MAJOR ARTICLE

Cerebrospinal Fluid Culture Positivity and Clinical Outcomes After Amphotericin-Based Induction Therapy for Cryptococcal Meningitis

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Background. Amphotericin-based combination antifungal therapy reduces mortality from human immunodeficiency virus (HIV)-associated cryptococcal meningitis. However, 40%–50% of individuals have positive cerebrospinal fluid (CSF) fungal cultures at completion of 2 weeks of amphotericin induction therapy. Residual CSF culture positivity has historically been associated with poor clinical outcomes. We investigated whether persistent CSF fungemia was associated with detrimental clinical outcomes in a contemporary African cohort.

Methods. Human immunodeficiency virus-infected individuals with cryptococcal meningitis in Uganda and South Africa received amphotericin (0.7–1.0 mg/kg per day) plus fluconazole (800 mg/day) for 2 weeks, followed by "enhanced consolidation" therapy with fluconazole 800 mg/day for at least 3 weeks or until cultures were sterile, and then 400 mg/day for 8 weeks. Participants were randomized to receive antiretroviral therapy (ART) either 1–2 or 5 weeks after diagnosis and observed for 6 months. Survivors were classified as having sterile or nonsterile CSF based on 2-week CSF cultures. Mortality, immune reconstitution inflammatory syndrome (IRIS), and culture-positive relapse were compared in those with sterile or nonsterile CSF using Cox regression.

Results. Of 132 participants surviving 2 weeks, 57% had sterile CSF at 2 weeks, 23 died within 5 weeks, and 40 died within 6 months. Culture positivity was not significantly associated with mortality (adjusted 6-month hazard ratio, 1.2; 95% confidence interval, 0.6–2.3; $P = .28$). Incidence of IRIS or relapse was also not significantly related to culture positivity.

Conclusions. Among patients, all treated with enhanced consolidation antifungal therapy and ART, residual cryptococcal culture positivity was not found to be associated with poor clinical outcomes.

Keywords. amphotericin; clinical outcome; cryptococcal meningitis; HIV.

Cryptococcal meningitis is the most common cause of adult meningitis in Africa and accounts for 20% of acquired immune deficiency syndrome (AIDS)-associated mortality in sub-Saharan Africa [1–4]. With amphotericin-based combination antifungal therapy, 10-week mortality from human immunodeficiency virus (HIV)-associated cryptococcosis can be as low as 20%–30% [5–7]. However, at the end of amphotericin treatment, 40%–50% of individuals continue to have viable *Cryptococcus neoformans* in their cerebrospinal fluid (CSF), which has historically been associated with 2- to 3-fold excess risk of detrimental clinical outcomes, including, death, immune reconstitution inflammatory syndrome (IRIS), or relapse [8–12].

After amphotericin induction therapy, consolidation therapy of fluconazole 400 mg/day is recommended to

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further suppress infection and support long-term recovery [13, 14]. However, 400 mg/day fluconazole is primarily fungistatic, which may allow persistence of the fungus through consolidation therapy [15]. Persistent culture positivity and failure to clear the organism is thought to be detrimental in HIV, setting the stage for paradoxical IRIS upon ART initiation and ultimately increasing the risk of neurologic deterioration and death [16–18]. However low-grade culture positivity might not be detrimental if fungicidal therapy is continued and sterilizes the CSF as the immune responses are restored with ART.

Few contemporary studies have investigated the detriments of residual cryptococcal infection after induction therapy. One recent study of 106 South Africans reported that residual fungemia was associated with a 3-fold higher hazard of paradoxical IRIS and an 8-fold higher hazard of culture-positive cryptococcal relapse when using traditionally recommended fluconazole 400 mg/day consolidation therapy [18]. During a recent clinical trial, we evaluated the association between persistent CSF culture positivity and detrimental clinical outcomes among participants treated with fluconazole 800 mg/day "enhanced consolidation" therapy.

METHODS

Study Population and Cryptococcal Treatment

The study population included individuals enrolled in the Cryptococcal Optimal Antiretroviral Therapy Timing (COAT) trial (www.clinicaltrials.gov: NCT01075152); a randomized clinical strategy trial of early antiretroviral therapy (ART) initiation (1–2 weeks after cryptococcal diagnosis; before hospital discharge) compared with deferred ART initiation (5 weeks after diagnosis; as an outpatient) among HIV-infected, ART-naive individuals with a first episode of cryptococcal meningitis [19]. The COAT trial enrollment began in November 2010 and ended in April 2012, during which time 177 individuals were randomized from 3 sites: (1) Mulago National Referral Hospital in Kampala, Uganda; (2) Mbarara National Referral Hospital in Mbarara, Uganda; and (3) GF Jooste Hospital in Cape Town, South Africa. Ethical approval for the trial was granted from the Uganda National Council of Science and Technology, South African Medicines Control Council, and the Institutional Review Boards at the University of Minnesota, Makerere University, University of Cape Town, and Mbarara University of Science and Technology.

