MAJOR ARTICLE

Lipid Formulations of Amphotericin B Significantly Improve Outcome in Solid Organ Transplant Recipients with Central Nervous System Cryptococcosis

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Background. Whether outcome of central nervous system (CNS) cryptococcosis in solid organ transplant recipients treated with lipid formulations of amphotericin B is different from the outcome of the condition treated with amphotericin B deoxycholate (AmBd) is not known.

Methods. We performed a multicenter study involving a cohort comprising consecutive solid organ transplant recipients with CNS cryptococcosis.

Results. Of 75 patients treated with polyenes as induction regimens, 55 (73.3%) received lipid formulations of amphotericin B and 20 (26.7%) received AmBd. Similar proportions of patients in both groups had renal failure at baseline ($P = .94$). Overall, mortality at 90 days was 10.9% in the group that received lipid formulations of amphotericin B and 40.0% in the group that received AmBd. In univariate analysis, nonreceipt of calcineurin inhibitors ($P = .034$), renal failure at baseline ($P = .016$), and fungemia ($P = .003$) were significantly associated with mortality. Compared with AmBd, lipid formulations of amphotericin B were associated with a lower mortality ($P = .007$). Mortality did not differ between patients receiving lipid formulations of amphotericin B with or without flucytosine ($P = .349$). In stepwise logistic regression analysis, renal failure at baseline (odds ratio [OR], 4.61; 95% confidence interval [CI], 1.02–20.80; $P = .047$) and fungemia (OR, 10.66; 95% CI, 2.08–54.55; $P = .004$) were associated with an increased mortality, whereas lipid formulations of amphotericin B were associated with a lower mortality (OR, 0.11; 95% CI, 0.02–0.57; $P = .008$).

Conclusions. Lipid formulations of amphotericin B were independently associated with better outcome and may be considered as the first-line treatment for CNS cryptococcosis in these patients.

Invasive fungal disease is a significant posttransplantation complication in solid organ transplant (SOT) recipients [1–3]. Whereas the overall incidence of opportunistic mycoses, particularly those caused by *Candida* and *Aspergillus* species, appears to have decreased

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with improvements in transplantation practices and wider use of antifungal prophylaxis, the incidence of cryptococcosis has remained unchanged over the past 2 decades [1, 4–6]. Currently, cryptococcosis is the third most common invasive fungal infection in SOT recipients, with an overall incidence of ~2.8% (range, 0.3%–5%) [7]. Central nervous system (CNS) involvement has been documented in 54%–68% of patients with cryptococcal disease, and mortality is 30%–51%, despite the expanding antifungal armamentarium [6–9].

Our previous study demonstrated that renal failure at baseline is associated with higher mortality among SOT recipients with cryptococcosis and that receipt of a calcineurin inhibitor agent was independently associated with lower mortality [10]. Whether these observations can also be applied to SOT recipients with CNS cryptococcosis is unknown. Although amphotericin B deoxycholate is active against a wide range of fungi, its use is limited by significant toxicity. Despite lack of nephrotoxicity, echinocandins, a potent antifungal class against yeasts, are not active against cryptococci [11]. On the other hand, lipid formulations of amphotericin B not only maintain the antifungal spectrum of amphotericin B deoxycholate but also have the advantages of less nephrotoxicity and less infusion-related pro-inflammatory toxicities and have emerged as the preferred treatment option for SOT recipients with cryptococcosis in the current era [6]. However, little is known about their efficacy for the treatment of CNS cryptococcosis in the SOT population. Flucytosine is a critical component of the treatment of CNS cryptococcosis in HIV-infected patients, but its use as induction therapy in SOT recipients appears to be decreasing, especially when induction therapy comprises lipid formulations of amphotericin B [6]. Whether such practice affects outcome deserves further investigation.

To our knowledge, no studies to date have probed these issues exclusively in the context of a SOT population. Thus, the goal of the present study was to prospectively assess the variables influencing mortality among SOT recipients with CNS cryptococcosis, including the effects of induction antifungal treatment regimens.

