MAJOR ARTICLE

Comparative Effectiveness of Induction Therapy for Human Immunodeficiency Virus-Associated Cryptococcal Meningitis: A Network Meta-Analysis

Jeffrey I. Campbell,[1] Steve Kanters,[2,3] John E. Bennett,[4] Kristian Thorlund,[5] Alexander C. Tsai,[1,6,7] Edward J. Mills,[2,8] and Mark J. Siedner[1,9]

[1]Harvard Medical School, Boston, Massachusetts; [2]Faculty of Health Sciences, University of Ottawa, and [3]School of Population and Public Health, University of British Columbia, Vancouver, Canada; [4]Laboratory of Clinical Infectious Diseases, National Institutes of Health, Bethesda, Maryland; [5]Faculty of Health Sciences, McMaster University, Hamilton, Canada; [6]Chester M. Pierce, MD Division of Global Psychiatry, Massachusetts General Hospital, Boston; [7]Mbarara University of Science and Technology, Uganda; [8]Stanford Prevention Research Center, Stanford University, California; [9]Division of Infectious Disease, Department of Medicine, Massachusetts General Hospital, Boston

Background. Multiple international treatment guidelines recommend amphotericin-based combination regimens for induction therapy of cryptococcal meningitis. Yet, only 1 trial has reported a mortality benefit for combination amphotericin-flucytosine, and none have reported a mortality benefit for combination amphotericin-fluconazole.

Methods. We conducted a Bayesian network meta-analysis to estimate the comparative effectiveness of recommended induction therapies for HIV-associated cryptococcal meningitis. We searched PubMed and Cochrane CENTRAL for clinical reports of induction therapy for HIV-associated cryptococcal meningitis. We extracted or calculated early (two-week) and late (six to 12-week) mortality by treatment arm for the following induction regimens: amphotericin B alone, amphotericin B + flucytosine, amphotericin B + triazoles, amphotericin B + flucytosine + triazoles, triazoles alone, triazoles + flucytosine, liposomal amphotericin B, and amphotericin B + other medicines.

Results. In the overall sample (35 studies, $n = 2483$), we found no evidence of decreased mortality from addition of flucytosine or triazoles to amphotericin B, compared with amphotericin B alone. Although we did find a nonsignificant benefit for addition of flucytosine to amphotericin B in studies including participants with altered levels of consciousness, we did not identify a benefit for combination therapy in restricted analyses in either resource-rich or resource-limited settings, studies conducted before or after 2004, and studies restricted to a high dose of amphotericin B and fluconazole.

Conclusions. Given considerations of drug availability and toxicity, there is an important need for additional data to clarify which populations are most likely to benefit from combination therapies for human immunodeficiency virus-associated cryptococcal meningitis.

Keywords. cryptococcal meningitis; HIV/AIDS; induction therapy; network meta-analysis; therapeutics.

Received 25 November 2014; accepted 16 January 2015.

Correspondence: Mark J. Siedner, MD, MPH, MGH Center for Global Health, 100 Cambridge St, 15th Flr, Boston, MA 02114 (msiedner@mgh.harvard.edu).

Open Forum Infectious Diseases

DOI: 10.1093/ofid/ofv010

Cryptococcal meningitis (CM) is among the leading causes of morbidity and mortality in sub-Saharan Africa [1, 2]. Induction therapy regimens are critical to optimal management of CM because mortality is highest early in the disease [3, 4]. Multiple guidelines recommend amphotericin B (AmB) combined with either flucytosine (5FC) or fluconazole [5–9] as induction therapy for human immunodeficiency virus (HIV)-associated CM. These recommendations are described

as A-I (ie, good evidence to support the recommendation based on randomized controlled trials) and B-I (ie, moderate evidence to support the recommendation based on randomized controlled trials) by the Infectious Disease Society of America (IDSA); and "Strong Recommendation" is described using the GRADE criteria by the World Health Organization. In addition, experts have recommended azoles in place of 5FC as a cost-effective alternative strategy for CM management when 5FC is unavailable [10].

However, no studies have shown a mortality benefit for the addition of a triazole (azole) to AmB, and only 1 study has shown a mortality benefit for the addition of 5FC to AmB [11]. Moreover, the majority of published studies of induction therapy for CM are relatively small and rely upon surrogate outcomes as primary endpoints [12–14], making definitive recommendations challenging [15]. In addition, although AmB is increasingly available in high-burden countries [17], 5FC remains largely unavailable. We conducted a Bayesian network meta-analysis (also called a multiple treatments meta-analysis) to estimate the comparative mortality of induction therapy regimens for HIV-associated CM [17], using all available data from published clinical trials.

METHODS

Data Sources and Searches

We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines for systematic reviews and meta-analyses [18]. We searched English-language abstracts for clinical trials that described induction-phase treatment of HIV-associated CM, using the combined search terms "cryptococcal meningitis," "treatment," and "trial" in the PubMed database and the terms "cryptococcal meningitis" and "treatment" in the Cochrane CENTRAL database. Searches included all results made available before July 5, 2012, and the search was updated on April 20, 2013. To ensure fidelity of our search terms, we compared our results with the references cited in the IDSA treatment guidelines [5].

Study Selection

We included both single-arm and comparative studies of induction therapy. We excluded studies that were not human clinical trials, did not report results for patients with HIV, did not report results specifically for CM, did not report mortality within our specified early or late time points, did not involve induction therapy, or described only surgical management of CM. A single study staff member (J. I. C.) reviewed all abstracts, which were independently confirmed by a second study member (M. J. S.). We reviewed full-text articles using the same criteria (J. I. C. and M. J. S.).

Data Extraction and Quality Assessment

We extracted the following data from articles that met criteria: (1) study site(s); (2) publication year; (3) inclusion criteria, with particular attention to inclusion of subjects with altered levels of consciousness; (4) study design; (5) antifungal agents and dosing; (6) sample size for each treatment arm; (7) early (2-week) mortality; (8) late (6- to 12-week) mortality; and (9) any other reported mortality. When calculations could be made based on presented data, we calculated mortality rates at early and late time points for studies that did not report them. We requested data directly from authors for articles that did not report mortality at either time point.

Data Synthesis and Analysis

We grouped treatment arms using the following categories: (1) AmB alone; (2) AmB + 5FC; (3) AmB + azole; (4) AmB + 5FC + azole; (5) azole alone; (6) azole + 5FC; (7) liposomal AmB; and (8) AmB + other medicine (rifampin, acetazolamide, or interferon-gamma, which we included for the purpose of increasing the sample size for AmB network meta-analyses). We used traditional DerSimonian–Laird random effects meta-analysis methods to create forest plots, estimate mortality rates by study arm, and summarize regimen-specific mortality across studies [19]. To describe the comparative effectiveness of all interventions, we conducted a Bayesian network meta-analysis using all 8 regimens [20]. The method of network meta-analysis provides better comparative evidence than conventional meta-analysis due to its combined use of both direct (ie, head-to-head comparative studies) and indirect evidence (single arm and noncomparative evidence), increasing the power of statistical comparisons while allowing for inferences about comparative effects between interventions that have not been included in the same head-to-head trial [17, 21]. We modeled comparative log odds ratios using the conventional logistic regression network meta-analysis setup [20]. All results for the network meta-analysis are reported as posterior medians with corresponding 95% credibility intervals, the Bayesian equivalent of classic confidence intervals (CIs).

