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ORIGINAL ARTICLE

Low Risk of Hepatitis B Virus Recurrence After Withdrawal of Long-Term Hepatitis B Immunoglobulin in Patients Receiving Maintenance Nucleos(t)ide Analogue Therapy

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Hepatitis B virus (HBV) recurrence rates of 0-16% had been reported in patients maintained on nucleoside analogues (NA) after hepatitis B immunoglobulin (HBIG) discontinuation after orthotopic liver transplantation (OLT). However, follow-up in most studies was short. We aimed to determine the long-term risk of HBV recurrence using this strategy. All HBV patients who received ≥7 doses of intravenous HBIG after OLT, with no HBV recurrence while receiving HBIG, and who eventually discontinued HBIG and were maintained on NA, were included. HBV recurrence was defined as HBsAg-positive or HBV DNA ≥5 log copies/mL on 2 consecutive occasions. Twenty-one patients met the inclusion criteria. Immediate post-OLT prophylaxis was combination HBIG and NA in 15 patients, whereas 6 patients received HBIG monotherapy for 62-109 months before NA was added. HBIG was discontinued a median of 26 (range, 0.2-121) months after OLT. Median follow-up post-HBIG discontinuation was 40 (range, 5-51) months. Only 1 patient, who had 12 months of HBIG and was noncompliant to NA therapy, had HBV recurrence, 34 months after HBIG discontinuation. One patient had HBV DNA of 3.3 log copies/mL 47 and 48 months after HBIG discontinuation but remained HBsAg-negative. Lamivudine-resistant mutations were detected in both patients. Probability of HBV recurrence was 0% and 9% at 2 and 4 years after HBIG discontinuation. Three patients had 1-2 episodes of transiently detectable HBV DNA. All were HBV DNA and HBsAg negative on repeated tests over a period of 2-36 months. Maintenance therapy with NA after discontinuation of long-term HBIG therapy is associated with a low risk of HBV recurrence after OLT in compliant HBV patients. ***Liver Transpl 13:374-381, 2007.*** © **2007 AASLD.**

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Before the advent of antiviral prophylaxis, the prognosis of patients undergoing orthotopic liver transplantation (OLT) for hepatitis B virus (HBV)-related liver disease was poor because of the high (80-100%) rate of graft reinfection after OLT.[1,2] Recurrence of hepatitis B in liver transplant recipients generally follows a more aggressive course than in immunocompetent patients, with a 2-year mortality of 50%. Fortunately, tremendous strides in antiviral prophylactic measures have been made in the last 20 years.[3] The administration of either high-dose intravenous (IV) hepatitis B immunoglobulin (HBIG) or lamivudine (LAM) for an indefinite duration after OLT have resulted in a marked reduction in 3-year HBV recurrence rates to 15-25% for HBIG[4-6] and 20-40% for LAM monotherapy.[7-9] In recent years, the combination of IV HBIG and LAM resulted in further reduction in HBV recurrence rates to less than 10% up to 3 years after OLT.[6,10-13]

However, IV HBIG is cumbersome to administer and expensive, with estimated costs of US$100,000 in the first year after OLT and US$50,000 in each subsequent year.[14] The cost of HBIG is lower in many countries outside the United States but remains substantial.[14,15] Therefore, long-term HBIG therapy poses a huge eco-

Abbreviations: OLT, orthotopic liver transplantation; NA, nucleos(t)ide analogue; HBIG, hepatitis B immunoglobulin; HBV, hepatitis B virus; IV, intravenous; IM, intramuscular; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; PCR, polymerase chain reaction; LAM, lamivudine; ADV, adefovir; FTC, emtricitabine; TDF, tenofovir.

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nomic burden on patients and on the health care system. Several studies have attempted to replace long-term HBIG with active immunization after varying durations of HBIG, with conflicting results.[16-19] Others have tried to lower the cost of HBIG by administering it intramuscularly (IM) at lower doses.[20-25] Finally, other investigators have tried to discontinue HBIG altogether after the initial post-OLT period and continue with antiviral monotherapy in patients at low risk for HBV recurrence (hepatitis B e antigen [HBeAg] negative with undetectable HBV DNA by non–polymerase chain reaction [PCR]-based assays at transplant), with recurrence rates varying from 0 to 16%.[26-28] These results are encouraging and may lead to cost-effective strategies permitting HBIG discontinuation in a select group of patients. However, the duration of follow-up after HBIG discontinuation in these studies was short (13-17 months). Thus, it is unclear if antiviral therapy alone will be sufficient in the long-term prevention of HBV recurrence after OLT.

