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Hepatitis B Core-related Antigen: An Alternative to Hepatitis B Virus DNA to Assess Treatment Eligibility in Africa

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Background. To eliminate hepatitis B virus (HBV) infection, it is essential to scale up testing and treatment. However, conventional tools to assess treatment eligibility, particularly nucleic acid testing (NAT) to quantify HBV DNA, are hardly available and affordable in resource-limited countries. We therefore assessed the performance of a novel immunoassay, hepatitis B core-related antigen (HBcrAg), as an inexpensive (US$ <15/assay) alternative to NAT to diagnose clinically important HBV DNA thresholds (≥2000, ≥20 000, and ≥200 000 IU/mL) and to select patients for antiviral therapy in Africa.

Methods. Using a well-characterized cohort of treatment-naive patients with chronic HBV infection in The Gambia, we evaluated the accuracy of serum HBcrAg to diagnose HBV DNA levels and to indicate treatment eligibility determined by the American Association for the Study of Liver Diseases, based on reference tests (HBV DNA, hepatitis B e antigen, alanine aminotransferase, liver histopathology, and/or FibroScan).

Results. A total of 284 treatment-naive patients were included in the analysis. The area under the receiver operating characteristic curve (AUROC), sensitivity, and specificity of serum HBcrAg were 0.88 (95% confidence interval [CI], .82–.93), 83.3%, and 83.9%, respectively, to diagnose HBV DNA ≥2000 IU/mL; and 0.94 (95% CI, .88–.99), 91.4%, and 93.2% for ≥200 000 IU/mL. A simplified treatment algorithm using HBcrAg without HBV DNA showed high AUROC (0.91 [95% CI, .88–.95]) with a sensitivity of 96.6% and specificity of 85.8%.

Conclusions. HBcrAg might be an accurate alternative to HBV DNA quantification as a simple and inexpensive tool to identify HBV-infected patients in need of antiviral therapy in low- and middle-income countries.

Keywords. hepatitis B core-related antigen; diagnostic test; validation studies; sensitivity and specificity; Africa.

Hepatitis B virus (HBV) infection is a major global health problem and is recognized as a public health priority by the United Nations Sustainable Development Goals. Subsequently, the World Health Organization (WHO) has developed a strategy to eliminate viral hepatitis by 2030; one of the objectives is to globally increase the treatment uptake in people with chronic HBV infection (CHB) eligible for antiviral therapy from 8% (2015) to 80% (2030) [1]. To achieve this goal, it is critical to scale up screening for hepatitis B surface antigen (HBsAg) and clinical staging for those carrying HBsAg to assess treatment eligibility.

Quantification of HBV DNA constitutes an essential element of clinical staging. The international guidelines define having high viremia (≥2000 or ≥20 000 IU/mL), in the presence of liver inflammation or fibrosis, to be one of the criteria to initiate antiviral therapy [2–5]. Moreover, the cutoff of ≥200 000 IU/mL is now used to select pregnant women for antiviral therapy to prevent mother-to-child transmission [2, 3]. However, the vast majority (>95%) of HBV-infected people live in low- and middle-income countries (LMICs) [6], and they have severely limited access to real-time polymerase chain reaction (PCR), a molecular assay to measure HBV DNA levels [7]. PCR is expensive, often restricted to large urban laboratories, and requires highly skilled laboratory technicians. Consequently, the WHO fully acknowledges an urgent need for a simple, low-cost assay to measure HBV replication [8].

Hepatitis B core-related antigen (HBcrAg), consisting of 3 viral proteins (HBV core antigen, hepatitis B e antigen [HBeAg], and a small core-related protein [p22cr]), is a novel serological marker of HBV replication [9]. Studies in Asia and Europe have confirmed a close correlation between serum HBcrAg levels and serum HBV DNA levels in treatment-naive patients with CHB [10–13]. Moreover, several studies also found a correlation of

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serum HBcrAg levels with intrahepatic covalently closed circular DNA (cccDNA), a transcriptional template of HBV [10, 11, 14, 15]. Because this immunoassay is cheaper (US$ <15 per assay) and simpler than the conventional real-time PCR (US$60–$200 per assay), this may represent an attractive alternative in LMICs. For hepatitis C virus (HCV) infection, a similar case has been already made. WHO now recommends the use of immunoassay (HCV core antigen [HCVcAg]) to diagnose chronic HCV infection when HCV RNA PCR is not accessible [8], as HCVcAg is an accurate and inexpensive alternative to HCV RNA [16–18].

We assessed the performance of serum HBcrAg levels to diagnose 3 clinically important HBV DNA thresholds (2000, 20 000, and 200 000 IU/mL) in a well-characterized cohort of treatment-naive CHB patients in The Gambia, West Africa. We also evaluated the associations of serum HBcrAg levels with significant liver fibrosis and inflammation, and the diagnostic accuracy of simplified treatment algorithms using HBcrAg as an alternative to HBV DNA, to correctly classify those eligible for antiviral therapy according to the conventional tests (HBV DNA, liver histology, or FibroScan) as a reference.

MATERIALS AND METHODS

Study Participants

In 2011–2014, the Prevention of Liver Fibrosis and Cancer in Africa (PROLIFICA) program recruited Gambian adults identified to carry HBsAg through community-based and blood bank screening using a rapid test (Determine, Alere; or OnSite Combo Rapid Test, CTK Biotech) [19, 20]. In addition, the program also recruited symptomatic patients with chronic liver disease referred from health facilities throughout the country [21]. After informed consent, HBsAg-positive participants systematically underwent the following clinical evaluations: fasting transient elastography (FibroScan 402, Echosens, France) [22], abdominal ultrasonography, hematology and biochemistry tests, HBeAg (ETI-EBK Plus, Diasorin, Italy), and HBV DNA (in-house real-time PCR, limit of detection: 50 IU/mL) [23]. All of these laboratory analyses were performed locally. A subset of patients underwent liver biopsy [24]. Patients consecutively recruited from April 2012 to October 2013 were included in the current analysis. We excluded from the analysis participants with hepatocellular carcinoma (HCC), prior or current antiviral therapy for HBV, or human immunodeficiency virus (HIV) coinfection, and those missing virological data.

Serum HBcrAg and HBsAg-HQ

Patients' serum samples at recruitment were stored at −80°C and shipped to Toshiba General Hospital, Tokyo, Japan, where HBcrAg was quantified using a fully automated chemiluminescent immunoassay (CLIA; Lumipulse G600II, Fujirebio, Tokyo, Japan) according to the manufacturer's instructions. The assay provided a reportable range of 3–7 log U/mL. Samples with HBcrAg >7 log U/mL were diluted and retested to quantify HBcrAg levels. HBsAg quantification was also made using a highly sensitive CLIA (Lumipulse HBsAg-HQ) with a limit of detection of 0.005 IU/mL. These measurements were performed by staff blinded to the reference test results.

