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Cryptococcus neoformans in Organ Transplant Recipients: Impact of Calcineurin-Inhibitor Agents on Mortality

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Abstract

Variables influencing the risk of dissemination and outcome of *C. neoformans* infection were assessed in 111 organ transplant recipients with cryptococcosis in a prospective, multicenter, international study. Sixty one percent (68/111) of the patients had disseminated infection. The risk of disseminated cryptococcosis was significantly higher for liver transplant recipients (adjusted HR 6.65, p = 0.048). Overall mortality rate at 90 days was 14% (16/111). Mortality rate was higher in patients with abnormal mental status (p = 0.023), renal failure at baseline (p= 0.028), fungemia (p=0.006) and disseminated infection (p = 0.035), and lower in those receiving a calcineurin-inhibitor agent (p= 0.003). In multivariate analysis, the receipt of a calcineurin-inhibitor agent was independently associated with a lower mortality (adjusted HR 0.21, p = 0.008), and renal failure at baseline with a higher mortality rate (adjusted HR 3.14, p = 0.037). Thus, outcome in transplant recipients with cryptococcosis appears to be influenced by the type of immunosuppressive agent employed. Additionally, discerning the basis for transplant type-specific differences in disease severity has implications relevant for yielding further insights into the pathogenesis of *C. neoformans* infection in transplant recipients.

INTRODUCTION

Invasive fungal infections occur in 15–42% of the organ transplant recipients (1,2). Refinements in surgical techniques and antifungal prophylaxis have led to a decline in the overall incidence of fungal infections in the early post-transplant period, particularly that of invasive candidiasis (3,4). The risk factors for cryptococcal infections, however, are poorly understood. Cryptococcosis generally occurs in the late post-transplant period, well beyond the usual period of employment of antifungal prophylaxis (5,6). Furthermore, most cases represent reactivation of latent infection (5,7–9) such that limiting the exposure is unlikely to curtail the risk of cryptococcosis.

Mortality rate in transplant recipients with cryptococcosis typically ranges from 15–20%, and approaches 40% in those with central nervous system infection (5,6,10), suggesting a need to better understand the variables that affect outcome in these patients. Factors that impact outcome in other hosts have yielded insights that are relevant to prognosis in transplant recipients as well (11–14). However, organ transplant patients are unique in that the calcineurin-inhibitor based immunosuppressive regimens employed in these patients have antifungal activity *in vitro* (15–17), and could potentially modify the extent of infection or its prognosis. Thus, assessment of characteristics and outcome of *C. neoformans* infection specifically in organ transplant recipients is important. In a multicenter study, we determined the extent to which the risk of dissemination and mortality in organ transplant recipients with cryptococcosis can be predicted by readily assessable clinical and laboratory variables.

METHODS

Patients

Study population included 111 organ transplant recipients with *C. neoformans* infection at the participating centers in the United States, Canada, Spain, France, and India. These patients represented 98.2%(111/113) of the cases of cryptococcosis in transplant recipients at our institutions during the study period; two patients diagnosed and followed at a site remote from the transplant center could not be enrolled. Patients included from France were transplant recipients who developed cryptococcosis during the study period and were enrolled in a

nationwide, multicenter, prospective study of the French Cryptococcosis Study Group. The study was conducted between December 1999 and March 2006; the timing of initiation at different sites varied. Institutional Review Board approval was obtained as per local requirements.

Definitions

C. neoformans infection was defined as per criteria proposed by the European Organization for Research and Treatment in Cancer and the Mycoses Study Group, i.e., positive cultures for *C. neoformans* in a clinical specimen, including blood cultures; histopathologic or cytopathologic examination of specimens of needle aspiration or biopsy showing encapsulated yeast cells; or positive cryptococcal antigen in the blood or cerebrospinal fluid in a patient with compatible clinical presentation (18). Variables assessed included demographic characteristics, immunosuppressive regimen at the time of diagnosis, rejection episodes or antifungal agent use within 6 months prior to the onset of infection, cytomegalovirus infection, renal failure (defined as creatinine ≥ 2 mg/dl) at the time of diagnosis, sites of infection, cerebrospinal fluid characteristics, antifungal therapy employed, and patient outcome. In all cases, the primary immunosuppressive agent at diagnosis was the patients' stable immunosuppressive regimen that had remained unchanged within the previous 6 months.

