New Approaches to the Diagnosis and Treatment of Cryptococcal Meningitis

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Abstract

Keywords

- ▶ cryptococcal meningitis
- ▶ human immunodeficiency virus
- ▶ acquired immunodeficiency syndrome
- ▶ sub-Saharan Africa

Cryptococcal meningitis remains one of the leading causes of morbidity and mortality among immunosuppressed individuals, particularly those with advanced acquired immunodeficiency syndrome. The greatest burden of disease is in sub-Saharan Africa and Asia where there is limited access to diagnostics and treatment for the disease. The authors review the available tools for diagnosing cryptococcal meningitis and review treatment for cryptococcal meningitis, highlighting the evidence behind current treatment guidelines.

The global burden of cryptococcal disease is high with 1 million new infections each year.¹ The greatest burden is in sub-Saharan Africa where cryptococcal meningitis is the fourth leading cause of mortality, accounting for over 600,000 deaths annually.¹ Disseminated cryptococcosis is the leading cause of meningitis in Zimbabwe,² and accounts for 40% of all cases of meningitis in Malawi.³ Cryptococcal meningitis is also associated with very high mortality rates, particularly in resource limited settings.^{4,5} Cryptococcosis is caused by an encapsulated yeast that belongs to the genus *Cryptococcus*. *Cryptococcus neoformans* and *Cryptococcus gattii* are responsible for the majority of cases of human cryptococcosis. *Cryptococcus neoformans* was originally classified into five groups (A–D and A/D) based on serology. Serologic typing classified *C. gattii* as *C. neoformans* serotype B and C, whereas serotypes A and D were *C. neoformans* var. *grubii* and *C. neoformans* var. *neoformans*, respectively. Molecular typing has enabled us to distinguish *C. neoformans* and *C. gattii* as two distinct species. Each species has been further divided into four major molecular subtypes,

often with distinct ecological niches (▶Table 1). *Cryptococcus neoformans* has a global distribution and has been found in association with pigeon droppings, soil, and decaying vegetation.^{6–8} *Cryptococcus gattii* has primarily been isolated in tropical and subtropical climates and is found in association with eucalyptus trees.^{9–12} Other *Cryptococcus* species that rarely cause human disease include *C. albidus*,^{13,14} *C. laurentii*,^{15,16} and *C. luteolus*.¹⁷

Epidemiology of Cryptococcal Disease

Cryptococcus was identified as a cause of human disease in 1894 when the organism was isolated from the tibia of a 31-year-old woman with disseminated disease. The first described case of meningoencephalitis was in 1905 by von Hansemann. Prior to the advent of the acquired immunodeficiency syndrome (AIDS) epidemic, *cryptococcus* rarely caused significant human disease. The onset of the AIDS epidemic resulted in a substantial increase in the number

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Table 1 Geographic distribution of cryptococcal species

Species	Serotype	Molecular type	Global distribution	Disease	Source
<i>C. Neoformans var grubii</i>	A	VNI VNII	Worldwide	Immunocompromised Pulmonary/ meningitis/ disseminated	Soil/avian environments
<i>C. gattii</i>	B C	VGI VGII VGIII VGIV	Tropical, subtropical, temperate	Immunocompromised/ Immunocompetent Less commonly causes meningitis	Woody materials, i.e., trees
<i>C. Neoformans var neoformans</i>	D	VNIV	Europe	Immunocompromised/ immunocompetent Skin disease	Soil/avian environments
<i>C. Neoformans</i>	A/D	VNIII	Worldwide	Immunocompromised Pulmonary/meningitis/ disseminated	

of cases of cryptococcal disease. In the United States in the pre-HAART (highly active antiretroviral treatment) era, 86% of cryptococcosis cases occurred in human immunodeficiency virus- (HIV-) infected individuals, and the annual incidence of the disease was markedly higher in individuals with HIV (up to 66 cases/1,000) compared with those without HIV (0.9 cases/100,000).¹⁸ Among individuals who did not have HIV, the greatest risk factors for developing cryptococcosis were malignancies, diabetes, steroid therapy, solid-organ transplant, and chronic medical diseases such as renal and liver failure.¹⁸ The introduction of HAART subsequently led to a substantial decline in incident cryptococcal infections in the developed world,^{19–21} though the prevalence among other immunocompromised individuals has remained stable.²⁰

In sub-Saharan Africa, cryptococcal disease continues to be one of the leading causes of mortality among individuals with HIV infection. Among adults with HIV in South Africa, the estimated incidence is 95 to 120 cases per 100,000^{22,23}; among individuals with AIDS (defined by CD4 < 200cells/mm³) the incidence is 14 cases per 1,000.²² In southeast Asia there are over 100,000 annual cases of cryptococcal disease.¹ The greatest burden of cryptococcal disease in Asia occurs in patients with AIDS, with cryptococcal meningoencephalitis accounting for up to a third of patients presenting with meningitis.²⁴ The epidemic of cryptococcal disease in sub-Saharan Africa is slowly evolving with some areas experiencing a decline in the number of cases, while other areas continue to see a large number of cases despite increasing access to ART.^{25,26}

