Cryptococcal Antigenemia in Advanced HIV: Pathophysiology, Epidemiology and Clinical Implications

Rachel M. Wake[1,2], Síle F. Molloy[1,3], Joseph N. Jarvis[4,5], Thomas S. Harrison[1,2,6,], Nelesh P. Govender[1,3,6,7,8]

1. Institute for Infection and Immunity, St George's University of London, London, United Kingdom
2. Clinical Academic Group in Infection and Immunity, St George's University Hospitals NHS Foundation Trust, London, United Kingdom
3. Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa
4. Department of Clinical Research, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom
5. Botswana Harvard AIDS Institute Partnership, Gaborone, Botswana
6. MRC Centre for Medical Mycology, University of Exeter, Exeter, United Kingdom
7. Centre for Healthcare-Associated Infections, Antimicrobial Resistance and Mycoses, National Institute for Communicable Diseases, a Division of the National Health Laboratory Service, Johannesburg, South Africa
8. Division of Medical Microbiology, University of Cape Town, South Africa; University of Cape Town, South Africa

Corresponding author: Dr Rachel Wake, Institute for Infection and Immunity, St George's University of London, Cranmer Terrace, London, SW17 0RE, United Kingdom; Email rmwake@gmail.com

Running title Cryptococcal Antigenemia: A Review

Abstract

Cryptococcal antigen (CrAg) is detectable in blood prior to the onset of symptomatic cryptococcal meningitis, a leading cause of death among people living with advanced HIV disease globally. Highly sensitive assays can detect CrAg in blood, and screening people living with HIV with low CD4 counts, followed by pre-emptive antifungal treatment, is recommended and widely implemented as part of a global strategy to prevent cryptococcal meningitis and end cryptococcal-related deaths. Cryptococcal antigenemia encompasses a spectrum of conditions from pre-clinical asymptomatic infection (CSF CrAg-negative), through subclinical (CSF CrAg-positive without overt meningism) to clinical symptomatic cryptococcal disease, usually manifesting as cryptococcal meningitis. This review summarizes current understanding of the pathophysiology, risk-factors for and clinical implications of cryptococcal antigenemia among people living with advanced HIV disease within this spectrum. It also provides an update on global prevalence, recommended screening and treatment strategies, and future considerations for improving outcomes among patients with cryptococcal antigenemia.

Key words: meningitis, cryptococcal; cryptococcosis; diagnostic screening programs; acquired immunodeficiency syndrome; HIV

Introduction

HIV-associated cryptococcal meningitis (CM) is responsible for over 180,000 deaths per year, with 75% occurring in African countries,(1). Cryptococcal antigen (CrAg) is detectable in blood prior to the onset of symptoms(2). Screening blood for CrAg and pre-emptive treatment of those who test CrAg-positive with fluconazole is now recommended and widely implemented, to prevent cryptococcal-related deaths among adults and adolescents living with HIV who have CD4 T-lymphocyte (CD4) counts of less than 200 cells/µL (3). This strategy was first recommended by World Health Organization (WHO) Rapid Advice in 2011(4), and has since been implemented in many high-burden countries.

Prospective data now indicate that targeted CrAg screening and pre-emptive fluconazole treatment reduces the incidence of cryptococcal meningitis and death(5). However, individuals with cryptococcal antigenemia still have a higher mortality risk than comparable individuals without antigenemia, despite anti-fungal treatment(5–7). The pathophysiological mechanism underlying this increased risk of death is not fully understood. However, a recent prospective study found that >70% of deaths were cryptococcal-related suggesting that fluconazole monotherapy is inadequate treatment(7). This review summarizes our current understanding of cryptococcal antigenemia, including susceptibility and pathophysiology of associated clinical conditions. It also provides an update on global prevalence, recommended screening approaches and treatment regimens, and future considerations for improving outcomes among patients with cryptococcal antigenemia. Although cryptococcosis occurs in the context of other immune defects, and less commonly in apparently-immunocompetent individuals, this review will focus on cryptococcal antigenemia among people living with advanced HIV disease, the main population affected by cryptococcosis.

Pathophysiology

Cryptococcus and Cryptococcal Antigen (CrAg)

Cryptococcus neoformans and *Cryptococcus gattii* are species-complexes of pathogenic yeasts that are ubiquitous in the environment and responsible for invasive cryptococcal disease or cryptococcosis. These fungi are commonly found in the decaying matter of soil and certain tree species, and avian excreta. Their survival in the environment is facilitated by a large gelatinous polysaccharide capsule made up of glucuronoxylomannan (90%-95%), galactoxylomannan (5%), and mannoproteins (<1%) (8).

Cryptococcal antigen is the term used for the predominant component of the cryptococcal capsule, glucuronoxylomannan. Biological fluid samples (blood, cerebrospinal fluid [CSF], pre-treated urine) can be tested for CrAg by latex agglutination (LA) tests, enzyme-linked immunosorbent assays (EIA) and lateral flow assays (LFA). The detection of CrAg in CSF samples is an accurate tool for diagnosing a first episode of cryptococcal meningitis, particularly in settings where laboratory facilities are limited(9). The Immuno-Mycologics (IMMY, Norman, OK) LFA is currently most widely used for CrAg screening. It uses two monoclonal antibodies, making it broadly reactive with all cryptococcal serotypes, encompassing both *C. neoformans* and *C. gattii* species-complexes, and is more sensitive than LA tests or EIA(9). Validation studies have found excellent concordance when using the LFA on serum or plasma, compared to CSF culture in patients with culture-confirmed cryptococcal meningitis(9). The IMMY CrAg LFA is also low cost, rapid and simple to use, enabling testing at the point of care, rather than in the laboratory(10).

