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Preventing Cryptococcosis—Shifting the Paradigm in the Era of Highly Active Antiretroviral Therapy

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Abstract Cryptococcosis remains a significant cause of morbidity and mortality among HIV-infected patients, especially in sub-Saharan Africa where it causes up to 20 % of AIDS-related deaths in HIV programs. A new, highly sensitive, and affordable point of care diagnostic test for cryptococcal infection, the lateral flow assay, can detect early sub-clinical cryptococcosis especially in areas with limited laboratory infrastructure. With a prevalence of detectable sub-clinical cryptococcal infection averaging 7.2 % (95 % CI 6.8–7.6 %) among 36 cohorts with CD4 <100 cells/ μ L in Africa, together with data showing that preemptive fluconazole prevents overt cryptococcal disease in this population, implementing a screen and treat strategy as part of HIV care practice among patients with CD4 <100 cells/ μ L could prevent the incidence of often fatal cryptococcal meningitis in the setting of the HIV pandemic.

Keywords HIV · Cryptococcosis · Cryptococcal antigen · Fluconazole · CD4 · CRAG screening · Preemptive therapy

Introduction

One of the most significant contributors to the increased burden of cryptococcosis worldwide is indisputably HIV infection. With 35.3 million individuals living with HIV and an estimated peak of 2.3 million HIV-associated deaths in 2012 [1], the rise in the incidence of opportunistic infections has been remarkable, resulting in tremendous strain on healthcare resources and lost lives and income especially in low- and middle-income countries (LMICs).

Prior to the global HIV epidemic, cryptococcosis was a disease primarily of individuals with low immunity, particularly after long-term use of immunosuppressive drugs and solid-organ transplantation, with other non-HIV risk factors including hematopoietic or other malignancies, innate immune defects, advanced liver or renal disease, sarcoidosis, rheumatologic disease, and diabetes mellitus [2–9]. One of the initial indicators of the HIV epidemic was the increase in

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cases of cryptococcal meningitis (CM) [10–12]. Although the incidence of CM has decreased in high-income countries with the advent of antiretroviral therapy (ART), sub-Saharan Africa continues to grapple with a high prevalence of HIV and opportunistic infections, with cryptococcal meningitis as the leading cause of meningitis among HIV-infected adults [3, 13, 14]. Over the last 3 decades of the HIV epidemic, efforts have been invested in improving cryptococcal care with revision of national guidelines focusing on diagnosis and management of cryptococcal meningitis but less so on prevention.

Fluconazole prophylaxis given to HIV-infected patients with CD4 <200 cells/ μ L before or during ART has been shown to reduce the risk of developing cryptococcal meningitis, including using thrice-weekly fluconazole dosing [15, 16]. However, this strategy is not as cost-effective as targeted cryptococcal antigen (CRAG) screening followed by preemptive therapy using fluconazole in the setting of cryptococcal antigenemia [17, 18]. Although currently only recommended in HIV-infected persons, it is also possible that CRAG screening and preemptive fluconazole therapy would be beneficial in solid-organ transplantation patients.

Preventing new HIV infections, early HIV diagnosis, implementing early linkage to care, ensuring timely initiation of ART with strict adherence, and adequate patient follow-up along the continuum of HIV care would ideally prevent incident cryptococcosis and other opportunistic infections. In this review, we outline the strategies for preventing cryptococcosis in the context of HIV infection and discuss the impact of new cryptococcal diagnostics on these strategies.

Burden of Cryptococcal Disease

Cryptococcus is an encapsulated yeast acquired by inhalation that can disseminate to cause a severe meningoencephalitis primarily in people with defective cell-mediated immunity. *Cryptococcus neoformans* is responsible for most human disease and has a worldwide distribution with an ecological niche in decaying organic matter and soil containing bird excrement [19].

