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MAJOR ARTICLE

Risk Factors for Invasive Fungal Infection in Lung Transplant Recipients on Universal Antifungal Prophylaxis

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Background. Many centers use universal antifungal prophylaxis after lung transplant, but risk factors for invasive fungal infection (IFI) in this setting are poorly described.

Methods. This retrospective, single-center cohort study including 603 lung transplant recipients assessed risk factors for early (within 90 days of transplant) invasive candidiasis (IC) and invasive mold infection (IMI) and late (90–365 days after transplant) IMI using Cox proportional hazard regression.

Results. In this cohort, 159 (26.4%) patients had 182 IFIs. Growth of yeast on donor culture (hazard ratio [HR], 3.30; 95% CI, 1.89–5.75) and prolonged length of stay (HR, 1.02; 95% CI, 1.01–1.03) were associated with early IC risk, whereas transplantation in 2016 or 2017 (HR, 0.21; 95% CI, 0.06–0.70; HR, 0.25; 95% CI, 0.08–0.80, respectively) and female recipient sex (HR, 0.53; 95% CI, 0.30–0.93) were associated with reduced risk. Antimold therapy (HR, 0.21; 95% CI, 0.06–0.78) was associated with lower early IMI risk, and female donor sex (HR, 0.40; 95% CI, 0.22–0.72) was associated with lower late IMI risk. Recent rejection was a risk factor for late IMI (HR, 1.73; 95% CI, 1.02–2.95), and renal replacement therapy predisposed to early IC, early IMI, and late IMI (HR, 5.67; 95% CI, 3.01–10.67; HR, 7.54; 95% CI, 1.93–29.45; HR, 5.33; 95% CI, 1.46–19.49, respectively).

Conclusions. In lung transplant recipients receiving universal antifungal prophylaxis, risk factors for early IC, early IMI, and late IMI differ.

Keywords. amphotericin B; antifungals; invasive fungal infection; lung transplant; prophylaxis.

Infection remains the most common cause of death in the first year post–lung transplant [1]. Among 11 US transplant centers, the cumulative incidence of IFI between 2001 and 2006 was 8.6% [2–5]. The majority of these were either invasive mold infections (IMIs) or invasive candidiasis (IC), both of which carry between a 22% and 27% 3-month mortality [2, 6].

Based on the high mortality and morbidity of IFIs in the lung transplant population, the International Society of Heart and Lung Transplantation (ISHLT), the American Society of Transplantation (AST), and the Infectious Diseases Society of America (IDSA) recommend preemptive treatment or universal antifungal prophylaxis following lung transplantation [7–10]. While several studies have examined risk factors for IFI among lung transplant recipients [10–19], risks for yeast and mold infections differ, and studies examining risks for IFI following lung transplantation have important limitations. First, antifungal prophylaxis strategies vary across these studies, and few are performed at centers that employ universal prophylaxis. Second, the studies vary in outcomes measured, with most focusing on invasive aspergillosis (IA). Finally, standardized criteria to define IFI are not used uniformly across these studies, complicating interpretation.

Our center adopted universal antifungal prophylaxis with inhaled amphotericin B lipid complex (iABLC) in lung transplant recipients in January 2000, with fluconazole + iABLC starting in December 2015. We recently published data demonstrating the safety and tolerability of this approach [20]. In this study, we aim to identify predictors of early (within 90 days of transplantation) and late (between 90 days and 1 year after transplantation) IC and IMI among 603 lung transplant recipients in whom universal antifungal prophylaxis was applied.

METHODS

We performed an uncontrolled, retrospective cohort study among consecutive lung transplant recipients between January 2012 and August 2017 at Duke University Hospital (DUH), a 924-bed, tertiary care hospital in Durham, North Carolina. This study was investigator-initiated and supported by a grant from Leadiant Biosciences, Inc., which manufactures Ablecet (amphotericin B lipid complex).

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Lung transplant recipients received universal antifungal prophylaxis with iABLC administered daily for 4 days starting immediately after transplant surgery, and weekly thereafter until hospital discharge or for at least 90 days post-transplant if the patient remained admitted. Beginning in December 2015, fluconazole (400 mg orally or intavenously daily) was also administered from the time of transplant until 90 days post-transplant. Recipients with known chronic airway fungal colonization before transplant may have received targeted systemic prophylaxis with a mold-active triazole (posaconazole, isavuconazole, or voriconazole) in lieu of fluconazole at the provider's discretion. Antifungal exposure is summarized in Supplementary Table 1. Although no routine surveillance for fungal colonization is performed at DUH, fungal cultures are often sent on surveillance bronchoscopies performed for rejection.

