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Point-of-Care Testing for Cryptococcal Disease among Hospitalized Human Immunodeficiency virus–Infected Adults in Ethiopia

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Abstract. In a cross-sectional study among hospitalized human immunodeficiency virus (HIV)–infected patients in Ethiopia, we sought to determine the rates and predictors of cryptococcal disease and evaluate the test performance of a recently introduced point-of-care test for *Cryptococcus neoformans* detection in various biological samples. We tested serum, urine, and fingerstick blood samples from each patient with a cryptococcal antigen lateral flow assay (CRAG LFA; Immuno Mycologic Inc., Norman, OK). Cerebrospinal fluid was collected at the discretion of the treating physician. Logistic regression was used to identify risk factors for a positive test result. Agreement between different sample types was also assessed. Among 198 hospitalized HIV-infected patients with a median CD4 count of 93 cells/mm^3, 18 patients (9.1%) had a positive serum CRAG LFA. Of these, 16 (8.1%) had confirmed cryptococcal meningitis (CM), all of whom had a positive fingerstick blood LFA result. There was a very high agreement between CRAG LFA tests in serum and fingerstick blood samples ($\kappa = 0.97$, 95% confidence interval [CI] = 0.91–1.00); this was higher than that between serum and urine samples ($\kappa = 0.76$, 95% CI = 0.58–0.93). A CD4 count < 100 cells/mm^3 was significantly associated with a positive CRAG LFA. The absence of fever, headache, meningismus, and neck stiffness had a negative predictive value of 100% for CM. In addition to finding high rates of cryptococcal disease, our study demonstrated that the use of the LFA on fingerstick whole blood is less invasive, and an effective method for CM case finding among hospitalized patients with HIV.

INTRODUCTION

Cryptococcal meningitis (CM) is an important and frequently unrecognized opportunistic infection that disproportionately affects human immunodeficiency virus (HIV)–infected persons in sub-Saharan Africa (SSA). A 2009 study estimated a burden of 720,000 HIV-associated CM cases annually in SSA along with a mortality rate of > 50%, which is considerably higher than the 10–20% mortality rate observed in most developed countries.[1,2] This high case fatality rate in SSA is likely due to a multitude of factors including patients presenting late into care, lack of optimal medications, and limited availability of diagnostic tests.[2] Many healthcare facilities in SSA and other resource-limited settings (RLS) have limited ability to diagnose cryptococcal disease and often rely on a clinical diagnosis and/or India ink microscopy of cerebrospinal fluid (CSF), which are insensitive diagnostics methods. Prompt and accurate cryptococcal testing would lead to earlier diagnosis and initiation of CM treatment, and may also prevent unnecessary workup and empiric treatment thus preserving precious resources.

The development of a lateral flow immunoassay (LFA; Immuno Mycologic Inc., Norman, OK) marked a major advancement in diagnostic testing for cryptococcal disease. The dipstick-based LFA is a low-cost and easy-to-perform test. It requires minimal infrastructure and training, and provides a result within 10 minutes.[3] The LFA meets the World Health Organization (WHO) ASSURED–based criteria for a point-of-care test (POCT) and, based on its performance in early studies, was approved for use in serum and CSF by the United States Food and Drug Administration and endorsed by the WHO as a preferred testing method for cryptococcal disease in 2011.[4] A recent meta-analysis found that the serum LFA had a sensitivity and specificity of 98% as compared with a standard including culture, latex agglutination (LA), and/or enzyme immunoassay for diagnosing cryptococcal disease.[5] LFA performance in CSF was similar with a sensitivity and specificity of 99%.[5] In an effort to use less-invasive samples, studies have also been carried out using urine, saliva, and fingerstick blood samples.[5–9] The performance of the LFA in urine has shown varying results and only one study has evaluated the LFA using fingerstick whole blood.[8]

