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ORIGINAL ARTICLE

Impact of Preoperative Hepatitis B Virus Levels on Prognosis After Primary and Repeat Hepatectomies for Hepatocellular Carcinoma Patients—a Retrospective Study

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Abstract

Background Chronic HBV plays an important role in hepatocellular carcinoma pathogenesis. Previously, most studies have been focusing on HBV DNA levels before the primary curative hepatectomy. However, the association of virus level before repeat hepatectomy with the degrees of inflammation and fibrosis on histopathology and prognosis has not been surveyed.

Methods From January 2002 to December 2009, all patients who were seropositive for hepatitis B surface antigen (HBsAg) were enrolled and assigned into four groups based on their HBV DNA levels before the primary and repeat hepatectomies. The cancer prognoses of these four groups of patients after the first and second operations were assessed and compared. The disease-free survival and overall survival were estimated by the Kaplan-Meier method. Univariate and multivariate analyses were performed to identify risk factors for the primary and repeat hepatectomies.

Results For the 385 patients in this study, a low level of serum HBV DNA before repeat hepatectomy, but not primary hepatectomy, was significantly associated with improvement in prognosis, in terms of tumor recurrence, liver fibrosis, and liver-related mortality.

Conclusion The levels of HBV DNA before hepatectomies were crucial prognostic risk factors of HBV-related hepatocellular carcinoma patients. Surveillance of serum HBV DNA levels at multiple time points, rather than at a single time point, and antiviral therapy to suppress the virus to a low level had beneficial effects for these patients.

Keywords Liver cancer · Antiviral · HBV · Hepatectomy · Risk factors

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Introduction

Hepatocellular carcinoma (HCC) is a major health problem, being the fifth most common malignancy in men and the eighth in women and the third leading cause of cancer-related death worldwide.[1–4] Hepatitis B virus (HBV) plays a pivotal role in hepatocarcinogenesis and has been linked epidemiologically to the development of HCC. The incidence of HCC is the highest in regions such as Asia and Africa where chronic HBV infection is endemic.[1,3] Chronic HBV infection (CHB) is responsible for approximately 54% of all cases of HCC and 70–90% of such HBV-related HCC arising from cirrhosis. Most of these patients undergo progressive chronic liver diseases, including hepatitis, fibrosis, cirrhosis, and finally HCC.[5,6] HBV viral replication is now recognized as the key driver of liver injury and liver cancer.[7]

Recent studies have identified a significant association between serum HBV DNA level (which reflects the level of

intrahepatic viral replication) and liver injury, liver fibrosis, cirrhosis, and liver failure in patients with HBV infection.[6,8–11] Higher serum HBV DNA levels are also found to be associated with increased incidence of HCC.[12,13] Consistently, antiviral therapy, which lowers HBV viral levels, is associated with the restraint of clinical and histological disease progressions.[2,14–17] Meta-analyses show the incidence of HCC in patients with CHB infection can even be reduced using antiviral therapy.[18,19]

Both preoperative serum HBV DNA levels and liver histology on inflammation and fibrosis in non-cancerous liver tissues have been identified to be independent risk factors in predicting prognosis after curative hepatectomy for HCC. However, whether there is a causal link between HBV DNA level and liver histology has not been well-established. Additionally, although high HBV DNA levels have frequently been linked to increased incidences of HCC and to recurrence after curative resection for HCC,[12,20] most previous studies were based on serum HBV DNA levels at a single time point. These observations did not take into account that HBV DNA levels fluctuate in the natural progression of chronic HBV infection and with antiviral therapy. Thus, the association of a static HBV DNA level with HCC progression is unreliable. A recent study demonstrated that persistently elevated serum HBV DNA levels documented at two time points strongly predicted the incidence of HCC.[21–23]

Here, we conducted a retrospective study to determine the serum HBV DNA levels before primary "curative" and repeat "curative" hepatectomies for patients with locally recurrent HBV-related HCC. The study aimed to correlate the influence of preoperative serum HBV DNA levels before the two operations with the degrees of inflammation and fibrosis in non-cancerous liver tissues on histopathology and prognosis after the operations.

Methods

Patient Identification

Between January 2002 and December 2009, all patients with recurrent HBV-related HCC after primary curative hepatectomy who underwent repeat curative hepatectomy at the Liver Surgery Unit were enrolled in this study. These patients were all seropositive for hepatitis B surface antigen (HBsAg) before the primary hepatectomy. A histopathological diagnosis of HCC was confirmed after each of the two hepatic resections. Curative hepatectomy was defined as the complete removal of all tumors detected preoperatively and intraoperatively with a clear microscopic margin.

The inclusion criteria for this study were as follows: (1) seropositivity for HBsAg before the primary hepatectomy; (2) histopathological diagnosis of HCC after the first and repeat hepatectomies; (3) no extrahepatic metastasis; (4) curative hepatectomies which were defined as the removal of all tumors detected preoperatively and intraoperatively with a negative resection margin using histopathological examination (R0 resection); (6) no radiologic evidence of invasion into major portal/hepatic venous branches; (7) good liver function with Child-Pugh class A and baseline serum alanine aminotransferase (ALT) levels of less than two times the upper limit of normal (reference range < 40 IU/L) and no history of encephalopathy, ascites which was refractory to diuretics, or esophagogastric variceal bleeding; (8) good renal function (a serum creatinine level < 133 μmol/L); and (9) no previous treatment for the HCC patients before the first hepatectomy. The exclusion criteria are listed in the patient flow chart in Fig. 1. This study was conducted at the time when the importance of routine antiviral therapy was not recognized to be important as part of treatment for patients with HBV-related HCC undergoing liver resection.

This retrospective study was approved by the ethics committee of the Eastern Hepatobiliary Surgery Hospital (Shanghai, China). Informed consent was obtained from all the patients for their data to be used for research purposes.

Perioperative Management

All patients had blood tests including serum HBsAg, hepatitis B virus e antigen (HBeAg) and its antibody, serum HBV DNA levels, hepatitis C virus antibody (HCV-Ab), serum α-fetoprotein (AFP), serum albumin, serum total bilirubin (TB), aspartate aminotransferase (AST), ALT, and prothrombin time (PT). Imaging studies included chest radiography (CR), abdominal ultrasonography (US), contrast computerized tomography (CT), and/or magnetic resonance imaging (MRI).

