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Lung Transplant Candidates With Pretransplant Gastroesophageal Reflux and Antibodies to Lung Self-antigens Have Shorter CLAD-free Survival After Transplant

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Background. Pre–lung transplant (LTx) gastroesophageal reflux (GER) and circulating antibodies against the lung self-antigens (SAbs) collagen V and K-alpha-1 tubulin may predispose recipients to chronic lung allograft dysfunction (CLAD). We aimed to study the association of pre-LTx GER or pre-LTx SAbs with CLAD. **Methods.** In this retrospective analysis of patients who underwent LTx between 2015 and 2019, pre-LTx GER and SAbs were dichotomously defined as present or absent. The study group comprised recipients with either GER, SAbs, or both, and the control group comprised recipients without GER or SAbs. Endpoints included CLAD and survival. **Results.** Ninety-five LTx recipients were divided into a study group (n = 71; 75%) and a control group (n = 24; 25%). Pretransplant GER was associated with pre-LTx SAbs (odds ratio [95% confidence intervals], 5.022 [1.419-17.770]; $P = 0.012$). In addition, the study group (either GER, SAbs, or both) had a higher risk of CLAD (hazard ratio [95% confidence intervals], 8.787 [1.694-45.567]; $P = 0.010$) and lower CLAD-free survival after LTx than the control group ($P = 0.007$); however, overall survival was similar between the 2 groups ($P = 0.618$). **Conclusions.** GER was associated with elevated SAbs in LTx candidates, and either GER, SAbs, or both were associated with CLAD in LTx recipients. This association suggests that GER may cause an immune response to normally sequestered lung-associated self-antigens that drives ongoing lung injury.

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INTRODUCTION

Lung transplantation (LTx) can be a life-extending option for patients with end-stage lung disease; however, long-term survival after LTx is shorter than long-term survival after other solid organ transplants, and mortality is driven by chronic lung allograft dysfunction (CLAD).[1] A number of risk factors for the development of CLAD have been identified and include primary graft dysfunction (PGD),[2] acute cellular rejection (ACR), development of donor-specific antibodies (DSAs), and recurrent infections.[1] Gastroesophageal reflux (GER) and the resultant aspiration may also be a risk factor for CLAD,[3,4] and pre-LTx GER may be associated with early allograft injury and higher 1-y mortality.[5,6] Furthermore, elevated titers of pre-LTx antibodies to the lung self-antigens (SAbs) collagen V (Col-V) and K-alpha-1 tubulin (Kα1T) have been

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

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identified in LTx recipients (LTxRs) with CLAD[7,8] and may have a dual role as both a biomarker and a propagator of lung injury. In this retrospective cohort study, we hypothesized that the presence of GER in LTx candidates may drive lung injury through a resultant expression of SAbs and that both GER and SAbs before transplant are a risk factor for the development of CLAD after transplant.

MATERIALS AND METHODS

Study Cohort

All included patients consented and were prospectively enrolled for research as part of a National Institutes of Health–approved study (NIH HL056643) in accordance with the principles of the Declaration of Helsinki. After institutional review board approval (PHXB-16-0027-10-18 dated March 7, 2016), we retrospectively retrieved pre-LTx SAb assay results for samples collected between 2015 and 2019. The esophageal disease center database was also queried to extract pre-LTx 24-h GER testing data at the time of LTx evaluation for these patients. From these data, patients with unavailable 24-h GER testing or 24-h GER testing done on acid suppression therapy and patients declined after LTx candidacy evaluation or remaining on the United Network for Organ Sharing waitlist as of March 31, 2020, were excluded. Serum samples obtained closest to the time of LTx listing were selected and analyzed for SAbs (Col-V and Kα1T) by ELISA as previously described.[8] Thus, samples serially obtained throughout the evaluation process, particularly among patients whose LTx listing was deferred, were discarded. This allowed for relative uniformity in the time frame between sample acquisition and LTx. Patients transplanted between January 2015 and June 2019 were included in the analysis. Patient selection for LTx was standardized and based on International Society for Heart and Lung Transplantation criteria.[9]

Study Groups

The presence of SAbs was dichotomously defined as present (anti-Col-V or anti-Kα1T titers ≥106 ng/mL and ≥116 ng/mL, respectively) or absent. The presence of GER was also dichotomously defined as present (DeMeester score ≥14.72) or absent. Uni- and multivariate analyses were performed to ascertain predictors of SAbs in LTx candidates. LTxRs with either GER, SAbs, or both were assigned to the study group, and recipients without GER or SAbs were assigned to the control group. Electronic charts of LTxRs were reviewed, and the study and control groups were compared for post-LTx outcomes and survival. The study group was further divided into 3 subgroups for stratified analysis of outcome: subgroup 1 = GER(+) SAbs(+); subgroup 2 = GER(–) SAbs(+); and subgroup 3 = GER(+) SAbs(–).

Endpoints

The primary endpoint of the study was time from LTx to first detection of CLAD. International Society for Heart and Lung Transplantation criteria were used to define CLAD.[1,10] Restrictive allograft syndrome and bronchiolitis obliterans syndrome were not separately defined for the study. The secondary endpoint was time from LTx to all-cause mortality and overall survival. All outcomes were adjudicated by transplant physicians blinded to GER results and SAb titers.

