Early versus Delayed Initiation of Antiretroviral Therapy for Concurrent HIV Infection and Cryptococcal Meningitis in Sub-Saharan Africa

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(See the editorial commentary by Meintjes and Wilkinson, on pages 1539–1541.)

Background. Cryptococcal meningitis (CM) remains a leading cause of acquired immunodeficiency syndrome–related death in sub-Saharan Africa. The timing of the initiation of antiretroviral therapy (ART) for human immunodeficiency virus (HIV)–associated CM remains uncertain. The study aimed to determine the optimal timing for initiation of ART in HIV-positive individuals with CM.

Methods. A prospective, open-label, randomized clinical trial was conducted at a tertiary teaching hospital in Zimbabwe. Participants were aged ⩾18 years, were ART naive, had received a first CM diagnosis, and were randomized to receive early ART (within 72 h after CM diagnosis) or delayed ART (after 10 weeks of treatment with fluconazole alone). Participants received 800 mg of fluconazole per day. The ART regimen used was stavudine, lamivudine, and nevirapine given twice daily. The duration of follow-up was up to 3 years. The primary end point was all-cause mortality.

Results. Fifty-four participants were enrolled in the study (28 in the early ART arm and 26 in the delayed ART arm). The median CD4 cell count at enrollment was 37 cells/mm^3 (interquartile range, 17–69 cells/mm^3). The 3-year mortality rate differed significantly between the early and delayed ART groups (88% vs 54%; $P<.006$); the overall 3-year mortality rate was 73%. The median durations of survival were 28 days and 637 days in the early and delayed ART groups, respectively ($P = .031$, by log-rank test). The risk of mortality was almost 3 times as great in the early ART group versus the delayed ART group (adjusted hazard ratio, 2.85; 95% confidence interval, 1.1–7.23). The study was terminated early by the data safety monitoring committee.

Conclusions. In resource-limited settings where CM management may be suboptimal, when compared with a delay of 10 weeks after a CM diagnosis, early initiation of ART results in increased mortality.

Trial registration. ClinicalTrials.gov identifier: NCT00830856.

Cryptococcal meningitis (CM) continues to be one of the most devastating AIDS-defining illnesses among people living with human immunodeficiency virus (HIV) infection [1–3]. CM is one of the leading causes of meningitis among hospitalized patients in sub-Saharan Africa [4] and the cause of death in as many as 20%–30% of patients with AIDS [5, 6]. Without antifungal therapy, the mortality rate for CM is 100%. In sub-Saharan Africa, azoles are the primary therapy and have led to a reduction in short-term mortality rates, even though long-term outcomes remain poor [7–9]. Until recently, fluconazole monotherapy at dosages of 200–400 mg per day had been the standard of care in much of sub-Saharan Africa. Recent data suggest that higher doses (eg, 800–1200 mg per day) are safe, well tolerated, and more rapidly fungicidal than are lower doses [7, 10, 11]

Amphotericin B with flucytosine results in improved outcomes, compared with azole monotherapy [12, 13]. In North America, the use of amphotericin B and flucytosine with aggressive management of elevated intracranial pressure has resulted in mortality rates of <10% [12, 14, 15]. In Africa, use of amphotericin B reduced

Received 6 December 2009; accepted 29 January 2010; electronically published 23 April 2010.

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Clinical Infectious Diseases 2010;50(11):1532–1538
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1058-4838/2010/5011-0017$15.00
DOI: 10.1086/652652

the 14-day mortality rate to 17%–36% [3, 16–18], but long-term follow-up of these participants has been limited. Because of its cost and the difficulty of administration and monitoring, amphotericin B and flucytosine are not routinely used in much of sub-Saharan Africa [19, 20], and fluconazole currently remains the only option available for most patients. The drug is provided for free through the Pfizer Diflucan Partnership Program [21].

