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The impact of routine cryptococcal antigen screening on survival among HIV-infected individuals with advanced immunosuppression in Kenya

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Abstract

OBJECTIVES To test the hypothesis that a screening and treatment intervention for early cryptococcal infection would improve survival among HIV-infected individuals with low CD4 cell counts.
METHODS Newly enrolled patients at Family AIDS Care and Education Services (FACES) in Kenya with CD4 $\leq$ 100 cells/μl were tested for serum cryptococcal antigen (sCrAg). Individuals with sCrAg titre $\geq$ 1:2 were treated with high-dose fluconazole. Cox proportional hazard models of Kaplan–Meier curves were used to compare survival among individuals with CD4 $\leq$ 100 cells/μl in the intervention and historical control groups.
RESULTS The median age was 34 years [IQR: 29,41], 54% were female, and median CD4 was 43 cells/μl [IQR: 18,71]. Follow-up time was 1224 person-years. In the intervention group, 66% (514/782) were tested for sCrAg; of whom, 11% (59/514) were sCrAg positive. Mortality was 25% (196/782) in the intervention group and 25% (191/771) in the control group. There was no significant difference between the intervention and control group in overall survival [hazard ratio (HR): 1.1 (95%CI:0.9,1.3)] or three-month survival [HR: 1.0 (95%CI:0.8,1.3)]. Within the intervention group, sCrAg-positive individuals had significantly lower survival rates than sCrAg-negative individuals [HR:1.8 (95%CI: 1.0, 3.0)].
CONCLUSIONS A screening and treatment intervention to identify sCrAg-positive individuals and treat them with high-dose fluconazole did not significantly improve overall survival among HIV-infected individuals with CD4 counts $\leq$ 100 cells/μl compared to a historical control, perhaps due to intervention uptake rates or poor efficacy of high-dose oral fluconazole.

keywords cryptococcus, screening, prevention, Africa, outcomes, cryptococcal meningitis

Introduction

In sub-Saharan Africa and South-East Asia, invasive cryptococcal disease is the second most common life-threatening HIV-associated opportunistic infection after tuberculosis and results in up to 20% of deaths (Tansuphasawadikul *et al.* 1999; Chariyalertsak *et al.* 2001; French *et al.* 2002; Lawn *et al.* 2008). A recent study estimates that cryptococcal meningitis may even be surpassing tuberculosis as the leading cause of death among individuals with HIV infection in sub-Saharan Africa (Park *et al.* 2009). This may be because case fatality (Mwaba *et al.* 2001; Bicanic *et al.* 2009) and prevalence rates (Tansuphasawadikul *et al.* 1999; Chariyalertsak *et al.* 2001; French *et al.* 2002; McCarthy *et al.* 2006) for invasive cryptococcal disease are higher in resource-limited settings.

Several developments have led to a growing interest in creating new approaches for the early treatment of invasive cryptococcal disease in resource-limited settings (World Health Organization 2011; Jackson & van der Horst 2012). Diagnostic testing and treatments for cryptococcal disease are now more widely available even in resource-limited settings. Both antiretroviral therapy (ART) and fluconazole are also increasingly available in sub-Saharan Africa (Collett & Parrish 2007; The Antiret-

roviral Therapy in Lower Income Countries (ART-LINC) Collaboration, ART Cohort Collaboration (ART-CC) groups 2006). In addition, serum cryptococcal antigen (sCrAg) is a well-established and highly sensitive and specific assay for invasive cryptococcal disease (Nelson *et al.* 1990) and a novel point-of-care cryptococcal antigen test that can be used on serum, plasma or urine has been recently evaluated (Jarvis *et al.* 2011; Lindsley *et al.* 2011).

Cryptococcal antigenaemia, or asymptomatic cryptococcal infection, is both a prevalent and highly morbid condition among HIV-infected individuals in Africa and South-East Asia. Five recent observational cohort studies from Uganda, South Africa, Cambodia and Thailand revealed that 6–13% of individuals entering HIV care with a CD4 cell count $\leq$ 100 cells/μl have asymptomatic cryptococcal infection (Liechty *et al.* 2007; Micol *et al.* 2007; Jarvis *et al.* 2009; Meya *et al.* 2010; Pongsai *et al.* 2010). Retrospective studies conducted in sub-Saharan Africa provide data on the clinical implications of untreated asymptomatic cryptococcal infection in HIV-infected individuals with CD4 cell counts $\leq$ 100 cells/μl. One demonstrated that asymptomatic cryptococcal infection was 100% sensitive for the development of cryptococcal meningitis during the first year of ART (Jarvis *et al.* 2009). A second study reported a population attributable risk for mortality of 18% among individuals initiating ART, comparable to that associated with active tuberculosis (Liechty *et al.* 2007). Thus, despite treatment with ART, asymptomatic cryptococcal infection in individuals with low CD4 cell counts is predictive of cryptococcal meningitis and death.

