Am J Cancer Res 2015;5(2):756-771
www.ajcr.us /ISSN:2156-6976/ajcr0003541

Original Article

Level of hepatitis B surface antigen might serve as a new marker to predict hepatocellular carcinoma recurrence following curative resection in patients with low viral load

Hua-Bang Zhou[1*], Qiao-Mei Li[1*], Zheng-Rong Zhong[2*], Jing-Yi Hu[3], Xiao-Lan Jiang[1], Hao Wang[1], Hui Wang[1], Bing Yang[1], He-Ping Hu[1]

[1]Department of Hepatobiliary I, Eastern Hepatobiliary Surgery Hospital, Second Military Medical University, Shanghai, China; [2]Department of Laboratory, First Affiliated Hospital, Bengbu Medical College, Anhui, China; [3]Department of Digestive Diseases, Huashan Hospital, Fudan University, Shanghai, China. [*]Equal contributors.

Received November 2, 2014; Accepted January 5, 2015; Epub January 15, 2015; Published February 1, 2015

Abstract: To investigate the association between preoperative HBsAg (hepatitis B surface antigen) level and risk of HCC (hepatocellular carcinoma) recurrence following curative resection, we enrolled 826 HBV-related HCC patients who underwent curative resection and received long-term follow-up at the Eastern Hepatobiliary Surgery Hospital (Shanghai, China). Multivariate analyses showed that serum HBsAg ≥ 2000 S/CO, seropositive hepatitis B e antigen (HBeAg), γ-glutamyl transpeptidase > 61 U/L, prothrombin time > 13 s, multinodularity, lager tumor size, and major portal vein invasion were independently associated with a increased risk of HCC recurrence. Compared with HCC patients with HBsAg level < 2000 S/CO, HCC patients with HBsAg level ≥ 2000 S/CO had a higher prevalence of seropositive HBeAg, antiviral therapy, and cirrhosis; were younger; and had a higher levels of alanine transaminase (ALT), aspartate aminotransferase (AST), and HBV viral load. Multivariable stratified analyses showed HCC patients with HBsAg level < 2000 S/CO tended to have a lower incidence of HCC recurrence in following subgroups of patients, including for noncirrhotic (HR, 0.561; 95% CI, 0.345-0.914), HBV DNA < 2000 IU/mL (HR, 0.604; 95% CI, 0.401-0.912), ALT ≤ 41 U/L (HR, 0.643; 95% CI, 0.440-0.942), AST ≤ 37 U/L (HR, 0.672; 95% CI, 0.459-0.983), and seronegative HBeAg (HR, 0.682; 95% CI, 0.486-0.958). When we evaluated HBeAg-negative patients with HBV DNA < 2000 IU/mL, HBsAg level still determined risk of HCC recurrence (p = 0.014), but not HBV DNA (p = 0.550) and ALT (p = 0.186). These results suggest high levels of HBsAg increase risk of HCC recurrence following curative resection. HBsAg level might serve as a new marker to complement HBV DNA level in predicting HCC recurrence, especially in HBeAg-negative patients with low viral load.

Keywords: Hepatitis B surface antigen, hepatocellular carcinoma, recurrence

Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide. Hepatitis B virus (HBV) infection, especially infection with a high HBV viral load, is a major risk factor for the development of HCC [1]. Surgery is considered the standard curative treatment option for HCC. However, despite advances in surgical modalities, survival after tumor resection remains poor mainly due to persistent high incidences of HCC recurrence [2, 3]. Many factors affect HCC recurrence risk after curative resection, including alanine transaminase (ALT) level; virological factors such as hepatitis B e antigen (HBeAg) status, HBV viral load, HBV genotype C, and antiviral treatment; cirrhosis; and tumor-associated variables such as serum α-fetoprotein (AFP) level, tumor number, tumor size, capsule formation, vascular invasion, Edmonson grading, and TNM stage [4-10]. Among these factors, HBV viral load is the most clinically correctable. Higher viral load has been reported to be an independent risk factor for HCC recurrence after surgery [11]. Nucleoside analogues are effective in suppressing HBV replication and ameliorating HBV-related liver disease. In addition, they have been shown to

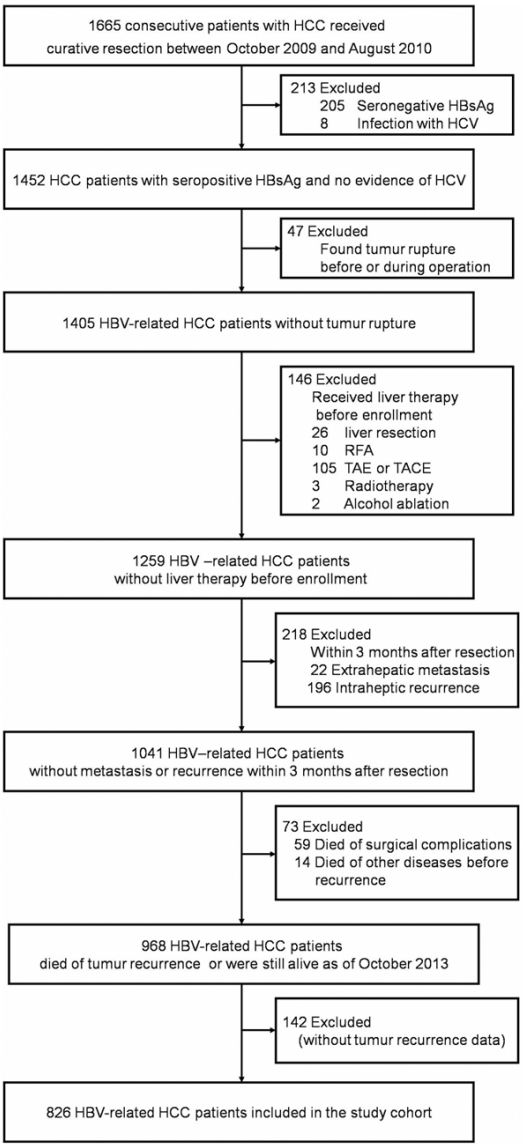


Figure 1. Flow chart of study cohort selection. HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; HBsAg, hepatitis B surface antigen; RFA, radiofrequency ablation; TAE, therapeutic arterial embolization; TACE, transcatheter arterial chemoembolization.

be associated with a lower risk of HCC development and postoperative HCC recurrence [8-10].

Recently, quantification of HBsAg levels has attracted much attention as an important marker for evaluating viral activity. Throughout the natural history of HBV infection, HBsAg levels vary markedly across different phases of chronic HBV infection and across different HBV genotypes [12-15]. Previous studies have shown a positive correction between HBsAg level and HBV viral load [16]. A lower HBsAg level was also shown to be associated with a higher chance of HBsAg loss and a lower risk of hepatitis activity in patients who were infected HBV genotype B or C [17]. One study has proposed that an HBsAg level < 1000 IU/mL and an HBV DNA level < 2000 IU/mL can be used together as a marker for inactive HBV genotype D carriers [15]. Furthermore, another recent study has shown that high levels of HBsAg increase the risk of HCC in patients with low HBV load [16]. However, it is still unclear whether higher levels of HBsAg increase the risk for HBV-related HCC recurrence following curative resection, especially in patients with low viremia.

To address this issue, we enrolled 826 HBV-related HCC patients who underwent curative resection and received long-term follow-up into the present study and sought to elucidate the association between HBsAg level at time of tumor resection and risk of HCC recurrence following resection in this cohort.

Materials and methods

Patient cohort

We identified all hospitalized patients who were admitted with a primary diagnosis of HCC and received curative liver resection (Admission Code: M81700/3) from Eastern Hepatobiliary Surgery Hospital of the Second Military Medical University (Shanghai, China) between October 2009 and August 2010. The diagnosis of HCC was confirmed by pathology.

Only HCC patients with HBV infection were included in our study cohort. We applied the following exclusion criteria: diagnosis of hepatitis C or other viral hepatitis; presence of malignant tumor; lack of data on tumor recurrence; tumor rupture before or during operation; and patients who received liver resection, transarterial chemoembolization, percutaneous ethanol injection, radiofrequency ablation, or liver transplantation before the index hospitalization. Patients with HCC recurrence in the first 3 months after the index hospitalization for liver resection were also excluded. Death before the recurrence of HCC was defined as competing mortality, and such patients were also excluded.

