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A predictive model for carcinogenesis in patients with chronic hepatitis B undergoing entecavir therapy and its validation

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Abstract

We created a model to predict the development of liver carcinogenesis in patients with chronic hepatitis B (CHB) undergoing entecavir (ETV) therapy and to validate the accuracy using an independent dataset.

A total of 328 CHB subjects were analyzed. Subjects were randomly assigned into 2 groups: the training group (n = 164) and the validation group (n = 164). Using data from the training group, we built a predictive model for liver carcinogenesis by performing univariate and multivariate analyses using variables associated with liver carcinogenesis. We subsequently assessed the applicability of the constructed model in the validation group.

The median (range) follow-up periods in the training and the validation groups were 5.03 years (1.03–9.98) and 4.84 years (1.10–9.97), respectively. The proportion of hepatitis B virus-DNA at 24 weeks <1.9 log IU/mL in the training group was 70.7% (116/164), while that in the validation group was 71.3% (117/164). For the entire cohort (n = 328), the median alpha-fetoprotein (AFP) value at 24 weeks (3.45 ng/mL; range, 0.9–102.7 ng/mL) significantly decreased compared to the baseline values (5.55 ng/mL; range, 0.9–1039.5 ng/mL), while the median alanine aminotransferase (ALT) value at 24 weeks (24 IU/mL; range, 6–251 IU/mL) also significantly decreased compared to baseline values (57 IU/mL; range, 7–1450 IU/mL). During the observation period, hepatocellular carcinoma (HCC) developed in 15 (9.1%) patients in the training group and in 17 (10.4%) patients in the validation group. The 3- and 5-year cumulative HCC incidence rates in the entire cohort were 4.48% and 9.52%, respectively. In the multivariate analysis of the training group, age ≥54 years ($P = 0.0273$), ALT level at 24 weeks ($P = 0.0456$), and AFP at 24 weeks ($P = 0.0485$) were found to be significant predictors linked to HCC. Using these independent predictors, the risk for HCC development was well stratified in the validation group (overall significance, $P < 0.0001$). Similar results were observed in subgroup analyses of patients with or without cirrhosis and HBe antigen positivity.

In conclusion, our predictive model was well verified; hence, it may be a promising model for the prediction of the development of liver carcinogenesis in CHB patients undergoing ETV therapy.

Abbreviations: AFP = alpha-fetoprotein, ALT = alanine aminotransferase, CHB = chronic hepatitis B, CHC = chronic hepatitis C, ETV = entecavir, HBs = hepatitis B surface, HBV = hepatitis B virus, HCC = hepatocellular carcinoma, H-group = high-risk group, IFN = interferon, I-group = intermediate-risk group, LAM = lamivudine, L-group = low-risk group, NAs = nucleoside analogs, TDF = tenofovir disoproxil fumarate.

Keywords: carcinogenesis, chronic hepatitis B, entecavir, predictive model, validation

1. Introduction

Chronic hepatitis B (CHB) infection is one of the major etiologies of hepatocellular carcinoma (HCC), especially in Asian countries where CHB accounts for approximately half of the HCC cases worldwide.[1–3] Previous reports have shown that up to 20% to 30% of patients with CHB eventually die from cirrhosis or HCC progression.[1,3–5]

The main goal for therapy in CHB is to prevent the development of cirrhosis, liver decompensation, and carcinogenesis.[3,6–11] Several antiviral therapies including interferon (IFN) or nucleoside analogs (NAs) have been developed for the purpose of ameliorating clinical outcomes in patients with CHB.[6–9] In daily clinical practice, therapeutic response is determined by the suppression of serum hepatitis B virus (HBV)-DNA quantification, HBe antigen seroconversion to HBe antibody, loss of hepatitis B surface (HBs) antigen, normalization of alanine aminotransferase (ALT) levels, and improvement in liver histological findings.[6–9] A previous study demonstrated that decreased HBV-DNA levels reduced the risk of HCC

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development in CHB patients with cirrhosis or advanced fibrosis treated with lamivudine (LAM).[12]

Currently, entecavir (ETV) and tenofovir disoproxil fumarate (TDF) are recommended as the first-line antiviral NAs in CHB patients due to their excellent viral suppression ability, their low risk for the development of antiviral resistance, and higher rate of HCC suppression as compared with LAM.[11,13–17] In the era of these novel antiviral therapies for CHB, predicting liver carcinogenesis for CHB patients is also of clinical importance, as use of NAs may lead to more favorable clinical outcomes. In patients receiving NAs (e.g., ETV and TDF), several factors including the HBV viral load, ALT, and alpha-fetoprotein (AFP) levels have changed, resulting in the improvement of liver inflammation, which is potentially linked to HCC suppression.[18,19]

