

# Cryptococcal Antigenemia in Immunocompromised Human Immunodeficiency Virus Patients in Rural Tanzania: A Preventable Cause of Early Mortality

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**Background.** Cryptococcal meningitis is a leading cause of death in people living with human immunodeficiency virus (HIV)/acquired immune deficiency syndrome. The World Health Organization recommends pre-antiretroviral treatment (ART) cryptococcal antigen (CRAG) screening in persons with CD4 below 100 cells/ $\mu$ L. We assessed the prevalence and outcome of cryptococcal antigenemia in rural southern Tanzania.

**Methods.** We conducted a retrospective study including all ART-naive adults with CD4 <150 cells/ $\mu$ L prospectively enrolled in the Kilombero and Ulanga Antiretroviral Cohort between 2008 and 2012. Cryptococcal antigen was assessed in cryopreserved pre-ART plasma. Cox regression estimated the composite outcome of death or loss to follow-up (LFU) by CRAG status and fluconazole use.

**Results.** Of 750 ART-naive adults, 28 (3.7%) were CRAG-positive, corresponding to a prevalence of 4.4% (23 of 520) in CD4 <100 and 2.2% (5 of 230) in CD4 100–150 cells/ $\mu$ L. Within 1 year, 75% (21 of 28) of CRAG-positive and 42% (302 of 722) of CRAG-negative patients were dead or LFU ( $P < .001$ ), with no differences across CD4 strata. Cryptococcal antigen positivity was an independent predictor of death or LFU after adjusting for relevant confounders (hazard ratio [HR], 2.50; 95% confidence interval [CI], 1.29–4.83;  $P = .006$ ). Cryptococcal meningitis occurred in 39% (11 of 28) of CRAG-positive patients, with similar retention-in-care regardless of meningitis diagnosis ( $P = .8$ ). Cryptococcal antigen titer >1:160 was associated with meningitis development (odds ratio, 4.83; 95% CI, 1.24–8.41;  $P = .008$ ). Fluconazole receipt decreased death or LFU in CRAG-positive patients (HR, 0.18; 95% CI, .04–.78;  $P = .022$ ).

**Conclusions.** Cryptococcal antigenemia predicted mortality or LFU among ART-naive HIV-infected persons with CD4 <150 cells/ $\mu$ L, and fluconazole increased survival or retention-in-care, suggesting that targeted pre-ART CRAG screening may decrease early mortality or LFU. A CRAG screening threshold of CD4 <100 cells/ $\mu$ L missed 18% of CRAG-positive patients, suggesting guidelines should consider a higher threshold.

**Keywords.** cryptococcal antigen; cryptococcal meningitis; diagnosis; mortality; prevention; retention in care; screening; sub-Saharan Africa.

*Cryptococcus neoformans* is a major cause of adult meningitis in sub-Saharan Africa and a leading cause of

mortality among people living with human immunodeficiency virus (PLHIV) [1, 2]. Cryptococcal meningitis

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(CM) is estimated to cause 13%–44% of HIV/acquired immune deficiency syndrome (AIDS) deaths in Africa [1,3] and is a major contributor of early mortality during the first year of antiretroviral treatment (ART), accounting for up to 20% of all deaths [4,5]. Treatment of cryptococcosis is suboptimal in most African settings, given the low availability of first-line antifungal drugs and low uptake of recommendations for management of intracranial pressure. As a result, cryptococcal-related mortality in Africa remains unacceptably high, ranging from 20% to 50% even in settings with available first-line drugs [6–10].

Early diagnosis of cryptococcal infection is the key to improving outcomes. Detectable cryptococcal antigen (CRAG) in peripheral blood precedes meningitis symptoms by weeks to months, opening a window of opportunity for early detection [3]. The World Health Organization (WHO) recommends pre-ART-targeted plasma CRAG screening for PLHIV with CD4 <100 cells/ $\mu$ L followed by preemptive fluconazole treatment in regions with high incidence of CRAG [11].

