Pretransplant *Aspergillus* Colonization of Cystic Fibrosis Patients and the Incidence of Post–Lung Transplant Invasive Aspergillosis

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Background. Invasive aspergillosis (IA) is an important cause of morbidity and mortality among patients undergoing lung transplant. Cystic fibrosis-lung transplant recipients (CF-LTRs) may be at greater risk of IA following lung transplantation because of the presence of *Aspergillus* in their airways before transplantation. This study evaluated the impact of pretransplant *Aspergillus* colonization on the risk for IA among CF-LTRs.

Methods. A single-center retrospective cohort study of CF-LTRs was conducted between 2006 and 2010. Respiratory tract cultures before transplantation were reviewed to identify patients with pretransplant *Aspergillus* colonization. Patients with positive *Aspergillus* sputum culture or positive bronchoalvelolar lavage (BAL) galactomannan after transplantation were classified as having colonization or disease according to the International Society of Heart and Lung Transplantation criteria.

Results. A total of 93 CF patients underwent lung transplantation. Seventy percent (65/93) of CF-LTRs had pretransplant *Aspergillus* colonization. Thirty-six patients had positive intraoperative *Aspergillus* culture from the native lung BAL. Overall, 22.5% (20/93) of CF-LTRs developed IA. Median time to IA was 42 days following transplantation. Positive intraoperative *Aspergillus* culture (OR 4.36, 95% CI 1.35–14.05, P=0.01) and treatment for acute cellular rejection within 90 days after transplantation (OR 3.53, 95% CI 1.03–12.15, P=0.05) were independent risk factors for IA. Antifungal prophylaxis was administered to 61% (57/93) of CF-LTRs. One-year mortality rate was 16% (15/93). IA was not associated with increased risk of death (OR 2.10, 95% CI 0.62–7.06, P=0.23).

Conclusion. Pretransplant *Aspergillus* colonization is frequent among CF-LTRs and a positive intraoperative *Aspergillus* culture produced a fourfold higher risk of developing IA.

Keywords: *Aspergillus*, Lung transplantation, Cystic fibrosis, Aspergillosis.

(*Transplantation* 2014;97: 351–357)

Aspergillus is a saprophytic fungus, frequently isolated from the respiratory secretions of patients with abnormal lung parenchyma. Up to 57% of patients with cystic fibrosis (CF) carry the organism in their respiratory tract (*1, 2*). Traditionally, the presence of *Aspergillus* in the respiratory tract

M.L. received consultant fees from Merck, Pfizer. C.R. received consultant fees from Astellas, Merck, and Pfizer; Speaker's Bureau for Astellas, Merck, Optimer, Pfizer, and Sunovion; and research support from Astellas, Merck, and Pfizer. S.H. received grant/research support from Pfizer, Merck, CSL Behring, and Astellas.

Preliminary findings of this study were presented at the International Society of Heart and Lung Transplantation—32nd Annual Meeting and Scientific Sessions. Prague, Czech Republic, April 18–21, 2012. Oral presentation #317.

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M.L., A.S., C.C., E.T., and S.H. contributed to the study design, data collection, institutional review board application, statistical analysis, data interpretation, and manuscript composition and revision. C.R. contributed to the study design, data collection, data interpretation, and manuscript revision. L.G.S. contributed to the data collection, data interpretation, and manuscript revision. V.W., S.A., and S.K. contributed to the data collection and manuscript revision.

Supplemental digital content (SDC) is available for this article. Direct URL citations appear in the printed text, and links to the digital files are provided in the HTML text of this article on the journal's Web site (www.transplantjournal.com).

Received 21 May 2013. Revision requested 10 June 2013.
Accepted 20 August 2013.