Clinical covariates of LTxRs were recorded throughout the study and included graft ischemia time, use of cardiopulmonary bypass or extracorporeal membrane oxygenation (ECMO), post-LTx length of hospital stay, baseline allograft function, pretransplant respiratory infections, PGD grade 3 at 72 h post-LTx, ACR (≥A2 or ≥B1R), and de novo development of DSAs (>1000 mean fluorescence intensity) to mismatched donor HLAs any time after LTx. Baseline lung allograft function was defined as the mean of the 2 best forced expiratory volumes in 1 s and forced vital capacity measurements during the first 6 mo after LTx. We did not differentiate between respiratory colonization and infection. Thus, pre-LTx respiratory infections were defined as having a respiratory specimen (sputum, tracheal aspirate, or bronchoalveolar lavage) with a positive bacterial culture or a nasopharyngeal swab or nasal wash with a positive viral polymerase chain reaction test within 6 mo before serum sample collection.

24-h pH Study

Ambulatory esophageal pH monitoring was performed using a catheter-based dual, proximal and distal esophageal, electrode probe (Digitrapper 400pH; Medtronic, Minneapolis, MN), and pH score was calculated using standard variables.[11] Briefly, the catheter-based pH probe was passed transnasally and positioned 5 cm above the upper border of the lower esophageal sphincter (defined on high-resolution manometry). Testing was done off acid suppression therapy (7 d for proton pump inhibitors and 3 d for H2 receptor blockers).

Detection of Circulating SAbs (Kα1T, Col-V) and DSAs to HLA

SAbs Kα1T and Col-V were detected via ELISA assay, as previously described.[8] In brief, ELISA plates were coated overnight at 4 °C with either recombinant Kα1T (1 μg/mL) or Col-V (1 μg/mL; Sigma-Aldrich, St. Louis, MI) in PBS and blocked for 2 h with 1% BSA. Samples from pre-LTx patients and healthy volunteers were diluted 1:1000 for Col-V and 1:1250 for Kα1T and loaded. Color was developed using tetramethylbenzidine substrate, and SAbs were detected using horseradish-peroxidase conjugated antihuman IgG (1:10,000) and read at 450 nm. SAbs (Kα1T, Col-V) were considered positive in readings 2 standard deviations above the mean of healthy controls (116 ng/mL for K-α1T and 106 ng/mL for Col-V). Antibody concentrations were calculated using standard curves of known concentrations of anti-Kα1T (Santa Cruz Biotechnology, Dallas, TX) or anti-Col-V (Abcam, Cambridge, United Kingdom). DSAs were detected using single antigen beads (One Lambda, ThermoFisher Scientific, Waltham, MA) using a Luminex platform.

Immunosuppression

Induction therapy included high-dose corticosteroids (methylprednisolone) before perfusion of each lung allograft and antilymphocyte therapy with basiliximab, rituximab, or antithymocyte globulin, with basiliximab as the induction agent of choice. The initial maintenance immunosuppressive regime was uniform across the study period and consisted of a corticosteroid (prednisone), an antiproliferative agent (mycophenolate mofetil or mycophenolic acid), and a calcineurin inhibitor (tacrolimus or cyclosporine).

Statistical Analysis

All analyses were performed using IBM SPSS Statistics for Windows, version 23.0 (IBM Corp, Armonk, NY), and R package 4.1.0 (R Foundation for Statistical Computing,

Vienna, Austria). Continuous variables were expressed as median (interquartile range [IQR]), and categorical variables were expressed as frequencies (percentage). Differences in continuous variables between study and control groups were assessed using the nonparametric Kruskal-Wallis test. The chi-squared or Fisher exact test was used to compare categorical variables between groups.

Univariate analysis was used to determine the predictors of SAbs in LTx candidates. Variables with $P < 0.2$ were included in a multivariate regression model. Included covariates were demographic characteristics of the LTx candidate, lung allocation score (LAS), underlying lung disease, pre-LTx hospitalization for respiratory infections, and pre-LTx GER. In addition, covariates relevant to the outcome (SAbs) in the literature, that is, type of transplantation,[12] were also included. Next, a univariate Cox regression was conducted with proportional hazard assumption for the endpoint of CLAD in LTxRs. For the multivariate Cox proportional hazard analysis, covariates based on statistical significance ($P < 0.2$ in univariate analysis) and variables known to be clinically important,[2,7] that is, PGD, DSA, and ACR, were purposefully selected. Significance was evaluated at the 0.05 alpha level. Confounding was defined as present if the change in the parameter estimate on the model adjusted for PGD, DSA, and ACR was >15% than the unadjusted model. Finally, stratified analysis was conducted to assess the relationship of CLAD to subgroups 1, 2, and 3 compared with the control group. The survival time was calculated from LTx to first detection of CLAD or death. Not meeting any of the 2 endpoints by the last clinical follow-up defined CLAD-free survival; time from LTx to death defined overall survival. Cumulative CLAD-free and overall survival was calculated using the Kaplan-Meier method. The log-rank test was used to compare survival rates between groups. A P value for the subgroups' survival analysis was adjusted using the Bonferroni method. Statistical significance was set at $P \leq 0.050$.