After each operation, the resected HCC and its surrounding liver tissues were examined both microscopically and macroscopically by a pathologist who was blinded to the patients' clinical and biochemical information. Histopathological characteristics of the tumor, including tumor number, tumor diameter, tumor encapsulation, macroscopic vascular invasion, microvascular invasion, and resection margins, were evaluated. The histological grade of HCC differentiation was determined by the Edmonson-Steiner classification. The histological grades of inflammation and fibrosis in the non-cancerous tissues were assessed according to the scoring system by Ishak and his associates.[24]

Surgical Procedures

The extent of liver resection was based on Couinaud's classification of liver segments. A major resection was

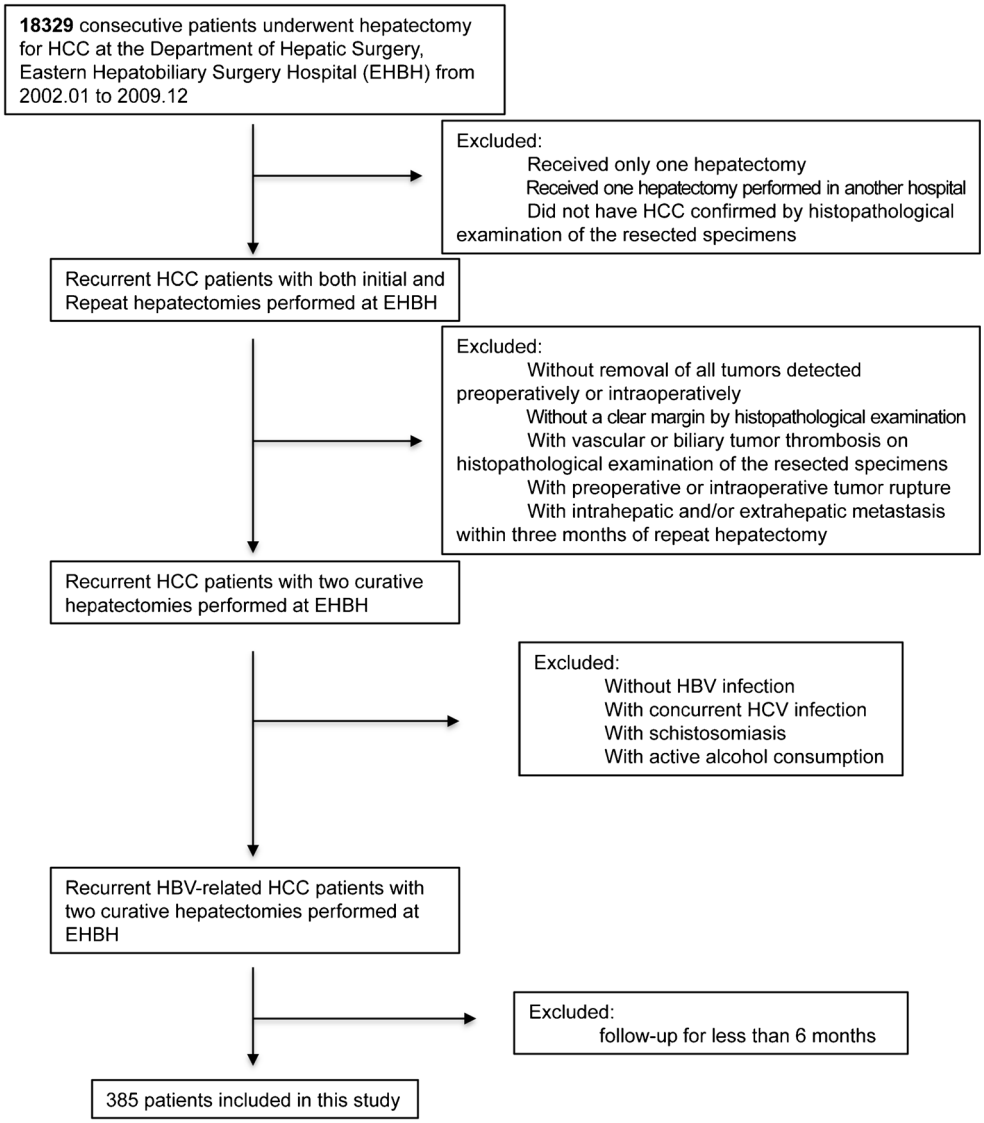


Fig. 1 The patient flow chart for this retrospective study. Of 18,329 patients, 385 patients were finally included in the study

defined as the resection of three segments or more, while a minor resection involved two segments or less. Repeat hepatectomy was recommended as the first choice for resectable recurrent HCC. The selection criteria and surgical procedures for repeat hepatectomy were the same as that for the primary tumor. Preoperative evaluation of resectability was determined by tumor factors, liver functional reserve, and general physiological condition of patients. During surgery, a bilateral subcostal incision was used. The abdominal cavity was then carefully inspected for the extent of local disease, extrahepatic metastases, and peritoneal seeding. After liver mobilization, intraoperative ultrasonography was performed routinely to determine the position and the size of tumors and to assess their relationship with vascular structures. Pringle maneuver was used to occlude the blood inflow of the liver, with clamp/unclamp cycles of 15/5 min. Liver transection was carried out by a clamp-crushing method.

Postoperative Management

All the patients received the same postoperative care by the same team of surgeons. They were all managed in the intensive care unit during the early postoperative period. Subsequent need for stay in the intensive care unit was determined by the patient's condition. Liver function tests and clotting profiles were monitored. No postoperative adjuvant therapies were given to the patients.

Follow-Up

After discharge from the hospital, the patients were followed up regularly at a monthly interval for the first year and then once every 3 months thereafter. Patients were examined by abdominal CT or MRI every 3–6 months. When recurrence or metastasis was suspected, further investigations, such as angiography and/or positron emission tomography-

computed tomography (PET-CT), were performed. The recurrence-free survival was defined as the interval between hepatectomy and diagnosis of recurrence. For further recurrence after repeat hepatectomy, radiofrequency ablation (RFA), transarterial chemoembolization (TACE), or percutaneous ethanol injection (PEI) was chosen according to the patient's liver function, location of recurrent tumor, and extent of intrahepatic metastasis. For patients with advanced or extrahepatic metastatic HCC, sorafenib or symptomatic treatment was given.

Statistical Analysis

Statistical analysis was performed using the SPSS 18.0 software. All values were expressed as mean ± standard deviation or median with range as appropriate. Quantitative values were compared using Student's t test or the Mann-Whitney non-parametric U test as appropriate. Frequencies were compared by Pearson's $\chi 2$ test. Paired observations were compared by Student's t test or the Wilcoxon signed-rank test as appropriate. Multivariate analysis was carried out by the Cox proportional hazard regression model. A two-tailed P value of less than 0.05 was considered statistically significant. Disease-free survival (DFS) and overall survival (OS) curves were estimated by the Kaplan-Meier method, and statistical comparisons were based on the log-rank test. When four curves were compared, a P value of less than 0.05 was considered statistically significant. When two of the four curves were compared, a P value of less than 0.001 was considered statistically significant.

Results

Patient Enrollment and HBV DNA Levels

From January 2002 to December 2009, partial hepatectomy was performed on 18,329 patients with a preoperative diagnosis of HCC at the Department of Liver Surgery, the Eastern Hepatobiliary Surgery Hospital, China. Of these patients, 17,944 (97.9%) were excluded from the study and the remaining 385 patients were included in this retrospective study according to the inclusion and exclusion criteria. They all underwent repeat hepatectomy for intrahepatic HCC recurrence (Fig. 1).

