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FUNGAL INFECTIONS IN PEDIATRIC LUNG TRANSPLANT RECIPIENTS: COLONIZATION AND INVASIVE DISEASE

M Liu¹, S Worley¹, GB Mallory², S Arrigain¹, J Robertson², MG Schecter², O Elidemir², and LA Danziger-Isakov¹

¹The Children's Hospital at Cleveland Clinic, Cleveland, OH

²Baylor College of Medicine & Texas Children's Hospital, Houston, TX

Abstract

To evaluate the epidemiology and investigate the impact of colonization and pulmonary fungal infections (PFI), we performed a retrospective analysis of 55 pediatric lung transplant recipients from 2002–2007 at a single institution. While 29 had positive pretransplant colonization, thirty-three (60%) were colonized post-transplant and 20% (11 subjects) developed proven or probable PFI. In a multivariable model, posttransplant fungal colonization was associated with older age (HR 2.9; 95% CI 1.1–7.6), CMV prophylaxis (5.6; 1.3–24.6) and respiratory viral infection prior to fungal colonization (2.9; 1.0–8.3). Neither fungal colonization nor PFI were associated with the development of chronic allograft rejection or death.

Keywords

Lung transplantation; Fungal infection; Pediatrics; Aspergillus

Introduction

Fungal infection remains a serious complication after lung transplantation, including in pediatric recipients (1). Invasive *Aspergillus* infection has been reported in 3–16% of adult lung transplant recipients with a mortality of 41–50% (2–7). In adults, donors have transmitted fungal infections (8), pretransplant colonization has been associated with posttransplant colonization (9,10), and decreased rates of infection but not colonization were seen with antifungal prophylaxis (5,11,12). Cystic fibrosis (CF) has been associated with increased risk of fungal infection (1,9).

Until recently, fungal infections in pediatric lung transplant recipients consisted of case series and reports of unique organisms (13–16). A large multicenter retrospective study showed pulmonary fungal infection (PFI) was associated with decreased survival at one year; however, this evaluation did not assess colonization and follow-up was truncated at one year (1).

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Correspondence: Lara A Danziger-Isakov, MD, MPH, Pediatrics / S-20, The Children's Hospital at Cleveland Clinic, 9500 Euclid Avenue, Cleveland, OH 44195, DANZIGL@ccf.org.

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Materials and Methods

A review of pediatric lung transplant recipients at Texas Children's Hospital was performed. After IRB approval, a comprehensive review of clinical, microbiologic and pathology records was executed in subjects from the inception of the program in 2002 until 2007. Data was collected from transplant until August 2007 unless the subject expired or transferred care.

Clinical-care regimens

All subjects received induction with basiliximab and triple-drug immunosuppressive therapy including a calcineurin inhibitor, mycophenolate and prednisone. Subjects received cytomegalovirus prophylaxis for 5.5 months with ganciclovir transitioned to valganciclovir when tolerating oral medications. Fungal prophylaxis aside from universal oral nystatin was individualized based on clinician choice. Surveillance bronchoscopy were performed at 2 and 6 weeks, and 3, 6, 9, 12 and 18 months after transplant.

Definitions

Pretransplant colonization—Evidence of a positive culture for fungus from sputum, trachea, bronchoalveolar lavage or explanted lung prior to or at transplantation.

Posttransplant colonization—Evidence of a positive culture for fungus or yeast from the bronchoalveolar lavage after transplantation without evidence of invasive infection as defined below.

Posttransplant infection—definitions are adapted from those proposed by the European Organization for Research and Treatment of Cancer/Mycoses Study Group (17) and previously reported studies (18). Proven fungal infections had either positive histopathology or a culture from a sterile site with new symptoms or radiographic infiltrate. Probable fungal infection was defined as positive culture from bronchoalveolar lavage with clinical evidence of infection.

Other posttransplant outcomes

Posttransplant outcome measures including **acute rejection (AR)** and **bronchiolitis obliterans syndrome (BOS)** were based on International Society for Heart and Lung Transplantation definitions (19–21).