We first identified 1665 potentially eligible HCC patients who received curative tumor resection. Using our inclusion and exclusion criteria (**Figure 1**), we excluded 839 patients because

Table 1. Characterization of 826 Patients with HCC

Variables	Patients, n (%)
Patient demographics	
Gender	
Male	717 (86.8)
Female	109 (13.2)
Age (years)	
< 50	361 (43.7)
50-59	293 (35.5)
≥ 60	172 (20.8)
Cirrhosis	534 (64.6)
Diabetes	46 (5.6)
Metformin use[a]	8 (1.0)
Viral factors	
Anti-HBV therapy (n = 691)[b]	483 (69.9)
Serum HBsAg level (S/CO)	
< 1000	99 (12.0)
1000-1999	38 (4.6)
2000-2999	42 (5.1)
3000-3999	49 (5.9)
≥ 4000	598 (72.4)
Seropositive HBeAg	232 (28.1)
Serum HBV DNA level (IU/mL) (n = 773)[b]	
< 1000	309 (40.0)
1000-1999	36 (4.7)
2000-19999	102 (13.2)
20000-199999	132 (17.1)
≥ 200,000	194 (25.1)
Biochemical factors	
ALT (> 41 U/L)	341 (41.3)
AST (> 37 U/L)	333 (40.3)
TBIL (> 18.8 μmol/L)	146 (17.7)
r-GT (> 61 U/L)	391 (47.3)
ALP (> 129 U/L)	97 (11.7)
ALB (< 34 g/L)	14 (1.7)
Prealbumin (< 170 g/L)	211 (25.5)
PT (> 13 s)	122 (14.8)
WBC (< $4.0*10^9$/L)	155 (18.8)
PLT (< $100.0*10^9$/L)	153 (18.5)
Tumor characteristics	
AFP (> 20 μg/L)	491 (59.4)
Tumor number	
single	677 (82.0)
multiple	149 (18.0)
Tumor size (cm)	
≤ 3	259 (31.4)
3~5	272 (32.9)
5~10	216 (26.2)

of seronegative HBsAg (n = 205), infection with HCV (hepatitis C virus) (n = 8), tumor rupture before or during operation (n = 47), receiving liver tumor therapy before enrollment (n = 146), having intrahepatic recurrence and extrahepatic metastasis within the first 3 months after the index hospitalization for liver resection (n = 218) [8], death prior to recurrence (n = 73), or no data on tumor recurrence (n = 142). Therefore, 826 HBV-related HCC patients were enrolled into the study. The project was approved by the Eastern Hepatobiliary Surgery Hospital Ethical Committee, China. All patients gave written informed consent to participate. The data do not contain any information that could identify the patients.

Biochemical and serological markers

Antibody against HBsAg (anti-HBs), HBeAg, antibody against HBeAg (anti-HBe), and antibody against HBcAg (anti-HBc) were tested using a radioimmunoassay kit (Roche, Mannheim, Germany). Antibody against HCV (anti-HCV) was measured by means of a second-generation enzyme immunoassay (Chemclin Biotech Co., Ltd., China). Serum biochemical tests were performed by a systemic multiautoanalyzer (Technicon SMAC, Technicon Instruments Corp., Tarrytown, NY). Serum alpha-fetoprotein (AFP) level was also measured by a radioimmunoassay (Serono Diagnostic SA, Coinsins, Switzerland).

Quantification of HBsAg and HBV DNA

Serum HBsAg was tested using a radioimmunoassay kit (Roche, Mannheim, Germany). The range of detection was from 0 S/CO to 4000 S/CO. HBV DNA was extracted from 100 μL of serum using a standard commercial diagnostic kit for the quantification of hepatitis B viral DNA (Shanghai Kehua Laboratory System Co., Ltd., China) according to the manufacturer's instructions with a final elution volume of 2 μL. The extracted DNA was then quantified with the same kit on the ABI7500 Fast Real-time PCR System (Applied Biosystems, Mortlake, USA) according to the manufacturer's instructions. To ensure the specificity of the test and to prevent diagnostic errors, the limit of detection was defined as 1000 IU/mL.

Follow-up

All patients were regularly followed for AFP measurement, ultrasonography (USG), and/or

> 10	79 (9.6)
Portal vein invasion	62 (7.5)
Cutting margin ≥ 1 cm (n = 590)[b]	112 (19.0)
Histo-pathological findings	
Capsule formation	691 (83.7)
Edmonson grading	
I	9 (1.1)
II	199 (24.1)
III	598 (72.4)
IV	20 (2.4)
Microvascular invasion	261 (31.6)
pTNM stage	
I	468 (56.7)
II	221 (26.8)
III	135 (16.3)
IVA	2 (0.2)

HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; WBC, white blood cell; PLT, platelet; AFP, alpha-fetoprotein; TBIL, total bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALB, albumin; PT, prothrombin time; γ-GT, γ-glutamyl transpeptidase; ALP, alkaline phosphatase. [a]Drug users indicate patients using a drug at least 1 day pen month on average. [b]Number of available data.

computed tomography (CT) or magnetic resonance imaging (MRI) scan every one to two months for the first six months after operation and every three months afterwards. Tumor recurrence was suspected when there was a progressive elevation of serum AFP and/or ultrasonographic evidence of a new hepatic lesion that was confirmed by dynamic CT scan, MRI or position emission tomography (PET). HCC recurrence was defined as re-hospitalization with a primary diagnosis of HCC after the index admission date and a treatment modality for HCC recurrence, such as surgery, transarterial chemoembolization, percutaneous ethanol injection, radiofrequency ablation, or liver transplantation, during the study period. Disease-free survival was measured from the date of surgery to the date of recurrence. Follow-up of patients was continued until HCC recurrence, death, or August 5, 2013. Causes of death were also investigated.

Statistical methods

Descriptive analyses of the variables were conducted using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Univariate analyses were performed using the Chi-squared test for categorical variables and the independent-samples t-test for discrete variables. Cumulative incidences were calculated using the Kaplan-Meier method. Clinicopathological prognostic factors were evaluated using the univariate Kaplan–Meier method and compared with the log-rank test to identify the prognostic predictors for recurrence. Multivariate regression analysis was performed using Cox proportional hazards models to identify the independent prognostic factors for recurrence. Variables to be entered into the multivariate analysis were selected based on the results of the univariate analyses ($p < 0.1$). In order to compare the predictive values of different factors for HCC recurrence, receiver operating characteristic (ROC) curve analysis was used to compute the area under the ROC curves for different factors. The performances of the factors in predicting HCC recurrence were compared using the Chi-squared test for categorical variables and the t-test for discrete variables. A value of $p < 0.05$ was considered statistically significant.

Results

Clinicopathological characterization of HCC patients

The demographic, biochemical, virological, surgical, and pathological data of the 826 patients are shown in **Table 1**. There were 717 men (86.8%) and 109 women (13.2%). The median age was 51.1 (range, 13-79) years. The median hospital stay was 15.2 (range, 7-72) days. Of the 826 patients, 232 (28.1%) were seropositive for HBeAg, 534 (64.6%) had cirrhosis, and 341 (41.3%) had alanine transarninase (ALT) levels > 41 U/L. Serum HBsAg levels in most patients (72.4%) were ≥ 4000 S/CO. HBV DNA levels in nearly half of the patients (44.6%) were < 2000 IU/mL. The tumor stage distribution according to the 7th edition of the AJCC/UICC staging system was as follows: stage I, 468 (56.7%); stage II, 221 (26.8%); stage III, 135 (16.3%); and stage IVA, 2 (0.2%). A total of 691 patients had accurate information on history of antiviral therapy. Of the 691 patients, 483 patients (69.9%) received antiviral therapy.

Cumulative incidence of HCC recurrence following curative resection

During a follow-up of 35.8 ± 9.9 months, 395 patients (47.8%) developed recurrent HCC.