RESULTS

Baseline Characteristics of Lung Transplant Recipients

In total, serum samples from 166 patients were analyzed for the presence of SAbs. Patients with inadequate GER testing (n = 27) and those on the United Network for Organ Sharing waitlist at the end of the study period (n = 44) were excluded; 95 LTxRs formed the study cohort (Figure 1). The median (IQR) patient age, body mass index, and LAS at the time of LTx were 65 y (56–71), 26 kg/m^2 (22–30), and 38 (34–45), respectively; 50.5% (n = 48) were men, and the most common indication for LTx was idiopathic pulmonary fibrosis (IPF). A bilateral LTx was performed in 96.8% (n = 92) of patients, and 4 had a redo-LTx.

Lung transplant recipients were assigned to the study group (n = 71, 74.7%) or the control group (n = 24, 25.3%). Baseline characteristics, including age, gender, body mass index, LAS, and underlying lung disease, were comparable between the study and control groups (Table 1). Post-LTx clinical outcomes including graft ischemic time, use of cardiopulmonary bypass, ECMO rescue for severe PGD, length of postoperative hospital stay, and baseline allograft function were also comparable between the study and control groups. The prevalence of PGD and DSA among the study group was significantly higher than the prevalence in the control group (64.8% versus 37.5%, $P = 0.019$, and 70.4% versus 37.5%, $P = 0.004$, respectively), whereas the prevalence of ACR was similar between the study and control groups (32.4% versus 20.8%, $P = 0.283$). Notably, the prevalence of CLAD was significantly higher in the study group than in the control group (42% versus 13%, $P = 0.008$; Table 1).

Lung Injury, Mediated by GER or Infection, Was Associated With Elevated SAbs

In LTx candidates, underlying lung disease, pretransplant GER, and history of pretransplant hospitalization for respiratory infections were associated with SAbs on univariate

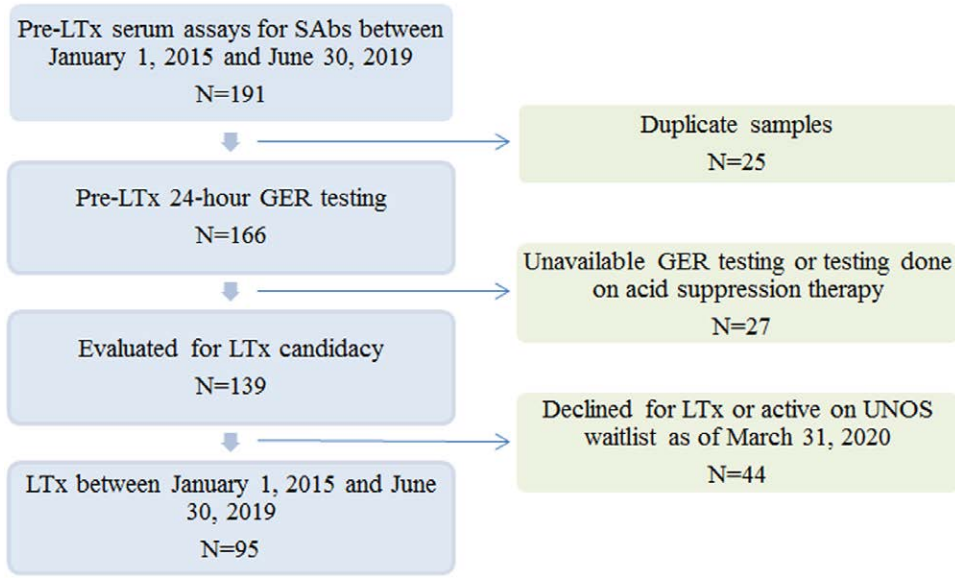

FIGURE 1. Patient selection flowchart. GER, gastroesophageal reflux; LTx, lung transplant; SAbs, antibodies to lung self-antigens; UNOS, United Network for Organ Sharing.

TABLE 1.

Baseline characteristics and peri- and post–lung transplant outcomes in study and control groups

