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CLINICAL RISK FACTORS FOR INVASIVE ASPERGILLOSIS IN LUNG TRANSPLANT RECIPIENTS: RESULTS OF AN INTERNATIONAL COHORT STUDY

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ABSTRACT

(1)Background: Invasive aspergillosis (IA) is a frequent complication in lung transplant recipients (LTRs). Clinical risk factors for IA have not been fully characterized, especially in the era of extensive antifungal prophylaxis.

(2)Objectives: The primary objective of this study was to evaluate the clinical risk factors associated with IA in LTRs. The secondary objective was to assess the mortality in LTRs who had at least one episode of IA compared with LTRs who never had experienced IA.

(3)Setting: University Hospitals

(4)Methods: We conducted an international, multicenter, retrospective cohort study of 900 consecutive adult LTRs transplanted between 2005 and 2008 with 4 years of follow-up. Risk factors associated with IA were identified using univariate and multiple regression Cox proportional hazards models.

(5)Results: 61.7% (555/900) of patients received antifungal prophylaxis. 79 patients developed 115 episodes of IA. The rate to development of first episode was 29.6 per 1000-person years. *Aspergillus fumigatus* was the most common species isolated (63%; 72/115 episodes). Through multivariate analysis, significant risk factors identified for IA development were single lung transplant (HR 1.84, p=0.02, 95% CI 1.09-3.10) and colonization with *Aspergillus* at one year post-transplantation (HR 2.11, p=0.003, 95% CI 1.28-3.49). Cystic fibrosis, pre-transplant colonization with *Aspergillus* spp., and use of antifungal prophylaxis were not significantly associated with the development of IA. Using time-dependent analysis, IA was associated with higher mortality rates.

(6)Conclusion: Incidence of IA remains high in LTRs. Single lung transplant and airway colonization with Aspergillus spp. at 1 year post-transplant were significantly associated with IA.

Introduction

Patients who have undergone lung transplantation have a high risk of developing invasive aspergillosis (IA).[1, 2] Decreased mucociliary clearance, impaired cough reflex, and continuous exposure of the lungs to the environment are among the factors that contribute to colonization with *Aspergillus* spp., which is found in 20 to 50% of patients.[3] However, incidence of IA is lower, ranging from 3 to 15%, depending on series reported. [4-7]

IA is associated with high morbidity and mortality rates. Thus, prophylactic strategies targeting *Aspergillus* spp. have been widely employed by lung transplant centers. The antifungal drugs used for prophylaxis have changed over time; currently voriconazole is the most widely used antifungal agent in LTRs.[8] However, the efficacy of antifungal prophylaxis, as well as the modalities of its use, are debated.[9, 10]

Some centers use universal prophylaxis for the first few months post-transplant in all LTRs, whereas others use targeted prophylaxis in patients with identified risk factors for IA.[3, 11] Thus, proper identification of risk factors for IA is critical for the management of LTRs. Several studies, mostly single center cohorts, have assessed risk factors for the development of IA in LTRs, but results are not always consistent. Given the number and the diversity of clinical parameters that need to be assessed, a multicenter study with a large number of patients is warranted. Additionally, it is possible that the respective impact of individual risk factors has changed compared to earlier studies due to evolutions in the management of LTRs over time. The aim of our study was to assess the risk factors for the development of IA in a large, multicenter cohort of LTRs in the current era of widespread antifungal prophylaxis use.

Materials and Methods

Study Design and Patients

This multicenter, retrospective cohort study was conducted at 14 lung transplant centers across 9 countries in Europe, North America, and Australia. This cohort of patients was initially studied to assess a different outcome, the occurrence of squamous cell carcinoma in lung transplant

recipients treated with voriconazole, and the study was powered accordingly[12]. We considered for inclusion all consecutive patients aged ≥ 18 years that underwent single-lung, double-lung, or heart-lung transplantation between January 1, 2005 and December 31, 2008. Patients with simultaneous or sequential abdominal organ transplant were excluded. At each site, the study protocol was approved by Institutional Review Boards and/or Independent Ethics Committees.

Data Collection

Patient-level data were collected from complete or partial electronic medical records and collated into an electronic database developed and maintained by the coordinating center, the University Health Network, Toronto, Canada.

