Int J Clin Exp Pathol 2014;7(7):4057-4066
www.ijcep.com /ISSN:1936-2625/IJCEP0000809

*Original Article*

# Clinicopathological features of hepatitis B virus recurrence after liver transplantation: eleven-year experience

Donghong Zhang, Zuoyi Jiao, Jixiang Han, Hongtai Cao

*Department of General Surgery, Lanzhou University Second Hospital, Lanzhou 730030, China*

Received May 16, 2014; Accepted May 31, 2014; Epub June 15, 2014; Published July 1, 2014

**Abstract:** Objective: We sought to investigate new changes in the clinical pathology of hepatitis B virus (HBV) recurrence after orthotopic liver transplantation (OLT) in era of new nucleoside or nucleotide analogues. Methods: One hundred and eighty-four adult patients who underwent OLT for HBV-related end-stage liver disease between 1999 and 2010 were enrolled in this study. Of these patients, 156 received lamivudine (LAM) plus hepatitis B immune globulin (HBIG) and 28 were treated with LAM. The liver function, serologic parameters and HBV-DNA of the 184 recipients were followed up, and clinical pathological characteristics of grafts with HBV recurrence were examined in this study. Results: One hundred and seventy-nine (97%) were alive at their last follow-up and eleven (6%) had developed HBV recurrence at a median of 22 (range 6 to 46) months post-OLT. Two of the 11 recipients were detected with HBV-S gene mutation, and 5 were tested with YMDD mutation. Four recipients who died of irreversible graft dysfunction secondary to HBV recurrence, developed fibrosing cholestatic hepatitis (FCH) because of no effective antiviral agents available in the early stages of HBV recurrence after OLT. Six recipients who received adefovir (ADV) (and Entecavir, ETV) in the early stages of HBV recurrence after OLT achieved improvement in hepatic histology. Conclusions: HBV recurrence post-OLT could be controlled at an acceptable level for a long time and the recipients could achieve long-term survival by using new antiviral agents, instead of advancing into FCH in the short term after HBV recurrence.

**Keywords:** Lamivudine, hepatitis B immunoglobulin, hepatitis B virus, recurrence, liver transplantation, fibrosing cholestatic hepatitis

## Introduction

Globally, an estimated 350-400 million suffer from chronic HBV infection which has been identified as one of the most important causes of cirrhosis, liver failure and hepatocellular carcinoma (HCC) [1]. The prevalence is high and estimated current HBV carriers in China run up to 93 million, including 20-30 million patients with chronic hepatitis B [2]. OLT is currently the most effective treatment for end-stage liver disease secondary to HBV infection in Asia, especially in China. HBV recurrence after OLT plays a key role in the post-transplant outcomes of the recipients, however, HBV is rarely possible to be eradicated after OLT in these recipients. HBV recurrence may cause a deadly liver failure, which is one of the main factors leading to death of liver recipients [3, 4].

HBIG was the first agent to show efficacy in preventing HBV recurrence. Although the introduction of HBIG reduced the recurrent HBV infection from 90% to 30-40% [5-7], HBIG monotherapy is almost never used for prophylaxis against post-transplant HBV recurrence as a result of the shortcomings of HBIG including high cost, inconvenient administration, adverse effects and the possible development of mutations. The introduction of Lamivudine (LAM) was a milestone in the treatment of chronic hepatitis B [8]. HBV recurrence rate ranged from 3.8% to 40.4% since the introduction of LAM monotherapy after OLT in the late 1990s and early 2000 [9-11]. Combination therapy with LAM and HBIG has achieved encouraging outcomes, with 1–10 years studies demonstrating a reduction in HBV recurrence rates in the early stage after OLT to less than 10% [12-17].

**Table 1.** Data on patients with HBV recurrence pre-OLT

| No. | Sex | Age (yr) | Child Grade | Diagnosis for OLT | HBeAg Pre-OLT | HBV-DNA On Admission (copies/ml) | HBV-DNA Pre-OLT (copies/ml) |
|---|---|---|---|---|---|---|---|
| 1 | M | 57 | C | Cirrhosis | – | $< 1.0\times10^3$ | $< 1.0\times10^3$ |
| 2 | M | 30 | B | Cirrhosis | + | $< 1.0\times10^3$ | $< 1.0\times10^3$ |
| 3 | M | 45 | C | Cirrhosis | + | $4.7\times10^4$ | $< 1.0\times10^3$ |
| 4 | M | 52 | C | Cirrhosis | + | $9.1\times10^6$ | $< 1.0\times10^3$ |
| 5 | M | 48 | C | Cirrhosis | + | $9.6\times10^6$ | $< 1.0\times10^3$ |
| 6 | M | 30 | C | Cirrhosis | + | $3.0\times10^7$ | $< 1.0\times10^3$ |
| 7 | F | 48 | B | Cirrhosis | – | $4.3\times10^4$ | $4.3\times10^4$ |
| 8 | F | 50 | B | Cirrhosis | – | $< 1.0\times10^3$ | $< 1.0\times10^3$ |
| 9 | M | 38 | C | Cirrhosis | – | $3.9\times10^4$ | $< 1.0\times10^3$ |
| 10 | M | 59 | B | Cirrhosis | – | $1.4\times10^4$ | $1.4\times10^4$ |
| 11 | M | 43 | C | Cirrhosis | – | $2.4\times10^5$ | $2.4\times10^5$ |

However, long-term use of LAM is associated with drug resistance leading to increasing rates of HBV recurrence, which if occur, may severely compromise the recipient survival and quality of life [18-21]. It has been reported that FCH is a severe clinical pathological manifestation of HBV recurrence after OLT [22-27]. Fortunately, new nucleoside or nucleotide analogues such as ADV and ETV have high efficacy and lower rates of resistance, increasing our ability to treat recurrent HBV infection after LT. However, there are limited data regarding the long-term efficacy of these agents in treating HBV recurrence in recipients after OLT, especially in a Chinese OLT population. In this study, we evaluated the new changes in the clinical pathology of HBV recurrence treated with new nucleoside or nucleoside analogues after OLT.

