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MAJOR ARTICLE

Evaluation of a Cryptococcal Antigen Lateral Flow Assay and Cryptococcal Antigen Positivity at a Large Public Hospital in Atlanta, Georgia

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Background. *Cryptococcus neoformans* is a major cause of morbidity and mortality among human immunodeficiency virus (HIV)-infected persons worldwide, and there are scarce recent data on cryptococcal antigen (CrAg) positivity in the United States We sought to determine the frequency of cryptococcal disease and compare the performance of a CrAg lateral flow assay (LFA) versus latex agglutination (LA) test.

Methods. All patients from Grady Health System in Atlanta who had a serum or cerebrospinal fluid (CSF) sample sent for CrAg testing as part of clinical care from November 2017 to July 2018 were included. Percentage positivity and test agreement were calculated.

Results. Among 467 patients, 557 diagnostic tests were performed; 413 on serum and 144 on CSF. The mean age was 44 years, and most were male (69%) and had HIV (79%). Twenty-four (6.4%, 95% confidence interval [CI] = 4.1–9.4) patients were serum CrAg positive, and 8 (5.8%, 95% CI = 2.6–11.2) individuals tested positive for CSF CrAg. Although overall agreement between the LA and LFA was substantial to high for CSF ($\kappa = 0.71$, 95% CI = 0.51–0.91) and serum ($\kappa = 0.93$, 95% CI = 0.86–1.00), respectively, there were important discrepancies. Five patients had false-positive CSF LA tests that affected clinical care, and 4 patients had discordant serum tests.

Conclusions. We found a moderately high proportion of cryptococcal disease and important discrepancies between the LA test and LFA. Clinical implications of these findings include accurate detection of serum CrAg and averting unnecessary treatment of meningitis with costly medications associated with high rates of adverse events.

Keywords. *Cryptococcus*; diagnostics; HIV; meningitis.

Cryptococcus neoformans, an environmental fungal pathogen, is a common opportunistic infection in people with human immunodeficiency virus (HIV) [1]. Globally, the prevalence of cryptococcal antigenemia among persons with HIV and a CD4 count ≤100 cells/mm^3 is estimated to be 6% [2]. Cryptococcal disease causes considerable morbidity and mortality worldwide, accounting for up to 15% of HIV-related deaths [3]. There exist scarce recent data on cryptococcal disease in the United States, especially in the Southeastern United States, where HIV rates are high. Previous studies have estimated that approximately half of all HIV diagnoses in the United States are located in the Southeast, with an HIV diagnosis rate of 25.7 per 100 000 person-years in Georgia [4]. A recent study of individuals with acquired immune deficiency syndrome (AIDS) in the United States found a prevalence of cryptococcal infection of 2.9%; however, there were no Southeastern sites included [5]. Another study found significant regional variation of cryptococcal disease across the United States, with an estimated incidence in Georgia of 5.1 per 100 000 persons [6]. However, although this study provided needed epidemiological information, it utilized data from 2000 to 2007; therefore, updated US estimates, especially for the Southeastern region, are needed.

A major contributor to the high morbidity and mortality of cryptococcal disease is delays in diagnosis of serum cryptococcal antigenemia, which, if untreated, can lead to cryptococcal meningitis (CM), the most serious manifestation of disease [7]. Early detection and prompt treatment has been shown to prevent the development of CM [8, 9]. Although the prevalence of cryptococcal antigenemia in the United States is lower than that in low-to-middle income settings, prior studies have found that cryptococcal screening is cost effective in settings with a prevalence ≥2% [10, 11]. This highlights the importance of measuring the burden of cryptococcal antigen (CrAg) in the United States

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Cryptococcal antigen testing can be performed using either a latex agglutination (LA) test or a lateral flow assay (LFA). The LFA is a point-of-care test developed in 2009 by IMMY, Inc. (Norman, OK) that has increased the feasibility of implementing CrAg testing. It is an immunochromatographic test for the qualitative or semiquantitative detection of *Cryptococcus* species polysaccharide antigens in serum or cerebrospinal fluid (CSF). The LFA is rapid, inexpensive, and does not require refrigeration. In contrast, the LA test requires more extensive laboratory infrastructure, refrigeration, and can take up to 45 minutes to perform. In validation studies, the LFA has performed extremely well, showing up to 100% accuracy in detecting true-positive and true-negative cases [12].