Data about the burden of cryptococcal disease in Tanzania are scarce. In a retrospective study in Dar es Salaam, analyzing 1144 cerebrospinal fluids (CSFs) specimens, *Cryptococcus neoformans* accounted for 98% of all positive CSF cultures among adults and 22% among children [12]. In another study in the Kilimanjaro region in 2005/2006, 27% (40 of 149) of consecutive HIV-infected adult inpatients presenting with headache or altered mental status were diagnosed with cryptococcal meningitis [13]. A study conducted in Mwanza, in northwestern Tanzania in 2009/2010, showed a 5% prevalence of CRAG by latex agglutination and 4.4% prevalence of cryptococcal meningitis among 333 PLHIV consecutively admitted in a medical ward [14]. Among the 93 patients with CD4 counts <100 cells/ $\mu$ L, the cryptococcal meningitis prevalence was 15%, and *Cryptococcus* accounted for 26% of all in-hospital deaths. A more recent cross-sectional study, aiming to evaluate the performance of CRAG lateral flow assay (LFA) in the Kilimanjaro region, showed a 5% prevalence among PLHIV with CD4 cell counts <100 cells/ $\mu$ L [15].

Taken together, these findings suggest that cryptococcosis is common among PLHIV in Tanzania and an important cause of preventable mortality. Aiming to explore the potential impact of the WHO recommendations on CRAG screening in Tanzania, we assessed the prevalence of CRAG and impact on survival and retention-in-care among a cohort of ART-naive PLHIV engaged in care between 2008 and 2012.

## METHODS

### Study Design and Setting

All data were prospectively collected from participants enrolled in the Kilombero and Ulanga Antiretroviral Cohort (KIULARCO). This ongoing, open, prospective cohort comprises all patients who visit the Chronic Diseases Clinic of Ifakara within

the Saint Francis Referral Hospital and provide their written informed consent. This is the largest healthcare facility in the Kilombero district of the Morogoro region in southern Tanzania, providing treatment and care for a population of approximately 600 000 inhabitants and an estimated 30 000 patients living with HIV/AIDS. Established in 2004, this was the first rural clinic accredited to be a Care and Treatment Centre of the National AIDS Control Program in the whole of Tanzania, and >7000 patients have been enrolled into care. Blood samples are drawn at routine clinic visits before, 2 weeks and 3 months after ART initiation, and every 6 months thereafter. Plasma is cryopreserved at  $-80^{\circ}\text{C}$ . Further details of the KIULARCO cohort are given elsewhere [16,17].

### Participants

We included all ART-naive patients >18 years-old of age enrolled in KIULARCO between January 1, 2008 and December 31, 2012 with CD4 counts <150 cells/ $\mu$ L who had an available cryopreserved pre-ART sample.

Cryptococcal Antigenemia Testing and Titration Cryptococcal antigenemia LFA (IMMY, Inc., Norman, OK) was performed according to the manufacturer's instructions in the most recent stored plasma sample collected within a maximum of 90 days before ART initiation. Semiquantitative LFA CRAG titers were measured in all CRAG-positive samples using 2-fold dilutions, as per the manufacturer's instructions.

### CD4 Counting

CD4 counts were measured after staining fresh whole blood samples with labeled antibodies: CD4, CD3, CD8, and CD45 in TruCount tubes (BD FACSCalibur, Franklin Lakes, NJ, U.S.A.).

### Statistical Analysis

All data were extracted from the electronic databases of the KIULARCO cohort. In addition, for patients with a positive CRAG test, medical records were reviewed for medication use, presenting symptoms, and follow-up status or cause of death.

The baseline was defined as the date of the pre-ART CRAG assessment. The time from baseline to the combined endpoint of death or LFU within 1 year was investigated. The time of the event was defined as the day of the last follow-up visit documented in the database within 1 year of follow-up. Patients who experienced an event after 1 year from baseline or were still retained-in-care were right-hand censored at 1 year. Log-rank test was used to compare the risk of developing the outcome of death or LFU by CRAG status.

