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Journal

ISSN

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Publication Date

DOI

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Human Leukocyte Antigens Antibodies after Lung Transplantation: Primary Results of the HALT Study

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Abstract

Donor-specific antibodies (**DSA**) to mismatched human leukocyte antigens (**HLA**) are associated with worse outcomes after lung transplantation. To determine the incidence and characteristics of DSA early after lung transplantation, we conducted a prospective multi-center observational study that used standardized treatment and testing protocols. Among 119 transplant recipients, 43 (36%) developed DSA: 6 (14%) developed DSA only to class I HLA, 23 (53%) developed DSA only to class II HLA, and 14 (33%) developed DSA to both class I and class II HLA. The median DSA mean fluorescence intensity (**MFI**) was 3197. We identified a significant association between the Lung Allocation Score and the development of DSA (HR = 1.02, 95% CI: 1.001 – 1.03, p = 0.047) and a significant association between DSA with an MFI ≥ 3000 and acute cellular rejection (**ACR**) grade ≥ A2 (HR = 2.11, 95% CI: 1.04 – 4.27, p = 0.039). However, we did not detect an association between DSA and survival. We conclude that DSA occur frequently early after lung transplantation, and most target class II HLA. DSA with an MFI ≥ 3000 have a significant association with ACR. Extended follow-up is necessary to determine the impact of DSA on other important outcomes.

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INTRODUCTION

Lung transplantation may prolong survival and improve quality of life for patients who have advanced lung disease (1–5). However, long-term outcomes after lung transplantation remain disappointing; according to the latest International Society for Heart and Lung Transplantation (**ISHLT**) Registry report, recipients from the most recent era had a median survival of approximately 6 years (6). The most common causes of death in the first year after transplantation are infection and allograft failure related to primary graft dysfunction (**PGD**), but allograft failure due to chronic lung allograft dysfunction (**CLAD**), including bronchiolitis obliterans syndrome (**BOS**) and restrictive allograft syndrome (**RAS**), accounts for over 40% of deaths and remains the main obstacle to better long-term outcomes after transplantation (6–10).

Conventional immunosuppression has primarily targeted T-cell proliferation and function for the prevention of allograft rejection in solid-organ transplantation. However, evidence supporting a role for humoral immunity in allograft rejection has grown over time. Hyperacute rejection due to preformed donor-specific antibodies (**DSA**) to mismatched human leukocyte antigens (**HLA**) has long been recognized as a cause of early allograft failure, and an association between HLA antibodies and CLAD has been recognized since the late 1990s (11–14). More recently, clinicians and investigators have better recognized antibody-mediated rejection (**AMR**) after lung transplantation, and an ISHLT working group has developed a consensus definition (15–20).

In previous retrospective single-center studies, the incidence of DSA after lung transplantation has varied between 13% and 61%, and these studies have not used a standardized approach to testing and management (21–28). We sought to examine the incidence of DSA and their characteristics in a prospective multi-center study that used a standardized approach to clinical management, monitoring, and DSA testing.

METHODS

Study Design and Eligibility Criteria

We conducted a prospective observational cohort study of lung transplant recipients at 6 centers in the United States to determine the cumulative incidence of post-transplant DSA, describe their characteristics, and assess their impact on clinical outcomes. The National Institutes of Health funded the study as a 2-year R34 grant (HL 10542). Each site aimed to prescreen/screen all lung transplant candidates placed on the United Network for Organ Sharing (**UNOS**) waiting list at the institution. Study personnel screened patients for eligibility (Table E1 in the online data supplement) at the time of listing for transplantation or within the first 10 days after transplantation. Enrollment was initiated on December 1, 2011 and completed on June 30, 2012; follow-up was completed on October 28, 2012. Local Institutional Review Boards at each center approved the study protocol, and all participants provided written informed consent.

Endpoints and Outcomes

Primary and secondary endpoints were defined during protocol development. The study used HALT-defined DSA (Table 1) as the primary endpoint, and the cumulative incidence of first DSA during the first 4 months after transplantation as the primary outcome. Protocol-defined secondary endpoints included antibody mediated rejection (**AMR**), acute cellular rejection (**ACR**), lymphocytic bronchiolitis (**LB**), and death. ACR and LB were defined according to the standard ISHLT criteria (29). However, for the diagnosis of AMR, we used a central blinded adjudication committee because the ISHLT definition was proposed after the study was completed (20). As part of the secondary endpoints, we also examined the association between DSA and the development of ACR and LB.

