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Field performance of the Determine HBsAg point-of-care test for diagnosis of hepatitis B virus co-infection among HIV patients in Zambia

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Abstract

Background—We evaluated the field performance of a rapid point-of-care (POC) test for hepatitis B surface antigen (HBsAg) that could support decentralization and scale-up of hepatitis B virus (HBV) diagnosis in Africa.

Objective—To determine the field performance of the Determine HBsAg POC test for diagnosis of HBV co-infection among HIV patients in Zambia.

Study design—Between 2013–2014, we screened HIV-infected adults for HBsAg at two urban clinics in Zambia. A subset were tested with the POC Determine HBsAg (Alere, USA) by finger prick in the clinic and HBsAg serology (Access2Analyzer, Beckman Coulter) at a reference laboratory. If either test was reactive, we determined HBV viral load (VL) and genotype. We described patient demographic and clinical characteristics (including liver fibrosis) and assessed the sensitivity, specificity, positive and negative predictive values (PPV and NPV) of the Determine test. In secondary analyses, we assessed sensitivity among patients with replicating HBV (i.e., VL>20 IU/ml) and with high HBV VL (i.e., >20,000 IU/ml).

Results—Among 412 participants with both HBsAg tests, median age was 34 years, 51% were women, and median CD4 was 208 cells/mm^3. By serology, 66 (16%) were HBsAg-positive. Overall Determine had 87.9% sensitivity, 99.7% specificity, 98.3% PPV, and 97.7% NPV. Six of 8 patients with false negative results had undetectable HBV VL and no evidence of significant liver fibrosis. Test sensitivity was 95.9% among the 51 with replicating HBV and 100% among the 28 with high HBV VL.

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Conclusions—Determine HBsAg is a cheaper alternative HBV testing option compared to the gold standard ELISA and has high specificity and good sensitivity in the field among HIV-infected individuals.

Keywords

HIV/AIDS; hepatitis B virus; point-of-care test; assay performance; liver fibrosis

Background

In Africa where the burden of hepatitis B virus is high,[1] rapid point-of-care (POC) tests are recommended to increase diagnostic capacity because they are low cost, temperature stable, and can be performed by lay health workers.[2,3] The World Health Organization recommends use of POC tests to identify patients with chronic HBV infection; however, real world data in Africa are limited on test performance and integration of HBV testing into the health system. Among available data two POC hepatitis B surface antigen (HBsAg) tests had sensitivities of 88.5 and 90.0% for diagnosis of HBV monoinfection in the Gambia when compared to a gold standard ELISA assay with >99% accuracy.[4] Building on this and several other studies,[5,6] we investigated the field performance of the Determine HBsAg test among HIV-infected individuals in Zambia, a group at increased risk for chronic HBV infection. Leveraging a prospective cohort where HBV virology and liver disease were well-characterized,[7,8] we evaluated assay performance as well as the clinical relevance of false negative results.

Objectives

Our objectives were to establish the field performance of the Determine HBsAg point-of-care test for diagnosis of hepatitis B virus co-infection among HIV patients in Zambia and to characterize patients with false negative test results in terms of HBV virology and liver fibrosis.

Study design

During 2013–2014, we established a prospective HIV cohort study in Lusaka, Zambia, to understand causes of liver disease.[7,9] At linkage and initial enrollment in HIV care, phlebotomy was performed for CD4+ count, creatinine, and HBsAg using a central laboratory Enzyme Linked Immunosorbent Assay (ELISA) that has ≥99.5% sensitivity and specificity (Access 2 Analyzer; Beckman Coulter Inc). HIV-infected adults (18+ years old) who were antiretroviral therapy (ART) eligible (CD4+ count <500 cells/mm^3 or WHO stage 3/4 conditions) were enrolled consecutively at two large public sector HIV clinics. At enrollment, regardless of programmatic HBsAg test results, a research nurse or research assistant re-tested each participant with Determine HBsAg test by finger prick sampling. Among HBsAg-positives by either test (ELISA or Determine), we measured the HBV viral load (VL; Roche COBAS AmpliPrep/COBAS Taqman HBV test, V 2.0) and sequenced HBV for determination of genotype.[10] We also screened each patient for alcohol consumption using the Alcohol Use Disorder Identification Test-Consumption (AUDIT-C)[11]

and assessed liver fibrosis/cirrhosis using transient elastography (Fibroscan 402, Echosens, Paris, France).[12,13]