Statistical Methods

The analysis of posttransplant fungal colonization excluded five patients with PFI prior to fungal colonization. Associations between risk factors and time to posttransplant colonization, PFI, and other outcomes were assessed using univariable Cox proportional hazards models. Events that occurred posttransplant were modeled as time-dependent covariates. The functional forms for age and transplant era were chosen by modeling quartiles of these variables as categorical variables and assessing the resulting parameter estimates. A multivariable model was also fit for the outcome posttransplant colonization using backwards selection, with a significance criterion of 0.05. The proportional hazards assumption for this model was assessed by entering risk-factor-by-time interactions into the model; this assumption was also assessed graphically using log-log-survival plots. There were too few patients who died (15) or had PFI (11) to fit multivariable models for those outcomes. All tests were two-tailed and performed at a significance level of 0.05. SAS version 9.2 (SAS Institute, Cary, NC) was used for all analyses.

Results

Demographics

Of the 55 pediatric lung transplant recipient, half were female (51%). The majority were Caucasian (71%), with the remainder Hispanic (16%) and African-American (4%). Fifty-two received bilateral lungs from a deceased donor. The majority of recipients had an underlying diagnosis of cystic fibrosis (62%), with pulmonary hypertension (9%), obliterative bronchiolitis (5%), interstitial pneumonitis (5%) and other etiologies (19%).

Pretransplant colonization

Three donors had positive lower respiratory cultures for *Candida* species. *C. albicans* and *C. tropicalis* recovered from one donor each, while an additional donor had *C. albicans* and *C. krusei*. Pretransplant colonization from the recipients was evaluated in 75% (41). Of those evaluated, 29 subjects (70%) were colonized pretransplant. Subjects with CF had increased rates of positive pretransplant fungal cultures (24/29) compared to subjects transplanted for other indications (5/12) (OR 6.7; 95% CI 1.5, 30.1). *Candida* species were most frequently recovered (21/29) followed by *Aspergillus* species (11/29). *Scedosporium* and *Basidiomycete* were recovered from one subject each.

Antifungal Prophylaxis

Fourteen subjects received fungal prophylaxis. Eleven of those with prophylaxis had pretransplant colonization, *Candida* (5) and *Aspergillus* (7). Eighteen with pretransplant colonization did not receive antifungal prophylaxis, *Aspergillus* (4) and *Candida* (16). Fungal prophylaxis was administered for a median of 51 days (range 14–272). Subjects with CF were as likely to receive fungal prophylaxis as those transplanted for another etiology.

Posttransplant colonization

After transplantation, 33 subjects had 73 distinct episodes of lower respiratory fungal colonization (range 1–9 episodes) at a median of 58 days. *Candida albicans* (13) and *Candida* species (9) were the most common while *Aspergillus* species (9) were also recovered. A variety of other molds (26) were recovered including *Basidiomycetes* (5), *Paecilomyces* (1) and *Scedosporium* (4). Six subjects had persistent colonization with the same organism recovered on two or more consecutive evaluations. Organisms included *C. albicans* (5), *C. glabrata* (1) and *Basidiomycetes* (1). No persistently colonized subject developed invasive fungal infection with the colonizing organism despite not being treated for their colonization, and posttransplant colonization with *Aspergillus* species was not detected prior to *Aspergillus* PFI in any patient. Univariable and multivariable Cox proportional hazard ratios assessed the risk for posttransplant colonization. In the univariable model (Table 1), risk of fungal colonization was associated with CMV donor-seropositivity (HR 3.1; 95% CI 1.2–8.0), receipt of CMV prophylaxis (3.3, 1.0–11.2), A2 or greater rejection or respiratory viral infection (RVI) prior to fungal colonization (3.5; 1.3–9.6 and 2.5; 0.97–6.2, respectively). The multivariable model revealed older age (2.9; 1.1–7.6), CMV prophylaxis – a marker for CMV seropositivity (5.6; 1.3–24.6) and RVI prior to colonization (2.9; 1.0–8.3) as independent predictors of posttransplant colonization. CF was not a risk for fungal colonization after transplantation and fungal colonization was not associated with death or retransplantation (HR 0.9; 95% CI 0.3–2.9).

