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Hepatitis B core-related antigen (HBcrAg): an alternative to HBV DNA to assess treatment eligibility in Africa

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Summary

Limited access to HBV DNA quantification represents a key barrier to global HBV elimination. We demonstrated high diagnostic accuracy of low-cost immunoassay, hepatitis B core-related antigen, to diagnose HBV DNA levels, and to select patients for antiviral therapy in Africa.

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Abstract

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 novel immunoassay, hepatitis B core-related antigen (HBcrAg), as an inexpensive (US$ <10-15/assay) alternative to NAT to diagnose clinically important HBV DNA thresholds (≥2,000; ≥20,000; and ≥200,000 IU/ml), and select patients for antiviral therapy in Africa.

Methods

Using well-characterized cohort of treatment-naïve 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 the reference tests (HBV DNA, HBV e antigen (HBeAg), alanine transaminase (ALT), liver histopathology and/or FibroScan).

Results

A total of 284 treatment-naïve 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% CI: 0.82-0.93), 83.3% and 83.9% to diagnose HBV DNA ≥2,000 IU/ml; and 0.94 (0.88-0.99), 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: 0.88-0.95)) with a sensitivity of 96.6% and specificity of 85.8%.

Conclusions

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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

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Introduction

Hepatitis B Virus (HBV) infection is a major global health problem, and recognized as a public health priority by the UN's Sustainable Development Goals. Subsequently, the World Health Organization (WHO) has developed a strategy to eliminate viral hepatitis by 2030, and 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 the clinical staging. The international guidelines define having high viremia (≥2,000 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 cut-off 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 low-cost simple assay to measure HBV replication [8].

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

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several studies also found a correlation of 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$ <10-15/assay) and simpler than the conventional real-time PCR (US$ 60-200/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 three clinically important HBV DNA thresholds (2,000, 20,000, and 200,000 IU/ml) in a well-characterized cohort of treatment-naïve 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, USA; or OnSite Combo Rapid Test, CTK Biotech, USA) [19,20]. In addition, the program also recruited symptomatic patients with chronic liver

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disease referred from health facilities throughout the country [21]. After informed consent, HBsAg-positive participants systematically underwent following clinical evaluation: 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 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, HIV co-infection, or missing virological data.

Serum HBcrAg and HBsAg-HQ

Patients' sera at the 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 Inc, 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 (HBsAg-HQ) with Lumipulse (limit of detection: 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), European Association for the Study of the Liver (EASL), and Asian Pacific Association for the Study of the Liver (APASL) largely rely on three factors: levels of viral replication by HBV

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DNA PCR and/or HBeAg sero-status, degree of liver inflammation based on liver histopathology and/or alanine aminotransferase (ALT) levels, and fibrosis staging by histopathology or liver stiffness measurement [2–4]. For these criteria, significant liver fibrosis and cirrhosis were defined as Metavir ≥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 guidelines provides criteria for LMICs where HBV DNA testing is not available: cirrhosis, diagnosed by physical examination or aspartate aminotransferase (AST)-to-platelet ration index (APRI) >2.0; or persistently elevated ALT [5]. Because the cross-sectional data was 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 guidelines (Supplementary Table 2).

Simplified treatment algorithms using HBcrAg

We developed three simplified algorithms using HBcrAg (Models 1-3) to select HBsAg-positive patients for antiviral therapy. Model 1 is exactly same as the conventional criteria (AASLD, EASL and APASL) except for HBV DNA which was replaced by HBcrAg, and liver histopathology replaced by FibroScan. Optimal HBcrAg cut-off levels equivalent to HBV DNA thresholds of ≥2,000 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 ≥2,000 IU/ml. The total point was obtained by adding: HBcrAg score, low (0 point) or high (1); and ALT score, <20 IU/L (0

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point), 20-39 (1), 40-79 (2) or ≥80 (3). 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-HQ were $\log_{10}$ transformed, and the detection limit of each assay was assigned to samples with undetectable result. The correlation between these markers was assessed using Pearson's correlation coefficient. The correlation was also evaluated by HBeAg sero-status and viral genotypes. The capability of HBcrAg levels to correctly discriminate clinically important HBV DNA levels at three different cut-offs (≥2,000, ≥20,000, and ≥200,000 IU/ml) was evaluated by the receiver operating characteristic (ROC) curve. The optimal cut-offs 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-HQ levels and HBeAg using area under the ROC curve (AUROC).