These 385 patients were divided into the following four groups according to the preoperative HBV DNA levels before the primary and the repeat hepatectomies (Table S1): The persistently low group (group A), with 122 patients with persistently low HBV DNA levels (< 2000 IU/mL); the elevated group (group B), with 62 patients with low HBV DNA levels (< 2000 IU/mL) before the first but high HBV DNA levels (≥ 2000 IU/mL) before the second hepatectomy; the decreased group (group C), with 130 patients with high HBV DNA levels (≥ 2000 IU/mL) before the first and low HBV DNA levels (< 2000 IU/mL) before the second hepatectomy; and the persistently high group (group D), with 71 patients with persistently high HBV DNA levels (≥ 2000 IU/mL) before the first and the second hepatectomies.

The clinicopathological characteristics of these 385 patients are presented in Tables S2 and S3. Before the primary hepatectomy, there were no significant differences in the variables between the patients in group A and in group B and in group C and in group D, respectively (Table S2). However, in the repeat hepatectomy, some of these variables became significantly different: when compared with group A, patients in group B had significantly higher levels of AST and ALT ($P < 0.001$), longer prothrombin time ($P = 0.016$), and different HBeAg-positivity profiles ($P = 0.003$). Similarly, patients in group D had significantly higher levels of ALT and AST ($P < 0.001$), longer PT ($P = 0.032$), and lower ALB ($P = 0.006$), when compared with group C (Table S3).

The Influence of Preoperative HBV DNA Levels on the Degrees of Inflammation and Fibrosis in Non-cancerous Liver Tissues

To determine whether there were any differences in the inflammation and fibrosis scores in non-cancerous liver tissues within the different individual groups of patients between the first and the repeat hepatectomies, Knodell inflammation score and Ishak fibrosis score were calculated. As shown in Fig. 2a, c, for patients in group A and group C who had low HBV DNA levels before the repeat hepatectomy, the liver inflammation and fibrosis scores in the non-cancerous tissue were significantly lower in the repeat than in the primary hepatectomies. For patients in group B and in group D who had high HBV DNA levels before the repeat hepatectomy, the fibrosis scores were significantly higher in the repeat hepatectomy, indicating that the livers developed degenerative changes associated with the high HBV DNA levels before the repeat hepatectomy (Fig. 2b, d). The representative HE images are shown in Fig. S1.

The liver inflammation and fibrosis scores were then compared among the four groups. In the non-cancerous tissues from the primary hepatectomy, no significant differences in Knodell inflammation score and Ishak fibrosis score were found between group A and group B and between group C and group D (Fig. 3a). However, in the non-cancerous tissues from the repeat hepatectomy, there were significant differences in Knodell inflammation score and Ishak fibrosis score between group A and group B ($P < 0.001$) and between group C and group D ($P < 0.001$) (Fig. 3b). These

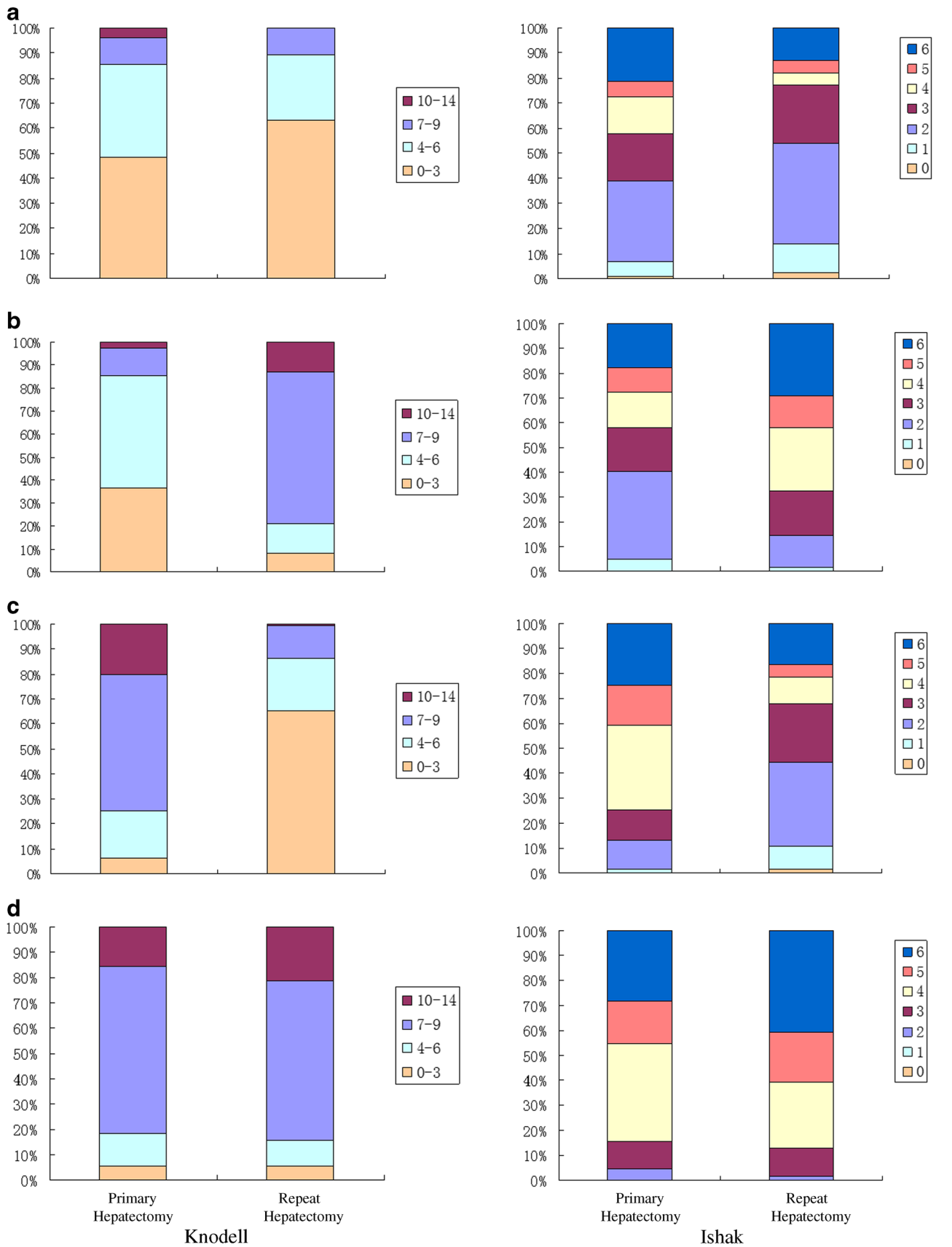


Fig. 2 Liver inflammation and fibosis before primary and repeat hepatectomies regarding the four groups of patients (**a**–**d**) were measured by Knodell and Ishak scoring system. The distribution of the Knodell (left) and Ishak (right) scores were shown

results suggested that there was a direct link between HBV DNA levels and the degrees of liver inflammation and fibrosis. The Knodell inflammation score and Ishak fibrosis score in group C were significantly higher than in group A ($P < 0.001$) in the non-cancerous tissues from the primary hepatectomy. However, there were no significant differences between group C and group A in the non-cancerous tissues from the repeat hepatectomy. Similarly, the Knodell inflammation score and Ishak fibrosis score in group D in the non-cancerous tissues from the primary hepatectomy were significantly higher than in group B ($P < 0.001$), but the differences between group B and group D for the non-cancerous tissues from the repeat hepatectomy were not significant even though the HBV DNA levels were high in these two groups (Fig. 3). In the non-cancerous tissues from both the primary and the repeat hepatectomies, the Knodell inflammation score and Ishak fibrosis score in group D were significantly higher than in group A ($P < 0.001$). In the non-cancerous tissues from the primary hepatectomy, the Knodell inflammation score and Ishak fibrosis score in group C were significantly higher than in group B ($P < 0.001$). These results were reversed in the non-cancerous tissues from the repeat hepatectomy (Fig. 3).