All participants underwent a lumbar puncture for cryptococcal diagnosis, confirmed by a positive CSF cryptococcal culture and/or cryptococcal antigen test. Induction therapy for cryptococcal meningitis consisted of amphotericin B deoxycholate (0.7–1.0 mg/kg) in combination with fluconazole 800 mg daily for 2 weeks, additional intravenous fluids, and electrolyte supplementation. Therapeutic lumbar punctures to manage intracranial pressure were recommended according to treatment guidelines [13]; in addition, lumbar punctures were conducted after approximately 7 and 14 days of amphotericin to monitor clearance of *C. neoformans*. Because the day 14 CSF culture result was not known at time of collection, all individuals received an enhanced consolidation regimen consisting of at least 3 weeks of fluconazole 800 mg/day. If individuals were found to have a positive culture at the end of amphotericin, the study protocol recommended 800 mg/day fluconazole be continued until the CSF was known to be sterile, at which time the fluconazole dose was reduced to 400 mg/day. Fluconazole was continued for an additional 8 weeks at 400 mg/day for consolidation therapy, and then fluconazole 200 mg/day for secondary prophylaxis for at least 1 year. Individuals initiated ART with efavirenz and lamivudine plus either zidovudine or stavudine. Stavudine was systematically switched to tenofovir 8 weeks after the cryptococcal diagnosis.

Cerebrospinal Fluid Fungal Burden and Sterility

All CSF samples had quantitative fungal cultures performed, as previously described [5]. In brief, CSF was plated onto Sabouraud Dextrose Agar and incubated at 30°C. Quantification of *C. neoformans* fungal burden was conducted using serial 10-fold dilutions of CSF, up to $1{:}10^5$ dilution, and counting colony-forming units (CFUs) of *C. neoformans* seen at the most diluted plate with growth [5]. Nonsterility was defined as no sterile cultures observed throughout induction therapy and with at least 1 CSF culture obtained after 12 days of amphotericin. Final culture positivity was unknown for 22 participants who survived induction therapy but who did not have a CSF culture after the 12th day of amphotericin. These 22 participants were excluded from the primary analysis. A sensitivity analysis was conducted with the full cohort using multiple imputation for those 22 persons, including the previous CSF culture values and the rate of early fungicidal activity. Cerebrospinal fluid cryptococcal antigen titers were measured using a lateral flow assay (Immy, Inc., Norman, OK) and $\log_2$ transformed for analysis.

The rate of cryptococcal clearance from the CSF, termed the early fungicidal activity [5], was calculated from the first quantitative culture to either a sterile culture or culture taken at the end of induction therapy, whichever occurred first. Subject-specific linear regression analysis was conducted using $\log_{10}$ CFU/mL as the dependent variable and days of amphotericin as the independent variable, as previously described [5]. The early fungicidal activity was estimated as the slope of the subject-specific regression equation multiplied by −1, such that a higher positive value denoted a faster rate of clearance.

Clinical Events

For IRIS events, a panel of 3 clinicians externally adjudicated possible IRIS cases, per the published case definition [20], with majority consensus. Cryptococcal relapse was defined as