International Treatment Guidelines

The conventional treatment criteria established by the international guidelines are summarized in Supplementary Table 1. The American Association for the Study of Liver Diseases (AASLD), the European Association for the Study of the Liver (EASL), and the Asian Pacific Association for the Study of the Liver (APASL) largely rely on 3 factors: (1) levels of viral replication by HBV DNA PCR and/or HBeAg serostatus; (2) degree of liver inflammation based on liver histopathology and/or alanine aminotransferase (ALT) level; and (3) fibrosis staging by histopathology or liver stiffness measurement [2–4]. For these criteria, significant liver fibrosis and cirrhosis were defined as Metavir score ≥F2 and F4 in those who had biopsy, and liver stiffness ≥7.9 kPa and ≥9.5 kPa in those without biopsy, respectively [24]. Family history of HCC was not used to define treatment eligibility due to its poor ascertainment in The Gambia [25]. The WHO guideline provides criteria for LMICs where HBV DNA testing is not available: cirrhosis, diagnosed by physical examination or aspartate aminotransferase to platelet ratio index >2.0; or persistently elevated ALT [5]. Because cross-sectional data were used in this study, the eligibility was considered on a single time point. We used upper limits of normal for ALT specifically defined in each guideline (Supplementary Table 2).

Simplified Treatment Algorithms Using HBcrAg

We developed 3 simplified algorithms using HBcrAg (models 1–3) to select HBsAg-positive patients for antiviral therapy. Model 1 is the same as the conventional criteria (AASLD, EASL, and APASL) except for HBV DNA, which was replaced by HBcrAg, and liver histopathology, which was replaced by FibroScan. Optimal HBcrAg cutoff levels equivalent to HBV DNA thresholds of ≥2000 and ≥20 000 IU/mL were applied to these conventional criteria (Supplementary Table 3). Model 2 is a simple score based on HBcrAg and ALT alone, which is similar to the Treatment Eligibility in Africa for HBV (TREAT-B) scoring system composed of HBeAg and ALT levels [26]. In this model, HBcrAg levels were dichotomized into high and low using an optimal threshold corresponding to HBV DNA levels of ≥2000 IU/mL. The total point was obtained by adding HBcrAg score (low [0 point] or high [1 point]); and ALT score (<20 IU/L [0 point], 20–39 [1 point], 40–79 [2 points], or ≥80 [3 points]). We considered the score of ≥2 to indicate treatment eligibility [26]. Model 3 only used the dichotomized HBcrAg levels.

Statistical Analyses

Quantified levels of serum HBV DNA, HBcrAg, and HBsAg were $\log_{10}$ transformed, and the detection limit of each assay was assigned to samples with an undetectable result. The correlation between these markers was assessed using Pearson correlation coefficient (r). The correlation was also evaluated by HBeAg serostatus and viral genotypes. The capability of HBcrAg levels to correctly discriminate clinically important HBV DNA levels at 3 different cutoffs (≥2000, ≥20 000, and ≥200 000 IU/mL) was evaluated by the receiver operating characteristic (ROC) curve. The optimal cutoffs for HBcrAg levels were selected to minimize the absolute difference between the sensitivity and specificity. The discrimination capabilities of HBcrAg levels were compared to those of HBsAg levels and HBeAg using the area under the ROC curve (AUROC).

Among the virological factors (HBcrAg, HBsAg, HBeAg, HBV DNA, and genotypes), those associated with liver inflammation (ALT ≥40 IU/L) and significant fibrosis were identified using logistic regression. The factors significantly associated with the outcome in the univariable analyses ($P < .05$) were further included in the multivariable model.

The performance of the simplified algorithms using HBcrAg (models 1–3) was evaluated for each of the international guidelines (AASLD, EASL, and APASL) as a reference. By using the AUROC, the discrimination capabilities of these algorithms were compared to the WHO criteria and TREAT-B. All the analyses were performed using Stata version 13.0 software (StataCorp, College Station, Texas). The study was approved by The Gambian government and Medical Research Council joint ethics committee, and reported in accordance with Standards for Reporting Diagnostic Accuracy (STARD) [27].

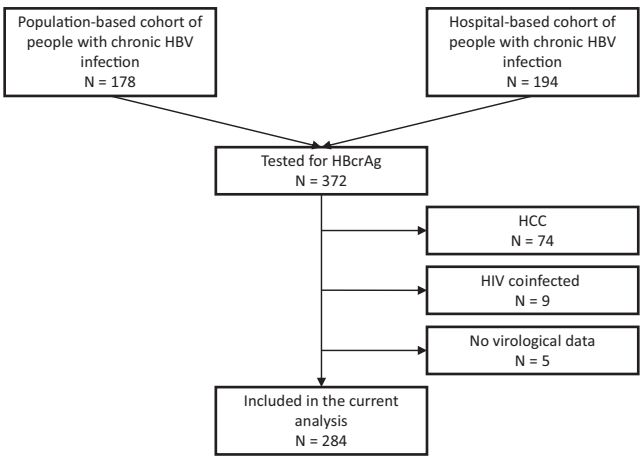

Figure 1. Flowchart of study participants. Abbreviations: HBcrAg, hepatitis B core-related antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HIV, human immunodeficiency virus.

RESULTS

Study Participants

Of 372 HBsAg-positive participants assessed for serum HBcrAg, 284 were included in the current analysis, after excluding 74 with HCC, 9 with HIV coinfection, and 5 with missing data (Figure 1). Their characteristics are described in Table 1. The median age was 36 years (interquartile range [IQR], 30–45 years), and 66% were men. Positive HBeAg, HBcrAg, and HBV DNA were observed in 36 (13%), 152 (53%), and 165 patients (58%), respectively. Median levels of HBsAg, HBcrAg, and HBV DNA were 3.6 (IQR, 2.9–4.1) log IU/mL, 4.0 log (IQR, 3.3–5.7) U/mL, and 2.9 (IQR, 2.2–5.0) log IU/mL, respectively, after excluding undetectable values. The majority harbored genotype

Table 1. Characteristics of Study Participants (N = 284)

Variable	Value
Median age, y	36 (30–45)
Male sex, no. (%)	188 (66)
Ever drunk alcohol, no. (%)	24 (9)
Median BMI, kg/m^2	22 (19–25)
Positive HBeAg, no. (%)	36 (13)
HBsAg, no. (%)	
0.005–999 IU/mL	83 (29)
1000–9999 IU/mL	109 (38)
≥10 000 IU/mL	92 (32)
HBcrAg, no. (%)	
Undetectable	132 (47)
3.0–3.9 log U/mL	75 (26)
≥4.0 log U/mL	77 (27)
HBV DNA, no. (%)	
Undetectable	119 (42)
50–1999 IU/mL	99 (35)
2000–19 999 IU/mL	21 (7)
20 000–199 999 IU/mL	10 (4)
≥200 000 IU/mL	35 (12)
HBV genotype, no. (%)	
A	38 (16)
E	198 (84)
Median liver stiffness, kPa	5.8 (4.5–10.7)
Liver cirrhosis, no. (%)	55 (19)
Median AST, IU/L	33 (26–53)
Median ALT, IU/L	25 (19–42)
Median GGT, IU/L	31 (22–70)
Median albumin, g/L	41 (36–44)
Median total bilirubin, IU/L	11 (8–18)
Median platelet count, 10^9 cells/L	180 (130–242)
Eligible for AASLD treatment criteria (2018), no. (%)	59 (21)
Eligible for EASL treatment criteria (2017), no. (%)	58 (20)
Eligible for APASL treatment criteria (2015), no. (%)	63 (22)
Eligible for WHO treatment criteria for LMICs (2015), no. (%)	140 (49)

Data are presented as median (interquartile range) unless otherwise indicated.