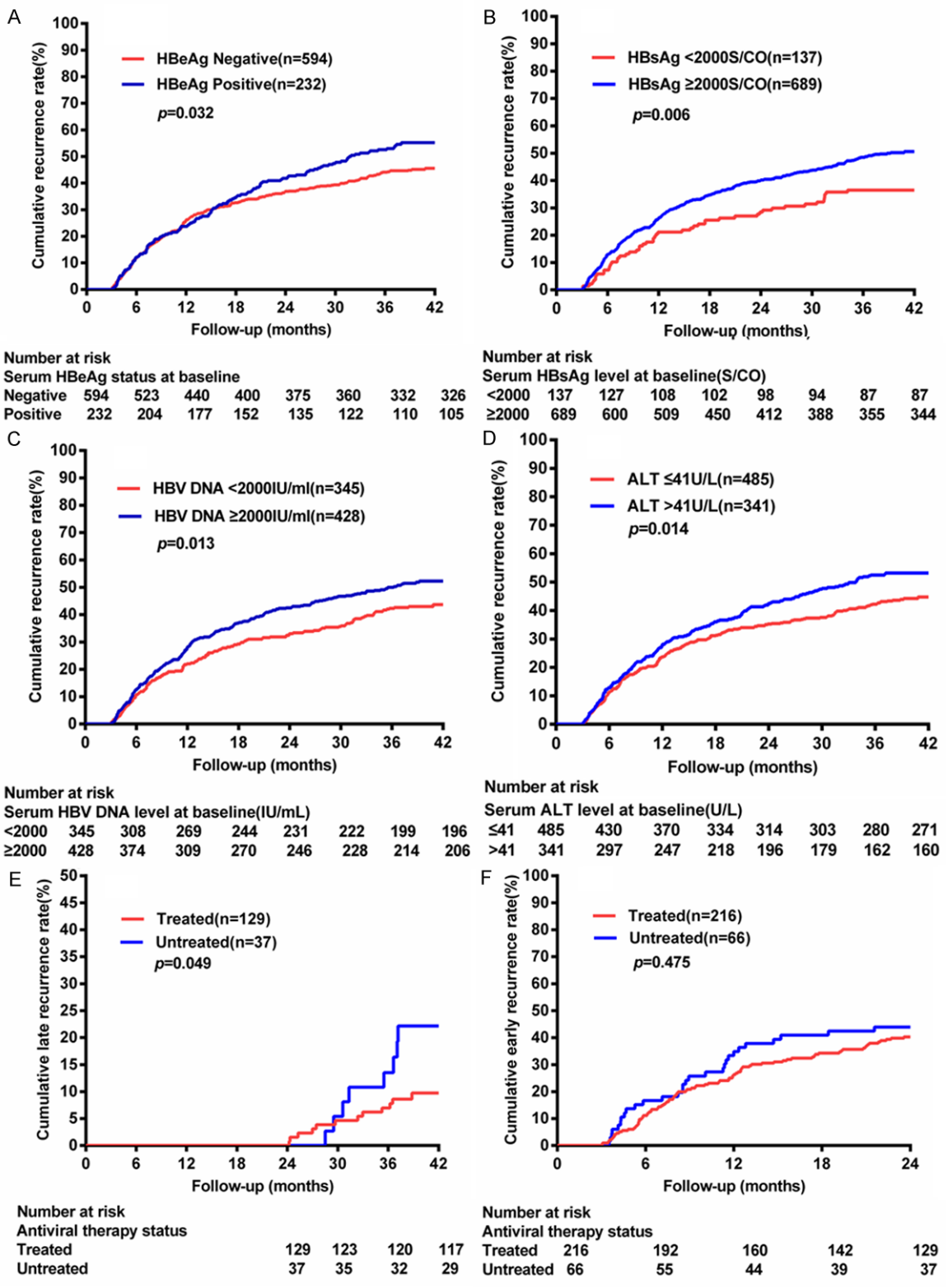


Figure 2. Cumulative incidences of hepatocellular carcinoma (HCC) recurrence following liver resection. (A-D) hepatitis B e antigen (HBeAg) seropositivity (A), and higher levels of serum hepatitis B surface antigen (HBsAg) (B), hepatitis B virus (HBV) viral load (C), and alanine transaminase (ALT) (D) were associated with a higher cumulative incidence of HCC recurrence. (E, F) Antiviral therapy was significantly associated with low risk of HCC late recurrence (E), but not HCC early recurrence (F).

Table 2. Univariate and multivariate analysis of factors associated with HCC recurrence

Factor	N	Recurrence rate (%)		Univariate analysis *(p value)*	Multivariate Analysis *(p value)*	Hazard ratio	95% CI
		1-year	3-year				
Gender				0.255	NA	NA	NA
Male	717	26.1	47.3				
Female	109	22.0	42.2				
Age				0.531	NA	NA	NA
< 50	361	26.6	48.8				
50-59	293	27.0	45.4				
≥ 60	172	20.9	44.2				
Cirrhosis				0.178	NA	NA	NA
Yes	534	25.8	48.5				
No	292	25.0	43.2				
Diabetes				0.441	NA	NA	NA
Yes	46	23.9	52.2				
No	780	25.6	46.3				
Metformin use[a]				0.574	NA	NA	NA
Yes	8	12.5	37.5				
No	818	25.7	46.7				
Anti-HBV therapy (n = 282)[b]				0.158	NA	NA	NA
Yes	216	26.4	44.4				
No	66	34.8	51.5				
Serum HBsAg level (S/CO)				0.006	0.026	1.538	1.054-2.243
< 2000	137	21.2	36.5				
≥ 2000	689	26.4	48.6				
Seropositive HBeAg				0.032	0.022	1.424	1.052-1.928
Negative	594	26.3	44.3				
Positive	232	23.7	52.6				
Serum HBV DNA level (IU/ml) (n = 773)[c]				0.013	0.910	1.017	0.761-1.359
< 2000	345	22.0	42.6				
≥ 2000	428	28.3	50.0				
ALT (U/L)				0.014	0.804	0.960	0.693-1.329
≤ 41	485	23.7	42.5				
> 41	341	28.2	52.5				
AST (U/L)				< 0.001	0.857	1.033	0.727-1.467
≤ 37	493	20.7	41.6				

> 37	333	32.7	54.1				
TBIL (μmol/L)				0.285	NA	NA	NA
≤ 18.8	680	24.9	45.7				
> 18.8	146	28.8	50.7				
γ-GT (U/L)				< 0.001	0.003	1.561	1.164-2.093
≤ 61	435	18.2	36.1				
> 61	391	33.8	58.3				
ALP (U/L)				0.036	0.053	0.648	0.417-1.006
≤ 129	729	24.0	45.5				
> 129	97	37.1	54.6				
AFP (μg/L)				0.020	0.546	1.092	0.821-1.453
≤ 20	335	17.9	43.0				
> 20	491	30.8	49.1				
ALB (g/L)				0.195	NA	NA	NA
< 34	14	14.3	28.6				
≥ 34	812	25.7	46.9				
Prealbumin (mg/L)				0.118	NA	NA	NA
< 170	211	33.2	50.7				
≥ 170	615	22.9	45.2				
PT (s)				0.043	0.025	1.451	1.047-2.011
≤ 13	704	25.1	45.0				
> 13	122	27.9	55.7				
WBC (*10^9/L)				0.676	NA	NA	NA
< 4	155	22.6	45.8				
≥ 4	671	26.2	46.8				
PLT (*10^9/L)				0.280	NA	NA	NA
< 100	153	26.1	51.6				
≥ 100	673	25.4	45.5				
Tumor number				< 0.001	0.003	1.621	1.183-2.222
single	677	21.0	42.2				
multiple	149	46.3	66.4				
Tumor size (cm)				< 0.001	0.012	1.219	1.044-1.424
≤ 3	259	13.1	35.1				
3~5	272	23.5	44.5				
5~10	216	33.3	56.9				
> 10	79	51.9	63.3				

Capsule formation				< 0.001	0.149	0.787	0.569-1.089
Yes	691	22.7	44.6				
No	135	40.0	57.0				
Edmonson grading				0.032	0.521	0.911	0.684-1.212
I	9	11.1	22.2				
II	199	13.1	39.7				
III	598	29.6	49.2				
IV	20	35.0	50.0				
Portal vein invasion				< 0.001	0.007	1.832	1.184-2.836
Yes	62	59.7	79.0				
No	764	22.8	44.0				
Microvascular invasion				< 0.001	0.083	1.285	0.968-1.705
Yes	261	41.0	58.6				
No	565	18.4	41.1				
Cutting margin (cm) (n = 590)[c]				0.001	0.119	0.735	0.499-1.082
< 1	478	25.9	46.7				
≥ 1	112	12.5	30.4				

HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; WBC, white blood cell; PLT, platelet; AFP, alpha-fetoprotein; TBIL, total bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALB, albumin; PT, prothrombin time; γ-GT, γ-glutamyl transpeptidase; ALP, alkaline phosphatase. [a]Drug users indicate patients using a drug at least 1 day pen month on average. [b]Only determined in patients with HBV DNA≥ 2000IU/mL and recieving antivirual therapy within 1 month pre-operation or within 3 months post-operation. [c]Number of available data.

