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Outpatient Cryptococcal Antigen Screening Is Associated With Favorable Baseline Characteristics and Improved Survival in Persons With Cryptococcal Meningitis in Uganda

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Background. It is unknown whether persons with symptomatic cryptococcal meningitis detected during routine blood cryptococcal antigen (CrAg) screening have better survival than persons presenting with overt meningitis.

Methods. We prospectively enrolled Ugandans with HIV and cryptococcal meningitis from December 2018 to December 2021. Participants were treated with amphotericin-based combination therapy. We compared outcomes between persons who were CrAg screened then referred to hospital with those presenting directly to the hospital with symptomatic meningitis.

Results. Among 489 participants with cryptococcal meningitis, 40% (194/489) received blood CrAg screening and were referred to hospital (median time to referral 2 days; interquartile range [IQR], 1–6). CrAg-screened persons referred to hospital had lower 14-day mortality than non–CrAg-screened persons who presented directly to hospital with symptomatic meningitis (12% vs 21%; hazard ratio, .51; 95% confidence interval, .32–.83; $P = .006$). Fewer CrAg-screened participants had altered mental status versus non–CrAg-screened participants (29% vs 41%; $P = .03$). CrAg-screened persons had lower quantitative cerebrospinal fluid (CSF) culture burden (median [IQR], 4570 [11–100 000] vs 26 900 [182–324 000] CFU/mL; $P = .01$) and lower CSF opening pressures (median [IQR], 190 [120–270] vs 225 [140–340] mmH$_2$O; $P = .004$) compared with non–CrAg-screened persons.

Conclusions. Survival from cryptococcal meningitis was higher in persons with prior CrAg screening than those without CrAg screening. Altered mental status was the most potent predictor for mortality in a multivariate model. We suggest that CrAg screening detects cryptococcal meningitis at an earlier stage, as evidenced by a favorable baseline risk profile and notably fewer persons with altered mental status.

Keywords. cryptococcal meningitis; cryptococcal antigenemia; advanced HIV disease; AIDS.

Cryptococcal meningitis is the most common cause of human immunodeficiency virus (HIV)–associated meningitis and remains a leading cause of death in persons with AIDS in sub-Saharan Africa [1, 2]. Subclinical infection precedes symptomatic meningitis and can be diagnosed by detecting circulating cryptococcal antigen (CrAg) in the blood [3]. In East Africa, approximately 8% of all persons with HIV with a CD4 count of 100 cells/μL or less have a positive blood CrAg test [4, 5]. An estimated 75% of persons

starting antiretroviral therapy (ART) who screen CrAg positive but who do not receive preemptive antifungal treatment subsequently die within 2 years [5]. Therefore, the World Health Organization recommends CrAg screening for patients who present with advanced HIV (CD4 <100 cells/μL) followed by preemptive fluconazole treatment for those found to be CrAg positive [6]. South Africa implemented a reflexive CrAg screening program in 2016, and subsequent studies have demonstrated both improved testing coverage (99%) [7] and a cost-effective advantage [8] as compared with provider-initiated screenings. Despite these recommendations, wider implementation of CrAg screening remains limited in sub-Saharan Africa due to logistical challenges, including limited CD4+ T-cell testing, stock-outs of CrAg assays and fluconazole, and timely patient follow-up and retention in care [9–11].

Even with an effective CrAg screening program, 20% to 30% of screened persons will develop fulminant cryptococcal

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meningitis [12]. While preemptive fluconazole therapy improves survival in blood CrAg-positive persons (particularly those with CrAg titers ≥1:160), mortality still routinely exceeds 20% [4, 13, 14]. In Ethiopia, mortality among 817 persons with HIV with asymptomatic cryptococcal antigenemia treated with fluconazole remained at 24% [13]. Similarly, in Uganda, among 94 asymptomatic and symptomatic CrAg-positive persons treated with preemptive fluconazole therapy, respectively, 27% of people were dead or lost to follow-up at 6 months [15]. In contrast, mortality from acute cryptococcal meningitis in the hospital setting (patients presenting directly to the hospital with frank meningitis) ranges between 30% and 50% in study settings [16–18]. It is unknown whether persons with cryptococcal meningitis detected during routine CrAg screening in the outpatient setting have better survival outcomes than those presenting directly to the hospital with symptomatic meningitis.

