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Original Article

Mac-2 binding protein glycosylation isomer at 5 years of antiviral therapy predict hepatocellular carcinoma and mortality beyond year 5 in chronic hepatitis B patients with cirrhosis

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Abstract: Whether serum Mac-2 binding protein glycosylation isomer (M2BPGi) level at year 5 of treatment could predict hepatocellular carcinoma (HCC) development and mortality beyond year 5 of entecavir or tenofovir disoproxil fumarate (TDF) treatment in chronic hepatitis B (CHB) patients with cirrhosis remain unclear. This retrospective study investigated the role of M2BPGi level at year 5 of treatment in predicting HCC and mortality beyond year 5 in CHB patients with cirrhosis. This study analyzed 1385 cirrhotic patients receiving entecavir or TDF treatment. Of them, 899 patients who did not develop HCC within the first 5 years of treatment were enrolled. In the entire cohort, there was no significant difference in the annual incidence of HCC before and after year 5 of entecavir or TDF treatment (P = 0.455). Multivariable Cox analysis identified old age, higher AFP and M2BPGi levels at 5 years of treatment as independent predictors of HCC occurrence beyond year 5. We developed the HCC risk prediction model, AMA, based on age, M2BPGi and AFP levels at 5 years of treatment, with the total score ranging from 0 to 8. The AMA model accurately categorized patients into low (≤2), medium (2-5), and high (≥5) risk groups in the development and validation groups (P<0.001) and exhibited good discriminant function in predicting HCC beyond year 5 in cirrhotic patients (AUROC: 0.743 at 5 years). The M2BPGi of 1.0 COI at 5 years of treatment stratified the risk of all-cause and liver-related mortality beyond year 5 (P<0.001). In conclusions, M2BPGi level at 5 years of treatment is a useful marker for predicting HCC development and mortality beyond year 5 of entecavir or TDF therapy in CHB patients with cirrhosis.

Keywords: AFP, chronic hepatitis B, cirrhosis, entecavir, hepatitis B virus, hepatocellular carcinoma, mortality, M2BPGi, risk model, tenofovir

Introduction

Entecavir and tenofovir disoproxil fumarate (TDF) have been recommended for first-line therapy of chronic hepatitis B (CHB) [1-3]. Long-term treatment with entecavir and TDF leads to improvement in liver histology, as well as reduced incidence of hepatocellular carcinoma (HCC) [4-7]. Although nucleos(t)ide analogue (NA) treatment could reduce the incidence of HCC, it does not eliminate its development, especially in cirrhotic patients [8].

The incidence of late HCC and its predictors in patients with cirrhosis receiving long-term NA therapy remain unclear. A previous study showed that CHB patients under long-term therapy with entecavir or TDF exhibited a reduced risk of HCC beyond year 5 of therapy [9]. However, we previously showed that the HCC incidence did not significantly decrease after 5 years of ETV therapy compared with the first 5 years of therapy [10]. Given that these studies included mixed populations of patients with or without cirrhosis, it remains unclear whether

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the HCC incidence decreases significantly after 5 years of NA therapy in patients with cirrhosis. Furthermore, it was shown that old age, low platelet count at baseline and year 5, and liver stiffness ≥12 kPa at year 5 were independently associated with HCC development beyond year 5 of entecavir or TDF therapy [9]. We previously demonstrated that the fibrosis-4 (FIB-4) index and alpha-fetoprotein (AFP) levels at year 5 were predictive of HCC development beyond year 5 of entecavir therapy [10].

The *Wisteria floribunda* agglutinin (WFA)-positive Mac-2 binding protein glycosylation isomer (M2BPGi) can induce the expression of Mac-2 protein in Kupffer cells [11]. Our recent study showed that M2BPGi level at 12 months of treatment was a useful marker for predicting HCC development in cirrhotic patients receiving long-term NA treatment [12]. It remains unclear whether M2BPGi level at 5 years of treatment could predict HCC beyond year 5 of NA therapy. Therefore, we aimed to investigate whether long-term NA therapy could reduce the risk of HCC beyond year 5 and to determine the risk factors, with focus on M2BPGi, associated with HCC development and mortality beyond year 5 in CHB patients with cirrhosis.

Patients and methods

Patients

This study retrospectively included a cohort of 1004 CHB patients with cirrhosis who initiated entecavir treatment between 2008 and 2018, and 381 CHB patients with cirrhosis who initiated TDF treatment between 2011 and 2018. The patients were enrolled from Kaohsiung Chang Gung Memorial Hospital (n = 982) and China Medical University Hospital (n = 403). In Taiwan, the costs of entecavir and TDF have been reimbursed for hepatitis B virus (HBV) treatment by Taiwan's National Health Plan since 2008 and 2011, respectively. The inclusion criteria were: (1) age >18 years and positive hepatitis B surface antigen (HBsAg) for more than 6 months before NA therapy; (2) entecavir or TDF monotherapy for at least 12 months before enrollment; (3) all patients fulfilled the diagnosis of cirrhosis either by liver histology (n = 220) or by repeated ultrasounds and clinical features, such as gastroesophageal varices, splenomegaly, thrombocytopenia or ascites. The exclusion criteria were: (1) evidence of alcoholic liver disease, autoimmune hepatitis, or coinfection with hepatitis C virus (HCV), hepatitis D virus or human immunodeficiency virus; (2) HCC or liver transplantation at baseline or within the first year of NA therapy.

Decompensated cirrhosis was defined based on a history or current evidence of ascites, variceal hemorrhage, or hepatic encephalopathy. All enrolled patients were randomly assigned to the models of development or validation group in a 2:1 ratio to construct the prediction model of HCC. The clinical parameters at baseline, 12 and 60 months of treatment were used to construct the HCC prediction model in the development cohort, and the validation cohort were used to examine its predictive performance.

The study was conducted in accordance with the Declaration of Helsinki of 1975, as revised in 2008. Informed consent was obtained from all patients before being included in the study, and the study was approved by the Research Ethics Committees of Chang Gung Memorial Hospital (approval number: 202101710B0) and China Medical University Hospital (approval number: CMUH102-REC1-113).

