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Diagnosis and management of cryptococcal meningitis in HIV-infected adults

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SUMMARY Cryptococcal meningitis is a leading cause of morbidity and mortality globally, especially in people with advanced HIV disease. Cryptococcal meningitis is responsible for nearly 20% of all deaths related to advanced HIV disease, with the burden of disease predominantly experienced by people in resource-limited countries. Major advancements in diagnostics have introduced low-cost, easy-to-use antigen tests with remarkably high sensitivity and specificity. These tests have led to improved diagnostic accuracy and are essential for screening campaigns to reduce the burden of cryptococcosis. In the last 5 years, several high-quality, multisite clinical trials have led to innovations in therapeutics that have allowed for simplified regimens, which are better tolerated and result in less intensive monitoring and management of medication adverse effects. One trial found that a shorter, 7-day course of deoxycholate amphotericin B is as effective as the longer 14-day course and that flucytosine is an essential partner drug for reducing mortality in the acute phase of disease. Single-dose liposomal amphotericin B has also

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been found to be as effective as a 7-day course of deoxycholate amphotericin B. These findings have allowed for simpler and safer treatment regimens that also reduce the burden on the healthcare system. This review provides a detailed discussion of the latest evidence guiding the clinical management and special circumstances that make cryptococcal meningitis uniquely difficult to treat.

KEYWORDS cryptococcal meningitis, cryptococcal antigen, amphotericin, flucytosine, fluconazole, lumbar puncture, antiretroviral therapy, HIV/AIDS

INTRODUCTION

Cryptococcus neoformans is the primary etiologic agent of cryptococcal meningitis, which is a leading cause of meningitis globally, with a disproportionate burden of disease in sub-Saharan Africa (1). In a recent analysis of UNAIDS data published through 2020, Rajasingham et al. found that cryptococcal disease was responsible for 19% of all global AIDS-related deaths (2). Despite advancements in screening, therapeutics, and access to antiretroviral therapy (ART), the proportion of AIDS-related deaths caused by cryptococcosis was essentially unchanged from a similar analysis of data through 2014 (3). While cryptococcal meningitis is the most recognizable clinical disease, *C. neoformans* causes disseminated infection throughout the body including the brain parenchyma (4). This understanding of the infection distribution has led many countries to develop screening programs to identify early cryptococcal disease using cryptococcal antigen (CrAg) testing and prevent the progression to meningitis. Cryptococcal meningitis occurs most commonly in HIV-infected persons with a $CD4^+$ T-cell count <100 cells/μL (3). Even when appropriate therapy is available, mortality during the acute manifestation of the disease is high but starkly disproportionate by geographic region, ranging from 10% to 15% in resource-rich countries to 60% in resource-poor countries (5–7). Furthermore, long-term complications can be debilitating including blindness, deafness, and neurocognitive impairment (8). In recent years, there have been exciting new developments in both diagnostics and treatment modalities that have led the WHO to revise their guidelines for cryptococcal meningitis management (9, 10).

DIAGNOSIS OF CRYPTOCOCCAL MENINGITIS

Diagnosis of cryptococcal meningitis has changed dramatically over time. Fungal culture of the cerebrospinal fluid (CSF) has historically been the gold standard for diagnosis with India ink being an essential adjunctive modality for decades. However, people with lower fungal burdens present a significant challenge to diagnosis. Typical CSF findings, which include a white blood cell count <50 cells/μL with a mononuclear predominance, slightly elevated protein and low to normal glucose, can be suggestive of cryptococcosis if suspicion is high, and classic diagnostic tests are negative (11, 12). However, with the advent of antigen testing, the sensitivity and specificity for cryptococcal meningitis can approach 100%, even with costs that are feasible in low-income settings and require minimal laboratory expertise (Table 1).

Cryptococcal culture

Historically, the gold standard for cryptococcal meningitis diagnosis has been the isolation of the *Cryptococcus* organism on fungal culture from the infected fluid or tissue. Sabouraud dextrose agar is the most commonly used culture medium for isolating the yeast. However, growth can take 3–7 days, and the sensitivity is limited by false-negatives, which are common in individuals with low fungal burden (13). In one large cohort, culture sensitivity was related to the input volume, where sensitivity was 82.4% with 10 μL of CSF increasing to 94.2% when 100 μL of CSF was used (6, 14). Blood cultures can also be useful as they are highly specific but are limited by a sensitivity of ~50% (15). While the time to result limits its utility for medical decision-making in the acute setting, CSF culture remains an important tool in confirming microbiologic control

TABLE 1 Overview of diagnostic modalities for cryptococcal meningitis

Methodology	Sensitivity	Specificity	Limitations
CSF culture	82.4–94.2%	100%	Time to diagnosis ~1–2 weeks. Limited sensitivity, especially in low fungal burden
Blood culture	50%	100%	Poor sensitivity
India ink staining	42–86%	100%	Requires laboratory expertise. Limited sensitivity, especially in low fungal burden
CSF cryptococcal antigen	99.1–100%	99.1%-100%	Prozone effect can reduce sensitivity in very high fungal burden cases. Test characteristics depend on the manufacturer.
Serum/plasma cryptococcal antigen	92–100%	86–100%	Prozone effect can reduce sensitivity in very high fungal burden cases. Test characteristics depend on the manufacturer.
CSF 1,3-β-D-glucan	89%	85%	Sensitivity reduced by low-level production in the fungal cell wall. Non-specific marker, limited specificity.
Molecular testing	50–96%	96–99%	Poor sensitivity especially in low fungal burden, high cost, requires technical laboratory skills

of *Cryptococcus* during induction therapy. CSF culture is also important in differentiating between meningitis relapse versus paradoxical immune reconstitution inflammatory syndrome (IRIS) after initial therapy (16). Furthermore, quantitative cryptococcal culture utilizes a specialized technique that can be useful in the research setting. Serial quantitative cultures have a linear $\log_{10}$ clearance rate, which can be used to calculate the early fungicidal activity (EFA) of different antifungal regimens. EFA has been shown to reliably predict cryptococcal-related mortality and has been used in numerous clinical trials to establish the utility of new drugs or evaluate various combinations of therapies for *Cryptococcus* (Table 2) (17, 18). The FDA has allowed for the use of surrogate endpoints in clinical trials in order to reduce the time and resources needed to conduct trials and identify new therapies (17). In a recent pooled cohort study evaluating data from three clinical trials, EFA < 0.2 $\log_{10}$ CFU/mL/day was shown to be an independent predictor of 18-week mortality (17). The use of EFA as a surrogate endpoint has allowed for much greater advancements in our understanding of cryptococcal disease through smaller, less resource-intensive studies that can be done within a shorter time scale (17).

India ink staining

India ink staining utilizes direct visualization and can be useful in reducing the time to diagnosis which complicates the utility of traditional culture methods. India ink takes advantage of the thick polysaccharide capsule which appears translucent against the darkly stained yeast under microscopy. While India ink preparation has a strong specificity of up to 100%, it is limited by poor sensitivity, ranging from 42% to 86% for initial or relapse diagnosis, decreasing with lower CSF fungal burden (14, 29, 30). Moreover, in one study in Uganda, where the prevalence of *Cryptococcus* was high, the use of India ink as the only diagnostic test had the potential of missing 8.8% of cryptococcal diagnoses (14).

Cryptococcal antigen

Given the many limitations of older diagnostic tools, the detection of CrAg has emerged as the new gold standard for diagnosing cryptococcal meningitis and is recommended by the WHO as the first-line diagnostic test (10). The CrAg can be detected in the blood or CSF using latex agglutination, enzyme-linked immunosorbent assay (ELISA), or lateral flow assay (LFA). Each of these modalities has varying utility depending on the circumstances, but all have sensitivity and specificity that range from 99.1% to 100% when testing CSF (14, 31, 32). While the ELISA and latex agglutination assays have shown major improvements in terms of sensitivity, specificity, and reduced intensity of

TABLE 2 Summary of clinical trials for induction therapy regimens[a]

Setting	Regimen	10 -week mortality	EFA	*N*	Reference
Vietnam	AMB monotherapy (28 days)	44.4%	0.31	99	(19)
	AMB + 5FC	30%	0.42	100	
	AMB + fluconazole (800 mg/day)	33%	0.32	99	
South Africa	AMB + 5 FC	30%	0.41	21	(20)
	AMB + fluconazole (800 mg/day)	33.3%	0.38	22	
	AMB + fluconazole (1,200 mg/day)	27.3%	0.41	23	
	AMB + voriconazole	25%	0.44	13	
Uganda	AMB (5 days) + fluconazole (1,200 mg/day)	28%	0.30	30	(21)
South Africa	AMB + 5FC	32%	0.49	30	(22)
	AMB + 5 FC + IFN-γ	30%	0.64	60	
Malawi	AMB (7 days) + fluconazole (1,200 mg/day)	37.5%	0.38	19	(23)
	AMB (7 days) + fluconazole (1,200 mg/day) + 5FC	46.2%	0.50	18	
Uganda	Fluconazole (1,200 mg/day)	48%	0.18	30	(24)
	Fluconazole (800 mg/day)	60%	0.07	30	
Uganda	AMB + fluconazole (800 mg/day) + sertraline	40% (18 weeks)	0.43	128	(25)
Malawi	Fluconazole (1,200 mg/day)	58%	0.11	20	(26)
	Fluconazole (1,200 mg/day) + flucytosine	43%	0.28	21	
Malawi, Zambia, Tanzania, Cameroon	AMB 1 week + either 5FC or fluconazole	36.2%	0.40	179	(27)
	AMB 2 week + either 5FC or fluconazole	39.7%	0.42	182	
	Fluconazole (1,200 mg/day) + flucytosine	35.1%	0.26	182	
	AMB + 5FC	31.1%	0.46	186	
	AMB + fluconazole (1,200 mg/day)	45%	0.36	175	
Botswana, Malawi, South Africa, Uganda, Zimbabwe	Single-dose liposomal Amphotericin B + 5 FC + Fluconazole (1,200 mg/day)	24.8%	0.40	407	(28)

[a]EFA = early fungicidal activity, AMB = Amphotericin B deoxycholate; 5FC = flucytosine at 100 mg/kg/day in ~4 divided doses. Amphotericin given for 2 weeks unless otherwise noted.

resources, they still require a cold chain for specimen transport and technical expertise (32). The CrAg LFA (Immy Inc., Norman, OK, USA) which was first released in 2011, can be performed at the bedside and requires minimal expertise (Fig. 1) (14, 32). This CrAg LFA developed and produced by Immy is FDA-approved and has been validated in multiple clinical studies and has emerged as the new gold standard for cryptococcal diagnosis. However, there are other non-FDA-approved CrAg LFAs available internationally from a

