Title – Invited Review

Cryptococcal meningitis: a review of cryptococcal antigen screening programs in Africa

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Greg Greene\*1, 2, David Lawrence\*2, 3, Alex Jordan4, Tom Chiller4, Joseph N. Jarvis2, 3

1 Centre for Healthcare-Associated Infections, Antimicrobial Resistance and Mycoses, National Institute for Communicable Diseases, a division of the NHLS, Johannesburg, South Africa

2Department of Clinical Research, Faculty of Infectious & Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom

3Botswana Harvard AIDS Institute Partnership, Gaborone, Botswana

4Mycotic Diseases Branch, Centers for Disease Control and Prevention, Atlanta, United States

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Abstract

Introduction

Cryptococcal meningitis remains a significant contributor to AIDS-related mortality despite widened access to antiretroviral therapy. Cryptococcal antigen (CrAg) can be detected in the blood prior to the development of meningitis. The development of highly sensitive and specific rapid diagnostic CrAg tests has helped facilitate the adoption of CrAg screening programs in 19 African countries.

Areas Covered

The biological rationale for CrAg screening and the programmatic strategies that have been adopted are reviewed. We describe the approach to the investigation of patients with cryptococcal antigenemia and the importance of lumbar puncture to identify individuals who may have cryptococcal meningitis in the absence of symptoms. The limitations of current treatment recommendations and the potential role of newly defined combination antifungal therapies are discussed. A literature review was conducted using a broad database search for cryptococcal antigen screening and related terms in published journal articles dating up to December 2019. Conference abstracts, publicly available guidelines and project descriptions were also incorporated.

Expert Opinion

As we learn more about the risks of cryptococcal antigenemia, it has become clear that the current management paradigm is inadequate. More intensive investigation and management are required to prevent the development of cryptococcal meningitis and reduce mortality associated with cryptococcal antigenemia.

Keywords (4-10)

Africa; AIDS; Antifungal therapy; Amphotericin; Cryptococcal antigen; Cryptococcal meningitis; Fluconazole; Flucytosine; Advanced HIV disease; Opportunistic infection.

Article Highlights

* Cryptococcal disease continues to cause significant mortality in sub-Saharan Africa.
* CrAg screening has been widely adopted into policy across African countries and the prevalence of cryptococcal antigenemia is now known in many key regions.
* Different approaches to CrAg screening have been developed and each can provide context-specific options for implementation. The operational benefits and challenges of these approaches are described.
* The presence of cryptococcal antigenemia warrants investigation for cryptococcal meningitis with diagnostic lumbar puncture, where feasible.
* Treatment outcomes among those with cryptococcal antigenemia treated only with oral fluconazole are suboptimal.
* New optimized oral or short-course treatment options for cryptococcal meningitis may be transferrable to patients with cryptococcal antigenemia.

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The authors report no conflicts of interest.Body of the Article

1. Introduction

Nearly two decades in to the era of antiretroviral therapy (ART), yearly AIDS-related deaths have fallen from an estimated 2.8 million in 1999 to just under 800 000 in 2018 [1, 2]. However, almost three-quarter of a million people are estimated to have died from preventable or treatable AIDS-related illnesses in 2019, and advanced HIV disease continues to be an issue even in countries such as South Africa and Botswana where rapid progress in ART scale-up has been achieved [3, 4]. Cryptococcal meningitis, an opportunistic fungal infection associated with advanced HIV, causes an estimated 15% of AIDS-related deaths globally, second only to tuberculosis [5]. Of the 223,000 cryptococcal meningitis cases estimated to have occurred in 2014, almost three-quarters were in sub-Saharan Africa. Typically more severe than tuberculosis infection, cryptococcal meningitis carries a mortality rate of 100% if untreated, and, even with currently recommended treatment, mortality often exceeds 40% [6–9]. In the past decade, screening for and pre-emptively treating early sub-clinical disease has emerged as a viable intervention with potential to reduce the contribution of cryptococcal meningitis to AIDS-related deaths. Cryptococcal antigen, or CrAg, is a highly specific biomarker of disseminated infection that is detectable in the blood weeks to months prior to the development of meningitis. In patients with very advanced HIV disease (CD4 count < 100 cells/µL), CrAg prevalence ranges from below 1% to nearly 23% across various country settings, with CrAg prevalence generally highest in the Africa Region where it averages 6-7% [10, 11].

1. Body
	1. – Areas Covered and Methods

This paper discusses cryptococcal screening programs in the African context, reviewing the biological and programmatic origins, outcomes, expert opinions on management, and emerging challenges. To compile this information, we conducted a non-systematic literature search using publicly available databases, including PubMed.gov, Google Scholar, and ClinicalTrials.gov. Search terms contained “cryptococcal antigen screening” or “CrAg screening” in combination with relevant topical terms. Peer-reviewed articles as well as conference abstracts, project reports, international and national guidance, and ongoing trial descriptions were included dating through December 2019.