There are currently no recent data on the performance of the LFA and the frequency of cryptococcal antigenemia in a large US city with a high burden of HIV infection. These data are needed to determine the utility of cryptococcal screening in the United States and in other similar settings. In this study, we sought to determine the proportion of cryptococcal disease among those tested within the Grady Health System and compare the performance of a CrAg LFA versus LA test.

METHODS

Study Participants

Participants consisted of patients ≥18 years seen at Grady Memorial Hospital or the Infectious Diseases Program Clinic (IDP), which is a free-standing HIV clinic in Atlanta, Georgia. All persons with a serum or CSF sample sent for CrAg testing as part of routine management by their provider at IDP between November 2017 and July 2018 were included. Routine practice recommends that patients enrolling or re-enrolling into the clinic with a CD4 count ≤150 cells/mm^3 should be screened for cryptococcal disease. Patients admitted to Grady Memorial Hospital who had a CrAg test performed as part of their work-up were also included.

Patient Consent Statement

This study was approved by the Emory University Institutional Review Board and the Grady Research Oversight Committee. Participant consent was waived because samples were collected for routine purposes.

Latex Agglutination Test

The LA test was performed according to the manufacturer's protocol and was the standard of care test in the Grady microbiology laboratory. The test utilizes latex particles coated with anti-CrAg antibodies. The CSF and serum samples were pretreated either with a heat treatment or incubated with a pronase solution, respectively. Samples were mixed with cryptococcal latex, and results were read. Specimens displaying granulations or clumps were compared against the manufacturer's positive control and specimen positivity was assessed.

Lateral Flow Assay

The LFA was performed on samples concurrently with the LA test by research staff, and results were not available to clinicians. The LFA was performed on all samples sent for CrAg LA testing according to manufacturer's instructions. This dipstick test uses gold-conjugated, monoclonal antibodies laid on an immunochromatographic test strip to detect cryptococcal capsular polysaccharide glucuronoxylomannan antigen for all 4 *C neoformans* serotypes (A–D). These gold-conjugated antibodies bind to cryptococcal antigen [13]. To perform the LFA, 1 drop of diluent was added to a container of patient specimen. The dipstick was placed into a container and incubated for 10 minutes. Tests were performed with a positive and negative control, according to the manufacturer's package insert and protocol. Lateral flow immunoassay kits were donated by IMMY, Inc. for this study.

Study Design and Data Collection

This was a prospective observational cohort. Demographic and clinical data on participants were abstracted from patient charts onto a standardized data collection form and then entered into a Research Electronic Data Capture (REDCap) database [14, 15].

Definitions

Serum cryptococcal antigenemia and CM were defined by a positive CrAg test on a blood or CSF sample, respectively, from an individual with or without symptoms. False-positive test results were defined using the following criteria: negative by other CrAg test, negative blood cultures (if available), negative India ink (if available), and clinical syndrome inconsistent with disease as reviewed by 2 clinical infectious disease physicians.

Statistical Methods

Data were summarized using proportions and median and interquartile ranges (IQRs). Baseline characteristics for all groups compared within the cohort were analyzed using the *t* test for continuous variables and χ^2 test for categorical variables. Proportions of disease were calculated. A kappa statistic was used to measure the agreement between the CrAg LFA and LA test with a value of 1 denoting perfect concordance and a value of 0 denoting agreement by chance alone. Statistical analyses were performed using SAS software, version 9.4 (SAS Institute Inc., Cary, NC) and R version 3.6.1 [16].

RESULTS

Between November 2017 and July 2018, a total of 467 patients underwent diagnostic testing for cryptococcal disease with serum and/or CSF antigen testing. The median age of participants was 44 years, and the majority (69%) were male (Table 1). At the time of diagnostic testing, most patients (62%) were hospitalized, with the remaining 38% tested in the outpatient

setting. Approximately 80% of individuals had HIV, with a median CD4 count of 73 cells/mm^3 (IQR = 19–184) and a median log viral load of 4.4 (IQR = 1.9–5.2). Twenty-three percent of individuals with HIV were on antiretroviral therapy (ART) at the time of CrAg testing. Forty-three individuals (9%) were immunosuppressed for reasons other than HIV, with the majority due to chronic diseases including kidney disease, hepatitis C infection, and systemic lupus erythematosus (SLE). There were 53 patients (11%) tested for CrAg who had no known risk factors or causes for immunosuppression.

