Laboratory Reflex and Clinic-Based Point-of-Care Cryptococcal Antigen Screening for Preventing Meningitis and Mortality Among People Living With HIV

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Introduction: Cryptococcosis remains a leading cause of meningitis and mortality among people living with HIV (PLHIV) worldwide. We sought to evaluate laboratory-based cryptococcal antigen (CrAg) reflex testing and a clinic-based point-of-care (POC) CrAg screening intervention for preventing meningitis and mortality among PLHIV in South Africa.

Methods: We conducted a prospective pre–post intervention study of adults presenting for HIV testing in Umlazi township, South Africa, over a 6-year period (2013–2019). Participants were enrolled during 3 phases of CrAg testing: CrAg testing ordered by a clinician (clinician-directed testing, 2013–2015); routine laboratory-based CrAg reflex testing for blood samples with CD4 ≤100 cells/mm^3 (laboratory reflex testing, 2015–2017); and a clinic-based intervention with POC CD4 testing and POC CrAg testing for PLHIV with CD4 ≤200 cells/mm^3 with continued standard-of-care routine laboratory reflex testing among those with CD4 ≤100 cells/mm^3 (clinic-based testing, 2017–2019). The laboratory and clinical teams performed serum CrAg by enzyme immunoassay and lateral flow assay (Immy Diagnostics, Norman, OK). We followed up participants for up to 14 months to compare associations between baseline CrAg positivity, antiretroviral therapy and fluconazole treatment initiation, and outcomes of cryptococcal meningitis, hospitalization, and mortality.

Results: Three thousand one hundred five (39.4%) of 7877 people screened were HIV-positive, of whom 908 had CD4 ≤200 cells/mm^3 and were included in the analyses. Laboratory reflex and clinic-based testing increased CrAg screening ($P < 0.001$) and diagnosis of CrAg-positive PLHIV ($P = 0.011$). When compared with clinician-directed testing, clinic-based CrAg testing showed an increase in the number of PLHIV diagnosed with cryptococcal meningitis (4.5% vs. 1.5%; $P = 0.059$), initiation of fluconazole preemptive therapy (7.2% vs. 2.5%; $P = 0.010$), and initiation of antiretroviral therapy (96.8% vs. 91.3%; $P = 0.012$). Comparing clinic-based testing with laboratory reflex testing, there was no significant difference in the cumulative incidence of cryptococcal meningitis (4.5% vs. 4.1%; $P = 0.836$) or mortality (8.1% vs. 9.9%; $P = 0.557$).

Conclusions: Laboratory reflex and clinic-based CrAg testing facilitated the diagnosis of HIV-associated cryptococcosis and fluconazole initiation but did not reduce cryptococcal meningitis or mortality. In this nonrandomized cohort, clinical outcomes were similar between laboratory reflex testing and clinic-based POC CrAg testing.

Key Words: HIV/AIDS, Cryptococcus sp., cryptococcal meningitis, screening, point-of-care testing, mortality

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INTRODUCTION

Cryptococcosis is an opportunistic fungal infection that causes approximately 15% of AIDS-related deaths worldwide, most of which occurs in sub-Saharan Africa.[1] In South Africa, cryptococcal infections account for approximately 63% of meningitis cases, partly because of the high prevalence of HIV/AIDS.[2] Cryptococcal antigens (CrAgs) can be detected before the onset of symptomatic cryptococcal meningitis,[3–5] and oral fluconazole significantly reduces the risk of cryptococcal meningitis and mortality.[6–9] For all antiretroviral therapy (ART)-naïve people living with HIV (PLHIV) with CD4 T-cell count <100 cells/mm^3, the WHO recommends CrAg screening[10] and for those with CD4

100–199 cells/mm^3, it recommends consideration for CrAg screening.[11]

Several sub-Saharan African countries have incorporated CrAg screening into guidelines, and some are evaluating laboratory reflex CrAg testing for blood samples with CD4 <100 cells/mm^3 to improve screening coverage.[1] Because survival benefit is related to prompt initiation of fluconazole therapy, minimizing the time to complete CrAg screening and treatment initiation is critical.[12] A rapid lateral flow assay (LFA) was developed to simplify CrAg testing for clinic-based point-of-care (POC) screening and has demonstrated good accuracy on serum and cerebrospinal fluid specimens.[13–16] A possible implementation strategy may be integrating clinic-based POC CrAg screening with fluconazole preemptive therapy when fungal burden is relatively low before the onset of symptomatic meningitis.[7,9,12]

In the Umlazi township of KwaZulu-Natal, South Africa, the prevalence of cryptococcal antigenemia is 1.2% among PLHIV initiating ART.[17] In 2015, routine laboratory-based reflex CrAg testing was implemented for people with CD4 ≤100 cells/mm^3. In this study, we sought to determine whether laboratory reflex CrAg testing improved the diagnosis of CrAg-positive PLHIV and initiation of fluconazole and reduced the incidence of cryptococcal meningitis or mortality. In addition, we sought to determine whether clinic-based POC CD4 and CrAg screening offered additional benefits for diagnosis, treatment, and clinical outcomes in comparison with standard-of-care (SoC) laboratory testing.