We analyzed data from a 2018–2021 prospective cohort of 489 persons with HIV and with cryptococcal meningitis in Uganda. This cohort was enrolled following implementation of a national CrAg screening program whereby persons with HIV with CD4 counts of less than 100 cells/μL or with virologic failure (HIV RNA >1000 copies/mL) were reflexively screened for blood CrAg. We sought to determine if patients with cryptococcal meningitis who were CrAg screened in the outpatient setting and referred to hospital had better clinical outcomes than persons not screened, instead presenting to a hospital directly due to symptomatic meningitis. Altered mental status, cerebrospinal fluid (CSF) with low white blood cell (WBC) count, increased intracranial pressure, and high fungal burden are associated with more advanced disease and unfavorable outcomes in cryptococcal meningitis [16, 19–21]. We hypothesized that persons who were identified by CrAg screening and thereafter referred to a hospital would present earlier in the course of cryptococcal disease, have favorable baseline characteristics, and have improved survival. We also evaluated whether recent ART initiation increased risk of mortality in CrAg-screened persons with cryptococcal meningitis given published data suggesting poorer outcomes in persons with unmasking cryptococcal meningitis [22].

METHODS

We prospectively enrolled persons with HIV-associated cryptococcal meningitis into an observational cohort from December 2018 to December 2021 in Kampala and Mbarara, Uganda. Participants were screened for symptoms of meningitis by our research team at Kiruddu National Referral Hospital or Mbarara Regional Referral Hospital after being referred from local health facilities. After informed consent was obtained, a lumbar puncture (LP) was performed on persons with suspected meningitis to confirm the diagnosis of cryptococcal meningitis by CrAg Lateral Flow Assay (LFA; IMMY; Norman, OK, USA). All participants who received LP were symptomatic. Additional diagnostics were performed as previously described [23]. Participants were followed until hospital discharge (typically, 14 days) or death. All participants received standard-of-care treatment with intravenous amphotericin B (0.7–1.0 mg/kg per day) and fluconazole 1200 mg per day for 14 days, followed by consolidation therapy with fluconazole 800 mg per day for an additional 8 weeks before transitioning to maintenance therapy with fluconazole 200 mg per day. A subset of participants (n = 314) were instead subsequently enrolled into the AMBITION-cm trial where they were evenly randomized to receiving single dose (10 mg/kg) liposomal amphotericin (AmBisome; Gilead Sciences, Inc; Foster City, CA, USA) with a 14-day backbone of flucytosine and fluconazole versus 7 days of amphotericin deoxycholate (1 mg/kg/day) and flucytosine, followed by 7 days of fluconazole, during induction therapy [24].

Patients with meningitis typically enter care by (1) presenting to a health facility with frank meningitis symptoms or (2) review of meningitis symptoms at time of a positive blood CrAg test, prompting further evaluation by LP. All participants diagnosed with first-episode cryptococcal meningitis and enrolled in our longitudinal meningitis study were included in this analysis. Both ART-naive and ART-experienced persons were included. Participants with prior cryptococcal meningitis or coinfection with tuberculous meningitis were excluded. Baseline data were collected on all participants and included demographics, cryptococcal history, ART status, and laboratory results. Additionally, we collected information about prior CrAg screening by patient history at time of enrollment. Dates of CrAg screening blood draw and results were presumed the same day for all participants as CrAg LFA is used in our referring clinics (IMMY).

We compared survival between the participants diagnosed with meningitis who had been referred to the hospital due to positive blood CrAg testing and those presenting with overt meningitis directly to the hospital—which we will refer to as CrAg-screened and non–CrAg-screened, respectively. We defined our primary outcome as all-cause mortality at 14 days. We additionally analyzed mortality at 10 weeks. We further categorized persons who had tested blood CrAg positive into 3 ART initiation categories of ART naive, ART 30 days or less, or ART more than 30 days, as prior data have demonstrated worse outcomes in persons with cryptococcal meningitis who had recently started ART [22].

Baseline characteristics and symptoms were compared by CrAg screening status using chi-square for proportions and Wilcoxon rank-sum tests for continuous variables. Survival was calculated from date of meningitis diagnosis to date of death or through 14 days; a secondary analysis included survival through 10 weeks. A Kaplan-Meier time-to-event model was

used to compare mortality between groups with log-rank testing. A univariable proportional hazards model was used to estimate the hazard ratio of those CrAg-screened and non–Crag-screened participants. Multivariable proportional hazards models were used to adjust for contributing risk factors of early mortality—including baseline Glasgow coma scale (GCS) score, CSF WBC count, CSF opening pressure, CSF fungal burden, seizures, ART use, randomization to AMBITION-cm intervention, age, and sex. All analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC). A P value of .05 or less was considered statistically significant.