HLA Testing

Before transplantation, candidates underwent low resolution HLA-A, B, C, DRB1, DRB3, DRB4, DRB5, and DQB typing by the transplant center's histocompatibility laboratory using DNA-based methods. Donors underwent similar typing at the transplant center. In cases where testing detected anti-DQA, DP or allele-specific antibodies, the lab conducted additional HLA-DQA, DP or allele level typing of donors and recipients. Before transplantation, candidates were tested for HLA antibodies using the LABScreen® Single Antigen assay (One Lambda, Canoga Park, CA), and donor organs were accepted for an allosensitized patient if the patient did not have HLA antibodies against the donor type (i.e., negative virtual crossmatch). However, the mean fluorescence intensity (**MFI**) cut-off to define pre-transplant HLA antibody positivity varied among sites. Candidates with pre-transplant DSA were eligible for enrollment if the DSA MFI < 5000 and the direct donor-recipient flow cytometry crossmatch was negative at the time of transplantation. The histocompatibility laboratory at the sites used a direct final prospective or a retrospective flow cytometry T- and B-cell crossmatch to determine donor-recipient HLA compatibility. The laboratories tested pre-transplant serum, and sera were tested in duplicate. Each laboratory used their protocols to perform sample preparation and standardization of instrument settings; instruments used by different laboratories were not cross-calibrated using reference materials. Flow crossmatch results were reported using median channel shift (**MCS**). The interpretation of the T- and B-cell crossmatch compatibility was based on cut off values established by each laboratory as defined in the HALT manual of operation. Prior to reporting, the crossmatch results were reviewed for consistency with past and current HLA antibody specificities, presence of autoantibody reactivity, or other potentially interfering substances. All laboratories considered patients with "positive" flow crossmatches according to the HALT eligibility criteria to participate in the study. In some centers, weak DSA (MFI value in the range of 1000–3000) may be undetected by the flow cytometry crossmatch if the sensitivity of the crossmatch is low due sample preparation or instrument setting. The HALT HLA Adjudication Committee adjudicated all flow crossmatch results as positive or negative.

After transplantation, recipients were tested for DSA using the LABScreen® Single Antigen assay at 10 (± 3), 30, 60, 90, and 120 (± 7) days after transplantation, and if they had clinical signs or symptoms of allograft dysfunction. The study classified post-transplant DSA as *de novo* or pre-formed (Table 1). *De novo* DSA was defined as a donor-specific antibody with

an MFI > 1000, and if the antibody was detectable pre-transplant with an MFI < 1000, the post-transplant MFI had to increase by   500 and > 30% compared to the pre-transplant MFI (Table 1). Among patients with pre-formed DSA (i.e., DSA MFI between 1000 – 5000 and a negative direct flow cytometry crossmatch), post-transplant DSA was defined as an MFI increase   500 and > 30% compared to the pre-transplant MFI (Table 1). The HALT HLA Adjudication Committee adjudicated all post-transplant DSA test results as present or absent. Additional details of HLA testing methodology are provided in the online data supplement.

Standardized Clinical Care

Prior to initiation of study enrollment, the sites agreed upon a standardized general clinical approach to induction and maintenance immunosuppression, infection prophylaxis, monitoring with pulmonary function testing (**PFT**) and bronchoscopy, and ACR treatment.

Statistical Analyses

The study used standard descriptive analyses for reporting. For comparisons of characteristics between subgroups, we used t-tests (or Wilcoxon-Rank sum tests if the data were not normally distributed) and chi-square (or Fisher's exact) tests. For the primary endpoint of the study (i.e., post-transplant DSA), we used the Kaplan-Meier method to estimate time to DSA development. We used Cox proportional hazards regression to identify risk factors for the development of DSA and to assess for associations between the development of DSA and ACR and LB. We performed these analyses post-hoc, and did not adjust for multiple comparisons. In the analyses of risk factors for the development of DSA, we only included episodes of ACR and LB that occurred before DSA, and in the analyses of the impact of DSA on ACR and LB, we only included episodes of ACR and LB that occurred after DSA. We confirmed model assumptions and performed regression diagnostics, and censored for death, re-transplant, end of study, and loss to follow-up. We used SPSS 22.0 (SAS, Inc., Chicago, IL, USA) for all analyses. The online data supplement provides additional details on data management and power calculations.

RESULTS

During the enrollment period, study personnel pre-screened 397 transplant candidates and recipients among the centers. Among the 397 potential participants, 227 (57%) were not transplanted during the study period. Of the 232 potentially eligible and consented patients, 126 underwent lung transplantation (Figure 1). Of these, the study excluded 7 patients because of treatment with intravenous immune globulin (**IVIG**) or cytomegalovirus (**CMV**) immune globulin for hypogammaglobulinemia or high-risk CMV status. The remaining 119 lung transplant recipients comprised the primary HALT study cohort (Figure 1). Only 51 of 397 (13%) potential participants were transplanted but not included in the study cohort. In general, study participants had similar demographics to contemporary U.S. lung transplant recipients (Table 2) (30). Interstitial lung disease was the most common indication for lung transplantation and accounted for 44% of patients. The mean Lung Allocation Score (**LAS**) at transplant was 45.59 ± 16.93 (median = 39.99). The median number of donor-recipient HLA mismatches at the A, B, C, DRB1, and DQB loci was 8 (Table 2).