Variables	Study group GER or SAbs (+), N = 71	Control group GER (–) SAbs (–), N = 24	*P*
Age at LTx, y[a]	64.9 (55.7–70.0)	66.7 (57.2–71.6)	0.631
Sex, male	34, 47.9	14, 58.3	0.376
Body mass index, kg/m^{2}[a]	25.5 (21.1–29.6)	27.4 (24.3–30.0)	0.176
Lung allocation score[a]	38.2 (34.1–43.9)	37.8 (33.3–49.9)	0.834
Underlying lung disease			
Obstructive lung diseases	26, 36.6	13, 54.2	0.131
Pulmonary hypertension	2, 2.8	1, 4.2	1.000
Cystic fibrosis	3, 4.2	0, 0	0.569
Restrictive lung diseases	40, 56.3	10, 41.7	0.213
Bilateral LTx	67, 94.4	24, 100	0.235
Graft ischemia time, min[a]	262 (213, 305)	262 (181.5, 323)	0.820
Cardiopulmonary bypass	5, 7	2, 8.3	1.000
ECMO salvage	9, 12.7	4, 16.7	0.732
Post-LTx LOS, d[a]	15 (12, 23)	11.5 (10, 19)	0.056
Baseline FEV_1, L[a]	2.4 (2.1, 2.9)	2.8 (2.3, 3.2)	0.098
Baseline FVC, L[a]	3.0 (2.4, 3.3)	3.2 (2.5, 3.8)	0.226
Primary graft dysfunction	46, 64.8	9, 37.5	**0.019**
Acute cellular rejection ≥A2	23, 32.4	5, 20.8	0.283
Donor-specific antibody	50, 70.4	9, 37.5	**0.004**
CLAD	30, 42.3	3, 12.5	**0.008**
Survival time, mo[a]	38.9 (24.9, 48.0)	41.1 (29.9, 47.2)	0.532
Mortality	15, 21.1	7, 29.2	0.416
Cause of death			
CLAD and respiratory failure	7	2	
COVID-19	0	2	
Cardiac, cerebrovascular, sepsis, multiorgan failure, cancer	8	3	

Values expressed as number, % unless otherwise specified. Bold indicates statistical significance ($P \leq 0.050$).
[a]Values expressed as median (interquartile range).
CLAD, chronic lung allograft dysfunction; COVID-19, coronavirus disease 2019; ECMO, extracorporeal membrane oxygenation; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; GER, gastroesophageal reflux; LOS, length of stay; LTx, lung transplant; SAbs, antibodies to lung self-antigens; (+), present; (–), absent.

analysis; however, only pretransplant GER (odds ratio [OR] [95% confidence interval (CI)], 5.022 [1.419-17.770]; $P = 0.012$) and history of pretransplant hospitalization for respiratory infections (OR [95% CI], 5.366 [1.940-14.843]; $P = 0.001$) remained significant on multivariate analysis (Table 2).

Anti-Col-V antibodies were seen in 42 patients with a median (IQR) titer of 153 (129, 224) ng/mL; 66.7% of patients with GER had anti-Col-V antibodies, whereas 33.4% of patients without GER had anti-Col-V antibodies (OR, 2.240 [0.969-5.179]; $P = 0.057$). Anti-Kα1T antibodies were seen in 34 patients with a median (IQR) titer of 185 (133–257) ng/

TABLE 2.

Univariate and multivariate analysis for pretransplant factors associated with antibodies to lung self-antigens in lung transplant candidates

	Univariate analysis			Multivariate analysis		
Variable	**Coefficient**	**95% CI**	***P***	**Odds ratio**	**95% CI**	***P***
Age	0.582		0.448			
Sex, male	0.248	–0.151 to 0.252	0.620			
BMI	0.320		0.573			
LAS	0.047		0.829			
Disease groups (ref: PHTN)	5.368		0.023			
RLD				4.032	0.293-55.522	0.297
OLD				1.922	0.136-27.108	0.628
CF				1.472	–1.255 to 87.632	0.999
Pre-LTx respiratory infections	5.886	0.049-0.494	0.017	5.366	1.940-14.843	**0.001**
Pre-LTx GER	9.994	0.114-0.500	0.002	5.022	1.419-17.770	**0.012**

Bold indicates statistical significance ($P \leq 0.050$).
BMI, body mass index; CF, cystic fibrosis; CI, confidence interval; GER, gastroesophageal reflux; LAS, lung allocation score; LTx, lung transplant; OLD, obstructive lung diseases; PHTN, pulmonary hypertension; Ref, reference; RLD, restrictive lung diseases.

mL; 77.1% of patients with GER had anti-Kα1T antibodies, whereas 22.9% of patients without GER had anti-Kα1T antibodies (OR [95% CI], 4.413 [1.724-11.296]; $P=0.001$). Therefore, anti-Kα1T antibodies were more strongly associated with GER than anti-Col-V antibodies. Finally, of 53 patients with GER, 28.3% (15 of 53) had both SAbs, and 45.3% (24/53) had 1 SAb, that is, either anti-Col-V antibody or anti-Kα1T antibody; however, the difference in the association of GER with subjects who had both SAbs versus subjects who had only 1 SAb was not statistically significant (OR [95% CI], 2.188 [0.605-7.906]; $P=0.227$).

Pretransplant GER and SAbs Were Associated With CLAD

Univariate Cox proportional hazard analysis showed an association of CLAD with the study group ($P=0.015$), SAbs ($P=0.143$), GER ($P=0.098$), PGD ($P=0.165$), ACR ($P=0.023$), and DSA ($P=0.011$), based upon our predefined P value of <0.2. Multivariate Cox proportional hazard analysis showed that the presence of either GER, SAbs, or both in the study group significantly increased the risk of CLAD compared with the control group (hazard ratio [95% CI], 8.787 [1.694-45.567]; $P=0.010$). Multivariate analysis also showed a significant association of ACR with CLAD ($P=0.002$; Table 3).