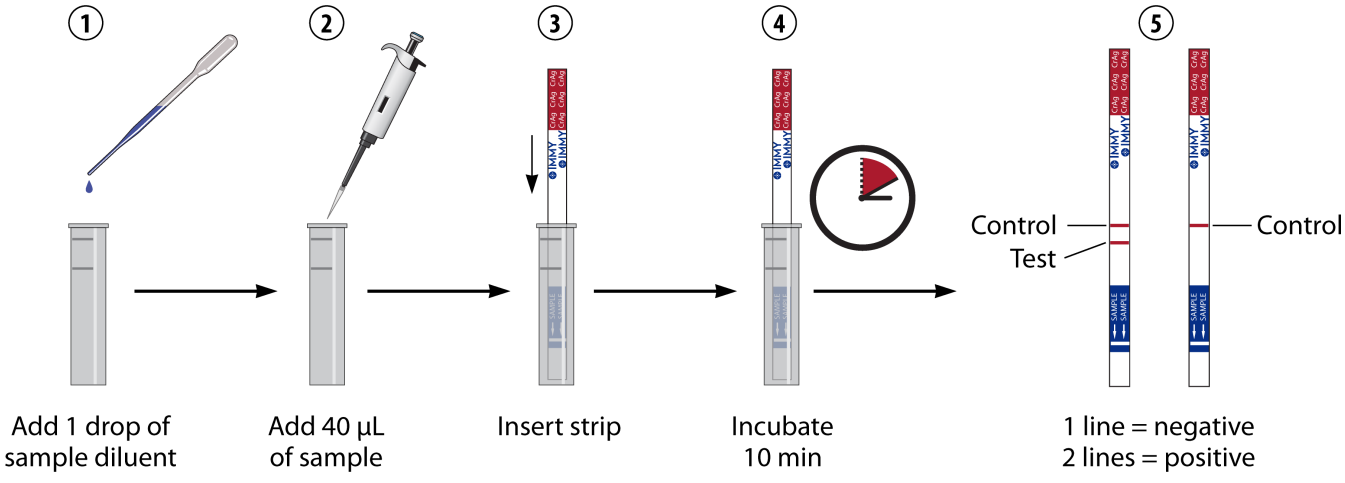

FIG 1 Instructions for cryptococcal antigen lateral flow assay (CrAg LFA) screening that can be completed at the bedside or as near-patient testing. (Courtesy of Immy, Inc., reproduced with permission.)

variety of manufacturers that should generally be avoided as clinical validation studies have revealed inferior performance (33–35).

In the largest validation study of CrAg diagnostics to date, the Immy CrAg LFA was validated in multiple clinical sites across Uganda and South Africa, which enrolled 832 persons with suspected meningitis and tested 666 CSF samples (14). Since the antigen tests have higher sensitivity than previously used diagnostics, a composite reference was used as the gold standard. This included CSF culture with positive cryptococcal growth or a negative culture with at least two other positive test results such as India ink, CrAg LFA, or CrAg latex agglutination, with no alternative diagnosis (14). When testing CSF, the CrAg LFA had a sensitivity of 99.3%, specificity of 99.1%, positive predictive value of 99.5%, and negative predictive value of 98.7% for diagnosing cryptococcal meningitis (14). The CrAg latex agglutination had a sensitivity of 97.8%, specificity of 85.9%, positive predictive value of 92.6%, and negative predictive value of 95.5% for diagnosing cryptococcal meningitis (14).

CrAg LFA and latex agglutination tests also performed well in diagnosing cryptococcal meningitis when testing peripheral fluid compartments including serum, plasma, and urine. Testing serum CrAg LFA had a sensitivity of 99.6% and specificity of 92%, while CrAg agglutination had a sensitivity of 98.3% in the serum (14). In the same study, plasma was retrospectively analyzed and found to have a sensitivity of 100%, but specificity was unable to be done (14). Capillary blood (i.e., fingerstick) CrAg LFA has been shown to have 100% agreement with whole blood, plasma, and serum CrAg. Fingerstick CrAg LFA also has a 100% negative predictive value for the exclusion of cryptococcal meningitis (36). The CrAg LFA had a sensitivity of 97% and specificity of 85% for cryptococcal meningitis when testing urine.

One potential rare limitation of the CrAg LFA is the influence of the "prozone phenomenon" that occurs when there is an excess of antigen relative to antibodies leading to the formation of antigen-antibody complexes that hinder the agglutination reaction resulting in a false negative result. For example, a study in South Africa showed a surprisingly low sensitivity of 92%; however, when paired with Gram staining to determine the presence of yeast coupled with a dilution of samples, sensitivity improved to 100% (37). Another example of the "prozone effect" was observed at a CrAg LFA titer of 1:1,310,000, where the yeasts were obvious on a Gram stain of the CSF (38). These studies show that the prozone phenomenon occurs only at exceedingly high CrAg titers and can be mitigated with the use of gram staining or serial dilutions when suspicion for cryptococcal meningitis is high.

Quantitative CrAg titers can be useful as a prognostic marker for cryptococcal mortality. In a study in Thailand, a CrAg latex agglutination titer was shown to have a moderate positive correlation with pre-therapy quantitative cultures ($R^2 = 0.5$) and a strong association with 2- and 10-week mortality (39). However, CrAg LFA was shown to have a strong positive association with quantitative cultures ($R^2 = 0.7$), and an increasing 2- and 10-week mortality with each twofold increase in CrAg LFA titer (40). Because quantitative CrAg titers are more difficult to perform than the simple point-of-care testing, a semi-quantitative CrAg LFA was developed to provide rapid prognostic data (38). The semi-quantitative CrAg-SQ (Immy Inc., Norman, OK, USA) provides 1+ to 5+ grades, which corresponded to median CrAg titers of 1:10, 1:60, 1:7,680, 1:81,920, and 1:1,474,000, respectively, when performed on CSF (38). The sensitivity and specificity of the CrAg-SQ test on CSF were 100% for cryptococcal meningitis (38). CSF CrAg-SQ of 3+ or higher was associated with 100% fungal culture positivity (38). Two-week mortality for individuals with CSF CrAg-SQ grades of 1+ to 3+ was 5% compared to 21% for those individuals with grades 4+ and 5+ (38). This new CrAg-SQ test largely maintains the ease of use, arguably improves the diagnostic accuracy of CrAg testing, and provides useful prognostic information for clinicians and patients.

While the baseline CrAg titer levels have been shown to strongly correlate with clinical outcomes, including 2- and 10-week mortality, as discussed above, the use of CrAg titers to monitor response to therapy has not been shown to be useful. In 1994,

Powderly et al. evaluated two clinical trials to evaluate the use of CSF CrAg to monitor response to therapy (41). They found that those who had a good response had higher rates of CSF CrAg titer decrease, but this difference failed to reach statistical significance (41). However, a rise in CSF CrAg titer was associated with cryptococcal relapse. While it is likely that persistently elevated CrAg titers indicate poorer prognosis, this has not been shown to be clearly useful in measuring treatment response (42).

The remarkable predictive value of CrAg testing has facilitated the large-scale expansion of CrAg testing into screening programs aimed at identifying individuals with cryptococcal antigenemia, who are at higher risk for developing cryptococcal meningitis. In a study conducted in rural Uganda, individuals with CD4 counts <100 cells/μL and symptoms of meningitis were found to have a CrAg positivity of 5.8% and a population-attributable risk for mortality of 18% (43). Subsequently, multiple studies demonstrated the utility of serum CrAg screening programs in reducing HIV-associated cryptococcal disease-related mortality, as discussed in the CrAg screening section below (44, 45).

1,3-β-D-glucan

The non-specific fungal marker (1, 3),-β-D-glucan is produced by *Cryptococcus* and can be a useful tool in the management of cryptococcal meningitis. In a population from Uganda and South Africa, the Fungitell β-D glucan (BDG) assay (Associates of Cape Cod Inc, East Falmouth, MA, USA) had 89% sensitivity and 85% specificity in CSF for cryptococcal meningitis diagnosis (46). Serum sensitivity and specificity were poor at 79% and 61%, respectively. Individuals with high fungal burdens [>10,000 colony forming units (CFU)/mL] had markedly improved BDG CSF sensitivity of 98%. High concentrations of BDG (>500 pg/mL) were associated with threefold higher 10-week mortality. The same study showed the CSF BDG rapidly normalizes within the first week of induction therapy, which compares favorably to CrAg levels which may remain persistently elevated despite appropriate therapy. Because of this, BDG may also be useful in differentiating relapse cryptococcal meningitis from IRIS, but this has not been systematically studied (46). Another study conducted during an outbreak of meningitis, showed that BDG had a 100% sensitivity and 98% specificity for diagnosing fungal meningitis, in general (47). A common misperception, propagated by the recommended, incorrect FDA warning label for the serum BDG assay, is that BDG offers no utility for diagnosing *Cryptococcus* (48, 49). However, these data indicate that while BDG is not a candidate for replacing CrAg testing, it can serve as a useful marker for prognosis and response to therapy.

Molecular testing

Finally, molecular testing has emerged as an alternative diagnostic modality, especially in resource-rich environments; however, it remains limited by poor sensitivity in individuals with low fungal burdens. The CSF BioFire FilmArray (BioFire Diagnostics, Salt Lake City, UT, USA) has included *Cryptococcus* on its panel of molecular diagnostics. In a validation study in Uganda, the FilmArray had a reasonable sensitivity and specificity of 96% for detecting *Cryptococcus* >100 CFU/mL (50). However, this sensitivity dropped to 50% in individuals with low fungal burdens (<100 CFU/mL). Similar findings were demonstrated in Los Angeles, California, where the BioFire panel was determined to have an overall sensitivity and specificity of 96.4% and 99.6%, respectively, when compared to culture (51). When compared to CrAg LFA, the sensitivity decreased to 83.8%, while specificity remained high. However, all false-negative results on the BioFire panel were associated with recurrent cryptococcal cases, indicating that the panel remains a good diagnostic tool for the initial diagnosis of cryptococcal meningitis. Still, the cost-intensive nature and failure to improve the diagnostic accuracy of simpler and cheaper tests will likely limit the usefulness of molecular testing in much of the world. Quantitative PCR is theoretically possible to estimate the quantitative CSF culture burden and predict culture sterility; however, this has not been developed.

Neuroimaging

Generally, lumbar puncture may safely be performed without prior neuroimaging in the absence of focal neurologic deficits, which may indicate a mass or space-occupying lesion (52). If focal deficits are present, neuroimaging by CT or MRI should be done prior to lumbar puncture. Mass lesions may be a contraindication to lumbar puncture in some cases and can suggest an alternative diagnosis, such as toxoplasmosis, tuberculosis, or lymphoma as neuro-cryptococcomas are relatively rare, especially in people with HIV (53). If imaging is consistent with hydrocephalus, placement of ventricular shunt may be required once the diagnosis of cryptococcal meningitis has been confirmed (54).

MANAGEMENT OF CRYPTOCOCCAL INFECTION

The management of cryptococcal infection is particularly challenging given the variety of available therapeutics with different levels of efficacy and the predominance of affected persons in resource-limited parts of the world. In recent years, there have been multiple ground-breaking clinical trials that have resulted in the WHO releasing updated guidelines for the diagnosis and management of cryptococcal meningitis in 2018 and 2022 (9, 10). In addition, the development of diagnostics with the capacity to identify cryptococcosis in the subclinical phase has led to a push for better screening, surveillance, and intervention programs. Given this, we would break down the management of cryptococcal meningitis into four key phases: screening, induction, consolidation, and maintenance phases (Table 3).