Organ sites involved were classified as central nervous system (CNS); pulmonary; skin, soft-tissue, osteoarticular; or other (5,19). Disseminated infection was defined as CNS infection or fungemia or involvement of ≥ 2 noncontiguous organ sites (5,19). As in previous studies on opportunistic mycoses, including cryptococcosis, the mortality rate was assessed at 90 days (11,20).

Statistical analysis

Stata (Intercooled Stata 9.2, College Station, TX) was used for all analyses. Logistic regression models were used to calculate odds ratios and confidence intervals for factors associated with disseminated infection; no adjustments were made for multiple comparisons. A multivariable model was developed to assess for the effect of several factors as risks for disseminated infection. For this model, backward selection was used with factors removed at $p > 0.20$. Interaction terms were generated and evaluated for the main effects factors in this model. Interaction terms were entered one at a time and dropped from the model if not statistically significant at $p < 1.0$. The Pearson goodness-of-fit was used to evaluate the final model. The Cox proportional hazards model was used to evaluate factors associated with mortality. Entry time was the date of diagnosis, and follow-up ended with death or 90 day post-diagnosis. A multivariable model was generated using backward selection with factors removed at $p > 0.20$. Interaction terms were generated and evaluated for the main effects factors in this model. Interaction terms were entered one at a time and dropped from the model if not statistically significant at $p < 1.0$. Schoenfeld residuals were used to test the proportional-hazard assumption. Treatment with amphotericin B was forced into the final model to adjust for potential effect of therapy.

RESULTS

The clinical and demographic characteristics of the study patients are outlined in Table 1. Cryptococcosis occurred a median of 21 months after transplantation; 68.5% of the infections developed >1 year post-transplant.

Disseminated infection

Of 111 patients, 54% (60/111) had pulmonary infection, 52.2% (58/111) had CNS, and 8.1% (15/111) had skin, soft-tissue, or osteoarticular infections (Table 2). Sixty-one percent (68/111)

of the patients had disseminated cryptococcosis and in 32.4% (36/111) of the patients, the infection was limited to the lungs. Patients receiving a calcineurin-inhibitor based regimen (tacrolimus or cyclosporine A) were significantly less likely to have CNS infection (48%, 46/96 vs. 80%, 12/15, p = 0.02), and were more likely to have cryptococcosis limited to the lungs (36.6%, 35/96 vs. 6.6%, 1/15, p = 0.02). CNS infection was present in 47.3% (36/76) of the tacrolimus recipients, 50% (10/20) of the cyclosporine A recipients, and 80% (12/15) of the patients who received azathioprine or mycophenolate mofetil, without a calcineurin-inhibitor agent (p = 0.004).

Univariable logistic regression analysis of factors associated with disseminated as compared with non-disseminated or localized infection is shown in Table 3. No association was found between rejection, cytomegalovirus infection, or time to onset post-transplant and dissemination (Table 3). However, the type of organ transplanted and the immunosuppressive agent employed appeared to be associated with the risk of dissemination, although statistical significance was not achieved (Table 3). A multivariable model was constructed to determine if the immunosuppressive regimen and the specific organ transplant type were independently associated with the risk of disseminated infection (Table 3). The effect of type of transplant was assessed in comparison to lung transplant recipients who had the lowest risk of dissemination in univariable analysis (Table 3). The risk of disseminated infection was significantly higher for liver transplant recipients (adjusted hazard ratio 6.65, 95% CI, 1.01 – 43.64, p = 0.048), even when controlled for the type of immunosuppression (Table 3). Of 28 liver transplant recipients, 61% (17/28) had hepatitis C virus or alcohol as underlying liver disease. The incidence of disseminated infection was 80% (8/10) in patients with hepatitis C with virus, 71% (5/7) in those with alcohol, and 64% (7/11) for patients with other underlying liver diseases (p = 0.71)

Mortality

Mortality rate in the patients at 90 days was 14% (16/111). Mortality was 7.9% (6/76) in patients receiving tacrolimus, 20% (4/20) in those receiving cyclosporine A, and 40% (6/15) in patients who received azathioprine or mycophenolate mofetil without the aforementioned agents (p = 0.004, Figure 1). When stratified by the site of involvement, the mortality rate was 19% (11/58) in patients with CNS infection, 20.6% (14/68) in those with disseminated infection, and 33.3% (8/24) in patients with fungemia. Mortality in patients with infection limited to the lungs was 2.8% (1/36).