Antiviral Treatment

Two hundred thirty-six patients with HBV levels ≥ 2000 IU/mL were given oral nucleoside analogs (lamivudine, 25 patients; adefovirdipivoxil, 135 patients; entecavir, 76 patients). No antiviral treatment other than nucleoside analogs was administrated in this study. One hundred six (25.7%) patients received antiviral treatment before the primary hepatectomy, and 130 (33.8%) patients received antiviral treatment before the repeat hepatectomy.

The Influence of Preoperative HBV DNA Levels on Disease-Free and Overall Survival Rates

There were 280 patients (280/385, 72.7%) who developed intrahepatic HCC recurrence and 234 patients (60.8%) who died within 48.9 + 26.9 months of follow-up. Most of these patients died of cancer recurrence ($n = 221$), and 13 died of liver failure. The mortality rate of liver failure for patients in groups B and D was significantly higher than in group A and group C (B/A, 6.5 vs 0.8%; D/C, 8.5 vs 1.5%, respectively).

For all the patients who were included in this study ($n = 385$), the disease-free survival rates for the 1st, 3rd, and 5th year after repeat hepatectomy were 87.3, 48.1, and 31.6%,

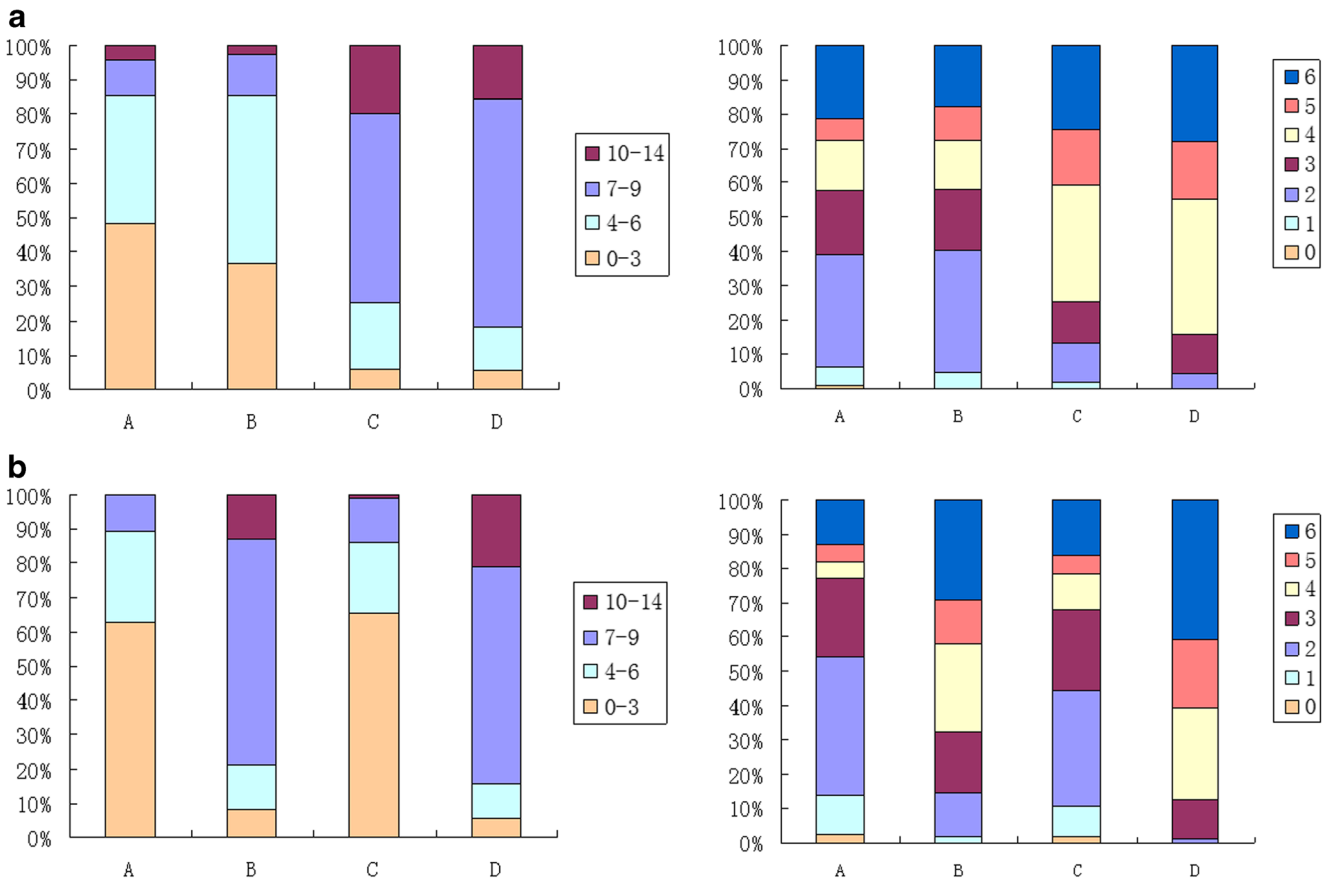


Fig. 3 **a** Distribution of Knodell and Ishak scores of the four patient groups associated with the primary hepatectomy. **b** Distribution of Knodell and Ishak scores of the four patient groups associated with the repeat hepatectomy

respectively. The corresponding overall survival rates were 95.6, 59.2, and 44.7%, respectively. Using the Kaplan-Meier survival analysis, the disease-free and overall survival rates in group A and group C were significantly better than in group B and group D (log-rank test, $P = 0.001$ and $P = 0.002$), suggesting that changes in the preoperative HBV DNA levels between the two operations influenced the prognosis of HCC patients after repeat hepatectomy (Fig. 4a, b).

Using the Kaplan-Meier analysis on the different groups of patients, the disease-free survival and overall survival rates of group A were significantly better than that of group B (log-rank test, $P = 0.003$, $P = 0.007$) (Fig. 5a). The disease-free survival rate in group C was significantly better than in group D (log-rank test, $P = 0.006$), but the overall survival rate was not significantly different (log-rank test, $P = 0.018$, Fig. 5b). As the HBV DNA levels in group D were persistently high before the first and the repeat hepatectomies, this suggested that the HBV DNA levels were independent factors of prognosis.