The majority of cryptococcal screening studies have been performed among HIV-infected patients initiating antiretroviral therapy (ART) or among HIV-infected patients suspected of having meningitis.[10] The benefit to screening HIV-infected patients early on is to catch and treat asymptomatic patients with cryptococcal disease early on and prevent CM. In 2011, WHO rapid advice (interim) guidelines recommended cryptococcal antigen (CRAG) screening among ART-naive HIV-infected adults with a CD4 count < 100 cells/mm.[3,4] However, there are no clear recommendations on who to test for cryptococcal disease and few studies have evaluated the rates and predictors of cryptococcal disease among hospitalized HIV-infected patients.[11]

Given the gaps in the current literature with regard to cryptococcal testing among hospitalized HIV-infected patients and to assess the utility of LFA testing for cryptococcal disease in a resource-limited area where HIV is prevalent, we evaluated the implementation of CRAG LFA testing for all HIV-infected patients admitted to a large university-affiliated referral hospital in Ethiopia. Our main study aims were to determine the prevalence and risk factors for cryptococcal disease including CM among adult HIV-infected inpatients and whether LFA testing among less-invasive urine and fingerstick blood samples would perform similar to serum and CSF LFA testing for the detection of CRAG. In addition, we sought to determine

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whether a universal versus a targeted approach for cryptococcal disease screening among HIV-infected patients was most optimal.

MATERIALS AND METHODS

Study setting and patients. We conducted a cross-sectional study among HIV-infected patients admitted to the Tikur Anbessa (Black Lion) Hospital in Addis Ababa, Ethiopia. Tikur Anbessa is the major teaching hospital affiliated with the Addis Ababa University (AAU) School of Medicine and is the largest hospital in Ethiopia. The hospital has 800 inpatient beds including 110 medical ward and six medical intensive care unit (MICU) beds. Before study implementation, the only available diagnostic test for cryptococcal disease was CSF India ink microscopy.

Adult patients (≥ 18 years) with HIV infection admitted to either the Internal Medicine wards or MICU regardless of CD4 count or admission diagnoses were eligible for enrollment. HIV testing was offered to all admitted patients upon admission (as the standard of care), and patients receiving a new diagnosis of HIV were eligible for enrollment. The study took place between June 2013 and May 2014. A study investigator approached eligible patients (i.e., HIV-infected adult patients on the Internal Medicine wards and MICU) for participation; written informed consent from the patient or next of kin was required for study enrollment. The institutional review boards of AAU, the National Research Ethics Review Committee of Ethiopia, and Emory University, Atlanta, GA approved the study.

Data collection. After providing written informed consent, patients were interviewed and examined by a study investigator. A standardized data collection form was completed to collect information on demographics, medical history, and selected clinical signs and symptoms. Medical chart abstraction was also performed at baseline and at discharge from the hospital to collect information on laboratory values, clinical diagnoses, hospital course, and patient status at time of hospital discharge. Data were entered into an online REDCap database.[12]

Cryptococcal testing. The following samples were obtained from each patient by trained ward nurses: 1) 3 ml venous blood, 2) 3 mL urine, and 3) approximately two drops of fingerstick blood. All samples were immediately delivered to the hospital ward laboratory where the LFA was performed by a trained laboratory technician according to the manufacturer's instructions.[13] After centrifugation, serum was obtained from the venous blood sample. One drop of standard diluent was added to each microtiter plate well along with 40 μL of patient specimen (serum and urine), and then the CRAG LFA test strip was inserted. The fingerstick blood sample was collected directly into a microcentrifuge tube prefilled with diluent after which the LFA test strip was inserted. After incubating at room temperature for 10 minutes, the test strip was read with a positive result indicated by the presence of a visible red control and test line. In patients with a positive serum or CSF (see below) LFA test, semiquantitative LFA titers were performed with 2-fold dilutions from 1:10 up to the greatest dilution with a positive reaction or until 1:2,560. A positive control was performed for each package kit.

A copy of each patient's serum LFA result was placed in their medical record, and the treating physician was verbally informed of the result. All management decisions including antifungal treatment and performing a lumbar puncture were at the discretion of the treating physician. Before study implementation, lectures were given by an infectious diseases specialist to hospital staff regarding cryptococcal management with an emphasis on guidelines from the WHO and Infectious Diseases Society of America.[4,14] For patients who had a lumbar puncture performed, the LFA was performed on a sample of CSF and positive samples were titrated. India ink microscopy was also performed. Manometers were not available.