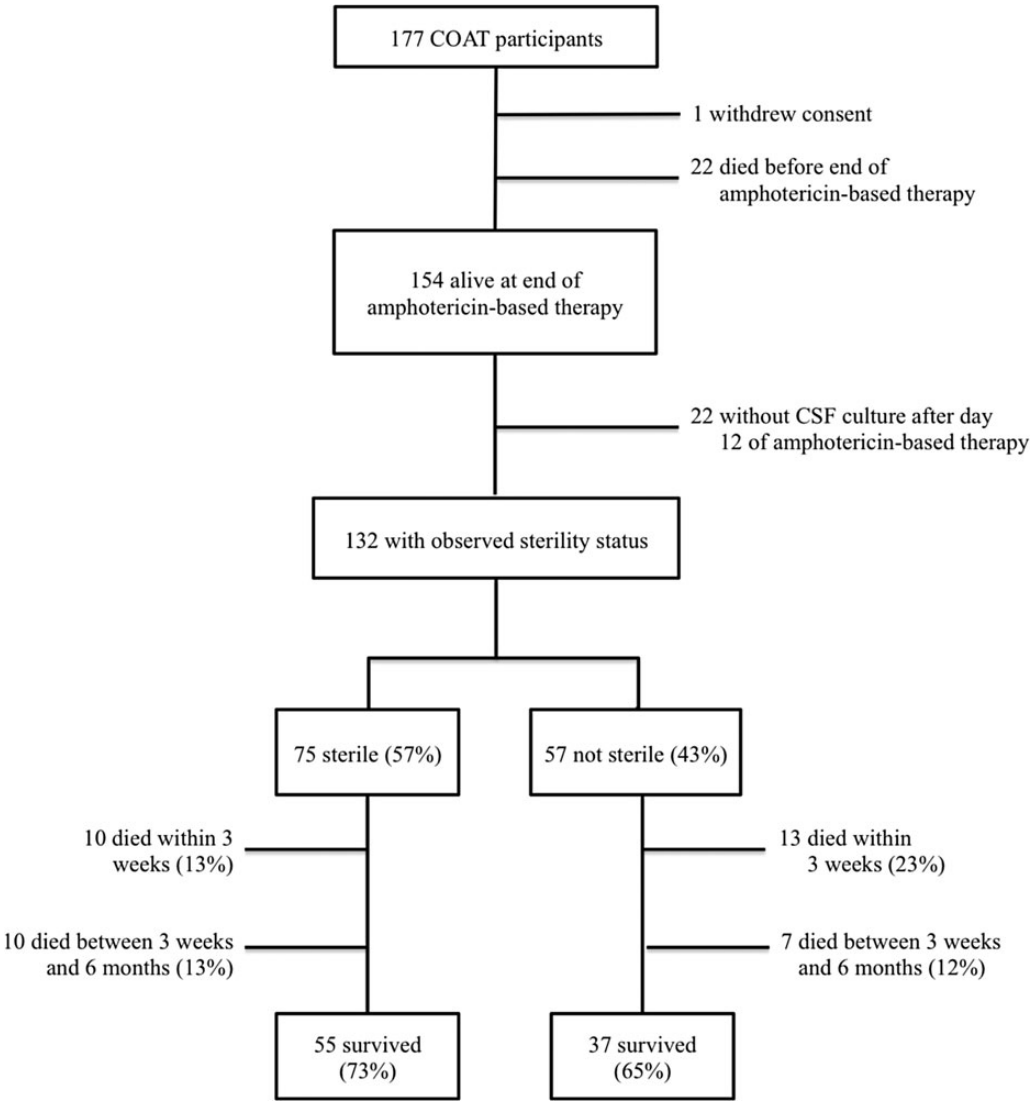

Figure 1. Cerebrospinal fluid (CSF) culture positivity and vital status outcomes for individuals with human immunodeficiency virus-associated cryptococcal meningitis in the Cryptococcal Optimal Antiretroviral Therapy Timing (COAT) trial.

any increasing quantitative count on CSF cultures after the end of induction therapy or any positive CSF culture 1 month after the end of induction therapy.

Statistical Analysis

For clinical and demographic characteristics, median values were compared using Wilcoxon rank-sum tests and categorical characteristics were compared using χ^2 tests. Two mortality endpoints were evaluated with Cox proportional hazards regression: (1) mortality between 2 and 5 weeks and (2) mortality between 2 weeks and 6 months. Differences in all-cause mortality by CSF sterility was assessed, and the proportional hazards assumption was suitable. Culture positivity were assessed for all endpoints, as a binary indicator (sterile vs nonsterile) and as a categorical variable (sterile, 10–99 CFU/mL, 100–999 CFU/mL, and ≥1000 CFU/mL). Univariate analyses are presented as are models adjusted for COAT trial arm and baseline cryptococcal quantitative culture count. Subgroup analysis was done by COAT randomization arm. Sensitivity analysis was conducted for the exclusion of participants with missing 2-week culture sterility using multiple imputation; imputing 40 datasets from a model including age, sex, weight, CSF white cell counts, ART timing, cryptococcal counts during amphotericin therapy, and days on amphotericin. The effect of fluconazole dosing after amphotericin was assessed using a Cox model with a time-varying covariate for fluconazole dose.

The cumulative incidences of cryptococcal-related IRIS, from the start of ART through 6 months of follow-up, and cryptococcal relapse, from the end of induction therapy through 6 months of follow-up, were compared by 2-week culture

Table 1. Baseline Characteristics and Survival, by CSF Culture Positivity at the End of 14 Days of Amphotericin Therapy, Among HIV-Infected Individuals With Cryptococcal Meningitis in the COAT Trial[a]

Characteristic	CSF Culture Negative at End of Amphotericin		CSF Culture Positive at End of Amphotericin		*P* Value
	N With Data	Median (IQR) or N (%)	N With Data	Median (IQR) or N (%)	
Study site	75		57		.40
Kampala, Uganda		43 (52%)		39 (48%)	
Mbarara, Uganda		16 (62%)		10 (38%)	
Cape Town, South Africa		16 (67%)		8 (33%)	
ART initiation Timing, N (%)[b]	75		57		.53
Earlier ART		37 (49%)		25 (44%)	
Deferred ART		38 (51%)		32 (56%)	
Age, y	75	34 (27, 40)	57	37 (30, 42)	.11
Males, N (%)	75	38 (51%)	57	38 (67%)	.07
Headache duration, d	71	14 (7, 28)	56	14 (7, 21)	.67
Glasgow Coma Scale score <15	75	22 (29%)	56	13 (23%)	.44
Fever, axillary temperature >37.5°C	75	19 (25%)	56	7 (13%)	.18
Randomization HIV parameters					
CD4 count, cells/μL	75	35 (10, 76)	57	17 (8, 70)	.16
HIV viral load, $\log_{10}$ copies/mL	74	5.4 (5.1, 5.8)	57	5.5 (5.1, 5.7)	.83
CSF parameters at diagnosis					
Opening pressure, mmH_2O	59	260 (150, 360)	52	305 (215, 437)	.05
Quantitative culture, $\log_{10}$ CFU/mL	70	4.6 (2.9, 5.4)	53	5.4 (4.7, 5.8)	.001
Cryptococcal antigen titer, 1:×	72	2000 (450, 7200)	55	4096 (1280, 12 800)	.01
White cells, per μL	72	50 (<5, 145)	53	7 (<5, 31)	.006
Early fungicidal activity through day 14, $\log_{10}$ CFU/mL per day	72	0.39 (0.33, 0.48)	57	0.24 (0.18, 0.30)	<.001
Mortality among 2-wk survivors					
5 wks	75	10 (13%)	57	13 (23%)	.17
6 mo	75	20 (27%)	57	20 (35%)	.34

Abbreviations: ART, antiretroviral therapy; CFU, colony-forming unit; COAT, Cryptococcal Optimal Antiretroviral Therapy Timing; CSF, cerebrospinal fluid; HIV, human immunodeficiency virus; IQR, interquartile range.