Abbreviations: AASLD, American Association for the Study of Liver Diseases; ALT, alanine aminotransferase; APASL, Asian Pacific Association for the Study of the Liver; AST, aspartate aminotransferase; BMI, body mass index; EASL, European Association for the Study of the Liver; GGT, gamma-glutamyl transpeptidase; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg-HQ, hepatitis B surface antigen; HBV, hepatitis B virus; LMICs, low- and middle-income countries; WHO, World Health Organization.

E (84%), followed by genotype A (16%). The proportion of patients eligible for antiviral therapy according to the AASLD, EASL, APASL, and WHO criteria for LMICs was 21%, 20%, 22%, and 49%, respectively.

Correlation of HBcrAg With HBV DNA and HBsAg

Correlation coefficient (r) was 0.75 ($P < .0001$) between HBcrAg and HBV DNA (Figure 2). The positive correlation was also confirmed in a subset of patients stratified by HBeAg serostatus ($r = 0.59$, $P = .0002$ for HBeAg positive; and $r = 0.57$, $P < .0001$ for HBeAg negative), and by genotype ($r = 0.69$, $P < .0001$ for genotype A; and $r = 0.76$, $P < .0001$ for genotype E) (Supplementary Figure 1). In contrast, the correlation was poor between HBcrAg and HBsAg ($r = 0.22$, $P = .0003$) and between HBsAg and HBV DNA ($r = 0.16$, $P = .006$) (Figure 2), irrespective of HBeAg positivity or viral genotype (Supplementary Figures 2–3).

Performance of HBcrAg to Diagnose Viral Load

The AUROC of HBcrAg to diagnose clinically important HBV DNA levels was 0.88 (95% confidence interval [CI], .82–.93)

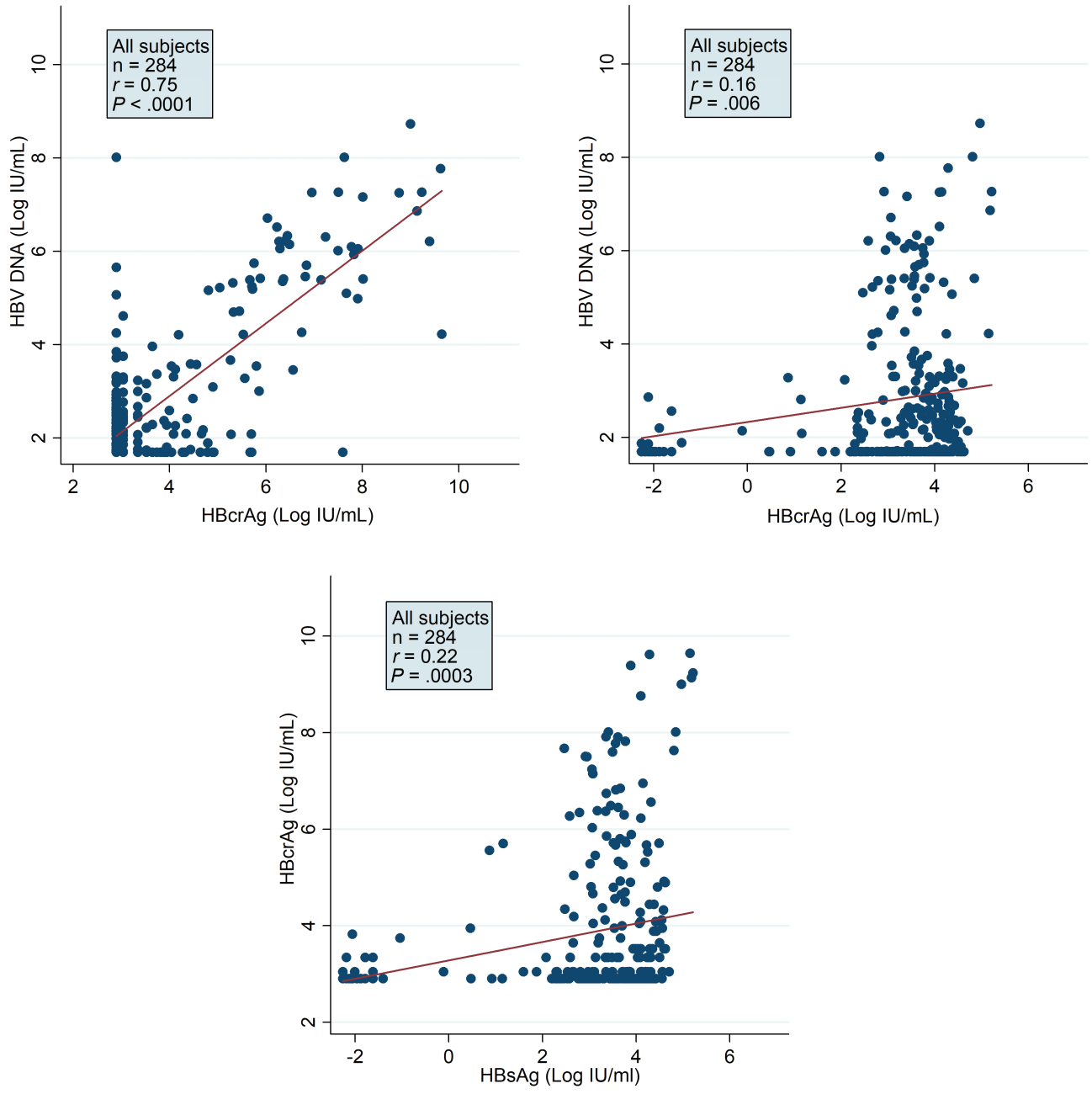

Figure 2. Correlation between hepatitis B core-related antigen (HBcrAg) and hepatitis B virus (HBV) DNA levels; hepatitis B surface antigen (HBsAg) and HBV DNA levels; and HBcrAg and HBsAg levels.