For this analysis, we excluded those without the HBsAg ELISA test and described the sociodemographic and clinical characteristics of those with both tests, stratified by HBsAg-serostatus. Chi square tests were used to compare categorical variables and Wilcoxon rank sum tests for continuous ones. We defined an elevated ALT as >33 IU/ml (laboratory upper limit of normal) and significant hepatic fibrosis/cirrhosis as liver stiffness measurement (LSM) >7.0 kPa.[14] Considering the ELISA test as the gold standard, we estimated the sensitivity (Se), specificity, positive and negative predictive values of Determine, as well as the positive and negative likelihood ratios. We also analyzed Se among patients with replicating HBV (HBV DNA >20 IU/ml) and with high HBV VL (>20,000 IU/ml). We compared HBV virological and liver disease between those with false negatives and true positives. Data analysis was performed using Stata version 13 (StataCorp, College Station, TX). All participants provided written informed consent and the study was approved by the ethics committees at University of Zambia (Lusaka, Zambia), and University of Alabama at Birmingham (Birmingham, USA).

Results

Among 798 HIV-infected adults enrolled, 412 (52%) with an HBsAg ELISA test were included in this analysis. Those under analysis had similar age, sex, and WHO stage compared to those excluded (all P>0.05). Baseline characteristics, stratified by HBsAg ELISA result, are displayed in Table 1. At enrollment, 66 patients (16.0%) had a positive HBsAg ELISA test and HBsAg-positives were more likely to have an elevated ALT (27.3% versus 19.0%; P=0.12) and had a higher median LSM (5.8 versus 4.9; P=0.007) compared to those who were HBsAg-seronegative. Median HBV VL was 6,350 IU/ml (interquartile range, 70–2,422,071), 51 (79.7%) had replicating HBV, and 28 (43.8%) had high HBV VL. We sequenced HBV in 42 patients; 22 had genotype A1, 19 had genotype E, and 1 had dual A1 and E infection.

Among 66 HBsAg-seropositives, Determine was positive in 58 and negative in 8; among 346 HBsAg-seronegatives, 1 had a positive Determine. These results translated to a Se of 87.9% (95% CI; 77.5–94.6), specificity of 99.7% (95% CI; 98.4–100), positive predictive value of 98.3% (95% CI; 90.9–100.0) and negative predictive value of 97.7% (95% CI; 95.6–99.0; Table 2). The positive likelihood ratio was 304 and the negative likelihood ratio was 0.12. In replicating HBV, Se was 95.9% (95% CI, 86.5–99.5) and among those with high HBV VL Se was 100%.

When compared to true positives (n=58), false negatives (n=8) tended to be male (75.0% versus 51.7%) and had lower median HBV VL (0 versus 19,142 IU/ml) with the exception of one patient with HBV VL of 5,600 IU/ml (Table 2). This patient had a mildly elevated ALT (43 IU/mL) and a relatively normal LSM at 5.9 kPa. Another patient with a false negative Determine had evidence of significant hepatic fibrosis/cirrhosis (LSM of 8.3 kPa) but a low HBV VL (119 IU/ml).

Discussion

When performed via finger prick sampling, Determine accurately diagnosed HBsAg positivity among HIV-infected adults when compared to the gold standard ELISA. Although nearly 1 in 10 had a false negative, sensitivity approached 100% when HBV viral load was detectable. Although absolute numbers were low, patients with false negative results did not tend to have substantial liver disease and 6 of 8 had undetectable HBV DNA. These data support the routine use of the Determine test among HIV-infected individuals in Africa at risk for HBV-related liver disease.

