HBcrAg-based risk score performs better than the HBV DNA-based scores for HCC prediction in grey zone patients who are HBeAg-negative

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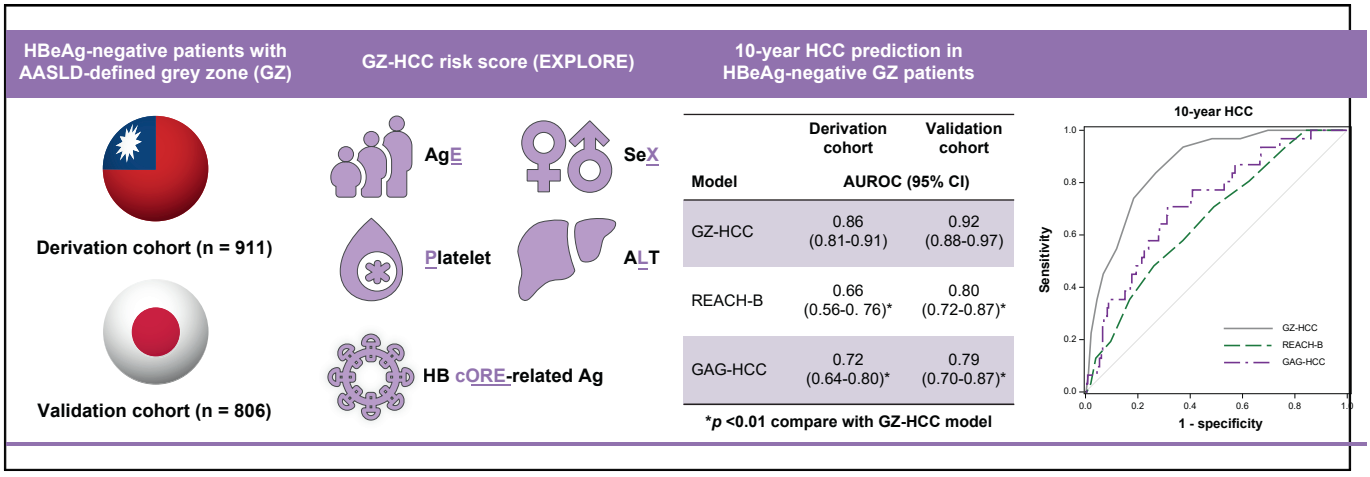
Graphical abstract



Highlights

- HCC risk is heterogenous in grey zone (GZ) patients with CHB who are HBeAg-negative.
- The GZ-HCC score shows superior predictive ability *vs.* HBV DNA-based risk scores for HCC in GZ patients who are HBeAg-negative.
- The GZ-HCC score derived from a Taiwanese cohort has been thoroughly validated in an independent Japanese cohort.
- The low-risk and high-risk GZ patients, stratified by a score of 8, had a similar HCC risk to patients with inactive CHB and immune-active CHB, respectively.
- This risk stratification approach can be used to optimise the clinical management of GZ patients who are HBeAg-negative.

Impact and implications

We have developed a risk score based on HBcrAg, which has shown better predictive ability for HCC compared with other risk scores based on HBV DNA. Using a score of 8, GZ patients can be classified into low- and high-risk groups, which can guide follow up and early treatment, respectively. This validated risk score is a valuable tool for optimising the management of GZ patients who are HBeAg-negative.

https://doi.org/10.1016/j.jhepr.2023.100956

Research article

JHEP|Reports

HBcrAg-based risk score performs better than the HBV DNA-based scores for HCC prediction in grey zone patients who are HBeAg-negative

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JHEP Reports **2024.** https://doi.org/10.1016/j.jhepr.2023.100956

Background & Aims: Risk scores have been designed to predict the development of hepatocellular carcinoma (HCC) in treatment-naive patients with chronic hepatitis B (CHB). However, little is known about their predictive accuracy in HBeAg-negative patients in the grey zone (GZ). We aimed to develop a HBcrAg-based HCC risk score and explore whether it outperforms other risk scores in GZ patients.
Methods: Two retrospective cohorts of HBeAg-negative patients with American Association for the Study of Liver Diseases-defined GZ were established for derivation and validation (Taiwanese, N = 911; Japanese, N = 806). All of them were non-cirrhotic at baseline and remained treatment-naive during the follow-up. The primary endpoint was HCC development.
Results: In a median follow-up period of 15.5 years, 85 patients developed HCC in the derivation cohort. We found that age, sex, alanine aminotransferase, platelet count, and HBcrAg, but not HBV DNA levels, were independent predictors and a 20-point GZ-HCC score was developed accordingly. The 10-year and 15-year area under the ROC curve (AUROC) ranged from 0.83 to 0.86, which outperformed the HBV DNA-based HCC risk scores, including REACH-B and GAG-HCC scores (AUROC ranging from 0.66 to 0.74). The better performance was also validated in EASL- and Asian Pacific Association for the Study of the Liver-defined GZ patients. These findings remained consistent in the validation cohort. Finally, the low-risk and high-risk GZ patients (stratified by a score of 8) had an HCC risk close to inactive CHB and immune-active CHB patients, respectively, in both cohorts.
Conclusions: The HBcrAg-based GZ-HCC score predicts HCC better than other HBV DNA-based risk scores in GZ patients who are HBeAg-negative patients, which may help optimise their clinical management.
Impact and implications: We have developed a risk score based on HBcrAg, which has shown better predictive ability for HCC compared with other risk scores based on HBV DNA. Using a score of 8, GZ patients can be classified into low- and high-risk groups, which can guide follow up and early treatment, respectively. This validated risk score is a valuable tool for optimising the management of GZ patients who are HBeAg-negative.