Table 2. Performance of Serum Hepatitis B Core-related Antigen Levels, Hepatitis B Surface Antigen Levels, and Hepatitis B e Antigen Positivity to Discriminate Clinically Important Hepatitis B Virus DNA Levels

	HBV DNA Level								
	≥2000 IU/mL			≥20 000 IU/mL			≥200 000 IU/mL		
Performance Measure	HBcrAg	HBsAg	HBeAg	HBcrAg	HBsAg	HBeAg	HBcrAg	HBsAg	HBeAg
AUROC (95% CI)	0.88 (.82–.93)	0.55 (.48–.62)	0.73 (.66–.79)	0.92 (.87–.98)	0.53 (.45–.61)	0.79 (.71–.86)	0.94 (.88–.99)	0.56 (.47–.66)	0.83 (.75–.91)
P value (compared to HBcrAg)	NA	<.001	<.001	NA	<.001	<.001	NA	<.001	.004
Cutoff	3.6 log U/mL	3.6 log IU/mL	Positive	4.8 log U/mL	3.6 log IU/mL	Positive	5.3 log U/mL	3.7 log IU/mL	Positive
Sensitivity, %	83.3	56.1	47.7	88.9	55.6	61.4	91.4	45.7	70.6
Specificity, %	83.9	49.5	97.6	92.9	49.0	96.0	93.2	53.0	94.9
PPV, %	61.1	25.2	86.1	70.2	17.0	75.0	65.3	12.0	66.7
NPV, %	94.3	78.8	85.5	97.8	85.4	92.8	98.7	87.4	95.7
Positive LR	5.2	1.1	19.6	12.5	1.1	15.5	13.4	1.0	13.9
Negative LR	0.2	0.9	0.5	0.1	0.9	0.4	0.1	1.0	0.3

Abbreviations: AUROC, area under the receiver operating characteristic curve; CI, confidence interval; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; LR, likelihood ratio; NA, not applicable; NPV, negative predictive value; PPV, positive predictive value.

for ≥2000 IU/mL; 0.92 (95% CI, .87–.98) for ≥20 000 IU/mL; and 0.94 (95% CI, .88–.99) for ≥200 000 IU/mL (Table 2 and Figure 3). The optimal cutoff of HBcrAg, sensitivity, and specificity at each HBV DNA level was 3.6 log U/mL, 83.3%, and 83.9%, respectively, to diagnose viremia ≥2000 IU/mL; 4.8 log U/mL, 88.9%, and 92.9% for ≥20 000 IU/mL; and 5.3 log U/mL, 91.4%, and 93.2% for ≥200 000 IU/mL.

In contrast to HBcrAg, HBsAg was not informative; the AUROC was 0.55 (95% CI, .48–.62), 0.53 (95% CI, .45–.61), and 0.56 (95% CI, .47–.66) for ≥2000, ≥20 000, and ≥200 000 IU/mL, respectively. The AUROC of HBeAg was modest: 0.73 (95% CI, .66–.79) for ≥2000 IU/mL; 0.79 (95% CI, .71–.86) for ≥20 000 IU/mL; and 0.83 (95% CI, .75–.91) for ≥200 000 IU/mL. HBcrAg performed significantly better than HBsAg and HBeAg for all of these HBV DNA thresholds (Table 2).

Association of HBcrAg With ALT and Fibrosis Stage

Box plots of HBcrAg according to ALT levels and fibrosis stage showed the positive correlation between these variables (Supplementary Figure 4). Of the virological factors, serum HBcrAg was the only variable independently associated with significant fibrosis (Table 3); compared to those with low HBcrAg levels (<3.6 log U/mL), its risk was 2.6 times (95% CI, 1.2–5.8) higher in those with 3.6–5.3 log U/mL, and 19.7 times (4.3–91.1) higher in those with ≥5.3 log U/mL (adjusted $P < .001$). Similarly, the statistically significant association with elevated ALT levels (≥40 IU/L) was only observed for HBcrAg after mutually adjusting for other viral factors. No statistically significant association was observed in the rest (HBsAg, HBeAg, HBV DNA, and genotype). Without any significant change in the standard errors of regression coefficients of the virological factors between the crude and adjusted analyses, collinearity between these was unlikely.

Performance of Simplified Treatment Algorithms Using HBcrAg

The AUROC of model 1, the algorithm using HBcrAg, HBeAg, ALT, and FibroScan, without HBV DNA, to select patients eligible for antiviral therapy was 0.91 (95% CI, .88–.95) for AASLD, 0.91 (95% CI, .88–.94) for EASL, and 0.96 (95% CI, .93–.98) for APASL (Table 4; Figure 4). The AUROC of model 2, the simplified score based on HBcrAg and ALT levels, was 0.90 (95% CI, .85–.94) for AASLD, 0.89 (95% CI, .84–.94) for EASL, and 0.96 (95% CI, .94–.98) for APASL. The AUROC did not significantly differ between models 1 and 2 across the guidelines (Supplementary Table 4). Model 3, which only uses HBcrAg, did not perform well compared to models 1 and 2, with the AUROC varying between 0.80 and 0.84.

TREAT-B, composed of ALT and HBeAg, showed AUROC of 0.87 (95% CI, .81–.92) for AASLD, 0.87 (95% CI, .81–.93) for EASL, and 0.95 (95% CI, .93–.98) for APASL. Compared to TREAT-B, the AUROC of model 1 was marginally higher to diagnose AASLD ($P = .09$) and EASL ($P = .07$), but no difference was observed for APASL ($P = .8$, Supplementary Table 4). The AUROC of model 2 was significantly higher than that of TREAT-B to indicate AASLD criteria ($P = .04$, Supplementary Table 4); however, there was no statistically significant difference for EASL ($P = .2$) and APASL ($P = .8$). The WHO criteria discriminated poorly: the AUROCs ranged between 0.73 and 0.80 and were significantly lower than those of any of the algorithms presented, except for model 3 to diagnose APASL criteria.

DISCUSSION

In developed countries, HBcrAg has recently emerged as a novel tool to monitor HBV-infected patients under nucleos(t)ide analogue therapy [28]. Although persistence of HBV cccDNA in the nucleus of infected hepatocytes determines the chronicity