Among the virological factors (HBcrAg/HBsAg-HQ/HBeAg/HBV DNA/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 ($p<0.05$) 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/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 13.0 (Stata Corporation, USA). The

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study was approved by the Gambian Government/MRC Joint Ethics Committee, and reported in accordance with the STARD [27].

Results

Study participants

Of 372 HBsAg-positive participants assessed for serum HBcrAg, 284 were included in the current analysis, after excluding 74 HCC cases, 9 HIV co-infection, and 5 with missing data (Figure 1). Their characteristics were described in Table 1. Median age was 36 years (interquartile range, IQR: 30-45), 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-HQ, HBcrAg, and HBV DNA were 3.6 log IU/ml (IQR: 2.9-4.1), 4.0 log U/ml (3.3-5.7), and 2.9 log IU/ml (2.2-5.0), respectively, after excluding undetectable values. Majority harbored genotype E (84%), followed by A (16%). 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-HQ

Correlation coefficient (r) was 0.75 ($p<0.0001$) between HBcrAg and HBV DNA (Figure 2). The positive correlation was also confirmed in a subset of patients stratified by HBeAg sero-status ($r=0.59$, $p=0.0002$ for HBeAg-positive; and $r=0.57$, $p<0.0001$ for HBeAg-negative), and by genotype ($r=0.69$, $p<0.0001$ for genotype A; and $r=0.76$, $p<0.0001$ for genotype E) (Supplementary Figure 1). In contrast, the correlation was poor between HBcrAg and HBsAg-

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HQ (r=0.22, p=0.0003), and between HBsAg-HQ and HBV DNA (r=0.16, p=0.006) (Figure 2), irrespective of HBeAg positivity or viral genotype (Supplementary Figures 2-3).

Performance of HBcrAg to diagnose viral load

AUROC of HBcrAg to diagnose clinically important HBV DNA levels were: 0.88 (95% CI: 0.82-0.93) for ≥2,000 IU/ml; 0.92 (0.87-0.98) for ≥20,000 IU/ml; and 0.94 (0.88-0.99) for ≥200,000 IU/ml (Table 2, Figure 3). The optimal cut-off of HBcrAg, sensitivity and specificity at each HBV DNA levels were: 3.6 log U/ml, 83.3% and 83.9% to diagnose viremia ≥2,000 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-HQ was not informative; the AUROC was 0.55 (95% CI: 0.48-0.62), 0.53 (0.45-0.61), and 0.56 (0.47-0.66), for ≥2,000, ≥20,000 and ≥200,000 IU/ml, respectively. AUROC of HBeAg was modest: 0.73 (95% CI: 0.66-0.79) for ≥2,000 IU/ml; 0.79 (0.71-0.86) for ≥20,000 IU/ml; and 0.83 (0.75-0.91) for ≥200,000 IU/ml. HBcrAg performed significantly better than HBsAg-HQ and HBeAg for all 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<0.001). Similarly, the statistically significant association with elevated ALT levels (≥40 IU/L)

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was only observed for HBcrAg after mutually adjusting for other viral factors. No statistically significant association was observed in the rest (HBsAg-HQ, 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