Table 3. Comparison of clinicopathological features of HCC patients according to the serum HBsAg level

	HBsAg level (S/CO)		*p* value
	< 2000 (n = 137)	≥ 2000 (n = 689)	
Age (mean ± SD, years)	54.6 ± 10.4	50.4 ± 10.6	< 0.001
Cirrhosis (%)	72 (52.6)	462 (67.1)	0.001
Anti-HBV therapy (%) (n = 691)[a]			< 0.001
No	61 (52.1)	147 (25.6)	
Yes	56 (47.9)	427 (74.4)	
Seropositive HBeAg (%)	25 (18.2)	207 (30.0)	0.005
Serum HBV DNA level (IU/mL) (%) (n = 773)[a]			< 0.001
≥ 2000	36 (28.8)	392 (60.5)	
< 2000	89 (71.2)	256 (39.5)	
ALT (U/L)			0.010
> 41	43 (31.4)	298 (43.3)	
≤ 41	94 (68.6)	391 (56.7)	
AST (U/L)			0.020
> 37	43 (31.4)	290 (42.1)	
≤ 37	94 (68.6)	399 (57.9)	
Prealbumin (mg/L)			0.054
< 170	26 (19.0)	185 (26.9)	
≥ 170	111 (81.0)	504 (73.1)	

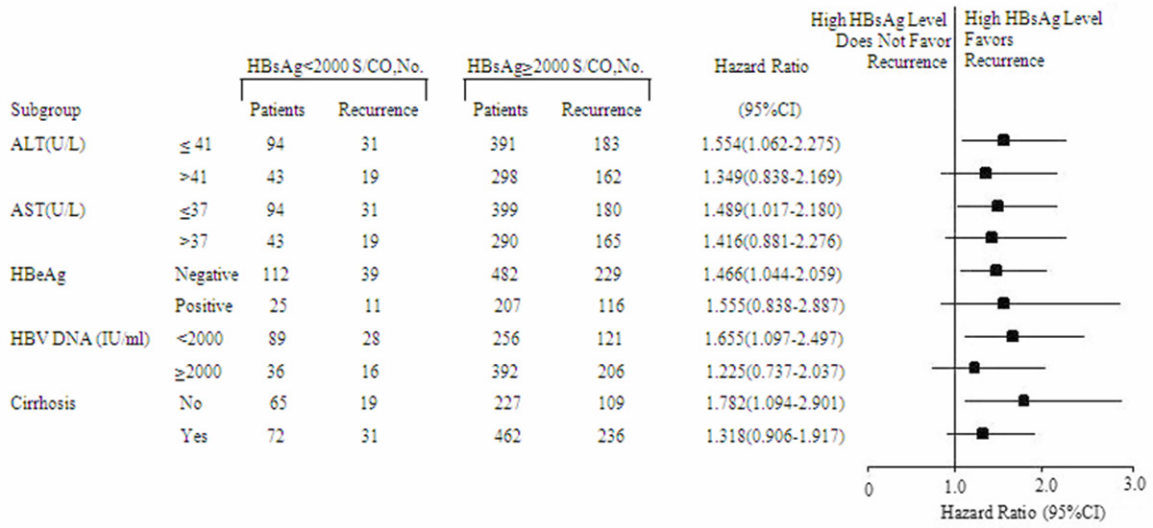
HCC: hepatocellular carcinoma; HBV: hepatitis B virus; HBsAg: hepatitis B surface antigen; HBeAg: hepatitis B e antigen; ALT: alanine transaminase; AST: aspartate aminotransferase.
[a]Number of available data.

Cumulative incidence of HCC recurrence at 1 year and 3 years after curative resection was 25.5% and 46.6%, respectively. Most recurrences (80.0%) clustered within two years of surgery (early recurrence). The tumor stage according to the 7th edition of the AJCC/UICC staging system was as follows: stage I, 468 (56.7%); stage II, 221 (26.8%); stage III, 135 (16.3%); and stage IVA, 2 (0.2%). The survival curves according to AJCC/UICC staging showed significant differences between stages I, II, III, and IV (p < 0.001). Univariate analysis revealed that HBeAg seropositivity, and higher levels of ALT, serum HBsAg, and HBV viral load were associated with a higher cumulative incidence of HCC recurrence (**Figure 2A-D**). In addition, other risk factors, including aspartate aminotransferase (AST) > 37 U/L,peptidase (GT) > 61 U/L, alkaline phosphatase (ALP) > 129 U/L, prothrombin time (PT) > 13 s, AFP > 20 μg/L, multinodularity, larger tumor size, cutting margin ≥ 1 cm, no capsule formation, portal vein invasion, microvascular invasion, and higher rank of Edmonson grading were found to be associated with HCC recurrence (**Table 2**). Those patients who received antiviral therapy tended to have a lower incidence of HCC recurrence than those who did not receive antiviral therapy, although the difference was not statistically significant ($p = 0.158$). In Supplementary Table 1, we stratified patients by the time of tumor recurrence. We found that antiviral therapy was significantly associated with a lower risk of HCC late recurrence (p = 0.049), but not early recurrence (p = 0.475) (**Figure 2E**, **2F**).

Multivariate analyses revealed that HBsAg level (95.0% CI: 1.054-2.243, $p = 0.026$), HBeAg status (95.0% CI: 1.052-1.928, $p = 0.022$), γ-GT (95.0% CI: 1.164-2.093, $p = 0.003$), PT (95.0% CI: 1.047-2.011, $p = 0.025$), tumor number (95.0% CI: 1.183-2.222, $p = 0.003$), tumor size (95.0% CI: 1.044-1.424, $p = 0.012$), and portal vein invasion (95.0% CI: 1.184-2.836, $p = 0.007$) were independent prognostic factors affecting cumulative incidence of HCC recurrence following curative resection (**Table 2**).

Correlation of serum HBsAg levels at time of resection with clinicopathological features

To further understand the impact of serum HBsAg levels at time of resection on HCC recurrence, the clinicopathological characteristics between HCC patients with HBsAg levels < 2000 S/CO and HCC patients with HBsAg levels ≥ 2000 S/CO were compared. Compared with HCC patients with lower HBsAg levels, those with HBsAg levels ≥ 2000 S/CO had a higher prevalence of seropositive HBeAg, history of antiviral therapy, and cirrhosis; were younger; and had higher levels of ALT, AST, and serum HBV viral load. There appeared to be a higher percentage of patients with prealbumin levels < 170 mg/L in the high-HBsAg group than in the low-HBsAg group, although the sta-



Subgroup		HBsAg<2000 S/CO,No. Patients	HBsAg<2000 S/CO,No. Recurrence	HBsAg≥2000 S/CO,No. Patients	HBsAg≥2000 S/CO,No. Recurrence	Hazard Ratio (95%CI)
ALT(U/L)	≤ 41	94	31	391	183	1.554(1.062-2.275)
	>41	43	19	298	162	1.349(0.838-2.169)
AST(U/L)	≤37	94	31	399	180	1.489(1.017-2.180)
	>37	43	19	290	165	1.416(0.881-2.276)
HBeAg	Negative	112	39	482	229	1.466(1.044-2.059)
	Positive	25	11	207	116	1.555(0.838-2.887)
HBV DNA (IU/ml)	<2000	89	28	256	121	1.655(1.097-2.497)
	≥2000	36	16	392	206	1.225(0.737-2.037)
Cirrhosis	No	65	19	227	109	1.782(1.094-2.901)
	Yes	72	31	462	236	1.318(0.906-1.917)

Figure 3. Multivariable stratified analyses on the association between HBsAg (hepatitis B surface antigen) level and HCC (hepatocellular carcinoma) recurrence. HCC patients with HBsAg levels < 2000 S/CO had lower cumulative incidences of recurrence than those with higher HBsAg levels when alanine transaminase (ALT) level was ≤ 41 U/L, aspartate aminotransferase (AST) level was ≤ 37 U/L, hepatitis B e antigen (HBeAg) status was seronegative, hepatitis B virus (HBV) DNA level was < 2000 IU/mL, or cirrhosis was absent.

tistical significance of the difference was just above the threshold (p = 0.054) (**Table 3**). Other factors including sex, hospital stay, diabetes, metformin use, PLT, WBC count, ALB, TBIL, γ-GT, ALP, and all the tumor-associated characteristics had no statistically significant difference between the two groups ($p > 0.05$) (Supplementary Table 2).

