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Original Research

Laboratory-reflex cryptococcal antigen screening is associated with a survival benefit in Tanzania

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Running Title: Lab-reflex CrAg screening in Tanzania

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ABSTRACT

Background: Cryptococcal antigen (CrAg) screening in persons with advanced HIV/AIDS is recommended to prevent death. Implementing CrAg screening only in outpatients may underestimate the true CrAg prevalence and decrease its potential impact. Our previous 12-month survival/retention in CrAg-positive persons not treated with fluconazole was 0%.

Methods: HIV testing was offered to all ART-naive outpatients and hospitalized patients in Ifakara, Tanzania, followed by laboratory-reflex CrAg screening for CD4<150 cells/μL. CrAg-positive individuals were offered lumbar punctures, and antifungals were tailored to the presence/absence of meningitis. We assessed the impact on survival and retention-in-care using multivariate Cox regression models.

Results: We screened 560 individuals for CrAg. The median CD4 count was 61 cells/μL (IQR 26-103). CrAg prevalence was 6.1% (34/560) among individuals with CD4 ≤150 and 7.5% among ≤100 cells/μL. CrAg prevalence was 2.3-fold higher among hospitalized participants than in outpatients (12% vs. 5.3%, p=0.02). We performed lumbar punctures in 94% (32/34), and 31% (10/34) had cryptococcal meningitis. Mortality did not differ significantly between treated CrAg-positive without meningitis and CrAg-negative individuals (7.3 vs 5.4 deaths per 100 persons-year, respectively, p=0.25). Independent predictors of 6-month death/lost to follow-up

were low CD4, cryptococcal meningitis (adjusted hazard ratio (aHR) 2.76, 95% CI 1.31-5.82)), and no ART initiation (aHR 3.12, 95%CI 2.16-4.50).

Conclusions: Implementing laboratory-reflex CrAg screening among outpatients and hospitalized-individuals resulted in a rapid detection of cryptococcosis and a survival benefit. These results provide a model of a feasible, effective and scalable CrAg screening and treatment strategy integrated into routine care in sub-Saharan Africa.

Keywords: cryptococcal meningitis; cryptococcal antigenemia; HIV; fluconazole; mortality; sub-Saharan Africa

INTRODUCTION

Cryptococcal meningitis accounts for ~15% of AIDS-associated deaths, being a leading cause of mortality among people living with HIV (PLHIV) recently enrolled into care and initiating antiretroviral therapy (ART) in sub-Saharan Africa [1-4]. To reduce this unacceptable high mortality in resource-limited settings, the World Health Organization (WHO) recommends early detection of subclinical cryptococcal antigen (CrAg) and pre-emptive treatment of CrAg-positive individuals without meningitis with fluconazole [5-9].

CrAg is detectable in plasma weeks to months prior to onset of clinical symptoms, thus allowing for effective interventions to prevent the development of meningitis and decrease mortality [10, 11]. In Tanzania, plasma CrAg prevalence has been reported to range from 3%-8% in different populations of PLHIV [4, 12-16], being consistent with that in other Sub-Saharan African countries [10].

However, even with the early roll-out of ART and implementation of the WHO guidelines, prospective data still report high mortality among CrAg-positive individuals [3, 4, 17, 18]. There are several potential reasons for this, including the increasing lack of available antifungal therapy, the implementation of these guidelines mostly among PLHIV presenting at outpatient HIV clinics, and the low uptake of diagnostic lumbar punctures (LP) for asymptomatic CrAg-positive individuals. Thus, this strategy may miss newly diagnosed hospitalized PLHIV who have been documented to have lower CD4 and therefore are at a higher risk of cryptococcosis [19-21] as well as pauci-symptomatic early meningitis cases. This may result in suboptimal antifungal treatment for those individuals with subclinical cryptococcal infection, putting them at risk of immune reconstitution inflammatory syndrome (IRIS) and death after early ART initiation [4].

A retrospective study conducted in 2008-2012 at our facility, in Tanzania, reported a 4.4% CrAg prevalence among ART-naïve adult outpatients with CD4 <100 cells/μL. We observed 75% mortality / loss to follow-up in CrAg-positive individuals [16]; similar as in Uganda [22]. In this study, the 12-month survival/retention in CrAg-positive persons not treated with fluconazole was 0%. Fluconazole receipt for any reason decreased death or loss to follow-up (LFU) among CrAg-positive individuals [16].

In October 2013, routine laboratory-reflex CrAg screening, routine lumbar punctures (LP) among CrAg-positive, and antifungal treatment tailored to the presence or absence of meningitis was implemented systematically at our institution among both hospitalized patients and outpatients. We hypothesized that the integration of this intervention into routine HIV care at our rural facility will lead to improved survival of individuals with cryptococcosis.

METHODS

Study Setting

This prospective study was nested in the Kilombero and Ulanga Antiretroviral Cohort (KIULARCO) among adults enrolled between October 2013 and July 2015. KIULARCO is an ongoing, open, prospective cohort of PLHIV, established in 2005. The KIULARCO cohort comprises of individuals visiting the Chronic Diseases Clinic of Ifakara (CDCI) at the Saint Francis Referral Hospital (SFRH) giving written informed consent to join the cohort. The SFRH 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 700,000 inhabitants and an estimated 40,000 PLHIV. The CDCI is the first rural clinic accredited to be a care and treatment center for PLHIV in Tanzania, with >9,000 patients enrolled. Further KIULARCO cohort details are published [23, 24].

