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The allograft injury marker CXCL9 determines prognosis of anti-HLA antibodies after lung transplantation

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Abstract

Despite the common detection of non-donor specific anti-HLA antibodies (non-DSAs) after lung transplantation, their clinical significance remains unclear. In this retrospective single-center cohort study of 325 lung transplant recipients, we evaluated the association between donor-specific HLA antibodies (DSAs) and non-DSAs with subsequent CLAD development. DSAs were detected in 30% of recipients and were associated with increased CLAD risk, with higher HRs for both de novo and high MFI (>5000) DSAs. Non-DSAs were detected in 56% of recipients, and 85% of DSA positive tests had concurrent non-DSAs. In general, non-DSAs did not increase CLAD risk in multivariable models accounting for DSAs. However, non-DSAs in conjunction with high BAL CXCL9 levels were associated with increased CLAD risk. Multivariable proportional hazards models demonstrate the importance of the HLA antibody-CXCL9 interaction: CLAD risk increases when HLA antibodies (both DSAs and non-DSAs) are detected in conjunction with high CXCL9. Conversely, CLAD risk is not increased when HLA antibodies are detected with low CXCL9. This study supports the potential utility of BAL CXCL9 measurement as a biomarker to risk stratify HLA antibodies for future CLAD. The ability to

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SUPPORTING INFORMATION

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DISCLOSURE

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discriminate between high versus low-risk HLA antibodies may improve management by allowing for guided treatment decisions.

Keywords

alloantibody; biomarker; bronchoalveolar lavage (BAL); cytokine receptors; cytokines; dysfunction; immunobiology; lung (allograft) function; lung transplantation / pulmonology; translational research / science

1 | INTRODUCTION

Chronic lung allograft dysfunction (CLAD) is the major factor limiting long-term survival after lung transplantation.[1] Since there are no known effective therapies for CLAD, the identification of key modifiable risk factors for CLAD is a crucial step toward improving post-transplant outcomes. Our understanding of the association between donor-specific human leukocyte antigen (HLA) antibodies (DSAs) and CLAD development has improved over the past several years. Studies have demonstrated increased CLAD risk for de novo DSAs (dnDSA)[2–6] and persistent DSAs.[7,8]

However, it remains controversial whether DSAs are the primary pathogenic mechanism causing HLA specific antibody-mediated lung allograft injury, or if they are more generally a marker of activated immunity, possibly in response to allograft injury. Antibody-mediated rejection (AMR) is defined by the presence of allograft dysfunction, DSA positivity and histopathologic changes including neutrophilic capillaritis and acute lung injury.[9,10] However, several studies have found that DSAs have a poor correlation with the histopathology of AMR,[11–14] and DSAs that existed prior to transplant may not be associated with CLAD.[15,16] Furthermore, DSAs are often accompanied by HLA antibodies not specific to donor HLA (non-DSAs),[17] with one smaller study reporting the presence of non-DSAs in 12/13 (92%) of lung transplant recipients with DSAs.[18] Despite their common occurrence after lung transplant, the incidence and clinical importance of non-DSAs have not been well studied to date.

We have previously demonstrated bronchoalveolar lavage (BAL) CXCL9 to be a marker of lung allograft injury, with elevated concentrations during acute lung injury (ALI),[19] as well as acute cellular rejection (AR)[20] and organizing pneumonia (OP).[21] In these studies, BAL CXCL9 elevations also predicted the risk of CLAD.[20,21] CXCL9 is not known to specifically be involved in AMR. However, we predicted that a marker of allograft injury (high BAL CXCL9) in conjunction with a marker of immune activation (anti-HLA antibodies) would be an especially high-risk marker for CLAD.

In this single-center retrospective study, we sought to evaluate the association of both DSAs and non-DSAs with the development of CLAD. We hypothesized that DSAs and non-DSAs were both markers of overall immune activation and would be associated with increased CLAD risk. We also hypothesized that BAL CXCL9 elevation in conjunction with DSAs and non-DSAs would reflect the extent of allograft injury and further prognosticate CLAD risk. The goals of this study were three-fold: (1) determine the association between

DSAs/non-DSAs and CLAD risk, (2) evaluate BAL CXCL9 levels during episodes of HLA antibodies, and (3) explore the interaction between HLA antibodies, BAL CXCL9 levels, and CLAD risk. The identification of a biomarker which can risk stratify HLA antibodies for future CLAD development may improve management by allowing optimal treatment for high-risk antibodies, while minimizing the treatment costs and side effects for low-risk antibodies.