The majority of individuals with HIV had CrAg testing performed only on their serum (80%), with 51 individuals who

Table 1. Demographic and Clinical Characteristics of Study Participants Tested for Cryptococcal Antigen

Characteristics	Total	(+) Serum CrAg[a]				Discordant Serum[b]	(–) CrAg[c]
		(+) CSF CrAg	(–) CSF CrAg	CSF CrAg NP	Discordant CSF[d]		
	N = 467	N = 8	N = 3	N = 11	N = 2	N = 4	N = 439
Age, median (IQR)	44 (32–54)	45 (42–55)	44 (39–45)	43 (30–53)	39 (29–48)	44 (32–51)	44 (32–54)
Male	322 (69)	6 (75)	3 (100)	8 (73)	2 (100)	3 (75)	301 (69)
Visit Type							
Hospitalized	290 (62)	8 (100)	2 (67)	4 (36)	2 (100)	2 (50)	272 (62)
Outpatient HIV Clinic	177 (38)	0 (0)	1 (33)	7 (64)	0 (0)	2 (50)	167 (38)
Re-enrollment visit	69 (15)	—	0 (0)	0 (0)	—	0 (0)	69 (16)
Follow-up visit	61 (13)	—	0 (0)	5 (45)	—	1 (25)	55 (13)
Urgent visit	38 (8)	—	1 (33)	2 (18)	—	1 (25)	34 (8)
Enrollment visit	9 (2)	—	0 (0)	0 (0)	—	0 (0)	9 (2)
HIV (+)	371 (79)	8 (100)	3 (100)	11 (100)	2 (100)	4 (100)	343 (78)
Receiving ART	107 (23)	2 (25)	1 (33)	5 (45)	0 (0)	2 (50)	97 (22)
CD4 count (cells/mm^3)[e], median (IQR)	73 (19–184)	18 (9–66)	14 (11–29)	124 (24–160)	56 (32–79)	21 (16–102)	73 (20–188)
Viral load (log_{10})[e], median (IQR)	4.4 (1.9–5.2)	4.4 (2.1–5.1)	3.9 (4.5–5.6)	4.4 (0–5.5)	4.7 (4.2–5.2)	0 (0–1.2)	4.5 (2.2–5.2)
Yrs. since HIV diagnosis, median (IQR)	9 (4,15)	18 (6–24)	2 (1–3)	6 (5–10)	12 (8–16)	16 (10–24)	9 (4–17)
Immunosuppressed[f]	43 (9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	43 (10)
Liver disease	9 (2)	—	—	—	—	—	9 (21)
Malignancy	5 (1)	—	—	—	—	—	5 (12)
Medications	2 (0.4)	—	—	—	—	—	2 (5)
HIV (–) and not immunosuppressed	53 (11)	—	—	—	—	—	53 (12)
Symptomatic[g,h]	—	7 (88)	1 (33)	7 (64)	2 (100)	3 (75)	—
Headache	—	6 (75)	1 (33)	1 (9)	1 (50)	2 (50)	—
Other[i]	—	5 (63)	1 (33)	7 (64)	2 (100)	3 (75)	—
Visual changes	—	3 (38)	0 (0)	0 (0)	0 (0)	0 (0)	—
Neck pain or stiff neck	—	2 (25)	0 (0)	0 (0)	0 (0)	0 (0)	—
Fever	—	1 (13)	0 (0)	2 (18)	0 (0)	2 (50)	—
(+) Blood culture	3 (0.6)	2 (25)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)
LP performed	142 (30)	8 (100)	3 (100)	0 (0)	2 (100)	1 (25)	128 (29)
(+) India Ink	1 (0.2)	1 (13)	0 (0)	—	0 (0)	0 (0)	0 (0)
(+) CSF culture	2 (0.4)	2 (25)	0 (0)	—	0 (0)	0 (0)	0 (0)
Had clinic visit before diagnosis[j]	—	7 (88)	0 (0)	8 (73)	2 (100)	4 (100)	—
Previous cryptococcal disease[g]	—	6 (75)	0 (0)	7 (64)	1 (50)	2 (50)	—
Cryptococcemia and meningitis	—	3 (38)	—	4 (36)	1 (50)	1 (50)	—
Cryptococcemia	—	0 (0)	—	3 (27)	0 (0)	1 (50)	—
Meningitis	—	3 (38)	—	0 (0)	0 (0)	0 (0)	—

Abbreviations: ART, antiretroviral therapy; CrAg, cryptococcal antigen; CSF, cerebrospinal fluid; HIV, human immunodeficiency virus; IQR, interquartile range; LP, lumbar puncture; NP, not performed.

NOTE: All values are shown as N (%) unless otherwise stated.

[a]Serum samples for which both the latex agglutination test and lateral flow assay were positive for cryptococcal antigen.