Since late 2013, provider-initiated HIV testing and counseling (PITC) was universally implemented in the medical wards of SFRH. Additionally, we introduced an enhanced package of care to improve cryptococcosis outcomes, including: (i) increased cryptococcal education and awareness; (ii) bedside finger-stick and laboratory-based plasma CrAg lateral flow assay (LFA, IMMY Inc, Norman, Oklahoma, USA) reflex testing for individuals with CD4 counts <150 cells/μL; (iii) routine diagnostic LP for all plasma CrAg-positive individuals; (iv) serial therapeutic LPs for cryptococcal meningitis and;**[**25] (v) optimized, standardized cryptococcosis treatment.

Study Design

This was a prospective longitudinal study of newly diagnosed PLHIV, ART-naïve adults ≥18 years-old, with CD4<150 cells/μL, consented and enrolled in KIULARCO from October 2013 to July 2015.

The exposure of interest was the CrAg status at baseline screening, and the main outcome was mortality or loss to follow-up (LFU) at six months after CrAg testing. The time of the event was defined as the date of death or last day of follow-up for participants with >60 days passed since last visit. Participants who were still retained -in-care after 6 months of follow-up from baseline were right-hand censored at 6 months.

Lab-Based Plasma CrAg Lateral Flow Assay

Following HIV diagnosis, all hospitalized patients and outpatients with CD4<150 cells/μL underwent reflex laboratory-based CrAg testing. CrAg LFA was performed in our laboratory according to the manufacturer's instructions from 40 μL plasma sample leftover drawn for CD4 count. Fingerstick CrAg LFA was also performed on unconscious hospitalized PLHIV by pricking a sterilized pad of the finger to produce 1 drop of whole blood placed directly onto the LFA test strip. The test strips were then placed in a 1.5-mL Eppendorf tube containing 2 drops of sample diluent, and allowed to incubate in an upright position at room temperature for 10 minutes before reading.

All CrAg-positive results were communicated by phone to the clinician in charge for immediate action. Semi-quantitative CrAg LFA titers were measured using 2-fold dilutions.

Clinical evaluation

During clinical visits, vital signs, and laboratory results were reviewed, and a structured physical examination was performed. Working diagnoses were captured through the 10th revision of the International Classification of Diseases and Health-Related problems (ICD-10) codes [26]. Treatment adherence, failure and toxicity, IRIS and other clinical details were assessed, recorded, and captured in the electronic databases of the cohort [23, 24]. Tuberculosis screening was performed in all individuals at baseline and during follow-up visits. Tuberculosis case diagnosis was defined by either: 1) positive microbiologic tests (e.g. Xpert MTB/RIF, or microscopy with acid-fast bacilli in sputum or other extra-pulmonary specimen), or 2) a chest radiograph suggestive of tuberculosis and at least one symptom of tuberculosis and receipt of anti-tubercular drugs.

Lumbar puncture and CSF Assay

All CrAg-positive participants were offered a diagnostic lumbar puncture after provision of verbal consent, regardless of neurological symptoms suggestive of meningitis. CSF CrAg LFA tests were performed twice, once by the clinical staff at the patient's bedside and a second time by a technician at the laboratory. For participants with CrAg detected in CSF, serial scheduled therapeutic lumbar punctures were conducted on days 1, 3, 7, 10, and 14 of antifungal therapy.

Antifungal Treatment

Participants with cryptococcal antigenemia and no meningeal involvement received pre-emptive fluconazole therapy consisting of 14 days of 800mg/day induction dose followed by 400mg/day for 8 weeks and then 200mg/day secondary prophylaxis. Due to the lack of

amphotericin or flucytosine in Tanzania, participants with CrAg detected in CSF were treated with high dose fluconazole monotherapy as per national guidelines [27] (fluconazole 1200mg/day for 14 days, followed by fluconazole 800mg/day for 8weeks and thereafter a secondary prophylaxis of 200mg/day). For those without evidence of meningitis, ART was initiated after 2 weeks, and for those with cryptococcal meningitis, ART was initiated at 6 weeks as per WHO guidelines [5, 28].

Data extraction and Statistical Analysis

All data were extracted from the KIULARCO cohort databases [23, 24]. Baseline was defined as the date of the first CrAg assessment. A window of +/- one month from this date was allowed to define baseline date for other laboratory parameters.

A descriptive analysis of the cohort computed frequencies and percentages with variables categorized based on clinically meaningful ranges. Thereafter, we compared the characteristics between CrAg-positive and CrAg-negative participants. We assessed binary categorical variables with Chi-square test and continuous variables with Kruskal-Wallis non-parametric test.

Incidence rate of death/LFU was calculated by dividing the number of death/LFU events by the person years at risk (PYR). Cox proportional hazard models determined the association between baseline characteristics and the time to death/LFU by estimating the respective hazard ratio. First, a univariate analysis estimated the unadjusted hazard ratio and 95% confidence intervals (95%CI) for the baseline characteristics. A multivariate model was then fitted using the backward criteria by including in the parsimonious model, using key parameters that did not exceed 10% of the cases. Only variables whose univariate association reached a statistical

significance ($p<0.1$) were selected. All models were adjusted for age and sex as selected *a priori* confounders. The likelihood ratio test compared the fit of the different models. Participants with missing data for a variable were excluded from models which included that variable. Lastly, Kaplan-Meier curves with log rank tests compared survival between CrAg-positive participants with and without CSF involvement with CrAg-negative participants.

All data were anonymized and analyzed using STATA version 14.2 (Stata statistical software, Stata Corporation, College Station, Texas, USA).