Etiology of cryptococcal antigenemia

Pathogenic cryptococci are ubiquitous and therefore exposure is common, probably near universal(11), through inhalation of desiccated yeast cells or basidiospores. Following inhalation, cryptococcal cell wall components are recognized by pattern recognition receptors (PRRs) on

immune cells triggering an innate immune response, including phagocytosis by alveolar macrophages, and granuloma formation. Since cryptococci are able to survive intracellularly following phagocytosis, they can evade effective immune responses, and reside latently in immunocompetent hosts(12).

In the context of immunosuppression, cryptococcal antigenemia likely occurs as a result of reactivation, rather than new infection through exposure to the fungus in the environment. When host immunity fails to suppress intracellular proliferation, fungal cells are released by cell lysis or vomocytosis (a non-lytic mechanism that avoids triggering a significant immune response), and disseminated hematogenously(13). It may be at this stage that antigen becomes detectable in blood. The initial lack of symptoms among patients with antigenemia might be due to low fungal burden and/or minimal inflammatory responses, particularly in the context of profound immune suppression.

Epidemiology in Advanced HIV

The global prevalence of cryptococcal antigenemia is estimated to be around 6% among adultswith CD4 counts ≤100(1) cells/μL and 2% among adults with CD4 counts 101-200 cells/μL(14). Although cryptococcal antigenemia is associated with lower CD4 counts(2), and prevalence varies geographically(1), no other demographic or environmental risk factors have been identified. Prior TB has been identified as a possible clinical risk factor for cryptococcal antigenemia (15), suggesting that a shared immunological defect, or prolonged duration of immune suppression may play a role in susceptibility to cryptococcal antigenemia.

Genetic Susceptibility to Cryptococcal Antigenemia

The occurrence of cryptococcal antigenemia in a relatively small subset of those at risk with advanced HIV disease, despite likely universal exposure, suggests a genetic predisposition to cryptococcosis. In people who are HIV-seronegative, FcγR and mannose-binding lectin (MBL)

polymorphisms may be important in cryptococcosis susceptibility(16,17). Among people living with HIV (mostly Caucasian males), targeted PCR-based genotyping identified the FcγR3A 158V allele as a risk factor, with homozygous expression conferring 21 times the risk of cryptococcal disease (p = 0.005)(18). In individuals of African descent, a genome-wide association study identified six loci upstream of the colony stimulating factor 1 (*CSF1*) gene to be associated with cryptococcosis, including in those with asymptomatic cryptococcal antigenemia(19).

Clinical Implications of Cryptococcal Antigenemia

Cryptococcal antigenemia constitutes a spectrum of clinical conditions, from pre-clinical asymptomatic infection (CSF CrAg-negative) through subclinical infection (CSF CrAg-positive, India Ink microscopy, or culture positive for *Cryptococcus* spp. but without overt meningism) to clinical symptomatic infection, usually presenting as fulminant meningitis. Around a third of individuals with asymptomatic cryptococcal antigenemia, have subclinical cryptococcal meningitis(19). Additionally, comprehensive screening of 67 asymptomatic CrAg-positive patients in South Africa revealed subclinical cryptococcal infection elsewhere (blood culture growth of *Cryptococcus neoformans* in 11/67 (16%) and pulmonary cryptococcosis in 2/32 (7%) who had samplescultured(7)).

Without treatment, the detection of CrAg in the blood heralds the onset of clinical symptomatic cryptococcal meningitis, although individuals with antigenemia can remain asymptomatic for weeks to months before clinical meningitis occurs(2,21–23). In South Africa, a cohort study of 707 patients initiating antiretroviral treatment (ART) demonstrated that retrospectively-determined and thus untreated baseline cryptococcal antigenemia predicted the development of subsequent cryptococcal meningitis within a year with 100% sensitivity and 96% specificity. No cases of meningitis occurred in 294 CrAg-negative patients with CD4 counts ≤100 cells/µl, within a year of testing(2). Retrospective testing of blood samples taken from patients with HIV-associated cryptococcal meningitis in Uganda found that cryptococcal antigenemia, preceded clinical symptoms by a median of 22 days (range, 5-

234)(21). Cryptococcal infection rarely develops in patients who are initially tested CrAg-negative, occurring in 19 (1.3%) of 1519 CrAg-negative participants of a primary prophylaxis trial in Uganda(24), mostly prior to ART commencement. Immune-reconstitution may be sufficient to clear asymptomatic cryptococcal infection in some CrAg-positive individuals, as observed in 11/21 (52%) patients who started ART but not antifungal therapy and remained disease-free, most with decreasing antigen titers during the following year(2).

Management of Cryptococcal Antigenemia

In view of the predictive power of antigenemia for cryptococcal meningitis among people living with advanced HIV disease, and recognition of a pre-symptomatic window, a strategy of screening and 'pre-emptive' treatment with fluconazole has been incorporated into national and international guidelines and implemented in more than 20 high-burden countries. In 2011, WHO Rapid Advice recommended CrAg screening in high prevalence areas among ART-naïve adults with CD4 counts of <100 cells/μL, and fluconazole treatment of CrAg-positive patients with no signs or symptoms of meningitis, at a dose of 800 mg daily for two weeks, followed by 400 mg for two months, and then 200 mg for at least a year pending immune reconstitution(4). This treatment approach was based on retrospective subgroup analyses finding no cases of cryptococcal meningitis in CrAg-positive patients who received even low doses of fluconazole (100 mg or 200 mg) for other reasons(23), and evidence that higher doses are well tolerated and more effective in cryptococcal meningitis (25). In addition, modelling identified a 'screen-and-treat' approach as the dominant strategy in health economic terms (it saved lives and money) over the standard of no screening, in areas with higher CrAg prevalence(26,27).

