table 1 and 2 legends for expansion of abbreviations.
[a]Time-dependent covariate.

Predictors for DSA Detection After Transplant

Given the relatively few DSA events in our study cohort, we limited our analysis to three predictors that were the strongest predictors for HLA antibodies after lung transplant: pretransplant HLA antibodies, CMV pneumonitis (prior to DSA detection), and the development of BOS (prior to DSA detection). In separate univariate analyses, pretransplant HLA antibodies and the development of BOS were significant and included in the multivariable model (Table 10). Both pretransplant HLA antibodies (HR, 3.68; 95% CI, 2.07-6.54; $P \leq .0001$) and the development of BOS (HR, 5.65; 95% CI, 2.16-14.8; $P = .0004$) remained significant predictors for the development of DSA after lung transplant (Table 11).

Discussion

In this large cohort of 441 lung transplant recipients, we have found a strong association between posttransplant HLA antibodies and the subsequent development of BOS and worse survival. Furthermore, the detection of DSA, particularly DSA class II, was associated with significantly worse survival than simply the presence of HLA antibodies. Although somewhat surprising, DSA was not associated with BOS. Our results add to the growing interest in the clinical significance of HLA antibodies by providing analysis of a large cohort, managed by protocol screening and with extended follow-up, which allowed for assessment of allograft function and survival.

There are several possible mechanisms by which antibodies may contribute to allograft dysfunction. Antibodies may promote complement deposition, directly damage the epithelium, or stimulate growth factors. Several reports have noted complement deposition in the allograft of recipients with circulating HLA antibodies.[7,20] Another potential mechanism, supported by in vitro work, suggests human epithelial airway cells exposed to anti-HLA antibodies lead to increased levels of defensins, which, in turn, induce growth factor production and epithelial proliferation in a manner similar to obliterative bronchiolitis.[21] Similarly, in vitro and animal fibrosis models indicate that HLA antibodies can promote profibrotic growth factors.[22,23]

Our findings that HLA-antibody detection is associated with the development of BOS is consistent

Table 7—*Multivariable Model of DSA Class I and Class II and Risk Factors for Death*

Risk Factors	HR (95% CI)	*P* Value
Age >56 y at transplant	1.24 (0.88-1.74)	.22
Cystic	1.06 (0.62-1.80)	.85
Restrictive	1.16 (0.82-1.64)	.40
PGD grade 3 at 72 h	1.21 (0.71-2.07)	.48
Development of CMV pneumonitis[a]	1.54 (1.09-2.17)	.02
Development of BOS[a]	12.96 (9.07-18.52)	<.0001
Development of DSA class I[a]	2.43 (1.34-4.41)	.003
Development of DSA class II[a]	3.12 (1.96-4.97)	<.0001

See Table 1 and 2 legends for expansion of abbreviations.
[a]Time-dependent covariate.

Table 8—*Univariate Analysis of Predictors for HLA Antibodies After Transplant*

Predictor	HR (95% CI)	*P* Value
Female sex	1.59 (1.14-2.22)	.006
White race	0.48 (0.31-0.75)	.001
Positive crossmatch	5.34 (1.68-16.9)	.004
Platelet transfusion within 30 d of transplant	1.79 (1.29-2.50)	.0006
Cryoprecipitate transfusion within 30 d of transplant	1.89 (1.72-2.80)	.0016
Pretransplant HLA-antibody positive	6.86 (4.88-9.63)	<.0001
Development of BOS[a]	3.77 (1.85-7.66)	.0003
Development of CMV pneumonitis[a]	2.04 (1.10-3.81)	.02

See Table 1 and 2 legends for expansion of abbreviations.
[a]Time-dependent covariate occurring before the detection of HLA antibodies.

Table 9—*Multivariable Analysis of Predictors for HLA Antibodies After Transplant*

Predictor	HR (95% CI)	*P* Value
Female sex	1.30 (0.92-184	.14
White race	0.69 (0.44-1.09)	.69
Positive crossmatch	1.13 (0.35-3.71)	.84
Platelet transfusion within 30 d of transplant	1.56 (1.05-2.33)	.03
Cryoprecipitate transfusion within 30 d of transplant	1.17 (0.73-1.89)	.51
Pretransplant HLA-antibody positive	6.19 (4.35-8.81)	<.0001
Development of BOS[a]	9.53 (5.40-16.8)	<.0001
Development of CMV pneumonitis[a]	2.03 (1.08-3.79)	.03

See Table 1 and 2 legends for expansion of abbreviations.
[a]Time-dependent covariate occurring before the detection of HLA antibodies.