Methods

Follow-up: During entecavir or TDF monotherapy, all patients were followed up every 1-3 months or more frequently if clinically indicated. HBV DNA levels were measured at baseline and every 6 months during treatment, and HBV DNA was also assessed in the event of biochemical breakthrough [8]. All patients were followed until the discontinuation of entecavir or TDF therapy or the last visit.

HCC surveillance was performed by abdominal ultrasonography and serum AFP measurement every 3 to 6 months. The diagnosis of HCC was based on the guidelines of the American Association for the Study of Liver Diseases [13].

Definitions: Diabetes mellitus (DM) was diagnosed according to the previous guideline [14]. Patients were also considered diabetic according to their medical history or if they had received insulin treatment or oral hypoglycemic agents. Hypertension was diagnosed according to the medical history or having received anti-hypertensive drugs. Cirrhotic events were

defined as new developments of hepatic encephalopathy, variceal bleeding, or ascites in patients without hepatic decompensation at the initiation of NA treatment.

Serology: Hepatitis B e antigen (HBeAg) and anti-HBe antibodies were detected using commercial assay kits (Abbott, North Chicago, IL, USA). Serum HBV DNA was quantified by the COBAS AmpliPrep/COBAS TaqMan HBV test with a detection limit of 20 IU/mL.

Measurement of WFA-positive M2BPGi: Serum WFA-positive M2BP (M2BPGi) level was measured based on a lectin-antibody sandwich immunoassay using the fully automatic immunoanalyzer, HISCL-2000i (Sysmex, Hyogo, Japan) [15]. The values of M2BPGi were expressed as cut-off index (COI).

Statistical analysis

Continuous variables are presented as the median ± interquartile range and were compared between groups using the Student's *t* test. Categorical variables were analyzed with the chi-squared test as appropriate. Kaplan-Meier analysis and the log-rank test were used to compare the cumulative incidences of HCC between subgroups. Factors associated with HCC were identified by Cox proportional hazards regression analyses. Variables with a *P* value of <0.2 in the univariate analysis were subjected to stepwise multivariable analyses.

Cox proportional hazards regression models with the forward method were used to determine independent factors, and variables with *P*<0.05 were kept in the models. Multiple imputation was used to deal with missing data [16]. The HCC risk score was constructed according to Cox proportional hazards regression model as described previously [16, 17]. The HCC risk was estimated with the equation: $1 - P_0^{\exp(\Sigma\beta age \times score - \Sigma\beta_i \times M_i)}$. The model discrimination was assessed with area under AUROC curves. AUROCs were calculated by time-dependent ROC curves for assessing the performance of the risk models and C-statistic was used to assess the overall performance of the risk model. AUROCs were used to evaluate the performance of the AMA, ASPAM-B and PAGE-B scores [12, 18]. The time-dependent receiver-operating characteristic (ROC) curve was analyzed to determine the best cutoff values for AFP and M2BPGi at initial or 5 years of treatment [19]. All statistical tests were two-sided, and a *P*-value of <0.05 was considered statistically significant.

Results

Clinical characteristics of the study population

Of the 1385 patients, 899 (64.9%) patients without HCC development within the first 5 years of therapy had continued beyond year 5 of treatment. In this study, early HCC development was defined as within the first 5 years of entecavir or TDF treatment and late HCC development was defined as beyond the first 5 years of entecavir or TDF treatment. One hundred and ninety (13.7%) of the 1385 patients developed HCC within the first 5 years of entecavir or TDF therapy, and 115 (12.8%) of the 899 patients without HCC development within the first 5 years of entecavir or TDF therapy developed HCC afterward. Patients with HCC development within the first 5 years had a higher proportion of decompensation, lower albumin and AFP levels and higher M2BPGi levels at initial treatment than those with HCC development beyond year 5 (**Table 1**).

In the entire cohort, the cumulative incidences of HCC at 5 and 12 years of entecavir or TDF treatment were 17.4% and 29.6%, respectively. In the entire cohort, the annual incidence of HCC was 3.48% within the first 5 years (annual incidence of early HCC development) and 2.03% within 5-12 years (annual incidence of late HCC development) of therapy. There was no significant difference in the annual incidence of HCC before and after year 5 of entecavir or TDF therapy (*P* = 0.455) (**Figure 1**).

Incidence and associated factors of HCC beyond year 5 of entecavir or TDF treatment

Table 2 presents the baseline characteristics of 899 patients with or without HCC development beyond year 5 of entecavir or TDF treatment. The median duration from year 5 to last follow-up was 249 weeks (range 6-627 weeks). Patients who developed HCC were older, and had higher proportions of entecavir treatment, hypertension, and lower albumin levels than those who did not develop HCC. The median duration from year 5 to last follow-up in entecavir or TDF group was 289 weeks (range 9-627

Table 1. Baseline characteristics of patients with HCC before or after year 5 of entecavir or TDF therapy

Variables	HCC before year 5 *n* = 190	HCC after year 5 *n* = 115	*P* value
Age (year)	59.0 ± 10.4	56.7 ± 10.5	0.065
Sex, male	149 (78.4%)	85 (73.9%)	0.367
Entecavir vs. TDF	152 vs. 38	100 vs. 15	0.120
HBeAg-positive status	44 (23.1%)	31 (26.9%)	0.455
Decompensation status	51 (26.8%)	18 (15.6%)	0.024
NA-naïve	158 (83.2%)	99 (86.1%)	0.496
Diabetes mellitus, yes	54 (28.4%)	26 (22.6%)	0.263
Hypertension, yes	70 (36.8%)	45 (39.1%)	0.689
HBV DNA, $\log_{10}$ IU/mL	5.51 ± 1.46	5.44 ± 1.59	0.694
AST, U/L	104.5 ± 182.0	115.1 ± 158.0	0.603
ALT, U/L	118.9 ± 241.4	136.7 ± 194.0	0.502
Total bilirubin, mg/dL	1.80 ± 2.68	1.94 ± 3.80	0.710
Albumin, g/dL	3.75 ± 0.66	3.97 ± 0.53	0.002
INR	1.20 ± 0.23	1.16 ± 0.24	0.200
Platelet, × 10^3/μL	119.0 ± 55.2	131.7 ± 55.3	0.051
M2BPGi, COI*	4.00 ± 4.06	2.69 ± 3.08	0.010
AFP, ng/mL	21.2 ± 40.8	42.6 ± 117.2	0.023

Abbreviations: AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; COI, cut-off index; HBeAg, hepatitis B e antigen; HCC, hepatocellular carcinoma; INR, international normalized ratio; M2BPGi, Mac-2 binding protein glycosylation isomer; NA, nucleos(t)ide analogue; TDF, tenofovir disoproxil fumarate. *Only 1085 patients had serum available for M2BPGi measurement at treatment initiation.

