

# Integrating Cryptococcal Antigen Screening and Pre-Emptive Treatment into Routine HIV Care

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**Abstract:** Cryptococcal meningitis is a leading cause of death in AIDS patients in sub-Saharan Africa. Cryptococcal antigen (CRAG) can be detected weeks before onset of symptoms, and those who are asymptomatic but CRAG positive have a high risk of subsequent cryptococcal meningitis and mortality. A new CRAG point of care immunochromatographic test is available that is remarkably easy to administer without laboratory infrastructure or expertise and has excellent sensitivity and specificity. We review the benefits of targeted CRAG screening, developments in CRAG diagnostics, and evidence regarding treatment options that can be implemented into routine HIV care in areas of high cryptococcal burden. Based on published CRAG+ prevalence rates of 2%–12%, the cost to save one life is between \$20 to \$140 in sub-Saharan Africa. We provide recommendations for implementation, pre-emptive treatment, and identify the gaps in our current knowledge.

**Key Words:** HIV, cryptococcosis, cryptococcal meningitis/DI, cost effectiveness, point-of-care systems, review

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## BURDEN OF CRYPTOCOCCAL INFECTION

Cryptococcal meningitis (CM) affects an estimated 957,900 people per year, causing approximately 624,700 deaths annually.<sup>1</sup> The overwhelming burden of disease is in sub-Saharan Africa, where there are an estimated 720,000 annual cases, with annual mortality equaling or exceeding that of tuberculosis. In multiple sub-Saharan African countries with high HIV prevalence, CM is the most common cause of meningitis accounting for 26% of cases in Malawi, 45% of cases in Zimbabwe, 63% of cases in South Africa.<sup>2–4</sup> The high prevalence of CM continues despite great improvements in availability of antiretroviral therapy (ART), as only 37% of those in sub-Saharan Africa who are eligible for ART are receiving ART.<sup>5</sup> As a result, much of the HIV-infected population still presents late to care with advanced AIDS.

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Although the majority of those who present with CM are ART naive, an increasing proportion of CM is being diagnosed in the first 3 months after ART initiation (20%–30%).<sup>6,7</sup>

Unfortunately, mortality from CM remains high even in referral health care centers with access to appropriate antifungal medications and ART, placing a substantial burden on health care resources. In Uganda, CM mortality is between 20% and 39% in a tertiary care referral hospital despite access to ART,<sup>8,9</sup> with only 40% alive after 6 months.<sup>10</sup> The experience in South Africa has been similar, with in-hospital mortality near 30%, and 6-month survival of 40%–60%.<sup>1,11</sup> Outcomes in routine clinical practice are likely substantially worse than in carefully conducted prospective research studies.

Medical care for patients with CM places immense strains on already overburdened health care systems. In one South African hospital, 31% of all inpatient days were secondary to CM within 32 weeks of initiating ART,<sup>12</sup> and the estimated cost for each admission was \$2883. Hospitalization is not only expensive but also requires complicated medical management including serial lumbar punctures to reduce intracranial pressure, administration of amphotericin, with subsequent monitoring of renal function and electrolytes, and frequent administration of IV fluids.<sup>13</sup> Amphotericin alone costs \$7/day in Uganda with the estimated cost for 2-weeks of hospital care with an amphotericin-based regimen being over \$400 per person with CM in Uganda—an unaffordable expense for the majority of patients. Typically, when amphotericin is out of stock or unaffordable, patients are treated with a substandard regimen of fluconazole alone. Manometers to measure intracranial pressure are unavailable outside of research studies. Furthermore, diagnosis of CM has historically relied on laboratory infrastructure and expertise to perform India ink staining or latex agglutination (LA), which are often unavailable outside of major health care centers. Given high mortality despite maximal available care, and the incredible burden on existing health care systems, public health strategies to prevent CM, or detect cryptococcosis before hospitalization are essential.