Data analysis. Data analyses were performed using SAS, version 9.3 (Cary, NC). For comparing characteristics among patients with a positive and negative serum cryptococcal LFA test, differences in categorical variables were tested using either the χ^2 or Fisher's exact test, and the Wilcoxon–Mann–Whitney test was used for comparing non-normally distributed continuous variables. A logistic regression model was used to estimate the independent association of potential risk factors with a positive serum cryptococcal LFA test. Logistic model building, covariate selection, and testing for confounding and interaction were based on the purposeful selection of patient-level factors as previously described.[15] In brief, variables with a P value < 0.20 in univariate analysis for a positive serum LFA result were included in an initial multivariate model, and the final model included variables with a $P \leq 0.10$ plus age given its prior association with cryptococcal antigenemia. We also checked for confounders, defined by a change in an odds ratio of ≥ 20% upon removal of a variable and rechecked all variables for inclusion in the final model. The degree of agreement between LFA results from different samples (serum, whole blood, and urine) was assessed using the kappa (κ), where $\kappa > 0.75$ represents excellent agreement, $\kappa = 0.4$–0.75 fair to good agreement, and $\kappa < 0.4$ poor agreement. The sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) for certain signs and symptoms for a positive serum cryptococcal LFA were determined. A P value of < 0.05 was considered statistically significant throughout analyses.

RESULTS

Study cohort. Among 2,310 adults admitted to the Internal Medicine wards or MICU during the study period, 210 (9%) were confirmed to be HIV infected; 198 (94%) were enrolled into the study. The 12 patients not included were due to refusal to consent, and either death or discharge before being approached for enrollment. The mean age of those enrolled was 36.7 years and 47% of patients were female; eleven patients (6%) were admitted to the MICU. The median duration of known infection with HIV was 12 months (interquartile range [IQR] = 2–59); 98 (49%) were not on ART or had received ART for less than 3 months at time of enrollment. Ninety percent of those enrolled had a CD4 count performed; the median CD4 count among this group was 93 cells/mm^3 (IQR = 40–214). Four patients (2%) had a history of cryptococcal disease (Table 1).

Cryptococcal screening. Among the 198 HIV-infected patients, 18 (9.1%) had a positive serum cryptococcal LFA result with titers ranging from 1:40 to 1:2,560. All 18 patients with a positive serum LFA had a lumbar puncture and 17 had their CSF tested with either LFA or India ink microscopy or both. Fifteen (88%) of 17 had either a positive CSF

TABLE 1
Comparison of characteristics between hospitalized HIV-infected patients with and without cryptococcal antigenemia

