Assay for Hepatitis B Core-related Antigen Identify Patients With High Viral Load: Systematic Review and Meta-analysis of Individual Participant Data

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Title

Hepatitis B core-related antigen to indicate high viral load: systematic review & meta-analysis of 10,397 individual participants

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Author Contributions

KY contributed through the acquisition of data, statistical analysis, and drafting of the manuscript.

AD contributed through the acquisition of data, analysis and interpretation of data, and critical revision of the manuscript for important intellectual content.

SFF contributed through the acquisition of data, analysis and interpretation of data, and critical revision of the manuscript for important intellectual content.

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YS contributed through the development of study concept and design, acquisition of data, statistical analysis, analysis and interpretation of data, and drafting of the manuscript.

List of Abbreviations

AUROC	Area under the receiver operating characteristic curve
cccDNA	Covalently closed circular DNA
CHB	Chronic HBV infection
HBcAg	Hepatitis B core antigen
HBcrAg	Hepatitis B core-related antigen
HBeAg	Hepatitis B e antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HDV	Hepatitis D virus
HCVcAg	HCV core antigen
IFN	Interferon
IPD	Individual participant data
IQR	Interquartile range
LMICs	Low- and middle-income countries
LOWESS	Locally weighted scatter plot smoothing
NA	Nucles(t)ide analogue
NAT	Nucleic acid testing
PRISMA-IPD	Preferred Reporting Items for a Systematic Review and Meta-Analysis of Individual Participant Data
QUADAS-2	Quality Assessment of Diagnostic Accuracy Studies
ROC	Receiver operating characteristic
RT-PCR	Real-time polymerase chain reaction
TMA	Transcription mediated amplification
WHO	World Health Organization
95% CI	95% confidence interval

Abstract (<260 words)

Background and Aims

To eliminate HBV infection, scale-up of testing and treatment in resource-limited countries is crucial. However, access to nucleic acid testing (NAT) to quantify HBV DNA, an essential test to examine treatment eligibility, remains severely limited. We assessed the performance of novel immunoassay, HBV core-related antigen (HBcrAg), as a low-cost (US$<15/assay) alternative to NAT to indicate clinically important high viremia in chronic HBV patients infected with different genotypes.

Methods

We searched Medline/Embase/Scopus/Web of Science until 06/27/2018. Three reviewers independently selected studies measuring HBV DNA and HBcrAg in the same blood samples. We contacted authors to provide individual participant data (IPD). We randomly allocated each IPD to derivation or validation cohort. We applied optimal HBcrAg cut-offs derived from the derivation set to the validation set to estimate sensitivity/specificity.

Results

Of 74 eligible studies, IPD were successfully obtained for 60 (81%). Meta-analysis included 5,591 IPD without antiviral therapy and 4,806 under antivirals. In untreated patients, pooled area under the receiver operating characteristic curve and optimal cut-offs (log U/ml) were: 0.88 (95%CI: 0.83-0.94) and 3.6 to diagnose HBV DNA level ≥2,000 IU/ml; and 0.96 (0.94-0.98) and 5.3 for ≥200,000 IU/ml, respectively. In the validation set, the sensitivity and specificity were 85.2% and 84.7% for ≥2,000 IU/ml, and 91.8% and 90.5% for ≥200,000 IU/ml, respectively. The performance did not vary by HBV genotypes. In patients under anti-HBV therapy the correlation between HBcrAg and HBV DNA was poor.

Conclusion

HBcrAg might be useful serological marker to indicate clinically important high viremia in treatment-naïve HBV-infected patients.

Keywords

Hepatitis B core-related antigen; diagnosis; sensitivity and specificity; systematic review and meta-analysis

Introduction

Viral hepatitis, the 7th leading cause of death worldwide, kills more people than any of the major infectious diseases (HIV/tuberculosis/malaria), and is now targeted by the United Nations Sustainable Development Goals.[1] Hepatitis B virus (HBV) infection accounts for more than half of these hepatitis-related deaths. In 2016, the World Health Organization (WHO) developed a global strategy to eliminate hepatitis B as a public health threat, and one of the goals is to increase antiviral treatment uptake from 8% in 2015 to 80% by 2030 in people with chronic HBV infection (CHB) who are eligible for antiviral therapy.[1] To achieve this objective, it is urgent to scale up both hepatitis B surface antigen (HBsAg) screening and clinical staging for people carrying HBsAg to assess their eligibility for anti-HBV therapy.

Following a positive HBsAg screening test, it is essential to quantify serum HBV DNA levels using nucleic acid test (NAT) to decide whether antiviral treatment should be initiated. According to the international guidelines, having high viremia (≥2,000 or ≥20,000 IU/ml) in the presence of liver inflammation or fibrosis indicates treatment eligibility.[2–5] Recently, the cut-off of ≥200,000 IU/ml has been applied to select pregnant women who would benefit most from antiviral prophylaxis to prevent mother-to-child transmission.[3,4] However, real-time polymerase chain reaction (RT-PCR), a standard NAT assay to quantify HBV DNA levels, is not affordable and accessible in many low- and middle-income countries (LMICs) because of its high cost (US$ 60-200/assay) and strict laboratory requirements for sophisticated equipment and well-trained staff.[1] Since the vast majority (>95%) of people with CHB live in LMICs,[6] the limited access to RT-PCR represents a major obstacle to achieve global hepatitis elimination, and WHO fully recognizes an urgent need for a low-cost simple assay to measure active HBV replication.[2]

Likewise, the limited access to NAT to diagnose chronic hepatitis C virus (HCV) infection remains an important barrier to expand anti-HCV treatment in LMICs. A systematic review was therefore undertaken to evaluate the accuracy of an immunoassay (HCV core antigen (HCVcAg)) as a low-cost

alternative to NAT, and found a comparable clinical sensitivity to the reference NAT assay.[7] Based on these data WHO now recommends the use of HCVcAg for the detection of HCV RNA when NAT is not accessible.[2]

HBV core-related antigen (HBcrAg) is a novel immunoassay to measure HBV replication. The assay quantifies HBV core antigen (HBcAg), e antigen (HBeAg) as well as p22cr and c-terminal modified HBcAg contained in the empty particle fraction in blood regardless of anti-HBc or anti-HBe antibodies.[8,9] A close correlation between serum HBcrAg levels and HBV DNA levels has been suggested in treatment-naïve patients with CHB.[10–14] Moreover, a correlation of serum HBcrAg levels with intrahepatic covalently closed circular DNA (cccDNA), a transcriptional template of HBV, has been also observed in several Asian and European studies.[10,11,15,16] Because this immunoassay is cheaper (US$ <15/assay) and simpler than the RT-PCR, HBcrAg may potentially represent an attractive alternative to HBV DNA PCR in resource-limited countries, and also for hard-to-reach populations living in high-income countries. However, its capability to discriminate between high and low serum HBV DNA levels across different HBV genotypes has never been formally assessed.

