MAJOR ARTICLE

Zygomycosis in Solid Organ Transplant Recipients: A Prospective, Matched Case-Control Study to Assess Risks for Disease and Outcome

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Background. Clinical characteristics, risks, and outcomes in solid organ transplant (SOT) recipients with zygomycosis in the era of modern immunosuppressive and newer antifungal agent use have not been defined.

Methods. In a matched case-controlled study, SOT recipients with zygomycosis were prospectively studied. The primary outcome measure was success (complete or partial response) at 90 days.

Results. Renal failure (odds ratio [OR], 3.17; $P = .010$), diabetes mellitus (OR, 8.11; $P < .001$), and prior voriconazole and/or caspofungin use (OR, 4.41; $P = .033$) were associated with a higher risk of zygomycosis, whereas tacrolimus (OR, 0.23; $P = .002$) was associated with a lower risk of zygomycosis. Liver transplant recipients were more likely to have disseminated disease (OR, 5.48; $P = .021$) and developed zygomycosis earlier after transplantation than did other SOT recipients (median, 0.8 vs 5.7 months; $P < .001$). Overall the treatment success rate was 60%. Renal failure (OR, 11.3; $P = .023$) and disseminated disease (OR, 14.6; $P = .027$) were independently predictive of treatment failure, whereas surgical resection was associated with treatment success (OR, 33.3; $P = .003$). The success rate with liposomal amphotericin B was 4-fold higher even when controlling for the aforementioned variables.

Conclusions. The risks identified for zygomycosis and for disseminated disease, including those that were previously unrecognized, have implications for further elucidating the biologic basis and for optimizing outcomes in SOT recipients with zygomycosis

Zygomycetes have emerged as important opportunistic pathogens in immunocompromised hosts [1–7]. Traditionally, mycelial fungi other than *Aspergillus* have accounted for ~2% of the mold infections in solid organ transplant (SOT) recipients [8]. However, 27% of the mold infections in SOT recipients in a recent study were due to these previously infrequently encountered fungi [8]. The mortality rate among SOT recipients with zygomycosis has typically ranged from 49% to 71% [1, 3]. Indeed, grave outcomes of zygomycosis in these patients are underscored by the fact that mortality associated with zygomycosis exceeded that attributable to all other invasive mold infections [8].

Transplantation practices and immunosuppressive regimens have continued to evolve. Emerging data suggest that potent T cell–depleting antibodies that are increasingly used as immunosuppressive agents in SOT recipients may be associated with a higher risk of invasive fungal infections [9, 10]. In patients receiving cancer chemotherapy and hematopoietic stem cell transplant recipients, growing prophylactic and empiric use of voriconazole has been proposed to increase the risk of zygomycosis [11–14]. Others have argued that

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the use of any antifungal agent to which the zygomycetes are innately resistant may predispose to this mold infection [15]. Currently, the roles of variables such as prior use of antifungal agents and T cell therapies in predisposing to zygomycosis after solid organ transplantation have not been fully defined. The goals of this study were to delineate the epidemiologic characteristics and to determine risk factors and outcomes associated with zygomycosis in SOT recipients in the current era.

METHODS

This was a prospective, matched case-controlled study involving consecutive SOT recipients with zygomycosis at our institutions from 2003 through 2007. Patients were included in the study if they had proven or probable zygomycosis as defined by previously proposed consensus criteria [16]. For each case patient, the next transplant recipient (closest to the case temporally with regard to time of transplantation) undergoing the same type of transplantation, but without zygomycosis, was selected as a control. Thus, control patients were matched with case patients for the type of transplant, time of transplantation (to ensure the same duration of follow-up), and transplant center. Timing of initiation of the study varied for different sites. Institutional review board approval was obtained at each site as per institutional requirements.