ISSN: 0041-1337/14/9703-351
DOI: 10.1097/01.TP.0000437434.42851.d4

of CF patients was considered nonpathogenic (*3*). However, recent data suggested that persistent *Aspergillus* colonization in patients with CF is associated with pulmonary exacerbation requiring hospitalization (*4*). A small subset of CF patients with *Aspergillus* colonization will go on to develop allergic bronchopulmonary aspergillosis, a syndrome characterized by respiratory deterioration and hypersensitivity to *Aspergillus* (*5, 6*). Although exceedingly rare, cases of invasive aspergillosis (IA) have been described in CF patients (*1*).

While *Aspergillus* is generally considered innocuous in CF patients, this may not be true after lung transplantation (*7, 8*). Following lung transplantation, impaired immunity precipitated by the use of immunosuppressive agent renders lung transplant recipients (LTRs) vulnerable to fungal infections. Furthermore, decreased cough reflex secondary to postsurgical denervation and local ischemia further facilitate colonization by fungal organisms. As a result, *Aspergillus* can colonize the airways, invade lung tissue, and ultimately cause serious or fatal disease (*9, 10*).

CF lung transplant recipients (CF-LTRs) appear to be at greater risk for IA compared to non-CF LTRs (*10*); however, the reason for this heightened risk is not fully understood. One hypothesis is that pretransplant *Aspergillus* colonization, frequently observed in CF patients, increases the risk for posttransplant IA. A number of authors have examined the significance of pretransplant *Aspergillus* on posttransplant outcomes. Earlier studies reported a higher incidence of bronchial anastomotic infection among LTRs with pretransplant *Aspergillus* colonization (*11, 12*). Others have not found an enhanced risk for posttransplant IA in patients with pretransplant colonization (*13–15*). Unfortunately, the majority of these studies failed to control for important variables such as antifungal prophylaxis use, had limited statistical power, and have produced conflicting results. Hence, the impact of pretransplant *Aspergillus* colonization on posttransplant outcome remains uncertain.

A study was conducted to assess epidemiologic trends and risk factors for IA among CF-LTRs. Specifically, we evaluated the clinical impact of pretransplant *Aspergillus* colonization on the risk of IA after transplantation. Understanding the epidemiology and risk factors for IA among CF-LTRs may facilitate the development of effective preventative strategies.

RESULTS

Population

Between January 1, 2005 and March 31, 2010, 93 CF patients underwent double lung transplantation at the University Health Network (Toronto, Ontario). Demographic details of the cohort are summarized in Table 1.

Pretransplant *Aspergillus*

Aspergillus was isolated from the respiratory tract in 70% (65/93) of patients at least once before transplantation (see **Table S1, SDC,** http://links.lww.com/TP/A892). Among these patients, 16.9% (11/65 with pretransplant *Aspergillus*) had their last positive culture more than 12 months before transplantation, 9.2% (6/65 with pretransplant *Aspergillus*) had their last positive culture between 6 and 12 months before transplantation, 18.5% (12/65 with pretransplant *Aspergillus*) had their last positive culture within 6 months

TABLE 1. Characteristics of 93 cystic fibrosis lung transplant recipients[a]

Characteristics	N=93 (%)
Pretransplant	
Age in years (median, interquartile range)	30 (25–36)
Male gender	55 (59.1)
Pretransplant diabetes	39 (41.9)
Cystic fibrosis–associated liver disease	17 (18.3)
Body mass index at transplantation (median, interquartile range)	19 (18.0–21.5)
Sinusitis/sinus polyposis	42 (45.2)
Pretransplant infection (at any time)	
• *Pseudomonas* spp.	83 (89.3)
• *Burkholderia* spp.	22 (23.7)
• *Stenotrophomonas* spp.	26 (28.0)
• *Staphylococcus* spp.	49 (52.7)
Pretransplant *Aspergillus* culture at any time before transplantation	65 (69.9)
Last pretransplant *Aspergillus* culture more than 12 mo before transplantation	11 (11.8)
Last pretransplant *Aspergillus* culture within 6–12 mo before transplantation	6 (6.5)
Last pretransplant *Aspergillus* culture within 0–6 mo before transplantation	12 (12.9)
Intraoperative *Aspergillus* BAL culture[a]	36 (38.7)
Perioperative	
Admission to ICU immediately before transplantation	6 (6.5)
Transplantation surgery with cardiopulmonary bypass	28 (30.1)
Total ischemic time in hours (median, interquartile range)	12.6 (10.6–15.1)
Primary graft dysfunction at 72 hr after surgery	30 (32.3)
Length of stay in ICU in days (median, interquartile range)	2 (1–6)
Length of stay in hospital in days (median, interquartile range)	18 (15–28)
Airways complication (stenosis/dehiscence/lesion/ necrosis)	12 (12.9)
Posttransplant	
Antifungal prophylaxis after transplantation	57 (61.3)
Voriconazole or itraconazole	36 (38.7)
Inhaled amphotericin B	21 (22.6)
Posttransplant *Aspergillus* colonization	12 (12.1)
CMV infection within 12 mo after transplantation	14 (15.1)
Treatment for rejection within 90 d after transplantation	48 (51.6)
Death within 12 mo of transplantation	15 (16.1)