The HBV DNA levels in group A and group C were low before the repeat hepatectomy although they were different before the primary hepatectomy. The DFS and OS rates were not significantly different between these two groups ($P = 0.400$, $P = 0.253$, respectively, Fig. 5c). Similarly, the DFS and OS rates of group B and group D showed no significant difference ($P = 0.798$, $P = 0.611$, respectively, Fig. 5d), suggesting that the HBV DNA levels before the repeat hepatectomy, rather than before the primary hepatectomy, were a predicting factor of prognosis.

Patients in group A and group D had vastly different HBV DNA levels before the two hepatectomies. However, the Kaplan-Meier analysis showed both the DFS and the OS in group A were significantly better than in group D (Fig. 5e). Interestingly, although patients in group C had a better DFS and OS rates than group B, the difference was not significant ($P = 0.017$ and $P = 0.082$, respectively, Fig. 5f). This indicated the HBV DNA levels before the primary hepatectomy contributed to prognosis less than the HBV DNA levels before the repeat hepatectomy.

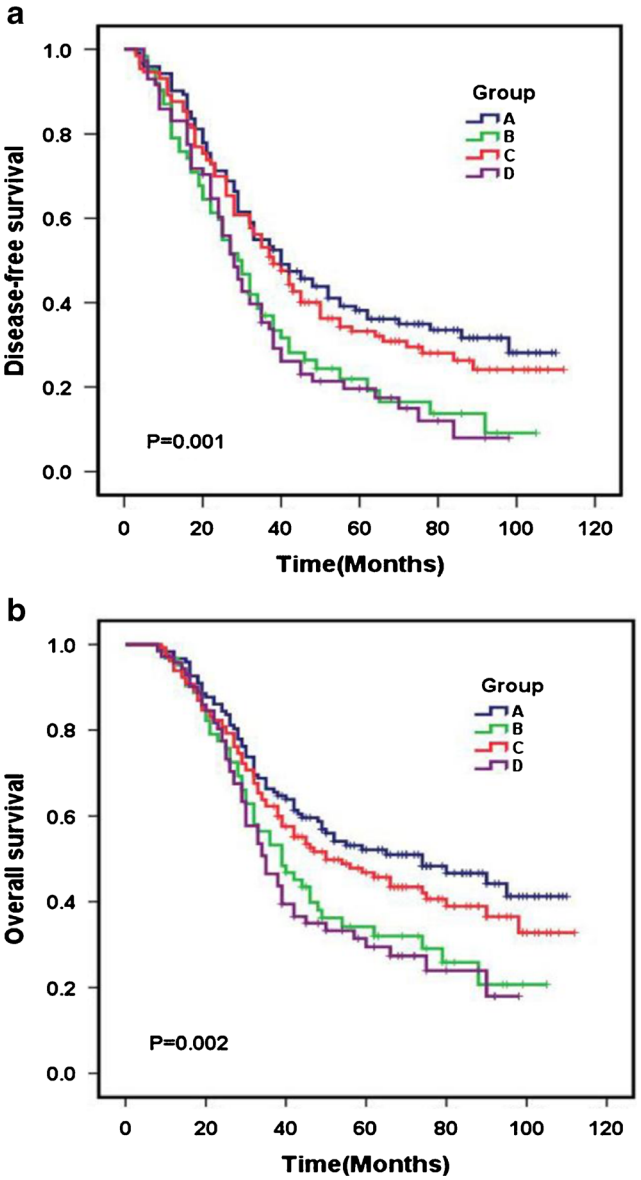
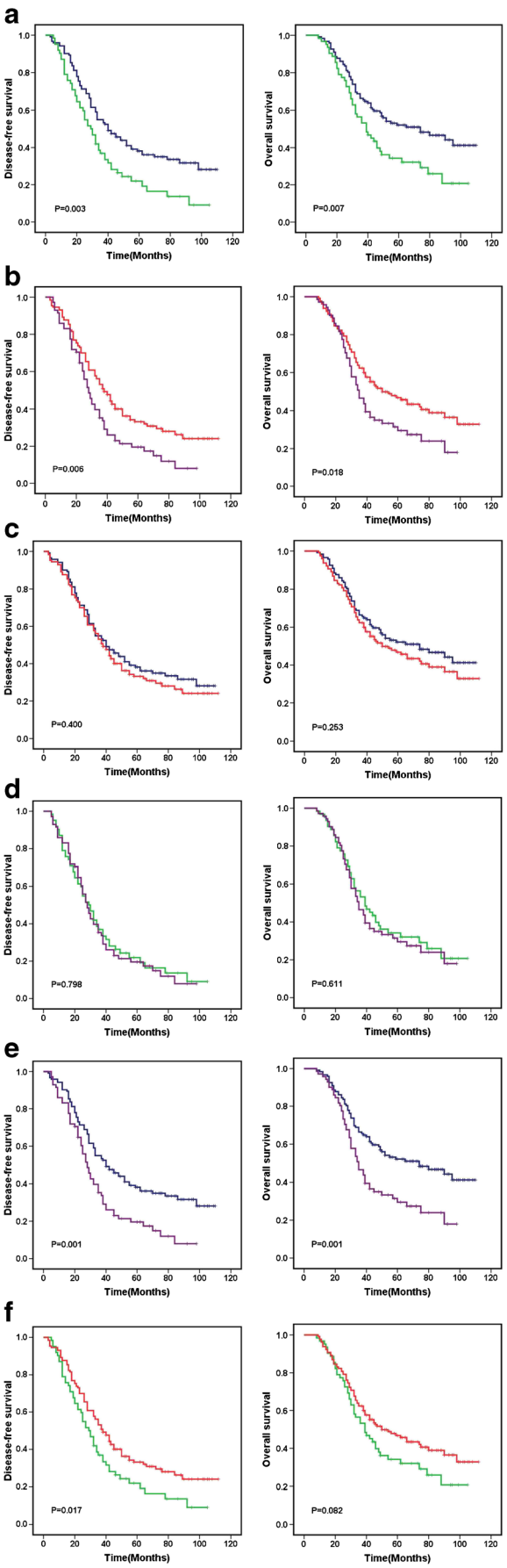


Fig. 4 Comparison of disease-free survival (**a**) and overall survival (**b**) among patient groups. Log-rank test was used to determine the significance of difference. *P* values were shown in each plot

The Risk Factors of HBV-Related HCC Recurrence After Repeat Hepatectomy

Univariate analysis was carried out for the 385 patients to identify the risk factors of HBV-related HCC recurrence. Tables S4 and S5 summarized the variable factors associated with the primary and repeat hepatectomies, respectively. Among the pretreatment characteristics of the primary hepatic resection, Ishak score > 3 ($P = 0.033$) and disease-free survival time ≥ 24 months ($P < 0.001$) were closely associated with the DFS rate of the repeat hepatectomy (Table S4). For parameters of the repeat hepatectomy, HBeAg positivity ($P = 0.003$), HBV DNA levels ≥ 2000 IU/mL ($P < 0.001$), ALT > 40 IU/L ($P = 0.003$), tumor size > 5 cm ($P = 0.038$), tumor numbers ($P < 0.001$), microvascular invasion ($P < 0.001$), tumor capsule formation ($P = 0.032$), Knodell score > 6 ($P < 0.001$), and Ishak score > 3 ($P < 0.001$) were correlated with worse disease-free survival after the repeat hepatectomy (Table S5).

