Evaluating the IMMY Semi-Quantitave CrAg LFA in HIV-Positive Patients in Botswana

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BACKGROUND

Higher cryptococcal antigen (CrAg) titers are strongly associated with mortality risk in individuals with HIV-associated cryptococcal disease.

Rapid tests to quantify CrAg level may provide important prognostic information and enable treatment stratification.

We performed a laboratory-based validation of the novel semi-quantitative IMMY CrAgSQ assay (IMMY, Norman, OK) against the current gold-standard CrAg tests.

METHODS

We assessed diagnostic accuracy of the CrAgSQ in HIV-positive individuals with CD4 ≤200 cells/μL undergoing CrAg screening in Gaborone, Botswana.

We determined the relationship between CrAgSQ scores and dilutional CrAg titers using CrAg-positive samples from the CrAg-screened cohort plus a collection of stored frozen plasma samples that had previously tested CrAg-positive using the IMMY CrAg LFA.

We assessed inter-rater reliability; and determined clinical correlates of CrAgSQ scores.

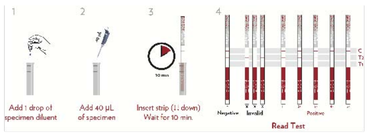

Figure 1. The IMMY semi-quantitative CrAgSQ lateral flow assay (IMMY, Norman, OK, USA). Scores indicating increasing cryptococcal antigen titers are derived from line intensity patterns as follows: T1<T2 = 1+; T1=T2 = 2+; T1>T2 = 3+; only T1 = 4+; only C = 5+. Only T2 and C = negative. A score of 1+ indicates "low positive", and a score of 5+ "very high positive".

RESULTS

872 plasma samples were tested using both CrAgSQ and conventional IMMY CrAg LFA tests:

- 692 sequential samples from HIV-positive individuals undergoing CrAg screening (cohort 1)
- an additional 180 known CrAg-positive plasma samples archived from prior studies (cohort 2)

Inter-rater agreement in CrAgSQ reading was excellent (98.17% agreement, Cohen's Kappa 0.962, p<0.001).

The semi-quantitative IMMY CrAgSQ cryptococcal antigen test had high sensitivity and specificity compared to current CrAg tests and provided quantitative CrAg results which were associated with both conventional CrAg titers and clinical outcomes

RESULTS (cont.)

Of the 692 samples tested in the sequentially-enrolled cohort, 43 (6.2%) were positive for CrAg using the IMMY CrAg LFA. Compared to the IMMY CrAg LFA as a reference standard, CrAgSQ (used as a qualitative test) was 93.0% sensitive (95% confidence interval [CI] 80.9% – 98.5%) and 93.8% specific (95% CI 91.7% – 95.6%).

In order to better classify potential false-positive and false-negative results, an additional analysis was performed in which the IMMY EIA test result was used as a "tie-breaker" for samples with discrepant test results between the CrAgSQ assay and IMMY CrAg LFA, and the reference test result reclassified accordingly. Following this adjustment to the reference standard, the **sensitivity of the CrAgSQ was 98.1% (95% CI 90.1 – 100%), and the specificity was 95.8% (95% CI 99.1% - 100%)**.

Table 1. Sensitivity, specificity, positive, and negative predictive value of the IMMY semi-quantitative LFA† (CrAgSQ) versus the conventional IMMY LFA test after reconciliation of discordant tests§ using EIA testing

	CrAgSQ +ve	CrAgSQ -ve	Total
CrAg +ve	53	1	54
CrAg -ve	27	611	638
Total	80	612	692
Sensitivity	98.1%	95% CI 90.1% - 100%	
Specificity	95.8%	95% CI 93.9% - 97.2%	
Positive predictive value	66.3%	95% CI 54.8% - 76.4%	
Negative predictive value	99.8%	95% CI 99.1% - 100%	

†All CrAgSQ results of 1+ and above were considered positive.

§The reference standard was a composite cryptococcal antigen result derived from the conventional IMMY qualitative lateral flow assay with discrepant LFA/SQ results reconciled using the IMMY EIA as the tie-breaker test.

27 (4.2%) of IMMY CrAg LFA|EIA negative samples were CrAgSQ positive, all at the lowest 1+ score, and classified as false-positive

1 of the IMMY CrAg LFA|EIA positive samples (at the lowest titer of 1:2) was negative on CrAgSQ testing, and classified as false-negative.

RESULTS (cont.)

Combining all 223 CrAg-positive samples from cohort 1 (n=43) and cohort 2 (n=180), median CrAg titers were:

- CrAgSQ **1+** category: **1:10** (IQR 1:5 – 1:20)
- CrAgSQ **2+** category: **1:40** (IQR 1:20 – 1:80)
- CrAgSQ **3+** category: **1:640** (IQR 1:160 – 1:2560)
- CrAgSQ **4+** category: **1:5120** (IQR 1:2560 – 1:30720)

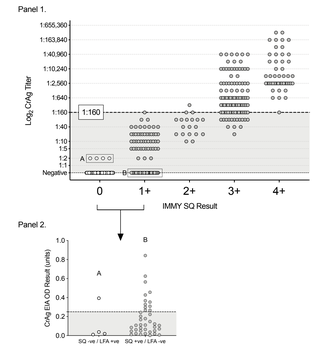

Figure 2. Relationship between CrAgSQ scores and cryptococcal antigen titers derived from serial dilutional testing with the IMMY lateral flow assay (Panel 1). Samples with discordant CrAgSQ and IMMY lateral flow assay positive / negative results were retested using the IMMY cryptococcal antigen enzyme immunoassay (EIA) (panel 2). Box A indicates samples that were positive on IMMY lateral flow assay testing and negative on CrAgSQ testing. Three of these four samples were negative on EIA testing at the optical density cut off of 0.265. Box B indicates the samples that were positive on CrAgSQ testing and negative on IMMY lateral flow assay testing. Thirteen of the forty samples were positive on EIA testing at the optical density cut off of 0.265.

CrAgSQ Score was associated with 10-week mortality.

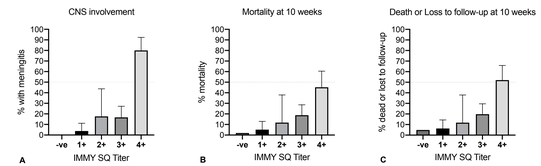

Figure 4. Associations between CrAgSQ score and A) baseline CNS disease (defined as positive cerebrospinal fluid CrAg) in the 189 CrAg-positive patients identified through reflex cryptococcal antigen screening; B) ten-week mortality in all participants; and C) ten-week mortality and loss to follow-up. Note that only 32% of CrAg-positive individuals underwent baseline CSF examination, thus these figures represent minimum estimates of baseline CNS disease. P for trend <0.001 in all cases.

CONCLUSIONS

The semi-quantitative CrAgSQ cryptococcal antigen test had **high sensitivity and specificity** compared to current IMMY CrAg LFA test and **provided quantitative CrAg results which were associated with both CrAg titers derived from dilutional testing and clinical outcomes.** The test provides an effective and practical method to stratify CrAg-positive patients according to CrAg levels and could provide the basis for differentiated management approaches.

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