## CRYPTOCOCCAL ANTIGEN

While the gold standard for diagnosis of cryptococcal disease is culture from bodily fluids, Cryptococcal antigen (CRAG) is used to presumptively diagnose cryptococcal disease with sensitivity and specificity near 100% in Sub-Saharan Africa in an HIV-infected population.<sup>9</sup> Notably, detectable CRAG in peripheral blood precedes symptoms of CM by an average of 22 days,<sup>14</sup> and approximately 11% of people will have antigen present >100 days before disease

onset. Prevalence of asymptomatic cryptococcal antigenemia has been studied in areas of high HIV burden (Fig. 1).<sup>12,15-19</sup> In these studies, antigenemia was infrequent in patients with a CD4 >100 cells per microliter. Because CRAG is detectable before symptomatic cryptococcal infection, CRAG can serve as a potential screening tool for cryptococcal disease, and targeting CRAG screening to patients with a CD4 count ≤100 cells/mcL would be of highest yield.

### Clinical Relevance of Asymptomatic Antigenemia

In otherwise asymptomatic HIV-infected persons, cryptococcal antigenemia independently predicts mortality. This was demonstrated by retrospectively CRAG testing samples in a community cohort of HIV-infected ART-naive patients, with a CD4 count ≤100 cells per microliter in rural Uganda.<sup>18</sup> Of 377 patients, 22 (5.8%) had a serum CRAG titer >1:2. Of those with a positive serum CRAG, 5 patients died within 12 weeks of initiating ART. In multivariate analysis, after controlling for CD4 count, viral load, body mass index, and active tuberculosis (TB), asymptomatic cryptococcal antigenemia was an independent predictor of mortality (relative risk = 6.6; 95% CI: 1.86 to 23.6) within the first 12 weeks of ART initiation. The population-attributable risk for mortality of a positive CRAG was 18%, similar to the attributable mortality due to tuberculosis (19%).

Similar mortality risks were reported in a TB HIV-coinfected cohort in Uganda (n = 302) where predictors of mortality included CRAG+ at enrollment [hazard ratio (HR) = 4.3, 95% CI: 1.5 to 12.1], which was only exceeded by no ART initiation (HR = 4.6, 95% CI: 2.4 to 9.0).<sup>20</sup> This cryptococcal mortality appears in other TB cohorts. In Cape Town, South Africa, the CM-related mortality exceeded that of paradoxical TB-immune reconstitution inflammatory syndrome (IRIS) in a HIV/TB cohort.<sup>21</sup> Thus, screening persons for cryptococcal antigenemia before initiating ART can identify those at highest risk for mortality due to cryptococcosis.

### Clinical Course of Asymptomatic Antigenemia

Few studies have described the clinical course of patients with asymptomatic cryptococcal antigenemia

(Table 1). One US study in the pre-ART era retrospectively investigated 10 persons who were serum CRAG+ but cerebrospinal fluid (CSF) CRAG and culture negative.<sup>22</sup> Six persons were empirically treated with fluconazole (unspecified dose) and had no evidence of subsequent CM. The seventh patient was not treated with fluconazole and developed cryptococcal liver disease, followed by meningitis, and died. Three of 10 patients were found to have disseminated cryptococcal disease from their initial evaluation. Two were fungemic and died. The third had pulmonary cryptococcosis with no reported outcome.<sup>22</sup>

CRAG was also retrospectively measured in 131 HIV-infected, ART-naive, asymptomatic patients in Thailand.<sup>19</sup> The prevalence of asymptomatic antigenemia was 12.9% for those with a CD4 count <100 cells per microliter and 3.6% with CD4 between 100 and 199 cells per microliter. Of serum CRAG+ patients, 33% developed cryptococcal infection, whereas 0.8% of CRAG negative developed cryptococcal infection over 1 year.<sup>19</sup>

In South Africa, CRAG has been retrospectively measured by LA in 707 HIV-infected patients who were about to start ART, of which 7% (46 of 707) were serum CRAG+.<sup>12</sup> If only those with a CD4 count ≤100 cells per microliter were screened, 42 of the total 46 patients with a positive CRAG would be detected, and the prevalence of CRAG+ was 13% in CD4 ≤100 cells per microliter. Positive serum CRAG by LA of >1:8 was 100% sensitive and 96% specific for predicting CM within 1 year. Of those who were CRAG+, 28% developed CM over the course of 1 year, whereas of the 661 who were CRAG negative, there were no cases of CM. Overall, 34% of CRAG+ versus 11% of CRAG-negative persons died in the first year of ART. CRAG+ was an independent predictor of mortality (HR: 3.2; 95% CI: 1.5 to 6.6), even after adjusting for CD4 count, viral load, age, and sex.<sup>12</sup> Mortality directly correlated with CRAG titer; those with a CRAG titer of 1:16 to 1:64 had 25% mortality, whereas those with a CRAG titer of >1:4096 had 67% mortality. Of those without a previous history of CM, 7 of 25 developed CM at a median of 35 days after starting ART. Of those with a prior history of CM, 29% (6 of 29) had an episode of culture-positive relapse at a median of 33 days (range: 5–70 days). Together this suggests that immune reconstitution with ART alone is insufficient to prevent CM when already CRAG+.

