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# Recent advances in AIDS-related cryptococcal meningitis treatment with an emphasis on resource limited settings

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**Abstract:**

**Introduction.** Recent advances in the treatment and prevention of cryptococcal meningitis have the potential to decrease AIDS-related deaths.

**Areas covered.** Targeted screening for asymptomatic cryptococcal antigenemia in persons with AIDS is a cost effective method for reducing early mortality in patients on antiretroviral therapy. For persons with symptomatic cryptococcal meningitis, optimal initial management with amphotericin and flucytosine improves survival compared to alternative therapies; however, amphotericin is difficult to administer and flucytosine has not been available in middle or low income countries, where cryptococcal meningitis is most prevalent.

**Expert Commentary.** Improved care for cryptococcal meningitis patients in resource-limited settings is possible, and new treatment possibilities are emerging.

**Keywords:** HIV; AIDS; Cryptococcal meningitis; CM-IRIS; Immune reconstitution inflammatory syndrome; Review; Antiretroviral therapy; Antifungal therapy; Sertraline

## 1. Introduction:

Cryptococcal meningitis causes 15-20% of AIDS-related mortality and is the most common cause of meningitis in Africa (1, 2). The vast majority of AIDS related cryptococcal meningitis is *Cryptococcus neoformans* although *Cryptococcus gattii* also can cause meningitis in people with HIV and distributions of subspecies vary by geographic location.(3) In Africa 94% of those reported from molecularly differentiated isolates were *C. neoformans*.(3) Presentation and treatment is the same for both Cryptococcus although characteristics like the minimum inhibitory concentration of amphotericin and Flucytosine differs.(4) In-hospital acute mortality from cryptococcal meningitis continues to remain high, ranging between 30-50%, even with antifungal therapy (5). Despite declines in long-term mortality overall from the introduction of antiretroviral therapy (ART) (6), in low-income countries ART distribution has not yet effectively reached all individuals needed to decrease the overall incidence of cryptococcal meningitis (7). In addition to high mortality, cryptococcal meningitis has substantial morbidity. Survivors can suffer from irreversible blindness and deafness, as well as neurocognitive impairments(8).

Fortunately, several recent innovations in the screening and treatment of both asymptomatic, high risk patients as well as those with meningitis are showing promise for improved care. This review will focus on these innovations, providing an update in the field of cryptococcal screening and management.

## 2. Screening Asymptomatic Patients at Risk for Meningitis

A growing body of evidence is showing that blood Cryptococcal antigen (CrAg) screening and treatment with fluconazole is an effective way of preventing meningitis (9-13) for patients with advanced HIV and positive cryptococcal antigenemia. The CrAg lateral flow assay (LFA) is the most sensitive and specific method for detecting early disseminated infection (14, 15), and is the preferred first-line recommended test for diagnosis (16, 17). Persons presenting with a CD4 count <100 cells/μl have the highest benefit from screening and treatment (18). These patients should be evaluated for meningitis, and if symptomatic receive a lumbar puncture. Those without meningitis are recommended to receive fluconazole (18, 19). The dose of fluconazole is not well established; however, 800 mg daily for two weeks followed by 400 mg daily for 10 weeks, with secondary prophylaxis at 200 mg daily for at least 6 months has been recommended (19, 20). Antiretroviral therapy initiation is recommended after at least 2 weeks of fluconazole preemptive therapy (18). Patients with higher antigenemia above CrAg LFA titers of ≥1:160 have progressively higher mortality (21). As such, there is need for research into more intensive treatment for individuals with high CrAg titers (≥1:140) including amphotericin or other adjuvant treatment. The general principle of cryptococcal screening is graphically displayed in **Figure 1**.

## 3. Management of Cryptococcal Meningitis:

The goal of meningitis therapy is rapid yeast clearance from the cerebrospinal fluid (CSF). Quantitative clearance from CSF, termed early fungicidal activity (EFA), is the rate of yeast clearance per mL of CSF per day. Slower rates of fungal clearance have been shown to be associated with increased mortality at both 2 and 10 weeks (22).

### *3.1 Induction Antifungal Therapy:*

Current guidelines recommend 2 weeks of amphotericin B deoxycholate (0.7–1.0 mg/kg per day) given intravenously in combination with flucytosine 100 mg/kg/day as first line therapy for treatment of cryptococcal meningitis (23). Lipid formulations of amphotericin B in either liposomal amphotericin (3-4 mg/kg/day) or amphotericin lipid complex (5 mg/kg/day) may be substituted. Liposomal amphotericin therapy has less toxicity but no increased efficacy (24). Combination therapy with amphotericin and flucytosine has been shown to be associated with a ~40% lower hazard of mortality at 10 weeks compared to amphotericin monotherapy (25). This effect persisted at 6 months and was associated with increased rates of fungal clearance as compared to four weeks of amphotericin monotherapy.

### *3.2 Middle Income and Low Income Countries*

Despite the superiority of combination therapy with amphotericin and flucytosine over alternative regimens, this regimen remains widely unavailable in most parts of the world with the highest burdens of disease. With only one U.S. manufacturer in 2014, the cost of flucytosine is ~$2000/day for a 70kg adult (26). This compares with a total treatment cost of $402 for two weeks of amphotericin with fluconazole (27). European generic flucytosine manufacturer Meda

Pharmaceuticals is to be acquired by Mylan Pharmaceuticals in 2016 (28), which may expand market access.

Amphotericin deoxycholate has been the only option in middle and low income countries. However lipid formulations are off-patent in 2016 (24). As such, lipid formulations may become more widely available and would be a significant advance provided that appropriate studies are performed to ensure bioequivalence with parent products, particularly important when carrier systems such as liposomes are used to reduce toxicity (29). Another strategy that could prove beneficial and would be applicable to resource-limited setting would be the use of short-course, high dose liposomal amphotericin B. A trial comparing alternative short course regimens of liposomal amphotericin B is currently underway at multiple sites in Africa (30).

