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Original Article

Incidence and predictors of hepatocellular carcinoma in Chinese hepatitis B virus-related cirrhotic patients receiving antiviral therapy: a retrospective cohort study

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Abstract: The aim of this study were: (1) to evaluate incidence and predictors of hepatocellular carcinoma (HCC) in Chinese hepatitis B virus (HBV)-related cirrhotic patients receiving antiviral therapy; (2) to develop preliminary nomograms for the risk of HCC in this population. Multivariable Cox proportional hazards models were used to evaluate independent prognostic factors for developing HCC. Nomograms were developed basing on Cox regression. A total of 207 HBV-related cirrhotic patients receiving antiviral therapy were included: 95 patients with compensated cirrhosis and 112 patients with decompensated cirrhosis. The cumulative incidence rate of HCC at months 24, 36, 48, 60 and 72 were 6.9%, 11.2%, 16.6%, 23.4% and 42.6%. In multivariable Cox regression analysis, the HCC risk was independently associated with older age (≥48 years) (HR: 2.42, 95% CI 1.05-5.58, p=0.038), male gender (HR: 2.85, 95% CI 1.12-7.25, p=0.028) , higher AST levels (≥55 U/L) (HR: 2.92, 95% CI 1.33-6.42, p=0.008) and lower platelet counts (<80×10^9/L) (HR: 2.86, 95% CI 1.20-6.80, p=0.017). Additionally, subgroup analyses identified resistance of nucleos(t)ide analogues (NAs) as an independent risk factor for patients with compensated cirrhosis (p=0.020) but not in those with decompensated cirrhosis. Our study indicated that risk of HCC remains high in hepatitis B virus-related cirrhotic patients, particularly for those with older age, male gender, higher AST levels and lower platelet counts. All cirrhotic patients should remain under HCC surveillance even treated with NAs. Further inquiries for more accurate nomograms are needed in the future.

Keywords: Hepatocellular carcinoma, liver cirrhosis, hepatitis B virus, nucleos(t)ide analogues

Introduction

Hepatitis B virus (HBV) infection is a major cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC) worldwide, which results in about 1 million deaths every year before the widely use of antiviral therapy [1]. Over the past three decades, several antiviral agents, especially oral nucleos(t)ide analogues (NAs), have been approved for the treatment of CHB patients and improved their long-time outcome dramatically. The principal goal of treatment for CHB patients is to achieve sustained suppression of HBV replication and prevention of cirrhosis, hepatic decompensation and HCC [2]. Clinical data has shown that antiviral therapy significantly reduces the incidence of HCC compared with CHB patients without antiviral treatment, including patients developing cirrhosis [3].

However, several studies also demonstrated that HCC still developed in CHB patients treated with antiviral agents. Furthermore, HCC risk remains increasing in patients with cirrhosis even if they have achieved virological remission [4, 5].

Nomograms, which are performed according to the contribution degree of variables in Cox regression model, can supply a numerical probability of clinical events by creating an intuitive graph of a statistical predictive model. Concordance index (C-index) is used to evaluate the predictive value of nomograms [6]. Nomograms have been widely used as reliable tools to quan-

tify risk by illustrating important factors for prognosis of some diseases [6, 7]. However, there are few studies involving nomograms for antiviral-treated cirrhotic patients with HBV infection.

The aims of our study were to evaluate the incidence of hepatitis B-related HCC and to explore independent predictors of HCC in Chinese HBV-related cirrhotic patients receiving antiviral therapy. Meanwhile, we also aimed to develop preliminary nomograms applying for the risk of HCC in this population.

Methods

Study population

This study retrospectively enrolled consecutive patients with HBV-related cirrhosis who were treated with oral NAs including lamivudine, adefovir, telbivudine, entecavir or tenofovir for at least 12 months between October 2008 and October 2016 at 2nd Hospital of Shandong University, Jinan, China. Patients treated initially with NAs plus interferon/peg-interferon were also included. All patients should have positive test results for hepatitis B surface antigen (HBsAg) for more than 6 months before antiviral therapy. Patients were excluded if they had (1) viral co-infections such as hepatitis C virus, hepatitis D virus, human immunodeficiency virus; (2) other liver diseases such as autoimmune hepatitis, drug-induced liver disease; (3) HCC at baseline or during the first 12 months of antiviral treatment. The study protocol abided by the guidelines of the 1975 Helsinki Declaration and had been approved by the Institutional Review Board of 2nd Hospital of Shandong University.

Definition

Compensated liver cirrhosis was defined by histopathological evidence (METAVIR F4) or ultrasonography/computed tomography (CT)/magnetic resonance imaging (MRI) findings associated with cirrhosis (spleen size >12 cm; portal vein >16 mm). Decompensated cirrhosis was defined by the following clinical parameters: (1) ascites confirmed by ultrasound/CT/MRI; (2) esophageal or gastric variceal bleeding; or (3) hepatic encephalopathy. HCC was defined by either (1) a tumor with a maximum diameter ≥2 cm and typical features of HCC observed with arterial hypervascularity and venous or delayed phase washout using CT or MRI, or (2) nodules of 1-2 cm were investigated with two coincident imaging technique (CT and MRI). Virological response (VR) was assessed at 6 and 12 months after NAs treatment and defined as an HBV DNA concentration <500 copies/ml.