Throughout the study period, induction immunosuppression included basiliximab, methylprednisolone, and either mycophenolate mofetil or azathioprine. Transbronchial biopsies were performed for routine rejection surveillance at 1, 3, 6, and 12 months after transplant. Acute cellular rejection (ACR) was typically treated with corticosteroids. For acute rejection without adequate response to steroids, clinicians typically administered antithymocyte globulin and, if still refractory, alemtuzumab. Patients treated with alemtuzumab received systemic antifungal prophylaxis with a mold-active azole.

Outcomes

This study had 3 primary outcomes: (1) IC within 90 days of lung transplantation (early IC), (2) IMI within 90 days of lung transplantation (early IMI), and (3) IMI between 90 days and 365 days of lung transplantation (late IMI). We were not able to analyze risk factors for IC occurring >90 days after transplantation because of the low frequency of late IC (n = 15). IMI and IC were defined using criteria for "proven" or "probable" IFI established by the European Organization for Research and Treatment of Cancer/Mycoses Study Group Educational and Research Consortium (EORTC/MSGERC) [21]. Patients were considered to be colonized with a fungal organism if it was isolated on culture but the patient did not meet EORTC/MSGERC criteria for "proven" or "probable" IFI. Microbiological data, histopathology reports, imaging results, and clinical documentation were reviewed, and cases were adjudicated by 1 of 3 clinicians (J.H., R.P., B.A.).

As a secondary outcome, we sought to determine whether IMI was independently associated with recovery of the same organism from the most recent surveillance bronchoscopy specimen preceding the IMI diagnosis.

Statistical Analysis

Cox proportional hazards models with time-dependent covariates were fit to test for associations between each of the 3 primary outcomes and potential risk factors. A complete list of covariates based on established or suspected risk factors for IFI in the absence of antifungal prophylaxis is provided in Supplementary Table 2. Multivariable models were fit starting with covariates that met a $P < .2$ marginal screening threshold in univariate analysis. Stepwise selection in both directions with the Akaike Information Criterion (AIC) as the selection criterion was used to build the final age-, underlying lung disease–, and transplant year–adjusted models. The proportional hazards assumption was tested using Schoenfeld residuals. To test for an association between IMI and respiratory colonization with the same organism recovered from the most recent prior surveillance bronchoscopy specimen, we fit a multivariable Cox proportional hazards model with time-dependent covariates adjusted for recipient age, underlying lung disease, and transplant year. Multiple imputation was performed as a sensitivity analysis for variables with high degrees of missingness.

This research was approved by the Duke University Institutional Review Board.

RESULTS

Patient Cohort

Between January 2012 and August 2017, 621 lung transplants were performed in 603 patients. For 18 patients who received 2 lung transplants during the study period, only the first transplant was included in the analysis.

The demographic data for the cohort are shown in Table 1. The participants were predominantly male (59.5%), White (91.5%), and non-Hispanic (98.5%). The median age (range) was 60 (16–78) years. The largest proportion of patients (44.6%) underwent lung transplantation for pulmonary fibrosis, followed by cystic fibrosis (19.2%) and chronic obstructive pulmonary disease (COPD; 15.8%). Few patients required mechanical ventilation (28, 4.6%), extracorporeal membrane oxygenation (ECMO; 33, 5.5%), or renal replacement therapy (RRT; 4, 0.7%) immediately before transplant.

Invasive Fungal Infection

One hundred eighty-two IFIs occurred in 159 patients (26.4%), yielding an incidence of 32.5 infections per 100 person-years. Infection characteristics are shown in Table 2. One-year mortality was significantly higher among patients with IFI compared with those without IFI (20.1% vs 10.9%; $P = .004$). Of 182 infections, 75 (41%) were due to *Candida*, 7 (4%) were due to non-*Candida* yeast, and 100 (55%) were due to mold. *Candida albicans* was the most common pathogenic yeast (36, 48%), and among molds, *Aspergillus fumigatus* (53, 53%) was the most commonly isolated pathogen. The majority of infections involved the lungs, including the pulmonary parenchyma (51, 28%), tracheobronchial tree (40, 22%), and pleural