The screening phase is focused on identifying persons with undetected disseminated cryptococcosis, or cases when *Cryptococcus* is affecting multiple organ systems which may or may not include the central nervous system (CNS). The target population are persons who are asymptomatic or with mild symptoms that have not advanced enough to result in detection by the medical system. The screening phase should include persons with CD4 counts <200 cells/μL (55). As discussed above, serum or plasma CrAg testing is sufficient to identify disseminated cryptococcosis, even in asymptomatic individuals.

The induction phase occurs for those who present with acute symptoms of cryptococcal meningitis, involves hospitalization, and emphasizes the use of combination

TABLE 3 Outline of the four-phases of management for cryptococcal meningitis

	Screening	Induction	Consolidation	Maintenance
Goal	Identify and treat individuals with undetected disseminated cryptococcosis	Rapidly reduce cryptococcal burden from the CNS	Control of cryptococcal organisms from the CNS	Prevent relapse of cryptococcal meningitis
Population	All individuals with HIV who have a $CD4^+$ T-cell count <200 cells/μL	All individuals with positive plasma or CSF CrAg testing and evidence of disseminated cryptococcosis	All individuals treated with induction therapy for cryptococcal infection	All individuals treated with consolidation therapy for cryptococcal infection
Diagnostic Intervention	Plasma CrAg ± CSF CrAg	Plasma and/or CSF CrAg	–	–
Therapeutic Intervention	Fluconazole 800 mg/day for 10 weeks OR (*Under investigation*) Fluconazole 1,200 mg/day plus flucytosine 100 mg/kg/day for 14 days OR Single-dose liposomal amphotericin B plus fluconazole 800 mg/day for 10 weeks	Single-dose liposomal amphotericin B plus fluconazole 1,200 mg/day and flucytosine 100 mg/kg/day OR Amphotericin B deoxycholate 1 mg/kg/day plus flucytosine 100 mg/kg/day for 7 days OR Fluconazole 1,200 mg/day plus flucytosine 100 mg/kg/day for 14 days	Fluconazole 800 mg/day for 8 weeks after induction therapy	Fluconazole 200 mg/day for up to 12 months or until immune system reconstitution

antifungal therapy to rapidly kill the fungus and management of elevated intracranial pressure (ICP). The consolidation phase occurs after discharge from the hospital, when antifungal therapy is deescalated, and ART is initiated. Finally, the maintenance phase further deescalates antifungal therapy in an effort to prevent the recurrence of cryptococcal meningitis until immune recovery.

CrAg screening and treatment of high-risk persons

In two large cohorts of persons with HIV and CD4 counts <100 cells/μL in both South Africa and Uganda, despite fluconazole administration for cryptococcal antigenemia, mortality at 6 months remained high at ~25% (56, 57). Individuals with cryptococcal antigenemia had a 3.3-fold increased risk of death compared to those without antigenemia (56, 57). Similar results were found in Uganda in a stepped-wedge cluster randomized trial, where a screen and treat program was implemented in a randomly selected cluster of Kampala Capital City Authority clinics every 2 months. Participants with CD4 counts <100 cells/μL and positive plasma CrAg without signs and symptoms of meningitis were treated with fluconazole 800 mg/day for 2 weeks, followed by 400 mg/day for 8 weeks (58). Of the CrAg-positive individuals who received fluconazole preemptive therapy, 7.9% developed cryptococcal meningitis, and 6-month survival was 79.6% overall. However, outcomes varied by baseline plasma CrAg LFA titer. Of CrAg-positive persons with baseline plasma CrAg titer ≥1:160, 36% failed preemptive fluconazole therapy, compared to 13% among participants with plasma CrAg titer ≤1:160. In a systematic review of all studies evaluating CrAg screening programs, Ford et al. found that 18.6% of CrAg-positive cases were identified in persons with a CD4 count of 101–200 cells/μL (55), which leads us to recommend screening in all individuals with CD4 count <200 cells/μL. Serum and plasma CrAg titers are approximately equivalent (14), yet recent CrAg screening programs have utilized plasma for CrAg testing from the leftover CD4 remnants, typically as reflexive tests when the CD4 <100 or <200 cells/μL.

Outcomes from CrAg screening programs indicate that fluconazole monotherapy is inadequate for treating disseminated cryptococcosis, even before there has been proven invasion of the CNS. Subsequent cohorts have evaluated the use of lumbar puncture to identify those with CNS involvement and found that those with CSF CrAg positivity had markedly higher mortality than those with only CrAg antigenemia (59, 60). In 2018, the WHO updated their *Cryptococcus* guidelines to recommend lumbar puncture for those with cryptococcal antigenemia, regardless of symptoms (9). Some experts disagree and believe that using CrAg titer to risk stratify is a better method that contains less risk and is more cost-effective.

CrAg screening programs have found that higher plasma CrAg titers predict the likelihood of CSF CrAg positivity and are independently associated with mortality, as shown in Fig. 2 and 3 (59–62). For example, nearly 60% of people with plasma CrAg titers of 1:1,280 or greater are also CSF CrAg-positive (4). Still, in these cohorts, 60% of individuals with asymptomatic cryptococcal antigenemia who died had a negative CSF CrAg at baseline (4). Therefore, it is crucial to recognize that early brain parenchymal CNS infection can be present in the absence of cryptococcal infection in the CSF anatomical space. Given the independent predictability for mortality of CrAg titers ≥1:640, these persons should be managed as CNS cryptococcal infection, regardless of CSF CrAg results. In these persons with high CrAg titers, the role of lumbar puncture becomes important not for diagnosis but as an adjunctive therapeutic measure to reduce elevated intracranial pressure, if present. Since fluconazole monotherapy alone is imperfect for those with higher plasma CrAg titers ≥1:160, further clinical trials are ongoing to optimize their management. Three strategies are possible. First, we recommend at a minimum, the use of fluconazole 800 mg/day for 10 weeks in asymptomatic CrAg-positive persons, followed by secondary prophylaxis until immune reconstitution (4). A second strategy to increase antifungal therapy is to use adjunctive flucytosine with fluconazole (4, 63). Molloy et al. demonstrated that this was safe and as effective as 2 weeks of IV amphotericin in cryptococcal meningitis (27). Unfortunately,

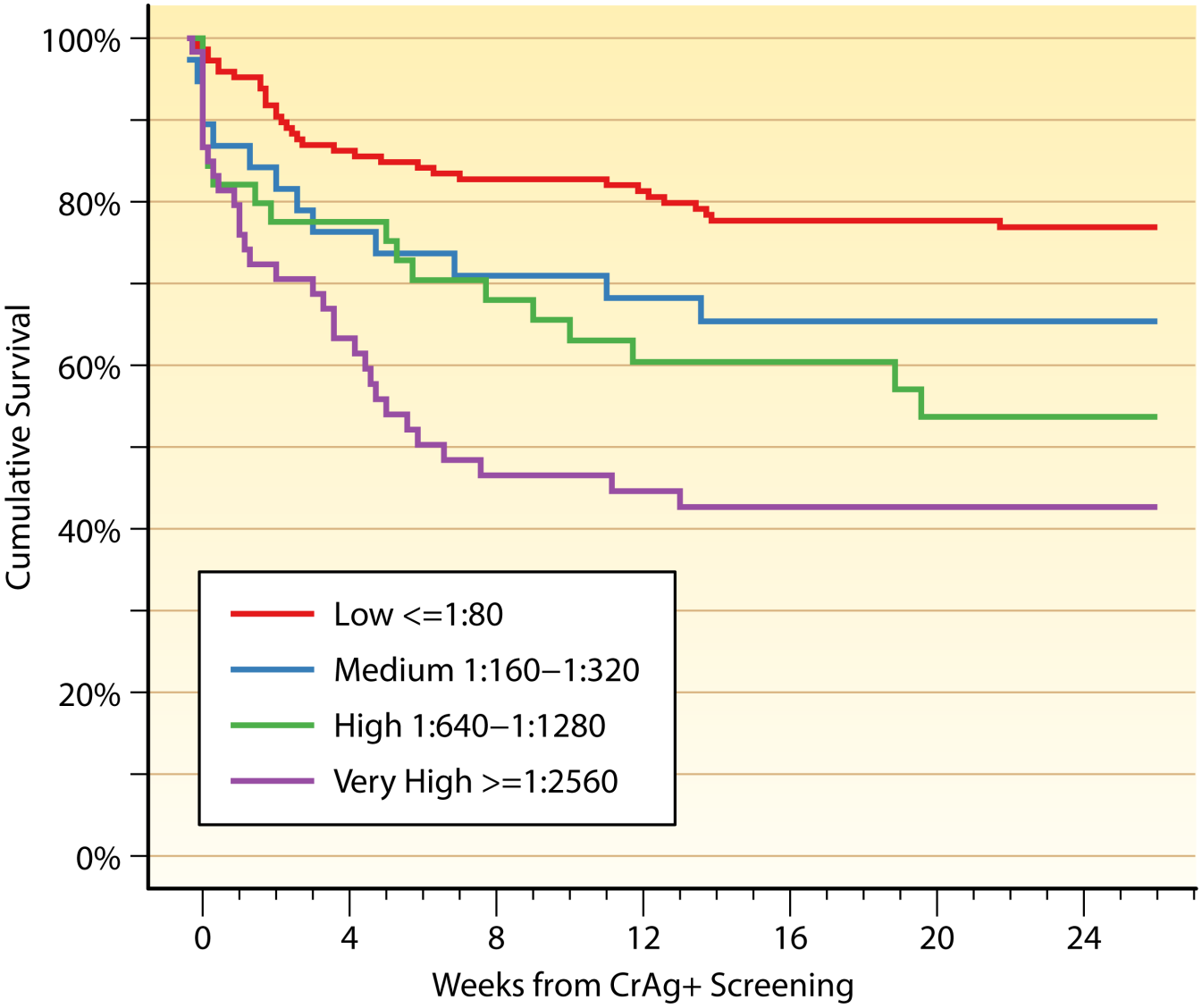

FIG 2 Survival in CrAg-positive persons by quantitative plasma cryptococcal antigen (CrAg) LFA titer. (Modified from reference 66.)

flucytosine remains widely unavailable in sub-Saharan Africa and prohibitively expensive in the United States ($425 /day) (4), although less expensive than a hospitalization. A third strategy being tested in a randomized clinical trial is giving single-dose liposomal amphotericin at 10 mg/kg, followed by standard fluconazole management for preemptive therapy of asymptomatic persons with high plasma CrAg titers (see registration no. NCT03945448 at Clinicaltrials.gov). This strategy is as effective as induction therapy in cryptococcal meningitis (28, 64). Regardless of the strategy, HIV therapy should be delayed 2 weeks after starting antifungals (65).