[b]Discordant serum samples in this study were all samples for which the latex agglutination (LA) test was negative and the lateral flow assay (LFA) was positive.

[c]Individuals who never tested positive for CrAg by either the LA test or LFA in their serum or CSF.

[d]Discordant CSF samples for which serum tested positive for CrAg were all samples that the LA test was negative and the LFA was positive.

[e]Values are from most recent date before or at CrAg testing.

[f]Immunosuppression for reasons other than HIV.

[g]Symptomaticity and cryptococcal disease history data were only collected for individuals who tested positive for CrAg.

[h]Symptomatic includes showing signs or symptoms including the following: fever, headache, neck pain, stiff neck, behavior change, visual changes, recent seizures.

[i]Other symptoms include the following: fatigue, nausea, vomiting, abdominal pain, night sweats, cough, dizziness, chest pain.

[j]Had at least 1 clinic visit in the previous 6 months.

had diagnostic testing performed on both serum and CSF (Figure 1). In contrast, those who were immunosuppressed for reasons other than HIV mainly had testing performed on CSF (60%). Likwise, testing was mainly performed on CSF (77%) for individuals with no cause for immunosuppression.

Among 467 individuals who had a diagnostic test, there were 557 samples tested using both the LA test and LFA (Figure 2). Four hundred thirteen tests were performed on serum and 144 tests were performed on CSF. Eighty-nine of the 140 individuals with a CSF sample tested did not have an associated serum test performed. Four serum and 9 CSF samples were tested with only the LA test, without a corresponding LFA.

There were 375 individuals who had both an LA test and LFA performed on their serum, and 24 tested positive with both methods, resulting in a proportion of serum cryptococcal antigenemia of 6.4% (95% CI = 4.1–9.4) (Table 1). There were 4 tests in which the LFA was positive and the LA test was negative, resulting in a proportion of serum cryptococcal antigenemia of 7.5% (95% CI = 5.0–10.6) using the LFA. Likewise, of 137 individuals who had diagnostic testing performed on their CSF, 8 tested positive with both methods, resulting in a proportion of CM of 5.8% (95% CI = 2.6–11.2). Overall, agreement between the 2 tests was high for serum (κ = 0.93, 95% CI = 0.86–1.00) and substantial for CSF (κ = 0.71, 95% CI = 0.51–0.91). Four individuals with HIV had discordant serum tests in which the LA test was negative and the LFA was positive (all with titers of 1:5). Two of these patients were on ART, previously diagnosed with cryptococcemia (1 also had CM), and were on fluconazole at the time of testing. The other 2 were not on ART and had CD4 counts <30 cells/mm^3; they had symptoms compatible with disease (ie, fever and headache) but were lost to follow-up after testing.

All patients who tested positive for CrAg had HIV, with similar demographics as the overall cohort (Table 1). The proportion of serum cryptococcal antigenemia and CM among individuals with HIV was 7.0% and 12.7%, respectively. In hospitalized patients with HIV, 8% of serum and 14% of CSF CrAg tests were positive based on both the LA test and LFA; 7% of serum tests were positive among individuals with HIV seen in clinic. Among those diagnosed with serum cryptococcal antigenemia, one third were on ART at the time of diagnostic testing, and median CD4 count ranged from 14 cells/mm^3 for individuals who also had a negative CSF CrAg to 124 cells/mm^3 for individuals who did not have testing performed on their