On the other hand, Yamada et al[20] reported that AFP levels 24 weeks after initiation of ETV therapy may be a predictor of HCC incidence in patients with CHB. Another previous study demonstrated that post-IFN treatment, ALT, and AFP levels were significantly associated with liver carcinogenesis in patients who had chronic hepatitis C (CHC).[21] In our previous study, we reported that a decrease in AFP levels predicted a reduced HCC incidence in patients with CHC undergoing IFN-based therapy.[22] However, to the best of our knowledge, there exists only 1 study that has examined the relationship between liver carcinogenesis and on-treatment factors such as changes of AFP or ALT values in CHB patients during ETV therapy, although several predictive models for liver carcinogenesis in CHB have been proposed.[20,23–27] Thus, clarifying the effects of on-treatment factors on the clinical outcomes of patients with CHB is a pressing issue.

A predictive model for liver carcinogenesis in patients with CHB undergoing ETV therapy would attract much attention. The aims of this study were to build a prognostic model for liver carcinogenesis in patients with CHB undergoing ETV therapy and to validate its accuracy using an independent dataset. Based on the abovementioned factors, we primarily focused on changes of AFP or ALT values during ETV therapy in this study.

2. Patients and methods

2.1. Patients

Between April 2006 and February 2015, 363 CHB patients who were initially treated with ETV and had no evidence of HCC on radiological findings were admitted to either the Division of Hepatobiliary and Pancreatic Diseases, Department of Internal Medicine, Hyogo College of Medicine, Hyogo, Japan or to the Department of Gastroenterology and Hepatology, Osaka Red Cross Hospital, Osaka, Japan, and they were enrolled in this study participation. In addition, all subjects were NA-therapy naïve, had HBs antigen positivity for at least 6 months, no clinical evidence of concurrent hepatitis C virus infection, and had no clear evidence of drug- or alcohol-related liver disease. All subjects were initially treated with ETV (0.5 mg/d). Subjects with HCC within 1 year after ETV therapy (n = 14), those with a follow-up period less than 1 year (n = 4), and those with insufficient clinical data available (e.g., lacking data for ALT at 24 weeks, AFP at 24 weeks, or HBV-DNA at 24 weeks) were excluded from this analysis (n = 17). Thus, a total of 328 patients were analyzed in this study. All analyzed subjects received ETV therapy for at least 12 months. Presence of cirrhosis was determined pathologically and/or radiologically. In patients without a liver biopsy, cirrhosis was defined by clinical characteristics of portal hypertension such as varices, ascites, or splenomegaly and by a shrunken and deformed liver with nodular surfaces as identified on liver imaging. The primary outcome measure in this study was the incidence of HCC.

Study subjects were randomly assigned into 1 of 2 groups: the training group or the validation group. In the training group, we initially examined variables known to be linked to liver carcinogenesis using univariate and multivariate analyses and subsequently built a model to predict the HCC development. Then, we evaluated the validity of the constructed model using a separate dataset. We retrospectively examined data of the patients in the training group and also tested data for the constructed predictive model in the independent validation set retrospectively.

Ethical approval for the present study protocol was obtained from the ethics committee in each hospital, and this protocol strictly adhered to all provisions of the Declaration of Helsinki.

2.2. HCC surveillance and follow-up

Follow-up after ETV therapy consisted of evaluation for HCC incidence by radiological findings using ultrasonography, computed tomography, and/or magnetic resonance imaging every 2 to 6 months and regular blood analyses, including HBV-related laboratory markers and tumor markers. In subjects with HCC incidence, the most appropriate therapy for each patient was determined based on the Japanese guidelines and through discussion with surgeons and radiologists.[28–30]

2.3. Serological studies

Detection of HBs antigen, determination of HBe antigen positivity, and HBV-DNA quantification were performed using commercial kits as reported previously.[31]

2.4. Statistical analysis

A prediction model to detect liver carcinogenesis was constructed using data from the subjects in the training group and verified in the independent validation group. First, a univariate analysis was performed in the training group to identify candidate variables among various clinical factors (e.g., age, gender, changes of ALT or AFP values during ETV therapy, serum albumin, total bilirubin, platelet count, presence of cirrhosis, pretreatment HBV-DNA or HBe antigen, and HBV-DNA levels at 24 weeks). For continuous variables other than AFP and ALT, variables were divided into 2 groups (using their respective median values at baseline as the cutoff points); they were subsequently analyzed as nominal variables. Variables with a P value less than 0.05 in the univariate analysis were entered into the multivariate analyses. Factors with a P value less than 0.05 in the multivariate analysis using the Cox proportional hazards model were finally chosen as components of the prediction model. Using these multivariate predictors, we derived a predictive model for the prediction of liver carcinogenesis in the training group. Next, we verified the prognostic accuracy of the model that had been derived from the training group by its implementation in a validation group.