Table 1. Characteristics of Lung Transplant Recipients

Characteristics	Total (n = 603), No. (%)
Age at transplant, median (IQR), y	60 (46–66)
Sex (female)	244 (40.5)
Race	…
Caucasian	552 (91.5)
African American	48 (8.0)
Asian	2 (0.3)
American Indian or Alaska Native	1 (0.2)
Ethnicity	…
Non-Hispanic	594 (98.5)
Hispanic	9 (1.5)
Bilateral lung transplant	469 (77.8)
Year of transplant	…
2012	104 (17.2)
2013	132 (21.9)
2014	108 (17.9)
2015	105 (17.4)
2016	95 (15.8)
2017	59 (9.8)
Underlying lung disease	…
Cystic fibrosis	116 (19.2)
Pulmonary arterial hypertension	11 (1.8)
Pulmonary fibrosis	269 (44.6)
Sarcoidosis	20 (3.3)
Alpha-1-antitrypsin deficiency	14 (2.3)
COPD	95 (15.8)
Other[a]	78 (12.9)
ECMO at time of transplant	33 (5.5)
RRT at time of transplant	4 (0.7)
Mechanical ventilation as bridge to transplant[b]	28 (4.6)
Length transplant hospitalization stay, median (IQR), d	19 (13–36)

Abbreviations: COPD, chronic obstructive pulmonary disease; ECMO, extracorporeal membrane oxygenation; IQR, interquartile range.

[a]Includes bronchiectasis (15), Eisenmenger's syndrome, acute interstitial pneumonia (1), nonspecific interstitial pneumonia (4), Kartagener's syndrome (1), bronchopulmonary dysplasia (3), rheumatoid disease (3), occupational lung disease (4), lymphangioleiomyomatosis (3), obliterative bronchiolitis (5), pulmonary veno-occlusive disease (4), thromboembolic pulmonary hypertension (2), granulomatosis with polyangiitis (2), scleroderma (2), common variable immunodeficiency (1), hypogammaglobulinemia (1), alveolar proteinosis (2), acute respiratory distress syndrome (2), idiopathic interstitial pneumonitis (1), eosinophilic granuloma (1), graft-vs-host disease (3), hypersensitivity pneumonitis (10), mixed connective tissue disorder (1), polymyositis (1), silicosis (1).

[b]Patients requiring mechanical ventilatory support immediately before transplant.

space (28, 15%); 21 (12%) involved multiple sites, which also frequently included the lung.

Of 60 early IC events, only 6 (10%) occurred after implementation of fluconazole prophylaxis in December 2015. Of 12 multifocal IC infections, 11 (92%) involved the pleural cavity, and 10 (91%) of these were accompanied by candidemia.

Risk Factors for Early Invasive Candidiasis

Of 75 IC episodes, 60 (80%) occurred within 90 days of transplant. The results of the univariate analysis are shown in Supplementary Table 3. On multivariable analysis, growth of yeast on donor culture (hazard ratio [HR], 3.30; 95% CI, 1.89–5.75), post-transplant RRT (HR, 5.67; 95% CI, 3.01–10.67), and length of stay (LOS; HR, 1.02; 95% CI, 1.01–1.03) were associated with increased hazard of early IC. Transplantation in the years 2016 (HR, 0.21; 95% CI, 0.06–0.70) or 2017 (HR, 0.25; 95% CI, 0.08–0.80) and female recipient sex (HR, 0.53; 95% CI, 0.30–0.93) were associated with reduced hazard of early IC (Table 3).

To ascertain the impact of the December 2015 transition to universal fluconazole prophylaxis on early IC, we modeled anti-*Candida* prophylaxis as a binary variable indicating receipt of any agent with activity against *Candida* species within 7 days of lung transplant surgery, since patients may have received a broader-spectrum agent if their providers perceived them to be at higher risk for mold infection. As shown in Table 3 and discussed above, we found no association between yeast-active prophylaxis and early IC in the transplant year–adjusted model. Results were similar when prophylaxis was modeled as receipt of fluconazole within 7 days of transplant and receipt of yeast-active antifungals within the last 7 days at time *t*. To rule out collinearity between transplant year and receipt of yeast-active prophylaxis, we assessed the correlation between each of the aforementioned prophylaxis exposure variables and transplant year. Although we did find moderate correlation between transplant year and each of these variables, inclusion of transplant year yielded a better model fit than models that included any of these variables to the exclusion of transplant year.

Risk Factors for Early Invasive Mold Infection

Of 100 total IMIs, 34 (34%) occurred within 90 days of transplant. The results of the univariate analysis are shown in Supplementary Table 4. On multivariable analysis, receipt of a mold-active antifungal agent (HR, 0.21; 95% CI, 0.06–0.78) was associated with reduced hazard of early IMI, and post-transplant RRT was associated with increased odds of early IMI (HR, 7.54; 95% CI, 1.93–29.45) (Table 4).