METHODS

Study Design and Participants

Following the World Health Organization and South African recommendations for CrAg screening among PLHIV with immunodeficiency, we conducted a prospective pre–post intervention study from September 12, 2013, to February 28th, 2019. During the initial phase of the study (September 12, 2013, to June 4, 2015), the clinical SoC was to conduct CrAg testing when ordered by a clinician, called clinician-directed testing, for PLHIV with CD4 <100 cells/mm^3, according to South African guidelines. During the second study phase (June 5, 2015, to November 8, 2017), the National Health Laboratory Service (NHLS) laboratory implemented routine CrAg reflex testing, whereby blood samples with CD4 <100 cells/mm^3 were reflexively tested for CrAg by an LFA called laboratory reflex testing. During the first 2 study phases, the study was observational, and there was no intervention. During the third study phase (November 9, 2017, to February 28, 2019), the clinical research team performed POC CD4 testing for all study participants, and those with CD4 <200 cells/mm^3 were tested for CrAg by a rapid LFA at the POC during routine clinic visits. The laboratory reflex CrAg testing continued during the third study phase and lasted until the end of the study period.

We recruited persons who presented for voluntary HIV testing at the iThembalabantu Clinic in the Umlazi township of KwaZulu-Natal, South Africa. The clinic provides free clinic-based and community-based HIV care and treatment for more than 15,000 PLHIV. Before conducting HIV testing, we enrolled English-speaking or Zulu-speaking adults aged 18 years or older, who were not pregnant, and who had not taken antifungal therapy in the preceding 3 months. All study participants provided written informed consent and received routine medical care, including ART, CD4 T-cell testing, and CrAg screening and treatment, according to local and national guidelines.[18] Throughout the study, all CrAg-positive participants were referred to a clinician for consideration of fluconazole therapy, lumbar puncture, and/or referral for hospitalization. Lumbar puncture was indicated for patients who were CrAg-positive and had headache for >24 hours, fever, neck stiffness, blurry or double vision, or confusion. The study was approved by the University of Washington's Institutional Review Board (IRB #49563), Partners Human Research Committee (#2013P002513), and the University of KwaZulu-Natal's Medical Research Ethics Committee (Protocol #BF052/13).

Data Collection

At enrollment, research assistants completed a sociodemographic questionnaire, and HIV counselors performed serial rapid HIV testing according to South African guidelines.[18] Among HIV-positive participants, research nurses obtained a medical history and administered a clinical symptom questionnaire. The research team contacted participants by phone and reviewed medical charts to record their treatment course and clinical outcomes. The procedures for recruitment, informed consent, administering a sociodemographic questionnaire, conducting HIV testing, and conducting phlebotomy for CrAg testing were the same across the 3 study periods.

At the end of the study, we reviewed each participant's medical chart and attempted up to 3 phone calls to participants and/or next of kin. We assessed local hospital records at Prince Mshiyeni Memorial Hospital, which is a single designated hospital serving the Umlazi catchment area, and all hospitalized participants had a hospital chart review to determine the cause of hospitalization. For individuals whose vital status could not be ascertained through direct contact or medical records, we searched the South African national death registry. All participants were followed up for up to 14 months after enrollment to assess vital status and other study outcomes.

Clinic-Based CD4 T-Cell and CrAg Testing

In the intervention phase, all participants received rapid clinic-based CD4 testing using an m-PIMA (Abbott, Chicago, IL). Individuals with a POC CD4 ≤200 cells/mm^3 had a venous blood draw for clinic-based CrAg testing. Trained research nurses conducted clinic-based screening on serum samples using a CrAg LFA (Immy Diagnostics, Norman, OK), according to the manufacturer's instructions.

In brief, a research nurse placed each CrAg LFA test strip in a 1.5-mL Eppendorf tube containing 2 drops of "specimen diluent," which was provided by the test manufacturer. All test tubes were rested upright at room temperature for 10 minutes before interpretation. All tests were independently read by 2 trained readers. We also performed positive control testing with a CrAg-spiked solution provided by the test manufacturer and according to their instructions, which were consistently positive.

Study Outcomes

The primary study outcomes were the diagnosis of cryptococcal meningitis, all-cause hospitalization, or all-cause death within 14 months of enrollment. Cryptococcal meningitis was defined as either CrAg-positive in cerebrospinal fluid or a recorded clinical diagnosis of cryptococcal meningitis by the treating medical team in hospital records. The secondary end points included hospitalization due to known cryptococcal meningitis diagnosis, mortality due to known cryptococcal meningitis diagnosis, initiation of ART, initiation of fluconazole prophylaxis, and treatment with intravenous amphotericin B. After 14 months since enrollment, all participants were categorized and censored as retained in care at the study clinic, transferred to another HIV clinic, lost to clinical follow-up, or deceased.

This study investigated process outcomes, including SoC CrAg testing and positivity to quantify background cryptococcal diagnosis and treatment and to determine the delivery of Cryptococcus sp. guidelines and the study intervention. Process outcomes included documented CrAg reflex testing, CrAg positivity among those tested or among the entire analysis set, and conducting the intervention POC PIMA CD4 and POC serum CrAg LFA testing.

Statistical Analyses

The analysis set included individuals who tested positive for HIV and who had a baseline CD4 count ≤200 cells/mm^3 as measured by the standard of care NHLS testing within 6 months of study enrollment. The demographic profile and clinical profile of each study group was quantified to assess how the study population changed over the study period and may have impacted participant outcomes. Demographics of interest included age, sex, employment status, income, marital status, number of children, and education level. Clinical history included in the analysis included symptoms that may be indicative of cryptococcal infection (headache, confusion, fever, neck stiffness, blurry or double vision, and seizure within 7 days of the baseline visit), baseline CD4 testing by NHLS blood testing and PIMA testing in the intervention group, and HIV and Cryptococcus sp. diagnosis and treatment.