[a] Medians and 25th to 75th percentile range (IQR) or frequency and column percentages are presented. *P* values from χ^2 test for frequencies and Wilcoxon rank-sum test for medians. Fisher's exact test *P* values reported for mortality comparisons.

[b] Column percentages are presented. Early ART initiation group began ART at 7–11 days after diagnosis. Antiretroviral therapy was initiated in the deferred ART group 5 weeks after meningitis diagnosis.

positivity. Comparisons of cryptococcal-related IRIS were stratified by timing of ART initiation, and the incidences of cryptococcal-related IRIS and relapse were formally evaluated using Gray's test for competing risks [21]. SAS version 9.3 (SAS Institute, Cary, NC) was used for all statistical analyses.

RESULTS

Study Population

Among 177 COAT trial participants, 154 survived to the end of 2 weeks of amphotericin-based induction therapy and 132 had observed CSF cultures available for analysis (Figure 1). The family of 1 participant withdrew consent, and vital status through 6 months was known for the remaining participants. A sterile CSF culture occurred in 57% (75 of 132) of participants by the end of amphotericin therapy, and 43% (57 of 132) had continued *C. neoformans* growth (Table 1). Individuals achieving 2-week CSF sterility had lower initial CSF quantitative cultures at diagnosis, lower cryptococcal antigen titers, greater CSF white cell counts, and more rapid early fungicidal activity compared with individuals who did not achieve CSF sterility by 2 weeks.

Mortality Outcomes

Among participants surviving to the end of induction therapy, 30% (40 of 132) died within 6 months. The median time to death was 34 days from diagnosis (interquartile range [IQR], 21–50 days). Because the majority of the deaths (23 of 40) occurred between weeks 2 and 5, this time period was examined in greater detail (Table 2). Mortality was slightly higher but not

Table 2. Risk of Death Based on CSF Culture Results 14 Days After Initiation of Amphotericin Therapy[a]

	Total	Died	Mortality (95%CI)	Crude Hazard Ratio (95% CI)	Adjusted Hazard Ratio (95% CI)[b]
Mortality between 2 and 5 wks					
Overall	132	23	17% (11%–24%)		
Sterile CSF	75	10	13% (5.6%–21%)	Reference	Reference
Nonsterile CSF	57	13	23% (12%–34%)	1.82 (.80, 4.14)	1.45 (.60, 3.48)
Sterile CSF	75	10	13% (5.6%–21%)	Reference	Reference
1–99 CFU/mL	28	6	21% (6.2%–37%)	1.74 (.63, 4.78)	1.63 (.54, 4.95)
100–999 CFU/mL	17	3	18% (.0%–36%)	1.33 (.37, 4.84)	0.87 (.23, 3.29)
≥1000 CFU/mL	12	4	33% (6.7%–60%)	2.76 (.86, 8.80)	2.14 (.66, 6.94)
Mortality between 2 wks and 6 mo					
Overall	132	40	30% (22%–38%)		
Sterile CSF	75	20	27% (17%–37%)	Reference	Reference
Nonsterile CSF	57	20	35% (23%–47%)	1.40 (.75, 2.61)	1.21 (.62, 2.34)
Sterile CSF	75	20	27% (17%–37%)	Reference	Reference
1–99 CFU/mL	28	9	32% (15%–49%)	1.29 (.59, 2.84)	1.22 (.52, 2.86)
100–999 CFU/mL	17	7	41% (18%–65%)	1.56 (.66, 3.70)	1.16 (.47, 2.85)
≥1000 CFU/mL	12	4	33% (6.7%–60%)	1.42 (.48, 4.15)	1.27 (.43, 3.77)

Abbreviations: ART, antiretroviral therapy; CFU, colony-forming unit; CI, confidence interval; CSF, cerebrospinal fluid.

[a] Conclusions were unaffected after a sensitivity analysis was conducted using multiple imputation for the 22 participants without CSF cultures after day 12 of amphotericin therapy.