Pre-LTx GER and SAbs Shortened CLAD-free Survival

We suspected a confounding association of PGD, DSA, and ACR between the study group and CLAD. The covariates were sequentially entered one at a time for adjustment, and the percent change in parameter estimate as compared with the unadjusted model was noted. PGD and ACR did not retain a confounding association; however, DSA was significantly related to CLAD and possibly made an important contribution in the presence of GER and SAbs (Table 4).

Irrespective of confounding, the probability of CLAD-free survival was significantly lower in the study group than in the control group (57.7% versus 87.5%, $P=0.007$; Figure 2). The median (IQR) CLAD-free survival in the study group and the control group was 22 (13–35) and 35 (22–40) mo, respectively.

Synergy Between GER and SAbs Was Not Identified

Next, a synergistic association between GER and SAbs was investigated for a possible dose response relationship with CLAD. The study group was further divided into 3 subgroups. Subgroup 1 (n = 39; 41%) included GER(+) SAbs(+) recipients, subgroup 2 (n = 18; 19%) included GER(–) SAbs(+) recipients, and subgroup 3 (n = 14; 15%) included GER(+) SAbs(–) recipients. CLAD-free survival in subgroups was compared with the control group. Stratified analysis showed the risk of CLAD was significantly higher among patients in subgroup 1 (OR [95% CI], 4.4 [1.1-17.2]; $P=0.035$), subgroup 2 (OR [95% CI], 5.6 [1.2-25.8]; $P=0.027$), and subgroup 3 (OR [95% CI], 7 [1.4-34.7]; $P=0.017$) than among patients in the control group (Table 5); however, a synergistic association between GER and SAbs with CLAD was not identified. The probability of CLAD-free survival of subgroups 1, 2, and 3 was significantly lower than that of the control group (61.5%, 55.6%, 50.0%, and 87.5%, respectively; $P=0.044$; Figure 3), with significant intergroup differences compared with the control group ($P=0.023$, 0.013, and 0.007, respectively). The median (IQR) CLAD-free survival of subgroup 1, 2, and 3 was 24 (14, 36), 21 (13, 39), and 22 (13, 29) mo, respectively.

Overall Survival in Study and Control Groups Was Comparable

Finally, the median (IQR) post-LTx follow-up time of the study cohort was 45 mo (33–50). The overall mortality in the cohort was 23% (n = 22). Mortality on index admission was 3.2% (n = 3); 1 patient had severe PGD requiring ECMO support, 1 had a posttransplant cerebrovascular accident and subsequent multiorgan system failure, and 1 had recurrent respiratory infections, delirium, multiorgan dysfunction, and resultant failure to thrive. At the end of follow-up, the deaths of 2 patients (both in the control group) were attributed to SARS-CoV-2 infection (Table 2). The probability of overall survival was comparable between the study group and the control group (78.9% versus 70.8%, $P=0.618$; Figure 4). The median (IQR) survival of the study group and the control group was also comparable (39 [25–48] and 41 [30–47] mo, respectively; $P=0.532$); however, the similarity of overall survival between groups may be related to short follow-up time.

DISCUSSION

Aspiration is the inhalation of oropharyngeal or gastric contents into the larynx and lower respiratory tract.[13] Asymptomatic microaspiration refers to aspiration of small

TABLE 3.

Univariate and multivariate Cox proportional hazard analysis for variables predicting chronic lung allograft dysfunction in lung transplant recipients

	Univariate analysis			Multivariate analysis		
Variable	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
SAbs (+) [Ref: SAbs (–)]	0.574	0.273-1.207	0.143	0.612	0.246-1.520	0.290
GER (+) [Ref: GER (–)]	0.542	0.262-1.120	0.098	0.965	0.392-2.377	0.938
Study group [Ref: control group]	4.389	1.337-14.405	0.015	8.787	1.694-45.567	**0.010**
Primary graft dysfunction	1.671	0.809-3.452	0.165	1.141	0.523-2.489	0.741
Acute cellular rejection ≥A2	0.447	0.224-0.894	0.023	3.290	1.560-6.936	**0.002**
Donor-specific antibodies	0.332	0.143-0.773	0.011	2.173	0.909-5.196	0.081
Pre-LTx respiratory infections	1.121	0.533-2.359	0.764			

Bold indicates statistical significance ($P \leq 0.050$).

CI, confidence interval; GER, gastroesophageal reflux; HR, hazard ratio; LTx, lung transplant; Ref, reference; SAbs, antibodies to lung self-antigens collagen- or Kα1-tubulin; (+), present; (–), absent.

TABLE 4.