The risk factors significantly associated with disease-free survival ($P < 0.05$) on univariate analysis were entered into multivariable analysis using the Cox proportional hazard regression model (Table 1). Of the parameters associated with the primary hepatic resection, only disease-free survival time ≥ 24 ($P = 0.009$) was an independent risk factor of HCC recurrence after the repeat hepatectomy. However, HBV DNA loading ≥ 2000 IU/mL ($P = 0.002$), tumor numbers ($P = 0.011$), microvascular invasion ($P < 0.001$), and Ishak score > 3 ($P = 0.042$) were independent



◀ **Fig. 5** Comparison of disease-free survival and overall survival among patient groups. The differences between group A and group B (**a**), group C and group D (**b**), group A and group C (**c**), group B and group D (**d**), group A and group D (**e**), and group B and group C (**f**) were determined by the log-rank test, respectively. *P* values were indicated in each plot

risk factors of cancer recurrence after the repeat hepatectomy (Table 1).

The Risk Factors of Overall Survival Rate After Repeat Hepatectomy

For all the 385 patients, univariable analysis was used to assess the risk factors related to OS after the repeat hepatectomy. Among the factors in the primary hepatic resection, Knodell score > 6 ($P = 0.026$), Ishak score > 3 ($P = 0.004$), and disease-free survival time ≥ 24 ($P < 0.001$) were significant independent risk factors (Table S6). As factors for the repeat hepatectomy, gamma-glutamyl transpeptidase (GGT) > 60 IU/L ($P = 0.021$), HBeAg positivity, HBV DNA ≥ 2000 IU/mL ($P < 0.001$), PT > 13 s ($P = 0.025$), tumor numbers ($P = 0.002$), tumor capsule formation ($P = 0.036$), microvascular invasion ($P = 0.002$), Knodell score > 6 ($P < 0.001$), and Ishak fibrosis score > 3 ($P < 0.001$) were poor prognostic factors of OS (Table S7).

Factors identified by univariable analysis ($P < 0.05$) were subjected to multivariable analysis using the Cox proportional regression model. Disease-free survival time ≥ 24 ($P = 0.025$) was the only independent risk factor related to the primary resection for OS. For the repeat hepatectomy, HBeAg positivity ($P = 0.035$), HBV DNA ≥ 2000 IU/mL ($P = 0.021$), number of nodules ($P = 0.035$), microvascular invasion ($P < 0.001$), and Ishak fibrosis score > 3 ($P = 0.022$) were significant risk factors associated with poor OS for patients with recurrent HCC (Table 2).

Discussion

Currently, surgical resection remains the optimal curative therapy for patients with hepatocellular carcinoma (HCC).[1,2,4] With advancement in surgical techniques and patient care, safety of curative resection has been dramatically improved. Perioperative mortality has been reduced from 15% in the 1980s to 3–5% nowadays.[25–27] Unfortunately, prognosis after hepatectomy is still unsatisfactory due to the high postoperative recurrence rate. Tumor recurrences occur in 43 to 65% of patients within 2 years of surgery and up to 85% within 5 years.[28–31] Repeat liver resection is still the best curative treatment for recurrent HCC after hepatectomy.[32] Studies have reported survival rates to range from 37 to 64% at 5 years, which is better than most other treatments.[23,29,33,34]

Many studies have now focused on identifying risk factors related to prognosis after repeat hepatectomy.[23,34–37] Female gender, young age, poor tumor grade, microscopic vascular invasion, recurrent tumors > 3 cm, and serum albumin level < 35 g/L have been found to be significant risk factors of poor prognosis after repeat hepatectomy. Unfortunately, the role of preoperative HBV DNA levels before repeat hepatectomy has not been fully investigated. A high HBV DNA load, besides being a major tumorigenesis factor, has been found to be significantly associated to liver injury, cirrhosis, and HBV-related HCC progression.[12,20,38,39] Wu et al. found high HBV DNA load (> 10^6copies/mL or > 20,000 IU/mL) to be an independent risk factor of late HCC recurrence after primary hepatic resection.[40] A high load of HBV virus is known to induce persistent hepatic inflammation and fibrosis, and ongoing hepatic inflammation and cirrhosis favor carcinogenesis. Furthermore, a high viral load promotes virus DNA integration into the host genome, resulting in hepatic gene mutation, chromosome instability, and tumor transformation.[41] Hence, antiviral therapy to maintain a low HBV viral load improves prognosis after liver resection by reducing HCC recurrence rates and alleviating liver damage from inflammation and fibrosis.[17]

The levels of HBV DNA change under certain circumstances. For instance, effective antiviral drugs significantly suppress viral levels, the immune system of the host may attack the virus after infection, or hepatic resections can significantly modulate or dampen the immune response of the host resulting in immuno-suppression and HBV reactivation in the perioperative period.[42,43] Thus, it is necessary to investigate the HBV DNA level at various time points for patients with HBV-related HCC.

In the present study, the patients were classified into four groups depending on the HBV DNA levels before the primary and the repeat hepatectomies. The HBV DNA levels in groups A and B were low before the primary hepatectomy, but they remained low in group A and were high in group B. Before the repeat hepatectomy, blood ALT and AST and Knodell and Ishak scores were significantly higher, and the disease-free survival and overall survival were significantly worse in group B. The high HBV DNA levels before the repeat hepatectomy could therefore be used to predict poor postoperative results. Similar results were obtained in group C and group D patients, with HBV DNA levels being high before primary hepatectomy, but they became low in group C and remained persistently high in group D before the repeat hepatectomy. Comparison of group A and group C patients who have different HBV DNA levels before the primary hepatectomy but both having low HBV DNA levels before the repeat hepatectomy showed no significant differences in the overall survival rates. Similarly, in group B and group D patients who had high HBV DNA levels before the repeat hepatectomy but different HBV DNA levels before the primary hepatectomy, the overall survival rates showed no significant differences. Taken together, while a high/low HBV DNA level before the primary hepatectomy was not important, this level before the repeat hepatectomy was an independent risk factor associated with cancer prognosis. These results further suggested that HBV DNA levels should be determined before and after each liver resection, rather than at a single time point. A low HBV DNA level before primary resection for HBV-related HCC may not necessarily mean a better prognosis. The HBV DNA level may subsequently rise after primary hepatectomy, resulting in further liver inflammation and fibrosis. A long-term suppression of virus using antiviral therapy should be considered. For patients who have a high HBV DNA level before primary hepatectomy, measures should be taken to reduce viral load

Table 1 Multivariable analysis of prognostic factors for DFS of patients with recurrent HCC