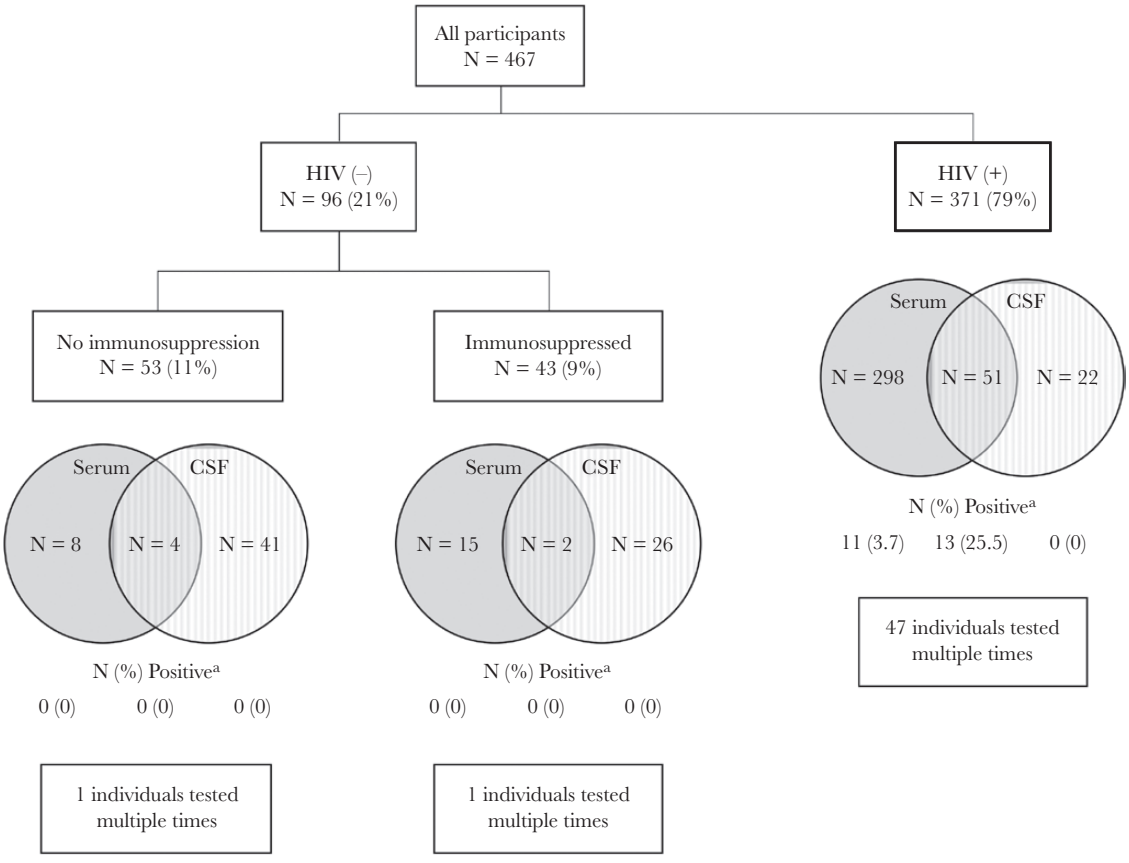
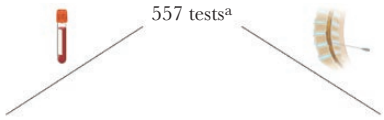


Figure 1. Distribution of study population and type of sample tested for cryptococcal antigen. All individuals had cryptococcal antigen diagnostic testing performed on serum and/or cerebrospinal fluid (CSF) samples, with several individuals tested multiple times over the study period. [a]Samples for which both the latex agglutination test and lateral flow assay were positive for cryptococcal antigen. HIV, human immunodeficiency virus.



Serum CrAg LA Test	Serum CrAg LFA (–)	(+)	Total
(–)	381	4	385
(+)	0	28	28
Total	381	32	413

CSF CrAg LA Test	CSF CrAg LFA (–)	(+)	Total
(–)	127	2	129
(+)	5	10	15
Total	132	12	144

Figure 2. Cryptococcal antigen test results. [a]Observations in which both the latex agglutination (LA) test and lateral flow assay (LFA) were performed. The figure was created with BioRender.com. CrAg, cryptococcal antigen; CSF, cerebrospinal fluid.

CSF. Among those with CM, one quarter were on ART, median CD4 count was 18 cells/mm^3, and median log viral load was 4.4. Symptoms were present in 71% of patients with serum cryptococcal antigenemia and 88% of patients with CM, with the most common symptoms being headache, and an "Other" category that included symptoms such as fatigue, nausea, vomiting, and abdominal pain. Asymptomatic disease was present in (1) 36% of patients seen in clinic, (2) 29% of those with serum cryptococcal antigenemia, and (3) 12% of those with CM. Blood cultures were positive in 12% of those with serum CrAg. Blood and CSF cultures were positive in 25% of those with CM.

A lumbar puncture (LP) was performed in 13 individuals with serum cryptococcal antigenemia; 8 individuals tested positive for CSF CrAg, and 3 tested negative. Two individuals had discordant CSF tests, with a positive LFA (titers of 1:5) and a negative LA test. One patient was previously treated for CM, with persistent detectable serum CrAg. The other individual tested positive for CSF CrAg by the LA test 9 months later. Of the 11 individuals with serum cryptococcal antigenemia who did not have an LP performed, 7 had a history of cryptococcemia (4 also had CM), 7 were symptomatic at the time of testing, and 9 were engaged in care at IDP after their positive test.

