

# Cryptococcal-related Mortality Despite Fluconazole Preemptive Treatment in a Cryptococcal Antigen Screen-and-Treat Program

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(See the Editorial Commentary by Rajasingham and Boulware on pages 1691–4.)

**Background.** Cryptococcal antigen (CrAg) screening and treatment with preemptive fluconazole reduces the incidence of clinically evident cryptococcal meningitis in individuals living with advanced human immunodeficiency virus (HIV) disease. However, mortality remains higher in CrAg-positive than in CrAg-negative patients with similar CD4+ T-lymphocyte counts.

**Methods.** We conducted a cohort study to investigate causes of morbidity and mortality during 6 months of follow-up among asymptomatic CrAg-positive and CrAg-negative (ratio of 1:2) patients living with HIV with CD4 counts <100 cells/μL attending 2 hospitals in Johannesburg, South Africa. When possible, minimally invasive autopsy (MIA) was performed on participants who died.

**Results.** Sixty-seven CrAg-positive and 134 CrAg-negative patients were enrolled. Death occurred in 17/67 (25%) CrAg-positive and 12/134 (9%) CrAg-negative participants (hazard ratio for death, adjusted for CD4 count, 3.0; 95% confidence interval, 1.4–6.7;  $P = .006$ ). Cryptococcal disease was an immediate or contributing cause of death in 12/17 (71%) CrAg-positive participants. Postmortem cryptococcal meningitis and pulmonary cryptococcosis were identified at MIA in all 4 CrAg-positive participants, 3 of whom had negative cerebrospinal fluid CrAg tests from lumbar punctures (LPs) at the time of CrAg screening.

**Conclusions.** Cryptococcal disease was an important cause of mortality among asymptomatic CrAg-positive participants despite LPs to identify and treat those with subclinical cryptococcal meningitis and preemptive fluconazole for those without meningitis. Thorough investigation for cryptococcal disease with LPs and blood cultures, prompt ART initiation, and more intensive antifungals may reduce mortality among asymptomatic CrAg-positive patients identified through screening.

**Keywords.** cryptococcus; acquired immunodeficiency syndrome; AIDS-related opportunistic infections; autopsy; cryptococcal meningitis.

Cryptococcal disease is a leading cause of AIDS-related death in sub-Saharan Africa [1]. Although cryptococcal antigen (CrAg) screening and preemptive treatment with fluconazole reduces the incidence of cryptococcal meningitis [2–5], individuals with cryptococcal antigenemia treated with fluconazole remain at higher risk of death than individuals without cryptococcal antigenemia with similar CD4+ T-lymphocyte (CD4) counts [2–4].

CrAg screen-and-treat strategies have been implemented in several countries where cryptococcal meningitis is a common opportunistic infection [6]. In South Africa, blood samples with CD4 counts <100 cells/μL are screened at central laboratories using the Immuno-mycologies (IMMY, Norman, OK) CrAg lateral flow assay (LFA). South African national guidelines stipulate that CrAg-positive patients are assessed for symptoms or signs of meningitis and, if present, investigated for cryptococcal meningitis with a lumbar puncture (LP). Asymptomatic patients are offered LPs if available at the screening site or nearby facility and started on preemptive fluconazole (800 mg daily for 2 weeks, followed by 400 mg daily for 2 months, and then 200 mg daily pending immune reconstitution) if cryptococcal meningitis is excluded or if LP is not performed. Antiretroviral therapy (ART) is delayed for 2 weeks in asymptomatic CrAg-positive patients and for 4–6 weeks if cryptococcal meningitis is diagnosed [7].

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The causes of excess mortality among CrAg-positive compared to CrAg-negative patients are as yet unclear. Previous studies have not fully investigated causes of death; no autopsies were performed [2–4, 8, 9]. Since very few (<5%) CrAg-positive patients were known to develop cryptococcal meningitis following screening and treatment [3, 4, 8], noncryptococcal causes were suspected.

We investigated morbidity and mortality during 6 months following screening among CrAg-positive and CrAg-negative adults living with human immunodeficiency virus (HIV) in Johannesburg, South Africa. Causes of death were investigated, including by minimally invasive autopsy (MIA).