The increased availability of antiretroviral therapy poses an opportunity to intervene and modify outcomes in individuals with HIV-associated opportunistic infections, such as CM. The timing of initiation of antiretroviral therapy (ART) in individuals with CM who are commencing antifungal therapy remains uncertain [22]. We conducted a prospective, open-label, randomized, 2-arm clinical trial to compare the 3-year mortality rates among participants allocated to receive early initiation of ART ($\leqslant$72 h after diagnosis of CM) or delayed initiation of ART (10 weeks after CM diagnosis).

METHODS

Study setting. The study was conducted at Parirenyatwa Central Hospital, a tertiary referral teaching hospital in Harare, Zimbabwe. Enrollment occurred from October 2006 through April 2008, and patients were observed through October 2009.

Study participants. Eligible participants were aged $\geqslant$18 years and HIV positive. HIV infection was determined using the national HIV testing protocol, by which HIV infection is documented by 2 positive results of different licensed rapid tests; in the event of a discordant result, a third rapid test is used as a tie breaker. All patients had CM confirmed by positive results of India ink identification of *Cryptococcus neoformans* in the cerebrospinal fluid (CSF) and/or a CSF cryptococcal polysaccharide antigen (CRAG) test (CALAS; Meridian Diagnostics). Participants were excluded if they had any of the following characteristics: patient had a previous diagnosis of or treatment for CM, was currently receiving ART, was receiving medications that affect the metabolism of fluconazole (especially rifampicin), was pregnant or lactating, or had a history of hepatic or renal dysfunction. To optimize retention and maximize follow-up, only participants who resided in a 50-km radius of Harare were enrolled.

Study design. A computer-generated randomization schedule was used to assign participants to the early ART and delayed ART arms of the study. This was an open-label study, and only the study statistician had access to the randomization schedule. The randomization sequence was concealed to the study nurse who was responsible for participant enrollment. After randomization assignment, physicians and nurses taking care of the participant were not blinded to the participant's treatment group.

All eligible participants were identified from the hospital's admission registry and microbiology laboratory, where CSF samples were processed. A chart review was performed for all patients in the adult medical wards who had CSF studies processed by the microbiology laboratory. Those whose India ink results were positive or whose chart review suggested meningitis (ie, the patient had headache, meningismus, fever, photophobia, and altered mental status) and met the eligibility criteria had CSF samples sent for CRAG testing. Participants whose CSF specimens yielded positive results of India ink examination and/or a CSF CRAG test were approached for recruitment, and informed consent was obtained in English or Shona (the local language) by the study nurse. Participants who could not consent for themselves or who did not have relatives available to consent for them were excluded. Consent had to be obtained $\leqslant$72 h after the diagnosis. At the time of enrollment, the next sequential enrollment number was allocated to the participant, and a sealed envelope bearing the corresponding number was opened by the participant to reveal the treatment group assignment.

Baseline investigations included complete blood cell counts; CD4 cell counts; determination of electrolyte, urea, and creatinine levels; liver function tests; microscopic examination and culture of CSF samples; and CSF CRAG testing. Serum and CSF samples were stored for assessment of viral loads and CRAG titers, cultures, and future studies.

Treatment protocol. All participants were inpatients at the time of enrollment and started taking fluconazole (800 mg once per day; Diflucan [Pfizer]) at the time of CM diagnosis. Participants were randomized to either initiate ART $\leqslant$72 h after diagnosis (the early ART arm) or to delay initiation of ART until after 10 weeks of fluconazole therapy (the delayed ART arm). Although participants were inpatients, they were treated by the primary medicine service, in accordance with the local standard of care; management included diagnostic lumbar punctures and measurement of CSF pressures, when manometers are available, but thereafter, CSF pressures are not monitored objectively. Worsening headaches and mental status may trigger repeated lumbar punctures.

