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Prevalence of antibodies to lung self-antigens (Kα1 tubulin and collagen V) and donor specific antibodies to HLA in lung transplant recipients and implications for lung transplant outcomes: Single center experience

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ABSTRACT

Purpose: For patients with end stage lung disease, lung transplantation (LT) remains the only definitive treatment option. Long term survival post LT is limited by acute and chronic allograft dysfunction. Antibodies to lung self-antigens Kα1Tubulin and collagen V (autoantibodies) have been implicated in adverse outcomes post LT. The aim of our study was to determine the prevalence of autoantibodies in pre- and post-transplant sera, evaluate the impact on post-transplant outcomes.

Methods: In a prospective observational cohort analysis, 44 patients were enrolled who received LT between 09/01/2014 and 10/31/2015. Pre- and post-transplant sera were analyzed using enzyme-linked immunosorbent assay (ELISA) for the presence of antibodies to collagen I, collagen V, and K-alpha 1 tubulin. The outcome variables are presence of primary graft dysfunction (PGD), cumulative acute cellular rejection (ACR), treatment with pulse steroids for clinical rejection, association with DSA, and onset of Bronchiolitis Obliterans Syndrome (BOS).

Results: In our cohort, 33 patients (75%) tested positive for the presence of autoantibodies. Pre-transplant autoantibodies were present in 23 patients (70%). Only a small percentage (26%) cleared these antibodies with standard immunosuppression. Some developed de novo post-transplant (n = 10). PGD was observed in 34% of our cohort, however the presence of autoantibodies did not correlate with increase in the incidence or severity of PGD. The prevalence of donor specific antibodies (DSA) in the entire cohort was 73%, with an increased prevalence of DSA noted in the autoantibody positive group (78.7% vs. 54.5%) than in the autoantibody negative group. BOS was observed in 20% of the cohort, with a median time to onset of 291 days' post-transplant. Patients with pre-transplant autoantibodies had a statistically significant decrease in BOS-free survival (p = 0.029 by log-rank test).

Conclusions: In our cohort, we observed a high prevalence of autoantibodies and DSA in lung transplant recipients. Pre-transplant autoantibodies were associated with de novo development of DSA along with a decrease in BOS-free survival. Limitations to our study include the small sample size and single center enrollment, along with limited time for follow-up.

1. Introduction

For patients with end-stage lung disease, lung transplantation serves as the only definitive treatment option. With a median post-transplant survival of approximately 5 years, survival for lung transplant recipients is the lowest among all solid organ transplant recipients. Infections and allograft failure are the leading causes of death in the first-year post-transplant, however the main barrier to long-term survival is chronic allograft dysfunction, which encompasses both restrictive allograft dysfunction and bronchiolitis obliterans syndrome (BOS) [1].

BOS is a clinical syndrome that refers to the progressive increase in airflow obstruction resulting from fibrous obliteration of the small airways [2]. Given the irreversible nature of this process, efforts to improve outcomes post-transplant must focus on delaying the onset of BOS. Several risk factors for the development of BOS have been identified, including both immune- and non-immune mediated factors. Viral

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infections and gastroesophageal reflux are well-known, non-immune-mediated risk factors for the development of BOS [3]. With respect to immune-mediated mechanisms, both the cellular and humoral immune responses have been implicated. Historically, cellular immunity has been regarded as the primary mechanism of graft rejection, and post-transplant immunosuppressive regimens have largely targeted T-cell proliferation. Acute cellular rejection is widely accepted as an independent risk factor for BOS, with increasing risk as the severity and frequency of rejection increases [2–4].

Recognizing the importance of cross-talk between the cellular and humoral immune responses, there has been increasing focus on the humoral immune response and the impact of antibody-mediated rejection on post-transplant outcomes. Antibody-mediated rejection (AMR) encompasses both alloimmunity and autoimmunity. Donor-specific antibodies against mismatched donor HLA (DSA) develop de novo post-transplant and have been linked with adverse outcomes, including acute cellular rejection, decreased freedom from BOS, and death [5–7]. On the other hand, development of antibodies directed against tissue restricted self-proteins (autoantibodies) that are expressed on lung parenchyma have also been associated with development of BOS. Autoantibodies can be detected both pre-and post-transplant. Antibodies directed against collagen type I (Col-I), collagen type V (Col-V), and Kα1 tubulin (Kα1T) have been associated with poor outcomes post-transplant, including development of DSA, earlier onset of BOS and increased mortality [8,9]. Furthermore, autoantibodies have been shown to increase the risk for primary graft dysfunction (PGD), which is a form of acute lung injury that occurs within the first 72 h' post-transplant. PGD has been shown to be an independent risk factor for the development of BOS and carries an increased risk of both short and long term mortality [3,10].

2. Objective

With mounting evidence supporting the role of humoral immunity in lung allograft rejection, additional studies are needed to further our understanding of the humoral immune response, with the hope of identifying additional targets for immunosuppression. The objective of this study was to determine the prevalence of autoantibodies in pre-and post-transplant sera, evaluate its effect on DSA, monitor patterns of clearance of autoantibodies along with DSA and analyze the impact on post-transplant outcomes, including PGD, cumulative acute cellular rejection, treatment with pulse steroids for clinical rejection, development of DSA, and onset of BOS.

3. Methods

3.1. Study population

The study was a collaborative effort with the UT Southwestern Medical Center, Dallas, Texas, Department of Surgery Washington University Medical Center in St. Louis, Missouri and the St. Joseph's Hospital and Medical Center Norton Thoracic Institute in Phoenix, Arizona. The study was approved by the UT Southwestern Medical Center Institutional Review Board. Written informed consent was obtained from all study participants.