Host Susceptibility to Cryptococcal Disease

Cryptococcus yeasts are ubiquitous in the environment and are acquired by inhalation. Primary pulmonary infection is likely acquired in childhood in a large proportion of individuals,^{27–29} although adult acquisition is also well documented.^{30,31} An effective cell-mediated immune response is important for recruiting and activating macrophages to con-

tain disease,^{32,33} resulting in clearance or establishment of a contained latent infection. A Th1 predominant cellular immune response with production of interferon- (IFN-) γ , tumor necrosis factor- (TNF-) α , interleukin- (IL-) 12, and IL-18 is important for decreasing fungal burden and preventing dissemination of disease.^{34–36} A Th2 response, however, is associated with disseminated disease.³⁷

In the immunocompromised host, reactivation of latent disease is believed to be responsible for the subsequent development of cryptococcal disease.^{38,39} In patients with AIDS, the depletion of CD4+ T cells affects the ability of the immune response to adequately contain cryptococcal infection. HIV infection results in a shift from a Th1 cytokine phenotype to a predominant Th2 phenotype, which is associated with disseminated cryptococcal disease.³⁷ In addition, HIV may infect alveolar macrophages, further limiting their ability to adequately contain cryptococcal infection.⁴⁰

Clinical Presentation of Cryptococcal Disease

The clinical presentation of cryptococcal disease is highly variable and can result in asymptomatic disease, localized pulmonary disease or disseminated disease. Disseminated disease can occur in any organ; however, there is a predilection for infection of the central nervous system (CNS), resulting in meningoencephalitis and occasionally causing focal intracerebral granulomas known as *cryptococcomas*. Patients with meningoencephalitis typically present with a severe headache. The headache may be present for several weeks to months, and can be accompanied by mental status changes, personality changes, fever, lethargy, and coma. Other complications of CNS involvement include hydrocephalus (both communicating and noncommunicating), papilledema that may lead to blindness, sudden onset of sensorineural deafness, cranial nerve palsies, motor and sensory deficits, cerebellar dysfunction, and seizures. **Table 2** summarizes some common clinical presentations of meningoencephalitis in

Table 2 Clinical presentation of meningoencephalitis among HIV-infected hospitalized patients

Signs and symptoms	HIV positive, USA (n = 89), N (%) ⁴¹	HIV positive, South Africa (n = 44) N (%) ⁴²	HIV positive, Zimbabwe N (%) ⁴³
Headache	65 (73)	37 (84)	73/76 (96)
Fever	58 (65)	21 (48)	38/74 (51)
Cough or dyspnea	28 (31)	ND	16/67 (24)
Nausea or vomiting	37 (42)	ND	31/76 (41)
Focal neurologic deficits	5 (6)	22 (50) ^a	6/80 (8)
Seizures	4 (4)	7 (16)	8/75 (11)
Altered mentation	25 (28)	16 (36)	10/67 (15) ^b
CSF parameters			
Opening pressure (≥ 200 mm H ₂ O)	33/50 (66)	ND	ND
Low glucose	21/89 (24)	27/44 (61) ^c	ND
Protein > 45 mg/dL	49/89 (55)	33/41 (81)	ND
WBC ≥ 20	19/89 (21) ^d	22/41 (54) ^e	ND ^f
India Ink positive	64/87 (74)	40/43 (93)	76/89 (85)

Abbreviations: CSF, cerebrospinal fluid; HIV, human immunodeficiency virus; ND, not determined; WBC, white blood count.

^aFocal neurologic signs included cranial nerve palsies, papilledema, hemiplegia, blindness, deafness, paraplegia.

^bDefined by a Glasgow Coma Score < 13 .

^cGlucose < 2.2 mmol/L (40 mg/dL).

^dWBC ≥ 20 cells/mm³.

^eWBC ≥ 5 cells/mm³.