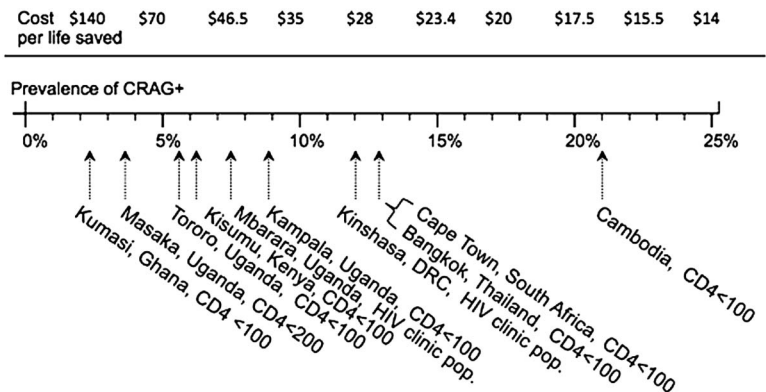


FIGURE 1. Prevalence of asymptomatic antigenemia with corresponding cost per life saved based on LFA cost of \$2.50 per test.

**TABLE 1.** Summary of Outcomes of Those With Asymptomatic Cryptococcal Antigenemia

First Author	Year	Country	Population	CRAG + Outcomes	Comments
French et al <sup>14</sup>	2002	Uganda	1372 HIV+, no ART	Average survival 26 days	Serum CRAG was a sensitive test. Detectable a median of 22 days before symptoms (100 days in 11%)
Liechty et al <sup>18</sup>	2007	Uganda	377 HIV+ CD4 ≤ 100, ART naive, asymptomatic	22 CRAG+ (5.8%). 23% died within 12 weeks of ART initiation	Serum CRAG+ was independent predictor of mortality (relative risk: 6.6) during first 12 weeks of ART
Feldmesser et al <sup>22</sup>	1996	USA	10 AIDS and asymptomatic +CRAG	Six of 7 with no invasive infection were treated with fluconazole, and survived. One untreated, died	3 of 10 found to have invasive cryptococcal infection
Jarvis et al <sup>12</sup>	2009	South Africa	707 HIV+, asymptomatic	46 CRAG + (7%). 28% developed CM in 1 year. 34% died in 1 year	100% sensitivity for CM within 1 year of ART. Independent predictor of mortality (HR: 3.2). Mortality correlated with CRAG titer
Pongsai et al <sup>19</sup>	2010	Thailand	131 HIV+ asymptomatic, ART naive	12 CRAG+ (9.2%). 33% developed CM in 1 year	—
Meya et al <sup>15</sup>	2010	Uganda	295 HIV + CD4 ≤ 100, ART naive	26 CRAG + (8.8%)	21 of 26 treated with fluconazole 200–400 mg. In fluconazole treated persons, 30-month survival was 71%. In untreated CRAG+, all 5 died within 2 months of ART initiation
Kendi et al <sup>23</sup>	2011	Kenya	1726 HIV infected, ART naive	108 CRAG+ (6.2%). 37.5% died. Only 59% of CRAG+ received fluconazole	Fluconazole given at: 1200 mg × 2 weeks; 800 mg × 8 weeks. Multivariate survival analysis, ART (HR = 0.08, <i>P</i> < 0.01) and fluconazole (HR = 0.15, <i>P</i> < 0.01) were significantly associated with improved survival