When flucytosine is unavailable, the combination of amphotericin with fluconazole is recommended (23). Pappas *et al.* demonstrated in an open label, three-arm, phase II trial with 143 patients that combination amphotericin with fluconazole 800 mg/day had numerically better long-term outcomes than amphotericin and fluconazole 400 mg/day or amphotericin alone (31). Day *et al.*, in a three group, open label, randomized trial with 299 patients did not find a statistically different survival benefit between amphotericin with fluconazole 800 mg/day and amphotericin alone, although fewer patients died in the combination arm (25). Loyse *et al.*. in a two week pharmacologic study did not find a statically significant difference in EFA of amphotericin with fluconazole at 800-1,200 mg/day (32). Therefore, current guideline favors the use of amphotericin in combination with fluconazole ≥800 mg/day when flucytosine is not available (23). Recommended treatment plan of cryptococcal meningitis in resource limited seen in **Table 1**.

Amphotericin is known to cause significant side effects including anemia, kidney insufficiency, hypokalemia, hypomagnesemia, and phlebitis. The administration of amphotericin requires inpatient hospitalization, intravenous administration, and a substantial nursing commitment. Therefore, administration of amphotericin over 14 days is not only costly but is also resource consuming. In a small study in Uganda, a short 5-day course of amphotericin with high dose fluconazole 1,200 mg/day had a superior EFA than either fluconazole at 800 mg/day or 1,200 mg/day alone (33). When flucytosine was added to a short, 7-day, course of amphotericin plus high dose fluconazole 1,200 mg/day, a greater EFA was observed than with amphotericin and fluconazole or fluconazole and flucytosine combinations (34). Short course (5-7 days) amphotericin with combined high dose fluconazole 1,200 mg/day is an alternative therapeutic option when 14 days of amphotericin is not feasible. Substantial life-threatening hypokalemia occurs during the second week, if not properly managed (35).

The use of voriconazole as a substitute for fluconazole in induction therapy has been studied and found to have similar EFA to amphotericin and fluconazole at both 800 mg/day and 1,200 mg/day doses (32). Although no benefit of voriconazole over fluconazole has been demonstrated for fluconazole-susceptible strains, a future role for newer antifungals for the treatment of cryptococcal meningitis might be anticipated, particularly in the context of increasing rates of fluconazole resistance, as costs come down, and as worldwide availability of these drugs increase (36, 37). Adjunctive Interferon-gamma (INF-γ) has also been shown to be an effective component of combination induction therapy. Jarvis *et al.* demonstrated a 30% increased rate of clearance with 2 doses of adjunctive INF-γ than with standard therapy of amphotericin and flucytosine (38). This is consistent with the same group's finding that CSF immune profiles which include higher levels of INF-γ in the CSF is protective against

cryptococcal meningitis and increased mortality.(39)

IDSA and WHO guidelines continue to recommend high dose fluconazole monotherapy at 1,200 mg/day for 10-12 weeks if amphotericin and flucytosine are not available (23, 40, 41). While clearly suboptimal compared to combination amphotericin therapy, fluconazole remains the only therapeutic option for the treatment of cryptococcal meningitis in much of the world, where amphotericin or flucytosine are unavailable. A recent study in Malawi looking at sixty patients with cryptococcal meningitis demonstrated high mortality (43% at 4 weeks) and treatment failure (77% at 1 year) with the use of 800 mg/day of fluconazole monotherapy for induction therapy (42). Fluconazole doses of 1,200 mg/day for the first two weeks of induction therapy were associated with an increased rate of CSF yeast clearance as compared with 800 mg/day, although no differences in mortality were seen at either 2 or 10 weeks however given this was only powered to look at fungal clearance not mortality given a sample size of just thirty-four (43). Comparison of CSF fungal clearance rates of different induction therapies is displayed in **Table 2** and **Figure 2**.

*3.3 Consolidation and Maintenance Therapy:*

Consolidation phase of therapy currently consists of fluconazole 400 – 800 mg/day for at least 8 weeks (23). Guidelines generally recommend starting consolidation therapy after 2 weeks of induction therapy. However, when possible, consolidation therapy should be started based on the individualized response to induction therapy. In a study in Uganda, 56% of patients treated with amphotericin-based therapy had positive cultures at the end of 2 weeks (44). Because fluconazole at 400 mg/day is fungistatic (45), ideally one would want the CSF to be sterile before reverting to a fungistatic fluconazole dose. Thus, a lumbar puncture and culture should be

done at 2 weeks to demonstrate CSF sterility. However, culture incubation takes time. When CSF sterility has been documented (often after 10-14 days of further culture incubation), the fluconazole dose should then be decreased from 800 mg/day to 400 mg/day. Guidelines support the use of longer durations of high dose fluconazole throughout the consolidation phase if using suboptimal induction therapy; mainly monotherapy with fluconazole or when CSF sterility has not been achieved (23).

After successful induction and consolidation therapy, culture-negative patients should be placed on fluconazole 200 mg/day for maintenance therapy (23). Recommendations for the use of long-term fluconazole stems from observations made in the pre-ART era of high relapse rates when therapy was discontinued (46). Fluconazole 200 mg/day was found to be superior when compared to weekly intravenous administration of amphotericin in preventing cryptococcal meningitis relapse (47). Comparison of fluconazole to itraconazole reproduced similar results (48). Historically, this switch to secondary prophylaxis was made after 8 weeks of consolidation therapy (i.e. 10 weeks after diagnosis). Our own experience prefers longer consolidation therapy, switching to secondary prophylaxis after 2-3 months of ART, which allows for time for immune recovery to occur on ART.

Secondary prophylaxis can be safely discontinued in patients on ART and with undetectable HIV RNA levels for greater than three months and CD4 cell counts ≥ 100 cells/μL (23). When HIV viral load testing is unavailable, the WHO recommends continuation of maintenance therapy for one year and discontinuation if CD4 counts are >200 cells/μL (40). Fluconazole maintenance therapy should be reinstituted in patients demonstrating immunologic failure, ART interruptions, or a fall in CD4 counts to below 100 cell/μL (23).

