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Risk of Lung Allograft Dysfunction Associated With *Aspergillus* Infection

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Background. We sought to determine whether invasive aspergillosis (IA) during the first year after lung transplantation increased the risk of chronic lung allograft dysfunction (CLAD). **Methods.** We retrospectively reviewed the records of 191 patients who underwent lung transplantation at our institution between January 2013 and December 2017. Screening for *Aspergillus* was with bronchial aspirates, bronchoalveolar lavage if indicated or during surveillance bronchoscopy, radiography, and computed tomography. We used Fine and Gray multivariable regression to identify potential risk factors for CLAD. **Results.** During the first posttransplant year, 72 patients had at least 1 deep-airway sample positive for *Aspergillus*; 63 were classified as having IA and were included in the study. Median number of endoscopies per patient during the first year was 9 (range, 1–44). Median time from transplantation to first *Aspergillus*-positive sample was 121 d. Bronchial aspirate samples and bronchoalveolar lavage fluid were positive in 71 and 44 patients, respectively. *Aspergillus fumigatus* (n = 36, 50%) predominated; bacterial samples were also positive in 22 (31%) patients. IA within 4 mo after transplantation was independently associated with CLAD development (subdistribution hazard ratio, 3.75; 95% confidence interval [CI], 1.61-8.73; *P* < 0.01) by regression analysis. Survival at 3 and 5 y conditional on 1-y CLAD-free survival was 37% (95% CI, 24%-58%), and 24% (95% CI, 11%-52%) in the IA <4 mo group compared to 65% (95% CI, 57%-73%) and 54% (95% CI, 43%-66%) in the non-IA group and to 69% (95% CI, 58%-83%) and 54% (95% CI, 35%-82%) in the IA ≥4 mo group, respectively (*P* < 0.01, logrank test). **Conclusions.** Our evaluation of de novo IA showed that this infection was most strongly associated with CLAD when found within 4 mo after transplantation.

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The main reason for failure to achieve long-term survival after lung transplantation (LTx) is the development of chronic lung allograft dysfunction (CLAD), manifesting mainly as bronchiolitis obliterans syndrome (BOS).[1] Among the many factors that have been implicated in the development of CLAD, infectious agents may play a substantial role.[2-6] Fungal

infections remain among the main causes of morbidity and mortality in lung-transplant recipients (LTxRs).[7] In LTxRs, the most common cause of fungal infection is *Aspergillus*, with a strong predominance of *Aspergillus fumigatus*.[8,9] When *Aspergillus* is present, invasive aspergillosis (IA) usually develops within the first posttransplantation year.[10] Although IA in

LxTRs has been associated with a reduction in 5-y survival,[11] it remains unclear whether the timing of the infection is associated with outcomes. A review of IA in LxTRs found higher mortality with late-onset compared to early-onset IA (57% versus 28%, P = 0.045).[12] However, this finding is difficult to interpret given the large proportion of single-lung recipients, in whom the native lung may have served as the source of infection. In one of the earliest studies of the effect of fungal infections and BOS development, fungal pneumonia in the first 100 posttransplant days was associated with BOS with a hazard ratio (HR) of 2.1 (95% confidence interval [CI], 1.1-4.0) compared to 1.5 (95% CI, 1.1-1.9) for fungal pneumonia during the late postoperative period.[13] However, all fungal infections were pooled, and limited information was provided on the causative agents.

Here, we used a retrospective observational cohort of LTxRs to investigate the potential association between the development of IA within the first year after LTx and the development of CLAD. To refine our analysis, we distinguished early IA, diagnosed within the first 4 mo, from IA diagnosed later in the first year.

MATERIALS AND METHODS

Study Design and Patients

We retrospectively included consecutive adults who underwent double-LTx or heart-LTx between January 2013 and December 2017 at our institution. Our institutional review board approved the study and waived the need for informed consent in compliance with French legislation on retrospective studies of anonymized data.

Collection of Baseline Data

We used standardized forms to record demographic data, the medical history, and the most recent lung function test (LFT) results. Spirometry was performed according to guidelines.[14] LFT results were recorded as percent of predicted values.[15]

Aspergillosis: Definitions, Screening, and Antifungal Treatment

We used the 2010 and 2015 International Society of Heart and Lung Transplantation Consensus Statements on standardized definitions of infections in cardiothoracic transplant recipients.[9,16] IA was defined as growth of *Aspergillus* in respiratory samples (aspirates and/or bronchoalveolar lavage [BAL]) or detection of galactomannan in BAL fluid in patients with recent-onset symptoms or recent-onset radiologic and/or endobronchial changes; or histologic changes consistent with fungal tissue invasion. *Aspergillus* colonization was defined as presence of the fungus in respiratory samples (aspirates and/or BAL fluid) or detection of galactomannan in the absence of symptoms or of radiologic or endobronchial changes. Preoperative aspergillosis screening as part of the listing workup included *Aspergillus* serology, bronchial endoscopy with tests for fungi, and thoracic computed tomography (CT). Postoperatively, screening for aspergillosis relied on testing bronchial aspirates and/or BAL fluid obtained when clinically indicated or during surveillance bronchial endoscopy, combined with chest radiographs and/or thoracic CT. Preoperatively, only patients with IA were treated with a mold-active oral azole drug. This treatment was continued until transplantation then stopped 1 mo after transplantation in the absence of invasive disease in the explanted lung. During the early postoperative period, preemptive targeted therapy consisting in nebulized amphotericin B was given to patients with ischemic injury of the proximal airways until this last improved significantly. During the first posttransplantation year, patients with IA or colonization received 3 mo of treatment, preferably with a regimen including an oral azole drug. The treatment was stopped if the workup, including thoracic imaging and tests on bronchoscopy samples, showed no evidence of *Aspergillus*. The calcineurin inhibitor (CNI) dosage was decreased by 50% at azole initiation then adjusted to blood CNI levels.