Step-wise analysis to study confounding association of chronic lung allograft dysfunction with primary graft dysfunction, donor-specific antibodies, and acute cellular rejection

Group	Coefficient	95% CI	*P*	% change
Study group, unadjusted	1.634	1.398-18.759	0.014	Ref
Study group[a]	1.554	1.267-17.661	0.021	4.8[b]
Study group[c]	1.347	1.003-14.729	0.049	17.6
Study group[d]	1.874	1.468-28.893	0.014	14.7[b]

[a]Model adjusted for primary graft dysfunction.
[b]Percent difference in parameter estimate <15% rules out theoretical confounding association.
[c]Model adjusted for primary graft dysfunction and donor-specific antibodies.
[d]Model adjusted for primary graft dysfunction, donor-specific antibodies, and acute cellular rejection.
CI, confidence interval.

volumes of oropharyngeal or gastric secretions into the lungs. GER and the resultant microaspiration are associated with lung disease and can contribute to recurrent exacerbations and lung disease progression.[4,14] In addition, data from LTx literature suggest that chronic microaspiration is associated with CLAD.[3] In fact, several studies have suggested that early fundoplication improves survival and decreases CLAD in LTxRs, presumably through reducing the frequency of microaspiration events.[15,16] In addition to direct airway and parenchymal damage, GER without aspiration may also produce functional changes in the respiratory tract.[17] Animal and human studies have shown that GER without aspiration (ie, GER affecting the distal esophagus alone) may increase airway resistance and promote airway inflammation by releasing proinflammatory mediators.[18,19] Our data suggest that GER can also trigger a pneumotoxic SAbs-mediated immune response.

Col-V and Kα1T are sequestered, yet immunogenic, self-antigens restricted to the lungs.[20] SAbs have been detected in both LTx candidates and LTxRs. In a sentinel study by Tiriveedhi et al,[8] pre- and posttransplant serum samples were analyzed from 317 LTxRs with underlying chronic obstructive pulmonary disease (n = 161), IPF (n = 50), cystic fibrosis (n = 55), or other lung diseases (n = 51) who underwent LTx between 2000 and 2011. This study demonstrated that 18% of LTxRs with chronic obstructive pulmonary disease (29 of 161; $P = 0.033$), 34% with IPF (17 of 50; $P = 0.0006$), 29% with cystic fibrosis (16 of 55; $P = 0.0023$), and 19.6% with other lung diseases (10 of 51; $P = 0.044$) had preexisting SAbs. Furthermore, patients with pre-LTx SAbs had a significantly higher post-LTx incidence of PGD (88% versus 54%, $P < 0.05$), DSA (70% versus 45%, $P < 0.01$), and bronchiolitis obliterans syndrome (90% versus 38%, $P < 0.001$) than patients without pre-LTx SAbs.

We have demonstrated that GER in LTx candidates may result in lung injury and lead to immune exposure to Col-V

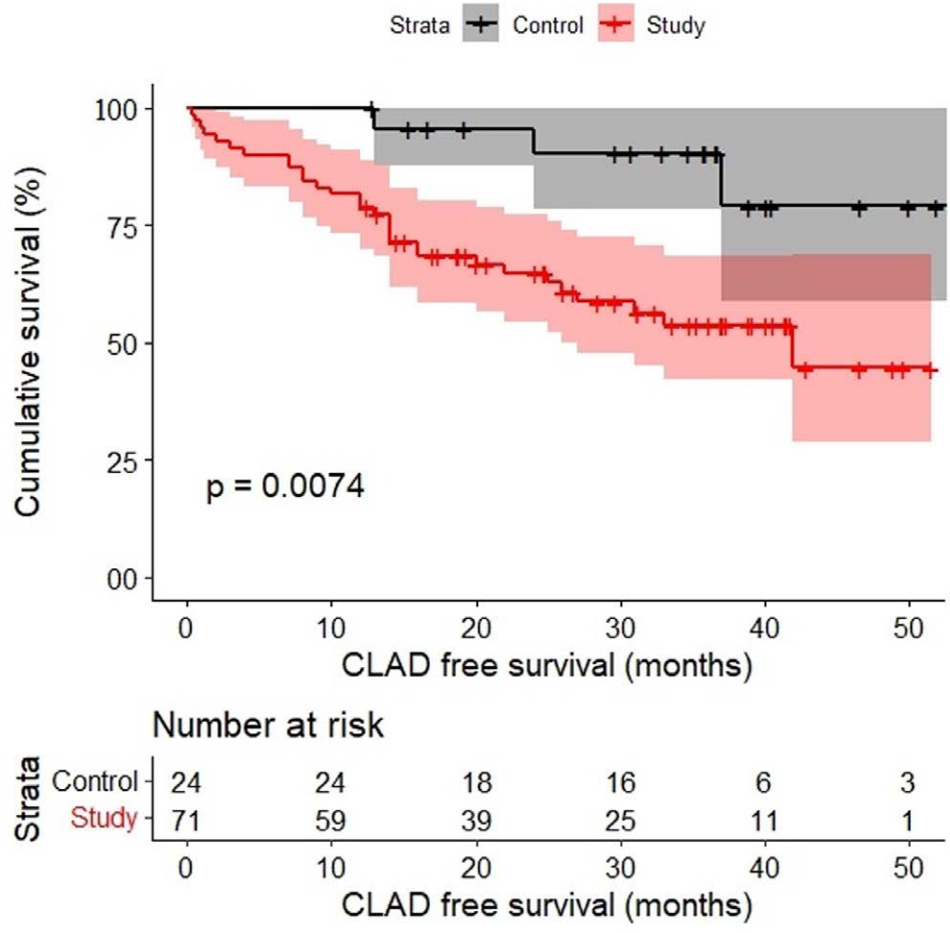

FIGURE 2. Kaplan-Meier curve shows the difference in CLAD-free survival between the study group and the control group. CLAD, chronic lung allograft dysfunction.