Follow-up of participants

Patients were followed routinely every 3-6 months for clinical assessment, including biochemistry examination (serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, serum albumin, international normalized ratio (INR), and α-fetoprotein (AFP) level), virological assessments (serum HBV DNA, hepatitis B e antigen (HBeAg)), and imaging techniques (ultrasound, CT, or MRI).

Follow-up time was calculated as the interval between the onset of therapy with NAs and the last available clinical information until October 2016. Analysis time was the time interval between the onset of therapy with NAs and the diagnosis of HCC or the end of follow-up in the absence of HCC development.

Laboratory tests

Liver biochemistry tests were detected with Beckman CX7 Chemistry Analyzer (Beckman Coulter, CA, USA) and original reagents. HBsAg, hepatitis B e antigen (HBeAg) and hepatitis B e antibody (HBeAb) levels were carried out using a commercial radioimmunoassay kit (Abbott Laboratories, Abbott Park, IL, USA). HBV DNA was measured by real-time PCR using suitable reagents (Sinomd Gene, Beijing, China) with the lowest detection limit of 500 copies/ml.

Statistical analysis

Statistical analyses to identify risk factors were performed by using SPSS 17.0 (SPSS, Chicago, IL). HBV DNA levels were logarithmically transformed for further analysis. Quantitative variables were compared using the t test or Mann-Whitney U test for variables according to different characteristics of distribution. Categorical data were compared using the Pearson X^2 test or Fisher's exact test. Survival curves were depicted using the Kaplan-Meier method and compared using the log-rank test. Multivariable Cox proportional hazards models were used to

Table 1. Baseline characteristics of the study population.

	Total (n=207)	Compensated cirrhosis (n=95)	Decompensated cirrhosis (n=112)	*P* value
Age, years	48.5±11.7	47.8±10.9	49.1±12.4	0.434
Gender, male (n, %)	143 (69.1%)	75 (78.9%)	68 (60.7%)	0.005
HBeAg-positive	123 (59.4%)	53 (55.8%)	70 (62.5%)	0.327
HBV DNA (log_{10} copy/ml)	5.7±1.3	5.8±1.3	5.7±1.3	0.698
ALT (U/L)	111.9±175.1	123.7±163.6	113.0±186.2	0.666
AST (U/L)	114.7±154.8	108.9±121.4	119.5±178.7	0.625
Bilirubin (umol/L)	56.6±77.0	35.8±47.1	74.2±91.9	0.000
Albumin (g/L)	32.1±16.6	34.8±6.5	29.9±21.6	0.036
Platelet count (10^9/L)	92.0±55.2	100.8±55.2	84.6±54.3	0.035
INR	1.2±0.4	1.2±0.3	1.4±0.4	0.000
AFP (ng/ml)	78.4±536.2	39.5±72.2	111.5±725.8	0.337
Initial NAs, n (%)				
ETV (n, %)	83 (40.1%)	33 (34.7%)	50 (44.6%)	
LAM (n, %)	42 (20.3%)	23 (24.2%)	19 (17.0%)	
ADV (n, %)	74 (35.7%)	36 (37.9%)	38 (33.9%)	0.147†
LDT (n, %)	5 (2.4%)	1 (1.1%)	4 (3.6%)	
LAM + ADV (n, %)	2 (1.0%)	1 (1.1%)	1 (0.9%)	
LAM + IFN (n, %)	1 (0.5%)	1 (1.1%)	0 (0.0%)	
Diabetes mellitus (n, %)	27 (13.0%)	12 (12.6%)	15 (13.4%)	0.871
Family history of HCC (n, %)	15 (7.2%)	11 (11.6%)	4 (3.6%)	0.027
NAs resistance (n, %)				0.143
M204I	12 (5.8%)	5 (5.3%)	7 (6.3%)	
M204I, L180M	12 (5.8%)	7 (7.4%)	5 (4.5%)	
A181V	6 (2.9%)	3 (3.2%)	3 (2.7%)	
L180M	1 (0.5%)	0 (0.0%)	1 (0.9%)	
V214A	2 (1.0%)	1 (1.1%)	1 (0.9%)	
N236T	2 (1.0%)	2 (2.1%)	0 (0.0%)	
Virological response				
VR6	88 (42.5%)	38 (40.0%)	50 (44.6%)	0.501
VR12	111 (53.6%)	50 (52.6%)	61 (54.5%)	0.792
Follow-up duration, months (range)	36.5±20.2 (12.0-96.2)	38.6±20.0 (12.1-92.9)	35.1±20.4 (12.0-96.2)	0.139

HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AFP, alpha-fetoprotein; INR, international normalized ratio; HCC, hepatocellular carcinoma; NAs, nucleos(t)ide analogues; ETV, entecavir; LAM, lamivudine; ADV, adefovir; LDT, telbivudine; IFN, interferon; VR6, virological response at 6 months; VR12, virological response at 12 months. †For initial ETV.

evaluate independent prognostic factors. The Enter method was used for multivariate analysis and a *P* value of 0.1 was used for including parameters. Some parameters were also included for multivariate analysis in subgroup analyses according to results of total population. *P*<0.05 (two-tailed) was considered to be statistically significant.

