Ann Surg Oncol (2015) 22:843–850
DOI 10.1245/s10434-014-4043-5

Annals of
SURGICAL ONCOLOGY
OFFICIAL JOURNAL OF THE SOCIETY OF SURGICAL ONCOLOGY

ORIGINAL ARTICLE – HEPATOBILIARY TUMORS

High Levels of Hepatitis B Surface Antigen are Associated with Poorer Survival and Early Recurrence of Hepatocellular Carcinoma in Patients with Low Hepatitis B Viral Loads

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ABSTRACT

Purpose. Recurrence is a disastrous outcome in patients with hepatitis-related hepatocellular carcinoma (HCC) who have undergone curative resection, and little is known about whether high levels of hepatitis B surface antigen (HBsAg) increase the risk of HCC recurrence.

Patients and Methods. This retrospective study included 1,360 HBsAg-positive postoperative HCC patients with hepatitis B viral (HBV) DNA levels < 2000 IU/mL, including 298 patients in a training cohort and 1,062 patients in a validation cohort. The prognostic value of the HBsAg level was evaluated using Cox regression and Kaplan–Meier analyses.

Results. We demonstrated that 1,000 IU/mL, but not 10 or 100 IU/mL, was a meaningful cutoff level for significantly discriminating these patients into an $HBsAg^{Low}$ group and an $HBsAg^{High}$ group based on correlations between the HBsAg level and liver cirrhosis ($p = 0.028$), tumor size ($p = 0.039$), and hepatitis B e antigen level ($p < 0.001$). The postoperative 1-, 3-, and 5-year overall survival (OS) rates of HCC patients in the $HBsAg^{Low}$ group were significantly higher than those of HCC patients in the $HBsAg^{High}$ group. Accordingly, the 5-year recurrence-free survival (RFS) rates of patients in the $HBsAg^{Low}$ group were markedly higher than those of HCC patients in the $HBsAg^{High}$ group. The HBsAg level was a prognostic indicator for OS ($p = 0.014$) and RFS ($p = 0.01$).

Conclusion. HBsAg level is correlated with more aggressive tumor behavior and serves as a prognostic indicator in patients with surgically resected HCC with low HBV load.

Liver cancer is one of the most commonly diagnosed neoplasms and is the most frequent cause of cancer death. Hepatocellular carcinoma (HCC) accounts for 70–85 % of the total liver cancer burden.[1] Hepatic resection remains the predominant treatment of choice for HCC in individuals without cirrhosis; however, 5 years after surgery, 70 % of cases are complicated by tumor recurrence, comprising true recurrence and de novo tumors.[2] Therefore, the classification of patients who have undergone radical hepatic resection is important for improving the postoperative prognosis.

Chronic infection with hepatitis B virus (HBV) is the primary risk factor in 80 % of HCC cases arising in eastern Asia.[2] HBV-related carcinogenesis is a multistep process involving numerous effects.[3] The large hepatitis B surface antigen glycoprotein has been shown to promote the proliferation of hepatoma and hepatic cells by activating the Src/PI3 K/Akt pathway.[4] HBV reactivation is a major risk factor for HCC recurrence,[5–7] and postoperative antiviral

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Electronic supplementary material The online version of this article (doi:10.1245/s10434-014-4043-5) contains supplementary material, which is available to authorized users.