Multivariate Cox proportional hazards models assessed the association between pre-ART CRAG status and death or LFU within 1 year from baseline. The following variables were considered a priori as potential cofactors or confounders and were included in the multivariate model: age, sex, body mass index (BMI), CD4 cell count, ART status, WHO stage (Stage 1/2 vs 3/4), and

tuberculosis (TB) status (suspected, on treatment, microscopy sputum smear positive vs no TB). Assumption of proportional hazards was confirmed by Schoenfeld's global test ( $P = .3$ ).

In a subgroup analysis of asymptomatic CRAG-positive participants, we evaluated the contribution of fluconazole to survival or retention-in-care using Cox proportional hazards models to assess the association between baseline variables and a combined endpoint of death or LFU within 1 year. Finally, the CRAG titer was analyzed for the presence of a threshold predicting the future development of cryptococcal meningitis, with statistical assessment by logistic regression.

All data were anonymized and analyzed using STATA version 12.1 (Stata Statistical Software, StataCorp, College Station, TX).

### Ethics Statement

Written informed consent was sought from all participants at registration at the Chronic Diseases clinic of Ifakara, and all data were deidentified. Samples were collected during routine clinical visits, and ethical approval for its cryopreservation and retrospective analysis was obtained from the Ifakara Health Institute institutional review board and from the National Health Research Ethics Review Committee of the National Institute for Medical Research of Tanzania.

## RESULTS

### Cohort Characteristics

A total of 750 ART-naive HIV-1-infected adults were included in the study (Figure 1). Table 1 summarizes the pre-ART baseline characteristics by CRAG status. Overall, 60% of participants were women with a median age at enrollment of 38.3 years (interquartile range [IQR], 32.5–45.2). Median BMI was 19.8 kg/m<sup>2</sup>

(IQR, 17.7–22.1), and median CD4 was 71 cells/μL (IQR, 36–109). Cryptococcal antigenemia-positive patients had lower CD4 cell counts, lower BMI, and had a higher proportion of WHO stage III/IV and males. Eighty-eight percent of patients started ART, with a median time of 5 days (IQR, 1–11) after enrollment.

### Prevalence and Characterization of Cryptococcal Antigenemia

The 750 participants enrolled contributed 488 person-years of follow-up time during the study period. Overall, 28 of 750 patients (3.7%; 95% confidence interval [CI], 2.4–5.1) had a positive CRAG LFA in plasma. CD4 stratum-specific CRAG prevalence was 6.3% (17 of 270; 95% CI, 3.4–9.2%) in CD4 <50, 4.4% (23 of 520; 95% CI, .5–4.3) in CD4 <100, and 2.2% (5 of 230; 95% CI, .3–4.1) in CD4 100–150 cells/μL. Overall, 18% of CRAG-positive persons had CD4 > 100 cells/μL (95% CI, 6.1%–37%). The median pre-ART CRAG LFA titer was 1:320 (IQR, 1:20–1:2560) (Supplementary Table).

### Outcome and Predictors of Death or Loss to Follow-Up

Of 28 CRAG-positive persons, 21 (75%) died or were lost compared with 302 of 722 (42%) CRAG-negative persons within the first year ( $P = .001$ ; Figure 2A). This proportion was similar across CD4 strata, being 76.5% (13 of 17), 83% (5 of 6), and 60% (3 of 5) among patients with ≤50, 51–100, and 101–150 CD4 cells/μL, respectively ( $P = .656$ ). The median survival time in CRAG-positive patients was 3.0 months (IQR, 0.5–6.82) compared with 12.0 months (IQR, 1.6–12.0;  $P = .001$ ) for CRAG-negative patients. Eighty-nine percent (25 of 28) of CRAG-positive persons initiated ART.