We conducted a systematic review and meta-analysis to estimate the sensitivity and specificity of HBcrAg test to diagnose three clinically important HBV DNA thresholds (≥2,000, ≥20,000, and ≥200,000 IU/ml) determined by the reference NAT assays in patients with CHB. We also conducted subgroup analyses according to viral genotypes. To synthesize these estimates, we sought individual participant data (IPD) from each eligible study, because aggregate data at these specific HBV DNA cut-offs were rarely reported in the previous literatures with few exceptions.[14]

Materials and Methods

Our systematic review followed a protocol registered at PROSPERO (CRD42017055440), and was reported according to the Preferred Reporting Items for a Systematic Review and Meta-Analysis of Individual Participant Data (The PRISMA-IPD) guidelines.[17] This meta-analysis was exempt from

ethical approval because the analysis only used anonymized data, and all the original studies had received ethics approval.

Data Sources and Searches

Ovid Medline, Ovid Embase, Scopus, and Web of Science were searched from 1st January, 2000 to 27th June, 2018. Following search terms and their variations were used: hepatitis B, core, antigen, and HBcrAg (see Supplementary Document 1 for detailed search strategy). A manual search through bibliographies was also conducted. Three authors (KY, AD, and SFF) independently screened the titles and abstracts of identified citations using pre-specified eligibility criteria. Full-text reading was performed for the potentially eligible citations to examine their eligibility. Disagreements were resolved by another reviewer (YS).

Study Selection

Studies that measured both HBV DNA and HBcrAg levels using the same blood sample from the same individuals with CHB were eligible for the analysis. These individuals needed to be stratified by the presence of concurrent antiviral treatment. Single case reports, letters, reviews, conference abstracts, and those included only patients with hepatocellular carcinoma (HCC) were excluded. Study inclusion was not restricted by language, sample size or participants' age.

Data Extraction and Quality Assessment

Two authors (KY and SFF) independently extracted the following information using a pre-piloted standardized form: study design, settings, objectives, number of participants, participants' selection methods and criteria, participants' characteristics, type and condition of samples used to measure HBcrAg and HBV DNA, and assay methods. Citations in foreign language were translated and the data was extracted by native speakers using the same data extraction sheet. Unless all the necessary individual-level data were reported in the original article, corresponding authors of the eligible papers were systematically invited to be a co-investigator of the current work by e-mail, and asked to provide the IPD using a standardized spreadsheet for the following variables: age, sex, HBcrAg levels, HBV

DNA levels, NAT type, HCC status, concurrent anti-HBV treatment, types and conditions of samples, HBeAg sero-status, HBV genotype, and HCV/HIV/HDV co-infection. In case of no response after four weeks, a reminder was sent. Studies without IPD were excluded from our analysis. The IPD were systematically examined for their integrity, including consistency and completeness. The risk of bias and quality assessment were performed using a tool adapted from the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2),[18] which is available in the Supplementary Document 2.

Data Synthesis and Analysis

Because some study centers generated multiple eligible studies, and these studies often used the data from the same individuals more than once, general characteristics of the IPD were presented by study center, rather than by each study, to avoid double counting. For the quantitative analysis, the quantification limit of the HBcrAg assay minus 0.1 (i.e., 2.9 log U/ml) was assigned to samples with undetectable HBcrAg. Virological characteristics were compared between those with and without antiviral therapy using Wald test with robust standard error to account for clustering within the centers. The linear correlation between HBcrAg levels and HBV DNA levels was evaluated quantitatively using Pearson's correlation coefficient, and visually using a locally weighted scatter plot smoothing (LOWESS). One step approach was used for the meta-analysis in which the IPD from all studies were modelled simultaneously while taking account for clustering of patients within the study centers.[19] The capabilities of HBcrAg to correctly discriminate serum HBV DNA levels at the cut-off of ≥2,000, ≥20,000, and ≥200,000 IU/ml were evaluated by using the receiver operating characteristic (ROC) curve. Our primary outcome measure was pooled area under the ROC curve (AUROC), which was obtained by the parametric two-stage model developed by Alonzo and Pepe[20] to account for the clustering within the centers. To estimate the 95% confidence interval (95% CI) of the AUROC, 1,000 bootstrap replications were used. Each non-treated individual was randomly allocated to derivation or validation cohort (1:1). Using the derivation set, the optimal HBcrAg cut-offs were determined to minimize the absolute difference between sensitivity and specificity. These cut-offs were then applied to the validation set to obtain the pooled sensitivity and specificity. The proportion correctly classified was computed as below: disease prevalence * sensitivity + (1 – disease prevalence) * specificity.

Subgroup analyses were conducted using the whole non-treated cohort. To compare the AUROC between the subgroups defined by sex, age, study region, HBeAg, HBV genotype, and HCV/HIV/HDV co-infection, the interaction test by Altman and Bland was conducted when comparing two groups,[21] and one-way analysis of variance when comparing more than two groups.[22] All the analyses were performed using STATA 13.0 (Stata Corporation, USA).

Results

Study Selection

A total of 10,863 titles and abstracts were screened, and 227 studies were assessed in full text for their eligibility (Figure 1). Of 74 studies that met our criteria, IPD were finally obtained for 60 studies (81%). The IPD were either reported in the original articles (4 studies) or shared by the corresponding authors (56 studies). After excluding 87 patients with HCC and 586 patients with incomplete information, the analysis finally included a total of 10,397 IPD: 5,591 patients without antiviral therapy (50 studies from 23 centers) and 4,806 patients under antivirals (39 studies from 19 centers). The vast majority of patients under treatment (94%, n=4,511) received nucles(t)ide analogues (NA), and only few (6%, n=278) had interferon-based therapy (IFN). Characteristics of the included studies by study center are summarized in Table 1. The study was conducted in Asia (n=50), Europe (n=9), and Africa (n=1). All the studies used serum samples to measure HBcrAg with a chemiluminescent immunoassay (Lumipulse®, Fujirebio Inc., Tokyo, Japan). HBV DNA was quantified by RT-PCR in majority of samples (10,103; 98%), using the following assays: COBAS TaqMan (n=8,044) and Amplicor (n=797) (Roche Diagnostics); RealTime/AccuGene (n=400) (Abbott); and in-house methods (n=862). The rest (157 samples; 2%) was tested using transcription mediated amplification (TMA): TMA, Chugai Diagnostics (n=104); and DNA Probe FR-HBV, Fujirebio (n=53).