*3.4 Identifying novel antifungal agents for cryptococcal meningitis:*

There are several shortcomings to drugs currently considered standard of care for the treatment of cryptococcal meningitis, as outlined above. Fluconazole is primarily fungistatic, and although it penetrates well into the CNS, even at high doses has relatively poor fungal clearance. Fluconazole is both dose-dependent and inoculum dependent (49). Amphotericin has better efficacy but substantial toxicity (50), needs to be administered intravenously, and is not readily available in low-resource countries despite inclusion on the WHO Essential Medication list. Flucytosine is currently not available in Asian and African countries that bear the largest burden of cryptococcal meningitis and has been associated with hematologic toxicity. There has therefore been a push for the development of new therapies that are 1) orally bioavailable, 2) low cost, 3) associated with low toxicity, and 4) fungicidal. While the antifungal activity of many novel compounds is currently being examined, the immediate and critical need has led some researchers to evaluate known compounds with the hope of identifying agents that can be repurposed as new antifungals (51). Although a neglected disease, cryptococcal meningitis is not currently listed as a neglected tropical disease for the FDA Tropical Disease Priority Review Vouchers program.

The antidepressant sertraline has been found to have potent fungicidal activity against *Cryptococcus* both *in vitro* and *in vivo* animal models (52). Sertraline reaches ~20 fold higher concentration in brain as well as ~65-fold higher concentrations in lung than in blood (53), and sertraline has a bidirectional synergistic effect with fluconazole (52, 54). There is currently a phase III randomized clinical trial underway to investigate 18-week survival of patients with adjunctive sertraline to standard therapy for the treatment of cryptococcal meningitis (ASTRO-

CM, clinicaltrials.gov NCT01802385). Rates of CSF *Cryptococcus* clearance by sertraline dose are summarized in **Figure 3**. Sertraline has a modest increase in fungal clearance which is hoped to decrease mortality in cryptococcal meningitis. Given the low cost and wide availability combined with the paucity of other therapeutic agents, we are hopeful sertraline will be a useful adjuvant therapy.

Other known agents with novel fungicidal activity include tamoxifen, INF-γ, and granulocyte-macrophage colony-stimulating factor (GM-CSF). Tamoxifen has demonstrated fungicidal activity both in vitro and in a mouse model via binding to *Cryptococcus* calmodulin (55). A sex difference in recovery after spinal cord injury in rodents pointed to a hormonal difference and led to the investigation of tamoxifen with the mechanism possibly related to anti-inflammatory, anti-oxidant properties.(56) INF-γ augments the type I T helper ($Th_1$) cell response increasing classical activation of macrophages resulting in significantly increase fungal CSF clearance when used as an adjuvant (38). GM-CSF has been shown important in cryptococcal meningitis as otherwise immunocompetent people with auto antibodies to GM-CSF are at risk for cryptococcosis (57). In vitro GM-CSF augments macrophage killing of *Cryptococcus* (58), although the clinical role is unclear. Another possibility includes a new tetrazole Viamet-1129 which has potent activity against *Cryptococcus* via inhibition of CYP51-mediated sterol synthesis (59). VT-1129 has the same target as traditional azoles; however, the tetrazole is highly selective for fungal CYP51 and has minimal interaction with human cytochrome P450 enzyme metabolism, resulting in a very low minimum inhibitory concentrations and very long half-life (59). Therapy updates and novel therapies displayed in **Table 3**.

One drug which has recently been shown to be harmful is dexamethasone. In a phase III randomized clinical trial, adjunctive dexamethasone started at time of cryptococcal diagnosis did not reduce mortality among patients with HIV-associated cryptococcal meningitis and was associated with a trend toward higher mortality, more adverse events, and more disability compared with placebo in the setting of amphotericin combination therapy (60).

*3.5 Management of Intracranial Pressure*:

Elevated intracranial pressure (ICP) is defined as CSF pressure ≥ 25 cm $H_2O$ and is a common complication of cryptococcal meningitis. The mechanism of elevated ICP is primarily due to a failure of CSF resorption via the arachnoid villa due to the physical obstruction by cryptococcal polysaccharide capsule and yeasts (61). ICP correlates with the burden of *Cryptococcus* in CSF (and thereby yeast present in the arachnoid granulations) and increasing size of the cryptococcal capsule (62). Severely elevated ICP can be characterized by headaches, vomiting, papilledema, reduction of visual acuity, blindness, cranial nerve palsy's (most commonly cranial nerve VI), confusion, altered mental status, and/or coma. Significantly elevated ICP, if not addressed, causes increased 10-14 day mortality (63-65). Traditional guidelines recommend measurement of CSF opening pressure with the initial lumbar puncture (LP), repeat LP at least daily if ICP is ≥25 cm $H_2O$, or with new symptoms are consistent with elevated ICP, and continued daily lumbar punctures until pressures have decreased or symptoms have resolved (23). These high-income country guidelines are often unrealistic in resource-limited settings, where manometers are expensive and/or absent. Additionally, even where manometers are available, measurement of ICP is not always performed in routine care when the diagnosis of cryptococcosis is made after the lumbar puncture is complete.

ICP can be measured with the opening pressure during an LP even in resource limited settings and should be done wherever possible. Rolfes *et al*. demonstrated the importance of aggressive ICP management, with a 69% relative survival benefit in the first 10 days with at least one therapeutic LP (median day 3) (64). This survival benefit was irrespective of initial ICP (**Figure 4**), meaning the survival benefit was observed regardless of opening pressure at baseline. Those with normal opening pressures <20cm $H_2O$ at baseline who did not receive a repeat therapeutic LP (which would be per guidelines) had higher 10-day mortality than those who received an additional therapeutic LP (64). Similar results were observed in Tanzania, where a strict protocol for serial lumbar punctures decreased 30-day mortality from 75% to 46% (66). In settings where manometers are not available, IV tubing can be used to measure ICP (66). Similarly, non-invasive methods such as handheld tonometers or ultrasound can measure intraocular pressure as a highly sensitive surrogate for detecting increased ICP (67). With persistently elevated ICP, such as during obstructive hydrocephalus, ventriculoperitoneal shunts can be used when conservative measures have failed. Other methods of decreasing ICP such as acetazolamide, mannitol, or corticosteroids should not be routinely used (68, 69).

If opening pressures cannot be measured, we recommend: 1) CrAg LFA screening by fingerstick as a point-of-care test prior to lumbar puncture; 2) for CRAG+ persons, presumptive removal of 20mL of CSF at diagnosis; 3) repeat LP in 48-72 hours with measurement of ICP with intravenous tubing if possible or removal of 20 mL; 4) strong consideration of lumbar punctures at 7 and 14 days, unless patients are fully asymptomatic.