TABLE 5.

Stratified analysis for association between GER, SAbs, and chronic lung allograft dysfunction among subgroups

Subgroup	Odds ratio	95% CI	*P*
1: GER (+) SAbs (+)	4.375	1.111-17.234	**0.035**
2: GER (–) SAbs (+)	5.600	1.218-25.751	**0.027**
3: GER (+) SAbs (–)	7.000	1.413-34.682	**0.017**
Control group	Reference		

Bold indicates statistical significance ($P \leq 0.050$).
CI, confidence interval; GER, gastroesophageal reflux; SAbs, antibodies to lung self-antigens; (+), present; (–), absent.

and Kα1T antigens, with resultant development of SAbs. This immune response is a potential marker of GER-induced lung injury in LTx candidates. GER and resultant microaspiration episodes may render the pulmonary microenvironment proinflammatory[21,22] and prone to antigen-antibody reactions, complement cascade activation, and release of proinflammatory cytokines;[7] however, in addition to GER, other factors such as infections and environmental exposures may trigger SAbs. The current study also suggests that pre-LTx GER and SAbs may drive lung allograft injury after LTx leading to CLAD.

CLAD is an important cause of mortality in LTxRs and is likely the end result of a variety of immune, infectious, and inflammatory injuries to the allograft. Our study suggests that causes of these injuries include pretransplant GER and SAbs, in addition to other well-known risk factors such as PGD, ACR, and DSA. Importantly, although DSA confounded the relationship between GER, SAbs, and CLAD (Table 4), an independent relationship between GER, SAbs, and CLAD was still identified. This suggests a mechanistic pathway between pre-LTx GER and SAbs, with SAbs being both a biomarker and propagator of lung injury. In addition, a review of the literature suggests that there may be a mechanistic relationship between SAbs and DSA posttransplant with SAbs driving lung injury leading to immunologic

