Postoperative hepatitis B virus reactivation in hepatitis B virus-related hepatocellular carcinoma patients with hepatitis B virus DNA levels <500 copies/mL

Zhi-Bo Xie1,2,*
Xiao-Bo Wang1,3,*
De-Liang Fu2
Jian-Hong Zhong1,4
Xia-Wei Yang1
Le-Qun Li1,4

1Department of Hepatobiliary Surgery, Affiliated Tumor Hospital of Guangxi Medical University, Nanning, 2Department of Pancreatic Surgery, Pancreatic Disease Institute, Huashan Hospital, Shanghai Medical College, Fudan University, Shanghai, 3Department of Hepatobiliary Surgery, Affiliated Minzu Hospital of Guangxi Medical University, 4Guangxi Liver Cancer Diagnosis and Treatment Engineering and Technology Research Center, Nanning, People’s Republic of China

*These authors contributed equally to this work

Introduction
Hepatocellular carcinoma (HCC) is the fifth most common type of cancer in the world and is the third leading cause of cancer-related death.1 Hepatitis B virus (HBV) infection is the major risk factor for HCC patients, especially in Asia-Pacific region.2,4 Because of the high incidence of postoperative HCC recurrence, overall survival (OS) of HCC patients remains relatively low, despite surgery being a curative therapy.5,6 However, postoperative HBV reactivation (PHR) is one significant factor7,8 that contributes to HCC recurrence. Several trials9–12 and our previous study13 found that PHR occurs after hepatectomy in HBV-related HCC (HBV-HCC) patients. Hepatitis B e antigen (HBeAg), preoperative HBV DNA level, liver cirrhosis degree, blood transfusion, operating time, and preoperative antiviral therapy are independent risk factors for PHR.14 PHR occurs not only in HCC patients with high HBV DNA levels but also in HCC patients with low HBV DNA levels.14,15 According to the guidelines from the Asian Pacific Association for the Study of the Liver,17 antiviral...
therapy was available for patients with detectable HBV DNA levels. However, there are no obvious suggestions about using antiviral therapies in patients with undetectable HBV DNA levels.

Here, we aimed to evaluate the incidence of PHR in HBV-HCC patients with undetectable HBV DNA levels, thus finding out the difference of the short-term and long-term outcomes between patients with PHR and patients without PHR, with undetectable HBV DNA levels.

**Patients and methods**

**Ethics statement**
This study was approved by the Institutional Review Board of Guangxi Medical University and conducted in accordance with the Declaration of Helsinki and internationally accepted ethical guidelines. The patients enrolled in this study signed written consent during their admission for surgery for their information to be stored in hospital databases and used for research. During data collection, patient records were anonymized, as we previously mentioned in our former study.13

**Patients**
A total of 258 consecutive patients admitted to our hospital from 2011 to 2012 were retrospectively enrolled in our study. The inclusion criteria were as follows: 1) 18–75 years old; 2) HBV-infected HCC patients and first diagnosed in our hospital;17 3) Child-Pugh stages A to B; 4) resectable HCC patients;18 and 5) HCC confirmed by postoperative pathology.

Patients were divided into two groups based on their HBV DNA levels (Group A: patients with HBV DNA levels \(<\) 500 copies/mL and Group B: patients with detectable HBV DNA levels). HCC was diagnosed based on the criteria of the European Association for the Study of the Liver.19

**Preoperative management**
A baseline assessment of HBV DNA; the presence of hepatitis B surface antigen, HBeAg, and against hepatitis B core antigen S1; serum levels of alanine aminotransferase (ALT), aspartate aminotransferase, total bilirubin (TBil), and albumin (ALB); prothrombin time (PT); levels of \(\alpha\)-fetoprotein (AFP); and proportions of several T-lymphocyte subpopulations (CD3\(^+\)CD4\(^+\) and CD3\(^+\)CD8\(^+\)) were tested within 1 week before hepatectomy.

Serum levels of HBV DNA were quantified using the PCR-based Care HBV V2 Assay Kit (Qiagen NV, Venlo, the Netherlands) in which the lower limit of detection was 500 copies/mL (1 IU/mL =1 copy/mL).

Entecavir (0.5 mg once a day; Tai-Tianqing Pharmaceutical Co., Jiangsu, People’s Republic of China) antiviral therapy was given to patients who fit the criteria of given antiviral therapy at least 3 days before hepatectomy.20

**Surgical management**
During hepatectomy, data on the surgical procedure, including tumor number, tumor diameter (the maximal tumor diameter), tumor rupture, complete capsule, surgical margin, tumor thrombus, hepatic inflow occlusion, operating time, blood loss, blood transfusion, and liver cirrhosis degree, were recorded.

**Postoperative management**
At the 1-month reexamination, the same blood tests and radiological examination as at baseline were repeated. Patients were monitored for PHR within postoperative 1 month. A tenfold increase in HBV DNA levels compared with preoperative levels as well as detectable level of HBV DNA postoperatively with undetectable level at baseline was defined as PHR.14 Patients with PHR were given antiviral therapy once upon the detection of reactivation.