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: 0.88-0.95) for AASLD, 0.91 (0.88-0.94) for EASL, and 0.96 (0.93-0.98) for APASL (Table 4, Figure 4). AUROC of Model 2, the simplified score based on HBcrAg and ALT levels was 0.90 (95% CI: 0.85-0.94) for AASLD, 0.89 (0.84-0.94) for EASL, and 0.96 (0.94-0.98) for APASL. The AUROC did not significantly differ between the Model 1 and 2 across the guidelines (Supplementary Table 4). Model 3 which only uses HBcrAg did not perform well compared to the Model 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: 0.81-0.92) for AASLD, 0.87 (0.81-0.93) for EASL, and 0.95 (0.93-0.98) for APASL. Compared to TREAT-B, the AUROC of Model 1 was marginally higher to diagnose AASLD (p=0.09) and EASL (p=0.07), but no difference was observed for APASL (p=0.8, Supplementary Table 4). The AUROC of Model 2 was significantly higher than that of TREAT-B to indicate AASLD criteria (p=0.04, Supplementary Table 4); however, there was no statistically significant difference for EASL (p=0.2) and APASL (p=0.8). 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 the Model 3 to diagnose APASL criteria.

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Discussion

In developed countries, HBcrAg has recently emerged as a novel tool to monitor HBV-infected patients under nucleos(t)ide analogues therapy [28]. Although persistence of HBV cccDNA in the nucleus of infected hepatocytes determines the chronicity 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)ides analogues, because these drugs almost invariably lead to undetectable serum HBV DNA by blocking reverse transcription, while cccDNA still persists in 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, since 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)ides analogues, HBcrAg was found to reduce to a similar extent to 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)ides 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].

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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 useful alternative to serum HBV DNA for the initial clinical assessment following HBsAg 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 (i) close correlation between HBcrAg and HBV DNA irrespective of HBeAg sero-status and HBV genotypes; (ii) excellent performance of HBcrAg to diagnose HBV DNA levels of ≥2,000, ≥20,000 and ≥200,000 IU/ml; and (iii) high accuracy of simplified treatment algorithm using HBcrAg serology. Moreover, accumulating evidence suggest that HBcrAg may not only serve as an "alternative", but even "superior" to HBV DNA in identifying treatment-naïve 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 1,031 treatment-naïve 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, while 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, recent advent of inexpensive automated point-of-care PCR assay, such as GeneXpert, may change the landscape of HBV diagnostics in LMICs. Further simplification of HBcrAg assay by

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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 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 2,000 or 20,000 IU/ml.

As a limitation, HBcrAg was measured in a laboratory in Japan using stored sera. 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 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 underway to assess the performance of HBcrAg in different HBV genotypes, with a wide range of viral load and HBeAg sero-positivity (registered at PROSPERO: 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.

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Conflict of Interest

MI, KM, KA are employees of Fujirebio Inc. YS has served as consultant, and MT has received grants from Gilead Sciences. The others authors declare no conflict of interest.

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Figure Legends

Fig. 1. Flow chart of study participants

Fig. 2. Correlation between HBcrAg & HBV DNA levels, HBsAg-HQ & HBV DNA levels, and HBcrAg & HQ-HBsAg levels

Fig. 3. Receiver operating characteristic curves for HBcrAg, HBsAg-HQ, and HBeAg to indicate serum HBV DNA levels

Fig. 4. Receiver operating characteristic curves for simplified algorithms to indicate treatment eligibility according to the international guidelines

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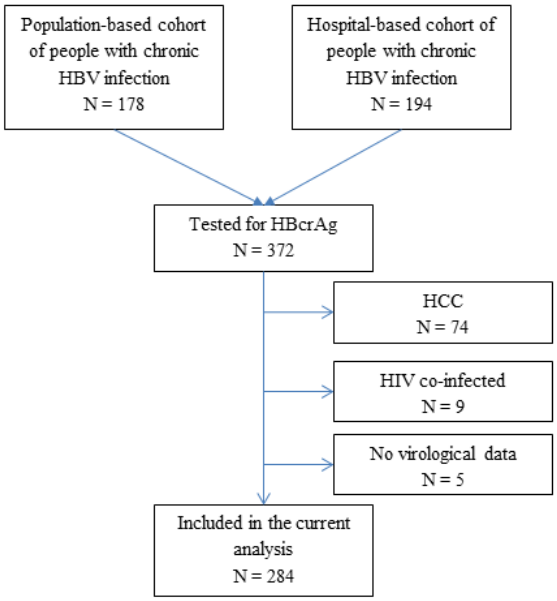