Since the introduction of this strategy, recommendations have adapted in response to prospective screening data(14,28,29). The criteria for considering screening is now adults and adolescents with CD4 counts of <200 cells/μL, and lumbar punctures (LPs) are advised to exclude subclinical cryptococcal meningitis in all CrAg-positive patients irrespective of symptoms(3). Southern African

guidelines recommend an increased induction fluconazole dose of 1200 mg and immediate ART initiation for those with CrAg-negative CSF (Figure 1)(30). In ART-experienced individuals in Uganda, cryptococcal antigenemia was detected in 4.2% of those with viral loads ≥5000 copies/mL. CrAg screening was therefore also suggested in the context of virological failure where CD4 counts are not performed (31).

Several prospective studies have shown the CrAg screen-and-treat approach to be effective at reducing the incidence of cryptococcal meningitis(5–7,31). In a multisite trial (REMSTART) in Tanzania and Zambia, HIV-infected adults with CD4 counts of <100 cells/μL were randomized to a strategy including community support and CrAg screening with pre-emptive fluconazole for CrAg-positive patients. The intervention reduced mortality risk by nearly a third, and the authors attributed half of this risk reduction to cryptococcal meningitis prevention due to CrAg screening(5). A systematic review and meta-analysis found that pre-emptive fluconazole initiated at 800 mg in patients with asymptomatic cryptococcal antigenemia, reduced the incidence of cryptococcal meningitis from 20% to 5%(20). The importance of setting national targets to achieve CrAg screening of 95% of eligible adults is emphasized in the Strategic Framework for Ending Cryptococcal Meningitis Deaths by 2030(32).

Cryptococcal Antigenemia is Associated with an Increased Risk of Mortality

Despite prevention of clinical cryptococcal meningitis using CrAg screen-and-treat strategies, cryptococcal antigenemia remains a risk factor for death amongpeople with advanced HIV disease (Figure 2). This was observed in retrospective studies prior to the introduction of CrAg screening and pre-emptive treatment (in South Africa, adjusted HR 3.2; 95% CI, 1.5-6.6(2); in Uganda, relative risk 6.6; 95% CI 1.86 – 23.61)(21); deaths following cryptococcal meningitis were not sufficient to account for excess mortality in either study(2,21). In prospective studies using fluconazole pre-emptive treatment, subsequent diagnoses of clinical cryptococcal meningitis are rare. However,

cryptococcal antigenemia was associated with a 2 to 3-fold increased risk of death within 6 months, compared to CrAg-negative patients with similar CD4 counts(5–7).

The excess mortality risk associated with cryptococcal antigenemia despite fluconazole treatment is not well understood, but a combination of suboptimal treatment and additional disease susceptibility is likely. Fluconazole monotherapy, known to be inferior induction-phase treatment of cryptococcal meningitis, may be undertreating CrAg-positive patients with undiagnosed subclinical cryptococcal meningitis or cryptococcaemia (blood culture growth of *Cryptococcus* spp.). Subclinical cryptococcal meningitis has an estimated prevalence of 33% (95% CI 21% – 45%) among asymptomatic CrAg-positive patients by meta-analysis of 10 studies(20). However, due to limited access, and poor uptake of LPs in this population(5,6,20), subclinical meningitis is likely to remain undiagnosed in the majority of cases. Even when LPs are used to screen for subclinical cryptococcal meningitis, and appropriate combination antifungals used for those with CrAg-positive CSF, fluconazole monotherapy fails to prevent some cryptococcal-related deaths in those who do not have subclinical cryptococcal meningitis at the time of screening. A study investigating causes of death following CrAg screening and treatment in South Africa, including use of minimally-invasive autopsies, attributed 71% (12/17) of deaths to cryptococcal disease as an immediate or contributing cause, including 8 patients who were known to die with cryptococcal meningitis(7). All 4 CrAg-positive patients with post-mortem samples were CSF CrAg-positive at the time of death. All had been asymptomatic and received fluconazole, and two, who had agreed to LP, were CSF CrAg negative at the time of screening(7).Furthermore, fluconazole monotherapy was associated with in-hospital mortality of 32% in CrAg-positive patients presenting to hospital in Uganda with meningism who had CrAg-negative CSF (likely early cryptococcal meningitis)(39).

Patients with cryptococcal antigenemia may be more susceptible to other pathologies, due to an underlying immune defect beyond CD4 depletion, possibly related to genetic predisposition. Animal and human studies have demonstrated a requirement for Th1-type T-cell mediated immunity with

pro-inflammatory cytokine production for successful cryptococcal clearance and improved chances of survival(40,41). Pathogen-specific immune responses in CrAg-positive and CrAg-negative patients with similar CD4 counts have not yet been characterized and compared.

In addition to the possibility of an underlying immune defect, *Cryptococcus* itself may lead to secondary immune perturbations; capsular and call wall components have multiple immunosuppressive effects, including suppression of pro-inflammatory responses (reviewed in (8)).