We used meta-regression to examine 2 potential sources of study heterogeneity: publication year and study setting. Study setting was defined dichotomously as resource-rich (wholly conducted in Europe, North America, or Australia) or resource-limited (at least partially conducted in sub-Saharan Africa, South America, or Southeast Asia). Because the deviance information criterion was minimized with the inclusion of study setting, suggesting that study setting was a significant source of heterogeneity, we adjusted for study setting in our network estimates. We also examined the following factors as potential sources of heterogeneity through subanalyses with restriction: AmB dose (≥0.7 mg/kg per day), fluconazole dose (≥800 mg/day), any itraconazole use, year of trial publication after 2004 (to reflect increasing antiretroviral therapy [ART] availability in sub-Saharan Africa), study setting (as defined above), and inclusion (versus not) of participants with altered levels of consciousness. For further details of the statistical analysis, please see the Supplementary Methods section. All

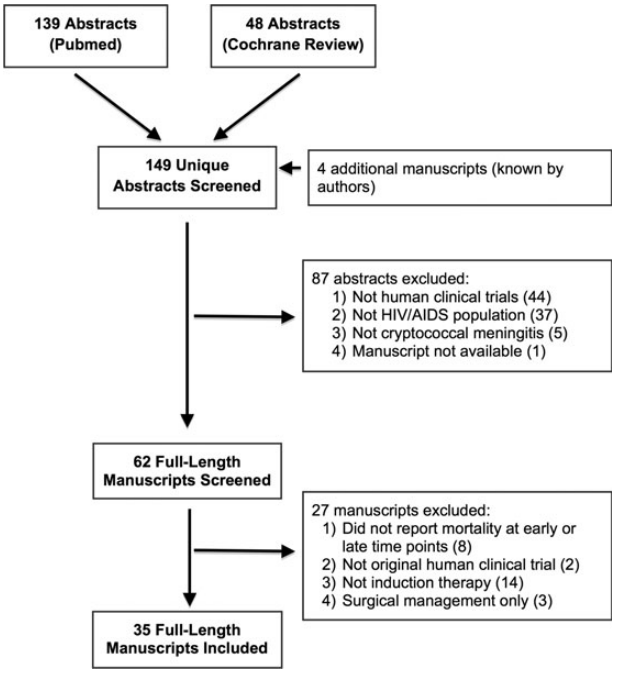

Figure 1. Schema of inclusion of studies for systematic review and meta-analysis for clinical trials of induction therapy for cryptococcal meningitis. Abbreviations: AIDS, acquired immune deficiency syndrome; HIV, human immunodeficiency virus.

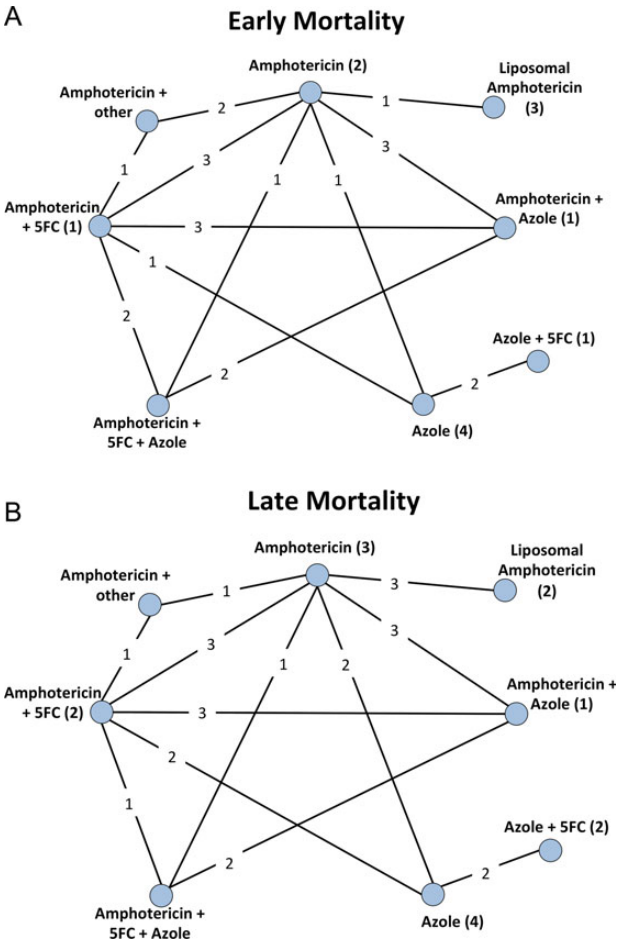

Figure 2. (A and B) Network diagrams for clinical studies of induction therapy for human immunodeficiency virus-associated cryptococcal meningitis. Blue nodes represent each antifungal therapy. The number in brackets next to each node indicates the number of monotherapy studies of that drug (including comparison trials of different doses or durations). The numbers on lines joining 2 nodes correspond to the number of comparative studies between those 2 drugs. (A) Network diagram for early (2-week) mortality. (B) Network diagram for late (6- to 12-week) mortality. Abbreviation: 5FC, flucytosine; azole, triazole.

analyses were conducted using WinBUGS version 1.4 (Medical Research Council Biostatistics Unit, Cambridge, Massachusetts), R version 3.0 (http://www.r-project.org/) and Stats Direct version 9.1 (www.statsdirect.com/).

Assessment of Bias

We assessed for bias within studies according to the Cochrane Handbook for Systematic Reviews of Interventions [22]. In particular, we assessed for evidence of the following: (1) selection bias through random sequence generation and allocation concealment, (2) performance bias through blinding of study participants, (3) detection bias through blinding of study personnel, (4) attrition bias at both early and late time points, and (5) reporting bias. Studies were deemed to be at risk for attrition if >10% of participants did not complete study therapy, were lost to follow-up, or were excluded after enrollment or randomization.

RESULTS

We identified 149 unique abstracts in our initial search, 62 full-length manuscripts for review, and 35 articles that met inclusion criteria and were included in our analyses (Figure 1; Supplementary Table S1). These represented 35 unique trials that reported mortality on 2466 participants. Twenty-four trials (69%) allocated therapy by random assignment, including 4 double-blinded trials. Twenty-two were conducted at least partly in resource-limited settings (Thailand [$n = 8$], South Africa [$n = 4$], Uganda [$n = 3$], Malawi [$n = 2$], Botswana [$n = 1$], Burundi [$n = 1$], India [$n = 1$], Vietnam [$n = 1$], Zimbabwe [$n = 1$]). Thirteen studies (37%) excluded potential participants because of altered mental status, and 7 studies (20%) excluded potential participants based on anticipated early mortality.

