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REVIEW ARTICLE

Cryptococcal Antigenemia in Advanced Human Immunodeficiency Virus Disease: Pathophysiology, Epidemiology, and Clinical Implications

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

Keywords. cryptococcal meningitis; cryptococcosis; diagnostic screening programs; acquired immunodeficiency syndrome; HIV.

Human immunodeficiency virus (HIV)–associated cryptococcal meningitis (CM) is responsible for more than 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 preemptive 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 <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 preemptive fluconazole treatment reduces the incidence of CM and death [5]. However, individuals with cryptococcal antigenemia still have a higher mortality risk than comparable individuals without antigenemia, despite antifungal treatment [5–7]. The pathophysiological mechanism that underlies 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]. In this review, we summarize our current understanding of cryptococcal antigenemia, including susceptibility and pathophysiology of associated clinical conditions. We also provide 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 focuses on cryptococcal antigenemia among people living with advanced HIV disease, the main population affected by cryptococcosis.

PATHOPHYSIOLOGY

Cryptococcus and Cryptococcal Antigen

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

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decaying matter of soil, 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].

CrAg is the term used for the predominant component of the cryptococcal capsule, glucuronoxylomannan. Biological fluid samples (blood, cerebrospinal fluid [CSF], pretreated urine) can be tested for CrAg using a latex agglutination (LA) test, enzyme-linked immunosorbent assay (ELISA), and lateral flow assay (LFA). The detection of CrAg in CSF samples is an accurate tool for diagnosing a first episode of CM, particularly in settings where laboratory facilities are limited [9]. The Immuno-Mycologics (IMMY, Norman, OK) LFA is currently the most widely used for CrAg screening. It uses 2 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 ELISA [9]. Validation studies have found excellent concordance when the LFA is used on serum or plasma compared with CSF culture in patients with culture-confirmed CM [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; 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 on immune cells that trigger 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 nonlytic 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 adults with CD4 counts ≤100 [1] cells/μL and 2% among adults with CD4 counts of 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 tuberculosis (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 polymorphisms may be important in cryptococcosis susceptibility [16, 17]. Among people living with HIV (mostly White males), targeted polymerase chain reaction–based genotyping identified the FcγR3A 158 V allele as a risk factor, with homozygous expression conferring 21 times the risk of cryptococcal disease ($P = .005$) [18]. In individuals of African descent, a genome-wide association study identified 6 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 preclinical 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 one-third of individuals with asymptomatic cryptococcal antigenemia have subclinical CM [19]. Additionally, comprehensive screening of 67 asymptomatic CrAg-positive patients in South Africa revealed subclinical cryptococcal infection elsewhere (blood culture growth of *C. neoformans* in 11 of 67 (16%) and pulmonary cryptococcosis in 2 of 32 (7%) who had samples cultured [7]).

Without treatment, the detection of CrAg in the blood heralds the onset of clinical symptomatic CM, although individuals with antigenemia can remain asymptomatic for weeks to months before clinical meningitis occurs [2, 20–22]. 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 CM within 1 year with 100% sensitivity and 96% specificity. No cases of meningitis occurred in 294 CrAg-negative patients with CD4 counts ≤100 cells/μL

within 1 year of testing [2]. Retrospective testing of blood samples taken from patients with HIV-associated CM in Uganda found that cryptococcal antigenemia preceded clinical symptoms by a median of 22 days (range, 5–234) [20]. Cryptococcal infection rarely develops in patients who initially test CrAg-negative, occurring in 19 (1.3%) of 1519 CrAg-negative participants of a primary prophylaxis trial in Uganda [23], mostly prior to ART commencement. Immune reconstitution may be sufficient to clear asymptomatic cryptococcal infection in some CrAg-positive individuals, as observed in 11 of 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 CM among people living with advanced HIV disease and recognition of a presymptomatic window, a strategy of screening and "preemptive" 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-naive adults with CD4 counts <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 2 weeks, followed by 400 mg for 2 months and then 200 mg for at least 1 year pending immune reconstitution [4]. This treatment approach was based on retrospective subgroup analyses that found no cases of CM in CrAg-positive patients who received even low doses of fluconazole (100 mg or 200 mg) for other reasons [22] and evidence that higher doses are well tolerated and more effective in CM [24]. In addition, modeling 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 [25, 26].