PATIENTS AND METHODS

Consecutive SOT recipients with cryptococcosis were prospectively enrolled at participating centers from 2001 through 2007, and patients with documented CNS cryptococcosis comprised the present study population. Patient management was in accordance with the standard of care at the participating centers. A detailed description of this cohort has been reported elsewhere [9, 10]. None of the patients were HIV positive. Cryptococcosis was defined according to criteria proposed by the European Organization for Research and Treatment in Cancer and the Mycoses Study Group [12]. Patients were considered to have CNS cryptococcosis if they had cerebrospinal fluid (CSF) culture positive for *Cryptococcus* species or positive CSF cryptococcal antigen test results. CSF or serum cryptococcal antigen level >1:512 was considered to represent high fungal load, as reported elsewhere [13, 14]. Data collected included demographic characteristics, type of organ transplant, immunosuppressive regimen at the time of diagnosis, renal failure at baseline (defined as serum creatinine level >2 mg/dL at the time of diagnosis), prior rejection, retransplantation, cytomegalovirus infection or disease, other sites of infection, antifungal therapy used, and mortality.

Data collected on antifungal therapy included antifungal agents used and duration of therapy. Lipid formulations of amphotericin B comprised liposomal amphotericin B and amphotericin B lipid complex. Primary therapy (or induction therapy) was defined as receipt of an antifungal agent as initial therapy for cryptococcosis as reported elsewhere [15]. An antifungal treatment regimen to which the patient was switched after induction and continued thereafter was considered to be maintenance therapy [15]. Mortality was assessed at 90 days after the treatment of CNS cryptococcosis.

Statistical analyses were performed using Intercooled Stata, version 9.2 (Stata). Categorical data were compared using the χ^2 test or Fisher's exact test. Continuous variables were compared using the rank-sum test. A maximum likelihood logistic model was used to estimate odd ratios (ORs) and 95% confidence intervals (CIs). A multivariable logistic model was used to estimate the effects of multiple variables on a dichotomous end point. A backward stepwise selection was used. All variables significant at $P < .20$ in the univariate analysis were entered in the model and then removed in a stepwise design if $P > .20$. Interactions among the main effects were examined, and the final model was checked with the Hosmer-Lemeshow goodness of fit test. A Cox proportional hazards regression model was used to estimate the survival curves for lipid formulations of amphotericin B and amphotericin B deoxycholate. The end point was date of death before day 90 or day 91 for survivors. A log-log plot was constructed to check for violations of the proportional hazards assumption.

RESULTS

CNS cryptococcosis was documented in a total of 80 patients, based on positive CSF culture results for 62 patients (77.5%) and positive CSF antigen test results for 18 patients (22.5%). The demographic and clinical characteristics of these 80 patients are presented in Table 1. CNS cryptococcosis developed a median of 25 months (interquartile range, 9–67 months) after transplantation, with 55 (68.7%) of 80 cases occurring 1 year after transplantation. In addition to the CNS, cryptococcal disease involved the lung in 33 patients (41.2%), skin in 15 (18.7%), and other sites in 3 (3.7%) (Table 2). Fungemia was documented in 29 (38.2%) of 80 cases, serum cryptococcal

Table 1. Demographic and Clinical Characteristics of 80 Solid Organ Transplant Recipients with Central Nervous System Cryptococcosis

Variable	Patients
Age, mean years (interquartile range)	51.5 (43–60)
Male sex	59 (73.8)
Type of transplant	
Kidney	44 (55.0)
Liver	17 (21.2)
Lung	4 (5)
Heart	4 (5)
Pancreas	2 (2.5)
Multiorgan	9 (11.3)
Kidney-pancreas	5 (6.2)
Kidney-heart	2 (2.5)
Kidney-liver	2 (2.5)
Primary immunosuppressive agent	
Tacrolimus	55 (68.7)
Cyclosporine A	12 (15.0)
Other[a]	13 (16.2)
Other immunosuppression	
Prednisone	77 (96.2)
Dose, median mg/day (interquartile range)	10 (5–10)
Mycophenolate mofetil	43 (53.7)
Anti–T cell antibody	6 (7.5)
Renal failure at baseline	24 (30.0)
Retransplantation	9 (11.2)
Rejection	18 (22.5)
Cytomegalovirus infection	16 (20.0)
Cytomegalovirus disease	7 (8.7)

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

[a] Included azothioprine (9 patients), mycophenolate mofetil and prednisone (2), rapamycin, mycophenolate mofetil and prednisone (1), and prednisone only (1).

antigen titer >1:512 in 28 (50.9%) of 55 cases, and CSF cryptococcal antigen titer >1:512 in 27 (36.5%) of 74 cases.