Neutropenia and hypogammaglobulinemia were defined as at least 1 episode of neutrophil count <1000 cells/µL and IgG level <4.5 g/L, respectively.

Immunosuppressive Therapy and General Management

Induction was indicated in patients with preoperative donor-specific antibodies producing a mean fluorescence intensity ≥2000 or in the case of delayed introduction of calcium-channel blocker therapy. Of the 191 patients, 79 (41%) received induction immunosuppressive therapy, which included either a monoclonal antibody against the interleukin-2 receptor (n = 49) or antithymocyte globulin (n = 30). Target trough tacrolimus and cyclosporin levels during the first posttransplantation year were 10–15 µg/L and 200–300 µg/L, respectively. Tacrolimus and cyclosporin overdosage was defined as baseline drug blood levels >20 and 400 µg/L, respectively. All patients received life-long *Pneumocystis* pneumonia prophylaxis with cotrimoxazole. Valganciclovir for cytomegalovirus (CMV) prophylaxis was given for 3 and 12 mo in recipient-positive and in donor-positive/recipient-negative patients, respectively.

Monitoring transbronchial biopsies were obtained routinely at 1 mo then as clinically indicated.

Patients with allograft dysfunction were investigated for acute cellular rejection, antibody-mediated rejection, lymphocytic bronchiolitis/neutrophilic reversible allograft dysfunction, and airway injury caused by infection or colonization. CLAD was diagnosed based on international criteria as previously described,[17,18] at routine outpatient assessments every 1–3 mo after a minimum of 3 mo posttransplantation. CLAD was suspected if the forced expiratory volume in 1 s (FEV_1) and/or forced vital capacity decreased to ≤90% of the baseline value for ≥3 wk. Comprehensive LFTs including spirometry and lung volume measurements, thoracic high-resolution CT (HRCT), and bronchoscopy with BAL and transbronchial biopsies were performed to look for causes of acute lung allograft dysfunction, including persistent acute rejection, azithromycin-responsive allograft dysfunction, infection, anastomotic stricture, and disease recurrence. CLAD encompasses a restrictive phenotype (restrictive allograft syndrome) with forced vital capacity ≤80% at baseline and CLAD diagnosed for ≥3 wk with interstitial infiltrates on thoracic HRCT, an obstructive phenotype (BOS) with FEV_1 ≤80% at baseline for ≥3 wk without interstitial infiltrates on thoracic HRCT, and a mixed phenotype (BOS and restrictive allograft syndrome) with FEV_1 ≤80% at baseline for ≥3 wk and interstitial infiltrates.[17,18]

Acute cellular rejection and antibody-mediated rejection were defined according to international guidelines.[19,20] Acute cellular rejection was treated by 15 mg/kg of intravenous methylprednisolone followed by a transient increase of 0.5 mg/kg of oral glucocorticoids over an additional 3-wk period. Depending on rejection severity, the multimodal treatment of antibody-mediated rejection would include 3 to 5 plasma exchange sessions, 1 g/kg of intravenous immunoglobulins, and a double dose of rituximab (375 mg/m^2) over a 14-d period, followed by additional courses of 1 g/kg of intravenous immunoglobulins every 4 wk until clearance of donor-specific antibodies.

Acute renal dysfunction was defined as a glomerular filtration rate drop of ≥25% compared to baseline.

Statistical Analyses

Quantitative baseline variables were described as mean ± SD if normally distributed and as median (range) (interquartile range) otherwise. Categorical variables were described as number (%). For bivariate analyses, continuous variables were compared using Student's t-test for independent samples if normality assumptions were satisfied (Shapiro-Wilks test) and the Welch t-test (if variances were heterogeneous) or the Mann-Whitney U test otherwise. Categorical variables were compared by applying Pearson's χ^2 test (with the Monte-Carlo method if counts were below 5). Overall and CLAD-free survival, as well as landmark analysis curves starting at 1-y posttransplantation to illustrate the importance of the time to IA, were analyzed using the Kaplan-Meier method with the logrank test for comparisons. Fine and Gray regression was performed to look for associations linking baseline variables to CLAD (with death in the absence of CLAD as a competing risk) and Kaplan-Meier multiple imputation to compare CLAD-free survival according to *Aspergillus* characteristics (IA, yes/no; IA < or ≥4 mo). Fine and Gray using

Kaplan-Meier multiple imputation was also applied to build the multivariate analyses, using co-variables associated with P values <0.1 by univariate analysis. The cumulative incidence function was chosen to assess associations linking IA to CLAD (with death as a competing risk); Gray's test was used for comparisons. Postoperative covariates, including CMV viremia, hypogammaglobulinemia, neutropenia, acute cellular rejection, and antibody-mediated rejection were recorded during the first posttransplant year. Two-tailed P values <0.05 were considered significant and (subdistribution) HRs were estimated with their 95% CIs. All statistical analyses and graphs were performed using R v3.6.0 with the "ggplot2," "survival," "cmprsk," and "kmi" packages.