^fMedian values for CSF parameters were provided as median WBC count 5/mm³ (range 0–1250, predominantly mononuclear cells), median glucose level 2.1 mmol/L (0.1–5.1), median protein level 1.1 g/dL (range 0.1–10.6).

three patient cohorts, in the United States,⁴¹ South Africa,⁴² and Zimbabwe.⁴³

Clinical presentation in sub-Saharan Africa is often delayed, with many patients presenting with advanced disease characterized by focal neurologic deficits and seizures. Cryptococcal meningitis is often the first AIDS-defining illness in many patients who present for care. Headache is the most common symptom on presentation, with meningismus present in most patients.^{2,43} Elevations in intracranial pressure are common, and may be due to fungal obstruction of the cerebrospinal fluid (CSF) drainage channels by accumulation in the arachnoid villi and subarachnoid spaces, resulting in communicating hydrocephalus.⁴⁴ High opening pressures are associated with poor clinical outcomes.⁴⁵ Ocular involvement may also be present, and most commonly occurs as a result of high intracranial pressures. Patients present with oculomotor palsies, papilledema, and complete vision loss.⁴⁶ Intraocular cryptococcosis is less frequent, and can cause endophthalmitis or direct invasion of the optic nerve.⁴⁷

Diagnosis of Cryptococcal Meningitis

The diagnosis of cryptococcal disease can be made by direct visualization, histopathology, culture, and or detection of cryptococcal antigens in blood, CSF, and urine. *Cryptococcus* exists in the blood, CSF, and tissues as a single-celled organism with a characteristic polysaccharide capsule. Microscopic identification of the organism can be done using India ink

staining or observation with a phase-contrast microscope. India ink staining has been the traditional method for identifying *Cryptococcus* organisms, particularly in resource limited settings. India ink stains the surrounding material blue, but not the fungal capsule, giving a characteristic “starry night” appearance. The sensitivity and specificity of India ink staining can be highly variable and often operator dependent, as lysed leukocytes can be mistaken for fungal elements.^{48,49} The organism can readily be cultured from most sites, using routine and automated culture systems.

Histopathologic diagnosis of *Cryptococcus* can be done with several readily available staining techniques. The Giemsa stain only partially stains the organisms and is not typically utilized. Hematoxylin and eosin does not stain the capsule and the yeast is only weakly stained. Gomori methamine silver stain binds to fungal aldehydes and stains the yeast a characteristic black color, without staining the capsule. Staining of the capsule can be done with mucicarmine or Alcian blue stains. The capsule takes up the red color of mucicarmine or the blue color of Alcian to facilitate visualization. In tissues, yeast are typically contained within granulomas, particularly in immunocompetent hosts. In immunocompetent individuals, the *Cryptococci* are taken up by macrophages and induce an inflammatory response with the formation of epithelioid granulomas with central giant cells and surrounding lymphocytes. These granulomas typically do not have caseous necrosis.⁵⁰ In immunocompromised individuals, full granuloma formation may not occur, and pseudocysts develop that

are filled with encapsulated yeasts and surrounded by macrophages and lymphocytes.^{44,50}

An evaluation of CSF parameters, such as cell counts, glucose, protein, and opening pressures, can aid in the diagnostic evaluation of cryptococcal meningoencephalopathy. In a case series of 40 patients with cryptococcal meningitis in the setting of diabetes, malignancies, sarcoidosis, and other rheumatologic disease, most patients presented with abnormal CSF parameters.⁵¹ Abnormal CSF cell counts were present in 97%. Cell counts ranged from 6 to 808 cells/mm³, with lymphocytes accounting for 8 to 100% of CSF white cells. Protein elevation was present in 90%, and a low CSF glucose in 55%. The opening pressure was elevated in 64%.⁵¹ In patients presenting with cryptococcal meningoencephalitis in the setting of advanced HIV infection, the classic findings of an elevated CSF white cell count, elevated protein, and low glucose are not always evident (► **Table 2**).

Detection of capsular antigen is the most reliable diagnostic tool for cryptococcosis. Cryptococcal antigen can be detected in serum, CSF, and urine specimens. Detection of capsular antigen can be done by latex agglutination (LA) assays, enzyme immunoassays (EIAs), or the novel lateral flow assay (LFA). The latex agglutination assay has been used for several decades for the detection of cryptococcal antigen and has a higher sensitivity and specificity than India ink staining.^{52,53} Latex particles are coated with anticryptococcal antibodies, and in the presence of cryptococcal antigen will agglutinate, forming visible clumps that are subjectively read on a predefined scale. The sensitivity and specificity of the LA tests vary with manufacturer and the use of pronase.^{54,55} A study comparing four commercially available LA tests and an EIA test to culture found a serum sensitivity of LA that ranged from 83 to 97%.⁵⁴ The tests with the lowest sensitivity did not use pronase on serum specimens. The specificity of the LA on serum ranged from 93 to 100%. The CSF sensitivity and specificity of the LA test was high and ranged from 93 to 100%, while the specificity range was 93 to 98%. The sensitivity and specificity on EIA testing of CSF was 100% and 98%, respectively.^{54,56} The sensitivity and specificity of the EIA on serum was 93% and 96%, respectively. Although the LA performs well when compared with EIA and culture,^{54,56} its primary limitation is that it is a cumbersome manual test with subjectivity in the interpretation of the result. The test also requires laboratory equipment and refrigeration of reagents, making it unsuitable for use in remote resource limited settings.