To assess how background SoC Cryptococcus sp. testing services may have impacted study outcomes, the association between study group and receipt of services was compared using the Fisher exact test to determine whether participants who enrolled during each study phase reported clinical symptoms. In addition, we conducted separate analyses for participants with CD4 <100 cells/mm^3 because this group is recommended for CrAg screening under current guidelines.[10,11]

Analyses comparing the percentage of study participants who experienced study outcomes between study groups were conducted in duplicate with one comparison between the intervention group and the clinician-directed treatment and another between the intervention group and laboratory reflex testing to isolate the effect of POC CrAg testing alone. Kaplan–Meier curves were conducted to compare time to hospitalization, cryptococcal meningitis, death, and fluconazole prophylaxis across study groups. Finally, we explored the association between CrAg positivity and primary study outcomes among participants who received laboratory-based reflex testing during all study phases and POC LFA CrAg testing during the intervention period. All analyses were conducted in SAS 9.4 (Cary).[19]

RESULTS

Overall, we screened and tested 7877 people for HIV and enrolled 3105 (39%) adults who were HIV-positive (Fig. 1). Among those, 908 (29%) participants had a laboratory-based CD4 ≤200 cells/mm^3 and were included in the analyses. During the clinician-directed testing period (phase 1), we enrolled 1031 participants, of which 323 (31.3%) had a CD4 ≤200 cells/mm^3. During the laboratory reflex testing period (phase 2), we enrolled 1354 PLHIV, of which 363 (26.8%) had a CD4 ≤200 cells/mm^3. During the clinic-based testing period (phase 3), we enrolled 720 PLHIV, of which 222 (30.8%) had a CD4 ≤200 cells/mm^3.

Among the complete cohort of 3105 PLHIV, the mean age was 33.2 (SD ± 9.3) years, and 1331 (43%) were women (Table 1). Six people reported a previous test showing a positive result for Cryptococcus sp. infection, and 4 of those people reported receiving treatment for cryptococcal infection. Participants commonly reported clinical symptoms that have been associated with cryptococcal infection or meningitis, including headache for >24 hours (23%), fever (23%), neck stiffness (16%), blurry or double vision (11%), confusion (7%), and having a seizure within the past 7 days (1%). The median CD4 count was 313 cells/mm^3 (interquartile range [IQR] 173–486 cells/mm^3), and the analyses were restricted to PLHIV with CD4 ≤200 cells/mm^3. Among this subset, the median CD4 was 107 cells/mm^3 (IQR 52–153 cells/mm^3). The baseline demographics and clinical presentations were not substantially different between the 3 enrollment periods.

CrAg Testing

During the clinician-directed testing period, only 10 of the 149 (6.7%) PLHIV with CD4 <100 cells/mm^3 were screened for CrAg, whereas 100% were screened during the laboratory reflex and clinic-based testing periods (Table 2). Among PLHIV with CD4 ≤200 cells/mm^3, 51.8% (188/363) and 45.9% (102/222) of the participants had CrAg screening during the laboratory reflex and clinic-based testing periods, respectively.

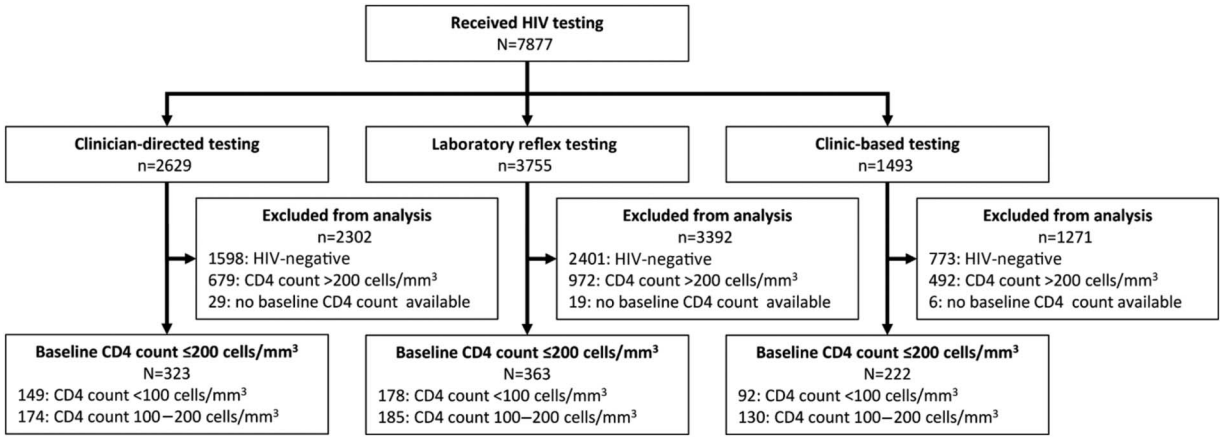

FIGURE 1. Participant enrollment and flowchart. "Clinician-directed testing" refers to the period (from September 12, 2013, to June 4, 2015) before implementation of routine laboratory-based reflex testing where the standard of care was testing among individuals suspected for cryptococcosis. "Laboratory reflex testing" refers to the period (from June 5, 2015, to November 8, 2017) in which the standard of care was laboratory-based reflex CrAg testing among all HIV-positive patients with CD4 count <100 cells/mm^3 in addition to suspected cryptococcosis cases. "Clinic-based testing" refers to the implementation of the study intervention that included clinic-based, point of care serum LFA CrAg testing among all HIV-positive patients with CD4 count ≤200 in the context of background standard of care laboratory-based reflex testing.

During the clinician-directed testing period, 3 of the 149 (2.0%) PLHIV with CD4 <100 cells/mm^3 were found to be CrAg-positive by laboratory-based CrAg testing, whereas 12 of the 178 (6.7%) and 6 of the 92 (6.5%) were found to be CrAg-positive among those receiving laboratory reflex testing and clinic-based testing, respectively. CrAg positivity was 1.4% (7/489) among people with CD4 100–200 cells/mm^3.