Univariate Cox regression analysis showed that the mortality rate was significantly higher in patients with abnormal mental status (HR 3.11, 95% CI, 1.17–8.31, p = 0.023), renal failure at baseline (HR 2.99, 95% CI, 1.12 – 7.98, p= 0.028), fungemia (HR 3.94, 95% CI 1.48–10.51, p=0.006) and disseminated infection (HR 4.93, 95% CI, 1.11 – 21.69, p = 0.035), and lower in patients receiving a calcineurin-inhibitor agent (HR 0.21, 95 % CI, 0.07 – 0.59, p= 0.001). Patients receiving a calcineurin-inhibitor compared to a non-calcineurin-inhibitor based regimen were older (mean 52 versus 41 years, p = 0.01), more likely to have cryptococcosis >1 year post-transplant (100% versus 63%, p = 0.003), and less likely to be kidney transplant recipients (46% versus 87%, p = 0.004). However, age, time to onset of infection, and type of transplant were not significantly associated with mortality (Table 4). Details of antifungal therapy have been discussed elsewhere (19) and are not the focus of this report. Briefly, 66.6% (74/111) of all patients and 90% (60/66) of those with disseminated infections were treated with amphotericin B preparations (amphotericin B deoxycholate or lipid formulations of amphotericin B). Fluconazole, on the other hand was employed primarily for localized infections. Of 27.9% (31/111) of the patients who received fluconazole, 80.6% (25/31) had pulmonary, skin/soft tissue or other single site involvement, and only 19.3% (6/31) had disseminated infections. When adjusted for the site of infection (disseminated versus localized)

there was no significant difference in outcome with the use of amphotericin B formulations as compared to fluconazole (Table 4).

A multivariate Cox regression model was constructed with abnormal mental status, disseminated infection, receipt of a calcineurin-inhibitor agent, and renal failure in the model. Since fungemia was considered a manifestation of disseminated infection (Methods), only the latter was included in the model. Renal failure and receipt of a calcineurin-inhibitor agent correlated independently and significantly with outcome even when controlled for disseminated infection and abnormal mental status at baseline (Table 4). Mortality was significantly higher in patients with renal failure (adjusted HR 3.14, 95% CI 1.06 – 9.26, p= 0.037), and lower in those receiving a calcineurin-inhibitor agent (adjusted HR 0.21, 95% CI, 0.06 – 0.66, p = 0.008) (Table 4). When amphotericin B as antifungal therapy was added to this model, the findings remained unchanged. Renal failure (adjusted HR 3.40, 95% CI, 1.14 – 10.06, p = 0.027) remained significantly associated with higher mortality and calcineurin-inhibitor agent use with a lower mortality rate (adjusted HR 0.16, 95% CI, 0.05 – 0.48, p = 0.001).

DISCUSSION

Several observations from our study are relevant with regards to cryptococcosis in transplant recipients. In all, 61% of the infections were disseminated and the risk of dissemination was significantly higher for liver transplant recipients even when controlled for the immunosuppressive regimen. A number of possible reasons could account for this. Liver disease *per se* appears to be associated with more severe presentation and poorer outcome in cryptococcosis. Cirrhotic patients were more likely to develop septic shock and cirrhosis of the liver was an independent predictor of mortality in cryptococcocemia (21). Specific deficits in chemotaxis, complement deficiency, and monocyte suppressor cell activity in liver dysfunction were proposed to be the basis for these findings (21).

While intact cell-mediated immunity is critical, antibody responses also contribute to the pathogenesis of cryptococcal disease (22–24). Transplant recipients with cryptococcosis had higher IgM and IgG titers to glucoronoxylomannan than those who did not develop this infection after transplantation (25). That antibody promotes disease expression may seem intuitively paradoxical, but is plausible since a prozone-like effect enhances the severity and increases mortality in experimental cryptococcosis (26,27). Liver transplant recipients have a lower frequency of posttransplant hypogammaglobulinemia due immunonusuppressive therapy than other transplant recipients (28–30). Given that hepatic sinusoidal and Kupffer cells play a role in the clearance of immunoglobulins (31), a decline in cryptococcal-specific or nonspecific immunoglobulins may be substantially less or protracted in liver than in other transplant recipients, thus enhancing their susceptibility (loss of resistance) to cryptococcosis. Finally, hepatic iron overload in liver transplant recipients may also enhance fungal virulence (32).