Multivariable stratified analysis

To eliminate confounding factors and further investigate the impact of perioperative HBsAg level on postoperative HCC recurrence, we stratified the patients according to HBeAg status, cirrhosis status, and levels of ALT, AST, and HBV viral load. We found that an HBsAg level < 2000 S/CO was associated with a reduced risk of HCC recurrence in the following categories: noncirrhotic (HR, 1.782; 95% CI, 1.094-2.901), HBV DNA < 2000 IU/mL (HR, 1.655; 95% CI, 1.097-2.497), ALT ≤ 41 U/L (HR, 1.554; 95% CI, 1.062-2.275), AST ≤ 37 U/L (HR, 1.489; 95% CI, 1.017-2.180), and seronegative HBeAg (HR, 1.466; 95% CI, 1.044-2.059) (**Figure 3**).

Factors affecting HCC recurrence risk in HBeAg-negative patients with low viral load

In HBeAg-negative patients with low viral load (HBV DNA level < 2000 IU/mL), univariate analysis showed that HCC recurrence was associated with diabetes, HBsAg level ≥ 2000 S/CO, AST level > 37 U/L, γ-GT level > 61 U/L, prealbumin level < 170 mg/L, multinodularity, larger tumor size, cutting margin ≥ 1 cm, lack of capsule formation, portal vein invasion, microvascular invasion, and higher rank of pTNM stage but not with ALT or HBV DNA level (**Figure 4A-C**). Of this population, noncirrhotic patients appeared to have a lower cumulative incidence of HCC recurrence than cirrhotic patients, although the difference was not statistically significant ($p = 0.098$) (**Table 4**). In HBeAg-negative and noncirrhotic patients with low viral load, we found that higher HBsAg level was associated with late recurrence of HCC ($p = 0.032$) (**Figure 4D**) but not with early recurrence. HBV DNA level was not associated with either early ($p = 0.206$) or late recurrence ($p = 0.557$).

Discussion

Although the association between HBsAg level and risk for HBV-related HCC development has been previously documented, the impact of HBsAg level on HBV-related HCC recurrence following curative resection remains unclear. In the present study, we not only showed that a higher level of HBsAg was associated with a higher cumulative incidence of postoperative

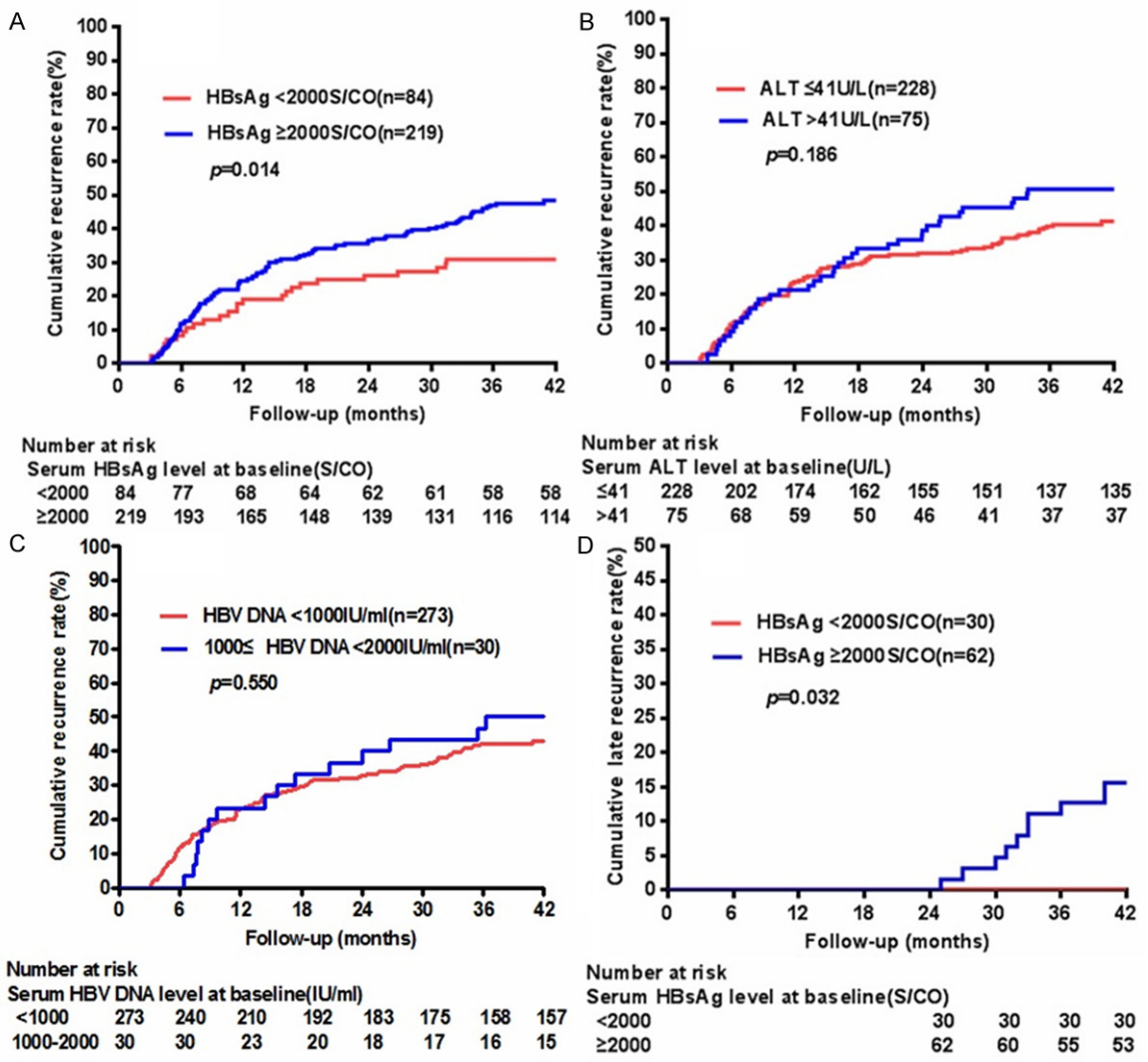


Figure 4. HCC recurrence in HBeAg-negative patients with low viral load. HCC recurrence was associated with HBsAg level ≥ 2000S/CO (A), but not with ALT (B) or HBV DNA level (C). (D) In HBeAg-negative and noncirrhotic patients with low viral load, higher HBsAg level was associated with late recurrence of HCC.

HCC recurrence but also found that HBsAg level among HBeAg-negative patients with low viral load, but not HBV DNA or ALT level, was still predictive of recurrence risk. These data suggested that HBsAg level might complement HBV DNA level in predicting HCC recurrence, especially in HBeAg-negative patients with low viral load.

Surgical resection has been an effective cure for some HCC patients. However, postoperative prognosis is poor because of the high recurrence rate. The 1- and 3-year cumulative incidences of HCC recurrence following surgical resection have been reported to be 9.4%-30.1% and 36.9%-62.3%, respectively [5, 6, 18-21]. These values are similar to those of our study. Many factors are reported to be associated with postoperative HCC recurrence, including hepatic functional parameters such as albumin level, PT, and Child-Pugh class; cirrhosis; and tumor characteristics such as AFP level, tumor number, tumor size, capsule formation, vascular invasion, Edmonson grading, and pTNM stage [4-7]. In the current study, we demonstrated that a higher cumulative incidence of postoperative HCC recurrence was associated with the following disease characteristics: elevated levels of ALT, AST, γ-GT, ALP, PT, and AFP, multinodularity, larger tumor size, cutting margin < 1 cm, lack of capsule formation, portal vein invasion, microvascular invasion, and higher rank of Edmonson grading or pTNM stage.