**Outcomes and follow-up**
The primary outcomes in our study were recurrence-free survival (RFS). We compared the difference of RFS between patients with HBV DNA levels \(>\) 500 copies/mL and \(<\) 500 copies/mL. Subgroup analysis of RFS was conducted based on the status of PHR. As secondary outcomes, we compared postoperative complications between two subgroups. These complications were evaluated using the Clavien-Dindo scoring system.21

**Statistical analysis**
All data analyses were performed using SPSS 21.0 (IBM Corporation, Armonk, NY, USA) and with \(P<0.05\) defined as the threshold of statistical significance. Normally distributed data were expressed as mean \(\pm\) SD, while asymmetrically distributed data were expressed as median (range). Differences in outcomes between two groups were assessed using independent samples \(t\)-tests for measurement data or \(\chi^2\) test for frequency of various attributes between groups. Factors significantly associated with PHR and HCC recurrence were identified first by univariate logistic regression, and then the significant univariate factors were examined by multivariate analysis using a stepwise logistic model. RFS curves were analyzed using the Kaplan–Meier method in which differences between curves were assessed using the log-rank test.
Comprehensive MEDLINE review

The following medical subject headings were comprehensively searched in the MEDLINE database: “hepatocellular carcinoma” or “primary liver carcinoma” or “primary liver cancer” or “liver cancer” or “liver tumor” and “hepatectomy” or “liver resection” or “hepatic resection” and “hepatitis B virus” and “reactivation”. Based on the following criteria, relevant references and review articles were manually searched: 1) evaluated PHR in HBV -HCC patients, 2) were published in English, and 3) outcomes were about survival outcomes, PHR rate, and HCC recurrence. We selected only the study with the largest number of participants when several studies were based on the same population.

Results

Characteristics of the study population

From January 2011 to 2012, 1,004 potentially eligible HCC patients were admitted to our hospital for hepatic resection. A total of 258 HCC patients satisfying the inclusion and exclusion criteria were enrolled. Of the 258 HCC patients, 159 patients with HBV DNA levels <500 copies/mL were defined as Group A and the remaining 99 patients with detectable HBV DNA levels were defined as Group B. Baseline characteristics were similar between the two groups (Table 1). Of the 258 HCC patients enrolled, 33 (12.8%) patients with detectable HBV DNA levels were given antiviral therapy. After hepatectomy, 50 (19.4%) patients (24 patients with HBV DNA levels <500 copies/mL and 15.1% and 26 patients with detectable HBV DNA levels, 26.3%) had PHR. Of the 33 patients with antiviral therapy, one (3.0%) patient had PHR.

Table 1 Characteristics of Chinese patients with HBV-related hepatocellular carcinoma who underwent hepatic resection

| Characteristics | Patients with HBV DNA <500 copies/mL (n=159) | Patients with detectable HBV DNA level (n=99) | P-value |
|-----------------|---------------------------------------------|----------------------------------------------|--------|
| Mean age ± SD (years) | 48.3 ± 11.3 | 49.6 ± 10.2 | 0.335 |
| Males, n (%) | 141 (88.7) | 83 (83.8) | 0.264 |
| Prophylactic antiviral therapy, n (%) | 0 (0.0) | 33 (33.3) | <0.001 |
| Postoperative HBV reactivation, n (%) | 24 (15.1) | 26 (28.9) | 0.027 |
| Positive for HBeAg, n (%) | 25 (15.7) | 15 (16.7) | 0.875 |
| Positive for HBV-cag s1, n (%) | 83 (52.2) | 56 (56.6) | 0.494 |
| PT (seconds) | 12.8 ± 1.22 | 13.3 ± 3.67 | 0.168 |
| TBil (μmol/L) | 10.30 (7.70–14.00) | 12.00 (8.70–16.10) | 0.982 |
| ALB (g/L) | 41.90 ± 4.48 | 40.95 ± 4.89 | 0.110 |
| ALT (IU/L) | 32.00 (22.00–45.00) | 39.00 (28.00–59.00) | 0.320 |
| AFP (ng/mL) | 459.00 (7.50–12,100.00) | 130.00 (11.00–12,100.00) | 0.948 |
| CD3+/CD4+ (%) | 33.5 ± 8.16 | 35.7 ± 10.41 | 0.497 |
| CD3+/CD8+ (%) | 23.12 ± 8.78 | 20.37 ± 5.67 | 0.076 |
| Tumor number | 1.39 ± 0.67 | 1.40 ± 0.70 | 0.872 |
| Tumor diameter (cm) | 7.06 ± 3.45 | 7.14 ± 3.77 | 0.781 |
| Tumor rupture, n (%) | 8 (5.0) | 5 (3.1) | 0.995 |
| Complete capsule, n (%) | 102 (64.2) | 63 (63.7) | 0.933 |
| Surgical margin (cm) | 2.38 ± 1.12 | 2.12 ± 1.05 | 0.613 |
| Tumor thrombus, n (%) | 37 (23.3) | 24 (24.2) | 0.337 |
| Hepatic flow occlusion, n (%) | 113 (71.1) | 72 (72.7) | 0.774 |
| Operating time (minutes) | 186.89 ± 62.45 | 198.22 ± 64.26 | 0.845 |
| Blood loss (mL) | 300.00 (200.00–500.00) | 350.00 (200.00–600.00) | 0.675 |
| Blood transfusion, n (%) | 32 (20.1) | 19 (19.2) | 0.855 |
| BCLC stage (A/B/C), n (%) | 82 (51.6)/40 (25.2)/37 (23.3) | 51 (51.5)/24 (24.2)/24 (24.2) | 0.978 |
| Degree of liver cirrhosis (0/1/2/3), n (%) | 25 (15.7)/69 (43.4)/48 (30.2)/17 (10.7) | 12 (12.1)/45 (45.5)/31 (31.3)/11 (11.1) | 0.886 |
| Cost (RMB) | 55,349.64 ± 11,649.67 | 56,649.16 ± 16,377.7 | 0.184 |
| Hospital stay (days) | 18.27 ± 7.24 | 19.16 ± 10.37 | 0.934 |
| Clavien-Dindo stage score | 1.89 ± 0.72 | 1.97 ± 0.87 | 0.436 |

Note: Values are shown as mean ± SD, n (%), or average (range), unless otherwise indicated.