2 | MATERIALS AND METHODS

With IRB approval (IRB# 13–000462), lung transplant recipients at UCLA were enrolled into a prospective observational study that included the collection of bronchoalveolar lavage (BAL) fluid for research purposes. Patients who received lung transplantation at UCLA between January 1, 2010 and September 1, 2017 were included in the study, with a retrospective collection of HLA antibody data. Lung transplant candidates received initial panel reactive antibody (PRA) testing with reflex single antigen testing during their pre-transplant evaluation and every 3 months while awaiting transplant. After transplant, recipients received surveillance single antigen testing according to the following protocol: posttransplant day 0, day 7, 1 month, 3 months, 6 months, then every 6 months thereafter. Additional HLA antibody testing was conducted at the discretion of a transplant pulmonologist for clinical suspicion of AMR. All HLA antibody testing was conducted at the UCLA Immunogenetics Center using solid phase microbead technology on a Luminex platform. Positive results were defined using a mean fluorescence intensity (MFI) cutoff of ≥1000 for the HLA-A, B, DR, and DQ loci and ≥2000 for HLA-C and DP loci due to their lower expression on cells.[22,23]

Recipients underwent surveillance bronchoscopy with BAL and transbronchial biopsy at 1, 3, 6, and 12 months posttransplant, as well as during episodes of clinical deterioration. Biopsies were interpreted by one of three pulmonary pathologists according to the International Society for Heart and Lung Transplantation criteria (acute cellular rejection [AR] and lymphocytic bronchiolitis [LB]).[24] The histopathologic findings OP, ALI, capillary neutrophilia, capillaritis, and endothelial cell C4d staining were considered “pathology compatible with antibody-mediated rejection (AMR)”.[25] TBBXs with no pathologic evidence of allograft injury were considered “healthy.” BAL samples were collected and stored at −80° C as previously described.[20] BALs collected closest to the date of the HLA antibody test, within a 14-day window, were thawed and CXCL9 levels were measured using Luminex microbead assays. HLA antibody levels were reported as mean MFI as per convention. CXCL9 levels were also reported as MFIs to minimize variability associated with standard curve interpolations.[26,27]

Immunosuppression, anti-microbial prophylaxis, and treatment of AR and AMR were administered in accordance with UCLA protocol as described previously (Supplement S1). UCLA’s protocol for AMR treatment included methylprednisolone 500 mg for 3 days, with daily plasma exchange (PLEX) for 5 days, followed by IVIG (2 g/kg total dose, divided over 2 to 4 days), and rituximab (1000 mg) on days 1 and 14. Treatment of DSAs without graft dysfunction or concerning histopathology was at the discretion of the treating transplant pulmonologist and ranged from observation (for stable or decreasing MFI) to the AMR

treatment protocol (usually for new or increasing MFI with clinical suspicion of AMR). Treatment was not administered for non-D SAs, in the absence of DSAs. Spirometry was performed serially on at least a quarterly basis. CLAD was defined as a sustained 20% drop in the forced expiratory volume in 1 s (FEV1) from the average of the two best posttransplant FEV1 measurements.[28]

To evaluate the association of BAL CXCL9 with HLA antibodies, mixed effects models were constructed taking into account repeated measurement from recipients with random intercepts assigned for each recipient. Chemokine levels were log2 transformed given the non-normal distribution. To evaluate the effect of HLA antibodies on CLAD risk, Cox proportional hazards models for time to CLAD were constructed with time-dependent dichotomous variables for each of the HLA antibody types. For example, the “Any DSA” variable started at 0 for each recipient and increased to 1 at the time of the first positive DSA test. It remained at 1 until the next test which showed no DSAs.

To determine the impact of BAL CXCL9 on the association between HLA antibodies and CLAD development, multivariable proportional hazards models were constructed to evaluate the interaction between HLA antibodies and CXCL9 levels. These models included three dummy variables for the combination of HLA antibody (yes or no) and CXCL9 level (high or low), compared with the reference group: “No antibody with low CXCL9.” The final multivariable models included additional variables known to be associated with CLAD: severe PGD, AR, LB, OP, and ALI. Severe PGD was defined as grade 2 or 3 PGD at 48 or 72 h posttransplant. The histopathologic findings (AR, LB, OP and ALI) were modeled as cumulative time-dependent counts.[19,20] For example, the AR variable started at 0 for each recipient and increased to 1 at the time of the first TBBX showing AR (grade ≥A2). It remained at 1 until the second episode of AR when it increased to 2. All analysis was performed using SAS v9.4 (Cary, NC).