Induction antifungal therapy for cryptococcal meningitis

Prior to 2018, the WHO recommended first-line induction therapy including 14 days of intravenous amphotericin B deoxycholate (0.7–1.0 mg/kg/day) with flucytosine 100 mg/kg/day in four divided doses (9). In 2018, Molloy et al. evaluated five antifungal combinations for induction therapy of cryptococcal meningitis in Zambia, Malawi, Cameroon, and Tanzania, including fluconazole (1,200 mg) plus flucytosine (100 mg/kg) for 14 days, deoxycholate amphotericin B (1 mg/kg) plus fluconazole (1,200 mg) or flucytosine (100 mg/kg) for 7 days, followed by fluconazole (1,200 mg) for 7 days, and

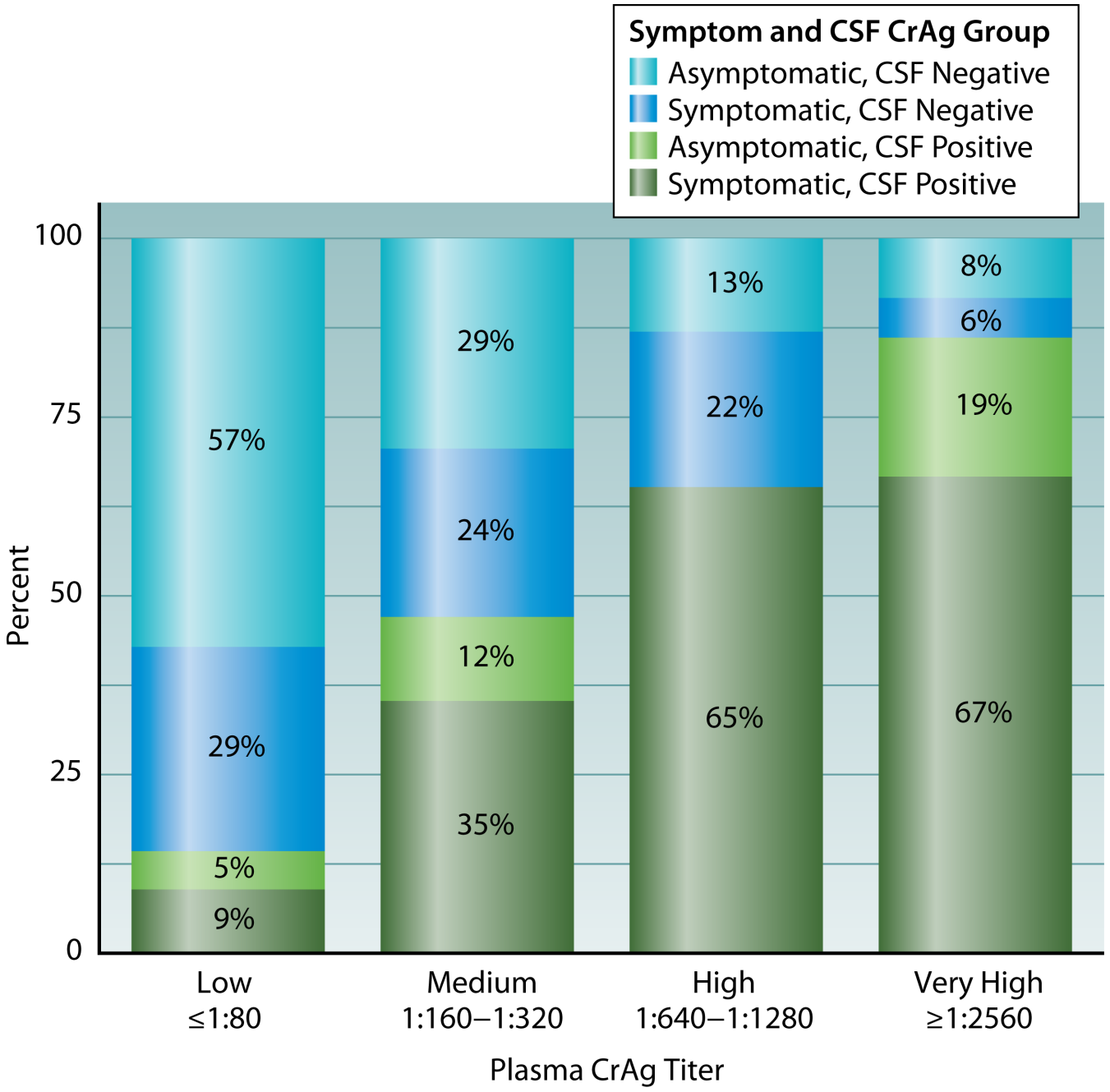

FIG 3 Plasma CrAg titer predicts CSF cryptococcal antigen (CrAg) titer positivity in symptomatic and asymptomatic individuals. Over 60% of persons with plasma titers ≥1:640 will have positive CSF titer. 19% of asymptomatic persons with plasma CrAg titer ≥1:2,560 will have positive CSF CrAg titer. (Based on data from reference 4.)

deoxycholate amphotericin B plus fluconazole (1,200 mg) or flucytosine (100 mg/kg) for 14 days (27). They found that all combinations of therapy that included flucytosine were superior to non-flucytosine-containing regimens (Fig. 4). They also found that 1 week of deoxycholate amphotericin was non-inferior to 2 weeks of deoxycholate amphotericin and resulted in fewer severe and life-threatening toxicities (i.e., grades 3–5 adverse events). The 10-week mortality in the deoxycholate amphotericin plus flucytosine group was 31% compared to 45% in the deoxycholate amphotericin plus fluconazole group (*P* value = 0.002) (27). The 1-week deoxycholate amphotericin plus flucytosine group had a 24% mortality compared to 38.3% mortality in the 2-week

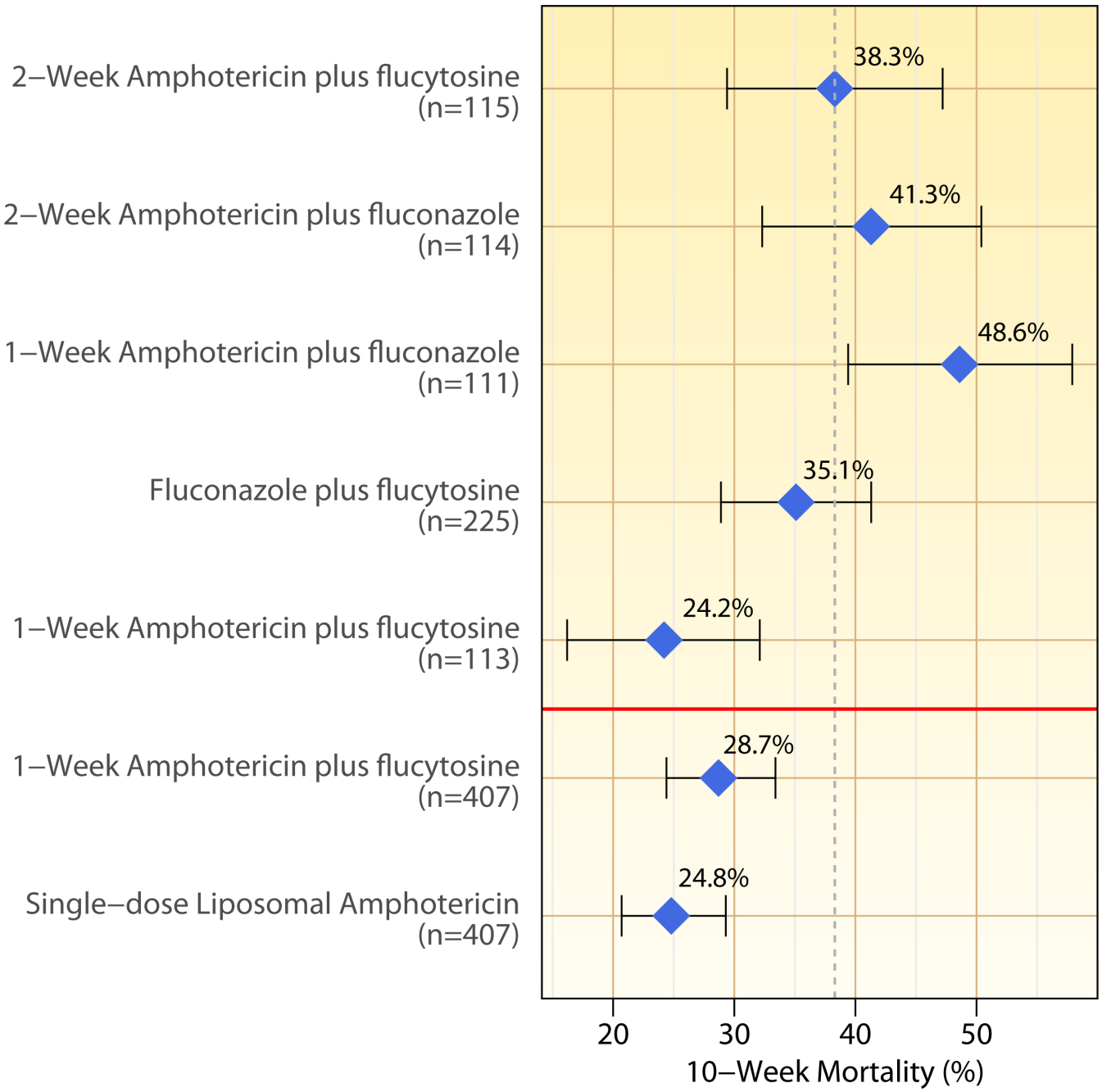

FIG 4 10-week mortality by antifungal regimen for cryptococcal meningitis from the clinical trial by Molloy et al. in top section (separated by red line) and Jarvis et al. in bottom section (27, 28). Proportions dying by 10 weeks for each group are represented by diamonds and 95% CI is represented by error bars.

deoxycholate amphotericin plus flucytosine group with a hazard ratio of 0.56 (95% CI = 0.35–0.91) (27). Finally, the all-oral regimen of 2 weeks flucytosine and fluconazole had a hazard ratio of 0.87 (95% CI = 0.6–1.27), which was non-inferior to the 2-week deoxycholate amphotericin and flucytosine group (27). The rate of grade 3 or 4 anemia in the 1-week deoxycholate amphotericin group was 13.8% compared to 26.3% in the 2-week deoxycholate amphotericin group (27). This high-quality trial confirmed the findings of many smaller studies that came before, that flucytosine is essential during the acute phase of cryptococcal infection for reducing mortality, and that a shorter course of amphotericin deoxycholate, with the appropriate partner drug, remains effective while reducing the incidence of drug-related adverse events. Flucytosine is a molecule that

is taken up into the fungal cell and converted to 5-fluorouracil and acts as a potent RNA and DNA synthesis inhibitor. Because the mechanism requires intracellular access, medications that disrupt the fungal cell wall like amphotericin and fluconazole have been thought to potentiate the effect of flucytosine (25, 67, 68).

The WHO updated their guidelines in 2018 to recommend a 7-day regimen of amphotericin B deoxycholate (0.7–1.0 mg/kg/day) with flucytosine 100 mg/kg in four divided doses, followed by 7 days of high-dose fluconazole 1,200 mg/day as the preferred first-line induction therapy regimen (9). Two weeks of fluconazole (1,200 mg/day) plus flucytosine (100 mg/kg/day) or 2 weeks of amphotericin B deoxycholate (0.7–1.0 mg/kg/day) were acceptable alternatives if resources limit the availability of first-line therapy.