In this prospective observational cohort analysis, we enrolled adult patients (age > 18) who underwent single or bilateral lung transplantation at UT Southwestern Medical Center between September 2014 and October 2015. Pre-and post-transplant sera were collected and analyzed for the presence of antibodies to lung self-antigens Col-I, Col V and Kα1T (autoantibodies) - in Dr. T. Mohanakumar's research lab initially at Washington University Medical Center and later at Norton Thoracic Institute, Phoenix.

3.2. Detection of autoantibodies

Antibodies to lung self-antigens were detected using an ELISA method [24]. In brief, 1 μg/ml Col I, Col V and Kα1T was suspended in phosphate-buffered saline and coated onto an enzyme-linked immunosorbent assay plate. This was then incubated overnight at 4 °C followed by addition of diluted patient and normal sera (Col I 1:250, Col V 1:1000 and Kα1T 1:1250). Detection was done using anti-human immunoglobulin G-HRP (1:10,000), developed using 3, 3′, 5, 5′-tetramethyl benzidine substrate, and read at 450 nm. A sample was considered positive if values were greater than the mean ± 2 standard deviations (20 ± 9 μg/ml Col- I, 82 ± 24 μg/ml and 66 ± 50 μg/ml Kα1T). Antibody concentration was calculated using a standard curve from known concentrations of Col-I (Abcam) Col-V (Abcam) and Kα1T antibodies (Santa Cruz Biotechnology, Santa Cruz, CA). Autoantibodies were screened once pre –transplant and then monthly for average of 6 months' post-transplant.

3.3. Clinical variables

Various demographic, clinical, operative, and outcome variables for the study population were extracted from the electronic medical record. Baseline characteristics including age, gender, race, smoking history, and indication for lung transplantation were recorded. Operative notes were reviewed to determine the type of transplant (single vs bilateral) and the use of cardiopulmonary bypass pump.

3.4. Post transplant management

All patients were screened for pre-formed HLA antibodies every 3 months prior to transplantation. Donor lungs were accepted only if the virtual crossmatch with all previously identified antibodies were compatible. At the time of transplantation, all recipients had direct crossmatch using serum obtained the day of surgery and results were available post operatively. Induction was not routinely used. Its use was limited to patients requiring Cardiopulmonary bypass (CPB), those with bleeding complications, coagulopathy, or hemodynamic instability. All other recipients received tacrolimus infusion at 30 micrograms/h initiated intraoperatively. The maintenance immunosuppression regimen consisted of tacrolimus, azathioprine, and prednisone. All recipients underwent surveillance bronchoscopy at 1, 3, 6, and 12 months after transplant. All patients were followed in the lung transplant clinic by a transplant physician as per our center protocol. Patients were seen in clinic twice a week for one month post discharge, then once a week for 2 weeks, every 2 weeks for three months and monthly for the first-year post transplant and then every three months for the rest of their time for follow up.

3.5. Outcome variables

The outcome variables included the presence of PGD which was determined based on the lowest PaO2/FiO2 ratio within the first 72 h' post-transplant. The severity of PGD was scored from 0 to 3 in accordance with the established ISHLT classification and grading system [11]. Additional outcome variables included cumulative acute cellular rejection (ACR), treatment with pulse steroids for clinical rejection, development of DSA, overall survival and BOS free survival. The presence of acute cellular rejection was determined based on review of pathology reports from trans bronchial biopsy specimens. In accordance with the established ISHLT classification of acute cellular rejection, the severity of rejection was graded from A1 (minimal) to A4 (severe) [12]. The cumulative acute rejection (CAR) score represents the sum of each individual acute rejection score. This score was then divided by the total number of trans bronchial biopsy specimens to determine the standardized CAR score. The need for pulse steroids for treatment of

