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Risk factors for invasive fungal disease in heart transplant recipients

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Abstract

BACKGROUND—Heart transplant (HT) recipients are at risk for invasive fungal disease (IFD), a morbid and potentially fatal complication.

METHODS—We performed a retrospective cohort study to evaluate the incidence and risk factors for IFD in HT recipients from 1995 to 2012 at a single center. IFD cases were classified as proven or probable IFD according to current consensus definitions of the European Organization for Research and Treatment of Cancer/Mycoses Study Group. We calculated IFD incidence rates and used Cox proportional hazards models to determine IFD risk factors.

RESULTS—Three hundred sixty patients underwent HT during the study period. The most common indications were dilated (39%) and ischemic (37%) cardiomyopathy. There were 23 (6.4%) cases of proven (21) or probable (2) IFD, for a cumulative incidence rate of 1.23 per 100 person-years (95% CI 0.78 to 1.84). *Candida* (11) and *Aspergillus* (5) were the most common etiologic fungi. Thirteen cases (56%) occurred within 3 months of HT, with a 3-month incidence of 3.8% (95% CI 2.2 to 6.4). Delayed chest closure (HR 3.3, 95% CI 1.4 to 7.6, $p = 0.01$) and the addition of OKT3, anti-thymocyte globulin or daclizumab to standard corticosteroid induction therapy (HR 2.7, 95% CI 1.1 to 6.2, $p = 0.02$) were independently associated with an increased risk of IFD.

CONCLUSIONS—IFD incidence was greatest within the first 3 months post-HT, largely reflecting early surgical-site and nosocomial *Candida* and *Aspergillus* infections. Patients receiving additional induction immunosuppression or delayed chest closure were at increased risk for IFD. Peri-transplant anti-fungal prophylaxis should be considered in this subset of HT recipients.

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Keywords

invasive fungal disease; heart transplantation; risk factors; *Aspergillus*; *Candida*

Heart transplant (HT) recipients are at risk for invasive fungal disease (IFD), a morbid and potentially fatal complication of transplantation. Surveillance studies of heart and other solid-organ transplant (SOT) recipients have suggested an overall IFD incidence of 1% to 12% with an attributable mortality of between 35% and 75%.^{1–7} Some studies have explored empirical anti-fungal prophylaxis after HT,^{6,8} but data regarding IFD incidence, timing and risk factors in the HT population are very limited. In this study, we sought to estimate IFD incidence and identify IFD risk factors in a cohort of HT patients to identify patients who may benefit from anti-fungal prophylaxis.

Methods

Study population

All patients receiving a first HT at Brigham and Women's Hospital (Boston, MA) between January 1, 1995 and December 31, 2012 were included in the study cohort and follow-up events were censored on March 1, 2013. We recorded the following data: patients' demographics; donor and recipient cytomegalovirus (CMV) serostatus; induction and maintenance immunosuppressive regimens; episodes of acute cellular allograft rejection treated with increased immunosuppressive therapy; and details of all IFD episodes. The study was approved by the Partners Human Research Committee.

Immunosuppression

All patients received methylprednisolone 1,000 mg by intravenous (IV) infusion in the operating room, followed by 125 mg IV every 8 hours for three doses, then a tapering dose of corticosteroids. Patients with substantial renal dysfunction at the time of HT, defined as pre-operative renal dysfunction, peri-operative renal injury or post-operative anuria, as adjudicated jointly by a staff transplant surgeon and transplant cardiologist within 24 to 48 hours of HT, received additional induction immunosuppression with OKT3 (1995 to 2001), daclizumab (2001 to 2010) or anti-thymocyte globulin (ATG) (2010 to 2012) to delay initiation of potentially nephrotoxic calcineurin inhibitors. Standard maintenance immunosuppression consisted of prednisone, cyclosporine and azathioprine from 1995 to 2002; prednisone, cyclosporine and mycophenolate mofetil (MMF) from 2002 to 2008; and prednisone, tacrolimus and MMF from 2008 to the end of the study period.