Characteristics of the included studies

Table 2 presents the patients' characteristics by the concurrent anti-HBV treatment. Median age (years) was 45 (IQR: 34-57) in non-treated and 56 (45-64) in treated group. HBeAg was positive in 37% of non-treated and 31% of treated group. Whilst proportion with undetectable HBV DNA was

significantly higher in treated (64%) than in non-treated (12%, $p<0.001$), the proportion with undetectable HBcrAg was similar in treated (26%) and non-treated (32%, $p=0.4$). Of note, for those with undetectable HBV DNA, only the minority had detectable HBcrAg in non-treated group (27%, 189/692), but the majority had detectable HBcrAg in treated group (69%, 2,114/3,080, $p=0.002$).

Risk-of-Bias Assessment

The risk-of-bias is summarized for all 60 included studies (Supplementary Figure 1) and for each study (Supplementary Figure 2). Only 16 studies enrolled a consecutive/random sample. Some studies restricted participants to be HBeAg-positive (n=13), HBeAg-negative (n=7), having high viral load (n=9), or low viral load (n=5), which raised concern about applicability of these studies. Except 13 studies, the objective of the original work was not to assess the correlation between HBcrAg and HBV DNA. No study, but one,[14] mentioned whether those examined HBcrAg were blinded to the results of HBV DNA, and vice versa.

Correlation between serum HBcrAg levels and HBV DNA levels

Figure 2 presents the scatter plot of HBcrAg and HBV DNA with the LOWESS in patients without antiviral therapy. The correlation was observed irrespective of viral genotypes. Pearson's correlation coefficient was 0.84 for all untreated subjects, and 0.83, 0.84, 0.83, 0.85, and 0.82 for genotype A, B, C, D, and E, respectively. The scatter plot of HBcrAg and HBV DNA in non-treated patients by HBeAg positivity is presented in the Supplementary Figure 3. In contrast with those without antiviral treatment, the correlation was poor in patients under treatment (r=0.54) (Supplementary Figure 4), and therefore the subsequent analyses were not performed in this group.

Performance of HBcrAg to diagnose high viral load in non-treated patients

In patients without antiviral therapy, the pooled AUROC was: 0.88 (95% CI: 0.83-0.94) for the diagnosis of HBV DNA levels of ≥2,000 IU/ml; 0.95 (0.93-0.97) for ≥20,000 IU/ml; and 0.96 (0.94-0.98) for ≥200,000 IU/ml (Figure 3). To obtain the optimal cut-offs, the non-treated cohort was randomly divided into two: derivation and validation set. These groups had similar characteristics

(Supplementary Table 1). The optimal cut-offs (log U/ml) were 3.6, 4.5, and 5.3 to diagnose HBV DNA levels (IU/ml) of ≥2,000, ≥20,000, and ≥200,000, respectively, using the derivation set. By applying these to the validation set, the sensitivity, specificity, and the proportion correctly classified were: 85.2%, 84.7% and 85.0% for ≥2,000 IU/ml, 90.4%, 91.9% and 91.1% for ≥20,000 IU/ml, and 91.8%, 90.5% and 91.1% for ≥200,000 IU/ml, respectively (Table 3).

Subgroup analysis in non-treated patients

The pooled AUROCs did not vary according to the sex, age groups (<30 or ≥30 years), study regions (Asia, Europe, or Africa), HBV genotypes (A/B/C/D/E), HCV, or HDV co-infection (Table 3). Across the different viral genotypes, the AUROCs were constantly ≥0.80, ≥0.93 and ≥0.95 to diagnose viremia of ≥2,000, ≥20,000 and ≥200,000 IU/ml, respectively. In 1,134 women of reproductive age (15-49 years), the AUROC was 0.98 (95% CI: 0.96-1.00) with a sensitivity of 94.1% and a specificity of 89.5% to diagnose HBV DNA thresholds requiring peripartum antiviral prophylaxis (i.e., viral load ≥200,000 IU/ml). A *post-hoc* analysis was performed in the women of reproductive age for the performance of HBeAg; although mean viral load (± standard deviation) was much higher in HBeA-positive women (7.18 ± 1.81 log IU/ml) than in HBeAg-negative women (3.07 ± 1.90 log IU/ml), the AUROC of HBeAg was 0.76 (95% CI: 0.64-0.88) to discriminate between high and low viral loads at the cut-off level of ≥200,000 IU/ml.

The performance of HBcrAg to diagnose viral load ≥2,000 IU/ml differed according to the HBeAg sero-status (*P* for interaction for AUROC <0.01); the sensitivity and specificity were 97.9% and 23.4% in HBeAg-positive and 66.0% and 87.2% in HBeAg-negative group, respectively. The low specificity in HBeAg-positive group and low sensitivity in HBeAg-negative group, however, had minimal impact on the overall misclassification in each sub-group. The high false positive rate (76.6%) in HBeAg-positive group was offset by the small prevalence (3.9%) of low viremia (<2,000 IU/ml) in this group, resulting in 95.0% of HBeAg-positive patients being correctly classified by HBcrAg (Table 3). Similarly, low sensitivity (66.0%) in HBeAg-negative group was relevant in only

the minority (37.0%) of those with high viremia (≥2,000 IU/ml), which led to 79.4% of HBeAg-negative patients being correctly classified.

Discussion

This systematic review successfully obtained the IPD from the majority of eligible studies (81%, 60/74). This allowed us to synthesize robust estimates using large, well-characterized datasets with varying virological and epidemiological background. Despite the heterogeneity among reference assays used to quantify HBV DNA across the studies, we found: (i) a close correlation between HBcrAg and HBV DNA and excellent performance of HBcrAg levels to indicate clinically important viral load irrespective of HBV genotypes in CHB patients without antiviral therapy; and (ii) lack of correlation in those treated with antivirals.

