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A CASE CONTROL STUDY OF THE RISK FACTORS FOR DEVELOPING ASPERGILLOSIS FOLLOWING CARDIAC TRANSPLANT

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KEY WORDS

Invasive aspergillosis, heart transplant, antifungal prophylaxis

ABSTRACT

Background: Invasive aspergillosis (IA) is a significant cause of morbidity and mortality following cardiac transplantation; however, data regarding the predictors of IA in this patient population is limited.

Methods: We conducted a case-control study to identify the risk factors for IA in patients who underwent cardiac transplantation at a single center from 1986 to 2008 (Cohort 1) and 2009 to 2015 (Cohort 2). Cases of IA were matched to two controls from the same year of transplantation and data was collected from the date of cardiac transplantation to the date of documented *Aspergillus* infection for each case, or for an equivalent number of days for each control. Univariate and multivariate logistic regression were used to identify independent predictors of IA in Cohort 1. After 2009, targeted anti-fungal prophylaxis with oral voriconazole was initiated in patients with risk factors for IA. The incidence of IA was compared pre- and post-intervention.

Results: IA was identified in 23 of 189 (8.0%) patients within Cohort 1. Significant risk factors for IA on multivariate analysis included an increased number of pre-transplant hospitalizations (OR 1.81, 95% CI 1.19 – 2.76) and post-transplant acute cellular allograft rejection (OR 1.99, 95% 1.06 – 3.75). Following the implementation of targeted anti-fungal prophylaxis in 2009, IA was identified in 2 of 107 (2.0%) patients in Cohort 2.

Conclusions: Increased pre-transplant hospitalizations and post-transplant acute cellular allograft rejection episodes represent significant risk factors for IA following cardiac transplant. Targeted anti-fungal prophylaxis in at risk patients reduces the incidence of IA.

Invasive fungal disease (IFD) is a significant cause of morbidity and mortality following cardiac transplantation. The incidence of IFD in cardiac transplant recipients ranges from 2% to 12% in epidemiologic studies, with up to 57% of infections occurring within 3 months of transplantation.[1-4] *Candida* infections and *Aspergillus* are the most common fungal infections in this patient population, and are associated with mortality rates of 28% and 67%, respectively.[4-6] The Infectious Diseases Society of American (IDSA) and European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Practice Guidelines recommend targeted prophylactic strategies against invasive aspergillosis (IA) in cardiac transplant recipients based on institutional epidemiology and the identification of one or more of the following risk factors: pre-transplant *Aspergillus* colonization, reoperation, post-transplant hemodialysis, post-transplant cytomegalovirus (CMV) infection, and an institutional outbreak of IA two months before or after transplantation.[7,8] The use of post-transplant extracorporeal membrane oxygenation has also been identified as a significant predictor of IA in a retrospective cohort study, and may represent an additional indication for anti-fungal prophylaxis in this patient population.[2]

While several studies have evaluated the efficacy of targeted anti-fungal prophylaxis following cardiac transplantation,[2,9,10] data regarding the incidence, timing, and predictors of IA is limited, and often extrapolated from heterogeneous solid organ transplant populations. The purpose of this study was to identify the risk factors associated with the development of IA utilizing two cohorts of cardiac transplant recipients to determine and assess the impact of targeted anti-fungal prophylaxis.

Methods

Patient population

Male and female patients ≥ 18 years of age who underwent single organ cardiac transplantation at the University of North Carolina Medical Center (Chapel Hill, NC) from 1986 to 2008 and 2009 to 2015 were included in Cohort 1 and Cohort 2, respectively. Immunologic serologies were not routinely collected and assessed in all patients prior to cardiac transplantation. Proven or probable cases of IA were identified from a systematic search of the paper and electronic medical

records and defined according to the European Organization for Research and Treatment of Cancer/Mycoses Study Group consensus definitions.[11] Each case of IA was matched to two randomized controls from the same year of cardiac transplantation. Data was collected from the date of cardiac transplant to the date of documented *Aspergillus* infection for each case, or for an equivalent number of days for each control. Data collected in all patients included baseline demographics, induction and maintenance immunosuppressive regimens, episodes of acute cellular allograft rejection (ACR) treated with increased immunosuppressive therapy, and post-transplant health status. Data collected for each case of IA included date of diagnosis, date of *Aspergillus* hospitalization, and mortality secondary to IA. The study protocol was approved by the Institutional Review Board and no informed consent was required.