[b] Models adjusted for timing of ART initiation and baseline CSF quantitative culture. Separate models were created for sterility as a binary variable (nonsterile vs sterile) and for categories of quantitative culture.

statistically significant between 2 and 5 weeks for those with nonsterile CSF compared with those with sterile CSF after amphotericin. No significant difference was seen in 6-month mortality by CSF culture positivity (adjusted hazard ratio [HR] = 1.2; 95% confidence interval [CI], .6–2.3; $P = .28$; Figure 2) when adjusted for COAT randomization arm and baseline CSF quantitative fungal burden. There was also no evidence of a statistical association between mortality and the degree of quantitative cryptococcal culture positivity after 2 weeks of amphotericin. The 5-week and 6-month HRs were unchanged after multiple imputation to include participants with missing 2-week sterility status (adjusted HR: 1.3, 95% CI, .6–3.1 and adjusted HR: 1.0, 95% CI, .6–1.9, respectively).

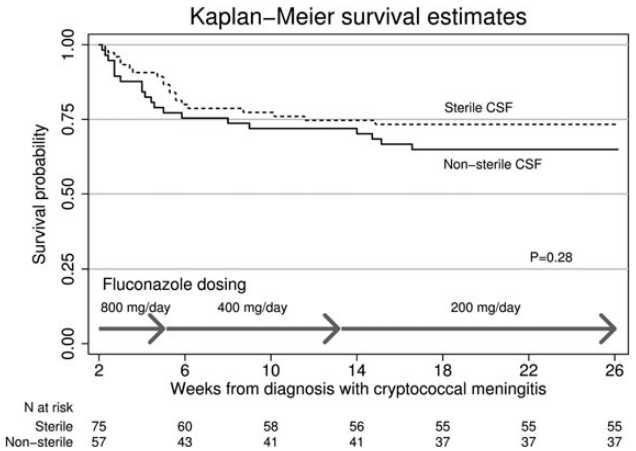

Figure 2. Kaplan-Meier survival probabilities starting at the end of 2 weeks of induction amphotericin therapy (week 0) through 6 months (26 weeks), by cerebrospinal fluid (CSF) culture positivity at the end of amphotericin therapy for individuals with human immunodeficiency virus-associated cryptococcal meningitis in the Cryptococcal Optimal Antiretroviral Therapy Timing trial.

Mortality after induction therapy was not found to be associated with the early fungicidal activity during induction therapy. Among those who died between 2 and 5 weeks, the median early fungicidal activity was 0.31 $\log_{10}$ CFU/mL per day (IQR: 0.22–0.39 $\log_{10}$ CFU/mL per day) compared with 0.34 $\log_{10}$ CFU/mL per day (IQR: 0.23–0.41 $\log_{10}$ CFU/mL per day) among those who survived ($P = .77$). The median early fungicidal activity for those who died within 6 months (0.32 $\log_{10}$ CFU/mL per day, IQR: 0.22–0.39 $\log_{10}$ CFU/mL per day) was also similar to those who survived beyond 6 months (0.34 $\log_{10}$ CFU/mL per day, IQR: 0.23–0.43 $\log_{10}$ CFU/mL per day; $P = .58$).

Mortality by Timing of Antiretroviral Therapy Initiation

Exploratory subgroup analysis was conducted to investigate the association between CSF culture positivity and mortality by COAT randomization, or the timing of ART initiation. By 5 weeks, individuals randomized to the early ART arm had been on ART for approximately 4 weeks, whereas those

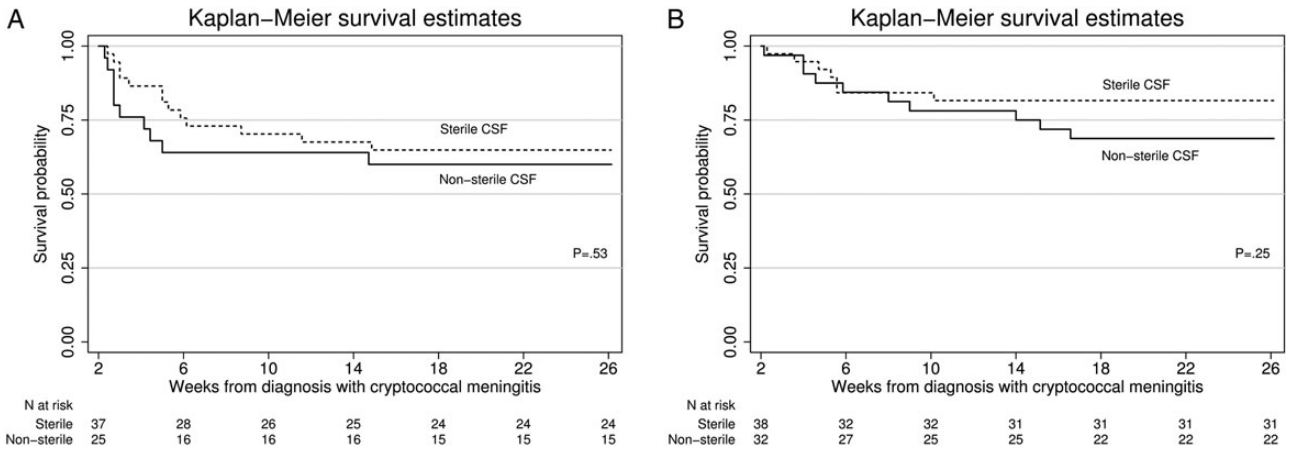

Figure 3. Kaplan-Meier survival probabilities by cerebrospinal fluid (CSF) culture positivity, at the end of amphotericin therapy, for the following: (A) 6-month mortality in the early antiretroviral therapy (ART) treatment arm (initiating ART 7–11 days after cryptococcal diagnosis), and (B) 6-month mortality in the deferred ART treatment arm (initiating ART 5 weeks after cryptococcal diagnosis) for individuals with human immunodeficiency virus-associated cryptococcal meningitis in the Cryptococcal Optimal Antiretroviral Therapy Timing trial.