In high-income country (HIC) settings, including the USA, Western Europe, and Australia, cryptococcosis emerged during the HIV epidemic in the 1980s as a cause of meningitis in 5–10 % of HIV-infected individuals [20, 21]. Rollout of ART starting in 1997 led to a dramatic decline in cases, illustrated by a greater than 90 % decreased incidence in a large UK cohort in 2006–2007 compared to 1996–1997 [14]. A review of over 200 million US hospital admissions from 1997 to 2009 similarly found a 5.8 % annual decline of HIV-associated CM and proportional rise in non-HIV CM over this period from 16 to 29 % of all cases (major risk factors including solid-organ transplant and long-term use of corticosteroids or other immunosuppressant drugs) [3]. Early case-fatality rate of CM in

HICs with access to amphotericin-based induction therapy (\pm flucytosine) is as low as 10 % in clinical trials, when excluding lost to follow-up [22, 23]. This decline is yet to be demonstrated in LMICs.

The vast majority of cryptococcal meningitis cases and deaths occur in LMICs with high HIV prevalence coupled with common delays in HIV diagnosis and ART initiation. Recent data suggests that well over 90 % of cryptococcal meningitis cases in LMICs are in people infected with HIV [13, 24–26]. In 2006, of an estimated 957,900 HIV-associated CM cases and 624,725 deaths worldwide, more than 90 % of cases and 95 % of deaths occurred in sub-Saharan Africa (720,000 cases, 504,000 deaths), South and Southeast Asia (120,000 cases, 66,000 deaths), and Latin America (54,400 cases, 29,900 deaths) [23]; however, these estimates are currently under revision. Cryptococcus is the leading cause of meningitis in much of Southern and Eastern Africa and causes up to 20 % of deaths in HIV-infected cohorts from sub-Saharan Africa [13, 27–29]. Health facilities in LMICs often lack amphotericin-based induction therapy (instead using high-dose fluconazole) and are under-resourced for performing serial lumbar punctures and monitoring common side effects of antifungal treatment, all of which contribute to poor treatment outcomes. Park et al. previously estimated a 3-month case-fatality rate in LMICs at 55 and 70 % in sub-Saharan Africa [23]. More recent reports suggest continued poor treatment outcomes, with *in-hospital* mortality of 27.9 % in a study from Burkina Faso, 52.0 % in Cameroon, 48.1 % in Ethiopia, 50.0 % in India, 35.8 % in Kenya, 62.2 % in Senegal, and 40.5 % in South Africa [24, 26, 30–34].

Asymptomatic Cryptococcosis

Cryptococcosis is one of the few infectious diseases in which the presence of disseminated infection can be demonstrated even while patients remain asymptomatic. Asymptomatic cryptococcosis (positive serum CRAG with absent or minimal symptoms) is a sub-clinical infectious state known to precede clinically apparent disease by weeks to months and is strongly associated with risk of incident meningitis and all-cause mortality [35, 36, 37]. Cryptococcal antigenemia is common in persons with AIDS and is inversely related to CD4 count (Table 1) [41, 43, 44, 49, 54]. In sub-Saharan Africa, patients with CD4 \leq 100 cells/ μ L have a CRAG prevalence reported between 2.2 and 21.0 % or up to 11.5 % in studies including only asymptomatic, ART-naïve patients [35, 36, 38, 39, 40–47, 55].

In Southeast Asia, CRAG prevalence in patients with CD4 \leq 100 cells/ μ L is reported between 4.0 and 20.6 % or up to 12.9 % in studies including only asymptomatic, ART-naïve patients [48–52]. In untreated asymptomatic antigenemic patients starting ART, over 25 % go on to develop incident

Table 1 Prevalence of Cryptococcal antigenemia in HIV-infected patients with CD4 ≤100 cells/μL