Titers for the LA test ranged from 1:4 to 1:512 in serum samples and 1:1 to 1:512 in CSF samples (Figure 3). There was a similarly wide range for LFA titers, with 1:10 to 1:2560 in serum and 1:5 to 1:2560 in CSF. All serum CrAg LFA titers were at 1:40 or above in patients who tested positive for CSF CrAg by the LFA.

Among CSF samples, there were 5 discordant tests in which the LA test was positive and the LFA was negative. These 5 LA tests were deemed to be false positives after negative CSF LFA testing, India Ink, CSF and fungal cultures, and clinical review and management of the cases (Figure 4). Serum CrAg testing for these patients was either negative or not performed. One patient was HIV negative and had SLE, whereas the remaining 4 were HIV positive. All individuals were hospitalized and initiated on treatment with amphotericin and flucytosine. Two patients had long hospital and treatment courses before confirmation of a false-positive test. One patient was treated in the hospital for 20 days before treatment was stopped. The other patient received 10 days of treatment in the hospital before being discharged on maintenance fluconazole that was halted approximately 2 weeks later after confirmation of his false-positive test during a follow-up clinic visit. Two patients were treated for ≤1 week, and 1 patient experienced a severe anaphylactic reaction to amphotericin that resulted in death while in the hospital.

DISCUSSION

In this study, we found a moderately high proportion of cryptococcal disease among all patients tested in our healthcare system with 24 individuals (6.4%; 95% CI = 4.1–9.4) diagnosed with serum cryptococcal antigenemia and 8 individuals (5.8%; 95% CI = 2.6–11.2) diagnosed with CM. Cryptococcal antigenemia was diagnosed only among patients with HIV, with high proportions seen in both the inpatient (8%) and outpatient (7%) settings. One third of those diagnosed with serum cryptococcal antigenemia and one quarter of those diagnosed with CM were on ART. Furthermore, the utilization of 2 different testing platforms resulted in important discrepancies, many with clinical implications. This study contributes to the limited data available examining the frequency of cryptococcal antigenemia in the Southeastern United States, despite the high rates of HIV in this region.

Important discrepancies existed between the LA test and LFA, with the LFA appearing to be more sensitive for serum CrAg and more specific for CM. Studies comparing the sensitivity and specificity of the 2 diagnostic tests have shown that the LFA has the best performance across both measures [17]. In our study, the LFA was able to rule out CM in 5 patients determined to have a false-positive LA. Likewise, the LFA was positive for 4 serum samples with titers of 1:5, whereas the LA test was negative; these were likely true positives for the 2 individuals with history of cryptococcal disease. Available data suggest that the LFA has better sensitivity at lower concentrations of CrAg compared with the LA test [18], which may result in a false-negative LA test result. False-negative LA tests have also been observed in cases of *Cryptococcus gatti*