Immunosuppression

All patients received an intra-operative intravenous infusion of methylprednisolone 500 mg, followed by intravenous methylprednisolone 125 mg every 8 hours for three consecutive doses, and a subsequent weight-based steroid taper, with a goal prednisone dose of 20 mg/day by post-operative days 14 – 30. Further biopsy-guided steroid weaning continued over the first year post-transplant. The standard maintenance immunosuppression regimen consisted of cyclosporine, azathioprine, and prednisone from 1986 to 1998; cyclosporine, mycophenolate mofetil, and prednisone from 1998 to 2006; and tacrolimus, mycophenolate mofetil, and prednisone from 2006 to 2015. Patients with an elevated panel reactive antibody > 75%; a positive B- or T-cell crossmatch; or pre- or post-operative renal impairment (serum creatinine > 1.5 mg/dL, creatinine clearance < 50 mL/min, or post-operative anuria) resulting in the delay of potentially nephrotoxic calcineurin inhibitors by > 48 – 72 hours were eligible for induction therapy with equine-derived antithymocyte globulin (ATGAM; 1986 – 2015), rabbit-derived antithymocyte globulin (ATG, 1986 – 2015), muromonab-CD3 (OKT3;1986 – 2010), daclizumab (1997 – 2009) or basiliximab (1998-2015).

Acute Cellular Allograft Rejection

Surveillance endomyocardial biopsies were generally performed at 1, 2, 3, 4, 6, and 8 weeks post-transplant, then monthly thereafter up through the first 8-12 months, then yearly for at least the subsequent 3 years. Additional biopsies were performed at the discretion of the treating physician in patients with a prior rejection episode, symptoms concerning for acute cellular rejection (ACR) on clinical presentation, a recent reduction in a component of the immunosuppression regimen, or concurrent infection. Biopsy specimens were assessed for histopathological evidence of ACR by the 1990 standard criteria of the International Society of Heart and Lung Transplantation (ISHLT), followed by the revised ISHLT criteria after 2005.[12] Patients with documented Grade 2 to 4 ACR on endomyocardial biopsy requiring increased doses of oral or intravenous corticosteroids and/or ATGAM, ATG, or OKT3, were considered to have treated ACR.

Anti-microbial and Anti-viral Prophylaxis

Pneumocystis jirovecii and *Toxoplasma gondii* prophylaxis with oral trimethoprim-sulfamethoxazole was provided to all patients for at least six months following cardiac transplantation. Glucose-6-phosphate dehydrogenase testing was used to guide the use of dapsone or atovaquone in patients with a documented sulfa allergy or intolerance to trimethoprim-sulfamethoxazole. Universal CMV prophylaxis with intravenous ganciclovir or oral valganciclovir was provided to moderate (CMV Donor/Recipient serostatus D+/R+, D-/R+) and high (D+/R-) risk patients for at least 3 and 6 months post-transplant, respectively. Herpes simplex virus prophylaxis with acyclovir was provided to patients at low risk of CMV (D-/R-) for at least one month post-transplant.