In high-income countries, HBcrAg is increasingly recognized as a surrogate marker for cccDNA amount and its transcriptional activity, and useful tool to monitor the treatment response.[16,23,24] Although quantification of intrahepatic amount of cccDNA, a key genomic form responsible for the persistence of infection, should be highly informative to manage CHB patients, this requires liver biopsy and therefore cannot be routinely performed. Instead, serum HBV DNA has been clinically used as a routine biomarker for HBV replication. In patients under antiviral therapy, however, discrepancies become apparent between intrahepatic cccDNA and serum HBV DNA levels; while nucleos(t)ides analogues inhibit reverse transcription and almost invariably lead to undetectable serum HBV DNA, intrahepatic cccDNA often persists despite long-term treatment.[25] Next commonly used marker for HBV replication is quantification of serum HBsAg. However, its correlation with cccDNA is also limited, particularly in HBeAg-negative patients, because HBsAg is derived not only from intrahepatic cccDNA but also from HBV DNA sequences integrated into the host genome.[26] Contrary to these conventional biomarkers, a close correlation between serum HBcrAg and intrahepatic cccDNA has been observed in treatment-naïve patients,[10,11,15,16,27–29] and also in those who underwent anti-HBV therapy in whom a magnitude of reduction in HBcrAg is well correlated with that of cccDNA.[10,11,15,27] Not surprisingly, in this meta-analysis, poor correlation was confirmed between

HBV DNA and HBcrAg in patients under treatment, and the majority (69%) of the treated patients with undetectable serum HBV DNA still carried detectable HBcrAg. These results are consistent with the previous findings, and support the utility of serum HBcrAg as an endpoint for novel anti-HBV drugs aiming at a complete cure of HBV infection.[16,24]

In addition to its great potential as a tool to monitor patients under treatment, serum HBcrAg may also be useful to indicate clinically important HBV DNA levels in treatment-naïve patients, as suggested by our meta-analysis. This may support its use as an alternative to NAT to select patients in need of antiviral therapy subsequent to a positive HBsAg screening result. A recent African study demonstrated a sensitivity of 96.6% and specificity of 85.8% for a simplified treatment algorithm using HBcrAg to indicate the treatment eligibility determined by the reference tests including HBV DNA quantification.[14] Furthermore, some studies suggested that HBcrAg might be even more accurate than HBV DNA levels to predict liver disease progression in treatment-naïve patients. A long-term follow-up of a large Japanese cohort of CHB patients without anti-HBV treatment found that quantification of HBcrAg was superior to serum viral load to predict the development of HCC,[30] and cirrhosis.[31] In a recent study in Taiwan also confirmed the independent association between serum HBcrAg levels and HCC development.[32]

A recent advent of inexpensive automated point-of-care PCR assays, such as GeneXpert, may help to overcome the limited access to the conventional NAT assays in LMICs. To further decentralize clinical staging of CHB, there is a need to develop an assay fully adapted to resource-limited primary healthcare settings without air-conditioning or uninterrupted electricity supply.[33] Indeed, the development of an inexpensive rapid test based on lateral-flow technology to detect HBcrAg is currently ongoing, and its evaluation will be performed in LMICs. Lowering the detection limit for such a test may be less relevant; for example, a rapid test detecting high HBcrAg levels of ≥5.3 log U/ml (equivalent to viral load of ≥200,000 IU/ml) should be extremely useful at antenatal care to select pregnant women for anti-HBV therapy to prevent mother-to-child transmission, because of its high clinical sensitivity (94.1%) and specificity (89.5%) to indicate HBV DNA levels at risk of

immunoprophylaxis failure. In addition, analytical sensitivity of HBcrAg has been recently improved (detection limit of highly sensitive HBcrAg assay: 2.1 log U/mL, personal communication), which may lead to future development of rapid test targeting lower thresholds equivalent to viral loads of ≥2,000 or ≥20,000 IU/ml.

As a limitation, most included studies are from high-income countries and mainly from Asia with an over-representation of HBV genotypes B and C. Studies are needed from other geographical regions, particularly those from resource-limited countries. Second, clinical sensitivity of HBcrAg for viral load ≥2,000 IU/ml was moderate (66.0%) in HBeAg-negative patients. This may have an impact in countries where the prevalence of HBeAg is low (e.g., in Africa). Nevertheless, by applying the data from a large population-based study in West Africa (96.7% of adults with CHB infection being negative for HBeAg, and 8.5% of those negative for HBeAg had viral load ≥2,000 IU/ml),[34] the use of HBcrAg would still correctly classify 85.4% of HBeAg-negative African people for diagnosing ≥2,000 IU/ml. New version of the HBcrAg assay with an improved analytical sensitivity (2.1 log U/mL) will be available in near future, and this should further increase the diagnostic sensitivity. Moreover, there are recent publications supporting the usefulness of HBcrAg in HBeAg-negative patients.[35,36] Third, we could not assess the performance of HBcrAg to indicate treatment eligibility *per se*. The treatment eligibility criteria, apart from those for pregnant women for the prevention of mother-to-child transmission, are composed of a combination of multiple factors, and HBV viral load merely represents one of these. In this study, we did not seek individual participant data for liver inflammation or fibrosis. Forth, the included studies suffered from the risk-of-bias. None, but one, reported that the index test was performed by staff blinded to the result of the reference test, and vice versa, because most of the original studies were not designed to evaluate the performance of HBcrAg to diagnose HBV viral load.

LMICs account for the highest HBV burden, yet, face enormous challenges in scaling up treatment services with the limited access to NAT assays. HBcrAg, an attractive alternative to HBV DNA RT-

PCR, has potential to contribute to the global elimination goals. Further studies, particularly those assessing the feasibility and cost-effectiveness of HBcrAg assays in LMICs, are highly deserved.