Data collected included demographic characteristics, immunosuppressive regimen at the time of diagnosis, rejection episodes occurring within 60 days before diagnosis, cytomegalovirus infection, antifungal agent use within 6 months of diagnosis, sites and types of invasive disease, antifungal therapy employed, surgical resection, and mortality at 90 days. The following variables were considered at baseline (or at time of diagnosis of disease): renal failure (defined as a creatinine level >2.0 mg/dL), requirement of dialysis, and retransplantation. T cell antibodies were categorized as nondepleting (interleukin [IL]-2 receptor antibodies, such as basiliximab or daclizumab) or depleting (alemtuzumab or antithymocyte globulin), depending on whether they reduce responsiveness to T cells with or without depleting them [17]. Disseminated zygomycosis was defined to be central nervous system disease or disease with >2 noncontiguous sites of involvement [8]. A granulocyte count <1000 cells/uL was considered to indicate neutropenia. Response to therapy was categorized as complete (resolution of all attributable signs and symptoms and radiographic abnormalities), partial (clinical improvement and 75% improvement in radiographic abnormalities), stable (no improvement in clinical manifestations and <50% improvement in radiographic findings), or failure (deterioration) as reported elsewhere [18, 19]. Complete and partial responses were considered to be successful outcomes, and failure, stable, or indeterminate responses were considered to be unsuccessful outcomes or failures [18]. The primary end point was a successful outcome at 90 days.

Statistical analyses. Intercooled Stata version 9.2 (StataCorp) was used for statistical analyses. The McNemar's χ^2 was used for examining categorical risks for disease between the case and control patients in pair-wise comparison. The Wilcoxon matched-pairs signed-ranks test was used to evaluate continuous variables. A logistic model clustered on pairing was used to estimate the effect of multiple variables. The dependent variable was zygomycosis, and the explanatory variables were factors found to be associated with disease in univariate analysis. Variables related to disease at $P < .1$ in univariate comparisons were entered into the logistic regression model. The χ^2 test for trend was used to appraise the increased risk of disease for the number of risk factors present in a patient. Risk factors for dissemination (compared with localized disease) were evaluated using the maximum likelihood estimate of the odds ratio (OR). Similarly, the maximum likelihood estimate of the OR was used to assess risk effects of multiple factors on outcome. The variables found to be associated with failure at $P < .1$ in univariate analysis were entered into the logistic regression model. Treatment differences were examined using the log likelihood ratio for categorical values and the Kruskal-Wallis rank test for continuous variables.

RESULTS

The study population comprised 100 SOT recipients that included 50 consecutive patients with zygomycosis (referred to as case patients) and 50 matched control patients. The demographic and clinical characteristics of the case patients are outlined in table 1. Zygomycosis developed a median of 5 months (interquartile range, 6 weeks to 12 months) after transplantation. Of 50 case patients, 44 (88%) had proven and 6 (12%) had probable zygomycosis. The most common clinical manifestation was pulmonary disease (24 [48%] of 50 case patients), and in 18 (39%) of 50 case patients, the lung was the only site of involvement (table 2). Of 13 patients with rhino-orbital-cerebral zygomycosis, 2 had central nervous system disease (table 2). Disseminated disease was documented in 13 (26%) of 50. *Rhizopus* species, *Mucor* species, and *Mycocladus corymbifer* (formerly *Absidia corymbifera*) were the most frequently cultured zygomycetes (table 2). Participating institutions contributed a median of 2 case (range, 0–6); 3 centers had 3 cases each, and 5 centers had 6 cases each during the study period.

Risk factor for zygomycosis. Candidate variables examined as risk factors for zygomycosis are depicted in table 3. A total of 25 (50%) of 50 case patients had received prior antifungal therapy that included fluconazole in 7 patients, itraconazole in 4, voriconazole in 4, caspofungin in 2, ketoconazole in 1, and a combination of an azole and caspofungin in 7 patients. In

Table 1. Demographic and Clinical Characteristics of the Patients with Zygomycosis

