Fungal Burden, Early Fungicidal Activity, and Outcome in Cryptococcal Meningitis in Antiretroviral-Naive or Antiretroviral-Experienced Patients Treated with Amphotericin B or Fluconazole

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(See the editorial commentary by Lortholary on pages 81–3)

In a prospective observational study of 54 patients with human immunodeficiency virus–associated cryptococcal meningitis, the early fungicidal activity of amphotericin B (1 mg/kg/day) was significantly greater than that of fluconazole (400 mg/day). Compared with antiretroviral therapy–naive patients, patients developing cryptococcal meningitis while already receiving antiretroviral therapy had lower baseline fungal burdens and a longer median duration of survival, but there were no differences observed in fungal clearance, cerebrospinal fluid proinflammatory cytokines, or 10-week mortality.

In African countries, prior to the availability of antiretroviral therapy (ART), survival after experiencing cryptococcal meningitis (CM) was poor, with an 88%–100% mortality rate at 6 months [1, 2]. Fluconazole was often the antifungal drug of choice. The advent of ART altered the long-term prognosis for patients with CM, although published data from the developing world are lacking. This neccesitates a fresh look at the benefits and risks of implementing amphotericin B (AmB)–based therapies in this context.

At Jooste Hospital (Cape Town, South Africa) in 2004, following expansion of the ART program, routine initial treatment for CM was switched from 400 mg/day of fluconazole to 1 mg/kg/day of AmB for 7 days, except for patients with a Glasgow Coma Score of <10, who continued to receive fluconazole, and except for times when the AmB supply was interrupted because of a worldwide shortage of AmB [3]. Access to ART may also have altered the pathophysiology and clinical course of CM in the proportion of patients now presenting with CM after initiating ART. We hypothesized that this group may mount a greater proinflammatory CSF cytokine response to CM. In prior work, we have demonstrated a correlation between such a proinflammatory response and baseline fungal burden, clearance, and outcome [4, 5].

Our aims were to determine and compare the early fungicidal activity (EFA) and toxicity of AmB 1 mg/kg/day versus fluconazole 400 mg/day for the initial treatment of HIV-associated CM. In addition, we compared the results of analysis of baseline organism load, EFA, CSF immune response, and long-term outcome among ART-naive and ART-experienced patients developing CM.

Methods. This study was a prospective observational study conducted at GF Jooste Hospital, and was approved by the Research Ethics Committee of the University of Cape Town (Cape Town, South Africa). From February to September 2005, after obtaining written informed consent from each person, we enrolled 54 consecutive HIV-infected adults aged ⩾21 years who were presenting with an episode of CM that had been diagnosed using CSF India ink or positive CSF cryptococcal antigen tests and confirmed with positive culture results for *Cryptococcus neoformans*. First and relapse episodes of CM in ART-naive and ART-experienced patients were included. There were no exclusion criteria.

Patients were treated according to hospital protocol. Patients with a baseline Glasgow Coma Score of ⩾10 received AmB deoxycholate (Fungizone; Bristol-Myers Squibb) at a dosage of 1 mg/kg/day for 7 days, thereafter switching to oral fluconazole (Diflucan; Pfizer) at a dosage of 400 mg/day for 8 weeks. Patients with a Glasgow Coma Score of <10 received fluconazole at a dosage of 400 mg/day, from the outset, for 10 weeks. All patients were switched to fluconazole at a dosage of 200 mg/day after 10 weeks. In patients who were receiving concomitant rifampicin, the fluconazole dose was increased by 50% [6].

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Patients received 1 L of 0.9% saline solution daily, as well as electrolyte supplementation as required. Follow-up lumbar punctures were performed on days 3, 7, and 14. Patients with an increased CSF opening pressure (>25 cm of water) underwent additional lumbar punctures, in accordance with current guidelines [7]. After patient discharge from the hospital, all participants not already receiving ART received counseling and initiated ART (stavudine plus lamivudine, plus efavirenz or nevirapine) not sooner than 4 weeks after the start of antifungal therapy. Patients were followed up for 1 year after enrollment into the study.

Altered mental status was defined as any reduction in Glasgow Coma Score. All participants underwent baseline hematological and biochemical testing, CD4 count and HIV viral load were determined, and subsequent twice-weekly biochemical and weekly hematological testing was performed to monitor for adverse effects of therapy. Quantitative fungal cultures of CSF samples were performed, as described elsewhere [4]. CSF immune parameters (IFN-γ, TNF-α, IL-1β, IL-2, IL-6 , IL-8, IL-10, G-CSF, MCP-1, MIP-1α, MIP-1β, and Regulated upon Activation, Normal T cell Expressed, and Secreted [RANTES] levels) were determined using the Luminex multianalyte system (Luminex) and cytokine kits (Bio-Rad), as described elsewhere [5], using a separate ELISA for IFN-γ and TNF-α (Quantikine; R&D Systems).