PFI epidemiology and risks

Fourteen proven or probable PFI were recorded in eleven subjects (20%, Table 2). Eleven (91.6%) of the twelve subjects with PFI had an underlying diagnosis of CF. The mean time to first PFI occurred at 271 days (range 6–925 days) after transplantation. Due to the number of

posttransplant PFI, multivariable modeling with an appropriate number of variables was not feasible; however, univariable analysis revealed increased risk of PFI with history of CMV prophylaxis (HR 4.0; 95% CI 1.2, 13.7) and CMV episode prior to PFI (7.7; 1.5–40.6). History of fungal prophylaxis approached statistical significance as a risk for PFI (3.2; 0.96–10.4; $p=0.06$). Pretransplant or posttransplant fungal colonization before the onset of PFI, AR and CF were not associated with PFI. PFI was not associated with increased risk of death or retransplantation (1.4; 0.3–6.7).

Discussion

Pulmonary fungal infections in adult lung transplant recipients cause significant morbidity and mortality. Compared to adult population reporting 30–40% lower respiratory colonization within one year after transplant (9,12), posttransplant colonization in pediatric recipients was higher at 60%; however, persistence of colonization was similar. Unlike adults, pretransplant colonization was not associated with posttransplant colonization or infection (22).

Interestingly, infectious fungal organisms described represent the diversity of potential pathogens in pediatric lung transplant recipients. Infection with *Aspergillus* species is not uncommon; however, reports of histologically proven *Candida* species pulmonary disease are unusual in adult subjects. The episodes of cryptococcosis, coccidiomycosis, pneumocystis and *Scedosporium* infection exemplify the variety of pathogens to be considered in evaluation and empiric therapy for presumed fungal infections. While the adult literature is mixed regarding the association between CMV and subsequent fungal infection (3,9), an episode of CMV was associated with increased the risk for subsequent PFI but not subsequent colonization. Interestingly, the receipt of CMV prophylaxis was associated with subsequent PFI. CMV prophylaxis functioned as a marker for donor or recipient CMV seropositivity as only these subjects received prophylaxis. This indicates that seropositivity alone posed a risk for colonization and PFI.

As with any retrospective review, limitations are inherent to the study design. While the number of subjects is limited, the uniform application of immunosuppression, CMV prophylaxis, and sample collection for fungal infections increases the ability to evaluate other aspects of risk. Although antifungal prophylaxis was not uniformly administered, the proportions of subjects with and without pretransplant colonization who received prophylaxis were similar. Finally, additional infections may have occurred that were not appropriately attributed. The adaptation of the ETROC definitions as used elsewhere (12,22) allows for comparison across the available literature.

In conclusion, pulmonary fungal colonization is common both before and after pediatric lung transplantation. Pretransplant colonization was not predictive of posttransplant colonization or disease. Posttransplant colonization was not associated with the development of invasive pulmonary disease. Unlike adult lung transplant recipients, proven *C. albicans* infections were not uncommon, even if occurring concurrently with invasive aspergillus infection.

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Colonization and PFI

Figure 1. Colonization and PFI

In Figure 1, patients are divided according to their colonization status (pre-transplant colonized, post-transplant de-novo colonization, or uncolonized). Further, patients with and without PFI after pediatric lung transplantation are identified for each category.