Ethical considerations

Written informed consent to participate in KIULARCO was sought from all participants at registration at the Chronic Diseases clinic of Ifakara, and all data were de-identified. The Ifakara Health Institute institutional review board, the Health Research Ethics Review Committee of the National Institute for Medical Research of Tanzania, the Tanzanian Commission of Science and Technology, and the Ethics Committee of the University and State of Basel, Switzerland, provided ethical approval for KIULARCO.

RESULTS

Between October 2013 and July 2015, 2023 individuals were diagnosed with HIV infection at the HIV clinic, outpatient clinics, and hospital medical wards. Of these, 713 (36%) patients did not consent to join the KILUARCO cohort. Of the 1,310 willing to join the cohort, 658 (50%) had baseline CD4 >150 cells/μL, 31 (2.3%) were <18 years-old, and 61 (4.6%) had reported

previous ART use. The remaining 560 ART-naive adult PLWHIV with CD4 ≤150 cells/μL were included in the study (Figure 1).

Table 1 summarizes the baseline characteristics of the 560 participants. Median CD4 count was 61 cells/μL (Interquartile Range (IQR), 26-103), and 74% had <100 cells/μL. Overall, 78% had anemia with hemoglobin <8 g/dL, and 9% had tuberculosis. Overall, 26% of the new HIV diagnoses occurred during hospitalization through the provider-initiated HIV testing introduced in the medical wards.

Cryptococcal antigen prevalence

CrAg prevalence was 6.1% (34/560) among those with CD4 ≤150 cells/μL and 7.5% (31/416) in those with CD4 ≤100 cells/μL. The concordance between the tests done at the bedside and at the laboratory was 100%. The CrAg prevalence among inpatients was 12.6% (16/127) vs 4.2% (18/433) among outpatients (p<0.001). Median CD4 cell counts were slightly lower among CrAg-positive compared to CrAg-negative participants (52 cells/μL vs 62 cells/μL, P=0.08). Tuberculosis was more prevalent among CrAg-positive individuals (22% vs 8%, P=0.01) (Table 1).

Overall, 31% (10/34) of the CrAg-positive participants had cryptococcal meningitis diagnosed by a positive CSF CrAg. At diagnosis, 44% (15/34) of the participants did not report any neurological symptoms, including one patient with proven meningitis. Plasma CrAg titers were available for 24 CrAg-positive participants (71%). Overall, 25% (6/24) had a plasma CrAg titer ≥1:160, including only 43% (3/7) of those with CrAg detected in CSF and 18% (3/17) of

participants without meningitis (P=0.20). Compared with CrAg-positive outpatients, CrAg-positive inpatients had higher CrAg titers (62.5% vs 18.75% ≥1:160, p=0.03).

Time sequence from cohort enrollment to treatment

The median time from diagnosis of HIV infection to enrollment into HIV care at the hospital was 1 day (interquartile range [IQR] 0-3 days); to CD4 testing, 1 day (IQR 0-2); and to CrAg testing, 1 day (IQR 0-2), with all CrAg-positive participants receiving a diagnostic LP on the same day. A consented LP was performed in 94% (32/34) of the CrAg-positive participants. Two participants not receiving LPs were diagnosed with HIV at the outpatient clinic but did not return to the clinic. Fluconazole tailored to meningitis-status was started in 82% of CrAg-positive patients overall (28/34), including 92% (22/24) of those with non-meningeal cryptococcosis and 60% (6/10) of those with meningitis. The overall time to fluconazole therapy was a median of three days after CrAg testing (IQR 1-30 days), including outpatients. ART was started in 68% (23/34) of CrAg-positive participants. Reasons for not having started ART were death (n=9) and lost to follow up (n=2). Those CrAg-positive participants without meningeal involvement started ART after a median time of 22 days (IQR 16, 41 days) vs 50 days (IQR 42, 56 days) for those with CrAg detected in CSF (Figure 2).

Cryptococcosis mortality and risk factors.

Outcome analysis was restricted to 508 participants after excluding 50 participants who were transferred to other HIV clinics and the 2 plasma CrAg-positive participants with unknown CSF status. These 508 participants contributed a total of 3,037 persons-year at risk follow up time.

Of these 508 participants (all CD4<150 cells/□L), 335 (66%) completed six-months of follow up, and 173 participants (34%) were dead or lost to follow-up. The overall six-month mortality rate in the cohort was 5.7 per 100 person-years (95%CI 4.9-6.6 per 100 person-years). Among CrAg-positive participants, the mortality rate was 10.7 per 100 person-years (95%CI, 6.6-17.5 per 100 person-years) overall, 7.3 per 100 person-years (95%CI, 3.7-14.6 per 100 person-years) for those without meningitis, and 20 per 100 person-years (95%CI, 1.02-40.6 per person-years) with CSF CrAg-positivity (**Table 2**).

We assessed risk factors associated with survival. In the univariate analysis, having CrAg detected in CSF was associated with 4-fold higher six-month mortality/LFU hazard compared to participants with non-meningeal cryptococcosis (Hazard Ratio = 4.1; 95%CI, 2-8.4; P<0.001). In contrast, among CrAg-positive participants without CSF involvement there was a 1.5-fold increase hazard of death/LFU vs CrAg-negative participants, which did not reach statistical significance (Hazard Ratio = 1.5; 95%CI, 0.8-3.1; P=0.2, **Figure 3**). Other predictors of mortality/LFU in the univariate analysis were anemia, low CD4, impaired renal function, and not having received ART (Table 2). Mortality tended to be higher among those participants with a high CrAg titer (above 1:160) versus those with titers below this threshold (36% vs 20%) but this difference did not reach statistical significance. In the univariate analysis, high titers were associated with a hazard of death/LFU of 2.1 without reaching statistical significance (p=0.236). Also, there was strong evidence suggesting that time from HIV and CrAg testing to initiation of fluconazole therapy were associated with up to 19% reduction in the risk of mortality (P=0.004, data not shown). However, the independent effect of these time factors was not further explored in the multivariate analysis due to the small number of observations.