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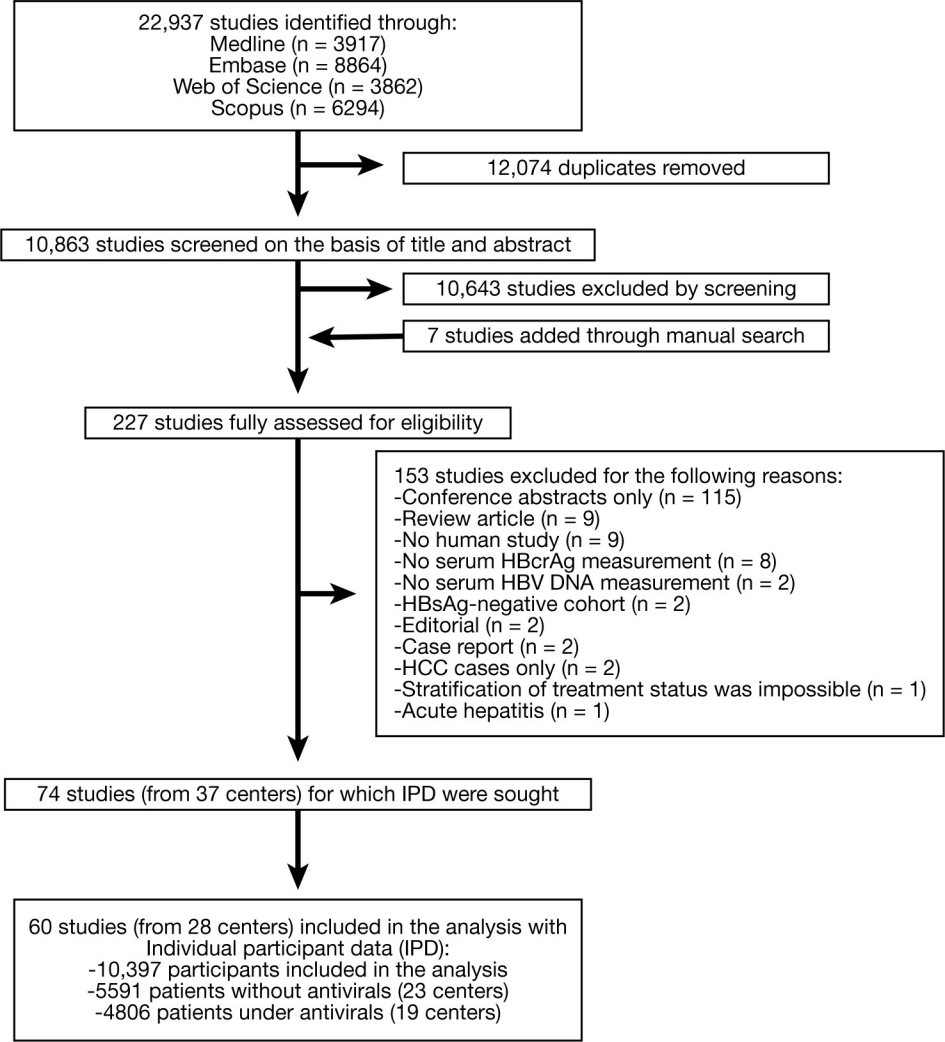
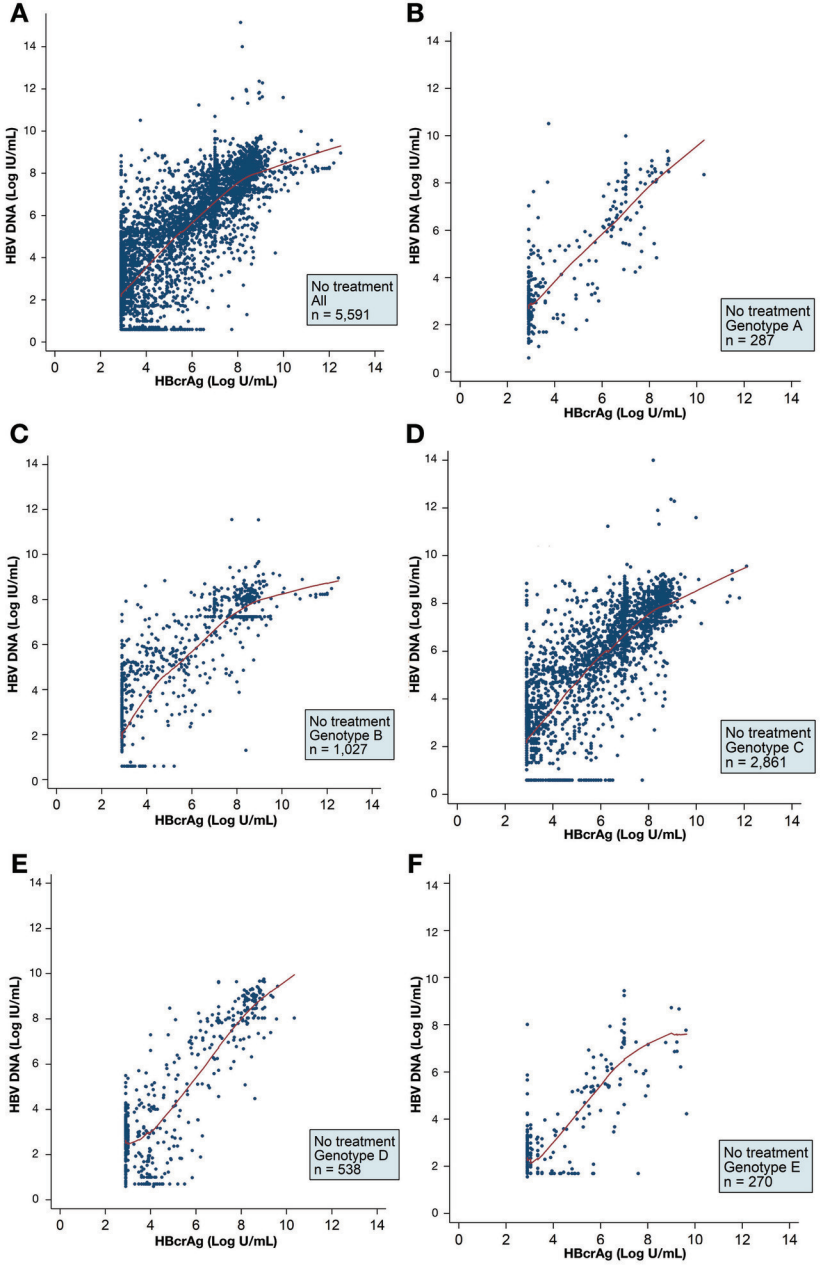
Figure Legends