* 1. – Background

*Cryptococcus sp.* is an invasive fungal infection most commonly causing disease in individuals who are immunocompromised due to HIV infection. *Cryptococcus sp.* is a ubiquitous encapsulated yeast and exposure to spores or desiccated yeasts by inhalation is almost universal. Clinically significant disease is primarily caused by reactivation of latent pulmonary infection; *Cryptococcus sp.* may or may not cause a respiratory syndrome before entering the blood stream and causing disseminated cryptococcal disease (cryptococcosis) [12, 13]. The most common site of end organ involvement is the central nervous system, resulting in cryptococcal meningitis. Throughout disease progression, *Cryptococcus* *sp.* sheds its polysaccharide fungal capsule, which can be detected using cryptococcal antigen (CrAg) tests. CrAg offers a highly specific biomarker for cryptococcal infection and remains present in the blood for months to years following initial disseminated infection [14]. Diagnosis of cryptococcal disease using CrAg has been revolutionized in the last ten years by the development of a number of easy-to-use and affordable lateral flow assays (LFAs). The most widely used of these tests is the IMMY LFA (Immuno-Mycologics, Norman, OK, USA), which was FDA-approved in July 2011, can be performed in under ten minutes with minimal laboratory infrastructure, and has a sensitivity and specificity of 99-100%, though this has been shown to vary in instances of particularly high or low antigen titer [15–19]. The LFA replaced the previously used latex agglutination test, which required cold-chain, laboratory equipment and cost over $16 per test. The LFA’s simplicity and significantly cheaper cost of only $2-$3 per test has facilitated large-scale screening in resource-limited settings [20, 21].

* 1. – Origins of and Rationale for CrAg Screening

One of the first studies to describe the natural history of cryptococcal antigenemia was a cohort study in Uganda from the pre-ART era in which stored serial serum samples from HIV-positive patients who developed cryptococcal meningitis were retrospectively tested for CrAg [14]. This study found that cryptococcal antigenemia was detectable a median of 22 days before the onset of meningitis symptoms, suggesting a window of opportunity for early identification of cryptococcal disease and pre-emptive treatment with the aim of preventing the development of meningitis. Subsequent research conducted in South Africa showed that the presence of cryptococcal antigenemia at the time of ART initiation was highly predictive of the development of cryptococcal meningitis, with no cases developing among CrAg-negative individuals (i.e. a 100% negative predictive value), and high rates of cryptococcal meningitis and death among those who were CrAg-positive [22]. Additionally, studies in Uganda described how cryptococcal antigenemia at the point of ART initiation was highly predictive of death [23].

Subsequent studies have shown that cryptococcal antigenemia is common in HIV-positive individuals with advanced HIV, and, as rapid diagnostic tests for cryptococcal antigenemia have become more widely available, it has been possible to generate robust data on the prevalence of CrAg among individuals with HIV at different strata of CD4 cell counts [5, 10, 11]. Meta-analyses by Temfack et al and Ford et al aiming to characterize the distribution of CrAg positivity have calculated the pooled global CrAg prevalence to be 6 and 6.5%, respectively, among adults with a CD4 <100 cells/µL and 2.0% among those with a CD4 of 101-200 cells/µL [10, 11]. The majority (81.4%) of CrAg-positive individuals were identified at or below a CD4 of 100 cells/µL, while 18.6% of cryptococcal antigenemia detected across studies occurred in individuals with a CD4 of 101 – 200 cells/µL [11]. CrAg prevalence has been demonstrated at fairly consistent rates across sub-Saharan Africa, with studies across various settings in east, west, and southern Africa having described CrAg prevalence ranging from 3.6% in Nigeria to 11% in Ethiopia and rural Lesotho [22, 24–33]. The geographic variation seen extends beyond regional differences, with prevalence measurements differing across areas within a country, as has been observed in Nigeria and South Africa [31, 34].

The optimal treatment for cryptococcal antigenemia is not known; however, the oral antifungal fluconazole (originally branded as Diflucan) was known to have activity against *Cryptococcus sp.* and to be safe and well-tolerated. From 2000, Pfizer supplied fluconazole cost-free to low- and middle-income countries for the treatment of cryptococcal meningitis and esophageal candidiasis through the Diflucan Partnership Program [35]. As a result, fluconazole became widely available across African contexts, quickly making fluconazole monotherapy the default treatment option for cryptococcal meningitis treatment despite sub-optimal efficacy. Fluconazole monotherapy is outperformed by all amphotericin B- and flucytosine-containing combination therapies in the treatment for cryptococcal meningitis [6, 36]. Patients treated with fluconazole 800mg monotherapy in a hospital study in Malawi experienced a mortality rate of 77% at one year compared to combination therapies with observed mortality rates of roughly 40% [37, 38]. Preliminary data regarding the possible impact of fluconazole therapy for cryptococcal antigenemia were available from a small cohort study in Uganda that recruited 609 ART-naïve patients between 2004-2006 [20]. Of these, 50 (8.2%) were CrAg-positive, 26 of whom had never had cryptococcal meningitis before. Of the 26, 21 were treated with fluconazole 200-400mg daily for two to four weeks. In this group, three developed cryptococcal meningitis, and the 30-month survival was 71%. All five who did not receive fluconazole died. Fluconazole was therefore considered as a therapeutic option for CrAg-positive individuals, with the term “pre-emptive treatment” preferred to “prophylaxis” as it is treatment for an established infection given to prevent progression to meningitis.

On the basis of these initial data, in 2011 the WHO published rapid advice on the diagnosis and management of cryptococcal disease and provisionally recommended CrAg screening for those with a CD4 <100 cells/µL [39]. The rapid advice stated that those who were positive should be assessed for signs and symptoms of meningitis and, if present, investigated with a lumbar puncture (LP). If cryptococcal meningitis were confirmed by CSF testing or suspected where an LP was not available or accepted, the rapid advice suggested treatment with amphotericin in combination with either flucytosine or fluconazole for two-weeks followed by an eight-week consolidation phase and subsequent maintenance treatment phase. If cryptococcal meningitis had been excluded, the recommendation was to treat with fluconazole 800mg daily for two weeks, followed by 400mg daily for eight weeks and then continued maintenance at 200mg a day. These treatment guidelines were issued on the basis of expert opinion, owing to the lack of robust evidence from randomized trials.