A controlled trial of 29 patients initially provided combination HBIG and LAM therapy for 1 month after OLT, then randomized to 17 months of LAM monotherapy (n = 14) or combination therapy (n = 15) showed excellent results with no recurrence 18 months after OLT.[28] However, a follow-up report later indicated that 4 patients, all of whom were receiving LAM monotherapy, eventually experienced HBV recurrence after 5 years of follow-up.[29] Another study found that HBV DNA persisted in the liver, peripheral blood mononuclear cells, and/or sera of 55% of patients who had been followed for 10 years after OLT with undetectable serum HBsAg while receiving HBIG therapy.[30] In these patients, overt HBV recurrence may have been suppressed by the presence of circulating hepatitis B surface antibodies. Thus, there is a possibility that HBV recurrence may manifest after discontinuation of HBIG, particularly in patients who have received LAM for many years.

The aims of the current study were to determine the rate of HBV recurrence in OLT recipients who were receiving maintenance nucleos(t)ide analogue (NA) therapy at the time HBIG was discontinued, and to identify pre- and post-OLT factors that are predictive of HBV recurrence in these patients.

METHODS

All patients who underwent OLT due to HBV-induced liver disease at the University of Michigan Health Care System between January 1, 1994, and December 31, 2005, and who had received at least 7 doses of high-dose (10,000 U) IV HBIG immunoprophylaxis after OLT with at least 3 months of follow-up after HBIG discontinuation were included. Patients who experienced HBV recurrence while receiving HBIG and patients who died while still receiving HBIG were excluded. This retrospective study was approved by our institutional review board.

Between 1994 and 1998, HBV prophylaxis at our center consisted of high-dose IV HBIG monotherapy (10,000 IU anhepatic, daily during the first week, and then monthly thereafter). In 1998, oral antiviral therapy consisting of LAM was administered before OLT to patients with detectable HBV DNA by hybridization assay or >4-5 log copies/mL by PCR assay and continued after OLT in conjunction with high-dose IV HBIG. In 2001, patients who had been followed for more than 1 year after OLT with no HBV recurrence were enrolled onto a study where the route of administration of HBIG was switched from IV to IM (1,000 U monthly) for 1 year, after which HBIG was discontinued and patients received maintenance antiviral therapy.[31] LAM was added when the HBIG administration route was switched from IV to IM in patients who were not receiving it at that time. In 2002, patients who were HBeAg negative with serum HBV DNA <5 log copies/mL at the time of OLT were enrolled onto a study where they received only 7 doses of IV HBIG after OLT (10,000 IU during the anhepatic and reperfusion phases, and 10,000 IU daily for 5 more days) while being maintained on NA therapy. Since 2003, patients with virologic breakthrough while receiving LAM received additional treatment with adefovir (ADV) or were switched from LAM to ADV monotherapy. All patients were tested for HBsAg and HBV DNA every month during the first year after OLT, every 3 months during years 2 and 3, and every 3-6 months thereafter.

Initial post-OLT immunosuppression consisted of prednisone, azathioprine, or mycophenolate mofetil, and tacrolimus or cyclosporine. Prednisone was discontinued 3-6 months after OLT; azathioprine or mycophenolate mofetil was discontinued 6-12 months after OLT.