For continuous parameters, we performed statistical analysis between the 2 groups by using either the Student t test, Mann–Whitney U test, or paired t test, as applicable. We compared categorical parameters by using Fisher exact test or

Table 1

Baseline characteristics in the entire cohort, the training, and validation groups.

Variables	All cases (n = 328)	Training group (n = 164)	Validation group (n = 164)	*P* value (training vs validation)
Age, y	50.9 ± 13.2	52.6 ± 13.0	49.2 ± 13.3	0.0180
Gender, male/female	206/122	100/64	106/58	0.8823
ALT, IU/L	114.3 ± 176.5	117.3 ± 184.6	111.2 ± 168.6	0.7566
Serum albumin, g/dL	4.1 ± 0.5	4.1 ± 0.4	4.1 ± 0.5	0.8823
Total bilirubin, mg/dL	1.0 ± 1.1	1.1 ± 1.4	1.0 ± 0.6	0.8318
Platelet count, $\times 10^4/mm^3$	17.1 ± 6.6	17.9 ± 6.7	16.3 ± 6.5	0.0362
Pretreatment alpha-fetoprotein, ng/mL	17.5 ± 68.8	15.1 ± 35.1	19.8 ± 90.8	0.2866
Presence of cirrhosis, yes/no	88/240	43/121	45/119	0.9009
HBV-DNA, log IU/mL	5.6 ± 1.2	5.5 ± 1.2	5.6 ± 1.2	0.8704
HBe antigen positivity, yes/no	159/169	74/90	85/79	0.2692

Data are expressed as number or mean ± standard deviation. ALT = alanine aminotransferase, HBV = hepatitis B virus.

Pearson χ^2 test, as applicable. Kaplan–Meier curves for liver carcinogenesis were created and compared by using the log-rank test. Time interval for HCC incidence was calculated from the date of ETV therapy until the date of the first confirmed HCC development. In subjects without HCC incidence, the follow-up period was defined as the time interval from the date of ETV therapy to the last follow-up date. Data are presented as number or means ± standard deviation unless otherwise stated. A *P* value less than 0.05 was considered to indicate a statistically significant difference between variables. We performed statistical analysis using the JMP 11 (SAS Institute Inc., Cary, NC).

3. Results

3.1. Baseline characteristics

The baseline characteristics of the training group (n = 164) and the validation group (n = 164) are shown in Table 1. The only statistically significant differences observed between the 2 groups were age ($P = 0.0180$) and platelet count ($P = 0.0362$) (Table 1). The median (range) follow-up periods in the training and the validation groups were 5.03 years (range, 1.03–9.98) and 4.84 years (range, 1.10–9.97), respectively.

3.2. Cumulative HCC incidence for all cases, the training group, and the validation group

During the observation period, HCC occurred in 15 (9.1%) patients in the training group and in 17 (10.4%) patients in the validation group. The median follow-up periods from the date of ETV therapy to the date of first confirmed liver carcinogenesis on radiological findings were 3.26 years in the training group and 3.72 years in the validation group. The 3-, 5-, and 7-year cumulative HCC incidence rates were, respectively, 4.48%, 9.52%, and 13.99% in all cases; 4.45%, 8.52%, and 12.81% in the training group; and 4.51%, 10.2%, and 15.27% in the validation group (Fig. 1A–C).