First Received: 9 April 2014;
Published Online: 1 October 2014

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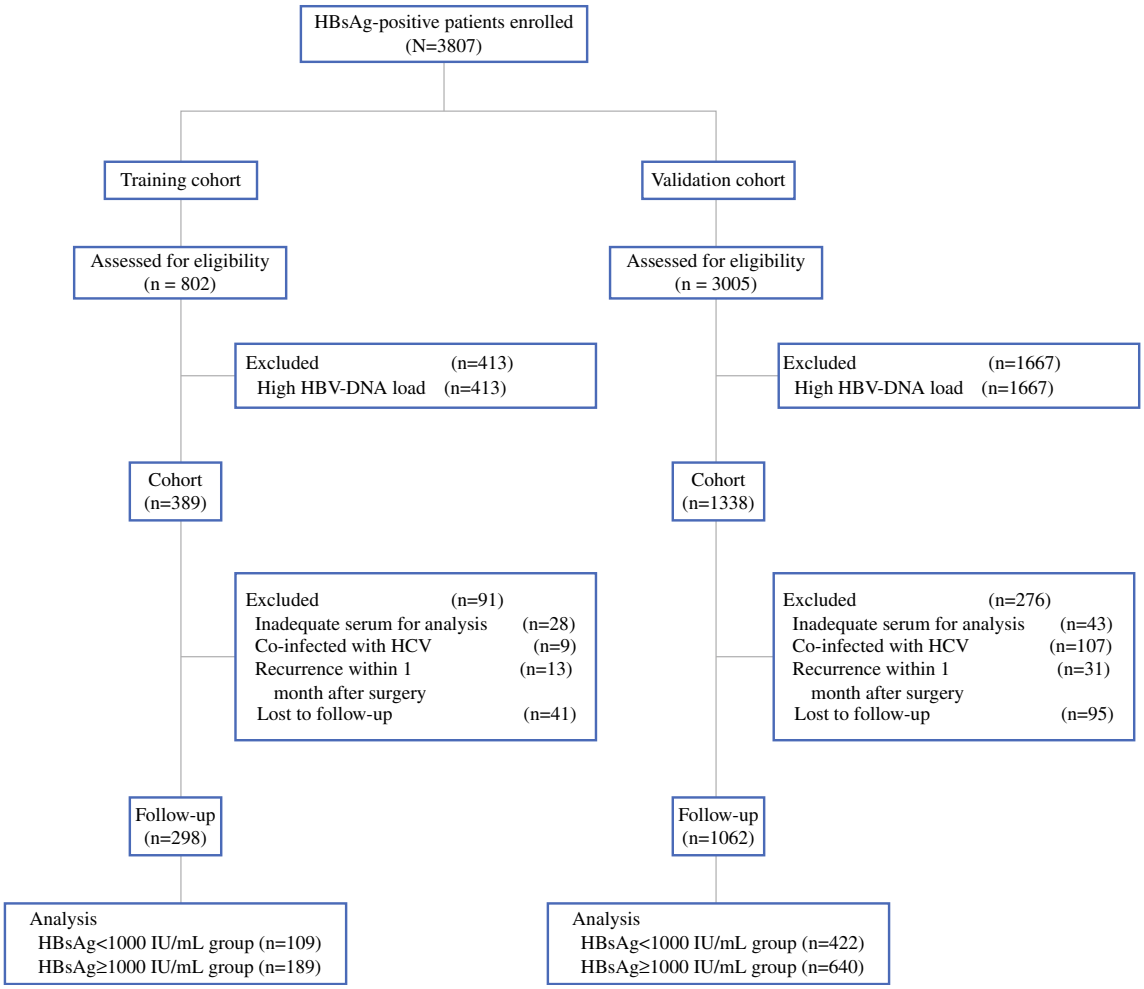

FIG. 1 Flowchart of study participants. *HBsAg* hepatitis B surface antigen, *HBV* hepatitis B virus, *HCV* hepatitis C virus

treatment has been demonstrated to improve the postoperative prognosis by reducing the viral load and relieving hepatic inflammation, resulting in better liver function and decreased HCC recurrence and mortality.[8] Nucleoside analogs may promote postoperative viral clearance and increased residual liver volume.[9]

Until now, HBsAg quantification, which reflects the concentration of covalently closed circular DNA, has increasingly been considered a marker for evaluating viral replication and predicting therapy outcome.[10–14] The combination of low HBV DNA (<2,000 IU/mL) and low HBsAg levels (<1,000 IU/mL) can predict an inactive carrier status and a low risk of HCC.[15] A lower HBsAg level usually indicates a better prognosis, and an HBV DNA level ≥ 2,000 copies/mL is significantly associated with decreases in overall survival (OS) and recurrence-free survival (RFS).[16] Therefore, for patients with low viral loads (HBV DNA < 2,000 IU/mL), it is clinically important to determine whether a higher HBsAg level is associated with a higher risk of recurrence in HCC patients after curative resection.

To obtain insights into this interesting issue, this study at Zhongshan Hospital of Fudan University enrolled two large cohorts—a training cohort that included 802 patients, and a validation cohort that included 3,005 patients. The primary aim of our study was to explore whether the HBsAg level could predict HCC recurrence in patients with low HBV DNA loads.