In 2009, a rapid point of care diagnostic test known as the lateral flow assay (LFA) was developed. The Cryptococcal antigen (CrAg) LFA is a capillary flow sandwich immunochromatographic assay that can be done as a point of care test. Gold-conjugated anticryptococcal monoclonal antibodies and control goat IgG antibodies are deposited on a membrane on the test strip. Specimen diluent is added to a tube to which 40 μ L of the patient specimen is added. The test strip is added to the tube and incubated at room temperature for 10 minutes before the result is read. The specimen migrates by capillary flow up the test strip, and in the presence of cryptococcal antigen will bind to the anticryptococcal monoclonal anti-

bodies. The bound antibodies will continue to flow up the dipstick to the detection lines. The first detection line contains immobilized anticryptococcal monoclonal antibodies, and the distal line contains immobilized bovine anti-goat IgG antibodies. A gold-conjugated anticryptococcal antibody that is bound to cryptococcal antigen will bind to the first detection line containing anticryptococcal antibody, creating a sandwich that is detected as a visible line at the test line site. The control goat IgG antibodies will continue to migrate up the test strip and bind to the bovine anti-goat IgG antibodies and be detected as a visible line in the control test line. A positive test is indicated by the presence of two lines (the test line and the control line); a negative test is indicated by the presence of the control line only. An invalid test is indicated by the absence of a control line. The sensitivity and specificity of the lateral flow assay is very high, with high levels of concordance with EIA and latex agglutination assays.^{57–59} In a study using archived specimens in Thailand, the LFA was positive in all blood culture positive specimens and had strong concordance with the EIA on testing of serum.⁵⁸ The LFA has also been shown to have high sensitivity in detection of all serotypes (A–D).^{60,61} The test has been approved by the Food and Drug Administration for use on serum and CSF.

The introduction of the lateral flow assay has the potential to revolutionize cryptococcal diagnosis in resource limited settings.⁶² It is as simple to perform as a urine pregnancy test, and therefore does not require trained laboratory personnel. It can be performed in the field and does not require sophisticated laboratory equipment, refrigeration and/or centrifugation.

Treatment of Cryptococcal Meningitis

In the absence of therapy cryptococcal meningoencephalitis is uniformly fatal.⁴ Early diagnosis and prompt treatment is critical to improve survival. The classes of antifungal drugs that have activity against *Cryptococcus* are the polyenes (amphotericin B formulations), the azoles, and flucytosine. Treatment of cryptococcal meningitis typically consists of a 2-week induction phase of therapy followed by 8 weeks of consolidation therapy, and additional maintenance therapy that acts as secondary prophylaxis against recurrence. The recommended treatment regimens are indicated in ► **Table 3**.

Combination therapy with amphotericin B and flucytosine was established as the superior regimen in the mid-1990s, and globally is the preferred regimen.^{63–65} However, both amphotericin B and flucytosine are associated with significant toxicities that included renal toxicity, anemia, and neutropenia, and require intravenous administration and monitoring of toxicities. In much of sub-Saharan Africa and parts of Asia flucytosine is either not registered or is too expensive for clinical use. Amphotericin B is not often available, particularly in remote settings far from central hospitals. The cost of amphotericin B, the monitoring and management of its associated toxicities, and the prolonged hospital admission make it an expensive treatment option

Table 3 Preferred treatment regimens for cryptococcal meningoencephalitis by different guidelines committees