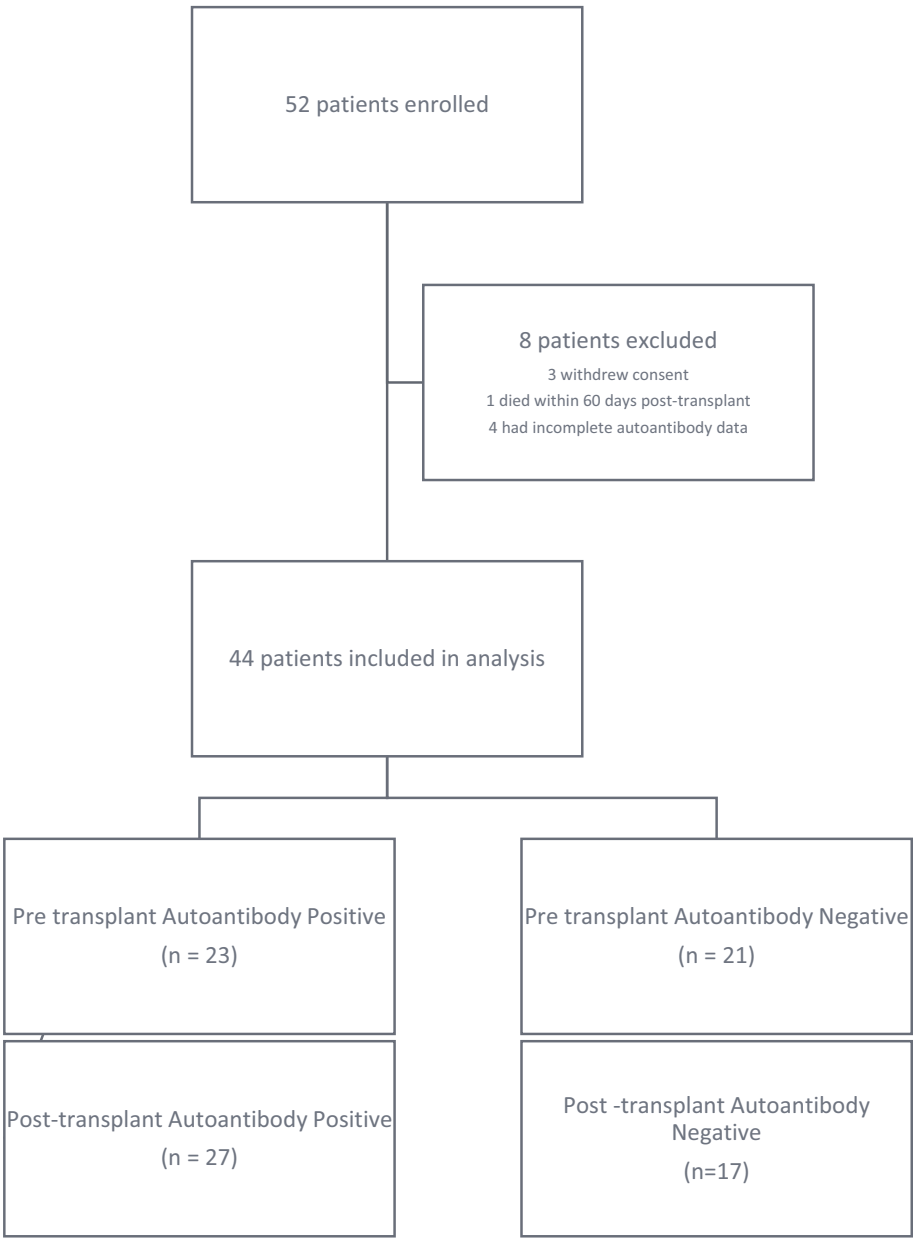

Fig. 1. Enrollment and presence of autoantibodies.

clinical rejection was determined based on review of the electronic medical record. Clinical rejection was defined by the use pulse steroids in the absence of pathologic confirmation of acute rejection. The detection of DSA in post-transplant sera was done using the Luminex single antigen bead (SAB) assay [13]. Post-transplant sera were screened every three months for the presence of DSA and quantified based on Mean Fluorescence Intensity (MFI). Spirometric data was reviewed to determine the baseline post-transplant FEV1. The two highest pre-bronchodilator FEV1 measurements were averaged to obtain the baseline post-transplant FEV1. The presence of BOS was defined as a decline in FEV1 to < 80% of the post-transplant baseline FEV1 and graded from 0 to 3 in accordance with ISHLT guidelines [14].

3.6. Statistical analysis

Summary statistics were used to characterize the distribution of the data. Differences in baseline characteristics between autoantibody groups were compared using the chi-square test or Fisher's exact test for categorical variables and Student's *t*-test or Wilcoxon rank-sum test for continuous variables. Chi-square test was used to examine if there is a significant difference in primary graft dysfunction between autoantibody groups. Wilcoxon rank-sum tests were used to compare continuous or ordinal outcomes such as grade of PGD, cumulative CAR score, standardized CAR score, hospital length of stay between autoantibody groups. Kaplan-Meier method was used to estimate the overall survival and BOS-free survival, and log-rank tests were conducted to compare overall survival and BOS-free survival between autoantibody groups. Statistical analysis was conducted with SAS 9.4 (SAS Institute, Cary, NC).

4. Results

4.1. Baseline characteristics

A total of 52 patients were enrolled. Three patients subsequently withdrew consent, and one patient died within 60 days after transplant

Table 1
Baseline characteristics comparing groups with and without autoantibodies.

Variable	Autoantibody Negative (N = 11)	Autoantibody Positive (N = 33)	Entire Cohort (N = 44)	p-value[a]
Age at transplant	63.5 ± 5.8	54.5 ± 13.7	56.8 ± 12.8	0.0043§
Race				0.8637*
Caucasian (N = 37)	11 (100.0%)	26 (78.79%)	37 (84.09%)	
African-American (N = 2)	0 (0.00%)	2 (6.06%)	2 (4.55%)	
Hispanic (N = 3)	0 (0.00%)	3 (9.09%)	3 (6.82%)	
Other (N = 2)	0 (0.00%)	2 (6.06%)	2 (4.55%)	
Sex				1.0000*
Male (N = 29)	7 (63.64%)	22 (66.67%)	29 (65.91%)	
Female (N = 15)	4 (36.36%)	11 (33.33%)	15 (34.09%)	
Smoking history				0.1620
Non-smoker (N = 20)	3 (27.27%)	17 (51.52%)	20 (45.45%)	
Smoker (N = 24)	8 (72.73%)	16 (48.48%)	24 (54.55%)	
Transplant type				0.2867*
Single (N = 14)	5 (45.45%)	9 (27.27%)	14 (31.82%)	
Bilateral (N = 30)	6 (54.55%)	24 (72.73%)	30 (68.18%)	
Diagnosis of transplant				0.0570*
COPD (N = 15)	6 (54.55%)	9 (27.27%)	15 (34.09%)	
IPF (N = 8)	0 (0.00%)	8 (24.24%)	8 (18.18%)	
CF (N = 5)	0 (0.00%)	5 (15.15%)	5 (11.36%)	
PAH (N = 2)	1 (9.09%)	1 (3.03%)	2 (4.55%)	
Other (N = 3)	2 (18.18%)	1 (3.03%)	3 (6.82%)	
Pulmonary Fibrosis, not IPF (N = 11)	2 (18.18%)	9 (27.27%)	11 (25.00%)	
Cardiopulmonary Bypass Time	42.2 ± 113.6	45.8 ± 84.8	44.909 ± 91.365	0.9106§

Note: § by t-test; * by Fisher's Exact test; Otherwise by Chi-Square test.

and was therefore not screened for the presence of DSA or autoantibodies post-transplant. Four additional patients were excluded due to incomplete autoantibody data. The remaining 44 patients were included in the final analysis.

A total of 33 patients (75%) tested positive for the presence of autoantibodies and 11 patients (25%) tested negative (Fig. 1). Within the cohort of patients who had autoantibodies, 23 patients (70%) tested positive for the presence of autoantibodies in the pre-transplant serum. Six of these patients had clearance of autoantibodies in the post-transplant serum, and 17 (73%) patients had persistence of autoantibodies post-transplant. 10 (22%) recipients developed de novo autoantibodies after lung transplantation and 9 among10 developed DSA as well.