During the clinic-based testing phase, all 222 PLHIV with a laboratory-based CD4 ≤200 cells/mm^3 received a POC CD4 test. Among those, 155 of the 222 (69.8%) had a POC CD4 test result ≤200 cells/mm^3 and, therefore, received clinic-based POC serum CrAg testing. Among those who received clinic-based POC serum CrAg testing, the CrAg test positivity was 6.7% (6/90) and 6.5% (10/155) among PLHIV with CD4 <100 cells/mm^3 and ≤200 cells/mm^3, respectively. Clinic-based testing identified significantly more people and a higher percentage of CrAg-positive PLHIV who would have been missed by laboratory reflex testing (P = 0.011).

Clinical Outcomes

During the follow-up period for 908 participants with a laboratory-based CD4 ≤200 cells/mm^3, 30 (3.3%) PLHIV were diagnosed with cryptococcal meningitis confirmed by medical or hospital records, 98 (10.8%) had been hospitalized, and 85 (9.4%) had died (Table 3). When compared with clinician-directed testing, an intervention of clinic-based testing showed an increase in the number of PLHIV diagnosed with cryptococcal meningitis (P = 0.059) but did not alter hospitalization or mortality rates. Comparing the clinic-based testing intervention with laboratory reflex testing, there was no significant difference in the cumulative incidence of cryptococcal meningitis (4.5% vs. 4.1%; P = 0.836) or mortality (8.1% vs. 9.9%; P = 0.557).

For secondary outcomes during the clinical follow-up period, 850 (93.6%) PLHIV initiated ART, 46 (5.1%) were started on fluconazole preventative therapy, 8 (0.9%) received intravenous amphotericin B treatment, and 113 (12.4%) were lost to follow-up. Clinic-based testing led to an increase in the initiation of ART (96.8% vs. 91.3%, P = 0.012) and fluconazole preemptive therapy (7.2% vs. 2.5%, P = 0.010) and reduced lost to follow-up (7.2% vs. 17.3%, P = 0.001), when compared with the clinic-directed testing period. There were no significant differences in secondary outcomes between the clinic-based testing and the laboratory reflex testing groups (Table 3).

Overall, when comparing the 3 periods, clinic-based CrAg testing significantly accelerated the time to initiation of fluconazole preemptive therapy (P = 0.027) and had a trend for accelerated diagnosis of cryptococcal meningitis (P = 0.093) but had no significant impact on time to hospitalization (P = 0.105) or time to mortality (0.685) (Fig. 2). The median follow-up time ranged from 364 to 367 days depending on the outcome of interest.

Association Between CrAg Positivity and Clinical Outcomes

During the laboratory-based CrAg testing period, CrAg positivity remained strongly associated with the diagnosis of cryptococcal meningitis, all-cause hospitalization, and all-cause mortality (Table 4). These strong associations persisted for the intervention period of clinic-based CrAg testing.

DISCUSSION

In this cohort of ambulatory adults in Umlazi township, South Africa, both laboratory reflex and clinic-based CrAg testing facilitated the diagnosis of HIV-associated cryptococcosis and fluconazole initiation, when compared with the previous

TABLE 1. Baseline Sociodemographic and Clinical Characteristics of Study Participants Included in the Analysis

		PLHIV With CD4 ≤200 cells/mm³		
	Cohort of PLHIV N = 3105	Clinician-Directed Testing N = 323	Laboratory Reflex Testing N = 363	Clinic-Based Testing N = 222
Baseline characteristics	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Sociodemographics				
Age (yr): mean (±SD)	33.2 (9.3)	35.1 (8.9)	35.7 (9.8)	36.0 (9.4)
Women	1331 (42.9)	165 (51.1)	198 (54.5)	114 (51.4)
Completed high school or higher degree	1525 (49.1)	112 (34.7)	162 (44.6)	136 (61.3)
Marital status				
Married	193 (6.2)	21 (6.5)	22 (6.1)	13 (5.8)
Single (never married)	2879 (92.7)	298 (92.3)	338 (93.1)	203 (91.4)
Widowed/divorced	33 (1.1)	4 (1.2)	3 (0.8)	6 (2.7)
Number of children (N = 3086)*				
No children	585 (19.0)	58 (17.9)	68 (18.9)	45 (20.5)
≥1 children	2501 (81.0)	264 (82.0)	292 (81.1)	174 (79.5)
Employed	1312 (42.3)	149 (46.1)	156 (43.0)	97 (43.7)
Income level ZAR <2000 (~US $150) (N = 3078)*	2226 (72.3)	246 (77.1)	262 (72.4)	127 (57.5)
HIV and medical history				
Previously received HIV testing	2390 (77.0)	220 (68.1)	228 (62.8)	209 (94.1)
Previously tested HIV-positive, among those tested (N = 2380)*	791 (33.2)	41 (18.9)	77 (33.9)	98 (46.7)
Partner HIV status (N = 3087)*				
HIV-positive	847 (27.4)	88 (27.4)	91 (25.2)	53 (24.8)
HIV-negative	508 (16.5)	40 (12.5)	48 (13.3)	18 (8.4)
Unknown	1732 (56.1)	193 (60.1)	222 (61.5)	143 (66.8)
Ever tested positive for Cryptococcus sp.	6 (0.2)	2 (0.6)	2 (0.6)	0 (0.0)
Ever received treatment of cryptococcal infection, among those positive	4 (0.1)	1 (0.3)	2 (0.6)	—
Clinical symptoms and signs				
Headache for >24 h	703 (22.6)	113 (35.0)	68 (18.7)	33 (14.9)
Fever	698 (22.5)	122 (37.8)	116 (32.0)	45 (20.3)
Neck stiffness	503 (16.2)	71 (22.0)	72 (19.8)	42 (18.9)
Blurry or double vision	325 (10.5)	39 (12.1)	52 (14.3)	46 (20.7)
Confusion	216 (7.0)	20 (6.2)	32 (8.8)	18 (8.1)
Seizure within the previous 7 d	26 (0.8)	6 (1.9)	3 (0.8)	3 (1.4)
CD4 T-cell count, cells/mm³				
Baseline CD4 count using laboratory-based test (N = 3057): median (IQR)	313 (173–486)	108 (51–151)	101 (45–147)	121 (65–161)
Baseline CD4 count using POC test (N = 720): median (IQR)†	361 (214–563)	—	—	147 (95–214)