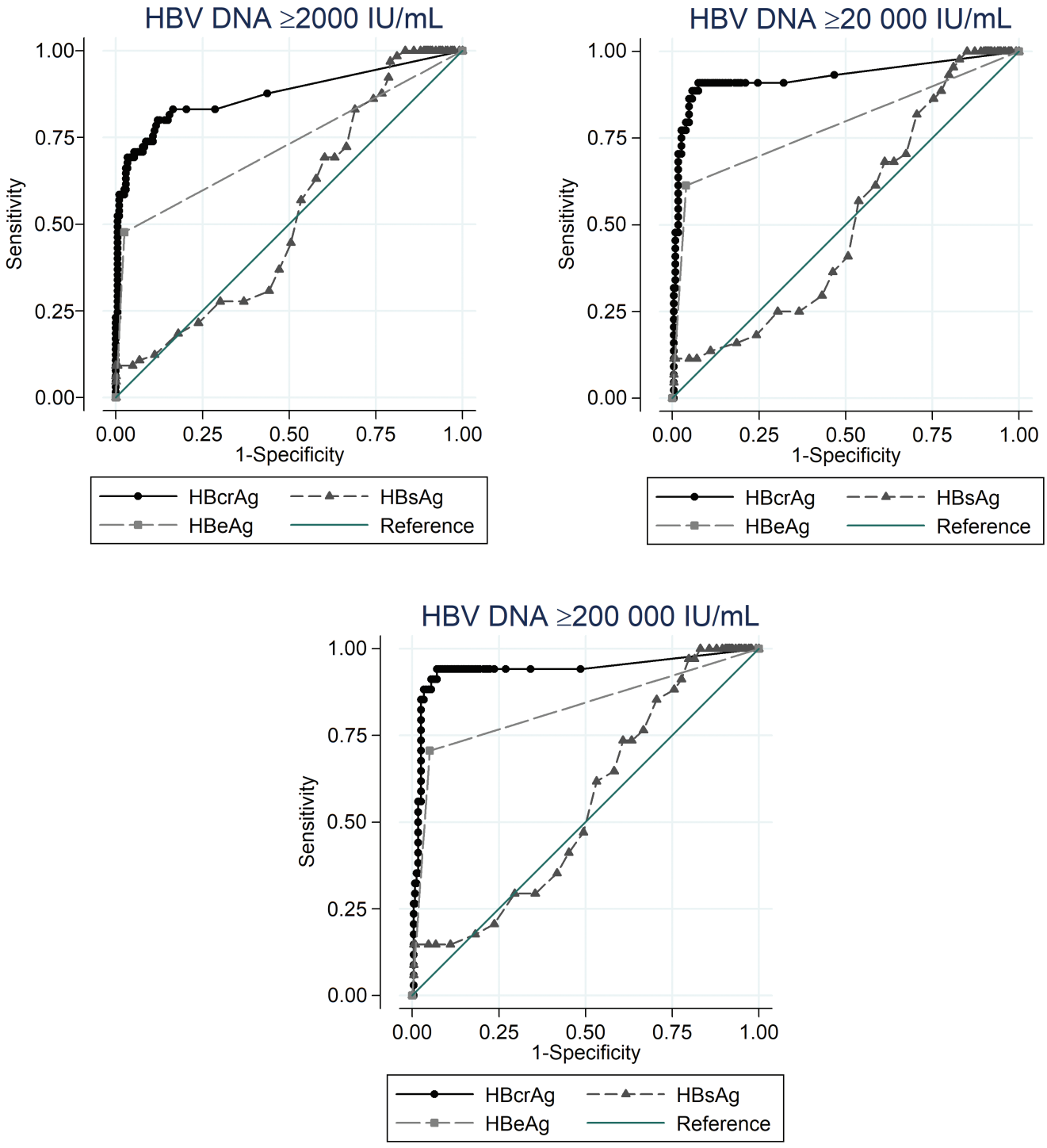

Figure 3. Receiver operating characteristic curves for hepatitis B core-related antigen (HBcrAg), hepatitis B surface antigen (HBsAg), and hepatitis B e antigen (HBeAg) to indicate serum hepatitis B virus (HBV) DNA levels.

of HBV infection and therefore represents a genuine marker of HBV replication, it is difficult to measure intrahepatic amount of cccDNA in routine clinical practice as this requires liver biopsy. Alternatively, serum HBV DNA is commonly used as a surrogate biomarker to evaluate HBV replication. However, its correlation with intrahepatic cccDNA is lost in patients treated with nucleos(t)ide analogues, because these drugs almost invariably lead to undetectable serum HBV DNA by blocking

Table 3. Virological Factors Associated With Significant Liver Fibrosis and Elevated Alanine Aminotransferase Levels

		Crude		Adjusted[a]	
Virological Factor	Significant Liver Fibrosis	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Association with significant liver fibrosis					
HBcrAg, log U/mL					
<3.6	20%	1.0	<.001	1.0	<.001
3.6–5.2	37%	2.3 (1.1–4.7)		2.6 (1.2–5.8)	
≥5.3	67%	8.2 (4.1–16.4)		19.7 (4.3–91.1)	
HBsAg, log IU/mL					
<3.6	35%	1.0	.2	…	
≥3.6	27%	0.7 (.4–1.1)		…	
HBeAg					
Negative	26%	1.0	<.001	1.0	.4
Positive	61%	4.6 (2.2–9.5)		0.6 (.2–2.1)	
HBV DNA, IU/mL					
Undetectable	25%	1.0	<.001	1.0	.8
50–1999 IU/mL	24%	1.0 (.5–1.8)		1.0 (.5–2.1)	
2000–199 999 IU/mL	39%	1.9 (.8–4.3)		0.7 (.2–2.0)	
≥200 000 IU/mL	60%	4.5 (2.0–9.8)		0.6 (.1–2.5)	
HBV genotype					
E	29%	1.0	1.0	…	
A	29%	1.0 (.5–2.2)		…	
Association with elevated ALT	ALT ≥40 IU/L				
HBcrAg, log U/mL					
<3.6	15%	1.0	<.001	1.0	.003
3.6–5.3	29%	2.3 (1.0–5.3)		1.9 (.8–4.7)	
≥5.3	73%	15.5 (7.3–32.9)		12.0 (2.8–50.6)	
HBsAg, log IU/mL					
<3.6	31%	1.0	.1	…	
≥3.6	23%	0.6 (.4–1.1)		…	
HBeAg					
Negative	20%	1.0	<.001	1.0	.7
Positive	69%	8.7 (4.0–19.0)		0.8 (.2–3.0)	
HBV DNA, IU/mL					
Undetectable	18%	1.0	<.001	1.0	.4
50–1999 IU/mL	15%	0.8 (.4–1.6)		0.8 (.4–1.8)	
2000–199 999 IU/mL	48%	4.2 (1.8–10.0)		2.0 (.7–5.8)	
≥200 000 IU/mL	71%	11.2 (4.7–26.8)		1.7 (.4–6.9)	
HBV genotype					
E	26%	1.0	.9	…	
A	27%	1.0 (.5–2.3)		…	

Abbreviations: ALT, alanine aminotransferase; CI, confidence interval; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; OR, odds ratio.