In comparing the autoantibody positive and negative groups, with the exception of age at transplant, there were no significant differences in baseline characteristics between the two groups. In our cohort, autoantibody positive patients were transplanted at a younger age compared to those without autoantibodies (54.5 ± 13.7 years vs. 63.5 ± 5.8 years, p = 0.0043). While there was no significant difference in autoantibody status based on the underlying diagnosis for transplant, it is worth noting that all patients in our cohort with IPF and CF were autoantibody positive (Table 1, p = 0.05).

4.2. PGD

Primary graft dysfunction was observed in 15 of 44 patients (34%). As PGD is an early finding post-transplant, we hypothesized that those with pre-transplant autoantibodies would be more likely to develop PGD, however we did not observe a difference between these two groups (7 of 23 patients with pre-transplant autoantibodies [30.4%] vs. 8 of 21 without pre-transplant autoantibodies [38.1%]; p = 0.59) (Table 2). Furthermore, among those with PGD, we found no significant difference in terms of severity of PGD based on pre-transplant autoantibody status (p = 0.95). 20% (6 of 29 recipients) with absent PGD and 26% (4 of 15 recipients) with PGD developed de novo autoantibodies post-transplant.

4.3. Acute cellular rejection

The median CAR score was not significantly different between the patients with autoantibodies and without autoantibodies (p = 0.46, Table 2). Even when comparing patients without autoantibodies to those with both pre- and post-transplant autoantibodies (and thus the highest total burden of autoantibodies), median CAR and standardized CAR scores were not significantly different (p = 0.67).

4.4. Pulse steroids for clinical rejection

In evaluating the need for pulse steroids for clinical rejection, we found no significant difference when comparing patients with autoantibodies to those without. Among the patients with autoantibodies, 15 of 33 (45%) were treated with pulse steroids for clinical rejection. In comparison, 6 of 11 patients (54%) without autoantibodies received pulse steroids for clinical rejection (p = 0.601).

Table 2
Outcomes – autoantibody subgroups: pre-transplant positive vs pre-transplant negative.

Outcome	Pre-Transplant Negative (N = 21)	Pre-Transplant Positive (N = 23)	p-Value[a]
Primary graft dysfunction			0.5923
Absent	13(61.90%)	16(69.57%)	
Present	8(38.10%)	7(30.43%)	
Grade of PGD			0.9538*
0	13(61.90%)	16(69.57%)	
1	3(14.29%)	3(13.04%)	
2	2(9.52%)	1(4.35%)	
3	3(14.29%)	3(13.04%)	
Median Cumulative CAR score	2.00	1.00	0.4681
Median Standardized CAR Score	0.40	0.25	0.4673
Median Overall Survival	Did not reach median	Did not reach median	0.3522
Median BOS-Free Survival (months)	Did not reach median	15.61	0.0291

Note: [a] Overall Survival and BOS-Free by log-rank Test; 'Primary graft dysfunction' and 'Grade of PGD' by Chi-Square test; * by Fisher's Exact test; Otherwise by Wilcoxon rank-sum test.