We compared baseline characteristics of groups using the χ^2 or Fisher's exact test for categorical variables, and the Mann-Whitney *U* test for continuous variables. Mortality at 10 weeks and 1 year was compared using Fisher's exact test. Survival curves were compared using the Mantel-Haenszel log-rank test. EFA was compared between groups using linear regression [4, 5]. Median cytokine concentrations were compared using the Mann-Whitney *U* test.

Results. Sixty-one patients met the inclusion criteria, of whom 54 gave informed consent and were enrolled. Table 1 shows baseline clinical and laboratory characteristics and outcomes. Forty-nine patients (91%) were treated with AmB, and 5 received fluconazole. Fluconazole was administered to 2 patients because of a nonavailability of AmB, to 1 patient because the patient refused hospital admission, to 1 patient because of renal impairment (baseline creatinine level, 273 μmol/L), and to 1 patient with a Glasgow Coma Score <10. The median follow-up of survivors was 14 months.

In the AmB group, the median duration of treatment was 7 days (interquartile range, 6–8 days). Renal impairment (creatinine level, >220 μmol/L), developed in 4 patients. Only 1 patient required discontinuation of AmB before 7 days because of renal impairment. No patients developed adverse events attributed to fluconazole. The fluconazole-treated group showed a trend towards more-severe disease: a higher baseline fungal burden (340,000 vs. 74,000 colony-forming units (CFU)/mL CSF; $P = .06$), a higher proportion of patients who experienced altered mental status (3 of 5 patients vs. 10 of 49; $P = .08$); and a higher viral load, compared with the AmB group (420,000 vs. 44,000 copies/mL; $P = .09$).

The EFA in patients treated with AmB was -0.48 ± 0.28 (mean $\pm$ SD) log CFU/mL of CSF per day ($n = 49$) when calculated over the whole of the initial 2 weeks of therapy; this value was -0.54 ± 0.3 when calculated over the first 7 days, when patients were receiving AmB for all or most of the time. Over 2 weeks, EFA was significantly higher for patients who received AmB than for patients who received fluconazole as initial therapy (EFA of fluconazole, -0.02 ± 0.05 log CFU/day; difference, 0.46 log CFU/day; 95% CI, 0.21–0.72; $P = .001$) (figure 1*A* and figure 1*B*). The difference remained significant ($P \leq .005$) if linear regression modeling was used to adjust for baseline organism load and/or for baseline CSF IFN-γ concentration—factors previously found to be associated with EFA [4, 5]—or for plasma viral load, altered mental status, prior receipt of ART, or if the experience was a first or a relapse episode of CM.

Overall mortality was 17% at 2 weeks and 37% at 10 weeks. Median survival was 110 days (153 days for AmB-treated patients and 61 days for patients treated initially with fluconazole; $P = .03$) (figure 1*C*).

In ART-naive patients, the median interval (interquartile range) from initiating antifungal therapy to initiating ART was 46 (36–57) days. In ART-experienced patients, the median interval (interquartile range) between initiating ART and presenting to a health care facility with CM was 30 (21–77) days. Compared with ART-naive patients, ART-experienced patients had a lower baseline fungal burden (34,000 vs. 235,000 CFU/mL CSF; $P = .03$), and displayed a trend towards higher WBC counts in CSF (24 vs. 7 cells/mm^3; $P = .19$).

There was no difference observed in EFA between the ART-naive and ART-experienced groups treated with AmB (mean $\pm$ SD, -0.5 ± 0.27 vs. -0.45 ± 0.31 log CFU/day; $P = .63$), even when linear regression modeling was used to adjust for baseline organism load or baseline CSF IFN-γ concentration. When comparing outcome in ART-naive patients with ART-experienced patients, 10-week mortality was similar (33% vs. 38%; $P = .8$), but 1-year mortality was significantly different (76% vs. 41%; $P = .03$) (table 1). Despite access to ART for ART-naive patients after undergoing treatment of an initial episode of CM, ART-experienced patients had a longer median survival (>1 year vs. 95 days; $P = .04$) (figure 1*D*). There were no significant differences observed between ART-naive and ART-experienced patients in the baseline CSF concentrations of any of the 12 cytokines and chemokines measured (data not shown), except for G-CSF (median level, 142 vs 307 pg/mL; $P = .003$).