## METHODS

Routine CrAg screening was performed on all individuals living with HIV with CD4 counts <100 cells/μL at Helen Joseph and Tambo Memorial hospitals, Johannesburg. HIV-seropositive adults (aged ≥18 years) with CD4 counts <100 cells/μL who were CrAg-positive were sequentially invited to participate when they attended the hospitals' HIV clinics or wards for results between June 2015 and October 2017. CrAg-negative patients with similar CD4 counts (±10 cells/μL) were concurrently recruited at a ratio of 2:1. Patients were excluded if they had symptoms or signs of cryptococcal meningitis (ie, severe headache or reduced level of consciousness) at any time since screening or if they were receiving antifungal treatment for previous cryptococcal meningitis. Written informed consent for participation and for MIA in the event of death was requested. The study was granted ethics approval by the University of the Witwatersrand and the London School of Hygiene & Tropical Medicine.

Training on the clinical algorithm for management of CrAg-positive patients [7] was provided to health workers at study sites by the principal investigator (R. M. W.). Study participants were assessed and managed by their usual clinicians following CrAg screening. A study nurse collected clinical data including symptoms, examination findings, medical diagnoses, and medication at face-to-face consultations, from clinical records at enrollment, and on 3 subsequent routine visits over 6 months. Time to treatment initiation was measured from the day that screening blood arrived in the laboratory. Adherence was assessed at participant visits and by review of pharmacy records. At enrollment, participants provided blood samples for prolonged (21 days) fungal culture and C-reactive protein (CRP) testing; pre- and/or postinduction sputum for fungal culture and tuberculosis (TB) testing (auramine staining and microscopy, liquid culture [BACTEC MGIT, Becton Dickinson, Franklin Lakes, NJ] and molecular testing [Xpert MTB/RIF, Cepheid, Sunnyvale, CA]); and urine for lipoarabinomannan (Determine TB LAM, Alere, Waltham, MA), *Histoplasma* antigen enzyme immunoassay (IMMY), and Xpert MTB/RIF Ultra testing. Sputum and blood culture results were provided to the participant's clinician if clinically significant. Further

assessment and TB treatment were recommended if the urine TB LAM test was positive. Participants were contacted by telephone every two weeks to discuss any new medical problems. If no contact was made, contact with a friend or relative named by the participant was attempted.

Cryptococcal disease was defined as any participant with cryptococcal meningitis (*Cryptococcus* identified by cerebrospinal fluid [CSF] microscopy with India ink, fungal culture, and/or CrAg testing), cryptococemia (*Cryptococcus* cultured from blood), or pulmonary cryptococcosis (*Cryptococcus* cultured from sputum). Asymptomatic cryptococcal antigenemia alone was not categorized as cryptococcal disease for the purposes of this study.

In the event of death, information regarding recent symptoms and hospital admissions was obtained from the participant's closest relative and clinical records. Permission was requested from the family to perform MIA if informed consent had been provided. MIAs were performed by the principal investigator (R. M. W.) with the assistance of a study nurse. An initial external examination of the body was performed, and organs were located using external anatomical landmarks or ultrasound. Skin was cleaned with iodine and half-centimeter incisions made at biopsy sites. Standardized multiple core biopsy samples were obtained from lungs, liver, spleen, and kidneys using a 14-gauge core biopsy needle. CSF from the cisterna magna (suboccipital approach) and blood from the subclavian vein were aspirated using 18-gauge needles and syringes. Bronchoalveolar lavage (BAL) was performed using normal saline delivered through a nasogastric tube into the bronchi through a tracheal incision. If abnormalities were clinically detected, aspirates of pleural and pericardial fluid and punch biopsies of any skin lesions (excluding on the face or neck) were taken.

Samples underwent microbiological testing at the National Institute for Communicable Disease and histological analysis at the University of the Witwatersrand. Investigators who performed analyses were not aware of the participants' ante-mortem CrAg status.

Tissue cores were preserved in formalin, embedded in paraffin, and stained with hematoxylin-eosin. Additional stains to assess for the presence of acid-fast bacilli (Ziehl-Neelsen), bacteria (Gram stain), and fungi (Grocott's methenamine silver) were performed if any features of inflammation were seen. Cytomegalovirus immunoperoxidase staining was performed when characteristic viral inclusions were noted.

Tissue cores, CSF, BAL, and pericardial fluid underwent bacterial, fungal, and mycobacterial cultures; bacterial and viral multiplex polymerase chain reaction; and Xpert MTB/RIF testing. Plasma, CSF, and BAL fluid were tested using the IMMY CrAg LFA.