Characteristic	Patients with zygomycosis (n = 50)
Age, median years (interquartile range)	53 (47–59)
Male sex	35 (70)
Type of transplant	
Kidney	16
Liver	11
Lung	9
Heart	5
Heart-kidney	4
Kidney-pancreas	2
Pancreas	1
Small-bowel	1
Multivisceral	1
Immunosuppression	
Tacrolimus-based	
All	33 (66)
Tacrolimus, mycophenolate mofetil, prednisone	19
Tacrolimus, prednisone	7
Tacrolimus, sirolimus, prednisone	3
Tacrolimus only	2
Tacrolimus, azathioprine, prednisone	1
Tacrolimus, mycophenolate mofetil	1
Cyclosporine A–based	
All	13 (26)
Cyclosporine A, mycophenolate mofetil, prednisone	7
Cyclosporine A, azathioprine, prednisone	6
Other	
All	4 (8)
Prednisone, mycophenolate mofetil	2
Sirolimus, mycophenolate mofetil, prednisone	1
Prednisone	
Any prednisone use	48 (96)
Prednisone only	1
Dose, median mg	15
Prior rejection	16 (32)
Retransplantation	16 (22)
Renal failure at baseline	28 (58)
Dialysis at baseline	19 (38)
Cytomegalovirus infection	7 (14)
Diabetes mellitus requiring insulin	19 (38)
APACHE II, median score (range)	16 (10–30)

NOTE. Data are no. or no. (%) of patients, unless otherwise indicated.

univariate analysis, retransplantation, renal failure at baseline, requirement of dialysis at baseline, insulin dependent diabetes mellitus, prior rejection, and prior use of voriconazole and/or caspofungin were significantly associated with zygomycosis, whereas tacrolimus was protective against zygomycosis (table 3). Prior use of voriconazole, although more common among case patients, was not statistically significantly different for case patients, compared with control patients (OR, 5.0 [95% confidence interval {CI}, 0.58–42.8]; $P = .14$). Because all 9 patients who had previously received caspofungin were case patients, the risk for the development of disease was incalculable with an OR approaching infinity. Age, cytomegalovirus infec-

Table 2. Patterns of Invasive Disease and Microbiologic Findings in Patients with Zygomycosis

Pattern or finding	No. or no. (%) of patients with zygomycosis (n = 50)
Type of invasive disease	
Proven	44 (88)
Probable	6 (12)
Site of involvement[a]	
Pulmonary	
Any	24 (48)
Only	18 (39)
Rhino-orbital-cerebral	
Any	13 (26)
Rhino-orbital	11
Rhino-orbital-cerebral	2
Cutaneous-soft tissue	
Any	11 (22)
Surgical wound site	4
Ulcerative/necrotic lesions	4
Vascular catheter site	2
Necrotizing fascitis	1
Gastrointestinal (any)	6 (12)
Disseminated disease[b]	13 (26)
Microbiologic finding	
Positive culture results	
Any	46 (92)
Rhizopus species	
All	16/46 (35)
Not speciated	10
Rhizopus microsporus	3
Rhizopus oryzae	2
Rhizopus arrhizus	1
Mucor species	17/46 (37)
Mycocladus corymbifer	6/44 (13)
Apophysomyces elegans	1
Cunninghamella bertholletiae	1
Blakeslea trispora	1
Species not identified	4
Histopathologic diagnosis only	4

[a] Some patients had >1 site of involvement.

[b] Sites of disseminated disease included central nervous system in 2 patients, cutaneous and pulmonary in 2, myositis and sinus in 1, gastrointestinal and lung in 1, kidney and spleen in 1, cutaneous and noncontiguous bone in 1, and multiple sites in 5 patients (4 of these cases were diagnosed at autopsy).

tion, neutropenia, and T cell depleting or nondepleting antibody use were not significantly associated with zygomycosis (table 3).

In logistic regression analysis (with retransplantation, renal failure, diabetes mellitus, prior voriconazole and/or caspofungin use, and tacrolimus in the model), renal failure, diabetes mellitus, and voriconazole and/or caspofungin use were significantly associated with a higher risk of zygomycosis, whereas tacrolimus was associated with a lower risk of zygomycosis in the study patients (table 3).

Risks for disseminated disease. Overall, disseminated disease occurred in 13 (26%) of 50 case patients; 55% of infections were in liver, 20% were in heart, 13% were in kidney, and 11% were in lung transplant recipients. When variables predictive of disseminated zygomycosis were examined (table 4), liver transplantation was associated with a 5-fold higher risk of disseminated disease (OR, 5.48 [95% CI, 1.3–23.1]; $P = .021$) that could not be explained by differences in other risk factors; renal failure (55% vs 59%; $P = .79$), dialysis (36% vs 33%; $P = .70$), diabetes mellitus (36% vs 39%; $P = .90$), rejection (37% vs 33%; $P = .70$), cytomegalovirus infection (18% vs 13%; $P = .65$), depleting T cell antibody use (9% vs 15%; $P = .48$), prior antifungal agent use (64% vs 44%; $P = .24$), or APACHE II scores (mean score, 22 vs 19; $P = .88$) did not differ for 11 liver transplant patients, compared with 39 other SOT recipients. Zygomycosis also occurred significantly earlier after transplantation in liver versus other organ transplant recipients (median, 0.8 vs 5.7 months after transplantation; $P < .001$).

