Cryptococcal-related mortality despite fluconazole pre-emptive treatment in a cryptococcal antigen (CrAg) screen-and-treat programme

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Key points: Asymptomatic cryptococcal antigenemia was a risk factor for mortality despite a screen-and-treat programme with pre-emptive fluconazole treatment. Cryptococcal disease was a significant cause of death on the basis of clinical and autopsy evidence. Fluconazole monotherapy may be inadequate pre-emptive treatment.

Abstract

BACKGROUND

Cryptococcal antigen (CrAg) screening and treatment with pre-emptive fluconazole reduces the incidence of clinically-evident cryptococcal meningitis in individuals with advanced 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 six-months follow-up, among asymptomatic CrAg-positive and CrAg–negative (ratio of 1:2) HIV-infected patients with CD4 counts <100 cells/μL attending two 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. Antiretroviral therapy (ART) was started 36 days (interquartile range 26–45) and 17 days (IQR 7–32) following screening in CrAg-positive and CrAg-negative participants respectively (p<0.001). 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% CI 1.4–6.7, p=0.006). Cryptococcal disease was an immediate or contributing cause of death in 11/17 (65%) CrAg-positive participants. Post-mortem cryptococcal meningitis and pulmonary cryptococcosis were identified at MIA in all four CrAg-positive participants, three of whom had negative cerebrospinal fluid CrAg tests from lumbar punctures (LPs) at the time of CrAg screening.

CONCLUSIONS

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

Key words: Cryptococcus; Acquired Immunodeficiency Syndrome; AIDS-related opportunistic infections; autopsy; meningitis, cryptococcal

Background

Cryptococcal disease is a leading cause of AIDS-related death in sub-Saharan Africa(1). Although cryptococcal antigen (CrAg) screening and pre-emptive treatment with fluconazole reduces the incidence of cryptococcal meningitis(2–5), individuals with cryptococcal antigenemia treated with fluconazole remain at higher risk of dying 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-mycologics (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 pre-emptive 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).

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), non-cryptococcal causes were suspected.

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

Methods

Routine CrAg screening was performed on all HIV-infected individuals with CD4 counts <100 cells/μL at Helen Joseph and Tambo Memorial Hospitals, Johannesburg. HIV-seropositive adults (≥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 (i.e. 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.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 and from clinical records at enrolment, and on three subsequent routine visits over six 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 enrolment, participants provided: blood samples for prolonged (21 days) fungal culture and C-reactive protein (CRP) testing; pre- and/or post-induction sputum for fungal culture and tuberculosis (TB) testing (Auramine staining and microscopy, liquid culture (BACTEC MGIT, Becton Dickinson,

Franklin Lakes, New Jersey), and molecular testing (Xpert MTB/RIF, Cepheid, Sunnyvale, California)); and urine for lipoarabinomannan (Determine TB LAM, Alere, Waltham, Massachusetts), *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 was 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 CSF microscopy with India ink, fungal culture, and/or CrAg testing); cryptococcemia (*Cryptococcus* cultured from blood); or pulmonary cryptococcosis (*Cryptococcus* cultured from sputum). Asymptomatic cryptococcal antigenemia alone was not categorised 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 next of kin 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.W.) with the assistance of a study nurse. An initial external examination of the body was performed, and organs 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. Cerebrospinal fluid (CSF) from the cisterna magna (sub-occipital approach) and blood from the subclavian vein was 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 had been 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 (NICD), and histological analysis at the University of the Witwatersrand. Investigators performing 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 (PCR), 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 six of the authors (infectious diseases physicians and pathologists) at 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 post-mortem samples were considered to be pathological if 1) the same organism was cultured from a pre-mortem sample, 2) there was clinical evidence of infection with the organism, or 3) histology showed consistent changes within the affected organ. Diagnoses were 'possible', 'probable' or 'likely' according to the data available, and categorised using the 'Coding of Causes of Death in HIV' (CoDe) Protocol(13).