Immediate and contributing causes of death were attributed by a panel of 6 of the authors (infectious diseases physicians and pathologists) at the University of the Witwatersrand Department

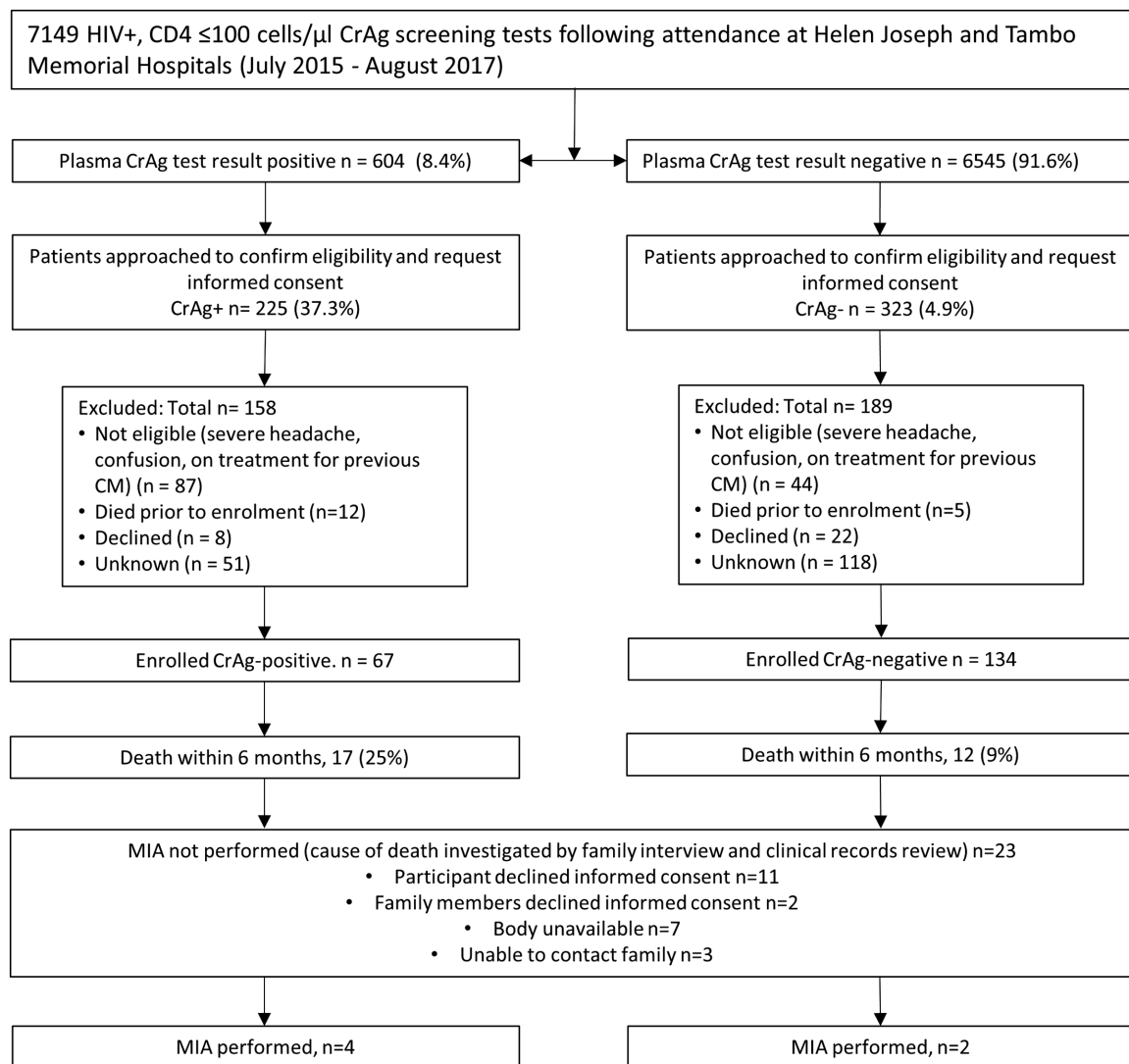
of Anatomical Pathology, which has extensive experience conducting MIA studies [10–12]. Procedures were similar to those described in previous MIA studies [10, 11]: organisms isolated on culture from postmortem samples were considered to be pathological if the same organism was cultured from a premortem sample, if there was clinical evidence of infection with the organism, or if histology showed consistent changes within the affected organ. Diagnoses were “possible,” “probable,” or “likely” according to the data available and categorized using the Coding of Causes of Death in HIV protocol [13].