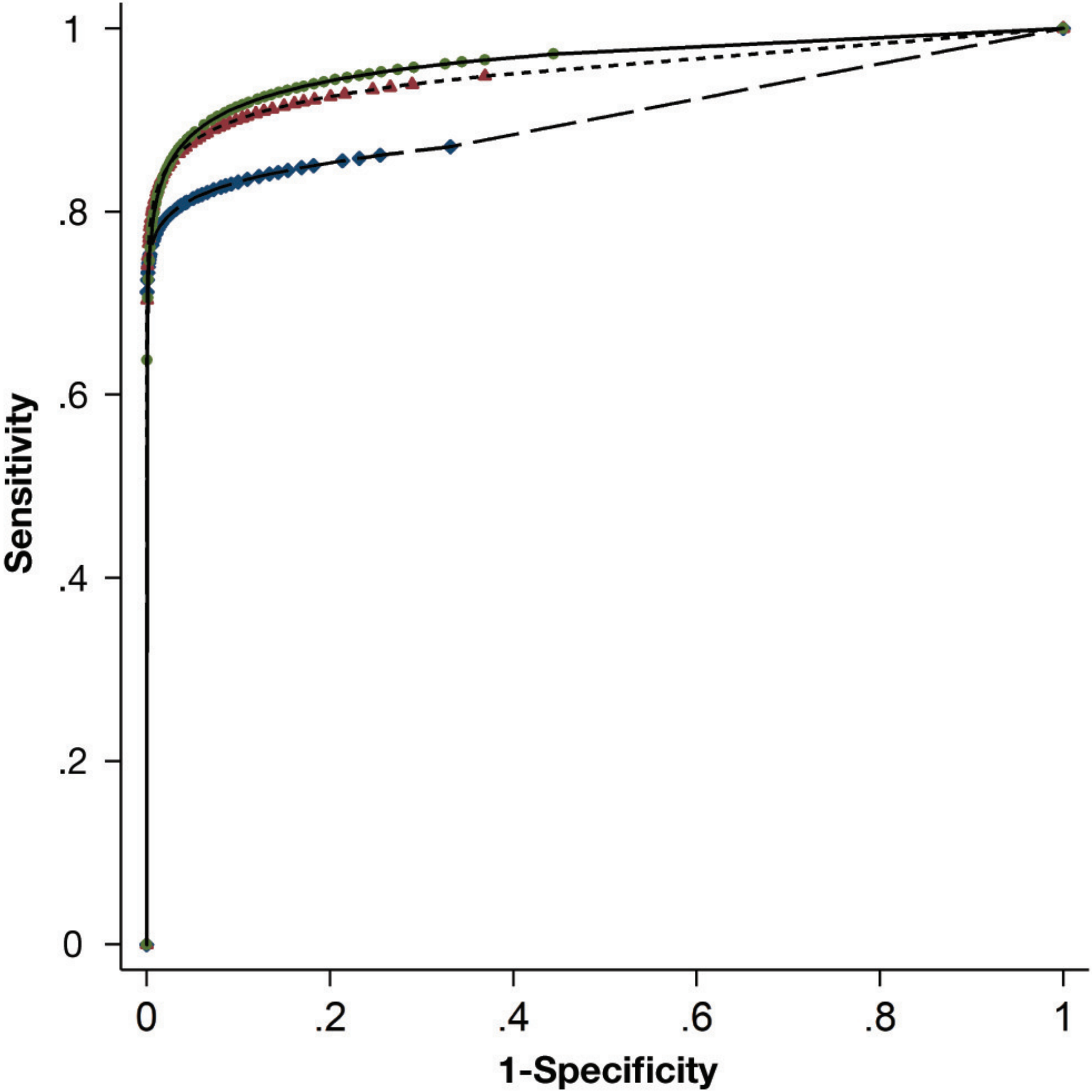
Figure 1. Flow diagram of study selection

Figure 2. Correlation between HBcrAg & HBV DNA levels with locally weighted scatter plot smoothing (LOWESS) in patients without treatment, by viral genotypes

Figure 3. Receiver operating characteristic curves for HBcrAg to diagnose clinically important HBV DNA levels (dash: ≥2,000 IU/ml; short dash: ≥20,000; solid: ≥200,000)


22,937 studies identified through:
Medline (n = 3917)
Embase (n = 8864)
Web of Science (n = 3862)
Scopus (n = 6294)
12,074 duplicates removed
10,863 studies screened on the basis of title and abstract
10,643 studies excluded by screening
7 studies added through manual search
227 studies fully assessed for eligibility
153 studies excluded for the following reasons:
-Conference abstracts only (n = 115)
-Review article (n = 9)
-No human study (n = 9)
-No serum HBcrAg measurement (n = 8)
-No serum HBV DNA measurement (n = 2)
-HBsAg-negative cohort (n = 2)
-Editorial (n = 2)
-Case report (n = 2)
-HCC cases only (n = 2)
-Stratification of treatment status was impossible (n = 1)
-Acute hepatitis (n = 1)
74 studies (from 37 centers) for which IPD were sought
60 studies (from 28 centers) included in the analysis with Individual participant data (IPD):
-10,397 participants included in the analysis
-5591 patients without antivirals (23 centers)
-4806 patients under antivirals (19 centers)


A
HBV DNA (Log IU/mL)
HBcrAg (Log U/mL)
No treatment
All
n = 5,591
B
HBV DNA (Log IU/mL)
HBcrAg (Log U/mL)
No treatment
Genotype A
n = 287
C
HBV DNA (Log IU/mL)
HBcrAg (Log U/mL)
No treatment
Genotype B
n = 1,027
D
HBV DNA (Log IU/mL)
HBcrAg (Log U/mL)
No treatment
Genotype C
n = 2,861
E
HBV DNA (Log IU/mL)
HBcrAg (Log U/mL)
No treatment
Genotype D
n = 538
F
HBV DNA (Log IU/mL)
HBcrAg (Log U/mL)
No treatment
Genotype E
n = 270


Sensitivity
1
.8
.6
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.2
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0
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.6
.8
1
1-Specificity

Table 1. Characteristics of 5,591 study participants without antiviral therapy by 23 study centers, and 4,806 study participants under antiviral therapy by 19 study centers

References*	Coordinating Center	Year published	Country	HBV DNA assay	No. of IPD	Median age (INR)	Male sex (%)	HIV (%)	HCV (%)	HDV (%)	HBe Ag (%)	HBV genotype (%)
Participants without antiviral therapy												
1–15	Shinshu University	2001-2018	Japan	TaqMan/Amplicor/Accugene/DNAprobe/in-house PCR/TMA	808	57 (46-63)	70	0	0	-	24	A:2, B:12, C:85, F:1
16–18	Nagoya City University	2006-2013	Japan	TaqMan/in-house PCR/TMA	521	56 (47-62)	49	-	0	-	7	A:2, B:52, C:46
19	St Marianna University	2010	Japan	Amplicor/DNAprobe	10	32 (24-40)	80	0	0	-	80	C:100
20	Nagasaki Medical Centre	2012	Japan	TaqMan	234	38 (30-47)	69	0	0	-	39	A:1, B:1, C:98
21–23	Ogaki Municipal Hospital	2013-2018	Japan	TaqMan	948	49 (37-59)	56	-	0	-	29	A:4, B:10, C:84, D:1, F:1
24–26	Osaka City University	2013-2018	Japan	TaqMan/Amplicor/TMA	102	35 (30-42)	87	-	0	-	81	A:4, B:1, C:95
27	Teine Keijinkai Hospital	2016	Japan	TaqMan	17	42 (35-55)	53	0	0	-	27	B:24, C:76
28	Kobe University	2017	Japan	TaqMan	27	34 (31-39)	59	-	0	-	67	B:7, C:93
29	Nippon Medical School	2018	Japan	TaqMan	218	58 (47-66)	51	-	0	-	10	A:8, B:20, C:71, D:1