Variables

Demographics and variables collected included recipient age at the time of transplant, gender, type of transplant (double lung transplant, single lung transplant, heart-lung transplant), re-transplantation, underlying disease, dialysis, number of episodes of acute rejection, number of episodes of neutropenia (neutrophils less than $1.5x10^9$/l), diabetes, CMV infection was defined according to the American society of transplantation criteria[13], immunosuppressive drugs, pre- and post-transplant colonization with *Aspergillus* spp., and use of anti-mold prophylaxis. Regarding immunosuppressive drugs, we collected data on the following medications for induction: monoclonal antibody directed against Interleukin-2 receptor (IL2RA), basiliximab, anti-thymocyte globulin (ATG) or monoclonal antibody against CD-52, alemtuzumab. For maintenance immunosuppressive regimens, we collected data on tacrolimus, cyclosporine, mycophenolic acid or mycophenolate mofetil (MMF), azathioprine, and sirolimus, if the drug was used for at least 30 days during the follow up period. For patients receiving calcineurin inhibitor drugs (CNI), we also collected the number of episodes of supra-therapeutic levels of CNI (elevated CNI levels were defined as cyclosporine trough >350 mcg/L or tacrolimus trough >20 mcg/L on at least one occasion). We collected the use of antifungal prophylaxis` (itraconazole, voriconazole, posaconazole, amphotericin and its lipidic formulations, echinocandins). Prophylaxis strategies were variable, with some centers using universal prophylaxis

and others using targeted prophylaxis in patients considered as high risk of IA. We collected data on Aspergillus colonization any time before transplantation, and colonization data for 4 years post transplantation.

Definition of Aspergillus sp. colonization and Invasive Aspergillosis (IA)

All the cases of Aspergillus colonization and Invasive Aspergillosis reported by the different centers were reviewed by the principal investigator and classified according to the International Society of Heart and Lung Transplantation criteria for invasive fungal infection[14]. In cases of ambiguity owing to the lack of data, corresponding centers were contacted and were classified only if all data-points were available.

Colonization was defined by the presence of *Aspergillus sp.* in the respiratory secretions (sputum of bronchoalveolar lavage (BAL)) detected by culture, polymerase chain reaction or biomarker (Galactomannan >1), or radiologic and endobronchial changes in the absence of symptoms.

Invasive Aspergillosis is defined by the presence of *Aspergillus sp.* in the respiratory secretions detected by culture, PCR or Galactomannan in the presence of symptoms and radiologic changes (new or progressive and persistent infiltrates, consolidation, cavitation, nodules) or endotracheal changes or presence of histologic changes consistent with fungal invasion of the tissue. Such identification of invasion in histology defines IA as proven, whereas other cases are referred as probable IA. We included cases of colonization up to 1-year post-transplantation.

Outcome

The primary outcome was occurrence of IA at 4 years post-transplant. IA was defined according to the International Society of Heart and Lung Transplantation criteria for invasive fungal infection[14]. The secondary outcome was all cause mortality at the end of the follow-up period.

Statistical analysis

Values were expressed as mean (standard deviation) or median (interquartile range) for continuous variables depending on the distribution, or as a count (percent) for categorical variables.

We compared those diagnosed with and without IA using the Student's t-test, chi-square, or Wilcoxon signed-rank tests, as appropriate for each variable. The criterion for statistical significance was set *a priori* at alpha=0.05, with all tests of significance being two-tailed.

Risk factors associated with IA were identified in univariate and multiple regression using Cox proportional hazards models. Variables with a p-value of <0.20 were subjected to a multivariate analysis with backward stepwise proportional hazard modeling. Additionally, we forced the inclusion of antifungal prophylaxis use in the final model as a variable of interest *a priori*. P-values <0.05 in the final model were considered statistically significant. The assumption of proportionality was graphically examined using log (cumulative hazard) plots and scaled Schoenfeld residuals. No important violations of the proportionality assumption were identified. The cumulative incidence curve for IA was produced using Gray's method, a class of K-sample tests for comparing the cumulative incidence of competing risk[15], considering death without IA as a competing risk. We compared survival rates at end of follow-up for patients with IA analyzed as a time-dependent variable using the Kaplan–Meier product limit method and the log-rank statistic to test the null hypothesis of no difference between survival curves. All data were analyzed using StataMP 12.1® (StataCorp LP, College Station, TX).

Results

Patient demographics and characteristics

Data for 900 LTRs were collected. Median period of follow up was 4 years (IQR: 1.7 to 4 years). Demographics and characteristics of patients are outlined in **Table 1**. Seventy nine percent of patients received a double lung transplant, 18% a single lung transplant, and 3% a heart and lung transplant. For 4.7% of patients, the procedure was a re-transplantation. The most frequent underlying disease was chronic obstructive pulmonary disease (28.7%), followed by idiopathic pulmonary fibrosis (24.4%) and cystic fibrosis (22.3%). ATG was used in 18.1% of patients. Pre-transplant colonization with *Aspergillus* spp. was identified in 3.6% of patients, and within 1-year post-transplant in 17.1% of patients. The strategies used for antifungal prophylaxis were variable between the different centers, as outlined in **Table 2**. Antifungal prophylaxis was used in 555 (61.7%) patients, as

universal prophylaxis in 442 (49.1%) of patients and targeted in 108 (12%) of patients. Indication was missing for 5 patients. While the percentage of patients receiving universal prophylaxis was not different according to the underlying disease, the use of targeted prophylaxis was higher in cystic fibrosis patients (22.9%). The percentage of patients who received prophylaxis was not different between transplant types (62.4% in double lung, 59.3% in heart/lung, 66% in single lung transplant). The most frequent oral antifungal used was voriconazole in 338 patients (37.6%), followed by itraconazole in 210 patients (23.3%) and posaconazole in 26 patients (2.9%). Inhaled Amphotericin B was used in 328 patients (36.4%).