randomized to deferred ART had not yet begun ART. Although mortality between 2 and 5 weeks was higher among those receiving early (15%) compared with deferred ART (7%), the association between CSF sterility and mortality was not found to differ by timing of ART initiation (early ART: HR = 1.6, 95% CI, .6–4.7; deferred ART: HR = 1.2, 95% CI, .3–5.3; interaction $P = .82$; Figure 3). There was also no evidence of interaction with 6-month mortality (early ART: HR = 1.1, 95% CI, .4–2.6; deferred ART: HR = 1.4, 95% CI, .5–3.8; interaction $P = .69$).

Cryptococcal Immune Reconstitution Inflammatory Syndrome, Relapse, and Intracranial Pressure

Overall, 18 cryptococcal-related IRIS events occurred; 9 among those with sterile and 9 among those with nonsterile CSF after 2 weeks of amphotericin. When stratified by timing of ART initiation, the cumulative incidence of cryptococcal-related IRIS among participants randomized to early ART was 11% (4 of 37) for those with sterile CSF and 24% (6 of 25) for those with nonsterile CSF ($P = .19$), and among participants randomized to deferred ART it was 15% (5 of 33) and 11% (3 of 27) for those with sterile and nonsterile CSF, respectively ($P = .69$). The incidence of culture-confirmed cryptococcal relapse was 4% (5 of 132) overall, through 6 months of observation, and relapse did not differ by CSF culture positivity at 2 weeks ($P = .90$). Cerebrospinal fluid opening pressure, as a measure of intracranial pressure, at the end of amphotericin therapy was >250 mmH_2O for 38% (19 of 50) of those with nonsterile CSF compared with 18% (10 of 57) of those with sterile CSF ($P = .02$).

Impact of Fluconazole Dosing

Fluconazole 800 mg/day, by protocol, was continued either through 5 weeks after cryptococcal diagnosis or until CSF cultures were known to be sterile, thus the duration of 800 mg/day fluconazole was intrinsically different for those with a sterile versus nonsterile CSF after amphotericin. The proportion of participants remaining on high-dose fluconazole for more than 5 weeks was greater among those with positive 2-week

Table 3. Timing of Switching From Enhanced Fluconazole 800 mg/day Consolidation to Lower Dose Fluconazole 400 mg/day After Amphotericin Induction Therapy[a]

Characteristic	Sterile at End of Amphotericin	Not Sterile at End of Amphotericin	*P* Value[b]
Individuals per group	75 (57%)	57 (43%)	
Switch to 400 mg/d fluconazole[a]			.008
Died before switching, N (%)	15 (20%)	13 (23%)	
Switched at 5 wks, N (%)	6 (8%)	10 (18%)	
Switched before 5 wks, N (%)	38 (51%)	13 (23%)	
Switched after 5 wks, N (%)	16 (21%)	21 (37%)	
Median days of fluconazole 800 mg/d, after amphotericin (IQR)	23 (21, 45)	40 (22, 54)	.04

Abbreviations: CSF, cerebrospinal fluid; IQR, interquartile range.

[a] *P* values calculated from χ^2 test of frequencies and Wilcoxon rank sum for medians.

[b] Protocol recommended switching to 400 mg/day fluconazole 3 weeks after the end of amphotericin, at time of outpatient clinic registration once the 2-week CSF culture status was known to be sterile. Persons with persistent CSF culture positivity were recommended to continue 800 mg/day fluconazole and switch to 400 mg/day only after CSF culture was confirmed to be sterile. For persons declining repeat lumbar puncture, higher dose fluconazole was recommended to be continued for at least 2 additional weeks.

CSF cultures (37%) compared with those with sterile 2-week cultures (21%; Table 3). None of the participants who died within 5 weeks were switched to the lower 400 mg/day fluconazole dose, and only 8 of the 40 (20%) who died within 6 months were ever switched to the lower fluconazole dose. Adjusting for dosage of fluconazole during follow up, in addition to ART timing and baseline CSF fungal burden, did not reveal an association between CSF culture positivity and 6-month mortality (HR = 0.9, 95% CI, .5–1.6, $P = .73$).

DISCUSSION

In the multisite COAT trial, 43% of individuals with cryptococcal meningitis who survived to the end of induction therapy had persistent CSF culture growth at 2 weeks. Unlike prior studies, showing markedly worse clinical outcomes with persistent CSF culture positivity, we did not find evidence of increased risk of mortality, paradoxical IRIS, or culture-positive relapse historically associated with positive CSF cultures, when using enhanced consolidation therapy.