Data is presented as number (%) unless otherwise specified.

[a] Among the 36 patients with positive intraoperative *Aspergillus* culture, one also had a previous positive culture more than 12 months before transplantation, two also had previous positive culture within 6–12 mo before transplantation, and 30 also had a previous positive culture within 0–6 mo before transplantation. Three patients did not have any previous positive *Aspergillus* culture.

BAL, bronchoalveolar lavage; ICU, intensive care unit; CMV, cytomegalovirus; ACR, acute cellular rejection.

before transplantation, and 55.4% (36/65 with pretransplant *Aspergillus*) had a positive intraoperative *Aspergillus* from the bronchoalvelolar lavage (BAL) culture of the native lung at the time of transplantation (see **Figure S1, SDC,** http://links.lww.com/TP/A892). Among patients with positive intraoperative *Aspergillus* culture, one also had a previously positive culture more than 12 months before transplantation, two patients also had a positive culture within 6 to 12 months before transplantation, and 30 also had a positive culture within 6 months before transplantation. Three patients without history of *Aspergillus* colonization before transplantation had a positive intraoperative culture.

Posttransplant *Aspergillus*

Thirty-two patients cultured *Aspergillus* or had a positive galactomannan (GM) from their BAL recorded after transplantation. However, only 22% (20/93) of patients developed IA. There were nine cases of pulmonary IA, two bronchitis IA, five bronchial anastomotic IA, and four mixed infections (one bronchopulmonary IA, two anastomopulmonary IA, and one endocarditis with cerebral abscess IA). Time-to-IA after transplantation is shown (see **Figure S2, SDC,** http://links.lww.com/TP/A892). Median time to first episode of IA among the entire cohort was 42 days (25%–75% IQR: 6, 102); the median time to IA among patients who received antifungal prophylaxis was 63 days (25%–75% IQR: 6, 103) while the median time to IA among patients who did not receive prophylaxis was 9 days (25%–75% IQR: 2, 186) (P=0.58). Eighty percent (16/20) of patients with IA were colonized with *Aspergillus* before transplantation. Sixteen patients developed *Aspergillus* colonization after transplantation; 12 patients remained well without progression to disease while four patients progressed to IA (three received antifungal therapy, one did not). Posttransplant *Aspergillus* colonization was not associated with increased risk for IA (OR 2.18, 95% CI 0.70–6.81, P=0.71).

Antifungal Prophylaxis

Sixty-one percent (57/93) of patients received antifungal prophylaxis immediately after transplantation (Fig. 1). Antifungal prophylaxis was administered to 75% (49/65) of patients with pretransplant *Aspergillus* colonization and 28.6% (8/28) of those without. Median duration of prophylaxis was 23 days (25%–75% IQR: 11–81). Twenty-two percent (13/57) of patients receiving prophylaxis developed IA compared to 19% (7/36) of those who did not receive prophylaxis (OR 1.22, 95% CI 0.44–3.43, P=0.70). Three patients developed breakthrough IA on prophylaxis. Two patients developed *Aspergillus* colonization during prophylaxis.