Baseline demographic and clinical variables were compared using  $\chi^2$ , Fisher exact, or Mann-Whitney *U* tests, as appropriate. Clinical outcomes during follow-up were compared using rate ratios with 95% confidence intervals (CIs). The effect of CrAg status on mortality, adjusted for baseline CD4 count, was estimated using Cox proportional hazards regression analysis.

Other explanatory variables were evaluated as risk factors for death, including age, sex, baseline CD4 count (dichotomized at a threshold of <50 cells/ $\mu$ L), ART status, low body mass index (BMI; <18 kg/m<sup>2</sup>), severe anemia (hemoglobin  $\leq$  8 g/dL), and active TB (diagnosed at enrollment, within the past 6 months, or if they remained on treatment). A sensitivity analysis was performed assuming that any participants lost to follow-up had died. A sample size of 63 CrAg-positive participants was required to detect, with 80% power and 5% significance level, a mortality difference of 18%, consistent with previous studies [2–4].

## RESULTS

Of 7149 participants screened at the sites during the study period, 604 (8.4%) were CrAg-positive. Of these, 67 (11%) CrAg-positive and 134 (2%) CrAg-negative patients were enrolled in the study (Figure 1, Supplementary Table 3). There were



**Figure 1.** Flow diagram showing patients included and excluded in the prospective cohort study. Abbreviations: CD4, CD4 T-lymphocyte count; CM, cryptococcal meningitis; CrAg, cryptococcal antigen; HIV, human immunodeficiency virus; MIA, minimally invasive autopsy.

no differences in demographic characteristics, prior or prevalent AIDS-defining illnesses (including TB and histoplasmosis following screening at enrollment), other infectious or noninfectious medical conditions, CRP, BMI, or hemoglobin at baseline between CrAg-positive and CrAg-negative participants (Table 1). CrAg-positive participants who had lower CD4 counts (27 cells/ $\mu$ L; interquartile range [IQR], 7–40 vs 41 cells/ $\mu$ L; IQR, 16–64;  $P = .002$ ), were more likely to have already started ART (24% vs 10%,  $P = .01$ ) and prophylactic co-trimoxazole (55% vs 24%,  $P < .001$ ) at the time of enrollment and less likely to be South African (42% vs 25%,  $P = .01$ ). Follow-up for 6 months or to time of death if earlier was completed for 193/201 (96%) of participants.

#### Evidence for Cryptococcal Disease at Baseline

Cryptococcal disease was found in 17/67 (25%; 95% CI, 16%–37%) asymptomatic CrAg-positive participants: subclinical cryptococcal meningitis in 11/57 (19%; 95% CI, 10%–32%) who

had LPs, cryptococcemia in 11/67 (16%) who had prolonged fungal blood cultures (4 had no evidence of cryptococcal meningitis on LP), and pulmonary cryptococcosis in 2/32 (7%) who had sputum fungal cultures (1 had a negative LP). Plasma CrAg titers were higher among those who had cryptococcal disease than those with asymptomatic cryptococcal antigenemia alone (median, 5120; IQR, 160–10,240 vs 10; IQR, 5–80;  $P < .001$ ) and in those with subclinical cryptococcal meningitis than those who had negative LPs (median, 10,240; IQR, 1280–81,920 vs 15; IQR, 5–160;  $P < .001$ ). No CrAg-negative participants had cryptococcal disease at baseline.

#### Treatment Received

Antifungal treatment in accordance with guidelines at the time [7] (fluconazole 800 mg daily for those without and amphotericin B 1 mg/kg daily with fluconazole 800 mg daily for those with cryptococcal meningitis) was commenced for 62/67 (93%) participants at a median of 7 (IQR, 3–12) days following