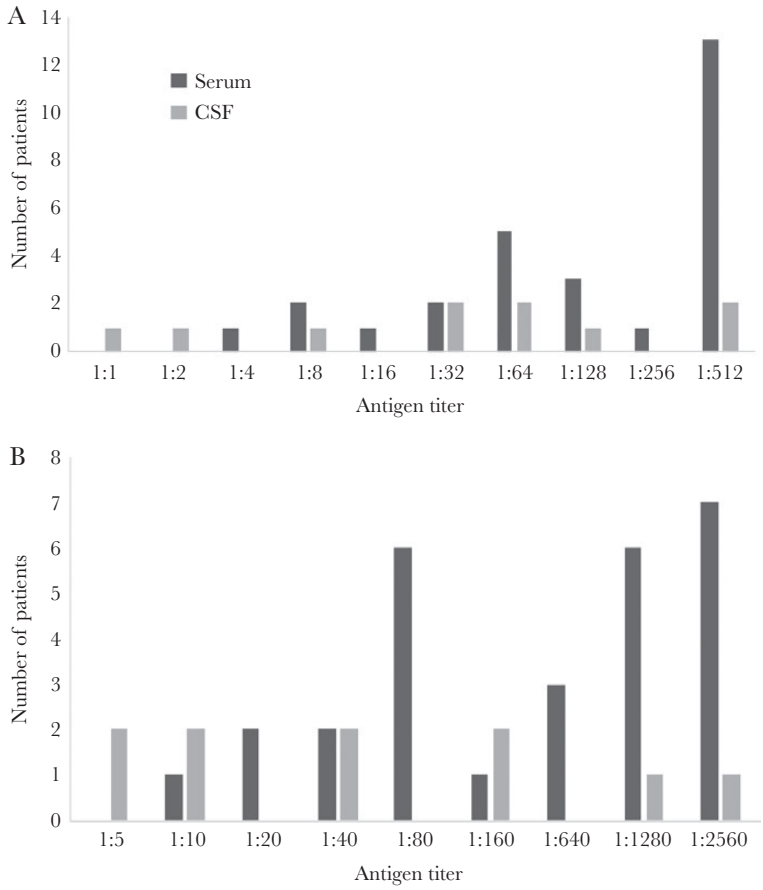


Figure 3. Distributions of cryptococcal antigen titers. (A) The latex agglutination test and (B) lateral flow assay diagnostic tests. N = 28 for tests performed on serum, and N = 10 for tests performed on cerebrospinal fluid (CSF).

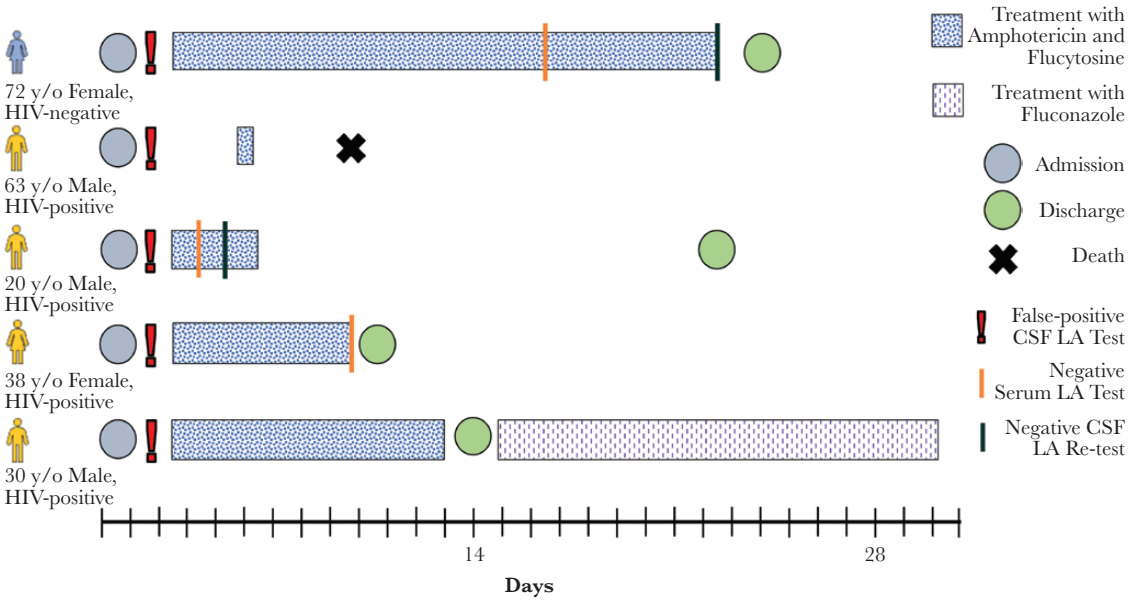


Figure 4. Hospitalization courses for 5 patients with false-positive cerebrospinal fluid (CSF) cryptococcal antigen (CrAg) latex agglutination (LA) tests. All patients had a negative CSF CrAg lateral flow assay (LFA), blood cultures, India ink, and CSF fungal cultures. Serum CrAg was either negative (N = 3) or not performed (N = 2). Two infectious disease physicians confirmed each false-positive result. HIV, human immunodeficiency virus; y/o, year-old.

infection, because the test has significantly reduced sensitivity for serotype C compared with other serotypes [19, 20]. Of note, in one study evaluating the accuracy of low-titer (1:2 and 1:5) LFA results, 34% were considered falsely positive, cautioning prompt interpretation of similar results [21]. Because the other 2 patients with low LFA titers and a negative LA test in our study both had advanced HIV, were not on ART, and presented with symptoms consistent with disease, it is likely that these tests were true positives. With regard to the false-positive LA tests in our study, it has been shown that the LA test displays cross-reactivity to other pathogenic fungi or minor contamination [22, 23]. Furthermore, there is higher potential for reader error because reading is more subjective, and consequences of missing CM are significant because delays in diagnosis are associated with mortality and long-term neurological deficits [24–26].

Taken together, these findings suggest that the LFA is able to perform better than the LA test when testing for cryptococcal disease. Furthermore, given the limited ability of India Ink, blood cultures, or CSF cultures to determine infection in this study, the LFA was the only test indicating infection for cases with discordant LA test and LFA results.