Twenty-one patients received inhaled amphotericin B for a median duration of 12 days (25%–75% IQR: 8–17). Nine percent (3/21) of those developed IA (see **Table S2, SDC,** http://links.lww.com/TP/A892). Thirty-six patients received systemic azole (voriconazole or itraconazole) for a median duration of 66 days (25%–75% IQR: 22–92). Twenty-seven percent (10/36) of these developed IA. Among 36 patients who did not receive prophylaxis, 44% (16/36) of patients had pretransplant *Aspergillus* colonization. Of these 16 patients, three received antifungal treatment for invasive fungal infection (anastomotic IA within 24 hr after transplant, necrotizing granuloma on the explanted lung, and early candidemia treated with caspofungin); nine patients did not receive prophylaxis because the presence of *Aspergillus* in the pretransplant culture was remote and not known to the treating physician at the time of transplantation. Only four patients with known history of pretransplant *Aspergillus* did not receive prophylaxis. It is unclear whether this was a voluntary or involuntary omission from the treating physician. Four of those (4/16) patients developed IA. Among the remaining 20 patients without pretransplant *Aspergillus* colonization and without antifungal prophylaxis, three developed IA.

Risk Factors for IA

In univariate analysis, pretransplant *Aspergillus* colonization within 12 months and 6 months before transplantation, intraoperative *Aspergillus* culture, pretransplant *Stenotrophomonas* sp. colonization, higher body mass index, prolonged ischemic time during surgery, primary graft dysfunction, prolonged length of stay in ICU, and to receive treatment for acute cellular rejection within the first 3 months after transplantation met the statistical threshold (P<0.2)

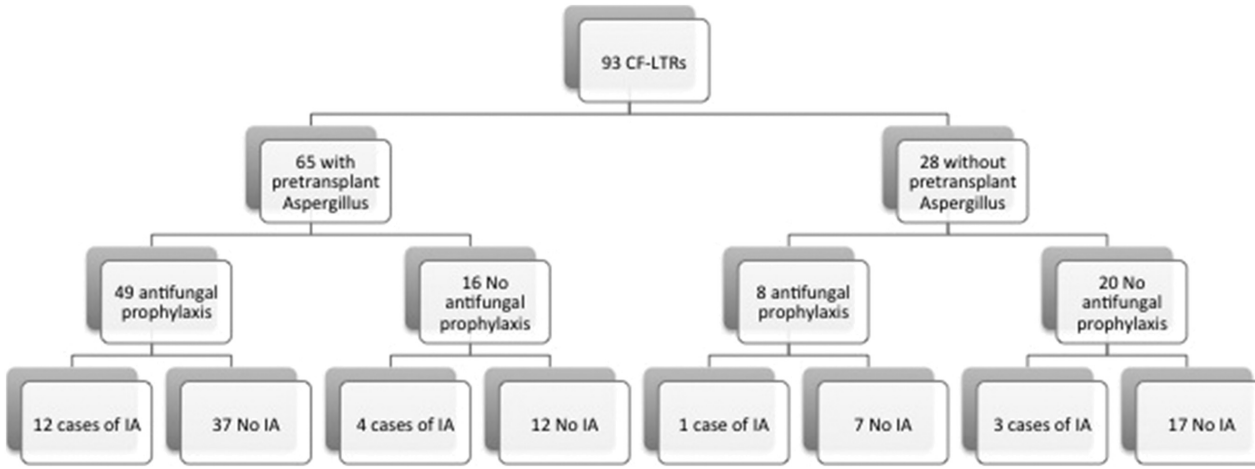

FIGURE 1. Distribution of cystic fibrosis lung transplant recipients with pretransplant *Aspergillus* colonization, antifungal prophylaxis, and posttransplant invasive aspergillosis.

for multivariable analysis (Table 2). Because the effect of pretransplant *Aspergillus* may be modified by antifungal therapy, the administration of antifungal prophylaxis was included in the multivariable logistic model despite the lack of statistical significance in univariate analysis. The final multivariable model demonstrated that intraoperative *Aspergillus* culture (OR 4.36, 95% CI 1.35–14.05, *P*=0.01) and treatment for acute cellular rejection within 90 days after transplantation (OR 3.53, 95% CI 1.03–12.15, *P*=0.05) were independently associated with increased risk of IA (Table 3).