**Table 1. Baseline Characteristics of the Cryptococcal Antigen (CrAg)-positive and CrAg-negative Patients**

Characteristic	CrAg-positive (n = 67)	CrAg-negative (n = 134)	P Value
Age, median (IQR), y	39 (32–47)	39 (33–48)	.63 <sup>a</sup>
Female (%)	37 (55)	62 (46)	.23
Self-assigned black race (%)	66 (99)	128 (96)	.4
Born outside of South Africa <sup>b</sup> (%)	28 (42)	33 (25)	.01
Non–Southern Africa Development Community (%)	3 (5)	3 (2)	.4
CD4 T-lymphocyte count cells/ $\mu$ L (median, IQR)	27 (7–40)	41 (16–64)	.002 <sup>a</sup>
<50 (%)	55 (82)	80 (60)	.001
Body mass index <18 kg/m <sup>2</sup> (%) <sup>c</sup>	9 (20)	20 (18)	1.0 <sup>d</sup>
Hemoglobin $\leq$ 8 g/dL (%) <sup>e</sup>	5 (9)	13 (11)	.8 <sup>d</sup>
C-reactive protein, mg/L (median, IQR) <sup>f</sup>	15 (3–49)	22 (4–56)	.4
Active TB at enrollment <sup>g</sup> (%)	19 (28)	33 (25)	.6
Previous TB <sup>h</sup> (%)	11 (16)	14 (10)	.3 <sup>d</sup>
Other previous or current ADI (%)	11 (16)	20 (15)	.8 <sup>d</sup>
Histoplasmosis <sup>i</sup>	3 (5)	3 (2)	.4 <sup>d</sup>
Other (non-ADI) medical conditions (%)	30 (45)	57 (43)	.8
Infectious disease (%)	18 (27)	39 (29)	.7
Hepatitis B <sup>j</sup>	6 (17)	11 (13)	.6 <sup>d</sup>
Immunocompromising (%)	2 (3)	4 (3)	1.0 <sup>d</sup>
Taking antiretroviral therapy (>1 day) (%)	16 (24)	13 (10)	.01 <sup>d</sup>
Taking co-trimoxazole (%)	37 (55)	32 (24)	<.001
Taking other antibiotics (%)	22 (33)	37 (28)	.4

Pearson  $\chi^2$  test used unless indicated.

Abbreviations: ADI, AIDS-defining illness; CrAg, cryptococcal antigen; IQR, interquartile range; TB, tuberculosis.

<sup>a</sup>Mann-Whitney  $U$  test.

<sup>b</sup>Data missing on birthplace for 1 patient.

<sup>c</sup>Data missing on BMI for 20 CrAg-positive and 23 CrAg-negative patients.

<sup>d</sup>Fisher exact test.

<sup>e</sup>Data missing on hemoglobin for 12 CrAg-positive and 15 CrAg-negative patients.

<sup>f</sup>Data missing on C-reactive protein for 5 CrAg-positive and 5 CrAg-negative patients.

<sup>g</sup>Active TB includes those diagnosed with TB within the past 6 months and/or on current TB treatment at enrollment (CrAg-positive, n = 12, 18%; CrAg-negative, n = 13, 10%), including those diagnosed on the day of enrollment (CrAg-positive, n = 7, 10%; CrAg-negative, n = 20, 15%). Diagnoses of TB were made clinically or on the basis of routine laboratory tests, or screening performed as part of the study.

<sup>h</sup>Previous TB includes those who were diagnosed more than 6 months prior to enrollment and who do not remain on TB treatment.

<sup>i</sup>Data missing on histoplasmosis screening (urine enzyme immunoassay for 1 CrAg-positive and 7 CrAg-negative patients);

<sup>j</sup>Data missing on hepatitis B for 32 CrAg-positive and 50 CrAg-negative patients.

a positive CrAg test. Good adherence to the recommended antifungal regimen was reported for 40/57 (70%) during the 6 months following CrAg screening. Three CrAg-positive participants had no record of receiving antifungals; 1 was lost to follow-up, 2 survived.

Fifty-three of 67 (79%) CrAg-positive and 128 (96%) CrAg-negative participants commenced ART during the study period (rate ratio [RR], 0.97; 95% CI, 0.71–1.34;  $P = .9$ ). The median delay between CD4 count and ART initiation was 36 (IQR, 26–45) days for CrAg-positive participants and 17 (IQR, 7–32) days for CrAg-negative participants ( $P < .001$ ). Among CrAg-positive participants, median delay to ART initiation was 21 (IQR, 8–57) days for participants with cryptococcal meningitis and 36 (IQR, 31–49) days for those without cryptococcal meningitis.

The proportions of CrAg-positive and CrAg-negative participants who took any antibiotic during the study period were similar (25/67, 37% vs 42/134, 31%;  $P = .4$ ).

### Clinical Outcomes

There were no significant differences between CrAg-positive and CrAg-negative participants in the proportion with undetectable HIV-1 viral loads within 6 months, BMI change, and incidence of TB or other infectious or noninfectious medical conditions during follow-up. Other AIDS-defining illnesses (not cryptococcosis or TB) developed in 8 (12%) CrAg-positive and 7 (5%) CrAg-negative participants (RR, 2.69; 95% CI, 0.98–7.42;  $P = .05$ ; Table 2).