[a]The variables significantly associated with the outcomes in the crude analyses ($P < .05$) were mutually adjusted.

reverse transcription, whereas cccDNA still persists in the majority of treated patients [29]. Another frequently used biomarker is serum HBsAg levels, but the degree of correlation with intrahepatic cccDNA is controversial, particularly for those negative for HBeAg, as HBsAg can be derived not only from cccDNA, but also from HBV DNA integrated into the host genome [30]. In contrast, serum HBcrAg was found to be closely correlated with the amount of intrahepatic cccDNA before antiviral therapy [10, 11, 14, 15, 31, 32]. After the initiation of nucleos(t)ide analogues, HBcrAg was found to reduce to a similar extent as the reduction in cccDNA [10, 11, 14, 31]. Moreover, the transcriptional activity of intrahepatic cccDNA, represented by pregenomic RNA, has been also shown to be correlated with serum HBcrAg levels in patients with [33] or without nucleos(t)ide analogues [15]. Consequently, HBcrAg is now proposed as a novel marker for treatment response monitoring and also as an endpoint for clinical trials of novel HBV drugs aiming at a functional cure of HBV infection [15, 28, 34].

In addition to its valuable and unique role in monitoring patients under HBV treatment, this study demonstrated for the first time that HBcrAg might be a useful alternative to serum HBV DNA for the initial clinical assessment following HBsAg

Table 4. Performance of Simplified Algorithm Using Hepatitis B Core-related Antigen, Treatment Eligibility in Africa for Hepatitis B Virus, and World Health Organization Criteria to Select Patients Eligible for Antiviral Therapy

	HBcrAg-based Algorithm				
Performance Measure	Model 1[a] (HBcrAg, HBeAg, FibroScan, ALT)	Model 2 (HBcrAg, ALT)	Model 3 (HBcrAg Alone)	TREAT-B (HBeAg, ALT)	WHO (APRI, ALT)
AASLD 2018					
AUROC (95% CI)	0.91 (.88–.95)	0.90 (.85–.94)	0.84 (.77–.91)	0.87 (.81–.92)	0.73 (.68–.79)
Cutoff	NA	2 points	3.6 log U/mL	2 points	NA
Sensitivity, %	96.6	89.3	83.1	81.8	86.4
Specificity, %	85.8	74.9	81.8	82.8	60.4
PPV, %	64.0	47.6	54.4	55.6	36.4
NPV, %	99.0	96.5	94.8	94.5	94.4
Positive LR	6.8	3.6	4.6	4.8	2.2
Negative LR	0.1	0.1	0.2	0.2	0.2
EASL 2017					
AUROC (95% CI)	0.91 (.88–.94)	0.89 (.84–.94)	0.84 (.78–.91)	0.87 (.81–.93)	0.73 (.68–.79)
Cutoff	NA	2 points	3.6 log U/mL	2 points	NA
Sensitivity, %	96.6	89.1	82.8	81.5	86.2
Specificity, %	85.4	74.5	81.4	82.4	60.2
PPV, %	62.9	46.7	53.3	54.3	35.7
NPV, %	99.0	96.5	94.8	94.5	94.4
Positive LR	6.6	3.5	4.5	4.6	2.2
Negative LR	0.1	0.1	0.2	0.2	0.2
APASL 2015					
AUROC (95% CI)	0.96 (.93–.98)	0.96 (.94–.98)	0.80 (.73–.87)	0.95 (.93–.98)	0.80 (.75–.84)
Cutoff	NA	2 points	3.6 log U/mL	2 points	NA
Sensitivity, %	96.8	100	74.6	96.6	95.2
Specificity, %	94.6	79.4	80.5	87.9	63.8
PPV, %	83.6	58.1	52.2	69.1	42.9
NPV, %	99.1	100	91.8	98.9	97.9
Positive LR	17.8	4.9	3.8	8.0	2.6
Negative LR	0.1	0	0.3	0.1	0.1

Abbreviations: AASLD, American Association for the Study of Liver Diseases; ALT, alanine aminotransferase; APASL, Asian Pacific Association for the Study of the Liver; APRI, aspartate aminotransferase to platelet ratio index; AUROC, area under the receiver operating characteristic curve; CI, confidence interval; EASL, European Association for the Study of the Liver; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; LR, likelihood ratio; NA, not applicable; NPV, negative predictive value; PPV, positive predictive value; TREAT-B, Treatment Eligibility in Africa for Hepatitis B Virus; WHO, World Health Organization.

[a]Model 1 is described in detail in Supplementary Table 3.

screening, to select patients in need of antiviral therapy in resource-limited settings. By comparing with serum HBV DNA PCR and treatment eligibility criteria centered by HBV viral load as references, we found (1) close correlation between HBcrAg and HBV DNA irrespective of HBeAg serostatus and HBV genotypes; (2) excellent performance of HBcrAg to diagnose HBV DNA levels of ≥2000, ≥20 000 and ≥200 000 IU/mL; and (3) high accuracy of the simplified treatment algorithm using HBcrAg serology. Moreover, accumulating evidence suggests that HBcrAg may not only serve as an alternative, but may even be superior to HBV DNA in identifying treatment-naive patients at elevated risk of liver disease. Tada et al found that HBcrAg was more accurate than HBV DNA to predict the development of HCC in a cohort of 1031 treatment-naive CHB patients after a median follow-up period of 10.7 years without antiviral treatment [35]. The same group also reported the superiority of HBcrAg to HBV DNA in predicting the progression to cirrhosis in patients without antiviral therapy [36]. Indeed, our study found that HBcrAg was independently associated with significant fibrosis and liver inflammation after adjusting for HBV DNA and HBeAg, whereas other HBV markers were not. These results support that the risk stratification based on HBcrAg might be more accurate than using HBV DNA to assess eligibility for antiviral therapy in CHB patients, although this needs to be further assessed in a longitudinal cohort study.

Compared to the conventional molecular assay, serological assay is better adapted to LMICs with limited laboratory capacity because this may be less expensive and simpler to perform. However, the recent advent of inexpensive, automated point-of-care PCR assays, such as GeneXpert, may change the landscape of HBV diagnostics in LMICs. Further simplification of the HBcrAg assay by developing a rapid diagnostic test with immunochromatographic lateral flow assay will be feasible and possible at a lower cost than the point-of-care HBV DNA PCR. Lowering the limit of detection may not be the priority for such a test; for example, a rapid test detecting