Discussion. The observational nature of our study, with few

Table 1. Baseline clinical and laboratory characteristics and clinical outcomes, by drug treatment group and antiretroviral therapy (ART) status.

Characteristic	All patients (n = 54)	Drug treatment group			ART status		
		AmB (n = 49)	Flu (n = 5)	P	ART naive (n = 36)	ART experienced (n = 18)	P
No. (%) of male patients	14 (26)	11 (22)	3 (60)		10 (28)	4 (22)	
Patient age, years	34 (29–39)	33 (29–38)	39 (37–51)		36 (29–39)	33 (30–39)	
Mean weight ± SD, in kg	55 ± 12	56 ± 12	49 ± 3		54 ± 12	58 ± 12	
No. (%) of patients with HIV infection status known at presentation	46 (85)	43 (88)	3 (60)		28 (78)	…	
No. (%) of patients with abnormal mental status	13 (24)	10 (20)	3 (60)	.08	9 (25)	4 (22)	
CD4 count, cells × 10^6/L	49 (21–71)	54 (21–72)	41 (22–45)	.22	41 (20–57)	65 (43–97)	.03
HIV load, copies/mL	55,000 (1060–250,000)	44,000 (830–230,000)	420,000 (212,000–750,000)	.09	170,000 (19,750–296,500)	155 (50–8688)	<.001
No. (%) of patients who experience a relapse of CM[a]	9 (17)	9 (18)	0 (0)		4 (11)	5 (28)	
No. (%) of patients receiving HAART	18 (33)	16 (33)	2 (40)		…	…	
CSF data							
OP, cm H20	28 (16–34)	26 (16–34)	31 (24–39)	.83	28 (15–35)	29 (18–34)	.87
WBC count, cells/mm^3	11 (1–56)	16 (2–59)	3 (0–10)	.13	7 (0–41)	24 (10–68)	.19
QC, CFU/mL CSF	115,500 (8050–550,000)	74,000 (7000–358,750)	340,000 (270,000–1,650,000)	.06	235,000 (12,425–1,076,875)	34,000 (3500–164,000)	.03
Deaths[b]							
Proportion (%) after 10 weeks of follow-up	19/52 (37)	16/48 (33)	3/4 (75)		13/34 (38)	6/18 (33)	.8
Proportion (%) after 1 year of follow-up	33/51 (65)	29/47 (62)	4/4 (100)		26/34 (76)	7/17 (41)	.03

NOTE. Data are median (interquartile range), unless otherwise indicated. AmB, amphotericin B 1 mg/kg/day; CFU, colony-forming units; CM, cryptococcal meningitis; Flu, fluconazole; OP, opening pressure; QC, quantitative culture.

[a] Relapse was defined as recurrent CM symptoms and positive CSF culture results in a patient with a documented, culture-confirmed prior episode of CM with complete clinical response to antifungal treatment. Five of these patients were included in a prior report of the sensitivity of *Cryptococcus neoformans* isolates in cases of relapse [6]. Patients were only included once, as either experiencing a first episode or a relapse case.

[b] Two patients were lost to follow-up at 10 weeks, and 3 patients were lost to follow-up at 1 year.