Characteristic	Total $N = 198$ (%)	Serum cryptococcal LFA positive $N = 18$ (%)	Serum cryptococcal LFA negative $N = 180$ (%)	P value
Demographics				
Mean age, years (SD)	36.7 (10.2)	34.2 (6.5)	36.9 (10.5)	0.13
Female	93 (47)	5 (28)	88 (49)	0.08
Current smoker	20 (10)	1 (6)	19 (11)	0.50
Current alcohol use	38 (19)	4 (22)	34 (19)	0.73
Illicit drug use	13 (7)	1 (6)	12 (7)	0.86
Diabetes	1 (1)	0	1 (1)	0.75
Malignancy	10 (5)	1 (6)	9 (5)	0.92
HIV and medical history				
Median duration of HIV months (IQR)	12.3 (2–59)	9.4 (2–36)	12.3 (2–60)	0.44
Prior CD4 count available	178 (90)	16 (89)	162 (90)	0.88
Median prior CD4 ($N = 176$) (IQR)	93 (40–214)	46 (23–74)	113 (45–236)	< 0.01
Known CD4 < 100 at time of admission	94 (47)	15 (83)	79 (43)	0.001
Current ART use	126 (64)	14 (78)	112 (62)	0.19
Median time on ART ($N = 130$), months (IQR)	59 (6–303)	23 (4–87)	62 (7–155)	0.11
No ART or on ART < 3 months at time of admission	98 (50)	7 (39)	91 (51)	0.35
Received steroid in prior 30 days	55 (28)	7 (39)	48 (27)	0.27
History of opportunistic infections				
Cryptococcal disease	4 (2)	2 (11)	2 (1)	< 0.01
Tuberculosis	83 (42)	8 (44)	75 (42)	0.82
Lymphoma	19 (11)	0	19 (11)	0.15
Hospital presentation				
Admitted to the ICU	11 (6)	0	11 (6)	0.28
Admitting syndrome				
Sepsis	16 (8)	1 (6)	15 (8)	0.68
Altered mental status	34 (17)	7 (39)	27 (15)	0.01
Seizure	16 (8)	0	16 (9)	0.19
Pulmonary syndrome	65 (32)	2 (11)	63 (35)	0.04
Hematologic disorder	33 (18)	2 (11)	33 (18)	0.44
Opportunistic infection	117 (59)	13 (72)	104 (58)	0.24
Symptoms				
Headache	77 (39)	15 (83)	62 (34)	< 0.01
Neck stiffness	21 (11)	7 (39)	14 (8)	< 0.01
Altered mental status	47 (24)	7 (39)	40 (22)	0.11
Photophobia	9 (5)	1 (6)	8 (4)	0.83
Fever	115 (58)	9 (50)	106 (59)	0.47
Nausea	84 (42)	10 (56)	74 (41)	0.24
Vomiting	91 (46)	11 (61)	80 (44)	0.18
Blurry vision	13 (7)	3 (17)	10 (6)	0.07
Night sweats	84 (42)	6 (33)	78 (43)	0.41
Cough	92 (47)	6 (33)	86 (48)	0.24
Shortness of breath	64 (32)	3 (17)	61 (34)	0.14
Signs				
Meningismus	20 (10)	5 (28)	15 (8)	< 0.01
Febrile (≥ 37.6 C° axillary temperature)	56 (28)	7 (39)	49 (27)	0.30
Cranial neuropathy	10 (5)	1 (6)	9 (5)	0.92
Median hemoglobin (IQR)	10.2 (8.4–12.5)	10.0 (7.6–14.7)	10.2 (8.5–12.3)	
Median white blood cell count (IQR)	7.5 (4.4–10.3)	8.4 (3.4–10.7)	7.5 (4.5–10.3)	
Hospital course				
Blood cultures performed	6 (3)	2 (11)	4 (2)	0.04
Antibiotics initiated	158 (80)	15 (83)	143 (80)	0.70

ART = antiretroviral therapy; HIV = human immunodeficiency virus; ICU = intensive care unit; IQR = interquartile range; LFA = lateral flow assay; SD = standard deviation. Values in parentheses indicate percentages unless otherwise indicated.

LFA (15 of 16) and/or India ink microscopy (11 of 15). A total of 14 patients had both a CSF LFA and India ink microscopy performed. Of these 14 patients, 13 had a positive LFA test and 11 had a positive India ink stain (Table 2). Thus, in comparison to the LFA, the India ink stain had 85% sensitivity in diagnosing CM. The one patient with a positive serum LFA test and no CSF LFA or India ink performed, had clinical signs and symptoms of meningitis. Thus, along with the one clinical diagnosis, there were a total of 16 patients (8.1%) with CM. The number of patients needed to be tested to detect one case of cryptococcal antigenemia and CM was 11 (198/18) and 13 (198/16), respectively. All 16 patients with CM had a positive fingerstick blood LFA, whereas only 12 (75%) had a positive urine LFA result.