Among the 119 study participants, 12 (10%) had pre-transplant DSA; 7 had DSA only to class I HLA, 3 had DSA only to class II HLA, and 2 had DSA to class I and class II HLA. The median class I DSA MFI was 1496, and the median class II DSA MFI was 1922. In the study cohort, 116 of 119 patients had a direct flow cytometry crossmatch performed. The T-cell crossmatch was negative in 115 (99%) recipients and positive in 1 (1%) with a median channel shift of 38. The B-cell crossmatch was negative in 99 (85%) recipients and positive in 17 (15%) with a mean MCS of 102. None of the recipients who had a positive T-cell or B-cell crossmatch had pre-transplant DSA, and the sites' clinical teams and the study's blinded adjudicators deemed all transplants HLA compatible.

Although the sites agreed upon a standardized general clinical approach to immunosuppression, deviations from this approach were allowed for patient-specific indications. Of the 119 recipients, 72 (60%) were treated with basiliximab and 2 (2%) were treated with rabbit anti-thymocyte globulin for induction immunosuppression, while 45 (38%) did not receive induction immunosuppression because they were deemed to be at increased risk of infectious complications (e.g., chronic infection with multi-drug resistant organisms, pre-transplant immunosuppression, or frailty). The maintenance immunosuppressive regimen on day 10 after transplantation consisted of prednisone (119/119; 100%), tacrolimus (116/119; 98%), and mycophenolate mofetil (112/119; 94%) or azathioprine (1/119; 1%).

During the 4 months of post-transplant follow-up, the cohort of lung transplant recipients had a first DSA cumulative incidence of 36% (43/119; 95% CI 28% – 45%; Figure 2). There was no statistically significant difference in the incidence of DSA between the 6 sites (p = 0.119, Table E2 in the online data supplement). Of the 43 patients who developed DSA, 26 (60.5%) developed DSA within the first 30 days of transplantation and 17 (39.5%) developed DSA beyond the first 30 days. Among those who developed DSA, 6 (14%) developed DSA only to class I HLA, 23 (53%) developed DSA only to class II HLA, and 14 (33%) developed DSA to class I and class II HLA. Overall, the mean number of DSA specificities among those who developed DSA was 2.0 ± 1.68 (median = 1). Of the 43 transplant recipients who developed DSA, 23 (54%) developed a single DSA, 12 (28%) developed 2 DSA, and 8 (18%) developed 3 or more DSA during follow-up (Figure 3A). DSA specificity for HLA-DQ was most frequent (n = 26, Figure 3B). The median MFI of the immunodominant DSA was 3197 (range 1068 – 18150); the DSA MFI stratified by HLA locus is shown in Figure 3C. Nine of the 43 (21%) recipients who developed DSA had detectable pre-transplant DSA; among these, 5 developed DSA with new specificities (*de novo* DSA) after transplant and 4 had the same pre-transplant specificities that increased in MFI and met the study definition of post-transplant DSA. We explored the incidence of DSA using different definitions of positivity. Among the 119 study participants, 32 (27%, 95% CI: 19% – 35%) developed DSA with an MFI 2000, and 24 (20%, 95% CI: 13% – 28%) developed DSA with an MFI 3000 (Table E3 in the online data supplement). During the study period, 9 patients cleared all DSA on follow-up testing, and all 9 had DSA initially identified in the first 30 days after transplantation.

Statistically significant univariable predictors of post-transplant DSA in Cox proportional hazards regression included pre-transplant DSA and a higher LAS at transplant (Table 3).

Although the magnitude of risk associated with LAS is small, this signifies a 2% higher risk of DSA development for every incremental increase in the LAS by 1. To make this relationship more easily interpretable, we analyzed the risk of DSA development with incremental increases of 10 in the LAS: HR = 1.22, 95% CI: 1.02 – 1.38, p = 0.027. We did not evaluate positive T-cell crossmatch as a risk factor for post-transplant DSA because only 1 patient had a positive T-cell crossmatch and developed DSA 16 days after transplantation. In a multivariable Cox proportional hazards regression model, LAS at transplant was a significant predictor of post-transplant DSA but pre-transplant DSA was not (Table 4). In separate analyses, we forced positive B-cell crossmatch, the number of donor-recipient mismatches at the HLA-DR and HLA-DQ loci, LB, ACR, PGD grade, age, and gender into the final model, but none of these covariates improved the model's −2 log likelihood or showed statistical significance (data not shown). In these models, the proportional hazards assumptions were met and we excluded nonlinearity using partial residual plots.