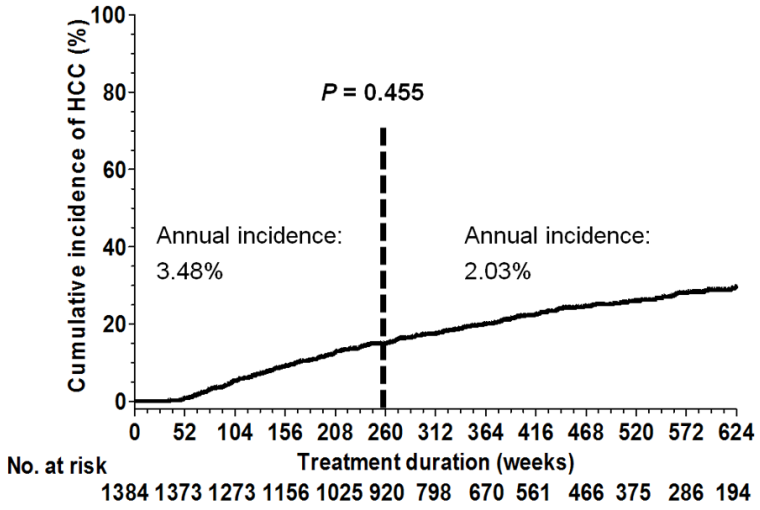

Figure 1. Cumulative incidences of hepatocellular carcinoma (HCC) within and beyond 5 years of entecavir or tenofovir disoproxil fumarate (TDF) therapy in chronic hepatitis B patients with cirrhosis.

weeks) and 187 weeks (range 6-383 weeks), respectively. The relatively smaller case number and shorter duration of follow-up might be the reasons why the case number of HCC was lower in TDF group compared to entecavir group (**Table 2**).

Of the 899 patients, 115 developed HCC beyond year 5 of entecavir or TDF treatment. The cumulative incidences of HCC at 1, 5 and 7 years beyond year 5 of treatment were 3.1%, 12.9% and 17.1%, respectively.

HCC risk predictors and prediction model of the development group

The clinical features of the development and validation cohorts are presented in Supplementary Table 1 and were similar across cohorts. The rates of HCC development were 2.9% versus 3.2% at 1 year, 13.3% versus 12.8% at 5 years and 15.7% versus 17.7% at 7 years beyond year 5 of treatment in the development (*n* = 620) and validation groups (*n* = 279), respectively (*P* = 0.860) (**Figure 2**).

We used factors at baseline, one and 5 years of treatment to predict the development of HCC beyond year 5 in the development group. The results of the multivariable Cox analysis showed that old age, higher M2BPGi and AFP levels at 5 years of treatment were independent predictors of HCC beyond year 5 of treatment (**Table 3**). The entecavir group was not a significant factor of HCC beyond year 5 of entecavir or TDF therapy (**Table 3**). The HCC risk prediction model was constructed on the basis of age, M2BPGi and AFP levels at 12 months of treatment, to develop the risk score, named as AMA (**Table 4**). We converted the regression coefficients of the independent risk factors to compute integer risk scores (**Table 4**). HCC predictive risk scores after 5 years of entecavir or

Table 2. Baseline characteristics of patients with or without HCC development beyond year 5 of entecavir or TDF therapy

Variables	HCC *n* = 115	No HCC *n* = 784	*P* value
Age (year)	56.6 ± 10.3	52.8 ± 11.6	0.001
Sex, male	85 (73.9%)	572 (72.9%)	0.910
Entecavir vs. TDF	100 vs. 15	556 vs. 228	<0.001
HBeAg-positive status	31 (26.9%)	180 (22.9%)	0.347
Decompensation status	19 (16.5%)	92 (11.7%)	0.170
NA-naïve	99 (86.1%)	653 (83.3%)	0.502
Diabetes mellitus, yes	26 (22.6%)	155 (19.8%)	0.458
Hypertension, yes	45 (39.1%)	214 (27.3%)	0.011
HBV DNA, log_{10} IU/mL	5.43 ± 1.58	5.47 ± 1.50	0.762
AST, U/L	112.4 ± 154.5	119.8 ± 249.5	0.757
ALT, U/L	129.3 ± 175.7	153.7 ± 337.9	0.447
Total bilirubin, mg/dL	1.96 ± 3.82	1.71 ± 3.05	0.429
Albumin, g/dL	3.95 ± 0.54	4.08 ± 0.57	0.018
INR	1.16 ± 0.24	1.18 ± 0.28	0.444
Platelet, × 10^3/μL	132.2 ± 55.5	141.2 ± 55.4	0.104
M2BPGi, COI*	2.79 ± 3.14	2.42 ± 3.22	0.311
AFP, ng/mL	43.0 ± 117.7	28.6 ± 135.0	0.280

Abbreviations: AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; COI, cut-off index; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; INR, international normalized ratio; M2BPGi, Mac-2 binding protein glycosylation isomer; NA, nucleos(t)ide analogue; TDF, tenofovir disoproxil fumarate. *Only 717 patients had serum available for M2BPGi measurement at treatment initiation.