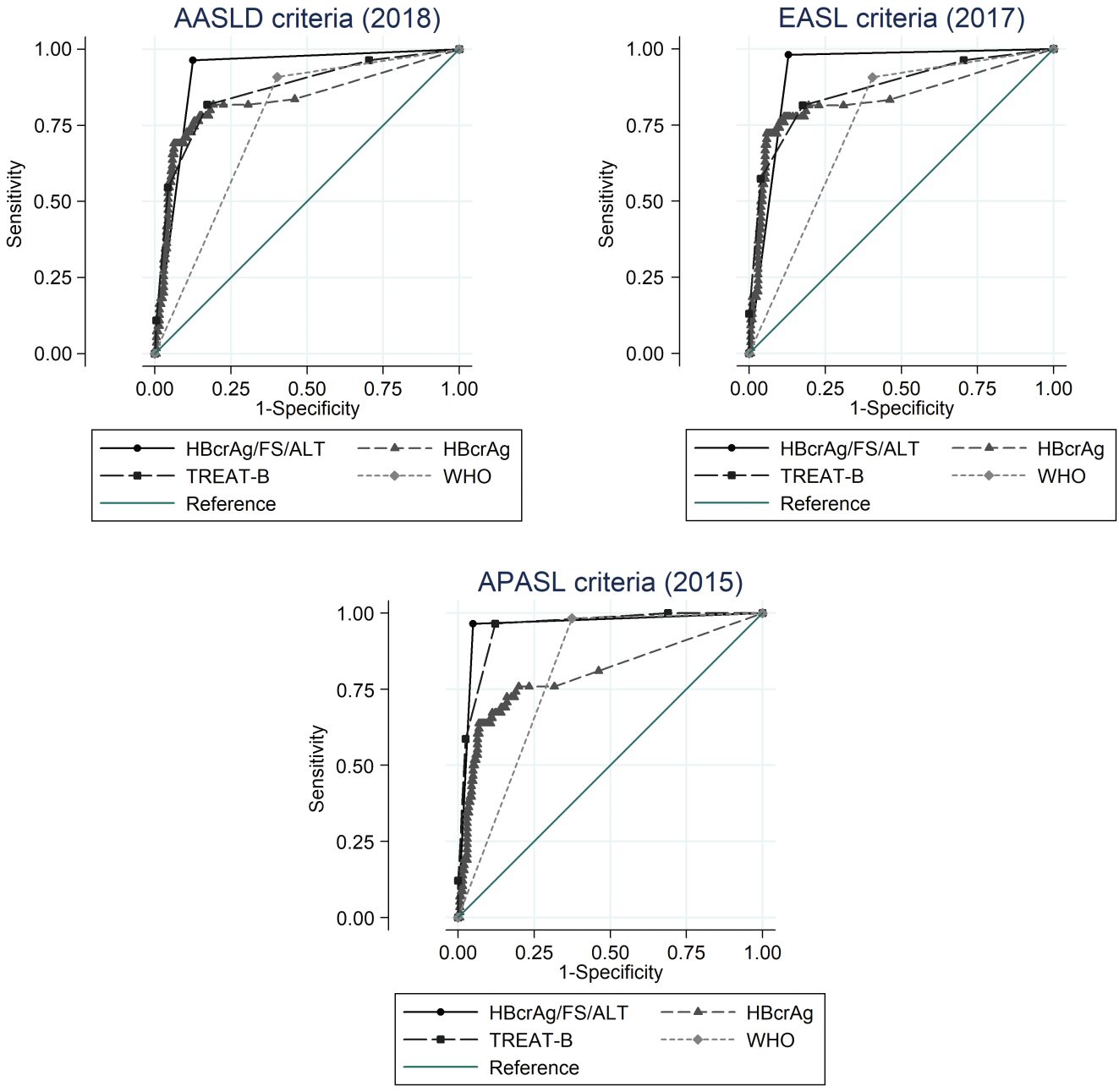

Figure 4. Receiver operating characteristic curves for simplified algorithms to indicate treatment eligibility according to international guidelines. Abbreviations: AASLD, American Association for the Study of Liver Diseases; ALT, alanine aminotransferase; APASL, Asian Pacific Association for the Study of the Liver; EASL, European Association for the Study of the Liver; FS, FibroScan; HBcrAg, hepatitis B core-related antigen; TREAT-B, Treatment Eligibility in Africa for Hepatitis B Virus; WHO, World Health Organization.

very high HBcrAg levels of 5.3 log U/mL (equivalent to serum HBV DNA levels of 200 000 IU/mL in this study) should be enough to identify pregnant women who would benefit most from antiviral therapy to prevent mother-to-child transmission [37], given the high diagnostic sensitivity (91.4%) and specificity (93.2%) to indicate viral load threshold associated with immunoprophylaxis failure [38]. Moreover, the improvement in analytical sensitivity of HBcrAg has been recently made (unpublished data), and this may also contribute to the future development of rapid HBcrAg test to diagnose lower thresholds equivalent to serum HBV DNA levels of 2000 or 20 000 IU/mL.

As a limitation, HBcrAg was measured in a laboratory in Japan using stored serum samples. We will soon start a field study to validate HBcrAg in a resource-limited African laboratory. Whether HBcrAg can be used for identifying African patients in

the inactive phase who have poor prognosis remains unknown. This question will be addressed through a longitudinal follow-up of the PROLIFICA cohort in West Africa. Our study was limited to HBV genotypes A and E, and the majority were HBeAg negative with low viral load; a meta-analysis is under way to assess the performance of HBcrAg in different HBV genotypes, with a wide range of viral load and HBeAg seropositivity (PROSPERO registration number CRD42017055440).

Without having simple, affordable, and reliable diagnostic tools to evaluate active HBV replication, it is unlikely to reach the WHO's global elimination goals [39]. HBcrAg, a promising alternative to HBV DNA PCR, warrants further validation.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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References