Since the introduction of this strategy, recommendations have adapted in response to prospective screening data [14, 27, 28]. The criteria for considering screening is now adults and adolescents with CD4 counts <200 cells/μL, and lumbar puncture (LP) is advised to exclude subclinical CM 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) [29]. 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 [30].

Several prospective studies have shown the CrAg screen-and-treat approach to be effective at reducing the incidence of CM [5–7, 30]. In a multisite trial in Tanzania and Zambia, adults living with HIV with CD4 counts <100 cells/μL were randomized to a strategy that included community support and CrAg screening with preemptive fluconazole for CrAg-positive patients. The intervention reduced mortality risk by nearly one-third, and the authors attributed half of this risk reduction to CM prevention due to CrAg screening [5]. A systematic review and meta-analysis found that preemptive fluconazole initiated at 800 mg in patients with asymptomatic cryptococcal antigenemia reduced the incidence of CM from 20% to 5% [31]. 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 CM using CrAg screen-and-treat strategies, cryptococcal antigenemia remains a risk factor for death among people living with advanced HIV (Figure 2). This was observed in retrospective studies prior to the introduction of CrAg screening and preemptive treatment (in South Africa, adjusted hazard ratio [HR], 3.2; 95% confidence interval [CI], 1.5–6.6 [2]; in Uganda, relative risk, 6.6; 95% CI, 1.86–23.61) [20]; deaths following CM were not sufficient to account for excess mortality in either study [2, 20]. In prospective studies that use fluconazole preemptive treatment, subsequent diagnoses of clinical CM are rare. However, cryptococcal antigenemia was associated with a 2- to 3-fold increased risk of death within 6 months compared with 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 an inferior induction-phase treatment of CM, may be undertreating CrAg-positive patients with undiagnosed subclinical CM or cryptococcemia (blood culture growth of *Cryptococcus* spp.). Subclinical CM has an estimated prevalence of 33% (95% CI, 21%–45%) among asymptomatic CrAg-positive patients by meta-analysis of 10 studies [31]. However, due to limited access and poor uptake of LPs in this population [5, 6, 31], subclinical meningitis is likely to remain undiagnosed in the majority of cases. Even when LPs are used to screen for subclinical CM and appropriate combination antifungals are used for those with CrAg-positive CSF, fluconazole monotherapy fails to prevent some cryptococcal-related deaths in those who do not have subclinical CM at the time of screening. An investigation of the causes of death following CrAg screening and treatment in South Africa, including use of minimally invasive autopsies, attributed 71% (12 of 17) of deaths to cryptococcal disease as an immediate or contributing cause, including 8 patients who were known to die with CM [7]. All 4 CrAg-positive patients with post-mortem samples were CSF