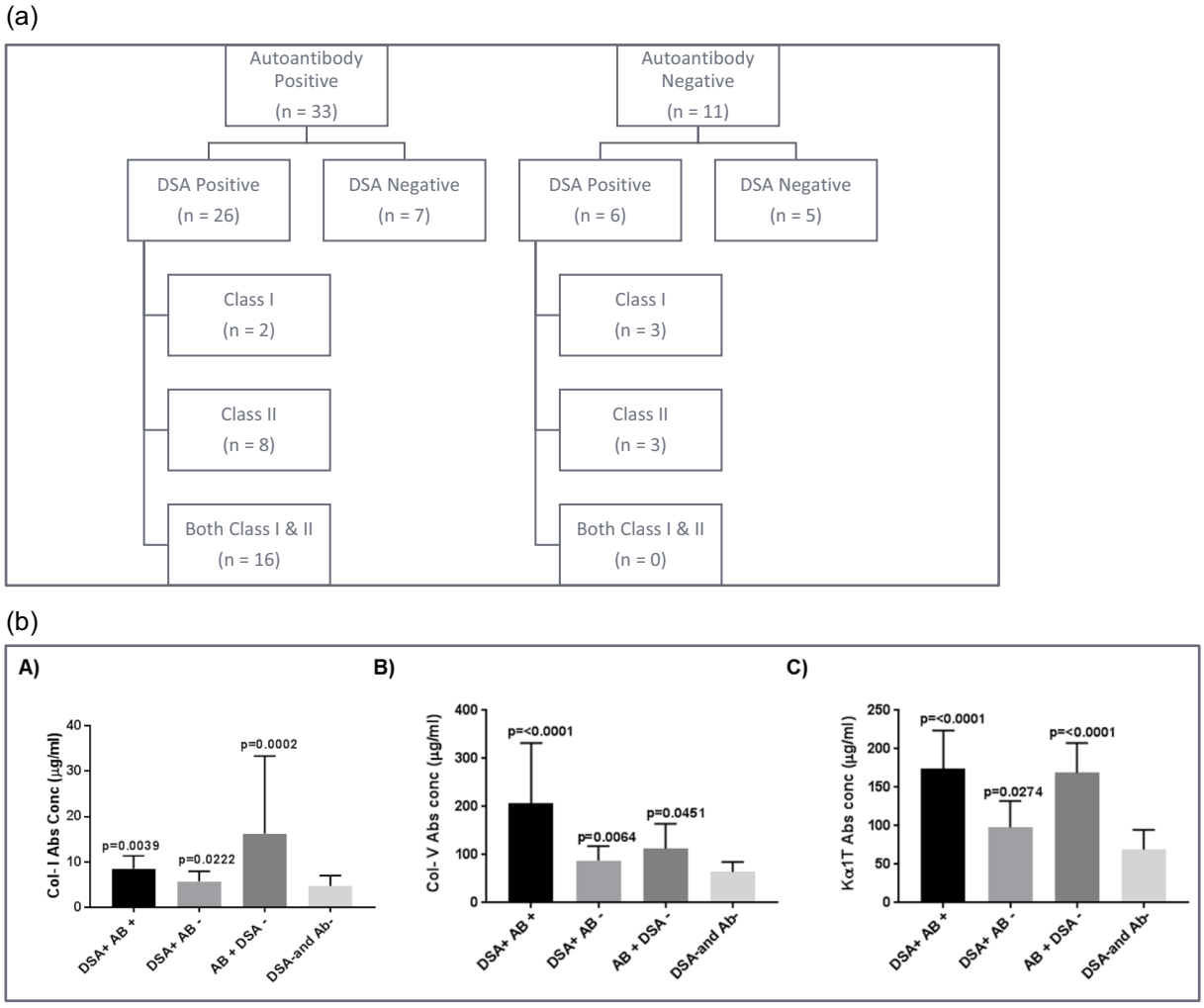

Fig. 2. a) Association of autoantibodies with DSA. b) Autoantibody concentration and DSA.

4.5. Association with DSA

Donor specific antibodies were detected in 32 of 44 patients (73%), and of those who were DSA positive, 16 patients (50%) had both class I and class II DSA. There was an increased prevalence of DSA in those with autoantibodies compared to those without (26 of 33 patients [78.7%] vs. 6 of 11 patients [54.5%], respectively) (Fig. 2a). Of the 26 patients with both autoantibodies and DSA, 17 patients (65%) had pre-transplant autoantibodies, and 13 patients (50%) had persistence of autoantibodies post-transplant. In addition, autoantibody concentration in the serum was higher in the group with DSA (Fig. 2b).

Serum was collected from recipients and analyzed for antibodies to lung self-antigens. Samples were divided into four groups 1) DSA + Ab +, 2) DSA + Ab-, 3) Ab+ DSA- and 4) DSA-Ab-. We observed that lung transplant recipients who developed de novo DSA had significantly higher concentration of antibodies to self-antigens in comparison to DSA-Ab- group. A) Antibodies to Collagen-I (8 ± 2.8 vs 5 ± 3.3; p value: 0.0039), B) antibodies to Collagen-V, (206 ± 126.1 vs 64 ± 20.8; p value: < 0.0001), C) antibodies to Kα1T (174 ± 50.1 vs 69 ± 25.7, p value: < 0.0001).

4.6. BOS

A total of 9 recipients (20%) developed BOS during the study period, 8 recipients had autoantibodies. Surprisingly, only 2 of these patients had PGD. The observed median time to onset of BOS was 291 days, and 5 of 9 patients had BOS grade 2 or higher (Fig. 3a). Patients who had pre-transplant autoantibodies were noted to have a decrease in BOS-free survival that was statistically significant (p = 0.0291 by log-rank test) (Fig. 3b).

4.7. Mortality

Four patients in the cohort died during the observation period. Each of these patients tested positive for the presence of autoantibodies, 3 of whom had pre-transplant autoantibodies. These 4 patients also developed DSA, and BOS was observed in 2 patients. Out of the 4 deaths, one was directly attributable to severe antibody-mediated rejection. Group with autoantibodies and DSA had a trend to worse survival but failed to meet statistical significance (p = 0.36) Fig. 4.

5. Discussion

The purpose of our study was to evaluate the prevalence of autoantibodies, follow clearance and assess their impact on several important post-transplant outcomes, namely PGD, ACR, treatment with pulse steroids for clinical rejection, association with DSA, and onset of BOS.

5.1. Autoantibodies, underlying lung disease

There is increasing new evidence [27,28] that autoantibodies play an important role in the progression of chronic lung disease. These