RESULTS

Patients

The 207 consecutive patients who underwent double-LTx or heart-LTx at our center during the study period were considered for eligibility. We excluded the 16 patients who died during the first posttransplantation month. The remaining 191 patients were considered (Figure 1). Table 1 reports their main features.

Of the 191 patients, 72 had at least 1 *Aspergillus*-positive lung sample, including 9 classified as having colonization and 63 as having IA. These 63 patients form the basis for our study. The median time from transplantation to the first positive sample was 121 d (4 mo). Of these 63 patients, 32 were diagnosed before and 31 after the first 4 mo. Compared to the group without IA, the number of patients given induction therapy was significantly higher in the IA group. Within the IA group, the number of CMV donor-positive/recipient-negative patients was significantly higher in the subgroup with IA ≥4 mo. Conversely, there was a trend toward a higher frequency of CLAD when IA occurred <4 mo (Table 1).
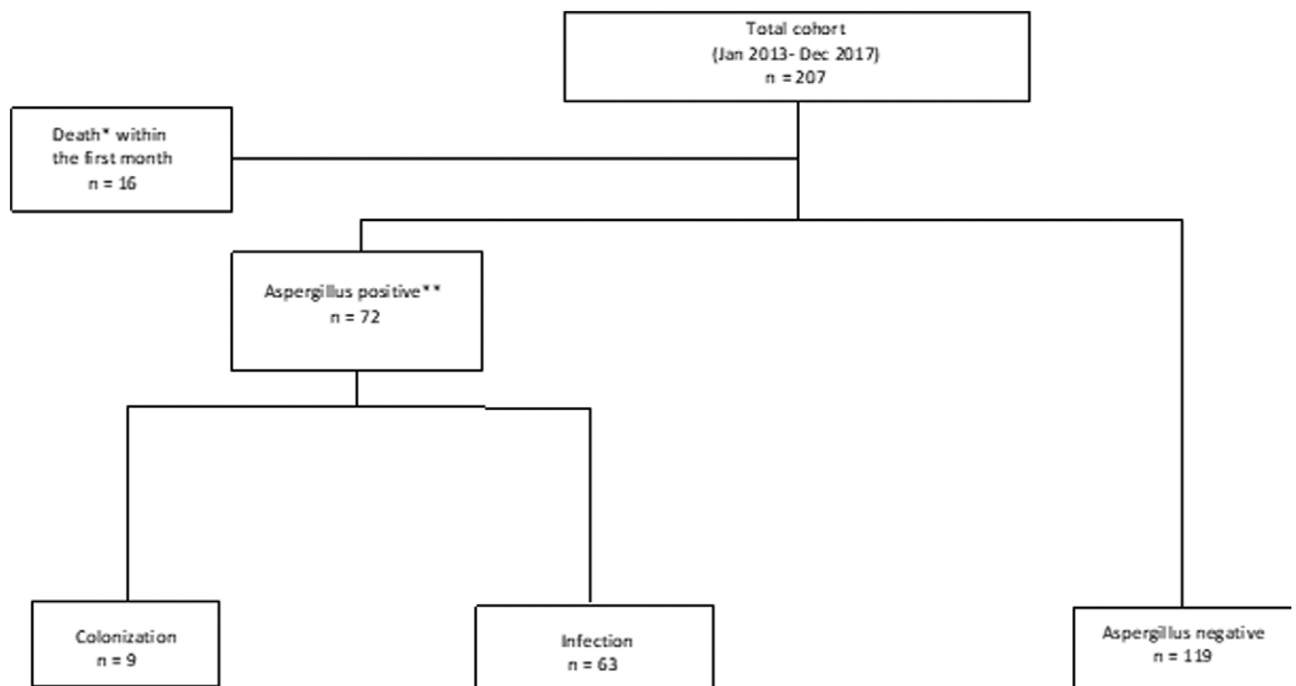

FIGURE 1. Flowchart. *Unrelated to *Aspergillus*. **Defined as at least 1 deep-airway sample positive for *Aspergillus* during the first posttransplant y.

TABLE 1.