After 10 weeks, fluconazole was reduced to a prophylactic dosage of 200 mg once per day. If there was clinical suspicion of treatment failure confirmed by positive India ink stain and/or culture results or if there were persistently elevated CRAG titers, the participant would resume or continue to receive fluconazole (800 mg once per day) until the CSF was clear. The ART regimen used consisted of a fixed-dose combination of stavudine (30 mg twice per day), lamivudine (150 mg twice per day), and nevirapine (200 mg twice per day, with a 200-mg once-daily 2-week lead-in dose), in accordance with the 2005 Zimbabwe national ART treatment guidelines. Adherence to fluconazole and ART was assessed by self-reports and pill counts at each visit.

Participants were observed at the Parirenyatwa Hospital outpatient HIV clinic at weeks 2, 4, 8, and 10. Thereafter, clinical evaluation was performed monthly, CD4 cell counts and complete blood cell counts were conducted every 3 months, and liver function tests were conducted every 6 months for up to 2 years. CSF was sampled at weeks 2, 4, and 10. CSF hypertension was reduced to <200 mm if it was found to be elevated at these visits or at any other visit necessitated by worsening headache. For patients who defaulted, telephone contacts and home visits were conducted.

The primary end point was all-cause mortality. Cause of death was ascertained through hospital records or contacts with next of kin if this occurred at home. Two investigators independently adjudicated and subsequently agreed on the cause of death.

Data safety and monitoring. On the basis of a number of factors, including feasibility and adequate statistical power, we chose a sample size per treatment arm of 100, for a total of 200 patients. This would provide adequate statistical power to be able to detect hazard ratios for the primary end point of mortality of at least 1.8.

An independent data safety monitoring committee was established with planned interim analysis after recruitment of 50% of participants. Initial estimated recruitment period was 24 weeks. However, recruitment was slow, and the first data safety monitoring committee review was conducted at 6 months when only 21 participants had been recruited. There were no safety or efficacy concerns, and the study was allowed to continue with enrollment. Recruitment continued to be slow, and the next data and safety review was conducted at 18 months, when 54 participants had been enrolled and all had commenced ART (either because they were in the immediate group or had reached week 10 after CM diagnosis). Analysis of the data showed a statistically significant difference in mortality between the 2 treatment groups, and it was decided to terminate study enrollment at that time.

Statistical analysis. All statistical analyses were based on the intention-to-treat principle. Baseline characteristics and mortality were compared between the 2 treatment arms. Continuous measures were summarized using means ($\pm$ standard deviations). Categorical variables were summarized using percentages. Differences between treatment arms were assessed using the χ^2 test or the Fisher exact test for categorical variables and a 2-sample *t* test or Wilcoxon 2-sample *t* test for continuous variables. Equality of variances was assessed to determine the appropriate test.

For each patient, survival time was calculated as the time from randomization entry until death for those who died, and those who were alive were censored at the date of the last clinical encounter or on 31 October 2009. Participants that were lost to follow-up were censored at their last follow-up visit. The Kaplan-Meier plot and log-rank test were used to compare differences in mortality and the median time to death between the 2 treatment arms. Incidence rates and corresponding 95% confidence intervals (CIs) under Poisson distribution were also calculated. After confirming that the assumption of proportionality of hazards was met, we ran a Cox model to compare the risk of death in the 2 treatment arms while adjusting for baseline CD4 cell count, which was modelled as a continuous variable. All statistical analyses were performed using Stata software, version 10 (Stata Corp). A P value <.05 was considered to indicate statistical significance.

Ethics approval. The study was approved by the Joint Research Ethics Committee of the University of Zimbabwe College of Health Sciences and Parirenyatwa Central Hospital, the Medical Research Council of Zimbabwe, and the Medicines Control Authority of Zimbabwe.