Abbreviations: AFP, α-fetoprotein; ALB, albumin; ALT, alanine aminotransferase; BCLC, Barcelona Clinic Liver Cancer; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HBV-cAg s1, against hepatitis B core antigen s1; PT, prothrombin time; TBil, total bilirubin.

A total of 258 HCC patients satisfying the inclusion and exclusion criteria were enrolled. Of the 258 HCC patients, 159 patients with HBV DNA levels <500 copies/mL were defined as Group A and the remaining 99 patients with detectable HBV DNA levels were defined as Group B. Baseline characteristics were similar between the two groups (Table 1). Of the 258 HCC patients enrolled, 33 (12.8%) patients with detectable HBV DNA levels were given antiviral therapy. After hepatectomy, 50 (19.4%) patients (24 patients with HBV DNA levels <500 copies/mL and 15.1% and 26 patients with detectable HBV DNA levels, 26.3%) had PHR. Of the 33 patients with antiviral therapy, one (3.0%) patient had PHR.

PHR

A total of 50 (19.4%) patients had PHR of whom one (1/33, 3.0%) patient was given antiviral therapy and the remaining 49 (49/225, 21.8%) patients were without antiviral therapy.
In patients with HBV DNA levels ≤ 500 copies/mL, 24 (15.1%) patients had PHR.

We conducted univariate and multivariate analyses and found the following significant factors related with PHR: without antiviral therapy (hazard ratio [HR] = 0.17, 95% CI 0.031–0.911, *P* < 0.001), HBeAg positivity (HR = 5.20, 95% CI 1.931–14.007, *P* = 0.001), against hepatitis B core antigen S1 positivity (HR = 2.54, 95% CI 1.116–5.762, *P* = 0.026), preoperative HBV DNA level of ≥ 500 copies/mL (HR = 1.28, 95% CI 1.085–2.884, *P* = 0.030), hepatic inflow occlusion (HR = 3.60, 95% CI 1.402–9.277, *P* = 0.008), moderate liver cirrhosis or more (HR = 2.26, 95% CI 1.001–5.121, *P* = 0.049), and blood transfusion (HR = 2.89, 95% CI 0.836–10.041, *P* = 0.043) (Table 2). Thus, HBV DNA levels ≥ 500 copies/mL and without preoperative antiviral therapy remained independent risk factors for PHR.

RFS

RFS (23.06 ± 2.46 months) was significantly lower in patients with PHR than in patients without PHR (29.30 ± 1.27 months, *P* = 0.014) (Figure 1). One-year, 2-year, and 3-year RFS rates for patients with PHR were 75.8%, 38.1%, and 28.9%, respectively, and those for patients without PHR were 84.5%, 56.5%, and 40.7%, respectively.

We conducted univariate and multivariate analyses and found the following significant risk factors for RFS: PHR (HR = 1.48, 95% CI 1.228–2.023, *P* = 0.047), HBeAg positivity (HR = 4.84, 95% CI 2.617–8.941, *P* < 0.001).

**Table 2** Univariate and multivariate analyses of prognostic factors for HBV reactivation in Chinese patients with HBV-related HCC

| Factor                       | Univariate analysis | Multivariate analysis |
|------------------------------|---------------------|-----------------------|
|                              | Patients, n (%)     | HR                    | 95% confidence interval | *P*-value | HR                    | 95% confidence interval | *P*-value |
| Antiviral therapy            |                     |                       |                       |           |                       |                       |           |
| Yes                          | 33 (12.8)           | 0.24                  | 0.055–1.030           | < 0.001   | 0.17                  | 0.031–0.911            | < 0.001   |
| No                           | 225 (87.2)          |                       |                       |           |                       |                       |           |
| Positive for HBeAg           |                     |                       |                       |           |                       |                       |           |
| Yes                          | 40 (15.5)           | 6.27                  | 3.021–13.001          | < 0.001   | 5.20                  | 1.931–14.007           | 0.001     |
| No                           | 218 (84.5)          |                       |                       |           |                       |                       |           |
| HBV-cAg S1                   |                     |                       |                       |           |                       |                       |           |
| Yes                          | 139 (53.9)          | 2.09                  | 1.085–4.007           | 0.028     | 2.54                  | 1.116–5.762            | 0.026     |
| No                           | 119 (46.1)          |                       |                       |           |                       |                       |           |
| Preoperative HBV DNA         |                     |                       |                       |           |                       |                       |           |
| >500                         | 99 (38.4)           | 1.81                  | 0.971–3.376           | 0.022     | 1.28                  | 1.085–2.884            | 0.030     |
| <500                         | 159 (61.6)          |                       |                       |           |                       |                       |           |
| Hepatic inflow occlusion     |                     |                       |                       |           |                       |                       |           |
| Yes                          | 185 (71.7)          | 3.93                  | 1.712–9.005           | 0.031     | 3.60                  | 1.402–9.277            | 0.008     |
| No                           | 73 (28.3)           |                       |                       |           |                       |                       |           |
| Degree of liver cirrhosis    |                     |                       |                       |           |                       |                       |           |
| Light (0–1)                  | 151 (58.5)          | 3.52                  | 1.834–6.736           | < 0.001   | 2.26                  | 1.001–5.121            | 0.049     |
| Moderate or more (≥ 2)       | 107 (41.5)          |                       |                       |           |                       |                       |           |
| Operating time (minutes)     |                     |                       |                       |           |                       |                       |           |
| >180                         | 80 (31.0)           | 4.25                  | 2.232–8.097           | 0.041     | 3.26                  | 1.418–7.508            | 0.055     |
| ≤180                         | 178 (69.0)          |                       |                       |           |                       |                       |           |
| Blood transfusion, n (%)     |                     |                       |                       |           |                       |                       |           |
| Yes                          | 51 (19.8)           | 4.85                  | 2.451–9.596           | < 0.001   | 2.89                  | 0.836–10.041           | 0.043     |
| No                           | 207 (80.2)          |                       |                       |           |                       |                       |           |

*Abbreviations:* HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HBV-cAg S1, against hepatitis B core antigen S1; HCC, hepatocellular carcinoma; HR, hazard ratio.