Mortality

The 1-year all-cause mortality rate was 16% (15/93) among CF-LTRs. As shown in Figure 2, the mortality rate among patients with IA and those without IA was 25% (5/20) and 13.7% (10/73), respectively (OR 2.10, 95% CI 0.62–7.06, *P*=0.23). None of the deaths were attributable to IA. Causes of death included sepsis secondary to *Burkholderia cepacia complex* (*Bcc*) (nine), bronchiolitis obliterans syndrome (one), pneumonia (one), adult respiratory distress syndrome (one), primary graft dysfunction (one), cerebral infarct (one), and unknown (one).

DISCUSSION

This study examined the impact of pretransplant *Aspergillus* colonization on the risk of IA after transplantation and demonstrates that while pretransplant *Aspergillus* colonization at any time before transplantation was not associated with increased risk for IA, *Aspergillus* airway colonization at the time of transplantation was associated with a fourfold increased risk of IA.

TABLE 2. Univariate analysis of risk factors for invasive aspergillosis among 93 cystic fibrosis lung transplant recipients[a]

Characteristics	IA=20	No IA=73	OR (95% CI)	*P*
Pretransplant				
Age (median, interquartile range) in years	27 (24–35)	30 (25–36)	—	0.51
Male gender	13 (65.0)	42 (57.5)	1.37 (0.49–3.84)	0.55
Pretransplant diabetes	8 (40.0)	31 (42.5)	0.90 (0.33–2.47)	0.84
Cystic fibrosis–associated liver disease	4 (20.0)	13 (17.8)	1.15 (0.33–4.02)	0.82
Body mass index, kg/m^2 (median, interquartile range)	20.7 (18.0–23.0)	19.7 (17.9–21.5)	—	0.16
Sinusitis/sinus polyposis	10 (50.0)	32 (43.8)	1.28 (0.48–3.45)	0.62
Pretransplant infection				
• *Pseudomonas* spp.	19 (95.0)	64 (87.7)	2.67 (0.32–22.45)	0.35
• *Burkholderia* spp.	3 (15.0)	19 (26.0)	0.50 (0.13–1.90)	0.30
• *Stenotrophomonas* spp.	9 (45.0)	17 (23.3)	2.70 (0.96–7.59)	0.06
• *Staphylococcus* spp.	13 (65.0)	36 (49.3)	1.91 (0.68–5.33)	0.21
Pretransplant *Aspergillus* sp. at any time before transplantation	16 (80.0)	49 (46)	1.96 (0.59–6.50)	0.27
Pretransplant *Aspergillus* sp. within 12 mo before transplantation[a]	15 (75.0)	39 (53.4)	2.62 (0.86–7.95)	0.19
Pretransplant *Aspergillus* sp. within 6 mo before transplantation[b]	13 (65.0)	32 (43.8)	2.38 (0.85–6.66)	0.10
Intraoperative *Aspergillus* BAL culture	12 (60)	24 (32.9)	3.06 (1.11–8.49)	0.02
Perioperative				
Admission to ICU immediately before transplantation	2 (10.0)	4 (5.5)	1.92 (0.32–11.31)	0.47
Transplantation surgery with cardiopulmonary bypass	8 (40.0)	20 (27.4)	1.77 (0.63–4.96)	0.28
Total ischemic time in hours (median, interquartile range)	12.6 (11.1–18.8)	12.6 (10.5–14.7)	—	0.45
Primary graft dysfunction at 72 hr after surgery	9 (45.0)	21 (28.8)	2.03 (0.73–5.60)	0.17
Length of stay in ICU in days (median, interquartile range)	5 (1–18)	2 (1–4)	—	0.01
Airways complication (stenosis/dehiscence/lesion/necrosis)	3 (12.0)	9 (12.3)	1.25 (0.31–5.15)	0.75
Posttransplant				
Antifungal prophylaxis after transplantation	13 (65.0)	44 (60.3)	1.22 (0.44–3.43)	0.70
Voriconazole or itraconazole	10 (50)	26 (35.6)	1.81 (0.67–4.91)	0.24
Inhaled amphotericin B	3 (15.0)	18 (24.7)	0.54 (0.14–2.05)	0.36
Posttransplant *Aspergillus* colonization	4 (20.0)	12 (16.4)	2.18 (0.70–6.81)	0.71
CMV infection within 12 mo after transplantation	2 (10.0)	12 (16.7)	0.56 (0.11–2.72)	0.46
Treatment for ACR within 90 d after transplantation	14 (70.0)	34 (46.6)	2.68 (0.93–7.73)	0.06
Death within 12 mo of transplantation	5 (25.0)	10 (13.7)	2.10 (0.62–7.06)	0.23