Invasive aspergillosis

79 patients (8.8%) developed 115 episodes of IA. The rate of development of first episode was 29.6 patients per 1000 person-years. Cumulative incidence rate of IA are shown in Figure 1. IA occurred at a median of 7.7 months (IQR: 2.1 to 21.1 months) post-transplant. **Figure 2** shows the time of occurrence of first episode of IA from transplant. The median time of occurrence of IA was not statistically different in the group of patient who received prophylaxis (7.3 months) compared to the patients who did not receive prophylaxis (9.4 month), p=0.95. *Aspergillus fumigatus* was the most frequent species (63% of positive samples), followed by *Aspergillus flavus* (18%), *Aspergillus niger* (10%) and other *Aspergillus* spp. (9%). Among 115 episodes, IA was classified as probable according to ISHLT guidelines in 93 cases, including 52 episodes of pneumonia (55.9%), 24 episodes of tracheobronchitis (25.8%), and 17 episodes of bronchial anastomosis infections (18.3%). 22 episodes were proven IA, including 10 episodes of pneumonia (45.5%), 9 bronchial anastomosis infections (40.9%), and 3 episodes of tracheobronchitis (13.6%). No case of disseminated IA was reported. In the universal prophylaxis group (n=471), there were 24 episodes of breakthrough IA while on prophylaxis. The majority were patients receiving lifelong itraconazole or inhaled amphotericin B (n=21) and only 3 patients on voriconazole.

Risk factors

In the univariate analysis as shown in **Table 3**, significant risk factors for the occurrence of IA were single lung transplant (p=0.01, HR 1.90, CI 95% 1.16-3.12), idiopathic pulmonary fibrosis as underlying disease (p=0.01, HR 2.32, CI 95% 1.24-4.36), more than 4 episodes of rejections (p=0.01, HR 2.60, CI 95% 1.28-5.28), and post-transplant colonization with *Aspergillus* spp. within 1 year (p<0.001, HR 2.15, CI 95% 1.34-3.45). Pre-transplant *Aspergillus* spp. colonization and cystic fibrosis as underlying disease were not significant risk factors in the univariate analysis. None of the immunosuppressive drugs were found to be associated with an increased risk of IA in univariate analysis. However, the occurrence of more than 3 episodes of supratherapeutic levels of calcineurin inhibitors (for more than 4 episodes p=0.03, HR 2.38, CI 95% 1.07-5.29) was a significant risk factor. The use of antifungal prophylaxis was not significantly associated with a risk reduction in univariate or multivariate analysis. When analyzing separately universal or targeted prophylaxis, none of them were protective either (results not shown).

Results of multivariate analyses are shown in **Table 3**. Factors significantly associated with increased risk of IA were single lung transplant (p=0.02, HR 1.84, CI 95% 1.09-3.10), and post-transplant colonization with *Aspergillus* spp. within 1 year (p=0.003, HR 2.11, CI 95% 1.28-3.49). As a sensitivity analysis, we analyzed the impact of colonization within 3 months post-transplant compared to our initial definition of colonization within 1-year post-transplant. On univariate analysis, colonization within 3-months was not statistically significantly associated with invasive aspergillosis (HR = 1.87; 95% CI: 0.93-3.75; p = 0.078), however, this changed when included in the multivariable model (HR = 2.22; CI: 1.08-4.57; p = 0.031). CMV infection was not a significant risk factor in multivariate analysis (p=0.055, HR 1.65, CI 95% 0.99-2.76).

Mortality

Mortality rates at 4 years post-transplant were not statistically significant between patients with or without IA (40.5% vs. 33.3%, p=0.19). However, in time dependent analysis, mortality was statistically higher in patients who experienced at least one episode of IA compared to patients with no IA (p=0.001), as shown in **Figure 3**.

Discussion

This large multicenter study shows that incidence of IA remains high in LTRs in the current era. Single lung transplant and *Aspergillus* colonization within 1 year post-transplantation were the only significant risk factors for occurrence of IA in multivariate analysis.

In this cohort of 900 LTRs, 8.8% of the patients experienced at least one episode of IA during the 4 years of follow up. This is consistent with the incidence of 8.9% reported in a large US registry with 1173 LTRs from 2001 to 2006.[1] Similar rates have also been reported in earlier European studies with incidence of IA of 7% in 335 LTRs between 1992 and 2003[10], and 8% in a cohort of 251 LTRs between 1991 and 2004.[7] This shows that rates of IA remain high despite widespread use of antifungal prophylaxis globally. At four years, although the mortality rates in patients with or without IA were similar, IA was associated with a higher mortality in time dependent analysis. This was primarily due to the separation of the survival curve during the first three months of transplantation, highlighting the fact that early invasive diseases is still associated with higher mortality.