### Cryptococcal Disease During Follow-up

One CrAg-positive participant who was diagnosed and treated for subclinical cryptococcal meningitis at enrollment developed a second episode 5 months later, despite reported adherence to fluconazole maintenance therapy and ART. Four of 11 (36%) participants with baseline subclinical cryptococcal meningitis died during follow-up; 3 following treatment with amphotericin B and fluconazole (13, 14, and 25 days after LP), 1 participant received fluconazole only and died 3 months later. Two of these participants also had cryptococemia at baseline, 1 had cryptococcal pneumonia identified at MIA.

Thirteen of 46 (28%) CrAg-positive participants died following negative LPs (none had cryptococemia, 1 had growth of *Cryptococcus neoformans* on sputum at baseline). Eight of these deaths were cryptococcal-related: 4 cases of cryptococcal meningitis (3 identified at MIA, 1 clinically diagnosed prior to death, at least 2 had good adherence to fluconazole), 1 case of pulmonary cryptococcal immune reconstitution inflammatory syndrome, 1 case of cryptococcal pneumonia, and 2 deaths in participants who defaulted preemptive fluconazole treatment (immediate cause of death unknown).

No cryptococcal disease was identified in any CrAg-negative participants during follow-up.

### Mortality

Death occurred in 14% (29/201, rate 0.4 deaths/person-year [d/PY]) of all participants within 6 months; 25% (17/67, 0.7 d/PY) CrAg-positive and 9% (12/134, 0.2 d/PY) CrAg-negative

**Table 2. Clinical Outcomes During Follow-up in Cryptococcal Antigen (CrAg)-positive and CrAg-negative Patients**

Clinical Outcome	Total (N = 201)	CrAg-positive (n = 67)	CrAg-negative (n = 134)	Rate Ratio in CrAg-positive vs CrAg-negative (95% CI)	P Value
New ADI, n (rate/1000 PY, 95% CI)	24 (0.8, 0.5–1.2)	10 (1.1, 0.6–2.1)	14 (0.7, 0.4–1.1)	1.68 (.75–3.78)	.2
Tuberculosis, <sup>a</sup> n (rate/1000 PY, 95% CI)	10 (0.3, 0.2–0.6)	2 (0.2, 0.1–1.0)	8 (0.4, 0.2–0.8)	0.54 (.12–2.66)	.5
Other <sup>b</sup> ADIs, n (rate/1000 PY, 95% CI)	15 (0.5, 0.3–0.8)	8 (0.9, 0.4–1.8)	7 (0.3, 0.2–0.7)	2.69 (.98–7.42)	.05
New (non-ADI) medical condition, n (rate/1000 PY)	51 (1.7, 1.3–2.2)	21 (2.3, 1.5–3.6)	30 (1.4, 1.0–2.0)	1.65 (.94–2.88)	.08
Infectious disease, n (rate/1000 PY)	25 (0.8, 0.6–1.2)	9 (1.0, 0.5–1.9)	16 (0.8, 0.5–1.2)	1.32 (.59–3.00)	.5
Body mass index change, <sup>c</sup> kg/month (median, IQR)	0.24 (0–0.24)	0.21 (–0.02 to 0.35)	0.24 (0.06–0.49)	...	.3 <sup>d</sup>
Undetectable viral load <sup>e</sup> (≤50 copies/mL) (%)	31 (27)	7 (28)	24 (27)	...	1.0 <sup>f</sup>
Deaths within 6 months (%)	29 (14)	17 (25)	12 (9)	3.28 (1.57–6.87)	.002
Median (IQR) days from CD4 to death (n = 29)	53 (20–74)	26 (14–52)	70 (37–95)		.02

Pearson  $\chi^2$  test used unless indicated.

Abbreviations: ADI, AIDS-defining illness; CI, confidence interval; CrAg, cryptococcal antigen; IQR, interquartile range; PY, person-years.

<sup>a</sup>Of those not diagnosed at enrollment: 60 CrAg-positive and 114 CrAg-negative patients. TB diagnosed during follow-up, not including at baseline.