PATIENTS AND METHODS

Patient Cohort

Figure 1 shows the inclusion and exclusion process for enrolling patients in our study. Ethical approval was obtained from the Zhongshan Hospital Research Ethics Committee, and written informed consent was obtained from each patient.

Data Collection

All patients underwent serological testing 1 week before surgery to determine the HBsAg, hepatitis B e antigen (HbeAg), anti-HBe, and anti-hepatitis C virus levels (Abbott Laboratories, Abbott Park, IL, USA), the HBV DNA load (Roche Diagnostics, Branchburg, NJ, USA), the α-fetoprotein (AFP) level, and liver function. The HBV genotype was determined using a real-time polymerase chain reaction (PCR)-based single-tube assay, as previously described.[17]

Clinicopathological Factors

Clinicopathological factors were based on our previous studies.[18] The clinicopathologic characteristics of the training cohort are summarized in supplementary Table S1. The follow-up data were summarized at the end of December 2011, with a median observation time of 52.2 months.

Follow-Up

The follow-up procedures were described in our previous study.[19]

Statistical Analyses

Preoperative clinical data, intraoperative and pathological findings, postoperative mortality, OS, and RFS were compared between the $HBsAg^{Low}$ and $HBsAg^{High}$ groups. A Chi square test or Fisher's exact test was performed to compare qualitative variables, survival was determined using the Kaplan–Meier method, and survival curves between different groups were calculated using the log-rank test. A Cox proportional hazards regression model was adopted for univariate and multivariate analyses, and a multivariate analysis was performed using a Cox regression model with a forward stepwise procedure. The statistical analyses were performed using SPSS 19.0 for Windows (IBM Corporation, Armonk, NY, USA). Medcalc (version 9.2.0.1; MedCalc, Mariakerke, Belgium) software was used to perform ROC and regression analysis. Probabilities with a two-tailed p value less than 0.05 were considered statistically significant.

RESULTS

Baseline Characteristics

Supplementary Table S1 shows the demographics of the 298 patients in the training cohort. Of these patients, 248 (83.2 %) were males; 53 (17.8 %) had an alanine aminotransferase (ALT) level > 42 U/L; 142 (47.7 %) had a GGT level > 54 U/L; 99 (31.2 %) were HBeAg-positive; and 228 (76.5 %) were infected with genotype B virus (data not shown).

TABLE 1 Correlation between the HBsAg level and clinicopathologic characteristics of the training cohort

Variable	HBsAg		p value[a]
	<1000 IU/ml	≥1000 IU/ml	
Sex			
Female	17	33	0.678
Male	92	156	
Age (years)			
≤50	37	86	0.051
>50	72	103	
AFP			
≤20	40	64	0.621
>20	69	125	
ALT			
≤42	95	150	0.090
>42	14	39	
GGT (U/L)			
≤54	65	91	0.056
>54	44	98	
Liver cirrhosis			
No	23	22	0.028
Yes	86	167	
Tumor size (cm)			
≤5	62	130	0.039
>5	47	59	
Tumor encapsulation			
Complete	67	99	0.128
None	42	90	
Microvascular invasion			
Absence	67	136	0.061
Present	42	53	
Tumor differentiation			
I + II	82	137	0.605
III + IV	27	52	
TNM stage			
I	90	148	0.377
II + III	19	41	
HBeAg[b]			
Negative	89	115	<0.001
Positive	20	73	

HBsAg hepatitis B surface antigen, *AFP* α-fetoprotein, *GGT* γ-glutamyl transferase, *ALT* alanine aminotransferase, *TNM* tumor-node-metastasis, *HBeAg* hepatitis B e antigen

[a] A p value < 0.05 was considered statistically significant. p values were calculated using the Pearson Chi square test