RESULTS

A total of 1328 CSF samples were processed in the Parirenyatwa Central Hospital Microbiology Laboratory during the recruitment period. Four hundred eight had positive results of CRAG tests or India ink examinations, and 93 (23%) met eligibility criteria. Twenty-eight participants were randomized to the early treatment arm, and 26 were randomized to the delayed treatment arm (Figure 1). The mean age of the participants was 37 $\pm$ 7.7 years, and 26 patients (48%) were women (Table 1). Typical symptoms at presentation included chronic headache, with acute worsening of headache and associated mental status changes. Other common presenting symptoms included weight loss, fever, night sweats, and decreased cognitive function. There were no significant differences in baseline characteristics, presenting symptoms, and previous opportunistic infections between patients in the 2 treatment arms (Tables 1 and 2).

The median CD4 cell count at enrollment was 37 cells/mm^3 (interquartile range, 17–69 cells/mm^3); there was no significant difference between the arms in the median CD4 cell count ($P = .289$) (Table 1). Baseline opening CSF pressures were not available for most participants, because the initial CSF opening pressures were done before study enrollment in the hospital's emergency department, where measurement of opening pressures was not routinely performed. All CSF samples tested negative for acid- and alcohol-fast bacilli by Ziehl-Neelsen staining.

The median duration of hospitalization was short, with most participants discharged from hospital within 1 week. Overall median follow-up time was 27 days (interquartile range, 9–571 days), with an overall 3-year mortality rate of 73%. The 3-year mortality rate was 88% in the early ART arm ($n = 23$) and 54% in the delayed ART arm ($n = 12$). Kaplan-Meier survival estimates for the 2 treatment groups revealed a median survival duration of 28 days (4 weeks) in the early ART arm and 637 days in the delayed ART arm ($P = .031$, by log-rank test) (Fig-

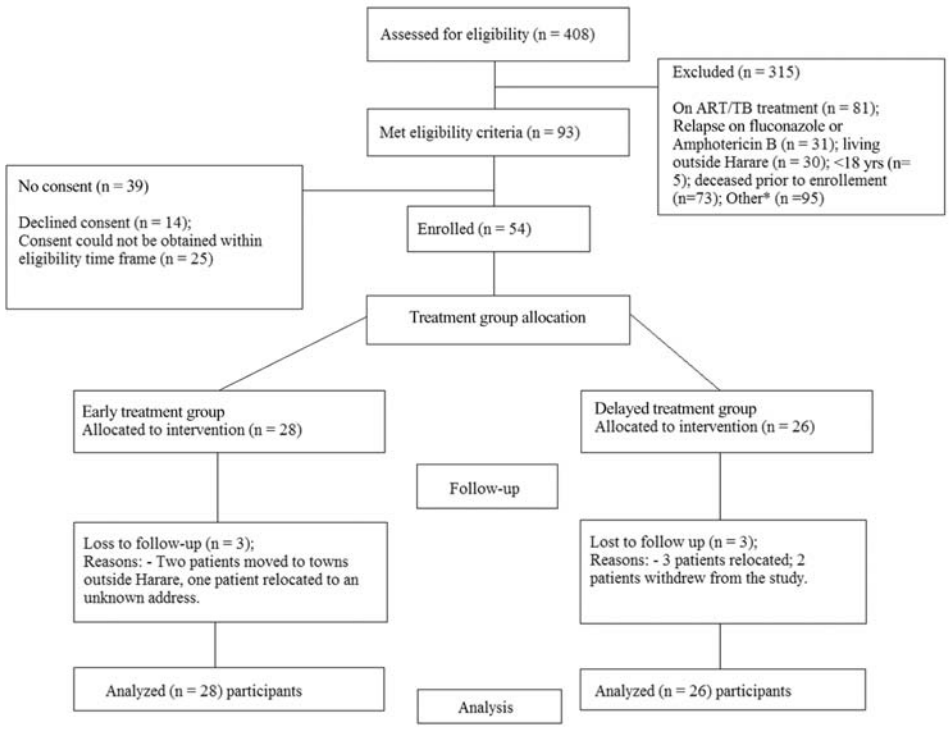

Figure 1. Study enrollment, treatment allocation, and analysis scheme. *Other included outpatients ($n = 75$), pregnant women ($n = 1$), patients with liver failure ($n = 3$), and persons enrolled in other studies ($n = 16$). ART, antiretroviral therapy; TB, tuberculosis.