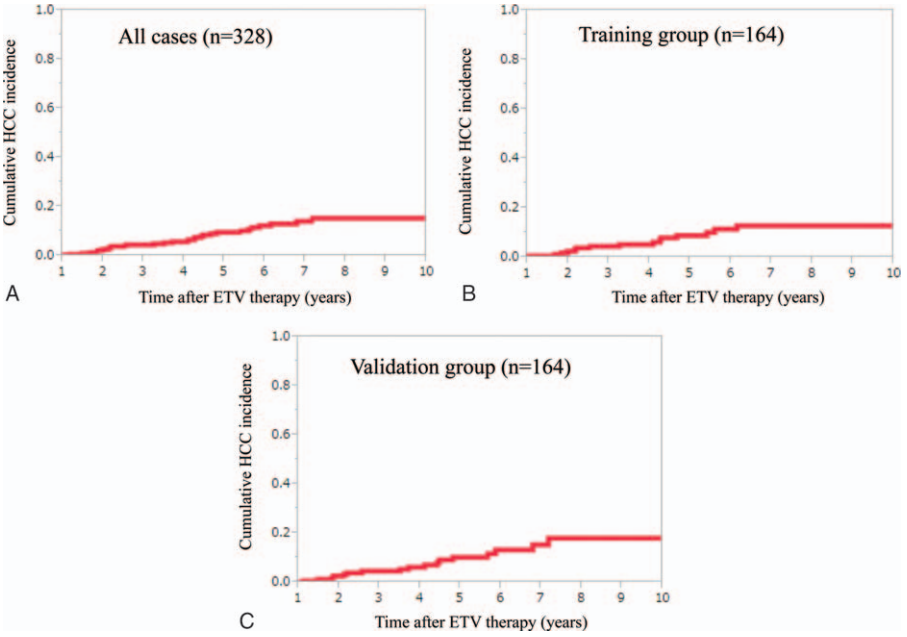

Figure 1. Cumulative hepatocellular carcinoma (HCC) incidence for all cases (A), the training group (B), and the validation group (C). The 3-, 5-, and 7-year cumulative HCC incidence rates were, respectively, 4.48%, 9.52%, and 13.99% in all cases; 4.45%, 8.52%, and 12.81% in the training group; and 4.51%, 10.2%, and 15.27% in the validation group.

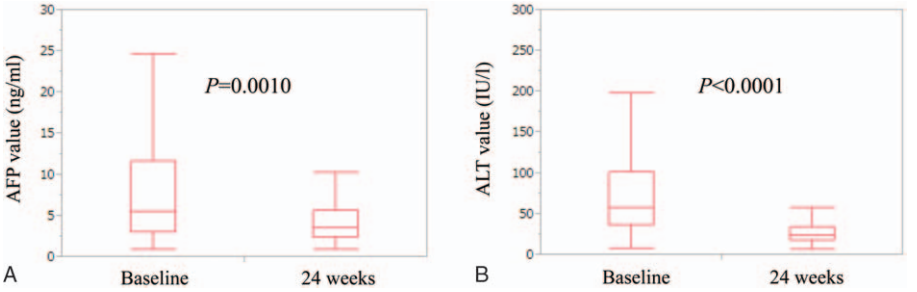

Figure 2. Box plots of alpha-fetoprotein (AFP) and alanine aminotransferase (ALT) levels at baseline and at 24 weeks after entecavir therapy for all cases (n = 328). (A) The median AFP value at 24 weeks (3.45 ng/mL; range, 0.9–102.7 ng/mL) significantly decreased as compared with that in baseline (5.55 ng/mL; range, 0.9–1039.5 ng/mL) ($P = 0.0010$). (B) The median ALT value at 24 weeks (24 IU/mL; range, 6–251 IU/mL) significantly decreased as compared with that in baseline (57 IU/mL; range, 7–1450 IU/mL, $P < 0.0001$).

3.3. Changes of AFP and ALT values during ETV therapy for all cases (n = 328)

The median AFP values at 24 weeks (3.45 ng/mL; range, 0.9–102.7 ng/mL) significantly decreased as compared to the baseline values (5.55 ng/mL; range, 0.9–1039.5 ng/mL), while the median ALT values at 24 weeks (24 IU/mL; range, 6–251 IU/mL) also significantly decreased as compared to the baseline values (57 IU/mL; range, 7–1450 IU/mL) (Fig. 2A and B). There were 6 patients with a baseline AFP value >100 ng/mL, whereas only 1 patient had an AFP value that was >100 ng/mL at 24 weeks. There were 28 patients with baseline ALT values >300 IU/mL, whereas no patient had an ALT value at 24 weeks that was >300 IU/mL.

3.4. Virological response in the training group and the validation group

The proportion of HBV-DNA at 24 weeks <1.9 log IU/mL in the training group was 70.7% (116/164), while that in the validation group was 71.3% (117/164).