3 | RESULTS

3.1 | Cohort characteristics

Table 1 describes the baseline patient characteristics for the overall cohort, as well as for those who developed DSAs and Non-DSAs. 96 (30%) recipients had at least one donor-specific antibody detected over a median follow-up of 2.3 years, while 183 (56%) recipients had at least one non-donor–specific antibody detected over a median follow-up of 1.9 years. 132 (41%) recipients never had HLA antibodies detected over a median follow-up of 1.3 years. Overall, recipients who developed DSAs and Non-DSAs were similar to recipients who did not develop HLA antibodies. The median number of HLA antibody tests as well as HLA antibody tests with concurrent BAL measurements were similar between these groups.

3.2 | Frequency of HLA antibodies detected

Table 2 describes the frequency of HLA antibodies detected by antibody type. In total, there were 2901 HLA antibody tests from 325 recipients. A total of 1639 (56%) tests from 241 recipients had no HLA antibodies, 1262 (44%) tests from 194 recipients had HLA antibodies detected with the following frequencies: HLA-A 143 (8%), HLA-B 294 (17%),

HLA-C 107 (6%), HLA-DP 21 (1%), HLA-DQ 416 (24%), and HLA-DR 754 (43%). 447 (15%) tests from 96 recipients had DSAs, while 1195 (41%) from 183 recipients had non-DSAs. In total, 380 (13%) tests from 84 recipients had concurrent DSA and non-D SAs ("concurrent DSA with non-DSA"). Thus, most episodes of DSAs (85%) had concurrent non-DSAs detected. Of the tests with positive HLA antibodies, the median number of HLA antibodies detected was 3 (IQR 1–10). Most DSAs were class II (79%) compared with class I (21%), while non-DSA had a similar frequency of class I (50%) and class II (50%) antibodies. In total, 306 (63%) DSAs were de novo and 179 (37%) were pre-existing, while 765 (52%) non-DSAs were de novo and 709 (48%) were pre-existing.

3.3 | Risk of CLAD after HLA antibody detection

To assess the impact of HLA antibodies on CLAD risk, univariable proportional hazards models for CLAD were constructed with time-dependent variables for each of the HLA antibody types (Table 3). Overall, the risk of CLAD development was higher for the detection of DSAs ("any DSA") compared with non-DSAs ("any non-DSA"). An episode of DSA was associated with a HR of 4.0 (95% CI 2.4–6.5), while an episode of non-DSA was associated with a HR of 1.8 (95% CI 1.2–2.7). Episodes of concurrent DSA with non-DSA were associated with higher risk with a HR for CLAD of 4.6 (95% CI 2.7–7.8). High-level antibodies (MFI >5000) was associated with a higher risk of CLAD for both DSA (HR 12.3 95% CI 6.7–22.4) and non-DSAs (HR 2.5 95% CI 1.5–4.0). De novo DSA (HR 5.5 95% CI 3.2–9.7) and de novo non-DSA (HR 1.7 95% CI 1.1–2.8) were both associated with increased CLAD risk, while pre-existing DSAs and pre-existing non-DSAs did not increase CLAD risk.

Multivariable proportional hazards models were constructed to determine the relative impact of the HLA antibody type on CLAD risk (Table 4). When "any DSA" and "any non-DSA" were included in the same model, DSAs predicted CLAD development (HR 3.5 95% CI 2.1–5.8), while non-DSAs lost significance (Model 1). Model 2 shows that the association between DSA and CLAD was explained mostly by episodes of DSA with concurrent non-DSAs. The HR for CLAD for an episode of "concurrent DSA with non-DSA" was 5.1 (95% CI 2.9–8.9), while "DSA only (without non-DSA)" and "Non-DSA only (without DSA)" episodes were not significant predictors of CLAD. It is worth noting that the HR for "DSA only (without non-DSA)" trended toward significance with a HR of 2.7 (95% CI 0.96–7.5), and may have been limited by the relatively small sample size ($n = 67$). Model 3 confirmed that episodes of "de novo DSA" predicted CLAD development (HR 5.0 95% CI 2.7–9.5), while "de novo non-DSA" did not.