Introduction

Chronic HBV infection is a major public health issue worldwide, with a recent estimate indicating that more than 292 million individuals are positive for HBsAg.[1] Patients with chronic hepatitis B (CHB) are at a higher risk of developing various adverse outcomes, including hepatocellular carcinoma (HCC).[2] Therefore, precise prediction of HBV-related HCC is crucial as it can help guide the timely decision of commencement of antiviral treatment.[2]

Currently, several risk scores exist that predict HCC risk in untreated patients with CHB, which are accompanied by clear management recommendations.[3] For example, the American Association for the Study of Liver Diseases (AASLD) guidelines recommend that patients with liver cirrhosis and those with immune-active CHB (HBV DNA ≥2,000 IU/ml and alanine aminotransferase [ALT] ≥2 × upper limits of normal [ULN]) should receive antiviral therapy. However, patients with inactive

Keywords: ERADICATE-B; HCC; Hepatocellular carcinoma; HBcrAg; Grey zone.
Received 29 August 2023; accepted 26 September 2023; available online 4 November 2023
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CHB (HBV DNA <2,000 IU/ml and ALT <ULN) only require HCC surveillance without antiviral treatment. Further HCC risk prediction in these well-established patient groups provides limited benefit in clinical practice as it will not change their management. In contrast, approximately half of patients who are HBeAg-negative fall short of these criteria and they are categorised as 'grey zone' (GZ) patients.[4,5] The optimal management for this particular subgroup remains unknown because of the poorly defined, heterogenous HCC risk.[5–8]

HBcrAg quantification is a useful biomarker for evaluating the transcriptional activity of covalently closed circular DNA.[9–11] Cohort studies also indicated that, in HBeAg-negative patients, serum HBcrAg levels better predict HCC risk in patients with CHB than HBV DNA levels.[12–15] We thus aimed to explore whether a HBcrAg-based risk score could achieve a more accurate prediction of HCC in GZ patients than other prediction models, which is the key to determining their clinical management pathways.

To achieve this, we first included GZ patients from Taiwan as a derivation cohort to develop a HBcrAg-based HCC risk score, that would outperform existing HBV DNA-based risk scores, including REACH-B,[16] GAG-HCC,[17] and CU-HCC scores.[18] Second, we confirmed our findings in an independent validation cohort from Japan. Lastly, we identified cut-offs to divide the GZ patients into groups with clinically significantly different HCC risk. Patients in the groups defined as low- or high-HCC risk, should have HCC risks similar to those of the inactive CHB patient group and immune-active CHB patient group, respectively. Hopefully, this risk stratification should optimise clinical management.

Patients and methods

Patient cohorts

The derivation cohort was composed of patients from the 'Elucidation of Risk Factors for Disease Control or Advancement in Taiwanese Hepatitis B Carriers' (ERADICATE-B) study,[12,19–22] and the inclusion and exclusion criteria are presented in Fig. S1A. We excluded 411 patients with liver cirrhosis at baseline, 412 patients who received antiviral therapy before HCC development or the end of follow up (including 102 GZ patients who were HBeAg-negative), and 516 patients who were HBeAg-positive. Among the 2,150 patients without liver cirrhosis who were treatment-naïve and HBeAg-negative, 911 patients met the criteria for AASLD-defined GZ with all viral and host variables available. All patients provided informed consent as approved by the research ethics committee of the National Taiwan University Hospital (ethics approval number: 202008021RIPC).

Fig. S1B illustrates the enrolment of Japanese patients in the validation cohort. Among the 1,312 individuals without liver cirrhosis who were treatment-naive and HBeAg-negative, 806 patients met the criteria for AASLD-defined GZ. Informed consent was obtained from each patient, and the study protocol complied with the ethical guidelines of the Declaration of Helsinki and the ethical guidelines for medical and health research involving human subjects of the Ministry of Health, Labour and Welfare in Japan. The Toranomon Hospital Ethics Committee approved the study (ethics approval number: 2223).

Data collection and serological marker assay

Baseline serological markers including HBsAg, HBeAg, anti-HBe, and anti-HCV, and also liver biochemical tests and alpha-foetoprotein levels were collected from all patients. The details of the serological assays are summarised in the Supplementary material. HCC surveillance, which included blood tests and abdominal ultrasonography, was performed at least every 6 months in both the derivation and validation cohorts. At each visit, serum samples were collected and stored at -20 °C (derivation cohort) or -80 °C (validation cohort) if available.

Quantification of HBV DNA, HBsAg, and HBcrAg serum levels

In the derivation cohort and the validation cohort when medical record data were unavailable, HBV DNA, HBsAg, and HBcrAg levels were retrospectively quantified using stored serum samples. HBV DNA levels were determined using commercial assays with a lower limit of detection of 15–20 IU/ml. HBsAg levels were determined using the Architect HBsAg QT (Abbott Laboratories, Abbott Park, IL, USA).[22–24] HBcrAg levels were determined using the Lumipulse G HBcrAg assay and the Lumipulse G1200 Analyzer (Fujirebio, Tokyo, Japan, with a dynamic range of 1,000 U/ml to 10,000,000 U/ml.[12]

Determination of HBV genotype

HBV genotype was determined using either a PCR-based assay or ELISA-based assay. The details of HBV genotyping assay are summarised in the supplementary material.

Definition of different disease stages in the HBeAg-negative phase

The AASLD 2018 HBV guidelines were adopted to categorise the clinical phases of patients who were HBeAg-negative. Inactive CHB was characterised by HBV DNA levels <2,000 IU/ml and ALT levels ≤1 × ULN, whereas immune-active CHB was identified by HBV DNA levels ≥2,000 IU/ml, coupled with ALT levels ≥2 × ULN. The AASLD guideline sets the ULN of ALT at 35 U/L for males and 25 U/L for females. Patients who were HBeAg-negative and who did not fit well within these two phases were designated as GZ phase. Beyond AASLD-defined GZ patients, we also analysed our data using GZ criteria defined by the EASL and APASL (Asian Pacific Association for the Study of the Liver) guidelines, respectively (Table S1).

Definition of HCC and cirrhosis

HCC was diagnosed either by histology/cytology results or by typical image findings (arterial enhancement and venous washout by contrast-enhanced computed tomography or magnetic resonance imaging) in hepatic nodules >1 cm.[25] Liver cirrhosis was diagnosed based on histology and ultrasonographic findings together with or without clinical features such as ascites, thrombocytopaenia, gastroesophageal varices, or laparoscopy (in the validation cohort only).[26,27]

Statistical analysis

Mean and standard deviation (SD) values were computed for continuous variables, whereas percentages were calculated for categorical variables.

The clinical follow up began at enrolment, and the person-years were censored on the earliest of the date of HCC identification, death, or the last follow-up date (June 30, 2017 for the derivation cohort and December 31, 2019 for the validation cohort). Patients were stratified using the log10 scale of HBcrAg, with the lower cut-off of 10,000 U/ml chosen based on our earlier findings.[12,15] We aimed to retain at least 5% of patients in each category across both cohorts, prompting the categorisation at 10,000 and 100,000 IU/ml. The study analysed the cumulative

incidence stratified by different scores or variables using the Kaplan–Meier curve analysis and the logrank test. The Cox proportional hazards regression model was used to assess the crude and adjusted hazard ratios (HRs) of each variable, including age, sex, ALT levels, platelet count, HBcrAg, HBV DNA, HBsAg levels, and HBV genotype.[12,19,20] Subsequently, only the factors that exhibited a statistically significant difference in the multivariable model were considered for the risk score calculation. The risk score was developed using Cox proportional hazards model to estimate the β regression coefficient for the selected risk factors. The β coefficient of each risk factor was divided by the β coefficient for a 5-year increase in age, and the number was rounded to an integer value to generate the risk score. The projected HCC risk was evaluated at 10-year and 15-year follow up.