Risk Factors for Late Invasive Mold Infection

Of 100 IMIs, 66 (66.0%) occurred between 91 and 365 days after transplant. The results of the univariate analysis are shown in Supplementary Table 5. On multivariable analysis, receipt of lungs from a female donor was associated with reduced hazard of late IMI (HR, 0.40; 95% CI, 0.22–0.72), whereas a recent episode of biopsy-proven ACR (HR, 1.73; 95% CI, 1.02–2.95) and post-transplant RRT (HR, 5.33; 95% CI, 1.46–19.49) were associated with increased hazard of late IMI (Table 5).

As a sensitivity analysis, we also performed Cox proportional hazard regression to explore risk factors for IMI at any point during the first year post-transplant, regardless of whether the IMI occurred within 90 days or beyond. The univariate analysis is shown in Supplementary Table 6. On multivariable analysis, female donor sex (HR, 0.55; 95% CI, 0.35–0.86) and

Table 2. Characteristics of Early (Within 90 Days) and Late (Between 91 and 365 Days) After Lung Transplant

Factor	Species	Total, No. (%)	Early (Within ≤90 Days of Transplant), No. (%)	Late (91–365 Days After Transplant), No. (%)	*P* Value
No.	…	182	97	85	
Invasive candidiasis	…	75 (41)	60 (62)	15 (18)	<.001
Blood	…	23 (31)	21 (22)	2 (2)	<.001
…	*C. albicans*	8 (35)	7 (33)	1 (50)	.97
	C. dubliniensis	1 (4)	1 (5)	0 (0)	
	C. glabrata	8 (35)	7 (33)	1 (50)	
	C. parapsilosis	2 (9)	2 (10)	0 (0)	
	C. tropicalis	3 (13)	3 (14)	0 (0)	
	C. albicans + C. parapsilosis	1 (4)	1 (5)	0 (0)	
Pleural	…	26 (35)	22 (23)	2 (2)	<.001
…	*C. albicans*	13 (50)	12 (55)	1 (50)	.25
	C. dubliniensis	5 (19)	5 (23)	0 (0)	
	C. glabrata	1 (4)	1 (5)	0 (0)	
	C. parapsilosis	2 (8)	1 (5)	1 (50)	
	C. tropicalis	3 (12)	3 (14)	0 (0)	
Incisional SSI	…	10 (13)	3 (3)	7 (8)	.13
…	*C. albicans*	7 (70)	1 (33)	6 (86)	.17
	C. krusei	1 (10)	1 (33)	0 (0)	
	C. parapsilosis	2 (20)	1 (33)	1 (14)	
Peritoneal	…	2 (3)	2 (2)	0 (0)	.18
…	*C. albicans*	2 (100)	2 (100)	0 (0)	
Tracheobronchial	…	2 (3)	1 (1)	1 (1)	.93
…	*C. albicans*	2 (100)	1 (100)	1 (100)	
Other[a]	…	2 (3)	0 (0)	2 (2)	.13
…	*C. albicans*	1 (50)	0 (0)	1 (50)	
…	Unknown[c]	1 (50)	0 (0)	1 (50)	
Multiple sites[b]	…	12 (16)	11 (11)	1 (1)	.006
…	*C. albicans*	2 (17)	2 (18)	0 (0)	.77
	C. dubliniensis	2 (17)	2 (18)	0 (0)	
	C. glabrata	3 (25)	2 (18)	1 (100)	
	C. lusitaniae	2 (17)	2 (18)	0 (0)	
	C. tropicalis	1 (8)	1 (9)	0 (0)	
	C. glabrata + C. tropicalis	1 (8)	1 (9)	0 (0)	
	C. albicans + C. tropicalis	1 (8)	1 (9)	0 (0)	
Noninvasive *Candida* Yeast infection	…	7 (4)	3 (3)	4 (5)	.57
Pleural	…	1 (14)	1 (1)	0 (0)	.35
…	*Trichosporon*	1 (100)	1 (100)	0 (0)	
Incisional SSI	…	1 (14)	0 (0)	1 (1)	.28
…	*Mallassezia*	1 (100)	0 (0)	1 (100)	
Tracheobronchial	…	1 (14)	1 (1)	0 (0)	.35
…	*Trichosporon*	1 (100)	1 (100)	0 (0)	
Multiple sites[b]	…	1 (14)	0 (0)	1 (1)	.28
…	*Cryptococcus*	1 (100)	0 (0)	1 (100)	
Lung	…	3 (43)	1 (1)	2 (2)	.48
…	*Cryptococcus*	3 (100)	1 (100)	2 (100)	
Invasive Mold Infection	…	100 (55)	34 (35)	66 (78)	<.001
Pleural	…	1 (1)	0 (0)	1 (1)	.28
…	*A. niger*	1 (100)	…	1 (100)	
Incisional SSI	…	5 (5)	2 (2)	3 (4)	.55
…	*A. fumigatus*	1 (20)	0 (0)	1 (33)	.29
	Mucor	1 (20)	1 (50)	0 (0)	
	Unknown[c]	1 (20)	1 (50)	0 (0)	
	Dematiaceous Mold	1 (20)	0 (0)	1 (33)	
	Exophiala dermatididis	1 (20)	0 (0)	1 (33)	