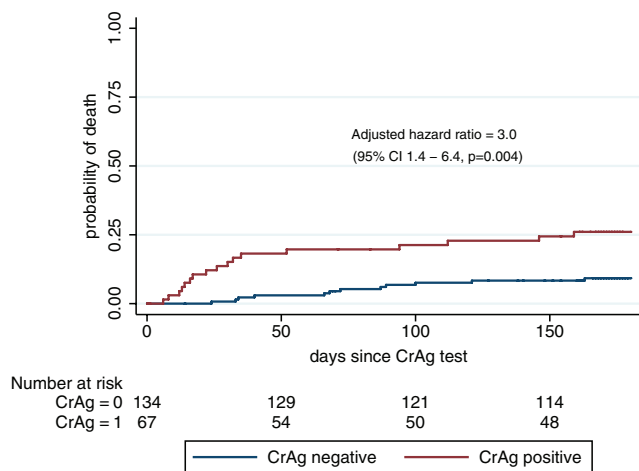
<sup>b</sup>ADIs other than cryptococcosis or tuberculosis (TB). CrAg-positive patients developed esophageal candidiasis (n = 2); cytomegalovirus (CMV) other than liver, spleen, nodes (n = 1); CMV retinitis (n = 2); herpes simplex (chronic ulcers) (n = 3); disseminated histoplasmosis (n = 1); Kaposi sarcoma (n = 1); *Mycobacterium avium* complex (n = 1); progressive multifocal leukoencephalopathy (n = 1); wasting syndrome (n = 1). CrAg-negative patients developed esophageal candidiasis (n = 2); CMV (n = 2); Kaposi sarcoma (n = 4); *Pneumocystis jirovecii* pneumonia (n = 2); recurrent pneumonia (n = 2); wasting syndrome (n = 1).

<sup>c</sup>Of those with subsequent weight measurements: 38 CrAg-positive and 90 CrAg-negative patients.

<sup>d</sup>Fisher exact test.

<sup>e</sup>Of those with human immunodeficiency virus type 1 viral load available between 140 and 240 days follow-up: 29 CrAg-positive and 93 CrAg-negative patients

<sup>f</sup>Mann-Whitney U test.



**Figure 2.** Kaplan–Meier curve showing mortality estimates in CrAg-positive and CrAg-negative patients within 6 months, adjusted for baseline CD4 T-lymphocyte count. Abbreviations: CI, confidence interval; CrAg, cryptococcal antigen.

(Table 2, Figure 2). Participants with cryptococcal antigenemia had 3.3 times increased risk of death (95% CI, 1.6–7.0;  $P < .001$ ) compared to CrAg-negative participants. This association remained significant when adjusted for baseline CD4 count ( $<50$  cells/ $\mu$ L; hazard ratio [HR], 3.0; 95% CI, 1.4–6.4;  $P = .004$ ) and if all those lost to follow-up were assumed to have died (HR, 2.4; 95% CI, 1.3–4.6;  $P = .008$ ). Of other baseline variables assessed, only active TB at the time of enrollment was associated with mortality (HR, 2.6; 95% CI, 1.2–5.4;  $P = .01$ ). Among CrAg-positive participants, death was associated with having a higher plasma CrAg titer (HR, 3.5 if titer  $>160$  vs  $\leq 160$ ; 95% CI, 1.4–9.2;  $P = .009$ ) but not with baseline cryptococcal disease. Time from CrAg test to death was a median 26 (IQR, 14–52) days in CrAg-positive and 70 (IQR, 37–95) days in CrAg-negative participants ( $P = .02$ ).

### MIA Results

MIAs were performed on 4 CrAg-positive and 2 CrAg-negative participants (Supplementary Table 2 and Figure 1). Postmortem cryptococcal meningitis was diagnosed in all 4 CrAg-positive participants by CSF CrAg test (plus culture for 1). Three of the participants had had negative LPs at the time of CrAg screening (7, 25, and 32 days prior to death); all became confused prior to death. The other participant died following treatment for subclinical cryptococcal meningitis. Cryptococcal pneumonia was identified histologically in lung tissue at MIA. BAL CrAg tests were positive in all 4 CrAg-positive participants. Although multiple other pathologies were identified from autopsy samples, there was no evidence of cryptococcal disease in either CrAg-negative participant.

### Causes of Death

Cryptococcosis was an immediate ( $n = 5$ ) or contributing ( $n = 7$ ) cause of death in 12/17 (71%) CrAg-positive participants (8/12, 67% had cryptococcal meningitis) and no CrAg-negative participants. Of all 53 causes of death attributed (Supplementary

Table 1), cryptococcosis (12, 23%) was most common, followed by sepsis (11, 21%) and TB (8, 15%).