	Patients, *n* (%)	Hazard ratio	95% confidence interval	*P*
Disease-free interval between the primary and repeat hepatectomies (month)				
< 24	159 (41.3)	0.714	0.554–0.921	0.009
≥ 24	226 (58.7)			
HBV DNA[a], IU/mL				
< 2000	252 (65.5)	1.554	1.182–2.044	0.002
≥ 2000	133 (34.5)			
Tumor number[a]				
Single	319 (82.9)	1.480	1.096–1.998	0.011
Multiple	66 (17.1)			
Microvascular invasion[a]				
Yes	316 (82.1)	1.812	1.336–2.457	< 0.001
No	69 (17.9)			
Ishak fibrosis score[a]				
≤ 3	150 (39.0)	1.318	1.010–1.720	0.042
>3	235 (61.0)			

[a] Factor associated with the repeat hepatectomy

Table 2 Multivariable analysis of prognostic factors for OS of patients with recurrent HCC

	Patients, *n* (%)	Hazard ratio	95% confidence interval	*P*
Disease-free interval between the primary and repeat hepatectomies (month)				
< 24	159 (41.3)	0.725	0.547–0.960	0.025
≥ 24	226 (58.7)			
HBeAg[a]				
Negative	275 (71.4)	1.346	1.020–1.776	0.035
Positive	110 (28.6)			
HBV DNA[a], IU/mL				
< 2000	252 (65.5)	1.434	1.055–1.948	0.021
≥ 2000	133 (34.5)			
Tumor number[a]				
Single	319 (82.9)	1.426	1.025–1.983	0.035
Multiple	66 (17.1)			
Microvascular invasion[a]				
Yes	316 (82.1)	1.999	1.444–2.765	< 0.001
No	69 (17.9)			
Ishak fibrosis score[a]				
≤ 3	150 (39.0)	1.412	1.010–1.050	0.022
>3	235 (61.0)			

[a] Factor associated with the repeat hepatectomy

to improve prognosis, and antiviral therapy to maintain a low HBV DNA level is beneficial to all patients with HBV-related HCC undergoing liver resection.

HBV induces inflammation, damages, and results in the rapid proliferation of hepatocyte cells. During regeneration and compensatory growth, the damage results in liver fibrosis, which eventually develops into cirrhosis. Previous studies have shown lamivudine and telbivudine therapies effectively suppress HBV DNA levels and improve liver function in patients with decompensated cirrhosis,[44,45] and the level of HBV is associated with liver inflammation and fibrosis. In this study, patient groups with low HBV DNA levels before the repeat hepatectomy had significantly lower hepatic inflammation and fibrosis scores. This study further showed advanced inflammation and fibrosis before the primary hepatectomy could be attenuated or even reversed as long as low HBV DNA levels were attained before the repeat hepatectomy. High serum HBV DNA levels before the repeat hepatectomy resulted in poor prognosis after surgery.

In this study, high correlations between HBV DNA levels and cancer recurrence and between Ishak score > 3 and disease-free/overall survival after the repeat hepatectomy could be explained by the majority of patients having cirrhosis or moderate to advanced fibrosis. Ko et al. also found liver fibrosis > 2 to be an independent risk factor of HCC recurrence.[46] As effective antiviral therapy suppresses HBV, reduces liver injury, improves liver function, and reduces the risk of cancer recurrence in patients with HBV-related HCC, a low HBV DNA load should be maintained after hepatic resection.

This study has limitations. First, this is a retrospective study which has the inherent defects of such a type of study; second, the antiviral therapies given to the patients were different. As antiviral treatment was only given to 25 and 33.8% of patients before the primary and the repeat hepatectomies, respectively, we were unable to draw any conclusions on the impact of the different types of antiviral therapies on the final outcomes of the patients.

In conclusion, this study measured the HBV DNA levels before primary and repeat hepatectomies, thus providing better prediction on prognosis. In addition, this study supports prolonged antiviral treatment for HBV-related HCC. Better oncological outcomes can therefore be expected based on frequent HBV surveillance and antiviral treatment before and after hepatic resection.