Aberrant host immune responses predisposing to, or induced by, cryptococcal antigenemia, may confer susceptibility to other opportunistic infections. Retrospective studies have found associations between prior TB and cryptococcosis (15,42) suggesting a shared immune defect. A prospective cohort study found CrAg-positive patients were more likely to develop other AIDS-defining illnesses than CrAg-negative patients (HR, 2.69; 95% CI, 0.98– 7.42; P = 0.05), and autopsies reveal multiple co-pathologies with cryptococcosis(7).

In addition to biological causes of excess mortality risk, screening does not work as seamlessly in the real world as it does in clinical trials. A prospective cohort study of ~2000 individuals reflexively screened as CrAg-positive in South Africa found that only around 50% who returned for care were started on fluconazole at a median time to treatment of 8 days. Around 20% of those assessed already had clinical symptoms of cryptococcal meningitis by the time they were assessed (unpublished, N.P. Govender, D.R. Boulware).

The Clinical Significance of Cryptococcal Antigen Titers

CrAg titers are an approximate measure of fungal burden and can be measured in blood as well as in CSF. Higher blood CrAg titers at the time of screening are associated with subsequent cryptococcal meningitis and death(2,23), and with concurrent cryptococcal meningitis in symptomatic and asymptomatic patients (6,28,35,43). Although no blood CrAg titer can accurately predict meningitis and LPs are recommended, a CrAg titer of greater than 80-160 indicates increased risk and is

suggested as a proxy for identifying those who urgently require a LP, or who could be considered for empirical cryptococcal meningitis treatment in settings where LP is not possible. This will be investigated in future trials of enhanced antifungal treatments for cryptococcal antigenemia.

CrAg titers can be determined by performing IMMY CrAg LFAs on serially diluted blood samples, although this is labor-intensive and expensive. Novel quantitative assays have been developed, though variable diagnostic accuracy has been observed with the CryptoPS (Biosynex, Strasbourg, France) (sensitivity 61% - 90%, specificity 94% - 97%(44–46)) and CrAgSQ (IMMY) (sensitivity 93% - 98%, specificity 94% - 100%(46,47)). Quantification scores correlated with IMMY LFA dilutional titers, cryptococcal meningitis and mortality(44–47), although LPs remain important to accurately determine CSF CrAg-status.

Enhanced Antifungal Treatment Regimens for Cryptococcal Antigenemia

Although fluconazole monotherapy appears to reduce the incidence of clinically-apparent cryptococcal meningitis, it is not sufficient to prevent cryptococcal-related deaths among all patients with cryptococcal antigenemia, even when screening LPs are performed(5,7). An ongoing trial (ACACIA) in Uganda is testing the efficacy of single-dose liposomal amphotericin B (L-AmB) 10 mg/kg plus fluconazole for pre-emptive treatment of patients with cryptococcal antigenemia (ClinicalTrials.gov ID: NCT03945448). Amphotericin B is superior to fluconazole in cryptococcal clearance from CSF(48) and expected to be effective in asymptomatic cryptococcal antigenemia due to lower fungal burdens. A single dose of L-AmB has recently been shown to be as effective as 7 days of amphotericin B deoxycholate in combination treatment of cryptococcal meningitis, with the benefit of reduced requirements for intravenous access and fewer adverse events(49). However, even a single intravenous treatment may be costly and challenging to implement, especially in primary-care settings. Another clinical trial (EFFECT) is comparing combination fluconazole and flucytosine to the current standard of fluconazole monotherapy (https://www.isrctn.com/ISRCTN30579828). Robust evidence from the ACTA trial has shown that

combining fluconazole with flucytosine for 2 weeks was as safe and as effective as 2 weeks' intravenous amphotericin B plus flucytosine for patients presenting with symptomatic CM, with mortality halved compared to historic cohorts treated with fluconazole monotherapy(50). In South Africa, recent programmatic data have shown that flucytosine-containing induction regimens were associated with a 53% reduced in-hospital cryptococcal meningitis mortality compared to regimens without flucytosine in a real-world setting(51). Flucytosine was historically expensive and inaccessible across most of Africa but following the ACTA trial results and subsequent inclusion of flucytosine in WHO preferred induction regimens for meningitis, costs are reduced with the introduction of new generic flucytosine products.

Although both combination treatments are known to be superior to fluconazole monotherapy in cryptococcal meningitis, prior trial findings cannot be generalized to ambulatory patients with asymptomatic antigenemia with likely lower fungal burdens. Furthermore, despite the risk of cryptococcal disease progression in a proportion of CrAg-positive patients, some clear their antigenemia with prompt initiation of ART alone(2). In the REALITY trial, a package of enhanced prophylaxis including relatively low doses of fluconazole for all those with a CD4 count <100 cells/µL was associated with a reduction in cryptococcal-related mortality(52). These trials will also ascertain if there is any difference in the effect of combination antifungal treatment in individuals with higher CrAg titers. The balance of risks and benefits of more intensive antifungal therapy in the CrAg-positive population are not known, and robust data on the impacts of combined treatment are urgently required.

Summary

Cryptococcal antigenemia is an intermediate-disease stage in which host immunity prevents progression to clinically-overt disease in some patients, and fails to do so in others. Individuals with cryptococcal antigenemia are within a spectrum of pre-clinical and asymptomatic (CSF CrAg-negative), subclinical (CSF CrAg-positive, no overt meningism) or clinical cryptococcal infection,

usually fulminant cryptococcal meningitis. Blood cryptococcal antigen titer and mortality risk correlate with these clinically-recognized conditions (Figure 2). While large-scale CrAg screening programs have been initiated in high-burden countries, implementation is variable and the effectiveness of reducing mortality at a population level has yet to be demonstrated. A more nuanced approach to identifying and treating patients with antigenemia at higher risk of disease progression needs to be tested. Clinical trials are underway to test enhanced pre-emptive treatment approaches given that fluconazole monotherapy may not be adequate to prevent progressive cryptococcosis and cryptococcal-related deaths.