	WHO ⁸⁹	SA HIV Clinicians ⁹⁰	IDSA ⁶⁷	IAS-USA ⁹¹
Preferred	Induction 2 wk	AmBd 0.7–1 mg/kg/d + fluconazole 100 mg/kg/d	AmBd 0.7–1 mg/kg/d + flucytosine 100 mg/kg/d ^a	LAmB 3–4 mg/kg/d + flucytosine 25 mg/kg qid
	Consolidation 8 wk	Fluconazole 400–800 mg/d	Fluconazole 400 mg/d	Fluconazole 400 mg/d
	Maintenance	Fluconazole 200 mg/d	Fluconazole 200 mg/d	Fluconazole 200 mg/d
Alternate regimen	Induction 2 weeks	N/A	AmBd 0.7–1 mg/kg/d or LAmB 3–4 mg/kg/d or ABLC 5 mg/kg/d (for 4–6 wk)	ABLC 5 mg/kg/d + flucytosine 25 mg/kg qid
	Consolidation 2 weeks	Fluconazole 400–800 mg/d	Not indicated	Fluconazole 400 mg/d
	Maintenance	Fluconazole 200mg/d	Fluconazole 200 mg/d	Fluconazole 200 mg/d
2 nd Alternate regimen	Induction 2 weeks	AmBd 0.7–1 mg/kg/d for 5–7 d + fluconazole 800 mg/d for 2 wk	AmBd + fluconazole 800 mg/d	AmBd 0.7–1 mg/kg/d + flucytosine 25 mg/kg qid
	Consolidation 8 wk	Fluconazole 800 mg/d	Fluconazole 800 mg/d	Fluconazole 400 mg/d
	Maintenance	Fluconazole 200 mg/d	Fluconazole 200 mg/d	Fluconazole 200 mg/d
3 rd Alternate regimen	Induction 2 wk	Fluconazole 1200 mg/d + flucytosine 100 mg/kg/d	Fluconazole 1200 mg/d + flucytosine 100 mg/kg/d for 6 wk	LAmB 3–4 mg/kg/d + fluconazole 800 mg/d
	Consolidation 8 wk	Fluconazole 800 mg/d	Not indicated	Fluconazole 400 mg/d
	Maintenance	Fluconazole 200 mg/d	Fluconazole 200 mg/d	Fluconazole 200 mg/d
4 th Alternate regimen	Induction 2 wk	Fluconazole 1200 mg/d	Fluconazole (800–2000 mg/ d) for 10–12 wk (≥ 1200 mg/ d is preferred)	AmBd (0.7–1 mg/kg/d) + fluconazole 800 mg/d
	Consolidation 8 wk	Fluconazole 800 mg/d	N/A	Fluconazole 400 mg/d
	Maintenance	Fluconazole 200 mg/d	Fluconazole 200 mg/d	Fluconazole 200 mg/d

(Continued)

Table 3 (Continued)

		WHO ⁸⁹	SA HIV Clinicians ⁹⁰	IDSA ⁶⁷	IAS-USA ⁹¹
5 th Alternate regimen	Induction 2 wk	N/A	N/A	Itraconazole 200 mg twice daily for 10–12 wk Use of this regimen discouraged.	LAmB 3–4 mg/kg/d alone
	Consolidation 8 wk				
	Maintenance				Fluconazole 400 mg/d
6 th Alternate regimen	Induction 2 wk	N/A	N/A	N/A	Fluconazole 200 mg/d
	Consolidation 8 wk				Fluconazole 400–800 mg/d + Flucytosine 25 mg/kg qid
	Maintenance				Fluconazole 400 mg/d
7 th Alternate Regimen	Induction 2 wk	N/A	N/A	N/A	Fluconazole 200 mg/d
	Consolidation 8 wk				Fluconazole 1200 mg/d
	Maintenance				Fluconazole 400 mg/d
					Fluconazole 200 mg/d

Abbreviations: HIV, human immunodeficiency virus; IAS-USA, International AIDS Society–United States; IDSA, Infectious Diseases Society of America; N/A, not applicable; qid; four times daily; WHO, World Health Organization.

^aLAmBd can be substituted for LAmB(3–4mg/kg/d) or AmB lipid complex ABLC 5 mg/kg/d. Different formulations of amphotericin B are designated as: AmBd = Amphotericin B deoxycholate; LAmB = liposomal amphotericin B, ABLC = amphotericin B lipid complex.

Table 4 Key studies evaluating different antifungal regimens for the treatment of cryptococcal meningitis in AIDS patients