The discrimination performance of each score was evaluated using the time-dependent receiver-operating characteristic (ROC) curve and area under the ROC curve (AUROC). We also compared the discrimination performance of the GZ-HCC score to different HBV DNA-based scores, including REACH-B, GAG-score (in both cohorts),[16,17] and CU-HCC score (in the validation cohort only because of missing bilirubin and albumin data in the derivation cohort).[18]

The calibration performance was evaluated by plotting the observed risk against the predicted risk determined by the Cox model to form a calibration chart. When the number of HCC cases was insufficient within a group having the same cumulative risk score, we merged that group with the adjacent groups to obtain a more accurate estimation of the HCC risk.[16]

The study evaluated the relationship between GZ-HCC score and HCC risk using the restricted cubic spline regression with different number of knots.[23,28] The best-fitting cubic spline model was determined according to the value of Akaike information criterion (AIC).

To validate the effectiveness of the GZ-HCC score in both the derivation and validation cohorts, we conducted three distinct sensitivity analyses. Firstly, we classified GZ patients according to EASL and APASL guidelines. Secondly, we excluded GZ patients who transitioned to the immune active phase within the initial year of follow up. Thirdly, we re-included GZ patients who received antiviral treatment during follow up, with the follow up being censored 1 year after treatment.[29]

Statistical testing with a significance level of 5% was used to provide statistical evidence for our findings. The time-dependent ROC analysis was conducted using TS1M7 version 9.4 statistical software (SAS Institute, Cary, NC, USA), using Uno *et al.*'s inverse probability of censoring weighting technique with 2,000 perturbation samples.[30] We performed all other analyses using Stata statistical software (version 13.0; Stata Corp, College Station, TX, USA).

Results

Comparison of baseline characteristics between the derivation and validation cohorts

Table 1 compares the baseline characteristics between 911 patients from the derivation cohort and 806 patients from the validation cohort. The patients in both cohorts were HBeAg-negative and non-cirrhotic at baseline and remained treatment-naive during the follow-up period. Patients in the derivation cohort were younger (median age: 41.8 *vs.* 43.5 years) than those in the validation cohort. In contrast, there were more patients with HBcrAg level <10,000 U/ml (54.8% *vs.* 80.5%) in the validation cohort. The dominant viral strain was genotype B in the derivation cohort (84.9%) and genotype C in the validation cohort (53.4%).

Comparison of HCC incidence between the derivation and validation cohorts

The median follow-up duration was 15.5 years and 8.1 years for the derivation and validation cohort (p <0.001), respectively. There was a higher cumulative incidence of HCC in the derivation cohort compared with the validation cohort (p = 0.014, Fig. S2) with 85 patients in the derivation cohort and 28 patients in the validation cohort developing HCC (Table 1).

Risk factors associated with HCC in GZ patients who were HBeAg-negative

We first analysed potential risk factors associated with HCC and found that HBcrAg level, but not HBV DNA or HBsAg level, was the only viral factor associated with HCC development (Table S2). We thus included independent risk factors, including age, sex, ALT, HBcrAg, and platelet count, into multivariable Cox proportional hazards model. The β regression coefficient estimates and the scores of each variable are shown in Table 2. The GZ-HCC score ranged from 0 to 20. The predicted HCC incidence according to each point of the GZ-HCC was detailed at 10 years and 15 years of follow up (Table 3).

Discrimination and calibration of the GZ-HCC score

In the derivation cohort, the AUROC of the GZ-HCC score for HCC development was 0.86 (95% CI: 0.81–0.91) and 0.83 (95% CI: 0.79–0.88) at 10 and 15 years of follow-up, respectively (Fig. 1A and B). The prediction performance was better than REACH-B and GAG scores at both time points (p <0.001). In the validation cohort, the AUROC of the GZ-HCC score for HCC development was 0.92 (95% CI: 0.88–0.97) and 0.90 (95% CI: 0.83–0.97), respectively (Fig. 1C and D). The prediction was consistently better than REACH-B, GAG, and CU-HCC scores at both time points.

The predicted and observed incidence rates of HCC were illustrated by the calibration chart. In the derivation cohort, the risk was well-calibrated at different time points (Fig. 2A and B) with a correlation coefficient of 0.984 at 10 years and 0.997 at 15 years. In the validation cohort, the correlation coefficient at 10 years and 15 years was 0.991 and 0.686, respectively (Fig. 2C and D).

Better prediction performance of the GZ-HCC score compared with other scores in GZ patients by different definitions

We also explored the discrimination performance of the GZ-HCC score in EASL- and APASL-defined GZ patients (number shown in Table S1) in Figs S3 and S4, respectively, and compared it with other prediction models. Consistent with the results in AASLD-defined GZ patients, the AUROC of the GZ-HCC score ranged from 0.8 to 0.9 in the derivation and validation cohorts, respectively. The prediction performance of the GZ-HCC score was better than other HBV-DNA-based risk scores at most time points in both cohorts.

Two sensitivity analyses: excluding patients developing immune-active CHB within 1 year and re-including patients receiving antiviral therapy during the follow-up period

Two sensitivity analysis were performed to validate our findings in both cohorts. We first excluded the patients who developed immune-active CHB within the first year of follow-up (N = 106

Table 1. Comparison of baseline characteristics and follow-up data between 911 GZ patients from the derivation cohort and 806 GZ patients from the validation cohort.