Main features of the study patients

	Overall (n = 191)	No invasive aspergillosis (n = 128)	Invasive aspergillosis (n = 63)	P[a]	Invasive aspergillosis		P[b]
					<4 mo (n = 32)	≥4 mo (n = 31)	
Female sex, n (%)	112 (59)	77 (60)	35 (56)	0.64	19 (59)	16 (52)	0.61
Recipient age, median (IQR)	49 (36–57)	49 (34–57)	48 (39–58)	0.60	48 (39–56)	47 (38–579)	0.64
Blood group 0/A/B/AB, n (%)	73 (38)/77(40)/33(17)/8(5)	45 (35)/54(42)/24(19)/5(4)	28 (44)/23(37)/9(14)/3(5)	0.59	15 (47)/13(41)/4(12)/0(0)	13 (42)/10(32)/5(16)/3(10)	0.34
Lung-transplant procedure DLT/HLT, n (%)	177 (93)/14(7)	117 (92)/11(8)	60 (94)/3(6)	0.39	31 (97)/1(3)	29 (94)/2(6)	0.61
Transplantation for PAH, fibrosis, COPD, other, n (%)	108 (57)/33 (17)/29 (15)/21 (11)	74 (58)/25 (20)/17 (13)/12 (9)	34 (54)/8 (13)/12 (19)/9 (14)	0.34	19 (59)/2(6)/6(19)/5(16)	15 (48)/6(19)/6(19)/5 (13)	0.47
CMV D+/R−, n (%)	39 (20)	23 (18)	16 (25)	0.34	7 (22)	16 (50)	0.03
CMV viremia, n (%)	41 (21)	28 (22)	13 (21)	1.00	6 (19)	7 (23)	0.76
High-emergency transplantation program, n (%), n/184	78 (42)	51 (41)	27 (44)	0.43	13 (41)	14 (48)	0.61
Ischemic time (min), median (IQR)							
Right	245 (210–285)	255 (211–286)	236 (211–286)	0.29	242 (207–291)	230 (211–276)	0.46
Left	350 (300–383)	354 (300–390)	335 (290–376)	0.35	344 (290–275)	323 (291–376)	0.84
Heart-lung	236 (220–248)	236 (222–244)	261 (233–290)	0.92	228 ± 7	261 ± 80	0.80
Induction, n (%)	73 (38)	39 (30)	34 (54)	0.02	16 (50)	18 (58)	0.61
Dialysis during ICU stay, n (%), n/189	37 (20)	27 (21)	10 (16)	0.44	4 (12)	6 (19)	0.51
PGD score grade 3 at 72 h, n (%), n/132	29 (22)	21 (22)	8 (22)	1.00	5 (20)	3 (19)	0.71
Ventilation time during ICU stay, median (IQR)	9 (2–20)	9 (2–20)	9 (2–22)	0.84	14 (3–26)	13 (1–13)	0.10
Hypogammaglobulinemia, n (%)	67 (35)	45 (35)	22 (35)	1.00	11 (34)	11 (35)	>0.99
Neutropenia, n (%), n/164	40 (24)	28 (26)	12 (21)	0.45	5 (16)	7 (26)	0.51
Diabetes, n (%), n/188	48 (25)	34 (27)	14 (23)	0.59	6 (19)	8 (27)	0.55
Acute cellular rejection ≥2 episodes, n (%), n/187	54 (29)	33 (26)	21 (34)	0.30	9 (28)	8 (26)	1.00
Antibody-mediated rejection, n (%), n/188	65 (35)	48 (38)	17 (27)	0.14	12 (37)	9 (31)	0.79
CLAD at last F-up, n (%)	43 (22)	28 (21)	15 (24)	0.85	11 (34)	4 (13)	0.07
BOS	35 (18)	22 (17)	11 (17)	0.99	7 (19)	4 (13)	0.56
RAS	4 (2)	2 (2)	2 (3)	0.58	2 (6)	0 (0)	0.45
Mixed CLAD	4 (2)	2 (2)	2 (3)	0.37	2 (6)	0 (0)	0.45
Bronchial endoscopy/patient, median (IQR)	9 (5–13)	8 (4–13)	9 (5–13)	0.56	9 (6–13)	9 (3–13)	0.60
Rigid bronchoscopy, n (%)	15 (8)	9 (7)	6 (10)	0.57	2 (6)	4 (13)	0.43

[a]P-value comparison between the groups with and without invasive aspergillosis.
[b]P-value comparison between the groups with invasive aspergillosis before vs after 4 mo posttransplantation.
BOS, bronchiolitis obliterans syndrome; CLAD, chronic lung allograft syndrome; CMV, cytomegalovirus; COPD, chronic obstructive pulmonary disease; DLT, double lung transplantation; F-up, follow-up; HLT, heart-lung transplantation; ICU, intensive care unit; IQR, interquartile range; PAH, pulmonary arterial hypertension; PGD, primary graft dysfunction; RAS, restrictive allograft syndrome.

Aspergillus, Microbiology, and Treatment

Table 2 reports the *Aspergillus* test results in the IA group. Of note, only 2 patients had *Aspergillus* colonization before transplantation. Interestingly, endobronchial samples were far more often positive than BAL fluid samples (98% and 27%, respectively). *A. fumigatus* (63%) was the predominant species. Bacterial samples were also positive in 21 (31%) patients as follows: *Staphylococcus aureus* in 5, *Pseudomonas aeruginosa* in 4, *Klebsiella pneumoniae* in 2, *Enterobacter cloacae* in 2, *Streptococcus pneumoniae* in 2, *Branhamella catarrhalis* in 2, and other in 4.