In 2022, Jarvis et al. published a phase 3, controlled, non-inferiority study in Botswana, Malawi, South Africa, Uganda, and Zimbabwe, that evaluated single-dose liposomal amphotericin B at 10 mg/kg in combination with flucytosine and fluconazole compared to the then-WHO-recommended first-line induction regimen of 1 week of amphotericin B deoxycholate plus flucytosine, followed by high dose fluconazole for 1 week (28). This study added single-dose liposomal amphotericin to the all-oral regimen, which had been found to be non-inferior to standard 2-week amphotericin (28). This was done in order to ensure that adequate therapy was being delivered even if single-dose amphotericin was ineffective and that flucytosine was not being used in the absence of a partner drug, since monotherapy is likely to induce resistance (69). They found that the single-dose liposomal amphotericin regimen had a 10-week mortality rate of 24.8% compared to 28.7% in the control group, which was statistically non-inferior (Fig. 4). The single-dose amphotericin group had a lower rate of grade 3 or 4 adverse events compared to the control group.

Following the evidence to support single-dose liposomal amphotericin, the WHO again updated the treatment guidelines in 2022 (10). The 2022 WHO guidelines recommended single-dose liposomal amphotericin B 10 mg/kg with flucytosine 100 mg/kg/day and fluconazole 1,200 mg/day for 14 days as the preferred first-line induction therapy where liposomal amphotericin is available (Fig. 5). US Health and Human Services Guidelines also recommend single-dose liposomal amphotericin as a co-equal first-line regimen along with traditional daily liposomal amphotericin with flucytosine for 14 days (15). However, despite these promising results, the Infectious Disease Society of America, the British HIV Association, and the European AIDS Clinical Society still recommend 14 days of liposomal amphotericin plus flucytosine, a regimen that has never been tested in rigorous clinical trials (15, 52, 70, 71). This regimen has been settled upon in high-income countries given the development of liposomal amphotericin, which is less toxic, based on the assumption that fungicidal activity will be equivalent between liposomal and deoxycholate amphotericin (69). However, in the only trial that compared liposomal and deoxycholate amphotericin, Hamill et al. compared liposomal versus deoxycholate amphotericin monotherapy and found that CSF sterility at 10 weeks was 60% in the commonly recommended dose of 3 mg/kg liposomal amphotericin compared to 79% in the deoxycholate amphotericin group (72). In addition, the AMBITION trial demonstrated a 77% culture sterility at 2 weeks (28, 69). Experts have suggested that the high initial dose of liposomal amphotericin may result in improved fungicidal effects compared to the daily dosing regimen (69). For these reasons, single-dose liposomal amphotericin is likely to be at least as fungicidal as the standard 14-day course with clear benefits in toxicity profile. Thus, we would recommend that single-dose liposomal amphotericin regimens should be preferred in low- and high-income settings when treating cryptococcal meningitis in people with HIV.

There have been numerous studies of alternative adjunctive therapies for cryptococcal meningitis, including interferon-gamma (IFN-γ), sertraline, tamoxifen, and dexamethasone, each found to have varying clinical utility. IFN-γ is an essential component of the host $CD4^+$ T-cell helper response to intracellular pathogens including *Cryptococcus* spp. IFN-γ secreted by type-1 helper T-cells directs the immune response to more

2022 WHO Antifungal Treatment Recommendations for Cryptococcal Meningitis

Medication	Admin	Week 1	Week 2	Week 3–10	Week 10+
If liposomal amphotericin B is available					
Liposomal amphotericin B (10 mg/kg)	IV	x 1 dose			
Flucytosine (100 mg/kg/day)	Oral				
Fluconazole (1200 mg/day)	Oral				
Without liposomal amphotericin B					
Amphotericin B deoxycholate (1 mg/kg/day)	IV				
Flucytosine (100 mg/kg/day)	Oral				
Fluconazole (1200mg/day)	Oral				
Consolidation therapy and secondary prophylaxis					
Fluconazole (800 mg/day)	Oral				
Fluconazole (200 mg/day)	Oral				

FIG 5 2022 WHO antifungal treatment recommendations for cryptococcal meningitis. Single-dose liposomal amphotericin is preferred when available; otherwise, amphotericin B deoxycholate for 7 days is an acceptable alternative. Therapeutic lumbar punctures and electrolyte supplementation are critical during the first 2-week period.

effectively target and clear *Cryptococcus*. Jarvis et al. evaluated the associations of cryptococcal-specific $CD4^+$ T-cell responses to various clinical outcomes and found that individuals with a predominantly IFN-γ response had a lower mortality rate compared to those with a more blunted response (73). In a small trial, they found that adding IFN-γ to the standard induction treatment regimen increased the rate of cryptococcal clearance from the CSF, but did not reduce the mortality rate – in a trial not powered for mortality (22). While these data are promising and merit further study, a better understanding of the host response during cryptococcal disease progression is needed to determine the appropriate targets and timing to intervene.

Sertraline, the most commonly prescribed antidepressant with good neurotropic effects, is also fungicidal against naturally occurring Cryptococcal isolates *in vitro* (67). In mouse models, sertraline alone or with fluconazole reduced the burden of *Cryptococcus* in the brain, kidney, and spleen (68). However, a phase 3 clinical trial in Uganda evaluating the use of adjunctive sertraline compared to the standard of care found no significant differences in the rate of fungal clearance or 18-week mortality between the two groups (25).

Tamoxifen, a selective estrogen receptor antagonist, commonly prescribed for breast cancer is a widely available, affordable medication that has also shown promise as a therapy for cryptococcal infection. Tamoxifen was found to have an *in vitro* median minimum inhibitory concentration (MIC) of 4 mg/L, which is comparable to flucytosine or fluconazole (68, 74, 75). However, in a small open-label phase 2 trial, the addition of tamoxifen to standard of care (amphotericin + fluconazole) did not improve the rate of fungal clearance as compared to standard of care alone (76).

Dexamethasone is a beneficial therapy in tuberculous and pneumococcal meningitis (77, 78). Given the complex interplay of inflammation and fungal burden in cryptococcal

meningitis, dexamethasone was thought to possibly be a beneficial adjunctive therapy. Beardsley et al. conducted a multisite randomized, placebo-controlled clinical trial evaluating the addition of dexamethasone for 6 weeks to the standard of care (79). The addition of dexamethasone to the standard of care for cryptococcal meningitis did not lead to a reduction in 10-week mortality (dexamethasone group 47% vs 41% placebo group; $P = 0.45$). In addition, the trial was stopped early for safety concerns as clinical adverse events were more common in the dexamethasone group (667 vs 494 total events), including an increased incidence of grade 3 or 4 infections, renal, and cardiac events (79). Furthermore, the EFA or rate of CSF fungal clearance was significantly lower in the dexamethasone group (0.21 vs 0.31 $\log_{10}$ CFU/mL/day). As we have discussed, this is associated with poorer mortality related to cryptococcosis (79). The findings from this trial emphasize the need to understand the host immune response during cryptococcal infection, such that an intervention is precisely timed on the damage-response framework (80, 81).

Consolidation antifungal therapy

The consolidation and maintenance phases of therapy are designed to continue clearance of *Cryptococcus* and prevent recurrence until there has been ART-related immune restoration. The consolidation phase of therapy typically starts 2 weeks after the start of induction therapy but can be altered depending on the individual's rate of fungal clearance during the induction phase. Despite appropriate induction therapy, a subset of individuals will not have achieved CSF sterility by 2 weeks (6). In a study in Uganda and South Africa, 43% of individuals were CSF culture positive after 2 weeks of induction therapy (82). However, it is important to note that the induction regimen evaluated in this study was deoxycholate amphotericin B (0.7–1 mg/kg/day) plus fluconazole (800 mg/day) for 14 days, which has been since been shown to be a sub-standard regimen. Given the high percentage of positive CSF cultures at 2 weeks, a higher dose of fluconazole (800 mg/day) was used as an enhanced consolidation regimen for 3 weeks or until CSF sterility was achieved (82). Thus, fluconazole 800 mg/day for the duration of the consolidation period has become the standard recommendation. While itraconazole can be used as an alternative in rare clinical scenarios, it is less efficacious compared to fluconazole due to its limited CNS penetration (83, 84). Voriconazole achieves good CNS levels, has excellent *in vitro* and *in vivo* activity against *Cryptococcus* and has been shown to have good clinical efficacy (85). However, these data primarily evaluated immunocompetent individuals and have limited generalizability for most people affected by *Cryptococcus*. There are no established breakpoints for fluconazole or other drugs against *Cryptococcus* and the clinical relevance of MIC is yet to be determined (86–88). Thus, MIC should not be used to routinely guide clinical decisions, except in cryptococcal relapse as discussed below.

Maintenance antifungal therapy

Individuals with advanced HIV disease are at risk of recurrent cryptococcal meningitis until immune function is restored. Following the completion of consolidation therapy, the WHO recommends fluconazole 200 mg/day (10). This should be continued for at least 1 year until the person is stable on ART, has a CD4 count >100 cells/μL and sustained HIV viral suppression or a CD4 count >200 cells/μL in case no HIV viral load monitoring is available. In the event that an individual has immunological failure underlined by interruption of ART or CD4 count falls below 100 cells/μL, fluconazole maintenance therapy should be resumed. These recommendations stem from a major study in 1991, which observed high rates of cryptococcal relapse when maintenance therapy was discontinued early (89). In a placebo-controlled double-blind study comparing fluconazole maintenance therapy to placebo, the placebo group had a 37% recurrence rate compared to 3% in the fluconazole group (89). The use of weekly amphotericin or daily itraconazole for maintenance therapy is not as efficacious as fluconazole in preventing cryptococcal meningitis relapse (90, 91).

Management of intracranial pressure

While pharmacological interventions are essential, equally important in the treatment of acute cryptococcal meningitis is the prompt management of elevated ICP. Approximately 50–75% of individuals present with elevated ICP, defined as CSF opening pressures >20 cmH_2O (5, 92, 93). The ICP should be measured on the initial diagnostic lumbar puncture and those with an initial pressure >20 cmH_2O should proceed with therapeutic CSF drainage on the same lumbar puncture. The goal is to reduce the ICP to <20 cmH_2O (10). Some guidelines recommend to only reduce by 50% on the initial lumbar puncture in cases of extreme elevation (10, 52); however, extensive clinical experience and several studies have demonstrated a survival benefit when reducing ICP to <20 cmH_2O (93, 94). Repeat lumbar puncture should be done daily for those with elevated ICP on opening pressure until it is normalized. Clinical symptoms of elevated ICP include headache, nausea, vomiting, confusion, papilledema, and visual acuity loss (95). Individuals with severely elevated opening pressure (>35 cmH_2O) have higher mortality even after receiving repeat lumbar punctures (93). Because of this, individuals with severely elevated ICP despite appropriate management after 1 week should be considered for ventricular or lumbar drain placement. The use of pharmacotherapeutics such as mannitol, acetazolamide, or corticosteroids to lower ICP is not recommended and may even cause harm (96, 97). The initial lumbar puncture is often done under emergent conditions and opening pressure is not measured. Lack of initial opening pressure is associated with fewer lumbar punctures during cryptococcal management and worse outcomes (11, 82, 94). Thus, we recommend a repeat lumbar puncture be done within 24 h to measure opening pressure if not measured on the initial lumbar puncture. In addition, individuals with normal ICP on the initial lumbar puncture generally get fewer lumbar punctures over the course of treatment. In a study in Uganda, 30-day mortality for persons with an initial opening pressure of <20 cmH_2O was similar to those with an opening pressure of >35 cmH_2O (93). Therefore, we also recommend that persons with normal initial opening pressure have a repeat lumbar puncture within the first week of therapy, regardless of symptoms as there is a survival benefit associated with therapeutic lumbar punctures (Fig. 6) (94). Among those with a baseline CSF opening pressure of <25 cm H_2O who received a second lumbar puncture, the ~10-day mortality was 0% (0/21) versus 16% (11/77) mortality for those who survived >24 h and did not receive a second lumbar puncture (94).