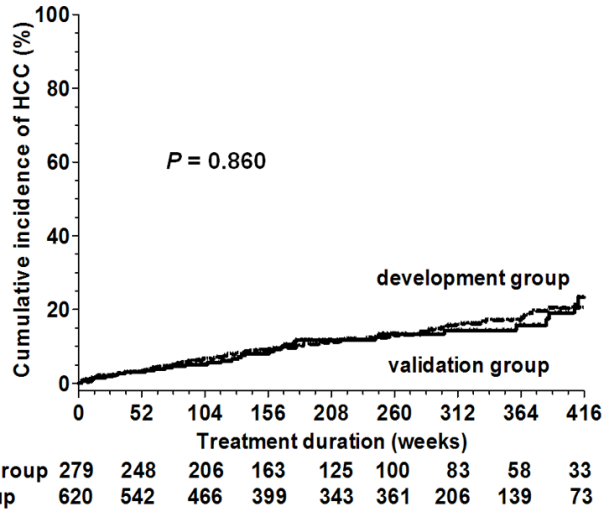

Figure 2. Comparison of the cumulative incidences of HCC between the development and validation groups (Treatment duration 0 represents 5 years of treatment).

TDF therapy are listed in **Table 4**. In this model, the total risk score ranged from 0 to 8. The C-statistic of the model was 0.710 (0.660-0.762).

We categorized the AMA score into three subgroups according to the total score: ≤2, 2-5 and ≥5, respectively. The cumulative HCC rates at 7 years beyond year 5 in the three subgroups were 9.1%, 20.4% and 39.6%, respectively (*P*<0.001, **Figure 3A**).

We compared the AUROCs among AMA, ASPAM-B and PAGE-B using our data at 5 years of treatment [12, 16]. The AUROCs for predicting 5-year risk of HCC beyond year 5 were 0.743, 0.665, and 0.610, for AMA, ASPAM-B and PAGE-B, respectively. The AUROCs at 5 years of treatment beyond year 5 between AMA and ASPAM-B (*P* = 0.047) and between AMA and PAGE-B (*P* = 0.01) were significantly different.

Validation of the HCC risk prediction model

According to the AMA score, the validation cohort was also categorized into low (≤2), medium (2-5), and high (≥5) risk. The cumulative HCC rates at 7 years beyond year 5 in the three subgroups were 3.4%, 18% and 39.2%, respectively (*P*<0.001, **Figure 3B**). The C-statistic of the risk model was 0.757 (0.829-0.684).

Predictive role of M2BPGi change during treatment and AFP cut-off at initial treatment and 5 years of treatment

Of 899 patients, 717 had M2GPBi data both at initial treatment and 5 years of treatment. The clinical characteristics of M2BPGi change are presented in **Table 5**. Patients with increased M2BPGi levels during treatment had lower rates of enetecavir used, HBeAg-positive status, decompensation, and had lower levels of

Table 3. Risk factors associated with hepatocellular carcinoma beyond year 5 of entecavir or TDF therapy in the development cohort (*n* = 620)

Variables	Univariate analysis		Multivariable analysis	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Baseline				
Age (year)	1.032 (1.013-1.052)	0.001		
Sex, male vs. female	1.156 (0.698-1.914)	0.574		
HBeAg, yes vs. no	1.146 (0.703-1.868)	0.586		
Decompensation, yes vs. no	1.557 (0.875-2.769)	0.132		
NA-naïve, yes vs. no	1.521 (0.785-2.948)	0.214		
TDF vs. entecavir	0.541 (0.284-1.029)	0.061		
Diabetes mellitus, yes vs. no	1.512 (0.886-2.580)	0.130		
Hypertension, yes vs. no	1.597 (1.015-2.514)	0.043		
HBV DNA, per $\log_{10}$ IU/mL	1.036 (0.894-1.200)	0.636		
ALT, per U/L	1.000 (0.999-1.000)	0.422		
Total bilirubin, per mg/dL	1.026 (0.971-1.084)	0.367		
Albumin, per g/L	0.635 (0.451-0.894)	0.009		
INR, per ratio	1.019 (0.456-2.283)	0.964		
Platelet, per 10^3/μL	0.997 (0.993-1.002)	0.219		
M2BPGi, per COI*	1.021 (0.956-1.090)	0.537		
AFP, per ng/mL	1.001 (1.000-1.002)	0.064		
12 months of treatment				
ALT, per U/L	1.007 (1.002-1.012)	0.006		
Total bilirubin, per mg/dL	1.011 (0.636-1.607)	0.963		
Platelet, per 10^3/μL	0.995 (0.991-1.000)	0.036		
M2BPGi, per COI**	1.059 (0.961-1.168)	0.247		
AFP, per ng/mL	1.009 (1.000-1.018)	0.064		
Five years of treatment				
Age (year)	1.032 (1.013-1.052)	0.001	1.033 (1.014-1.053)	0.001
ALT, per U/L	1.002 (0.990-1.013)	0.778		
Total bilirubin, per mg/dL	1.131 (0.938-1.364)	0.198		
Platelet, per 10^3/μL	0.994 (0.990-0.998)	0.009		
M2BPGi, per COI	1.140 (1.034-1.271)	0.007	1.117 (1.006-1.245)	0.038
AFP, per ng/mL	1.032 (1.013-1.052)	0.001	1.141 (1.083-1.203)	<0.001

Abbreviations: AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; COI, cut-off index; HBeAg, hepatitis B e antigen; INR, international normalized ratio; M2BPGi, Mac-2 binding protein glycosylation isomer; NA, nucleos(t)ide analogue; TDF, tenofovir disoproxil fumarate. *Only 717 patients had serum available for M2BPGi measurement at treatment initiation. **Only 781 patients had serum available for M2BPGi measurement at one year of treatment. Multivariable analysis did not include M2BPGi level at treatment initiation or one year of treatment due to missing data.

HBV DNA, AST, ALT, total-bilirubin, INR, M2GPGi and AFP levels, and had higher levels of albumin and platelet levels. The increased or persistent and reduced M2BPGi levels was not a significant factor predict HCC beyond year 5 of treatment.