Acute cellular rejection

Routine endomyocardial biopsies were performed at 1, 2, 3, 4 and 6 weeks, and 2, 3, 4, 5, 6, 8, 10, 12, 15 and 18 months post-HT, and annually thereafter, with more frequent biopsies in patients with recurrent rejection episodes. Biopsy specimens were assessed for evidence of acute cellular rejection by standard criteria of the International Society for Heart and Lung Transplantation.⁹ Patients who received an increased dose of oral corticosteroids, high-dose IV corticosteroids, OKT3, ATG or daclizumab for treatment of acute cellular rejection on biopsy were considered to have "treated acute cellular rejection."

Anti-fungal prophylaxis

No patients received systemic or inhaled anti-fungal prophylaxis over the study period, other than standard trimethoprim-sulfame-thoxazole *Pneumocystis jirovecii* pneumonia (PCP) prophylaxis for 6 months post-HT.

HEPA filtering

The air in all operating rooms and in all intensive-care unit and ward rooms was HEPA filtered over the study period.

IFD cases

IFD cases were identified via a systematic review of the electronic and paper longitudinal medical records of every HT recipient in our cohort, consisting of all provider notes, including all infectious diseases specialist consultation notes (at our hospital, all cases of suspected fungal infection are referred for transplant infectious diseases consultation), and results of all relevant radiology, pathology studies and clinical mycology data (cultures, serum fungal antigen testing). Using data on relevant host factors, imaging studies and mycology, we classified proven or probable IFD cases according to current consensus definitions of the European Organization for Research and Treatment of Cancer/ Mycoses Study Group (EORTC/MSG).¹⁰

Statistical methods

We determined incidence rates of IFD in the HT cohort and calculated actuarial estimates of time to first IFD episode and death using the Kaplan–Meier method. We developed Cox proportional hazards models to evaluate risk factors that we believed “a priori” could affect the risk of IFD, such as: age; gender; additional induction immunosuppression (which, by indication, was perfectly collinear with renal dysfunction at the time of HT); acute cellular rejection; ventricular assist device (VAD) pre-transplant; peri-transplant VAD placement due to allograft failure within 1 week of HT; reoperation within 10 days of initial HT; and delayed chest closure, defined as patients whose chest incision was not immediately closed after the HT procedure. Based on a review of the limited existing literature on risk factors for IFD in HT,^{8,11} we also assessed the risk of IFD after developing CMV disease. Acute cellular rejection was modeled as a time-varying covariate—we assumed an effect duration of 90 days for each acute cellular rejection episode treated with a transient increase in the dose of oral prednisone and a duration of 180 days for each episode treated with high-dose corticosteroids, OKT3, ATG or daclizumab. We verified the appropriateness of the proportional hazards assumption for each variable in our final multivariable model by plotting Schoenfeld residuals and by testing for an interaction between each of these variables and follow-up time. All analyses were performed using STATA version 11 (StataCorp, College Station, TX).

Results

Demographic and transplant characteristics of the 360 HT recipients are presented in Table 1. One hundred six patients (29.4%) underwent reoperation within 10 days of HT, most for delayed chest closure (Table 1). Although most patients received corticosteroids alone for

induction immunosuppression, 88 patients (24.4%) with renal dysfunction received additional induction immunosuppression with OKT3, daclizumab or ATG, to delay initiation of calcineurin-inhibitors post-HT. Median follow-up time for the cohort was 4.1 years (interquartile range [IQR] 1.3 to 15.2 years) after HT.

There were 23 cases of proven (21) or probable (2) IFD over the study period (Table 2). *Candida* (11) and *Aspergillus* (5) were the most common etiologic fungi. Of the *Candida* infections, there were 7 cases of *Candida fungemia* and 4 cases involving fungemia and surgical-site infections. Of the *Aspergillus* infections, there were 2 surgical-site infections, 2 cases of pneumonia and 1 case of disseminated aspergillosis.