After adjusting for age, sex, CD4 cell count, anemia, and kidney function, factors significantly associated with six-month mortality included: no ART initiation, CrAg detection in CSF, and low CD4 cell counts (Table 2). There was no evidence of any effect of age, sex, anemia or non-meningeal cryptococcosis on the risk of six-month mortality (Table 2). \

DISCUSSION

In this prospective cohort study of ART-naïve PLHIV with CD4<150 cells/μL in rural Tanzania, implementation of provider-initiated HIV testing coupled with laboratory-reflex CrAg testing of all persons entering into HIV care identified a high prevalence of cryptococcosis. This prevalence was 1.7-fold higher than when retrospectively testing outpatients only [16]. CrAg-positive individuals treated with fluconazole had a similar six-month survival as CrAg-negative individuals. After adjusting for the effect of age, sex, renal function, and hemoglobin level, CSF CrAg-positivity increased the risk of mortality / LFU by nearly three-fold while ART initiation reduced the risk by 71%. We confirmed our hypothesis that the pragmatic approach of combining routine HIV testing among both out-individuals and hospitalized individuals with laboratory-reflex CrAg testing, fluconazole pre-emptive treatment, and diagnostic LPs among CrAg-positive individuals would improve cryptococcal disease detection in our facility. Our historical survival was zero in CrAg-positive outpatients, retrospectively identified, who did not receive fluconazole [16].

We found a CrAg-positive prevalence of 7.5% among those individuals with CD4 counts <100 cells/μL and 2.1% in those with CD4 counts between 100 and 150. Importantly, we observed that survival among those with CD4 counts >100 cells/μL did not differ compared to those with CD4 between 51-100cells/μL (Table 3). This advocates for an increase in the CrAg

screening CD4 threshold as we had previously suggested [16]. CrAg prevalence was 3-fold higher among hospitalized patients vs outpatients (12.6% vs 4.2%). Indeed, half of the CrAg-positive individuals were diagnosed through provider-initiated HIV testing while hospitalized. Likely, these hospitalized patients may have otherwise been missed in absence of this strategy, and this suggests reconsidering expanding CrAg screening recommendations to hospitalized PLHIV with low CD4 counts. As the majority of PLHIV who are hospitalized have low CD4 counts, universal screening of hospitalized PLHIV should be considered in the absence of CD4 testing. Outpatient only CrAg screening may likely underestimate the CrAg burden and therefore miss opportunities to reduce AIDS-mortality.

One-third of CrAg-positive individuals (10/34) were diagnosed with meningitis by CSF CrAg positivity, including one asymptomatic individual. Overall, only 1 of 23 (4%) asymptomatic individuals was CSF CrAg-positive, and 9/10 participants with CSF CrAg-positivity were symptomatic with CNS symptoms. Our experience show a high LP acceptance once detailed information was given on its risks and benefits of LP. We also observed cryptococcal meningitis individuals had higher CrAg titers $\geq$1:160 as compared with CrAg-positive individuals with no CSF involvement (43% vs 18%) as expected [10, 17, 29-31]. However, due to the small sample size, this difference did not reach statistical significance ($p=0.20$). Nevertheless, these results may suggest that asymptomatic patients with lower CrAg titers may not need diagnostic LPs to rule out sub-clinical meningitis.

We implemented laboratory-reflex CrAg testing instead of relying on the clinical evaluation, favoring a consistent rate of testing and rapid communication of results vs provider initiated CrAg screening, as shown in previous studies [32-34]. Through this implementation, we found no statistically significant difference in survival between CrAg-negative individuals and those

with non-meningeal CrAg-positive treated with fluconazole, although the point-estimate in the univariate model pointed towards a 50% percent higher risk mortality/LFU. Our findings are in-line with the existing evidence on the survival benefit of integrating early CrAg screening and fluconazole pre-emptive therapy when fungal burden is low [10, 16].

In contrast, the observed six-month mortality/LFU among individuals with cryptococcal meningits treated with high dose fluconazole monotherapy was unacceptably high (80%, 8/10). Despite the implementation of an optimized meningitis clinical treatment protocol, including rapid CSF testing after CrAg screening, high-dose fluconazole, repeated LPs and intracranial pressure management, cryptococcal meningitis mortality was equal to that observed in the historical cohort [16]. This figure compares with other studies from Tanzania and sub Saharan Africa where recommended antifungals such as flucytosine and amphotericin B are widely unavailable [4, 14, 35]. Our findings therefore support the notion that approaches centered exclusively on HIV treatment are inadequate in reducing AIDS-related mortality and the undisputed need for available first-line antifungals for cryptococcal treatment [18, 36].

This study has several limitations. Selection bias is of concern as our analysis excluded individuals who were unable to provide ethical consent such as those who had an altered mental state throughout admission. Nevertheless, this may have underestimated the true prevalence of cryptococcosis among admitted individuals. Also, fluconazole was freely available at the hospital through the President's Emergency Plan for AIDS Relief (PEPFAR) support until late 2014, but thereafter individuals were required to purchase fluconazole. Fluconazole adherence was not verified, and individuals requiring a longer course may have defaulted treatment due to financial constraints. In addition, a composite outcome of death and loss to follow up was used due to suboptimal ascertainment of mortality in the cohort**.** Given the contribution of loss to

follow up to the 6-month outcome, the clinical endpoint of the study might have substantial misclassification. Indeed, in the absence of postmortem studies, the ascertainment of the cause of death was incomplete and based on clinical judgment. Furthermore, the power of this study to detect the true estimate is limited given the sample size. Finally, the lack of a survival difference between those with non-meningeal cryptococcosis and those CrAg-negative may suffer from a type-II statistical error due to lack of power, finding no difference when one truly exists, and thus, these results have to be interpreted with caution.