Overall, 75 of 80 patients received a polyene as primary therapy for CNS cryptococcosis; 5 patients treated with fluconazole were excluded from further analyses. The polyenes used were lipid formulations of amphotericin B in 55 (68.8%), which included liposomal amphotericin B in 26 (32.5%) and amphotericin B lipid complex in 29 (36.3%), and amphotericin B deoxycholate in 20 (25%) (Table 2). Renal failure at baseline was documented in 6 (29%) of 55 patients receiving lipid formulations of amphotericin B and 6 (30%) of 20 patients receiving amphotericin B deoxycholate ($P = .94$; regression model for this interaction, $P = .482$). Thirty-seven (67.3%) of the 55 SOT recipients who received lipid formulations of amphotericin B group concurrently used flucytosine; these included 16 patients in the liposomal amphotericin B group and 21 in the amphotericin B lipid complex group. Of 20 who patients received amphotericin B deoxycholate, 8 (40%) concurrently used flucytosine. Fourteen (18.7%) or 75 patients died while receiving primary therapy. Of 61 patients who survived after the induction period, 49 patients (82.0%) received fluconazole as maintenance therapy. Nine (14.7%) of 61 patients continued the initial therapy without changes, including amphotericin B deoxycholate in 3, amphotericin B lipid complex in 3, and liposomal amphotericin B in 3. The remaining 2 patients (3.3%) had no information available. Graft loss was observed in 6 (8%) of 75 patients, including 6 of 55 patients who received lipid formulations of amphotericin B and none of the 20 patients who received amphotericin B deoxycholate ($P = .18$).

CSF analysis was repeated at 2 weeks for 25 (31.2%) of 80 patients. Of 23 patients who initially had positive culture results, 13 (56.5%) had negative results of a second CSF culture. Overall, 64% of the patients who received a lipid formulation of amphotericin B were culture negative at 2 weeks, compared with 37% of those who received amphotericin B deoxycholate ($P = .22$).

Overall, the mortality rate at 90 days after treatment of CNS cryptococcosis was 18.7% (14 of 75 patients died). The mortality was 40% (8 of 20) among patients who received amphotericin B deoxycholate, 10.3% (3 of 29) among patients who received amphotericin B lipid complex, and 11.5% (3 of 26) among patients who received liposomal amphotericin B. The mortality rate was 20% among patients who had positive CSF culture results at 2 weeks and 8% among those with negative culture results ($P = .57$). In the group that received amphotericin B deoxycholate, deaths were considered to be due to cryptococcosis in 3 cases, due to unrelated events in 2 (cardiopulmonary arrest and myocardial infarction), and of undetermined or unknown etiology in 3. Of 6 patients who died in the group that received lipid formulations of amphotericin B, 2 died of cryptococcosis; 1 death each was related to myocardial infarction, intra-abdominal sepsis with acute respiratory distress syndrome and multiorgan failure, intracranial bleeding and progressive liver failure, and cardiomyopathy.

Candidate variables analyzed as predictors of mortality at 90 days after the treatment of cryptococcosis are outlined in Table 3. In univariate analysis, no receipt of calcineurin inhibitors (OR, 4.28; 95% CI, 1.11–16.49; $P = .034$), renal failure at baseline (OR, 4.48; 95% CI, 1.32–15.09; $P = .016$), and fungemia (OR, 8.627, 95% CI, 2.13–34.88; $P = .003$) were significantly associated with mortality. Compared with amphotericin B deoxycholate, lipid formulations of amphotericin B were associated with lower mortality (OR, 0.184; 95% CI, 0.05–0.62; $P = .007$). In addition, compared with amphotericin B deoxycholate, both liposomal amphotericin B (OR, 0.261; 95% CI, 0.06–1.04; $P = .058$) and amphotericin B lipid complex (OR, 0.115; 95% CI, 0.02–0.63; $P = .012$) were associated with lower mortality. Mortality did not differ between patients receiving lipid for-

Table 2. Characteristics of Central Nervous System (CNS) Cryptococcosis in 80 Solid Organ Transplant Recipients