A mold-active azole drug was used in 52 patients (Table 2). Of note, 11 patients received no treatment for IA, for the following reasons: comorbidities (n = 4), alternative diagnosis (n = 5: acute cellular rejection, n = 3; and bacterial pneumonia, n = 2) and death (n = 2 with 1 case each of septic shock, and sudden death). CNI overdosage, renal dysfunction, and other side effects occurred in 36 (62%), 18 (30%), and 11 (19%) patients, respectively.

Factors Associated With CLAD Development

At the end of follow-up, 43 patients (22%) had developed CLAD (Table 1). Overall 1-, 3- and 5-y CLAD-free survival estimates were 85% (95% CI, 80%-90%), 60% (95% CI, 53%-68%), and 47% (95%CI:39%-58%), respectively (Figure 2). Survival estimates at 3- and 5-y conditional on 1-y CLAD-free survival were 37% (95% CI, 24%-58%) and 24% (95% CI, 11%-52%) in the IA <4 mo group compared to 65% (95% CI, 57%-73%) and 54% (95% CI, 43%-66%) in the IA-negative group and to 69% (95% CI, 58%-83%) and 54% (95% CI, 35%-82%) in the IA ≥4 mo group, respectively ($P < 0.01$, logrank test) (Figures 2 and 3). By univariate analysis, only 2 variables were significantly associated with

CLAD, namely, B blood group and IA within 4 mo. There were also trends towards increased frequency of CLAD with CMV viremia and acute rejection ≥2. By multivariate analysis, only early IA was independently associated with CLAD development (Table 3).

Survival

Mean posttransplant follow-up was 34.1 ± 18.3 (range, 1.2–74.6) mo. At last follow-up, 139 patients were alive, 52 had died, and none were lost to follow-up. Overall 1-, 3-, and 5-y survival rates were 84% (95% CI, 79%-91%), 72% (95% CI, 66%-79%), and 70% (95% CI, 63%-78%), respectively. Although not significant, there was a slight trend toward better conditional on 1-y survival in the IA-negative group compared to the IA group, with 3- and 5-y survival rates of 73% (95% CI, 62%-79%) and 70% (95% CI, 59%-82%) versus 63% (95% CI, 56%-71%) and 63% (95% CI, 57%-75%), respectively ($P = 0.14$, logrank test). Most deaths were attributed to infections (n = 16) or to acute rejection or CLAD (n = 15).

DISCUSSION

Our longitudinal analysis of a cohort of double-LTx and heart-LTx recipients showed that early IA was independently associated with the development of CLAD.

CLAD is among the leading causes of death beyond the first posttransplantation year. CLAD is common[21] and infections are among the many factors reported to contribute to BOS.[22] However, nearly all the existing evidence derives from small retrospective single-center studies. One of the earliest studies found that fungal pneumonia in the first 100 posttransplant days was associated with BOS with a HR of 2.1 (95% CI,

Fungal microbiology and antifungal treatment

	Invasive aspergillosis (n = 63)	Invasive aspergillosis		
		<4 mo (n = 32)	≥4 mo (n = 31)	*P*
Pretransplant *Aspergillus*-positive endoscopic sample, n (%)	2 (3)	1 (3)	1 (3)	>0.99
Pretransplant *Aspergillus*-positive serology, n (%)	0 (0)	0 (0)	0 (0)	>0.99
Time from LTx to *Aspergillus* positivity (d), median (range)	121 (75–215)	75 (58–89)	223 (149–275)	<0.01
Diagnostic criteria, n (%)				
Respiratory symptoms	42 (67)	18 (62)	24 (70)	0.59
Endoscopic changes	50 (81)	26 (81)	24 (77)	0.11
Thoracic HRCT changes, n/22	11 (50)	6 (50)	5 (50)	>0.99
Aspergillus per endoscopic microbiology, n (%)				
Positive aspiration, n/62	61 (98)	31 (100)	30 (97)	>0.99
Positive BAL[a], n/41	11 (27)	5 (25)	6 (29)	>0.99
BAL *Aspergillus* Ag, n/16	2 (12)	1 (10)	1 (17)	0.48
Aspergillus, n (%), Fumigatus/Niger/Flavus/other	46 (63)/7 (11)/5 (8)/5 (8)	24 (75)/4 (13)/1 (3)/3 (9)	22 (71)/3 (10)/4 (13)/2 (6)	0.42
Bacterial coinfections, n (%)	21 (31)	11 (34)	10 (32)	>0.99
Treatment (Voriconazole/Posaconazole/none), n (%)	34 (54)/18 (29)/11 (17)	20 (62)/9 (28)/5 (21)	14 (45)/9 (29)/6 (19)	0.57
Treatment duration (d), median (range)	245 (89–172)	113 (86–175)	97 (91–153)	0.66
Calcineurin inhibitor overdosage[b], n (%) n/60	37 (62)	21 (68)	16 (59)	0.59
Acute renal failure[c], n (%), n/58	33 (57)	30 (58)	3 (50)	>0.99
Additional side effects[d], n (%), n/52	10 (6)	7 (25)	3 (11)	0.25