Baseline risk factors for death or LFU are presented in Table 2. The CRAG-positive status was found to be an independent predictor of increased risk of death or LFU compared with CRAG-negative status after adjusting for all prespecified confounders (HR, 2.50; 95% CI, 1.29–4.83;  $P = .006$ ). Additional variables found to be significantly associated with death or LFU were not having initiated ART (HR, 2.25; 95% CI, 1.03–4.88;  $P = .041$ ) and female gender (HR, 1.50; 95% CI, 1.05–2.15;  $P = .025$ ). Protective factors were older age (HR, 0.73 per 10 years increase; 95% CI, .60–.90;  $P = .003$ ) and higher BMI (HR, 0.9 per kg/m<sup>2</sup> increase; 95% CI, .85–.95;  $P < .001$ ). Higher CD4 counts were protective from death or LFU in univariable but not in multivariable analyses (Table 2).

### Predictors of Cryptococcal Meningitis and Mortality/Loss to Follow-Up Among Cryptococcal Antigenemia-Positive Patients

Seven CRAG-positive patients (25%) were recorded to have presented at baseline with pre-ART neurologic symptoms (eg, headache  $n = 7$ , suspected meningitis  $n = 1$ ), and 4 of 21 (19%) initially neurologically asymptomatic patients developed meningitis at a median of 6 weeks (range, 1–16 weeks) after starting ART. In total, 39% (11 of 28) of CRAG-positive patients were recorded to have neurological symptoms, and 9 were diagnosed clinically with cryptococcal meningitis during follow-up.

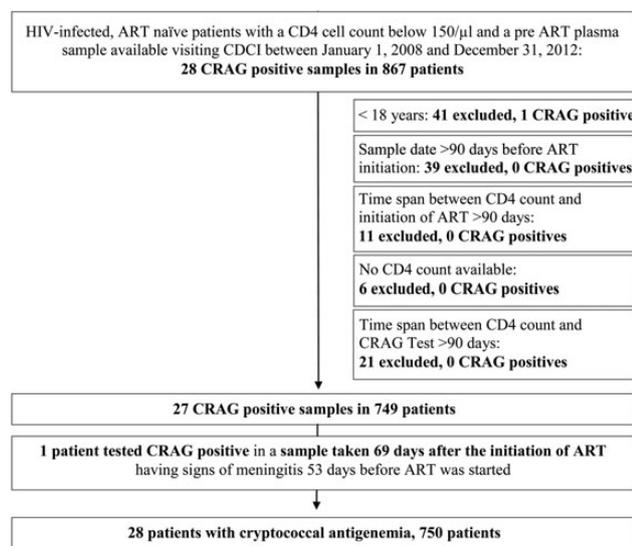


Figure 1. Study profile of included and excluded participants.