Table 4. Univariate and multivariate analysis of factors associated with HCC recurrence in HBeAg negative patients with low viral load

Factor	n	Recurrence rate (%)		Univariate analysis (*p value*)	Multivariate Analyses (*p* value)	Hazard ratio	95% CI
		1-year	3-year				
Gender				0.331	NA	NA	NA
Male	253	24.5	43.5				
Female	50	16.0	40.0				
Age				0.670	NA	NA	NA
< 50	118	22.9	39.8				
50-59	105	24.8	45.7				
≥ 60	80	21.3	43.8				
Cirrhosis				0.098	0.075	1.550	0.957-2.509
Yes	170	24.7	47.6				
No	133	21.1	36.8				
Diabetes				0.001	0.188	1.649	0.783-3.472
Yes	13	15.4	84.6				
No	290	23.4	41.0				
Serum HBsAg level (S/CO)				0.014	0.107	1.549	0.910-2.638
< 2000	84	19.0	31.0				
≥ 2000	219	24.7	47.5				
Serum HBV DNA level (IU/ml)				0.550	NA	NA	NA
< 1000	273	23.1	42.5				
1000-1999	30	23.3	46.7				
ALT (U/L)				0.186	NA	NA	NA
≤ 41	228	23.7	40.4				
> 41	75	21.3	50.7				
AST (U/L)				0.045	0.483	0.818	0.465-1.436
≤ 37	226	20.4	39.8				
> 37	77	31.2	51.9				
TBIL (μmol/L)				0.918	NA	NA	NA
≤ 18.8	252	22.2	43.3				
> 18.8	51	27.5	41.2				
γ-GT (U/L)				< 0.001	0.001	2.346	1.430-3.849
≤ 61	181	17.1	30.4				
> 61	122	32.0	61.5				
ALP (U/L)				0.103	NA	NA	NA
≤ 129	269	21.9	41.6				
> 129	34	32.4	52.9				
AFP (μg/L)				0.140	NA	NA	NA
≤ 20	135	14.8	40.0				
> 20	168	29.8	45.2				
ALB (g/L)				0.285	NA	NA	NA
< 34	2	0.0	0.0				
≥ 34	301	23.3	43.2				
Prealbumin (mg/L)				0.006	0.383	1.265	0.746-2.147
< 170	59	35.6	57.6				
≥ 170	244	20.1	39.3				
PT (s)				0.392	NA	NA	NA
≤ 13	270	21.9	42.2				
> 13	33	33.3	48.5				
WBC (*10^9/L)				0.825	NA	NA	NA
< 4	51	25.5	45.1				
≥ 4	252	22.6	42.5				

PLT (*10^9/L)				0.805	NA	NA	NA
< 100	44	25.0	45.5				
≥ 100	259	22.8	42.5				
Tumor number				< 0.001	0.941	1.020	0.606-1.716
single	250	19.2	38.4				
multiple	53	41.5	64.2				
Tumor size (cm)				< 0.001	0.230	1.178	0.901-1.541
≤ 3	95	8.4	30.5				
3~5	99	20.2	39.4				
5~10	82	35.4	56.1				
> 10	27	48.1	59.3				
Capsule formation				0.002	0.007	0.488	0.291-0.819
Yes	244	20.9	39.8				
No	59	32.2	55.9				
Edmonson grading				0.071	0.649	0.897	0.563-1.431
I	6	16.7	33.3				
II	70	10.0	32.9				
III	216	25.9	45.4				
IV	11	54.5	63.6				
Portal vein invasion				< 0.001	0.812	1.080	0.571-2.043
Yes	29	55.2	79.3				
No	274	19.7	39.1				
Microvascular invasion				< 0.001	0.001	2.215	1.367-3.589
Yes	104	42.3	58.7				
No	199	13.1	34.7				
Cutting margin (cm) (n = 224)[a]				0.004	0.067	0.470	0.210-1.055
< 1	187	25.7	45.5				
≥ 1	37	10.8	18.9				

HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; WBC, white blood cell; PLT, platelet; AFP, alpha-fetoprotein; TBIL, total bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALB, albumin; PT, prothrombin time; γ-GT, γ-glutamyl transpeptidase; ALP, alkaline phosphatase. [a]Number of available data.

Recently, the roles of virological factors such as HBV viral load, HBeAg status, and antiviral therapy in HCC recurrence have also been investigated [8-11]. Kubo et al first reported that high viral load is an independent risk factor for HCC recurrence after liver resection in HBV-related HCC patients [22]. Hung et al found that HBV viral load of > 2000 IU/mL is associated with HCC recurrence after liver resection with an odds ratio as high as 22.3 [11]. Our data also suggested that higher HBV viral load is significantly associated with an increased risk of postoperative HBV-related HCC recurrence. In addition, Sun et al reported that seropositive HBeAg is associated with higher risk of early recurrence and is an independent risk factor for postoperative recurrence of HBV-related HCC [23], and a recent meta-analysis study further confirmed the result [24]. Our data also demonstrated that HBeAg positivity is a significant independent risk factor for HCC recurrence. Nucleoside analogues have been shown to be associated with a lower risk of HCC and other cirrhosis-related complications in those with chronic hepatitis and cirrhosis [25-28]. Several recent studies further demonstrated that nucleoside analogues are associated with a lower risk of recurrence among patients with HBV-related HCC after liver resection [8-10]. Our data suggested that nucleoside analogues may significantly reduce the risk of HCC late recurrence and further confirmed that antiviral therapy may play an important role in reducing HCC recurrence after curative resection.

HBsAg is produced by HBV and serves as an important serological marker for the diagnosis and monitoring of patients with chronic hepatitis B. Recently, HBsAg quantification has become increasingly recognized as an important method for evaluating viral activity and possible host immune control over HBV infec-

tion as well as predicting HBsAg loss and virological response to antiviral therapy [12, 14, 17, 29-32]. A lower HBsAg level was shown to be associated with a higher chance of HBsAg loss and lower risk of hepatitis activity in patients with HBV genotype B or C infection [15, 17]. There is also a known positive correlation between HBsAg and HBV DNA levels [16]. This correlation has been shown to be higher at the HBeAg-positive phase, lower at the HBeAg-negative phase, and lowest at the lowly replicative phase [13, 14]. In our cohort, HCC patients with higher levels of HBsAg also had a higher prevalence of seropositive HBeAg as well as a higher level of HBV DNA and hepatitis activity. Another recent study suggested that a high level of HBsAg may increase the risk of HCC in patients with low HBV load [16]. Because a higher HBsAg level usually signifies a worse prognosis, it is of clinical interest to know whether a higher HBsAg level would also indicate a higher risk of HCC recurrence. Very recently, Sohn et al first reported that HBsAg levels were associated with late recurrence after curative resection in HBV-related HCC [33]. Huang et al further found a preoperative HBsAg level of 1000 IU/mL or greater is an independent risk factor for HCC recurrence in patients with low HBV DNA levels. In this study, we also found that a higher HBsAg level was an independent risk factor associated with HCC recurrence after curative resection [34]. Multivariable stratified analyses showed that HCC patients with higher HBsAg levels tended to have a higher incidence of HCC recurrence in several subgroups of patients with the following characteristics: noncirrhotic; low viral load; normal ALT or AST; and seronegative HBeAg. These observations further confirmed the association between higher levels of HBsAg and increased risk of HCC recurrence in HBV-related HCC patients after tumor resection. Furthermore, when we evaluated patients with negative HBeAg and low viral load, HBsAg level was still predictive of recurrence risk, while HBV DNA or ALT levels were not. These data suggested that HBsAg level might complement HBV DNA level in predicting HCC recurrence, especially in HBeAg-negative patients with low viral load.