This WHO-recommended regimen was tested in the REMSTART trial, the first randomized controlled trial (RCT) of CrAg screening in Africa [40]. The trial recruited 1999 ART-naïve participants in Tanzania and Zambia with a CD4 <200 cells/µL from 2012-2014 and randomized them to either standard clinic-based care or enhanced clinical care. Enhanced care was composed of CrAg screening with pre-emptive antifungal therapy – the fluconazole regimen recommended by the WHO – given to those who were CrAg-positive but did not have cryptococcal meningitis. This was in addition to home visits around the time of ART initiation. In this study, there was a 4% prevalence of CrAg positivity. Only 24% of those agreed to an LP, and 33% of those had cryptococcal meningitis, so the majority who were CrAg-positive received pre-emptive fluconazole for treatment of their cryptococcal antigenemia. The study found a 28% relative reduction in mortality (13% versus 18%, 95% confidence interval 10-43%) in the enhanced care group, providing the first RCT evidence backing the CrAg screen-and-treat approach as a life-saving intervention.

Cost-effectiveness modeling of CrAg screening and pre-emptive treatment further supported the adoption of such screen-and-treat programs. Cost-effectiveness analysis conducted using data from the 2004 prospective Ugandan cohort found that the number screened needed to save a life was 11.3 persons, and that the cost per DALY saved was $21 [41]. Further work modeled various approaches to cryptococcal meningitis prevention in Cape Town, comparing usual care to three scenarios: 1) screening and pre-emptive treatment for CrAg-positive asymptomatic patients with a CD4 <100 cells/µL, 2) screening and LP for all CrAg-positive patients, and 3) fluconazole primary prophylaxis for all ART-naïve patients [42]. The comparison found CrAg screening and pre-emptive fluconazole therapy to be the least costly overall approach and superior to the standard of care down to a CrAg prevalence of 0.6% in those with a CD4 <100 cells/µL.

* 1. – Screening Implementation

The data from the observational studies, the randomized controlled trial, and cost-effectiveness modelling provided sufficient justification for several countries to implement the recommendations of the WHO and incorporate CrAg screening and treatment in national policy and practice. At the time of writing, nearly 30 countries, 19 of which are in sub-Saharan Africa, have included CrAg screening and treatment in their national HIV treatment guidelines (**Figure 1**).

One of the first countries to implement screening was South Africa. In late 2011, screening began in two provinces under a *reflex* laboratory-based screening model in which remnant sera from CD4 samples were automatically, or *reflexively*, tested at CD4 laboratories from any sample with a CD4 <100 cells/µL [43]. The following year in the Western Cape Province, screening was similarly rolled out, but using a provider-initiated approach in which a clinician was responsible for ordering a subsequent CrAg test after a CD4 test <100 cells/µL had been received [44]. The parallel implementation of these two approaches allowed for comparison, yielding findings key to inform later national scale-up. Evaluation of the provider-initiated approach found that, despite modest improvements in coverage over time, only about one-third of eligible patients were screened [45]. The reflex approach achieved a coverage of over 95%, though this high coverage combined with the issue of test duplication for patients with multiple low CD4 counts increased reflex screening costs to $37,536 more per 100,000 CD4 tests than provider-initiated screening [44, 46–48]. Even with higher costs, the potential for lives saved and reduction in treatment costs by screening and pre-emptive treatment under the reflex system proved superior (30 additional lives saved and $55,165 in treatment costs saved per 100,000 CD4 tests), and in 2016 the South African National Department of Health and National Health Laboratory Service rolled out reflex CrAg screening at all CD4 laboratories across the country [44, 49]. In three years since the completion of rollout in October of 2016, South Africa’s national reflex CrAg screening program has achieved national coverage, screening over 200,000 individuals each year and identifying over 11,000 with cryptococcal disease [34]. However, provider action on CrAg test results and subsequent patient outcomes are not captured in the laboratory information system and remain to be evaluated.

Several other African countries have made considerable progress towards implementing routine reflex CrAg screening and pre-emptive fluconazole treatment. In 2014, eSwatini began piloting CrAg screening at a national referral hospital in Mbabane [50]. Following completion of this pilot, the eSwatini National AIDS Program (ENAP) recommended routine CrAg screening at a CD4 threshold of 100 cells/µL and pre-emptive fluconazole for CrAg-positive patients [51]. In 2018, ENAP began implementing routine reflexive CrAg screening at 16 hospitals, with plans to conduct intensive monitoring and evaluation of the program beginning in 2020 [52]. In Tanzania, the National AIDS Control Program (NACP), in partnership with implementing partners, began implementing the first phase of routine reflexive CrAg screening and pre-emptive treatment at 15 hospitals in 2018 [53]. In addition, the ongoing Translating Research into Practice (TRIP) cluster-randomized study aims to assess outcomes of CrAg-positive patients and feasibility of routine CrAg screening at 18 urban and rural health facilities in order to continuously inform implementation efforts in Tanzania [54].