**Figure 1** Recurrence-free survival between patients with HBV DNA levels <500 copies/mL and patients with detectable HBV DNA levels.

*Abbreviations:* HBV, hepatitis B virus; PHR, postoperative HBV reactivation.
surgical margin <1 cm (HR =0.53, 95% CI 0.290–0.964, P=0.038), moderate or severe liver cirrhosis (HR =1.88, 95% CI 1.040–3.377, P=0.036), and blood transfusion (HR =3.25, 95% CI 1.691–6.213, P<0.001) (Table 3).

Subgroup analysis of RFS depending on antiviral therapy was also conducted. We found that patients with and without antiviral therapy had similar RFS (28.13 months and 27.82 months, respectively, P=0.996).

Postoperative complications and recurrence

Table 3 Univariate and multivariate analyses of prognostic factors for recurrence-free survival in Chinese patients with HBV-related HCC

| Factor                              | Univariate analysis | Multivariate analysis |
|-------------------------------------|--------------------|-----------------------|
|                                    | Patients, n (%)    | HR                    | 95% confidence interval | P-value | HR                    | 95% confidence interval | P-value |
| Postoperative HBV reactivation      |                    |                      |                       |         |                      |                       |         |
| Yes                                 | 50 (19.4)          | 1.87                  | 1.119–3.103           | 0.017   | 1.48                  | 1.228–2.023            | 0.047   |
| No                                  | 208 (80.6)         |                       |                       |         |                       |                       |         |
| Positive for HBeAg                  |                    |                      |                       |         |                      |                       |         |
| Yes                                 | 40 (15.5)          | 6.64                  | 3.96–11.13            | <0.001  | 4.84                  | 2.617–8.941            | <0.001  |
| No                                  | 218 (84.5)         |                       |                       |         |                       |                       |         |
| Preoperative HBV DNA                |                    |                      |                       |         |                      |                       |         |
| >500                                | 99 (38.4)          | 10.30                 | 5.110–20.777          | <0.001  | 3.37                  | 1.424–7.962            | 0.006   |
| ≤500                                | 159 (61.6)         |                       |                       |         |                       |                       |         |
| Surgical margin (cm)                |                    |                      |                       |         |                      |                       |         |
| >1.0                                | 165 (64.0)         | 0.23                  | 0.141–0.380           | <0.001  | 0.53                  | 0.290–0.964            | 0.038   |
| ≤1.0                                | 93 (36.0)          |                       |                       |         |                       |                       |         |
| Hepatic inflow occlusion            |                    |                      |                       |         |                      |                       |         |
| Yes                                 | 185 (71.7)         | 1.22                  | 0.755–1.972           | 0.416   | –                     | –                     | –       |
| No                                  | 73 (28.3)          |                       |                       |         |                       |                       |         |
| Degree of liver cirrhosis           |                    |                      |                       |         |                      |                       |         |
| Light (0–1)                         | 151 (58.5)         | 2.41                  | 1.500–3.878           | <0.001  | 1.88                  | 1.040–3.377            | 0.036   |
| Moderate or more (≥2)               | 107 (41.5)         |                       |                       |         |                       |                       |         |
| Blood transfusion, n (%)            |                    |                      |                       |         |                      |                       |         |
| Yes                                 | 51 (19.8)          | 6.40                  | 3.938–10.404          | <0.001  | 3.25                  | 1.691–6.213            | <0.001  |
| No                                  | 207 (80.2)         |                       |                       |         |                       |                       |         |

Abbreviations: HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HR, hazard ratio.
Discussion

Many studies\textsuperscript{9,11,14,16,22} and our previous study\textsuperscript{13} figured out that PHR occurred after hepatectomy. However, the mechanism of PHR is not clear. We previously inferred that PHR may be associated with the immunosuppression induced by hepatectomy. Also, some other studies claimed that increased apoptosis of CD4\textsuperscript{+}/CD8\textsuperscript{+} T-cells and the immunosuppression associated with surgical injury.\textsuperscript{24,25} It is commonly believed that PHR remains the independent risk factor for HCC recurrence and RFS.\textsuperscript{14,23} Moreover, studies found that preoperative antiviral therapy could efficiently decrease the PHR rate and reduce the HCC recurrence, thus prolonging the RFS.\textsuperscript{14,16}

In our study, PHR rates are 3.0\% and 21.8\% in patients with and without antiviral therapy, respectively. The results were similar to those of our previous prospective study\textsuperscript{13} (total PHR rate, 19.3\%; PHR rate in patients with antiviral therapy, 2.2\%; PHR rate in patients without antiviral therapy, 27.8\%). In our literature review, total PHR rate varied from 6.1\% to 40.7\% and PHR rate varied from 0.0\% to 4.7\% in patients with antiviral therapy.\textsuperscript{9,12,14,16,22,23} For patients with antiviral therapy, PHR rate was significantly decreased. Studies claimed that antiviral therapy predicted good long-term survival.\textsuperscript{26,27} Other studies figured that antiviral therapy had no significant influence on HCC recurrence and OS.\textsuperscript{28,30} Hepatocarcinogenesis may be initiated by continuous HBV replication.\textsuperscript{31–33} Antiviral therapy may reduce HCC recurrence and prolong survival by suppressing and reducing continuous viremia and long-term hepatic inflammation. In our study, antiviral therapy remained the significant risk factor for PHR. However, antiviral therapy was not the independent risk factor for RFS and HCC recurrence. Also, we previously found that antiviral therapy failed to prolong short-term survival.\textsuperscript{34}