Variable	Patients
Time to onset of CNS cryptococcosis	
Median months (IQR)	25.0 (9–67)
0–30 days	2 (2.5)
31–90 days	7 (8.7)
91 days–1 year	16 (20)
>1 year	55 (68.7)
Involved site other than the CNS	
Lung	33 (41.2)
Skin	15 (18.7)
Other[a]	3 (3.7)
Fungemia	29 (38.2)
Median serum cryptococcal antigen level	1:512
Level ⩾1:512	28/55 (50.9)
Cerebrospinal fluid analysis	
White blood cell count, median cells/μL (IQR)	67 (13–160)
Glucose level, median mg/dL (IQR)	47 (31–69)
Cryptococcal antigen level, median (IQR)	64 (8–1024)
Level ⩾1:512	27/74 (36.5)
Positive culture result	62 (77.5)
Initial antifungal therapy[b]	
Lipid formulations of amphotericin B	55 (68.8)
Duration, median days (IQR)	
All	21 (14–32)
Amphotericin B lipid complex	18 (14–31)
Liposomal amphotericin B	25 (20–31)
Daily dose,[c] median mg/day (IQR)	
All	350 (300–400)
Amphotericin B lipid complex	350 (300–400)
Liposomal amphotericin B	375 (337.5–400)
Daily dose, median mg/kg/day (IQR)	
All	4.5 (3.75–5)
Amphotericin B lipid complex	5 (4.5–5)
Liposomal amphotericin B	4 (3.75–5)
Concurrent use of flucytosine	37/55 (67.3)
Duration of flucytosine use, median days (IQR)	14 (9–23)
Amphotericin B deoxycholate	20 (25.0)
Duration, median days (IQR)	20 (14–49)
Daily dose,[d] median mg/day (IQR)	50 (47.5–62.5)
Daily dose, median mg/kg/day (IQR)	1 (0.93–1.19)
Concurrent use of flucytosine	8/20 (40.0)
Duration of flucytosine use, median days (IQR)	37 (25–49)

NOTE. Data are no. or proportion (%) of patients, unless otherwise indicated. IQR, interquartile range.

[a] Included peritoneal fluid, bile duct, groin mass, and urine.
[b] Five patients treated with fluconazole alone were excluded from these analyses.
[c] Dose was available as mg/day for 41 patients and as mg/kg/day for 12 patients.
[d] Dose was available as mg/day for 7 patients and as mg/kg/day for 8 patients.

mulations of amphotericin B with or without flucytosine (OR, 0.441; 95% CI, 0.08–2.44; $P = .349$). Age, sex, type of organ transplant, receipt of anti–T cell antibody, prior rejection, retransplantation, year of cryptococcal diagnosis, time to onset of CNS cryptococcosis after transplantation, abnormal mental status, CSF opening pressure >20 cm, positive CSF culture results, time from onset of CNS cryptococcosis to initiation of treatment, and flucytosine use were not associated with mortality (Table 3).

Table 3. Variables Associated with Mortality at 90 Days After Treatment of Central Nervous System Cryptococcosis