[b] One patient with no HBeAg information was not calculated

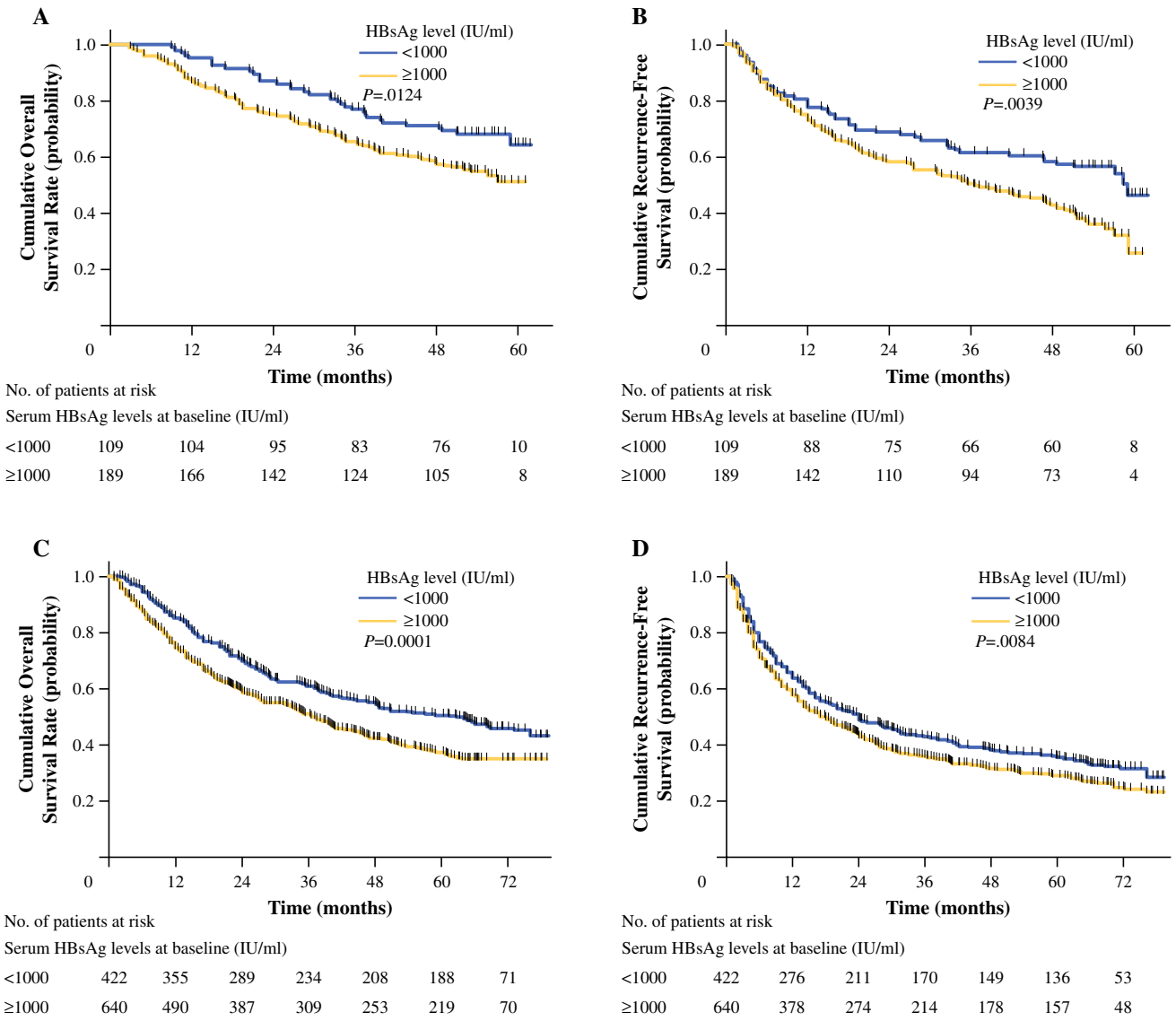

FIG. 2 Kaplan–Meier analysis of patient survival in the training and validation cohorts according to HBsAg level. **a** Comparison of overall survival rates between patients with an HBsAg level <1000 and ≥1000 IU/mL in the training cohort. **b** Recurrence-free survival between patients with an HBsAg level <1000 and ≥1000 IU/mL in the training cohort. **c** Overall survival between patients with an HBsAg level <1000 and ≥1000 IU/mL in the validation cohort. **d** Recurrence-free survival between patients with an HBsAg level <1000 and ≥1000 IU/mL in the validation cohort. *HBsAg* hepatitis B surface antigen

Correlations Between Hepatitis B Surface Antigen (HbsAg) Level and Clinicopathologic Characteristics

The correlations between patients' HBsAg levels and clinicopathologic characteristics are shown in Table 1. In the training cohort, the HBsAg level was positively correlated with liver cirrhosis ($p = 0.028$) and HBeAg level ($p < 0.001$), but negatively correlated with tumor size ($p = 0.039$). No other clinical characteristics were found to be significantly associated with the HBsAg level.