**PRE-EMPTIVE THERAPY**

Any screening test requires an effective intervention to prevent disease. In Uganda, a prospective cohort study assessed the impact of CRAG screening immediately before ART initiation.<sup>15</sup> Of 295 people with CD4 ≤100 cells per microliter and without prior CM, 26 (8.8%) were CRAG+. In this observational cohort, fluconazole therapy was not standardized. Of those CRAG+, 21 were treated with fluconazole (200–400 mg) for 2–4 weeks. Clinical CM developed in 3 fluconazole-treated persons, and 30-month survival was 71%. In the 5 CRAG+ persons with CD4 ≤100 cells per milliliter treated with ART but not fluconazole, all died within 2 months of ART initiation. In those with CD4 >100 cells per microliter, the CRAG+ incidence was 2.3% (7 of 298). Of these 7 CRAG+ persons, 86% (6 of 7) survived for >30 months on ART. Of the 6 survivors, 4 received fluconazole and 2 remained asymptomatic without fluconazole. The 1 person who died did not receive fluconazole.<sup>15</sup> These data demonstrated that pre-emptive therapy can prevent clinical disease, avoid hospitalization, and improve long term survival to be equivalent to CRAG-negative persons.

Conversely, concomitant medications are also important, particularly rifamycin. Among the aforementioned HIV/TB cohort in Uganda, those CRAG+ received fluconazole 400 mg daily. With rifampin-induced fluconazole metabolism, this is an effective fluconazole dose of ~250 mg daily. In this cohort, 4 of 7 CRAG+ persons died.<sup>20</sup> Implementation programs need to consider drug–drug interactions.

**CRAG LATERAL FLOW ASSAY**

Historically, CRAG has been detected by LA or enzyme immunoassay. However, these processes require heat inactivation, refrigeration of reagents, laboratory infrastructure for agglutination testing (eg, rotator), laboratory expertise, and labor. Given that the majority of cryptococcal disease occurs in resource-constrained settings, these facilities are often unavailable for diagnosis outside of urban settings or referral centers.

In July 2011, a lateral flow immunoassay was approved by the US Food and Drug Administration for detection of CRAG (Immy Inc, Norman, Oklahoma). The CRAG lateral flow assay (LFA) is a rapid diagnostic dipstick immunochromatographic assay that provides a definitive result for presence of CRAG within 15 minutes (Fig. 2). The procedure requires a drop (40 µL) of serum, plasma, urine, or CSF specimen to be placed in a reservoir with the LFA dipstick. If CRAG is present in the specimen, the specimen will bind to the gold-conjugated anticryptococcal antibodies on the test strip causing a visible line. The LFA can be run individually using an Eppendorf tube as a reservoir or can be batched on 96-well plates. The CRAG LFA is an ideal point of care test, as it can be performed by persons with minimal training, without any additional laboratory equipment other than a tube to hold the specimen. Only 1 drop of bodily fluid is required, and the assay can be performed at room temperature, not requiring refrigeration or heat inactivation.

The LFA test was validated in a Thai cohort of 701 HIV-infected patients hospitalized with acute respiratory

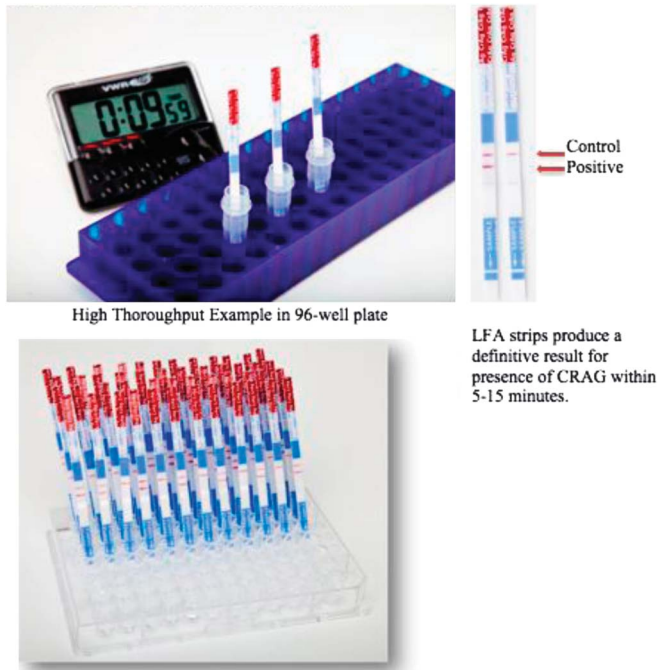


FIGURE 2. Example of LFA.