Fingerstick blood and urine samples were obtained for all patients. The agreement between serum and fingerstick blood LFA results was extremely high ($\kappa = 0.97$, 95% confidence interval [CI] = 0.91–1.00) and was higher than that between serum and urine LFA results ($\kappa = 0.76$, 95% CI = 0.58–0.93). Among the 18 patients with a positive serum LFA test, 17 (94%) had a positive fingerstick blood LFA result (Table 2). The patient with a negative fingerstick LFA result had cryptococcemia with a low LFA titer of 1:40 and no signs or symptoms of meningitis. Twelve (67%) of 18 patients with a positive serum LFA also had a positive

TABLE 2
Comparison of cryptococcal testing results among different sample types in patients with cryptococcal infection

Number	CD4 count (cells/mm^3)	Serum LFA	Fingerstick LFA	Urine LFA	CSF LFA	CSF India ink
1	67	1:40	Negative	Negative	Not done	Negative
2	216	1:2,560	Positive	Positive	1:80	Positive
3	69	1:10	Positive	Negative	1:40	Negative
4	16	1:2,560	Positive	Positive	1:640	Positive
5	47	1:80	Positive	Positive	1:1,280	Positive
6*	97	1:2,560	Positive	Positive	Not done	Not done
7	22	1:2,560	Positive	Positive	1:2,560	Positive
8	48	1:2,560	Positive	Positive	1:2,560	Positive
9	Unknown†	1:40	Positive	Positive	1:80	Positive
10	24	1:2,560	Positive	Positive	1:2,560	Positive
11	31	1:320	Positive	Positive	1:1,280	Positive
12	45	1:2,560	Positive	Positive	1:2,560	Positive
13	7	1:40	Positive	Negative	Negative	Negative
14	86	1:320	Positive	Negative	1:640	Positive
15	79	1:160	Positive	Negative	1:320	Negative
16	11	1:320	Positive	Positive	1:2,560	Not done
17	23	1:320	Positive	Positive	1:160	Not done
18	22	1:40	Positive	Negative	1:160	Positive

CSF = cerebrospinal fluid; LFA = lateral flow assay.
*A lumbar puncture but no LFA or India ink was performed.
†Patient had in-hospital mortality.

urine LFA result. Among the six patients with cryptococcal disease and a negative urine LFA test, four had a serum LFA titer ≤ 1:40 and the remaining two had titers of 1:160 and 1:320 (Table 3). There was one patient with a positive urine LFA and negative serum LFA result. Further test characteristics are shown in Table 2.

Risk factors for cryptococcal disease. In comparison to patients with a negative serum LFA, those with a positive LFA result were more likely to have a lower median CD4 count (46 versus 113 cells/mm^3, $P < 0.01$) and history of cryptococcal disease (11% versus 1%, $P < 0$ 0.01). Of note, one patient with CM had a CD4 > 200 cells/mm3; he presented with a deep venous thrombosis, pulmonary embolism, and heart failure, and his only sign consistent with CM was fever. Patients with a positive LFA were also more like to present with symptoms of headache (83% versus 34%, $P < 0.01$) or neck stiffness (39% versus 8%, $P < 0.01$) or have meningismus (28% versus 8%, $P < 0.01$) as compared with patients with a negative serum LFA. Further comparisons are shown in Table 1.

In multivariate analysis, a CD4 < 100 cells/mm^3 was associated with an increased risk (adjusted odds ratio [aOR] = 6.12, 95% CI = 1.42–26.32) of having cryptococcal disease based on a positive serum LFA test as was the presence of headache (aOR = 7.98, 95% CI = 1.76–36.24) or neck stiffness (aOR = 4.76, 95%CI = 1.14–19.81) (Table 4). Female gender was associated with a decreased risk for cryptococcal antigenemia (aOR = 0.16, 95% CI = 0.04–0.61) (Table 4).

TABLE 3
Performance of the cryptococcal fingerstick and urine lateral flow assays as compared with the serum cryptococcal lateral flow assay

	Fingerstick LFA	Urine LFA
True negative	180	179
True positive	17	12
False positive	0	1
False negative	1	6
Sensitivity	94.4 (84–100)	66.7 (45–88)
Specificity	100	99.4 (98–100)
Positive predictive value	100	92.3 (77–100)
Negative predictive value	99.5 (98–100)	96.7 (93–99)
Kappa	0.97 (0.91–1.00)	0.76 (0.58–0.93)

LFA = lateral flow assay. Values in parentheses indicate 95% confidence intervals.