Anti-fungal Prophylaxis

Prior to 2009, pharmacologic prophylaxis against *Aspergillus* was not provided to cardiac transplant patients at our institution. After 2009, cardiac transplant recipients possessing at least one clinical risk factor for IA received targeted anti-fungal prophylaxis with universal oral voriconazole 200 mg twice daily, titrated to a target trough concentration of 1 – 5.5 mcg/mL. Clinical risk factors

for IA included treatment for acute rejection with IV corticosteroids during the first 3-6 months post-transplant or repeated pulse steroids for recurrent rejection, and severe immunocompromised critical illness. Oral voriconazole prophylaxis was continued for a minimum of one month post-transplant during receipt of the standard weight-based prednisone taper and was discontinued at the discretion of the treating physician based on resolution of risk factors. A 30-day duration of antifungal prophylaxis was selected as no guidelines existed until nearing the end of the cohort 2 study period. The selection of the 30-day duration was largely due to the administration of high-doses of steroids as a component of a steroid taper scheduled to be completed 30 days post-transplant. Serum voriconazole trough concentrations were not routinely collected and were obtained at the discretion of the treating physician and clinical pharmacist. In general, levels were commonly obtained 12 hours following the last dose and after the patient has received at least 5-7 days of therapy. Oropharyngeal candidiasis prophylaxis with nystatin was provided for a minimum of one month post-transplant.

Statistical Methods

Patient characteristics between cases and controls in both cohorts were compared using the Fisher's exact test for continuous variables and the Wilcoxon rank-sum test for categorical variables. A series of logistic regression models were fit to all patients in Cohort 1 to identify the significant predictors of developing aspergillosis following cardiac transplant. Univariate models were fit for all important clinical factors to assess significance. Significant univariate predictors were then evaluated in a multivariate model to control for the effects of the other predictors. The small sample size of Cohort 2 precluded the use of comparative analyses between cohorts. Differences were considered significant at $p \leq 0.05$. SAS Version 9.4 (SAS Institute Inc., Cary, North Carolina) was used to perform all analyses.

Results

A total of 189 and 107 patients underwent cardiac transplantation at the University of North Carolina Medical Center from 1986 to 2008 and 2009 to 2015, respectively. A proven or probable case of IA was identified in 23 cardiac transplant patients within Cohort 1, conferring a cumulative incidence rate of 8.0%. *Aspergillus* infections occurred at a mean onset of 178 ± 463 days post-transplant and mortality attributable to IA occurred in 43% of infected patients. Ninety percent of deaths occurred within the index *Aspergillus* hospitalization. Following the implementation of targeted anti-fungal prophylaxis with oral voriconazole, a proven or probable case of IA was identified in 2 cardiac transplant patients within Cohort 2, representing a cumulative incidence rate of 1.9%. The onset of *Aspergillus* infections ranged from 90 – 113 days post-transplant and mortality attributable to IA occurred in 1 of 2 infected patients. All documented deaths occurred during the index *Aspergillus* hospitalization.

The baseline clinical characteristics and post-transplant demographics of Cohort 1 and Cohort 2 are presented in Table 1. Compared to matched controls, patients with a documented case of proven or probable IA in Cohort 1 possessed lower WBC counts following cardiac transplantation (4.2 ± 2.6 x 10^9 cells/L vs 6.3 ± 3.3 x 10^9 cells/L; $p = 0.01$) and lower serum albumin concentrations at the time of *Aspergillus* diagnosis (2.8 ± 0.7 g/dL vs 3.3 ± 0.7 g/dL; $p = 0.05$). No significant differences in the rates of induction therapy (30% vs 26%; $p = 0.78$), post-transplant hemodialysis (22% vs 7%; $p = 0.11$), post-transplant CMV infection (22% vs 20%; $p = 1.00$), lympholytic anti-rejection therapy (0% vs 0%; $p = 1.00$), maintenance immunosuppression (Table 2), or the remaining clinical characteristics and post-transplant demographics were observed in Cohort 1.

Older age at transplantation and the receipt of higher mean prednisone doses from one month post-transplant to the date of *Aspergillus* diagnosis were risk factors for IA following cardiac transplantation in Cohort 1 in univariate analysis, but not after adjustment for potential confounders in multivariate analysis (Table 3). Conversely, higher post-transplant white blood cell (WBC) counts and serum albumin concentrations at *Aspergillus* diagnosis were protective against IA in univariate and

multivariate analyses. Independent risk factors for IA in multivariate analysis included an increased number of pre-transplant hospitalizations (OR 1.81, 95% CI 1.19 – 2.76) and post-transplant ACR episodes (OR 1.99, 95% 1.06 – 3.75).