Medical records of eligible patients were reviewed. Demographic data, indication for OLT, dose and route of HBIG administration, and type and duration of antiviral therapy were recorded. Antibodies to hepatitis C virus and hepatitis D virus were determined at the time of listing. HBV markers (hepatitis B surface antigen [HBsAg], HBeAg, antibody to HBeAg [anti-HBe], HBV DNA, and hepatitis B surface antibody titers), hematology and chemistry results (blood counts, prothrombin time expressed as international normalized ratio of prothrombin time, albumin, total bilirubin, alkaline phosphatase, alanine and aspartate aminotransferases, and creatinine) at the time of listing for OLT, initiation of antiviral therapy, OLT, HBIG discontinuation, and every 6 months after HBIG was withdrawn were recorded. In addition, HBsAg and HBV DNA were tested every 1-3 months the first year after HBIG was withdrawn, and every 3-6 months thereafter. Since April 2003, serum HBV DNA was tested by PCR-based assays with lower limit of detection of 1.5-2.5 log copies/mL. Results from our center's laboratory were used in the analysis when available. Otherwise, results from outside laboratories were used.

HBV resistance mutations were determined in patient samples with detectable HBV DNA by direct sequencing of the HBV S and P genes at the DNA core sequencing facility of the University of Michigan Medical Center and by a line probe assay (INNO-Lipa DRv2; Innogenetics NV, Ghent, Belgium).[32]

TABLE 1. Characteristics of Patients at Time of Transplantation

Patient	Age	Gender	Race	OLT indication	HBV DNA (log copies/mL)	HBeAg	Antiviral therapy before OLT	Duration of pre-OLT antiviral therapy (months)
1	47	M	White	Cirrhosis	NA	Negative	LAM	3
2	51	M	White	Cirrhosis	Negative*	Negative	LAM	19
3	60	F	Asian	Cirrhosis	NA	Negative	LAM	30
4	40	M	White	Cirrhosis	5.7	Negative	LAM	28
5	39	M	White	HCC	7	Positive	LAM	1
6	25	M	White	Cirrhosis	Negative*	Negative	LAM	10
7	52	M	White	HCC	4.7	NA	LAM	4
8	52	M	Asian	Cirrhosis	3.5	Positive	LAM→ADV	40
9	57	F	White	Cirrhosis	Negative†	Negative	LAM	35
10	56	M	White	Cirrhosis	2.5	Negative	LAM	20
11	55	M	White	Cirrhosis	Negative†	Negative	LAM	27
12	65	M	White	Cirrhosis	NA	Positive	NA	NA
13	51	F	White	Cirrhosis	NA	NA	NA	NA
14	53	M	White	Cirrhosis	Negative*	Negative	NA	NA
15	34	M	White	Cirrhosis	NA	NA	NA	NA
16	41	M	White	Cirrhosis	NA	NA	NA	NA
17	51	M	White	Cirrhosis	4.3	Negative	NA	NA
18	48	M	White	HCC	Negative†	Negative	NA	NA
19	44	F	White	Cirrhosis	4	Positive	NA	NA
20	59	M	White	Cirrhosis	Positive*	Positive	NA	NA
21	40	M	White	Cirrhosis	NA	NA	NA	NA

Abbreviations: OLT, orthotopic liver transplant; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; NA, not available; LAM, lamivudine; ADV, adefovir.
*HBV DNA tested by hybridization.
†HBV DNA tested by polymerase chain reaction assay.

Study End Point

The end point of the study was HBV recurrence, defined as the presence of positive serum HBsAg or serum HBV DNA ≥5 log copies/mL (or detectable by hybridization assays) on 2 consecutive occasions.

Statistical Analysis

Continuous variables are expressed as mean ± SD unless specified otherwise. Categorical data are presented as number (percent). Kaplan-Meier analysis was used to estimate the cumulative probability of HBV recurrence. *P* values <0.05 were considered statistically significant.

RESULTS

A total of 886 liver transplantations were performed between January 1994 and December 2005 at our center, of which 40 (4.5%) were performed for HBV-related liver disease. Nineteen patients were excluded because of HBV recurrence (n = 4) and death while still receiving HBIG (n = 10), were still receiving HBIG at the time of analysis (n = 2), or were lost to follow-up (n = 3). Twenty-one patients (17 men) met the inclusion criteria. The mean age at the time of transplant was 48.6 ± 9.6 years. Eighteen patients received transplants for decompensated cirrhosis and 3 for hepatocellular carcinoma. At the time of OLT, only 3 of 15 patients tested had serum HBV DNA >5 log copies/mL, and 5 of 16 patients tested were positive for HBeAg (Table 1). Patient 7 had hepatitis C virus coinfection; patient 21 had both hepatitis C and D virus coinfection.