ure 2). Mortality typically occurred early in both treatment groups. Ten deaths (43.5%) in the early ART arm and 7 deaths (58.3%) in the delayed ART arm occurred ⩽2 weeks after enrolment. Six participants died during the initial hospitalization (ie, they were never discharged from the hospital; 3 in each treatment group). Thirteen participants in the early ART arm and 1 in the delayed ART arm died during subsequent rehospitalizations. Fifteen participants died at home. Univariate analysis revealed that the only significant predictor of mortality was early ART initiation ($P = .023$). Low baseline CD4 cell count and CSF CRAG titer were not significant predictors of mortality ($P = .067$ and $P = .72$, respectively). The results of the unadjusted Cox model showed that early ART was associated with more than twice the risk of mortality, comparison with delayed ART (hazard ratio, 2.34; 95% CI, 1.12–4.89). After adjusting for the potential confounding effects of age, sex, CSF CRAG titer, and CD4 cell count, the hazard ratio increased (hazard ratio, 2.85; 95% CI, 1.1–7.23).

The mortality rate adjusted for age, sex, and CD4 cell count was 40 deaths per 1000 person-weeks at risk (95% CI, 2.6–60.9 deaths per 1000 person-weeks at risk) in the early ART arm, compared with 11 deaths per 1000 person-weeks at risk (95% CI, 6.1–19.9 deaths per 1000 person-weeks at risk) in the delayed ART arm ($P<.05$). The cause of death for most participants was determined clinically at autopsy, and histopathological diagnoses were not available. The primary cause of death was CM in most patients (Table 3).

DISCUSSION

We conducted a 2-arm randomized clinical trial to determine whether the timing of initiation of ART in participants with concurrent HIV infection and CM has an impact on survival. This study, to our knowledge, is the first prospective, randomized study to address whether the timing of initiation of ART has an impact on mortality in patients with advanced CM. Although other studies of mixed patient cohorts or nonrandomized studies have suggested that early ART treatment would decrease mortality and possibly improve clinical outcomes [22, 23], the data from our study show that early ART results in increased mortality in individuals with HIV-associated CM.

In this study, mortality in both treatment groups was primarily due to complications related to CM, with most deaths occurring within the first 2 weeks after enrollment. Almost all deaths had occurred by week 4. The high mortality rates observed in this study are consistent with what has been observed in other studies and cohorts [8, 9]. However, the excess mortality associated with early initiation of ART suggest that early ART is detrimental in the treatment of patients with concurrent CM and HIV infection. Possible reasons for the detrimental effects of early ART in these participants include suboptimal

Table 1. Baseline Characteristics of Participants with Cryptococcal Meningitis, Stratified by Treatment Group

Characteristic	Early treatment arm (n = 28)	Delayed treatment arm (n = 26)	P
Age, mean years ± SD	36.6 ± 8.5	37.5 ± 6.9	.660
Female sex	14 (50)	12 (46)	.777
CD4 cell count, median cells/mm^3 (IQR)[a]	27 (17–69)	51.5 (25–69.5)	.289
Viral load, mean $\log_{10}$ copies/mL ± SD[b]	4.97 ± 0.61	5.21 ± 0.37	.215
ALT level, median U/L (IQR)[c]	17 (14–32)	20 (13–27)	.763
CSF glucose level <2.2 mmol/L	9 (32.1)	7 (26.9)	.675
CSF protein level <0.45 g/L	2 (7.1)	5 (19.2)	.243
Hemoglobin level <9.5 g/dL	3 (10.7)	4 (15.4)[d]	.699
White blood cell count, median ×10^3 cells/mm^3 (IQR)[e]	4.37 (2.52–5.95)	4.56 (3.67–6.07)	.768
CSF CRAG titer >1:128[f]	15 (65.2)	21 (87.5)	.071
Other WHO stage 4 conditions	12 (42.9)	8 (30.8)	.358

NOTE. Data are no. (%) of patients, unless otherwise indicated. ALT, alanine aminotransferase; CRAG, cryptococcal polysaccharide antigen; CSF, cerebrospinal fluid; IQR, interquartile range; SD, standard deviation; WHO, World Health Organization.