Baseline demographic and clinical variables were compared using chi-square, Fisher's exact, or Mann-Whitney U tests, as appropriate. Clinical outcomes during follow up, were compared using rate ratios with 95% confidence intervals (CI). 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/μL), ART status, low BMI (<18 kg/m^2), severe anemia (hemoglobin ≤8 g/dL), and active TB (diagnosed at enrolment, within the past six 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 7,149 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 no differences in demographic characteristics, prior or prevalent AIDS-defining illnesses (including TB and histoplasmosis following screening at enrolment), other infectious or non-infectious medical conditions, CRP, BMI or hemoglobin at baseline, between CrAg-positive and CrAg-negative participants (Table 1). CrAg-positive participants had lower CD4 counts (27 cells/μL (IQR 7–40) vs. 41 cells/μL (IQR 16–64), p=0.002), were more likely to have already started ART (24% vs. 10%, p=0.01) and prophylactic co-trimoxazole (55% vs. 24%, p<0.001) at the time of enrolment, and less likely to be South African (42% vs. 25%, p=0.01). Follow-up for six 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 (four had no evidence of cryptococcal meningitis on LP), and pulmonary cryptococcosis in 2/32 (7%) who had sputum fungal cultures (one 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<0.001) and in those with subclinical cryptococcal meningitis than those who had negative LPs (median 10,240, IQR 1,280–81,920 vs. 15, IQR 5–160, p<0.001). 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 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; one was lost to follow-up, two survived.

Fifty-three of 67 (79%) CrAg-positive and 128 (96%) CrAg-negative commenced ART during the study period (RR 0.97, 95% CI, 0.71-1.34, p=0.9). 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<0.001). 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.

There 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=0.4).

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; incidence of TB, or other infectious or non-infectious medical conditions during follow up. Other AIDS-defining illnesses

(not cryptococcosis or TB) developed in eight (12%) CrAg-positive and seven (5%) CrAg-negative participants (RR 2.69, 95% CI 0.98–7.42, p=0.05) (Table 2).

Cryptococcal disease during follow up

One CrAg-positive participant who was diagnosed and treated for subclinical cryptococcal meningitis at enrolment developed a second episode five 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; three following treatment with amphotericin B and fluconazole (13, 14, and 25 days after LP), one participant received fluconazole only and died three months later. Two of these participants also had cryptococcemia at baseline, one had cryptococcal pneumonia identified at MIA.

Thirteen of 46 (28%) CrAg-positive participants died following negative LPs (none had cryptococcemia, one had growth of *Cryptococcus neoformans* on sputum at baseline). Eight of these deaths were cryptococcal-related: four cases of cryptococcal meningitis (three identified at MIA, one clinically diagnosed prior to death, at least two had good adherence to fluconazole), one case of pulmonary cryptococcal immune reconstitution inflammatory syndrome (IRIS), one case of cryptococcal pneumonia and two deaths in participants who defaulted pre-emptive 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, Figure 2). Participants with cryptococcal antigenemia had 3.3 times increased risk of death (95% CI 1.6–7.0, p<0.001), compared to CrAg-negative participants. This association remained significant

when adjusted for baseline CD4 count (<50 cells/μL) (HR 3.0, 95% CI 1.4–6.4, p=0.004), and if all those lost to follow up were assumed to have died (HR 2.4, 95% CI 1.3–4.6, p=0.008). Of other baseline variables assessed, only active TB at the time of enrolment was associated with mortality (HR 2.6, 95% CI 1.2–5.4, p=0.01). Among CrAg-positive participants, death was associated with having a higher plasma CrAg titer (HR 3.5 if titer >160 vs. ≤160, 95% CI 1.4–9.2, p=0.009), but not with baseline cryptococcal disease. Time from CrAg test to death was median 26 (IQR 14–52) days in CrAg-positive and 70 (IQR 37–95) days in CrAg-negative participants (p=0.02).

Minimally invasive autopsy (MIA) results

MIAs were performed on four CrAg-positive and two CrAg-negative participants (supplementary Table 2 and Figure 1). Post-mortem cryptococcal meningitis was diagnosed in all four CrAg-positive participants by CSF CrAg test (plus culture for one). 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 four 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 (12/53, 23%) was most common, followed by sepsis (11/62, 18%) and TB (8/62, 13%).