3.5. Univariate and multivariate analyses of factors associated with HCC development in the training group

In this study, as described in the introduction section, we focused on changes of ALT or AFP values during ETV therapy. Regarding changes of ALT values during ETV treatment, we categorized the training group subjects into 1 of 3 groups: group A consisted of patients with an ALT value at 24 weeks above upper normal limit in each hospital (ALT-high at 24 weeks, n = 35), group B consisted of patients with a high ALT at baseline in each hospital and an ALT value at 24 weeks within normal range (ALT-normal at 24 weeks) in each hospital (n = 96), and group C consisted of patients with a normal ALT at baseline and at 24 weeks in each hospital (n = 33). Similarly, with regard to changes of AFP during ETV treatment, we categorized training group subjects into 3 groups: group D consisted of patients with AFP value at 24 weeks >10 ng/mL (AFP-high at 24 weeks, n = 16), group E consisted of those with AFP-high at baseline and AFP-normal at 24 weeks (n = 42), and group F consisted of patients with AFP-normal at baseline and at 24 weeks (n = 106) (Fig. 3). An AFP value of 10 ng/mL indicated the normal upper limit of an AFP value in each hospital.

In the univariate analysis, the following factors were identified to be significantly associated with HCC development for the training group: age ≥54 years ($P = 0.0024$), changes of ALT value during ETV therapy ($P = 0.0139$), serum albumin ≥4.1 g/dL ($P = 0.0191$), platelet count $\geq 17.4 \times 10^4/mm^3$ ($P = 0.0112$), changes of AFP value during ETV therapy ($P < 0.0001$), and presence of cirrhosis ($P < 0.0001$) (Table 2). The odds ratios and 95% confidence intervals in the multivariate analysis for the 6 factors with P value less than 0.05 in the univariate analysis are shown in Table 2. Age ≥54 years ($P = 0.0273$), group A ($P = 0.0456$), and group D ($P = 0.0485$) were significant predictors associated with HCC development.

3.6. Risk stratification for liver carcinogenesis in the training group

Using 3 significant predictors in the multivariate analysis, we divided training group subjects into 3 groups: high-risk group (H-group)—patients with 2 or more risk factors, intermediate-risk group (I-group)—patients with 1 risk factor, and low-risk group (L-group)—patients with none of these 3 risk factors. In the training group, the risk for HCC incidence was significantly stratified between each of the 2 groups except for the comparison between the I-group and L-group: H-group (n = 28) versus I-group (n = 75), $P = 0.0008$; I-group versus L-group (n = 61), $P = 0.1473$; and H-group versus L-group, $P < 0.0001$; overall significance, $P < 0.0001$ (Fig. 4A).

3.7. Prognostic accuracy of our proposed predictive model in the validation group and all cases

We tested the prognostic accuracy of our proposed predictive model in the validation group and also in the entire cohort (n = 328). In the validation group, the risk for HCC development was significantly stratified between each of the following 2

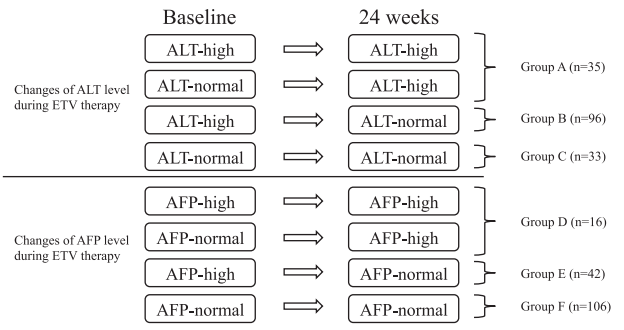

Figure 3. Classification based on alanine aminotransferase (ALT) or alpha-fetoprotein (AFP) value at baseline and ALT or AFP value at 24 weeks after entecavir therapy in the training group.

Table 2

Univariate and multivariate analyses of factors linked to the development of hepatocellular carcinoma in the training group (n = 164).

Variables	Number of each category	Univariate	Multivariate analysis		
		P	Odds ratio	95% CI	*P*
Age (y) ≥ 54, yes/no	85/79	0.0024	4.606	1.169–30.890	0.0272
Gender, female/male	100/64	0.3244			
Changes of ALT value during ETV therapy		0.0139			
Group A	35		3.958	1.027–17.621	0.0456
Group B	96		1.090	0.234–4.673	0.9083
Group C	33		Reference		
Serum albumin ≥4.1 g/dL, yes/no	85/79	0.0191	0.393	0.091–1.463	0.1671
Total bilirubin ≥0.8 mg/dL, yes/no	92/72	0.2024			
Platelet count $\geq 17.4 \times 10^4/mm^3$, yes/no	83/81	0.0112	0.621	0.116–2.894	0.5454
Changes of AFP value during ETV therapy		<0.0001			
Group D	16		3.988	1.060–17.873	0.0485
Group E	42		1.135	0.217–5.049	0.8713
Group F	106		Reference		
Presence of cirrhosis, yes/no	43/121	<0.0001	1.950	0.530–7.943	0.3186
Pretreatment HBV-DNA ≥5.8 log IU/mL, yes/no	84/80	0.9336			
HBV-DNA at 24 weeks <1.9 log IU/mL yes/no	116/48	0.3626			
Pretreatment HBe antigen positivity, yes/no	74/90	0.3049			