**Table 1. Baseline Characteristics of Study Population<sup>a</sup>**

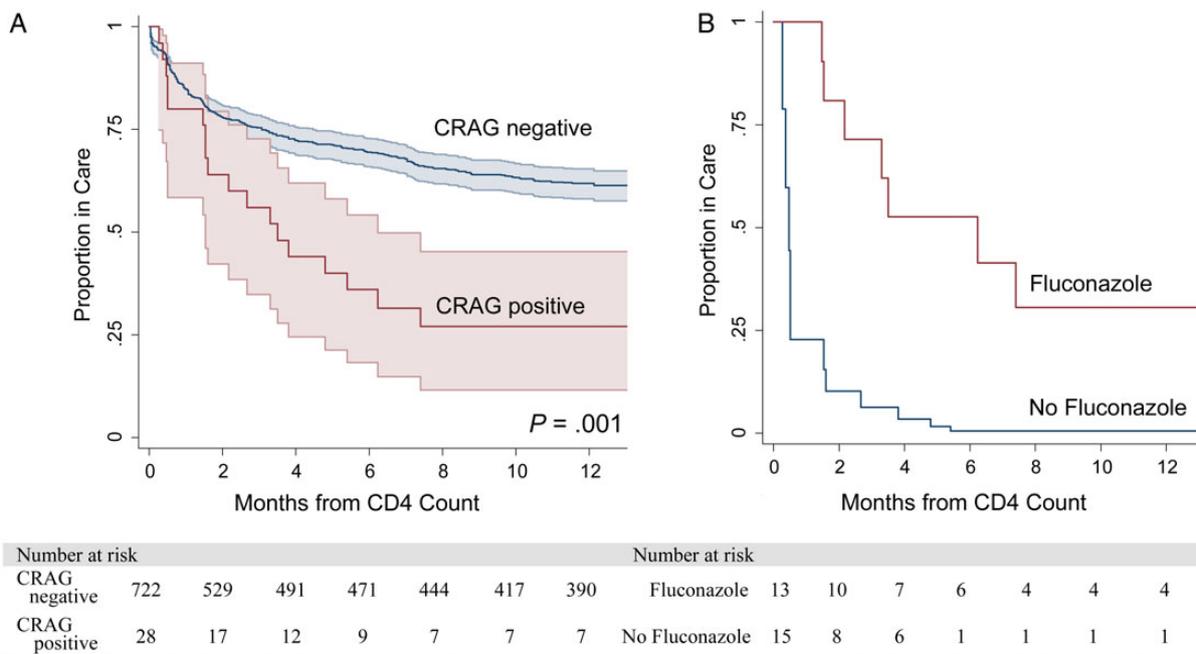
Variable	Total	CRAG <sup>-</sup>	CRAG <sup>+</sup>
N	750	722	28
Age, years - median (IQR)	38.3 (32.5–45.2)	38.3 (32.3–45.2)	36.7 (33.6–44.8)
Female	448 (60%)	436 (60%)	12 (43%)
BMI, kg/m <sup>2</sup> - median (IQR)	19.8 (17.7–22.1)	19.9 (17.7–22.1)	18.7 (17.5–19.7)
WHO - stage 3 or 4	297 (45%)	283 (45%)	14 (56%)
CD4 cells/μL - median (IQR)	71 (36–109)	71 (36–110)	37 (19–76)
Stratum 0–50 cells/μL	270 (36%)	253 (35%)	17 (61%)
Stratum 51–100 cells/μL	250 (33%)	244 (34%)	6 (21%)
Stratum 101–150 cells/μL	230 (31%)	225 (31%)	5 (18%)
CRAG titer, median (IQR)			320 (20–2560)
Stratum 1:5–1:20	...	...	8 (29)
Stratum 1:40–1:1280	...	...	12 (43)
Stratum 1:2560–1:10240	...	...	8 (29)
Hospitalized	...	...	9 (32%)
Fluconazole treatment	...	...	13 (46%)
Time to start ART, days - median (IQR)	5 (1–11)	5 (1–11)	1 (1–5)
Did not initiate ART	90 (12%)	87 (12%)	3 (11%)
Neurologic symptoms at baseline	...	...	7 (25%)
Active tuberculosis	77 (12%)	73 (12%)	4 (20%)

Abbreviations: ART, antiretroviral treatment; BMI, body mass index; CRAG, cryptococcal antigenemia; IQR, interquartile; WHO, World Health Organization.

<sup>a</sup> Data are presented as n (%) or median (IQR).

The risk of developing meningitis among the 21 initially asymptomatic CRAG-positive patients was significantly associated with a CRAG titer > 1:160 in a multivariable logistic

regression model, adjusted by CD4, gender, and age (odds ratio, 4.83; 95% CI, 1.24–8.41; *P* = .008). None of the 11 patients with an antigen titer of ≤1:160 developed symptoms of meningitis.



**Figure 2.** Left graph (A): Kaplan–Meier survival estimates of death or loss to follow-up by pre-antiretroviral treatment (ART) cryptococcal antigenemia (CRAG) status. Right graph (B): Time to death or loss to follow-up among CRAG-positive persons versus fluconazole receipt, adjusted by ART status and CD4 cell count.