Discussion

Cryptococcal antigenemia was a strong and independent predictor of mortality among HIV-infected adults 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 pre-emptive 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; a quarter had cryptococcal disease at the time of CrAg screening (19% had subclinical cryptococcal meningitis), four 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 emphasise 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 sub-optimal 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 10mg/kg liposomal amphotericin (L-AmB, effective fungicidal activity in CSF(23) and currently in a phase III 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 enrolment, we found no important 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 (+/- 10 cells/μL), convenience sampling resulted in the enrolment of CrAg-negative patients with higher CD4 counts, who were more likely to be ART naïve. 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 that 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 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 HIV-positive patients(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 pre-emptive fluconazole. Prompt initiation of ART and thorough screening for baseline cryptococcal disease is recommended in CrAg-positive patients. However, to fully realise the potential of CrAg screen-and-treat programmes for reducing cryptococcal-related mortality, adjunctive antifungal treatment should be considered.

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Conflict of Interest Statement:

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Table 1 Baseline characteristics of the CrAg-positive and CrAg-negative patients

	CrAg+ n=67	CrAg- n=134	P
Age (median, IQR)	39 (32-47)	39 (33–48)	0.63*
Female (%)	37 (55)	62 (46)	0.23
Self-assigned Black race (%)	66 (99)	128 (96)	0.4
Born in SA[1] (%)	38 (57)	131 (98)	0.01
SADC	25 (38)	0 (0)	<0.001
Non-SADC (%)	3 (5)	3 (2)	0.4
CD4 cells/μL (median, IQR)	27 (7–40)	41 (16–64)	0.002*
<50 (%)	55(82)	80 (60)	0.001
BMI <18 kg/m^2 (%)[2]	9 (20)	20(18)	1.0**
Hemoglobin ≤8g/dl (%)[3]	5 (9)	13 (11)	0.8**
CRP mg/L (median, IQR)[4]	15 (3-49)	22 (4-56)	0.4
Active TB at enrolment† (%)	19 (28)	33 (25)	0.6
Previous TB‡ (%)	11 (16)	14 (10)	0.3**
Other previous or current ADI (%)	11 (16)	20 (15)	0.8**
Histoplasmosis[5]	3 (5)	3 (2)	0.4**
Other (non-ADI) medical conditions (%)	30 (45)	57 (43)	0.8
Infectious disease (%)	18 (27)	39 (29)	0.7
Hepatitis B[6]	6 (17)	11 (13)	0.6**
Immunocompromising (%)	2 (3)	4 (3)	1.0**
Taking ART (>1 day) (%)	16 (24)	13 (10)	0.01**
Taking co-trimoxazole (%)	37 (55)	32 (24)	<0.001
Taking other antibiotics (%)	22 (33)	37 (28)	0.4

Abbreviations: CrAg = cryptococcal antigen; IQR = interquartile range; SA = South Africa; SADC = Southern Africa Development Community; CD4 = CD4 T-lymphocyte count; ART = antiretroviral therapy; BMI = body mass index; CRP= C-reactive protein; TB = tuberculosis; ADI = AIDS defining illness.

†Active TB includes those diagnosed with TB within past 6 months and/or on current tuberculosis treatment at enrolment (CrAg+ n=12 (18%), CrAg- n=13 (10%)), including those diagnosed on the day of enrolment (CrAg+ n=7 (10%), CrAg- 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.

‡Previous TB includes those who were diagnosed more than 6 months prior to enrolment, and who do not remain on tuberculosis treatment.

Data missing on: birthplace for 1 patient[1]; BMI for 20 CrAg-positive and 23 CrAg-negative patients[2]; hemoglobin for 12 CrAg-positive and 15 CrAg-negative patients[3]; CRP for 5 CrAg-positive and 5 CrAg-negative patients[4]; histoplasmosis screening (urine enzyme immunoassayfor 1 CrAg-positive and 7 CrAg-negative patients[5]; hepatitis B for 32 CrAg-positive and 50 CrAg-negative patients[6].

* Mann-Whitney U test

** Fisher's Exact Test

Pearson's chi square test used unless indicated.