Association Between the HBsAg Level and Outcome in the Training Cohort

We first categorized these patients with an HBV DNA level < 2,000 IU/mL using the following cutoff levels for HBsAg: 10, 100 IU/mL (supplementary Fig. S1a, b), and 1,000 IU/mL (Fig. 2a, b), and we analyzed the data using a Kaplan–Meier survival analysis. The 1,000 IU/mL level, but not the 10 or 100 IU/mL level, was shown to be a meaningful cutoff level for significantly discriminating these patients into two groups.

TABLE 2 Univariate and multivariate analyses of factors associated with survival and recurrence of HCC patients

Variable	OS				RFS			
	Univariate *p* value	Multivariate			Univariate *p* value	Multivariate		
		HR	95 % CI	*p* value[a]		HR	95 % CI	*p* value[a]
Sex (female vs. male)	0.163			NA	0.425			NA
Age, years (≤50 vs. >50)	0.490			NA	0.283			NA
AFP, ng/ml (≤20 vs. >20)	0.007			NS	0.030			NS
ALT, U/L (≤42 vs. >42)	0.036			NS	0.079			NA
GGT, U/L (≤54 vs. >54)	0.015			NS	0.009			NS
Liver cirrhosis (no vs. yes)	0.140			NA	0.053			NA
Tumor size, cm (≤5 vs. >5)	0.001	1.771	1.224–2.562	0.002	0.002	1.521	1.109–2.086	0.009
Tumor encapsulation (complete vs. none)	0.224			NA	0.706			NA
Microvascular invasion (no vs. yes)	0.003			NS	<0.001	1.598	1.145–2.231	0.006
Tumor differentiation (I–II vs. III–IV)	0.038			NS	0.041			NS
TNM stage (I vs. II–III)	0.001	1.796	1.204–2.678	0.004	0.001	1.627	1.143–2.316	0.007
HBeAg (negative vs. positive)	0.027			NS	0.007			NS
HBsAg, IU/mL (<1,000 vs. ≥1,000)	0.013	1.674	1.109–2.528	0.014	0.004	1.585	1.119–2.247	0.010

HCC hepatocellular carcinoma, *RFS* recurrence-free survival, *OS* overall survival, *AFP* α-fetoprotein, *ALT* alanine aminotransferase, *GGT* γ-glutamyl transferase, *TNM* tumor-node-metastasis, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HR* hazard ratio, *CI* confidential interval, *NA* not adopted, *NS* non-significant

[a] Cox proportional hazards regression

By the last follow-up, in December 2011, 57.0 % (170/298) of patients in the training cohort had experienced recurrence, and 40.6 % (121/298) had died. The 1-, 3-, and 5-year OS rates and cumulative recurrence rates in this cohort were 79.5, 37.9, and 63.1 %, and 51.0, 59.4, and 57.0 %, respectively. In addition, by Kaplan–Meier survival analysis, we found that the 1-, 3-, and 5-year survival rates of the $HBsAg^{Low}$ patients were significantly higher than those of the $HBsAg^{High}$ patients (87.2 vs. 75.1 %, 71.6 vs. 58.2 %, and 67.9 vs. 54.5 %, respectively; $p = 0.0124$) (Fig. 2a). Similarly, at 1, 3, and 5 years, the $HBsAg^{High}$ patients had a worse prognosis and lower RFS rates than the $HBsAg^{Low}$ patients (41.8 vs. 31.2 %, 56.6 vs. 41.3 %, and 63.5 vs. 45.9 %, respectively; $p = 0.0039$) (Fig. 2b). Finally, we evaluated the prognostic value of the ALT level, GGT level, and HBeAg positivity. The results indicated that higher ALT and GGT levels, as well as HBeAg positivity, were associated with a shorter OS ($p = 0.0337$, 0.0144, and 0.0239, respectively) (supplementary Fig. S2a, c, e). Additionally, a higher GGT level and HBeAg positivity, but not ALT, were associated with higher cumulative recurrence rates ($p = 0.0078$ and 0.0071, respectively) (supplementary Fig. S2d, f, b).

