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Evaluation of a Novel Point-of-Care Lateral Flow Assay To Detect Cryptococcal Antigen in Plasma and CSF

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Abstract

Background: Current diagnostics for Cryptococcal meningitis (CM) rely on India ink, CSF culture, or cryptococcal antigen (CRAG) latex agglutination test. India ink is relatively insensitive. Culture and CRAG require lab infrastructure. Recently, a novel point-of-care lateral flow assay (LFA) has been FDA-approved for diagnosis of *C. neoformans* in serum samples. The aim of this study was to evaluate the LFA performance in plasma and CSF samples.

Methods: ART-naïve subjects with and without cryptococcosis were enrolled in two prospective cohorts in Kampala, Uganda. Initial CM diagnosis was by CRAG, India ink, and quantitative CSF culture. Plasma and CSF were frozen at -80C. Quantitative CRAG titers and LFA testing with titers were conducted on stored CSF, plasma, and serum. Plasma samples were tested in 112 subjects (n=61 with CM, n=51 with no clinical OI). CSF was tested in 161 subjects with suspected meningitis (105 with CM) from 2006-2009 and on 102 (58 with CM) in 2011. LFA results were compared for concordance with CRAG latex agglutination, India ink, and CSF culture.

Results: The LFA was highly sensitive and specific with CSF and plasma samples compared to both culture and CRAG. CRAG and LFA assays had high Kappa concordance in plasma and CSF samples, 0.93 and 0.91, respectively. LFA titers were 2.5 – 10-fold higher than CRAG titers in CSF and in plasma, respectively; however high correlation existed between the two assays (Spearman's ρ=0.90 for plasma and 0.93 for CSF; p<0.001 for each). Culture and India ink had much lower concordance (Kappa 0.69 and 0.72, respectively) with LFA results (due to better LFA sensitivity), and titers were less correlated with quantitative culture results (ρ=0.71, p<0.001). Similar low concordance with culture and India ink were also seen with CRAG titers; suggesting the LFA results were not false-positives but more sensitive in detecting cryptococcal infection.

Conclusions: The LFA assay has excellent concordance with CRAG latex agglutination and titers in both plasma and CSF samples and is more sensitive than standard India ink and CSF culture. Because of the wide availability of plasma samples for other clinical assessments (e.g. CD4 testing), the excellent agreement with the established CRAG assay, and the ease of use, the cryptococcal LFA holds great promise for rapid diagnosis of CM as well as the potential for cryptococcal screening prior to ART initiation, particularly in resource-limited areas.

Background

-957,900 people develop Cryptococcal meningitis (CM) annually (1)
- 75% of burden is in sub-Saharan Africa, with up to 77% case-fatality
- Mortality from CM rivals or exceeds mortality from TB

-Current diagnostics include:
- CSF cultures, India ink staining, cryptococcal antigen detection (latex agglutination)
- Culture and India ink staining are relatively insensitive and prone to contamination
- Can be time consuming (48-72 hours for growth in culture) and requires lab infrastructure
- New point-of-care, lateral flow assay (LFA) to detect cryptococcal antigen is available
- Currently approved for serum and CSF

-Evaluations in South Africa: 100% sensitivity in serum, plasma, and urine compared to enzyme immunoassay (2)

-Evaluation in Thailand: 100% sensitivity in serum and 92% in urine, compared to blood culture (3)

-The aim of this study was to evaluate the LFA in plasma and CSF

Study Population

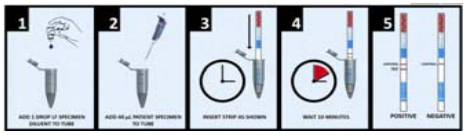
-All patients from Mulago Hospital and Infectious Diseases Institute (IDI), Makerere University, Kampala, Uganda
- All patients were HIV+ with CD4 <100 cells/μL

-In-patients with suspected meningitis screened for study of CM-related IRIS from 2006-2009 (4) combined with in-patients with suspected CM screened in 2011
- Samples from time of diagnostic LP
- 263 patients tested with CSF LFA had CSF culture &CSF latex agglutination results for analysis
  - 2006-2009: 161 in-patient samples (105 diagnosed with CM)
    - 61 also had available plasma LFA results
    - 2011: 102 in-patient samples (58 diagnosed with CM)

-Out-patients from IDI being evaluated for opportunistic infections (2006-2009)
- 51 patients tested with plasma LFA had serum latex agglutination results for analysis

Methods

-Lateral flow assay
- Immuno-chromatographic assay (IMMY, Inc.; Norman, OK)
- Detects cryptococcal capsule in clinical samples
- Approved for diagnostic use in serum and CSF
- Stored CSF and plasma samples tested according to manufacturer's protocol