The main strengths of this study were the integration of CrAg screening within routine clinical care and the prospective nature of the clinical and laboratory data collected, which allowed correct estimation of incidence rates and survival estimates. The electronic data collection, linking the KIULARCO database with the information from the laboratory, outpatient and medical wards, permitted estimation of time from HIV testing to CrAg testing, LP, fluconazole and ART initiation. To our knowledge, no previous studies have estimated the effectiveness of such a model incorporated into routine HIV care in rural sub-Saharan Africa.

In summary, these results confirm that implementing HIV testing in outpatients and in hospitalized individuals coupled with CrAg laboratory-reflex testing and early initiation of fluconazole pre-emptive treatment increase detection of cryptococcosis and may lead to a survival rate of non-meningeal cryptococcosis similar to that of CrAg-negative individuals with comparable CD4 counts. Cryptococcal mortality remains high in the absence of first-line antifungals despite timely diagnosis (median of 1 day from HIV testing), treatment with high dose fluconazole, and proper management of intracranial pressure as recommended by WHO. Recommended first-line antifungals are urgently needed to supplement these efforts.

Our study suggests a feasible, effective and scalable model for CrAg screening and pre-emptive treatment strategy to be integrated into routine care in rural sub-Saharan Africa. We further support the recently adopted higher CD4 threshold for CrAg screening and suggest to consistently extend these WHO recommendations among hospitalized individuals as part of the provider-initiated testing and counseling strategy. These recommendations are pertinent to reduce the unacceptably high AIDS-mortality among people with advanced HIV disease at a time were universal ART is being widely rolled-out in sub-Saharan Africa.

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REFERENCES

1. Jarvis JN, Meintjes G, Williams A, Brown Y, Crede T, Harrison TS. Adult meningitis in a setting of high HIV and TB prevalence: findings from 4961 suspected cases. BMC infectious diseases **2010**;10:67.
2. Durski KN, Kuntz KM, Yasukawa K, Virnig BA, Meya DB, Boulware DR. Cost-effective diagnostic checklists for meningitis in resource-limited settings. J Acquir Immune Defic Syndr **2013**;63:e101-8.

3. Rajasingham R, Smith RM, Park BJ, et al. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. The Lancet Infectious diseases **2017**;17:873-81.
4. Kapoor SW, Magambo KA, Kalluvya SE, Fitzgerald DW, Peck RN, Downs JA. Six-month outcomes of HIV-infected patients given short-course fluconazole therapy for asymptomatic cryptococcal antigenemia. AIDS **2015**;29:2473-8.
5. World Health Organization. Rapid advice: Diagnosis, prevention and management of cryptococcal disease in HIV-infected adults, adolescents and children. Available at: www.who.int/hiv/pub/cryptococcal_disease2011. Accessed 1 Jan.
6. Meyer AC, Kendi CK, Penner JA, et al. The impact of routine cryptococcal antigen screening on survival among HIV-infected individuals with advanced immunosuppression in Kenya. Tropical medicine & international health : TM & IH **2013**;18:495-503.
7. Jarvis JN, Govender N, Chiller T, et al. Cryptococcal antigen screening and preemptive therapy in patients initiating antiretroviral therapy in resource-limited settings: a proposed algorithm for clinical implementation. Journal of the International Association of Physicians in AIDS Care **2012**;11:374-9.
8. Jarvis JN, Lawn SD, Wood R, Harrison TS. Cryptococcal antigen screening for patients initiating antiretroviral therapy: time for action. Clin Infect Dis **2010**;51:1463-5.
9. Vallabhaneni S, Longley N, Smith M, et al. Implementation and Operational Research: Evaluation of a Public-Sector, Provider-Initiated Cryptococcal Antigen Screening and Treatment Program, Western Cape, South Africa. J Acquir Immune Defic Syndr **2016**;72:0000000000000976.

10. Rajasingham R, Meya DB, Boulware DR. Integrating cryptococcal antigen screening and pre-emptive treatment into routine HIV care. Journal of acquired immune deficiency syndromes **2012**;59:e85-91.

11. Jarvis JN, Percival A, Bauman S, et al. Evaluation of a novel point-of-care cryptococcal antigen test on serum, plasma, and urine from patients with HIV-associated cryptococcal meningitis. Clin Infect Dis **2011**;53:1019-23.

12. Wajanga BM, Kalluvya S, Downs JA, Johnson WD, Fitzgerald DW, Peck RN. Universal screening of Tanzanian HIV-infected adult inpatients with the serum cryptococcal antigen to improve diagnosis and reduce mortality: an operational study. Journal of the International AIDS Society **2011**;14:48.

13. Rugemalila J, Maro VP, Kapanda G, Ndaro AJ, Jarvis JN. Cryptococcal antigen prevalence in HIV-infected Tanzanians: a cross-sectional study and evaluation of a point-of-care lateral flow assay. Tropical medicine & international health : TM & IH **2013**;18:1075-9.

14. Magambo KA, Kalluvya SE, Kapoor SW, et al. Utility of urine and serum lateral flow assays to determine the prevalence and predictors of cryptococcal antigenemia in HIV-positive outpatients beginning antiretroviral therapy in Mwanza, Tanzania. Journal of the International AIDS Society **2014**;17.