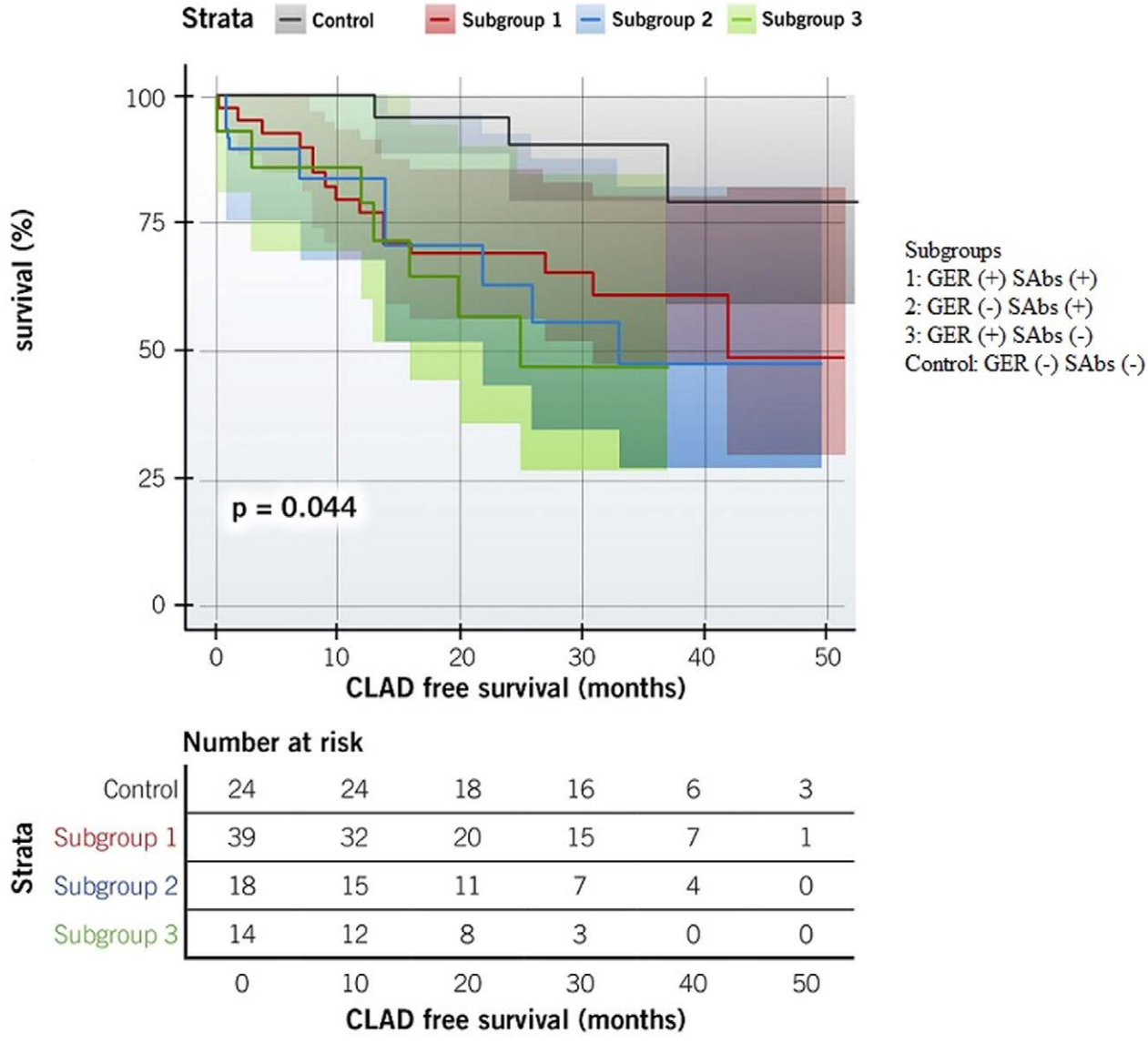

FIGURE 3. Kaplan-Meier curve shows the difference in CLAD-free survival between subgroups 1, 2, and 3 and the control group. CLAD, chronic lung allograft dysfunction; GER, gastroesophageal reflux; SAbs, antibodies to lung self-antigens; (+), present; (–), absent.

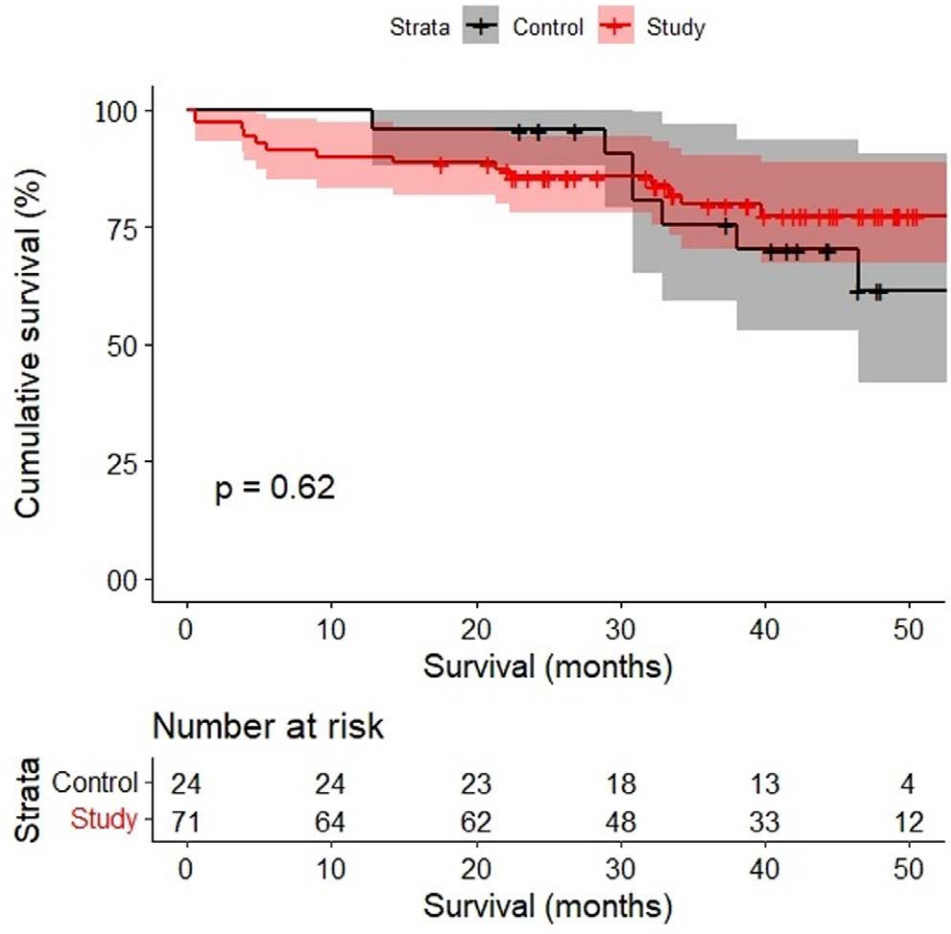

FIGURE 4. Kaplan-Meier curve shows the difference in overall survival between the study group and the control group.

exposure of HLA antigens and the resultant development of DSA.[8,20] The absence of synergy between GER and SAbs with CLAD may be due to the multifactorial, and potentially transient, nature of SAb elevation and CLAD, as both develop as a result of a variety of injuries.

Our study has limitations. This analysis is a single-center, retrospective cohort study, with a relatively small sample size, which makes establishing causation difficult; however, this study does illustrate a correlation between pre-LTx GER and SAbs with adverse post-LTx outcomes that warrants further evaluation in larger, multicenter trials. The results presented demonstrate a potentially important, clinically relevant finding that pre-LTx GER, in conjunction with SAbs to Col-V and Kα1T, predisposes recipients to CLAD after LTx. Although including SAbs in the pretransplant evaluation is feasible, additional validation is warranted before these serological markers impact clinical decision making.

In conclusion, Pre-LTx GER was associated with the presence of SAbs. Furthermore, pre-LTx GER and SAbs were associated with CLAD after LTx; however, the effect was not synergistic, likely due to the multifactorial nature of CLAD.

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