Outcome. The survival rate at 90 days was 62% (31 of 50 patients) among case patients, compared with 98% (49 of 50 patients) among control patients ($P = .001$). The success rate was 54% (13 of 24 patients) among case patients with any pulmonary involvement, 67% (12 of 18) among those with localized pulmonary disease, 69% (9 of 13) among those with rhino-orbital-cerebral disease, 83% (5 of 6) among those with cutaneous disease, and 0% (0 of 2) among those with gastrointestinal disease ($P = .24$). When analyzed by zygomycetes species, the success rate was 68% (11 of 16 patients) among patients infected with *Rhizopus* species, 59% (10 of 17) among those infected with *Mucor* species, 50% (3 of 6) among those infected with *Mycocladus* species, 100% for 1 patient infected with *Cunninghamella bertholletiae,* 100% for 1 patient infected with *Blakeslea trispora,* and 0% for 1 patient infected with *Apophysomyces elegans.*

Overall, the success rate among case patients was 60% (30 of 50 patients). Excluding 4 case patients in whom the diagnosis of zygomycosis was made at autopsy and 5 who received <7 days of antifungal therapy before death, the treatment consisted of liposomal amphotericin B (AmB) in 17 (42%), AmB lipid complex in 8 (20%), AmB deoxycholate in 5 (12%), posaconazole in 5 (12%), and a combination of antifungal agents as initial therapy in 6 (15%) of the 41 patients (table 5). Antifungal therapy consisted of liposomal AmB plus posaconazole in 5 of 6 patients who received combination therapy (table 5). The success rate with each of these regimens is outlined in table 5. Compared with liposomal AmB, the odds of successful treatment were lower with AmB deoxycholate (OR, 0.09 [95% CI, 0.006–1.4]; $P = .086$), AmB lipid complex (OR, 1.0 [95% CI,

Table 3. Risk Factors for Zygomycosis in the 50 Case Patients with Zygomycosis, Compared with Paired Control Patients

	Univariate analysis		Multivariate analysis	
Variable	OR (95% CI)	*P*	OR (95% CI)	*P*
Age	1.02 (0.99–1.04)	.213	…	
Retransplant	6.9 (1.6–31.0)	.011	5.67 (0.86–37.5)	.072
Diabetes mellitus	3.8 (1.51–9.39)	.004	8.11 (2.70–24.4)	<.001
Prior rejection	2.9 (1.1–7.5)	.03	2.62 (0.79–8.71)	.115
Renal failure at baseline	2.7 (1.3–5.4)	.006	3.17 (1.31–7.65)	.010
Dialysis at baseline	3.2 (1.4–7.5)	.007	…	
Cytomegalovirus infection	1.7 (0.42–6.88)	.451	…	
Prior voriconazole or caspofungin use	10.9 (1.4–85.2)	.002	4.41 (1.12–17.3)	.033
Immunosuppression				
Tacrolimus	0.52 (0.30–0.88)	.016	0.23 (0.09–0.57)[a]	.002
Cyclosporine A	1.5 (0.79–2.9)	.206	…	
Sirolimus	4.3 (0.43–42.0)	.215	…	
T cell antibodies				
Any	1.0 (0.5–1.9)	.99	…	
Depleting	1.2 (0.5–2.6)	.66	…	
Nondepleting	1.0 (0.5–2.1)	.99	…	

NOTE. CI, confidence interval; OR, odds ratio.

[a] Inverse risk (ie, risk of a lower rate of zygomycosis): OR, 4.41 (95% CI, 1.12–17.3); $P = .002$.