Author year	Study design (N)	Induction regimen	Consolidation and maintenance regimen	10-wk mortality	Outcome summary
Amphotericin B with flucytosine					
Van der Horst et al, 1997 ⁹²	Double blind multicenter RCT. United States.	AmB (0.7 mg/kg/d) × 2 wk (N = 179)	Fluconazole 400 mg/d	9.4%	Higher rates of CSF clearance in the group receiving AmB + 5FC however not statistically significant (51% vs. 60%, <i>p</i> = 0.06).
		AmB (0.7mg/kg/d) + 5-FC (100mg/kg/d) × 2 wk (N = 202)	Itraconazole 400 mg/d × 8 wk		
Bicanic et al, 2008 ⁶⁸	Randomized single-center study. South Africa	AmB (0.7 mg/kg/d) + 5-FC (100 mg/kg/d) (N = 30)	Fluconazole 400 mg/d × 8 wk followed by 200mg daily	21%	Greater early antifungal activity with high dose amphotericin compared with standard dose amphotericin B. There was no difference in survival between the two groups.
		AmB (1 mg/kg/d) + 5-FC (100mg/kg/d) (N = 34)		26%	
Liposomal amphotericin B					
Hamill et al, 2010 ⁹³	Double blind RCT. Multicenter study. USA and Canada.	AmB 0.7 mg/kg/d × 11–21 d (N = 87)	Fluconazole 400 mg/d till completion of 10 wk of antifungal therapy	11.5%	Fungal CSF clearance at 2 and 10 wk, clinical improvement and mortality was not significantly different across all 3 treatment groups. Safety and tolerance and nephrotoxicity was decreased in those receiving the L-AmB doses compared with Amb
		LAmB 3mg/kg/d × 11–21d (N = 86)		9.6%	
		LAmB 6mg/kg/d × 11–21 d (N = 94)		14%	
Amphotericin B only compared with Amphotericin B with fluconazole or voriconazole					
Brouwer et al, 2004 ⁹⁴	Randomized single-center study. Thailand	AmB (0.7 mg/kg/d) × 2 wk (N = 16)	Fluconazole 400 mg × 8 wk followed by 200 mg daily	19%	Mortality associated with high baseline fungal burden, seizures and decreased level of consciousness. AmB + 5-FC had significantly higher early fungicidal activity than the other combinations. Sample size too small to find a significant survival benefit of any regimen.
		AmB (0.7 mg/kg/d) + 5-FC (100 mg/kg/d) × 2 wk (N = 16)		7%	
		AmB (0.7 mg/kg/d) + Fluconazole (400 mg/d) × 2 wk (N = 16)		44%	
		AmB (0.7mg/kg/d) + 5-FC (100 mg/kg/d) + Fluconazole (400 mg/d) × 2 wk (N = 16)		19%	

(Continued)

Table 4 (Continued)

Author year	Study design (N)	Induction regimen	Consolidation and maintenance regimen	10-wk mortality	Outcome summary
Pappas et al, 2009 ⁹⁵	Phase II open label multicenter randomized trial to determine safety and tolerability of the combination therapy regimens. US and Thailand	AmB (0.7 mg/kg/d) × 2 wk (N = 47)	Fluconazole 400 mg/d × 8 wk	22.2%	In patients with CSF OP > 250 mortality was higher in the AmB arm only compared with the combination therapy arms at 32%, 19%, and 18%, respectively. Trend toward improved survival in the AmB + Fluc 800 mg arm compared with the AmB only, though not significant.
		AmB (0.7 mg/kg/d) + Fluconazole 400 mg/d × 2 wk (N = 48)	Fluconazole 400 mg/d × 8 wk	17%	
		AmB (0.7 mg/kg/d) + Fluconazole 800 mg/d × 2 wk (N = 48)	Fluconazole 800 mg/d × 8 wk	18.4%	
Loyse et al, 2012 ⁹⁶	Randomized study, 2 sites, South Africa	AmB (0.7–1 mg/kg/d) + 5-FC (100 mg/kg/d) × 2 wk (N = 21)	Fluconazole 400 mg/d × 8 wk then 200 mg daily	30%	There was no significant difference in mortality or early fungicidal activity between all four groups
		AmB (0.7–1 mg/kg/d) + Fluconazole 800 mg/d × 2 wk (N = 22)		33.3%	
		AmB (0.7–1 mg/kg/d) + Fluconazole 600 mg twice daily × 2 wk (N = 24)		27.3%	
		AmB (0.7–1 mg/kg/d) + Voriconazole 300 mg twice daily × 2 wk (N = 13)		25%	
Day et al, 2013 ⁹⁷	Randomized single-center trial. Vietnam	AmB (1 mg/kg/d) × 4 wk	Fluconazole 400 mg/d × 6 wk	43%	Higher mortality in the AmB only group compared with the other groups at 10 wk. The greatest survival benefit was with the use of AmB + 5-FC, and this benefit was sustained through till 6 months. Fewer deaths at 10 wk occurred in the AmB + Fluconazole group than in the AmB only group. Faster rate of clearance in AmB/5-FC group

Table 4 (Continued)