## Patients and methods

### *Patients*

Between 1999 and 2010, there were 184 patients who underwent OLT at Huaxi Liver Transplantation Center in Sichuan University because of HBV-related end-stage liver disease, and none had evidence of hepatitis C, D, HIV coinfection or suffering from Hepatocellular carcinoma (HCC). Eleven of these patients suffered from HBV recurrence after OLT. Grafts were all from voluntary donors who were negative for both HBsAg and HBV-DNA in serum. HBV recurrence was defined as HBsAg reappearance in serum or HBV-DNA level increased by > 2 log10 copies/mL after OLT. In the present study, 11 recipients who suffered from HBV recurrence after OLT were enrolled. The diagnosis for OLT was end-stage cirrhosis in 11 patients (**Table 1**). This study was approved by the Ethics Committee of West China Hospital of Sichuan University, and informed consents were obtained from all patients prior to study entry.

### *HBV recurrence prophylaxis protocol*

One hundred and eighty-four recipients were administered Lamivudine (LAM) monotherapy (28 recipients) or LAM and HBIG combination therapy (156 recipients) according to the availability of HBIG. HBV-DNA was evaluated in all patients at screening and again for subjects with waiting times not < 14 days before transplantation (RT-PCR, with a limit of detection of 1000 copies/mL). All patients with HBV-related liver disease who are on the waiting list (median, 18.5 days; range 7 to 29 days) were given oral LAM (100 mg daily; GlaxoSmithKline, Suzhou, China).

Eight of the 11 recipients (patients 1-8) received LAM (100 mg/day orally) monotherapy after OLT. Three (patients 9-11) received LAM and HBIG (Yuanda Shuyang, Sichuan, China) combination therapy: LAM (100 mg/day orally) after OLT and 2000 IU intramuscular (IM) HBIG in the anhepatic phase, followed by 800 IU IM daily for the next 7 days, followed by 800 IU IM weekly for 3 weeks, and at 400 to 1200 IU IM every 1 to 4 weeks thereafter to maintain the anti-HBs titer at > 100 IU/L.

### *Clinical follow-up and virologic monitoring*

Immunosuppression regimens consisted of prednisone, mycophenolate (or azathioprine)

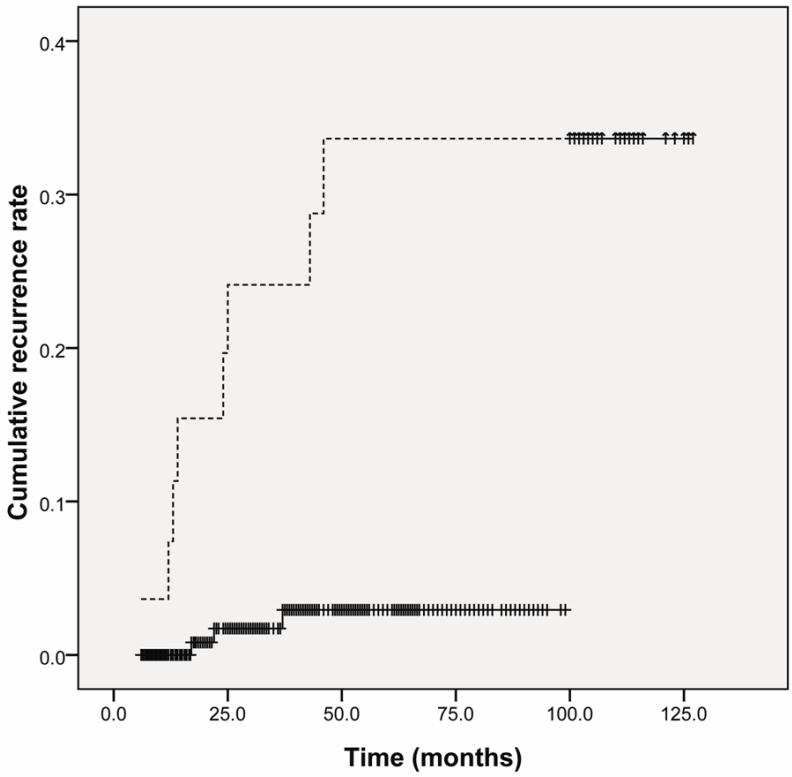


**Figure 1.** Cumulative recurrence rates in LAM montherapy group and LAM combined with HBIG therapy group. —, Combination Therapy; ---, LAM Montherapy; +, Combination Therapy censored; ↑, LAM Montherapy censored.

and cyclosporin A (or tacrolimus). Prednisone was generally discontinued within 3 months after OLT except for patients with graft rejection. Cyclosporine A levels ranged between 250 and 350 ng/mL for the first month after OLT, followed by 100~180 ng/mL within half a year, and then 50~150 ng/mL thereafter. The dose of tacrolimus was adjusted to maintain levels of 7~12 ng/mL for the first month after OLT and 3~7 ng/ml thereafter.

Serum HBV markers (HBsAg, HBsAb, HBeAg, anti-HBe, anti-HBc; ELISA), anti-HBs titer (Roche Elecsys anti-HBs Immunoassay), and serum HBV-DNA (real-time quantitative PCR, with a limit of detection of 1000 copies/mL) were monitored weekly in hospital. On discharge, all recipients were followed up weekly for the first two months, and then at increasing intervals until stable. HBV serum markers and HBV-DNA levels were monitored at least monthly. Anti-HBs titer was routinely measured every 1 to 4 weeks until the anti-HBs titer was stable at > 100 IU/L. Polymerase chain reaction-restriction fragment length polymorphism and Polymerase chain reaction-dideoxy chain termination method were used to detect wild-type and drug-induced HBV mutations in recipients with HBV recurrence [28, 29].