Clinical signs and symptoms and antigenemia. The predictive value of three candidate symptoms (reported fever, headache, and neck stiffness) and two signs (documented fever [≥ 37.6 C° axillary temperature] and meningismus) in detecting cryptococcal antigenemia are shown in Table 5. The large majority of study patients (142 of 198, 72%) had at least one of the clinical symptoms or signs, including 17 of 18 patients (94%) with cryptococcemia. The one patient with cryptococcemia and none of the candidate clinical symptoms or signs had an LFA titer of 1:40, a negative CSF LFA and India ink microscopy, and presented with sudden right visual loss. The sensitivity of any one symptom or sign ranged from 28% to 83%, whereas the specificity ranged from 39% to 92%. Although the PPV of any individual or combination of symptoms and signs was low, the NPV of headache and a combination of all signs and symptoms was 98%. With regard to CM, absence of reported or documented fever and headache as well as an absence of all candidate signs and symptoms both had a 100% NPV. If only patients with one of the candidate clinical signs or symptoms were tested for cryptococcal antigenemia, the number needed to test to detect one case would be nine (142/17).

Outcomes. A total of 61 (31%) of 198 HIV-infected patients died during the hospitalization; mortality did not significantly differ between those with and without cryptococcal disease (6 [33%] of 18 versus 55 [31%] of 180, $P = 0.81$). Sixteen patients received treatment with fluconazole (either 1,200 or 1,600 mg daily dose for patients with CM), whereas two patients were treated with amphotericin B. The two patients with cryptococcemia without meningitis survived. They were both receiving ART at the time of admission, and were treated with fluconazole 400 mg daily while in the hospital.

DISCUSSION

Utilizing a universal screening approach, we found a high prevalence (9.1%) of cryptococcal disease and meningitis

TABLE 4
Univariate and multivariate logistic regression analysis for risk factors for a positive serum cryptococcal LFA

Risk factor	Odds ratio	95% CI	*P* value	aOR	95% CI	*P* value
Age, per year	0.97	0.92–1.02	0.29	0.95	0.88–1.02	0.17
Female	0.40	0.14–1.16	0.09	0.16	0.04–0.61	**< 0.01**
Known CD4 < 100 at time of admission	6.39	1.79–22.86	< 0.01	6.12	1.42–26.32	**0.02**
No ART or on ART < 3 months at baseline	0.62	0.23–1.68	0.35			
History of cryptococcal disease	11.13	1.47–84.33	0.02			
Baseline symptoms						
Headache	9.52	2.65–34.12	< 0.01	7.98	1.76–36.24	**< 0.01**
Neck stiffness	7.55	2.52–22.52	< 0.01	4.76	1.14–19.81	**0.03**
Altered mental status	2.23	0.81–6.12	0.12			
Photophobia	1.27	0.15–10.73	0.83			
Fever	0.70	0.27–1.84	0.47			
Nausea	1.79	0.68–4.75	0.24			
Vomiting	1.96	0.73–5.30	0.18			
Blurry vision	3.40	0.84–13.71	0.09			
Night sweats	0.65	0.24–1.82	0.42			
Cough	0.55	0.20–1.52	0.25			
Shortness of breath	0.39	0.11–1.40	0.15			
Baseline signs						
Meningismus	4.23	1.32–13.48	0.01			
Febrile	1.70	0.62–4.64	0.30			
Cranial neuropathy	1.12	0.13–9.36	0.92			
Baseline laboratory values						
Hgb < 10 g/dL	1.31	0.49–3.46	0.59			
WBC < 4 K/μL	2.23	0.81–6.12	0.12	2.71	0.80–9.20	0.11

aOR = adjusted odds ratio; ART = antiretroviral therapy; CI = confidence interval; Hgb = hemoglobin; LFA = lateral flow assay; WBC = white blood cells. *P* values in bold denote statistical significance.