NOTES

Acknowledgements: thanks to Andrea Mazella for assistance with Figure 2.

Funding: This work is supported by Health Education England (HEE)/ National Institute for Health Research (NIHR) (CL-2019-16-001 to R.M.W). The views expressed in this publication are those of the author(s) and not necessarily those of the NIHR, NHS or the UK Department of Health and Social Care. This work was also supported by the NIHR through a Global Health Research Professorship (RP-2017-08-ST2-012) using UK aid from the UK Government to support global health research to J.N.J. N.P.G. was partly supported by a National Institutes of Health grant (1R01AI118511-01A1). This work was also supported by the Joint Global Health Scheme with funding from the UK Foreign, Commonwealth and Development Office, the UK Medical Research Council, the UK Department of Health and Social Care through the NIHR and Wellcome (grant ref. MR/V005731/1).

Potential conflicts of interest: RW no conflict; SM no conflict; JJ no conflict and reports grants or contracts from EDCTP and CDC (paid to institution), speaker fees from Gilead Sciences Inc, and the chair of DSMB for ARTIST trial, Harvest trial, and CASTLE trial; TH has received speaker fees from

Giliead sciences and Pfizer, and reports grants or contracts from MRC, WT, DFID Global Health Trials, and EFFECT Trial (paid to institution); NG no conflict. NG reports grants from NIHR, CDC, NIH, Gates Foundation, and NHLS Research Trust (paid to institution), payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from SA HIV Clinicians Society (paid to author), DSMB member for ACACIA trial, Advisory Board member for 5FC-Crypto, and Advisory Board chair for DREAMM; is the President and Council member for FIDSSA, a member of the CryptoMAG, Ambassador for GAFFI, a board member for Medical Mycology Society Nigeria, and a member for LIGHT Africa; and includes receipt of equipment, materials, drugs, medical writing, gifts or other services from Immy CrAg tests and OIDx Histoplasma antigen tests (paid to institution).

References

1. Rajasingham R, Smith RM, Park BJ, et al. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. Lancet Infect Dis. 2017;17(8):873–81.

2. Jarvis JN, Lawn SD, Vogt M, Bangani N, Wood R, Harrison TS. Screening for cryptococcal antigenemia in patients accessing an antiretroviral treatment program in South Africa. Clin Infect Dis. 2009 Apr;48(7):856–62.

3. World Health Organization. Consolidated guidelines on HIV prevention, testing, treatment, service delivery and monitoring: recommendations for a public health approach [Internet]. 2021 update. Geneva: World Health Organization; 2021 [cited 2022 May 19]. Available from: https://apps.who.int/iris/handle/10665/342899

4. WHO. Rapid Advice. Diagnosis, prevention and management of cryptococcal disease in HIV-infected adults, adolescents and children. Geneva, Switzerland: WHO; 2011.

5. Mfinanga S, Chanda D, Kivuyo SL, et al. Cryptococcal meningitis screening and community-based early adherence support in people with advanced HIV infection starting antiretroviral therapy in Tanzania and Zambia: an open-label, randomised controlled trial. The Lancet. 2015 May;385(9983):2173–82.

6. Longley N, Jarvis JN, Meintjes G, et al. Cryptococcal antigen screening in patients initiating ART in South Africa: a prospective cohort study. Clin Infect Dis. 2016 Mar 1;62(5):581–7.

7. Wake RM, Govender NP, Omar T, et al. Cryptococcal-related Mortality Despite Fluconazole Preemptive Treatment in a Cryptococcal Antigen Screen-and-Treat Program. Clin Infect Dis. 2019 Jun 8;1683–90.

8. May RC, Stone NRH, Wiesner DL, Bicanic T, Nielsen K. Cryptococcus: from environmental saprophyte to global pathogen. Nat Rev Microbiol. 2015 Dec 21;14(2):106–17.

9. Kozel TR, Bauman SK. CrAg lateral flow assay for cryptococcosis. Expert Opin Med Diagn. 2012 May;6(3):245–51.

10. Wake RM, Jarvis JN, Harrison TS, Govender NP. Brief Report: Point of Care Cryptococcal Antigen Screening. JAIDS J Acquir Immune Defic Syndr. 2018 Aug;78(5):574–8.

11. Goldman DL, Khine H, Abadi J, et al. Serologic evidence for Cryptococcus neoformans infection in early childhood. Pediatrics. 2001 May;107(5):E66.

12. Garcia-Hermoso D, Janbon G, Dromer F. Epidemiological Evidence for Dormant Cryptococcus neoformans Infection. J Clin Microbiol. 1999 Oct;37(10):3204–9.

13. Johnston SA, May RC. *Cryptococcus* interactions with macrophages: evasion and manipulation of the phagosome by a fungal pathogen: *Cryptococcus* interactions with macrophages. Cell Microbiol. 2013 Mar;15(3):403–11.

14. Ford N, Shubber Z, Jarvis JN, et al. CD4 Cell Count Threshold for Cryptococcal Antigen Screening of HIV-Infected Individuals: A Systematic Review and Meta-analysis. Clin Infect Dis. 2018 Mar 4;66(suppl_2):S152–9.