PHR rate was lower in our study than that in the study conducted by Huang et al\textsuperscript{10} (16.7\%). It is mainly because the HBV DNA lower limit is different. Our lower limit was 500 copies/mL (1 IU/mL =1 copy/mL according to the instruction of the kit), whereas their lower limit was 200 IU/mL (800 copies/mL, 1 IU/mL =4 copies/mL according to Chinese guidelines\textsuperscript{39}). In our result, HBV DNA load is still regarded as a risk factor, and a high HBV DNA load has been reported to be associated with an increased incidence of PHR.\textsuperscript{14,35} In our literature review, antiviral therapy was found to be a protective factor.\textsuperscript{9,11,12,14}

Due to the high incidence of HCC recurrence, OS of HCC patients still stays unsatisfied.\textsuperscript{36} In our study, patients with PHR suffered worse RFS (RFS: 23 months and 29 months for patients with and without PHR, respectively, and 1-year, 2-year, and 3-year RFS rates were 75.8\%, 38.1\%, and 28.9\% and 84.5\%, 56.5\%, and 40.7\% for patients with and without PHR, respectively). As described by Huang et al,\textsuperscript{14} PHR still remained the prognostic factor for RFS in multivariate regression analysis. Postoperative continuous HBV replication may constantly accelerate liver cirrhosis and give rise to HCC recurrence so as to decrease the RFS.\textsuperscript{37,38} Continuously, viremia may do harm to our immune system and cause multicentric carcinogenesis.\textsuperscript{39,40} Moreover, this replication may enhance tumor development and spread through the upregulation of adhesion molecules.\textsuperscript{39}

We found that HBeAg positivity was the independent risk factor for HCC recurrence and OS. Many studies claimed that HBeAg-positive patients have a higher risk of PHR and are associated with HCC recurrence.\textsuperscript{14,23} Also, a change from HBeAg-negative to HBeAg-positive status still related to PHR.\textsuperscript{51,52} The immune system has been broken down in HCC patients, especially in postoperative HCC patients. Resection induces immunosuppression and permits enhanced viral replication. Therefore, patients in the immune clearance phase, especially HBeAg-positive patients, were at risk for reactivation of viral replication after surgery.

Several limitations could be found in this study. First, our retrospective design of the study was the biggest limitation. However, the baseline characteristics were similar between the two groups. This may decrease the selection bias to some extent. Second, we did not have long follow-ups, and we took only RFS into consideration. Ideally, we would continue to follow up on these patients for decades. In future, a better designed trial with a large sample and a long follow-up need to be further established.

Conclusion

PHR indeed occurs after hepatectomy in HBV-HCC patients. Antiviral therapy could efficiently decrease the incidence of PHR. Patients with PHR are associated with HCC recurrence and a worse RFS. Patients with HBV DNA levels <500 copies/mL still have the risk of PHR.

Acknowledgment

This work was supported by grants from the National Major Special Science and Technology Project (no 2012ZX10002010001009).

Disclosure

The authors report no conflicts of interest in this work.
References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin. 2015;65(2):87–108.

2. de Martel C, Ferlay J, Franceschi S, et al. Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. Lancet Oncol. 2012;13(6):607–615.

3. McGlynn KA, Tsao L, Hsing AW, Devesa SS, Fraumeni JF Jr. International trends and patterns of primary liver cancer. Int J Cancer. 2001;94(2):290–296.

4. Kirk GD, Lesi OA, Mendy M, et al. The Gambia Liver Cancer Study: infection with hepatitis B and C and the risk of hepatocellular carcinoma in West Africa. Hepatology. 2004;39(1):211–219.

5. Poon RT, Fan ST, Lo CM, Wong LT, et al. Effects of antiviral therapy on hepatitis B virus replication in patients undergoing partial hepatectomy for hepatocellular carcinoma. J Gastroenterol Hepatol. 2012;27(1):158–164.

6. Huang L, Li J, Lau WY, et al. Perioperative reactivation of hepatitis B virus in patients undergoing partial hepatectomy for hepatocellular carcinoma. J Gastroenterol Hepatol. 2010;25(3):638–642.

7. Shah SA, Cleary SP, Wei AC, et al. Recurrence after liver resection of hepatocellular carcinoma: a retrospective study. J Gastroenterol. 2013;48(9):991–999.

8. Shah SA, Cleary SP, Wei AC, et al. Recurrence after liver resection for HBV-related small hepatocellular carcinoma. Australas Radiol. 2014;58(4):372–377.

9. Huang L, Li J, Lau WY, et al. Postoperative recurrence of hepatocellular carcinoma. Two hundred five consecutive patients who underwent hepatic resection in 15 years. Arch Surg. 1994;129(7):738–742.

10. Hung IF, Poon RT, Lai CL, Fung J, Fan ST, Yuen MF. Recurrence of hepatocellular carcinoma after partial hepatectomy: an analysis of 221 patients. J Hepatol. 1999;31(4):90–95.

11. Dan JQ, Zhang YJ, Huang JT, et al. Hepatitis B virus reactivation after radiofrequency ablation or hepatic resection for HBV-related small hepatocellular carcinoma: a retrospective study. Eur J Surg Oncol. 2013;39(8):865–872.

12. Lao XM, Luo G, Ye LT, et al. Effects of antiviral therapy on hepatitis B virus reactivation and liver function after resection or chemoembolization for hepatocellular carcinoma. Liver Int. 2013;33(4):595–604.