The overall IFD incidence rate was 1.23 per 100 person-years (95% confidence interval [CI] 0.78 to 1.84). Most IFD cases occurred shortly after HT, with 9 and 13 of the 23 IFD cases diagnosed within 3 weeks and 3 months of HT, respectively, including 8 cases of invasive candidiasis, 2 cases of invasive aspergillosis, 1 case of *Candida* and *Aspergillus* coinfection, 1 disseminated *Lichtheimia corymbifera* infection and 1 *Bipolaris spicifera* brain abscess.¹² The cumulative 3-month incidence was 3.8% (95% CI 2.2 to 6.4), cumulative 12-month incidence was 4.1% (95% CI 2.4 to 6.8) and cumulative 5-year incidence was 6.5% (95% CI 4.1 to 10.2). IFD incidence rates remained consistent over periods of different induction and maintenance immunosuppression regimens. IFD was associated with an increased risk of mortality compared to patients without IFD, with a hazard ratio (HR) of 5.1 (95% CI 2.8 to 9.2, $p < 0.001$).

In univariable analysis (Table 3), additional induction immunosuppression (HR 2.9, 95% CI 1.3 to 6.7, $p = 0.01$), reoperation within 10 days of HT (HR 2.3, 95% CI 1.02 to 5.3, $p = 0.05$), delayed chest closure (HR 3.5, 95% CI 1.5 to 8.2, $p = 0.003$) and peri-transplant VAD placement (HR 10.0, 95% CI 3.3 to 30.5, $p < 0.001$) were associated with an increased risk of IFD. Age, gender, pre-transplant VAD, and CMV disease were not associated with an increased risk of IFD. A time-dependent Cox model for acute cellular rejection failed to converge, as no IFD events occurred in the 90- to 180-day interval after treatment for acute cellular rejection.

In a multivariable Cox model, additional induction immunosuppression (HR 2.7, 95% CI 1.2 to 6.2, $p = 0.02$) and delayed chest closure (HR 3.3, 95% CI 1.4 to 7.6, $p = 0.006$) were predictive of IFD. Delayed chest closure, reoperation within 10 days of HT and peri-transplant VAD placement were highly collinear variables, so only delayed chest closure, a variable that encompassed almost all of the peri-transplant VAD placement cases, was included in the multivariable model. The median time from HT to chest closure was slightly longer in patients who developed IFD than in patients who did not ultimately develop IFD (6 days [IQR 3 to 13, range 2 to 17] and 2 days [IQR 1 to 4, range 1 to 42], respectively, $p = 0.005$). Figures 1 and 2 show freedom from IFD in our HT cohort by receipt of additional induction immunosuppression and delayed chest closure, respectively.

We assessed risk factors for invasive yeast ($n = 14$) and invasive mold ($n = 10$) infections separately (the patient with concurrent invasive candidiasis and invasive aspergillosis was counted in both analyses). In the univariable analysis, administration of additional induction

immunosuppression (HR 5.3, 95% CI 1.8 to 15.4, $p = 0.002$), delayed chest closure (HR 4.9, 95% CI 1.6 to 14.3, $p = 0.004$) and peri-transplant VAD placement (HR 12.6, 95% CI 3.2 to 48.8, $p = 0.001$) were associated with invasive yeast infections after HT. In the multivariable analysis, additional induction immunosuppression (HR 4.7, 95% CI 1.6 to 14.0, $p = 0.005$) and delayed chest closure (HR 4.3, 95% CI 1.4 to 12.8, $p = 0.010$) were both associated with invasive yeast infections.

Discussion

Invasive fungal disease is a major complication after SOT, and has been associated with increased morbidity and mortality rates. We conducted a comprehensive epidemiologic analysis of IFD in a large cohort of HT recipients and identified both additional induction immunosuppression and delayed chest closure as prominent risk factors for IFD after HT.