Table 2 Clinical outcomes during follow up in CrAg-positive and CrAg-negative patients

	Total (n=201)	CrAg-positive (n=67)	CrAg-negative (n=134)	Rate ratio in CrAg+ vs. CrAg- (95% CI)	P value
New ADI, n (rate/1000PY, 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 (0.75–3.78)	0.2
TB[1], n (rate/1000PY, 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 (0.12–2.66)	0.5
Other† ADIs, n (rate/1000PY, 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 (0.98–7.42)	0.05
New (non–ADI) medical condition, n (rate/1000PY)	51 (1.7, 1.3–2.2)	21 (2.3, 1.5–3.6)	30 (1.4, 1.0–2.0)	1.65 (0.94–2.88)	0.08
Infectious disease, n (rate/1000PY)	25 (0.8, 0.6–1.2)	9 (1.0, 0.5–1.9)	16 (0.8, 0.5–1.2)	1.32 (0.59–3.00)	0.5
BMI change[2], kg/month (median, IQR)	0.24 (0-0.24)	0.21 (-0.02-0.35)	0.24 (0.06–0.49)		0.3**
Undetectable VL[3] (<=50 copies/mL) (%)	31 (27)	7 (28)	24 (27)		1.0*
Deaths within 6 months (%)	29 (14)	17 (25)	12 (9)	3.28 (1.57–6.87)	0.002
Median (IQR) days from CD4 to death (n=29)	53 (20–74)	26 (14–52)	70 (37–95)		0.02

Abbreviations: CrAg = cryptococcal antigen; ADI = AIDS–defining illness; 1000PY = 1000 person years; TB = tuberculosis diagnosed during follow-up, not including at baseline; BMI = body mass index; kg = kilogram; VL = viral load; IQR = inter–quartile range

†ADIs other than cryptococcosis or TB. CrAg–positive patients developed: esophageal candidiasis (n=2); Cytomegalovirus other than liver, spleen, nodes (CMV) (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); *Pneumocytsis jirevecii* pneumonia (n=2), recurrent pneumonia (n=2); wasting syndrome (n=1)

1 Of those not diagnosed at enrolment: 60 CrAg-positive and 114 CrAg-negative patients

2 Of those with subsequent weight measurements: 38 CrAg–positive and 90 CrAg–negative patients

3 Of those with HIV–1 VL available between 140 and 240 days follow up: 29 CrAg–positive and 93 CrAg–negative patients

Pearson's chi square test used unless indicated.

* Mann-Whitney U test

** Fisher's Exact test

Figure legends

Figure 1 Flow diagram to show patients included and excluded in the prospective cohort study

Abbreviations: HIV = human immunodeficiency virus; CD4 = CD4 T-lymphocyte count; CrAg = cryptococcal antigen; CM = cryptococcal meningitis; MIA = minimally invasive autopsy

Figure 2 Kaplan Meier curve to show mortality estimates in CrAg-positive and CrAg-negative patients within 6 months, adjusted for baseline CD4 count

Abbreviations: CrAg = cryptococcal antigen.