Table 10—*Univariate Analysis of Predictors for DSA After Transplant*

Predictor	HR (95% CI)	*P* Value
Pretransplant detectable HLA antibody	4.14 (2.42-7.10)	<.0001
Development of BOS[a]	5.96 (2.34-15.2)	.0002
Development of CMV pneumonitis[a]	1.12 (0.42-2.98)	.83

See Table 1 and 2 legends for expansion of abbreviations.
[a]Time-dependent covariate and prior to the detection of DSA.

with other, smaller studies.[12,15] Surprisingly, our analysis did not find that DSA was a predictor for BOS, although it was associated with worse survival. This may reflect the competing risk for death and BOS in this group. We did consider the possibility that the increased risk of mortality was related to DSA treatment. However, only two of the 33 deaths were both within 1 year after increased immunosuppression and related to infection. Alternatively, this may indicate that our study is underpowered for BOS in the DSA cohort, given the relatively low incidence of DSA. Importantly, our incidence of DSA is similar to that of abdominal solid-organ transplant reports but notably lower than another study of 122 lung transplant recipients in which over one-half the cohort had DSA.[16,24,25] In addition, that particular study did not find an increase in BOS or worse survival with DSA; however, the follow-up period was considerably shorter than our analysis.

Leveraging a large number of samples, lung transplant recipients, and outcome events, we were able to identify specific risk factors for posttransplant HLA antibodies. Our finding of sex and pretransplant presence of HLA antibodies as risk factors for posttransplant HLA antibodies is not surprising. Prior reports noted more complications and worse survival in lung transplant recipients with pretransplant HLA antibodies.[13,26,27] In retrospect, this may have reflected posttransplant HLA-antibody presence, as our analysis found a strong relationship between pretransplant and posttransplant HLA antibodies. In considering HLA exposure, we assessed blood product transfusions within 30 days of transplant as risk factors for HLA-antibody development. We noted that platelets and cryoprecipitate transfusions were strong risk factors for the detection of HLA antibodies after transplant, likely reflecting the potential for HLA exposure of pooled donor blood products. Surprisingly, PGD grade 3 was not associated with pretransplant or posttransplant HLA-antibody detection. As PGD is associated with BOS and worse survival, we initially thought there may be a link to HLA antibodies. Our analysis did not confirm that, though we are limited by the relatively low incidence of PGD grade 3 in our cohort. However, our finding that the development of BOS and viral pneumonitis *prior to* HLA-antibody detection are risk factors for antibody detection is novel and highlights our limited understanding of the triggers for antibody production. This finding opens new avenues for understanding the mechanism of antibody-mediated rejection. However, the majority of the recipients had detectable HLA antibodies before they developed BOS or CMV pneumonitis. This calls for further exploration in other cohorts to validate the relationship.

Table 11—*Multivariable Analysis of Predictors for DSA After Transplant*

Predictor	HR (95% CI)	*P* Value
Pretransplant detectable HLA antibody	3.68 (2.07-6.54)	<.0001
Development of BOS[a]	5.65 (2.16-14.8)	.0004

See Table 1 and 2 legends for expansion of abbreviations.
[a]Time-dependent covariate and prior to the detection of DSA.

One limitation of our study is the exclusive focus on HLA antibodies to define possible humoral rejection. This is a widely accepted and reproducible measure of humoral immunity, and, therefore, our study is applicable to the majority of lung transplant recipients. However, antibodies to other allograft antigens, including endothelial antigens, collagen type V, and K-α tubulin, have been implicated in humoral rejection.[28-30] Recent reports in renal and heart transplant noted that complement-fixing HLA antibodies may offer more specificity in risk of graft dysfunction than just HLA-antibody measurements or mean fluorescence intensity.[31,32] Although controversial, pathology may also offer an alternative approach to define humoral rejection. The optimal antibody and assay for humoral rejection may still be evolving.

It is important to note that the technology of antibody detection changed and improved over the course of this study, most notably in 2005 with the addition of single-antigen bead assays, as described in e-Appendix 1. Potentially, there were earlier-era samples with HLA antibodies and/or DSA present but not detected in the older technology. This could have biased our analysis against finding a difference, and thus strengthens our finding of an association with any detectable HLA antibodies and worse outcomes. Another limitation is the focus on the first HLA-antibody detection after transplant regardless of clinical status. Although we followed a specific protocol for HLA-antibody testing, we did not prospectively record the clinical events that may have prompted additional testing.

Despite these limitations, this analysis represents the most comprehensive, systematic analysis of HLA antibodies after lung transplant to date. We confirmed that HLA antibodies after lung transplant are associated with an increased risk for BOS and worse survival, and that DSA development is associated with worse survival. HLA antibodies appear to be an integral

part of the immune response to the allograft both preceding graft dysfunction and after allograft dysfunction. A key question to address in further analysis is if the decrease or elimination of these antibodies correlates with improved outcomes.

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Dr Snyder: contributed to study design, data analysis, manuscript preparation, and served as principal author.
Ms Wang: contributed to data collection and analysis and review of the manuscript.
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