Eleven patients began LAM therapy 1-40 (median, 20) months before OLT, and all but one continued to receive LAM after OLT. Patient 8 experienced LAM breakthrough and was switched to ADV monotherapy 7 months before OLT, and continued to receive ADV after OLT. None of the patients cleared HBsAg while receiving antiviral therapy before OLT. The remaining 10 patients were either started on LAM immediately after OLT (n = 4) or after a median of 82 (range, 62-109) months after OLT (n = 6) as part of a prospective HBIG withdrawal protocol[31] (Table 2).

All patients received IV HBIG during the anhepatic phase and the first week after OLT. Three patients (patients 9, 17, and 18) who were deemed to be low-level replicators before OLT stopped HBIG after the first week after OLT. Among the 18 patients who continued to receive monthly IV infusions of HBIG, 15 received a median of 20 (range, 9-110) months of IV HBIG and were then switched to low-dose IM HBIG for 3-15 (median, 12) months before HBIG was discontinued, whereas the remaining 3 patients received 11-13

TABLE 2. Posttransplantation Hepatitis B Virus Prophylaxis and Outcome

Patient	Post-OLT antiviral therapy		Total duration of HBIG (months)	Switch to IM HBIG	Duration of post-HBIG follow-up (months)	Post-HBIG outcome	
	Type	Onset of therapy				HBsAg	HBV DNA
1	LAM→LAM+ADV*	Pre-OLT	12	Yes	45	Detected†	>5 log copies/mL†
2	LAM→FTC+TDF‡	Pre-OLT	26	Yes	50	UD§	>3 log copies/mL†
3	LAM	Pre-OLT	45	Yes	13	UD	UD
4	LAM	Pre-OLT	12	No	40	UD	UD
5	LAM	Pre-OLT	42	Yes	46	UD	UD
6	LAM	Pre-OLT	33	Yes	48	Transient‖	UD
7	LAM	Pre-OLT	25	Yes	34	UD	UD
8	ADV	Pre-OLT	26	Yes	5	UD	UD
9	LAM	Pre-OLT	0.2	No	32	UD	UD
10	LAM	Pre-OLT	24	Yes	6	UD	Transient‖
11	LAM	Pre-OLT	13	No	24	UD	Transient‖
12	LAM	Month 62 after OLT	75	Yes	51	UD	Transient‖
13	LAM	Month 91 after OLT	103	Yes	16	UD	UD
14	LAM	Immediately after OLT	31	Yes	29	UD	UD
15	LAM	Month 99 after OLT	111	Yes	13	UD	UD
16	LAM	Month 109 after OLT	121	Yes	9	UD	UD
17	LAM	Immediately after OLT	0.2	No	51	UD	UD
18	LAM	Immediately after OLT	0.2	No	44	UD	UD
19	LAM	Immediately after OLT	11	No	47	UD	UD
20	LAM	Month 67 after OLT	79	Yes	50	UD	UD
21	LAM	Month 73 after OLT	85	Yes	51	UD	UD

Abbreviations: OLT, orthotopic liver transplant; HBIG, hepatitis B immunoglobulin; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; LAM, lamivudine; FTC, emtricitabine; TDF, tenofovir; ADV, adefovir; UD, undetectable; PCR, polymerase chain reaction.

*ADV added to LAM at time of HBV recurrence.

†On 2 or more occasions.

‡LAM switched to FTC + TDF when LAM resistance was confirmed.

§UD indicates consistently negative HBsAg or HBV DNA by PCR-based assays.

‖ Transient detection of HBV DNA (with negative HBsAg) or HBsAg (with negative HBV DNA). All patients were negative for HBV DNA and HBsAg at last follow-up.

months of IV HBIG before it was discontinued. Four patients discontinued HBIG during year 1 (3 were deemed to be low-risk patients [patients 9, 17, and 18], and 1 missed the month 12 dose of HBIG [patient 19]), 4 in years 2-3, and 13 after year 3. The median duration of HBIG therapy (IV and IM) for the whole cohort was 26 (range, 0.2-121) months. At the time of HBIG discontinuation, all patients were negative for HBsAg and serum HBV DNA by PCR assay. None of the patients received treatment for acute rejection. Apart from the 3 patients who received only 1 week of HBIG, all patients had stopped receiving prednisone at the time HBIG was discontinued.