*Pathology assays*

Informed consent for liver biopsy was obtained from recipients upon HBV recurrence. Liver biopsy and histological examination were performed on clinical demand. And then all the liver specimens were fixed in 10% fomalin solution and embedded in paraffin wax. The expression of HBsAg and HBcAg in liver was tested by immunohistochemistry (Maixin Biotect, Fuzhou, China). The HBV-DNA in liver was detected by the in situ hybridization (ISH, Triplex Biosience, Xiamen, China). The liver fibrosis was evaluated by the Mallory trichrome (Yanyu Biotec, Shanghai, China). Chronic viral hepatitis was defined according to Scheuer and Desmet [30, 31].

*Statistical analysis*

SPSS 16.0 statistical software (SPSS Company, Chicago, IL) was used to analyze the relevant data. Cumulative patient HBV recurrence rates between LAM monotherapy group and combination therapy groups were described using Kaplan-Meier analysis, and the log-rank test was used to compare differences in cumulative recurrence rates between recurrence and non-recurrence groups. $P < 0.05$ was considered statistically significant.

## Results

*Characteristics of HBV recurrence*

HBsAg and HBV-DNA were detected negative in the serum of all recipients in the study within 3 weeks after OLT. One hundred and seventy-nine (97%) were alive at their last follow-up and eleven (6%) had developed HBV recurrence at a

**Table 2.** Data on patients with HBV recurrence post-OLT

| No. | Prophylaxis Protocol | Postoperative mutation | Treatment course | HBeAg at HBV recurrence | HBV-DNA (copies/mL) | | Status |
|---|---|---|---|---|---|---|---|
| | | | | | At HBV recurrence | 1 month post-treament | |
| 1 | LAM | S | ADV+ETV | + | $2.2\times10^7$ | $< 1.0\times10^3$ | Alive |
| 2 | LAM | YMDD | LAM | + | $5.2\times10^4$ | $5.5\times10^4$ | Dead† |
| 3 | LAM | YMDD | LAM | + | $3.7\times10^5$ | $6.5\times10^5$ | Dead† |
| 4 | LAM | – | LAM | + | $8.9\times10^3$ | $1.0\times10^4$ | Dead‡ |
| 5 | LAM | YMDD | LAM | + | $6.0\times10^4$ | $6.4\times10^4$ | Dead† |
| 6 | LAM | – | ADV | – | $1.0\times10^3$ | $< 1.0\times10^3$ | Alive |
| 7 | LAM | – | ADV | – | $4.5\times10^3$ | $< 1.0\times10^3$ | Alive |
| 8 | LAM | – | LAM | + | $6.8\times10^4$ | $8.1\times10^4$ | Dead† |
| 9 | LAM+HBIG | S | ADV+ETV | + | $2.0\times10^5$ | $< 1.0\times10^3$ | Alive |
| 10 | LAM+HBIG | YMDD | ADV+ETV | + | $2.0\times10^3$ | $< 1.0\times10^3$ | Alive |
| 11 | LAM+HBIG | YMDD | ADV+ETV | – | $7.6\times10^3$ | $< 1.0\times10^3$ | Alive |

†Fibrosing cholestatic hepatitis; ‡cerebral hemorrha.

median of 22 (range 6 to 46) months post-transplantation. The HBV recurrence rates in the LAM monotherapy group and combination therapy group were 28.6% (8/28) and 1.9% (3/156) respectively at their last follow-up. The difference in HBV recurrence rates after OLT between the two groups was statistically significant ($P$ = 0.006; log-rank test). **Figure 1** outlines the cumulative recurrence rates in the two groups using the Kaplan-Meier method. The majority (63.6%, 7/11) of recurrent HBV reinfection occurred 2 years after OLT. At the time of HBV recurrence, all (100%, 11/11) of these recipients tested positive for HBsAg, and 72.7% (8/11) were HBeAg positive; all recipients had detectable HBV-DNA levels ($\geq 1\times10^3$ copies/mL). Five of the recipients who developed recurrent HBV reinfection (45.5%, 5/11) developed YMDD mutants and two cases (18.2%, 2/11) developed S mutants. The primary result is shown in **Table 2**.

*Treatment for HBV recurrence*

Six of the 11 recipients who developed HBV recurrence had stable graft liver function, while the other 5 died during their follow-up. HBIG therapy was stopped immediately upon diagnosis of HBV recurrence. Of the 5 dead, except that 1 recipients died of cerebral hemorrha, the other 4 recipients who continued with LAM died of irreversible graft dysfunction secondary to HBV recurrence because of no effective therapy available at the time. In the remaining 6 recipients, two who were tested without YMDD or S mutant received ADV (10 mg/day, orally), while the other four who were detected with YMDD or S mutant received ADV (10 mg/day, orally) plus ETV (1 mg/day, orally). The 6 recipients achieved improvement in liver function, and their serum HBV-DNA level decreased from $10^5$ copies/mL to $10^3$ copies/mL.

*Clinical pathological features of HBV recurrence*

In the early stages of HBV recurrence, clinical pathology characterized by active HBV replication and mild-to-moderate viral hepatitis was detected in all the 11 patients who suffered from HBV recurrence during the follow-up after OLT. Liver cell swelling, ballooning degeneration, spotty or small necrosis, periportal with varying degrees of inflammatory cell infiltration and unconspicuous cholestatic bile duct could be found in recipients with HBV recurrence in the early stages (**Figure 2A**, **2B**). Six recipients who received ADV (and ETV) in the early stages of HBV recurrence achieved improvement in graft histology, which included HBsAg-positive or HBcAg-positive in fewer hepatocytes (**Figure 2C**, **2D**), detectable HBV-DNA only in partial hepatocyte nuclei (**Figure 2E**), unconspicuous proliferation of fibrous tissue (**Figure 2F**) and reduced inflammation and liver cells swelling (**Figure 2G**, **2H**).