			Derivation cohort (Taiwanese)	Validation cohort (Japanese)	*p* value
Age years, median (IQR)			41.8 (15.5)	43.5 (16.3)	0.025
Sex					0.120
Female			317 (34.8)	310 (38.5)	
Male			594 (65.2)	496 (61.5)	
Platelet (× 10^9/L)					0.245
≥150			804 (88.3)	726 (90.1)	
<150			107 (11.7)	80 (9.9)	
Serum ALT level*					0.047
≤ULN			513 (56.3)	415 (51.5)	
>ULN			398 (43.7)	391 (48.5)	
Serum HBcrAg level (U/ml)					<0.001
<10,000			499 (54.8)	649 (80.5)	
10,000–99,999			244 (26.8)	105 (13.0)	
≥100,000			168 (18.4)	52 (6.5)	
Serum HBV DNA level (IU/ml)					<0.001
<2,000			179 (19.7)	236 (29.3)	
2,000–19,999			401 (44.0)	355 (44.0)	
≥20,000			331 (36.3)	215 (26.7)	
Serum HBsAg level (IU/ml)					0.528
<1,000			424 (46.5)	362 (44.9)	
≥1,000			487 (53.5)	444 (55.1)	
HBV genotype†					<0.001
B			760 (84.9)	248 (33.5)	
C			135 (15.1)	432 (58.4)	
A				54 (7.3)	
Others (D, E, F, H)				6 (0.8)	
HBV DNA and ALT in AASLD-defined GZ patients					
Group	**HBV DNA (IU/ml)**	**ALT**			**<0.001**
1	<2,000	1–2 × ULN	129 (14.16)	175 (21.71)	
2	<2,000	2 × ULN	50 (5.49)	60 (7.44)	
3	2,000–19,999	≤ULN	317 (34.80)	278 (34.49)	
4	2000–19,999	1–2 × ULN	84 (9.22)	75 (9.31)	
5	≥20,000	≤ULN	196 (21.51)	138 (17.12)	
6	≥20,000	1–2 × ULN	135 (14.82)	80 (9.93)	
Follow-up years, median (IQR)			15.5 (5.4)	8.1 (11.3)	<0.001
HCC (n)			85	28	
Annual incidence (95% CI)			0.58 (0.47–0.72)	0.32 (0.21–0.45)	0.004

Data in parentheses are a percentage except when stated otherwise.
AASLD, American Association for the Study of Liver Diseases; ALT, alanine transaminase; FIB-4, fibrosis-4 index; ULN, upper limit of normal.
* Upper limit of normal ALT: 35 U/L for males and 25 U/L for females defined by AASLD guidelines.
† Undetermined genotype in 16 patients and 66 patients in the derivation and validation cohort, respectively.

and 77 for the derivation and validation cohorts, respectively). The AUROC remained ∼0.85 and 0.90 for the derivation and validation cohorts, respectively, which was comparable to the undeleted data (Table S3). Second, we re-included the GZ patients who received antiviral therapy during the follow-up period (N = 102 and 96 for the derivation and validation cohorts, respectively). The patients were all censored 1 year after antiviral treatment. The prediction performance at different time points was also similar to AUROC values derived from the treatment-naive patients (Table S4).

Applying the GZ-HCC score to stratify HCC risk

To streamline clinical management, the first aim was to identify a low-risk group with HCC risk indistinguishable from inactive CHB. The relationship between the GZ-HCC score and HCC risk in the derivation cohort was evaluated using the restricted cubic spline regression (Fig. S5) with the lowest AIC value (Table S5, three knots set at 10th, 50th, and 90th percentile). We found that HCC risk started to increase at a score of 8 and increased rapidly after a score of 13.

We thus decided to test three cut-offs in the GZ patients, including the scores of 8 (50.4% <8), 10 (71.5% <10), and 13 (92.0% <13), and plotted their risk alongside those of patients who had inactive CHB and immune-active CHB, and who remained treatment-naive throughout the follow-up period. We found that the HCC risk in the low-risk group using a score of 8 was close to inactive CHB (Fig. 3A). However, using cut-off values of 10 (Fig. S6A) or 13 (Fig S6B) did not reveal such a low risk. Interestingly, the HCC risk in patients with a score ≥8 was also close to that in patients who were immune-active. Compared with the patients with inactive CHB, the HR of GZ patients with a score <8 (N = 459), GZ patients with score ≥8 (N = 452), and immune-active CHB was 1.09 (95% CI: 0.49–2.40), and 8.92 (95% CI: 5.20–15.31), and 9.35 (95% CI: 5.01–17.45), respectively.

A score of 8 was then adopted to stratify HCC risk in the validation Japanese cohort. Compared with the patients with

Table 2. Hazard ratio, β coefficient, and the corresponding risk score from the derivation cohort using the Cox proportional hazards model.

Variable	Adjusted hazard ratio (95% CI)	β coefficient	*p* value	Risk score
Age (years)				
Per 5 years	1.40 (1.26–1.56)	0.34	<0.001	
<30				0
30–34				1
35–39				2
40–44				3
45–49				4
50–54				5
55–59				6
60–64				7
65–69				8
≥70				9
Sex				
Female	1.00	1.00	0	0
Male	2.16 (1.24–3.75)	0.77	0.006	2
Platelet count (10^9/L)				
≥150	1.00	1.00		0
<150	2.21 (1.35–3.61)	0.79	0.002	2
Serum ALT level*				
≤ULN	1.00	1.00		0
>ULN	1.97 (1.27–3.07)	0.68	0.003	2
HBcrAg (U/ml)				
<10,000	1.00	1.00		0
10,000–99,999	3.51 (1.96–6.30)	1.26	<0.001	4
≥100,000	5.63 (3.19–9.91)	1.73	<0.001	5

AASLD, American Association for the Study of Liver Diseases; ALT, alanine transaminase; HCC, hepatocellular carcinoma; ULN, upper limit of normal.
* Upper limit of normal ALT: 35 U/L for males and 25 U/L for females defined by the AASLD guidelines.

Table 3. Predicted HCC incidence within 10 years and 15 years according to each point of the GZ-HCC score.

Score	10 years	15 years
0	0.12%	0.28%
1	0.18%	0.40%
2	0.25%	0.56%
3	0.35%	0.79%
4	0.50%	1.12%
5	0.70%	1.58%
6	1.00%	2.22%
7	1.41%	3.13%
8	1.98%	4.40%
9	2.79%	6.17%
10	3.93%	8.62%
11	5.52%	11.98%
12	7.72%	16.52%
13	10.74%	22.55%
14	14.86%	30.35%
15	20.36%	40.06%
16	27.54%	51.53%
17	36.60%	64.12%
18	47.53%	76.55%
19	59.85%	87.16%
20	72.51%	94.52%

GZ-HCC, grey zone hepatocellular carcinoma.

inactive CHB, the HR of GZ patients with a score <8 (N = 496), GZ patients with a score ≥8 (N = 310), and immune-active CHB was 0.64 (95% CI: 0.16–2.55), 9.74 (95% CI: 3.99–23.7), and 11.4 (95% CI: 4.07–32.2), respectively. This re-affirmed that the low and high (HCC) risk was shown to be similar to those of patients with inactive CHB and immune-active CHB, respectively (Fig. 3B).

