Evaluation of Fingerstick Cryptococcal Antigen Lateral Flow Assay in HIV-Infected Persons: A Diagnostic Accuracy Study

Darlisha A. Williams,[1,2] Tadeo Kiiza,[2] Richard Kwizera,[2] Reuben Kiggundu,[2] Sruti Velamakanni,[1] David B. Meya,[1,2,3] Joshua Rhein,[1,2] and David R. Boulware[1]

[1]University of Minnesota, Minneapolis; [2]Infectious Diseases Institute, and [3]College of Health Sciences, Department of Medicine, Makerere University, Kampala, Uganda

Background. *Cryptococcus neoformans* is the most common cause of adult meningitis in sub-Saharan Africa. The cryptococcal antigen (CRAG) lateral flow assay (LFA) has simplified diagnosis as a point-of-care test approved for serum or cerebrospinal fluid (CSF). We evaluated the accuracy of the CRAG LFA using fingerstick whole blood compared with serum/plasma and CSF for diagnosing meningitis.

Methods. From August 2013 to August 2014, CRAG LFA (IMMY, Norman, Oklahoma) tests were performed on fingerstick whole blood, plasma/serum, and CSF in 207 HIV-infected adults with suspected meningitis in Kampala, Uganda. Venous blood was also collected and centrifuged to obtain serum and/or plasma. CSF was tested after lumbar puncture.

Results. Of 207 participants, 149 (72%) had fingerstick CRAG-positive results. There was 100% agreement between fingerstick whole blood and serum/plasma. Of the 149 fingerstick CRAG-positive participants, 138 (93%) had evidence of cryptococcal meningitis with a positive CSF CRAG. Eleven participants (5%) had isolated cryptococcal antigenemia with a negative CSF CRAG and culture, of whom 8 had CSF abnormalities (n = 3 lymphocytic pleocytosis, n = 5 elevated protein, n = 4 increased opening pressure). No persons with cryptococcal meningitis had negative fingersticks.

Conclusions. The 100% agreement between whole blood, serum, and plasma CRAG LFA results demonstrates that fingerstick CRAG is a reliable bedside diagnostic test. Using point-of-care CRAG testing simplifies screening large numbers of patients and enables physicians to prioritize on whom to measure CSF opening pressure using manometers.

Keywords. cryptococcal meningitis; cryptococcus; lateral flow assay; HIV; point-of-care systems.

Cryptococcal meningitis is the most common cause of adult meningitis in Africa and causes 20%–25% of AIDS-related deaths [1–4], accounting for 30%–60% of adult meningitis cases overall in the general population in eastern and southern Africa [3–7]. Typically, cryptococcal meningitis is diagnosed by performing a lumbar puncture (LP) with testing of cerebrospinal fluid (CSF) by either India ink microscopy, culture, or cryptococcal antigen (CRAG) [8, 9].

In 2011, a CRAG lateral flow immunochromatographic assay (LFA) (IMMY, Norman, Oklahoma), was approved by the US Food and Drug Administration. This point-of-care test has been validated in serum and CSF, with 99.3% sensitivity and >99.1% specificity in CSF [8]. Although the introduction of the CRAG LFA has made testing more feasible in resource-limited settings, a fundamental paradox remains

Received 5 January 2015; accepted 25 March 2015; electronically published 1 April 2015.

Correspondence: Darlisha A. Williams, MPH, Infectious Diseases Institute, Makerere University, P.O. Box 22418, Kampala, Uganda (darlisha@gmail.com).

Clinical Infectious Diseases® 2015;61(3):464–7

DOI: 10.1093/cid/civ263

that the diagnosis of cryptococcal meningitis cannot be confirmed until after the diagnostic LP. Intracranial pressure measurement and control are key components of cryptococcal management [10–12]. When the *Cryptococcus* diagnosis is made after the LP is completed, the opportunity to remove sufficient CSF volume to normalize the intracranial pressure is missed, unless manometers are always used. The known presence of CRAG in blood prior to obtaining a diagnostic LP shifts the pretest probability for cryptococcal meningitis significantly, alerting caregivers to the necessity of measuring LP opening pressure and allowing for more focused, cost-effective downstream CSF testing [3].