A nomogram was formulated by using the package of *rms* in R 3.3.1 (http://www.r-project.org) basing on the results of multivariable Cox analysis. C-index was used to evaluate the effectiveness of prognostic nomogram [6]. The detailed computerized procedures for programming nomogram with R software are listed in the **Appendix**.

Results

Baseline characteristics of the study population

A total of 217 chronic HBV patients with cirrhosis were initially identified for our study. Ten patients were excluded based on the inclusion and exclusion criteria. Six patients were treated with NAs for <12 months and four were diagnosed as HCC during the first 12 months of NAs treatment. Finally, 207 patients were analyzed

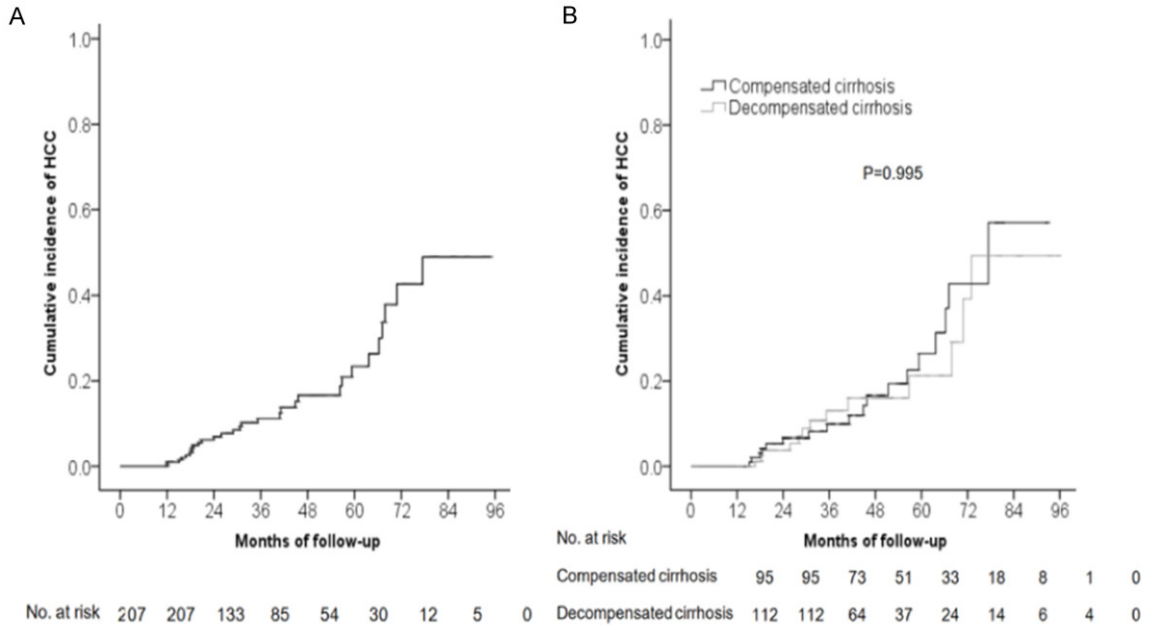

Figure 1. Cumulative incidence rate of hepatocellular carcinoma (HCC) according to liver status in patients with cirrhosis. A. Kaplan-Meier curve for HCC development in all patients. B. Kaplan-Meier curve for HCC development in patients with compensated cirrhosis and those with decompensated cirrhosis.

Table 2. Baseline characteristics and risk factors for incidence of HCC in all patients.