Figure 1

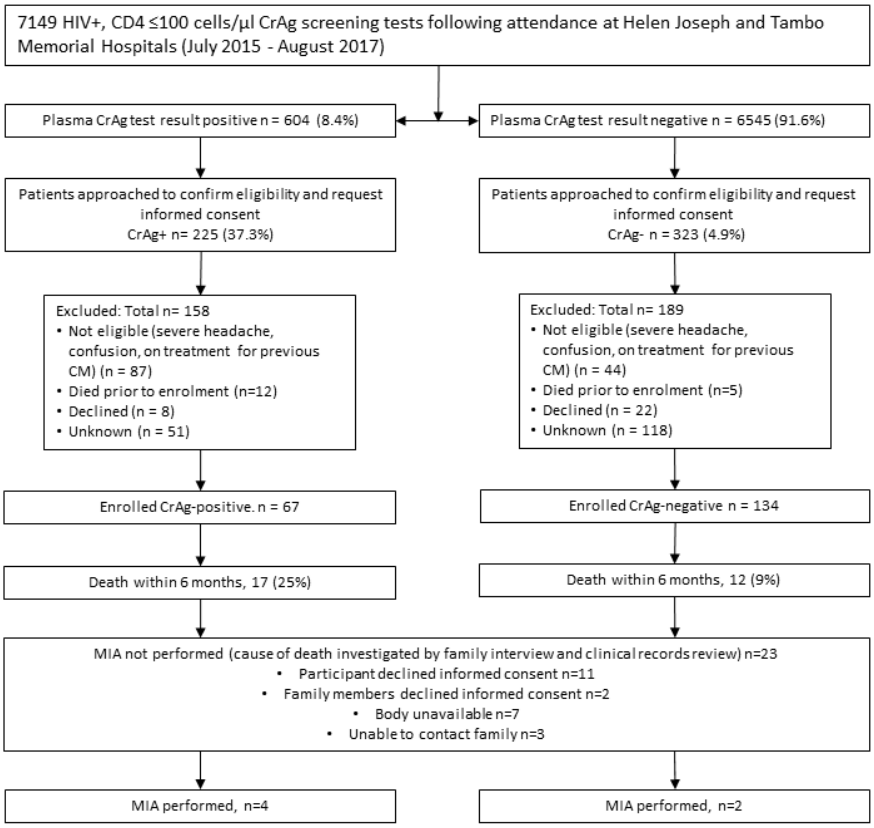
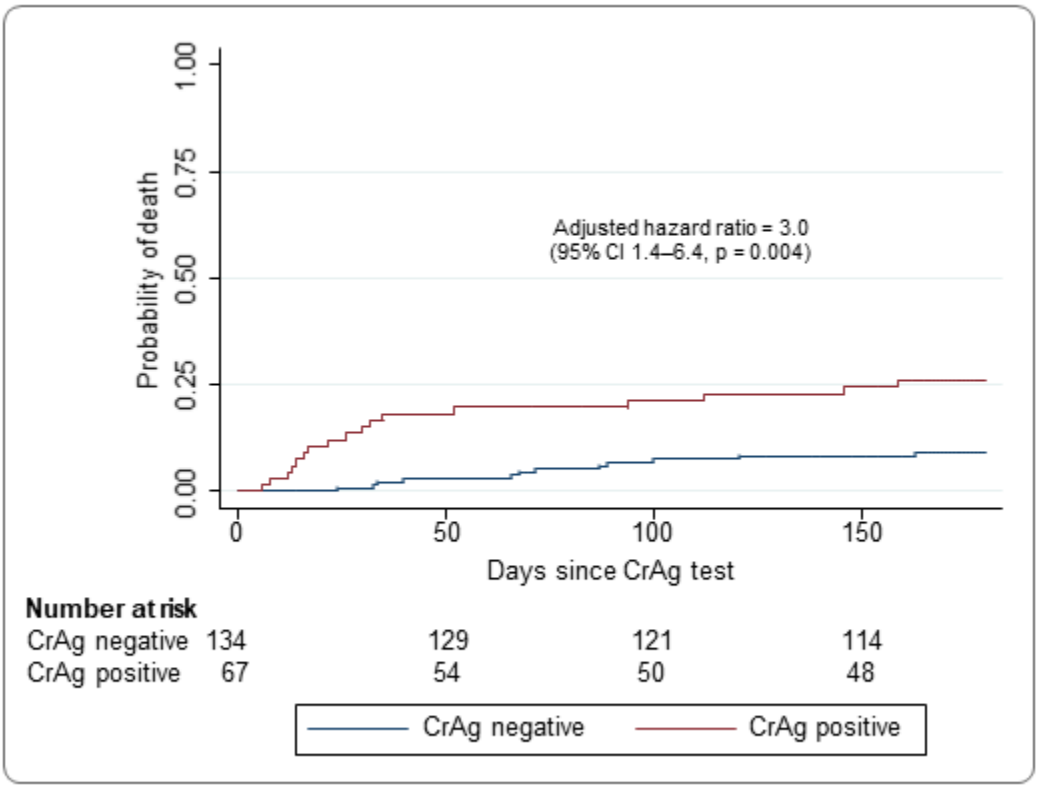


Figure 2



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