AFP = alpha-fetoprotein, ALT = alanine aminotransferase, CI = confidence interval, ETV = entecavir, HBV = hepatitis B virus. Group A consisted of patients with an ALT value at 24 weeks above upper normal limit in each hospital (ALT-high at 24 weeks). Group B consisted of patients with a high ALT at baseline in each hospital and an ALT value at 24 weeks within normal range (ALT-normal at 24 weeks) in each hospital. Group C consisted of patients with a normal ALT at baseline and at 24 weeks in each hospital. Group D consisted of patients with AFP value at 24 weeks >10 ng/mL (AFP-high at 24 weeks). Group E consisted of those with AFP-high at baseline and AFP-normal at 24 weeks. Group F consisted of patients with AFP-normal at baseline and at 24 weeks.

groups: H-group (n = 26) versus I-group (n = 81), $P = 0.0485$; I-group versus L-group (n = 57), $P = 0.0099$; and H-group versus L-group, $P < 0.0001$; overall significance, $P < 0.0001$ (Fig. 4B). In all cases, the risk for HCC development was also significantly stratified between each of the 2 groups: H-group (n = 54) versus I-group (n = 156), $P < 0.0001$; I-group versus L-group (n = 118), $P < 0.0001$; H-group versus L-group, $P < 0.0001$; overall significance, $P < 0.0001$ (Fig. 4C).

3.8. Subgroup analyses for cirrhotic and noncirrhotic patients

We also performed subgroup analyses in patients with cirrhosis and noncirrhosis. In patients with cirrhosis (n = 88), the risk for HCC development was significantly stratified between each of the 2 groups, except for the comparison between the H-group and I-group: H-group (n = 27) versus I-group (n = 43), $P = 0.0910$; I-group versus L-group (n = 18), $P = 0.0310$; H-group versus L-group, $P = 0.0028$; overall significance, $P = 0.0075$ (Fig. 5A). In patients without cirrhosis (n = 240), the risk for HCC development was significantly stratified between each of the 2 groups except for comparison between the I-group and L-group: H-group (n = 27) versus I-group (n = 113), $P = 0.0081$; I-group versus L-group (n = 100), $P = 0.1656$; H-group versus L-group, $P = 0.0002$; overall significance, $P = 0.0004$ (Fig. 5B).

3.9. Subgroup analyses for patients with and without HBe antigen positivity at baseline

Although HBe antigen positivity was not significant in the univariate analysis, predicting liver carcinogenesis according to baseline HBe antigen status may be clinically important. In

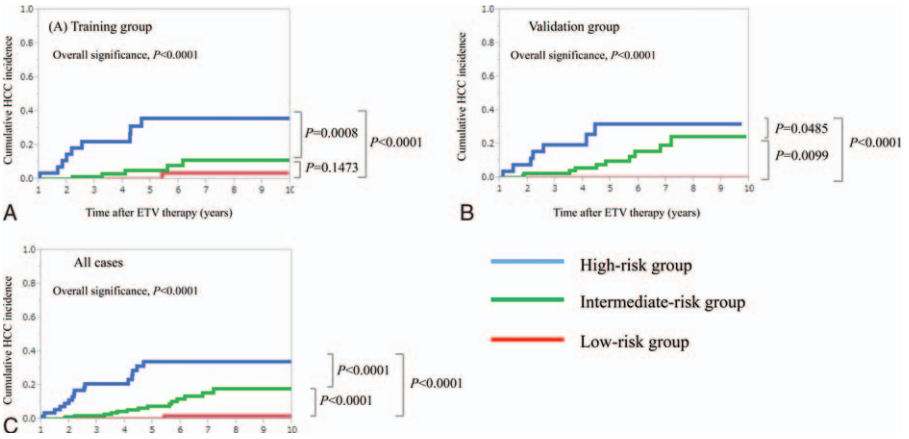

Figure 4. Kaplan–Meier curves for liver carcinogenesis in 3 risk groups (high-, intermediate-, and low-risk groups) in the training group (A), the validation group (B), and all cases (C).