Risk factors	Patients with HCC (n=30)	Patients without HCC (n=177)	Univariate *P* value¶	Multivariable cox regression analysis&		
				Hazard ratio (95% CI)	β value	*P* value
Age (years)†	53.1±9.5	47.8±11.9	0.019	2.42 (1.05–5.58)	0.88	0.038
Gender, male	24 (80.0%)	119 (67.2%)	0.035	2.85 (1.12–7.25)	1.05	0.028
HBeAg-positive	19 (63.3%)	104 (58.8%)	0.457			
HBV DNA (log_{10} copy/ml)	5.4±1.1	5.8±1.3	0.818			
ALT (U/L)	89.9±147.1	122.7±180.4	0.277			
AST (U/L)‡	93.4±146.9	118.3±156.3	0.002	2.92 (1.33-6.42)	1.07	0.008
Bilirubin (umol/L)	43.2±41.7	58.9±81.4	0.521			
Albumin (g/L)	31.7±7.9	32.2±17.7	0.191			
Platelet count§	75.5±40.7	94.8±56.9	0.094	2.86 (1.20–6.80)	1.05	0.017
INR	1.2±0.2	1.3±0.4	0.016	0.18 (0.02–1.43)	-1.71	0.105
AFP (ng/ml)	37.5±61.4	85.4±579.3	0.410			
Initial ETV (n, %)	7 (23.3%)	76 (42.9%)	0.527			
Virological response						
VR6	9 (30.0%)	79 (44.6%)	0.694			
VR12	11 (36.7%)	100 (56.5%)	0.424			
Diabetes mellitus (n, %)	5 (16.7%)	22 (12.4%)	0.760			
Family history of HCC (n, %)	4 (13.3%)	11 (6.2%)	0.224			
NAs resistance (n, %)	11 (36.7%)	24 (13.6%)	0.220			
Decompensated cirrhosis (n,%)	14 (46.7%)	98 (55.4%)	0.995			

HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AFP, alpha-fetoprotein; INR, international normalized ratio; HCC, hepatocellular carcinoma; NAs, nucleos(t)ide analogues. ETV, entecavir; VR6, virological response at 6 months; VR12, virological response at 12 months. †Age ≥48 years vs. <48 years. ‡AST ≥55 U/L vs. <55 U/L. §Platelet count <80×10^9/L vs. ≥80×10^9/L. ¶Log-rank test. &Multivariable cox regression analysis was performed with a enter method.

in our study. **Table 1** describes the baseline characteristics of the study population. The mean age of the patients was 48.5±11.7 years, and 69.1% of the patients were men. Overall mean follow-up duration of the study population was 36.5±20.2 months (range 12.0-96.2).