In our study, pre-transplant colonization with *Aspergillus* spp. and cystic fibrosis were not associated with an increased risk of IA. Cystic fibrosis has been inconsistently reported as a risk factor for IA[5, 6, 10, 16]. Several previous studies reported pre-transplant colonization with *Aspergillus spp.* as significantly associated with an increased risk of IA[17, 18], primarily in cystic fibrosis patients. Those patients are known to have high rate of pre-transplant colonization with *Aspergillus* spp.[6, 18-24] In a recent study of 93 cystic fibrosis patients[23], rates of pre-transplant colonization were 70%, however, the risk of IA was significant only in patients with positive intraoperative *Aspergillus* spp. culture, whereas the risk was not increased for patients with *Aspergillus* spp. colonization at any time, or colonization within 6 or 12 months prior to transplant. In our study, we did not differentiate intraoperative positive culture and previous colonization. Another parameter not assessed is the pathology of explanted lungs. Vadnerkar et al. reported invasive mold infection in 5% of explanted lungs in a cohort of 304 LTRs; invasive fungal infection developed in 43% of those patients, despite the use of voriconazole prophylaxis, compared to 14% in patients without fungal infection in explanted lungs.[5] Widespread use of prophylaxis in cystic fibrosis patients post-lung transplantation as reported previously[25, 26] may have contributed to our observation.

Single lung transplant was a significant risk factor for IA in our study. IA occurring in patients with single lung transplant has been associated with higher morbidity and poorer outcomes compared to patients with double lung transplant[26, 27]. Idiopathic pulmonary fibrosis was a significant risk factor in univariate analysis, but not in multivariate analysis, and was probably a surrogate marker of increased risk associated with single lung transplant. This observation is congruent with what has been reported previously in an earlier study[27], but not in more recent single center studies.[6, 16] This discrepancy may be due to modest sample size in single center studies.

Post-transplant risk factors may play a significant role in the development of IA. In our study, we did not find a correlation between the type of immunosuppressive drugs used and IA. In univariate, but not in multivariate analysis, some factors indicative of increased immunosuppression were statistically associated with increased risk of IA, such as more than 4 episodes of acute rejection, 3 or 4 episodes of neutropenia, and more than 3 episodes of supra-therapeutic CNI levels. This contrasts with other studies reported earlier, but those studies addressed specific patient populations or scenarios; for example, in cystic fibrosis patients, treatment for acute rejection within 90 days of transplant was associated with increased risk of IA.[23] In another study, ATG was not a risk factor in all patients, but was found to be a significant risk factor in patients with pre- or post-transplant *Aspergillus* spp. colonization.[16]

CMV infection was not a significant risk factor for IA in this study. The role of CMV infection as risk factor for IA has been debated. In a case control study[17] 14 LTRs with IA were compared to 57 controls without IA, and were found to be more likely to have CMV disease and/or CMV infection than controls with an OR of 4.2. However, this was not the case in subsequent studies[7, 9]. CMV infection has also been shown to be associated with increased risk of fungal infection in liver transplant patients[28]. Whether CMV infection reflects state of immunosuppression or has a specific role in susceptibility to fungal infections is unknown.

In our study, antifungal prophylaxis was not associated with a reduction in risk of IA. However, this result should be taken with caution because of the heterogeneity of practice between centers.

In our study, the most significant risk factor for IA was colonization with *Aspergillus* spp. within 1 year. This was also shown in a previous study with an OR of 6.69.[9] The use of universal prophylaxis has not been associated with a reduction in the rate of *Aspergillus* colonization.[10] This fact could argue for preemptive strategies that have been used in some centers. Hosseini-Moghaddam reported a single center experience with culture- directed preemptive treatment using voriconazole in 328 lung transplant recipients between 2006 and 2009.[16] Patients who effectively received 3 months of voriconazole had significantly less IA than patients colonized who did not receive preemptive treatment. However, half the IA in this cohort occurred in patients without known colonization pre- or post-transplant. Hence reliance solely on culture data may not be the most desirable strategy.

The main limitation of our study is its retrospective nature, as well as the variability of practices among centers, especially regarding antifungal prophylaxis. Even if we tried to adjust for this confounding factor, with the introduction of antifungal prophylaxis in the multivariate analysis, we cannot exclude that prophylaxis modified the association between some parameters and IA. This could be an explanation for the absence of increased risk of IA in patients with cystic fibrosis and pre-transplant colonization with *Aspergillus* spp., as those patients are probably more likely to receive targeted antifungal prophylaxis. However, we would argue that this was the largest multicenter study with 4 years follow-up, and had enough power to evaluate the risk factors associated with clinical practices in the current era. Similar antifungal prophylactic strategies that were in use at the time of transplant of our cohort are still employed currently. In a worldwide survey performed from September 2009 and January 2010 (*Neoh American Society of Transplant Surgeons 2011*), voriconazole was also noted as the most used agent for prophylaxis, with or without inhaled amphotericin B. In a recent international survey of 52 transplant centers in Europe, Oceania, North America and South America, the most common agents used for prophylaxis were voriconazole (43%) and inhaled amphotericin B ((29%), followed by posaconazole 13%) and itraconazole (11%), while caspofungin was used as first line agent for prophylaxis in only one center (2%)[29]. Moreover, this study clearly demonstrates that IA remains a significant problem despite widespread use of antifungal prophylaxis.