In the entire cohort, the cut-off value of AFP at initial treatment and at 5 years of treatment was 5.08 and 3.5 ng/mL for the prediction of HCC at 5 years beyond year 5 of treatment, respectively (AUROC: 0.58 versus 0.66, $P<0.05$).

Incidence and predictors of all-cause and liver-related mortality beyond year 5 of entecavir or TDF treatment

Of 899 patients, 57 and 37 experienced all-cause and liver-related mortality beyond year 5 of entecavir or TDF treatment during the

Table 4. Multivariable Cox regression analysis of on-treatment factors associated with hepatocellular carcinoma development in the development cohort

Variables	Hazard ratio (95% CI)	β coefficient	*P* value	Risk scores
Age at 60 months, years				
<50				0
50-60				1
60-70	1.532 (1.085-2.163)	0.426	0.015	2
>70				3
M2BPGi at 60 months, COI				
<0.37	1.000	0.636	0.035	0
0.37-1.5	1.889 (1.047-3.411)	1.225	<0.001	1.5
>1.5	3.400 (1.779-6.511)			3
AFP at 60 months, ng/mL				
≤3.9	1.000			0
>3.9	2.450 (1.521-3.947)	0.896	<0.001	2

The proportionality assumption of Cox models was examined, and the assumption was not violated. Abbreviations: AFP, alpha-fetoprotein; CI, confidence interval; COI, cut-off index; M2BPGi, Mac-2 binding protein glycosylation isomer.

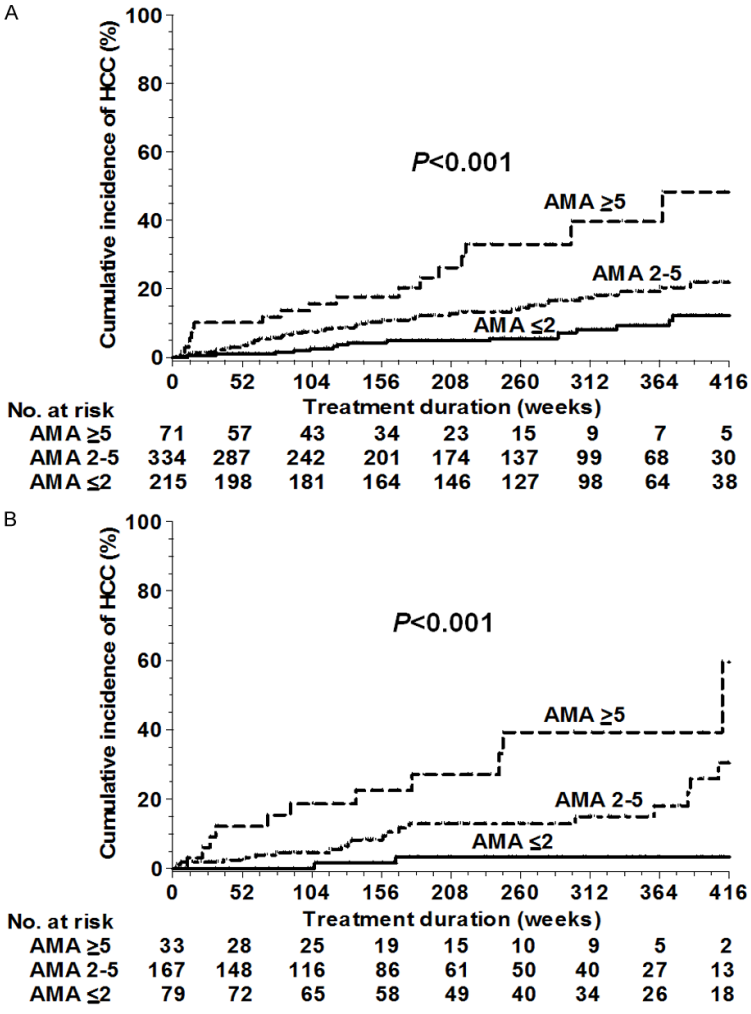

Figure 3. Cumulative incidences of HCC according to the AMA score in the (A) development and (B) validation groups (Treatment duration 0 represents 5 years of treatment).

median follow-up period of 73 months. The cumulative incidences of all-cause mortality at 1, 5 and 7 years beyond year 5 of treatment were 0.7%, 5.8% and 8.6%, respectively, and those of liver-related mortality were 0.6%, 4.0% and 5.6%, respectively.

We used factors at baseline, one and 5 years of treatment to predict the all-cause and liver-related mortality beyond year 5. The results of the multivariable analysis showed that old age, DM and higher M2BPGi levels at 5 years of treatment were independent predictors of all-cause mortality (Supplementary Table 2) and higher total bilirubin and M2BPGi levels at 5 years of treatment were independent predictors of liver-related mortality beyond year 5 (**Table 6**).

Because M2BPGi levels at 5 years of treatment were an independent factor predicting all-cause and liver-related mortality beyond year 5, the cutoff value for M2BPGi was determined using the time-dependent ROC curve. The cutoff

Table 5. Baseline characteristics of patients with M2BPGi levels change from 5 years of treatment to initial treatment

Variables	Increased M2BPGi *n* = 228	Persistent or reduced M2BPGi *n* = 489	*P* value
Age (year)	53.0 ± 11.5	53.3 ± 11.8	0.730
Sex, male	165 (72.3%)	359 (73.4%)	0.769
Entecavir vs. TDF	136 vs. 92	359 vs. 130	<0.001
HBeAg-positive status	38 (16.7%)	136 (27.8%)	0.001
Decompensation status	13 (5.7%)	76 (15.5%)	<0.001
NA-naïve	183 (80.2%)	414 (84.6%)	0.142
Diabetes mellitus, yes	39 (17.1%)	108 (22.1%)	0.124
Hypertension, yes	61 (26.7%)	146 (29.8%)	0.393
HBV DNA, $\log_{10}$ IU/mL	5.05 ± 1.36	5.65 ± 1.53	<0.001
AST, U/L	65.5 ± 129.2	147.0 ± 270.2	<0.001
ALT, U/L	82.9 ± 152.7	186.8 ± 364.1	<0.004
Total bilirubin, mg/dL	1.08 ± 0.82	2.07 ± 3.86	<0.001
Albumin, g/dL	4.21 ± 0.52	4.04 ± 0.58	<0.001
INR	1.13 ± 0.18	1.21 ± 0.30	<0.001
Platelet, × 10^3/μL	152.7 ± 57.2	136.2 ± 54.7	<0.001
M2BPGi, COI	0.97 ± 1.32	3.16 ± 3.57	<0.001
AFP, ng/mL	8.10 ± 18.0	41.1 ± 154.6	0.001