Early Mortality Analyses

Twenty-seven studies ($N = 1938$), comprising of 56 treatment arms, reported mortality estimates at 2 weeks (Figure 2*A* and Figure 3*A*). Estimates were derived from 15 trials that made direct head-to-head comparisons ($n = 1590$) and an additional 12 trials

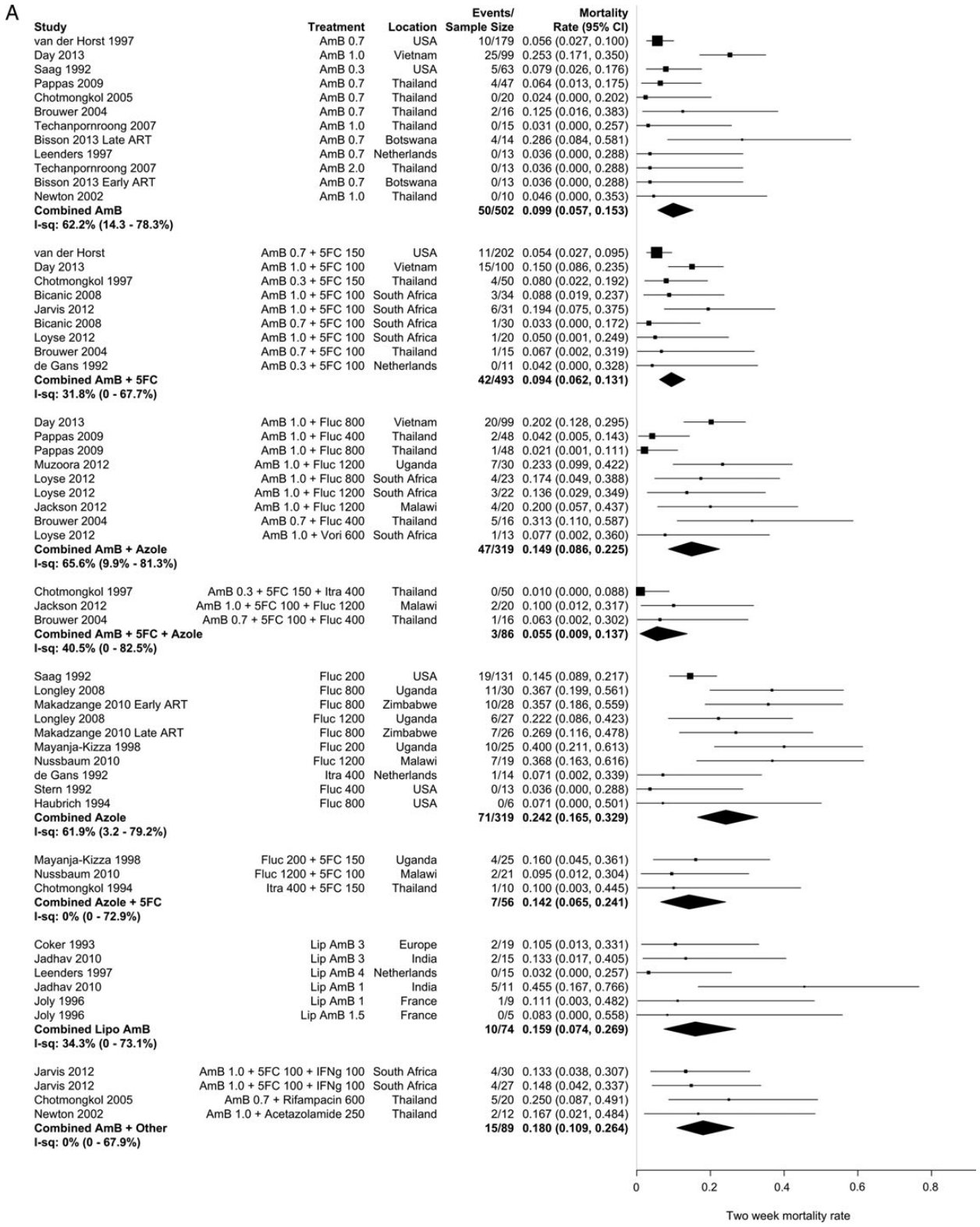

Figure 3. (A and B) Mortality rates by regimen for clinical studies of induction therapy for human immunodeficiency virus-associated cryptococcal meningitis. Estimates were obtained using DerSimonian–Laird random effects. In cases in which no events were observed, 0.5 was added to the numerator and 1 was added to the denominator. All results include 95% confidence intervals (CIs). (A) By arm forest plot of early (2-week) mortality rates. (B) By arm forest plot of late (6- to 12-week) mortality rates. Abbreviations: 5FC, flucytosine; AmB, amphotericin B; ART, antiretroviral therapy; azole, triazole; IFNg, interferon-gamma; Lip, liposomal.

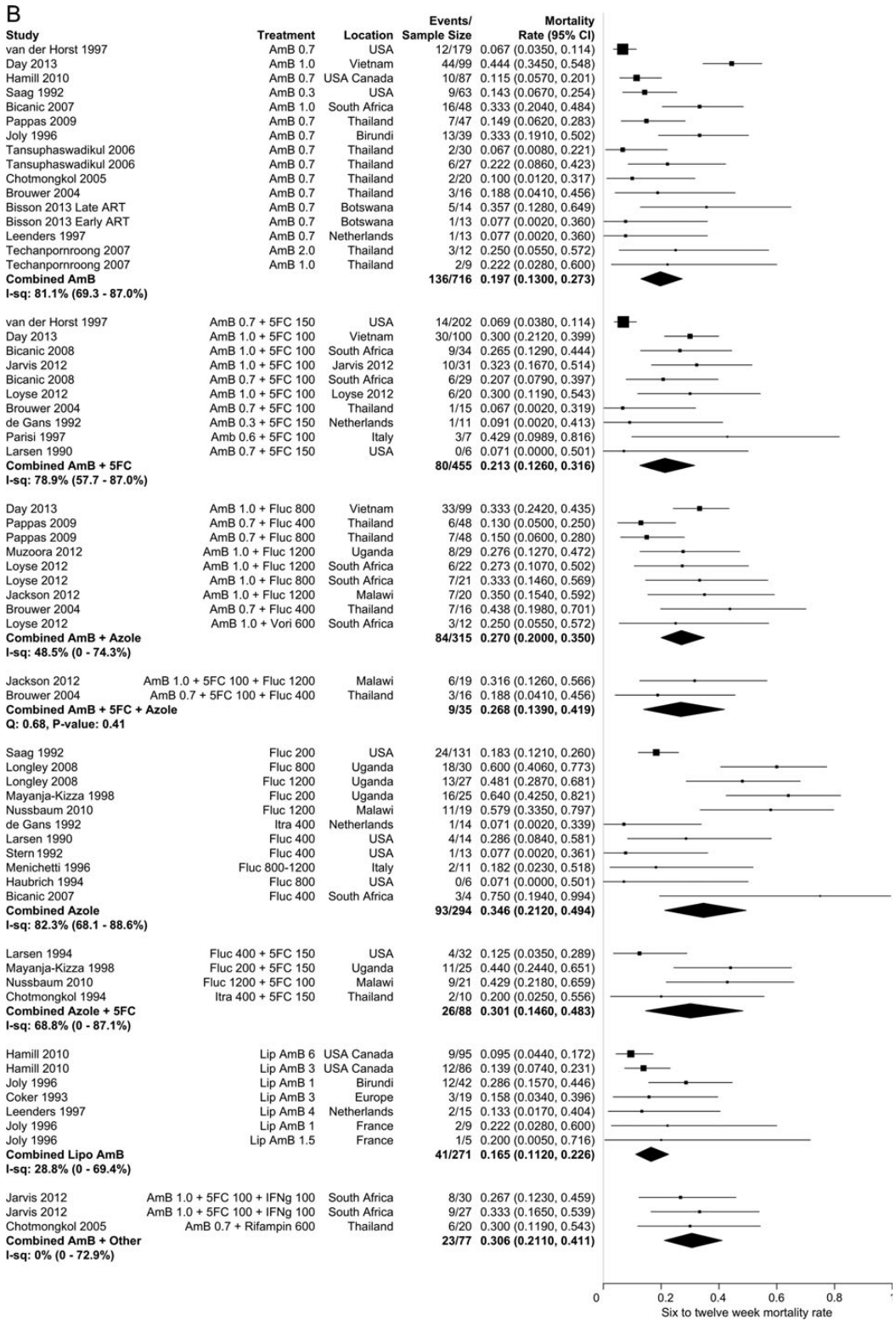

Figure 3 continued.