15. Wake RM, Ismail NA, Omar SV, et al. Prior Pulmonary Tuberculosis is a Risk Factor for Asymptomatic Cryptococcal Antigenemia in a Cohort of Adults living with Advanced HIV Disease. Open Forum Infect Dis. 2022 Apr 17;ofac202.

16. Ou XT, Wu JQ, Zhu LP, et al. Genotypes Coding for Mannose-Binding Lectin Deficiency Correlated With Cryptococcal Meningitis in HIV-Uninfected Chinese Patients. J Infect Dis. 2011 Jun 1;203(11):1686–91.

17. Hu XP, Wu JQ, Zhu LP, et al. Association of Fcγ Receptor IIB Polymorphism with Cryptococcal Meningitis in HIV-Uninfected Chinese Patients. Lafrenie R, editor. PLoS ONE. 2012 Aug 3;7(8):e42439.

18. Rohatgi S, Gohil S, Kuniholm MH, et al. Fc Gamma Receptor 3A Polymorphism and Risk for HIV-Associated Cryptococcal Disease. mBio. 2013 Aug 27;4(5):e00573-13-e00573-13.

19. Kannambath S, Jarvis JN, Wake RM, et al. Genome-Wide Association Study Identifies Novel Colony Stimulating Factor 1 Locus Conferring Susceptibility to Cryptococcosis in Human Immunodeficiency Virus-Infected South Africans. Open Forum Infect Dis. 2020 Nov 1;7(11):ofaa489.

20. Temfack E, Bigna JJ, Luma HN, et al. Impact of routine cryptococcal antigen screening and targeted pre-emptive fluconazole therapy in antiretroviral naive HIV-infected adults with less than 100 CD4 cells/µL: a systematic review and meta-analysis. Clin Infect Dis. 2019 Feb;68(4):688–98.

21. Liechty CA, Solberg P, Were W, et al. Asymptomatic serum cryptococcal antigenemia and early mortality during antiretroviral therapy in rural Uganda. Trop Med Int Health TM IH. 2007 Aug;12(8):929–35.

22. French N, Gray K, Watera C, et al. Cryptococcal infection in a cohort of HIV-1-infected Ugandan adults. AIDS Lond Engl. 2002 May 3;16(7):1031–8.

23. Letang E, Muller MC, Ntamatungiro AJ, et al. Cryptococcal Antigenemia in Immunocompromised Human Immunodeficiency Virus Patients in Rural Tanzania: A Preventable Cause of Early Mortality. Open Forum Infect Dis. 2015 Apr 28;2(2):ofv046.

24. Parkes-Ratanshi R, Wakeham K, et al. Primary prophylaxis of cryptococcal disease with fluconazole in HIV-positive Ugandan adults: a double-blind, randomised, placebo-controlled trial. Lancet Infect Dis. 2011 Dec;11(12):933–41.

25. Longley N, Muzoora C, Taseera K, et al. Dose response effect of high-dose fluconazole for HIV-associated cryptococcal meningitis in southwestern Uganda. Clin Infect Dis Off Publ Infect Dis Soc Am. 2008 Dec 15;47(12):1556–61.

26. Jarvis JN, Harrison TS, Lawn SD, Meintjes G, Wood R, Cleary S. Cost Effectiveness of Cryptococcal Antigen Screening as a Strategy to Prevent HIV-Associated Cryptococcal Meningitis in South Africa. Doherty TM, editor. PLoS ONE. 2013 Jul 19;8(7):e69288.

27. Meya DB, Manabe YC, Castelnuovo B, et al. Cost-Effectiveness of Serum Cryptococcal Antigen Screening to Prevent Deaths among HIV-Infected Persons with a CD4 $^{+}$ Cell Count $\leqslant$100 Cells/µL Who Start HIV Therapy in Resource-Limited Settings. Clin Infect Dis. 2010 Aug 15;51(4):448–55.

28. Wake RM, Britz E, Sriruttan C, et al. High Cryptococcal Antigen Titers in Blood Are Predictive of Subclinical Cryptococcal Meningitis Among Human Immunodeficiency Virus-Infected Patients. Clin Infect Dis. 2018 Feb 15;66(5):686–92.

29. Temfack E, Kouanfack C, Mossiang L, et al. Cryptococcal Antigen Screening in Asymptomatic HIV-Infected Antiretroviral Naïve Patients in Cameroon and Evaluation of the New Semi-Quantitative Biosynex CryptoPS Test. Front Microbiol. 2018;9:409.

30. Govender NP, Meintjes G, Mangena P, et al. Southern African HIV Clinicians Society guideline for the prevention, diagnosis and management of cryptococcal disease among HIV-infected persons: 2019 update. South Afr J HIV Med. 2019 Nov 8;20(1):16.

31. Mpoza E, Rajasingham R, Tugume L, Rhein J, Nabaggala MS, Ssewanyana I, et al. Cryptococcal antigenemia in Human Immunodeficiency Virus Antiretroviral Therapy-Experienced Ugandans With Virological Failure. CID. 2020 Oct 23;71(7):1726-1731

32. Ending Cryptococcal Meningitis Deaths by 2030 - Strategic Framework; 2021

33. Makadzange TA, Hlupeni A, Machekano R, et al. Survival following screening and preemptive antifungal therapy for subclinical cryptococcal disease in advanced HIV infection. AIDS. 2021 Oct 1;35(12):1929–38.