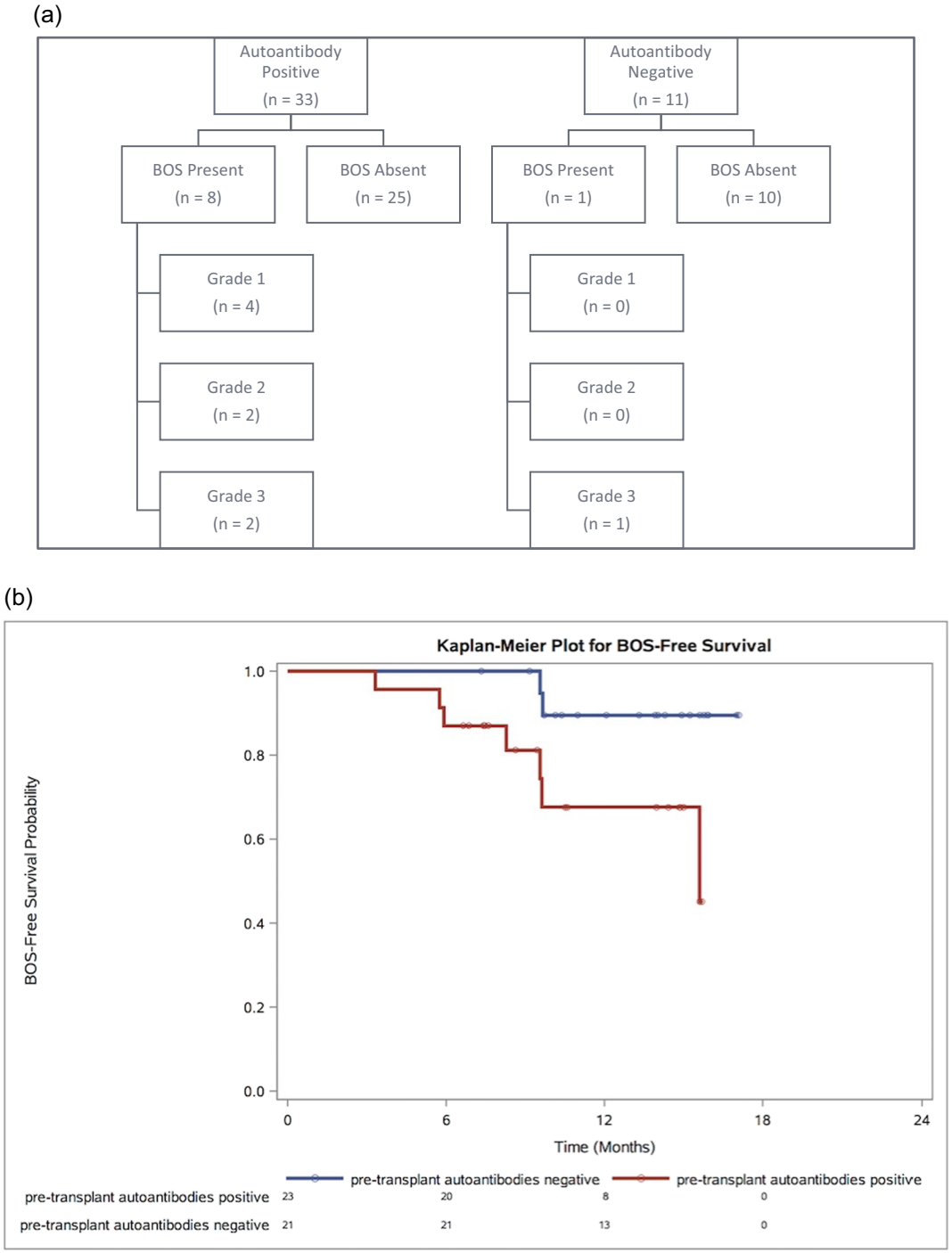

Fig. 3. a) Correlation of autoantibodies and development of BOS. b)Pre-existing autoantibodies decrease BOS free survival of lung transplant recipients.

autoantibodies provide new insight into complicated pathophysiology of lung disease and loss of regulation of lung injury. Interesting finding in our study was that all recipients with underlying diagnosis of IPF or CF had autoantibodies. Similar higher incidence of autoantibodies in IPF and CF was reported by earlier study [15,22,23]. IgG autoantibodies to human periplakin are reported in serum and Broncho alveolar lavage in patients with IPF (40%) [23]. IgA anti-Saccharomyces cerevisiae antibodies (ASCA) among patients with CF correlated with severity of lung disease and CF prognosis [22]. Recently published data from Patel et al. [27] show the presence of increased concentrations of circulating autoantibodies with specificities for Extracellular Matrix (ECM) in a 6-month smoking model of emphysema. Patel et al. used lungs from smoke-exposed mice and non-smoke exposed controls as donor organs in allogeneic pulmonary transplantations, and found ischemia-reperfusion injury and antibody/complement deposition were increased in recipients of the smoke-treated allografts. This report adds to the evidence that preformed autoantibodies are increased in a certain group of patients with chronic lung disease and contribute to allograft injury post-transplant. Such non-Human Leukocyte Antigen (HLA) antibodies are not evaluated during cross match process despite being contributors to allograft injury. Potential mechanisms of such autoantibodies leading to allograft injury, effect of immunosuppression on

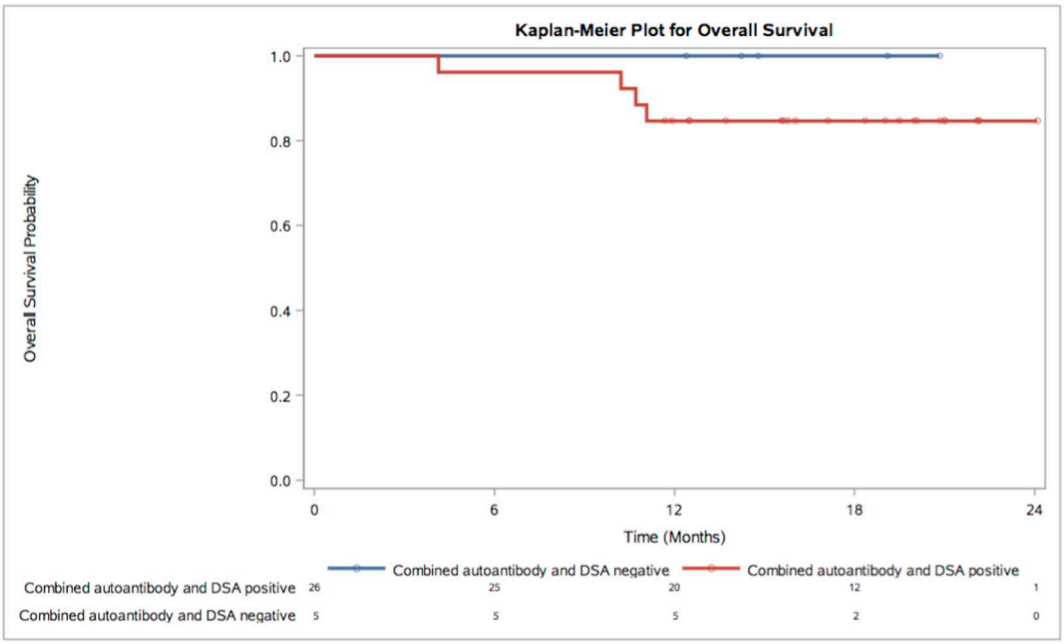

Fig. 4. Presence of autoantibodies and DSA and survival rate of Lung transplant recipients.

autoantibodies largely remains unexplored. In our study we report pre-transplant non HLA antibodies to Col I, Col V and Kα1T are associated with higher prevalence of DSA and decreased BOS free survival.