We note that a greater propensity of liver transplant recipients to develop disseminated infection has also been observed for aspergillosis. Historically, disseminated invasive aspergillosis has been documented in 50–60% of the liver as compared to 6–35% of the other organ transplant recipients (33–36). Notably, despite the requirement of a higher degree of immunosuppression, most *Aspergillus* infections in lung transplant recipients are limited to the lungs with disseminated infections occurring in ~6–16% of the patients (36,37). This suggests that immune defects that facilitate the evasion of host defenses by these opportunistic mycoses are greater in magnitude or that certain deficits occur uniquely in liver transplant recipients.

Tacrolimus (FK506) and cyclosporine exert their immunosuppressive effect by inhibiting calcineurin, a T-cell signaling molecule (16,38). Although highly conserved from man to yeast, calcineurin is also identified in fungi and plays a vital role in cell biology in pathogenic fungi, including cellular morphogenesis and virulence in *C. neoformans* (39,40). Calcineurin-inhibitor agents have potent *in vitro* antifungal activity against *C. neoformans* that is mediated through inhibition of fungal homologs of calcineurin (16,41). The minimum inhibitory concentration of FK506 at 37°C for *C. neoformans* was < 0.09 μg/mL and that for cyclosporine A was 0.39 – 5μg/mL (15). Despite *in vitro* activity against *C. neoformans*, cyclosporine A was associated with progressive infection in an animal model of cryptococcal meningitis (42). Cyclosporine A, however, penetrates the CNS less effectively than tacrolimus (5,42). Given that transplant recipients receiving calcineurin-inhibitor agents develop cryptococcosis, the immunosuppressive effect as compared to the antifungal effect appears to predominate in the clinical setting. However, the use of these agents appeared to confer a protective effect on mortality that was particularly notable for tacrolimus. Whether this association is due to antifungal attributes of tacrolimus or other unmeasured variables pertaining to the host or infection in our patients remains to be determined. The association of renal failure with poor outcome in opportunistic mycoses, including cryptococcosis has previously been reported (5).

Cryptococcosis has been reported following zoonotic exposure and in outbreak settings (43, 44). However, a vast majority of the cases are considered to be due to reactivation of strains acquired long before clinical disease – likely during early childhood, and sequestered in alveolar macrophages (7,9). Patients receiving a calcineurin-inhibitor agent in our study were less likely to have CNS involvement and more likely to have infection limited to the lungs. Thus, these agents might inhibit fungal calcineurin in strains emerging from the dormant phase and decrease dissemination from lungs and hilar lymph nodes to the CNS. However, the association of calcineurin-inhibitor agents with mortality was much stronger than the association of these agents with the risk of dissemination, suggesting that their protective effect on mortality may be mediated by other mechanisms as synergy with the azoles.

The combination of FK506 and fluconazole is synergistic *in vitro* for *C. neoformans* and resulted in ~30-fold decrease in the minimum inhibitory concentration of FK506 and 4-fold decrease in that of fluconazole for this yeast (45). Whether outcomes in patients receiving immunophilin-binding immunosuppressive agents can be further improved by employing therapeutic interventions that synergistically target calcineurin or signaling pathways distinct from it is an important question.

There are limitations of our study that deserve to be acknowledged. Since this was not a clinical trial, neither the immunosuppressive regimen nor antifungal therapy was randomized. There was also a significant difference in the time of onset of infection posttransplant with more patients who received a non-calcineurin inhibitor based regimen having later onset of cryptococcosis. However, this finding, if at all, would tend to bias the outcome in favor of the non-calcineurin inhibitor agent group as cummulative immunosuppression is generally lower and outcomes in opportunistic infections are better in the late posttransplant period. Although we found no statistically significant association between the time to onset and the risk of disseminated infection or mortality, it is possible that timing or yet unknown factors influenced the course of infection. Amongst the strengths of our study is that it included a large cohort of patients in a prospective, multicenter design which renders our findings generalizable to other transplant recipients with cryptococcosis.