In LAM monotherapy group, 4 recipients who continued with LAM developed FCH secondary to HBV recurrence. They died of irreversible graft failure because of gradually deepened jaundice and deterioration of liver function. Cell swelling, fatty degeneration and small necrosis could be found in liver nodules instead of nor-

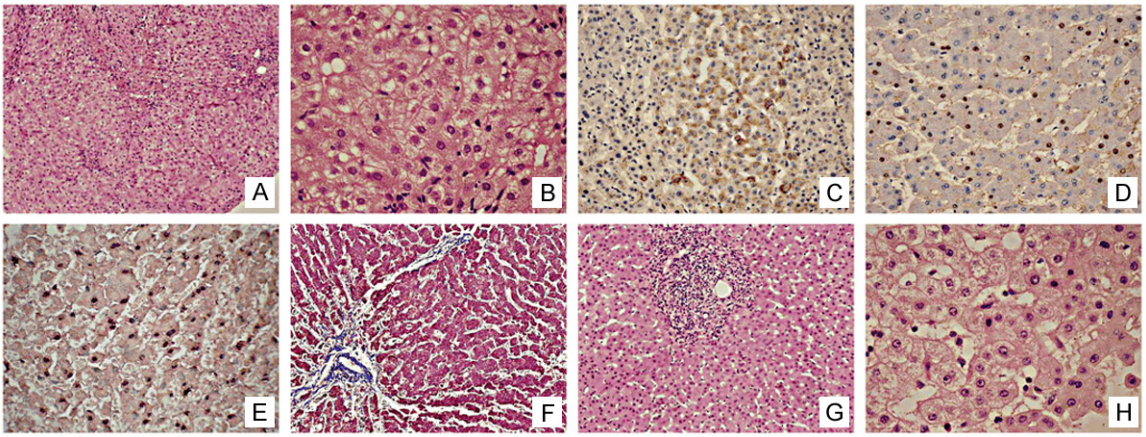


**Figure 2.** Clinical pathology for HBV recurrence after treatment: (A) Inflammatory cell infiltration without effective anti-HBV therapy (HE×100). (B) Liver cell swelling, ballooning degeneration without effective anti-HBV therapy (HE×400). (C) Positive expression of HBsAg with effective anti-HBV therapy (Immunohistochemistry staining×200). (D) Positive expression of HBcAg with effective anti-HBV therapy (Immunohistochemistry staining×200). (E) Positive expression of HBV-DNA with effective anti-HBV therapy (In situ hybridization×400). (F) Fibrous tissue proliferating inconspicuously with effective anti-HBV therapy (Mallory×200). (G) The number of inflammatory cell reduced with effective anti-HBV therapy (HE×200). (H) Liver cell swelling relieved with effective anti-HBV therapy (HE×400).

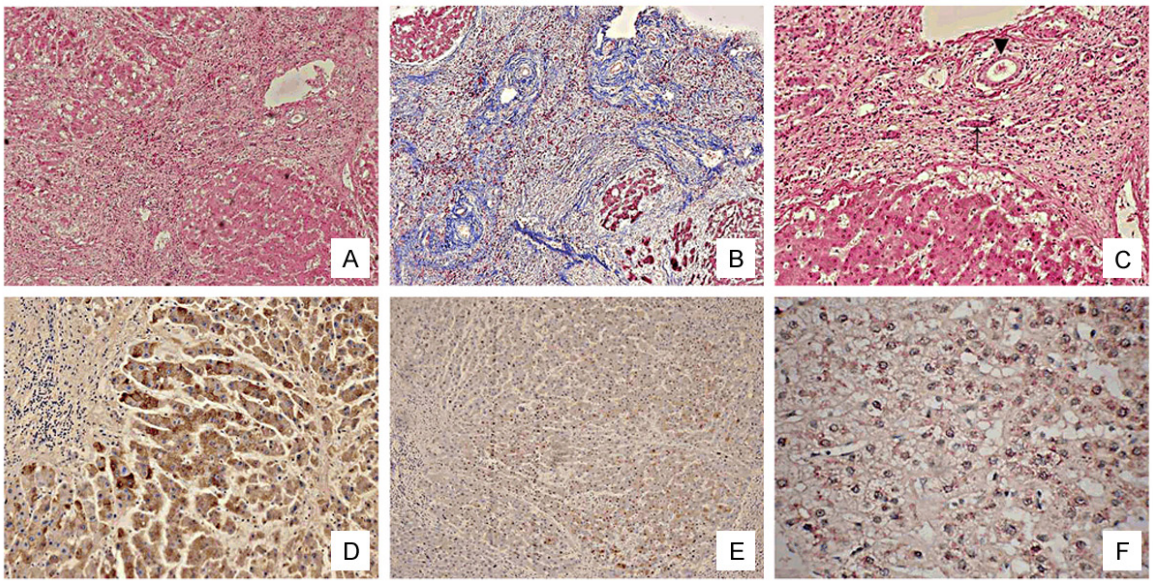


**Figure 3.** Clinical pathology of FCH: (A) Liver regeneration nodules, fibrosis of portal area and inflammatory cell infiltration (HE×100). (B) Hyperplastic fibrous tissue stained purple (Mallory×100). (C) Fibrosis of portal area, proliferation of bile duct epithelium (arrow), cholestasis in bile duct (arrowhead) (HE×200). (D) Positive expression of HBsAg stained brown (Immunohistochemistry staining×200). (E) Positive expression of HBcAg stained brown (Immunohistochemistry staining×200). (F) Positive expression of HBV-DNA (In situ hybridization×400).

mal hepatic lobule. Extensive fibrous tissue appeared in the periportal and nodules area with inflammatory cell infiltration (**Figure 3A**, **3B**). Bile duct hyperplasia and cellular and canalicular cholestasis were found markedly (**Figure 3C**). Rapid deterioration of liver function and active HBV replication were detected by HBsAg, HBcAg and HBV-DNA immunohistochemistry staining (**Figure 3D-F**).