LFA protocol (IMMY, Inc)

- **Cryptococcal culture and India Ink staining**
  - Conducted in Kampala at Makerere University-Johns Hopkins University (MU-JHU) laboratory
  - CSF samples from time of diagnostic LP (tested in real time)
  - Standard clinical lab protocols followed
  - 10 μL input volume (2006-2009) and 100 μL input volume (2011) used for CSF culture
- **Cryptococccal antigen latex agglutination**
  - Conducted at MU-JHU (in real time) and University of Minnesota (stored samples) according to manufacturer's protocol
  - For 2006-2009 cohort, samples considered positive if positive on latex from MU-JHU and UMN
  - CSF and serum samples tested among in-patients with suspected meningitis and serum tested among out-patients
- **Performance characteristics**
  - Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and Kappa concordance coefficient calculated
  - Qualitative CSF LFA results compared to qualitative CSF culture and latex agglutination results
  - Plasma LFA results compared to serum latex results among in-patients and out-patients
  - Semi-quantitative LFA titers, in plasma and CSF, compared to semi-quantitative latex titers and CSF quantitative culture with Spearman correlation

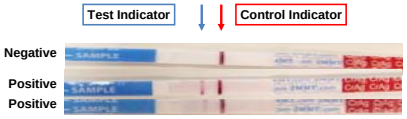
Conclusions/Implications

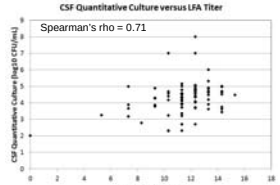
- LFA is accurate, simple, inexpensive point-of-care diagnostic for Cryptococcal meningitis
  - Stable at room temperature, 2-year shelf-life, and $2/test
- High sensitivity in samples collected for CSF and plasma, ideal for testing on samples collected for routine care
- Evidence that LFA may be more sensitive than current diagnostics
  - 85% of LFA +ve/culture -ve results were latex +ve
  - LFA titers in CSF and plasma were 2.5 – 10 fold higher than latex titers, respectively
- Clear implications for use of the LFA for screening
  - Among in-patients: screening could decrease the time from admission to CM diagnosis and treatment
  - Out-patients: screening, with fluconazole treatment, could reduce incidence of CM among ART-eligible individuals
- LFA needs further validation in screening populations
  - Among 51 out-patients, LFA had 67% sensitivity and 91% specificity (67% PPV and 98% NPV) compared to serum latex
- Field studies to validate LFA on whole blood and saliva are needed, particularly in order to use LFA for screening ART-eligible individuals

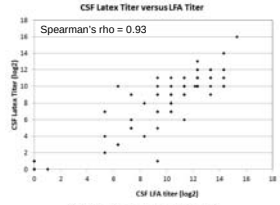
Results

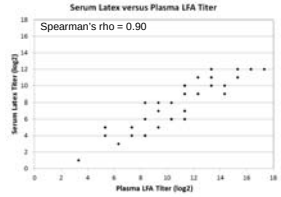
Comparison	N	Sensitivity	Specificity	PPV	NPV	Kappa
CSF LFA						
vs. CSF Culture						
2006-2009	161	98.8%	70.4%*	76.7%	98.3%	0.69
2011	102	100.0%	93.5%	94.9%	100.0%	0.94
vs. CSF Latex						
2006-2009	161	96.2%	96.4%	98.0%	93.0%	0.93
2011	102	100.0%	93.5%	94.9%	100.0%	0.94
Plasma LFA						
vs. Serum Latex						
2006-2009	112	98.5%	91.5%	--	--	0.91

* Likely reflective of the low yield of culture on 10 μL CSF









- LFA had >96% sensitivity in both CSF and plasma samples compared to classic diagnostics for cryptococcosis (including 97.5% sensitivity compared to India Ink)
- LFA had high specificity in both CSF and plasma samples
- 70% specificity of CSF LFA compared to CSF culture in 2006-2009 in patients
  - Possibly "false negatives" by culture
  - Of 27 samples LFA +ve/culture –ve, 46% India Ink +ve and 85% CSF latex +ve. The median latex titer was 1:256 and median LFA titer was 1:640.
  - Low culture input volume used from 2006-2009 (10 μL) may have limited ability to detect low fungal burden
  - Specificity was 93.5% compared to culture protocol using 100 μL input volume in 2011
  - Taken together, this suggests low sensitivity by the culture protocol in 2006-2009 resulted in *apparent* lower specificity of LFA rather than *true* lower specificity of LFA in CSF
- Semi-quantitative LFA titers strongly correlated with quantitative culture and latex titers
  - LFA titers 2.5-fold higher than latex titers in CSF
  - Plasma LFA titers 10-fold higher than latex in serum

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