Several studies have reported the association between 2-week CSF culture positivity and worse survival, all when using 400 mg/day fluconazole for consolidation therapy after amphotericin monotherapy or combination therapy [11, 18, 22]. Larsen and colleagues [11] reported, during the pre-ART era, that participants with nonsterile 2-week cultures had 25% 10-week mortality or lost-to-follow-up compared with 13% among participants with sterile cultures. In Thailand, a 3.6-fold greater relative risk of death was observed in individuals with nonsterile CSF (95% CI, 1.9–6.4) [22]. In a South African cohort, a non-statistical almost 2-fold higher hazard in 6-month mortality was observed when CSF was found to be nonsterile at a median of 18 days (14% mortality with sterile CSF vs 26% without sterility, $P = .13$) [18]. In addition, prior studies have reported positive cultures being associated with nearly 2-fold higher odds of treatment failure or relapse later during consolidation therapy [11, 12] and 3-fold increased risk of cryptococcal-related IRIS [18, 23].

Unlike prior studies, our data did not demonstrate significantly more detrimental outcomes after incomplete clearance of *Cryptococcus* in CSF. Using enhanced consolidation therapy with high-dose fluconazole (800 mg/day) for 3 additional weeks following the end of amphotericin for all patients and until the CSF culture was known to be sterile could be one reason we did not observe significant association. This dosage is modestly fungicidal, compared with 400 mg/day fluconazole used in previous studies, which is fungistatic [15, 24]. The higher dose fluconazole might have slowly eliminated the majority of any remaining fungus in the brain and CSF. Indeed, a phase II clinical trial conducted in Thailand and the United States demonstrated a trend toward lower 6-month mortality when 800 mg/day fluconazole was continued for 8 weeks after induction therapy compared with 400 mg/day fluconazole [25]. This study was not designed to test whether enhanced consolidation therapy led to better clinical outcomes than traditional consolidation therapy, thus future clinical trials are needed to understand which dosing of fluconazole is more favorable.

Alternative explanations and limitations exist for why we did not observe a significant association between CSF culture positivity and clinical outcomes after cryptococcal meningitis. First, although this study was larger than many prior studies reporting the risk of culture positivity, the relatively modest size of our cohort results in wide 95% CIs for the estimate of risk. Second, some prior studies were conducted before the combination ART era [11, 12, 22]. One hypothesis is that detrimental effects of culture positivity may only be seen in individuals who do not initiate ART promptly. However, in this cohort, the group who deferred ART initiation to 5 weeks had very little difference in mortality by culture positivity until later in follow-up. We did observe, in the group starting ART early, that mortality was non-significantly greater during 2 to 5 weeks of follow-up when the CSF was culture positive. This may be suggestive of increased risk of IRIS when *Cryptococcus* remains at ART initiation [18, 23].

Another important finding from our study was that the early fungicidal activity index was not found to be associated with mortality among those who survived amphotericin therapy. The early fungicidal activity has previously been associated with 2-week and 10-week mortality [9, 26] and, as such, has been used a surrogate marker in many contemporary clinical treatment trials for HIV-associated cryptococcal meningitis [7, 24, 27–29]. Most studies evaluating early fungicidal activity have included all participants, whereas we restricted analysis to those who survived 2 weeks of amphotericin induction therapy. This difference could account for the lack association between early fungicidal activity and morality in our cohort, but it should be explored further.

CONCLUSION

In conclusion, among individuals with HIV-associated cryptococcal meningitis in sub-Saharan Africa, we did not find that residual *Cryptococcus* in the CSF contributed to increased detrimental clinical outcomes when using higher dose fluconazole and initiating ART. Future studies, ideally randomized clinical trials, are needed to better understand the optimal dose and duration of consolidation therapy for patients with cryptococcal meningitis in resource-limited settings.