Discussion

It is known that GZ patients who are HBeAg-negative have an inconsistent prognosis, thus the optimal management remains unclear.[31] In this study, we developed a new HCC risk score (EXPLORE) by incorporating ag**e**, se**x**, **p**latelet, A**LT**, and hepatitis B c**ore**-related antigen. To the best of our knowledge, this is the first risk prediction model showing an HBcrAg-based HCC score that outperforms the HBV DNA-based HCC scores in GZ patients who are HBeAg-negative, as defined by AASLD, EASL, and APASL guidelines. Two sensitivity analyses also confirmed the robustness of this score. Furthermore, the prediction performance was validated in an independent cohort from Japan. Finally, for clinical translation, we proposed a GZ-HCC score of 8 to divide the GZ patients into high- and low-risk groups, where the HCC risk was similar to those of patients with immune-active CHB and inactive CHB, respectively.

There are three notable findings in this study. Firstly, this study clearly shows that the HBcrAg-based model predicts HCC development in GZ patients better than other HBV DNA-based prediction models. This fulfils the clinical unmet need for an HCC risk prediction model in GZ patients. To illustrate how this model is of clinical benefit, take a 53-year-old man with CHB who is HBeAg-negative with an ALT level of 40 U/ml, HBV DNA level of 10,000 IU/ml, and platelet count of 180,000 (10^9/L) for example (Table S6). Based on the REACH-B score, the predicted 10-year HCC risk is 5.2% (score of 10). However, according to the GZ-HCC score, his 10-year HCC risk could be 2.8%, 10.7%, or 14.9%, if his HBcrAg level is 1,000, 10,000, or 100,000 U/ml, respectively (Table S6). Such a huge variation in potential HCC risk based on GZ-HCC score on the same HBV DNA-based

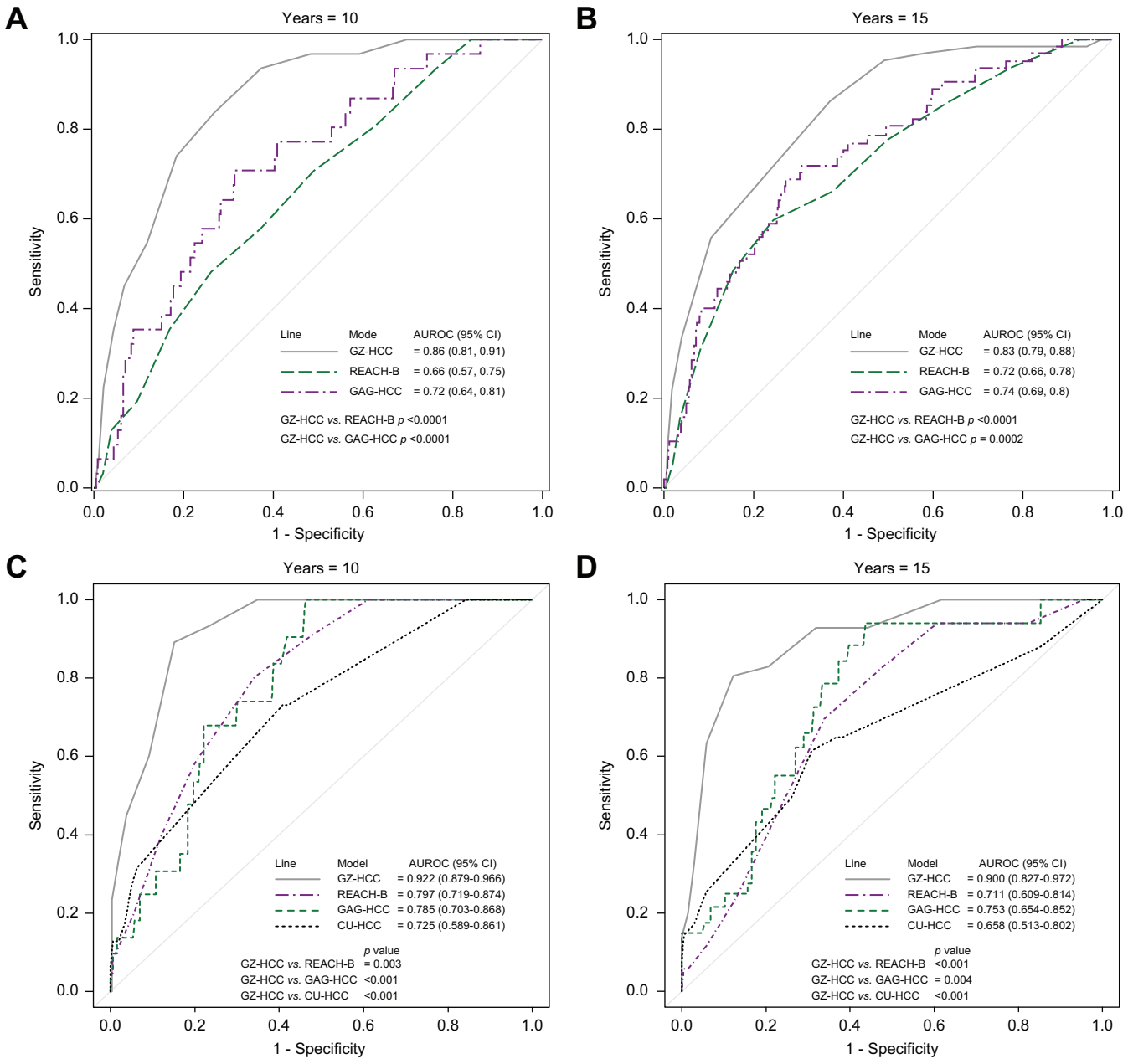


Fig. 1. Performance of GZ-HCC and HBV DNA-based risk scores for HCC prediction in GZ patients. Among the AASLD-defined GZ patients, time-dependent receiver-operating-characteristic (ROC) curves of GZ-HCC, REACH-B, GAG-HCC, and CU-HCC scores to predict (A) 10-year and (B) 15-year HCC in the derivation cohort as well as (C) 10-year and (D) 15-year HCC in the validation cohort. AASLD, American Association for the Study of Liver Diseases; GZ, grey zone; HCC, hepatocellular carcinoma.

score demonstrates how the GZ-HCC score is superior, and perhaps explains the clinical shortcoming of the HBV DNA-based score. Although HBV DNA and HBcrAg levels are generally thought to be highly correlated, the GZ cohort tends to exclude those with low viraemia and some high-viraemic patients, hence the predictive value of HBV DNA levels may be compromised. Therefore, HBcrAg levels remain an independent variable and could explain why the GZ-HCC score is a better prediction model than others.

Secondly, our previous data showed that the AUROC curve was ~0.70 using HBcrAg alone in predicting 10-year or 15-year risk of HCC. In this study, by incorporating age, sex, ALT, and platelet count, the prediction performance was largely improved with AUROC up to 0.8–0.9 in both cohorts. The GZ-HCC score comprehensively includes host, viral, liver fibrosis, and inflammation markers, hence provides a more accurate HCC prediction in GZ patients than viral markers alone.