5.2. Autoantibodies and DSA

We noted a higher incidence of de novo DSA development in our cohort. The prevalence across the entire cohort of patients was 73%, with an increased prevalence of DSA in the autoantibody positive group (78.7% vs. 54.5% in the autoantibody negative group). In a study done by Kauke et al. [26] recipients with de novo DSA that develop after lung transplantation had higher prevalence of BOS ($p = 0.002$). Pre-transplant HLA antibodies did not affect graft function and in most cases HLA antibodies detected before transplantation disappeared after transplantation. However, Kauke et al. did not evaluate non-HLA antibodies. In our study, we report that recipients with pre-transplant (non-HLA) autoantibodies (Col I, Col V and Kα1T) not only had higher prevalence of DSA but also those who developed DSA had higher concentration of autoantibodies and were less likely to clear them (autoantibodies) post-transplant. Saini et al. [24] analyzed serum from lung transplant patients who developed DSA. DSA were detectable on an average 3 months' post-transplant and antibodies to Col IV and Kα1T developed following DSA. In addition, autoantibodies persist despite DSA clearance. Our findings in our study suggest pre-transplant autoantibodies especially at higher concentration in DSA positive group. We believe that recipients with alloantibodies and autoantibodies may have higher levels of circulation pro-inflammatory cytokines as demonstrated by Tiriveedhi et al. [15]. This might result in a self-perpetuating process leading to antibody binding to exposed new epitopes after ischemia reperfusion injury. Antigen-antibody binding leads to complement activation which can then damage epithelial cell surface by complement mediated cytotoxicity as described in a mouse model by Patel et al. [27].

5.3. Autoantibodies before and after lung transplantation

Our study evaluated the effect of traditional immunosuppression on autoantibodies. Several of those who had pre-transplant autoantibodies did not clear post-transplant (17/23; 73%) with the traditional immunosuppression strategy. About 22% (n = 10) percentage also developed de novo autoantibodies post-transplant. Group with pre-transplant autoantibodies had less BOS free survival. Our findings suggest preexisting autoantibodies play a role in graft injury after lung transplantation and we speculate that these preexisting autoantibodies might be involved in underlying cause of chronic lung disease. Current methods of immunosuppression seem to have minimal impact on their clearance. Those autoantibodies that develop de novo post-transplant may be associated with DSA and did not seem to be associated as strongly with BOS.

PGD was observed in 34% of our cohort, however the presence of autoantibodies (including pre-transplant autoantibodies) was not shown to increase the incidence or severity of PGD. Our results differ from previously published studies [9,15] and may be attributable to differences in sample size, titer of antibodies and their synergy. However, the role of humoral immunity in primary graft dysfunction is yet to be understood.

The 20% incidence of BOS observed in our cohort was surprising given the short time for follow-up. Onset of BOS within two years' post-transplant has been associated with an increased risk of mortality [18]. Similarly, in our study 2 of the 9 patients with BOS died within the follow-up period. Kaplan-Meier analysis of combined DSA and autoantibody status showed a trend to worse BOS free survival but did not meet statistical significance. The pro-inflammatory milieu from binding of autoantibodies to new epitopes, in combination with DSA directly activate epithelial and endothelial cells to increase stress proteins, profibrotic growth factors. This can result in smooth muscle cell proliferation and fibrosis leading to.

BOS [16,17,25].

5.4. Future directions

There are several limitations to our study. The small sample size may have limited our ability to detect differences in the outcome variables of interest. In addition, the short time for follow-up post-transplant imposed a significant limitation in the assessment of BOS-free survival and mortality in our cohort.

While there are established therapies for the management of DSA that include IVIG, plasmapheresis, and rituximab, there are no consensus guidelines on when to initiate treatment [19–21] and there are no predictors of response to treatment. In our study, we found that standard immunosuppression did not clear most of the pre-transplant

autoantibodies. In our follow up study, we would like to evaluate the response of autoantibodies to current treatment strategies for DSA. Perhaps therapeutic strategies that inhibit complement activation or specific complement inhibitors such was Eculizumab may have a role in treating preexisting autoantibodies.

In conclusion, the host immune response to organ transplantation is highly complex, and many unanswered questions remain. The crosstalk between the cellular and humoral immune responses, the role of autoantibodies and its synergy with DSA and their impact on the outcomes for lung transplant recipients remains to be determined. However, our study provides evidence for a pathogenic role for autoantibodies in lung transplant recipients. We are hoping to confirm the preliminary findings in our study with a larger multi-center cohort.

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Competing interest statement

Authors have no competing interests to declare.

Submission declaration and verification

The work described has not been published previously (except in the form of an abstract), that it is not under consideration for publication elsewhere, that its publication is approved by all authors, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

Authorship

All authors made substantial contributions to all of the following: [1] the conception and design of the study, or acquisition of data, or analysis and interpretation of data, [2] drafting the article or revising it critically for important intellectual content, [3] final approval of the version to be submitted.

References

[1] R.D. Yusen, L.B. Edwards, A.I. Dipchand, et al., The registry of the international society for heart and lung transplantation: thirty-third official adult lung and heart-lung transplantation report–2016; focus theme: primary diagnostic indications for transplant, J. Heart Lung Transplant. 35 (2016) 1170–1184.

[2] K.C. Meyer, G. Raghu, G.M. Verleden, et al., An international ISHLT/ATS/ERS clinical practice guideline: diagnosis and management of bronchiolitis obliterans syndrome, Eur. Respir. J. 44 (2014) 1479–1503.

[3] D. Hayes Jr., A review of bronchiolitis obliterans syndrome and therapeutic strategies, J. Cardiothorac. Surg. 6 (2011) 92.

[4] R.R. Hachem, A.P. Khalifah, M.M. Chakinala, et al., The significance of a single episode of minimal acute rejection after lung transplantation, Transplant. 80 (2005) 1406–13.

[5] R. Hachem, Antibody-mediated lung transplant rejection, Curr. Respir. Care Rep. 1 (2012) 157–161.