Data is presented as number (%) unless otherwise specified.

[a] Patients with pretransplant *Aspergillus* colonization within 6 mo before transplantation were included within the group of patients with pretransplant *Aspergillus* colonization within 12 mo before transplantation.

[b] Patients with intraoperative *Aspergillus* were included within the group of patients with pretransplant *Aspergillus* colonization within 12 mo before transplantation.

BAL, bronchoalveolar lavage; ICU, intensive care unit; CMV, cytomegalovirus; ACR: acute cellular rejection.

TABLE 3. Final multivariable analysis for risk factors for invasive aspergillosis among cystic fibrosis lung transplant recipients

Risk factors	OR (95% CI)	*P*
Positive intraoperative *Aspergillus* culture	4.36 (1.35–14.05)	0.01
Treatment of ACR within 90 d after transplantation	3.53 (1.03–12.15)	0.05
Body mass index (per unit increase)	1.17 (0.95–1.44)	0.13
Length of stay in ICU (per day increase)	1.05 (1.00–1.11)	0.06

ACR, acute cellular rejection; ICU, intensive care unit; CF-LTR cystic fibrosis-lung transplant recipients; IA, invasive aspergillosis.

Aspergillus colonization before transplantation was previously recognized as a risk factor for posttransplant anastomotic invasive aspergillosis (*11, 12, 16*). Similarly, this study demonstrates an increased risk of anastomotic IA among patients with *Aspergillus* colonization at the time of transplantation, three of which occurred within 72 hr after transplantation. In addition, the presence of *Aspergillus* at the time of transplantation increased the risk for pulmonary disease, a finding that was not previously reported.

The results demonstrate that the timing of pretransplant *Aspergillus* is important to consider when assessing the risk of IA; transient remote pretransplant *Aspergillus* colonization may not have any impact after transplantation. In contrast, *Aspergillus* in the airways at the time of transplantation may recur after transplantation. It is unclear whether pretransplant *Aspergillus* colonization is a surrogate marker of the baseline risk exposure related to the patient's living condition. Nevertheless, this finding has important implication for the perioperative management of lung transplant recipients. First, routine screening for *Aspergillus* airway colonization at the time of transplantation may be useful to identify patients at greater risk of IA. Subsequently, such patients with positive intraoperative *Aspergillus* culture may benefit from close monitoring with bronchoscopic and radiologic evaluations for early and rapid detection of disease.