There are several limitations to the present study. First, the unit of HBsAg level is S/CO in our study instead of IU/mL. Thus, we could not use the cutoff value of ≥ 1000 IU/mL, the usefulness of which has been confirmed in previous studies [15, 16, 35, 36]. We recalculated the cutoff level of HBsAg with the unit of S/CO based on the ROC curves and found that Youden's index (YI) reached a maximum near HBsAg = 2000 S/CO. Second, we did not exclude patients who received antiviral therapy when we determined whether higher levels of HBsAg were associated with increased risk of recurrence in HBeAg-negative patients with low viral load. In this study, more patients in the high-HBsAg cohort had received antiviral therapy than in the low-HBsAg cohort. Because antiviral therapy is associated with lower risk of HCC recurrence, the higher baseline HBsAg level in the patients who received antiviral therapy may have led to a more conservative estimation of the association in the present study.

In conclusion, we found that higher levels of HBsAg were associated with a higher risk of HCC recurrence among patients with HBV-related HCC after curative resection. Among HBeAg-negative patients with low viral load, HBsAg level was still predictive of recurrence risk, but HBV DNA and ALT levels were not. Therefore, HBsAg level may be used as an additional marker to complement HBV DNA level in predicting HCC recurrence, especially in HBeAg-negative patients with low viral load.

Acknowledgements

This project is supported by National key scientific instrument and equipment development grant 2012YQ220113 (H.H.) and Natural Science Foundation of China (NSFC) grants 81372672 (H.H.) after highlight test.

Disclosure of conflict of interest

None to disclose.

Address correspondence to: He-Ping Hu, Department of Hepatobiliary I, Eastern Hepatobiliary Surgery Hospital, Second Military Medical University, Shanghai, China. Tel: +86-21-81875141; Fax: +86-21-81875141; E-mail: hp-hu@medmail.com.cn

References

[1] Chen CJ, Yang HI, Su J, Jen CL, You SL, Lu SN, Huang GT, Iloeje UH. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA 2006; 295: 65-73.

[2] Cho YK, Rhim H, Noh S. Radiofrequency ablation versus surgical resection as primary treatment of hepatocellular carcinoma meeting the

Milan criteria: a systematic review. J Gastroenterol Hepatol 2011; 26: 1354-1360.
[3] EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. J Hepatol 2012; 56: 908-943.
[4] Wu JC, Huang YH, Chau GY, Su CW, Lai CR, Lee PC, Huo TI, Sheen IJ, Lee SD, Lui WY. Risk factors for early and late recurrence in hepatitis B-related hepatocellular carcinoma. J Hepatol 2009; 51: 890-897.
[5] Imamura H, Matsuyama Y, Tanaka E, Ohkubo T, Hasegawa K, Miyagawa S, Sugawara Y, Minagawa M, Takayama T, Kawasaki S, Makuuchi M. Risk factors contributing to early and late phase intrahepatic recurrence of hepatocellular carcinoma after hepatectomy. J Hepatol 2003; 38: 200-207.
[6] Shah SA, Cleary SP, Wei AC, Yang I, Taylor BR, Hemming AW, Langer B, Grant DR, Greig PD, Gallinger S. Recurrence after liver resection for hepatocellular carcinoma: risk factors, treatment, and outcomes. Surgery 2007; 141: 330-339.
[7] Portolani N, Coniglio A, Ghidoni S, Giovanelli M, Benetti A, Tiberio GA, Giulini SM. Early and late recurrence after liver resection for hepatocellular carcinoma: prognostic and therapeutic implications. Ann Surg 2006; 243: 229-235.
[8] Wu CY, Chen YJ, Ho HJ, Hsu YC, Kuo KN, Wu MS, Lin JT. Association between nucleoside analogues and risk of hepatitis B virus-related hepatocellular carcinoma recurrence following liver resection. JAMA 2012; 308: 1906-1914.
[9] Chuma M, Hige S, Kamiyama T, Meguro T, Nagasaka A, Nakanishi K, Yamamoto Y, Nakanishi M, Kohara T, Sho T, Yamamoto K, Horimoto H, Kobayashi T, Yokoo H, Matsushita M, Todo S, Asaka M. The influence of hepatitis B DNA level and antiviral therapy on recurrence after initial curative treatment in patients with hepatocellular carcinoma. J Gastroenterol 2009; 44: 991-999.
[10] Wong JS, Wong GL, Tsoi KK, Wong VW, Cheung SY, Chong CN, Wong J, Lee KF, Lai PB, Chan HL. Meta-analysis: the efficacy of anti-viral therapy in prevention of recurrence after curative treatment of chronic hepatitis B-related hepatocellular carcinoma. Aliment Pharmacol Ther 2011; 33: 1104-1112.
[11] Hung IF, Poon RT, Lai CL, Fung J, Fan ST, Yuen MF. Recurrence of hepatitis B-related hepatocellular carcinoma is associated with high viral load at the time of resection. Am J Gastroenterol 2008; 103: 1663-1673.
[12] Brunetto MR. A new role for an old marker, HBsAg. J Hepatol 2010; 52: 475-477.
[13] Nguyen T, Thompson AJ, Bowden S, Croagh C, Bell S, Desmond PV, Levy M, Locarnini SA. Hepatitis B surface antigen levels during the natural history of chronic hepatitis B: a perspective on Asia. J Hepatol 2010; 52: 508-513.
[14] Jaroszewicz J, Calle SB, Wursthorn K, Deterding K, Schlue J, Raupach R, Flisiak R, Bock CT, Manns MP, Wedemeyer H, Cornberg M. Hepatitis B surface antigen (HBsAg) levels in the natural history of hepatitis B virus (HBV)-infection: a European perspective. J Hepatol 2010; 52: 514-522.
[15] Brunetto MR, Oliveri F, Colombatto P, Moriconi F, Ciccorossi P, Coco B, Romagnoli V, Cherubini B, Moscato G, Maina AM, Cavallone D, Bonino F. Hepatitis B surface antigen serum levels help to distinguish active from inactive hepatitis B virus genotype D carriers. Gastroenterology 2010; 139: 483-490.
[16] Tseng TC, Liu CJ, Yang HC, Su TH, Wang CC, Chen CL, Kuo SF, Liu CH, Chen PJ, Chen DS, Kao JH. High levels of hepatitis B surface antigen increase risk of hepatocellular carcinoma in patients with low HBV load. Gastroenterology 2012; 142: 1140-1149, e13-e14.
[17] Tseng TC, Liu CJ, Su TH, Wang CC, Chen CL, Chen PJ, Chen DS, Kao JH. Serum hepatitis B surface antigen levels predict surface antigen loss in hepatitis B e antigen seroconverters. Gastroenterology 2011; 141: 517-525, 521-525.
[18] Chen MS, Li JQ, Zheng Y, Guo RP, Liang HH, Zhang YQ, Lin XJ, Lau WY. A prospective randomized trial comparing percutaneous local ablative therapy and partial hepatectomy for small hepatocellular carcinoma. Ann Surg 2006; 243: 321-328.
[19] Huang J, Yan L, Cheng Z, Wu H, Du L, Wang J, Xu Y, Zeng Y. A randomized trial comparing radiofrequency ablation and surgical resection for HCC conforming to the Milan criteria. Ann Surg 2010; 252: 903-912.
[20] Feng K, Yan J, Li X, Xia F, Ma K, Wang S, Bie P, Dong J. A randomized controlled trial of radiofrequency ablation and surgical resection in the treatment of small hepatocellular carcinoma. J Hepatol 2012; 57: 794-802.
[21] Vivarelli M, Guglielmi A, Ruzzenente A, Cucchetti A, Bellusci R, Cordiano C, Cavallari A. Surgical resection versus percutaneous radiofrequency ablation in the treatment of hepatocellular carcinoma on cirrhotic liver. Ann Surg 2004; 240: 102-107.
[22] Kubo S, Hirohashi K, Tanaka H, Tsukamoto T, Shuto T, Yamamoto T, Ikebe T, Wakasa K, Nishiguchi S, Kinoshita H. Effect of viral status on recurrence after liver resection for patients with hepatitis B virus-related hepatocellular carcinoma. Cancer 2000; 88: 1016-1024.
[23] Sun HC, Zhang W, Qin LX, Zhang BH, Ye QH, Wang L, Ren N, Zhuang PY, Zhu XD, Fan J, Tang ZY. Positive serum hepatitis B e antigen is as-

sociated with higher risk of early recurrence and poorer survival in patients after curative resection of hepatitis B-related hepatocellular carcinoma. J Hepatol 2007; 47: 684-690.