that evaluated a single drug (including trials comparing different doses of 1 drug, and trials comparing timing of ART initiation [$n = 348$]). We identified 10 studies of AmB alone ($n = 502$), 8 studies of AmB + 5FC ($n = 493$), 6 studies of AmB + azole ($n = 319$), 8 studies of azole alone ($n = 319$), 3 studies of azole + 5FC ($n = 56$), and 3 studies of AmB + azole +5FC ($n = 86$).

In the network meta-analyses, inclusion of study setting in meta-regression minimized model variance (Supplementary Table S2), so we adjusted all estimates for study setting. There were no statistically significant differences in 2-week mortality for the combination of AmB + azole versus AmB alone (odds ratio [OR], 1.13; 95% CI, .54–2.75) or AmB + 5FC versus AmB alone (OR, 0.89; 95% CI, .47–2.07) (Table 1*A* and Figure 4). In contrast to AmB, the addition of 5FC to azole was associated with decreased mortality (OR, 0.27; 95% CI, .07–.94). The triple-drug regimen of AmB + 5FC + azole was superior to AmB alone (OR, 0.19; 95% CI, .03–.84), AmB + 5FC (OR, 0.21; 95% CI, .03–.84), and AmB + azole (OR, 0.16; 95% CI, .03–.64). We found a nonsignificant increased odds of mortality for azole alone versus AmB alone (OR, 1.99; 95% CI, .60–6.83) and decreased odds of 2-week mortality for azole + 5FC versus AmB alone (OR, 0.55; 95% CI, .10–3.01). In sensitivity analyses, there was a nonsignificant trend for a benefit of AmB + 5FC over AmB alone among studies that included participants with altered consciousness, but we found no other subgroups for which combination therapy seemed to be of benefit (Supplementary Table S3). Lastly, direct and indirect estimates (ie, comparing standard meta-analysis with the network analysis results) for early mortality were similar, suggesting little evidence of heterogeneity between the network (Supplementary Table S4).

Late Mortality Analyses

Thirty-one studies ($N = 2251$), comprising 62 treatment arms, reported HIV-associated CM mortality estimates 6–12 weeks after treatment initiation (Figure 2*B* and Figure 3*B*). Estimates were derived from 17 trials that made direct head-to-head comparisons ($n = 1889$) and an additional 14 trials that evaluated a single drug ($n = 375$). We identified 16 studies of AmB alone ($n = 723$), 9 studies of AmB + 5FC ($n = 456$), 6 studies of AmB + azole ($n = 319$), 10 studies of azole alone ($n = 294$), and 4 studies of azole + 5FC ($n = 88$).

Neither addition of 5FC to AmB (OR, 0.94, 95% CI; .64–1.48) nor addition of azole to AmB (OR, 1.05; 95% CI, .68–1.74) was associated with decreased odds of late mortality (Table 1*B* and Figure 4). The benefit of adding 5FC to azole was not significant at the late time point (OR, 0.61; 95% CI, .28–1.32), and we found no benefit of the triple-therapy regimen AmB + azole + 5FC versus AmB alone (OR, 0.86; 95% CI, .30–2.40), versus AmB + 5FC (OR, 0.91; 95% CI, .31–2.53), or versus AmB + azole (OR, 0.82; 95% CI, .29–2.12). As in the early analysis, late mortality was similar between arms of azole + 5FC and AmB alone (OR, 0.73; 95% CI, .32–1.74). Aside from a nonsignificant decrease in late mortality for addition of 5FC to AmB in studies including participants with altered mental status, we found no late mortality benefit from addition of azole or 5FC to AmB in restricted subanalyses (Supplementary Table S3).

Study Bias

We summarized risk of bias within each study in Supplementary Table S5 and Supplementary Figure S1. Eight of 35 (23%) studies evaluated a single-treatment arm and 3 studies (9%) used a nonrandomized treatment allocation to compare regimens. Of the 24 randomized trials, 15 (63%) concealed allocation. Although 4 trials were reported as double-blinded, only 2 trials clearly described methods of participant blinding, and none clearly described methods of study staff blinding. Six of 27 (22%) studies at the early time point and 8 of 31 (26%) studies at the late time point were at risk of attrition bias. We found no risk of reporting bias.

DISCUSSION

This network meta-analysis of induction-phase therapy for HIV-associated CM yields 2 critical findings: (1) in the overall sample, we found no mortality benefit from addition of azoles to AmB; and (2) we found that benefit for addition of 5FC to AmB seems to be limited to individuals with altered consciousness at treatment initiation. Important secondary findings include: (3) although limited by small sample size, the oral combination regimen of 5FC and fluconazole is a potentially promising alternative to AmB, which warrants further study; (4) consistent with current guidelines, AmB monotherapy seems to be advantageous compared with azole monotherapy; and (5) the 3-drug regimen of AmB + 5FC + azole had the lowest mortality among all regimens assessed, although comparative estimates were limited by small sample size.

Our results do not demonstrate a clear benefit for combination AmB + 5FC over single-agent AmB in populations without altered levels of consciousness. Although both animal and in vitro studies have shown synergistic effects from combining 5FC and AmB [23–27], only 1 of 3 randomized controlled studies in HIV-infected populations has shown mortality benefit for this combination [12, 27]. A trial of 400 participants in the United States, often cited as evidence for benefit of 5FC combination therapy, reported a nonsignificant ($P = .06$) benefit for 5FC in a retrospective analysis using a primary outcome of mycologic failure during recovery. More importantly, we report and include mortality rates from that study, which were nearly identical between the AmB and AmB + 5FC groups at both 2 and 10 weeks (5.5% vs 5.4% and 6.7% vs 6.9%, respectively) [27]. In contrast, a large study from Vietnam ($n = 298$), and the only randomized study to compare AmB with AmB + 5FC in the modern era, reported a mortality benefit for addition of 5FC at 10 weeks of therapy [28]. This study population had high rates of altered consciousness (30% had a Glasgow Coma Score <15) and the highest reported rate of mortality published in a randomized trial using AmB (~44% of participants died by 6 months), reinforcing that the addition of 5FC to AmB likely has benefit in patients presenting with advanced disease in