30–33	University of Hong Kong	2007-2017	China	TaqMan	652	45 (35-53)	68	0	0	0	48	B:45, C:55
34,35	Peking University	2016	China	TaqMan	111	25 (22-32)	77	0	0	0	100	B:34, C:66
36,37	Sichuan University	2017-2018	China	TaqMan	63	26 (23-33)	76	0	0	-	100	B:57, C:43
38	Academia Sinica	2018	Taiwan	TaqMan	316	37 (33-44)	64	-	0	-	100	B:47, C:53
39,40	Chulalongkorn University	2016-2017	Thailand	RealTime	167	37 (31-45)	70	0	0	0	28	A:1, B:19, C:79, G:1
41	Mongolian Academy of Sciences	2008	Mongolia	in-house PCR	57	40 (30-54)	52	-	25	42	14	A:4, C:6, D:90
42	University of Turin	2016	Italy	TaqMan	28	57 (52-59)	71	0	0	0	0	D:100
43,44	University of Pisa	2017-2018	Italy	TaqMan	253	46 (34-54)	59	0	4	40	3	A:5, B:1, D:92, E:1, F:1
45	Hannover Medical School	2015	Germany	TaqMan	283	33 (24-46)	58	0	0	0	40	A:23, B:9, C:4, D:64
46	Vall d'Hebron Hospital	2017	Spain	TaqMan	134	51 (38-59)	54	0	0	0	0	A:49, B:1, C:1, D:28, E:12, F:6, H:3
47	King's College	2017	UK	TaqMan	162	39 (32-47)	59	-	-	-	100	A:11, B:17, C:33, D:11, E:26, F:1, G:1
48	CHU de Lille	2018	France	TaqMan	13	28 (20-40)	62	8	-	-	15	A:40, B:20, C:10, D:20, E:10
49	Erasmus University	2016	Netherlands	TaqMan	174	30 (24-38)	71	0	0	0	100	A:7, B:19, C:43, D:31
50	MRC Unit, The Gambia	2019	Gambia	in-house PCR	293	37 (30-46)	65	3	3	5	13	A:15, E:85

References*	Coordinating Center	Year published	Country	HBV DNA assay	No. of IPD	Type of treatment	Median age (INR)	Male sex (%)	HIV (%)	HCV (%)	HDV (%)	HBe Ag (%)	HBV genotype (%)
Participants under antiviral therapy													
3–8,10–13,15	Shinshu University	2003-2018	Japan	TaqMan/Amplicor/Accugene/DNAprobe/in-house PCR/TMA	3304	NA: 3167 IFN: 120	58 (47-65)	74	2	0	-	29	A:1, B:10, C:87, F:2
16,18	Nagoya City University	2006-2013	Japan	TaqMan/in-house PCR/TMA	22	NA: 22	46 (39-52)	59	-	0	-	0	B:10, C:90
51,52	Nagasaki University	2009-2013	Japan	Amplicor	13	NA: 13	55 (48-59)	54	-	13	-	39	-
27,53	Teine Keijinkai Hospital	2009-2016	Japan	TaqMan	44	NA: 44	58 (49-62)	80	0	0	-	7	B:8, C:92
19	St Marianna University	2010	Japan	Amplicor/DNAprobe	12	IFN: 12	-	-	0	0	-	36	C:100
54	Okayama University	2011	Japan	TaqMan/ DNAprobe	14	NA: 14	50 (44-52)	93	0	14	-	54	C:100
24–26	Osaka City University	2013-2018	Japan	TaqMan/Amplicor/TMA	128	NA: 65 IFN: 63	35 (31-42)	81	-	0	-	63	A:3, B:2, C:95
55,56	Kanazawa University	2016-2018	Japan	TaqMan	281	NA: 281	60 (50-66)	65	-	0	-	23	B:11, C:88, D:1
28	Kobe University	2017	Japan	TaqMan	13	IFN: 13	32 (28-38)	77	-	0	-	85	B:15, C:85
57	Nagoya University	2018	Japan	TaqMan	38	NA: 38	52 (40-62)	61	11	4	-	50	A:17, B:5, C:78

30,31,33,58	University of Hong Kong	2007-2017	China	TaqMan	542	NA: 542	53 (43-61)	76	0	0	0	14	B:31, C:69
34	Peking University	2016	China	TaqMan	58	INF: 58	28 (23-36)	79	0	0	0	86	B:34, C:66
36,37	Sichuan University	2017-2018	China	TaqMan	32	NA: 32	27 (22-35)	75	0	0	-	100	B:66, C:34
59	Yonsei University	2016	Korea	Amplicor	110	NA: 110	50 (40-56)	62	0	0	0	41	C:100
42	University of Turin	2016	Italy	TaqMan	28	NA: 20 IFN: 8	57 (52-60)	71	0	0	0	0	D:100
44	University of Pisa	2017-2018	Italy	TaqMan	12	NA: 8 IFN: 4	47 (41-54)	58	0	25	100	17	D:100
60	Hannover Medical School	2016	Germany	TaqMan	12	NA: 12	49 (46-55)	67	0	0	0	0	B:17, C:8, D:75
47	King's College	2017	UK	TaqMan	94	NA: 94	42 (35-51)	60	-	-	-	100	A:12, B:12, C:33, D:13, E:28, F:1, G:1
48	CHU de Lille	2018	France	TaqMan	49	NA: 49	46 (40-54)	82	27	-	-	12	A:32, B:5, D:26, E:37

* The references of these studies are listed in the Supplementary Document 3.

Abbreviations: IFN, interferon-based therapy; NA, nucleos(t)ide analogue

Table 2. Characteristics of study participants by the concurrent antiviral therapy (N=10,397)

Variables		**Without antiviral therapy (n=5,591)**	**Under antiviral therapy (n=4,806)**
Median age (IQR)		45 (34-57)	56 (45-64)
Male sex (%)		3,482 (62%)	3,514 (73%)
Positive HBeAg (%)		2,032 (37%)	1,468 (31%)
HBV DNA (IU/ml)	Undetectable	692 (12%)	3,080 (64%)
	< 2,000	1,550 (27%)	1,256 (26%)
	2,000–19,999	506 (9%)	155 (3%)
	20,000-199,999	448 (8%)	116 (3%)
	≥ 200,000	2,395 (43%)	199 (4%)
HBcrAg (log U/ml)	Undetectable	1,811 (32%)	1,269 (26%)
	3.0 – 3.9	832 (15%)	1,074 (22%)
	4.0 – 4.9	423 (8%)	1,071 (22%)
	5.0 – 6.9	903 (16%)	1,201 (25%)
	≥ 7.0	1,622 (29%)	191 (4%)
HBV genotype	A	287 (6%)	74 (2%)
	B	1,027 (20%)	429 (11%)
	C	2,861 (57%)	3,392 (84%)
	D	538 (11%)	67 (2%)
	E	270 (5%)	34 (<1%)
	F	21 (1%)	61 (1%)
	G	2 (<1%)	1 (<1%)
	H	3 (<1%)	0 (0%)
Co-infection	HIV	10/2,240 (<1%)	16/991 (2%)
	HCV	49/4,465 (1%)	7/1,411 (<1%)
	HDV	156/2,031 (8%)	12/762 (2%)