Only a few small studies describe the outcomes of treatment with both antifungal medications and ART; mortality due to asymptomatic cryptococcal infection after treatment with ART and low-dose fluconazole is unacceptably high (Micol *et al.* 2007; Meya *et al.* 2010). There are no published studies of high-dose fluconazole for asymptomatic cryptococcal infection, although this has been studied for cryptococcal meningitis (Menichetti *et al.* 1996; Longley *et al.* 2008).

In summary, screening asymptomatic HIV-infected individuals with advanced immunosuppression for sCrAg clearly identifies a population at high risk of cryptococcal meningitis and death. Furthermore, routine sCrAg testing is feasible in resource-limited settings. While the mainstay of anticryptococcal therapy in resource-limited settings is oral fluconazole, preliminary evidence suggests that low-dose fluconazole is not an effective treatment.

In response to these findings, Family AIDS Care and Education Services (FACES), a decentralised outpatient HIV care and treatment programme in Kenya, initiated an intervention to screen HIV-infected individuals with advanced immunosuppression for early cryptococcal infection and treat those with cryptococcal infection with ART and high-dose fluconazole. The goal of this evaluation was to determine whether this intervention would improve survival among all adults with CD4 counts $\leq$ 100 cells/μl compared to a historical control. To our knowledge, there are no published reports of the outcomes of cryptococcal antigen screening in an outpatient HIV care setting, although there is one recent study from Tanzania reporting outcomes from screening HIV-infected individuals in an inpatient medical ward (Wajanga *et al.* 2011).

Methods

A screening and treatment intervention for early cryptococcal infection in HIV-infected adults with low CD4 + T-cell counts was initiated at FACES in November 2009. Founded in 2004, FACES has pioneered a family-centred model of HIV care, prevention and treatment and supports 83 HIV care and treatment sites across five districts in Nyanza Province, Kenya. As of July 2011, FACES had cumulatively enrolled 101 617 individuals and had 40 546 individuals on ART.

The screening and treatment intervention consisted of several components. FACES protocols require all HIV-infected adults who are newly enrolling in clinical care to have a CD4 + T-cell count and rapid plasma reagin (RPR) test. Laboratory tests are conducted centrally at five district laboratories. Laboratory protocols were developed so that all adults who had CD4 + T-cell count $\leq$ 100 cells/μl and had a serum sample (drawn for RPR) were automatically tested for the presence of sCrAg (Immuno-Mycologics, Norman, OK). If the RPR sample was not present and CD4 + T-cell count was $\leq$ 100 cells/μl, a request was sent back to the clinic site for a serum sample. If a sCrAg test was positive (defined as titre $\geq$ 1:2), then a treatment protocol was sent back to the health facility along with the test results.

Our treatment protocol for all patients with a positive sCrAg test recommended high-dose fluconazole for intensive and consolidation therapy (1200 mg per day for 2 weeks followed by 800 mg/day for 8 weeks). This was followed by maintenance fluconazole (200 mg/day). Fluconazole dose adjustments were recommended for individuals with renal failure or concurrent rifampicin treatment. If signs or symptoms of clinical meningitis were absent, the protocol recommended that ART be started at 2 weeks after initiating fluconazole treatment. If signs or symptoms of meningitis were present, ART was started at 6 weeks. Signs and symptoms of meningitis included: headache, stiff neck, photophobia, altered mental status, seizures or focal neurological deficits.

Our treatment protocol did not differentiate between asymptomatic and minimally symptomatic patients, although individuals with frank clinical meningitis as described above were not included in the intervention. Instead, we grouped asymptomatic and minimally symptomatic individuals together and defined this group as 'early cryptococcal infection'. Thus, individuals with early cryptococcal infection had a positive sCrAg test (titre $\geq$ 1:2) and may have had minimal symptoms of meningitis, but were well enough to be seeking medical care in an outpatient setting. We did not recommend different treatments for asymptomatic and minimally symptomatic patients because in our setting, even cryptococcal meningitis is treated primarily with fluconazole monotherapy (Kendi *et al.* 2012). Furthermore, in our setting, patients must pay for inpatient charges, while outpatient care and oral fluconazole are free. In addition, amphotericin is only intermittently available and only at central district hospitals. Flucytosine is not licensed in Kenya. Our experience was that patients would be reluctant to be admitted unless moribund, and even once admitted, would likely not receive anything more than oral fluconazole. Thus, we included minimally symptomatic patients in our outpatient treatment protocol.

Although the intervention was implemented across all FACES-supported clinical sites, because of the challenges of gathering the evaluation data, we selected one rural and one semi-urban district for this evaluation (Rongo and Kisumu districts, respectively). Inclusion criteria for both the control and intervention groups were adults $\geq$ 15 years of age, CD4 + T-cell count $\leq$ 100 cells/μl, enrolled in HIV care and treatment in one of the 18 clinics in Rongo or Kisumu districts. The intervention group was comprised of individuals enrolled in HIV care and treatment between 1 November 2009 and 31 May 2010, and the historical control group was comprised of individuals enrolled between 1 April 2009 and 31 October 2009. The primary endpoint was all-cause mortality.