15. Mfinanga S, Chanda D, Kivuyo SL, et al. Cryptococcal meningitis screening and community-based early adherence support in people with advanced HIV infection starting antiretroviral therapy in Tanzania and Zambia: an open-label, randomised controlled trial. Lancet **2015**;385:2173-82.

16. Letang E, Muller MC, Ntamatungiro AJ, et al. Cryptococcal antigenemia in immunocompromised HIV patients in rural Tanzania: A preventable cause of early mortality. Open Forum Infect Dis **2015**;2:ofv046.

17. Longley N, Jarvis JN, Meintjes G, et al. Cryptococcal antigen screening in patients initiating ART in South Africa: A prospective cohort study. Clin Infect Dis **2016**;62:581-7.

18. Tenforde MW, Mokomane M, Leeme T, et al. Advanced HIV disease in Botswana following successful antiretroviral therapy rollout: Incidence of and temporal trends in cryptococcal meningitis. Clin Infect Dis **2017**.

19. Andama AO, den Boon S, Meya D, et al. Prevalence and outcomes of cryptococcal antigenemia in HIV-seropositive patients hospitalized for suspected tuberculosis in Uganda. Journal of acquired immune deficiency syndromes **2013**;63:189-94.

20. Harris JR, Lindsley MD, Henchaichon S, et al. High prevalence of cryptococcal infection among HIV-infected patients hospitalized with pneumonia in Thailand. Clin Infect Dis **2012**;54:e43-50.

21. Katchanov J, Jefferys L, Tominski D, et al. Cryptococcosis in HIV-infected hospitalized patients in Germany: Evidence for routine antigen testing. The Journal of infection **2015**;71:110-6.

22. Meya DB, Manabe YC, Castelnuovo B, et al. Cost-effectiveness of serum cryptococcal antigen screening to prevent deaths among HIV-infected persons with a CD4+ cell count < or = 100 cells/microL who start HIV therapy in resource-limited settings. Clin Infect Dis **2010**;51:448-55.

23. Letang E, Kalinjuma AV, Glass TR, et al. Cohort profile: The Kilombero and Ulanga Antiretroviral Cohort (KIULARCO) - A prospective HIV cohort in rural Tanzania. Swiss Med Wkly **2017**;147:w14485.

24. Vanobberghen F, Letang E, Gamell A, et al. A decade of HIV care in rural Tanzania: Trends in clinical outcomes and impact of clinic optimisation in an open, prospective cohort. PLoS One **2017**;12:e0180983.

25. Rolfes MA, Hullsiek KH, Rhein J, et al. The effect of therapeutic lumbar punctures on acute mortality from cryptococcal meningitis. Clin Infect Dis **2014**;59:1607-14.

26. World Health Organization. WHO international statistical classification of diseases and related health problems: 10th edition.

27. Tanzania Ministry of Health and Social Welfare. National Guideline for Managment of HIV and AIDS. **2012**.

28. Boulware DR, Meya DB, Muzoora C, et al. Timing of antiretroviral therapy after diagnosis of cryptococcal meningitis. N Engl J Med **2014**;370:2487-98.

29. Kabanda T, Siedner MJ, Klausner JD, Muzoora C, Boulware DR. Point-of-care diagnosis and prognostication of cryptococcal meningitis with the cryptococcal antigen lateral flow assay on cerebrospinal fluid. Clin Infect Dis **2014**;58:113-6.

30. Vidal JE, Toniolo C, Paulino A, et al. Asymptomatic cryptococcal antigen prevalence detected by lateral flow assay in hospitalised HIV-infected patients in Sao Paulo, Brazil. Tropical medicine & international health : TM & IH **2016**;21:1539-44.

31. Jarvis JN, Lawn SD, Vogt M, Bangani N, Wood R, Harrison TS. Screening for cryptococcal antigenemia in patients accessing an antiretroviral treatment program in South Africa. Clin Infect Dis **2009**;48:856-62.

32. Cassim N, Schnippel K, Coetzee LM, Glencross DK. Establishing a cost-per-result of laboratory-based, reflex Cryptococcal antigenaemia screening (CrAg) in HIV+ patients with CD4 counts less than 100 cells/mul using a Lateral Flow Assay (LFA) at a typical busy CD4 laboratory in South Africa. PLoS One **2017**;12:e0171675.

33. Cassim N, Coetzee LM, Schnippel K, Glencross DK. Estimating the cost-per-result of a national reflexed Cryptococcal antigenaemia screening program: Forecasting the impact of potential HIV guideline changes and treatment goals. PLoS One **2017**;12:e0182154.

34. Larson BA, Rockers PC, Bonawitz R, et al. Screening HIV-Infected Patients with Low CD4 Counts for Cryptococcal Antigenemia prior to Initiation of Antiretroviral Therapy: Cost Effectiveness of Alternative Screening Strategies in South Africa. PLoS One **2016**;11:e0158986.

35. Boaz MM, Kalluvya S, Downs JA, Mpondo BC, Mshana SE. Pattern, Clinical Characteristics, and Outcome of Meningitis among HIV-Infected Adults Admitted in a Tertiary Hospital in North Western Tanzania: A Cross-Sectional Study. Journal of tropical medicine **2016**;2016:6573672.