**Table 2. Association With Death or Loss to Follow Up by 1 Year, Using a Cox Regression Model Among All Persons With CD4 <150 Cells/μL**

Variables	Unadjusted/Univariate Model		Adjusted/Multivariate Model (n = 137 Events)	
	HR (95% CI)	P Value	HR (95% CI)	P Value
CRAg <sup>+</sup>	2.43 (1.50–3.92)	<.001	2.50 (1.29–4.83)	.006
Age, per 10 years	0.96 (.86–1.09)	.553	0.74 (.60–.90)	.003
Female	1.42 (1.12–1.80)	.004	1.50 (1.05–2.14)	.025
Body mass index, per kg/m <sup>2</sup>	0.88 (.84–.93)	<.001	0.90 (.85–.95)	<.001
CD4 cell count, per 25/μL	0.87 (.81–.93)	<.001	0.93 (.84–1.04)	.205
Tuberculosis	1.38 (.96–1.99)	.080	1.19 (.72–1.97)	.505
No ART	3.79 (2.67–5.40)	<.001	2.25 (1.03–4.88)	.041

Abbreviations: ART, antiretroviral treatment; CI, confidence interval; CRAg, cryptococcal antigenemia; HR, hazards ratio.

Three of these patients died of unknown causes with an average time of 3.4 months after baseline.

A subanalysis was conducted to explore the contribution of fluconazole to survival or retention-in-care among the 21 neurologically asymptomatic CRAg-positive patients. Thirty-eight percent (8 of 21) of patients received fluconazole (100–200 mg/day) as per physician discretion, mostly for oral thrush (6 of 8). Among 8 CRAg-positive persons receiving any fluconazole, the median survival time was 6.5 (IQR, 2.4–12.0) months with 50% (4 of 8) 1-year survival. In contrast, only 2 of 13 persons (15%) survived 1 year among those CRAg-positive persons not receiving any fluconazole, with a median survival time of 1.6 (IQR, 0.5–4.8) months ( $P = .155$ ). After adjusting for CD4 cell count and ART status, receiving any fluconazole treatment reduced the hazard for death or LFU by 82% (95% CI, 22%–96%;  $P = .022$ ) during the first year of follow-up (Table 3; Figure 2B).

## DISCUSSION

Cryptococcal antigenemia was prevalent and an independent predictor of death or LFU in our cohort of ART-naive HIV-infected patients with a CD4 cell count <150 cells/μL in rural Tanzania. The development of signs compatible with cryptococcal meningitis among initially asymptomatic CRAg-positive patients was associated with CRAg LFA titers >1:160 in

plasma. Fluconazole, given incidentally as per physician discretion, most commonly for thrush, reduced the hazard of death or LFU in CRAg-positive persons. These results further support the implementation of a systematic CD4-targeted CRAg screening followed by preemptive treatment with fluconazole for CRAg-positive patients, as recommended by WHO and U.S. President’s Emergency Plan for AIDS Relief [11]. However, our results suggest reconsidering the CD4 threshold for CRAg screening, because 18% of CRAg-positive patients had a measured CD4 cell count >100 cells/μL along with a similarly high rate of poor survival.

We found a CRAg prevalence of 3.7% among ART-naive persons with a CD4 cell counts of <150 cells/μL, 4.4% among those with <100 cells/μL, and 6.7% among <50 cells/μL, comparable with other countries in sub-Saharan African settings [15, 18–21]. Cryptococcal antigenemia prevalence among those patients with CD4 counts between 100 and 150 cells/μL was 2.2% (95% CI, .3%–4.1%)

At this prevalence, the WHO CRAg screen and treat strategy would be cost saving in Tanzania compared with the cost of cryptococcal meningitis treatment [21, 22]. Due to the lower costs of CRAg LFA (USD 2/test), CRAg screening is cost-effective and likely cost-saving at a CRAg prevalence of ~1% [21, 23]. There are approximately 125 000 HIV-infected Tanzanians with CD4 <125 cells/μL not receiving effective ART [24]. Based on the

**Table 3. Association With Death or Loss to Follow Up by 1 Year, Using a Cox Regression Model Among 21 Asymptomatic CRAg-Positive Persons With CD4 <150 Cells/CD4 Cells >100 cells/μL**