Retrospective review of charts and home visits was performed between January 2010 and December 2011. Lists of newly enrolled patients during the study period were generated from an electronic medical record (when available) or from a review of paper records of new enrollees. The paper or online medical records were reviewed to determine age and CD4 + T-cell count. A standardised chart review was performed by trained abstractors for all cases that met inclusion criteria. Home visits were performed for all individuals who had not attended a clinic appointment within 3 months of the date of chart abstraction.

First, we calculated the percent uptake of the intervention using the following formula: 100* (no. sCrAg-positive individuals who received fluconazole during the intervention)/(expected number of sCrAg-positive individuals in the intervention group with perfect implementation). The expected number of sCrAg-positive individuals in the intervention group was calculated as follows: (total number of individuals who met inclusion criteria during the intervention period)* (proportion of individuals with CD4 T-cell count $\leq$ 100 cells/μl)* (proportion of individuals who were sCrAg positive). The latter two proportions were calculated using data from the intervention group but excluding those who were not tested from the denominator.

Descriptive statistics for the intervention and control groups were compared using Wilcoxon rank-sum tests for continuous variables and Fisher's exact tests for categorical variables. Demographic and clinical variables included: age, gender, body mass index (BMI), CD4 + T-cell count, receipt of ART, time between enrolment and initiation of ART, receipt of antituberculosis medication during follow-up period, serum cryptococcal antigen test results and whether an individual was hospitalised for cryptococcal infection.

We compared survival among all HIV-infected adults with CD4 + T-cell count $\leq$ 100 cells/μl (regardless of sCrAg test results) before and after implementation of the intervention. Our primary endpoints were all-cause mortality at 3 months and at the end of the follow-up period. We were not able to ascertain cause of death but date of death was either reported in the chart or given by a relative upon home visit. Survival was defined as time from enrolment until date of death or censoring. Individuals were censored at the date of the last clinic visit. We defined lost-to-follow-up as individuals who had not attended a clinic visit within 3 months of the date of chart abstraction. Individuals who were lost-to-follow-up and upon home visit were found to be alive were censored at the date of the home visit. Kaplan–Meier curves were generated for the intervention and historical control groups. Cox proportional hazard models were used to compare survival between the two groups.

Sensitivity analyses were conducted to account for incomplete uptake of the intervention and potential early initiation of the intervention. First, we excluded individuals who were not tested for sCrAg in the intervention group (n = 268). Then, we additionally excluded individuals who were sCrAg positive but did not have documentation of fluconazole therapy (n = 6). Finally, we excluded individuals in the control group who were tested for sCrAg but did not have documented signs or symptoms of meningitis (n = 96).

We also compared descriptive statistics and survival between individuals with and without early cryptococcal infection in the intervention group. Statistical analyses

were performed with STATA/SE 11.2 (StataCorp, College Station, TX). A significance level of $P < 0.05$ was used.

Power calculation

We estimated approximately 10% overall mortality in HIV-infected individuals with CD4 + T-cell counts $\leq$ 100 cell/μl within one year of enrolling in care (an average between similar cohorts reporting 16.8% (Jarvis *et al.* 2009) and 6.4% (Liechty *et al.* 2007). Mortality in sCrAg-positive individuals with CD4 + T-cell counts $\leq$ 100 cell/μl starting ART was estimated at 22% and 5.3% mortality among sCrAg-negative individuals (Liechty *et al.* 2007). We assumed a 10% sCrAg-positive rate in the FACES population. Assuming the maximum benefit of our intervention would be to decrease the mortality among sCrAg-positive individuals to the mortality rate of sCrAg-negative individuals, we estimated that the post-intervention mortality would be approximately 5.3%. The sample size needed to detect this difference, equivalent to a hazard ratio of 0.51, was 562 (assuming an alpha of 0.05, power of 0.8, 10% withdrawal rate and 1:1 ratio of cases to controls). After we had the actual sample size and mortality rate in our population, we additionally performed a *post hoc* power calculation assuming a sample size of 770 in each group, 75% probability of survival in the control group at the end of the follow-up period and alpha of 0.05. This calculation suggests that we are 80% powered to detect a hazard ratio of 0.74. This is roughly equivalent to a 26% reduction in mortality, or using our sample size, a reduction from 192 deaths (25% mortality) to 142 deaths, a difference of 50 deaths. However, our observed mortality rate in sCrAg-negative individuals was substantially higher that that reported in prior studies (24% *vs.* 5.3% (Liechty *et al.* 2007) and 14% (Jarvis *et al.* 2009)), while our mortality rate of 39% in sCrAg-positive individuals was higher than previously reported rates of 37% (Jarvis *et al.* 2009) and 22% (Liechty *et al.* 2007). Our intervention only reduced mortality in those who were sCrAg positive, which in our sample was 59 individuals. At best, if we reduced the mortality in the sCrAg-positive group from 39% to 24%, we would only reduce the number of deaths from 23 to 14. Thus, because of the higher baseline mortality, in this programmatic evaluation, we were underpowered to detect an impact on overall mortality.