13. Xie ZB, Zhu SL, Peng YC, et al. Postoperative hepatitis B virus reactivation and surgery-induced immunosuppression in patients with hepatitis B-related hepatocellular carcinoma. J Surg Oncol. 2015;112(6):634–642.

14. Huang G, Lai EC, Lau WY, et al. Posthepatectomy HBV reactivation in hepatitis B-related hepatocellular carcinoma influences postoperative survival in patients with preoperative low HBV-DNA levels. Ann Surg. 2015;261(1):56–66.

15. Li X, Zhong X, Chen ZH, et al. Hepatitis B virus DNA negativity acts as a favorable prognostic factor in hepatocellular carcinoma patients. Asian Pac J Cancer Prev. 2014;15(22):9635–9641.

16. Huang G, Lau WY, Shen F, 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(9):2370–2376.

17. Liaw YF, Leung N, Kao JH, et al. Chronic Hepatitis B Guideline Working Party of the Asian-Pacific Association for the Study of the Liver. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. Hepatol Int. 2008;2(3):263–283.

18. Zhong JH, Ke Y, Gong WF, et al. Hepatic resection associated with good survival for selected patients with intermediate and advanced-stage hepatocellular carcinoma. Ann Surg. 2014;260(2):329–340.

19. Bruix J, Sherman M, Llovet JM, et al; EASL Panel of Experts on HCC. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. J Hepatol. 2001;35(3):421–430.

20. Chinese Society of Hepatology and Chinese Society of Infectious Diseases, Chinese Medical Association. The guideline of prevention and treatment for chronic hepatitis B (2010 version). Zhonghua Gan Bing Za Zhi. 2011;19(1):13–24.

21. Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. Ann Surg. 2004;240(2):205–213.

22. Huang G, Lau WY, Wang ZG, et al. Antiviral therapy improves postoperative survival in patients with hepatocellular carcinoma: a randomized controlled trial. Ann Surg. 2015;261(1):56–66.

23. Sohn W, Paik YH, Cho JY, 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(6):539–550.

24. Dietz A, Heilmich F, Daniel V, Polazar H, Weidauer H, Maier H. Immuno-modulating effects of surgical intervention in tumors of the head and neck. Otolaryngol Head Neck Surg. 2000;123(1 Pt 1):132–139.

25. Fong DY, Wong WK, Tse CH, et al. Early virological suppression is associated with good maintained response to adefovir dipivoxil in lamivudine resistant chronic hepatitis B. Aliment Pharmacol Ther. 2007;25(8):891–898.

26. Kuzuya T, Katano Y, Kumada T, et al. Efficacy of antiviral therapy with lamivudine after initial treatment for hepatitis B virus-related hepatocellular carcinoma. J Gastroenterol Hepatol. 2007;22(11):1929–1935.

27. Chuma M, Hige S, Kamiyama T, et al. 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(9):991–999.

28. Ni L, Lai EC, Shi J, et al. A comparative study of antiviral therapy after resection of hepatocellular carcinoma in the immune-active phase of hepatitis B virus infection. Ann Surg Oncol. 2010;17(1):179–185.

29. Colombo M, Sangiovanni A. Etiology, natural history and treatment of hepatocellular carcinoma. Antiviral Res. 2003;60(2):145–150.

30. Lai SH, Hildt E. Hepatitis B virus-induced oncogenesis. World J Gastroenterol. 2007;13(17):74–81.

31. Chen JD, Yang HH, Iloeje UH, et al; Risk Evaluation of Viral score in HCC patients with undetectable HBV DNA levels. Am J Gastroenterol. 2000;195(10):1141–1147.

32. Shah SA, Cleary SP, Wei AC, et al. Antiprotis and surgical trauma: dysregulated expression of death and survival factors on peripheral lymphocytes. Arch Surg. 2000;135(10):1141–1147.

33. Sohn W, Paik YH, Cho JY, 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(6):539–550.

34. Dietz A, Heilmich F, Daniel V, Polazar H, Weidauer H, Maier H. Immuno-modulating effects of surgical intervention in tumors of the head and neck. Otolaryngol Head Neck Surg. 2000;123(1 Pt 1):132–139.

35. Delogu G, Moretti S, Antonucci A, et al. Apoptosis and surgical trauma: dysregulated expression of death and survival factors on peripheral lymphocytes. Arch Surg. 2000;135(10):1141–1147.

36. Shah SA, Cleary SP, Wei AC, et al. Antiprotis and surgical trauma: dysregulated expression of death and survival factors on peripheral lymphocytes. Arch Surg. 2000;135(10):1141–1147.

37. Sohn W, Paik YH, Cho JY, 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(6):539–550.

38. Dietz A, Heilmich F, Daniel V, Polazar H, Weidauer H, Maier H. Immuno-modulating effects of surgical intervention in tumors of the head and neck. Otolaryngol Head Neck Surg. 2000;123(1 Pt 1):132–139.

39. Delogu G, Moretti S, Antonucci A, et al. Apoptosis and surgical trauma: dysregulated expression of death and survival factors on peripheral lymphocytes. Arch Surg. 2000;135(10):1141–1147.

40. Sohn W, Paik YH, Cho JY, 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(6):539–550.

41. Dietz A, Heilmich F, Daniel V, Polazar H, Weidauer H, Maier H. Immuno-modulating effects of surgical intervention in tumors of the head and neck. Otolaryngol Head Neck Surg. 2000;123(1 Pt 1):132–139.