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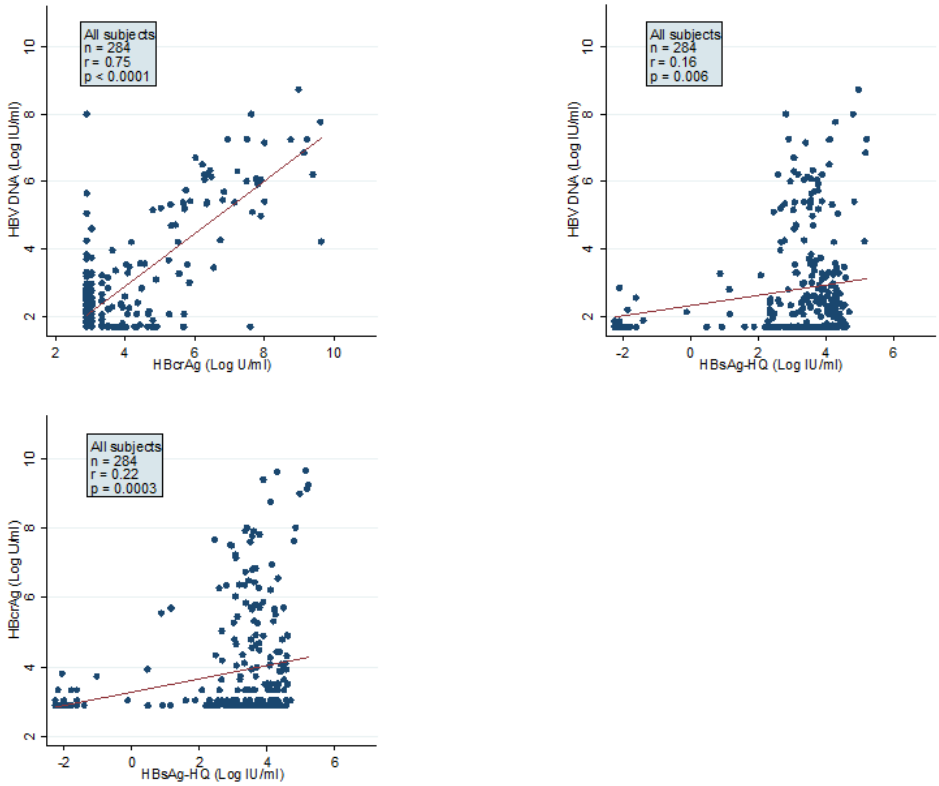

Fig. 2. Correlation between HBcrAg & HBV DNA levels, HBsAg-HQ & HBV DNA levels, and HBcrAg & HQ-HBsAg levels

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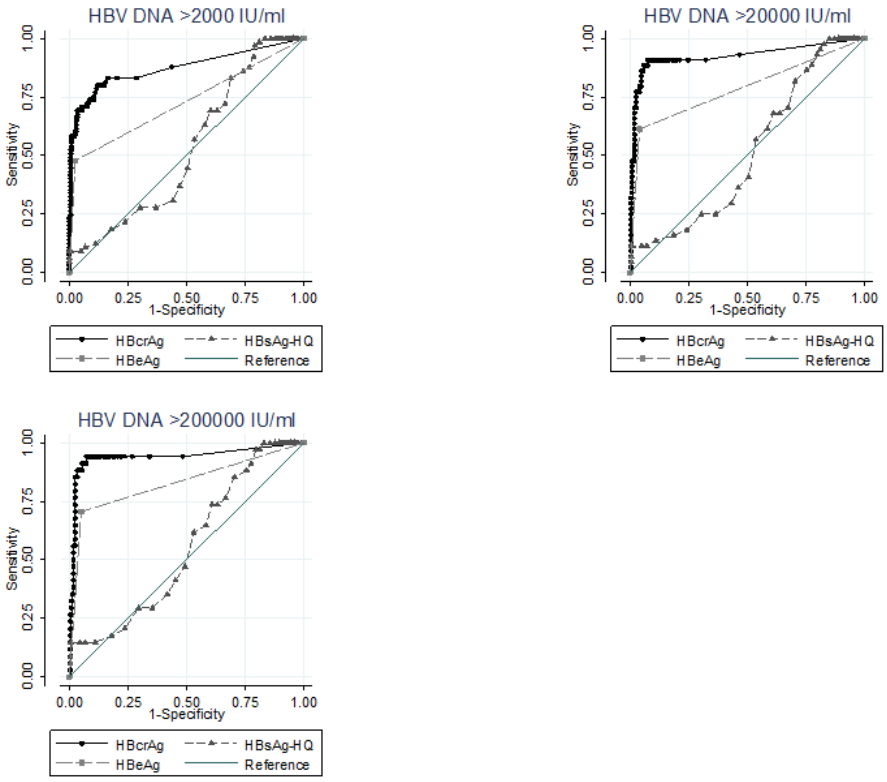