Ethics

This non-research protocol using routinely collected FACES programme data for evaluation purposes was approved by the University of California San Francisco Committee on Human Research, the Kenya Medical Research Institute Ethical Review Committee and the Centers for Disease Control and Prevention.

Results

Of 12 211 individuals enrolling in HIV care and treatment at FACES-supported sites in Kisumu and Rongo districts, Nyanza Province, Kenya, 1601 adults had CD4 + T-cell counts $\leq$ 100 cells/μl. Charts were available for 1553 (97%) (Figure 1) of these. The rate of loss-to-follow-up was 23% [95% confidence interval (CI): 21, 25] (360/1553) (Figure 1). Home visits were attempted for all individuals lost-to-follow-up, and vital status was determined for all but 8% [95%CI: 6, 9] (118/1553). Among the 118 individuals with unknown vital status, 86% [95%CI: 79, 92] (101/118) had given false directions to their home.

Overall, there were 1224 person-years of follow-up time; 691 person-years in the control group and 532 person-years in the intervention group. The median follow-up period in the control was 331 days [range: 1–981] and 237.5 days [range: 1–736] in the intervention group. Overall mortality during the follow-up period in both groups was 25%, and mortality within the first 3 months after enrolment into HIV care and treatment was 16% in the control group and 17% in the intervention group. In the control group, median survival time among individuals who died was 52 days [95%CI: 44, 70] and in the intervention group, 57 days [95%CI: 45, 65].

Overall uptake of the intervention was 52% [95%CI: 42, 62] (51/98). In the intervention group, 4757 adults were enrolled, of whom, 92% [95%CI: 91, 92] (4354/4757) had a CD4 T-cell count performed. Of the 782 adults with a CD4 T-cell count $\leq$ 100 cells/μl, 66% [95%CI: 62, 69] (514/782) had sCrAg tests performed, of whom, 11% [95%CI: 9, 15] (59/514) were positive, defined as early cryptococcal infection. Of the individuals with early cryptococcal infection, 86% (51/59) [95%CI: 75, 94] received fluconazole.

The intervention and control groups were not significantly different with regard to mortality rates, age, gender, BMI, CD4 + T-cell count, proportion started on ART during the follow-up period or the time between enrolment and initiation of ART (Table 1). Individuals in the intervention group were significantly more likely to have sCrAg test checked ($P < 0.001$) and have lower rates of sCrAg-positive tests ($P < 0.001$) (Table 1). Importantly, the median time between enrolment and having sCrAg tested was less than one week for the intervention group and was 12 weeks for the control group ($P < 0.001$). These findings reflect the change in clinical

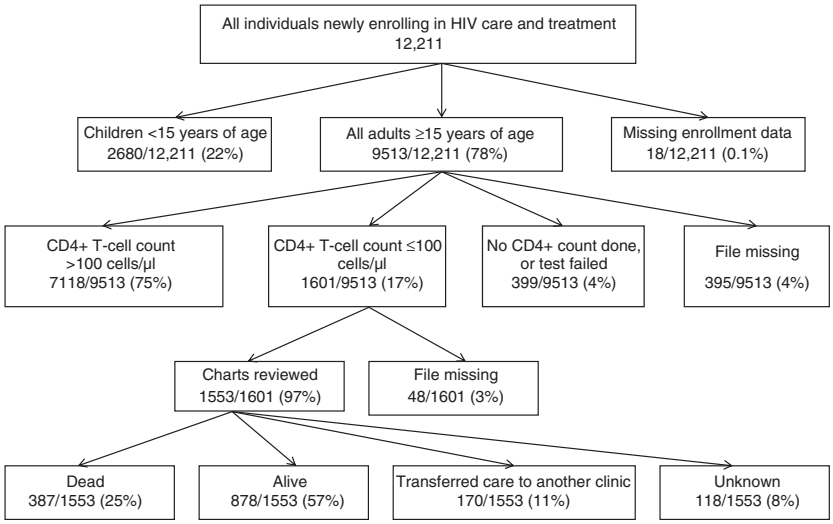

Figure 1 Flow chart describing eligibility and outcomes of HIV-infected individuals newly enrolling in care and treatment at Family AIDS Care and Education Services in Kenya in 2009–2010.