42. Delogu G, Moretti S, Antonucci A, et al. Apoptosis and surgical trauma: dysregulated expression of death and survival factors on peripheral lymphocytes. Arch Surg. 2000;135(10):1141–1147.
40. Kubo S, Hirohashi K, Tanaka H, et al. Virologic and biochemical changes and prognosis after liver resection for hepatitis B virus-related hepatocellular carcinoma. *Dig Surg.* 2001;18(1):26–33.

41. Yoshiba M, Sekiyama K, Sugata F, Okamoto H, Yamamoto K, Yotsumoto S. Reactivation of precore mutant hepatitis B virus leading to fulminant hepatic failure following cytotoxic treatment. *Dig Dis Sci.* 1992;37(8):1253–1259.

42. Bird GL, Smith H, Portmann B, Alexander GJ, Williams R. Acute liver decompensation on withdrawal of cytotoxic chemotherapy and immunosuppressive therapy in hepatitis B carriers. *Q J Med.* 1989;73(270):895–902.
Supplementary materials

Table S1 Postoperative complications between patients with and without PHR

| Postoperative complications | Patients with PHR (n=50) | Patients without PHR (n=208) | P-value |
|-----------------------------|--------------------------|-----------------------------|---------|
| Fever, n (%)                | 32 (64.0)                | 83 (39.9)                   | 0.002   |
| Nausea, vomiting, n (%)     | 5 (10.0)                 | 18 (8.7)                    | 0.764   |
| Pain, n (%)                 | 17 (34.0)                | 79 (38.0)                   | 0.601   |
| Liver function impairment, n (%) | 39 (78.0)          | 129 (62.0)                  | 0.033   |
| Kidney function impairment, n (%) | 4 (8.0)            | 27 (13.0)                   | 0.468   |
| Liver failure, n (%)        | 8 (16.0)                 | 15 (7.2)                    | 0.049   |
| Bile leakage, n (%)         | 2 (4.0)                  | 7 (3.4)                     | 0.548   |
| Gastrointestinal hemorrhage, n (%) | 0 (0.0)              | 1 (0.5)                     | 0.806   |
| Infection, n (%)            | 11 (22.0)                | 21 (10.1)                   | 0.022   |
| Refractory ascites, n (%)   | 3 (6.0)                  | 7 (3.4)                     | 0.302   |
| Pulmonary complication, n (%) | 7 (14.0)              | 17 (8.2)                    | 0.203   |
| Postoperative fat liquefaction of incisions, n (%) | 7 (14.0)            | 12 (5.8)                    | 0.045   |
| Urinary tract infection, n (%) | 1 (2.0)              | 2 (1.0)                     | 0.477   |
| Pressure ulcers, n (%)      | 1 (2.0)                  | 1 (0.5)                     | 0.351   |
| Clavein-Dindo stage score, mean ± SD | 2.01±0.55          | 1.78±0.57                   | 0.038   |
| Overall incidence, n (%)    | 41 (82.0)                | 141 (67.8)                  | 0.048   |

Abbreviations: HBV, hepatitis B virus; PHR, postoperative HBV reactivation; SD, standard deviation.

Table S2 Univariate and multivariate analyses of prognostic factors for HCC recurrence in Chinese patients with HBV-related HCC

| Factor                              | Univariate analysis | Multivariate analysis |
|-------------------------------------|---------------------|-----------------------|
|                                     | Patients, n (%)     | HR                    | 95% confidence interval | P-value | HR                    | 95% confidence interval | P-value |
| Positive for HBeAg                   |                      |                       |                        |         |                       |                        |         |
| Yes                                 | 40 (15.5)            | 6.06                  | 2.961–12.419           | <0.001  | 5.36                  | 1.523–18.863            | 0.009   |
| No                                  | 218 (84.5)           |                       |                        |         |                       |                        |         |
| Portal vein thrombus, n (%)         |                      |                       |                        |         |                       |                        |         |
| Yes                                 | 61 (23.6)            | 2.00                  | 1.105–3.616            | 0.022   | 1.70                  | 0.227–3.135             | 0.047   |
| No                                  | 197 (76.4)           |                       |                        |         |                       |                        |         |
| Blood transfusion, n (%)            |                      |                       |                        |         |                       |                        |         |
| Yes                                 | 51 (19.8)            | 64.05                 | 23.319–175.904         | <0.001  | 218.15                | 21.946–2,168.391        | <0.001  |
| No                                  | 207 (80.2)           |                       |                        |         |                       |                        |         |

Abbreviations: HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HR, hazard ratio.

Table S3 Perioperative changes in patients with HBV-related HCC

| Index                              | Preoperative levels | P-value | Postoperative levels | P-value |
|------------------------------------|---------------------|---------|----------------------|---------|
|                                    | PHR (n=50)          | Non-PHR (n=208) | PHR (n=50)          | Non-PHR (n=208) |
|                                    |                     |           |                     |           |
| PT (seconds)                       | 13.51±1.64          | 12.73±1.41 | 0.876               | 13.91±1.57   | 13.61±1.67   | 0.821   |
| TBil (μmol/L)                      | 13.00 (6.50–16.70)  | 10.10 (4.50–14.20) | 0.264               | 18.90 (11.30–22.50) | 17.60 (9.80–20.60) | 0.862   |
| ALB (g/L)                          | 36.01±2.27          | 41.32±4.79 | 0.001               | 31.75±5.79   | 36.10±4.26   | 0.032   |
| ALT (IU/L)                         | 38.30 (22.30–46.50) | 34.10 (20.10–42.90) | 0.291               | 130.50 (79.00–282.30) | 115.20 (75.00–210.60) | 0.086   |
| AFP (ng/mL)                        | 616.20 (8.00–12,100.00) | 125.00 (6.50–12,100.00) | 0.101               | 352.50 (6.30–4,940.00) | 106.50 (4.50–3,630.00) | 0.063   |
| CD3<sup>+</sup>CD4<sup>+</sup> (%) | 31.17±8.83          | 38.46±8.71 | 0.041               | 18.94±7.14   | 39.97±11.02  | <0.001  |
| CD3<sup>+</sup>CD8<sup>+</sup> (%) | 21.34±7.28          | 22.39±7.55 | 0.621               | 13.74±5.49   | 25.43±7.46   | <0.001  |

Note: Values are shown as mean ± SD, n (%), or average (range), unless otherwise indicated.