[a]BAL *Aspergillus* antigen was negative in all 25 patients in the *Aspergillus*-negative group who underwent the test
[b]Tacrolimus and cyclosporin overdosages were defined by baseline drug blood levels above 20 μg/L and 400 μg/L, respectively.
[c]Acute renal failure was defined as a glomerular filtration rate drop of ≥25% compared to baseline.
[d]Additional side effects included hepatitis and peripheral neuropathy.
Ag, antigen; BAL, bronchoalveolar lavage; HRCT, high-resolution computed tomography; LTx, lung transplantation.
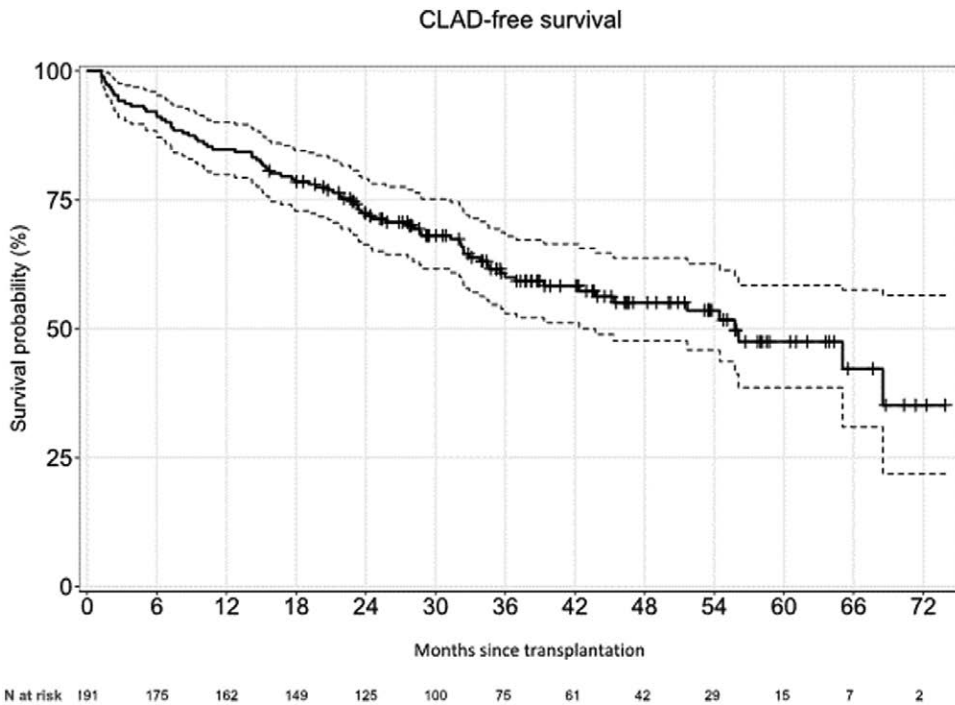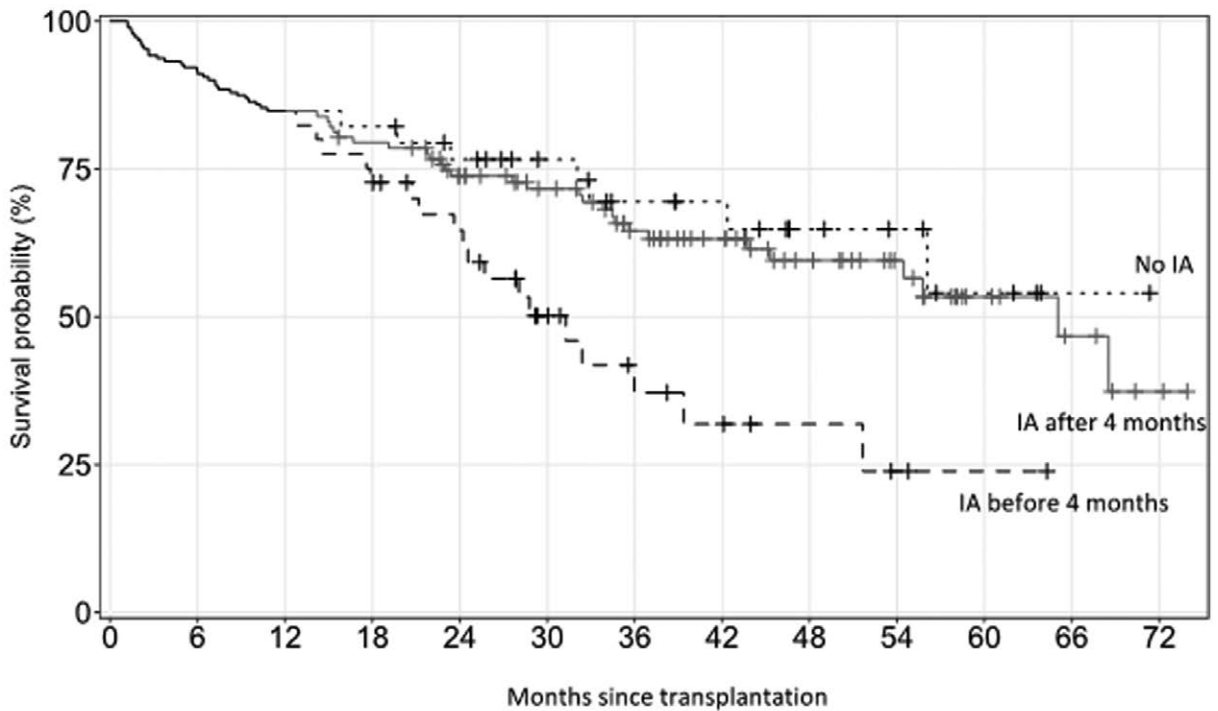
FIGURE 2. Kaplan-Meier chronic lung allograft dysfunction-free survival estimates from the date of transplantation in the overall population of 191 patients. Chronic lung allograft dysfunction-free survival rates were 85% (95% CI, 80%-90%), 60% (95% CI, 53%-68%), and 47% (95% CI, 39%-58%), respectively. CI, confidence interval.