## DISCUSSION

Cryptococcal antigenemia was a strong and independent predictor of mortality among adults living with HIV without severe headache or reduced consciousness at the time of CrAg screening. This is consistent with previous studies that have found an increased mortality risk, irrespective of CD4 cell count and despite preemptive fluconazole treatment for CrAg-positive patients [2–4]. We found clinical and pathological evidence of cryptococcosis as a significant cause of morbidity and mortality among CrAg-positive participants; one-quarter had cryptococcal disease at the time of CrAg screening (19% had subclinical cryptococcal meningitis), 4 participants developed cryptococcal meningitis following negative baseline LPs, and more than two-thirds of deaths were attributed to cryptococcosis as an immediate or contributing cause.

Our findings emphasize the need to thoroughly investigate asymptomatic CrAg-positive patients for cryptococcal disease with LPs and blood cultures. However, we found that several cryptococcal-related deaths occurred despite the exclusion of cryptococcal disease at the time of screening. This indicates that fluconazole monotherapy, which is known to be suboptimal treatment for cryptococcal meningitis [14–17], is also inadequate for preventing deaths among CrAg-positive patients; enhanced treatment strategies are needed.

One approach would be to use adjunctive antifungal treatment for all CrAg-positive patients or for those with higher blood CrAg titers, identified by quantitative CrAg assays [18, 19]. We found blood CrAg titers of  $>160$  to be predictive of death, consistent with previous studies [5, 20, 21]. Adjunctive treatment options include flucytosine (shown to be effective for treating cryptococcal meningitis in the Advancing Cryptococcal meningitis Treatment for Africa (ACTA) trial [22]) or a single dose of 10 mg/kg liposomal amphotericin (effective fungicidal activity in CSF [23] and currently in a phase 3 trial for treating cryptococcal meningitis [24]). Both treatments are safe and feasible to administer in outpatient settings; randomized, controlled trials are required to establish if they would reduce mortality in asymptomatic CrAg-positive patients.

Delayed ART commencement may have contributed to increased mortality in CrAg-positive participants. Delay was longer in participants without subclinical cryptococcal meningitis, despite fluconazole being started relatively promptly after CrAg screening. Although point-of-care CrAg testing might improve linkage to ART [25–29], continued health worker education is imperative to ensure CrAg-positive patients initiate ART after the recommended 14 days of fluconazole 800 mg daily.

Previous studies have suggested that excess mortality among CrAg-positive patients might be explained by increased

susceptibility to other infections such as TB [2, 3, 30]. Despite enhanced screening for TB at enrollment, we found no evidence of an association between cryptococcal antigenemia and TB. CrAg-positive patients were more likely to develop other AIDS-defining illnesses, and it is likely that these contributed to increased mortality.

The study was limited by several factors. Despite attempts to match CD4 counts ( $\pm 10$  cells/ $\mu$ L), convenience sampling resulted in the enrollment of CrAg-negative patients with higher CD4 counts who were more likely to be ART-naive. It is notable, however, that CrAg-positive participants remained around three times more likely to die than CrAg-negative participants when adjusted for baseline CD4 cell count. Although the sample size was sufficient to detect a mortality difference, the relatively small cohort did not allow adjustment for more than one confounder simultaneously.

The study was also limited by varying levels of clinical information available for participants during follow-up and at the time of death; it was only possible to perform MIAs on a small proportion of those who died. MIAs identified several pathologies that were not diagnosed prior to death, including cryptococcal meningitis and pulmonary cryptococcosis, using CSF/BAL CrAg tests. It is possible that CrAg detection in post-mortem samples might reflect contamination or leakage from blood. However, it is pertinent that all participants who had CrAg-positive CSF at MIA developed confusion prior to death. CSF CrAg testing is a standard autopsy procedure [31] and has been used in similar studies [10, 11]. Furthermore, previous autopsy and BAL studies have found pulmonary cryptococcosis to be underdiagnosed among patients living with HIV [32–35].

This study provides valuable insight into the causes of excess mortality in CrAg-positive compared to CrAg-negative patients, with cryptococcosis remaining a leading cause despite preemptive fluconazole. Prompt initiation of ART and thorough screening for baseline cryptococcal disease are recommended in CrAg-positive patients. However, to fully realize the potential of CrAg screen-and-treat programs for reducing cryptococcal-related mortality, adjunctive antifungal treatment should be considered.

### Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

### Notes

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