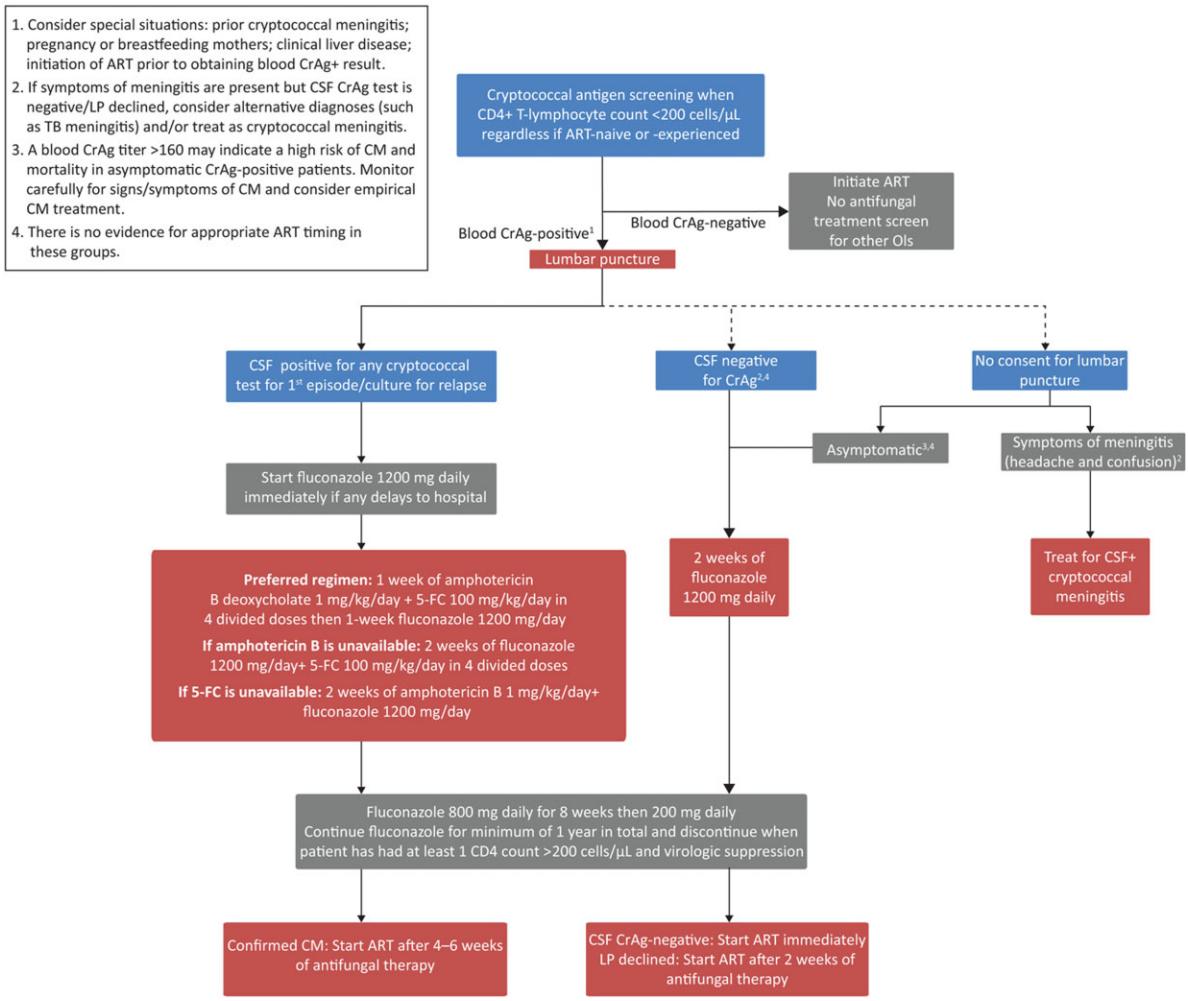


Figure 1. Cryptococcal antigen screening and treatment algorithm from the Southern African HIV Clinicians Society 2019 guideline for the prevention, diagnosis, and management of cryptococcal disease among HIV-infected persons [29]. Abbreviations: ART, antiretroviral therapy; CM, cryptococcal meningitis; CrAg+, cryptococcal antigen-positive in blood; CSF, cerebrospinal fluid; LP, lumbar puncture; pOI, opportunistic infection; pTB, tuberculosis; 5-FC, flucytosine.

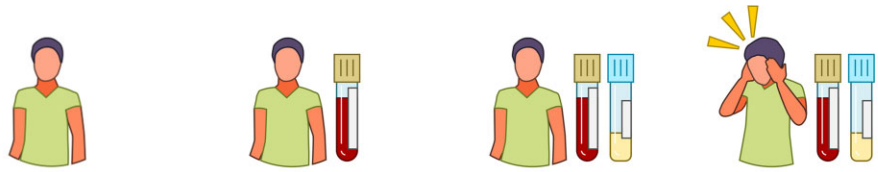
CrAg-positive at the time of death. All had been asymptomatic and received fluconazole, and 2, who had agreed to LP, were CSF CrAg-negative at the time of screening [7]. Furthermore, fluconazole monotherapy was associated with in-hospital mortality of 32% in CrAg-positive patients who presented to the hospital in Uganda with meningism and had CrAg-negative CSF (likely early CM) [33].