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

References

1. Corbett EL, Churchyard GJ, Charalambos S, et al. Morbidity and mortality in South African gold miners: impact of untreated disease due to human immunodeficiency virus. Clin Infect Dis **2002**; 34:1251–8.
2. Durski KN, Kuntz KM, Yasukawa K, et al. Cost-effective diagnostic checklists for meningitis in resource-limited settings. J Acquir Immune Defic Syndr **2013**; 63:e101–8.
3. Lawn SD, Harries AD, Anglaret X, et al. Early mortality among adults accessing antiretroviral treatment programmes in sub-Saharan Africa. AIDS **2008**; 22:1897–908.
4. Okongo M, Morgan D, Mayanja B, et al. Causes of death in a rural, population-based human immunodeficiency virus type 1 (HIV-1) natural history cohort in Uganda. Int J Epidemiol **1998**; 27:698–702.
5. Brouwer AE, Rajanuwong A, Chierakul W, et al. Combination antifungal therapies for HIV-associated cryptococcal meningitis: a randomized trial. Lancet **2004**; 363:1764–7.
6. Day JN, Chau TT, Wolbers M, et al. Combination antifungal therapy for cryptococcal meningitis. N Engl J Med **2013**; 368:1291–302.
7. Loyse A, Wilson D, Meintjes G, et al. Comparison of the early fungicidal activity of high-dose fluconazole, voriconazole, and flucytosine as second-line drugs given in combination with amphotericin B for the treatment of HIV-associated cryptococcal meningitis. Clin Infect Dis **2012**; 54:121–8.
8. Dromer F, Bernede-Bauduin C, Guillemot D, Lortholary O. Major role for amphotericin B-flucytosine combination in severe cryptococcosis. PLoS One **2008**; 3:e2870.
9. Jarvis JN, Bicanic T, Loyse A, et al. Determinants of mortality in a combined cohort of 501 patients with HIV-associated Cryptococcal meningitis: implications for improving outcomes. Clin Infect Dis **2014**; 58:736–45.
10. Kambugu A, Meya DB, Rhein J, et al. Outcomes of cryptococcal meningitis in Uganda before and after the availability of highly active antiretroviral therapy. Clin Infect Dis **2008**; 46:1694–701.
11. Robinson PA, Bauer M, Leal MA, et al. Early mycological treatment failure in AIDS-associated cryptococcal meningitis. Clin Infect Dis **1999**; 28:82–92.
12. Van Der Horst C, Saag M, Cloud G, et al. Treatment of cryptococcal meningitis associated with the acquired immunodeficiency syndrome. N Engl J Med **1997**; 337:15–21.
13. Perfect JR, Dismukes WE, Dromer F, et al. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the Infectious Diseases Society of America. Clin Infect Dis **2010**; 50:291–322.
14. Panel on Opportunistic Infections in HIV-Infected Adults and Adolescents. Guidelines for the prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from the CDC, the NIH, and the HIV Medicine Association of the Infectious Diseases Society of America. **2014**: M1–10. Available at: http://www.info.nih.gov/contentfiles/lvguidelines/adult_oi.pdf. Accessed 13 June 2013.
15. Bicanic T, Meintjes G, Wood R, et al. Fungal burden, early fungicidal activity, and outcome in cryptococcal meningitis in antiretroviral-naive or antiretroviral-experienced patients treated with amphotericin B or fluconazole. Clin Infect Dis **2007**; 45:76–80.
16. Barber DL, Andrade BB, Sereti I, Sher A. Immune reconstitution inflammatory syndrome: the trouble with immunity when you had none. Nat Rev Microbiol **2012**; 10:150–6.
17. Boulware DR, Meya DB, Bergemann TL, et al. Clinical features and serum biomarkers in HIV immune reconstitution inflammatory syndrome after cryptococcal meningitis: a prospective cohort study. PLoS Med **2010**; 7:e1000384.
18. Chang CC, Dorasamy AA, Gosnell BI, et al. Clinical and mycological predictors of cryptococcosis-associated Immune reconstitution inflammatory syndrome (C-IRIS). AIDS **2013**; 27:2089–99.
19. Boulware DR, Meya DB, Muzoora C, et al. Timing of antiretroviral therapy after diagnosis of cryptococcal meningitis. N Engl J Med **2014**; 370:2487–98.
20. Haddow LJ, Colebunders R, Meintjes GA, et al. Cryptococcal immune reconstitution inflammatory syndrome in HIV-1-infected individuals: proposed clinical case definitions. Lancet Infect Dis **2010**; 10:791–802.
21. RJ Gray. A class of K-sample tests for comparing the cumulative incidence of a competing risk. Ann Stat **1988**; 16:1141–54.
22. Pitisuttithum P, Tansuphasawadikul S, Simpson AJ, et al. A prospective study of AIDS-associated cryptococcal meningitis in Thailand treated with high-dose amphotericin B. J Infect **2001**; 43:226–33.
23. Jarvis JN, Meintjes G, Rebe K, et al. Adjunctive interferon-γ immunotherapy for the treatment of HIV-associated cryptococcal meningitis: a randomized controlled trial. AIDS **2012**; 26:1105–13.
24. Longley N, Muzoora C, Taseera K, et al. Dose response effect of high-dose fluconazole for HIV-associated cryptococcal meningitis in Southwestern Uganda. Clin Infect Dis **2008**; 47:2–7.
25. Pappas PG, Chetchotisakd P, Larsen RA, et al. A phase II randomized trial of amphotericin B alone or combined with fluconazole in the treatment of HIV-associated cryptococcal meningitis. Clin Infect Dis **2009**; 48:1775–83.
26. Bicanic T, Muzoora C, Brouwer AE, et al. Independent association between rate of clearance of infection and clinical outcome of HIV-associated cryptococcal meningitis: analysis of a combined cohort of 262 patients. Clin Infect Dis **2009**; 49:702–9.
27. Muzoora CK, Kabanda T, Ortu G, et al. Short course amphotericin B with high dose fluconazole for HIV-associated cryptococcal meningitis. J Infect **2012**; 64:76–81.
28. Bicanic T, Wood R, Meintjes G, et al. High-dose amphotericin B with flucytosine for the treatment of cryptococcal meningitis in HIV-infected patients: a randomized trial. Clin Infect Dis **2008**; 47:123–30.
29. Molefi M, Chofle AA, Molloy SF, et al. AMBITION-cm: intermittent high dose AmBisome on a high dose fluconazole backbone for cryptococcal meningitis induction therapy in sub-Saharan Africa: study protocol for a randomized controlled trial. Trials **2015**; 16:276.