This study was not designed to assess antifungal prophylaxis efficacy; however, some observations are worth discussing. Antifungal prophylaxis did not reduce the rate of IA. However, interpretation of this finding is difficult because of the comparison between two nonequivalent groups (85% of patients receiving prophylaxis had pretransplant *Aspergillus* colonization vs. 44% of patients not receiving prophylaxis had pretransplant *Aspergillus* colonization), the use of different antifungal agents over the study period, and the small sample size. Antifungal prophylaxis appeared to delay the median time of onset of IA after transplantation (42 days) as the majority of IA occurred after cessation of prophylaxis. It is possible the duration of antifungal prophylaxis (23 days) given in the study was insufficient to prevent IA. To date, optimal antifungal drug for prophylaxis and duration remains uncertain. Currently available studies are retrospective and yield conflicting results (*17–22*). Many questions remain unanswered regarding the prophylactic antifungal regimen including who may benefit the most from this strategy.

This study is limited by its retrospective design and relatively small sample size. However, this is the largest cohort study among CF lung transplant recipients with *Aspergillus* infection. The study accounted for antifungal prophylaxis as well as for pretransplant *Aspergillus* colonization, two important risk factors for IA often incompletely reported. Variation in antifungal prophylaxis practices may or may not have had an impact on the association between pretransplant *Aspergillus* and *Aspergillus* at the time of transplantation, and the risk for subsequent disease. However, data regarding the effectiveness and tolerability of antifungal prophylaxis after lung transplantation is limited (*24*). The analysis was conducted based on the assumption that any kind of prophylaxis was protective against invasive disease (based on limited evidence) to provide the most conservative estimate of the association between pretransplant *Aspergillus* colonization and posttransplant disease. Finally, another detractor was that the incidence of pretransplant *Aspergillus* colonization was reported among CF patients based on sputum culture positivity. The frequency of sputum culture testing varied between patients, and multiple testing among selected patients may have biased the results (compared to fewer testing among patients from out of town who have follow-up at other CF clinics).

The findings suggest that routine pretransplant fungal sputum culture, and most importantly, intraoperative BAL culture from the native lung, is useful for identifying patients at higher risk of IA. Identifying at-risk patients is important to develop efficient targeted preventative strategies to decrease the morbidity and mortality associated with IA.

MATERIALS AND METHODS

A retrospective cohort study was conducted of all adult CF patients who were followed by the Adult CF program at St. Michael's Hospital (Toronto, Ontario) and subsequently underwent lung transplantation at the University Health Network (Toronto, Ontario) between January 1, 2005 and January 31, 2010. Follow-up data were collected for 1 year after lung transplantation. Demographic, clinical characteristics, surgical data, immunosuppressive agents, infection, rejection, bronchoscopic findings, and antifungal use were collected by database review. The study was approved

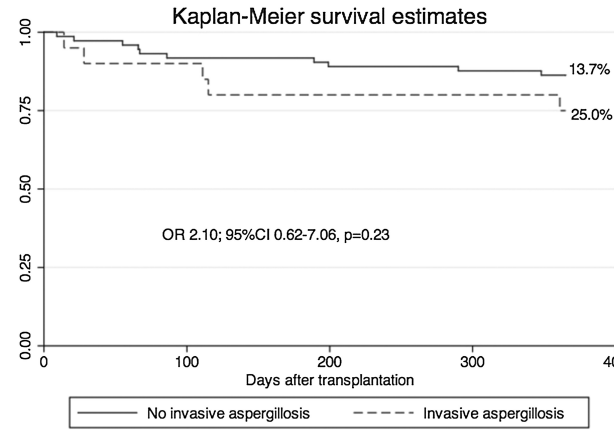

FIGURE 2. One-year survival after lung transplantation among cystic fibrosis patients with and without posttransplant invasive aspergillosis.

by the Research Ethics Board of each institutions (UHN-REB-11-0220-AE, SMH-REB-04-077).

Immunosuppressive Regimen

The immunosuppressive regimen consisted of high-dose methylprednisolone intraoperatively, followed by maintenance therapy with a calcineurin inhibitor, prednisone, and an antiproliferative agent (azathioprine or mycophenolate mofetil). Induction therapy with T-cell-depleting agent or IL-2 antagonist was not used.