Table 1. Early (2-Week) and Late (6- to 12-Week) Mortality Odds Ratios for HIV-Associated Cryptococcal Meningitis by Induction Therapy Regimen[a]

Regimen	AmB Alone	AmB + 5FC	AmB + Azole	AmB + 5FC + Azole	Azole Alone	Azole + 5FC	Liposomal AmB
A. Early (2-week) mortality odds ratios for HIV-associated cryptococcal meningitis by induction therapy regimen							
AmB + 5FC	0.89 (0.47, 2.07)						
AmB + Azole	1.13 (0.54, 2.75)	1.26 (0.57, 2.76)					
AmB + 5FC + Azole	**0.19 (0.03, 0.84)**	**0.21 (0.03, 0.84)**	**0.16 (0.03, 0.64)**				
Azole alone	1.99 (0.60, 6.83)	2.22 (0.55, 7.96)	1.76 (0.41, 6.93)	**10.68 (1.65, 89.00)**			
Azole + 5FC	0.55 (0.10, 3.01)	0.60 (0.09, 3.48)	0.48 (0.07, 2.87)	2.96 (0.30, 31.87)	**0.27 (0.07, 0.94)**		
Liposomal AmB	0.78 (0.02, 24.13)	0.85 (0.02, 27.53)	0.68 (0.01, 22.46)	4.30 (0.07, 189.60)	0.39 (0.01, 14.65)	1.44 (0.02, 68.23)	
AmB + Other	1.88 (0.60, 7.77)	2.08 (0.67, 7.40)	1.65 (0.47, 7.02)	10.20 (1.80, 85.68)	0.94 (0.19, 5.88)	3.46 (0.49, 31.87)	2.46 (0.07, 141.11)
B. Late (6- to 12-week) mortality odds ratios for HIV-associated cryptococcal meningitis by induction therapy regimen							
AmB + 5FC	0.94 (0.64, 1.48)						
AmB + Azole	1.05 (0.68, 1.74)	1.11 (0.69, 1.81)					
AmB + 5FC + Azole	0.86 (0.30, 2.40)	0.91 (0.31, 2.53)	0.82 (0.29, 2.12)				
Azole alone	1.19 (0.66, 2.17)	1.26 (0.65, 2.37)	1.13 (0.55, 2.23)	1.38 (0.45, 4.61)			
Azole + 5FC	0.73 (0.32, 1.74)	0.77 (0.31, 1.92)	0.69 (0.27, 1.76)	0.85 (0.23, 3.22)	0.61 (0.28, 1.32)		
Liposomal AmB	0.90 (0.54, 1.54)	0.96 (0.48, 1.83)	0.86 (0.43, 1.67)	1.05 (0.34, 3.31)	0.76 (0.35, 1.64)	1.25 (0.46, 3.26)	
AmB + Other	1.14 (0.49, 2.65)	1.20 (0.54, 2.64)	1.08 (0.44, 2.56)	1.34 (0.38, 4.82)	0.97 (0.36, 2.50)	1.57 (0.49, 4.94)	1.26 (0.48, 3.38)

Abbreviations: 5FC, flucytosine; AmB, amphotericin B; Azole, triazole; HIV, human immunodeficiency virus.

[a] An odds ratio >1.00 indicates an estimated increased odds of mortality for the regimen along the vertical axis in the first column, whereas an odds ratio <1.00 indicates an estimated decreased odds of mortality for the regimen along the vertical axis in the first column. Estimates are adjusted by meta-regression for study setting (resource-rich vs resource-limited). Bolded results indicate statistically significant relationships.

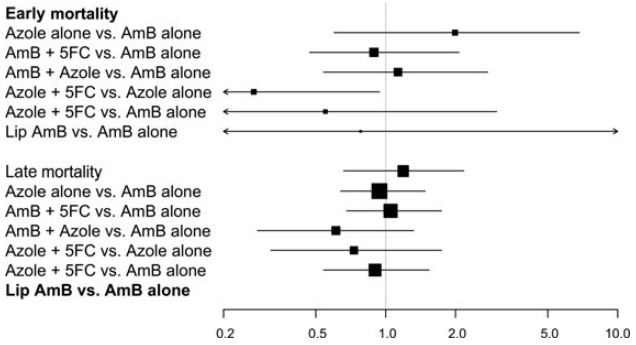

Figure 4. Forest plot comparing mortality in a network analysis of human immunodeficiency virus-associated cryptococcal meningitis by treatment regimen at early (2-week) and late (6- to 12-week) time points. Legend: Comparative groups are not consistent. Odds ratios on the left favor the first listed treatment group in each comparison. Abbreviations: 5FC, flucytosine; AmB, amphotericin B; azole, triazole; Lip, liposomal.

resource-limited settings. This hypothesis is supported by the ~50% decreased odds of mortality for addition of 5FC to AmB we found among studies that included subjects with altered levels of consciousness [4, 28], and it should motivate advocacy efforts to avail 5FC in such settings [29]. Our analyses, combining data from more than 1000 patients in a large variety of settings, did not show support for this combination in terms of improved mortality in other populations, eg, those without altered consciousness or studies in resource-rich settings. These results highlight the need to further evaluate which populations are most likely to benefit from AmB + 5FC over AmB alone to optimize the specificity of the HIV-associated CM treatment guidelines.

Most guidelines support use of AmB + azole as induction therapy for CM when 5FC is unavailable [6]. Unlike AmB + 5FC, in vitro and animal studies of AmB + azole have shown antagonistic and null effects [30–32]. Likewise, none of 3 randomized controlled trials demonstrated a mortality benefit for the addition of azole to AmB. One of the 3 studies was not powered to do so [12]. A second study conducted partially in Thailand and partially in the United States ($n = 150$) noted similar mortality between arms of AmB alone, AmB + fluconazole 400 mg/day, and AmB + fluconazole 800 mg/day at 2 and 10 weeks (6.4%, 4.2%, 2.1% and 14.9%, 12.5% vs 14.6%, respectively). The recent randomized trial in Vietnam ($n = 298$) also compared AmB with AmB + fluconazole 800 mg/day and found no statistically significant difference in mortality at either 2 or 10 weeks (20.2% vs 25.3% and 44.4% vs 33.3%, respectively). Although data on the addition of azole to AmB in resource-rich settings are lacking, our results failed to show a benefit for this combination at early or late endpoints, including in studies evaluating higher doses of AmB and fluconazole and studies including participants with altered consciousness. The null effects, which were independent of setting, dosing, and geography, should promote further discussion of this combination as recommended in current guidelines [5, 6, 9].

In contrast to AmB-based regimens, we did find evidence of benefit from addition of 5FC to azole-based regimens. Two randomized studies have reported decreased mortality for 5FC + azole versus azole alone [33, 34]. We found significantly decreased mortality at 2 weeks and nonsignificantly reduced mortality at later time points, with consistent estimates in sensitivity analyses. Although no trial has directly compared AmB alone with azole + 5FC regimens, our network analysis findings demonstrated similar mortality between these regimens and provide preliminary evidence that an oral regimen for CM, sparing AmB, might be a possible alternative to the current standard of care. Availing generic formulations of 5FC in resource-limited settings will be an important step towards testing this hypothesis [29]. Unexpectedly, the 3-drug combination regimen of AmB + 5FC + azole demonstrated the lowest mortality rate at 2 weeks (5.5%; 95% CI, .9%–13.9%), superior to AmB alone, azole alone, and AmB + 5FC. Nevertheless, these estimates were limited by small sample sizes, so they offer only very preliminary support for its use. Further pharmacokinetic or clinical data are warranted to corroborate the comparative efficacy of this regimen.