(8.1%) among HIV-infected persons hospitalized at a university-affiliated hospital in Addis Ababa, Ethiopia. Among patients with signs and/or symptoms of meningitis, the prevalence of cryptococcal disease was much higher (12%), and screening only this group would have captured 94% (17 of 18) of patients with cryptococcal disease including all patients (16 of 16, 100%) with CM. Furthermore, we demonstrated that the cryptococcal LFA performed extremely well using fingerstick blood samples as compared with serum and thus, this may offer an attractive, less-invasive approach to rapidly diagnosing patients with cryptococcal disease and triaging which patients need a lumbar puncture for diagnosis and treatment of CM. Our findings argue for the implementation of a broader testing approach for cryptococcal disease among HIV-infected patients admitted to the hospital. A rapid scale-up of the low-cost cryptococcal LFA (~$2 USD) should vastly improve the diagnosis and hence management of cryptococcal disease in RLS.

A universal cryptococcal screening strategy among HIV inpatients has only been reported in one other study, which was carried out in a Tanzanian referral hospital.[11] In this prospective study, 333 HIV-infected patients were screened with an LA test, and 17 patients were found to have cryptococcemia, 15 with CM.[11] It is unclear if our higher rate of cryptococcal disease was due to a higher background burden of *Cryptococcus* in the community or if it was mostly related to differences in HIV presentation and ART use. Interestingly, our prevalence (9.1%) of cryptococcal disease was similar to that found in a prior study evaluating the rate of cryptococcemia (8.4%) among HIV outpatients in Addis Ababa.[16] Other studies evaluating cryptococcal disease among hospitalized HIV patients have targeted select populations including tuberculosis (TB) suspects, meningitis suspects, and patients with pneumonia. A multisite study in South Africa and Uganda screened over 800 hospitalized HIV-infected persons with suspected meningitis and found 63% had CM.[17] In contrast, retrospective cryptococcal screening studies among TB suspects in Uganda (6 and 7%) and patients with pneumonia in Thailand (13%) found lower but still high rates of cryptococcal disease albeit most was nonmeningeal disease.[12,18,19] What is clear from the above studies including ours is that rates of cryptococcal

TABLE 5
The performance of certain signs and symptoms in detecting patients with cryptococcal antigenemia and cryptococcal meningitis

	Sensitivity (%)		Specificity (%)		PPV (%)		NPV (%)	
Sign(s)/symptom(s)	CrAg	CM	CrAg	CM	CrAg	CM	CrAg	CM
Fever	56	56	39	40	8	8	90	91
Headache	83	88	66	65	20	18	98	98
Neck stiffness	39	44	92	92	33	33	94	95
Meningismus	28	31	92	92	28	25	93	94
Fever* or headache	94	100	31	31	12	11	98	100
Fever* or stiffness	78	81	38	38	11	10	94	96
Fever* or meningismus	67	69	38	39	10	9	92	93
All four symptoms	94	100	31	31	12	11	98	100

CM = cryptococcal meningitis; CrAg = cryptococcal antigenemia; NPV = negative predictive value; PPV = positive predictive value.
*Fever = subjective history of fever or documented fever (≥ 37.6°C axillary temperature).

disease are high in RLS, and further strategies to optimize screening approaches among hospitalized HIV-infected patients are needed.