3.4 | Frequency of HLA antibodies with associated transbronchial biopsies

Table 5 describes the frequency of histopathologic findings by the HLA antibody results, for TBBX obtained within a 14-day window of the antibody tests. Only 452 (16%) of 2901 HLA antibody tests had TBBX collected within the 14-day window. In total, 238 (53%) of these antibody tests had no HLA antibodies, while 68 (15%) had DSA and 146 (32%) had non-DSAs only (non-DSAs only without concurrent DSAs). In this limited sample set, 24 (35%) of TBBXs associated with DSAs had pathology compatible with AMR (organizing pneumonia, acute lung injury, capillary neutrophilia, capillaritis, or capillary endothelial

C4D staining), while 36 (25%) of TBBXs associated with “non-DSA only” had pathology compatible with AMR ($p = .04$). The frequency of acute rejection (AR, ≥A2), lymphocytic bronchiolitis (LB), organizing pneumonia (OP) and capillary neutrophilia/capillaritis were similar in biopsies associated with DSAs compared with “non-DSA only.” The frequency of acute lung injury (ALI) however, was higher in biopsies associated with DSAs compared with “non-DSA only” ($p < .005$). Capillary endothelial C4D staining was infrequently observed in only 4 (6%) of DSA biopsies and 2 (1%) of non-DSA only biopsies ($p = .063$).

3.5 | Frequency of HLA antibodies with associated BALs

Table 6 describes the frequency of HLA antibody results that had BALs available within a 14-day window for CXCL9 measurement. There were 486 samples from 234 recipients in total. 275 (57%) tests from 143 recipients had no HLA antibodies, 68 (14%) tests from 45 recipients had DSAs, 200 (41%) tests from 112 recipients had non-DSAs, and 57 (12%) tests from 41 recipients had concurrent DSA with non-DSAs. Forty-four (59%) of DSAs were de novo, while 131 (54%) of non-DSAs were de novo.

3.6 | BAL CXCL9 levels by HLA antibody type

In general, HLA antibodies were associated with higher BAL CXCL9 levels compared with no HLA antibodies, although the differences were not all significant. Median CXCL9 MFIs for DSA, non-DSA and concurrent-DSA samples were 1182 ($p = .11$), 1129 ($p < .02$), and 1303 ($p < .03$), respectively, compared with 699 for no HLA antibody samples (Table 7). DSAs with high MFIs (≥5000) had higher CXCL9 levels than DSA with low MFIs (<5000): 2006 vs. 570, respectively ($p < .04$). Non-DSA with high MFIs had higher CXCL9 levels than non-DSA with low MFI, but the difference was not significant. De novo DSA had higher CXCL9 levels than pre-existing DSAs: 1740 vs. 394, respectively ($p < .01$). De novo non-DSA had higher CXCL9 levels than pre-existing non-DSAs, but the difference was not significant.

3.7 | Impact of BALF CXCL9 during HLA antibodies on CLAD risk

To determine the impact of BAL CXCL9 on the association between HLA antibodies and CLAD development, multivariable proportional hazards models were constructed using three dummy variables for the combination of HLA antibody (yes or no) and CXCL9 level (high or low), compared to the reference group: “No antibody with low CXCL9.” These models demonstrate a strong interaction between the HLA antibodies and high CXCL9 levels. For each of the HLA antibody types: “Antibodies with low CXCL9” and “No antibodies with high CXCL9” did not increase CLAD risk. However, the interaction term “Antibodies with high CXCL9” was strongly associated with increased CLAD risk (Table 8). Thus, “DSA with low CXCL9” and “No DSA with high CXCL9” were not associated with CLAD, while “DSA with high CXCL9” significantly increased CLAD risk (HR 4.3 95% CI 2.2–8.3). Similarly, “Non-DSA with low CXCL9” and “No non-DSA with high CXCL9” was not associated with CLAD, while “Non-DSA with high CXCL9” increased CLAD risk (HR 3.4 95% CI 1.9–6.0). This relationship persisted for the models involving the interaction variables “Any antibody with high CXCL9” (HR 3.2 95% CI 1.8–5.6) and “Any high antibody with high CXCL9” (HR 5.5 95% CI (3.0–10.3). Of note, even episodes of “non-DSAs only,” which excluded samples with concurrent DSA, were a significant

predictor of CLAD when there was high CXCL9: “Non-DSA only with high CXCL9” had a HR for CLAD of 2.6 (95% CI 1.4–5.0).

Finally, we evaluated the association between “antibodies with high CXCL9” and CLAD, adjusted for other known CLAD risk factors: severe PGD, AR≥A2, LB, OP, and ALI, (Table 9). These multivariable models show the persistence of the “antibody with high CXCL9” and CLAD association, after adjustment for these risk factors. In model 1, “DSA with high CXCL9,” AR and ALI were associated with CLAD development with HRs of: 5.1 95% CI 2.6–9.7, 2.3 95% CI 1.4–3.9, and 2.0 95% CI 1.2–3.4, respectively. Similarly, in model 2 “non-DSA with high CXCL9,” AR and ALI were all associated with CLAD with HRs of: 3.4 95% CI 2.0–5.8, 2.2 95% CI 1.3–3.7, and 1.9 95% CI 1.2–3.2, respectively. Even episodes of “non-DSA only with high CXCL9” which excluded samples with concurrent DSA, remained associated with CLAD. The HRs for “non-DSA only with high CXCL9,” AR and ALI were: 2.5 95% CI 1.3–4.7, 2.2 95% CI 1.3–3.7, and 2.1 95% CI 1.3–3.4, respectively.