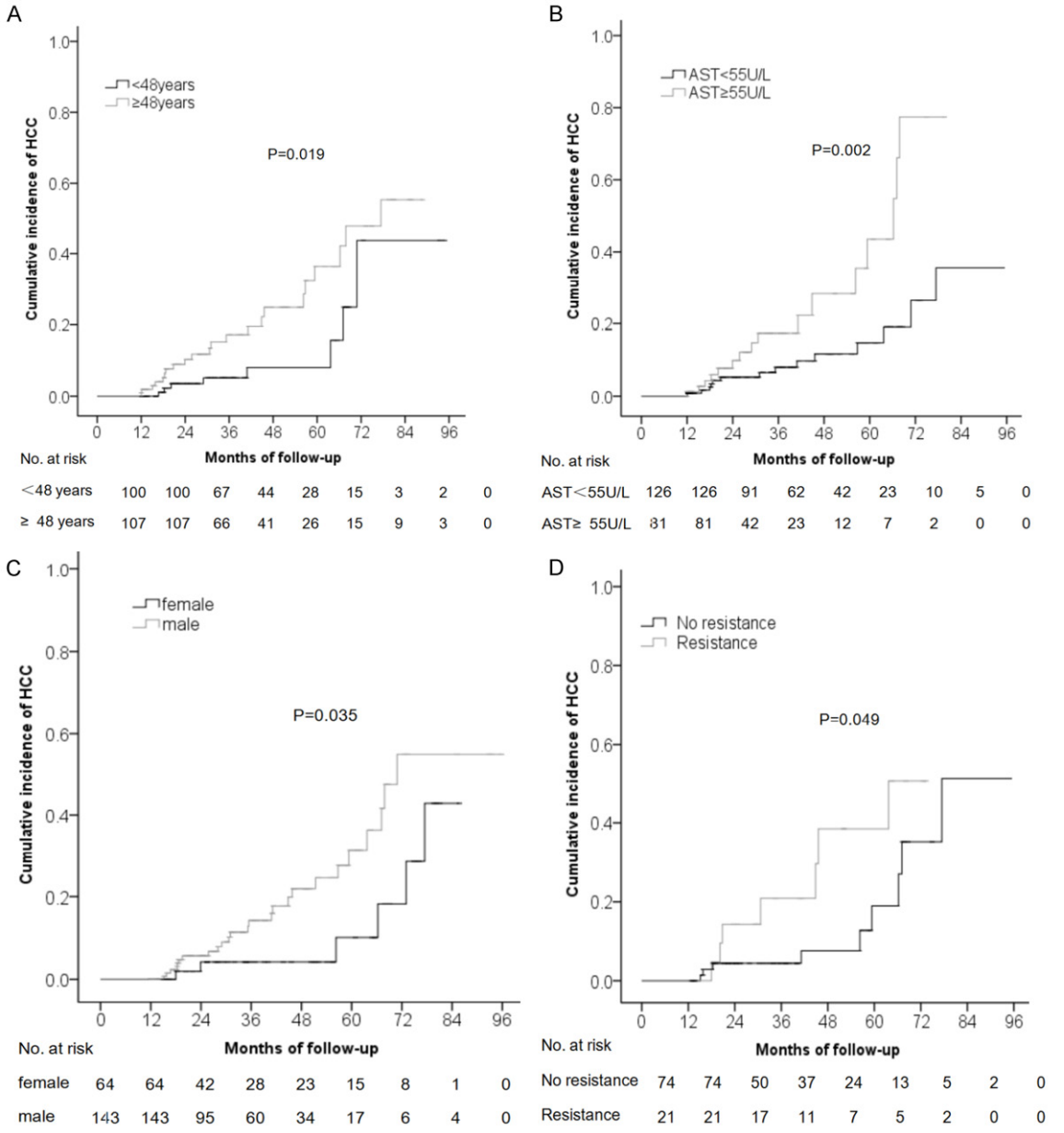

Figure 2. Kaplan-Meier curve for cumulative incidence of hepatocellular carcinoma (HCC) according to the main risk factors. A. Older patients (age ≥48 years) showed significant higher HCC incidence in all patients. B. Patients with higher AST levels (AST ≥55 U/L) showed significant higher HCC incidence in all patients. C. Male patients had showed significant higher HCC incidence in all patients. D. Patients with resistance of NAs showed significant higher HCC incidence in patients with compensated cirrhosis.

In total, 83 patients (40.1%) were treated initially with entecavir, 74 (35.7%) and 42 (20.3%) patients were treated with adefovir and lamivudine respectively, 2 (1.0%) patients were treated initially with lamivudine plus adefovir, and 1 patient (0.5%) with lamivudine plus interferon. Eighty-nine patients had data of HBV genotype and most of them were genotype C patients (87/89). The study population consisted of 95 patients with compensated cirrhosis (45.9%) and 112 patients (54.1%) with decompensated cirrhosis. Patients with decompensated cirrhosis had less male gender, higher total bilirubin level and international normalized ratio (INR), lower serum albumin levels and platelet counts at baseline comparing with patients with compensated cirrhosis. There were no statistical differences for the comparison of age, HBeAg

Table 3. Risk factors for incidence of HCC in compensated or decompensated cirrhotic patients.

Risk factors	Patients with compensated cirrhosis						Patients with decompensated cirrhosis					
				Multivariable cox regression analysis¶						Multivariable cox regression analysis¶		
	Patients with HCC (n=16)	Patients without HCC (n=79)	Univariate *P* value	Hazard ratio (95% CI)	β value	*P* value	Patients with HCC (n=14)	Patients without HCC (n=98)	Univariate *P* value	Hazard ratio (95% CI)	β value	*P* value
Age (years)†	53.2±9.2	46.8±10.9	0.183	7.10 (1.30–38.47)	1.96	0.023	52.9±10.2	48.6±12.7	0.117	1.18 (1.01–2.15)	0.17	0.013
Gender, male	12 (75%)	63 (79.7%)	0.317	8.20 (1.12–60.27)	2.10	0.019	12 (85.7%)	56 (57.1%)	0.017	6.78 (1.35–34.05)	1.91	0.002
HBeAg-positive	9 (56.3%)	44 (55.7%)	0.708				10 (71.4%)	60 (61.2%)	0.400			
HBV DNA (log_{10} copy/ml)	5.5±0.9	5.8±1.4	0.802				5.3±1.3	5.7±1.3	0.913			
ALT (U/L)	65.8±46.0	135.4±176.2	0.084				117.5±210.5	112.4±183.9	0.769			
AST (U/L)‡	66.3±40.8	117.6±130.4	0.039	12.05 (2.65–55.56)	2.53	0.001	124.4±210.1	118.8±175.0	0.977			
Bilirubin (umol/L)	27.1±14.3	37.5±51.1	0.083				61.6±54.4	76.0±96.1	0.486			
Albumin (g/L)	30.8±5.1	34.3±6.7	0.049	1.10 (0.95–1.27)	0.10	0.201	25.8±6.3	30.5±22.9	0.366			
Platelet count§	88.9±44.4	103.2±57.1	0.296	14.10 (2.72–73.14)	2.65	0.000	60.1±30.8	88.1±56.1	0.184			
INR	1.1±0.1	1.2±0.3	0.076	0.00 (0.00–0.31)	-5.81	0.053	1.3±0.2	1.4±0.4	0.217			
AFP (ng/ml)	49.8±81.3	37.4±70.6	0.543				23.5±19.7	124.0±775.6	0.399			
Initial type of NAs, n (%)												
ETV (n, %)	3 (18.8%)	30 (38.0%)	0.633&				4 (28.6%)	46 (46.9%)	0.714&			
Other NAs (n, %)	13 (81.2%)	49 (62.0%)					10 (71.4%)	52 (53.1%)				
Virological response												
VR6	4 (25%)	34 (43.0%)	0.468				5 (35.7%)	45 (45.9%)	0.897			
VR12	6 (37.5%)	44 (55.7%)	0.597				5 (35.7%)	56 (57.1%)	0.526			
Diabetes mellitus (n, %)	3 (18.8%)	9 (11.4%)	0.867				2 (14.3%)	13 (13.3%)	0.904			
Family history of HCC (n, %)	4 (25%)	7 (8.9%)	0.047	3.51 (0.80-15.41)	1.26	0.096	0 (0.0%)	4 (4.1%)	0.633			
NAs resistance (n, %)	7 (43.8%)	11 (13.9%)	0.049	5.71 (1.31–24.83)	1.74	0.020	4 (28.6%)	13 (13.3%)	0.985			

HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AFP, alpha-fetoprotein; INR, international normalized ratio; HCC, hepatocellular carcinoma; NAs, nucleos(t)ide analogues. †Age ≥48 years vs. <48 years. ‡AST ≥55 U/L vs. <55 U/L. §Platelet count $<80\times10^9$/L vs. $\geq80\times10^9$/L. ¶Multivariable cox regression analysis was performed with a enter method. &for initial ETV

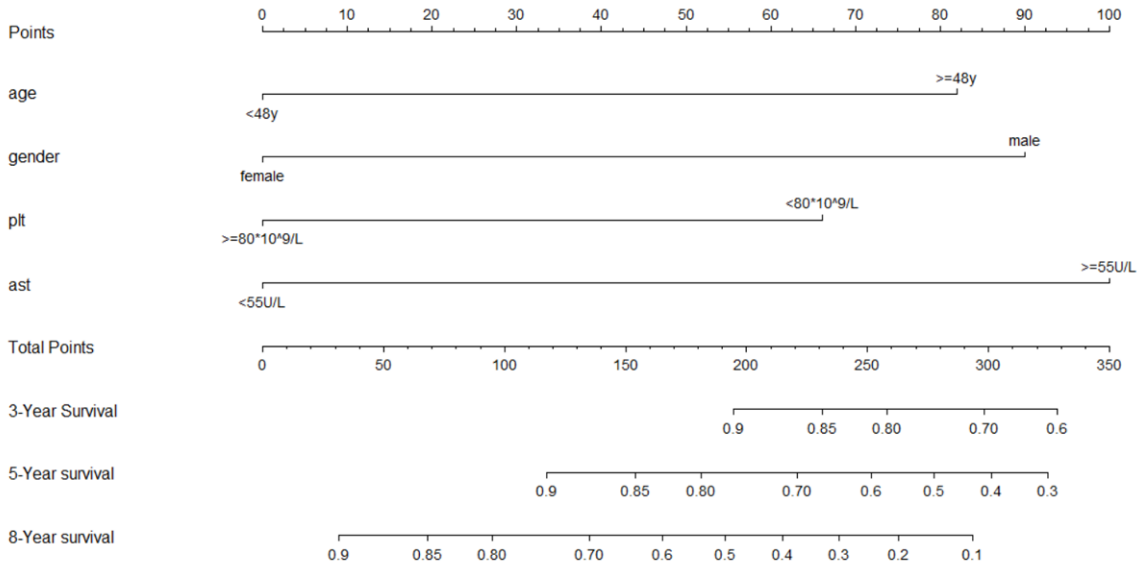

Figure 3. Nomogram developed for incidence of HCC. In the nomogram, we can add the points identified on the points scale for each risk factor (age, gender, plt and ast). The total points projected on survival scales on the bottom show the probability of 3-year, 5-year and 10-year survival rate. "Survival" means no incidence of HCC.

status, HBV DNA, initial NAs, resistance of NAs and follow-up duration in the two subpopulations.

Cumulative incidence of HCC

Figure 1 shows the Kaplan-Meier curve for the Cumulative incidence rate of HCC. HCC developed in 30 (14.5%) of the 207 cirrhotic patients. The cumulative incidence rates of HCC at months 24, 36, 48, 60 and 72 were 6.9%, 11.2%, 16.6%, 23.4% and 42.6% for the included cirrhotic patients respectively (**Figure 1A**). For patients with compensated cirrhosis and patients with decompensated cirrhosis, the cumulative incidence rates of HCC at months 24, 36, 48, 60 and 72 were 7.0%, 8.7%, 16.0%, 23.9%, 39.6% and 6.7%, 14.0%, 16.9%, 22.1%, 44.3%, respectively. Notably, there was no significant difference for the incidence of HCC in the two subpopulations (p=0.995) (**Figure 1B**). **Table 2** shows the baseline characteristics of patients with HCC and patients without HCC. HCC patients tended to be older (p=0.021 by t test), less with entecavir (p=0.043 by X^2 test), more often with NAs resistance (p=0.002 by X^2 test) and less achieving VR at 12 months (p=0.044 by X^2 test). The median ALT, AST, total bilirubin levels, serum albumin levels, platelet counts, INR, AFP and HBV DNA in the two groups were comparable (data not shown).