We hypothesized that performing a fingerstick CRAG LFA is a simple, low-cost point-of-care method that can be used to rapidly identify persons with cryptococcosis. This information can then determine which patients with suspected meningitis require measurement of the CSF opening pressure during an LP with a manometer or an improvised manometer using intravenous tubing and a meter stick [13]. Capillary fingerstick whole-blood collection has the additional advantage of being less invasive than obtaining venous blood and does not require a centrifuge for serum or plasma separation, and bedside testing can be performed rapidly during informed consent for the LP. We evaluated the accuracy of the CRAG LFA using fingerstick whole blood to screen for meningitis compared with serum/plasma or CSF.

METHODS

From August 2013 to August 2014, we enrolled a prospective cohort of 207 human immunodeficiency virus (HIV)–infected adults with symptoms of suspected meningitis who were admitted to Mulago National Referral Hospital in Kampala, Uganda. Screening was performed as part of the Adjunctive Sertraline for the Treatment of HIV-Associated Meningitis (ASTRO-CM) study (ClinicalTrials.gov identifier NCT01802385). Institutional review board approvals occurred at all relevant institutions in Uganda and Minnesota. Inclusion criteria included physician-suspected meningitis, age ≥18 years, and written informed consent.

Fingerstick Assay

After obtaining verbal consent, a fingerstick CRAG LFA was performed. The pad of the index finger was sterilized with an alcohol swab, and a lancet was used to prick the finger and produce 1 drop of whole blood. The patient's finger was quickly placed directly on the tip of the LFA test strip so that the blood sample (approximately 40 μL) could be absorbed directly onto the LFA strip. The test strip was then placed in a 1.5-mL Eppendorf tube containing 1–2 drops of sample diluent, and allowed to incubate in an upright position at room temperature for 10 minutes. CRAG LFAs were read by trained study personnel. This included the phlebotomist and the study medical officers. Supplementary video demonstrates how the fingerstick CRAG LFA was performed. As the fingerstick CRAG LFA incubated, written informed consent was obtained for permission to conduct an LP and participate in the study. The CRAG LFA result was used to prioritize on whom to use manometers to measure CSF opening pressure. LPs were then performed in the lateral decubitus position.

Parallel Testing of Serum and Plasma

Serum (n = 206) and plasma (n = 27) were collected via venipuncture by a phlebotomist. Serum and/or plasma were tested separately for CRAG LFA by adding 40 μL of specimen to Eppendorf tubes containing 1 drop of sample diluent. CRAG LFA dipsticks were placed into each tube and read after 10 minutes. Serum and plasma were interchangeably tested, per prior published equivalence [8].

CSF Analysis

All CSF samples had a CRAG LFA performed at bedside, collecting 1 drop of CSF into an Eppendorf tube with 1 drop of sample diluent. All CSF CRAG LFA tests were performed twice, once by the clinical staff on the hospital ward and a second time by a microbiologist upon processing the CSF sample. Quantitative fungal cultures were performed using 5 serial 10-fold dilutions of CSF [8, 14]. Further analysis was performed on CSF samples to determine the white blood cell (WBC) count, WBC differential, and protein measurements. Persons with negative fingerstick and CSF CRAG also had Gram stain and enhanced tuberculosis meningitis diagnostics performed through a streamlined diagnostic approach [3]. These diagnostics included GeneXpert, acid-fast bacilli smear microscopy, and tuberculosis cultures.

Statistical Analysis

The diagnostic performance (ie, sensitivity and specificity) of fingerstick CRAG was compared against a composite reference standard of either serum/plasma or CSF CRAG positivity. The relationship between positive fingerstick CRAG, CSF CRAG, and CSF culture was also examined to determine the concordance and positive predictive value of fingerstick testing.