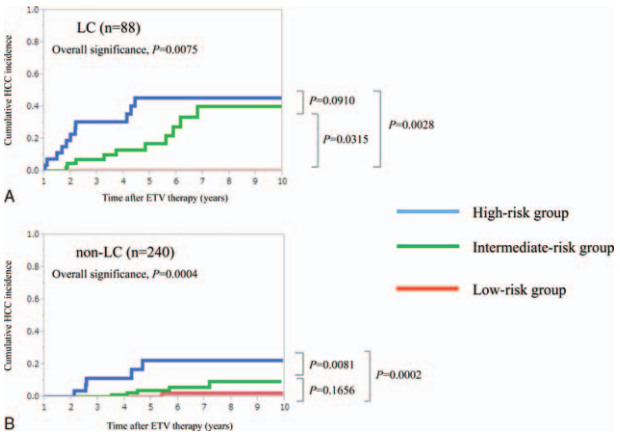

Figure 5. Subgroup analysis in patients with cirrhosis (A) and noncirrhosis (B). Kaplan–Meier curves for liver carcinogenesis in 3 risk groups (high-, intermediate-, and low-risk groups) are demonstrated.

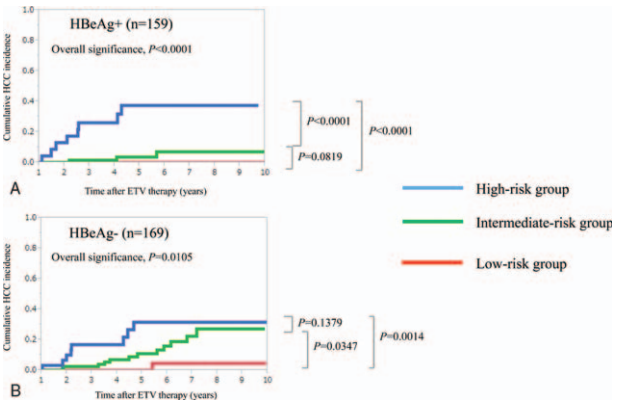

Figure 6. Subgroup analysis in patients with HBe antigen positive at baseline (A) and HBe antigen negative at baseline (B). Kaplan–Meier curves for liver carcinogenesis in 3 risk groups (high-, intermediate-, and low-risk groups) are demonstrated.

patients with HBe antigen positivity at baseline (n = 159), the risk for HCC development was significantly stratified between each of the 2 groups except for the comparison between the I-group and L-group: H-group (n = 23) versus I-group (n = 68), $P < 0.0001$; I-group versus L-group (n = 68), $P = 0.0819$; H-group versus L-group, $P < 0.0001$; overall significance, $P < 0.0001$ (Fig. 6A). In patients without HBe antigen positivity at baseline (n = 169), the risk for HCC development was significantly stratified between each of the 2 groups except for the comparison between the H-group and I-group: H-group (n = 31) versus I-group (n = 88), $P = 0.1379$; I-group versus L-group (n = 50), $P = 0.0347$; H-group versus L-group, $P = 0.0014$; overall significance, $P = 0.0105$ (Fig. 6B).

4. Discussion

To the best of our knowledge, the proposed predictive model is the newest scoring system for liver carcinogenesis in patients with CHB undergoing ETV therapy. Although ETV therapy in patients with CHB can reduce the incidence of HCC, identifying patients at high risk for HCC incidence is also clinically beneficial since it impacts clinical outcomes.[16,20] The potential benefit of ETV for CHB raises questions regarding the validity of HCC risk stratifications, since most previous reports were derived from baseline data in NA-therapy-naïve CHB patients.[23–27,32,33] We therefore conducted this study, mainly focusing on the on-treatment factors. The cumulative HCC incidence for all subjects was in accordance with those in data presented by Yamada et al[20] (4.48%, 9.52%, and 13.99% in 3, 5, and 7 years in our data vs 6.0%, 9.6%, and 17.2% in 3, 5, and 7 years in Yamada et al data). Since we excluded patients with HCC incidence within 1 year after ETV therapy from our analysis, we believe that AFP elevation at baseline or at 24 weeks did not reflect AFP elevation caused by HCC itself. Our observation that the median follow-up periods from the date of ETV therapy to the date of first confirmed HCC incidence were more than 3 years may also support this hypothesis.