[a] Data are for 23 patients in the early treatment arm and 20 patients in the delayed treatment arm.
[b] Data are for 13 patients in the early treatment arm and 15 patients in the delayed treatment arm.
[c] Data are for 23 patients in the early treatment arm and 21 patients in the delayed treatment arm.
[d] Data are for 24 patients.
[e] Data are for 23 patients in each arm.
[f] Data are for 23 patients in the early treatment arm and 24 patients in the delayed treatment arm.

management of CM via fluconazole monotherapy, inadequate CSF pressure management, drug-drug interactions between fluconazole and ART, and immune reconstitution inflammatory syndrome (IRIS) in the context of early initiation of ART.

Early introduction of nevirapine-based ART has been reported to result in adverse events, such as serious hepatotoxicity due to nevirapine-fluconazole drug interaction [24, 25]. Coadministration of fluconazole and nevirapine results in increased levels of nevirapine, with minimal effects on fluconazole drug levels [25]. The only nevirapine-related toxicity event observed in the study was a desquamating rash. Liver function test results remained normal throughout the study for participants in both treatment groups.

Early initiation of ART most likely resulted in increased rates of cryptococcal IRIS and the excess mortality observed in the early ART arm. However, in this study, we did not have objective criteria (eg, decreasing viral loads or increasing CD4 cell counts) to confirm IRIS. Suboptimal treatment of CM may increase the risk of CM IRIS, because the organism is cleared more slowly. The early initiation of ART may have altered central nervous system and peripheral cytokine profiles, thus limiting central nervous system clearance [26]. Dysregulation of the homeostatic balance in T cell subsets likely plays a role in the development of IRIS [27–29]. We postulate that early initiation of ART may have altered this balance resulting in a pro-inflammatory state that may have contributed to the significant increase in mortality in the early ART arm.

Our data provide strong evidence that, in the acute care setting, it is important to clear cryptococcal infection prior to initiation of ART. Early initiation of ART most likely resulted

Table 2. Clinical Presenting Symptoms in Participants with Cryptococcal Meningitis, Stratified by Treatment Group

Symptom	Early treatment arm (n = 28)	Delayed treatment arm (n = 26)	P[a]
Headache	28 (100)	26 (100)	.342
Vision loss	1 (3.6)	0 (0)	.519
Confused or poor cognitive function	3 (10.7)	7 (26.9)	.169
Vomiting	14 (50)	14 (53.9)	.793
Fever	13 (46.4)	12 (46.2)	.98
Weight loss	10 (10.4)	10 (9.6)	.835
Night sweats	10 (35.7)	11 (42.3)	.619
Cough	4 (14.3)	7 (26.9)	.249
Dyspnea	2 (7.1)	3 (11.5)	.663

[a] Determined by the Fisher exact test.

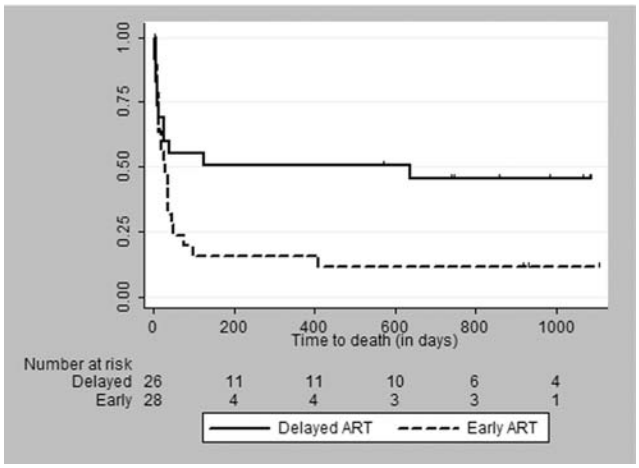

Figure 2. Kaplan-Meier survival estimates by treatment group. Early treatment was associated with increased mortality and a median survival time of 28 days, compared with delayed with median survival time of 637 days ($P = .031$, by log-rank test). ART, antiretroviral therapy.