[24] Qu LS, Zhu J, Chen H, Jin F, Ni RZ, Lu CH. Effects of hepatitis B e-antigen on recurrence of hepatitis B-related hepatocellular carcinoma after curative resection: A meta-analysis. Hepatol Res 2013; 43: 347-354.

[25] Wong GL, Chan HL, Mak CW, Lee SK, Ip ZM, Lam AT, Iu HW, Leung JM, Lai JW, Lo AO, Chan HY, Wong VW. Entecavir treatment reduces hepatic events and deaths in chronic hepatitis B patients With liver cirrhosis. Hepatology 2013; 58: 1537-47.

[26] Hosaka T, Suzuki F, Kobayashi M, Seko Y, Kawamura Y, Sezaki H, Akuta N, Suzuki Y, Saitoh S, Arase Y, Ikeda K, Kobayashi M, Kumada H. Long-term entecavir treatment reduces hepatocellular carcinoma incidence in patients with hepatitis B virus infection. Hepatology 2013; 58: 98-107.

[27] Lai CL, Yuen MF. Prevention of hepatitis B virus-related hepatocellular carcinoma with antiviral therapy. Hepatology 2013; 57: 399-408.

[28] Huang G, Lai EC, Lau WY, Zhou WP, Shen F, Pan ZY, Fu SY, Wu MC. Posthepatectomy HBV reactivation in hepatitis B-related hepatocellular carcinoma influences postoperative survival in patients with preoperative low HBV-DNA levels. Ann Surg 2013; 257: 490-505.

[29] Manesis EK, Hadziyannis ES, Angelopoulou OP, Hadziyannis SJ. Prediction of treatment-related HBsAg loss in HBeAG-negative chronic hepatitis B: a clue from serum HBsAg levels. Antivir Ther 2007; 12: 73-82.

[30] Brunetto MR, Moriconi F, Bonino F, Lau GK, Farci P, Yurdaydin C, Piratvisuth T, Luo K, Wang Y, Hadziyannis S, Wolf E, McCloud P, Batrla R, Marcellin P. Hepatitis B virus surface antigen levels: a guide to sustained response to peginterferon alfa-2a in HBeAg-negative chronic hepatitis B. Hepatology 2009; 49: 1141-1150.

[31] Moucari R, Mackiewicz V, Lada O, Ripault MP, Castelnau C, Martinot-Peignoux M, Dauvergne A, Asselah T, Boyer N, Bedossa P, Valla D, Vidaud M, Nicolas-Chanoine MH, Marcellin P. Early serum HBsAg drop: a strong predictor of sustained virological response to pegylated interferon alfa-2a in HBeAg-negative patients. Hepatology 2009; 49: 1151-1157.

[32] Tangkijvanich P, Komolmit P, Mahachai V, Sa-Nguanmoo P, Theamboonlers A, Poovorawan Y. Comparison between quantitative hepatitis B surface antigen, hepatitis B e-antigen and hepatitis B virus DNA levels for predicting virological response to pegylated interferon-alpha-2b therapy in hepatitis B e-antigen-positive chronic hepatitis B. Hepatol Res 2010; 40: 269-277.

[33] Sohn W, Paik YH, Kim JM, Kwon CH, Joh JW, Cho JY, Gwak GY, Choi MS, Lee JH, Koh KC, Paik SW, Yoo BC. HBV DNA and HBsAg levels as risk predictors of early and late recurrence after curative resection of HBV-related hepatocellular carcinoma. Ann Surg Oncol 2014; 21: 2429-2435.

[34] Huang G, Lau WY, Zhou WP, Shen F, Pan ZY, Yuan SX, Wu MC. Prediction of Hepatocellular Carcinoma Recurrence in Patients With Low Hepatitis B Virus DNA Levels and High Preoperative Hepatitis B Surface Antigen Levels. JAMA Surg 2014; [Epub ahead of print].

[35] Yang HI, Sherman M, Su J, Chen PJ, Liaw YF, Iloeje UH, Chen CJ. Nomograms for risk of hepatocellular carcinoma in patients with chronic hepatitis B virus infection. J Clin Oncol 2010; 28: 2437-2444.

[36] Yang HI, Yuen MF, Chan HL, Han KH, Chen PJ, Kim DY, Ahn SH, Chen CJ, Wong VW, Seto WK. Risk estimation for hepatocellular carcinoma in chronic hepatitis B (REACH-B): development and validation of a predictive score. Lancet Oncol 2011; 12: 568-574.

Supplementary Table 1. Antiviral therapy and early and late recurrence of HCC following curative resection

Antiviral therapy	Early recurrence rate (%)				Late recurrence rate (%)		
	n	1-year	2-year	*p*	n	3-year	*p*
Yes	216	26.4	40.3	0.475	129	7.0	0.049
No	66	34.8	43.9		37	13.5	

HCC: hepatocellular carcinoma.

Supplementary Table 2. Comparison of clinicopathological features of HCC patients according to the serum HBsAg level

	HBsAg level (S/CO)		
	< 2000 (n = 137)	≥ 2000 (n = 689)	*p* value
Gender (M/F)	117/20	600/89	0.595
Hospital stay (days)	15.7 ± 6.4	15.1 ± 4.4	0.197
Diabetes (%)	4 (2.9)	42 (6.1)	0.139
Metformin use (%)[a]	0 (0.0)	8 (1.2)	0.365
TBIL (μmol/L)			0.587
> 18.8	22 (16.1)	124 (18.0)	
≤ 18.8	115 (83.9)	565 (82.0)	
r-GT (U/L)			0.141
> 61	57 (41.6)	334 (48.5)	
≤ 61	80 (58.4)	355 (51.5)	
ALP (U/L)			0.752
> 129	15 (10.9)	82 (11.9)	
≤ 129	122 (89.1)	607 (88.1)	
AFP (μg/L)			0.300
> 20	76 (55.5)	415 (60.2)	
≤ 20	61 (44.5)	274 (39.8)	
ALB (g/L)			0.486
< 34	1 (0.7)	13 (1.9)	
≥ 34	136 (99.3)	676 (98.1)	
PT (s)			0.951
> 13	20 (14.6)	102 (14.8)	
≤ 13	117 (85.4)	587 (85.2)	
WBC (*10^9/L)			0.108
< 4.0	19 (13.9)	136 (19.7)	
≥ 4.0	118 (86.1)	553 (80.3)	
PLT (*10^9/L)			0.195
< 100.0	20 (14.6)	133 (19.3)	
≥ 100.0	117 (85.4)	556 (80.7)	
Tumor number			0.165
single	118 (86.1)	559 (81.1)	
multiple	19 (13.9)	130 (18.9)	
Tumor size (cm)			0.879
≤ 3	45 (32.8)	214 (31.1)	
3~5	43 (31.4)	229 (33.2)	
5~10	34 (24.8)	182 (26.4)	
> 10	15 (10.9)	64 (9.3)	

Capsule formation	109 (79.6)	582 (84.5)	0.156
Edmonson grading			0.619
I	2 (1.5)	7 (1.0)	
II	29 (21.2)	170 (24.7)	
III	104 (75.9)	494 (71.7)	
IV	2 (1.5)	18 (2.6)	
Portal vein invasion	14 (10.2)	48 (7.0)	0.187
Microvascular invasion	46 (33.6)	215 (31.2)	0.585
Cutting margin (cm) (n = 590)[a]			0.534
< 1	82 (78.8)	396 (81.5)	
≥ 1	22 (21.2)	90 (18.5)	
pTNM stage			0.575
I	78 (56.9)	390 (56.6)	
II	33 (24.1)	188 (27.3)	
III	25 (18.2)	110 (16.0)	
IVA	1 (0.7)	1 (0.1)	

HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; WBC, white blood cell; PLT, platelet; AFP, alpha-fetoprotein; TBIL, total bilirubin; ALB, albumin; PT, prothrombin time; γ-GT, γ-glutamyl transpeptidase; ALP, alkaline phosphatase. [a]Number of available data.