Abbreviations: AFP, α-fetoprotein; ALB, albumin; ALT, alanine aminotransferase; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; PHR, postoperative HBV reactivation; PT, prothrombin time; TBil, total bilirubin.
Table S4 Characteristics and outcomes of recent literature and our study

| Study          | Country       | Design     | Patients, n | Antiviral therapy, n | PHR, n (%) | DFS                  |
|----------------|---------------|------------|-------------|----------------------|-------------|----------------------|
| Dan et al      | People's Republic of China | Retrospective | 93          | 35                   | 13 (14.0) | 1 (2.9) 12 (20.7) |
| Huang et al    | People's Republic of China | Retrospective | 164         | 126                  | 10 (6.1)  | 2 (1.6) 8 (21.2) |
| Huang et al    | People's Republic of China | Retrospective | 1,609       | 150                  | 308 (19.1)| 7 (4.7) 301 (20.6) |
| Huang et al    | People's Republic of China | Retrospective | 84          | 40                   | 15 (17.9)| 1 (2.5) 14 (31.8) |
| Huang et al    | People's Republic of China | Retrospective | 1,602       | 227                  | 175 (10.9)| 4 (2.2) 170 (12.4) |
| Huang et al    | People's Republic of China | Retrospective | 200         | 100                  | 20 (10.0)| 1 (1.0) 19 (9.0) |
| Lao et al      | People's Republic of China | Retrospective | 204         | 83                   | 19 (9.3) | 0 (0.0) 19 (15.7) |
| Sohn et al     | Korea         | Retrospective | 130         | 64                   | 53 (40.7)| – – – |
| Our study      | People's Republic of China | Retrospective | 258         | 33                   | 50 (19.4)| 1 (3.3) 49 (21.8) |

Abbreviations: DFS, disease-free survival; HBV, hepatitis B virus; PHR, postoperative HBV reactivation.

Table S5 Prognostic factors of PHR, recurrence, and RFS in recent literature and our study

| Study          | Risk factors for PHR | Risk factors for HCC recurrence | Risk factors for RFS |
|----------------|----------------------|---------------------------------|----------------------|
| Dan et al      | Without antiviral therapy and hepatic resection | – | – |
| Huang et al    | Without antiviral therapy and preoperative HBV DNA <10^9 copies/mL | – | – |
| Huang et al    | HBeAg positivity, HBV DNA level of ≥200 IU/mL, Ishak inflammation score of ≥3, preoperative TACE, operation time of >180 minutes, and blood transfusion | – | HBeAg positivity, HBV DNA level of ≥200 IU/mL, tumor diameter of >5 cm, presence of satellite nodules, presence of portal vein tumor thrombus, blood transfusion, resection margin of <1.0 cm, and HBV reactivation |
| Huang et al    | – | Tumor size of >5 cm, surgical margin of <1 cm, tumor encapsulation, presence of microsatellite nodules, and presence of microportal vein tumor thrombus | – |
| Lao et al      | Without antiviral therapy and hepatic inflow occlusion | – | – |
| Sohn et al     | Without antiviral therapy | | |
| Our study      | Without antiviral therapy, HBeAg positivity, HBV-cag s1 positivity, preoperative HBV DNA level of ≥500 copies/mL, hepatic inflow occlusion, moderate liver cirrhosis or more, operating time >180 minutes, and blood transfusion | HBeAg positivity, tumor number >1, microvascular invasion, and HBV reactivation | PHR, HBeAg positivity, surgical margin <1 cm, moderate liver cirrhosis or more, and blood transfusion |

Abbreviations: HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HBV-cag s1, against hepatitis B core antigen s1; HCC, hepatocellular carcinoma; PHR, postoperative HBV reactivation; RFS, recurrence-free survival; TACE, transarterial chemoembolization.
References
1. Dan JQ, Zhang YJ, Huang JT, et al. Hepatitis B virus reactivation after radiofrequency ablation or hepatic resection for HBV-related small hepatocellular carcinoma: a retrospective study. *Eur J Surg Oncol*. 2013;39:865–872.
2. Huang L, Li J, Lau WY, et al. Perioperative reactivation of hepatitis B virus replication in patients undergoing partial hepatectomy for hepatocellular carcinoma. *J Gastroenterol Hepatol*. 2012;27:158–164.
3. Huang G, Lai EC, Lau WY, et al. 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.
4. Huang L, Li J, Yan J, et al. Antiviral therapy decreases viral reactivation in patients with hepatitis B virus-related hepatocellular carcinoma undergoing hepatectomy: a randomized controlled trial. *J Viral Hepat*. 2013;20:336–342.
5. Huang G, Lau WY, Shen F, 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.
6. Huang G, Lau WY, Wang ZG, et al. Antiviral therapy improves postoperative survival in patients with hepatocellular carcinoma: a randomized controlled trial. *Ann Surg*. 2015;261:56–66.
7. Lao XM, Luo G, Ye LT, et al. Effects of antiviral therapy on hepatitis B virus reactivation and liver function after resection or chemoembolization for hepatocellular carcinoma. *Liver Int*. 2013;33:595–604.
8. Sohn W, Paik YH, Cho JY, 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.