In summary, our data show that cryptococcosis remains a significant complication in organ transplant recipients. The outcome, and to some extent the spectrum of infection appears to be influenced by the receipt of calcineurin-inhibitor based immunosuppression. Calcineurin-

inhibitor agents remain the mainstay of immunosuppression; however, long-term outcomes in transplant recipients receiving these drugs are suboptimal. Renal dysfunction, metabolic toxicity, and cardiovascular complications due to cumulative exposure to calcineurin-inhibitor agents (46,47) have spawned a growing interest in the use of induction therapy with the aim of achieving calcineurin-free/sparing maintenance immunosuppression after transplantation (48,49). The impact of these evolving strategies on the spectrum of infectious complications, including cryptococcosis remains to be determined. Finally, future studies to discern the precise basis for organ-specific differences in disease expression and severity of opportunistic mycoses have the potential to yield further insights into the pathogenesis of these infections in transplant recipients.

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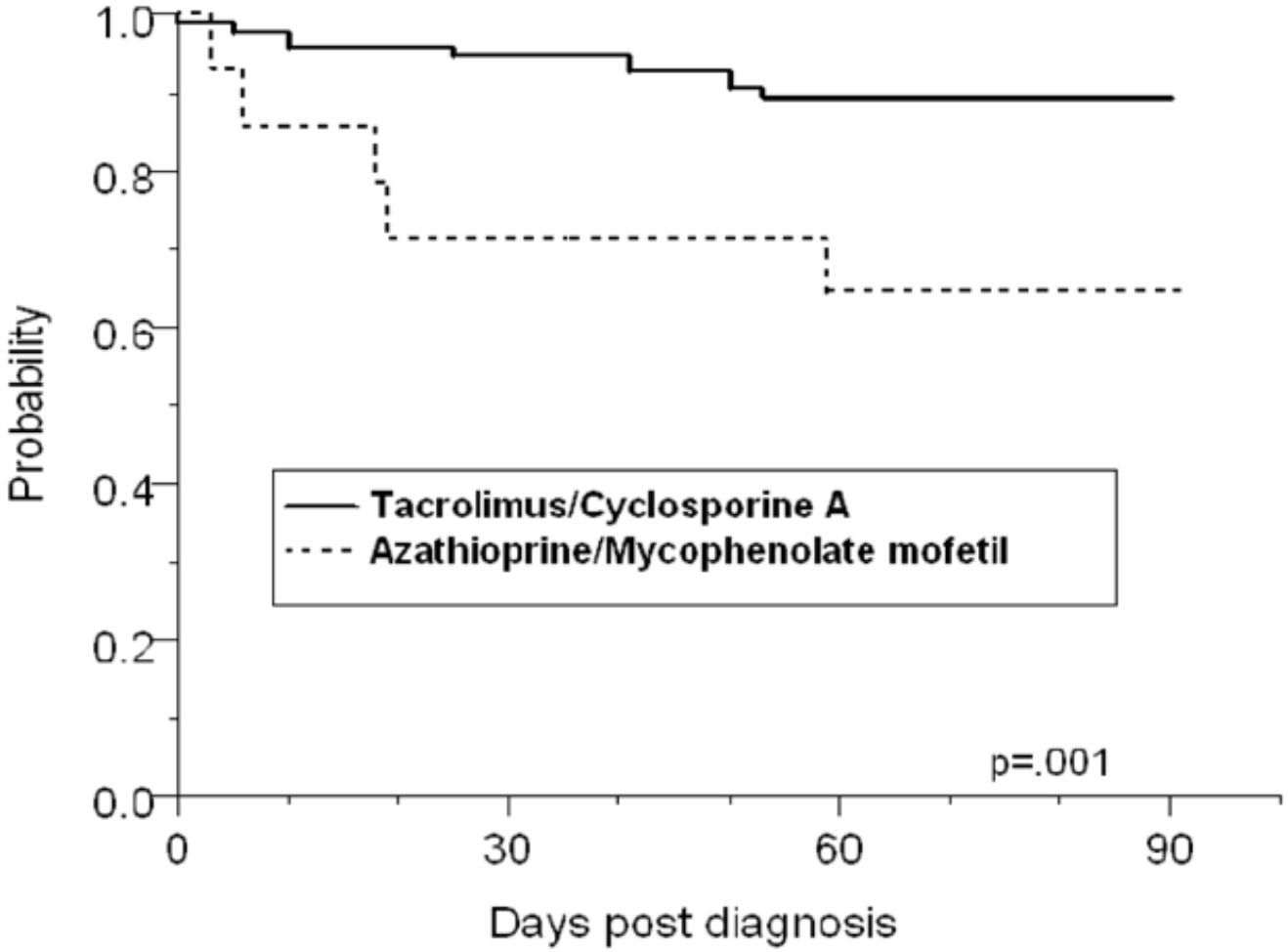

Figure 1.
Kaplan-Meier survival analysis showing that the probability of survival after the diagnosis of cryptococcosis was significantly higher in patients who received a calcineurin-inhibitor agent (tacrolimus or cyclosporine A) as compared to those who received azathioprine or mycophenolate mofetil without a calcineurin-inhibitor agent (p=.001, log rank test).