We analyzed various combinations of signs and symptoms to evaluate whether targeted cryptococcal testing of certain patients may be of higher yield than universal screening. Such an approach has been implemented in screening for pulmonary TB among HIV-infected persons in RLS, where the absence of four symptoms has been found to have a high NPV for disease and thus abdicates the need for further workup.[20,21] We chose symptoms and signs that are characteristic of CM and readily attainable by clinicians. Although the specificity and PPV were low, the absence of fever (reported or documented), neck stiffness, headache, and meningismus had a 98% NPV for cryptococcemia and 100% for CM. Although not calculated, the cryptococcal screening study from Tanzania supports our findings as all patients with CM had at least two of the four following symptoms: fever, headache, neck stiffness, and altered mental status.[11] By limiting cryptococcal screening to patients with at least one candidate sign or symptom, we would have reduced the number needed to test to diagnose one case of cryptococcal disease from 11 to nine patients. Given the LFA cost in RLS is approximately $2 USD, this would equate to an $18 USD test cost to diagnose one case of cryptococcal disease. Similar to Boulware and others, we were also able to show the LFA can easily be implemented in a busy hospital ward laboratory and thus, additional labor costs should be minimal.[17] We also confirmed the well-known risk factor for cryptococcal disease of CD4 count < 100.[3,4] Further limiting screening patients with CD4 < 100 may be a cost-effective approach in settings with low resources. Although a clinical screening algorithm would simplify cryptococcal screening, it would likely miss some cases. Our patient with a CD4 count of 216 cells/mm^3 and presenting with a deep vein thrombosis, pulmonary embolus, and congestive heart failure who was found to have CM would have not been tested using our screening algorithm. A clinical screening algorithm would be most useful for CM as many cases of cryptococcemia and cryptococcal pneumonia lack traditional signs and symptoms of cryptococcal disease as demonstrated in studies performed among TB suspects and patients with pneumonia.[12,18,19] Multisite studies in RLS would be helpful to validate optimal clinical screening algorithms.

Our study is only the second published study to evaluate the performance of the LFA using a fingerstick whole blood sample. From a prior study among HIV-infected patients with suspected meningitis from Uganda, fingerstick blood LFA results were 100% in agreement with serum and plasma LFA results and also had a 100% NPV in ruling out CM.[8] Similarly, we found excellent agreement between fingerstick blood and serum LFA results (κ = 0.97) and a 100% NPV for CM. In addition, given we had a large population without meningitis or cryptococcal disease, we were also able to show the fingerstick LFA had a 100% specificity for cryptococcal disease. Once approved for use on fingerstick whole blood samples, the LFA can be implemented at bedside to help determine which patient needs a lumbar puncture. Early CSF pressure management in patients with CM may improve outcomes as indicated by recent studies.[22,23] In contrast to the excellent performance of fingerstick whole blood, there were six patients with negative urine LFA as compared with serum results (sensitivity 67%). This is in line with one prior study (70%) and on the lower end of the sensitivity range from a recent meta-analysis (70–98%).[5,7] The lower sensitivity in urine is likely due to lower quantities of CRAG in the urine, which were up to 22-fold lower compared with serum among patients with CM.[6] A more recent study casts further doubt on the utility of urine LFA testing finding a sensitivity of 57% as compared with the serum cryptococcal LFA as well as 16 patients with a positive urine and negative serum LFA, which were considered to be false-positive tests.[24] Our study also supports the superiority of CSF LFA to India ink microscopy in diagnosing CM. If India ink microscopy were used alone, 15% of cases would have been missed. Our sensitivity of 85% is similar to that found in a multisite validation study which found India ink microscopy to have an 86% sensitivity in diagnosing CM, which decreased to < 50% among persons with a low fungal burden by culture.[17]

Our study is subject to limitations including limited sample size, and lack of culture and titers for all specimen types. Numerous studies have shown the serum LFA has excellent accuracy in diagnosing cryptococcal disease compared with culture, and culture is not available in most RLS.[13,17,25,26] Performing titers for positive urine and fingerstick specimens would have allowed us to evaluate the correlation between specimen types; however, they were not performed due cost constraints. In addition, our study was performed at only one hospital thus potentially limiting the generalizability of the findings to other hospitals in Ethiopia and other RLS countries.

In conclusion, using a universal screening approach and a novel POCT, we found a high prevalence of CM among hospitalized HIV-infected patients in Ethiopia. Our data also suggest a more targeted screening approach that combines CD4$^+$ cell count threshold and a clinical algorithm and utilization of less-invasive samples such as fingerstick blood may be an effective method to optimize cryptococcal screening in the inpatient setting. The advent of the LFA marks a new era in cryptococcal diagnosis and, with rapid scale, could have a significant impact on improving CM management.

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