In summary, our multicenter study shows that single lung transplant and post-transplant *Aspergillus* colonization are the only two clinical factors that increase the risk of IA in lung transplant recipients. Perhaps a combination of clinical risk factors and biomarkers[30, 31] may be able to stratify the subsequent development of IA more accurately.

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2015; **211**(10): 1646-57.

Figure 1. Cumulative incidence rate for IA.

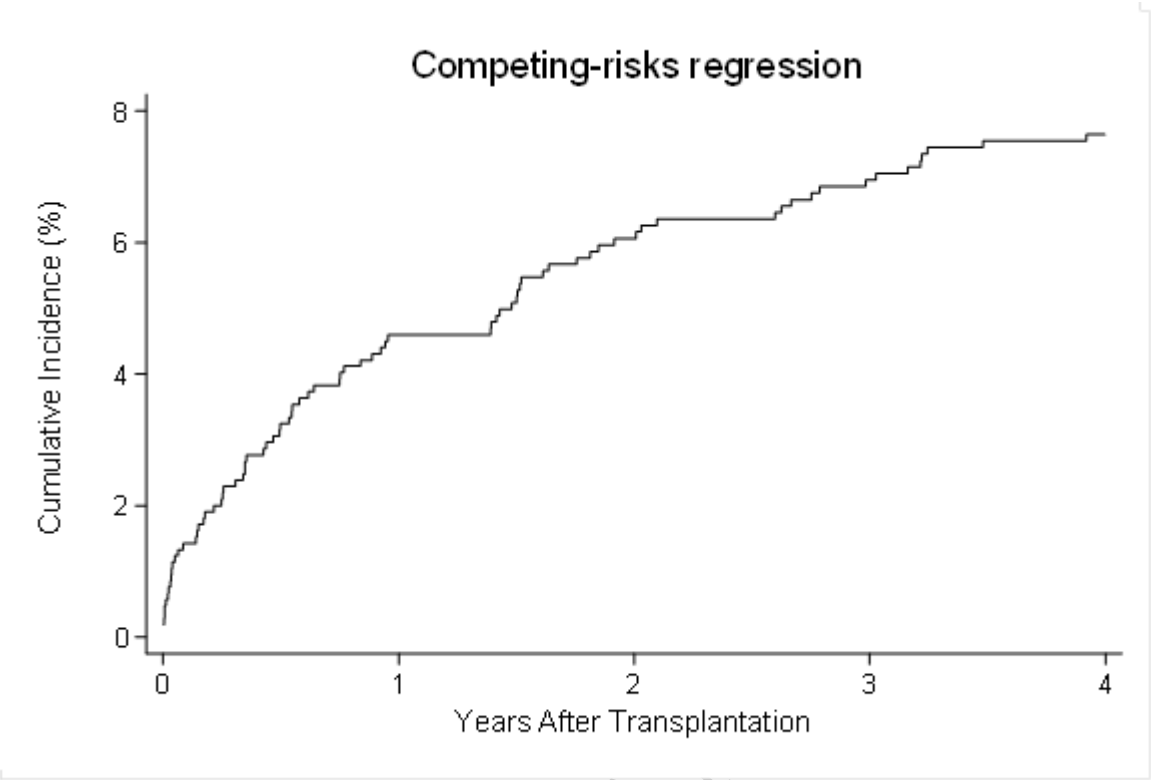

Figure 2. Proportion of patients diagnosed with invasive aspergillosis by time post-transplantation (n=79)