The overall results showed that each of the 3 risk groups (e.g., H-group, I-group, and L-group) were well stratified for liver carcinogenesis for the entire cohort, the training group, and the validation group, although differences in several 2 groups did not reach significance. Furthermore, similar results were obtained in all subgroup analyses according to cirrhosis status or HBe antigen seropositivity. These results indicate that the proposed predictive model was helpful for predicting liver carcinogenesis in CHB patients treated with ETV. A major strength of this study is that our predictive model was verified in an independent validation cohort, although the validation group was selected retrospectively. Another strength is that our study subjects were randomly assigned into 1 of the 2 groups.

Previously proposed predictive models reported from Asian countries for HCC development in patients with CHB have examined several clinical factors such as age, ALT, serum albumin, total bilirubin, HBV-DNA, presence of cirrhosis, and HBe antigen status.[23–27] However, all of these factors were baseline factors. In the era of new NA therapy for CHB, predictive model including on-treatment factors can be more beneficial since improvement in liver inflammation activity by NA therapy may be linked to the suppression of HCC development.[20,34,35] AFP and ALT levels at 24 weeks after ETV therapy can be key points for clinical outcomes.[20–22]

In comparison between the results from Yamada et al[20] and ours, the major difference is that the presence of cirrhosis was found to be an independent predictor in Yamada et al[20] study, while it was not an independent predictor in our study. Other confounding factors for cirrhosis may have diminished the effects of cirrhosis status on carcinogenesis in our multivariate analysis. Indeed, in the training group, out of 10 cirrhotic patients with HCC incidence, 9 (90%) patients were 54 years of age or older.

It is of note that in liver cirrhosis patients, no patients in the L-group developed HCC during the observation period, and in patients with HBe antigen positivity at baseline, no patients in the L-group developed HCC during the observation period. The presence of cirrhosis and HBe antigen positivity are, in general, both adverse predictors associated with liver carcinogenesis.[24–27] Ameliorated liver inflammatory activity in younger CHB patients can completely suppress liver carcinogenesis even in patients with advanced fibrosis or HBe antigen positivity. These results may provide useful information for clinicians and may shed light on liver carcinogenesis in CHB patients undergoing ETV therapy.

According to American Association for the Study of Liver Disease practice guidelines for the management of HBV, the goal of NA therapy is to decrease serum HBV-DNA levels to undetectable levels for suppression of HCC incidence.[11] However, despite the close relationship between the HBV-

DNA level and the risk for HCC development, it is still uncertain whether the HBV-DNA level may be a useful predictor linked to HCC development in CHB patients undergoing NA therapy.[10] In our analysis, neither the pretreatment HBD-DNA level nor the HBV-DNA level at 24 weeks was significant predictor. Further research is required to confirm these results.

AFP is a key biomarker of HCC. It can be a reliable, independent predictor of long-term HCC risk in patients with CHB.[36] On the other hand, an increase of serum AFP level in liver diseases has been interpreted to indicate dedifferentiated hepatic regeneration.[37] Serum AFP elevation, along with ALT elevation, is frequently found in CHB or CHC patients with severe liver inflammation activity in the absence of HCC, which potentially leads to carcinogenesis.[20–22] The aims of this research were partly based on these previous reports.[20–22] Furthermore, a recent study demonstrated that AFP mediates HBx-induced carcinogenesis in the hepatocyte cytoplasm.[38] Although the AFP level in the hepatocyte cytoplasm was not tested in our study, their study results may be associated with our current results.

There are several limitations to our study. First, our study had a retrospective nature, and therefore our current data should be cautiously interpreted. Second, only internal validation was conducted; our findings need prospective external validation. Third, our study cohort was limited to patients initially treated with ETV, hence whether our results could be extrapolated to patients initially treated with other NAs such as LAM or TFV requires further research. Fourth, the study was based on a Japanese population, and additional studies using different ethnic populations are required to further validate our proposed predictive model and to extrapolate it to non-Japanese populations. Finally, the small number of patients with HCC incidence in our study was inadequate for analysis. *P* value may be susceptible to 1 HCC incidence. However, our present results showed that the prediction model performed well as a screening method for selecting CHB patients with liver carcinogenesis.

In conclusion, we present a simple prediction model, mainly based on on-treatment factors, to develop a screening method for picking up CHB patients with liver carcinogenesis. In addition, this novel model may be a promising model for predicting liver carcinogenesis in CHB patients undergoing ETV therapy.

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