Management of amphotericin-related toxicities

In the past few years, studies discussed in this review by Molloy et al. and Jarvis et al. have greatly improved the induction therapy regimen to limit the side effects of amphotericin B (27, 28). Both the single-dose liposomal amphotericin B and the 7-day amphotericin B deoxycholate regimens are better tolerated than the 14-day amphotericin deoxycholate course. However, management of amphotericin B-related toxicities remains a crucial factor in the appropriate management of cryptococcal meningitis. In a regional referral hospital in Uganda, universal, standardized amphotericin management improved 14-day survival (98). Unfortunately, the cost of monitoring, which requires regular renal function tests and full blood counts, and the nursing staff for safe administration of amphotericin with pre- and post-load fluids can be difficult in resource-limited settings.

Toxicities related to amphotericin B include infusion-related reactions (local or systemic), renal impairment, anemia, and electrolyte abnormalities, especially hypokalemia and hypomagnesemia (98–100). The 2022 WHO treatment guidelines outline a detailed approach to laboratory monitoring, electrolyte and blood product repletion and management of infusion-related reactions (10). In general, peripheral IV lines should be routinely flushed and rotated to prevent phlebitis. Persons should be given pre- and post-infusion fluids, supplemented with potassium, if reasonable in the clinical situation. This is routine for single-dose or daily dose infusion of liposomal or deoxycholate

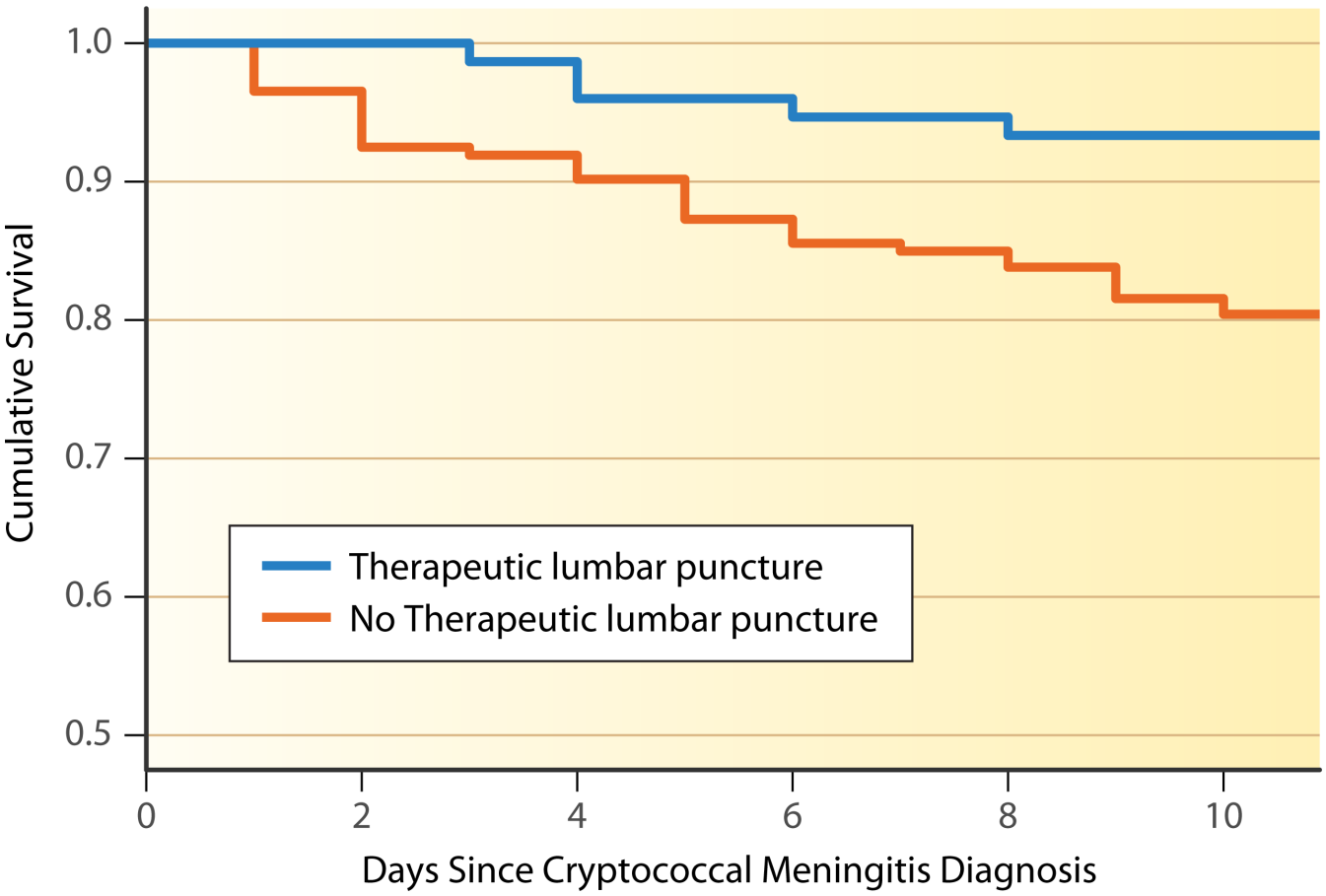

FIG 6 Cumulative survival in Ugandan and South African individuals with cryptococcal meningitis who did or did not receive a therapeutic lumbar puncture. The adjusted relative risk of mortality in those who received a therapeutic lumbar puncture was 0.31 (95% CI: 0.12–0.82), implying a 69% relative risk of mortality reduction with a lumbar puncture conducted in the first week (94). The median time to the second lumbar puncture was ~2 days (IQR: 1–4). (Based on data from reference 87.)

formulations of amphotericin B. Individuals receiving single-dose liposomal amphotericin B should receive supplementation of potassium chloride and magnesium or close monitoring for the first 3 days (10, 28). For those receiving 7 days of amphotericin, potassium and magnesium should be supplemented or closely monitored daily, while those receiving 14 days of amphotericin may need supplementation for up to 3 days after treatment completion (10). Electrolyte, renal function, and hemoglobin monitoring are essential throughout treatment in order to tailor supplementation to the individual needs of each person. Close monitoring for infusion-related reactions, phlebitis and secondary skin infections are also crucial to ensure rapid intervention.

Acute kidney injury and anemia are more common with cumulative doses of amphotericin B (27, 100). In the event of renal injury, doses of amphotericin B may be held or adjusted and additional fluids can be delivered (10, 98). Red blood cell transfusions are critical and potentially lifesaving for people with significant anemia and should be given to all those with a hemoglobin <7 g/dL or with symptomatic anemia. If red blood cell transfusions are not available, or significant renal toxicity develops the benefits of continued amphotericin beyond 7 days do not outweigh the risks of worsening toxicity (27, 101).

Management of other treatment-related toxicities

While the management of amphotericin B toxicities is the most intensive and difficult, especially in resource-limited settings, other treatment-related toxicities are also important to keep in mind. Flucytosine can cause significant bone marrow suppression including anemia and leukopenia as well as hepatic impairment (102, 103). Laboratory monitoring can follow the same approach as that for amphotericin described above with the addition of liver function monitoring. People with underlying hematologic disorders or who have received radiation or myelosuppressive medications are at higher risk of bone marrow suppression, and should be monitored more closely (102). In the event significant and sustained leukopenia occurs, decreased doses or switching to fluconazole should be considered; however, there is limited evidence to guide these decisions.

Therapeutic drug monitoring of flucytosine can be useful in managing and preventing toxicities, when available (104). Higher peak concentrations of flucytosine have been linked to worsened myelotoxicity and hepatotoxicity (104, 105). In one clinical trial, serum flucytosine levels > 100 µg/mL were significantly associated with higher rates of toxicity (105). Monitoring can also be useful in trying to minimize the development of resistance, which has been shown to develop in *Candida* when exposed to lower concentrations lower concentrations of flucytosine (106). Thus therapeutic monitoring to ensure peak levels <100 µg/mL and troughs >20–40 µg/mL can be considered, when available (104). It is also essential to adjust flucytosine dosing levels based on creatinine clearance (CrCl), especially if therapeutic monitoring is unavailable. In general, no adjustment is needed for CrCl >40 mL/min, but the dose should be reduced by 50% for every 50% reduction in CrCl <40 mL/min (15, 52).

Fluconazole and voriconazole may be used as alternative agents during induction therapy or during consolidation and maintenance phases. Fluconazole, even at high doses used during induction therapy, is relatively safe compared to the other therapies discussed. However, hepatotoxicity and increased serum transaminases can rarely occur and should be routinely monitored (107). Hepatotoxicity is a greater concern in voriconazole as the frequency of occurrence is more common (107). Fluconazole and voriconazole are both associated with prolonged QT and cardiac arrhythmias (108). Voriconazole is associated with visual disturbances and hallucinations (109, 110). Dose reduction or switching to an alternative agent is usually effective in resolving these adverse events (108).

Identifying novel antifungal therapeutics

While the addition of single-dose liposomal amphotericin to the regimen for induction therapy of cryptococcal meningitis is the first major innovation in many years, there are multiple clinical trials investigating novel approaches to cryptococcal meningitis management. There is an ongoing trial using preemptive treatment with single-dose liposomal amphotericin B of CrAg-positive persons, which aims to improve the efficacy of screening and treatment of persons with asymptomatic cryptococcal meningitis. A second trial is investigating adjunctive flucytosine added to fluconazole for asymptomatic CrAg-positive persons.

Novel therapeutics include an orally bioavailable formulation of amphotericin B (111). This innovative formulation uses a lipid nanocrystal (LNC) to encase the amphotericin B to be orally bioavailable and delivered to monocytes and macrophages (111, 112). In mouse models, the experimental oral amphotericin with flucytosine was found to be equally efficacious compared to the intravenous formulation with flucytosine (112). LNC-amphotericin B has the potential to be used in lieu of intravenous amphotericin for induction therapy, with the goal of maintaining efficacy and improving the safety profile even further (113). The phase 1 trial for oral amphotericin B showed a promising safety profile (111). When given in four to six divided doses up to 2.0 g/day, 95% of participants completed all doses without grade 3 or worse adverse events (111). In a qualitative survey of participants, 96% preferred their experience with oral amphotericin

compared to previous IV amphotericin (111). The phase 2 trial was recently completed and enrolled a total of 80 participants to receive LNC-amphotericin B (40 received two loading doses of IV amphotericin B and 40 received all oral LNC-amphotericin B) and 41 control participants received IV amphotericin B and flucytosine (114). They found a similar EFA amongst all groups with 0.41 $\log_{10}$ *Cryptococcus* CFU/mL/day for the all-oral LNC-amphotericin B group, 0.42 $\log_{10}$ *Cryptococcus* CFU/mL/day for the oral LNC-amphotericin B with IV loading dose, and 0.46 $\log_{10}$ *Cryptococcus* CFU/mL/day for the control group (114). The 18-week survival was 85% for the all-oral LNC-amphotericin B group, 90% for the oral amphotericin B with IV loading dose and 85% for the control group (114). Grades 3–4 adverse events occurred in 41% of participants for the oral LNC-amphotericin B groups and 61% in the control group ($P = 0.05$) (114). This trial provides promising evidence for this novel oral amphotericin B and phase 3 trials are being planned.