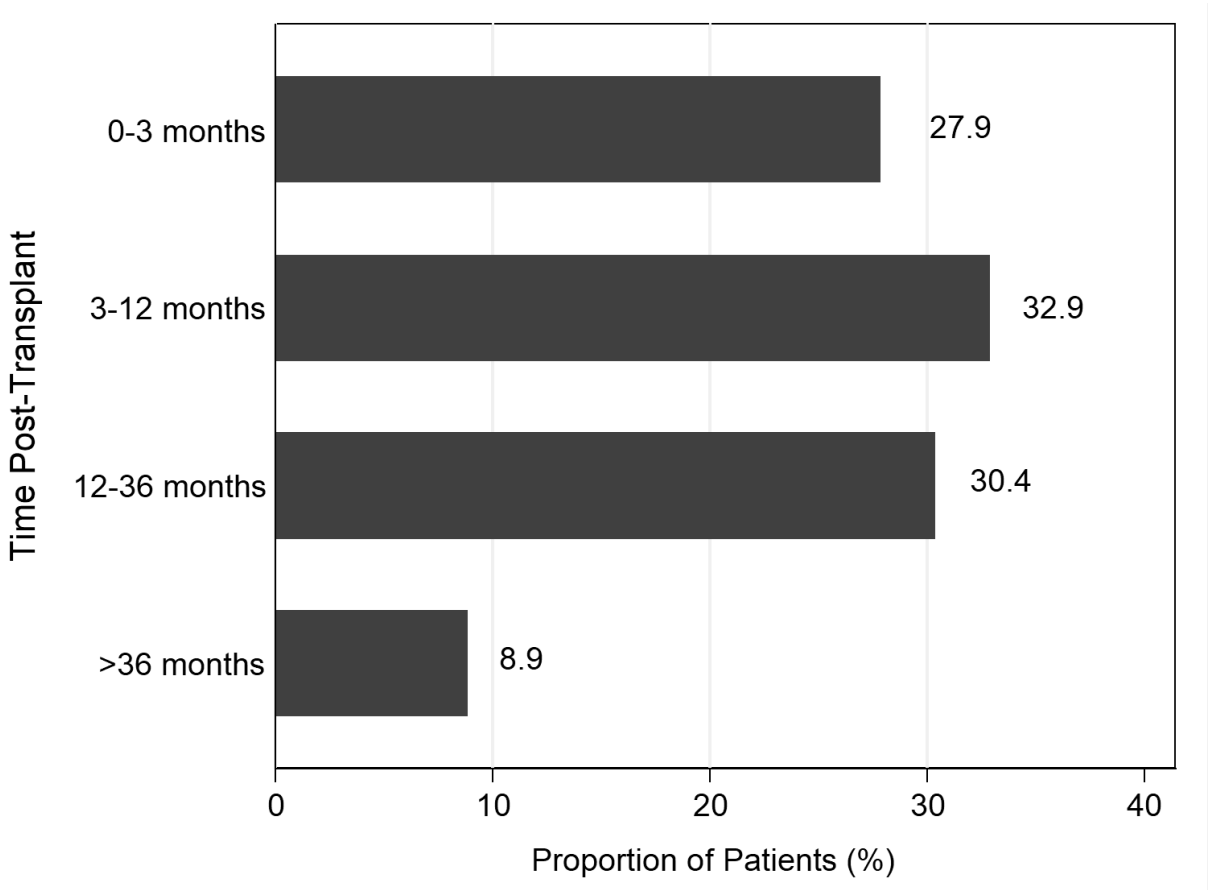

Figure 3. Kaplan-Meier survival estimates.

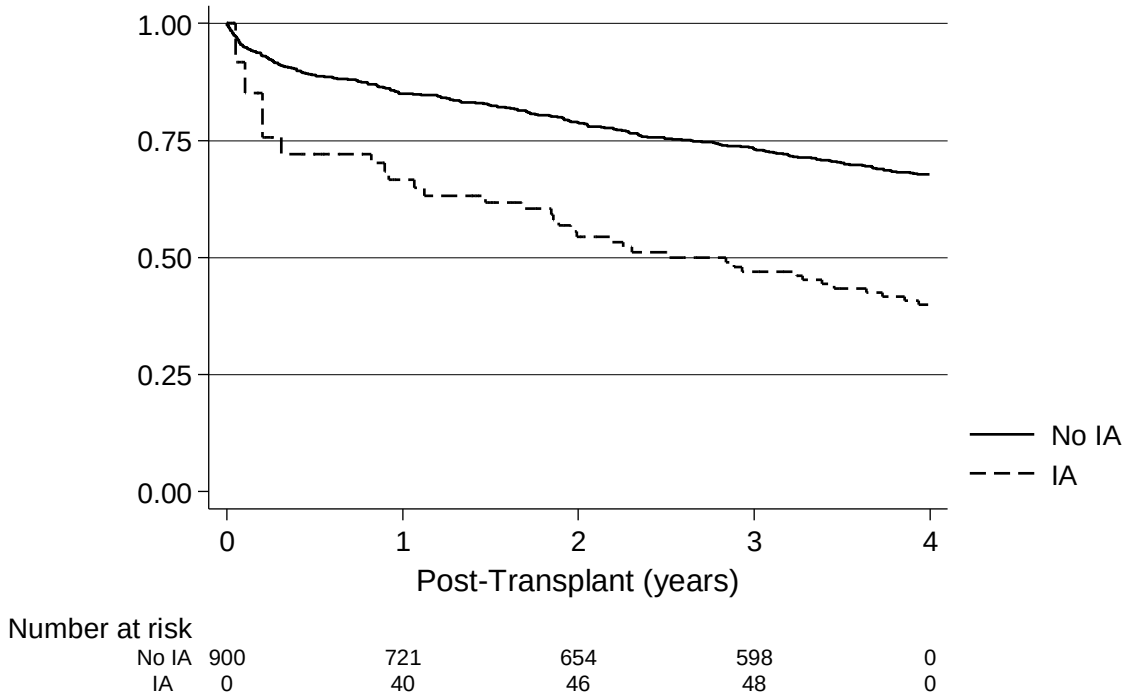

Table 1. Patient demographics.