Fig. 3. Receiver operating characteristic curves for HBcrAg, HBsAg-HQ, and HBeAg to indicate serum HBV DNA levels

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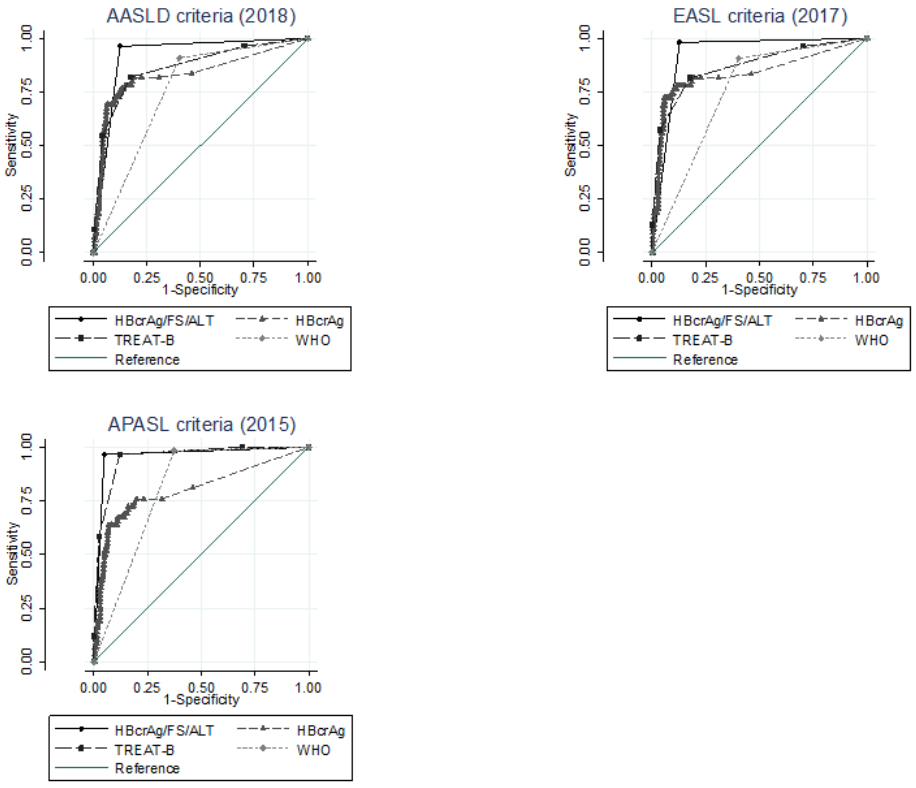

Fig. 4. Receiver operating characteristic curves for simplified algorithms to indicate treatment eligiblity according to the international guidelines

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Table 1. Characteristics of study participants (n=284)