34. Vallabhaneni S, Longley N, Smith M, et al. Implementation and Operational Research: Evaluation of a Public-Sector, Provider-Initiated Cryptococcal Antigen Screening and Treatment Program, Western Cape, South Africa. JAIDS J Acquir Immune Defic Syndr. 2016 Jun;72(2):e37–42

35. Hurt WJ, Tenforde MW, Molefi M, et al. Prevalence and Sequelae of Cryptococcal Antigenemia in Antiretroviral Therapy–Experienced Populations: An Evaluation of Reflex Cryptococcal Antigen Screening in Botswana. Clin Infect Dis. 2021 May 18;72(10):1745–54.

36. Bozzette SA, Larsen RA, Chiu J, et al. A Placebo-Controlled Trial of Maintenance Therapy with Fluconazole after Treatment of Cryptococcal Meningitis in the Acquired Immunodeficiency Syndrome. N Engl J Med. 1991 Feb 28;324(9):580–4

37. Rothe C, Sloan DJ, Goodson P, et al. A Prospective Longitudinal Study of the Clinical Outcomes from Cryptococcal Meningitis following Treatment Induction with 800 mg Oral Fluconazole in Blantyre, Malawi. PLOS ONE. 2013 Jun 28;8(6):e67311.

38. Kanyama C, Molloy SF, Chan AK, et al. One-year Mortality Outcomes From the Advancing Cryptococcal Meningitis Treatment for Africa Trial of Cryptococcal Meningitis Treatment in Malawi. Clin Infect Dis. 2020 Jan 16;70(3):521–4.

39. Ssebambulidde K, Bangdiwala AS, Kwisera R, Kandole TK, Tugume L, Kiggundu R, et al. Symptomatic Cryptococcal Antigenemia Presenting as Early Cryptococcal Meningitis With Negative Spinal Fluid Analysis. CID. 2019 May 30;68(12):2094-2098

40. Wormley FL, Perfect JR, Steele C, Cox GM. Protection against Cryptococcosis by Using a Murine Gamma Interferon-Producing Cryptococcus neoformans Strain. Infect Immun. 2007 Mar 1;75(3):1453–62.

41. Jarvis JN, Casazza JP, Stone HH, et al. The Phenotype of the Cryptococcus-Specific CD4+ Memory T-Cell Response Is Associated With Disease Severity and Outcome in HIV-Associated Cryptococcal Meningitis. J Infect Dis. 2013 Jun 15;207(12):1817–28.

42.Jarvis JN, Harrison TS, Corbett EL, Wood R, Lawn SD. Is HIV-associated tuberculosis a risk factor for the development of cryptococcal disease?: AIDS. 2010 Feb;24(4):612–4.

43Rajasingham R, Wake RM, Beyene T, Katende A, Letang E, Boulware DR. Cryptococcal Meningitis Diagnostics and Screening in the Era of Point-of-Care Laboratory Testing. Kraft CS, editor. J Clin Microbiol. 2019 Jan 1;57(1):e01238-18.

44. Blasich NP, Wake RM, Rukasha I, Prince Y, Govender NP. Association of semi-quantitative cryptococcal antigen results in plasma with subclinical cryptococcal meningitis and mortality among patients with advanced HIV disease. Med Mycol. 2021 Oct 4; 59(10):1041-1047.

45. Tenforde MW, Boyer-Chammard T, Muthoga C, et al. Diagnostic Accuracy of the Biosynex CryptoPS Cryptococcal Antigen Semiquantitative Lateral Flow Assay in Patients with Advanced HIV Disease. Diekema DJ, editor. J Clin Microbiol. 2020 Dec 17;59(1): e02307-20

46. Skipper C, Tadeo K, Martyn E, et al. Evaluation of Serum Cryptococcal Antigen Testing Using Two Novel Semiquantitative Lateral Flow Assays in Persons with Cryptococcal Antigenemia. J Clin Microbiol. 2020 Mar 25;58(4):e02046-19.

47. Jarvis JN, Tenforde MW, Lechiile K, et al. Evaluation of a Novel Semiquantitative Cryptococcal Antigen Lateral Flow Assay in Patients with Advanced HIV Disease. Hanson KE, editor. J Clin Microbiol. 2020 Aug 24;58(9):e00441-20

48. Bicanic T, Meintjes G, Wood R, et al. Fungal burden, early fungicidal activity, and outcome in cryptococcal meningitis in antiretroviral-naive or antiretroviral-experienced patients treated with amphotericin B or fluconazole. Clin Infect Dis. 2007 Jul 1;45(1):76–80.

49. Jarvis JN, Lawrence DS, Meya DB, et al. Single-Dose Liposomal Amphotericin B Treatment for Cryptococcal Meningitis. N Engl J Med. 2022 Mar 24;386(12):1109–20.

50. Molloy SF, Kanyama C, Heyderman RS, et al. Antifungal Combinations for Treatment of Cryptococcal Meningitis in Africa. N Engl J Med. 2018 Mar 15;378(11):1004–17.

51. Mashau R, Meiring S, Quan V, et al. Outcomes of flucytosine-containing combination treatment for cryptococcal meningitis in a South African national access programme. 2022. Lancet Infect Dis. 2022;In press.

52. Hakim J, Musiime V, Szubert AJ, et al. Enhanced Prophylaxis plus Antiretroviral Therapy for Advanced HIV Infection in Africa. N Engl J Med. 2017 Jul 20;377(3):233–45.