Table 2. Continued

Factor	Species	Total, No. (%)	Early (Within ≤90 Days of Transplant), No. (%)	Late (91–365 Days After Transplant), No. (%)	*P* Value
Tracheobronchial	…	37 (37)	15 (15)	22 (26)	.081
…	*A. fumigatus*	17 (46)	7 (47)	10 (45)	.52
	A. flavus	2 (5)	1 (7)	1 (5)	
	Rhizopus	1 (3)	0 (0)	1 (5)	
	Fusarium	1 (3)	0 (0)	1 (5)	
	Paecilomyces	3 (8)	2 (13)	1 (5)	
	Unknown[c]	1 (3)	0 (0)	1 (5)	
	A. versicolor	2 (5)	0 (0)	2 (9)	
	A. ochraceus	1 (3)	0 (0)	1 (5)	
	A. fumigatus + *A. versicolor*	1 (3)	1 (7)	0 (0)	
	Paecilomyces + *A. versicolor*	1 (3)	1 (7)	0 (0)	
	A. fumigatus + *Paecilomyces*	1 (3)	0 (0)	1 (5)	
	A. versicolor + *Acremonium* species	1 (3)	0 (0)	1 (5)	
	A. fumigatus + *A. ochraceus* + *A. versicolor*	1 (3)	1 (7)	0 (0)	
	A. nidulans + *A. versicolor*	1 (3)	1 (7)	0 (0)	
	A. terreus + *A. ochraceus*	1 (3)	0 (0)	1 (5)	
	A. terreus + *A. niger*	1 (3)	0 (0)	1 (5)	
	Rhinocladiella aquaspera	1 (3)	1 (7)	0 (0)	
Lung	…	48 (48)	14 (14)	34 (40)	<.001
…	*A. fumigatus*	23 (48)	2 (14)	21 (62)	.050
	A. flavus	5 (10)	3 (21)	2 (6)	
	Rhizopus	1 (2)	0 (0)	1 (3)	
	Fusarium	3 (6)	2 (14)	1 (3)	
	Pseudallescheria boydii/ *Scedosporium apiospermum*	1 (2)	1 (7)	0 (0)	
	Paecilomyces	3 (6)	2 (14)	1 (3)	
	Unknown[c]	2 (4)	2 (14)	0 (0)	
	A.fumigatus + *Acrophialophora* species	1 (2)	0 (0)	1 (3)	
	A. versicolor	1 (2)	1 (7)	0 (0)	
	A. fumigatus + *A. niger*	3 (6)	1 (7)	2 (6)	
	A. fumigatus + *Fusarium*	1 (2)	0 (0)	1 (3)	
	A. ochraceus	1 (2)	0 (0)	1 (3)	
	Unknown + *Alternaria*	1 (2)	0 (0)	1 (3)	
	Paecilomyces + *A. flavus* + *A. terreus*	1 (2)	0 (0)	1 (3)	
	Dematiaceous mold	1 (2)	0 (0)	1 (3)	
Other[a]	…	1 (1)	0 (0)	1 (1)	.28
…	*Alternaria*	1 (100)	0 (0)	1 (100)	
Multiple sites[b]	…	8 (8)	3 (3)	5 (6)	.36
…	*A. fumigatus*	3 (38)	1 (33)	2 (40)	.40
	Rhizopus	1 (13)	1 (33)	0 (0)	
	Unknown[c]	1 (13)	1 (33)	0 (0)	
	A. fumigatus + *A. ochraceus*	1 (13)	0 (0)	1 (20)	
	Exserohilum species	1 (13)	0 (0)	1 (20)	
	Arthrographis species	1 (13)	0 (0)	1 (20)	

Abbreviation: SSI, surgical site infection.