Risk factors for incidence of HCC

The baseline clinical features and laboratory characteristics were also assessed as potential risk factors for incidence of HCC. The univariate analysis by log-rank test showed the cumulative incidence of HCC was higher in male patients (p=0.035), patients with older age (≥48 years, p=0.019), patients with higher AST levels (≥55 U/L, p=0.002) and patients with lower INR levels (p=0.016). Cumulative incidences of HCC also trended higher for patients with lower platelet counts, however, there was no significant difference (p=0.094). In multivariable Cox regression analysis, the following independent risk factors for HCC incidence in all the patients were: older age (≥48 years) (HR: 2.42, 95% CI 1.05-5.58, p=0.038), male gender (HR: 2.85, 95% CI 1.12-7.25, p=0.028), higher AST levels (≥55 U/L) (HR: 2.92, 95% CI 1.33-6.42, p=0.008) and lower platelet counts (<80×10^9/L) (HR: 2.86, 95% CI 1.20-6.80, p=0.017) (**Table 2**, **Figure 2**). Also, the multivariable Cox regression analysis showed older age, male gender, lower platelet levels, higher AST levels and resistance of NAs were independent risk factors for patients with compensated cirrhosis. Only older age and male gender were shown to be as significant risk factors for the development of HCC in patients with decompensated cirrhosis. HBeAg

status, type of initial NAs and VR at month 6 or 12 were not significant in both compensated and decompensated cirrhotic patients (**Table 3**).

Prognostic nomograms for incidence of HCC

We integrated 4 independent risk factors for incidence of HCC according to the results of multivariable Cox regression analysis and performed the prognostic nomogram (**Figure 3**). Calibration of the nomogram for 3-, 5-, and 8-year survival (no incidence of HCC) was also performed. The C-index for HCC prediction was 0.53 (95% CI 0.49-0.57).

Discussion

Antiviral therapy is targeted at CHB and cirrhotic patients to reduce the risk of disease progression to hepatic decompensation and HCC. Incidence of HCC decreases if HBV replication could be suppressed. However, HCC still developed in CHB patients experiencing antiviral treatment, furthermore, incidences and risk factors for HCC in HBV-related cirrhotic patients are not consistent in different studies [4, 8-10].

We retrospectively included 207 patients with hepatitis B-related cirrhosis and indicated that HCC still developed under long-term antiviral therapy, even if patients had achieved VR. The cumulative incidence rates of HCC at years 3, 5 and 8 were 11.2%, 23.4% and 49.0%, respectively. Several baseline clinical features and laboratory characteristics were assessed as potential risk factors for HCC in our study and we found that older age (≥48 years, HR 2.42), male gender (HR 2.85), higher AST levels (≥55 U/L, HR: 2.92) and lower platelet counts ($<80\times10^9$/L, HR 2.86) were significant risk factors for developing HCC in all hepatitis B-related cirrhotic patients. We also developed a preliminary nomogram for the risk of HCC in this population.

The 5-year cumulative incidence of HCC was 23.4% in our study, which was consistent with the previous study performed by Kim *et al* [8] and higher than those in some studies [9, 10]. Similarly with the baseline clinical features of patients included by Kim *et al* [8], most patients in our study had genotype C, which was associated with a higher risk for developing HCC than other genotypes [4]. Meanwhile, only 40.1% of included patients in our study were treated with entecavir and no patient were treated with tenofovir, although we found no significant association with entecavir and lower incidence of HCC (p=0.527), the deficiency of antiviral drugs with high resistance barrier might be another reason for a higher HCC incidence rate. Furthermore, most patients (181/207) in our study were diagnosed as cirrhosis by clinical indicators and ultrasonography/CT/MRI findings, the relatively low sensitivity might be a reason for missed diagnosis of early stage of cirrhosis. Moreover, distribution of HCC in different regions or ethnic groups may be another reason for a higher HCC incidence rate [4].

Owing to our long-term follow-up (range 12.0-96.2 months), we calculated that the 8-year cumulative incidence of HCC was 49.0% in our study. This high HCC incidence indicated the relatively poor prognosis of cirrhotic patients, even if they had experienced antiviral therapy and achieved VR. Periodic monitoring for disease progression and hepatocarcinogenesis was very important for this population, especially for patients with high risk factors.

We found that the risk of HCC was 2.85 times as high among male patients as it was among female patients, which was consistent with previous study [4, 8, 11]. Meanwhile, previous epidemiological investigation found that the greatest proportional increase in cases of HCC among Hispanics and whites was between 45 and 60 years of age [4], similarly, our study also discovered older age as an independent risk factor for developing HCC and the cutoff boundary was 48 years (HR 2.42). Additionally, chronic inflammation, a driving factor in many types of cancers, is an important pathogenetic mechanism for development of HBV-related HCC. Our study found that a higher pretreatment AST level (≥55 U/L) was an independent risk factor for HCC in all cirrhotic patients and patients with compensated cirrhosis, but not in patients with decompensated cirrhosis. This finding indicated that inflammation might play a more important role in enhancing the risk of HCC in patients with early stage of cirrhosis than those with late stage of cirrhosis. The mechanism of this indication was unknown and further studies were needed to confirm our result.