During the study period, 112 recipients had transbronchial lung biopsies for evaluation of ACR, while 7 did not because of various clinical contraindications. Among the 112, 72 (64%) had at least one episode of ACR grade A1, 45 (40%) had at least one episode of ACR grade A2, and 18 (16%) had at least one episode of LB grade B1R. We did not detect a significant association between HALT-defined DSA and ACR (Table 5). We examined associations between DSA and ACR using different DSA MFI thresholds, and identified a trend to an increased risk of ACR grade A1 associated with DSA with an MFI 2000 and MFI 3000 and a statistically significant association between DSA with an MFI 3000 and ACR grade A2 (Table 5). Furthermore, we detected a significant association between the number of DSA specificities and ACR grade A2 (Table 5). We examined associations between DSA class and ACR and identified an increased risk of ACR grade A2 associated with DSA to both class I and class II HLA (Table 5). We did not detect any significant associations between any definition of DSA and LB.

During the study period, 4 of the 43 (9%, 95% CI: 1% – 18%) recipients who developed DSA developed AMR (Table 6). All 4 patients had DSA to class I and class II HLA, and the median MFI of the immunodominant DSA was 4939 (range 2167 – 9572). The central blinded adjudication committee evaluated these cases and confirmed the diagnosis of AMR in 2 cases but deemed the other 2 cases as probably not AMR (Table 6). According to the recent ISHLT consensus definition (20), 2 cases were consistent with possible AMR and 2 were consistent with probable AMR. All patients with AMR were treated with various combinations of high-dose corticosteroids, IVIG, and rituximab, and all experienced a clinical improvement. Because study follow-up was limited to 4 months after transplantation, we did not examine the impact of DSA on BOS or CLAD development. We did not detect a significant difference in survival during the study period between recipients who developed DSA and those who did not; 4 of the 76 (5%) recipients who did not develop DSA died, and none of the 43 recipients who developed DSA died during the study period (log rank p = 0.128). However, study follow-up was limited to 4 months after transplantation.

DISCUSSION

In this prospective multicenter study that utilized a standardized approach to patient management and DSA testing, we examined the incidence and characteristics of DSA after lung transplantation. DSA developed frequently and early after transplantation, with an incidence of 36% in the first 4 months. The majority of DSA were directed at class II HLA, and a minority was directed solely at class I HLA. We identified higher LAS at transplant as an independent risk factor for the development of DSA. The early development of DSA, particularly in the first 2 weeks after transplantation, suggests a memory response though most patients did not have identifiable DSA before transplantation. Nonetheless, although pre-transplant DSA was a significant risk factor for developing DSA after transplantation in univariable analysis, it was no longer significant after adjusting for LAS. We detected an association between DSA with an MFI 3000 and ACR, though we did not detect a significant association between DSA with a lower MFI and ACR, and DSA had no significant impact on allograft survival. However, we note that the short follow-up period in this study limits our ability to fully characterize the impact of DSA on CLAD and allograft survival.

The cumulative incidence of DSA of 36% in this study is similar to previous single-center reports where the incidence ranged between 13% and 61% (21–28). Differences in the incidence of DSA among studies are likely related to different DSA testing protocols, DSA definitions, patient characteristics, and clinical regimens (e.g., immunosuppression, blood product transfusion). We used a low threshold MFI to define DSA positivity in this study in an attempt to detect DSA early. Previous studies have consistently shown an association between DSA and adverse clinical outcomes after lung transplantation, and we reasoned that early detection would identify patients at increased risk for ACR, LB, CLAD, and allograft failure (23–28, 32, 33). Not surprisingly, changing the definition of DSA positivity by increasing the threshold MFI resulted in a lower incidence of DSA. Ultimately, the definition of DSA should be based on the association between DSA development and clinical outcomes, and our results suggest that a threshold MFI 3000 is associated with a significantly increased risk of ACR. However, longer follow-up may demonstrate that DSA with lower MFI may increase the risk of ACR and LB. In addition, up to 9% of recipients who develop DSA develop possible or probable AMR according to the ISHLT definition. Nonetheless, a longer duration of follow-up is necessary to identify the MFI threshold that may be associated with the development of CLAD. Although our findings identified a significant association between DSA and ACR, this does not indicate that DSA have a causal role in the development of ACR. Rather, this underscores a synergy between humoral and cellular immune responses. Furthermore, the association between the number of DSA specificities and the risk of ACR suggests that the breadth of the humoral immune response correlates with the risk of cellular rejection. We did not identify a significant association between DSA to class II HLA and ACR as described in some previous studies, likely due to the relatively small sample size. In contrast, we did find an association between DSA to both class I and class II HLA and an increased risk of ACR, and we suspect the number of DSA specificities drove this relationship.

The study protocol did not include antibody-directed therapy after DSA identification because this remains controversial (21, 34, 35). Although retrospective cohort studies have suggested that treatment of asymptomatic DSA may improve clinical outcomes, the optimal regimen is unknown and spontaneous DSA clearance may occur (27–28, 34, 35). Thus, we propose that the efficacy and safety of treatment for asymptomatic DSA should be investigated in the context of a randomized controlled trial.