Outcomes

Median follow-up after HBIG discontinuation was 40.2 (range, 4.5-51.1) months. The number of patients followed for more than 12, 24, 36, and 48 months after HBIG discontinuation were 18, 15, 11, and 6, respectively. All patients were still alive at the time of analysis.

A single patient (patient 1) developed HBV recurrence, with positive serum HBsAg and an HBV DNA of 7.9 log copies/mL at 34.2 months after HBIG discontinuation. This patient began LAM treatment 3 months before OLT and had an HBV DNA level of 3.5 log copies/mL and negative serum HBeAg at the start of LAM treatment. HBV DNA was not determined at the time of transplantation. After OLT, this patient initially received a combination of IV HBIG and LAM for 9 months, after which IV HBIG was switched to IM HBIG because of lack of insurance coverage. After 3 months on IM HBIG and LAM, this patient was lost to follow-up despite numerous attempts to contact the patient. This patient was not seen in the clinic, and no laboratory results were available between 12 and 46 months after OLT. At month 46 after OLT, this patient returned to clinic with fatigue but had no evidence of hepatic decompensation. Laboratory tests showed the following: alanine aminotransferase 164 U/L, bilirubin 1 mg/dL, positive serum HBeAg and HBsAg, and serum HBV DNA 7.9 log copies/mL. This patient claimed to have continued LAM throughout, but admitted to stopping HBIG after month 12. Resistance testing performed at the time of HBV recurrence revealed the presence of LAM-resistant mutations (leucine to methionine substi-

tution at codon 180 [rtL180M], methionine to valine at codon 204 [rtM204V], and valine to leucine at codon 173 [rtV173L] of the polymerase gene). Mutations in the "a" determinant of the HBV surface protein were not detected. ADV was added to LAM and serum HBV DNA decreased to 5.4 log copies/mL and alanine aminotransferase to 47 U/L 4 months later. The cumulative probability of developing HBV recurrence was 0%, 0%, 9.1%, and 9.1% at 1, 2, 3, and 4 years after HBIG discontinuation, respectively.

One patient (patient 2) had transiently detectable but unquantifiable HBV DNA (<2.3 log copies/mL) on one occasion 16 months after HBIG discontinuation. HBV DNA was undetectable on 9 subsequent occasions. However, HBV DNA became detectable again at 3.3 log copies/mL 46.6 months after HBIG discontinuation. This patient had no symptoms and had negative serum HBsAg and normal liver chemistries. He was compliant with follow-up and insisted that he never missed a single dose of LAM. Repeat tests a month later showed that serum HBV DNA remained detectable at the same level, but HBsAg was still not detected, and liver chemistries were normal. Resistance testing revealed the presence of LAM-resistant mutations (rtL180M and rtM204V), and antiviral therapy was switched to a combination of emtricitabine and tenofovir. This patient was HBeAg-positive with HBV DNA of 6.2 log copies/mL when LAM was started 19 months before OLT, with loss of HBeAg and undetectable serum HBV DNA by hybridization assay at the time of OLT.

Two patients (patients 10 and 12) had 1 episode and one patient had 2 episodes (patient 11) of transiently detectable HBV DNA as assessed by PCR-based assays after HBIG was discontinued, but with negative HBsAg. These 3 patients denied missing any dose of LAM and had no change in immunosuppressive therapy at the time of transient HBV DNA detection. All instances of detectable HBV DNA were <3 log copies/mL except for one occasion in a single patient (3.8 log copies/mL; patient 10). Serum samples were available in 2 of 4 instances, and resistance testing did not detect the presence of antiviral drug–resistant mutations. All 3 patients remained HBsAg negative with normal liver chemistries throughout. Each patient had HBV DNA and HBsAg retested a median of 5 (range, 1-6) times over a period of 2-36 months since the last detectable HBV DNA, with undetectable HBV DNA and HBsAg on all occasions. One patient (patient 6) had detectable HBsAg once 9 months after HBIG discontinuation, but HBV DNA was persistently undetectable with normal liver chemistries. This patient was repeatedly negative for HBsAg up to the last visit, 39 months after the transient detection of HBsAg. The remaining 15 patients remained HBsAg negative with undetectable HBV DNA and normal liver chemistries throughout the follow-up period, 5-51 months after HBIG was stopped.