The study received institutional review board approval from both Uganda and University of Minnesota regulatory authorities. Written informed consent was obtained from all participants; those without capacity at the time of screening had surrogate consent obtained.

RESULTS

We enrolled 489 Ugandan adults with HIV-associated cryptococcal meningitis over the study period. The median age was 35 years (interquartile range [IQR], 29–42 years), and 57% were men. Overall, 194 (40%) of persons with meningitis had received previous blood CrAg screening compared with 295 (60%) who presented to the hospital without any prior CrAg screening. The median time from prior outpatient CrAg screening to meningitis diagnosis was 2 days (IQR, 1–6 days). Important baseline characteristics differed between the 2 groups (Table 1). More CrAg-screened participants were already receiving ART at baseline (47%; median duration, 71 days; IQR, 20–448 days) as compared with non–CrAg-screened participants (37%; median duration, 88 days; IQR, 17–981 days; $P = .03$). More CrAg-screened participants had prior fluconazole use (49%; median duration, 2 days; IQR, 2–8 days) as compared with non–CrAg-screened participants (11%; median duration, 2 days; IQR, 1–5 days; $P < .001$). Of the 489 total participants, 314 participants were subsequently enrolled into the AMBITION-cm trial, including 109 (56%) from the CrAg-screened group and 205 (70%) in the non–CrAg-screened group ($P = .003$). Among those enrolled in AMBITION-cm, 29% (56/194) of CrAg-screened participants were randomized to receive single-dose AmBisome while 34% (101/295) of non–CrAg-screened participants were randomized to the same intervention ($P = .21$). Participants who received CrAg screening were less likely to present with altered mental status compared with non–CrAg-screened participants (29% vs 41% with GCS score <15, respectively; $P = .02$). Additionally, fewer CrAg-screened participants experienced baseline seizures (11%) than non–CrAg-screened participants (23%) ($P = .004$). Cerebrospinal fluid fungal burden was lower in CrAg-screened persons compared with non–CrAg-screened persons (4570 colony-forming units [CFU]/mL

Table 1. Baseline Characteristics of Participants With Cryptococcal Meningitis by CrAg Screening Status

Variable	CrAg Screened	Non–CrAg Screened	P
No. per group	194 (40%)	295 (60%)	–
Age, y	36 [30, 42]	35 [29, 42]	.57
Men	109 (56%)	169 (57%)	.81
Receiving ART	91 (47%)	107 (37%)	.03
Glasgow Coma Scale score = 15	137 (71%)	171 (59%)	.02
Baseline seizures	21 (11%)	67 (23%)	.004
CD4+ cell count/μL[a]	27 [10, 57]	23 [9, 53]	.37
Hemoglobin, g/dL	10.8 [9.0, 12.6]	11.5 [10.0, 13.2]	.01
Prior fluconazole	95 (49%)	32 (11%)	<.001
Prior fluconazole duration, days	2 [2, 8]	2 [1, 5]	.36
CSF white cells/μL	<5 [<5, 55]	<5 [<5, 45]	.98
CSF white cells <5 cells/μL	110 (60%)	162 (58%)	.55
CSF protein, mg/dL	86 [51, 121]	74 [35, 115]	.01
Opening pressure, mmH$_2$O	190 [120, 270]	225 [140, 340]	.004
Opening pressure >250 mmH$_2$O	52 (28%)	122 (43%)	.001
CSF culture, CFU/mL	4570 [11, 100 000]	26 900[182, 324 000]	.01
Sterile CSF culture	43 (23%)	45 (17%)	.10
Received single-dose AmBisome[b]	56 (29%)	101 (34%)	.21

Data are presented as n (%) or median [IQR]. P values from chi-square or Wilcoxon rank-sum tests.

Abbreviations: ART, antiretroviral therapy; CFU, colony-forming units; CrAg, cryptococcal antigen; CSF, cerebrospinal fluid; IQR, interquartile range.