Variable	Reference group	Univariate analysis		Multivariate analysis[a]	
		OR (95% CI)	*P*	OR (95% CI)	*P*
Age	Continuous variable	1.00 (0.955–1.05)	.941	…	
Female ($n = 20$)	Male ($n = 55$)	0.398 (0.08–1.96)	.258	…	
Liver transplant ($n = 13$)	Renal transplant ($n = 43$)	0.687 (0.13–3.67)	.661	…	
Lung transplant ($n = 4$)	Renal transplant	1.26 (0.12–13.60)	.849	…	
Heart transplant ($n = 4$)	Renal transplant	1.26 (0.12–13.60)	.849	…	
Pancreas transplant ($n = 2$)	Renal transplant	UC[b]	.325	…	
Multiorgan transplant ($n = 4$)	Renal transplant	1.26 (0.12–13.60)	.849	…	
No CNI ($n = 12$)	CNI ($n = 63$)	4.28 (1.11–16.49)	.034	…	
Anti–T cell antibody ($n = 6$)	No anti–T cell antibody ($n = 69$)	UC[c]	.586	…	
Renal failure at baseline ($n = 22$)	No renal failure at baseline ($n = 53$)	4.48 (1.32–15.09)	.016	4.61 (1.02–20.80)	.047
Prior rejection ($n = 16$)	No prior rejection ($n = 59$)	0.560 (0.11–2.80)	.480	…	
Retransplantation ($n = 9$)	No retransplant ($n = 66$)	1.286 (0.23–6.98)	.771	…	
Year of cryptococcal diagnosis	Continuous variable	0.849 (0.66–1.09)	.209	…	
Time to onset after transplantation	Continuous variable	1.01 (0.99–1.02)	.216	…	
Abnormal mental status ($n = 36$)	Normal mental status ($n = 39$)	2.267 (0.68–7.56)	.183	…	
Fungemia ($n = 28$)	No fungemia ($n = 43$)	8.627 (2.13–34.88)	.003	10.66 (2.08–54.55)	.004
OP $\geqslant$20 cm ($n = 33$)	OP <20 cm ($n = 10$)	0.55 (0.08–3.58)	.533	…	
Positive CSF culture result ($n = 60$)	Negative CSF culture result ($n = 15$)	3.872 (0.46–32.25)	.211	…	
CSF antigen titer $\geqslant$1:512 ($n = 26$)	CSF antigen titer <1:512 ($n = 44$)	1.071 (0.31–3.70)	.913	…	
Time from onset to treatment	Continuous variable	0.996 (0.97–1.02)	.789	…	
Lipid formulations of AmB ($n = 55$)	AmBd ($n = 20$)	0.184 (0.05–0.62)	.007	0.11 (0.02–0.57)	.008
L-AmB ($n = 26$)	AmBd ($n = 20$)	0.261 (0.06–1.04)	.058	…	
ABLC ($n = 29$)	AmBd	0.115 (0.02–0.63)	.012	…	
Flucytosine use ($n = 45$)	No flucytosine use ($n = 30$)	0.865 (0.27–2.80)	.809	…	
Lipid formulations of AmbB with flucytosine ($n = 37$)	Lipid formulations of AmbB without flucytosine ($n = 18$)	0.441 (0.08–2.44)	.349	…	

NOTE. ABLC, amphotericin B lipid complex; AmB, amphotericin B; AmBd, amphotericin B deoxycholate; CI, confidence interval; CNI, calcineurin inhibitor; CSF, cerebrospinal fluid; L-AmB, liposomal amphotericin B; OP, opening pressure (43 [57.3%] of 75 patients had opening pressure recorded); OR, odds ratio; UC, unable to calculate.

[a] Variables significant at $P < .20$ level in the univariate analysis (ie, receipt of CNIs, renal failure at baseline, abnormal mental status, fungemia, and receipt of lipid formulations of AmbB) were entered into the model and then removed in a stepwise design if $P > .20$. Receipt of CNIs and abnormal mental status were removed from the multivariate model because $P > .20$.

[b] OR was not calculable because of zero value: mortality among pancreas transplant recipients and renal transplant recipients was 20.9% (0 of 2 patients vs 9 of 43 patients).

[c] OR was not calculable because of zero value: mortality among patients with and without anti–T cell antibody was 20.3% (0 of 6 patients vs 14 of 69 patients).

With use of stepwise logistic regression analysis with variables significant at $P < .2$ in the model (ie, receipt of calcineurin inhibitors, renal failure at baseline, abnormal mental status, fungemia, and the use of lipid formulations of amphotericin B), only renal failure at baseline (OR, 4.61; 95% CI, 1.02–20.80; $P = .047$) and fungemia (OR, 10.66; 95% CI, 2.08–54.55; $P = .004$) were independently associated with increased mortality, whereas use of lipid formulations of amphotericin B was associated with a lower mortality (OR, 0.11; 95% CI, 0.02–0.57; $P = .008$) (Table 3). The Hosmer-Lemeshow goodness of fit for this model showed an overall good fit ($P = .627$), and the receiver operating characteristic curve value was 0.857. The outcome in study patients was also analyzed with a Cox proportional hazards regression model (Figure 1). When adjusted for fungemia and renal failure at baseline, the 90-day survival probability with the receipt of lipid formulations of amphotericin B was significantly higher than that with the receipt of amphotericin B deoxycholate ($P = .008$). We also evaluated a Cox model by creating log-log plots, and the lines were parallel, indicating that the proportion-hazards assumption was not violated.