Variables	Unadjusted/Univariate Model		Adjusted/Multivariate Model (n = 14 Events)	
	HR (95% CI)	P Value	HR (95% CI)	P Value
CD4 cells >100 cells/μL	0.64 (.14–2.86)	.556	0.28 (.05–1.50)	.137
No ART	2.41 (.53–11.13)	.256	1.05 (.21–5.21)	.955
Fluconazole treatment	0.28 (.08–1.04)	.051	0.18 (.04–.78)	.022

Abbreviations: ART, antiretroviral treatment; CI, confidence interval; CRAg, cryptococcal antigenemia; HR, hazards ratio.

data herein, approximately 5000 CRAG-positive Tanzanians annually would potentially benefit from having access to pre-ART CRAG LFA screening (testing cost = \$250 000). High-burden countries, such as Tanzania, should prioritize reliable access to this CRAG LFA test and the implementation of the WHO screening recommendations to enable early diagnosis of cryptococcal infection and improve survival. The optimal CD4 screening threshold remains unknown. In our cohort, 18% of all CRAG-positive persons had CD4 counts >100 cells/ $\mu$ L, with 4 of 5 between 100 and 125 cells/ $\mu$ L. This proportion of CRAG-positive patients with a CD4 cell count >100 cells/ $\mu$ L lies at the upper end of published data, which ranges from 1% to 21% [25–28]. Setting the CRAG-screening threshold at 125 CD4 cells/ $\mu$ L would capture 96% of CRAG-positive persons in our population. Unlike the published outcomes experience in Uganda [25], the survival outcome in patients with CD4 >100 cells/ $\mu$ L did not differ from those with CD4 <100 CD4 cells/ $\mu$ L, although the sample size was small. Of note, the only two 1-year survivors who were CRAG-positive and untreated with fluconazole in our study had CD4 >100 cells/ $\mu$ L. Thus, based on resources available and accuracy of CD4 testing, the threshold for screening may need to be raised to CD4 125 cells/ $\mu$ L.

Among initially asymptomatic CRAG-positive persons, the development of cryptococcal meningitis was associated with the CRAG titer in plasma. Having a CRAG LFA titer >1:160 was associated with an approximately 5-fold increase in the odds of developing meningitis, and none of the patients with a CRAG titer  $\leq$ 1:160 developed meningitis. However, 3 of these patients died of unknown reasons, and we cannot exclude cryptococcosis as the cause of death. Using this cutoff titer would have required 1 single additional CRAG LFA test per patient. Thus, for the screening of 750 patients, 28 additional test strips would have been needed to determine whether the CRAG titer was above or below the breakpoint, with an additional cost of approximately \$56.00 per 750 screened patients, \$0.07 per patient screened. Our results support the findings of a study showing differences in CRAG titer between CRAG-positive patients with and without proven meningitis (1:128 to 1:1024 vs 1:8 to 1:128, respectively). Likewise, in another study conducted in South Africa [29], using a cutoff CRAG titer of 1:8 by latex agglutination (equivalent to approx. 1:40 titer by LFA LA screening [30]) had 100% sensitivity and 96% specificity for predicting incident CM among all patients with CD4 counts <100 cells/ $\mu$ L. In asymptomatic CRAG-positive patients with low CRAG titers, fluconazole alone without lumbar puncture may be sufficient for treatment. Although the specific CRAG titer cutoff remains uncertain, it may be approximately 1:100 (or within  $\pm$ 2-fold dilution thereof). Our results warrant further validation in larger studies.

Receiving any dose of fluconazole during follow-up given incidentally at physician discretion significantly improved survival and retention-in-care within the first year (50% vs 8% without fluconazole). A reduction in mortality due to

fluconazole treatment was also seen in a noncontrolled observational study by Meya et al [25], in which low-dose fluconazole treatment of asymptomatic CRAG-positive persons resulted in a 76% 5-year survival vs 0% 4-month survival without fluconazole treatment [31]. In a Cambodian CRAG study, 80% (8 of 10) survival occurred with fluconazole at 200 mg/day with no deaths related to meningitis [28]. Another study done in Thailand showed 100% survival in asymptomatic CRAG-positive patients given preemptive fluconazole [26]. These data suggest that even low-dose fluconazole treatment may lower early mortality in asymptomatic CRAG-positive PLHIV initiating ART and further supports the need for implementing a systematic screening followed by preemptive fluconazole treatment in Tanzania and other African countries. Again, these results should be interpreted with caution due to the limited sample size.