## Discussion

The development of prophylactic treatments has significantly reduced the post-transplant

recurrence of HBV and has markedly improved prognoses of OLT. However, HBV recurrence in liver recipients is still a challenge. Hepatitis B relapse occurred in 8 of 28 (28.6%) recipients in the LAM monotherapy group at their last follow-up, which was similar to articles published earlier [9, 32-34]. Several studies have shown that combination therapy with HBIG and LAM reduces rates of recurrent hepatitis B to < 10% [21, 35, 36]. In our combination therapy group, hepatitis B relapsed in 3/156 (1.9%) recipients, which are similar to those of the above studies but significantly lower than that in the LAM monotherapy group. However, the drug resistance to this combination therapy has also emerged. In aggressive clinical course, when a rapid suppression of viral replication is required, high potency antiviral agents should be used [37]. The availability of ADV and ETV changed the clinical course of 6 recipients suffering from LAM-resistant HBV recurrence, and they proved to be effective and safe in treating HBV recurrence in the present study. Previous studies also showed that ADV could act as the rescue therapy for LAM-resistant HBV recurrence post-OLT [35, 38-41]. Several studies in OLT recipients reported lower rates of clinical resistance with ETV plus HBIG than with LAM, and a more favorable safety profile than ADV [42-44]. However, ETV is not a good choice for LAM-resistant recipients after OLT, although ETV has been tried in some LAM resistant recipients after OLT [45, 46]. Interestingly, a recent study demonstrated that ETV therapy is safe and efficient for recipients with ADV resistant HBV infection [45]. In this study, ADV (10 mg/day, orally) plus ETV (1 mg/day, orally) were used to treat HBV recurrence with YMDD or S mutant, and the 4 recipients achieved stable graft liver function. Our study may suggest that drug-resistant rates can be decreased significantly due to a mechanism by using ADV plus ETV.

HBV recurrence is a common cause of graft dysfunction in recipients transplanted for HBV-related end-stage liver disease. It has been reported that graft can suffer from pathological damage of various properties and degrees, such as mild self-limited hepatitis, chronic active hepatitis, fulminant hepatitis, and FCH [47-51]. FCH could rapidly progressed to hepatic failure, which was originally described in HBV-infected recipients after a liver transplantation [47]. Antiviral therapy to reduce the viral loads played an important role in the treatment of FCH which resulted from the direct toxicity of massive HBV loads [25, 48, 50]. Fortunately, with the availability of more potent antiviral therapy and better surveillance of patients after transplant for HBV-DNA and HBSAb titer in those receiving HBIG, recurrent hepatitis B could be controlled at a acceptable level for a long time post-OLT, instead of advancing into FCH in the short term after HBV recurrence. In our study, 4 recipients in the LAM monotherapy group without effective antiviral therapy at early stage of recurrent hepatitis B died of FCH, which was characterized by marked hepatocyte ballooning (swelling), intracellular and canalicular cholestasis, and periportal and/or perisinusoidal collagen deposition. Six recipients achieved improvement in liver function and hepatic histology after receiving ADV (and ETV) instead of LAM in the early stages of HBV recurrence after OLT.

The potential sources of HBV recurrence include peripheral blood mononuclear cells, bone marrow, spleen and pancreas, as viral DNA has been demonstrated in these tissues [52, 53]. HBV that is present in the recipient's blood at the time of graft implantation and released from extrahepatic reservoirs can infect the graft at any time after OLT [54]. The risk factors for HBV recurrence include a high viral load (HBV-DNA > 5 log10 copies/mL) at transplantation, immunosuppression because of steroids and/or chemotherapy, mutation of the YMDD nucleotide-binding locus of the HBV-DNA polymerase [55]. HBV resistance to nucleoside analogues in post-operative patients is the leading cause for reinfection. In this series, 5 cases of HBV-YMDD mutations were detected in the 11 recipients who experienced HBV recurrence during the follow-up period. Compared with previous studies, the rate of YMDD mutation appears to be lower in our study [19, 56]. Immunosuppression may have a great influence on the development of YMDD mutation, which is consistent with an earlier report that the LAM resistance was detected lower in immunocompetent patients compared with those under immunosuppression within the first treatment year [57]. Some studies have reported that LAM can be used to lower the pre-transplant high viral load, which affects recurrence [19, 56, 58]. The results in this study further reinforce this point. Previous studies reported that antiviral therapy could apply selective pressures on HBV in infected individu-

als leading to the generation and accumulation of mutations in the S gene [29, 59, 60]. We also found that S mutations was tested in 2 of 11 recipients, which might contribute to HBV recurrence. In addition, HBeAg positivity was the apparent cause of 5 recipients with HBV recurrence in the study, which suggested that HBeAg positivity was another important risk factor contributing to HBV recurrence in Chinese recipients.

In summary, we suggested that HBV recurrence post-OLT could be controlled at a acceptable level for a long time and the recipients could achieve long-term survival by using new antiviral agents, instead of advancing into FCH in the short term after HBV recurrence. The study should be investigated continuously because the number of cases studied is fairly limited.