DISCUSSION

Despite substantial attributable nephrotoxicity and infusion-related reactions, amphotericin B deoxycholate has been the mainstay of therapy for CNS cryptococcosis for decades. Lipid formulations of amphotericin B retain the antifungal spectrum of amphotericin B deoxycholate with improved toxicity profile and offer an attractive alternative therapeutic option for cryptococcosis. Current guidelines for the management of cryptococcal disease in SOT recipients recommend amphotericin B deoxycholate in combination with flucytosine as the first-line therapy for CNS cryptococcosis and suggest that lipid for-

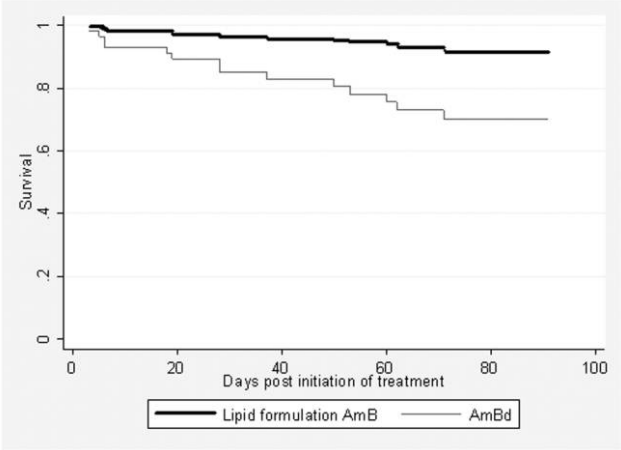

Figure 1. Cox proportional hazards regression survival curves for the receipt of lipid formulations of amphotericin B (AmB) and AmB deoxycholate (AmBd). Receipt of lipid formulations of AmB remained associated with a significantly improved 90-day survival after adjustment for 2 other factors associated with increased mortality: renal failure at baseline and fungemia ($P = .008$).

mulations of amphotericin B may be useful for patients with cryptococcal meningitis and renal insufficiency [16]. The upcoming updated guidelines, on the other hand, preferentially recommend lipid formulations of amphotericin B as induction therapy for CNS and severe non-CNS disease in SOT recipients [17]. The basis of this recommendation is largely the lower incidence of drug-associated nephrotoxicity, as evidence-based data on improved outcomes with these agents are largely unavailable at present [17]. To our knowledge, our study is the first to systematically evaluate the efficacy of lipid formulations of amphotericin B for CNS cryptococcosis exclusively in SOT recipients.

To date, published reports comparing the efficacy of lipid formulations of amphotericin B with amphotericin B deoxycholate in patients with cryptococcal meningitis remain limited [18, 19]. In a randomized study involving 55 patients with HIV-associated cryptococcal meningitis, those treated with amphotericin B lipid complex had a clinical response rate of 86%, compared with 65% among patients treated with amphotericin B deoxycholate [18]. In another small randomized trial of 28 HIV-positive patients with cryptococcal meningitis, liposomal amphotericin B sterilized CSF cultures significantly more rapidly than did amphotericin B deoxycholate (7–14 days vs 21 days), although the clinical response rates (86% vs 80%) were similar [19]. An unpublished randomized, double-blind trial involving 267 HIV-infected patients with cryptococcal meningitis showed no difference between the efficacy of liposomal amphotericin B (3 mg/kg/day or 6 mg/kg/day) and that of amphotericin B deoxycholate as measured by CSF culture conversion at 2 weeks (58.3% or 48% vs 47.5%) and success at 10 weeks (37% or 49% vs 53%); however, more nephrotoxicity was observed with the higher dose of liposomal amphotericin B [20, 21]. In a prospective observational study, no differences in outcome existed in patients with CNS cryptococcosis when the combination of amphotericin B plus flucytosine included deoxycholate or one of its lipid formulations [22].

Nevertheless, a meta-analysis showed that lipid formulations of amphotericin B significantly reduced all-cause mortality risk of systemic fungal infections, including cryptococcosis, by an estimated 28%, compared with amphotericin B deoxycholate (OR, 0.72; 95% CI, 0.54–0.97) [23]. Our data show that the use of lipid formulations of amphotericin B was independently associated with a lower mortality among SOT recipients with CNS cryptococcosis (Table 3). The precise reason why lipid formulations of amphotericin B have better efficacy than do amphotericin B deoxycholate is not known, although animal model data support some hypotheses. Lipid formulations of amphotericin B reduced the fungal burden to a significantly greater degree and achieved higher survival rates than did amphotericin B deoxycholate in animal models [24, 25]. In murine cryptococcal meningitis, 20 or 30 mg/kg of liposomal amphotericin B completely cleared the yeast from the brains in 44% and 78% of mice, respectively, whereas 3 mg/kg of amphotericin B deoxycholate did not [24]. In another study, mice with cryptococcal meningitis treated with 10 mg/kg of liposomal amphotericin B had a better survival rate on day 5 than did those treated with 1 mg/kg of amphotericin B deoxycholate (50%–60% vs 12.5%–30%) [25].