4. **Optimal Timing of ART Initiation**:

The timing of ART initiation is an important consideration for persons with cryptococcal meningitis, as with advanced immunosuppression people are at high risk of AIDS progression and death (70). However, ART initiation should be balanced against the risk for development of paradoxical immune reconstitution inflammatory syndrome (IRIS). A consensus case definition of cryptococcal paradoxical IRIS defines the clinical syndrome as one occurring after treatment of the initial cryptococcal meningitis followed by ART initiation with subsequent clinical deterioration manifesting as one of the following: aseptic meningitis, intracranial lesions, lymphadenopathy, pneumonitis or pulmonary nodules, or cutaneous soft tissue lesions (71). The reported incidence of paradoxical cryptococcal IRIS is highly variable in incidence, ranging between 8-49%, presenting as soon as 4 days and up to 6 years after ART initiation, and carrying a mortality rate of 0-36% (71-73). Better microbiologic therapy and achieving CSF sterility is a key principle at reducing the risk of IRIS (74). In Uganda, the incidence of CNS events decreased from 30% to 13% by adding fluconazole 800 mg/day to the induction therapy and continuing for 4-6 weeks until ART initiation (50, 75). Further addition of sertraline decreased the incidence of paradoxical IRIS to $\leq$5% (54), with a randomized trial ongoing to confirm this preliminary result.

Regarding timing of ART initiation, a multisite, randomized trial conducted in Uganda and South Africa found 15% higher 26 week mortality in individuals initiating ART at 1-2 weeks from diagnosis as compared with those initiating ART 4-6 weeks after meningitis diagnosis (50). Three other smaller trials showed: increased risk of death with earlier ART with fluconazole monotherapy (76), increased risk of IRIS with amphotericin therapy (77), and no differences (70). Timing of ART remains somewhat controversial as three African trials showed increased harm with earlier ART whereas a small U.S. based trial showed no difference. No trial has

demonstrated a benefit of earlier ART with cryptococcal meningitis. Based on randomized clinical trial data (50), we recommend completion of induction therapy, verification that the CSF culture at 14-days is sterile, with an aim to initiate ART at approximately 4-6 weeks. Persons lacking CSF pleocytosis at diagnosis are at high risk of IRIS (78), and these persons in particular are at higher risk of death when starting ART at <2 weeks (50).

Finally, increased ART availability in resource-limited settings, coupled with a lack of pre-ART CrAg screening has led to a greater proportion of patients developing cryptococcal meningitis after initiating ART (54, 79). In places where cryptococcal meningitis once manifested primarily as an AIDS-defining illness in ART-naïve individuals, the occurrence of cryptococcal meningitis after initiating ART has now become common. In two cohorts from Uganda and South Africa, individuals already receiving ART at time of diagnosis had higher CD4 counts and lower fungal burdens, but outcomes were not improved (54, 80). Furthermore, individuals in the Ugandan study who developed cryptococcal meningitis within 14 days of initiating ART had significantly higher 2-week mortality (43% compared with those on ART for 15 days to 4 months (16%), >4 months (10%), or ART-naïve (25%); p=0.05). This study underscores the detrimental effect of immune recovery in the setting of an untreated CNS infection and the importance of pre-ART cryptococcal antigen screening to prevent cryptococcal meningitis occurring early after ART initiation (18, 20). Potentially this is due to unmasking IRIS or the cryptococcal meningitis becoming apparently only after starting ART and immune recovery.

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**5. Cryptococcal Meningitis Relapse**:

Cryptococcal meningitis relapse, or microbiological relapse, is the recurrence of meningeal symptoms with recovery of organism on CSF culture (81). Microbiological relapse must be distinguished from paradoxical immune reconstitution syndrome in which symptoms recur but CSF cultures are found to be sterile. In a South African study, fluconazole non-adherence or non-prescription was found to be the primary cause of relapse (82). During a recent Ugandan trial, we have found similar challenges with prescribing long term fluconazole secondary prophylaxis in routine HIV care (54). Thus although 5% of relapse cases can occur with optimal therapy (50), more often this is a systems failure.

Therapy for cryptococcal meningitis relapse consists of reinitiating induction therapy with amphotericin (1 mg/kg/day) and higher dose fluconazole (800-1,200 mg/day) (23). Voriconazole and INF-γ have been used in case reports for salvage therapy in cases of cryptococcal meningitis refractory to standard therapy (83).

Cryptococcal meningitis relapse should be differentiated from persistent infection, or treatment failure. Whereas relapse occurs after documentation of sterile cultures, a person with persistent infection will continue to have positive cultures after 4 weeks of standard therapy at effective doses. Susceptibility testing should be done on isolates to assess fluconazole resistance if persistent infection is suspected, and brain imaging should be considered to rule out cryptococcoma. Fluconazole resistance should be considered whenever the minimum inhibitory concentration (MIC) is $\geq 64$ μg/mL (81).

*5.1 Diagnosis and Treatment of IRIS:*

The presentation of recurrent symptomatic meningitis after the treatment of first-episode of cryptococcal meningitis and post ART initiation should raise concern for disease relapse,

treatment failure or development of paradoxical IRIS. Symptomatic relapse may be secondary to either persistent infection due to fluconazole resistance, ineffective primary therapy or presence of a cryptococcoma, whereas microbiological relapse has been shown to be mainly due to non-adherence of secondary fluconazole prophylaxis (81). Distinguishing from treatment failure/relapse and paradoxical IRIS can be difficult, and the two entities are not always mutually exclusive. A positive cryptococcal culture, virologic failure, and lower CSF inflammatory profile supports the diagnosis of cryptococcal meningitis relapse whereas a sterile culture and higher CSF WBC supports the diagnosis of paradoxical IRIS (71, 78).