FIGURE 3. Kaplan-Meier estimates of survival conditional on 1-y chronic lung allograft dysfunction-free survival from the date of transplantation in the overall population according to timing of invasive aspergillosis occurrence. Conditional on 1-y chronic lung allograft dysfunction-free survival at 3 and 5 y was 37% (95% CI, 24%-58%) and 24% (95% CI, 11%-52%) in the invasive aspergillosis <4 mo group compared to 65% (95% CI, 57%-73%) and 54% (95% CI, 43%-66%) in the no invasive aspergillosis group and to 69% (95% CI, 58%-83%) and 54% (95% CI, 35%-82%) in the invasive aspergillosis ≥4 mo group, respectively (*P* < 0.01, logrank test). CI, confidence interval; CLAD, chronic lung allograft dysfunction; IA, invasive aspergillosis.

1.1-4.0).[13] In our study, IA before 4 mo was independently associated with CLAD. Within the IA group, only CMV donor-positive/recipient-negative status differed between the subgroups with IA before versus after 4 months. We also found that endobronchial samples were far more often positive than BAL fluid samples. We are not aware of other studies differentiating endobronchial from alveolar samples.[23-25] This higher prevalence of endobronchial sample positivity may suggest that IA mainly occurred as tracheobronchitis, in keeping with earlier data.[10] Interestingly, small-conidia *Aspergillus* species (eg, *Aspergillus fumigatus*) predominated in our patients and have been reported to be associated with a higher BOS rate compared to large-conidia species.[26] Conceivably, presence within the bronchi of *A. fumigatus* early after transplantation may result in epithelial injury followed by dysregulation of repair mechanisms responsible ultimately for chronic fibro-proliferation and progressive graft dysfunction.[27]

An important strength of our study is that it specifically addressed associations linking postoperative IA to outcomes, as all but 2 patients were *Aspergillus*-negative before transplantation. However, although IA usually occurs in up to 15% of patients,[10] the frequency of IA diagnosed during the first posttransplant year in our cohort was about 33%. Although this high frequency remains incompletely understood, induction therapy was used significantly more often in the IA group, suggesting that greater severity of immunosuppression may have contributed to IA development.

IA in LTxRs has been associated with a reduction in the 5-y survival rate.[11] In our study, survival conditional on 1-y survival was not significantly different in the IA and non-IA groups, although this result may be ascribable to limited statistical power.

Voriconazole is currently the drug of choice for aspergillosis[28,29] In a randomized controlled trial in 144 patients (including 6.2% of solid organ transplant recipients), voriconazole was associated with improved survival (70.8%) compared to amphotericin (57.9%).[30] However, in immunosuppressed patients, voriconazole was associated with a significant risk of skin cancer, which should lead to close monitoring. In our cohort, 83% of IA patients received antifungal treatment, including two-thirds who received voriconazole. Although antifungal treatment was consistently successful, IA remained the main risk factor for CLAD. This suggests that universal antifungal prophylaxis might decrease the risk of IA

TABLE 3.

Univariate and multivariate analyses of variables associated with CLAD development