Some countries have adopted a modified CrAg screening approach using CrAg-testing at the point of care in hospitals and HIV-clinics. Designed as a point-of-care (POC) testing platform, the CrAg LFA has high potential for POC use, with near-perfect agreement between use on whole blood compared to laboratory-based serum testing when conducted by trained study technicians using transfer pipettes and reading after 20 minutes [55, 56]. However, comprehensive training and thorough oversight are required if the POC approach is to be used, with preliminary findings suggesting that POC testing may not perform as well in routine clinic settings, particularly if the LFA test strip is applied directly to a finger-prick blood spot, with resulting sensitivity as low as 20-38% when compared to the gold standard laboratory EIA testing [55, 57]. Using a pipette to transfer larger volumes of blood to diluent before CrAg LFA testing and reading results after 20 minutes is therefore essential if the POC method is to be effective.

In 2017 the Malawi Ministry of Health (MOH), in partnership with the Malawi Department of HIV/AIDS (DHA), began planning routine implementation of CrAg screening and pre-emptive treatment using a POC approach. A pilot of POC CrAg screening and pre-emptive treatment was initiated at five health facilities (three hospitals, two primary clinics) in 2018 in order to inform the MOH and DHA on the burden of cryptococcal disease and the feasibility of a routine screening program. This pilot concluded in August 2019, with preliminary results revealing a high burden of CrAg in both newly diagnosed and ART-experienced patients with a history of poor adherence or defaulting treatment. POC screening and pre-emptive therapy were also found to be feasible in the Malawi context, evidenced by high CrAg testing coverage, rapid turn-around times of test results and prompt initiation of pre-emptive treatment [58]. In 2018, DHA formally recommended CrAg screening for those with a CD4 <200 cells/µL or a WHO clinical stage 3 or 4 illness as well as in individuals with danger signs or admitted to hospital, with pre-emptive fluconazole therapy for CrAg-positive patients without cryptococcal meningitis [59]. The DHA plans to continue expanding routine implementation using a phased approach through 2020. However, many African countries have not yet incorporated CrAg screening into their national guidelines, in part due to a lack of clear evidence for improved patient outcomes and specific guidance on how best to integrate CrAg screening into health systems.

* 1. – Current Understanding – Clinical

Despite evidence demonstrating that CrAg screening reduces the incidence of cryptococcal meningitis and related mortality, there remains a persistent association between CrAg positivity and death, even among those who receive pre-emptive fluconazole therapy as per the WHO guidelines [25, 29, 60–62]. This may be a result of under-investigating those with antigenemia and missing cases of cryptococcal meningitis who require more intensive antifungal treatment, or because fluconazole alone is not enough to prevent the development of life-threatening cryptococcal disease in a subset of CrAg-positive individuals.

There is evidence that *Cryptococcus sp.* can be present in the central nervous system without causing overt symptoms, i.e. *subclinical meningitis*. Studies have found this to be present in 25-40% of asymptomatic individuals with antigenemia [25, 63, 64]. One way to determine this is to perform an LP in all patients with antigenemia, irrespective of symptoms, and the 2018 WHO guidelines now recommend this approach [65]. However, there are significant operational issues related to a lack of access to trained healthcare workers, facilities, and equipment as well as a high rate of LP refusal [40, 66]. In situations where LP is not feasible or is refused, it has been suggested that blood CrAg titers could help stratify management. Subclinical meningitis has been shown to be strongly associated with CrAg titers of >1:160, and titers of ≥1:160 have also been shown to be associated with increased mortality in a study in Uganda [67]. It is possible that CrAg titer level could help to stratify those who are in particular need of an LP or who could be managed with more intensive antifungal therapy [25, 63]. To help facilitate this approach, there are a number of semi-quantitative lateral flow assays that are currently being developed and validated [32, 68, 69].

Additional evidence that high-dose fluconazole alone may be inadequate for treatment of cryptococcal antigenemia has recently emerged out of South Africa. A minimally invasive autopsy study there identified cases of cryptococcal meningitis in patients with cryptococcal antigenemia who had CrAg-*negative* CSF on LP performed after CrAg screening [62]. This may provide insight into suboptimal outcomes experienced in some patients currently prescribed 800mg daily dosed fluconazole for pre-emptive therapy, and enhanced treatment regimens for cryptococcal antigenemia may also need to be considered. The high tolerability of fluconazole and evidence for increased efficacy of daily doses of 1200mg compared to 800mg in treatment of cryptococcal meningitis has led the Southern Africa HIV Clinicians Society Guidelines to now recommend an initial two week treatment with 1200mg daily [70, 71].

Current advances in the management of cryptococcal meningitis could be transferrable to patients with antigenemia. The recently completed ACTA trial tested five different antifungal combinations and found that an oral combination arm of flucytosine and fluconazole performed well, resulting in a 10-week mortality of 35% (95% CI 29-41%) [6]. This oral combination arm may be a suitable treatment option for cryptococcal antigenemia. The ongoing AMBITION phase 3 trial is testing a single high dose of liposomal amphotericin for the treatment of cryptococcal meningitis with results expected in early 2021 [69, 72]. This regimen has been taken forward specifically for the management of cryptococcal antigenemia in a study in Uganda and recruitment is ongoing [73].

* 1. – Current Understanding – Operational

Translating research findings to public health programs comes with significant difficulties, and as experience in the implementation of CrAg screening grows in sub-Saharan Africa, several operational issues have emerged. In South Africa, effectively interfacing clinics and laboratories remains a programmatic challenge for CrAg screening and treatment. The South African NHLS conducts routine CD4 testing on over 3 million samples per year with a typical turnaround time of 24-48 hours [46, 74]. However, how to effectively translate rapidly available laboratory results to clinical action remains poorly understood, especially in the context of a reflex testing system where providers do not directly order the CrAg test.