DISCUSSION

Our data demonstrate that discontinuing HBIG in the presence of maintenance therapy with an NA was associated with a low risk of HBV recurrence (9%) during a median follow-up of 40 months. The lone patient who experienced HBV recurrence in our study was noncompliant and was lost to follow-up for an extended period of time. None of the compliant patients developed HBV recurrence. Our data are consistent with earlier published reports where the duration of follow-up after HBIG withdrawal was shorter than in our study.[26-28,33] Dodson et al.[26] prospectively followed 16 patients who were switched to LAM monotherapy after 24 months of IV HBIG. No HBV recurrence was noted a median of 13 months after HBIG was stopped. One study from Europe randomized 24 patients to receive either HBIG or LAM monoprophylaxis after at least 6 months of HBIG monotherapy. HBV recurrence occurred in 2 of 12 patients in the LAM group; both had received <12 months of HBIG before being switched to LAM monoprophylaxis.[27] Two other studies used relatively short durations of LAM and HBIG combination therapy (1-4 weeks) followed by LAM monotherapy, with HBV recurrence rates of 0-10% at 18 months after OLT.[28,33] Two reports published as abstracts showed higher rates of recurrence after longer follow-up. Buti et al.[29,34] followed up on their initial report and showed an HBV recurrence rate of 19% (4 of 21) in patients maintained on LAM monotherapy 5 years after OLT. Two of their patients who developed recurrence were noncompliant. Similarly, Wang et al.[35] reported HBV recurrence rates of 15% vs. 9% at 5 years after OLT in patients given 6 ± 2 vs. 12 ± 2 months of combination HBIG and antiviral therapy, respectively, followed by antiviral monotherapy.

Discontinuing HBIG therapy after the first 2-3 years after OLT may translate into marked reduction in health care costs for HBV patients who undergo OLT. Although there is a wide range in the dose of HBIG used across different transplant centers and a marked variation in the price of HBIG in different countries,[14,15] the costs of indefinite HBIG therapy remain high. Estimated charges for 1 year of IV (10,000 U/mo) and IM (1,000 U/mo) HBIG therapy in our center are US$125,000 and US$36,000 per patient, respectively. In comparison, the estimated costs for 1 year of LAM, ADV, or entecavir are US$2,800, US$7,700 and US$8,500, respectively (http://www.drugstore.com/). A recent cost-effective analysis suggested that the use of LAM monotherapy with ADV rescue for recurrence resulted in substantial savings per HBV recurrence prevented over a regimen of indefinite IV or IM HBIG with LAM.[14] However, this was at the expense of a higher HBV recurrence rate in patients provided LAM monotherapy. A strategy involving a finite course of combination HBIG and NA followed by maintenance NA therapy would be more cost-effective if the HBV recurrence rate is similar to prophylaxis with indefinite combination of HBIG and NA.

The increased availability of highly sensitive PCR-based HBV DNA assays has enabled the detection of low amounts of virus in post-OLT patients with no serologic evidence of HBV reinfection (undetectable serum HBsAg). From a purely virological standpoint, the