Region	Country	Setting	Year	Prevalence	No Hx Crypto	ASx	Other information	Test	Reference
Africa	Cape Town	Outpatient	2002–2005	6.7 % (21/312)	Yes	Yes	ART naive	LA	[36•]
	Mbarara	Inpatient and outpatient	~2003	10.7 % (21/197) ^a	NS	No	ART naive; 61.9 % (13/21) CRAG+ patients had confirmed CM	LA	[38]
	Tororo	Outpatient	2003–2004	5.8 % (22/377)	Yes	Yes	ART naive	LA	[35]
	Kampala	Outpatient	2004–2006	8.8 % (26/295)	Yes	Yes	ART naive	LA	[39•]
	Kusami	Outpatient	2008–2009	2.2 % (2/92)	No	Yes	% on ART NS but likely low (78 % samples tested within 1 week of HIV diagnosis)	LA	[40]
	Kampala	Inpatient and outpatient	2009–2010	18.8 % (69/367)	Yes	No	ART naive; of 30 patients who consented to LP, 24 had confirmed CM (≥34.8 % of all CRAG+ patients)	LA	[41]
	Rongo and Kisumu	Outpatient	2010–2011	11.5 % (59/514)	No	Yes	ART naive	LA	[42]
	Addis Ababa	Outpatient	2011	11.2 % (13/116)	Yes	No	68 % on ART	LA	[43]
	Benin City	Outpatient	2011	21.0 % (17/81)	Yes	NS	ART naive	LA	[44]
	Moshi	Outpatient	2011–2012	4.8 % (6/124)	Yes	Yes	ART naive (or on ART <6 months)	LA / LFA	[45]
Mwanza	Outpatient	2012–2013	8.2 % (6/73)	Yes	Yes	ART naive	LFA	[46]	
Ekurhuleni and Johannesburg	Outpatient	2012–2014	4.5 % (839/18,544)	No	No	% on ART unknown	LFA	[47]	
Asia	Bangkok	Outpatient	2003–2007	12.9 % (11/85)	Yes	Yes	ART naive	LA	[48]
	Phnom Penh	Inpatient and outpatient	2004	20.6 % (58/282)	Yes	No	ART naive	LA	[49]
	Multi-site	Outpatient	2005–2007	10.7 % (9/84)	Yes	NS	ART naive	LA	[50]
	Bandung	Outpatient	2007–2011	7.1 % (58/810)	Yes	Yes	ART naive	LFA	[51]
	Hanoi and Ho Chi Minh City	Outpatient	2009–2012	4.0 % (9/226)	Yes (in part of cohort)	NS	ART naive	LFA	[52]
	Europe								
	London	Inpatient	2004–2010	5.0 % (8/157)	No	No	ART naive; 7/8 CRAG-positive patients with CM	LA	[53]
	USA								
	Multi-site	Outpatient	1986–2012	2.9 % (55/2872)	NS	NS	% on ART NS	LFA	[54]

Adapted from Meyer AC and Jacobson MA 2013 23715897

ART antiretroviral therapy, ASx asymptomatic, CM cryptococcal meningitis, CRAG cryptococcal antigen, Hx history, LA latex agglutination, LFA lateral flow assay, LP lumbar puncture, NS not specified

^a CRAG prevalence limited to patients with CD4 count <50 cells/μL

cryptococcal meningitis within 12 months versus as few as 0 % of CRAG-negative patients [36•]. Baseline cryptococcal antigenemia is also a strong independent predictor of mortality in the first year of ART after adjusting for baseline clinical (e.g., CD4 count) and demographic factors, with a study from Indonesia, including only patients without initial symptoms of meningitis, showing an adjusted hazard ratio for death of 2.6 (95 % CI 1.4–4.6) and a study from South Africa showing an adjusted hazard ratio of 3.2 (95 % CI 1.5–6.6) [36•, 51]. A Ugandan study of ART-naïve, TB/HIV co-infected patients followed for 2 years found an adjusted hazard ratio for death of 4.27 (95 % CI 1.5–12.1) in those who were CRAG positive at baseline [56]. For pre-ART asymptomatic CRAG-positive patients, another Ugandan study found a relative risk of death (within 12 weeks after ART initiation) of 6.6 (95 % CI 1.9–23.6) compared to pre-ART CRAG-negative patients [35].

Robust evidence is lacking to guide the optimal management of patients found to have asymptomatic cryptococcal antigenemia, but expert guidelines suggest using 800 mg of fluconazole for 2 weeks followed by 400 mg for 8 weeks [57]. These guidelines have been adopted with some variation by several countries (Table 2).