*Does not sum to column total because of missing data.
†Intervention period was clinic-based testing period only.

practice of clinician-directed CrAg testing. When compared with clinician-directed testing, clinic-based CrAg testing showed an increase in the number of PLHIV diagnosed with cryptococcal meningitis but did not alter hospitalization or mortality rates. When comparing the laboratory reflex testing and clinic-based testing, there were no significant differences in the diagnosis of cryptococcal meningitis, hospitalization, mortality, or any secondary outcomes. The results of this nonrandomized study support laboratory reflex or clinic-based CrAg testing to facilitate the diagnosis of HIV-associated cryptococcosis and early initiation of fluconazole preemptive therapy for those CrAg-positive.

The estimated prevalence of cryptococcal antigenemia in our cohort was consistent with other studies of PLHIV in sub-Saharan Africa.[1,20] An earlier study in Cape Town reported a higher CrAg prevalence of 12% (42/336) among pre-ART PLHIV with CD4 ≤100 cells/mm³[5]; the overall incidence of HIV-associated cryptococcosis has been declining throughout South Africa since 2006.[21] In our cohort, most (95%) of the participants who developed cryptococcal meningitis had a baseline CD4 ≤200 cells/mm³.[11] Although these data also support increasing the CrAg screening threshold to CD4 ≤200 cells/mm³ among newly diagnosed PLHIV, the study was not designed to address this important

TABLE 2. Cryptococcus sp. Diagnosis and Treatment Delivery by Study Phase and Baseline CD4 Count

	Clinician-Directed Testing	Laboratory Reflex Testing	Clinic-Based Testing	
	n/N (%)	n/N (%)	n/N (%)	*P*
Standard of care diagnosis and treatment				
Received laboratory-based SoC CrAg testing				
Laboratory CD4 <100 cells/mm^3	10/149 (6.7)	178/178 (100.0)	92/92 (100.0)	**<0.001**
Laboratory CD4 ≤200 cells/mm^3	11/323 (3.4)	188/363 (51.8)	102/222 (45.9)	**<0.001**
CrAg-positive by laboratory-based SoC CrAg testing				
Laboratory CD4 <100 cells/mm^3	3/149 (2.0)	12/178 (6.7)	6/92 (6.5)	0.096
Laboratory CD4 ≤200 cells/mm^3	3/323 (0.9)	16/363 (4.4)	9/222 (4.1)	**0.011**
CrAg-positive from laboratory-based SoC CrAg testing, among those who received testing				
Laboratory CD4 <100 cells/mm^3	3/10 (30.0)	12/178 (6.7)	6/92 (6.5)	0.053
Laboratory CD4 ≤200 cells/mm^3	3/11 (27.3)	16/188 (8.5)	9/102 (8.8)	0.131
Study intervention clinic-based CD4 and CrAg testing*				
Received POC CD4 test				
Laboratory CD4 <100 cells/mm^3	—	—	92/92 (100.0)	—
Laboratory CD4 ≤200 cells/mm^3	—	—	222/222 (100.0)	—
POC CD4 count ≤200 cells/mm^3				
Laboratory CD4 <100 cells/mm^3	—	—	90/92 (97.8)	—
Laboratory CD4 ≤200 cells/mm^3	—	—	155/222 (69.8)†	—
Received POC serum CrAg LFA, if POC CD4 count ≤200 cells/mm^3				
Laboratory CD4 <100 cells/mm^3	—	—	90/90 (100.0)	—
Laboratory CD4 ≤200 cells/mm^3	—	—	155/155 (100.0)	—
CrAg-positive by POC serum CrAg LFA				
Laboratory CD4 <100 cells/mm^3	—	—	6/90 (6.7)	—
Laboratory CD4 ≤200 cells/mm^3	—	—	10/155 (6.5)	—

Bold font indicates a statistically significant *P*-value < 0.05.
*Intervention period was clinic-based testing period only. Denominators reflect the results from laboratory-based CD4 testing.
†Results indicate that 67 people had a POC CD4 >200 cells/mm^3, who had a laboratory CD4 ≤200 cells/mm.^{3}