Author year	Study design (N)	Induction regimen	Consolidation and maintenance regimen	10-wk mortality	Outcome summary
Bicanic et al, 2007 ⁹⁸	Prospective observational study. Single-center study. South Africa	AmB (1 mg/kg/d) + 5-FC (100 mg/kg/d) × 2 wk	Fluconazole 400 mg/d × 8 wk	28%	Median survival was higher in those treated with AmB compared with those receiving fluconazole monotherapy (153 d vs 61 d, $p = 0.03$)
		AmB (1 mg/kg/d) + Fluconazole 400 mg twice daily × 2 wk	Fluconazole 400 mg/d × 8 wk	31%	
		AmB (1 mg/kg/d) × 7 d (N = 49) Fluconazole 400 mg/d × 10 wk (N = 5)	Fluconazole 400 mg/d × 8 wk then 200 mg daily	37%	
Muzoora et al, 2012 ⁹⁹	Prospective observational study. Single center study in Uganda.	AmB (1 mg/kg/d) × 5 d + Fluconazole 1200 mg/d × 2 wk (N = 30)	Fluconazole 800 mg/d X 2 wk then 400 mg/d × 6 wk followed by 200 mg daily	28%	Noted continued CSF clearance despite discontinuation of amphotericin B at 5 d of therapy.
Short-course amphotericin with fluconazole ± flucytosine					
Jackson et al, 2012 ¹⁰⁰	Randomized single-site study. Malawi	Fluconazole 1200mg/d × 2 wk + AmB (1 mg/kg/d) × 7 d (N = 21)	Fluconazole 800 mg/d × 2 wk then Fluconazole 400mg/d × 6 wk then Fluconazole 200 mg daily	30%	Higher rate of CSF clearance in the AmB + Fluc + 5FC arm than in the AmB + Fluconazole. No significant difference in survival – study not adequately powered.
		Fluconazole 1200 mg/d + 5-FC (100 mg/kg/d) × 2 wk plus AmB (1 mg/kg/d) × 7d (N = 22)		35%	
Fluconazole flucytosine compared with fluconazole only					
Nussbaum et al, 2010 ¹⁰¹	Randomized single center study. Malawi	Fluconazole 1200 mg/d × 2 wk (N = 20)	Fluconazole 800 mg/d × 2 wk, then Fluconazole 400 mg/d × 6 wk, followed by 200 mg daily	43%	No statistically significant difference in mortality at 2 and 10 wk. Significant difference in early fungicidal activity. Combination therapy had higher rates of early fungicidal activity as compared with fluconazole monotherapy.
		Fluconazole 1200 mg/d + 5-FC (100 mg/kg/d) × 2 wk (N = 21)		58%	

(Continued)

Table 4 (Continued)

Author year	Study design (N)	Induction regimen	Consolidation and maintenance regimen	10-wk mortality	Outcome summary
High-dose fluconazole monotherapy					
Longley et al, 2008 ¹⁰²	Prospective cohort study. (N = 60)	Fluconazole 800 mg/d × 2 wk (N = 30)	Fluconazole 400 mg/d × 8 wk then 200 mg/d	60%	Rapid rate of CSF clearance at higher doses of fluconazole. No significant difference in mortality between the 2 groups.
		Fluconazole 1200 mg/d (N = 30)	Fluconazole 400 mg/d × 8 wk then 200 mg/d	48%	

Abbreviations: AIDS, acquired immunodeficiency syndrome; CSF, cerebrospinal fluid; Note. All studies prior to the use of high dose amphotericin B and studies containing largely non-HIV patients have been excluded.

in resource-limited settings. Several studies have assessed the efficacy of alternative regimens that either contain Amphotericin B alone, amphotericin with fluconazole, fluconazole with flucytosine, fluconazole monotherapy or short courses of amphotericin B. The results of the key studies are summarized in ►Table 4. The greatest limitation is that most of the studies are small, single center studies that are not adequately powered to assess mortality benefit of a given regimen. Most patients in sub-Saharan Africa continue to receive induction therapy with fluconazole monotherapy despite the associated high mortality rates.⁶⁶

In addition to antifungal therapy, optimal management of raised intracranial pressure is required to reduce morbidity and mortality. Patients who have CSF opening pressures ≥ 25 cm of H₂O should undergo repeat lumbar puncture until CSF pressures have normalized. Daily lumbar punctures may be required. If pressures remain persistently elevated a lumbar drain should be considered.⁶⁷

Management of Toxicities Associated with Cryptococcal Meningitis Treatment

Amphotericin has significant associated toxicities. These include headaches, chills, fever, and some local site reactions that can be minimized by the use of analgesics as well as slow infusion of amphotericin B over 4 to 6 hours. Renal toxicities are common and may result in significant decreases in glomerular filtration rate, necessitating early termination of the drug. Renal dysfunction will typically resolve after discontinuation of amphotericin B.⁶⁸ Other renal associated toxicities include hypokalemia and hypomagnesaemia. Ample prehydration with isotonic fluid is recommended to minimize amphotericin-induced nephrotoxicity. Monitoring of electrolytes should be done at least twice weekly, with appropriate replacement of potassium and magnesium. Anemia is another common side effect of amphotericin B therapy,⁶⁹ and monitoring of hemoglobin at least once a week during amphotericin B therapy is recommended. Flucytosine can lead to marrow toxicity resulting in neutropenia, thrombocytopenia, anemia, or pancytopenia. Discontinuation of the drug will often result in resolution. Finally, interactions between therapies directed against cryptococcal meningitis and those targeted to treat comorbidities and other infections (i.e., tuberculosis) pose significant challenges to optimal pharmacologic therapy and must be monitored.