0.008–1.23]; $P = .073$), or posaconazole (OR, 0.09 [95% CI, 0.006–1.4]; $P = .086$), although these differences were not statistically significant. On the other hand, compared with liposomal AmB, the success rate was significantly lower with combination therapy (OR, 0.06 [95% CI, 0.005–0.8]; $P = .035$). However, patients receiving combination antifungal therapy were more likely to have disseminated disease than were those receiving any of the monotherapy regimens for the treatment of zygomycosis (4 [67%] of 6 vs 3 [9%] of 35 patients; $P < .005$) (table 5).

Sixteen of 41 patients had a change in initial antifungal therapy; the reasons for the change included step down to oral therapy in 8, progressive disease in 5, and nephrotoxicity attributable to antifungal therapy in 3 patients. The mean duration of initial and the total duration of antifungal therapy were 44 and 60 days, respectively (table 5). The success rate was not significantly different for patients who had no change in initial antifungal therapy (17 [66%] of 25), compared with those in whom initial treatment was changed because of toxicity or step down therapy (9 [81%] of 11) or progressive disease (4 [80%] of 5; $P = .52$).

Candidate variables analyzed as predictors of outcome (treatment failure) are outlined in table 6. In univariate analysis, renal failure ($P = .05$), disseminated disease ($P = .01$), T cell depleting antibody use ($P = .067$) were associated with treatment failure, whereas surgical resection ($P = .001$) and initial antifungal treatment with liposomal AmB ($P = .07$) were protective against treatment failure (or associated with success). In a logistic regression analysis that included the aforementioned variables in the model, only renal failure ($P = .023$) and disseminated disease ($P = .027$) were independently predictive of treatment failure, whereas surgical resection was associated with success ($P = .003$) (table 6).

DISCUSSION

Within the past decade, a number of reports of zygomycetes infection, primarily in patients with hematologic malignancy or neutropenia, have highlighted the growing relevance of zygomycetes and grave outcomes associated with infection due to these molds. There are several observations from our study that focused solely on SOT recipients, a patient population in which few systematically conducted studies on zygomycosis currently exist. Although still an infrequently occurring disease, notable changes in the epidemiologic characteristics of this disease in SOT recipients have occurred. In 2 previous reviews that summarized the world's literature on zygomycosis through 1995 and 2002, rhino-orbital-cerebral zygomycosis was the most frequently occurring form of disease, accounting for 57% and 31% of the cases in these reports, respectively [3, 6]. Our data show that, currently, pulmonary disease is the most frequent form of zygomycosis among these patients, and that in ~40% of these cases, the disease is limited to the lungs (table 2).

We also show that liver transplant recipients were at a significantly higher risk of disseminated disease and developed zygomycosis earlier after transplantation than did all other types of SOT recipients. This propensity could not be explained by known risk factors for invasive fungal infection (tables 3 and

4) and may be related to unique host defense defects or iron overload in liver transplant recipients [20, 21]. Iron is not only a pivotal growth factor but also compromises critical host defenses against zygomycetes, including suppression of interferon-γ mediated macrophage and monocyte function, reduction of nitrous oxide in macrophages, and impairment of phagocytic activity [20, 22–24]. Iron also decreases the proliferation of T cells and alters T helper response towards Th2 predominance that facilitates disease progression [20]. Previous studies have shown that liver transplant recipients with iron overload are predisposed to early onset opportunistic infections, including invasive fungal infection [21, 25]. Although iron overload is potentially amenable to reversal on liver transplantation [20], this may not happen immediately, as evidenced by our data showing development of zygomycosis early after transplantation in liver transplant recipients.

Calcineurin inhibitors remain the mainstay of immunosuppression in SOT recipients. An unexpected observation from our study is that tacrolimus was associated with a 4-fold reduction in the risk of zygomycosis. Although used for its immunosuppressive effects, calcineurin plays a vital role in the virulence and pathogenicity of at least 3 other major opportunistic fungi, such as *Candida, Cryptococcus,* and *Aspergillus* species [26–28]. In SOT recipients with cryptococcosis, tacrolimus was associated with a lower risk of disseminated disease and overall mortality [29]. To our knowledge, the role of the calcineurin signaling pathway in the pathogenesis of zygomycetes has not been reported. However, calcineurin homolog genes have been identified in zygomycetes (Soo Chan Lee and J. Heitman, personal communication). Data from present and previous studies also show that diabetes mellitus independently increased the risk of zygomycosis even in patients with additional risk factors, such as SOT recipients receiving iatrogenic immunosuppressives or neutropenic patients with hematologic malignancies [11]. Other variables previously known to be risk factors for invasive fungal infection, such as retransplantation and renal failure, were also significantly associated with zygomycosis in our study.