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 proinflammatory cytokine production for successful cryptococcal clearance and improved chances of survival [34, 35]. 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 proinflammatory 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, 36], 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, .98– 7.42; $P = .05$), and autopsies revealed multiple copathologies 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



	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 preemptive treatment, 84% (22) ART initiated, no fluconazole, 14%–28% (2,21) ART initiated, fluconazole 800 mg daily, 0%–6% (5,27) ART initiated, fluconazole 800 mg 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 preemptive treatment, 100% (22) ART initiated, no preemptive treatment, 19%–34% (2,21) ART initiated, fluconazole 800 mg daily, 23%–30% (5,33,35) ART initiated, fluconazole 800 mg daily, screening LP, 18%–24% (7,28)	Induction treatment with AMB and fluconazole 800 mg (2 weeks), 22%–46% (28,33)	Induction treatment with fluconazole 800 mg daily, 77% (37) Induction treatment with AMB and fluconazole 800 mg (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 1200 mg (2 weeks), 25% (49)*

Figure 2. Cryptococcal antigen titers, risk of subsequent CM 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 CM and overt/clinical CM. Abbreviations: AMB; amphotericin B deoxycholate, ART; antiretroviral therapy, CD4; CD4 T-lymphocyte cell count, CM; cryptococcal meningitis, CrAg; cryptococcal antigen, HIV; human immunodeficiency virus, IQR; interquartile range, LP; lumbar puncture, L-AmB; liposomal amphotericin B, N/A; not applicable.

approximately 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 CM by the time they were assessed (unpublished, N.P. Govender, D.R. Boulware).

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 CM and death [2, 22] and with concurrent CM in symptomatic and asymptomatic patients [6, 27, 37, 38]. Although no blood CrAg titer can accurately predict meningitis and LPs are recommended, a CrAg titer of >80–160 indicates increased risk and is suggested as a proxy for identifying those who urgently require an LP or who could be considered for empirical CM 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% [39–41]) and CrAgSQ (IMMY; sensitivity 93%–98%, specificity 94%–100% [41, 42]). Quantification scores correlated with IMMY LFA dilutional titers, CM, and mortality [39–42], 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 CM, 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 in Uganda is testing the efficacy of single-dose liposomal amphotericin B (L-AmB) 10 mg/kg plus fluconazole for preemptive treatment of patients with cryptococcal antigenemia (ClinicalTrials.gov: NCT03945448). Amphotericin (AmB) is superior to fluconazole in cryptococcal clearance from CSF [43] 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 AmB deoxycholate in combination treatment of CM, with the benefit of reduced requirements for intravenous access and fewer adverse events [44]. However, even a single intravenous treatment may be costly and challenging to implement,

especially in primary care settings. Another clinical trial is comparing combination fluconazole and flucytosine to the current standard of fluconazole monotherapy [45]. 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 of intravenous AmB plus flucytosine for patients who present with symptomatic CM, with mortality halved compared with historic cohorts treated with fluconazole monotherapy [46]. In South Africa, recent programmatic data have shown that flucytosine-containing induction regimens were associated with a 53% reduced in-hospital CM mortality compared with regimens without flucytosine in a real-world setting [47]. Flucytosine was historically expensive and inaccessible across most of Africa. However, following release of the ACTA trial results and subsequent inclusion of flucytosine in WHO-preferred induction regimens for meningitis, costs declined with the introduction of new generic flucytosine products.

Although both combination treatments are known to be superior to fluconazole monotherapy in CM, 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 Reduction of early mortality in HIV-infected African adults and children starting antiretroviral therapy 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 [48]. 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 is 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 preclinical and asymptomatic (CSF CrAg-negative), subclinical (CSF CrAg-positive, no overt meningism), or clinical cryptococcal infection, usually fulminant CM. Blood CrAg 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 preemptive treatment approaches given that fluconazole monotherapy may not be adequate to prevent progressive cryptococcosis and cryptococcal-related deaths [49–53].

Notes

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