Abbreviations: AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; COI, cut-off index; HBeAg, hepatitis B e antigen; HCC, hepatocellular carcinoma; INR, international normalized ratio; M2BPGi, Mac-2 binding protein glycosylation isomer; NA, nucleos(t)ide analogue; TDF, tenofovir disoproxil fumarate.

value for M2BPGi at 5 years of treatment was 1.007 COI (AUROC: 0.82 and 0.83 for predicting all-cause and liver-related mortality, respectively). The cumulative incidence rates of all-cause and liver-related mortality at 7 years beyond year 5 in patients with M2BPGi>1.0 COI and those with M2BPGi≤1.0 COI were 21.1% and 3.8%, respectively (*P*<0.001), and 14.5% and 2.2%, respectively (*P*<0.001) (**Figure 4**).

Discussion

In this cohort, of 899 patients without HCC within the first 5 years of therapy who had continued treatment, the cumulative incidences of HCC and liver-related mortality at 7 years of treatment beyond year 5 were 11.7% and 5.6%, respectively.

A previous study demonstrated that the HCC risk decreased beyond year 5 of entecavir or TDF therapy in Caucasian patients with CHB (annual HCC incidence rate: 1.22% versus 0.73% before and after year 5, *P* = 0.05), particularly in those with compensated cirrhosis (3.22% versus 1.57%, *P* = 0.039) [9]. Similarly, a study from Korea showed that the annual incidence rate of HCC significantly decreased after 4 years of therapy with entecavir or TDF compared to the first 4 years of therapy (0.3% versus 1.9%, *P*<0.001) [20]. However, our previous study and another study from Korea showed that the incidence rates of HCC in patients with CHB did not differ significantly before and after year 5 of entecavir therapy, including non-cirrhotic and cirrhotic patients [10, 21]. In the current study enrolling only cirrhotic patients, there was no significant difference in the incidence of HCC before and after year 5 of entecavir or TDF therapy (*P* = 0.455). The risk of HCC remains substantial in patients with cirrhosis despite prolonged NA therapy. As such, we suggest that the same HCC surveillance program should be implemented beyond year 5 of NA therapy.

To date, some risk factors have been reported to predict HCC development beyond year 5 in patients receiving long-term NA therapy. A previous study showed that old age, low platelet count at baseline or year 5, and liver stiffness ≥12 kPa at year 5 were independently associated with HCC development beyond year 5 of entecavir or TDF therapy [9]. We previously showed that old age, FIB-4 and AFP levels at year 1 or 5, and genotype C were independent risk factors for HCC occurrence beyond year 5 [10]. Considering that these studies included mixed populations of patients with or without cirrhosis, the risk factors of HCC development beyond year 5 in cirrhotic patients receiving long-term NA therapy remain to be investigated.

In recent years, it has been demonstrated that serum M2BPGi levels correlate with the stage of liver fibrosis in patients with CHB and that serum M2BPGi level is a useful marker for predicting HCC in CHB patients receiving NA therapy [22-25]. Our recent study showed that M2BPGi level at 12 months of treatment was

Table 6. Univariate and multivariable analyses of factors associated with liver-related mortality

Variables	Univariate analysis		Multivariable analysis	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Baseline				
Age (year)	1.015 (0.986-1.045)	0.319		
Sex, male vs. female	0.644 (0.328-1.265)	0.201		
HBeAg, yes vs. no	0.868 (0.397-1.899)	0.723		
Decompensation, yes vs. no	2.354 (1.110-4.992)	0.026		
NA-naïve, yes vs. no	0.879 (0.386-2.003)	0.759		
TDF vs. entecavir	0.780 (0.320-1.899)	0.584		
Diabetes mellitus, yes vs. no	1.361 (0.642-2.885)	0.421		
Hypertension, yes vs. no	1.281 (0.652-2.517)	0.472		
HBV DNA, per $\log_{10}$ IU/mL	0.896 (0.724-1.108)	0.309		
AST, per U/L	1.000 (0.998-1.001)	0.657		
ALT, per U/L	0.998 (0.995-1.001)	0.184		
Total bilirubin, per mg/dL	0.942 (0.795-1.116)	0.491		
Albumin, per g/L	0.520 (0.316-0.856)	0.010		
INR, per ratio	2.147 (1.078-4.693)	0.055		
Platelet, per 10^3/μL	0.994 (0.987-1.000)	0.068		
M2BPGi, per COI*	1.110 (1.033-1.191)	0.004		
AFP at baseline, per ng/mL	0.994 (0.983-1.006)	0.337		
12 months of treatment				
ALT, per U/L	1.012 (1.007-1.017)	<0.001		
Total bilirubin, per mg/dL	2.010 (1.292-3.128)	0.002		
Platelet, per 10^3/μL	0.994 (0.988-1.001)	0.090		
M2BPGi, per COI**	1.260 (1.156-1.374)	<0.001		
AFP, per ng/mL	1.012 (0.999-1.025)	0.071		
Five years of treatment				
ALT, per U/L	1.014 (1.008-1.020)	<0.001		
Total bilirubin, per mg/dL	1.372 (1.231-1.529)	<0.001	1.193 (1.048-1.358)	0.008
Platelet, per 10^3/μL	0.996 (0.991-1.002)	0.214		
M2BPGi, per COI	1.377 (1.259-1.505)	<0.001	1.305 (1.176-1.449)	<0.001
AFP, per ng/mL	1.038 (1.005-1.072)	0.023		