Platelet counts was considered as a serum marker for fibrosis and/or cirrhosis. Previous

studies showed that lower platelet counts was an independent predictor for HCC risk in HBV-related patients experiencing antiviral therapy [9, 12, 13]. Our study also concluded that lower platelet counts was an independent risk factor for developing HCC (HR 2.86), indicating the role of significant fibrosis in disease progression and hepatocarcinogenesis. However, this association was also not found in patients with decompensated cirrhosis. In view of the small number of patients with decompensated cirrhosis, HCC rates in these subgroups need confirmation in larger cohorts.

Stage of cirrhosis was considered as an important independent risk factor for developing HCC [4]. However, we found no significant difference for the incidence of HCC in patients with compensated cirrhosis or decompensated cirrhosis. In our study, patients with compensated cirrhosis were more often male (78.9% versus 60.7%, p=0.005) and had higher prevalence of family history of HCC (11.6% versus 3.6%, p=0.027) at baseline comparing with patients with decompensated cirrhosis. This might partially explain the comparable incidence of HCC between the two groups. Additionally, in our study, liver cirrhosis was primarily defined on the basis of clinical findings; therefore, patients with early-stage liver cirrhosis may not be included in this study. So this conclusion should be re-evaluated.

Recent studies have shown that a reduction of HBV DNA to low or undetectable levels reduced the risk of liver-related events and/or HCC [8, 10]. However, some studies found considerable rates of HCC despite long-term viral suppression [12, 13]. In the current study, we were unable to confirm the association between time to VR and a reduction of HCC incidence. Reasons for different results are currently unclear, but may come down to differences in HBV genotype distribution, time since infection and previous treatment exposure in different study cohorts. In our study, 40.1% of patients were treated initially with entecavir, while 35.7% and 20.3% of patients were treated with adefovir and lamivudine respectively. We did not find type of initial NAs was a significant risk factor for incidence of HCC, which was consistent with previous studies [14, 15]. However, we confirmed resistance of NAs as an independent risk factor for developing HCC in patients with compensated cirrhosis, indicating that NAs with high resistance barrier should be selected for patients with HBV infection to avoid potential treatment failure and risk for HCC.

We developed a preliminary nomogram for the risk of HCC in antiviral-treated cirrhotic patients with HBV infection. This was an innovation of our study. However, the C-index for HCC prediction was only 0.53, indicating a relatively low model discrimination ability. Considering the few independent risk factors (age, gender, AST and platelet counts) integrated in the prognostic nomogram, this gloomy conclusion is not surprising. Moreover, it indicates that we may not simply apply these risk factors above to make an early warning. These baseline risk factors may not be suitable or adequate for the conformation of prognostic nomogram. More valuable risk factors for HCC should be evaluated and integrated in future studies.

The present study had a few limitations. First, it was a retrospective study. Therefore, we did not directly show the advantage of antiviral treatment for HCC development in patients with cirrhosis. Second, as mentioned above, patients with early-stage liver cirrhosis may not be included in this study because of the relatively low sensitivity of clinical diagnostic methods. Future prospective study with a large number of patients who have received potent antiviral treatment such as entecavir or tenofovir will be needed to confirm our results.

In conclusion, antiviral therapy did not entirely eliminate the risk of developing HCC in HBV-related cirrhotic patients. Periodic monitoring for HCC development is warranted for patients with cirrhosis receiving antiviral therapy, particularly for those with older age (≥48 years), male gender, higher AST levels (≥55 U/L) or lower platelet counts ($<80\times10^9$/L). Meanwhile, further inquiries for more accurate nomograms are needed in the future.

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Disclosure of conflict of interest

None.

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Appendix

The detailed computerized procedure for programming nomograms with R software.

For nomogram

```
coxm <- cph(Surv(time,censor==0)~age+gender+plt+ast,x=T,y=T,data=pancer,surv=T)

coxm

surv <- Survival(coxm)

surv1 <- function(x)surv(1*36,lp=x)

surv2 <- function(x)surv(1*60,lp=x)

surv3 <- function(x)surv(1*96,lp=x)

plot(nomogram(coxm,fun=list(surv1,surv2,surv3),lp=F,funlabel=c('3-Year Survival','5-Year survival','8-Year survival'),maxscale=100,fun.at=c('0.9','0.85','0.80','0.70','0.6','0.5','0.4','0.3','0.2','0.1')),xfrac=30)
```

For calculation of the C-Index

```
S<-Surv(hcc$time,hcc$censor)

rcorrcens(S~predict(coxm))
```