A matter of significant and unresolved controversy is whether broad spectrum antifungal agents, in particular voriconazole, have led to increased rates of zygomycosis in immunocompromised hosts. In a matched, case-controlled study of patients with leukemia or allogeneic stem cell transplant recipients, voriconazole prophylaxis was significantly associated with zygomycosis (OR, 1.43 [95% CI, 1.11–1.85]) [11]. Additionally, voriconazole enhanced the virulence of zygomycetes in animal models [11, 30]. Pre-exposure to voriconazole in mice infected with *Rhizopus oryzae* was associated with higher mortality ($P = .01$). Anecdotal cases of zygomycosis after voriconazole use in SOT recipients have been reported [31]. However, existing data have not incontrovertibly documented an association between voriconazole and zygomycosis in this patient population. In a report published, thus far, only in abstract form, antifungal prophylaxis with voriconazole in 635 liver and multivisceral transplant recipients was associated with a reduction in invasive aspergillosis, compared with a control cohort of 538 patients (0.15% vs 2.2%; $P < .001$), but no selection of non-*Aspergillus* myecilial species [32]. A possible explanation for these observations may be that, unlike most other immunocompromised hosts, virtually all SOT recipients receive calcineurin-inhibitor agents that have shown synergistic interactions with voriconazole and other triazoles against zygomycetes in vitro [33, 34]. The clinical relevance of these findings, however, remains to be determined. Voriconazole use did not correlate significantly with zygomycosis in our study. However, the small number of patients who had previously received voriconazole may have precluded meaningful interpretation of these data. Nevertheless, prior antifungal agent use of voriconazole and/or caspofungin was significantly associated with a higher risk of zygomycosis in our patients.

Overall mortality rates in patients with zygomycosis who were receiving AmB deoxycholate have ranged from 39% to 57% [35, 36]. Currently, the polyenes, in particular the lipid formulations of AmB, are regarded as the first-line therapy for zygomycosis [37–40]. An analysis of 64 cases of zygomycosis,

Table 4. Risk factors for Disseminated Zygomycosis in the Study Patients

Variable	OR[a] (95% CI)	*P*
Age[b]	0.98 (0.9–1.04)	.69
Male sex[c]	1.6 (0.4–6.4)	.44
Retransplant	0.93 (0.2–4.1)	.93
Diabetes mellitus	0.6 (0.2–2.5)	.53
Renal failure[d]	1.2 (0.3–4.4)	.76
Dialysis	0.65 (0.16–2.5)	.53
Rejection	0.55 (0.13–2.4)	.42
Prior voriconazole/caspofungin use	1.38 (0.34–5.6)	.65
Cytomegalovirus infection	0.4 (0.05–3.9)	.45
Immunosuppression		
Tacrolimus	1.5 (0.4–5.9)	.52
T cell antibodies		
Any	1.9 (0.5–7.4)	.33
Depleting	2.5 (0.5–12.9)	.27
Neutropenia	1.8 (0.14–21.9)	.63
Type of transplant		
Liver	5.48 (1.3–23.1)	.02
Kidney	0.30 (0.06–1.6)	.15
Heart	2.06 (0.30–13.9)	.46
Lung	0.30 (0.03–2.7)	.28

NOTE. CI, confidence interval; OR, odds ratio.

[a] Odds of developing disseminated versus localized disease.

[b] Age was analyzed as a continuous variable.

[c] Odds are presented in comparison to female sex.

[d] Creatinine level ⩾2 mg/dL at diagnosis.