4 | DISCUSSION

This study evaluated the association between HLA antibodies (DSAs and non-DSAs) and the development of CLAD. We find that both DSAs and non-DSAs were a common occurrence after lung transplantation, detected in 30% and 56% of recipients, respectively. Similar to prior studies, we find that DSAs strongly increase CLAD risk. High MFI (>5000) and de novo antibodies both further increased CLAD risk, while pre-existing antibodies were not associated with CLAD. In the subset of antibody tests with a paired BAL sample, DSAs were associated with elevated levels of BAL CXCL9. Furthermore, multivariable modeling with interaction terms demonstrate the importance of the DSA and BAL CXCL9 interaction: DSAs concurrent with high BAL CXCL9 levels are associated with increased CLAD risk (HR 5.1 95% CI 2.6–9.7), while DSAs with low BAL CXCL9 are not.

Despite the common occurrence of non-DSAs after lung transplant, there have been a paucity of studies evaluating their clinical significance. Lobo et al detected non-DSAs in 22 (50%) of 44 of recipients, and 12 (92%) of 13 recipients with DSAs.[18] They reported CLAD development in 14 (64%) of 22 recipients in the non-DSA group compared with 7 (32%) of 22 recipients in the group without non-DSAs ($p = .069$). More recently, Verleden et al. reported a lower frequency of non-DSA detection in 37 (10%) of 362 recipients.[17] There was no difference in CLAD development or graft survival associated with non-DSAs in their study.

We found that in general, non-DSAs were not associated with increased CLAD risk in multivariable models accounting for DSAs. However, there was a strong interaction between non-DSAs and BAL CXCL9: non-DSAs concurrent with high BAL CXCL9 levels were associated with increased CLAD risk (HR 3.4 95% CI 2.0–5.8, while non-DSAs concurrent with low CXCL9 were not. In fact, we found that any HLA antibody, regardless of the antibody type (DSA, non-DSAs, “Any HLA antibody,” “Any high HLA antibody”), in conjunction with high BAL CXCL9 was associated with increased CLAD risk, while these antibodies in conjunction with low CXCL9 levels was not. This applied even to

"isolated non-DSAs," excluding episodes of concurrent DSA/non-DSA: The HR for CLAD for "non-DSA only" with high CXCL9 was 2.5 95% CI 1.3–4.7. The association between HLA antibodies with high CXCL9 and CLAD development persisted after multivariable adjustment for other known risk factors for CLAD such as: severe PGD, AR, LB, OP, and ALI.

We hypothesized that HLA antibodies (DSA and non-DSA) are markers of overall immune activation and may not be the primary mechanism causing allograft injury. Several lines of reasoning support this hypothesis. First, the strong association between high CXCL9 non-DSAs and CLAD argues against antibody mediated injury, since the HLA epitope recognized by the antibody should not be present in the allograft. Second, similar to Lobo et al,[18] we found that most episodes of DSA (85%) had concurrent non-DSAs detected. Of the tests with positive HLA antibodies, the median number of HLA antibodies detected was 3. This common co-occurrence of DSA with non-DSA supports a non-specific immune response. Finally, the poor correlation between DSAs and histopathology consistent with AMR[11,12,14] also argues against a primary HLA-specific antibody mediated injury. Roux et al in their study of 206 lung transplant recipients, found that only 22 (21%) of 106 DSA positive recipients had histopathologic findings of AMR, while 84 (79%) DSA positive recipients had no histopathologic findings of AMR.[13] Similarly, in our study we found that only 24/68 (35%) of DSA-positive recipients had pathology compatible with AMR, while 35/68 (51%) of DSA-positive recipients had no pathologic findings.

Conversely, we hypothesize that HLA antibodies are often the consequence of allograft injury caused by a number of possible insults including: infection, acute cellular rejection, as well as antibody-mediated rejection. The allograft injury triggers immune activation, upregulation of antigen presentation and recruitment of T cells, NK-cells, and B cells, leading to both donor and non-donor–specific antibody production. CXCL9 is a potent chemoattractant of activated T cells and NK-cells, and a major mediator of the Th1 immune response. Our group has previously demonstrated the key role of CXCL9/CXCR3 biology in the pathogenesis of allograft injury in animal models.[29,30] We have also demonstrated the utility of BAL CXCL9 as a biomarker of allograft injury, with elevated concentration during acute lung injury (ALI),[19] acellular rejection (AR),[20] and organizing pneumonia (OP).[21] Furthermore, these studies showed the prognostic importance of BAL CXCL9, with elevations during allograft injury predicting subsequent CLAD development.