Management of IRIS, once the diagnosis has been made, includes management of elevated intracranial pressures with lumbar puncture and large volume drainage of CSF. Recommendations for therapeutic modalities are mainly based on expert opinion and clinical experience. For severe cases of IRIS with CNS complications, including increased intracranial pressure or neurological deterioration, the current IDSA guidelines recommends 0.5-1 mg/kg of prednisone equivalent to be tapered over a 2-6 week period, although the duration of the taper may be individualized based on clinical status (23). There have been several case reports documenting neurologic improvement with the use of thalidomide, a tumor necrosis factor-alpha (TNF-α) inhibitor, in steroid dependent or refractory cases of IRIS (84). Adalimumab, a human monoclonal antibody that binds to TNFα, blocking its anti-inflammatory actions demonstrated neurological improvement in a patient with IRIS associated cryptococcoma (85). Both thalidomide and adalimumab were used after documented sterility of the CSF.

## 6. Expert Commentary:

Cryptococcal meningitis remains a prevalent opportunistic infection with high mortality and morbidity. Combination amphotericin and flucytosine is the best present induction therapy; however, the cost of flucytosine is prohibitive in most settings where cryptococcal meningitis occurs and is presently unavailable. In the absence of flucytosine, concomitant fluconazole 800-1200 mg/day is recommended. After induction therapy, using enhanced consolidation therapy with fluconazole 800 mg/day until documentation of CSF sterility and ART initiation, should decrease the risk of persistent infection, disease relapse, or IRIS. Based on the timing of CSF sterility and immune recovery, longer duration of fluconazole consolidation therapy may be needed, often for 3-4 months. In ART-naïve persons, ART should be initiated 4-6 weeks after the diagnosis of cryptococcal meningitis. Earlier initiation of ART prior to 2 weeks after diagnosis has been associated with increased mortality, particularly in those lacking CSF inflammation.

The CrAg lateral flow assay has been a significant recent breakthrough in cryptococcal diagnosis. This point-of-care assay allows for quick diagnosis of meningitis from CSF but also allows the identification of asymptomatic patients who have cryptococcal antigenemia enabling prevention of meningitis with preemptive fluconazole. The ability to CrAg screen and initiate preemptive treatment has the potential to prevent much morbidity and mortality in the future. Current preemptive therapy recommendations are not perfect. Among persons with high cryptococcal titer ($\geq$1:160 CrAg LFA titer by Immy), mortality with fluconazole monotherapy remains high (25-30%). Combination therapy using short course amphotericin or sertraline in addition to fluconazole may be useful and evaluations of such additional treatment is underway.

One day patients may present earlier with HIV reducing their risk of cryptococcal meningitis and the need for new treatments. However, until that day, continued advances in treatment for cryptococcal meningitis are needed.

## 7. Five Year View

Over the next five years the roll out of CrAg testing and treatment should continue. Ideally this would shift meningitis cases to earlier presentation and prevent many cases. However, in practice cryptococcal meningitis will remain a major problem given the difficulties with accessing HIV therapy, retention-in-care, and virologic failure.

As persons with asymptomatic cryptococcal antigenemia who have low CD4 and high fungal burden are known to do have high mortality in spite of proactive treatment with fluconazole prior to symptomatic disease. As such new strategies for their treatment are needed and hopefully will be further developed over the next 5 years. This could further reduce cases of meningitis and overall mortality from cryptococcal disease.

There are a number of possibilities for new access to old treatments and possible new treatments as noted above in section 3.4. If a lipid formulation of amphotericin and/or flucytosine could become available in low income countries that would have a significant impact as the combination is known to be the most efficacious given increased ability to tolerate therapy. Of the many novel treatments, sertraline is the only one currently available in resource limited settings at present. If the current phase III randomized clinical trial comparing the 18-week survival of patients with adjunctive sertraline to standard therapy for the treatment of cryptococcal meningitis (ASTRO-CM, clinicaltrials.gov NCT01802385) has positive results that could be rolled out widely. Also the new azole VT-1129 would be quite useful if introduced but timeline or certainty of the introduction is not known yet. For novel compounds to move

forward, cryptococcal meningitis would need to be added to FDA's list of neglected tropical diseases. Finally, while treatments such as tamoxifen, INF-γ, and granulocyte-macrophage colony-stimulating factor are all promising, they are very early in development and not close to being available in clinical practice.

## 8. Key Issues

- Screening of patients with low CD4<100 for cryptococcal antigenemia with subsequent preemptive treatment is effective in reducing meningitis and overall mortality.
- Asymptomatic cryptococcal antigenemic patients with CD4 <50 and high fungal burdens are known to have high mortality. Further work to reduce this mortality is needed.
- Amphotericin remains necessary for successful treatment of cryptococcal meningitis.
- Adjunctive treatment with amphotericin using flucytosine (if available) or fluconazole has mortality benefit.
- Amphotericin duration is 1-2 weeks depending on fungal burden and availability of supportive care for monitoring and managing toxicity.
- Subsequent consolidation therapy with high dose fluconazole 800 mg is recommended until CSF culture is sterile and the patient is started on ART, longer if the CSF fungal culture remains positive or is unknown.
- Optimal dose and duration of consolidation therapy are unclear. In persons with high burdens of initial infection, lacking CSF sterility at 2 weeks,
- Secondary prophylaxis with fluconazole 200 mg is recommended for at least a year or until the CD4 has been greater than 200 cells/μL for more than 6 months.

- Managing intracranial pressure is a critical component for effective treatment of meningitis. At least one repeat lumbar puncture has a survival benefit regardless of initial intracranial pressure.
- A number of novel antifungal agents are in development including sertraline, new azoles, novel oral amphotericin formulations, and immunologic treatments. Adding cryptococcal meningitis to the FDA list of neglected tropical diseases is essential to the commercialization of novel agents in development.

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**Declaration of interest**

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## Reference Annotations

Letang, et al demonstrated there was high mortality in CRAG positive patients with HIV and CD4 less than 150, with 75% of participants having died within 1 year. However, treatment with fluconazole significantly improved mortality with a hazard ratio of 0.18.

Mfinanga, et al showed that those with HIV and a CD4 count under 100 and CRAG positive who got fluconazole and adherence support had a 28% mortality reduction at 12 months.

Morawski, et al reported that there was still significant mortality in those with HIV and CD4 under 100 and asymptomatic CRAG antigenemia who get fluconazole preemptively. Overall 6-month mortality was 22% and those with a CRAG titer greater or equal to 1:160 had 32% mortality.