Discussion

Invasive *Aspergillus* infections were a severe complication following cardiac transplantation in our retrospective analysis encompassing thirty years of secular trends in immunosuppression and anti-infective prophylaxis. Prior to the implementation of targeted anti-fungal prophylaxis at our institution, a documented case of proven or probable IA was identified in approximately 8% of patients who underwent cardiac transplantation, while mortality attributable to IA occurred in 43% of patients. These rates are comparable to the incidence rates of 5% – 9% and mortality rates of 35% – 67% reported in previous studies of cardiac transplant recipients.[3, 9, 10]

Although risk factors for the development of IFD following solid organ transplantation have been incorporated into published practice guidelines governing the therapeutic application of targeted anti-fungal prophylaxis, data regarding the predictors for invasive *Aspergillus* infections in a homogenous population of cardiac transplant recipients is limited. [2, 9, 13, 14] In a multivariate model, we found that an increased number of pre-transplant hospitalizations and post-transplant ACR episodes were independent risk factors for developing IA following cardiac transplantation in Cohort 1. These findings were likely reflective of more aggressive immunosuppression to treat ACR and the higher baseline severity of illness, where 74% of patients were listed as United Network for Organ Sharing Status 1A or 1B. Higher post-transplant WBC counts and serum albumin concentrations at the time of *Aspergillus* diagnosis were protective factors, further emphasizing the association between aggressive immunosuppression, greater severity of illness, and developing IA.

In other studies, post-transplant hemodialysis and post-transplant CMV infection have been identified as independent risk factors for developing early-onset IA following cardiac transplantation. In a retrospective analysis of 278 patients who underwent cardiac transplant between 1988 and 2002, 24 of 278 (8.6%) patients developed IA at a median onset of 50 ± 63 days post-transplant. Post-

transplant hemodialysis (relative risk [RR] 4.9, 95% CI 1.2 – 18) and post-transplant CMV infection (RR 5.2, 95% CI 2 – 13.9) increased the risk of developing IA by approximately 5-fold.[9] We were unable to replicate these results in our analysis, a discordance which may be partially explained by our small sample size and the 178 ± 463 day mean onset of IA observed in our study. While post-transplant hemodialysis (OR 3.2, 95% CI 1.3 – 8.1) and post-transplant CMV infection (OR 2.3, 95% CI 1.1 – 4.9) remained independent predictors of early-onset IA in a case-control study of 11,014 solid organ transplant recipients, they were not found to be independent risk factors for late-onset IA in multivariate analysis.[13] Additional research should be conducted to assess whether risk factors differ between early- and late-onset invasive *Aspergillus* infections in a homogenous population of cardiac transplant recipients so that patient risk stratification and timing or duration of targeted anti-fungal prophylactic strategies may be optimized.

Following the implementation of targeted anti-fungal prophylaxis with oral voriconazole at our institution, the cumulative incidence of invasive *Aspergillus* infections following cardiac transplantation declined from 8% to 2%. An equivalent reduction in aspergillosis incidence was observed in a prospective cohort of 133 patients who underwent cardiac transplant from 2003 to 2010.[15] Targeted anti-fungal prophylaxis with an echinocandin (caspofungin, anidulafungin, or micafungin) was administered to 13 of 133 (9.8%) patients upon identification of risk factors for IA and continued for a median of 30 (23.5 – 30) days beyond risk factor resolution. Targeted antifungal prophylaxis was effective in all but one patient who should have received a higher weight-based dose of caspofungin and reduced the incidence of IA (8.6% vs. 2.2%; p=0.01) and *Aspergillus* mortality (5.8% vs. 1.5%; p=0.06) despite an aspergillosis outbreak in the ICU. These results were corroborated in an analysis of 85 patients who underwent cardiac transplant from 2004 to 2012.[2] Following the implementation of targeted anti-fungal prophylaxis with IV caspofungin in 2010, given as a 70 mg loading dose on day one, followed by a 50 mg daily maintenance dose until 2 weeks after risk factor resolution, the rate of IFD declined from 10% to 4%. Administration of caspofungin prophylaxis was also associated with a 75% reduction in 30-day mortality (HR 0.25, 95% CI 0.09 – 0.8) when compared to patients who did not receive targeted anti-fungal prophylaxis. Universal anti-fungal