In addition, lipid formulations of amphotericin B have unique immunomodulatory characteristics, compared with amphotericin B deoxycholate, that may be beneficial during the treatment of cryptococcosis [26]. Liposomal amphotericin B and amphotericin B lipid complex either down-regulate or have no effect on inflammatory cytokine gene expression [26–28]. Liposomal amphotericin B induces Toll-like receptor (TLR) 4–dependent signaling in neutrophils, and amphotericin B deoxycholate induces TLR2-mediated inflammatory cascade in human monocytes and macrophages [29, 30]. TLR4 activation is associated with a more anti-inflammatory pattern of cytokine production and, subsequently, lesser degrees of inflammatory tissue damage during fungal infection [30]. These anti-inflammatory properties might be derived from the ability of liposomes to lessen the extravasation of neutrophils into sites of inflammation by modifying the intracellular signaling [26, 31, 32]. In addition, empty liposomes improve fungal clearance and survival of corticosteroid-treated mice with invasive pulmonary aspergillosis by attenuating the immunopathology [33]. It has been increasingly recognized that surviving an infection requires a tightly controlled immune system that straddles a fine line between successful eradication of the invading pathogen and limiting the damage to tissues from a dysregulated

immune response [34]. It is probable that liposomes not only facilitate delivery of amphotericin B to the site of infection but also limit the subsequent immune-related tissue damaged caused by the antifungal agent.

Flucytosine plays a pivotal role in the treatment of cryptococcal meningitis. It has been well documented that amphotericin B deoxycholate plus flucytosine has significantly greater early fungicidal activity than does amphotericin B deoxycholate alone for cryptococcal meningitis in HIV-positive patients not receiving highly active antiretroviral therapy [35, 36]. In addition, lack of flucytosine for induction therapy is independently associated with lack of CSF sterilization at week 2 in HIV-positive patients with cryptococcal meningitis (OR, 24.4; 95% CI, 4.8–123.5; $P < .001$) and with mycological failure at week 2 in HIV-positive or HIV-negative patients with cryptococcosis (OR, 3.8; 95% CI, 1.9–7.8; $P < .001$) [14]. In patients with meningoencephalitis, lack of induction therapy with amphotericin B formulations and flucytosine, compared with any other induction therapies, was an independent factor of treatment failure at week 2 (OR, 51.25; 95% CI, 9.67–271.52; $P < .001$) [22]. Furthermore, prescription of flucytosine for <14 days was independently associated with treatment failure of cryptococcosis at month 3 [22]. It is not clear why flucytosine did not have such profound effects in SOT recipients with CNS cryptococcosis in our study. The patient populations in the aforementioned studies comprised primarily HIV-infected persons (77%) who typically have higher fungal burden (CSF antigen level >1:512) [14, 22]. Because flucytosine expedites CSF sterilization in the early stage of treatment, this effect might not be prominent or clinically relevant in the context of patients with low fungal burden, such as SOT recipients [9]. In addition, most studies used CSF sterilization at week 2 as a measure of the efficacy of treatment with flucytosine, whereas the current study used mortality at 90 days after the treatment. The small number of our patients receiving flucytosine may also have precluded meaningful analysis.

Several weaknesses of our study deserve to be acknowledged. The data regarding outcomes were systematically assessed using standardized criteria; however, our results should be interpreted with caution because this was not a randomized trial evaluating therapeutic efficacy of antifungal regimens. In addition, although the data analyses controlled for all potential confounders, it is plausible that unknown or unmeasured factors could have influenced outcomes, such as management of immunosuppression after diagnosis of cryptococcosis, development of renal failure during therapy with various agents, or contribution of other opportunistic infections. We note, however, that the comparison group in our study was concurrent and contemporaneous, thus rendering our result more relevant than if a historic comparator was used.

In summary, the lipid formulations of amphotericin B as primary therapy, regardless of flucytosine use, were independently associated with improved survival in SOT recipients with CNS cryptococcosis. Given significant implications of these data for the management of CNS cryptococcal disease not only in SOT recipients but also in other hosts, future studies to validate our findings are warranted.

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