In the current study, we find that a marker of allograft injury (high BAL CXCL9), in conjunction with immune activation (anti-HLA antibodies) is associated with increased CLAD risk, regardless of the antibody type (DSA, non-DSA, any antibody). However, immune activation alone without allograft injury did not associate with subsequent CLAD. Our study design does not allow for the assessment of causality between our markers of immune activation and allograft injury. We feel that future studies evaluating HLA antibody formation after histopathologic allograft injuries are warranted.

The major limitation of this study is the potential for confounding given the retrospective single-center design. For example, patients with clinical deterioration may have received more frequent HLA antibody testing and bronchoscopic evaluation. Importantly,

multivariable adjustment for all known risk factors for CLAD (e.g., respiratory infections) and treatments received for HLA antibody results were beyond the scope of this analysis. At our institution, recipients received solumedrol, PLEX, IVIG, and rituximab for the clinical diagnosis of AMR. Treatment of DSAs without allograft dysfunction or collaborating histopathology was left to the discretion of the transplant pulmonologist. There was no treatment administered for non-DSAs, in the absence of DSAs. This lack of adjustment in our models for this treatment difference may have attenuated the results for DSAs and exaggerated them for non-DSAs.

Despite these limitations, our results are unique in demonstrating the potential utility of BAL CXCL9 measurement as a biomarker to risk stratify HLA antibodies for future CLAD development. The ability to discriminate between high versus low-risk HLA antibodies may improve management by allowing optimal treatment for high-risk antibodies, while minimizing the treatment costs and side effects for low-risk antibodies. Our findings suggest that future multi-center studies evaluating the interaction between HLA antibodies, allograft histopathology, and BAL CXCL9 levels are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Abbreviations:

BAL	bronchoalveolar lavage
BOS	bronchiolitis obliterans syndrome
CLAD	chronic lung allograft dysfunction
CT	chest tomography
DSA	donor-specific antibody
FEV1	forced expiratory volume in 1 second
FVC	forced vital capacity
HLA-Ab	anti-HLA antibody

ISHLT	International Society of Heart and Lung Transplantation
LTR	lung transplant recipients
SAS	Statistical Analysis Software
TBBX	transbronchial biopsy
UCLA	University of California Los Angeles

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Table 1:

Baseline Patient Characteristics Among Pts with Antibodies and Cytokines

	All Patients		Patients with DSAs		Patients with Non-DSAs	
	n	**%**	**n**	**%**	**n**	**%**
Number of Patients	325	100%	96	30%	183	56%
Median Age at Transplant (Q1,Q3)	62	(53–67)	61	(51–67)	62	(53–67)
Male Gender	199	61%	52	54%	96	52%
Single lung transplant	289	89%	87	91%	162	89%
Native Lung Disease						
Restrictive ILD	217	67%	61	64%	123	67%
COPD/AAT	67	21%	21	22%	38	21%
CF / bronchiectasis	21	6%	7	7%	10	5%
Other	20	6%	7	7%	12	7%
Ethnicity						
White	257	79%	72	22%	141	43%
Black	15	5%	8	2%	11	3%
Asian	13	4%	4	1%	8	2%
Other	40	12%	12	4%	23	7%
Median HLA Antibody Tests (Q1,Q3)	7	(4,12)	11	(7–16)	9	(5–14)
Median Tests with Concurrent BALs	2	(1–3)	2	(1–3)	2	(1–3)

Table 2:

Frequency of Antibodies Detected by Type

	Samples		Patients	
	n	%	n	%
Total Samples	2901		325	
No HLA antibody	1639	56%	241	74%
DSA	447	15%	96	30%
Non-DSA	1195	41%	183	56%
Concurrent DSA / Non-DSA	380	13%	84	26%
High DSA (>5000)	150	5%	41	13%
High Non-DSA (>5000)	545	19%	94	29%
DSA				
Class I	105	21%	27	23%
Class II	398	79%	88	77%
Non-DSA				
Class I	775	50%	126	49%
Class II	783	50%	133	51%
DSA				
De novo	306	63%	83	76%
Pre-existing	179	37%	26	24%
Non-DSA				
De novo	765	52%	158	66%
Pre-existing	709	48%	82	34%

Table 3:

Cox Proportional Hazards Model for CLAD Univariable Models:

	# of Events	HR	p-value	95% CI	
DSA					
Any DSA	447	4.0	0.0001	2.4	6.5
High MFI (>5000)	150	12.3	0.0001	6.7	22.4
De novo	306	5.5	0.0001	3.2	9.7
Pre-Existing	179	1.8	0.1531	0.8	4.2
Non-DSA					
Any Non-DSA	1195	1.8	0.0044	1.2	2.7
High MFI (>5000)	545	2.5	0.0002	1.5	4.0
De novo	765	1.7	0.0163	1.1	2.8
Pre-Existing	709	1.5	0.1082	0.9	2.4
Concurrent DSA / Non-DSA	380	4.6	0.0001	2.7	7.8

Table 4:

Cox Proportional Hazards Model for CLAD Multivariable Models:

	# of Events	HR	p-value	95% CI	
Model 1:					
Any DSA	447	3.5	0.0001	2.1	5.8
Any Non-DSA	1195	1.4	0.1244	0.9	2.2
Model 2:					
DSA Only (without Non-DSA)	67	2.7	0.0600	0.96	7.5
Non-DSA Only (without DSA)	815	1.3	0.2657	0.8	2.2
Concurrent DSA / Non-DSA	380	5.1	0.0001	2.9	8.9
Model 3:					
De novo DSA	306	5.0	0.0001	2.7	9.5
De novo Non-DSA	765	1.2	0.5363	0.7	2.0

Table 5:

Frequency of Antibodies Detected by Type With Concurrent BAL CXCL9 Measurement

	Samples		Patients	
	n	%	n	%
Total Samples	486		234	
No Anti-HLA antibody	275	57%	143	61%
DSA	68	14%	45	19%
Non-DSA	200	41%	112	48%
Concurrent DSA / Non-DSA	57	12%	41	18%
DSA [1]				
De novo	44	59%	34	67%
Pre-exist	31	41%	17	33%
Non-DSA [2]				
De novo	131	54%	84	60%
Pre-exist	112	46%	56	40%

[1] There were 7 samples which had both denovo and pre-existing DSA.

[2] There were 43 samples which had both denovo and pre-existing non-DSA.

Table 6:

Median BAL CXCL9 Levels By Antibody Status

		CXCL9		
	n	Median	Q1 - Q3	p-value [1]
No Anti-HLA Antibody	275	699	(215 – 2332)	
Any DSA	68	1182	(338 – 2905)	0.1063
No Anti-HLA Antibody	275	699	(215 – 2332)	
Any Non-DSA	200	1129	(344 – 3390)	0.0177
No Anti-HLA Antibody	275	699	(215 – 2332)	
Any Dual-DSA	57	1303	(493 – 3871)	0.0203
DSA with low MFI [2]	46	570	(264 – 2022)	
DSA with high MFI [2]	22	2006	(1159 – 8081)	0.0364
Non-DSA with low MFI [2]	115	1083	(347 – 2881)	
Non-DSA with high MFI [2]	85	1260	(340 – 3652)	0.2607
Pre-existing DSA	24	394	(227 – 884)	
De novo DSA	44	1740	(495 – 4935)	0.0032
Pre-existing Non-DSA	69	782	(246 – 2570)	
De novo Non-DSA	131	1258	(391 – 3814)	0.5109

[1] P-values compare 2 groups by mixed effects modeling.

[2] High antibody level defined as ≥5000 MFI.

Table 7:

Cox Proportional Hazards Model for CLAD Using HLA Antibodies by CXCL9 Levels Multivariable Models:

	# of Events	HR	p-value	95% CI	
DSA [1]					
DSA with low CXCL9	32	1.4	0.4932	0.5	3.6
No DSA with high CXCL9	184	1.4	0.1920	0.8	2.5
DSA with high CXCL9	36	4.3	0.0001	2.2	8.3
Non-DSA [2]					
Non-DSA with low CXCL9	95	1.1	0.8028	0.6	2.1
No Non-DSA with high CXCL9	115	0.9	0.6796	0.4	1.8
Non-DSA with high CXCL9	105	3.4	0.0001	1.9	6.0
Any Anti-HLA Antibody [3]					
Any antibody with low CXCL9	105	0.9	0.8379	0.5	1.8
No antibody with high CXCL9	114	0.7	0.4532	0.3	1.6
Any antibody with high CXCL9	106	3.2	0.0001	1.8	5.6
Any High HLA Antibody [4]					
Any high antibody with low CXCL9	40	0.8	0.6660	0.3	2.2
No antibody with high CXCL9	172	1.1	0.6600	0.6	2.0
Any high antibody with high CXCL9	48	5.5	0.0001	3.0	10.3
Non-DSA Only [5]					
Non-DSA only with low CXCL9	73	0.8	0.5012	0.4	1.6
No Non-DSA with high CXCL9	150	1.3	0.3273	0.8	2.4
Non-DSA only with high CXCL9	70	2.6	0.0033	1.4	5.0