Loyse, et al showed that when used with amphotericin B fluconazole had no statistical difference to flucytosine in terms of efficacy or mortality. This is important as fluconazole is generally available in Africa while flucytosine is not.

Boulware, et al reported the unexpected finding that early start of antiretroviral therapy after cryptococcal meningitis has increased mortality. As such it is advised to wait 6 weeks following meningitis to start antiretroviral therapy.

Rhein, et al described a phase 2 study that demonstrated sertraline can increase the rate of fungal clearance in conjunction with amphotericin B and fluconazole.

Rolfes, et al is important as there is a mortality benefit to frequent lumbar punctures regardless of intracranial pressure.

**Figure 1.** Rate of CSF *Cryptococcus* culture clearance for induction antifungal regimens.

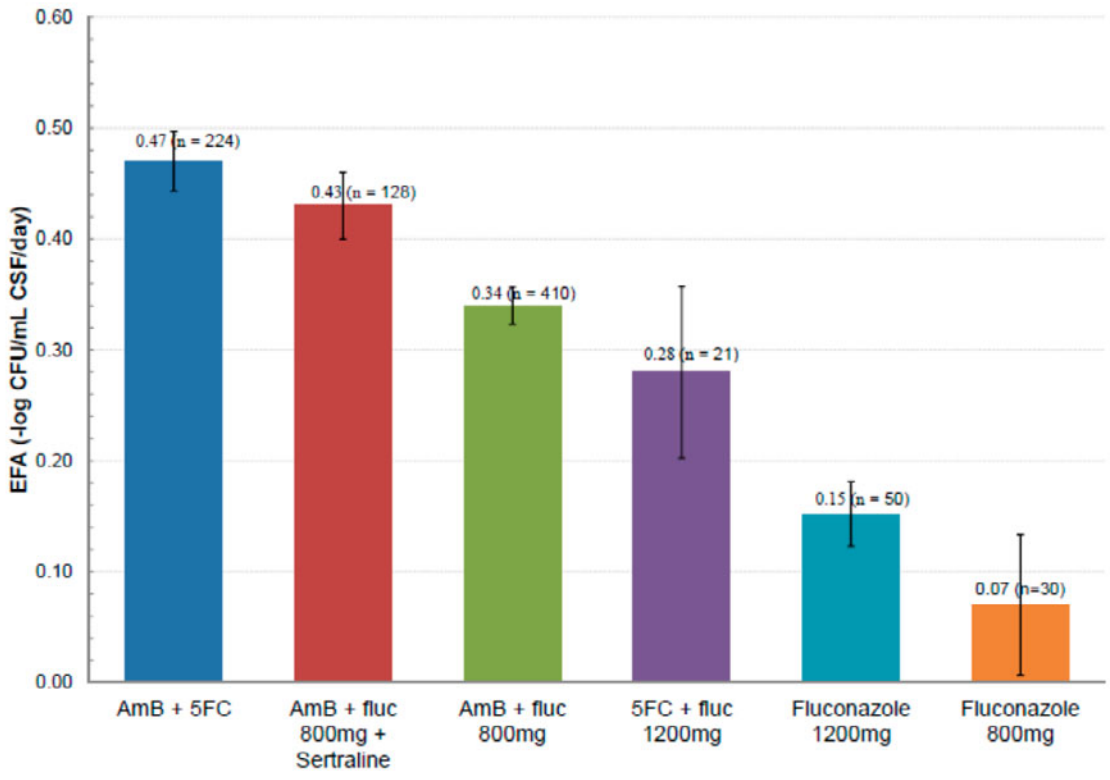

Figure 1 displays the early fungicidal activity (EFA) of induction regimens for the treatment for cryptococcal meningitis, measured as $\log_{10}$ clearance of *Cryptococcus* yeasts per mL of CSF per day using quantitative CSF cultures and calculated via linear regression. Values are the means with 95% confidence intervals as pooled from Table 2. Abbreviation: AmB, amphotericin; fluc, fluconazole; CFU, colony forming units; CSF, cerebrospinal fluid.