Our analyses offer modest support for preference of AmB alone over azole alone for CM induction therapy. Although mortality differences were not statistically significant in the full network model, we estimated a more-than-doubling odds of mortality for patients receiving azoles alone versus AmB alone at the early time point and a statistically significant increase in mortality at the late time point in resource-limited settings.

Finally, although studies have demonstrated both reduced toxicity [35] and reduced treatment discontinuation [36] with liposomal AmB, we found no evidence to suggest a mortality benefit for use of liposomal versus standard AmB. Although the IDSA guidelines list liposomal AmB in combination with 5FC as a preferred induction therapy, our search did not return any comparative clinical trials that evaluated the benefit of adding 5FC to liposomal AmB.

Discordance between our findings and conclusions drawn from prior work might be partly explained by the use of surrogate outcomes in most CM clinical trials. Early fungicidal activity (EFA), or the rate of change in fungal culture colony-forming units (CFU) during the first 14 days of treatment, was the primary outcome in 9 studies [12, 13, 34, 37–42], and 8 additional studies used negative cerebrospinal fluid culture as the primary outcome [35, 43–49]. Although others have shown in pooled analyses that improved EFA (at a rate of $\leq$0.33 log CFU/day) is associated with decreased mortality [50], of the 9 studies that compared regimens by EFA in our review [12, 13, 34, 37–40, 51, 52], only 2 [12, 37] found differences in EFA that crossed this identified threshold for mortality. Results from a recent randomized controlled study also challenged the predictive

value of EFA for mortality after finding statistically significantly improved EFA with AmB + 5FC versus AmB + azole (−0.42 vs −0.31; $P < .001$) but no difference in 2-week or 10-week mortality between these regimens (15% vs 20%, and 31% vs 33%, respectively). Although EFA demonstrates in vivo fungicidal activity, its accuracy might be limited by deaths early in treatment (which preclude repeated measures required to estimate EFA). EFA also fails to account for drug toxicity, which might have delayed effects on outcomes. In contrast, because mortality is a common outcome of HIV-associated CM, regimens with even modest mortality benefit should be amenable to study without expansive sample sizes.

Our findings should be interpreted with the following limitations in mind. First, our estimates are subject to the general limitations of all meta-analyses, including heterogeneity of study design, population, regimen dosing, and outcome assessment. We investigated between-study heterogeneity and found that study context contributed significantly to this variance; therefore, we adjusted for it in our analytic estimates. We also conducted multiple subgroup analyses and found that our estimates were consistent across time periods, inclusion or not of participants with altered levels of consciousness, dosing of AmB or azoles, and resource-rich or resource-limited study setting. More importantly, data on levels of consciousness, intracranial pressure measurement, and use of diagnostic lumbar puncture, a critical element of CM management [53], varied greatly across studies (Supplementary Table 1). The variability of these study characteristics serves as one possible source of bias in our analyses. Other factors that varied between studies that also might bias our estimates include: duration of induction therapy, regimen and dosing of consolidation therapy, and timing of ART initiation. For the latter, few studies initiated ART during the 10-week observation period, with the exception of 2 that specifically considered the question of timing of ART [51, 56]. Second, sample sizes of included trials were generally small. Only 6 studies enrolled more than 100 subjects [14, 27, 35, 54, 55]. However, sample size limitations should be understood in the context of challenges to recruiting, consenting, and observing patients with a high-acuity disease that occurs during advanced stages of HIV. We increased our statistical power by including previously unpublished data from 2 of the largest randomized controlled studies of induction therapy for CM [14, 27], and our meta-analysis represents the largest combined sample of patients with CM studied in clinical trial settings.

Third, nearly one half of the included studies (25% of the total sample size) were single-arm trials. Although such trials are not included in conventional meta-analyses, when we compared our estimates with those from a conventional meta-analysis, we found similar point estimates but greater precision. Fourth, mortality was the primary reported outcome in only 3 studies [33, 56], and we restricted our analyses to studies that reported mortality at prespecified time points.

CONCLUSIONS

In summary, in a Bayesian network meta-analysis of induction therapy for CM, we failed to identify a mortality benefit for addition of azole to AmB-based regimens, and we identified a potential benefit for addition of 5FC to AmB primarily in those with altered consciousness. These results raise questions about the specificity of generally accepted regimens to treat CM, and highlight the need to advance well designed, adequately powered studies using clinical endpoints to expand evidence for treatment of this disease in more varied subpopulations. Future studies can help to discern optimal regimens through selection of clinical outcomes and concomitant use of cerebrospinal fluid pressure monitoring. Nonstandard regimens, including AmB-sparing regimens (eg, azole + 5FC) and 3-drug combinations (AmB + 5FC + azole), might hold promise in this regard and should be further studied in hopes of reducing the morbidity and mortality associated with this disease.

Supplementary Data

Supplementary material is available online at *Open Forum Infectious Diseases* (http://OpenForumInfectiousDiseases.oxfordjournals.org/).

Acknowledgments

We acknowledge the Mycoses Study Group and the AIDS Clinical Trials Group for provision of mortality data from their work [28].

Author contributions. J. I. C. contributed to literature search, data collection, data interpretation, figure and table production, writing, and editing of the manuscript. S. K. contributed to data analysis, data interpretation, figure and table production, writing, and editing of the manuscript. J. E. B. contributed to data interpretation, writing, and editing of the manuscript. K. T. contributed to data analysis, data interpretation, figure and table production, writing, and editing of the manuscript. A. C. T. contributed to data interpretation, writing, and editing of the manuscript. E. J. M. contributed to study design, data analysis, data interpretation, writing, and editing of the manuscript. M. J. S. contributed to study design, literature search, data collection, data analysis, data interpretation, figure and table production, writing, and editing of the manuscript.

Disclaimer. Funding agencies had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Financial support. M. J. S. receives salary support from that National Institutes of Health (NIH) grant number K23MH099916. J. I. C. received financial support from the Harvard Medical School Scholars in Medicine Office, an Infectious Disease Society of America Medical Student Scholarship, and a Benjamin H. Kean Traveling Fellowship in Tropical Medicine from the American Society of Tropical Medicine and Hygiene. A. C. T. receives salary support from NIH K23MH096620 and the Robert Wood Johnson Health and Society Scholars Program.