The Changing Landscape of Cryptococcal Diagnostics

Historically, cryptococcal infection has been diagnosed by India ink microscopy, latex agglutination for cryptococcal antigen, or culture. Culture is ultimately considered to be the gold standard; however, delays in obtaining a result make culture clinically unhelpful for initial management decisions. CRAG assays include enzyme immunoassay (EIA), latex agglutination (LA), or a recently developed point-of-care lateral flow assay (LFA) (IMMY, Inc., Norman, Oklahoma), which was approved by the US Food and Drug Administration for use with CSF or serum in July 2011. The test utilizes an immunochromatographic test strip containing gold-conjugated monoclonal antibodies that bind to glucuronoxylomannan (GXM) cryptococcal antigen and is capable of detecting all cryptococcal serotypes (A–D). One drop (~40 µL) of the patient sample is mixed with one drop of diluent and a binary readout (two bands—test and control—for a positive test) is produced within 10 min if antigen is detected. The assay has a number of qualities that make it ideal for diagnostic testing in resource-limited settings, including low cost, high sensitivity/specificity, point-of-care testing, stability of diluent and test strips at room temperature with a long shelf life (up to 2 years), minimal training requirement, and no need for processing of samples (e.g., pretreatment, heat inactivation) or specialized laboratory equipment. Furthermore, titers can be done using this assay for CRAG quantification.

Using serum or plasma samples, the LFA has excellent sensitivity and specificity and a high agreement with results from other antigen-based tests [45, 65, 66•, 67, 68]. Serum LFA had a sensitivity of 99.6 to 100 % in published studies including patients with laboratory-confirmed cryptococcal disease and a specificity of 92 % in a study of patients hospitalized with suspected meningitis (although this may be an understatement as several of the “false positives” on LFA may have actually been false negatives on reference tests) [65, 69, 70•, 71]. Plasma LFA testing appears to perform equivalently to serum testing [70•, 71]. Additionally, serum LFA and LA had a 100 % agreement in a study from Tanzania evaluating CRAG prevalence in asymptomatic, ART-naïve patients, supporting LFA as a good alternative to other antigen tests for use in CRAG screening for prevention [45]. Unpublished data from Uganda and South Africa suggests that there is 100 % agreement between whole blood, serum, and plasma CRAG LFA testing, demonstrating that fingerstick is a viable option for bedside detection of CRAG, particularly in settings where phlebotomy is unavailable [72].

The LFA performs sub-optimally, however, with urine and saliva samples. Although sensitive, LFA in urine lacks specificity, leading to a poor positive predictive value for cryptococcosis [46, 65, 69, 70•, 71]. Attempts to improve the specificity of the urine assay by altering the diluent have been unsuccessful, leading to a reduction in test sensitivity compared to serum LFA as reference and continued sub-optimal specificity (sensitivity decreasing from 100 % to 80 % and specificity increasing from 73.8 % to 91.5 %) [46]. Using samples of saliva, a recent Ugandan study found excellent specificity but poor sensitivity (88 % in symptomatic patients and only 27 % in asymptomatic patients) of the LFA in detecting cryptococcal antigenemia using serum/plasma samples for reference [73]. In a review of the published literature addressing performance of the LFA assay, median CSF sensitivity was 100 %, and median specificity was 97.7 %. In serum, median sensitivity was again 100 %, with median specificity of 99.5 % [74].

Cost-Effectiveness of CRAG Screening

The cost per sample using the CRAG LFA is substantially lower than other antigen test methodologies or culture. The manufacturer offers the test for US\$2 per sample in resource-limited settings with a current delivered in-country real world cost from local distributors of US\$3–4. Further efficiencies in distribution are possible. The cost in high-income countries is \$5 per LFA. Cost-effectiveness analyses of a CRAG “screen and treat” approach in antiretroviral therapy initiators with CD4 counts <100 cells/µL have been performed in varied settings, including Cambodia, South Africa, Uganda, and Vietnam [17•, 18•, 39•, 52]. All studies have shown this

Table 2 Countries that have revised their national guidelines following the WHO 2011 rapid advice and the individual fluconazole regimens proposed