illness and without meningitis.<sup>24</sup> Seventeen of 701 persons had positive blood cultures for *Cryptococcus*. All 17 had a positive serum LFA (100% sensitivity). When compared with the cryptococcal enzyme immunoassay (EIA) test, 87 of the 91 EIA-positive samples were detected by serum LFA after 15 minutes (96% sensitivity). Kappa agreement of EIA and LFA for sera was 0.96 after 15 minutes. Urine LFA had lower sensitivity of 92% as compared with blood cultures and 71% sensitivity when compared with EIA-positive serum samples. Patients with CM likely have a higher burden of circulating organisms and thus may be more likely to have detectable antigen.

The LFA was recently validated in 62 patients with CM or a recent history of CM in South Africa.<sup>25</sup> Serum and urine LFA results were positive in 61 of 62 with a positive enzyme-linked immunosorbent assay, and correlation of LFA and enzyme-linked immunosorbent assay was 0.93 ( $P < 0.001$ ) for serum, 0.94 ( $P < 0.001$ ) for plasma, and 0.94 ( $P < 0.001$ ) for urine. The concentration of cryptococcal polysaccharide capsule was 22-fold lower in the urine compared with serum; however, the urine LFA was still highly sensitive. Further unpublished data from a Ugandan CM cohort also found a >99% sensitivity of the LFA in CSF or plasma (unpublished data, Boulware Feb 2012).<sup>10</sup> In this cohort, the LFA was 5-fold more sensitive than standard LA when cross comparing semiquantitative titers by serial dilution (eg, a 1:8 CrAg titer by LA is positive at 1:40 titer by LFA).

Notably, the LFA has not yet been prospectively validated as a screening tool for asymptomatic patients with cryptococcal antigenemia; however, based on our unpublished experience above, the 5-fold greater LFA sensitivity is not anticipated to be a problem for CrAg detection.

## Cost-Effectiveness of CRAG Screening

The cost-effectiveness of serum CRAG screening and pre-emptive therapy was evaluated in the 609-person cohort from Kampala, Uganda.<sup>15</sup> In the cohort with 8.2% CRAG+ prevalence, the number need to test and treat to detect one person with asymptomatic antigenemia was 11.3, at a cost of \$190 in a population with a CD4 count  $\leq 100$  cells per microliter. At that time in 2010, the CRAG LA test cost \$16.75 in a CAP-certified independent clinical laboratory in Uganda. The number needed to prevent 1 death was 15.9 at a cost of \$266. The authors assumed an average increase in life expectancy of 12.5 years with ART, which equated to \$21 per disability-adjusted life year saved. This was a conservative analysis as it did not incorporate hospitalization or avoided medication costs (as most people died rapidly without accruing additional health care costs).

Micol et al<sup>26</sup> also analyzed the cost-effectiveness of CRAG screening in asymptomatic people with CD4 count  $\leq 100$  cells per microliter in Cambodia, where the prevalence of asymptomatic antigenemia is 21%. Using data from Cambodian cohorts to construct a mathematical model to compare the cost effectiveness of CRAG screening versus fluconazole primary prophylaxis in everyone with a CD4 count  $\leq 100$  cells per microliter, they found the cost-effectiveness of CRAG screening (based on a LA test cost of \$6.60) compared with no intervention was \$180 per life-year gained, and the cost-effectiveness of fluconazole primary prophylaxis versus CRAG screening was \$511 per life-year gained. Although both strategies can reduce the incidence of clinical cryptococcosis, targeted screening and treatment of those with a CD4  $\leq 100$  cells per microliter was found to be more cost-effective than primary fluconazole prophylaxis.