[a]Other includes for invasive candidiasis: superinfected biloma (*C. albicans*) and esophagitis (unknown); for invasive mold infection: sinusitis.

[b]Multiple sites includes for invasive candidiasis: blood + pleural space (10), skin/soft tissue + pleural space (*C. albicans*), skin/soft tissue + blood (*C. glabrata*); for noninvasive *Candida* yeast infection: lung + blood; for invasive mold infection: tracheobronchial + lung (*A. fumigatus* × 3, *A. fumigatus* + *A. ochraceus*, *Rhizopus*), skin/soft tissue + bone (*Arthrographis* species), lung + heart (unknown), and skin/soft tissue + sinus (*Exserohilum* species).

[c]Invasive fungal infection diagnosed on pathology specimen with no growth on culture.

receipt of a mold-active azole at the time of IFI (HR, 0.52; 95% CI, 0.29–0.94) were associated with reduced odds of IMI. Post-transplant RRT was associated with increased odds of IMI (HR, 5.69; 95% CI, 2.31–13.98) (Supplementary Table 7).

Prior Colonization and Invasive Mold Infection

We examined the relationship between IMI and isolation of the same organism from the most recent bronchoscopy culture. Only *Aspergillus fumigatus* was recovered frequently enough

Table 3. Association Between Risk Factors and Early (Within 90 Days of Lung Transplant) Invasive Candidiasis Using Multivariable Cox Proportional Hazard Regression

Variable	Hazard Ratio	95% CI	*P* Value
Post-transplant RRT	5.67	(3.01–10.67)	<.0001
Yeast on donor culture	3.30	(1.89–5.75)	<.0001
LOS, d	1.02	(1.01–1.03)	.0004
Recipient sex (female)	0.53	(0.30–0.93)	.0281
Recipient age, y	1.03	(1.00–1.05)	.0541
Underlying lung disease	…	…	
Cystic fibrosis	Reference	-	-
Pulmonary arterial hypertension	1.48	(0.34–6.39)	.6003
Pulmonary fibrosis	0.41	(0.16–1.02)	.0545
Sarcoidosis	0.93	(0.29–3.03)	.9083
Alpha-1-antitrypsin deficiency	1.07	(0.28–4.07)	.9248
COPD	0.30	(0.09–1.06)	.0615
Other[a]	0.49	(0.19–1.30)	.1521
Transplant year	…	…	
2012	Reference	-	-
2013	0.78	(0.35–1.77)	.5570
2014	1.33	(0.57–3.12)	.5116
2015	1.34	(0.65–2.75)	.4254
2016	0.21	(0.06–0.70)	.0109
2017	0.25	(0.08–0.80)	.0201

Abbreviations: COPD, chronic obstructive pulmonary disease; LOS, length of stay; RRT, renal replacement therapy.

[a]See Table 1.

Table 4. Association Between Risk Factors and Early (Within 90 Days of Lung Transplant) Invasive Mold Infection Using Multivariable Cox Proportional Hazard Regression

Variable	Hazard Ratio	95% CI	*P* Value
Receipt of mold-active azole	0.21	(0.06–0.78)	.0200
Post-transplant RRT	7.54	(1.93–29.45)	.0037
Recipient age, y	1.01	(0.98–1.03)	.6077
Underlying lung disease	…	…	
Cystic fibrosis	Reference	-	-
Pulmonary fibrosis	1.02	(0.26–4.04)	.9798
Sarcoidosis	2.11	(0.35–12.56)	.4114
Alpha-1-antitrypsin deficiency	1.26	(0.11–14.14)	.8487
Other[a]	3.00	(0.85–10.53)	.0867
Transplant year	…	…	
2012	Reference	-	-
2013	0.61	(0.20–1.80)	.3687
2014	0.61	(0.21–1.83)	.3792
2015	0.90	(0.33–2.50)	.8432
2016	0.31	(0.07–1.32)	.1137
2017	0.17	(0.02–1.29)	.0868

Abbreviations: COPD, chronic obstructive pulmonary disease; RRT, renal replacement therapy.

[a]See Table 1.