Country	Screening recommendations	Fluconazole regimen	Comments on implementation
Rwanda [58] (2011 guidelines)	Patients with advanced immunosuppression are at higher risk than others of having an asymptomatic or symptomatic cryptococcal infection; therefore, it is recommended to screen for cryptococcal disease in every patient with CD4 <200 cells/ μ L using CRAG testing on plasma	Fluconazole 800–1200 mg daily for 2 weeks as an induction phase for patients with asymptomatic cryptococcal infection (with antigenemia only)	None
Kenya [59] (2011 guidelines)	CRAG screening is recommended in PLHIV prior to starting ART if CD4 is <100 cells/ μ L	No mention is made of the preemptive therapy to be used after screening	It is not possible (and neither desirable) for all facilities providing ART services to perform all the laboratory tests required for HIV care and treatment. If a facility does not have on-site capacity to carry out any particular test, arrangements should be made to transport specimens to a local or regional reference laboratory. Facilities are encouraged to join or form regional networks of laboratory services to improve access to these tests
Botswana [60] (2012)	Screening of asymptomatic ART-naïve individuals with CD4 count <100 cells/ μ L is recommended and should be done with a CRAG test using latex agglutination tests (LA) or lateral flow assays (LFA) on serum, plasma, or CSF. A lumbar puncture should be offered to individuals who screen positive for cryptococcal antigen, as a positive cryptococcal antigen may precede the onset of clinical cryptococcal meningitis by many weeks	Fluconazole 800 mg daily for 2 weeks, then fluconazole 400 mg daily for 8 weeks, followed by maintenance therapy with fluconazole 200 mg daily until CD4 >200 cells/ μ L for 6 months	No mention is made of CRAG screening Serum CRAG positive: if available recommend LP; If CSF CRAG negative, manage for cryptococcal meningitis; if CSF CRAG negative, treat with fluconazole Timing of ART for individuals with asymptomatic cryptococcal antigenemia is unknown. We recommend initiation of ART 2–4 weeks after initiation of antifungal therapy in individuals who screen positive for serum CRAG without any evidence of disseminated cryptococcal meningitis
Zimbabwe [61] (2013)	All PLHIV with CD4 <100 cells/ μ L should be screened for cryptococcal antigen using serum or plasma irrespective of symptoms	Fluconazole 800 mg daily for 2 weeks followed by 400 mg daily for 8 weeks	CRAG screening is to be done either by CRAG LA or LFA (on serum or plasma) Initiate ART 2 weeks after starting preemptive therapy
Uganda [62] (2013 guidelines)	HIV-infected adults with CD4 ⁺ T lymphocyte count <100 cells/ μ L	Fluconazole 800 mg daily for 2 weeks as outpatient, fluconazole 400 mg daily for 2 months then 200 mg daily. Continue fluconazole for minimum of 1 year in total and discontinue when patient has had 2 CD4 counts >200 cells/ μ L taken at least 6 months apart	Screen for cryptococcal antigenemia on serum or plasma by reflex laboratory or clinician-initiated testing. If clinician-initiated testing is performed, screening should be restricted to ART-naïve adults with no prior CM. Either the LA or LFA may be used as a screening test
South Africa [63] (2015 guidelines)			
USA [64]			Although fluconazole and itraconazole have been shown to reduce frequency of primary cryptococcal disease among those who have CD4 cell counts <100 cells/ μ L, primary prophylaxis for cryptococcosis is not routinely recommended in this group with advanced medical care. This recommendation is based on the relative infrequency of cryptococcal disease, lack of survival benefits, possibility of drug-drug interactions, creation of direct antifungal drug resistance, medication compliance, and costs

Ethiopia and Mozambique have also incorporated CRAG screening into revised HIV care guidelines

approach to be extremely cost-effective, even using a low-range prevalence estimate of 2 %. As these studies have CRAG testing cost between US\$4.13 per test and US\$16.75 per test, as a screen and treat strategy, the LFA at US\$2.50 per test would further increase cost-effectiveness.