TABLE 3. Clinical Outcomes by Study Phase

		Intervention Group			Standard-of-Care Groups					
	Total (N = 908)	Clinic-Based Testing (N = 222)			Clinician-Directed Testing (N = 323)			Laboratory Reflex Testing (N = 363)		
Postbaseline Clinical Outcomes	N	% (CI)	n	% (CI)	n	% (CI)	*P**	n	% (CI)	*P**
Primary outcomes										
Cryptococcal meningitis diagnosis	30	3.3 (2.3 to 4.7)	10	4.5 (2.4 to 8.2)	5	1.5 (0.6 to 3.7)	0.059	15	4.1 (2.5 to 6.8)	0.836
All-cause hospitalization	98	10.8 (8.9 to 13.0)	21	9.5 (9.5 to 14.1)	28	8.7 (6.0 to 12.3)	0.762	49	13.5 (10.3 to 17.4)	0.151
All-cause mortality	85	9.4 (7.6 to 11.4)	18	8.1 (5.1 to 12.5)	31	9.6 (6.8 to 13.3)	0.648	36	9.9 (7.2 to 13.5)	0.557
Secondary outcomes										
Hospitalization due to known cryptococcal infection	11	1.2 (0.7 to 2.2)	4	1.8 (0.5 to 4.7)	4	1.2 (0.4 to 3.3)	0.721	3	0.8 (0.2 to 2.5)	0.436
Mortality due to known cryptococcal infection	9	1.0 (0.5 to 1.9)	4	1.8 (0.5 to 4.7)	1	0.3 (0.0 to 1.9)	0.164	4	1.1 (0.3 to 2.9)	0.486
Initiation of antiretroviral therapy	850	93.6 (91.8 to 95.0)	215	96.8 (93.5 to 98.6)	295	91.3 (87.7 to 94.0)	**0.012**	340	93.7 (90.6 to 95.8)	0.121
Received fluconazole preventative therapy†	46	5.1 (3.8 to 6.7)	16	7.2 (4.4 to 11.5)	8	2.5 (1.2 to 4.9)	**0.010**	22	6.1 (4.0 to 9.1)	0.607
Received intravenous amphotericin B	8	0.9 (0.9 to 3.1)	3	1.4 (0.3 to 4.1)	4	1.2 (0.4 to 3.3)	1.000	1	0.3 (0.0 to 1.7)	0.156
Lost to follow-up	113	12.4 (10.5 to 14.8)	16	7.2 (4.4 to 11.5)	56	17.3 (13.6 to 21.9)	**0.001**	41	11.3 (8.4 to 15.0)	0.116

Bold font indicates a statistically significant *P*-value < 0.05.
**P* value represents the Fisher exact test of the comparison between each standard of care group with the intervention group.
†Oral fluconazole was indicated for people with serum cryptococcal antigenemia but without cryptococcal meningitis.

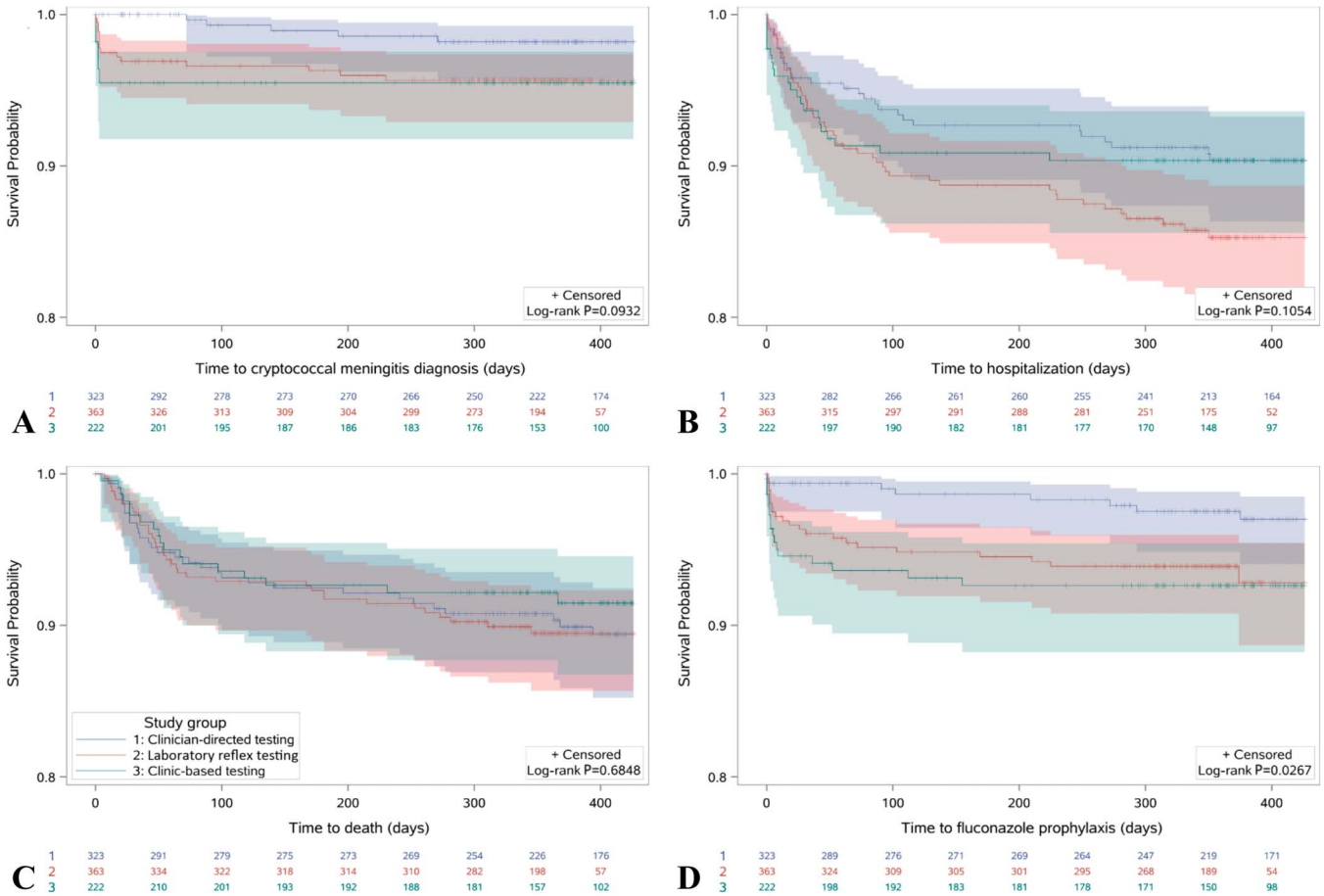

FIGURE 2. Time to (A) cryptococcal meningitis diagnosis, (B) hospitalization, (C) death, and (D) fluconazole prophylaxis by study group. Participants were followed up for 1 year, and all events were censored at 14 months. Y-axes range from a survival probability of 0.8 to 1.0. Lines in blue represent clinician-directed testing (phase 1), those in red represent laboratory reflex testing (phase 2), and those in green represent clinic-based testing (phase 3), and bands around lines indicate 95% confidence intervals (CIs). full color online

research question. Either clinic-based or laboratory reflex CrAg screening remains an important component of clinical care for PLHIV with advanced disease.