[1] Any donor specific antibody (DSA)

[2] Any non-donor specific antibody (Non-DSA)

[3] Any anti-HLA antibody (DSA or non-DSA)

[4] Any high HLA antibody (MFI > 5000)

[5] Non-DSA without concurrent DSA

TABLE 8

Cox proportional hazards model for CLAD using HLA antibodies by CXCL9 levels multivariable models

	# of Events	HR	*p*-value	95% CI	
DSA[a]					
DSA with low CXCL9	32	1.4	.4932	0.5	3.6
No DSA with high CXCL9	184	1.4	.1920	0.8	2.5
DSA with high CXCL9	36	4.3	.0001	2.2	8.3
Non-DSA[b]					
Non-DSA with low CXCL9	95	1.1	.8028	0.6	2.1
No non-DSA with high CXCL9	115	0.9	.6796	0.4	1.8
Non-DSA with high CXCL9	105	3.4	.0001	1.9	6.0
Any anti-HLA antibody[c]					
Any antibody with low CXCL9	105	0.9	.8379	0.5	1.8
No antibody with high CXCL9	114	0.7	.4532	0.3	1.6
Any antibody with high CXCL9	106	3.2	.0001	1.8	5.6
Any high HLA antibody[d]					
Any high antibody with low CXCL9	40	0.8	.6660	0.3	2.2
No antibody with high CXCL9	172	1.1	.6600	0.6	2.0
Any high antibody with high CXCL9	48	5.5	.0001	3.0	10.3
Non-DSA Only[e]					
Non-DSA only with low CXCL9	73	0.8	.5012	0.4	1.6
No non-DSA with high CXCL9	150	1.3	.3273	0.8	2.4
Non-DSA only with high CXCL9	70	2.6	.0033	1.4	5.0

[a]Any donor specific antibody (DSA).

[b]Any non-donor specific antibody (non-DSA).

[c]Any anti-HLA antibody (DSA or non-DSA).

[d]Any high HLA antibody (MFI >5000).

[e]Non-DSA without concurrent DSA.

TABLE 9

Cox proportional hazards model for CLAD using HLA antibodies by CXCL9 levels final multivariable models

	# of Events	HR	*p*-value	95% CI	
Model 1: DSA					
DSA with low CXCL9	32	1.4	.5097	0.1	3.5
No DSA with high CXCL9	184	1.4	.1998	0.8	2.3
DSA with high CXCL9	36	5.1	.0001	2.6	9.7
Severe PGD[a]	23	0.9	.8146	0.5	1.7
Acute cellular rejection (≥A2)	74	2.3	.0014	1.4	3.9
Lymphocytic bronchiolitis	137	1.2	.5509	0.7	1.8
Organizing pneumonia	47	0.7	.2890	0.4	1.3
Acute lung injury	63	2.0	.0048	1.2	3.4
Model 2: Non-DSA					
Non-DSA with low CXCL9	95	1.8	.7940	0.6	2.0
No Non-DSA with high CXCL9	115	0.9	.8006	0.5	1.8
Non-DSA with high CXCL9	105	3.4	.0001	2.0	5.8
Severe PGD[a]	23	1.1	.8713	0.6	2.0
Acute cellular rejection (≥A2)	74	2.2	.0039	1.3	3.7
Lymphocytic bronchiolitis	137	1.0	.8653	0.6	1.7
Organizing pneumonia	47	0.7	.2347	0.4	1.3
Acute lung injury	63	1.9	.0101	1.2	3.2
Model 3: Non-DSA Only[b]					
Non-DSA with low CXCL9	73	0.1	.5989	0.4	1.7
No non-DSA with high CXCL9	150	1.5	.1034	0.9	2.6
Non-DSA with high CXCL9	70	2.5	.0065	1.3	4.7
Severe PGD[a]	23	0.9	.6493	0.5	1.6
Acute cellular rejection (≥A2)	74	2.2	.0036	1.3	3.7
Lymphocytic bronchiolitis	137	1.0	.8715	0.7	1.6
Organizing pneumonia	47	0.7	.2308	0.4	1.3
Acute lung injury	63	2.1	.0041	1.3	3.4

[a]Primary graft dysfunction grades 2 or 3 at 48 or 72 h post-transplant.

[b]Non-DSA without concurrent DSA.