RESULTS

Overall, 207 participants with suspected meningitis were enrolled, of whom 72% (n = 149) had evidence of cryptococcosis. Sixty percent (125/207) of participants were male, with a median age of 36 years (interquartile range [IQR], 30–42 years). Participants were HIV infected with a median CD4 T-cell count of 25 cells/μL (IQR, 9–73 cells/μL; maximum, 660 cells/μL) and with

51% (105/207) receiving antiretroviral therapy at diagnosis. Among patients reporting antecedent headache at diagnosis (n = 193), the median headache duration was 14 days (IQR, 7–21 days). The Glasgow Coma Scale score was <15 in 47% (98/207) of patients at presentation. The median CSF white cell count was <5 cells/μL (IQR, <5–60 cells/μL), with 67% having <5 white cells/μL. Median CSF protein at diagnosis was 50 mg/dL (IQR, 20–99 mg/dL). Approximately 141 participants had their CSF opening pressures measured at screening; the median opening pressure was 280 mm H_2O (IQR, 180–430 mm H_2O). Of persons without cryptococcosis, 1 participant had *Streptococcus pneumoniae* meningitis by Gram stain with polymerase chain reaction confirmation (0.5% prevalence). Tuberculosis meningitis was confirmed by GeneXpert and/or culture in 15 participants. The remaining CRAG-negative participants had aseptic/viral meningitis of predominantly unknown etiology.

Of the 207 CRAG LFA tests performed on fingerstick whole blood, 149 (72%) were positive, of which 138 (93%) were also CSF CRAG positive. Among the 207 participants with CRAG LFA tests performed in serum or plasma, 149 (72%) were positive (Figure 1). There was 100% concordance between plasma and serum CRAG results (n = 26; where both serum and plasma were available). Among those fingerstick CRAG-positive, 93% (138/149) were also CSF CRAG positive. Of those CSF CRAG positive, 91% (127/139) also had positive cryptococcal culture growth (range, 10 colony-forming units [CFU]/mL to >15 million CFU/mL). No persons who tested fingerstick (or CSF) CRAG negative grew *Cryptococcus* in culture (100% negative predictive value). Furthermore, there was 100% concordance between fingerstick whole blood and serum/plasma (κ = 1.0; 95% confidence interval lower bound, 0.979). The positive predictive value of fingerstick LFA for the detection of cryptococcal infection in blood was 100% (149/149) and for cryptococcal meningitis was 93% (138/149). Eleven (5%) participants had isolated cryptococcal antigenemia without proven CSF *Cryptococcus* infection that was represented by CRAG-positive fingerstick and CRAG-positive serum/plasma, but negative CSF CRAG and negative culture. Of these 11 fingerstick CRAG-positive and CSF CRAG-negative participants, 8 had CSF abnormalities (n = 3 with CSF lymphocytic pleocytosis, n = 5 with increased CSF protein [>45 mg/dL], and n = 4 with elevated opening pressure >200 mm H_2O).

The time of point-of-care testing at the hospital bedside (10 minutes) was less than our prior experience of a median of 4 hours 50 minutes for laboratory-based CRAG testing at the same site [8].