Table 5. Antifungal Treatment and Outcomes in Patients with Zygomycosis

	Outcome					Change in therapy	
Treatment	Disseminated disease	Surgical resection	APACHE II score >20	Duration of initial therapy, mean days (IQR)	Success rate	Step down or salvage for toxicity	Salvage for worsening disease
AmB deoxycholate ($n = 5$)	0/5 (0)	2/5 (40)	1/5 (20)	45 (20–50)	3/5 (60)	1/5 (20)	1/5 (20)
AmB lipid complex ($n = 8$)	0/8 (0)	4/8 (50)	4/8 (50)	55 (10–102)	5/8 (63)	2/8 (25)	1/8 (13)
Liposomal AmB ($n = 17$)	2/17 (12)	12/17 (71)	4/17 (23)	52 (21–59)	16/17 (94)	6/17 (35)	3/17 (18)
Posaconazole ($n = 5$)	1/5 (20)	2/5 (40)	2/5 (40)	34 (10–90)	3/5 (60)	0/5 (0)	…
Combination therapy[a] ($n = 6$)	4/6 (67)	4/6 (67)	1/6 (17)	15 (7–21)	3/6 (50)	2/6 (33)	…
P[b]	.01[c]	.58[d]	.58	.23	.10	.41	.42

NOTE. Data are proportion (%) of patients, unless otherwise indicated. Amb, amphotericin B; IQR, interquartile range.

[a] Combination therapy comprised liposomal AmB and posaconazole in 5 case patients (with successful outcome in 2 of 5) and liposomal AmB, caspofungin, and voriconazole in 1 case patient (with successful outcome).

[b] P values represent overall differences between the groups.

[c] Patients receiving combination therapy, compared with monotherapy, had a higher rate of disseminated disease (67% vs 9%; $P < .005$). There was no difference in the frequency of disseminated disease between the 4 monotherapy groups ($P = .83$).

[d] There was no significant difference in the rate of surgical resection between patients receiving monotherapy vs combination therapy (67% vs 57%; $P = .65$) or between the 4 monotherapy groups ($P = .12$).

Table 6. Variables associated with outcome (failure) in patients with zygomycosis.

Variable	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*P*	Adjusted OR (95% CI)	*P*
Age	1.02 (0.97–1.08)	.36	…	
Sex	1.00 (0.3–3.4)	.99	…	
Immunosuppression				
Tacrolimus-based	0.9 (0.3–3.1)	.72	…	
Cyclosporine based	1.5 (0.4–5.0)	.53	…	
T cell antibody				
Any	2.2 (0.67–7.5)	.21	…	
Depleting	4.7 (0.8–27.0)	.067	1.7 (0.09–32.9)	.70
Renal failure	3.4 (1.0–11.8)	.05	11.3 (1.3–92.6)	.023
Dialysis	3.6 (1.0–11.1)	.047	…	
Diabetes mellitus	1.1 (0.4–3.7)	.81	…	
Rejection	0.5 (0.2–2.0)	.39	…	
Cytomegalovirus infection	.21 (0.02–1.9)	.16	…	
Site of infection				
Pulmonary (only)	0.64 (0.2–2.1)	.47	…	
Pulmonary (any)	1.6 (0.5–5.0)	.42	…	
Disseminated	5.3 (1.3–21.0)	.01	14.6 (0.03–1.55)	.027
Prior voriconazole/caspofungin use	1.4 (0.4–5.0)	.60	…	
Neutropenia	0.78 (0.06–9.3)	.85	…	
Definite infection	1.38 (0.2–8.4)	.72	…	
Initial treatment				
AmB deoxycholate	1.3 (0.2–8.6)	.77	…	
Liposomal AmB	0.29 (0.07–1.1)	.07	0.23 (0.03–1.55)[a]	.132
AmB lipid complex	1.1 (0.2–5.6)	.86	…	
Posaconazole	1.3 (0.19–8.6)	.80	…	
Surgical resection	0.11 (0.03–0.41)	.001	0.03 (0.004–0.33)[b]	.003

NOTE. AmB, amphotericin B; CI, confidence interval; OR, odds ratio.

[a] Inverse risk (ie, risk of success): OR, 4.0 (95% CI, 0.63–26); $P = .132$.