Antimicrobial Prophylaxis

Following transplantation, patients routinely received antibiotics for 14 days as prophylactic therapy. If the patient was receiving antibiotics for an infectious exacerbation immediately before transplantation, the same antibiotics were continued for 14 days. If the patient was not receiving antibiotics before transplantation, antibiotics were chosen based on sputum cultures before transplantation. If sputum cultures before transplantation were not available, a combination of intravenous (IV) ceftazidime 2 g three times daily, meropenem 2 g IV three times daily, and inhaled tobramycin 160 mg twice daily were prescribed. If patients had sputum culture positive for *Bcc* before transplantation, the antibiotic regimen was prolonged for at least 21 days until there was no clinical evidence of infection. Lifelong trimethoprim-sulfamethoxazole was administered to all patients for prevention of *Pneumocystis jirovecii* pneumonia (dapsone or inhaled pentamidine was used for sulfa-allergic patients). Ganciclovir 5 mg/kg IV daily followed by oral valganciclovir 900 mg daily was administered to patients at risk of cytomegalovirus (CMV) infection and was continued for 3 months for CMV seropositive patients and 6 months for patients with CMV mismatch (seropositive donor/seronegative recipient). Oral acyclovir was administered for 3 months to patients who were both donor CMV seronegative and recipient CMV seronegative.

Antifungal Prophylaxis

Prior to 2008, patients with known pretransplant *Aspergillus* colonization received targeted antifungal prophylaxis with inhaled amphotericin B deoxycholate 20 mg twice daily within 72 hr after transplantation until discharge from hospital. From 2008 onwards, the prophylaxis regimen was modified to intravenous voriconazole 6 mg/kg IV twice daily times two doses followed by oral voriconazole 200 mg twice daily for 3 months. Additionally, antifungal prophylaxis could be given at the discretion of the treating physician if a patient was considered at high risk for a fungal infection (i.e., severe respiratory insufficiency, treatment for acute cellular rejection). Other antifungal agents could be used if the patient developed intolerance to the initial agent. Prophylaxis could be stopped earlier if the risks were felt to exceed the benefits.

Posttransplant Protocol

Following discharge from hospital, patients underwent weekly assessment blood-work and pulmonary function tests for the first 3 months, then monthly thereafter. A surveillance chest CT scan and bronchoscopy were performed at 2, 6, 12, 24, 36, and 52 weeks after transplantation. BAL fluid was routinely cultured for fungus and after 2008 was also routinely tested for GM. Additional CT scans of the chest and bronchoscopies were performed for suspicion of rejection or infection.

Definitions

Pretransplant *Aspergillus*

All available sputum cultures were reviewed. Patients with a positive *Aspergillus* culture from sputum before transplantation or from the native lung BAL at the time of transplantation were considered to have pretransplant *Aspergillus* colonization.

Posttransplant *Aspergillus*

The primary outcome was occurrence of IA within 1 year after lung transplantation. Cases of posttransplant IA were defined according to the International Society of Heart and Lung Transplantation criteria for invasive fungal infection (*24*). Patients with positive sputum or BAL culture for *Aspergillus* sp. not meeting the criteria for probable or proven IA were considered to have colonization.

Statistical Analysis

Descriptive statistics were used to characterize the study population. Comparison between patients with and those without IA was conducted by univariate analysis of the variables included in Table 1 to determine potential risk factors associated with IA. Differences between the groups were determined using chi-square test, Student *t* test, and Wilcoxon rank-sum test for categorical, continuous parametric, and continuous nonparametric variables, respectively. An exploratory multivariable logistic regression was performed with forward and backward selection analysis to determine independent risk factors for IA. Variables with *P* value less than 0.2 and those with *P* values less than 0.1 (predetermined statistical likelihood thresholds) were included in the forward and backward selection model, respectively. Antifungal prophylaxis was included in the model irrespective of the *P* value because of its inherent biological impact on the research question. A *P* value of less than 0.05 was considered statistically significant in the final multivariable logistic model. Kaplan-Meier analysis was performed to calculate cumulative 1-year survival rate among patients with and without IA. All analyses were performed using STATA version 12.0 software.

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