36. Williamson PR. The relentless march of cryptococcal meningitis. The Lancet Infectious Diseases **2017**;17:790-1.

Figure 1: Study profile of Cohort

Figure 2. Timing from HIV testing through ART initiation

Figure 2: Timing from HIV diagnosis to ART initiation. P-values compare the median time between CrAg positive and CrAg negative individuals. Overall, the median time from entry into

HIV care to CD4 testing, CrAg testing, and referral for lumbar puncture was 1 day in total with prioritization of addressing advanced HIV disease.

Figure 3. Kaplan-Meier survival estimates of Death/ Lost to Follow-up by CrAg Status

Figure: 3a (Top left): Kaplan-Meier survival estimates of death or loss to follow-up among CrAg negative versus Non-meningeal CrAg positive individuals. 3b (Top right): Kaplan-Meier survival estimates of death or loss to follow-up among CrAg negative versus Cryptococcal meningitis individuals. 3c (Bottom left): Kaplan-Meier survival estimates of death or loss to follow-up by CrAg status.3d (Bottom right): Kaplan-Meier survival estimates of death or loss to follow-up among CrAg negative versus CrAg positive by ART status.

Table 1. Distribution of baseline characteristics of study participants

Factor	Total	CrAg Negative	CrAg Positive	P-value [a]
Overall, n(%)	560 (100)	526 (100)	34 (100)	
Women, n(%)	311 (56)	291 (55)	19 (59)	0.7
Age, years	38 (33-46)	38 (33-46)	41 (35-49)	0.3
Hemoglobin g/dL	9.7 (8.3-11.2)	9.7 (8.3-11.2)	9.4 (8.2-0.9)	0.4
CD4, cells/µL, median (IQR)	61 (26-103)	62 (26-105)	52 (28-105)	0.08
0-50, n(%) 51-100 , n(%) 101-150, n(%)	229 (41) 187 (33) 144 (26)	213 (40) 172 (33) 141 (27)	16 (47) 15 (44) 3 (9)	0.06
Tuberculosis Treatment, n(%)	51 (9)	44 (8)	7 (22)	0.01
Neurological symptoms, n(%)	94 (17)	75 (14)	19 (56)	<0.001
Procedence, n(%) Outpatient Inpatient	 420 (74) 144 (26)	 399 (76) 127 (24)	 17 (50) 17 (50)	0.001
Cryptococcal Meningitis, n(%) Positive CSF CrAg Negative CSF CrAg LP not performed	 10 (2) 22 (4) 528 (94)	 -- -- 526 (100)	 10 (29) 22 (65) 2 (6)	 - - -
ART Initiated, n(%)	461 (82)	438 (83)	23 (68)	0.021
6-month outcome, n(%) Alive Dead LFU Transferred	 335 (60) 56 (10) 119 (21) 50 (9)	 320 (61) 43 (8) 114 (22) 49 (9)	 15 (44) 13 (38) 5 (15) 1 (3)	 <0.001

Numbers are n (column %) or median (interquartile range).

Abbreviations: GFR, estimated Glomerular Filtration Rate; BMI, Body mass index; LP, Lumbar puncture; ART, antiretroviral therapy; LFU: Lost to follow-up

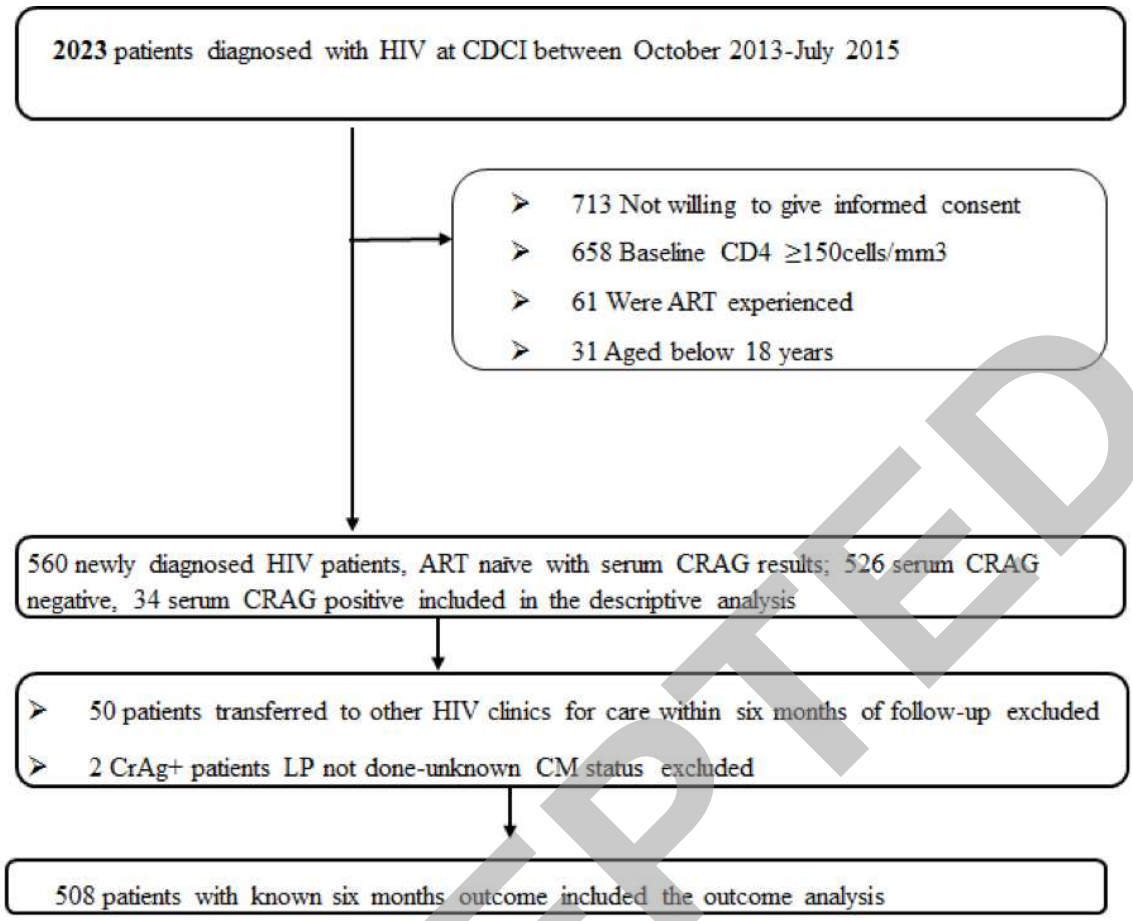
[a] P-value from Chi-square test or Kruskal–Wallis rank test