[a]CD4+ T-cell counts were only available for 353 participants. Total available data by variable are listed in Supplementary Table 2.

[b]liposomal amphotericin B 10mg/kg, Gilead Sciences, Inc.

[IQR, 11–100 000] vs 26 900 CFU/mL [IQR, 182–324 000]; $P = .01$) by quantitative cryptococcal culture. Similarly, CSF opening pressure also differed between the 2 groups, with CrAg-screened participants having significantly lower opening pressures compared with non–CrAg-screened participants (190 mmH$_2$O [IQR, 120–270] vs 225 mmH$_2$O [140–340]; $P = .004$). Table 1 includes a full comparison of baseline and laboratory characteristics between CrAg-screened and non–CrAg-screened groups. Supplementary Table 1 compares the clinical presentations between CrAg-screened and non–CrAg-screened groups, which were generally similar aside from symptoms attributed to altered mental status.

We assessed differences in outcomes between CrAg-screened and non–CrAg-screened groups. Among CrAg-screened persons, 12% (23/194) died at 14 days after meningitis diagnosis compared with 21% (63/295) in the non–CrAg-screened group ($P = .005$) (Figure 1). Two-week mortality by unadjusted proportional hazards modeling demonstrated that persons who were CrAg screened were more likely to survive than persons who were not CrAg screened (hazard ratio = .51; 95% confidence interval [CI], .32–.83; $P = .006$). At 10 weeks, mortality by unadjusted proportional hazards modeling demonstrated that persons who were CrAg
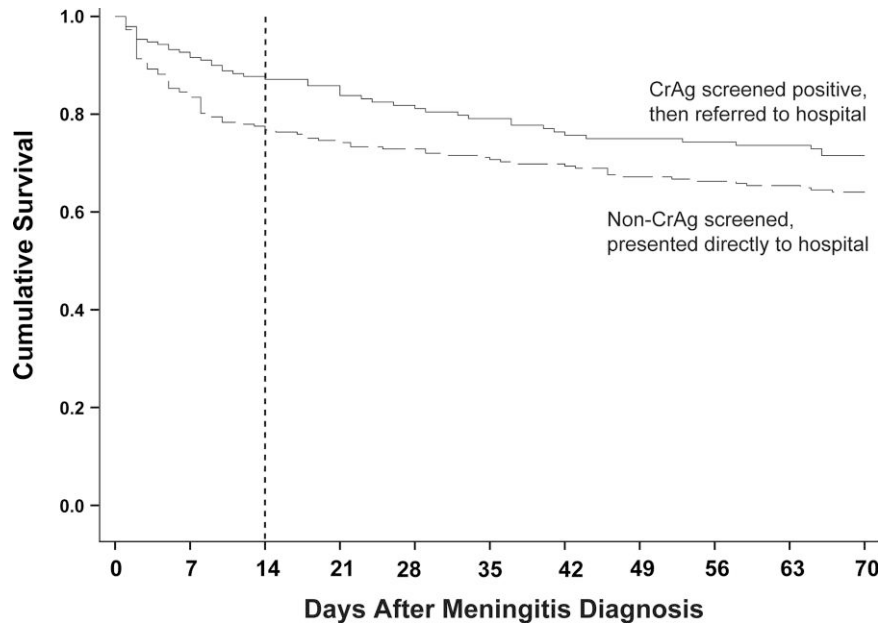
Figure 1. Survival among persons with HIV-associated cryptococcal meningitis, by CrAg screening status in a Kaplan-Meier model. Mortality by subgroup was as follows: at 14 days (dashed line), mortality in those CrAg-screened participants in the outpatient setting and referred to the hospital was 12% (23/194) versus those non–CrAg-screened participants presenting with overt meningitis directly to the hospital at 21% (63/295) (log-rank $P = .005$). At 10 weeks, mortality in those CrAg-screened participants in the outpatient setting and referred to the hospital was 24% (47/194) versus non–CrAg-screened participants presenting with overt meningitis directly to the hospital at 32% (94/295) (log-rank $P = .04$). Abbreviations: CrAg, cryptococcal antigen; HIV, human immunodeficiency virus.

screened were more likely to survive than non–CrAg-screened persons (hazard ratio = .70; 95% CI, .49–.99; $P = .04$).