Abbreviations: AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; COI, cut-off index; HBeAg, hepatitis B e antigen; INR, international normalized ratio; M2BPGi, Mac-2 binding protein glycosylation isomer; NA, nucleos(t)ide analogue; TDF, tenofovir disoproxil fumarate. *Only 717 patients had serum available for M2BPGi measurement at treatment initiation. **Only 781 patients had serum available for M2BPGi measurement at one year of treatment. Multivariable analysis did not include M2BPGi level at treatment initiation or one year of treatment due to missing data.

an independent predictor for HCC development. We developed the HCC risk prediction model, the ASPAM-B score, based on age, sex, platelet count, AFP and M2BPGi levels at 12 months of treatment for predicting HCC occurrence [12]. The ASPAM-B model exhibited good predictive performance for HCC occurrence in cirrhotic patients who received long-term entecavir or TDF treatment [12, 17]. It remains unclear whether M2BPGi level at 5 years of treatment could predict HCC occurrence beyond year 5 of NA therapy in CHB patients with cirrhosis. In this study, we demonstrated that old age, higher M2BPGi and AFP levels at 5 years of treatment were independent predictors of HCC occurrence beyond year 5. The M2BPGi level measured at year 5 was a better predictor than at the baseline or one year of treatment. Furthermore, we established a new prediction model, the AMA score, according to the predictors for predicting HCC development beyond year 5. The AMA model improved the

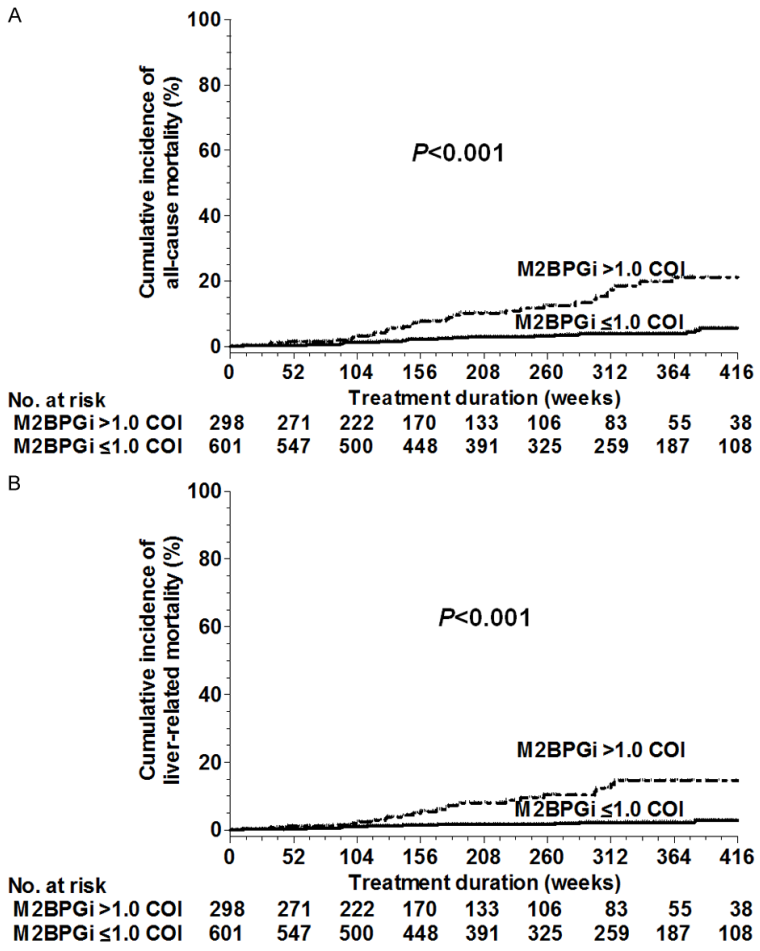

Figure 4. Cumulative incidences of (A) all-cause mortality and (B) liver-related mortality beyond year 5 of entecavir or TDF therapy by M2BPGi levels at 5 years of treatment (Treatment duration 0 represents 5 years of treatment).

discriminative performance for HCC occurrence. Furthermore, risk stratification of HCC into three distinct subgroups through the AMA risk model may facilitate implementation of precision surveillance strategy in the future [26]. The risk of HCC may be modulated by ongoing NA therapy over the course of treatment and on-treatment predictors may outperform baseline predictors for risk stratification of HCC. Herein we demonstrated that the risk model at year 5 outperformed the risk model established at year one, although we did not address the predictors at other treatment time points. Thus, we propose that year 5 of NA treatment may represent a feasible time point for future refinement of the optimal risk model to predict HCC occurrence beyond year 5 of NA treatment.

In our study, 717 patients had M2GPBi data both at initial treatment and 5 years of treatment. Patients with higher liver function test, HBV DNA, M2BGPi and APF levels were associated with persistent or reduced M2BPGi levels during NA treatment. The change of M2BPGi levels during NA therapy was not a significant factor predict HCC beyond year 5 of treatment. Based on our finding, it is the absolute level of M2BPGi at year 5, instead of the magnitude of its decline from one to 5 years, which matters most in determining the subsequent risk of HCC. In additional, AFP cut-off value for the prediction of HCC was different at initial treatment and at 5 years of treatment. AFP at 5 years of treatment was a better marker to predict HCC development beyond year 5 of treatment than AFP at initial treatment.

Our study also demonstrated that M2BPGi at 5 years of treatment was an independent factor predicting all-cause and liver-related mortality beyond year 5. The M2BPGi level measured at year 5 was a better predictor than at the baseline or one year of treatment. This is in line with our previous studies demonstrating that M2BPGi levels at 12 months of treatment predict liver-related mortality in patients with cirrhosis receiving long-term NA treatment [12] and can be accounted for by the notion that M2BPGi may reflect the severity of cirrhosis or hepatocyte dysfunction [27].

There are several limitations to this study. First, it is a retrospective study and selection bias may have been present. External validation with an independent cohort is needed. Second, cirrhosis was mostly diagnosed via ultrasonography and clinical features. Thus, patients with early cirrhosis might have been excluded. Third, metabolic risk factors other than DM and hypertension were not available for analyzing

their associations with HCC. Fourth, our study only included Asian patients, among which genotypes B and C are predominant through vertical transmission. Whether similar predictors are applicable to other ethnicities or HBV genotypes remains to be verified.