In contrast to previous studies, we did not identify an association between PGD and DSA (36, 37). However, the cohort had a small number of patients who developed PGD grade 2 or grade 3, and the study did not have sufficient power to detect a small but potentially important association. Nevertheless, our findings demonstrate a significant association between the LAS at transplant and DSA development. This is consistent with a recent study that reported a more than 2-fold higher risk of DSA among patients with an LAS > 60 (28). The LAS reflects severity of illness before transplantation, and a higher LAS is associated with increased morbidity that includes PGD, acute kidney injury, infections, respiratory failure, and prolonged critical illness early after transplantation (38–40). These comorbidities may upregulate pro-inflammatory mediators that increase the risk of DSA development (36, 41). Unfortunately, we did not collect data regarding these early post-transplant complications, and we are unable to examine their impact on the risk of DSA development in this cohort.

Despite the standardization of DSA testing, patient assessments, treatment approaches, and data collection among sites, some methodology limitations likely affected the results of the HALT study. The short follow-up duration, limited to 4 months after transplantation, almost certainly underestimates the incidence of DSA after transplantation. Although most patients developed DSA within 3 months of transplantation in some studies (21, 25–26), a significant number of patients developed DSA beyond the first year after transplantation in other studies (23, 24, 27). In addition, the short follow-up did not allow an appropriate assessment of the impact of DSA on CLAD and survival. The study duration also likely underestimated the incidence of AMR. Furthermore, the short follow-up duration limited our ability to assess the frequency of DSA persistence, resolution, recurrence, MFI changes, and profile switching. The study sample size influenced the precision of our estimates of the cumulative incidence of first DSA, though the 95% CI (28% – 45%) suggests that DSA occur commonly. The small sample size likely also impacted our ability to identify risk factors for DSA development. Lastly, we did not validate antibody testing results in a separate or core laboratory.

We conclude that DSA occur commonly early after lung transplantation, and that DSA are more frequently directed at class II HLA. We strongly support the move toward standardized DSA testing and reporting (42). Future studies should further clarify the association between DSA and long-term outcomes after lung transplantation and the potential role of preemptive antibody-directed therapy for transplant recipients who develop DSA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

ABBREVIATIONS

ACR	Acute cellular rejection
AMR	Antibody-mediated rejection
BOS	Bronchiolitis obliterans syndrome
CLAD	Chronic lung allograft dysfunction
CMV	Cytomegalovirus
DSA	Donor-specific antibodies
HLA	Human leukocyte antigens
ISHLT	International Society for Heart and Lung Transplantation
IVIG	Intravenous immunoglobulin
MCS	Median channel shift
MFI	Mean fluorescence intensity
PFT	Pulmonary function testing
PGD	Primary graft dysfunction
RAS	Restrictive allograft syndrome
UNOS	United Network for Organ Sharing