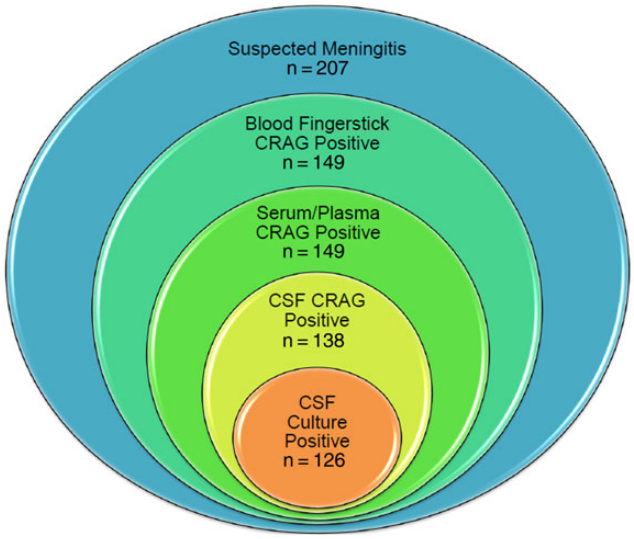

Figure 1. Distribution of cryptococcal diagnostics in blood and cerebrospinal fluid (CSF). The concordance of fingerstick cryptococcal antigen (CRAG) testing was κ = 1.0 for blood (P = .99) and κ = 0.947 for CSF representing 5% of participants (n = 11) having isolated cryptococcal antigenemia in peripheral blood with early disseminated cryptococcal infection but without microbiologically proven meningeal involvement. Of these 11 blood CRAG-positive and CSF CRAG-negative participants, 8 participants had abnormal CSF profiles with CSF inflammation or increased opening pressure. Fifty-eight participants were negative for all cryptococcal testing in blood and CSF. No person had CSF cryptococcal involvement by CRAG or culture who was CRAG-negative by fingerstick or in peripheral blood.

DISCUSSION

CRAG testing of whole blood by capillary fingerstick had 100% concordance with serum or plasma CRAG results collected by venipuncture. Fingerstick CRAG had 100% negative predictive value for excluding cryptococcal meningitis. The ability to conduct reliable fingerstick CRAG testing overcomes a clinical management paradox. Previous studies have found a significant improvement in acute mortality when patients are able to receive at least 1 therapeutic LP to normalize intracranial pressure [11]. However, a major challenge to managing cryptococcal meningitis is that the diagnosis is typically confirmed only after an LP is performed, thus making it difficult to measure and manage intracranial pressure, unless CSF opening pressures are universally measured. Manometers are rarely available in low-income countries, and not always used in high-income countries where available. Improvised measurement of CSF opening pressure can be conducted using intravenous tubing and a meter stick [13]; however, this requires additional physician time. By CRAG screening prior to the LP, one can prioritize on whom to measure opening pressure or empirically remove 20–25 mL CSF, which was the median amount removed in this cohort [11, 13]. Additionally, healthcare workers in both rural and urban areas can also easily screen persons with HIV or suspected meningitis using fingerstick, and quickly exclude cryptococcosis or refer symptomatic CRAG-positive persons to the hospital for LPs.

Another challenge with traditional diagnostic methods (eg, CRAG latex agglutination, culture) is the necessary infrastructure and laboratory skilled labor required for testing. The CRAG LFA represents an important diagnostic advance, providing an affordable point-of-care test that can be performed at the bedside on blood to influence pretest probability and repeated at time of LP to confirm the diagnosis of cryptococcal meningitis. The CRAG LFA is easy to use, reliable, and versatile. It can be performed on serum, plasma, whole blood, urine, or CSF [8, 9]. Notably, CRAG testing of saliva performs less optimally (88% sensitivity and 98% specificity) [15]. LFA tests performed at the bedside also expedite antifungal treatment.

Although the CRAG LFA has been previously validated in serum and CSF [8], we wanted to investigate the accuracy of using fingerstick whole blood, which is easier to collect and provides a quicker method for diagnosis compared with collecting CSF or serum/plasma. The fingerstick CRAG LFA showed a 100% agreement with serum and/or plasma. Of participants who were fingerstick CRAG positive, 93% were also CSF CRAG positive, with the others having isolated cryptococcal antigenemia, which also requires treatment [16]. No test is 100% perfect, and a larger sample size may have eventually uncovered false positives or false negatives; however, in real-world use, fingerstick testing performed very well.

Given the poor outcomes of patients with cryptococcal meningitis [17], typical delays in diagnosis [18], and the documented approximately 70% relative survival benefit at 10 days of repeated therapeutic LPs to reduce ICP [11], using the fingerstick CRAG LFA as a rapid initial step toward establishing a diagnosis can enable better treatment. Fingerstick CRAG testing represents an important and simple tool for meningitis diagnosis worldwide.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We thank Dr Thomas Kozel for his work in developing monoclonal antibodies and Dr Sean Baumann and colleagues for developing the cryptococcal antigen (CRAG) lateral flow assay (LFA). We also thank Nathan Yueh for creating the fingerstick CRAG LFA video. We thank Bozena Morawski, MPH, for data management, and Drs Thomas Harrison and Tihana Bicanic for sharing their quantitative cerebrospinal fluid culture protocol and training of laboratory staff in Uganda. We appreciate institutional support from Drs Paul Bohjanen and Andrew Kambugu.