Thirdly, a recent clinical trial has shown that antiviral treatment may reduce the risk of fibrosis progression in GZ patients.[32] However, it definitely increases the economic burden largely as around half of the patients who are HBeAg-negative are designated as GZ.[4,5] To address this issue, we propose that the GZ-HCC score should be calculated in all the GZ patients defined by different treatment guidelines. These patients can be categorised

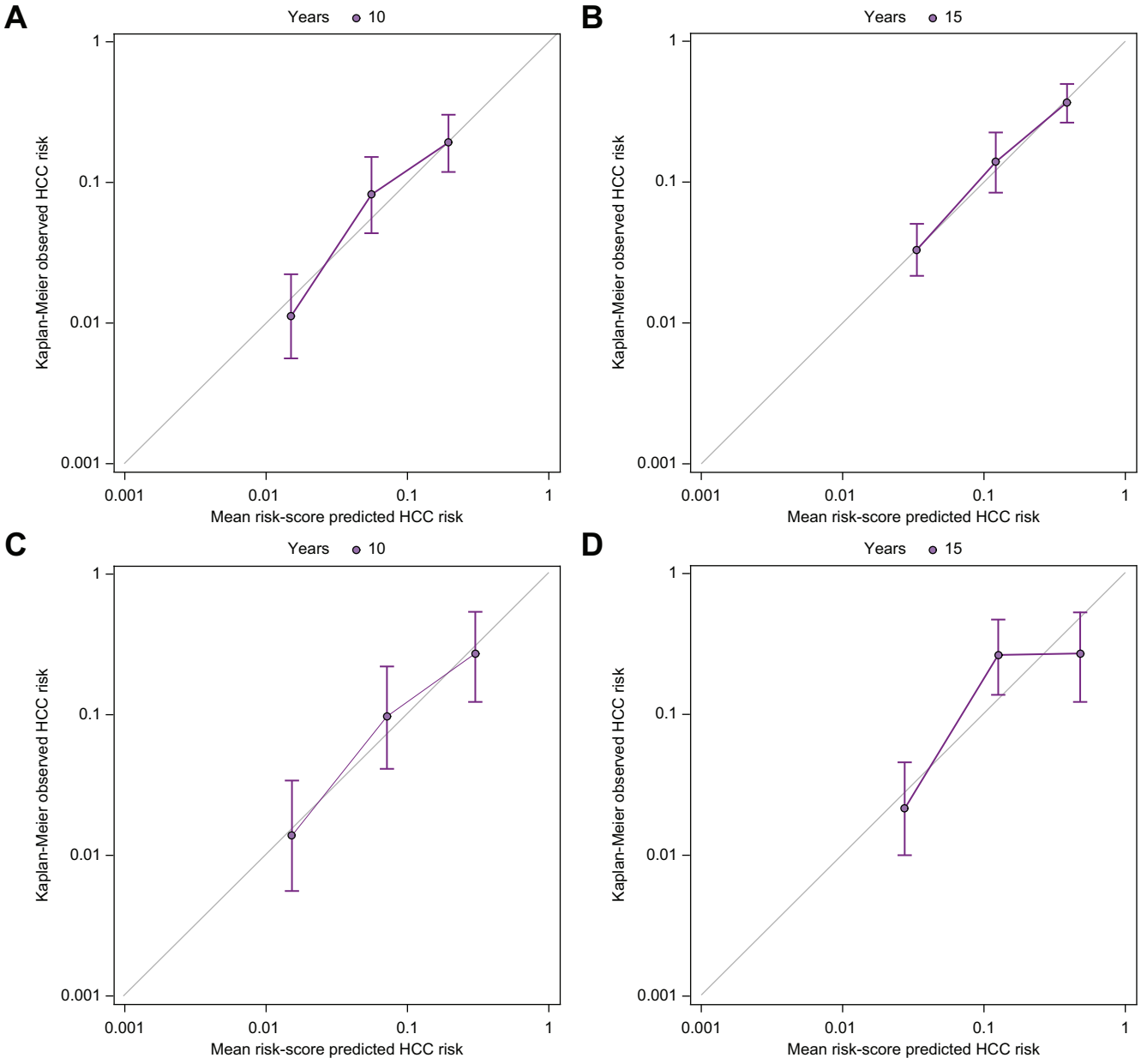
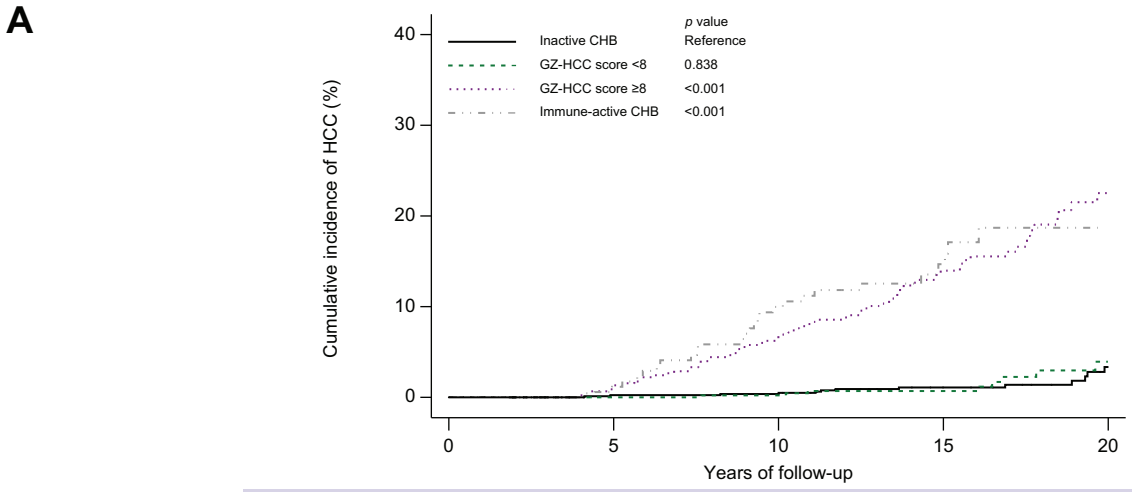