1. World Health Organization. Global health sector strategy on viral hepatitis 2016–2021. Geneva, Switzerland: WHO, **2016**.
2. Terrault NA, Lok ASF, McMahon BJ, et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. Hepatology **2018**; 67:1560–99.
3. European Association for the Study of the Liver. EASL 2017 clinical practice guidelines on the management of hepatitis B virus infection. J Hepatol **2017**; 67:370–98.
4. Sarin SK, Kumar M, Lau GK, et al. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. Hepatol Int **2016**; 10:1–98.
5. World Health Organization. Guidelines for the prevention, care and treatment of persons with chronic hepatitis B infection. Geneva, Switzerland: WHO, **2015**.
6. Razavi-Shearer D, Gamkrelidze I, Nguyen MH, et al. Global prevalence, treatment, and prevention of hepatitis B virus infection in 2016: a modelling study. Lancet Gastroenterol Hepatol **2018**; 3:383–403.
7. Andriamandimby SF, Olive MM, Shimakawa Y, et al. Prevalence of chronic hepatitis B virus infection and infrastructure for its diagnosis in Madagascar: implication for the WHO's elimination strategy. BMC Public Health **2017**; 17:636.
8. World Health Organization. Guidelines on hepatitis B and C testing. Geneva, Switzerland: WHO, **2017**.
9. Kimura T, Rokuhara A, Sakamoto Y, et al. Sensitive enzyme immunoassay for hepatitis B virus core-related antigens and their correlation to virus load. J Clin Microbiol **2002**; 40:439–45.
10. Wong DK, Tanaka Y, Lai CL, Mizokami M, Fung J, Yuen MF. Hepatitis B virus core-related antigens as markers for monitoring chronic hepatitis B infection. J Clin Microbiol **2007**; 45:3942–7.
11. Suzuki F, Miyakoshi H, Kobayashi M, Kumada H. Correlation between serum hepatitis B virus core-related antigen and intrahepatic covalently closed circular DNA in chronic hepatitis B patients. J Med Virol **2009**; 81:27–33.
12. Maasoumy B, Wiegand SB, Jaroszewicz J, et al. Hepatitis B core-related antigen (HBcrAg) levels in the natural history of hepatitis B virus infection in a large European cohort predominantly infected with genotypes A and D. Clin Microbiol Infect **2015**; 21:606.e1–10.
13. Riveiro-Barciela M, Bes M, Rodríguez-Frías F, et al. Serum hepatitis B core-related antigen is more accurate than hepatitis B surface antigen to identify inactive carriers, regardless of hepatitis B virus genotype. Clin Microbiol Infect **2017**; 23:860–7.
14. Wong DK, Seto WK, Cheung KS, et al. Hepatitis B virus core-related antigen as a surrogate marker for covalently closed circular DNA. Liver Int **2017**; 37:995–1001.
15. Testoni B, Lebossé F, Scholtes C, et al. Serum hepatitis B core-related antigen (HBcrAg) correlates with covalently closed circular DNA transcriptional activity in chronic hepatitis B patients. J Hepatol **2019**; 70:615–25.
16. Duchesne L, Njouom R, Lissock F, et al. HCV Ag quantification as a one-step procedure in diagnosing chronic hepatitis C infection in Cameroon: the ANRS 12336 study. J Int AIDS Soc **2017**; 20:1–8.
17. Mohamed Z, Mbwambo J, Shimakawa Y, et al. Clinical utility of HCV core antigen detection and quantification using serum samples and dried blood spots in people who inject drugs in Dar-es-Salaam, Tanzania. J Int AIDS Soc **2017**; 20:21856.
18. Freiman JM, Tran TM, Schumacher SG, et al. Hepatitis C core antigen testing for diagnosis of hepatitis C virus infection: a systematic review and meta-analysis. Ann Intern Med **2016**; 165:345–55.
19. Lemoine M, Shimakawa Y, Njie R, et al; PROLIFICA Investigators. Acceptability and feasibility of a screen-and-treat programme for hepatitis B virus infection in The Gambia: the prevention of liver fibrosis and cancer in Africa (PROLIFICA) study. Lancet Glob Health **2016**; 4:e559–67.
20. Shimakawa Y, Lemoine M, Njai HF, et al. Natural history of chronic HBV infection in West Africa: a longitudinal population-based study from The Gambia. Gut **2016**; 65:2007–16.
21. Shimakawa Y, Lemoine M, Bottomley C, et al. Birth order and risk of hepatocellular carcinoma in chronic carriers of hepatitis B virus: a case-control study in The Gambia. Liver Int **2015**; 35:2318–26.
22. Lemoine M, Shimakawa Y, Njie R, et al. Food intake increases liver stiffness measurements and hampers reliable values in patients with chronic hepatitis B and healthy controls: the PROLIFICA experience in The Gambia. Aliment Pharmacol Ther **2014**; 39:188–96.
23. Ghosh S, Sow A, Guillot C, et al. Implementation of an in-house quantitative real-time polymerase chain reaction method for hepatitis B virus quantification in West African countries. J Viral Hepat **2016**; 23:897–904.
24. Lemoine M, Shimakawa Y, Nayagam S, et al. The gamma-glutamyl transpeptidase to platelet ratio (GPR) predicts significant liver fibrosis and cirrhosis in patients with chronic HBV infection in West Africa. Gut **2016**; 65:1369–76.
25. Shimakawa Y, Bah E, Wild CP, Hall AJ. Evaluation of data quality at The Gambia national cancer registry. Int J Cancer **2013**; 132:658–65.
26. Shimakawa Y, Njie R, Ndow G, et al. Development of a simple score based on HBeAg and ALT for selecting patients for HBV treatment in Africa. J Hepatol **2018**; 69:776–84.
27. Bossuyt PM, Reitsma JB, Bruns DE, et al. STARD 2015: an updated list of essential items for reporting diagnostic accuracy studies. Radiology **2015**; 277:826–32.
28. Mak LY, Wong DK, Cheung KS, Seto WK, Lai CL, Yuen MF. Review article: hepatitis B core-related antigen (HBcrAg): an emerging marker for chronic hepatitis B virus infection. Aliment Pharmacol Ther **2018**; 47:43–54.
29. Lai CL, Wong D, Ip P, et al. Reduction of covalently closed circular DNA with long-term nucleos(t)ide analogue treatment in chronic hepatitis B. J Hepatol **2017**; 66:275–81.
30. Cornberg M, Wong VW, Locarnini S, Brunetto M, Janssen HLA, Chan HL. The role of quantitative hepatitis B surface antigen revisited. J Hepatol **2017**; 66:398–411.
31. Chen EQ, Feng S, Wang ML, et al. Serum hepatitis B core-related antigen is a satisfactory surrogate marker of intrahepatic covalently closed circular DNA in chronic hepatitis B. Sci Rep **2017**; 7:173.
32. Chuaypen N, Posuwan N, Chittmittraprap S, et al. Predictive role of serum HBsAg and HBcrAg kinetics in patients with HBeAg-negative chronic hepatitis B receiving pegylated interferon–based therapy. Clin Microbiol Infect **2018**; 24:306.e7–13.
33. Honda M, Shirasaki T, Terashima T, et al. Hepatitis B virus (HBV) core-related antigen during nucleos(t)ide analog therapy is related to intra-hepatic HBV replication and development of hepatocellular carcinoma. J Infect Dis **2016**; 213:1096–106.
34. Seto WK, Lo YR, Pawlotsky JM, Yuen MF. Chronic hepatitis B virus infection. Lancet **2018**; 392:2313–24.
35. Tada T, Kumada T, Toyoda H, et al. HBcrAg predicts hepatocellular carcinoma development: an analysis using time-dependent receiver operating characteristics. J Hepatol **2016**; 65:48–56.

36. Tada T, Kumada T, Toyoda H, Kobayashi N, Akita T, Tanaka J. Hepatitis B virus core-related antigen levels predict progression to liver cirrhosis in hepatitis B carriers. J Gastroenterol Hepatol **2018**; 33:918–25.
37. Shimakawa Y, Seck A, Nayagam S, Toure-Kane C, Lemoine M. Screening strategies to prevent mother-to-child transmission of hepatitis B in sub-Saharan Africa. Lancet Gastroenterol Hepatol **2018**; 3:222–3.
38. Wen WH, Chang MH, Zhao LL, et al. Mother-to-infant transmission of hepatitis B virus infection: significance of maternal viral load and strategies for intervention. J Hepatol **2013**; 59:24–30.
39. Seck A, Ndiaye F, Maylin S, et al. Poor sensitivity of commercial rapid diagnostic tests for hepatitis B e antigen in Senegal, West Africa. Am J Trop Med Hyg **2018**; 99:428–34.