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Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Bosch FX, Ribes J, Díaz M, Cléries R: Primary liver cancer: worldwide incidence and trends. Gastroenterology 2004;127:S5–S16.
2. Davis GL, Dempster J, Meler JD, Orr DW, Walberg MW, Brown B, et al.: Hepatocellular carcinoma: management of an increasingly common problem. Proc (Bayl Univ Med Cent) 2008;21:266–280.
3. Parkin DM: Global cancer statistics in the year 2000. Lancet Oncol 2001;2:533–543.
4. Simonetti RG, Camma C, Fiorello F, Politi F, D'Amico G, Pagliaro L: Hepatocellular carcinoma. A worldwide problem and the major risk factors. Dig Dis Sci 1991;36:962–972.
5. Macaron C, Hanouneh IA, Zein NN: Incidence and risk factors of hepatocellular carcinoma in patients with primary biliary cirrhosis. Hepatology 2010;52:2239 author reply 2239–2240.
6. Fattovich G, Stroffolini T, Zagni I, Donato F: Hepatocellular carcinoma in cirrhosis: incidence and risk factors. Gastroenterology 2004;127:S35–50.
7. El-Serag HB: Hepatocellular carcinoma. N Engl J Med 2011;365:1118–1127.
8. del Olmo JA, Serra MA, Rodriguez F, Escudero A, Gilabert S, Rodrigo JM: Incidence and risk factors for hepatocellular carcinoma in 967 patients with cirrhosis. J Cancer Res Clin Oncol 1998;124:560–564.
9. Mahtab MA, Kamal M, Rahman S: Hepatic necroinflammation and severe liver fibrosis in patients with chronic hepatitis B with undetectable HBV DNA and persistently normal alanine aminotransferase. Bangladesh Med Res Counc Bull 2014;40:92–96.
10. Praneenararat S, Chamroonkul N, Sripongpun P, Kanngurn S, Jarumanokul R, Piratvisuth T: HBV DNA level could predict significant liver fibrosis in HBeAg negative chronic hepatitis B patients with biopsy indication. BMC Gastroenterol 2014;14:218.
11. Bonnard P, Sombié R, Lescure F-X, Bougouma A, Guiard-Schmid JB, Poynard T, et al.: Comparison of elastography, serum marker scores, and histology for the assessment of liver fibrosis in hepatitis B virus (HBV)-infected patients in Burkina Faso. Am J Trop Med Hyg 2010;82:454–458.
12. Huang G, Lau WY, Zhou W-P, Shen F, Pan Z-Y, Yuan S-X, et al.: 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;149:519–527.
13. Kremsdorf D, Soussan P, Paterlini-Brechot P, Brechot C: Hepatitis B virus-related hepatocellular carcinoma: paradigms for viral-related human carcinogenesis. Oncogene 2006;25:3823–3833.
14. Sohn W, Paik YH, Cho JY, Choi GS, Kim JM, Kwon CH, et al.: Influence of hepatitis B virus reactivation on the recurrence of HBV-related hepatocellular carcinoma after curative resection in patients with low viral load. J Viral Hepat 2015;22:539–550.
15. Chong CCN, Wong GLH, Lai PBS: Impact of antiviral therapy on post-hepatectomy outcome for hepatitis B-related hepatocellular carcinoma. World J Gastroenterol 2014;20:6006–6012.
16. Yin J, Li N, Han Y, Xue J, Deng Y, Shi J, et al.: Effect of antiviral treatment with nucleotide/nucleoside analogs on postoperative prognosis of hepatitis B virus-related hepatocellular carcinoma: a two-stage longitudinal clinical study. J Clin Oncol 2013;31:3647–3655.
17. Huang G, Lau WY, Wang Z-G, Pan Z-Y, Yuan S-X, Shen F, et al.: Antiviral therapy improves postoperative survival in patients with hepatocellular carcinoma: a randomized controlled trial. Ann Surg 2015;261:56–66.
18. Zhou Y, Zhang Z, Zhao Y, Wu L, Li B: Antiviral therapy decreases recurrence of hepatitis B virus-related hepatocellular carcinoma after curative resection: a meta-analysis. World J Surg 2014;38:2395–2402.
19. Wong JS-W, Wong GL-H, Wong VW-S, Cheung SY-S, Chong CN, Wong J, et al.: 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.
20. Huang G, Lau WY, Shen F, Pan Z-Y, Fu S-Y, Yang Y, et al.: Preoperative hepatitis B virus DNA level is a risk factor for postoperative liver failure in patients who underwent partial hepatectomy for hepatitis B-related hepatocellular carcinoma. World J Surg 2014;38:2370–2376.
21. Chen C-J, Iloeje UH, Yang H-I: Long-term outcomes in hepatitis B: the REVEAL-HBV study. Clin Liver Dis 2007;11:797–816 viii.
22. Lopez P, Marzano E, Piardi T, Pessaux P: Repeat hepatectomy for liver metastases from colorectal primary cancer: a review of the literature. J Visc Surg 2012;149:e97–e103.
23. Zhou Y, Sui C, Li B, Yin Z, Tan Y, Yang J, et al.: Repeat hepatectomy for recurrent hepatocellular carcinoma: a local experience and a systematic review. World J Surg Oncol 2010;8:55.
24. Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, et al.: Histological grading and staging of chronic hepatitis. J Hepatol 1995;22:696–699.
25. Watters DA, Hollands MJ, Gruen RL, Maoate K, Perndt H, McDougall RJ, et al.: Perioperative mortality rate (POMR): a global indicator of access to safe surgery and anaesthesia. World J Surg 2014;39:856–864.
26. Schroeder RA, Marroquin CE, Bute BP, Khuri S, Henderson WG, Kuo PC: Predictive indices of morbidity and mortality after liver resection. Ann Surg 2006;243:373–379.
27. Bozzetti F, Gennari L, Regalia E, Bignami P, Montalto F, Mazzaferro V, et al.: Morbidity and mortality after surgical resection of liver tumors. Analysis of 229 cases. Hepatogastroenterology 1992;39:237–241.
28. Lee PH, Lin WJ, Tsang YM, Hu RH, Sheu JC, Lai MY, et al.: Clinical management of recurrent hepatocellular carcinoma. Ann Surg 1995;222:670–676.
29. Kanematsu T, Matsumata T, Takenaka K, Yoshida Y, Higashi H, Sugimachi K: Clinical management of recurrent hepatocellular carcinoma after primary resection. Br J Surg 1988;75:203–206.
30. Sugioka A, Kanai T: Postresection prognosis of patients with hepatocellular carcinoma. Surgery 1993;113:612–618.
31. Chen MF, Hwang TL, Jeng LB, Jan YY, Chen SC: Postoperative recurrence of hepatocellular carcinoma. Two hundred five consecutive patients who underwent hepatic resection in 15 years. Arch Surg 1994;129:738–742.
32. Bodzin AS: Hepatocellular carcinoma (HCC) recurrence and what to do when it happens. Hepatobiliary Surg Nutr 2016;5:503–505.
33. Itamoto T, Nakahara H, Kohashi T, Ohdan H, Tashiro H: Repeat hepatectomy for recurrent hepatocellular carcinoma. Surgery 2007;141:589–597.
34. Maehara S, Tanaka S, Shimada M, Sugimachi K: Repeat hepatectomy is the most useful treatment for recurrent hepatocellular carcinoma. J Hepatobiliary Pancreat Surg 2001;8:410–416.
35. Chan DL, Morris DL, Chua TC: Clinical efficacy and predictors of outcomes of repeat hepatectomy for recurrent hepatocellular carcinoma—a systematic review. Surg Oncol 2013;22:e23–30.
36. Shimada M, Takenaka K, Taguchi K, Fujiwara Y, Gion T, Kajiyama K, et al.: Prognostic factors after repeat hepatectomy for recurrent hepatocellular carcinoma. Ann Surg 1998;227:80–85.
37. Wang R-Y, Chen L, Chen H-Y, Hu L, Li L, Sun H-Y, et al.: MUC15 inhibits dimerization of EGFR and PI3K-AKT signaling and is associated with aggressive hepatocellular carcinomas in patients. Gastroenterology 2013;145:1436–48.e1–12.
38. Cheung Y-S, Chan HLY, Wong J, Lee K-F, Poon TCW, Wong N, et al.: Elevated perioperative transaminase level predicts intrahepatic recurrence in hepatitis B-related hepatocellular carcinoma after curative hepatectomy. Asian J Surg 2008;31:41–49.

39. Huang G, Yang Y, Shen F, Pan Z-Y, Fu S-Y, Lau WY, et al.: Early viral suppression predicts good postoperative survivals in patients with hepatocellular carcinoma with a high baseline HBV-DNA load. Ann Surg Oncol 2013;20:1482–1490.
40. Wu J-C, Huang Y-H, Chau G-Y, Su C-W, Lai C-R, Lee P-C, et al.: Risk factors for early and late recurrence in hepatitis B-related hepatocellular carcinoma. J Hepatol 2009;51:890–897.
41. Bréchot C: Pathogenesis of hepatitis B virus-related hepatocellular carcinoma: old and new paradigms. Gastroenterology 2004;127: S56–61.
42. Kubo S, Nishiguchi S, Hamba H, Hirohashi K, Tanaka H, Shuto T, et al.: Reactivation of viral replication after liver resection in patients infected with hepatitis B virus. Ann Surg 2001;233:139–145.
43. Su I-J, Hsieh W-C, Tsai H-W, Wu H-C: Chemoprevention and novel therapy for hepatocellular carcinoma associated with chronic hepatitis B virus infection. Hepatobiliary Surg Nutr 2013;2:37–39.
44. Liaw YF, Leung NW, Chang TT, Guan R, Tai DI, Ng KY, et al.: Effects of extended lamivudine therapy in Asian patients with chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. Gastroenterology 2000;119:172–180.
45. Chan HLY, Chen YC, Gane EJ, Sarin SK, Suh DJ, Piratvisuth T, et al.: Randomized clinical trial: efficacy and safety of telbivudine and lamivudine in treatment-naïve patients with HBV-related decompensated cirrhosis. J Viral Hepat 2012;19:732–743.
46. Ko S, Kanehiro H, Hisanaga M, Nagao M, Ikeda N, Nakajima Y: Liver fibrosis increases the risk of intrahepatic recurrence after hepatectomy for hepatocellular carcinoma. Br J Surg 2002;89:57–62.