With the CRAG LFA, the cost considerations have further improved. The CRAG LFA is priced at \$2 when ordered directly from the manufacturer ([www.immy.com](http://www.immy.com)) for institutions in resource-limited areas. International shipping to sub-Saharan Africa in bulk is \$.03 per test (personal communication, Sean Bauman, Immy, Inc.). We assume an additional \$0.47 for local personnel costs, laboratory overhead for a total test cost of \$2.50. Assuming a total cost of \$2.50 per test, for every CM admission in South Africa that is estimated at \$2883,<sup>27</sup> one could assay 1153 CRAG LFA tests. Using results of Meya et al<sup>15</sup> from Uganda, the cost of detecting 1 person with asymptomatic antigenemia with the LFA would be \$28.37 and the cost of saving one life would be \$39.73 (95% CI: \$28 to \$60). Assuming an average increase in life expectancy of 18 years for a 30-year-old Ugandan initiating ART with a CD4 count  $\leq 100$  cells per microliter,<sup>28</sup> this equates to \$1.57 per disability adjusted life year saved. According to the World Health Organization Commission on Macroeconomics and Health, the LFA is very cost-effective as it is far under the GDP per capita of sub-Saharan African countries. This cost-effectiveness varies by regional prevalence (Fig. 1), but remains  $< \$140$  per life saved where CRAG+ prevalence is  $> 2\%$ .

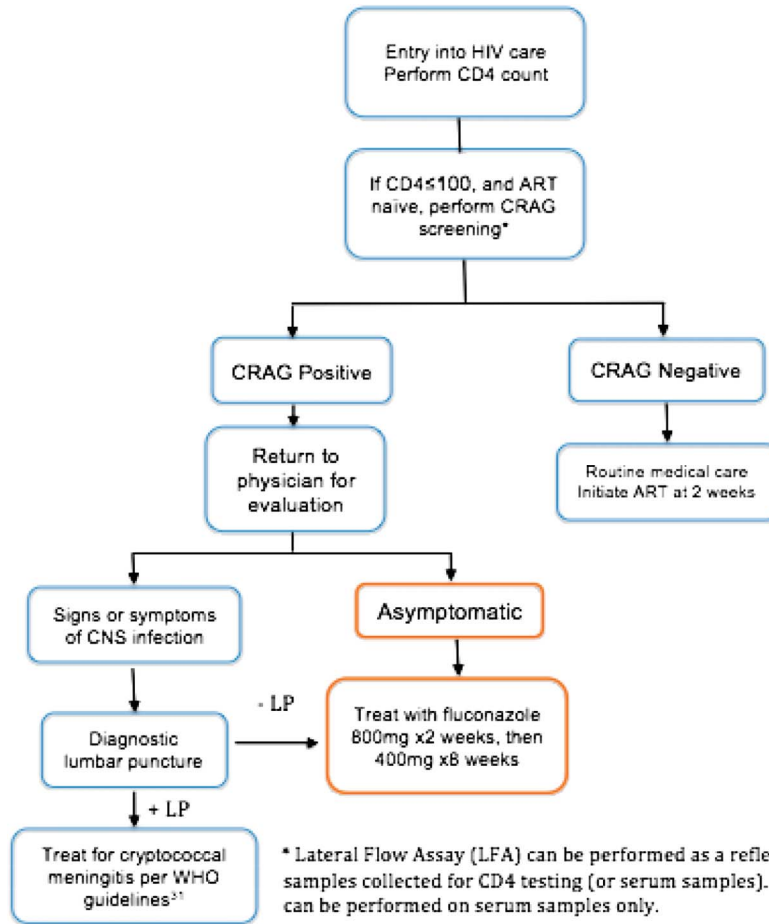


FIGURE 3. Algorithm for CRAG screening upon entry into HIV care.

### LFA APPLICATION

The potential applications for a highly sensitive dipstick test that yields definitive results in 5–15 minutes without the need for laboratory infrastructure are many. Implementation of the LFA as a point-of-care test to detect CRAG will certainly be valuable in symptomatic patients as a triage tool to confirm diagnosis of cryptococcal infection and initiate timely intervention (Fig. 3). An LFA may also guide the need for using a manometer to measure opening pressure. If performed immediately before a lumbar puncture (on a urine or finger stick specimen), a CRAG+ result indicates the need for measurement of CSF opening pressure. If a manometer is unavailable, 15–20 mL of CSF could be taken off therapeutically in CRAG+.<sup>29</sup> When intracranial pressure is managed aggressively, survival is similar, regardless of increased intracranial pressure.<sup>29</sup>