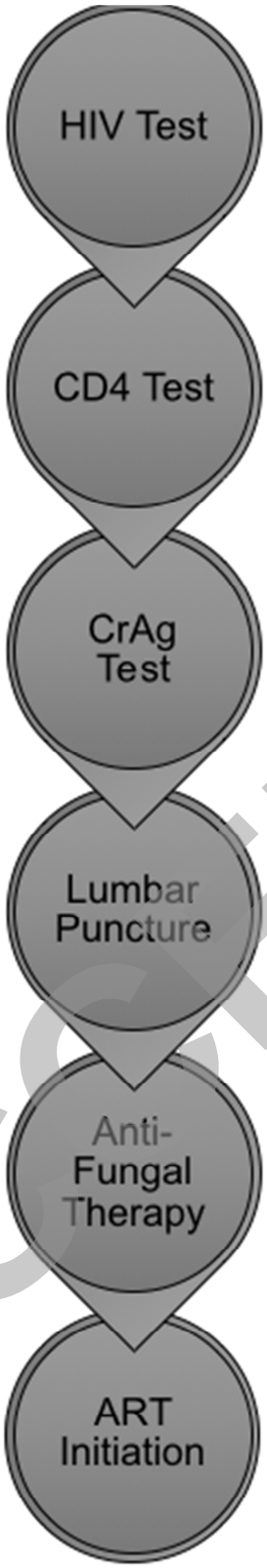
Table 2 Univariate and multivariate analysis of factors associated with death/loss to follow-up at six-month.

	Death or Lost to Follow-up	Person-Years Exposure	Rate /100 Person years	Univariate Analysis		Multivariable Analysis (N=498) [a]	
				Hazard Ratio (95% CI)	*p*	Hazard Ratio (95%CI)	*p*
Age, years	--		--	1.01 (0.99-1.02)	0.32	1.01 (0.99-1.03)	0.19
Sex							
Men	79	13.6	5.8	1.0	0.88	1.0	0.81
Women	94	16.7	5.6	1.02 (0.76-1.38)		1.04(0.75-1.44)	
CD4, cells/μL							0.02[b]
0-50	89	12.1	7.4	1.0	0.04	1.0	
51-100	55	10.7	5.1	0.69 (0.49-0.96)		0.68 (0.48-0.96)	
>100	29	7.6	3.8	0.66 (0.43-1.00)		0.66 (0.43-1.02)	
Anemia							
No	130	24.5	5.3	1.0	0.06	1.0	0.60
Yes	43	5.9	7.3	1.40(0.99-1.99)		1.11(0.76-1.61)	
Non-CNS CrAg+							
Negative	157	28.9	5.4	1.0	0.25	N/A	N/A
Positive	8	1.1	7.3	1.53 (0.75-3.12)			
Meningitis in CrAg+							
Negative	8	1.1	7.3	1.0	<.001	1.0	0.01
Positive	8	0.4	20	4.08 (2.00-8.37)		2.77 (1.31-5.83)	
ART Initiation							
No	55	3.9	14.2	1	<.001	1	<.001
Yes	118	26.5	4.5	0.29(0.20-0.41)		0.32(0.22-0.46)	
Provider-Initiated HIV Test							
No	125	23.1	5.4	1	0.13	N/A	N/A
Yes	48	7.2	6.6	1.29(0.92-1.81)			
eGFR					0.03		
≥90	129	24.12	5.3	1		1	0.52[b]
30 - 89.9	32	4.9	6.5	1.38(0.93-2.04)		1.19(0.79-1.80)	
< 30.0	12	1.3	9.2	2.22(1.22-4.05)		1.38(0.72-2.65)	

BMI, per kg/m2							
≥25	11	2.5	4.5	1	0.22	N/A	N/A
18.5-24.9	88	17.2	5.1	1.37(0.71-2.66)			
<18.5	56	9.0	6.3	1.70(0.86-3.36)			
TB co-infection					0.48		
No	153	27.9	5.5	1		N/A	N/A
Yes	20	2.5	8.1	1.18(0.74-1.90)			

Abbreviations; PYR, person years at risk; CI, confidence Interval; HR, Hazard ratio; CrAg cryptococcal antigen; ART, antiretroviral; eGFR, estimated Glomerular Filtration Rate.
Variables included in the multivariable model were age at CrAg test as a continuous variable, sex, CD4, anemia, Cryptococcal meningitis, ART initiation and eGFR. P-value from Wald test for binary variables and likelihood ratio test for categorical variables. [B] P-value for trend.





	Days Median (IQR)		P-value
1	(0-2)		0.126
1	(0-2)		0.21
1	(0-2)		0.57
7	(2,30)		0.004
22	(16,41)	CrAg+	0.77
50	(42,56)	meningitis	0.66