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. Liechty CA, Solberg P, Were W, et al. Asymptomatic serum cryptococcal antigenemia and early mortality during antiretroviral therapy in rural Uganda. Trop Med Int Health **2007**; 12:929–35.
2. Park BJ, Wannemuehler KA, Marston BJ, Govender N, Pappas PG, Chiller TM. Estimation of the current global burden of cryptococcal

meningitis among persons living with HIV/AIDS. AIDS **2009**; 23: 525–30.
3. Mwaba P, Mwansa J, Chintu C, et al. Clinical presentation, natural history, and cumulative death rates of 230 adults with primary cryptococcal meningitis in Zambian AIDS patients treated under local conditions. Postgrad Med J **2001**; 77:769–73.
4. French N, Gray K, Watera C, et al. Cryptococcal infection in a cohort of HIV-1-infected Ugandan adults. AIDS **2002**; 16:1031–8.
5. 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 lnfect Dis **2010**; 50:291–322.
6. Govender N, Meintjes G. Southern African HIV Clinicians' Society Guidelines for the Prevention, Diagnosis, and Management of Cryptococcal Meningitis among HIV-infected Persons. Available at: http://www.sahivsoc.org/upload/documents/Crypto%20Guidelines%20Update_08-04-2013.pdf. Accessed 20 April 2013.
7. Portegies P, Solod L, Cinque P, et al. Guidelines for the diagnosis and management of neurological complications of HIV infection. Eur J Neurol **2004**; 11:297–304.
8. Nelson M, Dockrell D, Edwards S, et al. British HIV Association and British Infection Association guidelines for the treatment of opportunistic infection in HIV-seropositive individuals 2011. HIV Med **2011**; 12: (Suppl 2):1–140.
9. World Health Organization. Rapid Advice: Diagnosis, Prevention and Management of Cryptococcal Disease in HIV-infected Adults, Adolescent and Children. Available at: www.who.int/hiv/pub/cryptococcal_disease2011/en/. **2011**. Accessed 29 December 2014.
10. Rajasingham R, Rolfes MA, Birkenkamp KE, Meya DB, Boulware DR. Cryptococcal meningitis treatment strategies in resource-limited settings: a cost-effectiveness analysis. PLoS Med **2012**; 9:e1001316.
11. Day JN, Chau TT, Wolbers M, et al. Combination antifungal therapy for cryptococcal meningitis. New Engl J Med **2013**; 368:1291–302.
12. Brouwer AE, Rajanuwong A, Chierakul W, et al. Combination antifungal therapies for HIV-associated cryptococcal meningitis: a randomised trial. Lancet **2004**; 363:1764–7.
13. 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 lnfect Dis **2012**; 54:121–8.
14. 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 lnfect Dis **2009**; 48:1775–83.
15. Farlow AW. The challenges and possibilities of reducing deaths from cryptococcal meningitis in sub-Saharan Africa. PLoS Med **2012**; 9: e1001318.
16. Indian Ministry of Health and Family Welfare. National List of Essential Medicines of India. **2011**. Available at: http://apps.who.int/medicinedocs/documents/s18693en/s18693en.pdf. Accessed 28 Jan 2014.
17. Mills EJ, Thorlund K, Ioannidis JP. Demystifying trial networks and network meta-analysis. BMJ **2013**; 346:f2914.
18. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med **2009**; 6:e1000097.
19. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials **1986**; 7:177–88.
20. Lu G, Ades AE. Combination of direct and indirect evidence in mixed treatment comparisons. Stat Med **2004**; 23:3105–24.
21. Mills EJ, Ioannidis JP, Thorlund K, Schunemann HJ, Puhan MA, Guyatt GH. How to use an article reporting a multiple treatment comparison meta-analysis. JAMA **2012**; 308:1246–53.
22. Higgins JPT, Altman DG, Sterne JAC. Chapter 8: Assessing risk of bias in included studies. In: Higgins JPT, Green S (ed). *Cochrane Handbook for Systematic Reviews of Interventions* (version 5.1.0). Available at: www.cochrane-handbook.org. Accessed 1 March 2014.
23. Te Dorsthorst DT, Verweij PE, Meletiadis J, et al. In vitro interaction of flucytosine combined with amphotericin B or fluconazole against thirty-five yeast isolates determined by both the fractional inhibitory concentration index and the response surface approach. Antimicrob Agents Chemother **2002**; 46:2982–9.
24. Schwarz P, Dromer F, Lortholary O, Dannaoui E. In vitro interaction of flucytosine with conventional and new antifungals against *Cryptococcus neoformans* clinical isolates. Antimicrob Agents Chemother **2003**; 47: 3361–4.
25. Schwarz P, Dromer F, Lortholary O, Dannaoui E. Efficacy of amphotericin B in combination with flucytosine against flucytosine-susceptible or flucytosine-resistant isolates of *Cryptococcus neoformans* during disseminated murine cryptococcosis. Antimicrob Agents Chemother **2006**; 50: 113–20.
26. Schwarz P, Janbon G, Dromer F, Lortholary O, Dannaoui E. Combination of amphotericin B with flucytosine is active in vitro against flucytosine-resistant isolates of *Cryptococcus neoformans*. Antimicrob Agents Chemother **2007**; 51:383–5.
27. van der Horst CM, Saag MS, Cloud GA, et al. Treatment of cryptococcal meningitis associated with the acquired immunodeficiency syndrome. National Institute of Allergy and Infectious Diseases Mycoses Study Group and AIDS Clinical Trials Group. N Engl J Med **1997**; 337:15–21.
28. Hakim JG, Gangaidzo IT, Heyderman RS, et al. Impact of HIV infection on meningitis in Harare, Zimbabwe: a prospective study of 406 predominantly adult patients. AIDS **2000**; 14:1401–7.
29. Milani B, Ford N. Responding to the evidence for improved treatment for cryptococcal meningitis in resource-limited settings. S Afr Med J **2012**; 102:196–7.
30. Sugar AM. Use of amphotericin B with azole antifungal drugs: what are we doing? Antimicrob Agents Chemother **1995**; 39:1907–12.
31. Barchiesi F, Schimizzi AM, Caselli F, et al. Interactions between triazoles and amphotericin B against *Cryptococcus neoformans*. Antimicrob Agents Chemother **2000**; 44:2435–41.
32. Ghannoum MA, Fu Y, Ibrahim AS, et al. In vitro determination of optimal antifungal combinations against *Cryptococcus neoformans* and *Candida albicans*. Antimicrob Agents Chemother **1995**; 39: 2459–65.
33. Mayanja-Kizza H, Oishi K, Mitarai S, et al. Combination therapy with fluconazole and flucytosine for cryptococcal meningitis in Ugandan patients with AIDS. Clin lnfect Dis **1998**; 26:1362–6.
34. Nussbaum JC, Jackson A, Namarika D, et al. Combination flucytosine and high-dose fluconazole compared with fluconazole monotherapy for the treatment of cryptococcal meningitis: a randomized trial in Malawi. Clin lnfect Dis **2010**; 50:338–44.
35. Hamill RJ, Sobel JD, El-Sadr W, et al. Comparison of 2 doses of liposomal amphotericin B and conventional amphotericin B deoxycholate for treatment of AIDS-associated acute cryptococcal meningitis: a randomized, double-blind clinical trial of efficacy and safety. Clin lnfect Dis **2010**; 51:225–32.
36. Sharkey PK, Graybill JR, Johnson ES, et al. Amphotericin B lipid complex compared with amphotericin B in the treatment of cryptococcal meningitis in patients with AIDS. Clin lnfect Dis **1996**; 22: 315–21.
37. 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 lnfect Dis **2007**; 45:76–80.
38. 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 lnfect Dis **2008**; 47:123–30.
39. Jarvis JN, Meintjes G, Rebe K, et al. Adjunctive interferon-gamma immunotherapy for the treatment of HIV-associated cryptococcal meningitis: a randomized controlled trial. AIDS **2012**; 26:1105–13.
40. Longley N, Muzoora C, Taseera K, et al. Dose response effect of high-dose fluconazole for HIV-associated cryptococcal meningitis in southwestern Uganda. Clin lnfect Dis **2008**; 47:1556–61.
41. 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.