Univariate analysis revealed that the HBsAg level was associated with OS [$p = 0.013$; hazard ratio (HR) 1.643; 95 % confidence interval (CI) 1.108–2.435] and RFS ($p = 0.004$; HR 1.619; 95 % CI 1.162–2.256). The AFP level, GGT level, tumor size, microvascular invasion, tumor differentiation, TNM stage, and HBeAg level were predictors for OS and RFS; however, the ALT level was associated with OS only. Other characteristics, including sex, age, liver cirrhosis, and tumor encapsulation, showed no prognostic significance for OS or RFS. The individual clinicopathologic features that demonstrated significance in the univariate analysis were further analyzed as covariates in a multivariate Cox proportional hazards model. The results indicated that the HBsAg level was a prognostic indicator for OS ($p = 0.014$; HR 1.674; 95 % CI 1.109–2.528) and RFS ($p = 0.01$; HR 1.585; 95 % CI 1.119–2.247) (Table 2).

We further investigated the predictive value of the HBsAg level within subgroups and found that the prognostic significance of the HBsAg level was retained. The 5-year RFS rates of the $HBsAg^{High}$ patients were lower than those of the $HBsAg^{Low}$ patients in the tumor size < 5 cm group ($p = 0.0033$; Fig. 3a), the single tumor group ($p = 0.0029$; Fig. 3b), the no vascular invasion group ($p = 0.0367$; Fig. 3c), the TNM stage I group ($p = 0.0029$; Fig. 3d), the ALT < 75 U/L group ($p = 0.0162$; Fig. 3e), the HBeAg-negative group ($p = 0.0149$; Fig. 3f), the Edmondson stage III–IV group ($p = 0.0057$; supplementary Fig. S3a), the vascular invasion group ($p = 0.0067$; supplementary Fig. S3b), the AFP > 20 ng/mL group ($p = 0.008$; supplementary Fig. S3c), the GGT > 54 U/L group ($p = 0.0422$; supplementary Fig. S3d), and the BCLC 0 + A group ($p = 0.0036$; supplementary Fig. S3e).