In multiple studies, CrAg LFA testing has proven accurate when compared with CrAg EIA and latex agglutination testing in serum and/or cerebrospinal specimens.[13–16,22,23] However, one study has reported false-negative results from CrAg LFA testing on serum due to a prozone effect.[24] We have previously reported on the diagnostic accuracy of clinic-based POC CrAg LFA testing, when compared with laboratory-based serum CrAg EIA testing.[17] In this study, POC CrAg LFA testing was feasible and may be an alternative to laboratory-based reflex CrAg testing for clinics that have the capacity to conduct POC CD4 testing. In addition, CrAg screening for HIV-positive adults with CD4 100–200 cells/mm^3 identified additional people with cryptococcal antigenemia at risk of poor clinical outcomes.

Although our study showed improvements in clinical diagnosis of cryptococcal meningitis and proportion who initiated preemptive fluconazole therapy, there was no observed impact on mortality. Another report from South Africa found no reduction in the annual case fatality ratio for cryptococcal meningitis, which was attributed to delays in diagnosing HIV-associated cryptococcal infections.[25] In the REALITY trial, the provision of fluconazole improved outcomes for PLHIV with low CD4 count, regardless of CrAg screening results.[9] The absolute benefits were still greater for CrAg-positive patients, and the authors concluded that CrAg screening should be routine for PLHIV with CD4 <100 cells/mm^3.[9] In our cohort, many people who were serum CrAg-positive during clinic-based testing already had evidence of cryptococcal meningitis. Therefore, although clinic-based testing may have accelerated time to initiation of fluconazole and diagnosis of cryptococcal meningitis, the accelerated treatment may have already been too late to significantly reduce mortality rates.

This study had several limitations and strengths. The study was conducted over a 6-year period and included a significant number of immunosuppressed PLHIV at substantial risk for cryptococcal meningitis or mortality. The study was implemented as a pre–post study design, which does not provide a direct comparison between study arms.

TABLE 4. Association Between CrAg Positivity and Study Outcomes Among Patients

	CrAg−		CrAg+		
Postbaseline Clinical Outcomes	**n/N**	**% (CI)**	**n/N**	**% (CI)**	***P***
Standard of care laboratory testing*					
Cryptococcal meningitis diagnosis					
<100 cells/mm^3	3/259	1.2 (0.2 to 3.5)	19/21	90.5 (69.9 to 98.6)	**<0.001**
≤200 cells/mm^3	3/273	1.1 (0.2 to 3.3)	25/28	89.3 (72.0 to 97.1)	**<0.001**
All-cause hospitalization					
<100 cells/mm^3	43/259	16.6 (12.5 to 21.6)	8/21	38.1 (20.7 to 59.2)	**0.033**
≤200 cells/mm^3	47/273	17.2 (13.2 to 22.2)	11/28	39.3 (23.5 to 57.6)	**0.010**
All-cause mortality					
<100 cells/mm^3	30/259	11.6 (8.2 to 16.1)	7/21	33.3 (17.1 to 54.8)	**0.012**
≤200 cells/mm^3	35/273	12.8 (9.3 to 17.3)	10/28	35.7 (20.6 to 54.3)	**0.004**
Intervention clinic-based testing†					
Cryptococcal meningitis diagnosis					
<100 cells/mm^3	1/84	1.2 (0.0 to 7.1)	6/6	100.0 (55.7 to 100.0)	**<0.001**
≤200 cells/mm^3	1/145	0.7 (0.0 to 4.2)	9/10	90.0 (57.4 to 100.0)	**<0.001**
All-cause hospitalization					
<100 cells/mm^3	13/84	15.5 (9.1 to 24.8)	3/6	50.0 (18.8 to 81.2)	0.067
≤200 cells/mm^3	16/145	11.0 (6.8 to 17.3)	4/10	40.0 (16.7 to 68.8)	**0.026**
All-cause mortality					
<100 cells/mm^3	11/84	13.1 (7.3 to 22.1)	4/6	66.7 (29.6 to 90.8)	**0.007**
≤200 cells/mm^3	12/145	8.3 (4.7 to 14.0)	5/10	50.0 (23.7 to 76.3)	**0.002**

Bold font indicates a statistically significant *P*-value < 0.05.
*Among patients who were documented receiving a SoC laboratory-based CrAg test. It included participants from all 3 study periods.
†Intervention period was clinic-based testing period only.

However, conducting a randomized trial in which one arm does not adequately provide CrAg testing for immunosuppressed PLHIV would be considered unethical. Despite the large sample size, the relatively fewer participants with cryptococcal antigenemia and/or CD4 immunosuppression reflected real-world practice in South Africa[25] but was a limitation for assessing differences between study periods, with progression to cryptococcal meningitis, hospitalization, and death as the primary outcome measures. The average CD4 count among PLHIV presenting for initiation of care and treatment may have changed during the 6-year study period, but analyses focused on PLHIV with CD4 ≤200 cells/mm^3 for the entire study duration.