Table 1

Univariable Hazard Ratios for risk of fungal colonization or fungal infection (PFI)

Risk factor	Fungal Colonization			Fungal Infection (PFI)		
	N	Hazard ratio (95% CI)	P-value	N	Hazard ratio (95% CI)	P-value
Age at transplant verses 16.5–20.9 years	50		0.20	55		0.85
		0.2–6.6 years 6.6–13.6 years 13.6–16.5 years	0.38 (0.13, 1.1) 0.38 (0.13, 1.09) 0.66 (0.25, 1.8)		0.00 (0.00, .) 0.50 (0.11, 2.3) 0.69 (0.17, 2.8)	
Year of transplant verses 2006	50		0.17	55		0.49
		2002–2004 2004–2005 2005–2006	1.7 (0.42, 7.1) 2.7 (0.82, 8.8) 3.4 (1.09, 10.8)		1.6 (0.26, 10.3) 0.45 (0.04, 5.0) 2.1 (0.41, 11.1)	
Cystic Fibrosis Etiology	50		0.17	55		0.83
		1.8 (0.78, 4.3)			0.87 (0.24, 3.2)	
Donor CMV positive	38		0.020	50		0.47
Recipient CMV positive	45	3.1 (1.2, 8.0) 1.3 (0.59, 2.8)	0.54	41	0.64 (0.19, 2.2) 1.02 (0.26, 4.0)	0.98
Positive pretransplant fungal culture	37		0.20	53		0.28
CMV Prophylaxis	48	1.9 (0.70, 5.4) 3.3 (1.00, 11.2)	0.050	55	3.1 (0.39, 24.1) 4.0 (1.2, 13.7)	0.027
Fungal Prophylaxis	50	0.79 (0.30, 2.1)	0.64	55	3.2 (0.96, 10.4)	0.06
A2 rejection prior to fungal event	50		0.016	55		0.85
Respiratory viral infection prior to fungal event	50	3.5 (1.3, 9.6) 2.5 (0.97, 6.2)	0.057	55	1.1 (0.31, 4.2) 2.0 (0.56, 7.5)	0.28
CMV prior to fungal event	50	2.8 (0.32, 25.6)	0.35	55	7.7 (1.5, 40.6)	0.016

CMV: cytomegalovirus

Table 2

Summary of Proven and Probable PFI in Pediatric Lung Transplant Recipients

Subject	Episode	Type	Time to event (days)	Organisms	Therapy	Pre-transplant culture	Prophylaxis
1	10y/o M Pneumonitis	Proven	42	<i>C. Albicans</i> <i>A. fumigatus</i>	Amphotericin Voriconazole	Not done	Fluconazole
1	10y/o M Pneumonitis	Proven	99	<i>C. neoformans</i>	Fluconazole Voriconazole	Not done	Fluconazole
2	17y/o M CF	Proven	88	<i>A. fumigatus</i>	Amphotericin(i) Voriconazole	Negative	
3	7y/o F Hispanic CF	Proven	864	<i>P. jirovecii</i>	Bactrim	<i>C. albicans</i>	
4	18y/o M CF Expired	Proven	360	<i>Aspergillus sp.</i>		<i>C. albicans</i>	
5	14y/o F CF	Proven	85	<i>A. flavus</i>	Voriconazole	<i>A. flavus</i>	Voriconazole
6	13y/o F CF	Proven	6	<i>C. albicans</i>	Voriconazole	<i>C. albicans</i>	Voriconazole
6	13y/o F CF	Proven	88	<i>C. immitis</i>	Voriconazole	<i>C. albicans</i> <i>A. fumigatus</i>	Voriconazole
7	8y/o M CF	Proven	100	<i>A. fumigatus</i> <i>C. albicans</i>	Voriconazole Amphotericin Caspofungin	<i>C. albicans</i> <i>A. flavus</i>	
7	8y/o M CF	Probable	925	<i>A. terreus</i>		<i>C. albicans</i> <i>A. flavus</i>	
8	17y/o F CF	Probable	41	Yeast	Amphotericin(i) Voriconazole	<i>C. parapsilosis</i> (i)	Amphotericin
9	19y/o M CF	Probable	409	<i>S. aptospermum</i>	Voriconazole	Negative	Fluconazole
10	14y/o F CF	Probable	412	<i>A. niger</i> <i>A. terreus</i> <i>C. albicans</i>	Voriconazole	<i>C. albicans</i>	
11	16y/o M CF	Probable	278	<i>A. fumigatus</i>		Negative	

Negative M: Male, F: female, CF cystic fibrosis, (i) inhaled