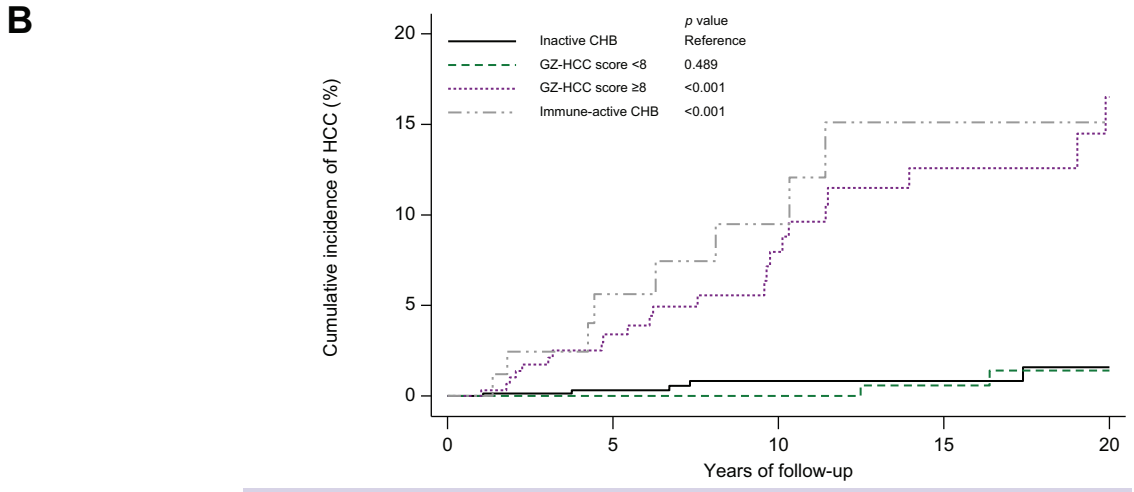
Fig. 2. Predicted HCC incidence calibrated against observed HCC estimates. Predicted HCC incidence calibrated against observed HCC estimates using the Kaplan–Meier method in the derivation cohort over (A) 10 years and (B) 15 years, and in the validation cohort over (C) 10 years and (D) 15 years. All patients were stratified into three groups based on GZ-HCC scores: 0–10, 11–12, and 13–20. GZ, grey zone; HCC, hepatocellular carcinoma.

into low- and high-risk groups using a GZ-HCC score of 8. Low-risk GZ patients could be managed similarly to patients with inactive CHB with regular follow up and antiviral treatment could be prioritised for the high-risk patients, if further evidence emerges supporting HCC risk reduction after antiviral treatment in this specific clinical setting.

There are a few limitations to this study. Firstly, the predicted HCC risk was not well calibrated at year 15 in the validation cohort, which could be attributed to a short duration of follow up. A cohort with a longer duration of follow-up is thus required to address the issue. Secondly, it is inevitable that some of the patients received antiviral therapy during the follow-up. These patients were initially excluded but were re-included for sensitivity analysis. The prediction performance remained similar in both cohorts. Thirdly, GZ patients may meet treatment criteria shortly after enrolment as ALT levels can vary rapidly. We thus excluded GZ patients who transitioned to the immune-active CHB phase within the first year of follow-up. The GZ-HCC score still worked well in predicting HCC. Fourthly, we did not include fibrosis-4 index as a variable as many components overlap with



N° at risk					
Inactive CHB	824	822	776	469	175
GZ-HCC score <8	459	459	435	257	87
GZ-HCC score ≥8	452	446	407	248	74
Immune-active CHB	175	170	151	72	21



N° at risk					
Inactive CHB	776	494	260	167	95
GZ-HCC score <8	496	337	209	141	84
GZ-HCC score ≥8	310	209	119	68	40
Immune-active CHB	89	55	38	23	14

Fig. 3. Using a GZ-HCC score of 8 to stratify HBeAg-negative GZ patients into high-risk and low-risk groups. HCC risk in the high-risk and low-risk groups closely resembled the risk in patients with immune-active CHB and inactive CHB, respectively, in (A) the derivation cohort and (B) the validation cohort. CHB, chronic hepatitis B, GZ, grey zone; HCC, hepatocellular carcinoma.

it in this study, including age, ALT, and platelet count. Finally, only Asian patients were enrolled and further validation in treatment-naive patients of other ethnicities with genotype A or D infection is warranted.

In summary, an easy-to-use risk score combining host, viral, and liver variables for the estimation of HCC risk in Asian GZ patients with CHB is developed. This HBcrAg-based score achieves a better HCC prediction than HBV DNA-based scores. A validated score of 8 divides the patients into two different HCC-risk groups, which may optimise the management of GZ patients who are HBeAg-negative, in daily practice.

Abbreviations

AASLD, American Association for the Study of Liver Diseases; AIC, Akaike information criterion; ALT, alanine aminotransferase; CHB, chronic hepatitis B; GZ, grey zone; HCC, hepatocellular carcinoma; HR, hazard ratio; ROC, receiver operating characteristic; ULN, upper limit of normal.

Financial support

The Taiwanese research team was supported by grants from the National Taiwan University Hospital (109-N4644, 109-P05, 110-N4850, 110-P06, and 111-N0067), the Ministry of Science and Technology, Executive Yuan, Taiwan (MOST 109-2314-B-002-086-MY3 and MOST

110-2314-B-002-040-MY3), and Gilead Sciences (IN-TW-988-5987). The Japanese research team was supported in part by the Program for Basic and Clinical Research on Hepatitis, the Japan Agency for Medical Research and Development (AMED) (No. 22HC1001) (No. JP21fk0210084).

Conflicts of interest

T-CT has served on speaker's bureaus for Fujirebio, Bristol-Myers Squibb, and Gilead Sciences and received grant support from Gilead Sciences. TH served as a speaker for Gilead Sciences, Eisai Co., Ltd, and Fujirebio. FS served as a speaker for Gilead Sciences. HK served as a speaker for Gilead Sciences, AbbVie Inc., MSD K.K., Eisai Co., Ltd, and Sumitomo Pharma.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Study concept and design: T-CT, J-HK. Acquisition of data: W-TY, C-JL, H-CY, T-HS, C-HL, P-JC, J-HK, TH, FS, HK. Analysis and interpretation of data: W-TY, T-CT, TH, J-HK. Drafting of the manuscript: T-CT, TH. Critical review of the manuscript for important intellectual content: J-HK. Statistical analysis: CC, W-TY, TH, T-CT. Obtained funding: T-CT, J-HK. Technical or material support: C-MH, T-CT. Study supervision: J-HK. Article guarantor: TH, J-HK. Approved the final version of the manuscript: all authors.

Data availability statement

All reagents, antibodies and resources used in this research can be found in the CTAT table.