Table 1

Demographic and clinical characteristics of the study patients (n = 111)

Age, years, median (range)	52 (19–77)
Gender, male	67% (74)
Type of transplant	
Kidney	51% (57)
Liver	25% (28)
Heart	8% (9)
Lung	7% (8)
Other/multiorgan	8% (9)
Kidney-pancreas	(5)
Kidney-heart	(2)
Kidney-liver	(1)
Small bowel-pancreas	(1)
Immunosuppressive regimens	
Tacrolimus based	69% (76)
Tacrolimus + mycophenolate mofetil + prednisone	36
Tacrolimus + prednisone	21
Tacrolimus + azathioprine + prednisone	10
Tacrolimus + mycophenolate mofetil	4
Tacrolimus only	5
Cyclosporine A based	18% (20)
CsA + mycophenolate mofetil + prednisone	11
CsA + azathioprine + prednisone	4
CsA + prednisone	4
CsA + mycophenolate mofetil	1
Other	14% (15)
Azathioprine + prednisone	10
Mycophenolate mofetil + prednisone	5
T-cell agent use	3% (4/111)
As induction therapy	
Antithymocyte globulin	1
As rejection therapy	
Antithymocyte globulin	2
Campath-1H	1
Prednisone dose mg/qd, median*	10
Retransplant**	1% (2)
Rejection†	30% (33)
Cytomegalovirus infection	27% (30)
Renal failure at baseline††	26% (29)
Prior antifungal agent use§	7% (6/11)
Antifungal therapy	
Amphotericin B	67% (74)
Fluconazole	28% (31)

Other[§§]	5% (6)

Numbers represent actual values unless identified as percentages.

[*] In those receiving prednisone.

[**] Retransplant implies prior receipt of an organ transplant.

[†] Episodes occurring within 6 months prior to the onset of cryptococcosis.

[††] Renal failure refers to creatinine ≥ 2 mg/dl at the time of diagnosis of infection.

[§] Only one of these patients had received fluconazole.

[§§] Includes 3 patients who received no therapy and 3 who received a triazole agent.

Table 2

Characteristics of *C. neoformans* infection in the study patients (n = 111)

Sites of infection	
Central nervous system	52.2% (58)
Pulmonary	54% (60)
Skin, soft-tissue, osteoarticular	18% (20)
Other	3.6% (4)
Renal abscess	2
Abdominal abscess	1
Spinal and iliac mass	1
Disseminated infection	61% (68)
Central nervous system	52.2% (58)
Fungemia*	20.7% (23)
≥ 2 noncontiguous sites**	9% (10)
Serum cryptococcal antigen titer	
Median	1:64
Interquartile range	1:4–1:512
Cerebrospinal fluid values (in patients with CNS infection)	
White blood cell, median (interquartile range)	81 (2–131)
No. with positive culture of the cerebrospinal fluid	88% (49/56)
Cryptococcal antigen titer, median (interquartile range)	1:64 (1:2 – 1:1024)
Time to onset of infection post-transplant	
Median (interquartile), months	21 (9.4 – 53)
Infection occurring within	
0–30 days	2.7% (3)
31–90 days	5.4% (6)
91days – 1 year	23.4% (26)
> 1 year post-transplant	68.5% (76)

* These included 20 patients with central nervous system infection.