[b] Inverse risk (ie, risk of success): OR, 33.0 (95% CI, 4.2–255); $P = .003$.

including 10 cases in SOT recipients from the Collaborative Exchange of Antifungal Research database, documented an overall treatment response rate of 72% and 69% with the use of AmB lipid complex as primary and salvage therapy, respectively [41]. More recently in patients with rhino-orbital-cerebral zygomycosis, the success rate was 68% among patients receiving liposomal AmB or AmB deoxycholate, compared with 32% among those who received AmB lipid complex [42]. Difference in central nervous system concentrations achievable with AmB lipid complex, compared with liposomal AmB were considered to account for these findings. To date, posaconazole has largely been employed as salvage therapy for zygomycosis. Of 91 patients with zygomycosis who received this agent for intolerance or because of failure of therapy, the success rate was 60% [19]. Posaconazole as salvage therapy was associated with successful response in 13 (57%) of the 27 SOT recipients with invasive fungal infection, including 1 of 2 with zygomycosis [43].

A successful outcome was documented in 30 (60%) of the 50 SOT recipients with zygomycosis in our study. The success rate was significantly lower among our patients with disseminated, compared with localized, disease but did not differ for various types of localized disease, such as disease of pulmonary, cutaneous, or gastrointestinal sites. In addition, there was no difference in outcome when stratified by the infecting zygomycetes species (likely because of the small number of patients with disease due to specific species). The success rate among our patients receiving AmB lipid complex was 60% (3 of 5 patients), which was similar to that (3 [50%] of 6 patients) reported previously with this agent in kidney transplant recipients with zygomycosis [5]. In all, 60% (3/5) of our patients receiving posaconazole as primary therapy had successful outcome. These data are among the first to document the efficacy of posaconazole as primary treatment for zygomycosis, because posaconazole, to our knowledge, has thus far been used as primary therapy for zygomycosis in only a case report [44]. Liposomal AmB was associated with a 4-fold higher success rate even when controlling for other variables that influence outcomes, such as renal failure, disseminated disease, and sur-

gical resection (table 6). A previous study comprising 5 SOT recipients with zygomycosis, including 2 with rhino-orbital disease, also reported a successful outcome in all with the use of liposomal AmB [2].

More recently, promising results with the use of a polyene plus an echinocandin for rhino-orbital-cerebral zygomycosis has garnered considerable interest in the role of combination therapy for the treatment of zygomycosis [42]. In our study, combination therapy was more likely to be employed in patients with disseminated disease, and the most frequently used combination was liposomal AmB and posaconazole. Although a beneficial role of this combination has been suggested anecdotally [45], experts have cautioned against the use of a polyene with posaconazole for zygomycosis, given the potential for antagonistic interactions [40]. Indeed, in an animal model of *R. oryzae,* posaconazole in combination with liposomal AmB did not significantly reduce the fungal burden, compared with the latter agent alone [46]. The success rate in our patients receiving a combination of liposomal AmB and posaconazole was 40% (table 5).

To date, reports of zygomycosis in organ transplant recipients have comprised retrospective case reports or case series delineating largely single-center experience. The present study using a prospective design was conducted at geographically diverse transplant centers. Our findings therefore may be considered generalizable to a wider population of SOT recipients. Several weaknesses of our study, however, deserve to be acknowledged. We were unable to assess the precise impact of iron overload on the development of zygomycosis in our patients, particularly in liver transplant recipients. Serum ferritin levels, although readily measurable, are not accurate predictors of iron overload, given that ferritin is an acute phase reactant and may be elevated during acute inflammatory conditions [47]. A potentially more reliable measure of iron overload is stainable iron content of the hepatic explant. Although the data regarding efficacy of antifungal regimens were systematically assessed using standardized criteria for outcome and controlled for potential confounders, our results should be interpreted with caution because this was not a randomized trial evaluating therapeutic options.

In summary, we show that liver transplant recipients have more fulminant disease expression and develop zygomycosis significantly earlier than do other SOT recipients. The precise basis for this observation remains to be defined. Prior use of voriconazole or caspofungin portended a higher risk, whereas immunosuppressive regimens including the calcineurin inhibitor agent, tacrolimus, appeared to confer a lower risk of zygomycosis. Overall outcomes, although improved compared with those historically reported in organ transplant recipients with zygomycosis, are still suboptimal. Future investigations to explore novel therapeutic strategies, including assessment of synergistic interactions between antifungal and immunosuppressive agents, warrant consideration.

AFFILIATIONS

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