	No IA, n=821 (%)	IA, n=79 (%)	Total, n=900 (%)	P value
Age				0.38
18-29	132 (16.1)	13 (16.5)	145 (16.1)	
30-49	227 (27.6)	24 (30.4)	251 (27.9)	
50-59	245 (29.8)	28 (35.4)	273 (30.3)	
>60	217 (26.4)	14 (17.7)	231 (25.7)	
Gender: Male	437 (53.2)	41 (51.9)	478 (53.1)	0.82
Lung Type				0.06
Double	656 (79.9)	55 (69.6)	711 (79.0)	
Heart/Lung	25 (3.0)	2 (2.5)	27 (3.0)	
Single	140 (17.1)	22 (27.8)	162 (18.0)	
Retransplantation	40 (4.9)	2 (2.5)	42 (4.7)	0.35
Underlying Disease				0.09
Cystic fibrosis	188 (22.9)	13 (16.5)	201 (22.3)	
COPD	243 (29.6)	15 (19.0)	258 (28.7)	
AAT	44 (5.4)	3 (3.8)	47 (5.2)	
IPF	193 (23.5)	27 (34.2)	220 (24.4)	
PPH	24 (2.9)	4 (5.1)	28 (3.1)	
Scleroderma	16 (1.9)	3 (3.8)	19 (2.1)	
Other	113 (13.8)	14 (17.7)	127 (14.1)	
Dialysis	24 (2.9)	3 (3.8)	27 (3.0)	0.66
Acute Rejection (number of episodes)				0.01
0	419 (51.0)	34 (43.0)	453 (50.3)	

1-2	293 (35.7)	27 (34.2)	320 (35.6)	
3-4	78 (9.5)	9 (11.4)	87 (9.7)	
>4	31 (3.8)	9 (11.4)	40 (4.4)	
Neutropenia (number of episodes)				0.09
0	318 (38.7)	22 (27.8)	340 (37.8)	
1-2	263 (32.0)	29 (36.7)	292 (32.4)	
3-4	109 (13.3)	17 (21.5)	126 (14.0)	
>4	131 (16.0)	11 (13.9)	142 (15.8)	
Diabetes	191 (23.3)	14 (17.7)	205 (22.8)	0.39
CMV Serostatus				0.46
D-R-	171 (20.8)	18 (22.8)	189 (21.0)	
D+R+	227 (27.6)	27 (34.2)	254 (28.2)	
D-R+	177 (21.6)	11 (13.9)	188 (20.9)	
D+R-	155 (18.9)	13 (16.5)	168 (18.7)	
Missing	91 (11.1)	10 (12.7)	101 (11.2)	
CMV Infection	159 (19.4)	24 (30.4)	183 (20.3)	0.02
IL2-RA Use				0.89
No	465 (56.6)	44 (55.7)	509 (56.6)	
Yes	273 (33.3)	28 (35.4)	301 (33.4)	
Missing	83 (10.1)	7 (8.9)	90 (10.0)	
Alemtuzumab Use				0.10
No	619 (75.4)	67 (84.8)	686 (76.2)	
Yes	121 (14.7)	5 (6.3)	126 (14.0)	
Missing	81 (9.9)	7 (8.9)	88 (9.8)	

ATG Use	148 (18.0)	15 (19.0)	163 (18.1)	0.83
Cyclosporine Use	407 (49.6)	39 (49.4)	446 (49.6)	0.97
Tacrolimus Use	555 (67.6)	58 (73.4)	613 (68.1)	0.29
MMF Use	567 (69.1)	61 (77.2)	628 (69.8)	0.13
Azathioprine Use	247 (30.1)	32 (40.5)	279 (31.0)	0.06
Sirolimus Use	51 (6.2)	5 (6.3)	56 (6.2)	0.97
Pre-Transplant colonization with *Aspergillus* spp.	29 (3.5)	3 (3.8)	32 (3.6)	0.90
Colonization with *Aspergillus* spp. within 1-year post transplant	129 (15.7)	25 (31.6)	154 (17.1)	<0.001
Antifungal prophylaxis	507 (61.8%)	48 (60.8%)	555 (61.7)	0.86

COPD: Chronic Obstructive Pulmonary Disease; AAT: Alpha 1 Antitrypsin deficiency; IPF: Idiopathic Pulmonary Fibrosis; PPH: Primary Pulmonary Hypertension; IL2RA: Antibody against Interleukin 2 receptor; ATG: Anti-thymocyte globulin; MMF: Mycophenolate Mofetil or Mycophenolic acid