In asymptomatic patients, the LFA will be valuable in screening for cryptococcal antigenemia, especially in smaller clinics and rural areas before initiating ART. The LFA can be run on plasma (unlike the LA), thus LFA testing could be incorporated as a laboratory reflex testing when CD4 < 100 using the same plasma sample collected for CD4 testing. Using urine to detect CRAG clearly eliminates the need for phlebotomy; however, validation of LFA in urine, serum, and plasma has yet to be performed in persons with asymptomatic

cryptococcal antigenemia. Likely, there is a threshold of antigen burden, which predicts meningitis. Based on current limited knowledge, a CRAG LFA titer >1:40 (or >1:8 by LA)<sup>12</sup> would be the current threshold above which subsequent meningitis becomes increasingly likely.

### Areas of Future Research

In September 2011, the Copenhagen Consensus expert panel of epidemiologists, economists, and demographers concluded that prevention of CM is an “overlooked policy with merit” and should be a public health priority in sub-Saharan Africa given substantial potential benefit, at minimal cost. Yet, many implementation science questions remain regarding how best to scale-up screening and the most appropriate management for CRAG+ persons. First, the sensitivity and specificity of the LFA needs to be determined in the context of screening an asymptomatic population at high risk of developing CM. Sensitivity will not be a problem; however, the threshold LFA titer at which one is likely to develop meningitis has not yet been firmly established (ie, positive predictive value). Published data suggest CrAg LA titers lower than 1:8 are low risk,<sup>12</sup> and this extrapolates to LFA titers lower than 1:40. Once identified as CRAG+, the appropriate diagnostic work up remains ill defined. For example, if

an asymptomatic patient is found to be CRAG+, do they require a lumbar puncture to rule out CNS infection? If those with asymptomatic antigenemia are treated with antifungal therapy without further work up, is there potential risk of missing underlying CM, and what are the consequences, if so? If further work up is deemed necessary, the acceptability of lumbar punctures in asymptomatic individuals could present a barrier to which local solutions will be needed based on local culture and concepts of disease.

In terms of optimal treatment once identified as CRAG+, ART alone does not adequately treat antigenemia.<sup>12,15</sup> Fluconazole treatment in combination with ART reduces mortality<sup>15</sup>; however, the optimal dose and duration have not been prospectively studied. In a Ugandan study, fluconazole was given at doses between 200 mg and 400 mg daily for 2–4 weeks. Although those who were treated with fluconazole had lower mortality compared with CRAG+ persons who were untreated (29% vs. 75%), higher doses of fluconazole (between 800 mg and 1200 mg daily) have been shown to have greater fungicidal activity<sup>30</sup> without greater side effects and will likely demonstrate superior survival benefit. What is the role of short course amphotericin? Likely there are 3 key components which will influence optimal treatment as follows: CD4 count, antigen burden, and presence of meningitis with increased intracranial pressure.

In Kenya, CRAG screening was implemented in a multisite clinic network among those with CD4 counts  $\leq 100$  cells per microliter.<sup>23</sup> The CRAG+ prevalence was 6.2% (108 of 1726). Recommendations were made for fluconazole treatment 1200 mg for 2 weeks, 800 mg for 8 weeks, and then secondary prophylaxis. Those who did not receive ART or fluconazole had a median survival of 2.5 weeks. In the interim multivariate survival analysis, ART (HR = 0.08,  $P < 0.01$ ) and high-dose fluconazole (HR = 0.15,  $P < 0.01$ ) were significantly associated with improved survival. However, overall mortality of CRAG+ persons was still 37.5%. Several problems were observed with implementation. First, the uptake of screening was only 46%. Barriers to uptake included limited laboratory capacity for LA testing, and limited access to phlebotomy. In this scenario, it is possible that implementation of the LFA may reduce the burden on laboratory capacity and phlebotomy. Second, only 59% of CRAG+ actually received fluconazole possibly secondary to limited access to clinicians in a busy clinic system. This emphasizes the need for a health systems-based approach to implementation. Third, symptomatic patients had approximately twice the hazard of death as asymptomatic persons, thus caution is warranted for clinical evaluation of CRAG+ individuals to assess whether meningitis is present.