Costs of Cryptococcal Meningitis Management

In comparison to the costs of screening, the costs of management of symptomatic cryptococcal meningitis are substantially greater. Costs of cryptococcal meningitis vary by country but include costs of medications, healthcare personnel for 2-week inpatient hospitalization, laboratory monitoring for medication toxicity, and finally costs of serial lumbar punctures including manometers if available. In Uganda, total cost of medical care for 2 weeks of induction therapy with amphotericin and flucytosine is estimated at \$467.48 [75]. Given that flucytosine is unavailable, the total cost of care with what is considered the next best regimen of amphotericin + fluconazole is \$402.07, with the cost of medication making up a small percentage of this total cost (23 %). In South Africa, the estimated cost for care of cryptococcal meningitis is \$2883 [18]. This variability is likely due to much higher cost of personnel in South Africa. Estimated US costs for 14 days of inpatient management of cryptococcal meningitis is \$50,000 with flucytosine alone costing approximately \$9000 for a 70-kg adult [76].

Integrating Cryptococcal Antigen Screening and Fluconazole Therapy into Routine Care—Challenges and Opportunities

Integrating CRAG screening into routine HIV care could potentially provide a platform for improved linkage to care, promoting continuity in care while diminishing attrition, and contributing to improved patient outcomes. As an integrated practice, healthcare facilities could focus on the more severely immunosuppressed patients, by having dedicated staff to follow up CD4 and CRAG test results, and ensure that this population of patients (with CD4 <100 cells/ μ L) returns to the clinic in a timely manner to receive the appropriate intervention, including fluconazole, if CRAG positive. The timing of ART initiation among patients with asymptomatic cryptococcal antigenemia has not been studied; however, current guidelines recommend initiating ART after the 2 weeks of high-dose fluconazole. Following the release of the 2011 WHO rapid advice guidelines on the diagnosis, prevention, and management of cryptococcal disease in HIV-infected individuals, several countries have revised their national guidelines to reflect these changes; however, actual implementation of these guidelines has been varied (Table 2) [57].

Several operational challenges must be addressed as screen and treat programs are implemented. First, cryptococcal screening must be integrated into routine management algorithms for patients who require rapid ART initiation. Second, for the program to succeed, patients found to have cryptococcal antigenemia must be immediately traced, assessed, and initiated on antifungal treatment before they develop meningitis or die. Laboratory reporting and clinic tracing systems need to be enhanced. Third, supply, procurement, and distribution of CRAG tests and antifungal drugs to clinics must be adequate and consistent, and nurses who identify patients with antigenemia need to have the ability to initiate antifungal treatment.

From the experience of an ongoing CRAG screening study in Kampala Uganda, challenges of rolling out the CRAG screening program include lack of adequate staff training across all levels of health cadres, from laboratory, clinical, and administrative staff, which limits the appreciation of program ownership; lack of adequate logistics and the irregular supply of CD4 testing reagents, which slows the screening process; lack of CRAG screening tests at health facilities; lack of a steady supply of fluconazole for preemptive treatment; and, finally, poor referral systems in the absence of relevant manpower to handle this very sick population. More often than not, health facilities are ill equipped with the required drugs and tests to manage patients with breakthrough meningitis.

Conclusions

Significant improvement in cryptococcal diagnosis has occurred along with a better understanding of cryptococcal management. By integrating CRAG screening and fluconazole preemptive therapy as recommended by the WHO, the morbidity and mortality occasioned by cryptococcal disease could be drastically reduced. In the long term, with a subsiding number of newly diagnosed HIV-infected individuals presenting to HIV care with lower CD4 counts, increasing numbers of patients started on ART at CD4 counts >500 cells/ μ L, and improved linkage to long-term HIV care, screen and treat programs will become less expensive with fewer persons requiring CRAG testing and fluconazole therapy. There still remains the benefit of saving money to preemptively treat patients who would otherwise develop cryptococcal meningitis. Ideally, with perfect CRAG screening programs, one could envision elimination of cryptococcal meningitis using this screen and treat strategy, especially in sub-Saharan Africa. However, challenges remain on how best to implement such a strategy and a better understanding of risk factors for breakthrough cryptococcal meningitis remains to be elucidated.

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Compliance with Ethics Guidelines

Conflict of Interest David Meya, Radha Rajasingham, Elizabeth Nalintya, Mark Tenforde, and Joe Jarvis declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal studies performed by the authors.

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