Table 2. Prophylaxis strategies used in the different centers

CENTRE	Universal	Targeted/ Pre-emptive	Other	Description
Royal Adelaide Hospital, Australia	0	30	0	Targeted usually with voriconazole
Hopital Européen Georges Pompidou, France	0	27	0	Targeted with voriconazole for 12 months but stopped if explant negative
Hannover medical school, Germany	195	0	0	(1) All CF pts and patients colonized pre-Tx receive voriconazole for 4-6 months then lifelong intraconazole; (2) Non-CF pts receive itraconazole lifelong
University of Insubria, Italy	0	26	0	Pre-emptive
Hospital Puerta de Hierro Madrid, Spain	48	0	0	Universal for life with inhaled liposomal amphotericin B
University Medical Center Groningen, Netherlands	0	91	0	Culture-directed pre-emptive (usually voriconazole)
University of Pittsburgh, USA	128	0	0	All patients receive voriconazole
University of Lausanne, Switzerland	0	0	17	All patients receive inhaled amphoteracin B post-Tx during ICU stay; Patients receive voriconazole if colonized pre/post-Tx
University of Texas Health Center San Antonio, USA	14	0	0	No details
University of San Francisco, USA	39	0	0	All patients receive 3 months voriconazole
Univertsity Health Network, Canada	0	228	0	Targeted and pre-emptive
Universiy of Pennsylvania, USA	0	10	0	Targeted and pre-emptive with 3-6 months of voriconazole
University of Southern California, USA	11	0	0	All patients receive micafungin in ICU then voriconazole for 6 months
Universidad de Valencia, Spain	36	0	0	All patients receive amphotericin b lipid complex for 3 to 6 months
Total	**471**	**412**	**17**	

CF: Cystic Firbosis; AAT: Alpha 1 Antitrypsin deficiency; Tx: transplant; ICU: Intensive Care Unit

Table 3. Univariate and multivariate analyses evaluating risk factors for IA

	Univariate		Multivariate	
	HR (95% CI)	**p-value**	**HR (95% CI)**	**p-value**
Age >60 years	0.71 (0.33-1.51)	0.37		
Gender: Male	0.99 (0.63-1.53)	0.95		
Transplant procedure				
Heart/Lung transplant	1.16 (0.28-4.87)	0.84	1.10 (0.25-4.83)	0.90
Single Lung transplant	1.90 (1.16-3.12)	0.01	1.84 (1.09-3.10)	0.02
Underlying Disease (reference: COPD)				
Cystic fibrosis	1.09 (0.52-2.3)	0.81		
AAT	1.15 (0.33-3.95)	0.83		
IPF	2.32 (1.24-4.36)	0.01		
PPH	2.48 (0.83-7.4)	0.11		
Scleroderma	2.63 (0.83-8.32)	0.10		
Retransplantation	0.55 (0.14-2.15)	0.39		
ATG use	1.12 (0.64-1.96)	0.70		
IL2-RA Use	1.17 (0.73-1.88)	0.51		
MMF Use	1.14 (0.68-1.93)	0.62		
Azathioprine use	1.40 (0.89-2.19)	0.14		
Sirolimus use	0.89 (0.36-2.21)	0.80		
Cyclosporine use	0.92 (0.59-1.43)	0.70		
Tacrolimus use	1.04 (0.63-1.72)	0.86		
Supratherapeutic level of calcineurin inhibitor (number of episodes)				
1-2	1.35 (0.79-2.29)	0.27		
3-4	2.26 (1.19-4.28)	0.01		

>4	2.38 (1.07-5.29)	0.03		
Acute Rejection (number of episodes)				
1-2	0.97 (0.58-1.6)	0.90	0.95 (0.57-1.57)	0.83
3-4	1.13 (0.55-2.33)	0.74	0.90 (0.42-1.93)	0.79
>4	2.60 (1.28-5.28)	0.01	1.94 (0.90-4.20)	0.09
Neutropenia (number of episodes)				
1-2	1.39 (0.8-2.42)	0.24		
3-4	1.77 (0.95-3.32)	0.07		
>4	0.97 (0.47-2.01)	0.93		
Diabetes	0.70 (0.39-1.25)	0.22		
Dialysis	2.00 (0.59-6.71)	0.26		
CMV Infection	1.57 (0.98-2.53)	0.06	1.65 (0.99-2.76)	0.055
Pre-Transplant colonization with *Aspergillus* spp.	0.91 (0.3-2.76)	0.86		
Colonization with *Aspergillus* spp. within 1-year post transplant	2.15 (1.34-3.45)	<0.001	2.11 (1.28-3.49)	0.003
Antifungal prophylaxis	0.94 (0.6-1.47)	0.78	0.86 (0.54-1.39)	0.54

AAT: Alpha 1 Antitrypsin deficiency; IPF: Idiopathic Pulmonary Fibrosis; PPH: Primary Pulmonary Hypertension; IL2RA: Antibody against Interleukin 2 receptor; ATG: Anti-thymocyte globulin; MMF: Mycophenolate Mofetil or Mycophenolic acid