Table 1 Comparison of key baseline clinical and demographic variables between the control and intervention groups in an evaluation of a screening and treatment intervention for early cryptococcal infection in HIV-infected individuals in Kenya in 2009–2010

	Intervention n = 782	Control n = 771	P
Overall mortality	25% (196) [22, 28]	25% (191) [22, 28]	0.91
Three-month mortality	17% (131) [14, 19]	16% (122) [13, 18]	0.63
Age (years)	35 [29, 41]	34 [29, 42]	0.96
Female	55% (414) [49, 56]	55% (424) [51, 59]	0.44
Body mass index (kg/m^2)*	18.8 [17.0, 20.9]	18.6 [16.8, 20.7]	0.43
CD4 + T-cell count (cells/μl)	41 [18, 70]	45 [18, 72]	0.35
Started on ART during follow-up period	77% (601) [74, 80]	76% (586) [73, 79]	0.72
Time between enrolment and initiation of ART (weeks)†	4 [2,7]	4 [2,6]	0.10
Received antituberculous medication	23% (183) [20, 26]	27% (209) [24, 30]	0.10
Hospitalised for cryptococcal infection	0.3% (2) [−0.1, 0.6]	1% (8) [0.3, 1.8]	0.06
sCrAg testing			
Tested for sCrAg	66% (514) [62, 69]	15% (114) [12, 17]	<0.001
Positive sCrAg test (titre $\geq$ 1:2)‡	11% (59) [9, 14]	28% (32) [20, 36]	<0.001
Time between enrolment and sCrAg test (weeks)**	0 [0, 2]	12 [2, 25]	<0.001

ART, antiretroviral therapy; sCrAg, serum cryptococcal antigen.
Data are% (n) [95% confidence interval] of patients or median [interquartile range] unless otherwise indicated. Two-sided Fisher's exact test was used for categorical variables, and Wilcoxon rank-sum tests were used to compare continuous variables.
*n = 1499, control n = 741, intervention n = 758.
†n = 1133, control = 557, intervention = 576.
‡n = 628, control = 114, intervention = 514.
**n = 627, control = 113, intervention = 514.

practice from ordering sCrAg tests only for individuals with suspected meningitis to a routine screening test.

Kaplan–Meier curves were generated for the intervention and control groups (Figure 2). There were no significant differences between the intervention and control groups with regard to overall survival [HR: 1.1; 95%CI: 0.9, 1.3] or survival at three months [HR: 1.0; 95%CI: 0.8, 1.3]. Sensitivity analyses were conducted to account for potential early implementation of the intervention by excluding individuals in the control group who were tested for sCrAg but did not have documentation of signs or symptoms of meningitis. In

addition, sensitivity analyses were performed to account for incomplete uptake of the intervention by excluding individuals in the intervention group who were not tested for sCrAg and individuals in the intervention group who were sCrAg positive but did not have documentation of fluconazole therapy. Even after exclusion of these groups, no significant differences in survival were demonstrated between the intervention and control groups (Table 2).

Within the intervention group, individuals with positive sCrAg had significantly higher overall mortality and mortality at three months (Table 3). There were few significant differences in key demographic or clinical characteristics between individuals with positive sCrAg, negative sCrAg or those who were not tested. However, individuals with positive sCrAg had significantly lower CD4 + T-cell counts, significantly higher rates of hospitalisation for cryptococcal infection and were more likely to be treated with antituberculosis medications.

Kaplan–Meier curves were generated for the 508 individuals in the intervention group who underwent sCrAg tests (Figure 3). In the intervention group, individuals with early cryptococcal infection had significantly lower overall survival [HR: 1.8 (95%CI: 1.2, 2.9); $P = 0.009$] and survival at three months [HR: 1.8 (95%CI: 1.0, 3.0); $P = 0.03$]. Similar results were obtained if individuals who had early cryptococcal infection but did not have documentation of fluconazole therapy were excluded for overall survival [HR: 1.8 (95%CI: 1.1, 2.9); $P = 0.01$] and survival at three months [HR: 1.8 (95%CI: 0.9, 3.1); $P = 0.05$].

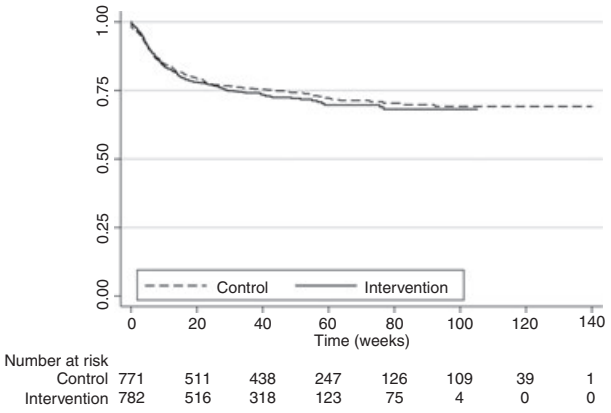

Figure 2 Kaplan–Meier survival curves for overall survival in the control and intervention groups in an evaluation of a screening and treatment intervention for early cryptococcal infection in Kenya in 2009–2010.