In assessing risk factors accounting for the improved survival observed in CrAg-screened participants, we conducted a multivariate model adjusting for previously identified mediators impacting mortality in cryptococcal meningitis, including baseline GCS score, CSF WBC, CSF opening pressure, CSF fungal burden, seizures, ART use, randomization to

AMBITION-cm intervention, age, and sex. In this multivariate model, the effect of CrAg screening on 2-week mortality was attenuated (hazard ratio = .63; 95% CI, .37–1.08; $P = .09$), yet not fully accounted for (ie, hazard ratio = 1.0) (Table 2). Results were similar when analyzing mortality at 10 weeks (Supplementary Table 2). A GCS score of less than 15 at baseline was consistently identified as the variable carrying the largest mortality hazard in our multivariate models, and fewer

Table 2. Proportional Hazards Model for 14-Day Mortality of Participants With Cryptococcal Meningitis By CrAg Screening Status

	Univariate			Multivariate		
Variable	Hazard Ratio	95% CI	P	Hazard Ratio	95% CI	P
CrAg screened	.51	.32–.83	.006	.63	.37–1.08	.09
Glasgow Coma Scale score <15	4.87	3.05–7.76	<.001	5.22	3.03–8.98	<.001
CSF WBC count <5 cells/μL	1.83	1.13–2.96	.01	2.15	1.23–3.77	.007
CSF culture, per $\log_{10}$ CFU/mL	1.18	1.05–1.33	<.001	1.11	.98–1.26	.11
CSF opening pressure >250 mmH$_2$O	1.60	1.05–2.45	.03	1.25	.76–2.05	.38
Baseline seizure	1.82	1.24–2.67	.002	1.13	.70–1.84	.61
Receiving ART at baseline	.93	.60–1.43	.73	1.53	.94–2.49	.09
Randomized to AmBisome[a]	1.00	.64–1.56	.99	1.23	.75–2.01	.42
Age, per year	1.01	.99–1.03	.36	1.01	.99–1.03	.40
Male	.89	.58–1.37	.60	.71	.43–1.16	.17

A multivariate proportional hazards model for 14-day mortality was used to adjust for contributing meningitis risk factors. CrAg screening was no longer significantly associated with mortality after multivariate adjustment, while Glasgow Coma Scale score <15 and CSF WBC count <5 cells/μL remained significantly associated.

Abbreviations: ART, antiretroviral therapy; CFU, colony forming units; CI, confidence interval; CrAg, cryptococcal antigen; CSF, cerebrospinal fluid; WBC, white blood cell.

[a]liposomal amphotericin B 10mg/kg by Gilead Sciences, Inc.

CrAg-screened participants presented with altered mental status (29%) than non-screened participants (41%).

Given the potential impact of ART initiation on the phenomenon of unmasking cryptococcal meningitis, we compared 3 ART timing cohorts in Table 3. Baseline characteristics were similar between the 3 groups, with the exception of the ART-naive group having lower CD4 cell counts (16 cells/μL; IQR, 8–42 cells/μL) versus ART-less-than-30-days and ART-more-than-30-days groups (39 cells/μL [IQR, 11–56] and 36 cells/μL [IQR, 16–78], respectively; $P = .04$). Prior fluconazole use also differed, with the ART-naive group having fewer participants with prior use (38%) versus the ART-less-than-30-days and ART-more-than-30-days groups (63% and 56% respectively; $P = .02$). Mortality at 2 weeks did not differ across the 3 ART timing groups ($P = .22$).

DISCUSSION

We found that individuals who were CrAg screened prior to hospitalization for cryptococcal meningitis experienced a relative approximately 50% decrease in 14-day mortality compared with non–CrAg-screened participants before adjustment. However, CrAg screening was not independently associated with survival after multivariate adjustment. A GCS score less than 15 (hazard ratio = 5.22; 95% CI, 3.03–8.98) and CSF WBC less than 5 cells/μL (hazard ratio = 2.15; 95% CI, 1.23–3.77) remained strong predictors of harm in our adjusted models. Our models suggest that, while CrAg screening may not directly improve survival in cryptococcal meningitis, it indirectly leads to benefit through the mitigation of mediators on a causal pathway, most notably evidenced by 12% fewer persons with altered mental status, a potent risk factor for mortality. In the context of existing literature, numerous studies have demonstrated that altered mental status, CSF with low WBC count, increased intracranial pressure, and high fungal burden are associated with increased mortality in cryptococcal meningitis [16, 18–21].