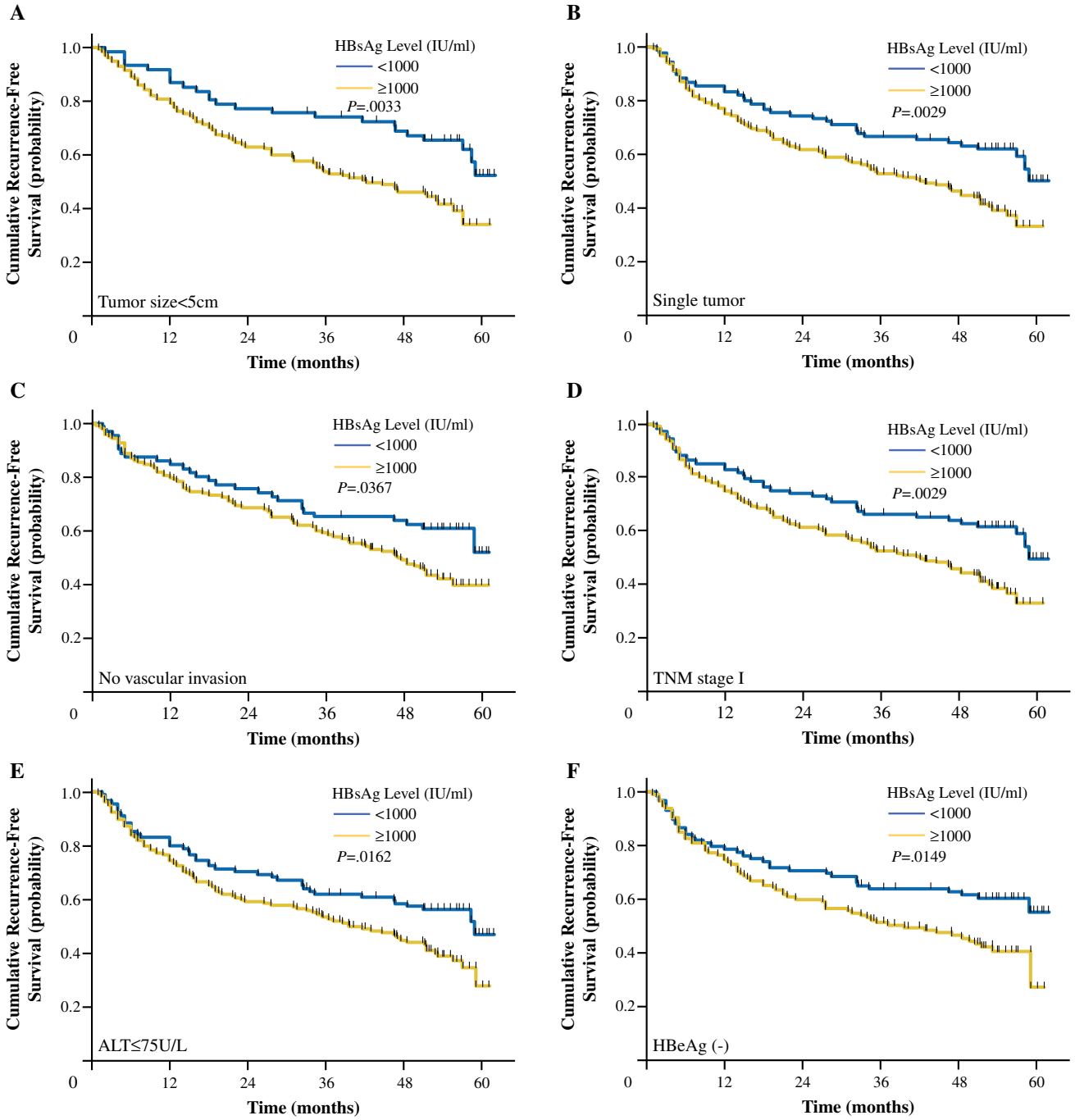

FIG. 3 Kaplan–Meier analysis of patient survival according to HBsAg level in different subgroups of the training cohort. **a** Tumor size <5 cm; **b** single tumor; **c** no vascular invasion; **d** TNM stage I; **e** ALT ≤75 U/L; **f** HBeAg-negative. *HBsAg* hepatitis B surface antigen, *ALT* alanine aminotransferase, *HBeAg* hepatitis B e antigen

Validation Analysis in the Validation Cohort

To validate our findings, we studied a validation cohort that included 1,062 patients after curative resection. Briefly, we found that the 1-, 3-, and 5-year OS rates of the $HBsAg^{High}$ patients were 63.0, 44.8, and 37.5 %, respectively, and were significantly lower than those of the $HBsAg^{Low}$ patients (70.9, 53.3, and 46.4 %, respectively) (Fig. 2c). The 1-, 3-, and 5-year cumulative recurrence rates of the $HBsAg^{High}$ patients were 55.6, 67.3, and 72.2 %, respectively, and were significantly higher than those of the $HBsAg^{Low}$ patients (48.8, 60.9, and 66.1 %, respectively) (Fig. 2d).

Predictive Value of HBsAg Quantification

The area under the curve (AUC) for HBsAg quantification in predicting OS was 0.727, with a sensitivity of 90.7 % and specificity of 60.47 % ($p < 0.001$; 95 % CI 0.662–0.785) (supplementary Fig. S4a). Furthermore, the AUC for HBsAg quantification in predicting recurrence was 0.777, with a sensitivity of 82.22 % and specificity of 76.25 % ($p < 0.001$; 95 % CI 0.716–0.831) (supplementary Fig. S4b).

DISCUSSION

For HCC patients after curative resection, recurrence is the primary barrier to an improved prognosis. It is critical to identify risk factors for HCC recurrence in clinical practice. In the present study, we investigated clinical relevance in two large cohorts of surgically resected HCC patients that included 298 and 1,062 patients, respectively.

We discovered that a high HBsAg level was significantly associated with unfavorable characteristics of HCC patients. This finding was based on the markedly more frequent detection of aggressive features, such as liver cirrhosis, large tumor size, and HBeAg positivity, in the $HBsAg^{High}$ patients. This finding indicated that the HBsAg level might be a potential powerful prognostic indicator for HCC. To determine the value of the HBsAg level for predicting OS and recurrence, we categorized the study patients, who had an HBV DNA level < 2,000 IU/mL, by applying the following cutoff levels for HBsAg: 10, 100, and 1,000 IU/mL, and we analyzed the data using a Kaplan–Meier survival analysis. The results revealed that only 1,000 IU/mL was a meaningful cutoff level for predicting prognosis. These interesting observations were confirmed in the validation cohort. Furthermore, the prognostic significance of the HBsAg level persisted in the subgroup analysis. In line with the subgroups with more malignant phenotypes such as Edmonson III–IV, vascular invasion, AFP > 20 ng/ml, and GGT > 54 U/L, HBsAg quantification was also statistically in discriminating those patients who were at risk for early recurrence due to the fact that HBV could contribute to HCC progression and recurrence.