Multiple promising potential compounds are in pre-clinical development as of 2023 but have yet to enter human phase 1 trials (115). Some of these include the repurposing of old drugs, such as the antihelminthic benzimidazoles or the antibacterial macrolide antibiotics. The benzimidazole, fenbendazole has *in vitro* activity against *C. neoformans* and *C. gattii* and similar findings were shown in a mouse model. This drug was found to reduce growth, macrophage inhibition, and reduced animal mortality related to cryptococcosis similar to amphotericin B (116). Macrolides were shown to reduce capsule formation of *C. gattii in vitro* which may promote the innate immune response (117). Clofazamine, a broad-spectrum antifungal and antimycobacterial agent, has also been shown to work synergistically with other antifungals by inducing cell membrane stress (115). Even more novel approaches have identified new compounds and monoclonal antibodies to target various essential components of the *Cryptococcus* cell wall or metabolism. The monoclonal antibody 18B7 has been identified in mouse models and binds to a cryptococcal polysaccharide that enhances antifungal immune system activity by activating the complement pathway (118). A novel agent called APX001 is an *N*-phosphonoxymethyl prodrug that is completely metabolized to an active agent by systemic alkaline phosphatases. APX001 targets a fungal enzyme that is essential in the localization of cell wall mannoproteins and thus fungal cell wall integrity (119). It has *in vitro* and *in vivo* activity that reduces the cryptococcal fungal burden comparable to currently used therapies (119). Finally, lenalidomide is a TNF-α inhibitor that is already in use as an anti-neoplastic agent and is being considered as an immunomodulatory agent that may improve outcomes in cryptococcal meningitis (120). A recent small interventional study, conducted on 14 people with HIV and cryptococcal meningitis, was given lenalidomide after successful induction therapy for cryptococcal meningitis. Eleven of the 14 participants completed follow-up, of whom all had sustained clinical remission and rapid resolution of symptoms by week 4 of follow-up. However, there was no control group or comparison making it difficult to determine whether lenalidomide was essential in the outcomes of these participants. While these compounds demonstrate varying degrees of promise, all are yet to enter into human clinical trials and would not be recommended for therapeutic use at this time.

TIMING OF ANTIRETROVIRAL THERAPY INITIATION

In general, immune restoration with early ART initiation has been the preferred strategy in the management of HIV/AIDS-related opportunistic infections. This has been shown in large clinical trials to improve mortality (121). However, in cryptococcal meningitis, the general approach to timing of ART initiation is not nearly as clear. Determining the optimal time for ART initiation involves balancing the survival benefit of reconstituting the immune system against the risk of paradoxical cryptococcal IRIS. Paradoxical cryptococcal IRIS is defined as the worsening or recurrence of cryptococcal symptoms in the same or new anatomic sites after successful cryptococcal therapy and ART initiation (6, 122, 123). This occurs in up to one third of persons treated for cryptococcal meningitis and has a mortality rate of up to 36% (122, 124).

In two small clinical trials, early ART initiation was demonstrated to result in an increased incidence of paradoxical cryptococcal IRIS and an overall increase in mortality (125, 126). Boulware et al. completed a landmark Cryptococcal Optimal ART Timing (COAT) trial in Uganda and South Africa to better understand how best to optimize the timing of ART initiation while balancing these risks (65). The trial randomized individuals with cryptococcal meningitis to initiate ART 1–2 weeks versus 4–6 weeks after starting antifungal therapy. They found that those with a delayed ART initiation had a ~15% lower 30-day, 6-month, and 1-year mortality compared to the earlier ART initiation group (65). In this trial, no persons were lost to follow-up through 1 year. In 2023, publication of a smaller case-control study from pooled cohorts in high-income countries demonstrated the absence of an increased mortality risk when starting ART immediately (127). In this case-control study, 69% of participants with cryptococcal meningitis were either lost within 30 days or excluded from analysis, which fundamentally casts doubts on the validity of the analysis (128). International guidelines and cryptococcal experts continue to recommend ART initiation at approximately 4–6 weeks after initial antifungal treatment.

SPECIAL CIRCUMSTANCES

Cryptococcus gattii infection

Cryptococcus gattii is a separate species from the more commonly recognized *C. neoformans* infection that we have been discussing thus far. It causes a similar clinical syndrome to *C. neoformans* with meningitis being the predominant and most severe form of the disease, especially in those who are infected with HIV (129). Mortality rates in people infected with HIV are comparable to cases of *C. neoformans* infections and have been recorded up to 36% in a South African hospital and no different than meningitis due to C. *neoformans* in a hospital in Botswana (129, 130). However, *C. gattii* infections occur more commonly in immunocompetent people (131, 132). While *C. gattii* has been noted to be endemic in tropical and subtropical regions of the world, recent decades have seen its emergence in British Columbia, Canada, and the Pacific Northwest region of the United States (133–136).

Treatment of *C. gatii* infections is generally extrapolated from data on *C. neoformans* and expert opinion as there are no robust clinical trials evaluating the most appropriate treatment regimen. For treatment of meningitis due to *C. gattii*, the IDSA guidelines recommend following the same induction, consolidative, and suppressive regimens as that used for *C. neoformans* (52). Cryptococcomas and hydrocephalus, which are more common in *C. gattii* should be monitored more closely with diagnostic imaging and follow-up (52). Others have suggested a more prolonged course of induction therapy of up to 6 weeks depending on the individual clinical circumstances (137). In a cohort of Australian patients, 88% received amphotericin B and 78% received flucytosine for a median of 6 weeks (137). This group had a similar mortality but improved clinical response at 12-month follow-up compared to those who received alternative regimens (137). Serial lumbar punctures to manage elevated intracranial pressures remain a cornerstone of treatment for *C. gattii* neurologic infections (138).

Cryptococcal meningitis during pregnancy

High-dose fluconazole and flucytosine are both potentially teratogenic compounds, making the therapy of cryptococcal meningitis particularly challenging during pregnancy. In a 2011 safety announcement, the FDA classified fluconazole at doses over 400 mg/day as category D, which indicates positive evidence of human fetal risk (139). Flucytosine is considered category C, which indicates evidence exists in animal studies of teratogenicity, but there is insufficient human data to determine the risk (140). Amphotericin B, on the other hand, is considered category B, which indicates that no risk of teratogenicity has been observed in animal or human data (140). In a large Danish cohort involving more than 8,000 pregnancies exposed to fluconazole and over 968,000

non-exposed pregnancies, doses of fluconazole ranging from 150 to 300 mg were generally safe in all stages of pregnancy (141). There was three times increased odds of tetralogy of Fallot; however, this still only occurred in 0.1% of exposed pregnancies (141). The risks and benefits of antifungal therapy and under-treated cryptococcosis need to be weighed on an individual person level.

With any critical illness, maternal mortality and fetal demise are unfortunately common, thus low absolute risks of teratogenicity need to be put in the context of a highly lethal infection. Pastick et al. described the largest case series to date of 12 cases of cryptococcosis during pregnancy or the postpartum period (142). The maternal meningitis survival rate at hospital discharge was 75% (9/12) with amphotericin B induction monotherapy, and neonatal/fetal survival rate was 44% (4/9) among the mothers who survived (142). In general, induction therapy with amphotericin and flucytosine should be completed regardless of the trimester. Consolidation therapy during the first trimester can be continued with weekly amphotericin B. In the second or third trimesters of pregnancy, the benefit of fluconazole used as consolidation therapy at 400 mg/day doses generally outweigh the risks (142). Given the risk of recurrent cryptococcal meningitis in those not treated with fluconazole, the benefit of fluconazole doses of 200 mg/day for maintenance therapy likely outweighs the risks in all stages of pregnancy. For pregnant women detected with subclinical cryptococcal antigenemia, we recommend a customized approach. Those with low plasma CrAg LFA titers of ≤1:80, we recommend fluconazole 200 mg/day for 4 weeks with immediate ART-initiation. For those with moderate titers of 1:160 to 1:320, we recommend a 10-week fluconazole duration. For those with plasma CrAg LFA titers ≥1:640, we recommend excluding meningitis and giving liposomal amphotericin 10 mg/kg once with fluconazole 200 mg/day for 10 weeks. This strategy is currently being validated in clinical trials.

Cryptococcal meningitis relapse

Individuals who have symptoms of cryptococcal meningitis after appropriate management and initial improvement must be distinguished from five clinical entities: (i) persistent cryptococcal meningitis inadequately initially treated; (ii) culture-positive microbiological relapse; (iii) paradoxical IRIS; (iv) complications of persistent elevated intracranial pressure in the absence of inflammation or relapse; and (v) different CNS infection. Persistent cryptococcal meningitis applies to those whose CSF culture remains positive during the course of treatment while relapse occurs in those who had sterile CSF and subsequent culture-positive CSF. Paradoxical IRIS occurs in those who have a recurrence of cryptococcal symptoms, typically accompanied with increased CSF pleocytosis without positive CSF culture. Persistent infection is generally defined as positive CSF culture beyond 4 weeks of appropriate therapy and is managed similarly to relapse infection (52).

Early studies of cryptococcal meningitis relapse in New York demonstrated that the strain of *Cryptococcus* responsible for disease relapse was the same strain during primary cryptococcal infection rather than a reinfection with a new strain (143). The serial isolates were not found to have increased antimicrobial resistance (144); however, resistance is possible. Similar findings were demonstrated in Uganda, using the same genetic typing techniques, individuals with serial positive CSF cryptococcal cultures had identical sequential isolates and low rates of fluconazole resistance (137, 145). Still, if fluconazole monotherapy is used for the induction regimen, fluconazole resistance has been shown to be as high as 76% in one study that defined resistance as fluconazole MIC ≥64 μg/mL (146). Relapse isolates should have fluconazole susceptibility profiles checked. Treatment-emergent resistance to amphotericin B is rare.

Individuals presenting with symptoms of cryptococcal meningitis relapse or persistent infection beyond 4 weeks of therapy should first be evaluated for appropriate therapeutic regimen and adherence to fluconazole during the consolidation and maintenance phases (10). Lumbar puncture should be performed to assess for elevated opening pressure, obtain cryptococcal cultures (which should be incubated for at least

2 weeks), and to rule out concomitant infections. In those with positive cryptococcal cultures, induction therapy should be re-initiated. The consolidation and maintenance phases should use a higher dose of fluconazole (e.g., 1,200 mg/day), if tolerated. If antifungal susceptibility testing is available, fluconazole resistance testing should be done. Itraconazole, voriconazole, or weekly amphotericin B can be considered for salvage therapy for consolidation and maintenance phases in those with fluconazole resistance.