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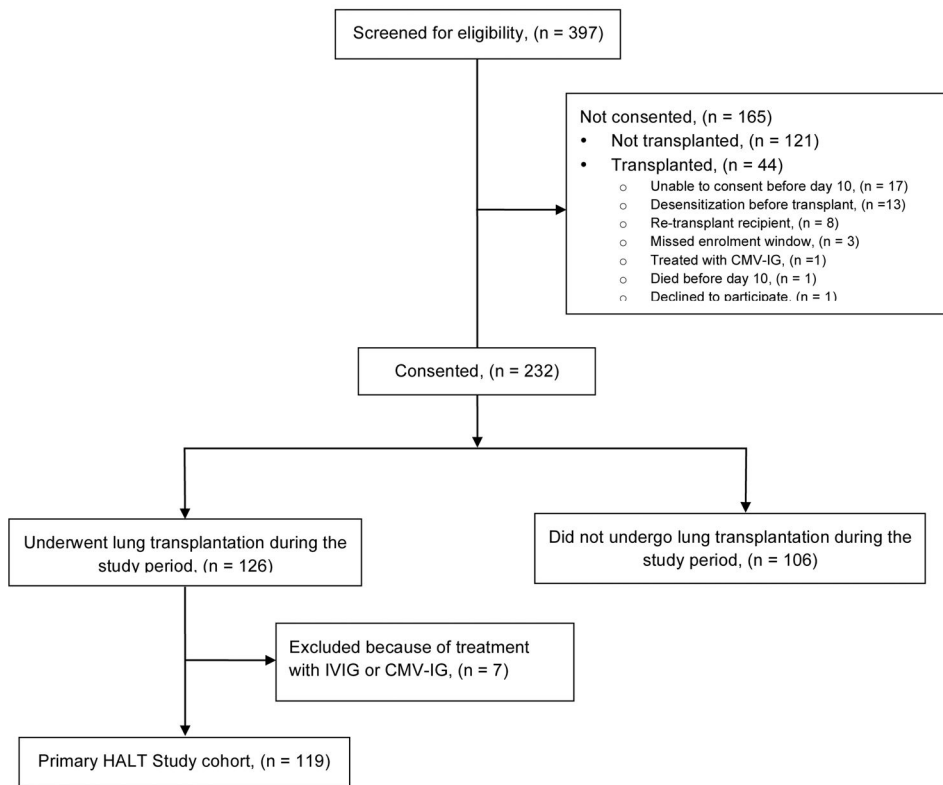
Figure 1.
Flow diagram: study screening and eligibility. IVIG: intravenous immune globulin. CMV-IG: cytomegalovirus immune globulin.
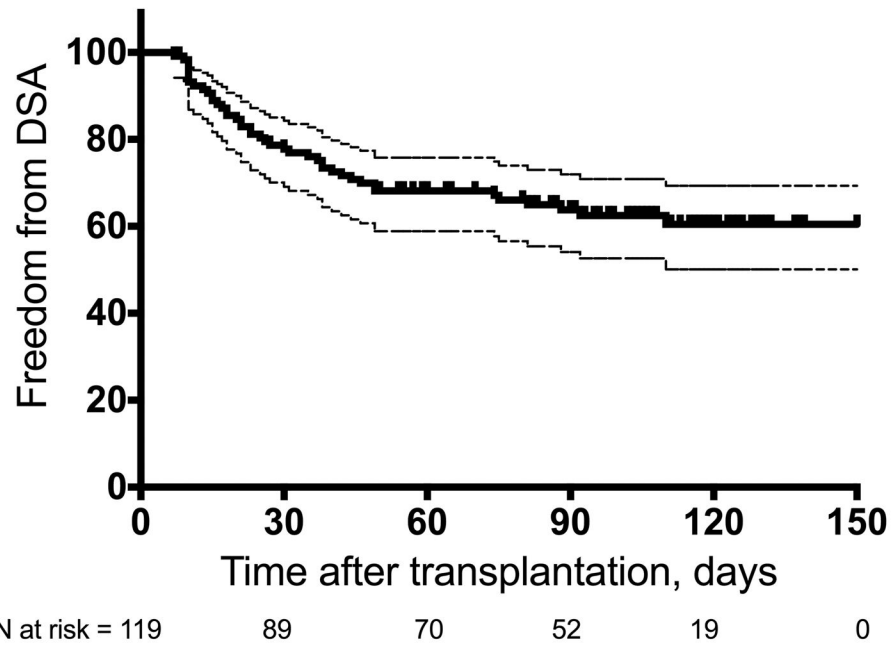
Figure 2.
Kaplan-Meier freedom from post-transplant donor-specific HLA antibodies (DSA) during the study period. Thin dashed lines depict upper and lower bounds of 95% confidence interval.
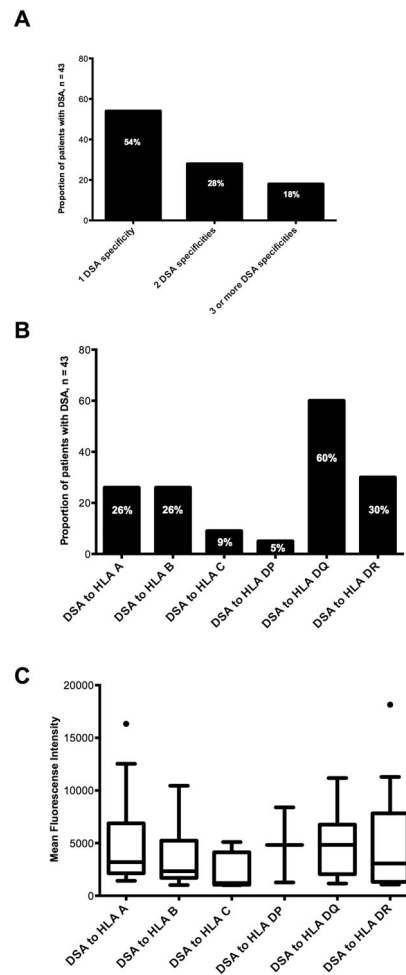
Figure 3.

A. The proportion of patients who developed post-transplant donor-specific HLA antibodies (**DSA**) stratified by the number of DSA specificities.

B. The proportion of patients who developed post-transplant DSA stratified by DSA specificity.

C. Post-transplant DSA mean fluorescence intensity (**MFI**) stratified by HLA locus. Boxes extend from the 25th to the 75th percentiles, whiskers extend to the largest and smallest values within 1.5 times box lengths, and the line within the boxes represents the median. Solid circles represent outlier cases between 1.5 and 3 box lengths from the upper edge of the boxes.

Table 1

HALT Study definition of post-transplant donor-specific HLA antibody (**DSA**) positivity.