**Figure 2.** Rate of CSF *Cryptococcus* Clearance by Sertraline Dose

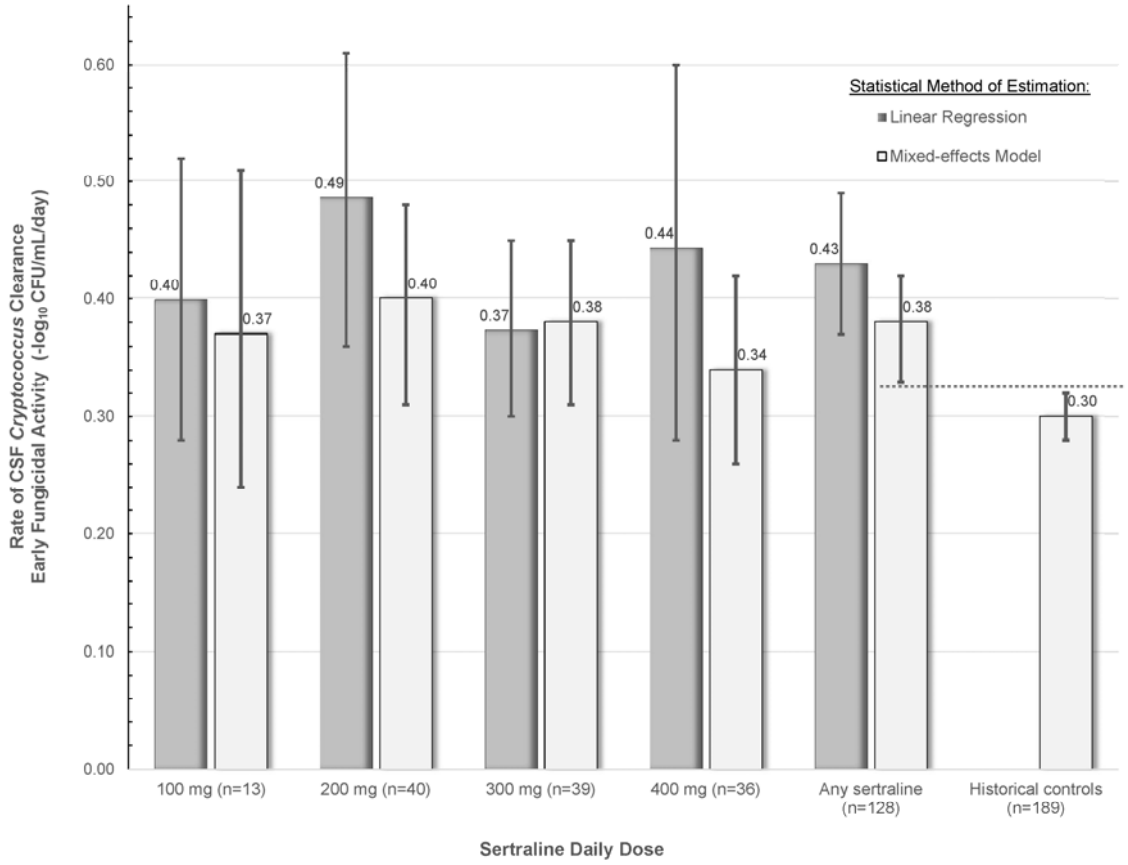


Figure 2 displays the CSF early fungicidal activity when receiving increases doses of sertraline coupled with a background regimen of amphotericin B deoxycholate and fluconazole 800 mg/day. EFA is estimated by simple linear regression or by a mixed effects regression model (54). The comparison historical controls were from the same study site in Uganda during the Cryptococcal Optimal ART Timing (COAT) trial who also received amphotericin B and fluconazole 800 mg/day ($P$=0.04) (50).

**Figure 3.** Survival after Therapeutic Lumbar Puncture or No Therapeutic Lumbar Puncture

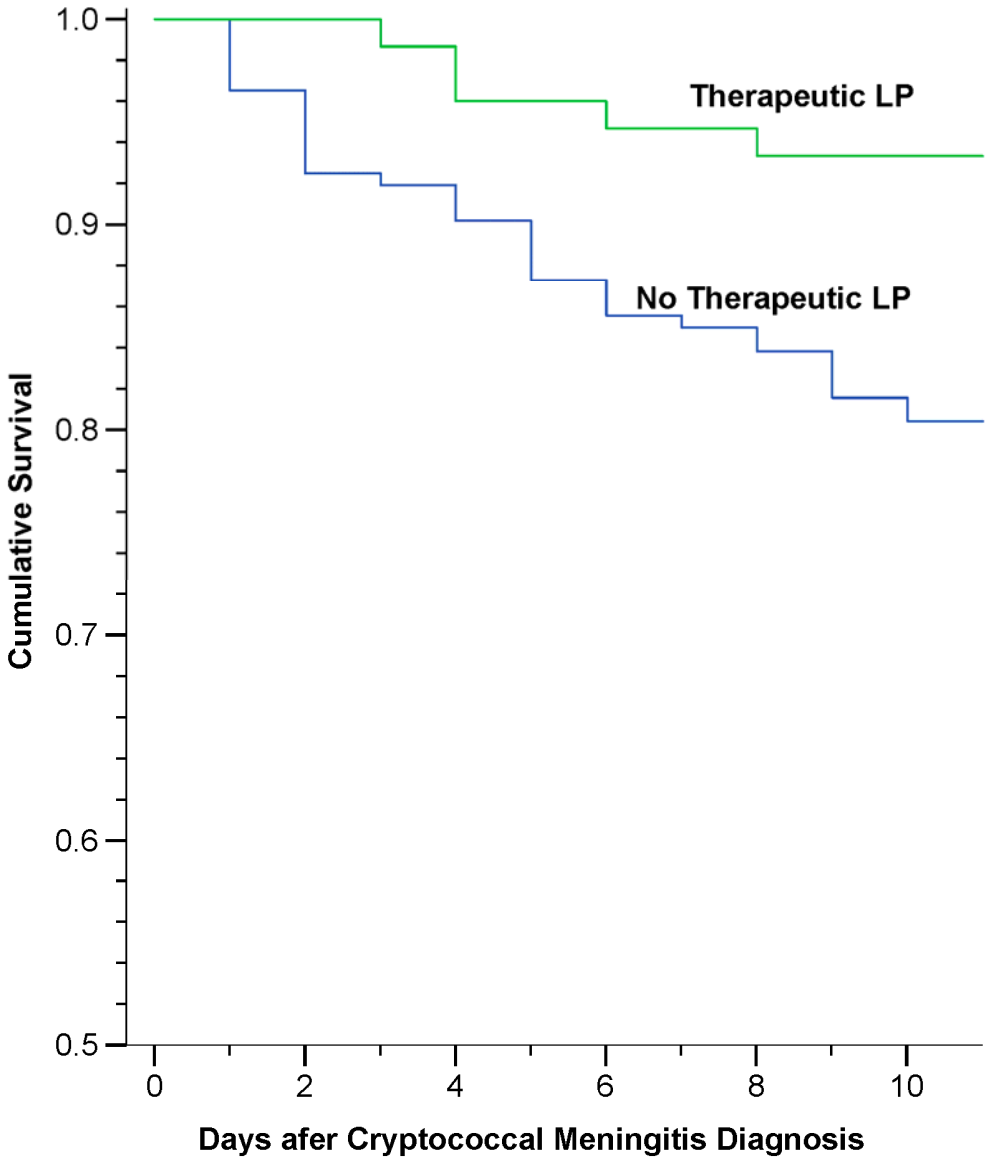


Figure 3 displays the survival curves of those receiving a therapeutic lumbar puncture in the first 10 days of therapy (median 2 days after diagnosis) versus no therapeutic lumbar puncture, as reported by Rolfes *et al*. (64)

**Table 1.** Recommended Treatment for Cryptococcal Meningitis in Resource-Limited Settings.

| **Medication and Dose** | **1-2 weeks** [b] | **10-12 weeks** | **52 weeks** |
|---|---|---|---|
| Amphotericin (0.7-1.0 mg/kg/day) + second adjunctive agent [a] | | | |
| Fluconazole 800-1200 mg daily | Continue until CSF is known sterile [c] | | |
| Fluconazole 400 mg daily | | | ± [c] |
| Fluconazole 200 mg daily | | | until CD4 >200 for ≥ 6 months |
| **Treatment Phase** | **Induction** | **Consolidation** | **Secondary Prophylaxis** |