Data from South Africa’s initial implementation of CrAg screening found that, of 624 CrAg-positive cases diagnosed at enhanced surveillance sites between September 2012 and April 2014, 96 (15%) never returned for their CD4 and accompanying CrAg test results.[75] Additional data from the Western Cape Province in 2012 found that, of 7,964 patients with a CD4 <100 cells/µL, 34% did not subsequently initiate ART [76]. Following later nationwide implementation in South Africa, an audit at a large hospital in Durban reviewed records of CrAg-positive patients at referring clinics between June 2015 and May 2016; of 190 CrAg-positive patients, only 5 (2.6%) were followed up and admitted for LP, as per the South African HIV treatment guidelines [49, 77]. Thirty-eight (20%) other CrAg-positive screened patients underwent LP, but only following presentation with overt cryptococcal meningitis symptoms rather than being referred based on CrAg screening results. Though loss to follow-up is an issue not confined to CrAg-screened patients, follow-up and appropriate action on CrAg screening results remain weak links in reflex testing, even in South Africa where centralized CD4 laboratory testing returns over 90% of results within 48 hours [78]. The ongoing Cryptococcal Antigen Screening and Treatment National Evaluation Team (CAST-NET) study is expected to produce more comprehensive findings regarding care received by CrAg-positive patients in sampled areas across all 9 of South Africa’s provinces in 2020 [79].

A potential means of avoiding the losses between CrAg testing and treatment is implementation of POC testing [44, 80, 81]. As described above, care must be taken to ensure that the correct POC testing technique is used so that adequate sample volumes are collected [55]. Pilot data from several African countries have shown the feasibility of nurse-led CrAg screening at the health facility level and potential for enhanced retention between testing and treatment [28, 82]. In the Malawian POC pilot study at five health centers across three regions of Malawi, 1255 of 1304 (96.2%) eligible patients with advanced HIV disease were successfully screened and treated [83]. This high coverage is comparable to that achieved by reflex programs elsewhere, though unlike reflex programs, patients in the Malawi POC screening system can be immediately followed up on the same day before leaving the health facility. Additional piloting of POC LFA testing in Lesotho by Médecins Sans Frontières (MSF) explored the possibility of task-shifting for very low-resource settings [28]. Lay counsellors at three rural primary care clinics were trained to administer POC CrAg testing to all HIV-infected patients with CD4 < 100 cells/µL based on POC PIMA CD4 testing (Abbott, Chicago, IL, USA). Lay workers collected finger-prick specimens and were responsible for running CrAg LFA tests as well as positive and negative controls for each batch, while nurses were trained in recognition of cryptococcal meningitis symptoms and referral pathways. Over 1 year of screening, 128 of 129 eligible patients were screened (99% coverage), and 12 of 14 (86%) asymptomatic CrAg-positive patients correctly received fluconazole treatment. Follow-up of CrAg-positive results was not optimal, but the pilot demonstrates the feasibility of this method in areas where human resources are limited.

In addition to issues around the care cascade, several HIV-related policy shifts have added complexity to the implementation of CrAg screening, notably the shift to universal ART access (“treat all”), the subsequent decline in baseline CD4 testing rates, and implementation of same-day ART initiation [84, 85]. CD4 testing is essential for identification of individuals at risk of cryptococcal infection who would benefit from CrAg screening, and if baseline CD4 testing is discontinued, the opportunity for CrAg screening is lost. Even in settings where baseline CD4 testing is continued, if individuals are initiated on ART immediately pending CD4 results, the effectiveness of the CrAg screening intervention may be compromised. Both the initial WHO 2011 rapid advice and the more recent WHO 2018 updated cryptococcal guidelines recommend delaying ART initiation for two weeks in patients with cryptococcal antigenemia in order to mitigate the risk of immune reconstitution inflammatory syndrome (IRIS) in potentially meningitic patients [39, 65]. It is currently not known what risk same-day ART initiation poses to asymptomatic serum CrAg-positive patients. However, the overall benefits of rapid ART initiation have been well-documented, and care must be taken to ensure that implementation of CrAg screening does not delay ART initiation in the majority of patients. With this in mind, the 2019 Southern Africa HIV Society guidelines recommend initiating ART immediately in CrAg-positive individuals who have an LP that excludes cryptococcal meningitis, and to continue ART in patients who have already initiated and for whom meningitis can be excluded [70]. Those who decline an LP or for whom an LP is contraindicated should, however, start ART after at least 2 weeks of antifungals. Patients who are diagnosed with cryptococcal meningitis should not start ART until 4-6 weeks after starting antifungal therapy.

The ORCAS trial, the first study assessing CrAg screening implementation, highlighted the potential impact of many of these challenges. A stepped-wedge cluster-randomized trial, the ORCAS study aimed to assess differences in survival between observational (pre-CrAg screening) and interventional (CrAg screening and pre-emptive treatment) phases at 11 urban and 6 rural outpatient HIV clinics in Uganda [67]. The study found that 6-month mortality did not significantly differ between the pre-screening and screening periods (24.8% vs 30.4%, HR = 1.34, 95% CI: 0.86 to 2.10, *p* = 0.20). A major potential confounder in the study that may have contributed to the lack of difference in survival observed between the pre-screening and screening arms was the surge in clinic patient volumes and the lower patient return for ART initiation rates that were experienced as the study progressed and Uganda markedly expanded its ART program. Return for follow-up and ART initiation dropped by 13% between study phases, from 84% in the observational phase to 73% in the intervention phase (*p* < 0.001). Though overall CrAg screening showed little improvement in this operational study, these findings do underscore the point that any CrAg screening intervention should not delay ART initiation.