FIGURE LEGENDS

Figure 1 Cryptococcal antigen screening and treatment algorithm from the Southern African HIV Clinicians Society 2019 guideline for the prevention, diagnosis and management of cryptococcal disease among HIV-infected persons (30). Abbreviations: ART, antiretroviral therapy; CrAg+, cryptococcal antigen-positive in blood; CSF, cerebrospinal fluid; LP, lumbar puncture; TB, tuberculosis; CM, cryptococcal meningitis; OI, opportunistic infection; 5-FC, flucytosine

Figure 2 Cryptococcal antigen titers, risk of subsequent cryptococcal meningitis and mortality among people living with advanced HIV disease without cryptococcal antigenemia, and with cryptococcal antigenemia at different stages of the clinical spectrum: asymptomatic, subclinical cryptococcal meningitis and overt/clinical cryptococcal meningitis. Abbreviations: HIV; human immunodeficiency virus, CD4; CD4 T-lymphocyte cell count, CrAg; cryptococcal antigen, N/A; not applicable, IQR; interquartile range, CM; cryptococcal meningitis, ART; antiretroviral therapy, LP; lumbar puncture, AMB; amphotericin B deoxycholate, L-AMB; liposomal amphotericin B.

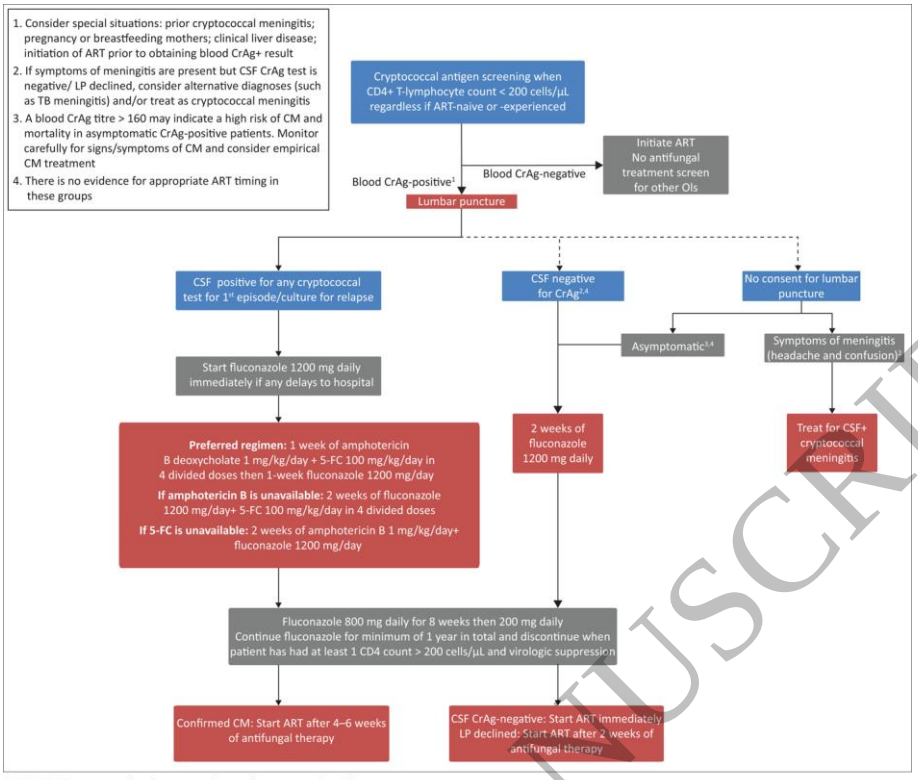
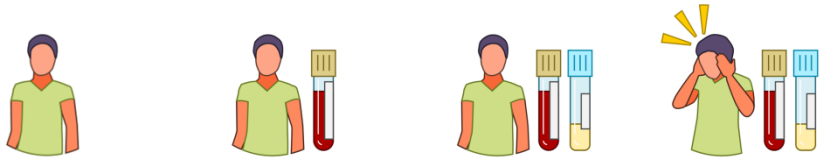


FIGURE 1: Cryptococcal antigen screening and treatment algorithm.

Figure 2

120x104 mm (x DPI)



	HIV+ CD4<100 CrAg-negative	Asymptomatic Cryptococcal Antigenemia	Subclinical Cryptococcal Meningitis	Overt/Clinical Cryptococcal Meningitis
Blood CrAg titer	N/A	Median 40 (IQR 10-160) (28)	Median 1440 (IQR 320 – 10240)(43)	Median 2560 (IQR 160-20480)(49)
Risk of subsequent CM	No ART, 0-2%(22,34) ART initiated, 0%(2,5)	No ART or pre-emptive treatment, 84%(22) ART initiated, no fluconazole, 14-28%(2,21) ART initiated, fluconazole 800mg daily, 0-6%(5,27) ART initiated, fluconazole 800mg daily, screening LP, 0-4%(6,7)	unknown	No maintenance therapy, 15% (36) Fluconazole maintenance therapy, 0% (36)
Mortality **(at 6 months - 1 year)** ***at 10 weeks**	ART initiated, 9-15%(2,6,7,33)	No ART initiated or pre-emptive treatment, 100% (22) ART initiated, no pre-emptive treatment, 19-34%(2,21) ART initiated, fluconazole 800mg daily, 23-30%(5,33,35) ART initiated, fluconazole 800mg daily, screening LP, 18-24%(7,28)	Induction treatment with AMB and fluconazole 800mg (2wks), 22-46%(28,33)	Induction treatment with fluconazole 800mg daily, 77% (37) Induction treatment with AMB and fluconazole 800mg (2 weeks), 50% (38) Induction treatment with AMB and flucytosine (1 week), 28% (38) Induction treatment with single-dose L-AMB and flucytosine and fluconazole 1200mg (2 weeks), 25% (49)*

Figure 3

254x190 mm (x DPI)