De novo post-transplant DSA
Compared to pre-transplant bead reactivity [*]:
•   Increase in DSA MFI > 30%, and
•   Increase in DSA MFI ≥ 500, and
•   DSA MFI > 1000
Pre-transplant DSA with MFI: 1000 – 5000 and negative flow cytometry crossmatch
Compared to pre-transplant bead reactivity:
•   Increase in DSA MFI > 30%, and
•   Increase in DSA MFI ≥ 500

DSA: donor-specific HLA antibody

MFI: mean fluorescence intensity

[*] LABScreen® Single Antigen assay (One Lambda, Canoga Park, CA)

Table 2

Demographics of HALT study participants.

Variable	N = 119
Age, mean ± standard deviation (SD)	57 ± 13
Female recipient, n (%)	49 (41%)
Diagnosis, n (%)	
COPD	38 (32%)
Interstitial lung disease / pulmonary fibrosis	53 (44%)
Cystic fibrosis (CF) or non-CF bronchiectasis	18 (15%)
Pulmonary hypertension	2 (2%)
Other	8 (7%)
Lung allocation score at transplant, mean ± SD	45.59 ± 16.93
Pre-transplant ECMO support	2 (2%)
Bilateral lung transplant, n (%)	90 (76%)
Single lung transplant, n (%)	29 (24%)
Intraoperative cardiopulmonary bypass, n (%)	69 (58%)
Ischemic time (minutes), mean ± standard deviation	285 ± 80
Post-transplant ECMO support, n (%)	1 (1%)
Identical donor-recipient ABO status, n (%)	107 (90%)
Compatible donor-recipient ABO status, n (%)	12 (10%)
Recipient CMV seropositive, n (%)	53 (45%)
Recipient CMV seronegative and donor seropositive, n (%)	35 (29%)
Pre-transplant DSA, n (%)	12 (10%)
Number of donor-recipient mismatches at HLA A, B, C, DRB1, and DQB loci, median (IQR)	8 (3)
Number of donor-recipient mismatches at HLA-DRB1 locus, median (IQR)	2 (1)
Number of donor-recipient mismatches at HLA-DQB locus, median (IQR)	1 (1)
Positive Flow Cytometry B-cell crossmatch, n (%)	17 (15%)
PGD grade at time 0 [*], n (%)	
PGD 0	69 (60%)
PGD 1	26 (23%)
PGD 2	10 (9%)
PGD 3	10 (9%)
PGD grade at 72 hours [**], n (%)	
PGD 0	49 (47%)
PGD 1	49 (47%)
PGD 2	3 (3%)
PGD 3	4 (4%)
Blood product transfusion during transplant hospitalization, n (%)	63 (53%)
Induction immunosuppression, n (%)	
None	45 (38%)
Basiliximab	72 (60%)
Rabbit anti-thymocyte globulin	2 (2%)

COPD: chronic obstructive pulmonary disease, with or without alpha 1-antitrypsin deficiency

ECMO: extracorporeal membrane oxygenation

CMV: cytomegalovirus

PGD: primary graft dysfunction

*
Immediately after transplantation (time 0), 115 recipients had sufficient data to calculate PGD grades

**
At 72 hours, 105 recipients had sufficient data to calculate PGD grades

Table 3

Univariable Cox proportional hazards models of risk factors for post-transplant donor-specific HLA antibody (**DSA**) development.

Variable	HR	95% CI	p value
Recipient age	1.01	0.99 – 1.04	0.297
Recipient female gender	0.91	0.49 – 1.67	0.753
Diagnosis (reference = COPD)			0.381
Interstitial lung disease	1.28	0.65 – 2.52	0.469
Cystic fibrosis or bronchiectasis	0.38	0.11 – 1.35	0.135
Pulmonary hypertension	1.25	0.16 – 9.55	0.831
Other	0.80	0.18 – 3.54	0.766
Lung allocation score at transplant	1.02	1.002 – 1.03	0.027
Pre-transplant ECMO support	1.19	0.16 – 8.64	0.865
Pre-transplant DSA	2.34	1.08 – 5.05	0.030
Single lung transplant (reference = bilateral)	0.93	0.46 – 1.89	0.931
Ischemic time	1.00	0.99 – 1.01	0.589
Intraoperative cardiopulmonary bypass	1.18	0.64 – 2.18	0.595
Compatible donor-recipient ABO status (reference = identical)	1.78	0.79 – 4.00	0.162
Number of donor-recipient HLA mismatches	0.99	0.83 – 1.17	0.881
Number of donor-recipient mismatches at HLA-DRB1 locus	1.34	0.78 – 2.29	0.289
Number of donor-recipient mismatches at HLA-DQB locus	1.12	0.70 – 1.78	0.636
Positive B-cell crossmatch	1.75	0.84 – 3.67	0.136
PGD grade at time 0 (reference = PGD 0)			0.968
PGD 1	1.27	0.62 – 2.59	0.514
PGD 2	1.07	0.32 – 3.57	0.907
PGD 3	1.16	0.40 – 3.33	0.790
PGD grade at 72 hours (reference = PGD 0)			0.999
PGD 1	1.05	0.54 – 2.06	0.887
PGD 2	0.98	0.13 – 7.40	0.988
PGD 3	1.11	0.15 – 8.32	0.923
Blood product transfusion	0.76	0.42 – 1.39	0.378
Induction agent (reference = none)			0.177
Basiliximab	0.65	0.35 – 1.19	0.162
Rabbit anti-thymocyte globulin	1.94	0.45 – 8.32	0.371
Acute cellular rejection grade A1[*]	1.43	0.54 – 3.82	0.470
Acute cellular rejection grade A2[*]	1.88	0.65 – 5.46	0.244
Lymphocytic bronchiolitis grade B1R[*]	2.43	0.80 – 7.39	0.117