Discussion

An intervention to screen HIV-infected individuals with CD4 + T-cell count $\leq$ 100 cells/μl for early cryptococcal infection and treat with high-dose fluconazole did not demonstrate significant differences in overall or three-month survival. Potential explanations for why this intervention was not effective are limited uptake of the intervention, inclusion of individuals with minimal signs or symptoms of meningitis, inadequate power or poor efficacy of high-dose oral fluconazole. Sensitivity analyses to account for uptake of the intervention or possible early implementation of the intervention in the control group did not alter the results. However, our study was not powered to detect differences in overall survival in a programmatic setting. The mortality rates in our control population were substantially higher that those observed in prior studies; thus, a post hoc power calculation suggests that we are underpowered to detect a reduction in

Table 2 Comparison of overall and three-month survival between the control and intervention groups in an evaluation of a screening and treatment intervention for early cryptococcal infection in HIV-infected individuals in Kenya in 2009–2010

	Overall survival hazard ratio [95% CI]	Three-month survival hazard ratio [95% CI]
Overall sample ($n = 1553$)	1.1 [0.9, 1.3]	1.0 [0.8, 1.3]
Sensitivity analysis excluding:		
Poor uptake		
Individuals in the intervention group who were not tested for sCrAg ($n = 1285$)	1.1 [0.8, 1.3]	1.0 [0.8, 1.4]
AND Individuals in the intervention group who were sCrAg positive but did not have documentation of fluconazole therapy ($n = 1277$)	1.0 [0.8, 1.3]	1.0 [0.8, 1.3]
Early implementation		
AND Individuals in the control group who were tested for sCrAg but did not have documentation of signs or symptoms of meningitis ($n = 1181$)	0.99 [0.8, 1.2]	0.99 [0.7, 1.3]

sCrAg, serum cryptococcal antigen.

Table 3 Comparison of outcomes, clinical and demographic variables among individuals who had sCrAg testing performed in the intervention group in an evaluation of a screening and treatment intervention for early cryptococcal infection in HIV-infected individuals in Kenya in 2009–2010

	sCrAg positive (titre $\geq$ 1:2) n = 59	sCrAg negative n = 455	P
Overall mortality	39% (23) [26, 52]	24% (107) [20, 27]	0.02
Mortality within 3 months of enrolment	27% (16) [16, 39]	16% (71) [12, 19]	0.04
Age (years)	36 [29, 42]	35 [28, 42]	0.64
Female	54% (32) [41, 67]	50% (228) [46, 55]	0.58
Body mass index (kg/m^2)*	18.9 [16.3, 19.9]	18.7 [16.9, 20.8]	0.22
CD4 + T-cell count (cells/μl)	23 [9, 56]	41 [19, 70]	0.002
Started on ART during follow-up period	68% (40) [56, 80]	78% (355) [74, 82]	0.06
Time between enrolment and initiation of ART (weeks)†	4.5 [2.5, 8]	4 [2, 7]	0.29
On anti-TB medication during follow-up period	37% (22) [25, 50]	24% (109) [20, 28]	0.04
Hospitalised for cryptococcal infection	4% (2) [−1, 8]	0% (0)	0.01

ART, antiretroviral therapy; ND, not done; sCrAg, serum cryptococcal antigen; TB, tuberculosis.
Data are% (n) [95% confidence interval] of patients or median [interquartile range] unless otherwise indicated. Two-sided Fisher's exact test was used for categorical variables, and Wilcoxon rank-sum tests were used to compare continuous variables.
*sCrAg + (n = 57), sCrAg − (n = 442).
†sCrAg + (n = 40), sCrAg − (n = 34).

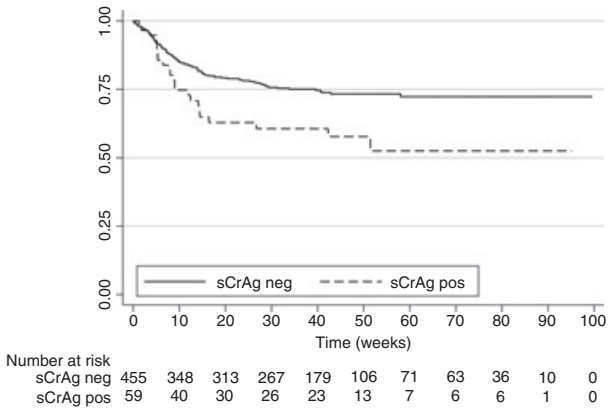

Figure 3 Kaplan–Meier survival curves for overall survival in serum cryptococcal antigen (sCrAg)-positive and (sCrAg)-negative individuals identified as part of a screening and treatment intervention for early cryptococcal infection in Kenya in 2009–2010.

the overall risk of mortality due to the effect of this intervention.