Duration of Maintenance Therapy

Fluconazole maintenance therapy is recommended to prevent recurrence.⁷⁰ Maintenance therapy should be given with fluconazole 200 mg orally once daily. Maintenance therapy or secondary prophylaxis should be continued until immune reconstitution and viral suppression have been achieved.^{71,72} Typically prophylaxis should be continued until the viral load is suppressed and the CD4 count is > 200 cells/mm³ for at least 3 to 6 months.

Diagnosis and Treatment of Cryptococcal Immune Reconstitution Inflammatory Syndrome

Cryptococcal immune reconstitution inflammatory syndrome (IRIS) can occur in 8 to 49% of individuals with HIV starting ART.⁷³ IRIS can occur as a paradoxical reaction in those with known disease who are either currently on effective therapy or who have previously successfully completed treatment. It can also occur in the setting of “unmasking” previously undiagnosed subclinical cryptococcal disease. IRIS likely occurs as a result of a dysregulated immune response to cryptococcal antigens.⁷⁴ The risk factors for developing IRIS include high fungal burden, low baseline CD4 count, early introduction of antiretroviral therapy prior to sufficient CSF sterilization, and initial rapid decline in viral load.^{75,76} The syndrome can occur weeks to months following initiation of ART and can be associated with significant morbidity and mortality. Treatment for IRIS includes continuation of ART and antifungal therapy, and in severe cases corticosteroids may be considered.⁶⁷

Timing of ART in Cryptococcal Disease

The exact timing of ART initiation in patients with cryptococcosis and HIV coinfection is yet to be determined. In patients with opportunistic infections such as *Pneumocystis jiroveci*, delayed initiation of ART is associated with higher rates of AIDS progression and/or death than early initiation of ART.⁷⁷ Early initiation of ART in patients with tuberculosis is associated with increased risk of IRIS, but decreased mortality, particularly in those with low CD4 counts.^{78–80} In patients with tuberculous meningitis, early ART initiation is not associated with improved clinical outcomes.⁸¹ Early initiation of ART in patients with cryptococcal meningitis is associated with poor clinical outcomes. In a study conducted in Zimbabwe, patients were randomized to early initiation of ART (within 72 h of diagnosis) or delayed initiation (at 10 wk). Patients who were initiated on ART early had significantly higher 3-year mortality than those in whom ART was delayed (88% vs. 54%, $p = 0.006$). All patients were treated with fluconazole monotherapy at 800 mg once daily.⁸² Early ART was also associated with increased mortality in Ugandan patients with cryptococcal meningitis treated with amphotericin B therapy who were randomized to early ART initiation within 1 to 2 weeks of starting antifungal therapy compared with those in whom ART was delayed and initiated at 5 to 6 weeks (D Bouleware, CROI 2013⁸³). Most experts would recommend delaying initiation of ART in patients with cryptococcal meningitis and HIV infection for at least 4 weeks, particularly in patients who are treated with fluconazole monotherapy.

Prevention of Cryptococcal Disease

The greatest burden of cryptococcal disease is in resource limited settings where it is associated with HIV infection in severely immunosuppressed individuals with CD4 counts

< 100 cells/mm³.⁸⁴ Significant reductions in cryptococcal disease incidence can be achieved by decreasing HIV incidence, improving access to ART, and initiation of ART at higher CD4 counts. In individuals who access care late at CD4 T cell counts below 100 cells/mm³, screening for cryptococcal disease by cryptococcal antigen testing, exclusion of cryptococcal meningitis in those who screen positive, and pre-emptive treatment for asymptomatic cryptococcal disease may decrease cryptococcal associated morbidity and mortality.^{85,86} However, implementing these strategies prospectively has yet to show a mortality benefit.⁸⁷ Where available, all individuals with CD4 counts < 100 cells/mm³ who are being evaluated for ART initiation should be screened for cryptococcal disease and offered appropriate therapy, although evidence for optimal therapy for asymptomatic disease detected by screening is lacking.⁸⁸

In conclusion, cryptococcal meningitis remains one of the leading causes of AIDS associated death, and one of the most common fungal mycoses among immunocompromised individuals. Significant progress has been made in simplifying the diagnosis of cryptococcosis and thereby enabling diagnostic testing to be available in resource limited settings with limited laboratory infrastructure. Widespread implementation of screening programs in some sub-Saharan countries will hopefully lead to substantial decreases in the number of infected people. Further research now needs to focus on identifying cheaper, less toxic, oral antifungal agents that are as efficacious as amphotericin B and flucytosine in reducing cryptococcal associated mortality. In the interim, registration of, reducing the cost of, and increasing access to amphotericin B and flucytosine must become a priority for governments in sub-Saharan Africa.

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