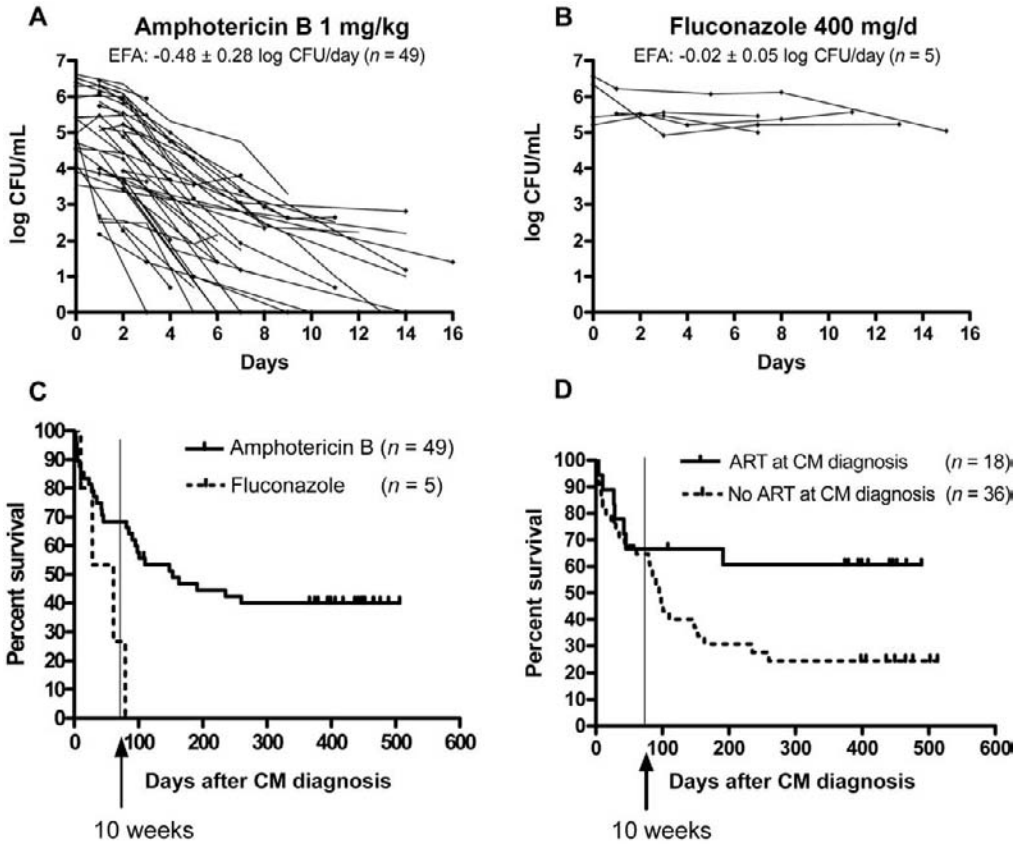

Figure 1. *A* and *B,* the decrease in CSF *Cryptococcus neoformans* colony-forming units (CFU) over time, by treatment group. The decrease in log CFU per mL of CSF per day was calculated for each patient using the slope of the linear regression of log CFU against time. For each treatment group, early fungicidal activity (EFA) is shown as the mean ± SD rate of decrease in log CFU counts. EFA was significantly greater for amphotericin B, compared with fluconazole ($P = .001$). *C,* Kaplan-Meier survival curves, from diagnosis of cryptococcal meningitis (CM), by drug treatment group. Median survival was significantly greater for amphotericin B than for fluconazole (153 days vs. 61 days; $P = .03$ with the χ^2 test). *D,* Kaplan-Meier survival curves from cryptococcal meningitis diagnosis by antiretroviral therapy (ART) status at presentation. Median survival was significantly greater for patients who presented while receiving ART (>1 year vs. 95 days for those not receiving ART; $P = .04$ with the χ^2 test). CM, cryptococcal meningitis.

patients with more-severe disease receiving fluconazole, means that differences in median survival between treatment groups may be subject to bias. However, the marked difference in EFA of fluconazole and AmB, which was significant even when markers of severity of disease or host immunity shown to be associated with clearance were controlled for [4, 5], likely represents a real difference in the activity of the drugs. At a dosage of 400 mg/day, fluconazole dosage is almost static over the first 2 weeks of therapy.

Given a previous study linking CSF proinflammatory cytokines—IFN-γ in particular—to EFA [5], the lack of any difference in CSF immune parameters between ART-naive and ART-experienced patients (except for G-CSF, a finding that needs to be confirmed in an independent series) is consistent with the fact that we also found no difference in EFA between these 2 groups. There is ongoing debate as to the risks and benefits of immediate or early institution of ART in the setting of acute CM in ART-naive patients. Our findings of similar acute CM-related mortality but large differences in 1-year survival between ART-naive and ART–experienced patients presenting with CM would suggest that such a strategy may not reduce acute CM-related deaths, but may reduce deaths due to other HIV infection–related causes. The risks of administration of earlier ART, particularly of increased rates and severity of immune reconstitution reactions [8–10], remains unclear.

It is interesting to note that a recent study [11] found no evidence for a decrease in acute (3-month) mortality among patients presenting with CM in France in the ART era, compared with the pre-ART era. However, in that study, after the introduction of ART, only 21% of French patients with CM were receiving ART at the time of CM diagnosis, compared with 100% of the ART-experienced group who presented with CM in our study.

The marked difference in EFA and survival in patients treated

with AmB versus fluconazole lends further support to the use of AmB to treat CM wherever feasible, even if resources limit the duration of AmB to <2 weeks. At 1mg/kg/day, AmB was very well tolerated in this setting. When safe adminstration of AmB is not possible, these results underline the urgent need for studies, currently underway, to determine the efficacy of higher doses of fluconazole. This study confirms that, when acute infection has ended and an ART regimen has been established, patients in resource-limited areas who have HIV infection–associated CM have a good prognosis. The challenge remains to improve acute disease management, thereby increasing the proportion of patients who survive the critical initial months.

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