1. Conclusion

Nearly a decade has passed since the development of the CrAg LFA test and inclusion of CrAg screening and treatment in international guidelines. In this time, CrAg screening and pre-emptive treatment has been incorporated into the national HIV treatment guidelines of many African countries. Trial evidence from Tanzania and Zambia has demonstrated the CrAg screen-and-treat approach’s potential as a life-saving intervention when coupled with a strong ART delivery system, and the rollout of large-scale screening programs such as that of South Africa have shown the feasibility of incorporating CrAg screening into African health systems. However, implementation of CrAg screening has not been without its challenges. Laboratory-based reflex screening has struggled with issues common to the HIV treatment cascade regarding high loss to follow-up and specific issues with timely communication of laboratory results and appropriate clinical action. Factors driving uptake of CrAg screening results remain poorly understood. In addition, extensive emerging data now suggest that subclinical cryptococcal meningitis is relatively common in asymptomatic CrAg-positive individuals, and fluconazole pre-emptive therapy is not sufficient to prevent disease progression and death in many asymptomatic CrAg-positive patients, with further robust clinical trial data needed to define optimal management strategies.

Along with these challenges, opportunities for improved management of cryptococcal antigenemia have also arisen. Higher CrAg titers have been strongly linked to subclinical meningitis, opening up the possibility for eventual use of newly developed semi-quantitative CrAg LFA tests to better guide treatment. Demonstrations of feasible POC screening offer a potentially superior option for countries lacking the comprehensive laboratory infrastructure required for national reflex approaches, and the recent development and validation of a POC semi-quantitative CD4 rapid test now opens up the possibility for low-tech rapid testing of both CD4 *and* CrAg at the point of care [86, 87]. The proven effectiveness and tolerability of new combinations of antifungal drugs for treatment of cryptococcal meningitis offers the clear possibility of better treatment options for CrAg-positive patients.

Further challenges lie ahead, with the longstanding Diflucan Partnership Program donation program ending in 2021, and many international initiatives focusing resources on expanding and streamlining ART care for stable patients. Hopefully mitigating some of these issues, funding for advanced HIV disease and CrAg screening have been announced by international donors such as PEPFAR and UNITAID, and the 2017 WHO advanced HIV disease guidelines have now incorporated CrAg screening into a consolidated package of care [88–90]. This more holistic view, coupled with renewed funding efforts, offers the opportunity for existing and future CrAg screening programs to expand and innovate, now better able to adapt to specific contexts and evolve as new findings from clinical studies guide better patient care.

1. Expert Opinion (500 words)

Cryptococcal antigen screening remains an important component of HIV care. Baseline CD4 counts are essential to identify patients who should be screened for cryptococcal antigenemia. CrAg screening programs should be tailored to the specific context, health service and laboratory infrastructure (**Table 1**). All patients with a CD4 <100 cells/µL should undergo CrAg screening. Screening in patients with CD4 up to 200 cells/µL should additionally be considered where feasible, depending on the cost and burden of disease across CD4 strata in this category. Where CD4 testing is centralized, reflex testing may be the most convenient approach, but ensuring appropriate communication of results and patient follow-up and appropriate subsequent management can be challenging and should receive sufficient resources and support. Where CD4 testing is decentralized or where POC testing is available, provider-initiated POC CrAg testing is feasible and can facilitate prompt provider action and rapid initiation of pre-emptive therapy or ART, though care should be taken that healthcare workers administering POC tests are adequately trained and optimal procedures followed.

Treatment must also be tailored based on patient condition as well as treatment and LP access and acceptability (**Table 2**). All individuals with blood cryptococcal antigenemia should be offered an LP, as a lack of symptoms does not rule out cryptococcal meningitis. Based on expert opinion, if patients have already initiated ART and are CSF-negative, then they should continue ART and begin antifungal treatment, while those who are not currently on ART and are CSF-negative should begin ART immediately along with antifungal treatment. For treatment of blood CrAg-positive and CSF-negative patients for whom cryptococcal meningitis has been excluded, antifungal treatment, based on current expert opinion, should consist of 2 weeks of induction therapy with fluconazole 1200mg followed by 8 weeks of consolidation therapy with fluconazole 800mg and at least 1 year of maintenance therapy with fluconazole 200mg. If a patient is CSF-positive, then a provider may decide whether or not to continue ART, as current expert opinion, given the limited evidence, is that there is clinical equipoise between decisions to continue or interrupt ART. CSF-positive adult patients should be started as quickly as possible on one week of induction therapy with a combination of amphotericin B (1 mg/kg/day using the deoxycholate formulation) and flucytosine (100 mg/kg/day) followed by one week of fluconazole 1200 mg, or in settings where flucytosine is not available, two weeks of amphotericin B and fluconazole 1200 mg combination therapy. Where amphotericin B is not available, flucytosine and fluconazole combination therapy provides an alternative. Subsequent consolidation and maintenance therapy should be administered in line with that of CrAg-positive, CSF-negative patients, as described above.

Where CrAg titer testing is available, particular effort should be made to encourage patients with a titer >1:160 to undergo an LP. If an LP is refused or unavailable, these patients should be treated as if they have cryptococcal meningitis. Where cryptococcal meningitis is excluded by LP, patients should be treated with 1200mg fluconazole daily for two weeks, followed by 800mg for eight weeks and fluconazole 200mg daily until CD4 >200 cells/µL. Fluconazole monotherapy for pre-emptive treatment is likely to be replaced by more intensive antifungal therapy in future guidelines as evidence from planned and ongoing trials is reported and global access to antifungal drugs improves, with possible options being fluconazole and flucytosine combination antifungal therapy (an all-oral combination treatment), or short-course high-dose liposomal amphotericin B.