**Notes:** [a] Flucytosine (5FC) 100 mg/kg/day preferred where available, otherwise fluconazole at 800–1200 mg/day in divided doses. KCl 40–60 mEq/day should be given with amphotericin (35);

[b] optimal duration of initial induction therapy is unknown. In resource-limited regions, the cost-benefit is likely maximal for one week induction with amphotericin B at 1 mg/kg/day coupled with 4 weeks of fluconazole 1200 mg kg/day (27);

[c] We recommend continuing fluconazole at 800-1200 mg/day until the CSF culture result is known to be sterile and ART has been initiated. We strongly recommend longer duration of consolidation therapy if 2 week CSF culture is positive or culture status is unknown. Optimal duration of consolidation therapy is unclear. Traditional duration of 8 weeks for consolidation is likely inadequate for those with a high burden of initial infection, who may take 3-4 weeks to sterilize their CSF.

**Table 2:** Trials comparing early fungicidal activity of CSF *Cryptococcus* clearance with induction antifungal treatment regimens

| Induction Regimen | EFA | ± SD | n | Source |
|---|---|---|---|---|
| Amphotericin | -0.31 | 0.15 | 99 | (25) |
| Amphotericin + 5FC | -0.42 | 0.10 | 100 | |
| Amphotericin + fluconazole (800 mg/day) | -0.32 | 0.10 | 99 | |
| Amphotericin + 5FC | -0.41 | 0.22 | 21 | (32) |
| Amphotericin + fluconazole (800 mg/day) | -0.38 | 0.18 | 22 | |
| Amphotericin + fluconazole (1200 mg/day) | -0.41 | 0.35 | 23 | |
| Amphotericin + voriconazole | -0.44 | 0.20 | 13 | |
| Amphotericin (5 days) + fluconazole (1200 mg/day) | -0.30 | 0.11 | 30 | (33) |
| Amphotericin + 5FC | -0.49 | NA | 30 | (38) |
| Amphotericin + 5FC + INF-γ | -0.64 | NA | 60 | |
| Amphotericin (7 days) + fluconazole (1200 mg/day) | -0.38 | 0.20 | 19 | (34) |
| Amphotericin (7 days) + fluconazole (1200 mg/day) + 5FC | -0.50 | 0.15 | 18 | |
| Fluconazole (1200 mg/day) | -0.18 | 0.11 | 30 | (43) |
| Fluconazole (800 mg/day) | -0.07 | 0.17 | 30 | |
| Amphotericin (1 mg/kg/day) | -0.48 | 0.28 | 49 | (45) |
| Fluconazole (400 mg/day) | -0.02 | 0.05 | 5 | |
| Amphotericin + fluconazole (800 mg/day) | -0.36 | 0.25 | 223 | (50) |
| Amphotericin + fluconazole (800 mg/day) + sertraline | -0.43 | 0.39 | 128 | (54) |

**Abbreviations:** EFA = early fungicidal activity ($log_{10}$ CFU/mL CSF/day) calculated by linear regression except for Day et al which is calculated with mixed regression model (25); amphotericin B deoxycholate 0.7 or 1 mg/kg/day or as indicated; 5FC = flucytosine (25 mg/kg 4 times daily); Voriconazole (300 mg twice daily; 400 mg twice on day 1); INF-γ = interferon-gamma (100 μg subcutaneously, 2 or 6 doses).

**Table 3.** Cryptococcal Anti-fungal Therapy Options: Current and Future

| Therapy | Dose | Comments |
|---|---|---|
| Amphotericin B deoxycholate | 0.7-1.0 mg/kg/d | Traditional duration of 14 days. Favorable outcomes with 5-7 days of therapy (33, 34). Ongoing clinical trial (ACTA) testing 7 vs 14 days. |
| Amphotericin, Lipid formulation | 3 mg/kg/d | 3 mg/kg/day equivalent to 6 mg/kg/day (24) U.S. Patent expires in August 2016 (86) |
| | 10 mg/kg x 1-3 | Ongoing trial testing 10 mg/kg dose(s) (30) |
| Fluconazole | 800-1200 mg/d | Fungicidal activity poor with monotherapy (43) Used as second adjunctive agent where 5FC is not available. Optimal dose unclear. |
| Flucytosine (5FC) | 100 mg/kg/d | Cost prohibitive in the U.S. (~$2000/day)(26) Generic available in Europe, India (28, 87) |
| | ***Novel Use*** | |
| Sertraline | Interferes with protein translation. Synergistic with fluconazole in vitro and in vivo; (52, 88) increases rate of CSF clearance. (54) | |
| Tamoxifen | Binds to Cryptococcus calmodulin; In vitro and in vivo model fungicidal activity (55); Phase II trial in Vietnam in 2017. | |
| Interferon-gamma (IFN-g) | Showed to significantly increase fungal clearance (38) Auto-antibodies against IFN-g increase risk for cryptococcosis | |
| Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) | Auto-antibodies against GM-CSF a risk for disease (57). GM-CSF increases macrophage killing of yeast (58); can precipitate unmasking disease (89); Unclear clinical role. | |
| | ***Novel Agents*** | |
| VT-1129 | Novel tetrazole blocks sterol 14-alpha-demethylase CYP51 enzyme; undergoing phase I trials in 2016 (59), phase II trials in 2017. Active against fluconazole resistant yeast. | |
| MAT2203 (Oral Amphotericin) | Encochleated Formulation of amphotericin B which is a lipid-crystal nanoparticle formulation that is orally absorbed; has been studied in animal model studies of candidiasis, cryptococcal meningitis, aspergillosis, and visceral leishmaniasis. Entering into probable phase II trials in 2017. | |
| | ***Not Recommended*** | |
| Dexamethasone | Increased mortality and does not improve intracranial pressure (69); May have roll in IRIS reactions and non-HIV persons. | |
| Acetazolamide | Ineffective at intracranial pressure control, increased electrolyte abnormalities.(68) | |