Variables	Reference	Modality	Univariate			Multivariate		
			sdHR	95 CI	P	sdHR	95 CI	P
Sex	Male	Female	1.09	0.64-2.01	0.77			
Recipient age (y)			1.02	0.98-1.02	0.97			
Blood group	A	0	1.17	0.59-2.34	0.65	1.18	0.15-9.15	0.87
		B	2.24	1.04-4.80	0.04	1.88	0.84-4.20	0.13
		AB	0.74	0.10-5.64	0.77	1.13	0.49-2.63	0.78
CMV donor-positive/recipient-negative	Yes	No	1.18	0.58-2.41	0.65			
CMV viremia	No	Yes	1.81	0.96-3.43	0.07	1.82	0.84-3.93	0.13
Lung-transplant procedure	HLT	DLT	0.73	0.28-1.95	0.53			
Lung-transplant indication	COPD	PAH	0.72	0.34-1.52	0.39			
		Fibrosis	0.48	0.17-1.40	0.18			
		Other	0.38	0.10-1.45	0.16			
High-emergency transplant program	No	Yes	0.74	0.40-1.36	0.33			
Ischemic time right (min)			1.00	0.99-1.00	0.27			
Ischemic time left (min)			1.00	0.99-1.00	0.34			
Cardiopulmonary bypass	No	Yes	0.84	0.38-1.84	0.66			
Induction	No	Yes	1.08	0.59-1.97	0.80			
Dialysis during ICU stay	No	Yes	1.24	0.59-2.63	0.56			
PGD score grade 3 at 72 h	No	Yes	1.32	0.58-3.02	0.51			
Ventilation time during ICU stay	1–7 d	0	0.00	0.00-0.00	<0.01			
		8–14 d	0.48	0.17-1.34	0.16			
		15–30 d	0.93	0.43-2.00	0.85			
		>30 d	0.37	0.09-1.60	0.18			
Hypogammaglobulinemia	No	Yes	1.07	0.57-2.02	0.82			
Neutropenia	No	Yes	1.45	0.73-2.91	0.82			
Diabetes	No	Yes	1.26	0.63-2.53	0.52			
Postoperative tracheotomy	No	Yes	1.18	0. 47-2.97	0.71			
Aspergillus infection	Negative	Positive	1.39	0.76-2.52	0.57			
	Negative	Positive before 4 mo	2.56	1.32-4.97	<0.01	3.75	1.61-8.73	<0.01
	Negative	Positive after 4 mo	0.45	0.14-1.43	0.18			
Azole treatment	No	Yes	1.29	0.68-2.47	0.44			
Acute cellular rejection ≥2	No	Yes	1.67	0.92-3.04	0.09	1.96	0.98-3.93	0.06
Antibody-mediated rejection	No	Yes	1.27	0.70-2.28	0.43			

95% CI, 95% confidence interval; CLAD, chronic lung allograft dysfunction; COPD, chronic obstructive pulmonary disease; DLT, double lung transplantation; HLT, heart-lung transplantation; ICU, intensive care unit; PAH, pulmonary arterial hypertension; PGD, primary graft dysfunction; sdHR, subdistribution hazard ratio.

occurrence and therefore the risk of CLAD. In a metaanalysis in LTxRs, no significant reduction in IA or *Aspergillus* colonization was found with universal anti-*Aspergillus* prophylaxis.[31] Additionally, breakthrough IA can occur despite antifungal prophylaxis.[32] Furthermore, the use of azoles is limited by side effects (hepatotoxicity, neurotoxicity, QT prolongation), drug interactions, and emerging resistance.[33] In our experience, despite the initial decrease and subsequent adjustment of CNI dosages, azole agent initiation resulted in CNI overdosage, acute renal dysfunction, and additional side effects in 62%, 30%, and 19% of patients, respectively. Close monitoring of azole-CNI interactions is crucial.

Interestingly, 31% of our patients had a positive bacterial sample, often for *P. aeruginosa*. Although interactions between *P. aeruginosa* and *Aspergillus* may be complex, some clinical evidence obtained in patients with cystic fibrosis suggests a synergistic effect of the combined infection that translates into a poorer prognosis for the patient.[34]

There are several limitations of our study that may influence the general applicability of our results. First, outcomes may have been biased by the absence of anti-*Aspergillus* treatment in 11 of the 63 IA patients. However, it is important to note that these untreated patients were fairly evenly distributed between the early and late IA subgroups. It is therefore unlikely that they skewed the associations linking early versus late IA to outcomes. Furthermore, although all 63 IA patients underwent chest imaging, only 22 had a chest HRCT. Consequently, infiltrates may have been missed, leading to infection being misclassified as colonization. However, since only 9 patients were classified as having *Aspergillus* colonization, any such misclassification can only have been limited. In the absence of a standardized definition of early ischemic complications in our program, early airway complications could not be included in the analysis. However, the number of rigid bronchoscopies performed to treat early airway complications was not different among groups. In addition, due to limited availability, the galactomannan antigen assay was performed in a limited number of patients. This fact may have resulted in some cases of *Aspergillus* positivity being missed. However, *Aspergillus* antigen was negative in BAL fluid in all 25 patients in the *Aspergillus*-negative group who underwent the test. Furthermore, although it has been postulated that gastroesophageal reflux may be associated with CLAD, in the absence of a standardized approach to report gastroesophageal reflux in the posttransplantation period in our cohort, we were not able to include this variable in our analysis.

Last, the limited sample size of our study and the large number of variables included in the regression model may have affected our statistical analysis, thereby limiting the general applicability of our results. Thus, in the absence of previous data in the literature, we postulated that the observed association between B blood group and CLAD risk may reflect Type I error related to multiple testing.

In conclusion, our results indicate that IA is significantly associated with a higher risk of CLAD. More importantly, by its ability to investigate de novo IA, our study showed that early infection had the strongest association with CLAD, indicating a need for regular early screening. Randomized controlled trials should be undertaken to determine whether the universal use of antifungal agents improves outcomes.

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