Standard practice is for fluconazole to be initiated immediately following a positive CrAg test result, where preemptive fluconazole treatment is associated with increased survival [5]. Our CrAg-screened cohort experienced an average of 2 days between positive CrAg test and confirmed diagnosis of meningitis during which time half were treated with fluconazole. Although suboptimal as monotherapy for cryptococcal meningitis, the additional fluconazole may confer a disease-mitigating effect when compared with no antifungal exposure over the same preceding period. Additionally, although mortality did not differ significantly based on ART status, more CrAg-screened participants were receiving ART at baseline than non–CrAg-screened participants. Together, the greater prevalence of ART and fluconazole exposure in the CrAg-screened group suggests improved health-seeking behavior and retention in care. CrAg-screened participants were more likely to have a GCS score of 15, lower CSF opening pressures, lower CSF fungal burden, and prior fluconazole and ART use—ultimately suggesting that the benefit of CrAg screening may be due to the detection of persons with earlier (ie, less advanced) cryptococcal disease.

Studies of CrAg screening programs have demonstrated all-cause mortality benefit, most notably in a randomized trial in Tanzania and Zambia [25]. In Uganda, a prospective observational cohort found that a dedicated CrAg screening program improved 6-month survival compared with historical controls [15]. Yet, this is the first study to specifically examine if CrAg screening impacts risk factors for mortality in those who still develop meningitis. CrAg screening appears to be beneficial to our population by leading to the treatment of earlier cryptococcal meningitis. Our data add to the growing body of literature supporting CrAg screening programs as a critical component of advanced HIV packages of care, particularly in sub-Saharan Africa. Unfortunately, routine CrAg screening is still suboptimal in many regions of sub-Saharan Africa, where test and reagent stock-outs, delay in test result communication, and general provider awareness remain barriers to consistent implementation. We support continued advocacy for the study and adoption of CrAg screening programs, particularly as a component of a package of care for advanced HIV disease.

Early ART initiation remains an important component in advanced HIV disease, when not clinically contraindicated. Current guidelines recommend initiating ART 2 weeks after starting preemptive therapy for a positive blood CrAg test in the initial absence of clinical meningitis [6]. Rhein et al [22] found that persons with subclinical meningitis at the time of ART initiation are at high risk of death, due to unmasking cryptococcal meningitis. To account for the possible risk of recent ART initiation in our cohort, we stratified CrAg-screened persons by ART timing categories. ART-naive participants had lower CD4 counts and less prior fluconazole exposure, but baseline characteristics were otherwise similar, and in-hospital mortality was not higher in the CrAg-screened participants who recently initiated ART.

One limitation of this study is our focus on acute in-hospital mortality. We did so to maximize the sample size to include those not enrolled in follow-up studies. Some additional mortality occurs post-hospitalization, particularly in the first 18 weeks [14], although we are able to demonstrate the same trends existed in our subset who had 10 weeks of data. Participant survival would be expected to decline over time, possibly disproportionately affecting non–CrAg-screened persons with more advanced disease. Some participants were excluded when performing multivariate analysis, due to missing covariate data, decreasing the sample size from 489 to 428 and thus decreasing our statistical power in our multivariate models. Unfortunately, we are missing baseline CD4 data for

Table 3. Baseline Characteristics and Outcomes of Participants With Cryptococcal Meningitis Who Were CrAg Screened By ART Timing Status

Variable	CrAg Screened (ART Naive)	CrAg Screened (ART <30 Days)	CrAg Screened (ART >30 Days)	P
Characteristics				
No. per group	87	30	77	–
Age, y	37 [30, 43]	36 [30, 40]	35 [30, 40]	.25
Men	50 (58%)	21 (70%)	38 (49%)	.15
CD4+ cell count/μL	16 [8, 42]	39 [11, 56]	36 [16, 78]	.04
Glasgow Coma Scale score = 15	58 (67%)	22 (73%)	57 (74%)	.71
Baseline seizures	9 (10%)	2 (7%)	10 (13%)	.32
Hemoglobin, g/dL	11.3 [9.1, 13.0]	11.2 [8.4, 12.6]	10.3 [8.8, 12.4]	.31
Prior fluconazole	33 (38%)	19 (63%)	43 (56%)	.02
CSF white cells/μL	<5 [<5, 70]	<5 [<5, 30]	<5 [<5, 65]	.51
CSF white cells <5 cells/μL	50 (60%)	20 (69%)	40 (57%)	.55
CSF protein, mg/dL	90 [60, 124]	88 [58, 130]	82 [40, 114]	.51
Opening pressure, mmH$_2$O	190 [120, 280]	230 [130, 270]	180 [130, 240]	.68
Opening pressure >250 mmH$_2$0	28 (34%)	8 (28%)	16 (21%)	.20
CSF culture, CFU/mL	7240 [96, 115 000]	3630 [11, 34 700]	603 [0, 85 100]	.35
Sterile CSF culture	16 (19%)	7 (23%)	20 (27%)	.42
Randomized to AmBisome[a]	28 (32%)	9 (30%)	19 (25%)	.56
Outcomes				
14-Day mortality	14 (16%)	3 (10%)	6 (8%)	.22
Died during first hospitalization	14 (16%)	3 (10%)	12 (16%)	.71