Our study demonstrated significantly lower survival among individuals with early cryptococcal infection than those without early cryptococcal infection, even when excluding those who did not receive high-dose fluconazole. However, the magnitude of the survival difference is substantially below that reported in prior research of individuals with *untreated* asymptomatic cryptococcal infection. A study from Uganda noted a relative risk of death of 6.6 [95%CI: 1.86, 23.61] (Liechty *et al.* 2007) and a study from South Africa noted a hazard ratio of 4.75 [95% CI: 2.6, 8.8] and an adjusted hazard ratio of 3.2 [95% CI: 1.5. 6.6] for individuals with asymptomatic cryptococcal infection (Jarvis *et al.* 2009). Lower survival among sCrAg-positive individuals may have been due to significantly lower CD4 + T-cell counts or possibly due to significantly higher rates of concurrent tuberculous infection. Nonetheless, because we included individuals with minimal signs and symptoms of cryptococcal meningitis, we would expect larger hazard ratios than those reported in studies of individuals with asymptomatic cryptococcal infection if fluconazole treatment was not effective. Thus, our findings suggest that treatment with high-dose fluconazole may have some benefit. Further study of high-dose fluconazole in a controlled research setting is essential to determine its efficacy.

Furthermore, sCrAg-positive individuals had higher rates of hospitalisation for cryptococcal infection. However, it is difficult to know how to interpret this because of the way data were acquired. As part of the intervention, clinicians caring for sCrAg-positive individuals received a special form with treatment information as well as a request for information such as whether the individual was referred for hospitalisation and whether the individual had signs and symptoms of meningitis. Individuals who were sCrAg negative did not receive this form, so it is unclear whether the higher hospitalisation rate is real or due to improved documentation of hospitalisations for sCrAg-positive individuals.

Nonetheless, mortality rates among HIV-infected individuals with advanced immunosuppression remain

unacceptably high both in our sample of HIV-infected individuals and similar cohorts in sub-Saharan Africa (Lawn *et al.* 2008; The Antiretroviral Therapy in Lower Income Countries (ART-LINC) Collaboration, ART Cohort Collaboration (ART-CC) groups 2006). Thus, interventions to identify HIV-infected individuals earlier in the course of their disease, initiate ART and improve health outcomes among this vulnerable population remain critically important.

There are several key limitations of this programme evaluation. The uptake of 52% likely reduced the effect of the intervention on mortality. Second, because of the study design, our outcome measure was mortality among all individuals with CD4 + T-cell count $\leq$ 100 cells/μl (including individuals with and without early cryptococcal infection). This likely diluted the effects of the intervention, as most of the individuals in the intervention group were sCrAg negative. For ethical reasons, we could not use a control group of individuals with early cryptococcal infection who did not receive fluconazole treatment. Because we used a historical control, there may have been other unmeasured differences between the groups that led to confounding, although analysis of key clinical and demographic characteristics demonstrated that the groups were similar. Also, providing the same treatment to individuals with and without minimal signs or symptoms of meningitis may have diluted the effects of the intervention as we would expect individuals with cryptococcal meningitis to have poorer outcomes from monotherapy with fluconazole than those with positive sCrAg but no cryptococcal meningitis (Micol *et al.* 2007). However, determining whether individuals who had minimal signs and symptoms had cryptococcal meningitis was not possible for this analysis.

Because this was a programme evaluation using available clinical records, some information was missing due to missing enrolment information [0.1% (18/12, 211)] or missing files at clinic sites [4% (395/9513)]. Finally, documentation of fluconazole treatment was poor. We had documentation of at least some fluconazole treatment in 86% of individuals with early cryptococcal infection; however, clear documentation of fluconazole dose, duration and adherence was not available.

A programme evaluation of a screening and treatment intervention for early cryptococcal infection does not demonstrate significant improvement in overall survival among HIV-infected individuals with advanced immunosuppression as compared to a historical control group. Potential explanations include inadequate power, low uptake of the intervention, inclusion of individuals regardless of mild signs or symptoms of meningitis or poor efficacy of high-dose oral fluconazole for the treatment of early cryptococcal infection. However, based on this evaluation, it cannot be concluded that systematic pre-emptive fluconazole therapy for asymptomatic and minimally symptomatic patients does not reduce cryptococcal-specific mortality or overall mortality in an adequately powered study. Furthermore, the lower hazards of death observed in this evaluation among individuals with early cryptococcal infection as compared to studies of untreated individuals suggest that there is some benefit to this intervention. Future controlled studies in research settings are critical to identify efficacious treatments for early cryptococcal infection. Identifying ways to improve uptake of similar interventions and improve access to inpatient treatment for individuals with signs and symptoms of meningitis is crucial to improve outcomes for this high-risk population in decentralised HIV care and treatment programmes.

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References

Bicanic T, Muzoora C, Brouwer AE *et al.* (2009) Independent Association between Rate of Clearance of Infection and Clinical Outcome of HIV-Associated Cryptococcal Meningitis:

analysis of a Combined Cohort of 262 Patients. *Clinical infectious diseases* **49**, 702–9.

Chariyalertsak S, Sirisanthana T, Saengwonloey O & Nelson KE (2001) Clinical presentation and risk behaviors of patients with acquired immunodeficiency syndrome in Thailand, 1994–1998: regional variation and temporal trends. *Clinical Infectious Diseases* **32**, 955–962.