## Disclosure of conflict of interest

None.

**Address correspondence to:** Dr. Zuoyi Jiao, Department of General Surgery, Lanzhou University Second Hospital, 82 Cuiyingmen, Lanzhou, Gansu Province, China. Tel: 0931-8943709; Fax: 0931-8943829; E-mail: jiaozx@lzu.edu.cn

## References

[1] Mahboobi N, Agha-Hosseini F, Mahboobi N, Safari S, Lavanchy D, Alavian SM. Hepatitis B virus infection in dentistry: a forgotten topic. J Viral Hepat 2010; 17: 307-316.

[2] Lu FM, Li T, Liu S, et al. Epidemiology and prevention of hepatitis B virus infection in China. J Viral Hepat 2010; 17: 4-9.

[3] Roche B, Samuel D. Evolving strategies to prevent HBV recurrence. Liver Transpl 2004; 10: S74- S85.

[4] Rao W, Wu X, Xiu D. Lamivudine or lamivudine combined with hepatitis B immunoglobulin in prophylaxis of hepatitis B recurrence after liver transplantation: a meta-analysis. Transpl Int 2009; 22: 387-394.

[5] Samuel D, Muller R, Alexander G, Fassati L, Ducot B, Benhamou JP, Bismuth H. Liver transplantation in European patients with the hepatitis B surface antigen. N Engl J Med 1993; 329: 1842-1847.

[6] McGory RW, Ishitani MB, Oliveira WM, Stevenson WC, McCullough CS, Dickson RC, Caldwell SH, Pruett TL. Improved outcome of orthotopic liver transplantation for chronic hepatitis B cirrhosis with aggressive passive immunization. Transplantation 1996; 61: 1358-1364.

[7] Terrault NA, Zhou S, Combs C, Hahn JA, Lake JR, Roberts JP, Ascher NL, Wright TL. Prophylaxis in liver transplant recipients using a fixed dosing schedule of hepatitis B immunoglobulin. Hepatology 1996; 24: 1327-1333.

[8] Lai CL, Chien RN, Leung NW, Chang TT, Guan R, Tai DI, Ng KY, Wu PC, Dent JC, Barber J, Stephenson SL, Gray DF. A one-year trial of lamivudine for chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. N Engl J Med 1998; 339: 61-68.

[9] Lo CM, Cheung ST, Lai CL, Liu CL, Ng IO, Yuen MF, Fan ST, Wong J. Liver transplantation in Asian patients with chronic hepatitis B using lamivudine prophylaxis. Ann Surg 2001; 233: 276-281.

[10] Mutimer D, Dusheiko G, Barrett C, Grellier L, Ahmed M, Anschuetz G, Burroughs A, Hubscher S, Dhillon AP, Rolles K, Elias E. Lamivudine without HBIg for prevention of graft reinfection by hepatitis B: long-term follow-up. Transplantation 2000; 70: 809-815.

[11] Perrillo RP, Wright T, Rakela J, Levy G, Schiff E, Gish R, Martin P, Dienstag J, Adams P, Dickson R, Anschuetz G, Bell S, Condreay L, Brown N. A multicenter United States-Canadian trial to assess lamivudine monotherapy before and after liver transplantation for chronic hepatitis B. Hepatology 2001; 33: 424-432.

[12] Markowitz JS, Martin P, Conrad AJ, Markmann JF, Seu P, Yersiz H, Goss JA, Schmidt P, Pakrasi A, Artinian L, Murray NG, Imagawa DK, Holt C, Goldstein LI, Stribling R, Busuttil RW. Prophylaxis against hepatitis B recurrence following liver transplantation using combination lamivudine and hepatitis B immune globulin. Hepatology 1998; 28: 585-589.

[13] Han SH, Ofman J, Holt C, King K, Kunder G, Chen P, Dawson S, Goldstein L, Yersiz H, Farmer DG, Ghobrial RM, Busuttil RW, Martin P. An efficacy and cost-effectiveness analysis of combination hepatitis B immune globulin and lamivudine to prevent recurrent hepatitis B after orthotopic liver transplantation compared with hepatitis B immune globulin monotherapy. Liver Transpl 2000; 6: 741-748.

[14] Marzano A, Salizzoni M, Debernardi-Venon W, Smedile A, Franchello A, Ciancio A, Gentilcore E, Piantino P, Barbui AM, David E, Negro F, Rizzetto M. Prevention of hepatitis B virus recurrence after liver transplantation in cirrhotic patients treated with lamivudine and passive immunoprophylaxis. J Hepatol 2001; 34: 903-910.

[15] Steinmüller T, Seehofer D, Rayes N, Müller AR, Settmacher U, Jonas S, Neuhaus R, Berg T, Hopf U, Neuhaus P. Increasing applicability of