** 2 of 10 patients also had fungemia.

Table 3

Variables associated with disseminated versus localized cryptococcosis

Variable	Odds ratio	95% CI	P-value
Univariable analysis			
Age	0.99	0.97 – 1.03	0.98
Type of transplant			
Liver	1.19	0.76 – 4.84	0.17
Heart	0.81	0.20 – 3.18	0.75
Kidney	1.58	0.65 – 3.81	0.31
Lung	0.19	0.04 – 1.01	0.05
Multiorgan	0.47	0.12 – 1.88	0.29
Renal failure at baseline	1.77	0.72 – 4.35	0.21
Cytomegalovirus infection	0.68	0.29 – 1.58	0.36
Rejection	1.04	0.45 – 2.39	0.93
Time from onset to diagnosis	0.99	0.99 – 1.00	0.35
Months post-transplant to diagnosis	1.10	0.48 – 2.52	0.81
Onset < 1 year post-transplant	1.17	0.51 – 2.66	0.71
Receipt of prednisone	4.32	0.79 – 1.13	0.09
Prednisone, dose	1.03	0.97 – 1.10	0.34
Receipt of a calcineurin inhibitor agent*	0.35	0.09 – 1.32	0.12
Receipt of tacrolimus**	0.34	0.08 – 1.31	0.12
Receipt of cyclosporine A†	0.37	0.079 – 1.76	0.21
Multivariable analysis			
Receipt of a calcineurin-inhibitor agent	0.37	0.09 – 1.52	0.17
Receipt of prednisone	1.03	0.96 – 1.10	0.34
Type of Transplant††			
Liver	6.65	1.01 – 43.64	0.048
Heart	3.21	0.38 – 26.75	0.28
Kidney	4.07	0.69 – 23.88	0.11
Multiorgan	1.46	0.15 – 13.46	0.73

*Includes tacrolimus or cyclosporine. Comparison is made with the receipt of a non-calcineurin-inhibitor based regimen (azathioprine or mycophenolate mofetil).

**Compared to non-tacrolimus regimen.

†Compared to non-cyclosporine A regimen.

††Comparison is made with lung transplant recipients as reference.

Table 4

Variables associated with mortality at 90 days in the study patients based on Cox proportional hazard analysis

Variable	Hazard ratio (95% C.I.)	P-value
Univariable analysis		
Age	0.98 (0.94 – 1.02)	0.35
Gender, female	1.16 (0.39 – 3.40)	0.78
Type of transplant (compared to the lung)		
Liver	1.10 (0.12 – 9.65)	0.95
Heart	0.89 (0.06 – 14.24)	0.94
Kidney	1.36 (0.17 – 10.65)	0.77
Retransplant	0,56 (0.07 – 4.29)	0.58
Rejection	0.75 (0.24 – 2.33)	0.62
Cytomegalovirus infection	0.35 (0.08 – 1.52)	0.16
Renal failure at baseline	2.99 (1.12 – 7.98)	0.028
Infection within 1 year post-transplant	1.28 (0.46 – 3.53)	0.63
Duration of symptoms prior to therapy	0.99 (0.98 – 1.01)	0.69
Site of infection		
Central nervous system	2.15 (0.75 – 6.20)	0.15
Pulmonary	0.89 (0.33 – 2.36)	0.81
Skin, soft-tissue, osteoarticular only	1.08 (0.14 – 8.20)	0.94
Fungemia	3.94 (1.48 – 10.51)	0.006
Disseminated infection	4.93 (1.11 – 21.69)	0.035
Abnormal mental status at presentation	3.11 (1.17 – 8.31)	0.023
Primary immunosuppressive agent		
Calcineurin-inhibitor agent (compared to non-calcineurin-inhibitor agent use)	0.21 (0.07 – 0.59)	0.003
Tacrolimus	0.15 (0.05 – 0.49)	.001
CsA	0.45 (0.13 – 1.59)	.21
Antifungal therapy with amphotericin B (compared to fluconazole)		
Localized infection	1.85 (0.11 – 29.64)	0.66
Disseminated infection	1.06 (0.13 – 8.21)	0.95
5 flucytosine use as initial therapy (compared to no 5 flucytosine use)		
Localized infection*	--	--
Disseminated infection	0.66 (0.22 – 1.97)	0.46
Multivariable analysis**		
Disseminated infection	4.13 (0.92 – 18.42)	0.063
Receipt of a calcineurin-inhibitor agent	0.21 (0.06 – 0.66)	0.008
Renal failure	3.14 (1.06 – 9.26)	0.037

* Unable to calculate as there were no deaths in this group. Variables included in the model were abnormal mental status, renal failure, disseminated infection, and receipt of calcineurin-inhibitor agent. Using backward selection with factors removed at 0.20, abnormal mental status fell from the model.

** There were no significant interactions and no significant violation of the proportional hazard assumption.