This testing can be time and resource-intensive as the Clinical & Laboratory Standards Institute (CLSI) recommends broth microdilution to determine the MIC (68). MIC testing for fungal infections has been shown to poorly correlate with clinical outcomes, following what is known as the "90–60 rule" (147). This rule states that infections due to susceptible isolates will respond to therapy ~90% of the time, while those due to resistant isolates will respond to therapy ~60% of the time (147). In addition, there are no established breakpoints by the CLSI or European Committee on Antimicrobial Susceptibility Testing (EUCAST). Neither CLSI nor EUCAST has established breakpoints for antifungals specifically in *Cryptococcus* (87, 88). These factors make interpreting MIC data in *Cryptococcus* very difficult. However, epidemiologic cutoff (ECOFF) values are often used to infer resistance to antifungals for *Cryptococcus* (148). ECOFF is determined by describing the typical MIC distribution for a population and identifying the value at which resistance mutations are likely to exist (148). ECOFFs do not predict clinical success but can be used to determine if specific isolates are likely to carry resistance. Given these complexities, we do not recommend routine antifungal susceptibility testing to guide therapy with the exception of relapse cases when fluconazole resistance may be playing an important role and alternative azole options are feasible.

Paradoxical immune reconstitution inflammatory syndrome

Those with recurrence of cryptococcal symptoms despite appropriate therapy and initiation of ART, but negative CSF cultures are considered to have paradoxical IRIS. While there is a paucity of data to guide recommendations, management of elevated intracranial pressure is a critical aspect of cryptococcal IRIS (122, 123). Mild cases of IRIS, especially those with normal intracranial pressure usually improve without any specific interventions (52). The 2010 IDSA guidelines recommend those with severe IRIS including elevated ICP or neurological deterioration receive corticosteroids with 1 mg/kg/day of prednisone equivalent (52). Duration is dependent on clinical response, but generally the corticosteroids should be tapered over a 2-to-6-week period. Thalidomide, which is hypothesized to reduce TNF-alpha, was shown in a small case series to result in rapid clinical improvement in three cases of refractory, steroid-dependent cryptococcal IRIS (149). Anti-TNF monoclonal antibodies have also been used for refractory cases.

Unmasking disease

Unmasking cryptococcal disease is the phenomenon where asymptomatic or subclinical disease becomes manifest after the reconstitution of the immune system with ART (150). Unmasked cryptococcal meningitis is common in sub-Saharan Africa and is associated with higher mortality (151). In a large prospective cohort in Uganda, 46% of all persons presenting with cryptococcal meningitis were receiving ART at presentation with a median of 17 weeks since initiation (151). While overall mortality did not differ between ART-naive and ART-experienced groups, those who initiated ART within 14 days had a much higher 2-week mortality of 47% compared to 23% for those who initiated ART more than 14 days prior to diagnosis. Improving these poor outcomes is a primary goal of "test and treat" strategies discussed above.

Cerebral cryptococcoma

Cerebral cryptococcoma is a rare phenomenon of cryptococcosis that is characterized by localization of *Cryptococcus* in the brain parenchyma. Cryptococcomas may appear

as a mass or ring-enhancing lesion on neuroimaging and can be confused with other pathogens such as toxoplasmosis or CNS tumor (52). Cryptococcomas are exceedingly rare in people living with HIV who are not receiving effective ART. However, cryptococcomas may present as an "unmasking" manifestation in persons with previously unrecognized disseminated cryptococcal infection who are started on ART without first receiving antifungal therapy. Failure to respond to antifungal therapy should prompt a biopsy of the lesion to rule out other causes. The therapeutic regimen consists of an extended induction regimen with amphotericin B and flucytosine for six weeks, followed by fluconazole for a consolidation and maintenance period of up to 18 months (52). Individuals with extensive surrounding edema should receive adjunctive corticosteroids. Large (≥3 cm), accessible lesions that cause mass effect should be referred for neurological intervention (52).

Symptomatic neurologic cryptococcal antigenemia

A subset of individuals with HIV-associated cryptococcal disease have cryptococcal antigenemia with meningitis symptoms, but CSF CrAg is negative (152). This presentation is on the continuum of cryptococcal disease progression from asymptomatic cryptococcal antigenemia to overt cryptococcal meningoencephalitis. Without evidence of CNS disease, management of these individuals with fluconazole monotherapy resulted in 32% mortality (152). Many individuals with CrAg antigenemia have hyponatremia, which is an independent risk factor for progression to meningitis and mortality (153). Combination antifungal therapy with single-dose liposomal amphotericin, followed by fluconazole and flucytosine is currently being investigated as a treatment option for individuals with cryptococcal antigenemia with or without symptoms of meningitis. Individuals with neurologic symptomatic cryptococcal antigenemia are likely to have early cryptococcal meningoencephalitis given that metagenomic next-generation sequencing detected *Cryptococcus* in some of these individuals (154).

CONCLUSION

Cryptococcal meningitis continues to be a leading cause of meningitis globally. It is also a significant cause of mortality in people with HIV and advanced HIV disease with a disproportionate burden of disease following on those in resource-limited countries. Prompt diagnosis and therapeutic intervention are essential to reducing the mortality associated with cryptococcosis. In addition, campaigns to screen at-risk populations with cryptococcal antigen testing can prevent progression to severe CNS disease and reduce mortality on a population scale.

Recent large, high-quality clinical trials have led to marked changes in our understanding of the optimal induction therapy. A shorter, 7-day course of deoxycholate amphotericin B is as effective as a 14-day course with improved safety outcomes; and a single high-dose of liposomal amphotericin B in combination with flucytosine and fluconazole is as effective as the 7-day course of deoxycholate amphotericin B and flucytosine. Flucytosine is a critical adjunctive therapy that has a survival benefit. Therapeutic lumbar punctures are an essential aspect of management and improve survival when implemented appropriately. We recommend therapeutic lumbar punctures on all persons with cryptococcal meningitis at days 3 and 7 of therapy, regardless of baseline opening pressure (93). Given the severe nature of IRIS in the central nervous system, delayed initiation of ART remains preferred, generally 4–6 weeks after antifungal therapy has been initiated. Relapse of cryptococcal meningitis or paradoxical IRIS cannot be clinically distinguished and requires lumbar punctures with CSF culture, with distinct approaches to management.

While there now exists a rich body of evidence to guide diagnostic and therapeutic decisions in managing cryptococcal meningitis, significant gaps in knowledge still exist. The best approach to screening is still under investigation as CrAg testing has greatly improved early diagnosis of cryptococcosis. However, all the regimens studied thus far have not shown a significant benefit in reducing cryptococcal-related mortality. A

clinical trial evaluating single-dose amphotericin followed by fluconazole is underway in Uganda (Clinicaltrials.gov: NCT03945448). The optimal approach to therapeutic lumbar punctures is still not clear. While we present compelling evidence for multiple lumbar punctures to be performed, regardless of initial opening pressure, we are unable to suggest the best approach beyond this general recommendation. The AMBITION trial, which demonstrated the benefit of single-dose amphotericin B, has already revolutionized care of cryptococcosis in resource-limited settings. However, its use in high-income settings has been resisted. We discuss compelling reasons that this regimen is likely to be generalizable to these settings, as outlined by Harrison et al. (69). Further studies of this regimen in these settings are likely to improve its acceptability by practitioners. Finally, the new frontier of cryptococcal management is likely to be better understanding of the inflammatory response that is responsible for a significant amount of disease. Further immunological studies to describe the pathophysiology will improve our ability to identify new therapeutics.

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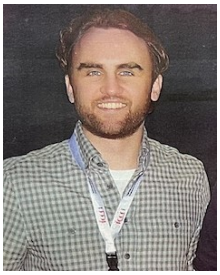

Thomas McHale is an infectious disease doctor in his final year of fellowship training at the University of Minnesota. He completed internal medicine training at Montefiore Medical Center in the Bronx, New York during the COVID-19 pandemic. He has an interest in improving access to care for underserved populations and better understanding neglected diseases with a particular interest in spatial epidemiology, public health, and fungal infections. Since 2021 he has worked with a clinical trials team focusing on cryptococcal meningitis in Uganda and completed several projects to better understand the resistance profile and pharmacodynamics of flucytosine.

David Boulware is an infectious disease physician-scientist and Professor of Medicine at the University of Minnesota. While he may best be known for the pioneering of remote, internet-based COVID-19 clinical trials on hydroxychloroquine testing post-exposure prophylaxis, early treatment, and pre-exposure prophylaxis, the actual focus of his research is meningitis in resource-limited areas including diagnosis, prevention, treatment, and quality improvement initiatives incorporating cost-effectiveness analyses in order to translate knowledge into improved care to impact guidelines. His collaborative team has particular interest in TB meningitis and in cryptococcal meningitis as Cryptococcus is the most common cause of adult meningitis in Sub-Saharan Africa causing 15-20% of AIDS-related mortality globally.

John Kasibante is a research medical doctor and clinical immunologist at the Infectious Diseases Institute, Makerere University. He has four years of research experience in HIV-associated meningitis clinical trials in Uganda. Among the groundbreaking clinical trials he has worked on is the AMBITION-cm clinical trial. This trial provided evidence of the efficacy of single-dose amphotericin B for the treatment of cryptococcal meningitis which has since been adopted for the updated 2022 World Health Organisation cryptococcal meningitis treatment guidelines. He has also worked on EnACT clinical trial, the first oral amphotericin B clinical trial for the treatment of cryptococcal meningitis in sub-Saharan Africa. He has extensive research experience in managing tuberculous meningitis and has worked on two tuberculous meningitis clinical trials. Dr Kasibante conducts translational laboratory research describing cellular and cytokine immunophenotypes and antibody repertoires in HIV-associated meningitis and how these parameters are applicable for diagnosis, prognosis, and immunotherapy targeting.

Kenneth Ssebambulidde serves as a medical officer at the Infectious Diseases Institute, Makerere University, and holds a visiting Postdoctoral fellowship in the Laboratory of Clinical Immunology and Microbiology of the National Institute Allergy and Infectious Diseases at the U.S. National Institutes of Health. He is a valued member of a collaborative team dedicated to conducting clinical trials focused on HIV-associated cryptococcal, and tuberculosis meningitis based in Uganda. These clinical trials have yielded crucial insights into the prevention, screening, and management of advanced HIV disease-associated meningitis. His special interests are immunological mechanisms underlying diseases particularly the intricate connections between communicable and non-communicable central nervous system disorders.

Caleb Skipper is an infectious diseases doctor with an assistant professor position at the University of Minnesota, focusing on an academic clinical research tract. He is interested in improving healthcare in resource limited settings through impactful clinical research focused on infectious diseases, particularly those related to HIV/AIDS. He is currently working toward developing a diverse skillset that will build the foundation needed to be a successful independent researcher in global health. His specific focuses are on the impact of viral co-infections on persons with advanced HIV and concomitant opportunistic infections, and designing clinical trials that are feasible and relevant in sub-Saharan Africa.

Mahsa Abassi is an infectious disease doctor and assistant professor at the University of Minnesota. Her research focuses on HIV-associated cryptococcal meningitis with a particular interest in understanding the pathophysiology of neurological impairment. In 2018, she won the Peter G. Pappas Young Investigators Award. She has extensive experience working on clinical trials in Uganda and her future work will look at targeting neuropathogenesis of altered mental status to improve survival in cryptococcal meningitis.