Variables		**Values**
Median age (years)		36 (30-45)
Male sex, n (%)		188 (66)
Ever drunk alcohol, n (%)		24 (9)
Median BMI (kg/m^2)		22 (19-25)
Positive HBeAg, n (%)		36 (13)
HBsAg-HQ, n (%)	0.005 – 1,000 IU/ml	83 (29)
	1,000 – 10,000 IU/ml	109 (38)
	≥ 10,000 IU/ml	92 (32)
HBcrAg, n (%)	Undetectable	132 (47)
	3.0 – 4.0 log U/ml	75 (26)
	≥ 4.0 log U/ml	77 (27)
HBV DNA, n (%)	Undetectable	119 (42)
	50 – 2,000 IU/ml	99 (35)
	2,000 – 20,000 IU/ml	21 (7)
	20,000 – 200,000 IU/ml	10 (4)
	≥ 200,000 IU/ml	35 (12)
HBV genotype, n (%)	A	38 (16)
	E	198 (84)
Median liver stiffness (kPa)		5.8 (4.5-10.7)
Liver cirrhosis, n (%)		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 platelets (10^9/L)		180 (130-242)
Eligible for AASLD treatment criteria (2018), n (%)		59 (21)

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Eligible for EASL treatment criteria (2017), n (%)	58 (20)
Eligible for APASL treatment criteria (2015), n (%)	63 (22)
Eligible for WHO treatment criteria for LMICs (2015), n (%)	140 (49)

Continuous variables are presented as median (interquartile range).

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Table 2. Performance of serum HBcrAg levels, HBsAg-HQ levels and HBeAg to discriminate clinically important HBV DNA levels

	HBV DNA levels								
	2,000 IU/ml			**20,000 IU/ml**			**200,000 IU/ml**		
	HBcrAg	**HBsAg**	**HBeAg**	**HBcrAg**	**HBsAg**	**HBeAg**	**HBcrAg**	**HBsAg**	**HBeAg**
AUROC (95% CI)	0.88 (0.82-0.93)	0.55 (0.48-0.62)	0.73 (0.66-0.79)	0.92 (0.87-0.98)	0.53 (0.45-0.61)	0.79 (0.71-0.86)	0.94 (0.88-0.99)	0.56 (0.47-0.66)	0.83 (0.75-0.91)
P-value (compared to HBcrAg)	N/A	<0.001	<0.001	N/A	<0.001	<0.001	N/A	<0.001	0.004
Cut-off	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
Positive predictive value (%)	61.1	25.2	86.1	70.2	17.0	75.0	65.3	12.0	66.7
Negative predictive value (%)	94.3	78.8	85.5	97.8	85.4	92.8	98.7	87.4	95.7
Positive likelihood ratio	5.2	1.1	19.6	12.5	1.1	15.5	13.4	1.0	13.9
Negative likelihood ratio	0.2	0.9	0.5	0.1	0.9	0.4	0.1	1.0	0.3

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Table 3. Virological factors associated with significant liver fibrosis and elevated ALT levels

1. Association with significant liver fibrosis		**Significant liver fibrosis (%)**	**Crude**		**Adjusted***	
			OR (95% CI)	**p-value**	**OR (95% CI)**	**p-value**
HBcrAg (log U/ml)	< 3.6 log U/ml	20%	1.0	<0.001	1.0	<0.001
	3.6 – 5.3 log U/ml	37%	2.3 (1.1-4.7)		2.6 (1.2-5.8)	
	≥ 5.3 log U/ml	67%	8.2 (4.1-16.4)		19.7 (4.3-91.1)	
HBsAg-HQ (log IU/ml)	< 3.6 log IU/ml	35%	1.0	0.2		
	≥ 3.6 log IU/ml	27%	0.7 (0.4-1.1)			
HBeAg	Negative	26%	1.0	<0.001	1.0	0.4
	Positive	61%	4.6 (2.2-9.5)		0.6 (0.2-2.1)	
HBV DNA (IU/ml)	Undetectable	25%	1.0	<0.001	1.0	0.8
	50–2,000	24%	1.0 (0.5-1.8)		1.0 (0.5-2.1)	
	2,000–200,000	39%	1.9 (0.8-4.3)		0.7 (0.2-2.0)	
	≥200,000	60%	4.5 (2.0-9.8)		0.6 (0.1-2.5)	
HBV genotype	E	29%	1.0	1.0		
	A	29%	1.0 (0.5-2.2)			
2. Association with elevated ALT (≥40 IU/L)		**ALT ≥40 IU/L (%)**	**Crude**		**Adjusted***	
			OR (95% CI)	**p-value**	**OR (95% CI)**	**p-value**
HBcrAg	< 3.6 log U/ml	15%	1.0	<0.001	1.0	0.003
	3.6 – 5.3 log U/ml	29%	2.3 (1.0-5.3)		1.9 (0.8-4.7)	