[6] S. Safavi, D.R. Robinson, S. Soresi, M. Carby, J.D. Smith, De novo donor HLA-specific antibodies predict development of bronchiolitis obliterans syndrome after lung transplantation, J. Heart Lung Transplant. 33 (2014) 1273–1281.

[7] M.R. Morrell, J.M. Pilewski, C.J. Gries, et al., De novo donor-specific HLA antibodies are associated with early and high-grade bronchiolitis obliterans syndrome and death after lung transplantation, J. Heart Lung Transplant. 33 (2014) 1288–94.

[8] R.R. Hachem, V. Tiriveedhi, G.A. Patterson, A. Aloush, E.P. Trulock, T. Mohanakumar, Antibodies to K-alpha 1 tubulin and collagen V are associated with chronic rejection after lung transplantation, Am. J. Transplant. 12 (2012) 2164–2171.

[9] A. Bharat, D. Saini, N. Steward, et al., Antibodies to self-antigens predispose to primary lung allograft dysfunction and chronic rejection, Ann. Thorac. Surg. 90 (2010) 1094–1101.

[10] Y. Sukuzi, E. Cantu, J.D. Christie, Primary Graft Dysfunction, Semin. Respir. Crit. Care Med. 34 (2013) 305–319.

[11] J.D. Christie, M. Carby, R. Bag, et al., Report of the ISHLT working group on primary lung graft dysfunction part II: definition. A consensus statement of the international society for heart and lung transplantation, J. Heart Lung Transplant. publ. Int.Soc. Heart Transplant. 24 (10) (2005) 1454–1459.

[12] A.C. Roden, D.L. Aisner, T.C. Allen, et al., Diagnosis of acute cellular rejection and antibody-mediated rejection on lung transplant biopsies: a perspective from members of the pulmonary pathology society, Arch. Pathol. Lab. Med. 141 (3) (2017 Mar) 437–444.

[13] R. Pei, J.H. Lee, N.J. Shih, M. Chen, P.I. Terasaki, Single human leukocyte antigen flow cytometry beads for accurate identification of human leukocyte antigen antibody specificities, Transplant. 75 (1) (2003) 43–49.

[14] K.C. Meyer, G. Raghu, G.M. Verleden, et al., An international ISHLT/ATS/ERS clinical practice guideline: diagnosis and management of bronchiolitis obliterans syndrome, Eur. Respir. J. 44 (2014) 1479–1503.

[15] V. Tiriveedhi, B. Gautam, N.J. Sarma, et al., Pre-transplant antibodies to Kalpha1 tubulin and collagen-V in lung transplantation: clinical correlations, J. Heart Lung Transplant. 32 (2013) 807–814.

[16] J.L. Todd, S.M. Palmer, Bronchiolitis obliterans syndrome: the final frontier for lung transplantation, Chest 140 (2011) 502–508.

[17] T.A. Goers, S. Ramachandran, A. Aloush, E. Trulock, G.A. Patterson, T. Mohanakumar, De novo production of K-α1 tubulin-specific antibodies: role in chronic lung allograft rejection, J. Immunol. 180 (7) (2008) 4487–4494.

[18] C.A. Finlen Copeland, L.D. Snyder, D.W. Zaas, W.J. Turbyfill, W.A. Davis, S.M. Palmer, Survival after bronchiolitis obliterans syndrome among bilateral lung transplant recipients, Am. J. Respir. Crit. Care Med. 182 (2010) 784–789.

[19] R.R. Hachem, R.D. Yusen, B.F. Meyers, et al., Anti-human leukocyte antigen antibodies and preemptive antibody-directed therapy after lung transplantation, J. Heart Lung Transplant. 29 (2010) 973–980.

[20] A.H. Daoud, A.D. Betensley, Diagnosis and treatment of antibody mediated rejection in lung transplantation: a retrospective case series, Transpl. Immunol. 28 (2013) 1–5.

[21] D.J. Levine, A.R. Glanville, C. Aboyoun, et al., Antibody-mediated rejection of the lung: a consensus report of the international society for heart and lung transplantation, J. Heart Lung Transplant. 35 (2016) 397–406.

[22] F. Lachenal, K. Nkana, R. Nove-Josserand, N. Fabien, I. Durieu, Prevalence and clinical significance of auto-antibodies in adults with cystic fibrosis, Eur. Respir. J. 34 (2009) 1079–1085.

[23] C. Taille, S. Grootenboer-Mignot, C. Boursier, et al., Identification of periplakin as a new target for autoreactivity in idiopathic pulmonary fibrosis, Am. J. Respir. Crit. Care Med. 183 (2012) 759–766.

[24] D. Saini, J. Weber, S. Ramachandran, et al., Alloimmunity-induced autoimmunity as a potential mechanism in the pathogenesis of chronic rejection of human lung allografts, J. Heart Lung Transplant. 30 (2011) 624–631.

[25] T.A. Goers, S. Ramachandran, A. Aloush, et al., De novo production of K-alpha1 tubulin-specific antibodies: role in chronic lung allograft rejection, J. Immunol. 180 (2008) 4487–4494.

[26] T. Kauke, N. Kneidinger, B. Martin, et al., Bronchiolitis obliterans syndrome due to donor-specific HLA-antibodies, Tissue Antigens 86 (3) (2015 Sep) 178–185.

[27] K.J. Patel, Q. Cheng, S. Stephenson, et al., Emphysema associated autoreactive antibodies exacerbate post lung transplant ischemia reperfusion injury, Am. J. Respir. Cell Mol. Biol. (Dec 2018) 20, https://doi.org/10.1165/rcmb.2018-0224oc [online ahead of print].

[28] S.R. Duncan, A. Gaggar, What lies beneath preformed autoantibodies and lung transplantation, Am. J. Respir. Cell Mol. Biol. (2019 Jan 16), https://doi.org/10.1165/rcmb.2018-0415ED.