In conclusion, laboratory reflex and clinic-based CrAg testing facilitated the diagnosis of HIV-associated cryptococcosis and fluconazole initiation but did not reduce cryptococcal meningitis or mortality. As programs move toward same-day ART initiation, implementing either clinic-based or laboratory reflex CrAg testing may be helpful in identifying those with cryptococcal antigenemia for fluconazole therapy because clinician-directed CrAg testing will likely miss many more cases. Overall, CrAg testing was feasible when performed by trained nurses at the clinical point of care and led to more people being diagnosed with cryptococcosis and initiated on fluconazole therapy. Although programs should emphasize early ART initiation to prevent severe immunosuppression, CrAg testing will help accelerate diagnosis and treatment of HIV-associated cryptococcal infections to reduce HIV-associated cryptococcal mortality.

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REFERENCES

1. Rajasingham R, Smith RM, Park BJ, et al. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. *Lancet Infect Dis.* 2017;17:873–881.
2. Jarvis JN, Meintjes G, Williams A, et al. Adult meningitis in a setting of high HIV and TB prevalence: findings from 4961 suspected cases. *BMC Infect Dis.* 2010;10:67.
3. French N, Gray K, Watera C, et al. Cryptococcal infection in a cohort of HIV-1-infected Ugandan adults. *AIDS.* 2002;16:1031–1038.
4. Liechty CA, Solberg P, Were W, et al. Asymptomatic serum cryptococcal antigenemia and early mortality during antiretroviral therapy in rural Uganda. *Trop Med Int Health.* 2007;12:929–935.
5. Jarvis JN, Lawn SD, Vogt M, et al. Screening for cryptococcal antigenemia in patients accessing an antiretroviral treatment clinic in South Africa. *Clin Infect Dis.* 2009;48:856–862.
6. 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;385:2173–2182.
7. Kapoor SW, Magambo KA, Kalluvya SE, et al. Six-month outcomes of HIV-infected patients given short-course fluconazole therapy for asymptomatic cryptococcal antigenemia. *AIDS.* 2015;29:2473–2478.
8. 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.

9. Pett SL, Haddow L, Nhema R, et al. CrAg status and effects on benefits from enhanced prophylaxis in the REALITY trial. Abstracts presented at Conference on Retroviruses and Opportunistic Infections, 2018; March 5, 2018; Boston, MA. Poster #784.
10. World Health Organization. *Rapid Advice: Diagnosis, Prevention and Management of Cryptococcal Disease in HIV-Infected Adults, Adolescents and Children*. Geneva, Switzerland: WHO; 2011.
11. World Health Organization. *Guidelines for the Diagnosis, Prevention, and Management of Cryptococcal Disease in HIV-Infected Adults, Adolescents and Children*. Geneva, Switzerland: World Health Organization; 2018.
12. 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–e91.
13. 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;53:1019–1023.
14. Rugemalila J, Maro VP, Kapanda G, et al. Cryptococcal antigen prevalence in HIV-infected Tanzanians: a cross sectional study and evaluation of a point-of-care lateral flow assay. *Trop Med Int Health.* 2013;18:1075–1079.
15. Klausner JD, Chiller TV. Sensitivity and specificity of a new cryptococcal antigen lateral flow assay in serum and cerebrospinal fluid. *MLO Med Lab Obs.* 2013;45:16–20.
16. McMullan BJ, Halliday C, Sorrell TC, et al. Clinical utility of the cryptococcal antigen lateral flow assay in a diagnostic mycology laboratory. *PLoS One*. 2012;7:e49541.
17. Drain PK, Hong T, Krows M, et al. Validation of clinic-based cryptococcal antigen lateral flow assay screening in HIV-infected adults in South Africa. *Sci Rep.* 2019;9:2687.
18. Republic of South Africa Department of Health. *The South African Antiretroviral Treatment Guidelines*. 2017.
19. Cohen JF, Korevaar DA, Altman DG, et al. STARD 2015 guidelines for reporting diagnostic accuracy studies: explanation and elaboration. *BMJ Open.* 2016;6:e012799.
20. Ford N, Shubber Z, Jarvis JN, et al. CD4 cell count threshold for cryptococcal antigen screening of HIV-infected individuals: a systematic review and meta-analysis. *Clin Infect Dis.* 2018;66(suppl 2):S152–S159.
21. Govender NP. *Declining Incidence of HIV-Associated Cryptococcosis in South Africa, 2005-2015*. Abstracts presented at Conference on Retroviruses and Opportunistic Infections, 2018; March 5, 2018; Boston, MA. Poster #789.
22. Beyene T, Woldeamanuel Y, Asrat D, et al. Comparison of cryptococcal antigenemia between antiretroviral naïve and antiretroviral experienced HIV positive patients at two hospitals in Ethiopia. *PLoS One*. 2013;8:e75585.
23. Hansen J, Slechta ES, Gates-Hollingsworth MA, et al. Large-scale evaluation of the immune-mycologics lateral flow and enzyme-linked immunoassay for detection of cryptococcal antigen in serum and cerebrospinal fluid. *Clin Vaccin Immunol.* 2013;20:52–55.
24. Williams DA, Kiiza T, Kwizera R, et al. Evaluation of fingerstick cryptococcal antigen lateral flow assay in HIV-infected persons: a diagnostic accuracy study. *Clin Infect Dis.* 2015;61:464–467.
25. Temfack E, Kouanfack C, Mossiang L, et al. Cryptococcal antigen screening in asymptomatic HIV-infected antiretroviral naïve patients in Cameroon and evaluation of the new semi-quantitative Biosynex CryptoPS Test. *Front Microbio.* 2018;9:409.