HBsAg quantification has become increasingly used for therapeutic candidate selection, therapeutic response surveillance, and drug resistance detection.[20–24] For HBeAg-negative patients with an HBV DNA level < 2,000 IU/mL, we determined that an HBsAg level ≥ 1,000 IU/mL, but not the HBV DNA level, was a risk factor for HCC progression.[15] An HBsAg level < 1,000 IU/mL in combination with a low level of HBV DNA (<2,000 IU/mL) has been shown to predict the development of HBeAg-negative hepatitis, hepatitis flares, and cirrhosis.[14] A combination of LHBs, HBsAg, and HBV DNA was reported to facilitate the efficient and early prediction of virological response (VR) and serological response (SR) to antiviral therapy.[25] Nevertheless, our study was the first to apply HBsAg quantification to predicting the prognosis of HCC patients after curative resection.

HBV DNA load is known to be significantly associated with decreased OS and RFS.[8] However, because patients with low viral loads (HBV DNA < 2,000 IU/mL) are usually described as low-risk HBV carriers,[26,27] we focused on that group of patients. We found that for patients with low viral loads (HBV DNA < 2,000 IU/mL), an HBsAg level ≥ 1,000 IU/mL was a risk factor for relapse.

The present study had some unique characteristics. In contrast to HBV DNA quantification, HBsAg quantification is less expensive and largely automatic, with high-throughput capacity. The dynamic range of the HBsAg level is wider than that of the HBV DNA level (0.05–>10,000 IU/mL vs. 15–2,000 IU/mL, respectively). Thus, for patients with an HBV DNA level < 2,000 IU/mL, the HBsAg level is more likely to accurately identify patients who are prone to recurrence.[15]

HBV reactivation was associated with worse hepatic function and decreased postoperative survival.[28] Antiviral treatment has produced obvious effects in decreasing HCC recurrence.[16,29–32] Nucleotide/nucleotide analog (NA) treatment was shown to improve OS and RFS in a non-randomized retrospective cohort and randomized controlled trial (RCT).[8] Routine prophylactic antiviral treatment prior to partial hepatectomy has been proposed due to the common occurrence of HBV reactivation after partial hepatectomy for HBV-related HCC, even in patients with a low preoperative HBV DNA level < 2,000 IU/mL.[28] However, because the average annual rate of HBsAg loss was reported to be 0.62 %,[13] the termination of antiviral treatment in postoperative HCC patients is controversial. The HBsAg level remains more stable than the HBV DNA level; therefore, an HBsAg level < 1,000 IU/mL in combination with an HBV DNA level < 2,000 IU/mL may be a more feasible indicator for antiviral treatment termination.

The major limitation of the present study was its retrospective nature and the 'lost to follow-up' records. Moreover, RFA, PEI, TACE, or external radiotherapy selection for those patients with HCC recurrence might influence the result of OS.

CONCLUSIONS

To the best of our knowledge, this is the first study to demonstrate the implications of HBsAg level in HCC prognosis prediction. Moreover, given the importance of the HBsAg level in HCC recurrence, the consideration of HBsAg in antiviral treatment will pave the way for RCT

applications and clinical therapeutic guidance. Rather than being a substitute, HBsAg quantification may be an ideal complement to HBV DNA-level measurement in prognosis prediction and therapeutic guidance for HCC surgical patients. Finally, further investigation and validation are warranted by other HBV registries or institutions in China.

ACKNOWLEDGMENT This work was supported by Grants from the National Natural Science Foundation of China (No. 81272389 and 81030038), National Key Sci-Tech Project (2012ZX10002011-002), and Scholarship Award for Excellent Doctoral Student granted by the Ministry of Education (JFF152005).

CONFLICT OF INTEREST Nothing to report.

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