42. Brouwer AE, van Kan HJ, Johnson E, et al. Oral versus intravenous flucytosine in patients with human immunodeficiency virus-associated cryptococcal meningitis. Antimicrob Agents Chemother **2007**; 51: 1038–42.
43. Jadhav MP, Bamba A, Shinde VM, et al. Liposomal amphotericin B (Fungisome) for the treatment of cryptococcal meningitis in HIV/AIDS patients in India: a multicentric, randomized controlled trial. J Postgrad Med **2010**; 56:71–5.
44. Joly V, Geoffray C, Reynes J, et al. Amphotericin B in a lipid emulsion for the treatment of cryptococcal meningitis in AIDS patients. J Antimicrob Chemother **1996**; 38:117–26.
45. Larsen RA, Bozzette SA, Jones BE, et al. Fluconazole combined with flucytosine for treatment of cryptococcal meningitis in patients with AIDS. Clin lnfect Dis **1994**; 19:741–5.
46. Pappas PG, Bustamante B, Ticona E, et al. Recombinant interferon-gamma 1b as adjunctive therapy for AIDS-related acute cryptococcal meningitis. J Infect Dis **2004**; 189:2185–91.
47. Parisi A, Malfitano A, Bruno R, et al. Efficacy of a short-term amphotericin B+ flucytosine combination therapy followed by itraconazole monotherapy in acute and chronic AIDS-associated cryptococcosis. Mycoses **1997**; 40:203–7.
48. Chotmongkol V, Arayawichanont A, Sawanyawisuth K, Thavornpitak Y. Initial treatment of cryptococcal meningitis in AIDS. Southeast Asian J Trop Med Public Health **2005**; 36:170–3.
49. Techapornroong M, Suankratay C. Alternate-day versus once-daily administration of amphotericin B in the treatment of cryptococcal meningitis: a randomized controlled trial. Scand J Infect Dis **2007**; 39:896–901.
50. 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 lnfect Dis **2009**; 49:702–9.
51. Bisson GP, Molefi M, Bellamy S, et al. Early versus delayed antiretroviral therapy and cerebrospinal fluid fungal clearance in adults with HIV and cryptococcal meningitis. Clin lnfect Dis **2013**; 56: 1165–73.
52. Jackson AT, Nussbaum JC, Phulusa J, et al. A phase II randomized controlled trial adding oral flucytosine to high-dose fluconazole, with short-course amphotericin B, for cryptococcal meningitis. AIDS **2012**; 26:1363–70.
53. Boulware DR, Meya D, Muzoora C, et al. ART initiation within the first 2 weeks of cryptococcal meningitis is associated with higher mortality: a multisite randomized trial. In: 20th Conference on Retroviruses and Opportunistic Infections. Atlanta, GA, 3–6 March **2013**.
54. Saag MS, Powderly WG, Cloud GA, et al. Comparison of amphotericin B with fluconazole in the treatment of acute AIDS-associated cryptococcal meningitis. The NIAID Mycoses Study Group and the AIDS Clinical Trials Group. New Engl J Med **1992**; 326:83–9.
55. Chotmongkol V, Sukeepaisarncharoen W, Thavornpitak Y. Comparison of amphotericin B, flucytosine and itraconazole with amphotericin B and flucytosine in the treatment of cryptococcal meningitis in AIDS. J Med Assoc Thai **1997**; 80:416–25.
56. Makadzange AT, Ndhlovu CE, Takarinda K, et al. Early versus delayed initiation of antiretroviral therapy for concurrent HIV infection and cryptococcal meningitis in sub-saharan Africa. Clin lnfect Dis **2010**; 50:1532–8.
57. Miller J. The inverse of the Freeman-Tukey double arcsine transformation. Am Stat **1978**; 32:138.
58. Bucher HC, Guyatt GH, Griffith LE, Walter SD. The results of direct and indirect treatment comparisons in meta-analysis of randomized controlled trials. J Clin Epidemiol **1997**; 50:683–91.
59. Walter SD. Choice of effect measure for epidemiological data. J Clin Epidemiol **2000**; 53:931–9.
60. Larsen RA, Leal MA, Chan LS. Fluconazole compared with amphotericin B plus flucytosine for cryptococcal meningitis in AIDS. A randomized trial. Ann Intern Med **1990**; 113:183–7.
61. de Gans J, Portegies P, Tiessens G, et al. Itraconazole compared with amphotericin B plus flucytosine in AIDS patients with cryptococcal meningitis. AIDS **1992**; 6:185–90.
62. Tansuphaswadikul S, Maek-a-Nantawat W, Phonrat B, et al. Comparison of one week with two week regimens of amphotericin B both followed by fluconazole in the treatment of cryptococcal meningitis among AIDS patients. J Med Assoc Thai **2006**; 89:1677–85.
63. Coker RJ, Viviani M, Gazzard BG, et al. Treatment of cryptococcosis with liposomal amphotericin B (AmBisome) in 23 patients with AIDS. AIDS **1993**; 7:829–35.
64. Joly V, Aubry P, Ndayiragide A, et al. Randomized comparison of amphotericin B deoxycholate dissolved in dextrose or Intralipid for the treatment of AIDS-associated cryptococcal meningitis. Clin lnfect Dis **1996**; 23:556–62.
65. Leenders AC, Reiss P, Portegies P, et al. Liposomal amphotericin B (AmBisome) compared with amphotericin B both followed by oral fluconazole in the treatment of AIDS-associated cryptococcal meningitis. AIDS **1997**; 11:1463–71.
66. Newton PN, Thai le H, Tip NQ, et al. A randomized, double-blind, placebo-controlled trial of acetazolamide for the treatment of elevated intracranial pressure in cryptococcal meningitis. Clin lnfect Dis **2002**; 35:769–72.
67. Haubrich RH, Haghighat D, Bozzette SA, Tilles J, McCutchan JA. High-dose fluconazole for treatment of cryptococcal disease in patients with human immunodeficiency virus infection. The California Collaborative Treatment Group. J Infect Dis **1994**; 170:238–42.
68. Menichetti F, Fiorio M, Tosti A, et al. High-dose fluconazole therapy for cryptococcal meningitis in patients with AIDS. Clin lnfect Dis **1996**; 22:838–40.
69. Stern JJ, Pietroski NA, Buckley RM, Rinaldi MG. Parenteral and oral fluconazole for acute cryptococcal meningitis in AIDS: experience with thirteen patients. Ann Pharmacother **1992**; 26:876–82.
70. Chotmongkol V, Jitpimolmard S. Treatment of cryptococcal meningitis with combination itraconazole and flucytosine. J Med Assoc Thai **1994**; 77:253–6.