HBcrAg: hepatitis B core-related antigen; HBeAg: hepatitis B e antigen; HBV: hepatitis B virus; HCV: hepatitis C virus; HDV: hepatitis D virus; IQR: interquartile range

Table 3. Performance of serum HBcrAg to diagnose clinically important HBV DNA levels in untreated patients*

		HBV DNA ≥2,000 IU/ml					HBV DNA ≥20,000 IU/ml					HBV DNA ≥200,000 IU/ml				
		AUROC	Sen	Spe	CC	*p*†	AUROC	Sen	Spe	CC	*p*†	AUROC	Sen	Spe	CC	*p*†
Derivation set (2,796)		0.89 (0.84-0.94)	84.5	84.5	84.5	N/A	0.95 (0.93-0.97)	89.3	89.6	89.5	N/A	0.96 (0.94-0.98)	91.1	91.1	91.1	N/A
Validation set (2,795)		0.88 (0.83-0.93)	85.2	84.7	85.0	N/A	0.95 (0.93-0.97)	90.4	91.9	91.1	N/A	0.96 (0.94-0.99)	91.8	90.5	91.1	N/A
Women of reproductive age (1,134)		0.91 (0.85-0.96)	87.3	78.5	84.4	N/A	0.96 (0.93-0.99)	92.2	87.1	90.0	N/A	0.98 (0.96-1.00)	94.1	89.5	91.8	N/A
Sex	Males (3,482)	0.89 (0.84-0.94)	84.9	82.7	84.1	0.6	0.94 (0.92-0.97)	88.5	89.1	88.8	0.3	0.96 (0.93-0.98)	90.1	89.0	89.5	0.1
	Females (2,098)	0.87 (0.81-0.93)	83.5	87.0	85.1		0.96 (0.94-0.98)	91.9	91.8	91.8		0.98 (0.96-0.99)	93.1	93.3	93.2	
Age group	<30 years (892)	0.92 (0.87-0.98)	91.6	64.4	85.9	0.2	0.97 (0.94-1.00)	95.0	81.6	91.1	0.2	0.98 (0.95-1.00)	96.3	83.7	91.9	0.3
	≥30 years (4,679)	0.87 (0.81-0.93)	83.1	86.4	84.5		0.95 (0.92-0.97)	89.2	91.2	90.2		0.96 (0.94-0.98)	90.1	91.5	90.9	
Region	Asia (4,251)	0.89 (0.83-0.94)	84.8	87.2	85.7	0.9	0.94 (0.92-0.97)	89.0	90.1	89.5	0.5	0.96 (0.93-0.98)	89.6	90.0	89.8	0.5
	Europe (1,047)	0.86 (0.69-1.00)	83.4	74.7	80.0		0.98 (0.93-1.00)	95.4	91.4	93.4		0.99 (0.95-1.00)	96.9	93.3	94.9	
	Africa (293)	0.85 (0.77-0.93)	80.2	83.9	83.0		0.92 (0.85-0.99)	89.8	89.8	89.8		0.94 (0.88-1.00)	92.8	92.5	92.5	
HBeAg	Positive (2,032)	0.91 (0.87-0.96)	97.9	23.4	95.0	<0.01	0.87 (0.82-0.93)	98.8	21.0	93.3	0.7	0.86 (0.81-0.91)	98.1	25.6	88.7	0.08
	Negative (3,407)	0.74 (0.63-0.85)	66.0	87.2	79.4		0.89 (0.84-0.95)	77.2	94.5	90.3		0.93 (0.88-0.98)	79.5	97.0	94.4	
HBV genotype	A (287)	0.83 (0.71-0.94)	75.8	89.8	82.5	0.7	0.93 (0.84-1.00)	85.6	93.3	90.4	0.9	0.95 (0.87-1.00)	90.5	92.9	92.2	0.9
	B (1,027)	0.87 (0.79-0.96)	85.0	93.6	88.5		0.95 (0.91-0.99)	91.6	94.8	93.2		0.97 (0.94-1.00)	92.3	93.0	92.7	
	C (2,861)	0.90 (0.85-0.96)	86.8	84.6	86.0		0.95 (0.92-0.99)	91.0	88.4	89.9		0.96 (0.94-0.99)	91.2	88.6	89.9	

	D (538)	0.80 (0.55-1.00)	78.2	63.6	70.8		0.96 (0.89-1.00)	91.4	89.4	90.1		0.98 (0.92-1.00)	93.2	94.0	93.7	
	E (270)	0.88 (0.60-1.00)	85.4	85.9	85.7		0.95 (0.91-0.99)	94.6	87.8	89.8		0.96 (0.89-1.00)	95.1	90.5	91.8	
HCV	Positive (49)	0.92 (0.82-1.00)	89.8	78.3	81.6	0.5	0.97 (0.92-1.00)	94.8	82.9	85.1	0.5	0.98 (0.94-1.00)	95.6	84.5	85.6	0.2
	Negative (4,416)	0.89 (0.83-0.94)	84.9	85.4	85.1		0.95 (0.93-0.98)	90.3	90.6	90.4		0.96 (0.94-0.98)	91.3	90.9	91.1	
HDV	Positive (156)	0.88 (0.81-0.96)	85.3	83.4	83.7	0.9	0.96 (0.93-0.99)	92.0	89.3	89.5	0.9	0.97 (0.94-0.99)	93.3	90.3	90.4	0.9
	Negative (1,875)	0.89 (0.84-0.94)	85.2	88.4	86.1		0.95 (0.93-0.98)	90.8	91.2	91.0		0.97 (0.95-0.99)	91.7	91.0	91.4	

* Optimal cut-offs for serum HBcrAg derived from the derivation set were 3.6 log U/ml to diagnose HBV DNA ≥2,000 IU/ml, 4.5 log U/ml for ≥20,000 IU/ml, and 5.3 log U/ml for ≥200,000 IU/ml.

† *p* for interaction for AUROC

AUROC: area under the receiver operating characteristic curve; CC, correctly classified; CI: confidence interval; HBcrAg: hepatitis B core-related antigen; HBeAg: hepatitis B e antigen; HBV: hepatitis B virus; HCV: hepatitis C virus; HDV: hepatitis D virus; Sen, sensitivity; Spe, specificity