in increased rates of cryptococcal IRIS in the early ART arm. HIV-associated tuberculosis is yet another important opportunistic infection for which the timing of initiation of ART in relation to disease specific therapy remains incompletely answered. Data from an on-going South African study have shown that comparison of integrated (ART during tuberculosis therapy) versus sequential (ART following completion of tuberculosis therapy) resulted in a 55% lower mortality in the integrated treatment group [30]. We eagerly await results of the comparison of the 2 components of the integrated treatment group (early versus delayed ART), which best mirrors the comparison in our study.

Our study had limitations, including the small sample size, the lack of blinding of clinicians to the randomization arm of the study, and the fact that the study was conducted in a setting where the overall management of CM is suboptimal. The study was nested in the routine standard of care in a tertiary care hospital in resource-limited circumstances. The researchers had no control over the general management of inpatients, including the measurement and management of elevated CSF pressures and the timing of discharge. Fluconazole drug susceptibility testing was also not performed in our study. Recent studies suggest a growing rate of resistance to fluconazole among cryptococcal isolates in Africa, prompting several clinicians to use increasingly higher doses of fluconazole [7, 31, 32]. Nonetheless, all but 2 participants who had survived until week 10 had negative India ink CSF staining and culture results at 10 weeks. Of the 2 participants who had not cleared their CSF of the organism, one was suspected to have developed tuberculosis-related IRIS, and the other—who had persistently positive CSF India ink and culture results—ultimately died of the disease several months after initiation of therapy. Despite these limitations, the data are highly applicable to the clinical setting prevalent in many resource-limited countries where the treatment of CM remains suboptimal.

Our study clearly shows that CM, when treated with fluconazole monotherapy (800 mg per day), does not favor early initiation of ART. We therefore recommend that, where fluconazole monotherapy is used for treating CM, initiation of

Table 3. Cause of Death among Study Participants

Cause of death (other diagnosis)	No. of patients (no. of hospital deaths/ no. of deaths during first admission)	
	Early treatment arm (n = 23)	Delayed treatment arm (n = 12)
Cryptococcal meningitis plus finding below		
None	14 (7/2)	8 (3/2)
TB	1 (1/0)	…
Abdominal TB	1 (1/0)	…
Pulmonary TB	1 (1/0)	…
Falciparum malaria	1 (1/1)	…
Desquamating rash	1 (1/0)	…
Pelvic inflammatory disease	…	1 (1/1)
Jaundice	…	1 (0/0)
Cerebrovascular accident	…	1 (0/0)
Disseminated TB	1 (1/0)	…
Severe gastroenteritis	1 (1/0)	…
Lobar pneumonia	1 (1/0)	…
Severe anaemia	1 (1/0)	…
Pneumonia vs TB IRIS (gastroenteritis)	…	1 (0/0)

NOTE. IRIS, immune reconstitution inflammatory syndrome; TB, tuberculosis.

ART should be delayed until the CM is adequately treated and, if possible, culture results are negative or titers have decreased significantly. On the basis of our data, outcomes are improved if treatment is initiated at least 10 weeks after initiation of treatment for CM.

Acknowledgments

We are grateful to all study participants who volunteered to participate in this study. We are also grateful to both the clinical and laboratory staff at the Parirenyatwa Central Hospital and its Family Care Centre (the outpatient HIV Clinic).

Financial support. The AIDS Care Research in Africa (ACRiA) program and the small grants funding program from the Infectious Disease Society of America.

Potential conflicts of interest. All authors: no conflicts.

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