Data are presented as n (%) or median [IQR]. P values from chi-square or Wilcoxon rank-sum tests.

Abbreviations: ART, antiretroviral therapy; CFU, colony-forming units; CrAg, cryptococcal antigen; CSF, cerebrospinal fluid; IQR, interquartile range.

[a]liposomal amphotericin B 10 mg/kg by Gilead Sciences, Inc.

136 participants, which would have resulted in excess case exclusion if included in the multivariate model. The routine measuring of CD4 count is often suboptimal in resource-limited settings, with a recent study showing that only 36% of persons with newly diagnosed HIV received a CD4 test in Uganda [26].

Another potential limitation of this study was heterogeneity in treatment, yet this is also a strength of the study, increasing the generalizability. Participants either enrolled into a cohort providing standard-of-care therapy or into the AMBITION-cm trial, where some were randomized to receive single 10-mg/kg dose AmBisome [24]. The benefit of CrAg screening remained consistent with or without adjustment for induction antifungal treatment received. This is logical as CrAg screening detected persons with earlier stage meningitis that was less severe and easier to treat, leading to better outcomes regardless of the treatment received. Thus, our finding should be generalizable.

Finally, we did not formally evaluate health-seeking behavior across the 2 groups. One could assume that persons who had a CrAg test prior to hospitalization were more likely to have interfaced with the healthcare system and therefore had increased health-seeking behavior compared with those who presented to a hospital without prior CrAg testing, as evidenced by the greater proportion having prior ART and fluconazole exposure at baseline. Increased health-seeking behavior likely leads to earlier detection of disease, supporting our assessment that CrAg screening is associated with more favorable outcomes due to earlier diagnosis and treatment, thereby mitigating causal pathway mediators, such as altered mental status.

In summary, CrAg screening is likely detecting persons with early cryptococcal meningitis, where induction antifungal therapy has greater benefit. In contrast, persons presenting to the hospital with frank meningitis have more advanced disease as evidenced by higher CSF fungal burden and a greater proportion with altered mental status. The high mortality from cryptococcal meningitis in low- and middle-income countries highlights a failure of timely diagnosis, linkage to care, access to treatment, and retention in care. Given these shortcomings, CrAg screening and preemptive treatment is an essential component of a routine package of care for people presenting with AIDS in Uganda to both prevent progression to meningitis but also to mitigate mortality in those who develop meningitis.