Collett G & Parrish A (2007) Fluconazole donation and outcomes assessment in cryptococcal meningitis. *South African Medical Journal* **97**, 175–176.

French N, Gray K, Watera C *et al.* (2002) Cryptococcal infection in a cohort of HIV-1-infected Ugandan adults. *AIDS* **16**, 1031–1038.

Jackson A & van der Horst C (2012) New insights in the prevention, diagnosis and treatment of cryptococcal meningitis. *Current HIV/AIDS reports* **9**, 267–277.

Jarvis J, Lawn S, Vogt M, Bangani N, Wood R & Harrison T (2009) Screening for cryptococcal antigenemia in patients accessing an antiretroviral treatment program in South Africa. *Clinical Infectious Diseases* **48**, 856–862.

Jarvis J, Percival A, Bauman S *et al.* (2011) Evaluation of a novel point-of-care cryptococcal antigen test on serum, plasma, and urine from patients with HIV-associated cryptococcal meningitis. *Clinical Infectious Diseases* **53**, 1019–1023.

Kendi C, Penner J, Koech J *et al.* (2012) Predictors of outcome in routine care for Cryptococcal meningitis in Western Kenya: lessons for HIV outpatient care in resource-limited settings. *Postgraduate Medical Journal* 1–5.

Lawn S, Harries A, Anglaret X, Myer L & Wood R (2008) Early mortality among adults accessing antiretroviral treatment programmes in sub-Saharan Africa. *AIDS* **22**, 1897–1908.

Liechty CA, Solberg P, Were W *et al.* (2007) Asymptomatic serum cryptococcal antigenemia and early mortality during antiretroviral therapy in rural Uganda. *Tropical medicine & international health* **12**, 929–935.

Lindsley M, Mekha N, Baggett H *et al.* (2011) Evaluation of a newly developed lateral flow immunoassay for the diagnosis of cryptococcosis. *Clinical Infectious Diseases* **53**, 321–325.

Longley N, Muzoora C, Taseera K *et al.* (2008) Dose response effect of high-dose fluconazole for HIV-associated cryptococcal meningitis in southwestern Uganda. *Clinical Infectious Diseases* **47**, 1556–1561.

McCarthy KM, Morgan J, Wannemuehler KA *et al.* (2006) Population-based surveillance for cryptococcosis in an antiretroviral-naive South African province with a high HIV seroprevalence. *AIDS* **20**, 2199–2206.

Menichetti F, Fiorio M, Tosti A *et al.* (1996) High-dose fluconazole therapy for cryptococcal meningitis in patients with AIDS. *Clinical infectious diseases* **22**, 838–840.

Meya DB, Manabe YC, Castelnuovo B *et al.* (2010) 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. *Clinical Infectious Diseases* **51**, 448–455.

Micol R, Lortholary O, Sar B *et al.* (2007) Prevalence, determinants of positivity, and clinical utility of cryptococcal antigenemia in Cambodian HIV-infected patients. *Journal of Acquired Immune Deficiency Syndromes* **45**, 555–559.

Mwaba P, Mwansa J, Chintu C *et al.* (2001) Clinical presentation, natural history, and cumulative death rates of 230 adults with primary cryptococcal meningitis in Zambian AIDS patients treated under local conditions. *Postgraduate Medical Journal* **77**, 769–773.

Nelson MR, Bower M, Smith D, Reed C, Shanson D & Gazzard B (1990) The value of serum cryptococcal antigen in the diagnosis of cryptococcal infection in patients infected with the human immunodeficiency virus. *Journal of Infection* **21**, 175–181.

Park B, Wannemuehler K, Marston B, Govender N, Pappas P & Chiller T (2009) Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. *AIDS* **23**, 525.

Pongsai P, Atamasirikul K & Sungkanuparph S (2010) The role of serum cryptococcal antigen screening for the early diagnosis of cryptococcosis in HIV-infected patients with different ranges of CD4 cell counts. *Journal of Infection.* **60**, 474–477.

Tansuphasawadikul S, Amornkul PN, Tanchanpong C *et al.* (1999) Clinical presentation of hospitalized adult patients with HIV infection and AIDS in Bangkok, Thailand. *Journal of acquired immune deficiency syndromes* **21**, 326–332.

The Antiretroviral Therapy in Lower Income Countries (ART-LINC) Collaboration, ART Cohort Collaboration (ART-CC) group (2006) Mortality of HIV-1-infected patients in the first year of antiretroviral therapy: comparison between low-income and high-income countries. *Lancet* **367**, 817–824.

Wajanga B, Kalluvya S, Downs J, Johnson W, Fitzgerald D & Peck R (2011) 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* **14**, 48.

World Health Organization (2011) Rapid advice: diagnosis, prevention and management of cryptococcal disease in HIV-infected adults, adolescents and children. World Health Organization, Geneva, Switzerland.

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