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(log U/ml)	≥ 5.3 log U/ml	73%	15.5 (7.3-32.9)		12.0 (2.8-50.6)	
HBsAg-HQ (log IU/ml)	< 3.6 log IU/ml	31%	1.0	0.1		
	≥ 3.6 log IU/ml	23%	0.6 (0.4-1.1)			
HBeAg	Negative	20%	1.0	<0.001	1.0	0.7
	Positive	69%	8.7 (4.0-19.0)		0.8 (0.2-3.0)	
HBV DNA (IU/ml)	Undetectable	18%	1.0	<0.001	1.0	0.4
	50–2,000	15%	0.8 (0.4-1.6)		0.8 (0.4-1.8)	
	2,000–200,000	48%	4.2 (1.8-10.0)		2.0 (0.7-5.8)	
	≥200,000	71%	11.2 (4.7-26.8)		1.7 (0.4-6.9)	
HBV genotype	E	26%	1.0	0.9		
	A	27%	1.0 (0.5-2.3)			

* The variables significantly associated with the outcomes in the crude analyses ($p<0.05$) were mutually adjusted.

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Table 4. Performance of simplified algorithm using HBcrAg, TREAT-B and WHO criteria to select patients eligible for antiviral therapy

	HBcrAg-based algorithm			**TREAT-B:**	**WHO:**
	***Model 1*:** **HBcrAg** **HBeAg** **FibroScan** **ALT**	***Model 2*:** **HBcrAg** **ALT**	***Model 3*:** **HBcrAg alone**	**HBeAg** **ALT**	**APRI** **ALT**
AASLD 2018					
AUROC (95% CI)	0.91 (0.88-0.95)	0.90 (0.85-0.94)	0.84 (0.77-0.91)	0.87 (0.81-0.92)	0.73 (0.68-0.79)
Cut-off	N/A	2 points	3.6 log U/mL	2 points	N/A
Sen (%)	96.6	89.3	83.1	81.8	86.4
Spe (%)	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
PLR	6.8	3.6	4.6	4.8	2.2
NLR	0.1	0.1	0.2	0.2	0.2
EASL 2017					
AUROC (95% CI)	0.91 (0.88-0.94)	0.89 (0.84-0.94)	0.84 (0.78-0.91)	0.87 (0.81-0.93)	0.73 (0.68-0.79)
Cut-off	N/A	2 points	3.6 log U/mL	2 points	N/A
Sen (%)	96.6	89.1	82.8	81.5	86.2
Spe (%)	85.4	74.5	81.4	82.4	60.2
PPV (%)	62.9	46.7	53.3	54.3	35.7

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NPV (%)	99.0	96.5	94.8	94.5	94.4
PLR	6.6	3.5	4.5	4.6	2.2
NLR	0.1	0.1	0.2	0.2	0.2
APASL 2015					
AUROC (95% CI)	0.96 (0.93-0.98)	0.96 (0.94-0.98)	0.80 (0.73-0.87)	0.95 (0.93-0.98)	0.80 (0.75-0.84)
Cut-off	N/A	2 points	3.6 log U/mL	2 points	N/A
Sen (%)	96.8	100	74.6	96.6	95.2
Spe (%)	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
PLR	17.8	4.9	3.8	8.0	2.6
NLR	0.1	0	0.3	0.1	0.1

* Model 1 is described in details in Supplementary Table 3.

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