Supplementary Data

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

Notes

References

1. 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.
2. Rajasingham R, Smith RM, Park BJ, et al. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. Lancet Infect Dis **2017**; 17:873–81.
3. Rajasingham R, Meya DB, Boulware DR. Integrating cryptococcal antigen screening and pre-emptive treatment into routine HIV care. J Acquir Immune Defic Syndr **2012**; 59:e85–91.
4. Faini D, Kalinjuma AV, Katende A, et al. Laboratory-reflex cryptococcal antigen screening is associated with a survival benefit in Tanzania. J Acquir Immune Defic Syndr **2019**; 80:205–13.
5. 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.
6. World Health Organization. Guidelines for the diagnosis, prevention and management of cryptococcal disease in HIV-infected adults, adolescents and children. **2018**. Available at: www.who.int/publications/i/item/9789241550277. Accessed 26 July 2022.
7. Govender NP, Meintjes G, Mangena P, et al. Southern African HIV Clinicians Society guideline for the prevention, diagnosis and management of cryptococcal disease among HIV-infected persons: 2019 update. South Afr J HIV Med **2019**; 20:1030.
8. 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.
9. Lofgren SM, Nalintya E, Meya DB, Boulware DR, Rajasingham R. A qualitative evaluation of an implementation study for cryptococcal antigen screening and treatment in Uganda. Medicine (Baltimore) **2018**; 97: e11722.
10. Ramachandran A, Manabe Y, Rajasingham R, Shah M. Cost-effectiveness of CRAG-LFA screening for cryptococcal meningitis among people living with HIV in Uganda. BMC Infect Dis **2017**; 17:225.
11. Rajasingham R, Meya DB, Greene GS, et al. Evaluation of a national cryptococcal antigen screening program for HIV-infected patients in Uganda: a cost-effectiveness modeling analysis. PLoS One **2019**; 14:e0210105.
12. Meya DB, Kiragga AN, Nalintya E, et al. Reflexive laboratory-based cryptococcal antigen screening and preemptive fluconazole therapy for cryptococcal antigenemia in HIV-infected individuals with CD4 <100 cells/μL: a stepped-wedge, cluster-randomized trial. J Acquir Immune Defic Syndr **2019**; 80:182–9.
13. Beyene T, Zewde AG, Balcha A, et al. Inadequacy of high-dose fluconazole monotherapy among cerebrospinal fluid cryptococcal antigen (CrAg)-positive human immunodeficiency virus-infected persons in an Ethiopian CrAg screening program. Clin Infect Dis **2017**; 65:2126–9.
14. Butler EK, Boulware DR, Bohjanen PR, Meya DB. Long term 5-year survival of persons with cryptococcal meningitis or asymptomatic subclinical antigenemia in Uganda. PLoS One **2012**; 7:e51291.
15. Nalintya E, Meya DB, Lofgren S, Huppler Hullsiek K, Boulware DR, Rajasingham R. A prospective evaluation of a multisite cryptococcal screening and treatment program in HIV clinics in Uganda. J Acquir Immune Defic Syndr **2018**; 78:231–8.
16. Kambugu A, Meya DB, Rhein J, et al. Outcomes of cryptococcal meningitis in Uganda before and after the availability of highly active antiretroviral therapy. Clin Infect Dis **2008**; 46:1694–701.
17. Rhein J, Huppler Hullsiek K, Tugume L, et al. Adjunctive sertraline for HIV-associated cryptococcal meningitis: a randomised, placebo-controlled, double-blind phase 3 trial. Lancet Infect Dis **2019**; 19:843–51.
18. Bicanic T, Meintjes G, Wood R, et al. Fungal burden, early fungicidal activity, and outcome in cryptococcal meningitis in antiretroviral-naive or antiretroviral-experienced patients treated with amphotericin B or fluconazole. Clin Infect Dis **2007**; 45:76–80.
19. Pitisuttithum P, Tansuphasawadikul S, Simpson AJ, Howe PA, White NJ. A prospective study of AIDS-associated cryptococcal meningitis in Thailand treated with high-dose amphotericin B. J Infect **2001**; 43:226–33.
20. Anekthananon T, Manosuthi W, Chetchotisakd P, et al. Predictors of poor clinical outcome of cryptococcal meningitis in HIV-infected patients. Int J STD AIDS **2011**; 22:665–70.
21. Siddiqui AA, Brouwer AE, Wuthiekanun V, et al. IFN-gamma at the site of infection determines rate of clearance of infection in cryptococcal meningitis. J Immunol **2005**; 174:1746–50.
22. Rhein J, Hullsiek KH, Evans EE, et al. Detrimental outcomes of unmasking cryptococcal meningitis with recent ART initiation. Open Forum Infect Dis **2018**; 5: ofy122.
23. Ellis J, Bangdiwala AS, Cresswell FV, et al. The changing epidemiology of HIV-associated adult meningitis, Uganda 2015-2017. Open Forum Infect Dis **2019**; 6:ofz419.
24. Jarvis JN, Lawrence DS, Meya DB, et al. Single-dose liposomal amphotericin B treatment for cryptococcal meningitis. N Engl J Med **2022**; 386:1109–20.
25. 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.
26. Meya DB, Tugume L, Nabitaka V, et al. Establishing targets for advanced HIV disease: a call to action. South Afr J HIV Med **2021**; 22:1266.