The majority of individuals with serum cryptococcal antigenemia and/or CM were not on ART at the time of testing. Median CD4 counts for those who tested positive for serum CrAg and who also had an LP performed were ≤100 cells/mm^3. These results highlight the importance of ART in the prevention of disease, and they also highlight a group at higher risk for disease that may benefit from expanded screening practices. Previous studies have shown significant benefit in screening individuals for CrAg who have CD4 counts ≤100 cells/mm^3 before ART initiation [27, 28]. Increased screening in this group can help to identify individuals at risk of CM, reducing both time to treatment and mortality.

Our study comprised mainly individuals with HIV who were not on ART at the time of testing, with over half of testing performed in the inpatient setting, which is a unique study population when comparing these results to previous US studies on cryptococcal disease. Patients more likely to have disease may have been tested more frequently in this study, although comparable proportions of positive serum CrAg were observed in the outpatient setting. With this in mind, we found higher estimates compared with other US-based estimates. Among patients with AIDS from 1986 to 2012, prevalence of CrAg positivity was 2.9% [5]. It is notable that the prevalence of cryptococcal antigenemia among patients with HIV in Ethiopia is estimated to be 8.4% [29], 8.1% in Argentina [30], 7% in South Africa [27], and 5% in the United Kingdom [31]. We found that 29% of individuals with serum cryptococcal antigenemia and 12% of those with CM were asymptomatic at the time of testing. For both serum cryptococcal antigenemia and CM, only 12% of people presented with a fever. Likewise, one quarter of patients with CM presented with a stiff neck or neck pain. Asymptomatic CrAg positivity has been observed in several other high-burden HIV settings [32, 33]. The variable clinical presentation and lack of CNS symptoms in many cases highlights that routine CrAg screening may be beneficial in certain settings.

We found that all patients with serum CrAg LFA titers at 1:40 or above also had CM. This highlights the potential for setting a cutoff for serum CrAg titers at which an LP should be performed to screen for CM, before the manifestation of meningitis symptoms. There is evidence to suggest the utility of a cutoff in other settings [9, 34, 35]. In a study from South Africa, approximately one third of asymptomatic CrAg-positive patients had CM, and serum CrAg titers could be used as guidance in determining who to target for an LP to avoid mortality due to undiagnosed CM [33]. This study found that a titer cutoff of 1:40 in asymptomatic patients had a sensitivity of detecting concurrent CM of 97.1% [33].

Among patients tested who were immunosuppressed for reasons other than HIV, none were diagnosed with cryptococcal disease—excluding 1 patient who had a false-positive CSF CrAg test. In a similar analysis in the Southeast, researchers found that 36% of individuals with cryptococcosis did not have HIV nor were a transplant recipient [36]. This highlights the variation in disease distribution, even within the Southeast. Furthermore, no patients with no known cause for immunosuppression were diagnosed with cryptococcal disease in our study. Cryptococcal testing among patients with no known cause for immunosuppression and immunosuppressed for reasons other than HIV is relevant for diagnostic stewardship [37, 38]. To avoid these situations, being more cognizant about important high-risk groups in the population in which one is working can aid in narrowing down who clinicians decide to test to maximize pretest probability.

There are several limitations to our study. First, because information was abstracted from patient charts, not all variables were captured for all participants. Second, the decision to test patients for cryptococcal antigenemia likely varied by physician; however, due to the total number tested in our study and the high awareness of cryptococcal disease in our setting, it is unlikely that many cases were missed. Third, due to how patients were sampled, we were unable to estimate the true prevalence of cryptococcal disease, and we were only able to calculate the frequency of positive tests among the individuals tested in our setting. Fourth, our study population is unique, and it is important to note that there were no positive CrAg tests among individuals without HIV. This limits the applicability of these results to centers in which cryptococcal disease may be more frequently detected among other immunosuppressed populations, such as a large transplant center [39]. Finally, not all symptomatic individuals with positive serum CrAg had an LP performed, which precluded us from determining risk factors for progression to and concurrent CM.

CONCLUSIONS

The results from our study show that there is a moderately high proportion of cryptococcal disease among people with advanced HIV in Atlanta. Screening for cryptococcal disease is an important priority among this population to avoid serious manifestations of disease. Clinical implications of these findings include accurate detection and treatment of serum CrAg and averting unnecessary treatment of meningitis with costly medications associated with high rates of adverse events. Finally, it is crucial to consider the important risk groups in the setting in which one is working to avoid testing and treating those with a low probability of disease.

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