An interesting area of future study is the optimal time to initiate ART in those who are CRAG+ versus CRAG negative and the influence of antigen burden. It is plausible that those who are CRAG+ with a high antigen burden are at greater risk of cryptococcal immune reconstitution inflammatory syndrome and delaying ART may be of benefit in this population. Yet, delaying ART in persons with low antigen burden is likely also unwarranted as there is ongoing mortality due to AIDS during any delay in ART initiation. ART should likely not be delayed beyond 2 weeks.

**TABLE 2.** Key Principles of CRAG Screening

**CRAG can be detected before onset of disease in asymptomatic individuals**

- The majority of CRAG+ persons have CD4 count  $\leq 100$  cells/mL
- Those who are CRAG+ are at high risk of infection and mortality, particularly within 12 weeks of initiating ART
- CRAG screening is cost-effective in areas of high CM burden<sup>15</sup>

**The CRAG LFA**

- A rapid diagnostic point of care assay that is highly sensitive and specific for CRAG<sup>26</sup>
- Requires no laboratory infrastructure or expertise
- Highly cost-effective at \$2 per assay (Immy.com)
- LFA titer is approximately 5  $\times$  more sensitive than standard LA
  - A 1:8 titer by LA is equivalent to a 1:40 LFA titer

**Screening recommendations**

- Target those with a CD4 count  $\leq 100$  cells/mL who are ART naive or have defaulted
- Ensure patient is without signs or symptoms of invasive infection
- LFA titers  $> 1:40$  (LA  $> 1:8$ ) are at high risk for meningitis<sup>19</sup>

**Treatment recommendation**

- Treat CRAG+ persons with a minimum dose of Fluconazole 800 mg daily for 2 weeks, followed by 400 mg for 8 weeks<sup>31</sup>
  - Exclude symptomatic meningitis. When in doubt, perform lumbar puncture
  - Consider longer duration of antifungal treatment for CD4  $< 50$  cells/mL
  - Consider longer duration for higher fungal burden, by quantitative titer
- Efavirenz preferred over nevirapine due to lack of fluconazole drug–drug interaction
- Rifampin induces metabolism of fluconazole by 50%. Increase fluconazole dose

Finally, implementing a new screening test into routine HIV care may bring about challenges to medical providers who struggle to provide comprehensive HIV and primary care in areas of scarce resources. For example, it is unclear whether health care providers should initiate CRAG screening as a point of care test, or if this would have greater uptake if reflexively performed by laboratory personnel for those with a CD4 count  $\leq 100$  cells per microliter as part of clinic protocols. Loss-to-follow-up after a CRAG+ result will need to be addressed if an intervention is to have mortality benefit. A point-of-care CRAG test helps eliminate delay in reporting but is physician dependent. Difficulties with implementation will likely be site specific and will only be evident once routine screening is started.

**RECOMMENDATIONS FOR CLINICAL CARE**

In areas of high burden of cryptococcal disease, we recommend CRAG screening before initiating ART in those with a CD4 count  $\leq 100$  cells per microliter (Table 2). All persons who are CRAG+ should be clinically evaluated to rule out symptomatic CM. If they have symptoms concerning for meningitis, then lumbar puncture is essential to diagnose or exclude meningitis. Those who remain asymptomatic should be considered to have subclinical cryptococcal infection and receive pre-emptive antifungal treatment, typically with fluconazole.<sup>31</sup> The newly released LFA is the ideal point

of care test—sensitive, inexpensive, requires minimal laboratory infrastructure, at a total cost of <\$40 to save one person's life. We posit that CRAG screening and treatment should be integrated into routine HIV care, targeting those with a CD4 count  $\leq 100$  cells per microliter before starting ART. Though further studies are needed to determine the optimal treatment dose and duration for those with asymptomatic antigenemia, based on the available data<sup>15,23,30</sup> and expert opinion, we recommend preemptive treatment with a minimum fungicidal dose of fluconazole 800 mg daily for 2 weeks followed by fluconazole 400 mg for 8 weeks to reduce mortality at the time of ART initiation, in line with a conditional World Health Organization recommendation.<sup>31</sup>

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