Acknowledgements

We thank Fujirebio Company for providing quantitative HBcrAg kits for the Taiwanese research team. We thank the staff of the Department of Medical Research, National Taiwan University Hospital for the Integrated Medical Database (NTUH-iMD), who provided the follow-up data. We also thank the Cancer Registry, Cancer Administration and Coordination Center, NTUH for providing the cancer registration data to confirm our diagnoses. Finally, we are grateful for the support received from Gilead Sciences and the Liver Disease Prevention & Treatment Research Foundation, Taiwan.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2023.100956.

References

[1] Nguyen MH, Wong G, Gane E, et al. Hepatitis B virus: advances in prevention, diagnosis, and therapy. Clin Microbiol Rev 2020;33:e00046–e119.
[2] Tseng TC, Kao JH. Elimination of hepatitis B: is it a mission possible? BMC Med 2017;15:53.
[3] Hsu YC, Tseng CH, Huang YT, et al. Application of risk scores for hepatocellular carcinoma in patients with chronic hepatitis B: current status and future perspective. Semin Liver Dis 2021;41:285–297.
[4] Di Bisceglie AM, Lombardero M, Teckman J, et al. Determination of hepatitis B phenotype using biochemical and serological markers. J Viral Hepat 2017;24:320–329.
[5] Huang DQ, Li X, Le MH, et al. Natural history and hepatocellular carcinoma risk in untreated chronic hepatitis B patients with indeterminate phase. Clin Gastroenterol Hepatol 2022;20:1803–1812.
[6] Zhou K, Wahed AS, Cooper S, et al. Phase transition is infrequent among North American adults with e-antigen-negative chronic hepatitis B and low-level viremia. Am J Gastroenterol 2019;114:1753–1763.
[7] Bonacci M, Lens S, Mariño Z, et al. Anti-viral therapy can be delayed or avoided in a significant proportion of HBeAg-negative Caucasian patients in the grey zone. Aliment Pharmacol Ther 2018;47:1397–1408.
[8] Lee HW, Kim SU, Baatarkhuu O, et al. Progression of untreated minimally active chronic HBV infection compared to inactive infection. Clin Gastroenterol Hepatol 2019;17:2808–2810.e2802.
[9] 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.
[10] Suzuki F, Miyakoshi H, Kobayashi M, et al. 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.
[11] Dusheiko G, Agarwal K, Maini MK. New approaches to chronic hepatitis B. N Engl J Med 2023;388:55–69.
[12] Tseng TC, Liu CJ, Hsu CY, et al. High level of hepatitis B core-related antigen associated with increased risk of hepatocellular carcinoma in patients with chronic HBV infection of intermediate viral load. Gastroenterology 2019;157:1518–1529.e1513.
[13] To WP, Mak LY, Wong DK, et al. Hepatitis B core-related antigen levels after HBeAg seroconversion is associated with the development of hepatocellular carcinoma. J Viral Hepat 2019;26:1473–1480.
[14] 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.
[15] Tseng TC, Hosaka T, Liu CJ, et al. Hepatitis B core-related antigen stratifies the risk of liver cancer in HBeAg-negative patients with indeterminate phase. Am J Gastroenterol 2022;117:748–757.
[16] Yang HI, Yuen MF, Chan HL, et al. Risk estimation for hepatocellular carcinoma in chronic hepatitis B (REACH-B): development and validation of a predictive score. Lancet Oncol 2011;12:568–574.
[17] Yuen MF, Tanaka Y, Fong DY, et al. Independent risk factors and predictive score for the development of hepatocellular carcinoma in chronic hepatitis B. J Hepatol 2009;50:80–88.
[18] Wong VW, Chan SL, Mo F, et al. Clinical scoring system to predict hepatocellular carcinoma in chronic hepatitis B carriers. J Clin Oncol 2010;28:1660–1665.
[19] Tseng TC, Liu CJ, Su TH, et al. Fibrosis-4 index helps identify HBV carriers with the lowest risk of hepatocellular carcinoma. Am J Gastroenterol 2017;112:1564–1574.
[20] Tseng TC, Liu CJ, Yang HC, et al. High levels of hepatitis B surface antigen increase risk of hepatocellular carcinoma in patients with low HBV load. Gastroenterology 2012;142:1140–1149.
[21] Tseng TC, Liu CJ, Yang WT, et al. Serum hepatitis B core-related antigen level stratifies risk of disease progression in chronic hepatitis B patients with intermediate viral load. Aliment Pharmacol Ther 2021;53:908–918.
[22] Tseng TC, Liu CJ, Yang HC, et al. Serum hepatitis B surface antigen levels help predict disease progression in patients with low hepatitis B virus loads. Hepatology 2013;57:441–450.
[23] Tseng TC, Liu CJ, Su TH, et al. Serum hepatitis B surface antigen levels predict surface antigen loss in hepatitis B e antigen seroconverters. Gastroenterology 2011;141:517–525.
[24] Tseng TC, Liu CJ, Yang HC, et al. Determinants of spontaneous surface antigen loss in hepatitis B e antigen-negative patients with a low viral load. Hepatology 2012;55:68–76.
[25] Heimbach JK, Kulik LM, Finn RS, et al. AASLD guidelines for the treatment of hepatocellular carcinoma. Hepatology 2018;67:358–380.
[26] Tseng TC, Liu CJ, Yang HC, et al. Higher proportion of viral basal core promoter mutant increases the risk of liver cirrhosis in hepatitis B carriers. Gut 2015;64:292–302.
[27] Tseng TC, Liu CJ, Chang CT, et al. HEV superinfection accelerates disease progression in patients with chronic HBV infection and increases mortality in those with cirrhosis. J Hepatol 2020;72:1105–1111.
[28] Harrell FE. Regression modeling strategies: with applications to linear models, logistic regression, and survival analysis. Berlin: Springer; 2001.
[29] Kim GA, Han S, Choi GH, et al. Moderate levels of serum hepatitis B virus DNA are associated with the highest risk of hepatocellular carcinoma in chronic hepatitis B patients. Aliment Pharmacol Ther 2020;51:1169–1179.
[30] Uno H, Cai T, Pencina MJ, et al. On the C-statistics for evaluating overall adequacy of risk prediction procedures with censored survival data. Stat Med 2011;30:1105–1117.
[31] Jeng WJ, Lok AS. Should treatment indications for chronic hepatitis B be expanded? Clin Gastroenterol Hepatol 2021;19:2006–2014.
[32] Hsu YC, Chen CY, Chang IW, et al. Once-daily tenofovir disoproxil fumarate in treatment-naive Taiwanese patients with chronic hepatitis B and minimally raised alanine aminotransferase (TORCH-B): a multicentre, double-blind, placebo-controlled, parallel-group, randomised trial. Lancet Infect Dis 2021;21:823–833.