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\*\*World Health Organization. Guidelines for the diagnosis, prevention, and management of cryptococcal disease in HIV-infected adults, adolescents and children. Geneva; 2018 Mar.

This WHO publication is currently the leading international guidance on cryptococcal screening and treatment.

\*\*Govender NP, Meintjes G, Mangena P, Nel J, Potgieter S, Reddy D, 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 [Internet]. 2019 Nov 8 [cited 2019 Dec 10];20(1). Available from: http://www.sajhivmed.org.za/index.php/HIVMED/article/view/1030

This guidance, issued in 2019 by the Southern African Clinicians Society, incorporates the latest research findings to inform the most up-to-date guidance on cryptococcal antigen screening as well as cryptococcal meningitis treatment.

\*\*Molloy SF, Kanyama C, Heyderman RS, Loyse A, Kouanfack C, Chanda D, et al. Antifungal Combinations for Treatment of Cryptococcal Meningitis in Africa. N Engl J Med. 2018 15; 378(11):1004–17.

The ACTA trial demonstrated the noninferiority of a short course (1 week) of amphotericin B plus flucytosine in the treatment of cryptococcal meningitis as well as the effectiveness of fluconazole plus flucytosine, an all-oral treatment regime.

\*\*Mfinanga S, Chanda D, Kivuyo SL, Guinness L, Bottomley C, Simms V, 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, randomized controlled trial. The Lancet. 2015 May; 385(9983):2173–82.

The REMSTART trial was the first randomized controlled trial to demonstrate the effectiveness of CrAg screening when paired with community adherence support, providing much of the basis for stronger WHO recommendations advocating for the intervention.

\*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 1; 48(7):856–62.

Jarvis’s 2009 retrospective study of CrAg screening in South Africa established CrAg screening’s utility in identifying patients at risk for cryptococcal meningitis at ART initiation.

\*Ford N, Shubber Z, Jarvis JN, Chiller T, Greene G, Migone C, et al. CD4 Cell Count Threshold for Cryptococcal Antigen Screening of HIV-Infected Individuals: A Systematic Review and Meta-analysis. Clin Infect Dis Off Publ Infect Dis Soc Am. 2018 Apr 1; 66(Suppl 2):S152–9.

Ford’s systematic review and meta-analysis examines the evidence from studies conducting CrAg screening across all CD4 thresholds, making the argument for key cutoffs.

\*Larson BA, Rockers PC, Bonawitz R, Sriruttan C, Glencross DK, Cassim N, et al. Screening HIV-Infected Patients with Low CD4 Counts for Cryptococcal Antigenemia prior to Initiation of Antiretroviral Therapy: Cost Effectiveness of Alternative Screening Strategies in South Africa. PLOS ONE. 2016 Jul 8; 11(7):e0158986.

The cost-effectiveness study compares provider-initiated screening to a laboratory-based reflex approach in South Africa, finding that, due to far superior coverage of reflex screening, the reflex approach has potential to save both lives and money.

\*Wake RM, Jarvis JN, Harrison TS, Govender NP. Point of Care Cryptococcal Antigen Screening: Pipetting Finger-Prick Blood Improves Performance of Immuno-Mycologics Lateral Flow Assay. J Acquir Immune Defic Syndr 1999. 2018 Aug 15; 78(5):574.

Wake’s assessment of POC CrAg screening suggests several key considerations for best practices when implementing POC screening using finger-prick and, thought with a small sample, demonstrates the potential cost when not performed optimally.