[*]Analyzed as a time-dependent variable.

COPD: chronic obstructive pulmonary disease, with or without alpha 1-antitrypsin deficiency

ECMO: extracorporeal membrane oxygenation

DSA: donor-specific HLA antibody

HLA: human leukocyte antigen

PGD: primary graft dysfunction

Table 4

Multivariable Cox proportional hazards model of risk factors for post-transplant donor-specific HLA antibody (**DSA**) development.

Variable	HR	95% CI	p value
Lung allocation score at transplant	1.02	1.001 – 1.03	0.047
Pre-transplant DSA	1.67	0.73 – 3.82	0.226

Model Chi-square = 6.83, −2 log likelihood = 371.02, p = 0.033

Table 5

Univariable Cox proportional hazards regression analyses of donor-specific HLA antibody (**DSA**) as predictors of outcome (acute cellular rejection [**ACR**] grade ≥ A1, ACR ≥ A2, or lymphocytic bronchiolitis [**LB**] grade ≥ B1R).

DSA definition	ACR grade ≥ A1			ACR grade ≥ A2			LB grade ≥ B1R		
	HR	95% CI	p value	HR	95% CI	p value	HR	95% CI	p value
HALT-defined DSA *	1.42	0.85 – 2.38	0.178	1.42	0.75 – 2.69	0.279	1.18	0.42 – 3.34	0.757
DSA MFI ≥ 2000 *	1.74	0.99 – 3.04	0.052	1.54	0.76 – 3.12	0.230	0.95	0.27 – 3.29	0.929
DSA MFI ≥ 3000 *	1.80	0.98 – 3.28	0.057	2.11	1.04 – 4.27	0.039	1.19	0.34 – 4.12	0.788
Number of DSA ** specificities	1.22	0.96 – 1.54	0.105	1.32	1.02 – 1.72	0.038	1.19	0.78 – 1.80	0.423
DSA Class **			0.668			0.159			0.964
No DSA (reference)	1.00			1.00					
Class I only	0.84	0.33 – 2.11	0.705	1.01	0.31 – 3.31	0.993	0.82	0.11 – 6.33	0.851
Class II only	1.06	0.59 – 1.91	0.842	0.94	0.43 – 2.06	0.869	1.18	0.38 – 3.67	0.772
Class I and Class II	1.66	0.70 – 3.92	0.252	2.71	1.11 – 6.60	0.029	0.70	0.09 – 5.39	0.732

ACR: acute cellular rejection

LB: lymphocytic bronchiolitis

MFI: mean fluorescence intensity

*
Analyzed as a time-dependent variable

**
DSA defined according to HALT study criteria (i.e., HALT-defined DSA)

Table 6

Clinical presentation and diagnostic certainty of cases of suspected antibody-mediated rejection (AMR).

Case	Time After Transplant	Allograft Dysfunction	DSA Locus & MFI*	Pathology	C4d Deposition	Adjudication Committee Determination	ISHLT Consensus Statement Diagnostic Certainty
1	46 days	Dyspnea, decline in spirometry	A (MFI = 1451) DRB1 (MFI = 2167)	Acute cellular rejection (A2B0), capillaritis	Negative	Definitely AMR	Probable AMR
2	22 days	Hypoxemic respiratory failure	B (MFI = 3380) DQB1 (MFI = 3705) DQB1 (MFI = 3320)	Acute lung injury, capillaritis	Negative	Definitely AMR	Probable AMR
3	92 days	Dyspnea, decline in spirometry	A (MFI = 9572) DQB1 (MFI =1461)	Acute cellular rejection (A2B0)	Negative	Probably not AMR	Possible AMR
4	75 days	Hypoxemic respiratory failure	A (MFI = 3008) B (MFI = 2245) DQB1 (MFI =4312)	Not done	Not done	Probably not AMR	Possible AMR

MFI: mean fluorescence intensity