Disclaimer. Immuno-Mycologics did not provide any financial support for this study.

Financial support. Financial support for this research was provided by the National Institute of Allergy and Infectious Diseases (grant numbers U01AI089244, R01NS086312, K24AI096925, T32AI055433, and R25TW009345).

Potential conflicts of interest. All authors: No potential conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Park BJ, Wannemuehler KA, Marston BJ, Govender N, Pappas PG, Chiller TM. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. AIDS **2009**; 23:525–30.
2. Group for Enteric, Respiratory and Meningeal disease Surveillance in South Africa. GERMS-SA annual report 2012. Available at: http://www.nicd.ac.za/units/. Accessed 7 April 2015.
3. Durski KN, Kuntz KM, Yasukawa K, Virnig BA, Meya DB, Boulware DR. Cost-effective diagnostic checklists for meningitis in resource-limited settings. J Acquir Immune Defic Syndr **2013**; 63:e101–8.
4. Jarvis JN, Meintjes G, Williams A, Brown Y, Crede T, Harrison TS. Adult meningitis in a setting of high HIV and TB prevalence: findings from 4961 suspected cases. BMC Infect Dis **2010**; 10:67.
5. Cohen DB, Zijlstra EE, Mukaka M, et al. Diagnosis of cryptococcal and tuberculous meningitis in a resource-limited African setting. Trop Med Int Health **2010**; 15:910–7.
6. Hakim JG, Gangaidzo IT, Heyderman RS, et al. Impact of HIV infection on meningitis in Harare, Zimbabwe: a prospective study of 406 predominantly adult patients. AIDS **2000**; 14:1401–7.
7. Siddiqi OK, Ghebremichael M, Dang X, et al. Molecular diagnosis of central nervous system opportunistic infections in HIV-infected Zambian adults. Clin Infect Dis **2014**; 58:1771–7.
8. Boulware DR, Rolfes MA, Rajasingham R, et al. Multisite validation of cryptococcal antigen lateral flow assay and quantification by laser thermal contrast. Emerg Infect Dis **2014**; 20:45–53.
9. 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**; 53:1019–23.
10. Perfect JR, Dismukes WE, Dromer F, et al. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the Infectious Diseases Society of America. Clin Infect Dis **2010**; 50:291–322.
11. Rolfes MA, Hullsiek KH, Rhein J, et al. The effect of therapeutic lumbar punctures on acute mortality from cryptococcal meningitis. Clin Infect Dis **2014**; 59:1607–14.
12. Bicanic T, Brouwer AE, Meintjes G, et al. Relationship of cerebrospinal fluid pressure, fungal burden and outcome in patients with cryptococcal meningitis undergoing serial lumbar punctures. AIDS **2009**; 23:701–6.
13. Meda J, Kalluvya S, Downs JA, et al. Cryptococcal meningitis management in Tanzania with strict schedule of serial lumber punctures using intravenous tubing sets: an operational research study. J Acquir Immune Defic Syndr **2014**; 66:e31–6.
14. Bicanic T, Meintjes G, Wood R, et al. Fungal burden, early fungicidal activity, and outcome in cryptococcal meningitis in antiretroviral-naive or antiretroviral-experienced patients treated with amphotericin B or fluconazole. Clin Infect Dis **2007**; 45:76–80.
15. Kwizera R, Nguna J, Kiragga A, et al. Performance of cryptococcal antigen lateral flow assay using saliva in Ugandans with CD4 <100. PLoS One **2014**; 9:e103156.
16. World Health Organization. Rapid advice: diagnosis, prevention and management of cryptococcal disease in HIV-infected adults, adolescents and children. Geneva, Switzerland: WHO, **2011**.
17. Boulware DR, Meya DB, Muzoora C, et al. Timing of antiretroviral therapy after diagnosis of cryptococcal meningitis. N Engl J Med **2014**; 370:2487–98.
18. Trachtenberg JD, Kambugu AD, McKellar M, et al. The medical management of central nervous system infections in Uganda and the potential impact of an algorithm-based approach to improve outcomes. Int J Infect Dis **2007**; 11:524–30.