This study has several limitations. First, although clinical data were collected prospectively, we conducted retrospective CRAG testing of cryopreserved plasma samples. Of note, physicians managing these cases were unaware of the CRAG result, because CRAG LFA was not available in our facility before 2013. Medical records of CRAG-positive patients were then reviewed to retrieve additional data on symptoms and management. Based on these records, 11 of 28 CRAG-positive patients presented with neurologic symptoms either pre-ART (n = 7) or after ART initiation (n = 4), of whom 9 were clinically diagnosed with cryptococcal meningitis. Indeed, the outcomes between symptomatic and asymptomatic CRAG-positive patients did not seem to be different, reinforcing that asymptomatic CRAG-positive patients who do not receive antifungal therapy will eventually die. Second, fluconazole was given as per physician criteria, mostly for other conditions such as oral thrush or tinea with different doses and treatment duration. This limits any possible conclusion from our data beyond the recognition that having received any fluconazole improved survival or retention-in-care. In addition, due to a suboptimal ascertainment of mortality in our cohort, we had to use a composite outcome of death or LFU, limiting our conclusions regarding the impact of CRAG screening and fluconazole treatment on mortality. However, considering the low CD4 counts of our study population, it is likely that the majority of those lost actually died [32]. There was incomplete ascertainment on the cause of death. Therefore, the characterization of the course of untreated CRAG was limited to the proportion of those who were clinically diagnosed with cryptococcal meningitis and those who died of any cause or were loss to follow-up. Finally, as already mentioned, the subanalysis performed among CRAG-positive patients should be interpreted with caution due to the low sample size.

There are several implications of our findings. First, there is a need to raise awareness about the prevalence and high mortality associated with CRAG in Tanzania and other sub-Saharan African countries. The CRAG prevalence found in our study justifies the adoption of the WHO recommendations on CRAG

screening in Tanzania, which would be cost-effective at this prevalence based on previous studies [21, 23]. Moreover, the observed improved outcomes among patients that received fluconazole further justify the implementation of this low-cost, simple recommendation. Optimal fluconazole dosing is unknown [11]. However, the observation of similarly poor outcomes of the CRAG-positive patients who had measured CD4 counts >100 cells/ $\mu$ L raises concerns about what is the optimal CD4 threshold for CRAG screening. Although data on CRAG prevalence and outcome of CD4 >100 cells/ $\mu$ L is still limited, our results may support increasing the CD4 threshold to 125 or 150 cells/ $\mu$ L. Finally, we observed that CRAG-positive patients with low CRAG titers did not develop meningitis, which suggests a potential role of using titers for risk stratification among asymptomatic CRAG-positive patients.

## CONCLUSIONS

In summary, CRAG was prevalent and an independent predictor of early mortality on ART. Three-quarters of CRAG+ patients died within 1 year. This unacceptably high mortality needs to be urgently addressed, and it could be easily prevented by increasing access to CRAG LFA and adopting the CRAG-screening guidelines recommended by the WHO for high-burden countries.

## Supplementary Material

Supplementary material is available online at *Open Forum Infectious Diseases* (<http://OpenForumInfectiousDiseases.oxfordjournals.org/>).

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**Author contributions.** E. L., D. R. B., M. C. M., and T. R. G. conceived and designed the study. M. C. M., E. L., N. K., and A. J. N. performed the experiments. M. C. M. and T. R. G. analyzed the data. E. L., D. R. B., M. C. M., T. R. G., M. B., H. F., C. H., and M. T. interpreted the results. E. L., M. C. M., D. R. B., and T. R. G. wrote the paper. M. B., H. F., D. F., C. H., and M. T. reviewed the manuscript. All authors reviewed and approved the final version.

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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