In conclusion, HCC incidence tended to decrease but did not change significantly before and after year 5 of entecavir or TDF therapy in CHB patients with cirrhosis. The M2BPGi level at 5 years of treatment was a useful marker for predicting HCC development and mortality beyond year 5 in cirrhotic patients receiving NA treatment. The AMA risk model at 5 years of treatment exhibited good discriminant function in predicting HCC occurrence beyond year 5. HCC surveillance should be continued in those who receive long-term entecavir or TDF therapy without developing HCC by year 5.

Acknowledgements

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Disclosure of conflict of interest

Cheng-Yuan Peng has served as an advisory committee member for AbbVie, Bristol-Myers Squibb, Gilead, Merck Sharp & Dohme, and Roche.

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Supplementary Table 1. Clinical characteristics at baseline and 5 years of treatment in the development and validation groups

Variables	Development group *n* = 620	Validation group *n* = 279	*P* value
Age (year)	52.8 ± 11.7	54.3 ± 11.0	0.175
Sex, male	451 (72.7%)	206 (73.8%)	0.732
HBeAg-positive status	147 (23.7%)	64 (22.9%)	0.801
Decompensation status	77 (12.4%)	34 (12.2%)	0.922
NA-naïve	517 (83.8%)	235 (84.2%)	0.752
Diabetes mellitus, yes	117 (18.9%)	64 (22.9%)	0.159
Hypertension, yes	169 (27.2%)	90 (32.2%)	0.082
HBV DNA, $\log_{10}$ IU/mL	5.51 ± 1.46	5.38 ± 1.41	0.244
AST, U/L	125.0 ± 257.5	105.2 ± 193.0	0.252
ALT, U/L	161.4 ± 353.8	126.7 ± 234.6	0.136
Total bilirubin, mg/dL	1.78 ± 3.29	1.64 ± 2.84	0.537
Albumin, g/dL	4.07 ± 0.57	4.08 ± 0.57	0.815
INR	1.18 ± 0.29	1.17 ± 0.25	0.425
Platelet, × 10^3/μL	139.0 ± 55.7	142.5 ± 55.1	0.383
M2BPGi, COI	2.44 ± 3.36	2.50 ± 2.87	0.817
AFP, ng/mL	27.8 ± 100.9	36.3 ± 185.6	0.375
Five years of treatment			
ALT, U/L	28.8 ± 23.1	28.0 ± 17.8	0.602
Total bilirubin, mg/dL	1.11 ± 1.20	1.08 ± 0.68	0.680
Platelet, × 10^3/μL	156.8 ± 65.4	158.0 ± 68.8	0.789
M2BPGi, COI	1.14 ± 1.59	1.26 ± 1.62	0.304
AFP, ng/mL	3.38 ± 2.64	3.88 ± 4.64	0.175

Abbreviations: AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; COI, cut-off index; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; INR, international normalized ratio; M2BPGi, Mac-2 binding protein glycosylation isomer; NA, nucleos(t)ide analogue.

Supplementary Table 2. Univariate and multivariable analyses of factors associated with all-cause mortality

Variables	Univariate analysis		Multivariable analysis	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Baseline				
Age (year)	1.046 (1.002-1.071)	<0.001	1.043 (1.020-1.067)	<0.001
Sex, male vs. female	0.642 (0.289-1.106)	0.110		
HBeAg, yes vs. no	0.558 (0.397-1.199)	0.144		
Decompensation, yes vs. no	2.173 (1.170-4.037)	0.014		
NA-naïve, yes vs. no	1.269 (0.601-2.681)	0.532		
TDF vs. entecavir	0.712 (0.332-1.528)	0.383		
Diabetes mellitus, yes vs. no	2.150 (1.239-3.773)	0.007	1.826 (1.049-3.180)	0.033
Hypertension, yes vs. no	1.491 (0.878-2.553)	0.138		
HBV DNA, per $\log_{10}$ IU/mL	0.908 (0.765-1.708)	0.271		
AST, per U/L	1.000 (0.999-1.001)	0.862		
ALT, per U/L	0.999 (0.995-1.001)	0.271		
Total bilirubin, per mg/dL	0.995 (0.795-1.116)	0.485		
Albumin, per g/L	0.480 (0.323-0.713)	<0.001		
INR, per ratio	2.174 (1.137-4.154)	0.019		
Platelet, per 10^3/μL	0.994 (0.988-0.999)	0.027		
M2BPGi, per COI*	1.100 (1.036-1.167)	0.002		
AFP at baseline, per ng/mL	0.999 (0.996-1.002)	0.678		
12 months of treatment				
ALT, per U/L	1.010 (1.004-1.016)	0.001		
Total bilirubin, per mg/dL	1.459 (0.923-2.306)	0.106		
Platelet, per 10^3/μL	0.995 (0.989-1.000)	0.056		
M2BPGi, per COI**	1.220 (1.129-1.319)	<0.001		
AFP, per ng/mL	1.011 (0.999-1.023)	0.067		
Five years of treatment				
ALT, per U/L	1.011 (1.003-1.018)	0.007		
Total bilirubin, per mg/dL	1.345 (1.209-1.497)	<0.001		
Platelet, per 10^3/μL	0.997 (0.993-1.002)	0.264		
M2BPGi, per COI	1.329 (1.226-1.441)	<0.001	1.379 (1.261-1.507)	<0.001
AFP, per ng/mL	1.032 (1.002-1.063)	0.038		

Abbreviations: AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; COI, cut-off index; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; INR, international normalized ratio; M2BPGi, Mac-2 binding protein glycosylation isomer; NA, nucleos(t)ide analogue; TDF, tenofovir disoproxil fumarate. *Only 717 patients had serum available for M2BPGi measurement at treatment initiation. **Only 781 patients had serum available for M2BPGi measurement at one year of treatment. Multivariable analysis did not include M2BPGi level at treatment initiation or one year of treatment due to missing data.