liver transplantation for patients with hepatitis B-related liver disease. Hepatology 2002; 35: 1528-1535.
[16] Jiang L, Yan L, Li B, Wen T, Zhao J, Jiang L, Cheng N, Wei Y, Yang J, Xu M, Wang W. Prophylaxis against hepatitis B recurrence posttransplantation using lamivudine and individualized low-dose hepatitis B immunoglobulin. Am J Transplant 2010; 10: 1861-1869.
[17] Tanaka T, Benmousa A, Marquez M, Therapondos G, Renner EL, Lilly LB. The long-term efficacy of nucleos(t)ide analog plus a year of low-dose HBIG to prevent HBV recurrence post-liver transplantation. Clin Transplant 2012; 26: E561-E569.
[18] Tzakis AG, Gordon RD, Makowka L, Esquivel CO, Todo S, Iwatsuki S, Starzl TE. Clinical considerations in orthotopic liver transplantation. Radiol Clin North Am 1987; 25: 289-297.
[19] Zheng S, Chen Y, Liang T, Lu A, Wang W, Shen Y, Zhang M. Prevention of hepatitis B recurrence after liver transplantation using lamivudine or lamivudine combined with hepatitis B Immunoglobulin prophylaxis. Liver Transpl 2006; 12: 253-258.
[20] Papatheodoridis GV, Sevastianos V, Burroughs AK. Prevention of and treatment for hepatitis B virus infection after liver transplantation in the nucleoside analogues era. Am J Transplant 2003; 3: 250-258.
[21] Papatheodoridis GV, Cholongitas E, Archimandritis AJ, Burroughs AK. Current management of hepatitis B virus infection before and after liver transplantation. Liver Int 2009; 29: 1294-1305.
[22] Todo S, Demetris AJ, Van Thiel D, Teperman L, Fung JJ, Starzl TE. Orthotopic liver transplantation for patients with hepatitis B virus-related liver disease. Hepatology 1991; 13: 619-626.
[23] O'Grady JG, Smith HM, Davies SE, Daniels HM, Donaldson PT, Tan KC, Portmann B, Alexander GJ, Williams R. Hepatitis B virus reinfection after orthotopic liver transplantation. Serological and clinical implications. J Hepatol 1992; 14: 104-111.
[24] Lo CM, Cheung ST, Ng IO, Liu CL, Lai CL, Fan ST. Fibrosing cholestatic hepatitis secondary to precore/core promoter hepatitis B variant with lamivudine resistance: successful retransplantation with combination adefovir dipivoxil and hepatitis B immunoglobulin. Liver Transpl 2004; 10: 557-563.
[25] Chan TM, Wu PC, Li FK, Lai CL, Cheng IK, Lai KN. Treatment of fibrosing cholestatic hepatitis with lamivudine. Gastroenterology 1998; 115: 177-181.
[26] Harrison RF, Davies MH, Goldin RD, Hubscher SG. Recurrent hepatitis B in liver allografts: a distinctive form of rapidly developing cirrhosis. Histopathology 1993; 23: 21-28.
[27] Zanati SA, Locarnini SA, Dowling JP, Angus PW, Dudley FJ, Roberts SK. Hepatic failure due to fibrosing cholestatic hepatitis in a patient with pre-surface mutant hepatitis B virus and mixed connective tissue disease treated with prednisolone and chloroquine. J Clin Virol 2004; 31: 53-57.
[28] European Association For The Study Of The Liver. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. J Hepatol 2012; 57: 167-185.
[29] Sloan RD, Ijaz S, Moore PL, Harrison TJ, Teo CG, Tedder RS. Antiviral resistance mutations potentiate hepatitis B virus immune evasion through disruption of its surface antigen a determinant. Antivir Ther 2008; 13: 439-447.
[30] Scheuer PJ. Classification of chronic viral hepatitis: a need for reassessment. J Hepatol 1991; 13: 372-374.
[31] Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. Hepatology 1994; 19: 1513-1520.
[32] Dickson RC, Terrault NA, Ishitani M, Reddy KR, Sheiner P, Luketic V, Soldevila-Pico C, Fried M, Jensen D, Brown RS Jr, Horwith G, Brundage R, Lok A. Protective antibody levels and dose requirements for IV 5% Nabi Hepatitis B immune globulin combined with lamivudine in liver transplantation for hepatitis B-induced end stage liver disease. Liver Transpl 2006; 12: 124-133.
[33] Degertekin B, Han SH, Keeffe EB, Schiff ER, Luketic VA, Brown RS Jr, Emre S, Soldevila-Pico C, Reddy KR, Ishitani MB, Tran TT, Pruett TL, Lok AS. Impact of virologic breakthrough and HBIG regimen on hepatitis B recurrence after liver transplantation. Am J Transplant 2010; 10: 1823-1833.
[34] Dienstag JL, Goldin RD, Heathcote EJ, Hann HW, Woessner M, Stephenson SL, Gardner S, Gray DF, Schiff ER. Histological outcome during long-term lamivudine therapy. Gastroenterology 2003; 124: 105-117.
[35] Schiff E, Lai CL, Hadziyannis S, Neuhaus P, Terrault N, Colombo M, Tillmann H, Samuel D, Zeuzem S, Villeneuve JP, Arterburn S, Borroto-Esoda K, Brosgart C, Chuck S. Adefovir dipivoxil for wait-listed and post-liver transplantation patients with lamivudine-resistant hepatitis B: final long-term results. Liver Transpl 2007; 13: 349-360.
[36] Vierling JM. Management of HBV Infection in Liver Transplantation Patients. Int J Med Sci 2005; 2: 41-49.
[37] Terrault N, Roche B, Samuel D. Management of the hepatitis B virus in the liver transplantation setting: a European and an American perspective. Liver Transpl 2005; 11: 716-732.