Our cumulative incidence of IFD at 1 year (4.1%) was comparable to the 2.2% to 3.4% incidence rate reported in previous multicenter cohort studies of HT recipients.^{1,2} Comparison to other studies is limited, as incidence rates were inconsistently reported and the reports included heterogeneous SOT populations.^{6,8,13} Although the incidence of IFD was relatively low in our cohort of HT patients, those with IFD had a crude 5-fold increase in mortality compared to patients without IFD. Fifty-seven percent of cases (13 of 23) occurred within the first 3 months after HT, including 4 cases of fatal mediastinitis from *Candida* or *Aspergillus* species. Late sporadic infections, sometimes years after transplant, were often due to less common pathogens such as *Phoma* or *Scopulariopsis* and endemic fungi.

Although earlier studies have identified reoperation, post-transplant hemodialysis and CMV disease as risk factors for IFD in SOT recipients,^{14–16} data have been limited in the HT population. In a multivariable model, we found that additional induction immunosuppression in patients with impaired renal function and delayed chest closure were strong risk factors for IFD, potentially reflecting greater underlying illness in this subset of patients, more complex surgical procedures, and a predisposition to fungal colonization with delayed sternal closure, exposure to ambient fungal conidia, and use of broad-spectrum antibacterial agents.¹⁷

We were unable to separate the effect of impaired renal function from the effect of exposure to additional immunomodulating agents at the time of HT; by indication, patients judged to have renal dysfunction at the time of HT received these agents to delay initiation of potentially nephrotoxic calcineurin inhibitors. Notably, poor renal function alone has been linked to immune dysregulation associated with an increased risk of infection.¹⁸

Our findings raise important questions regarding peri-operative management of HT patients at highest risk for IFD. Two studies have analyzed the impact of empirical anti-fungal prophylaxis in HT patients.^{6,8} Paniagua Martin and colleagues retrospectively compared no fungal prophylaxis to universal prophylaxis with either itraconazole capsules or inhaled amphotericin for prevention of aspergillosis during the 3 months after HT.⁶ Subjects were not stratified according to risk of IFD. The investigators showed a reduction in the incidence

of pulmonary aspergillosis with prophylactic itraconazole (1.4%) or inhaled amphotericin (0%) when compared with no fungal prophylaxis (5%). Muñoz and colleagues conducted a study of targeted anti-fungal prophylaxis in a cohort of HT recipients, in which they administered systemic anti-fungal therapy to patients who underwent reoperation, required post-transplantation hemodialysis, or developed CMV disease.⁸ Compared with no anti-fungal therapy, targeted anti-fungal prophylaxis was associated with a relative reduction in the incidence of invasive aspergillosis (8.6% vs 2.2%).

Our results help identify HT recipients at highest risk for IFD and suggest that anti-fungal prophylaxis may be indicated for patients receiving additional induction immunosuppression agents due to impaired renal function and for patients with delayed chest closure. A rational approach could target patients within the first 3 weeks of HT with these risk factors, as 9 of 13 early cases of IFD (within 3 months of HT) occurred within this 3-week interval.

There are limitations to the current study. Our investigation was conducted at a single center and our findings may not be generalizable to other heart transplant centers. The cases were identified by a comprehensive chart review and classified according to current EORTC/MSG criteria; however, “possible” cases of IFD were excluded, leading to potential underestimation of IFD incidence. Although we did not identify any significant risk factors for invasive mold infection alone, our ability to detect a relationship between intra-operative and transplant factors and the risk of mold infection was limited as there were only 3 early surgical-site mold infections and just a few sporadic late cases.

Although the overall incidence of IFD was low in our HT cohort, we found a substantially increased risk of IFD in patients who received additional OKT3, daclizumab or ATG due to renal impairment at HT and in those with delayed chest closure. These patients comprise a sub-population that may be targeted for peri-transplant anti-fungal prophylaxis, although further research is necessary to understand the efficacy and safety of such an approach in reducing post-HT IFD infections and their associated morbidity and mortality.

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Figure 1.
Freedom from invasive fungal disease by induction immunosuppression regimen.

Figure 2.
Freedom from invasive fungal disease by delayed chest closure.