Table 5. Association Between Risk Factors and Late (Between 90 and 365 Days After Lung Transplant) Invasive Mold Infection Using Multivariable Cox Proportional Hazard Regression

Variable	Hazard Ratio	95% CI	*P* Value
Donor sex (female)	0.40	(0.22–0.72)	.0026
Recent rejection	1.73	(1.02–2.95)	.0428
Post-transplant RRT	5.33	(1.46–19.49)	.0114
Non-CT surgical procedure	0.30	(0.07–1.34)	.1148
Recipient age, y	1.01	(0.99–1.04)	.3554
Underlying lung disease	…	…	
Cystic fibrosis	Reference	-	-
Pulmonary arterial hypertension	1.11	(0.13–9.60)	.8506
Pulmonary fibrosis	1.16	(0.39–3.44)	.7860
Sarcoidosis	1.62	(0.35–7.40)	.5360
Alpha-1-antitrypsin deficiency	1.44	(0.29–7.15)	.6530
COPD	.96	(0.30–3.11)	.9488
Other[a]	1.60	(0.59–4.32)	.3579
Transplant year	…	…	
2012	Reference	-	-
2013	2.10	(0.93–4.74)	.0759
2014	1.67	(0.64–4.38)	.2939
2015	0.81	(0.27–2.48)	.7178
2016	1.88	(0.76–4.67)	.1723
2017	2.05	(0.75–5.63)	.1618

Abbreviations: COPD, chronic obstructive pulmonary disease; RRT, renal replacement therapy.

[a]See Table 1.

Table 6. Association Between Prior *Aspergillus fumigatus* Colonization Invasive Mold Infection Using Multivariable Cox Proportional Hazard Regression Adjusted for Age, Underlying Lung Disease, and Transplant Year

Variable	Hazard Ratio	95% CI	*P* Value
Prior colonization with A. fumigatus	1.15	(0.42–3.17)	.7874
Recipient age, y	1.02	(1.00–1.05)	.0784
Underlying lung disease	…	…	
Cystic fibrosis	Reference	-	-
Pulmonary arterial hypertension	1.00	(0.11–9.06)	.99981
Pulmonary fibrosis	0.68	(0.21–2.21)	.5247
Sarcoidosis	2.01	(0.56–7.25)	.2850
Alpha-1-antitrypsin deficiency	0.64	(0.07–6.19)	.6978
COPD	0.47	(0.12–1.78)	.2670
Other[a]	1.26	(0.43–3.66)	.6746
Transplant year	…	…	
2012	Reference	-	-
2013	1.58	(0.73–3.44)	.2478
2014	1.03	(0.40–2.63)	.9527
2015	0.20	(0.04–0.95)	.0425
2016	1.04	(0.41–2.62)	.9315
2017	0.82	(0.25–2.66)	.7396

Abbreviation: COPD, chronic obstructive pulmonary disease.

[a]See Table 1.

to meaningfully analyze the relationship between prior colonization and subsequent invasive infection. In an age-, underlying lung disease–, and transplant year–adjusted multivariable model, colonization with *A. fumigatus* from the prior surveillance bronchoscopy was not associated with a higher hazard of IMI (HR, 1.15; 95% CI, 0.42–3.17) (Table 6). As colonization with a mold, regardless of whether it was isolated

from the most recent lower respiratory specimen, may be significant with respect to future IFI, we also tested for an association between mold colonization within 90 days and IMI, but the results were similar.

DISCUSSION

In this large cohort of lung transplant recipients, we assessed potential risk factors for IFI and found important differences in risk for IC vs IMI and for early vs late IMI. As in past studies, we found that IFI after lung transplant was associated with elevated 1-year mortality.

Fluconazole was added to our universal prophylactic antifungal regimen after an epidemiological study at our center found elevated rates of IC in the first 180 days after lung transplantation [3, 22–24]. In the present study, only 6 of 60 (10%) early IC episodes occurred after implementation of fluconazole prophylaxis, but it was interesting to note that in the transplant year–adjusted multivariate model, receipt of fluconazole or another antifungal agent with activity against *Candida* species within the first week post-transplant was not associated with lower odds of early IC. We performed several sensitivity analyses to determine whether this finding was the product of the prophylaxis variable definition or due to collinearity between the date variables and the timed introduction of fluconazole prophylaxis, but we found a unique association between transplant year and lower odds of early IC. These findings suggest the introduction of an unmeasured protective factor in late 2015. To our knowledge, there were no other strategies implemented at the same time as fluconazole prophylaxis aimed at mitigating rates of IC.