This study builds on other African data where Determine HBsAg had Se of 88–95%[4,15,1,] although there was some concern that Se may be reduced among HIV-infected individuals due to antiretroviral drugs.[6] Most studies did not measure HBV VL and we hypothesize that differences in HBV DNA levels, which sometimes correlate with quantitative HBsAg levels, could explain some of the differences in test performance. Although false negatives occurred ~10% of the time, the majority of patients with false negatives had undetectable HBV DNA and/or did not have elevated fibrosis markers similar to other reports.[4,16–18]

These results are relevant in other LMIC where laboratory capacity is low because testing was performed in a typical public sector facility in Zambia using finger prick sampling. Another strength of this study was measurement of HBV VL and genotype, providing supportive information on the test performance and partial explanation of false negatives. The study also had weaknesses that warrant discussion. Half of cohort participants were excluded for lack of HBsAg serologic tests due to incomplete uptake of guidelines; however, we suspect missing data occurred at random since patients excluded had similar characteristics to those included.[19] Unfortunately we failed to sequence one-third of samples including one from a patient with negative Determine and relatively high HBV VL. Quantification of HBsAg would have better characterized false negatives.

POC tests like Determine HBsAg could be integrated into existing public health programs where other rapid POC tests are used in antenatal care, HIV counseling and testing, and population-based surveys. Determine HBsAg had a high PPV and positive likelihood ratio suggesting that individuals testing positive can be immediately linked to care and treatment without need for a confirmatory test.[20] These results also support WHO recommendations that low cost rapid HBsAg tests can be used to address the lack of HBV burden data as HBV is not part of routine surveillance in many African countries.[20] Cost savings are another advantage of this test when compared to a laboratory ELISA assay. In conclusion, rapid POC tests will play an important role in scale-up of treatment and prevention of HBV in sub-Saharan Africa settings. At urban HIV clinics in Lusaka, Zambia, Determine HBsAg test detected 88% of HBV coinfections overall and a greater percentage among those with higher HBV DNA levels.

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Table 1

Sociodemographic and clinical characteristics of HIV-infected adults tested for hepatitis B surface antigen at antiretroviral therapy initiation in Zambia

	HBsAg-seropositive (n=66)	HBsAg-seronegative (n=346)	P value
Age, in years			
18–29	19 (28.8)	80 (23.1)	0.45
30–39	31 (47.0)	159 (46.0)	
40+	16 (24.2)	107 (30.9)	
Sex			
Male	34 (51.5)	167 (48.3)	0.63
Female	32 (48.5)	179 (51.7)	
WHO clinical stage			
1 or 2	40 (61.5)	198 (57.4)	0.32
3 or 4	25 (38.5)	147 (42.6)	
Tuberculosis	9 (13.6)	51 (14.7)	0.83
Alcohol consumption *			
None/Moderate	33 (50.0)	211 (61.0)	0.10
Hazardous	33 (50.0)	135 (39.0)	
Median ALT, in U/L	21 (14–37)	19 (14–28)	0.20
Median CD4+ count, in cells/mm^3	237 (129–347)	204 (112–324)	0.30
LSM, in kiloPascals	5.8 (4.4–6.4)	4.9 (4.4–5.8)	0.01

All values are median (IQR) or number (%). Abbreviations: WHO, World Health Organization; ALT, alanine transaminase; LSM, liver stiffness measurement; CD4, cluser of differentiation 4.

* Hazardous drinking was defined as AUDIT-C score of >2 for women and >3 for men.

Table 2

Characteristics of HIV-HBV coinfected patients with true positive and false negative Determine HBsAg point-of-care tests

Group	Sex	Age (yrs)	HBV VL (IU/ml)	ALT (U/L)	AUDIT-C score	LSM (kPa)	CD4 count
True positives (n = 58)	30-M, 38-W	34 (27–38)	19142 (154–2810000)	19 (13–37)	3 (0–9)	5.6 (4.4–6.4)	163 (76–266)
False negatives (n=8)	6-M, 2-W	36 (31–39)	0 (0–60)	30 (27–40)	1 (0–10)	6 (5.2–6.6)	216 (156–309)
Individuals with false negatives							
1.	F	32	0	33	0	6.1	129
2.	M	30	0	27	0	4.0	174
3.	M	24	0	27	11	5.9	-
4.	F	37	0	12	0	6.8	244
5.	M	35	0	37	11	6.4	275
6.	M	38	0	63	9	4.4	317
7.	M	41	119	27	2	8.3	233
8.	M	54	5600	43	0	5.9	224

All values are number or median (interquartile range)

Abbreviations: HIV, human immunodeficiency virus; HBV, hepatitis B virus; ALT, alanine aminotransferase; AUDIT-C, alcohol use disorder identification test-consumption; LSM, liver stiffness measurement; CD4, cluster of differentiation 4