Table 1**Cohort Characteristics (N = 360)**

Recipient age, years (IQR, range)	53 (44–60, 20–71)
Male gender [<i>n</i> (%)]	268 (74.4)
Indication for HT [<i>n</i> (%)]	
Dilated cardiomyopathy	141 (39.2)
Ischemic cardiomyopathy	134 (37.2)
Valvular cardiomyopathy	24 (6.7)
Hypertrophic cardiomyopathy	23 (6.4)
Adriamycin-induced cardiomyopathy	18 (5.0)
Congenital heart disease	5 (1.4)
Restrictive heart disease	4 (1.1)
Other ^a	11 (3.1)
Induction immunosuppression [<i>n</i> (%)]	
Methylprednisolone alone	272 (75.6)
Methylprednisolone and OKT3/daclizumab/ATG	88 (24.4)
Cytomegalovirus disease post-transplant [<i>n</i> (%)]	10 (2.8)
1 episode of acute cellular rejection post- transplant [<i>n</i> (%)]	148 (41.1)
Pre-operative VAD [<i>n</i> (%)]	134 (37.2)
Reoperation within 10 days of HT [<i>n</i> (%)]	106 (29.4)
Delayed chest closure [<i>n</i> (%)]	86 (23.9)
Peri-operative VAD placement [<i>n</i> (%)]	17 (4.7)
Post-operative hemorrhage [<i>n</i> (%)]	16 (4.4)
Other [<i>n</i> (%)] ^b	3 (0.8)

ATG, anti-thymocyte globulin; HT, heart transplant; IQR, interquartile range; VAD, ventricular assist device.

^a Arrhythmogenic right ventricular cardiomyopathy (3), acute viral myocarditis (3), amyloidosis (3) or giant cell myocarditis (2).

^b Reoperation for post-HT pneumomediastinum/air leak (1), abdominal dehiscence (1) or placement of an extracorporeal membrane oxygenation circuit (1).

Table 2Cases of Proven or Probable IFD^a

Genus	EORTC/MCS proven IFD cases (n)	EORTC/MCS probable IFD cases (n)	Total IFD cases, EORTC/MCS proven or probable (n)
<i>Candida</i>	11	—	11
<i>Aspergillus</i>	4	1	5
<i>Lichtheimia</i>	1	—	1
<i>Cryptococcus</i>	1	—	1
<i>Bipolaris</i>	1	—	1
<i>Scopulariopsis</i>	1	—	1
<i>Phoma</i>	1	—	1
<i>Histoplasma</i>	—	1	1
<i>Candida</i> and <i>Aspergillus</i>	1	—	1
Total cases	21	2	23

EORTC/MSG, European Organization for Research and Treatment of Cancer/Mycoses Study Group; IFD, invasive fungal disease.

^aProven or probable cases were classified according to current consensus definitions of the EORTC/MSG.¹⁰

Table 3**Risk Factors for Invasive Fungal Disease**

	Univariable HR (95% CI), <i>p</i> -value	Multivariable HR (95% CI), <i>p</i> -value
Age, per decade	1.04 (0.74–1.47), 0.83	—
Male gender	0.75 (0.31–1.84), 0.51	—
Pre-transplant VAD	0.87 (0.27–2.84), 0.82	—
CMV disease	1.39 (0.19–10.3), 0.75	—
Additional induction immunosuppression	2.95 (1.28–6.77), 0.01	2.68 (1.16–6.20), 0.02
Reoperation within 10 days	2.33 (1.02–5.33), 0.05	—
Delayed chest closure	3.54 (1.53–8.20), 0.003	3.26 (1.40–7.60), 0.006
Peri-transplant VAD placement	10.0 (3.27–30.5), <0.001	—

CI, confidence interval; CMV, cytomegalovirus; HR, hazard ratio; HT, heart transplant; IFD, invasive fungal disease; VAD, ventricular assist device.

* A time-dependent Cox model for acute cellular rejection failed to converge—there were no IFD events 90 to 180 days after treatment for acute cellular rejection in the study cohort. Reoperation within 10 days of HT, peri-operative VAD placement and delayed chest closure were collinear variables, so we included only delayed chest closure in the multivariable model.