We identified yeast isolated on a donor culture at the time of transplant as a risk factor for early IC. It is possible that the recipient's pleural cavity is contaminated with donor-derived yeast during implantation of the allograft, resulting in post-transplant infection. Supporting this hypothesis, among 60 early IC episodes in our cohort, 36 (60.0%) involved the pleura or clamshell incision. Unfortunately, our microbiology lab does not report species of yeast isolated on respiratory culture, limiting our ability to speculate further about a donor origin of these infections.

Our finding that transplant admission LOS was associated with higher odds of early IC lends support to past studies identifying LOS, and ICU LOS in particular, as a predictor of IC [25, 26].

Interestingly, other studies have reported an association between male sex and increased risk of IC [27, 28]. While we do not expect that sex-specific behaviors or exposures should play a role in early post-transplant IC, sex-related variability in immunologic response to fungi may. For example, a murine model found that higher levels of testosterone decreased resistance to *Candida* infection. Such sex-related variability warrants further investigation [29, 30].

Most studies examining risks for IMI in lung transplant recipients have limited their focus to IA, and there is little consensus with respect to risks across these studies, likely owing to variability in infection definitions as well as prophylaxis practices [11–19]. A relationship between augmentation of immunosuppression, often in response to rejection, is well described in the literature [15, 19, 31, 32]. We found an association between late IMI and ACR on the most recent biopsy specimen regardless of whether ACR treatment was given. We did not, however, capture augmentation of the recipients' daily immunosuppressive regimen that may have occurred in response to these events.

While we did find an association between receipt of a mold-active antifungal agent and lower risk of early IMI, we found no association between late IMI and antimold therapy in our study. However, administration of antifungal prophylaxis beyond 90 days post-transplant is largely provider-driven at our center, and this may have impacted our findings. While we do recommend re-initiation of mold-active azole prophylaxis after administration of alemtuzumab for allograft rejection, decisions regarding prophylaxis in the setting of other rejection therapies and in response to fungal colonization are made on a case-by-case basis following transplant hospitalization. Further, most patients have been discharged by this point, and antifungal exposure is more difficult to accurately ascertain in the outpatient setting, which may also have affected our ability to show an impact on receipt of mold-active prophylaxis and IMI risk.

We found that female donor sex was the only variable independently associated with reduced risk of late IMI. In light of epidemiological data showing a higher incidence of IFI among men [27, 30], it is possible that lungs recovered from male donors place recipients at sustained immunologic or, as discussed above, hormonal risk for IMI following transplant.

Interestingly, RRT post-transplant was a risk factor for early IC, early IMI, and late IMI. RRT is a well-known risk factor for IC, and past studies have described a relationship between RRT and risk of IMI in heart and liver transplantation [33–35]. This association may in part reflect the deleterious effect of end-stage renal disease on the innate and adaptive arms of the immune system, but RRT may also act as a surrogate marker for a more complicated post-transplant course [36].

As a secondary aim, we investigated the relationship between IMI and prior colonization with *Aspergillus fumigatus*. While colonization was associated with higher risk of later infection, the association was not statistically significant. Three of 5 studies included in the previously mentioned meta-analysis identified *Aspergillus* isolated from a pretransplant, intraoperative, or post-transplant culture as a risk factor for subsequent IA. Two of these studies were performed at centers that provide targeted antifungal prophylaxis, and the third was a multicenter study including hospitals with varied prophylactic strategies [11, 16, 18, 19]. It is

likely that universal mold-active antifungal prophylaxis and provider-level decisions regarding treatment of positive surveillance cultures affect this association after transplant.

This study has several limitations. It is a single-center study, limiting its generalizability, and as a retrospective study, it is subject to unmeasured confounding. Difficulty in accurately capturing outpatient administration of antimicrobial or immunosuppressive therapy may have affected our ability to detect associations between these variables and the outcomes of interest. Our center does not routinely obtain surveillance cultures, which limits our ability to assess for associations between fungal colonization and infection. Because of low numbers of events, we were unable to comment on risk factors for late IC or an association between colonization with molds other than *A. fumigatus* and subsequent infection. Finally, prophylaxis and surveillance strategies vary from center to center, which may further limit the generalizability of these results.

The large size of our cohort and number of IFIs provided the opportunity to investigate multiple risks for developing IC and IMI separately. We were able to provide further support for previously described associations between RRT and IFI risk and between rejection and late IMI risk. We found new associations between recipient and donor sex and risk of early IC and late IMI, respectively. The observation that yeast on donor culture predisposes to early IC has not previously been described and warrants further investigation.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Acknowledgments

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

Patient consent. This study does not include factors necessitating patient consent.

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