prophylaxis with oral itraconazole, given as a 600 mg loading dose for three consecutive days, followed by a 400 mg maintenance dose for 3 to 6 months post-transplant, was also shown to confer significant reductions in the risk of IA (RR 0.2, 95% CI 0.07 – 0.9) and improvements in 1-year survival (RR 0.5, 95% CI 0.3 – 0.8) in a study of 278 patients who underwent cardiac transplantation between 1988 and 2002.[9]

Our study has several important limitations. Given the retrospective nature of this study, reliability of data collection for proven or probable cases of IA and matched controls may have been affected by variability in documentation given the transition from paper to electronic medical records. Additionally, associations between Aspergillus colonization or re-operation were not specifically evaluated. Secular changes in immunosuppression protocols over the last thirty years and building construction at our institution between 1990 - 2000 have likely affected the incidence of invasive *Aspergillus* infections and the independent risk factors associated with developing IA following cardiac transplantation. We were unable to confirm this hypothesis as the small sample size of Cohort 2 limited analysis to detect risk factors and precluded comparative analyses between Cohort 1 and Cohort 2. However, this limitation may have been a direct result of the reduced incidence of invasive *Aspergillus* infections observed in Cohort 2 following the implementation of targeted anti-fungal prophylaxis. Lastly, the study's single center design may limit the generalizability of our findings to other cardiac transplant centers.

Beyond risk factors outlined by the IDSA guidelines, our study characterized several additional risk factors that may assist with identifying cardiac transplant recipients at highest risk for invasive *Aspergillus* infections. In addition, our study findings confirm the role of targeted anti-fungal prophylaxis in reducing the incidence of this life-threatening infection. An increased number of pre-transplant hospitalizations and post-transplant ACR episodes were independent risk factors for developing IA in our population of single-organ cardiac transplant recipients. In contrast, higher white blood cell counts and serum albumin concentrations were protective factors. Our findings should be validated in a prospective, multicenter study with a focus on the timing and duration of anti-fungal prophylaxis.

Disclosure Statement

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Dr. Jo Ellen Rodgers has served on an advisory board and as a heart failure consultant for Novartis Pharmaceuticals. The remaining authors do not have any conflicts of interest to disclosure.

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Table 1. Comparison of Clinical Characteristics and Transplant Demographics between Cases of Aspergillosis and Matched Controls in Heart Transplant Recipients from Cohorts 1 and 2

	Cohort 1 (1986 - 2008)		**Cohort 2 (2009 – 2015)**	
Characteristic	Case (n=23)	Control (n=46)	Case (n=2)	Control (n=4)
Recipient age, years	54 ± 9	46 ± 14	53 ± 1	54 ± 12
Male	74	74	50	75
Caucasian	78	54	0	50
UNOS status 1/2	74/22	61/35	100/0	100/0
Hospitalizations 1 year pre-transplant, *n*	2.4 ± 2.4	1.6 ± 1.6	3.0 ± 1.4	3.0 ± 0.5
Induction immunosuppression	30	26	0	50
Diabetes pre-transplant	30	17	50	0
Diabetes post-transplant	57	50	100	50
CMV post-transplant	22	20	50	0
Baseline SCr > 1.5 mg/dL	39	39	0	0
Hemodialysis post-transplant	22	7	50	0
Lowest WBC count post-transplant, x 10^9 cells/L	4.2 ± 2.6	6.3 ± 3.3*	4 ± 4.5	6.9 ± 5.1
Albumin, g/dL[†]	2.8 ± 0.7	3.3 ± 0.7*	2.9 ± 0.7	—[‡]
Patients with treated ACR episode	52	52	100	50
Total number of ACR episodes, *n*	31	37	2	2
Rejections per person	1.4 ± 0.98	0.8 ± 1.6	1.0 ± 0.0	0.4 ± 0.6
ISHLT grade 3A/3B rejections	45	46	100	25
Time to initial rejection, days	31 ± 22	24 ± 25	8 ± 1	18 ± 13
Median (range)	18 (8 – 81)	20 (6 – 90)	9 (8 – 9)	18 (9 – 27)
Anti-rejection lympholytic therapy	0	0	0	0