[38] Xiong X, Flores C, Yang H, Toole JJ, Gibbs CS. Mutations in hepatitis B DNA polymerase associated with resistance to lamivudine do not confer resistance to adefovir in vitro. Hepatology 1998; 28: 1669-1673.
[39] Limquiaco JL, Wong J, Wong VW, Wong GL, Tse CH, Chan HY, Kwan KY, Lai PB, Chan HL. Lamivudine monoprophylaxis and adefovir salvage for liver transplantation in chronic hepatitis B: a seven-year follow-up study. J Med Virol 2009; 81: 224-229.
[40] Akyildiz M, Karasu Z, Zeytunlu M, Aydin U, Ozacar T, Kilic M. Adefovir dipivoxil therapy in liver transplant recipients for recurrence of hepatitis B virus infection despite lamivudine plus hepatitis B immunoglobulin prophylaxis. J Gastroenterol Hepatol 2007; 22: 2130-2134.
[41] Wai CT, Prabhakaran K, Wee A, Lee YM, Dan YY, Sutedja DS, Mak K, Isaac J, Lee KH, Lee HL, Da Costa M, Lim SG. Adefovir dipivoxil as the rescue therapy for lamivudine-resistant hepatitis B post liver transplant. Transplant Proc 2004; 36: 2313-2314.
[42] Perrillo R, Buti M, Durand F, Charlton M, Gadano A, Cantisani G, Loong CC, Brown K, Hu W, Lopez-Talavera JC, Llamoso C. Entecavir and hepatitis B immune globulin in patients undergoing liver transplantation for chronic hepatitis B. Liver Transpl 2013; 19: 887-895.
[43] Ueda Y, Marusawa H, Kaido T, Ogura Y, Ogawa K, Yoshizawa A, Hata K, Fujimoto Y, Nishijima N, Chiba T, Uemoto S. Efficacy and safety of prophylaxis with entecavir and hepatitis B immunoglobulin in preventing hepatitis B recurrence after living-donor liver transplantation. Hepatol Res 2013; 43: 67-71.
[44] Schreibman IR, Schiff ER. Prevention and treatment of recurrent Hepatitis B after liver transplantation: the current role of nucleoside and nucleotide analogues. Ann Clin Microbiol Antimicrob 2006; 5: 8.
[45] Kamar N, Milioto O, Alric L, El Kahwaji L, Cointault O, Lavayssière L, Sauné K, Izopet J, Rostaing L. Entecavir therapy for adefovir-resistant hepatitis B virus infection in kidney and liver allograft recipients. Transplantation 2008; 86: 611-614.
[46] Sherman M, Yurdaydin C, Sollano J, Silva M, Liaw YF, Cianciara J, Boron-Kaczmarska A, Martin P, Goodman Z, Colonno R, Cross A, Denisky G, Kreter B, Hindes R. Entecavir for treatment of lamivudine-refractory, HBeAg-positive chronic hepatitis B. Gastroenterology 2006; 130: 2039-2049.
[47] Davies SE, Portmann BC, O'Grady JG, Aldis PM, Chaggar K, Alexander GJ, Williams R. Hepatic histological findings after transplantation for chronic hepatitis B virus infection, including a unique pattern of fibrosing cholestatic hepatitis. Hepatology 1991; 13: 150-157.
[48] Jung S, Lee HC, Han JM, Lee YJ, Chung YH, Lee YS, Kwon Y, Yu E, Suh DJ. Four cases of hepatitis B virus-related fibrosing cholestatic hepatitis treated with lamivudine. J Gastroenterol Hepatol 2002; 17: 345-350.
[49] Roche B, Samuel D, Feray C, Majno P, Gigou M, Reynes M, Bismuth H. Retransplantation of the liver for recurrent hepatitis B virus infection: the Paul Brousse experience. Liver Transpl Surg 1999; 5: 166-174.
[50] Tillmann HL, Bock CT, Bleck JS, Rosenau J, Böker KH, Barg-Hock H, Becker T, Trautwein C, Klempnauer J, Flemming P, Manns MP. Successful treatment of fibrosing cholestatic hepatitis using adefovir dipivoxil in a patient with cirrhosis and renal insufficiency. Liver Transpl 2003; 9: 191-196.
[51] Wyatt JI. Liver transplant pathology - messages for the non-specialist. Histopathology 2010; 57: 333-341.
[52] Pontisso P, Poon MC, Tiollais P, Brechot C. Detection of hepatitis B virus DNA in mononuclear blood cells. Br Med J (Clin Res Ed) 1984; 288: 1563-1566.
[53] Davison F, Alexander GJ, Trowbridge R, Fagan EA, Williams R. Detection of hepatitis B virus DNA in spermatozoa, urine, saliva and leucocytes, of chronic HBsAg carriers. A lack of relationship with serum markers of replication. J Hepatol 1987; 4: 37-44.
[54] Coffin CS, Mulrooney-Cousins PM, van Marle G, Roberts JP, Michalak TI, Terrault NA. Hepatitis B virus quasispecies in hepatic and extrahepatic viral reservoirs in liver transplant recipients on prophylactic therapy. Liver Transpl 2011; 17: 955-962.
[55] Laryea MA, Watt KD. Immunoprophylaxis against and prevention of recurrent viral hepatitis after liver transplantation. Liver Transpl 2012; 18: 514-523.
[56] Gane EJ, Angus PW, Strasser S, Crawford DH, Ring J, Jeffrey GP, McCaughan GW. Lamivudine plus low-dose hepatitis B immunoglobulin to prevent recurrent hepatitis B following liver transplantation. Gastroenterology 2007; 132: 931-937.
[57] Lok AS, Heathcote EJ, Hoofnagle JH. Management of hepatitis B: 2000--summary of a workshop. Gastroenterology 2001; 120: 1828-1853.
[58] Marzano A, Gaia S, Ghisetti V, Carenzi S, Premoli A, Debernardi-Venon W, Alessandria C, Franchello A, Salizzoni M, Rizzetto M. Viral load at the time of liver transplantation and risk of hepatitis B virus recurrence. Liver Transpl 2005; 11: 402-409.
[59] Huang X, Qin Y, Li W, Shi Q, Xue Y, Li J, Liu C, Hollinger FB, Shen Q. Molecular analysis of the hepatitis B virus presurface and surface gene

in patients from eastern China with occult hepatitis B. J Med Virol 2013; 85: 979-986.
[60] Villet S, Pichoud C, Villeneuve JP, Trépo C, Zoulim F. Selection of a multiple drug-resistant hepatitis B virus strain in a liver-transplanted patient. Gastroenterol 2006; 131: 1253-1261.