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 or 2 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

Received 16 June 2015; accepted 19 October 2015.

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Open Forum Infectious Diseases

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DOI: 10.1093/ofid/ofv157

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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 1:10⁵ 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 CFU 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 log₂ 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 log₁₀ 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
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 χ² 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...
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 significantly so compared with participants who survived longer than 5 weeks from diagnosis.

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

| Characteristic                        | CSF Culture Negative at End of Amphotericin | CSF Culture Positive at End of Amphotericin | P Value |
|---------------------------------------|---------------------------------------------|---------------------------------------------|---------|
| Study site                            | N With Data | Median (IQR) or N (%) | N With Data | Median (IQR) or N (%) |         |
| Kampala, Uganda                       | 75 | 43 (52%) | 39 (48%) | .40 |
| Mbarara, Uganda                       | 16 (62%) | 16 (67%) | 8 (33%) |         |
| Cape Town, South Africa               | 75 | 37 (49%) | 25 (44%) | .53 |
| ART initiation Timing, N (%)          | 75 | 38 (51%) | 32 (56%) | .11 |
| Deferred ART                          | 75 | 34 (27, 40) | 37 (30, 42) | .11 |
| Age, y                                | 75 | 38 (51%) | 38 (67%) | .07 |
| Headache duration, d                  | 75 | 14 (7, 29) | 14 (7, 21) | .67 |
| Glasgow Coma Scale score < 15         | 75 | 22 (29%) | 13 (23%) | .44 |
| Fever, axillary temperature > 37.5°C  | 75 | 19 (25%) | 7 (13%) | .18 |

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 \( x^2 \) 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.
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).

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 \(0.31 \log_{10} \text{CFU/mL per day} (IQR: 0.22–0.39 \log_{10} \text{CFU/mL per day})\) compared with \(0.34 \log_{10} \text{CFU/mL per day} (IQR: 0.23–0.41 \log_{10} \text{CFU/mL per day})\) among those who survived (*P* = .77). The median early fungicidal activity for those who died within 6 months \((0.32 \log_{10} \text{CFU/mL per day}, IQR: 0.22–0.39 \log_{10} \text{CFU/mL per day})\) was also similar to those who survived beyond 6 months \((0.34 \log_{10} \text{CFU/mL per day}, IQR: 0.23–0.43 \log_{10} \text{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

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

| Mortality between 2 and 5 wks | Total | Died | Mortality (95% CI) | Crude Hazard Ratio (95% CI) | Adjusted Hazard Ratio (95% CI)b |
|-------------------------------|-------|------|-------------------|-----------------------------|--------------------------------|
| Overall                       | 132   | 23   | 17% (11%–24%)     | Reference                   | Reference                      |
| Sterile CSF                   | 75    | 10   | 13% (5.6%–21%)    | Reference                   | Reference                      |
| Nonsterile CSF                | 57    | 13   | 23% (12%–34%)     | 1.82 (0.80, 4.14)           | 1.45 (0.60, 3.48)              |
| Sterile CSF                   | 75    | 13   | 13% (5.6%–21%)    | Reference                   | Reference                      |
| 1–99 CFU/mL                   | 28    | 6    | 21% (6.2%–37%)    | 1.74 (0.63, 4.78)           | 1.63 (0.54, 4.95)              |
| 100–999 CFU/mL                | 17    | 3    | 18% (3.0%–36%)    | 1.33 (0.37, 4.84)           | 0.87 (0.23, 3.29)              |
| ≥1000 CFU/mL                  | 12    | 4    | 33% (6.7%–60%)    | 2.76 (0.86, 8.80)           | 2.14 (0.66, 6.94)              |

| Mortality between 2 wks and 6 mo| Total | Died | Mortality (95% CI) | Crude Hazard Ratio (95% CI) | Adjusted Hazard Ratio (95% CI)b |
|---------------------------------|-------|------|-------------------|-----------------------------|--------------------------------|
| Overall                         | 132   | 40   | 30% (22%–38%)     | Reference                   | Reference                      |
| Sterile CSF                     | 75    | 20   | 27% (17%–37%)     | Reference                   | Reference                      |
| Nonsterile CSF                  | 57    | 20   | 35% (23%–47%)     | 1.40 (0.75, 2.61)           | 1.21 (0.62, 2.34)              |
| Sterile CSF                     | 75    | 20   | 27% (17%–37%)     | Reference                   | Reference                      |
| 1–99 CFU/mL                     | 28    | 9    | 32% (15%–49%)     | 1.29 (0.59, 2.84)           | 1.22 (0.52, 2.86)              |
| 100–999 CFU/mL                  | 17    | 7    | 41% (18%–65%)     | 1.56 (0.66, 3.70)           | 1.16 (0.47, 2.85)              |
| ≥1000 CFU/mL                    | 12    | 4    | 33% (6.7%–60%)    | 1.42 (0.48, 4.15)           | 1.27 (0.43, 3.77)              |

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

* 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.

* 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.

**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 Anti-retroviral 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, 0.6–4.7; deferred ART: HR = 1.2, 95% CI, 0.3–5.3; interaction P = .82; Figure 3). There was also no evidence of interaction with 6-month mortality (early ART: HR = 1.1, 95% CI, 0.4–2.6; deferred ART: HR = 1.4, 95% CI, 0.5–3.8; 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 mmH2O 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

| Characteristic                              | Sterile at End of Amphotericin | Not Sterile at End of Amphotericin | P Valueb |
|---------------------------------------------|--------------------------------|-----------------------------------|----------|
| Individuals per group                       | 75 (57%)                       | 57 (43%)                          | .008     |
| Switch to 400 mg/d fluconazolea             |                                |                                   |          |
| 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 χ² 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, 0.5–1.6, 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 as 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.

**Acknowledgments**

We thank Drs. Jason Baker, Tihana Bicanic, and Lewis Haddow for serving on the external adjudication committee for clinical events. We thank Dr. Thomas Harrison for input on enhanced consolidation therapy and Dr. Tihana Bicanic for training of laboratory personnel in quantitative
culture methodology. We thank Drs. Claudia Muñoz-Zanzi, Alan Lifson, and James Neaton for critical review of the manuscript. We thank the National Institutes of Health support from Drs. Trinh Ly, Chris Lambros, Karen Reese, and Neal Wetherall. We thank institutional support from Drs. Andrew Kambu, Alex Coutinho, Henry Kajumbula, Aaron Friedman, and the Infectious Diseases Institute DataFax team of Mariam Namaweje and Mark Sennono for data management with support from the National Institute of Allergy and Infectious Diseases Office of Cyberinfrastructure and Computational Biology, Kevin Newell, and Dr. Steven Reynolds.

Financial support. Financial support for this research was provided by the National Institute of Allergy and Infectious Diseases (grants U01AI089244; K23AI073192; K24AI096925; T32AI055433) and Wellcome Trust (grants 081667; 098316; to G. M.).

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

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

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