Abbreviations: ACR, acute cellular rejection; CMV, cytomegalovirus; ISHLT, International Society for Heart and Lung Transplantation; SCr, serum creatinine; UNOS, United Network for Organ Sharing; WBC, white blood cell.

Values are reported as mean ± standard deviations or percentages unless otherwise indicated.

[*] *p*-value ≤ 0.05 for cases vs. controls in Cohort 1.

[†] At the time of aspergillosis diagnosis.

[‡] Serum albumin concentrations were not obtained at the time of aspergillosis diagnosis.

Table 2. Immunosuppression Before and At Time of Aspergillosis Diagnosis in Cardiac Transplant Recipients from Cohorts 1 and 2

	Cohort 1 (1986 – 2008)		**Cohort 2 (2009 – 2015)**	
Immunosuppression	Case[†] (n=23)	Control[‡] (n=46)	Case (n=2)	Control (n=4)
From 1 Month Post-Transplant to *Aspergillus* Diagnosis				
Cyclosporine trough concentration (ng/mL)	284 ± 81	330 ± 95	—	—
Tacrolimus trough concentration (ng/mL)	11 ± 3	10 ± 2	9 ± 2	8 ± 3.2
Prednisone dose (mg/kg/day)	0.6 ± 0.6	0.4 ± 0.2	0.3 ± 0.1	0.2 ± 0.1[§]
At Time of *Aspergillus* Diagnosis				
Cyclosporine trough concentration (ng/mL)	246 ± 128	331 ± 156	—	—
Tacrolimus trough concentration (ng/mL)	15 ± 6	14 ± 6	6.4 ± 0.6	8.9 ± 2.5
Prednisone dose (mg/kg/day)	0.4 ± 0.3	0.3 ± 0.2	0.2 ± 0.1	0.2 ± 0.04[§]

Values are reported as mean ± standard deviations unless otherwise indicated.

[†]*n* = 8, 15, and 23 for cyclosporine, tacrolimus, and prednisone, respectively.

[‡]*n* = 17, 29, and 26 for cyclosporine, tacrolimus, and prednisone, respectively.

[§]*n* = 3.

Table 3. Predictors for Developing Aspergillosis Following Cardiac Transplant in Cohort 1 (*n* = 69)

Risk Factor	Univariate Analysis OR (95% CI)	Multivariate Analysis OR (95% CI)
Recipient age, years	1.05 (1.00 – 1.11)	1.06 (0.97 – 1.14)
Average prednisone dose from 1 month post-transplant to *Aspergillus* diagnosis	5.91 (1.13 – 30.82)	25.13 (0.86 – 737.43)
Lowest WBC count prior to *Aspergillus* diagnosis, x 10^9 cells/L	0.80 (0.66 – 0.96)	0.74 (0.57 – 0.97)
Serum albumin concentration at *Aspergillus* diagnosis, g/dL[†]	0.37 (0.16 – 0.87)	0.13 (0.03 – 0.52)
Number of hospitalizations 1 year pre-transplant	1.25 (0.95 – 1.63)	1.81 (1.19 – 2.76)
Number of ACR episodes prior to *Aspergillus* diagnosis	1.46 (0.97 – 2.19)	1.99 (1.06 – 3.75)

Abbreviations: ACR, acute cellular rejection; CI, confidence interval; OR, odds ratio; WBC, white blood cell.

[†]*n* = 67.