1. Figures and Tables

**Figure 1:** Countries with CrAg screening in national guidelines and their respective CD4 thresholds employed



**Table 1:** Considerations for CrAg screening program design

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|   | **Ideal Setting** | **Benefits** | **Key Considerations** | **Supportive Data** |
| **Screening Approach** |   |
| Laboratory-based reflexive | - Centralized laboratory infrastructure- Rapid lab turnaround time- Functional result delivery system | - Integrated easily into existing testing and reporting systems- Can be monitored through laboratory information systems- Can achieve high coverage | - Providers must be aware and adequately trained on action on results- Additional costs from duplicate testing for patients with multiple CD4 counts- Results can only be acted on during patient's return visit | - Tenforde et al, Wellcome Open Res 2019 [81]- Coetzee, PLoS One 2018 [47]- Larson, PLoS One 2016 [48]- Govender & Glencross, SAMJ 2018 [44] |
| Point-of-care | - Decentralized laboratory infrastructure- Other rapid tests performed onsite | - Action on results can be delivered same-day where POC CD4 available- Low-tech and requires minimal laboratory infrastructure- Can be performed by broad range of healthcare workers | - Need regular refresher training and oversight to ensure adequate sample collection and test interpretation | - Drain et al, Scientific Reports 2019 [57]- Wake et al, Clin Infect Dis 2019 [28]- Rick, PLoS One 2018 [28] |
| Provider-initiated | - Centralized or decentralized laboratory infrastructure | - Ease and low cost of implementation | - Coverage historically low- Providers must be made aware and must remember to order tests when appropriate- Long turnaround time and requires multiple patient visits | - Larson, PLoS One 2016 [48]- Govender & Glencross, SAMJ 2018 [44] |
| **CD4 Threshold** |   |   |   |   |
| ≤ 100 cells/µL | - | - Majority of CrAg cases fall below this threshold (~80%)- Higher CrAg prevalence means higher yield- Strongest evidence for screening | - CrAg cases at higher CD4 counts likely to be missed- Higher proportion of patients likely to have overt or subclinical CM | - Ezeanoloue, JAIDS 2016 [31]- Ford, Clin Infect Dis 2018 [11]- Jarvis PLoS One 2013 [42] |
| ≤ 150 cells/µL | - | - Studies in South Africa, Ethiopia and Nigeria suggest may capture 90% or more of CrAg-positive cases | - Limited evidence supporting this threshold- Higher cost and greater workload than CD4 100 cells/µL threshold | - Tufa, Open Forum Inf Dis 2017 [91]- Govender, HIV Med 2015 [92]- Ezeanoloue, JAIDS 2016 [31] |
| ≤ 200 cells/µL | - | - Most comprehensive coverage - Multi-country randomized controlled trial demonstrated benefit- May account for misclassification observed in POC CD4 testing | - Highest cost and greatest workload of all thresholds- Lower prevalence at higher CD4 | - Mfinanaga, The Lancet 2015 [40]- Beyene PLoS One 2013 [93]- Luchters, J Clin Micro 2019 [86]- Pham BMC Inf Dis 2016 [94] |
| **Titer-based Screening** |   |   |   |   |
| Titer testing with 1:160 CM cut-off  | - Settings with high LP refusal rate- Settings with continued high mortality in patients receiving fluconazole pre-emptive treatment | - Evidence that titer cutoff predicts sub-clinical meningitis in otherwise asymptomatic patients- Can better guide providers in additional treatment and referral decisions | - Greater expense of testing- Currently requires laboratory infrastructure, though new rapid semi-quantitative LFA tests have recently emerged- CrAg titer does not yield a definitive CM diagnosis and does not negate need for LP | - Wake, Clin Infect Dis 2018[63]- Rajasingham, J Clin Microbiol 2019[80]- Gassiep, J Medical Microbiol 2018[95] |
| No titer-based testing | - Resource-limited settings where cost of infrastructure for titer testing is prohibitive | - Simplified treatment algorithm compared to titer testing- Requires less laboratory infrastructure and technical expertise | - LP is only option to confirm or exclude CM, and refusal rates are often high- May risk undertreating some CrAg-positive patients | - Thakur, Neurology 2015[66] |

**Table 2:** Treatment guidance for CrAg-screened patients

|  |  |  |  |
| --- | --- | --- | --- |
| **Screening Result** | **Condition** | **Treatment** | **Guidance and Evidence** |
| Blood CrAg-positive | LP done, CSF-negative | * Antifungal therapy
	+ Induction*:* Fluconazole 1200mg for 2 weeks
	+ Consolidation*:* 800mg for 8 weeks
	+ Maintenance*:* 200mg for at least 1 year and until CD4 > 200 and virologic suppression (maintenance)
* Antiretroviral therapy
	+ Rapid ART initiation if not currently on ART and no clinical symptoms of meningitis
	+ ART continuation if already initiated
 | Southern African HIV Clinicians Society *guideline for the prevention, diagnosis, and management of cryptococcal disease among HIV-infected persons*: *2019 update*REMSTART TrialWHO *Guidelines for Managing Advanced HIV Disease and Rapid Initiation of Antiretroviral Treatment (2017)*Wake et al, 2019 |
| LP unavailable or patient does not consent to LP, AND either: * CM symptoms *not present* and no titer available, *or*
* CrAg titer ≤ 1:160
 |
| LP done, CSF-positive | * Antifungal therapy
	+ Induction*:* 1 week amphotericin B deoxycholate1 1mg/kg/day + 5-FC 100mg/kg/day in 4 doses then 1 week fluconazole 1200mg

If **amphotericin** is *not available*, 2 weeks of fluconazole 1200mg + 5-FC 100 mg/kg/day in 4 dosesIf **5-FC** is *not available*, 2 weeks of amphotericin B 1mg/kg/day + fluconazole 1200mg/day* + Consolidation*:* Fluconazole 800mg/day for 8 weeks
	+ Maintenance*:* Fluconazole 200mg/day for minimum 1 year and CD4 > 200 + virologic suppression
* Antiretroviral therapy
	+ Delay ART by 4-6 weeks if not currently on treatment
	+ ART continuation if already initiated, though risk of IRIS exists
 | WHO CM guidelinesSouthern African HIV Clinicians Society *guideline for the prevention, diagnosis, and management of cryptococcal disease among HIV-infected persons*: *2019 update*ACTA TrialCOAT StudyWake et al, 2019 |
| LP unavailable or patient does not consent to LP, AND either: * CM symptoms *present* and no titer available*, or*
* [[1]](#footnote-1)CrAg titer > 1:160
 |
| Blood CrAg-negative | N/A | * Antiretroviral therapy
	+ Rapid ART initiation if not currently on ART and no other contraindication
	+ ART continuation if already initiated
 | WHO Advanced HIV GuidelinesSTART TrialTEMPRANO Trial |

1. Liposomal amphotericin B (L-AmB) is preferable to amphotericin B deoxycholate and, where available, can be given in place of amphotericin B deoxycholate at a dosage of 3-4mg/kg/day. However, this formulation is rarely available in sub-Saharan African settings due to its high comparative cost. [↑](#footnote-ref-1)