reappearance of serum HBV DNA may be interpreted as an early sign of HBV recurrence, as it is possible that detection of HBV DNA may precede the reappearance of serum HBsAg.[36] Several studies have reported that HBV DNA can be detected in the liver, peripheral blood mononuclear cells, or sera of post-OLT patients who have no serologic evidence of HBV reinfection, indicating that some of these patients may have occult or subclinical reinfection.[4,13,27,28,30] However, serum HBV DNA can also be detected, albeit in low concentrations, in patients who have spontaneously recovered from acute HBV infection.[37] Therefore, the question remains as to whether the detection of any level of serum HBV DNA in post-OLT patients should be taken as an early sign of HBV recurrence. In our series, HBV DNA was detected transiently in 4 patients, of whom 3 remained negative for HBV DNA by PCR a median of 12 months after the last detection of HBV DNA, and HBsAg remained negative with normal liver chemistries. This confirms previous reports that transient detection of low levels of serum HBV DNA after OLT may not necessarily signify HBV recurrence and emphasizes the need for serial monitoring.[13,27] The fourth patient (patient 2) had detectable serum HBV DNA of 3.3 log copies/mL on 2 consecutive occasions. Sequencing of the HBV genome in this patient revealed the presence of LAM-resistant mutations, so therapy was switched from LAM to combination of emtricitabine and tenofovir.

The significance of the detection of antiviral-resistant mutations in post-OLT patients who test negative for HBsAg has been questioned.[27,38] Naoumov et al.[27] showed that HBsAg and HBV DNA (hybridization assay) remained negative 10-22 months after the initial detection of LAM-resistant mutations in 3 of their post-OLT patients who continued LAM monotherapy after the discontinuation of HBIG. However, these patients had very low level viremia (2.5-2.7 log copies/mL) when LAM-resistant mutations were detected. The decreased replication fitness of LAM-resistant mutants may account for the low HBV DNA level and undetectable HBsAg when the mutants first emerge. In addition, some mutations in the polymerase gene lead to changes in the overlapping surface gene that may decrease recognition of the surface protein in serology assays resulting in false negative HBsAg test results.[39] Regardless, continuation of LAM may eventually select for additional mutations that restore replication fitness of LAM-resistant mutants leading to virus rebound, hepatitis flares, and hepatic failure.[40-42] Furthermore, severe hepatitis flares and death can occur in patients who develop LAM resistance after OLT.[42-45] Therefore, initiation of rescue therapies seems prudent in post-OLT patients who have persistent detection of antiviral-resistant HBV.

Our favorable results may not be applied to all HBV patients. Most (67%) of our patients had received ≥24 months of HBIG before it was discontinued. In addition, most patients were not receiving corticosteroids and were receiving a single calcineurin inhibitor when HBIG was withdrawn. Although none of the 4 patients who received <12 months of HBIG therapy in our study experienced HBV recurrence, all of them had low virus replication immediately before OLT (<5 log copies/mL), making them less likely to experience HBV recurrence. Therefore, it is unknown whether the same results can be achieved in patients who stopped HBIG earlier, and in those with high virus replication before OLT. Unlike most studies on HBIG discontinuation, where most patients were at low risk for HBV recurrence after OLT (HBeAg-negative and negative HBV DNA by hybridization assay),[26-28] we included 6 patients (29%) who would have been considered high risk at the time of OLT (Table 1). On the other hand, we acknowledge that by excluding patients who experienced HBV recurrence while they were still receiving HBIG, we may have selected for patients who are less likely to have HBV recurrence regardless of HBIG discontinuation. Additional prospective randomized studies are needed to define the optimal timing and to identify the best candidates for HBIG withdrawal.

In conclusion, we have demonstrated excellent long-term results in compliant patients in whom HBIG was discontinued after >24 months after OLT. It is possible that similar results can be obtained in patients given a shorter duration of HBIG therapy after OLT with the use of newer antiviral therapies, such as entecavir, that are more potent and have lower rates of drug resistance. We found that transient detection of low-level serum HBV DNA is not uncommon and is not necessarily associated with HBV recurrence. However, persistent detection of HBV DNA levels of >3 log copies/mL and the detection of antiviral therapy–resistant mutations may be harbingers of HBV recurrence and may warrant intervention before reappearance of HBsAg in order to prevent hepatitis flares.

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REFERENCES

1. 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.
2. O'Grady JG, Smith HM, Davies SE, Daniels HM, Donaldson PT, Tan KC, et al. Hepatitis B virus reinfection after orthotopic liver transplantation. Serological and clinical implications. J Hepatol 1992;14:104-111.
3. 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.
4. Terrault NA, Zhou S, Combs C, Hahn JA, Lake JR, Roberts JP, et al. Prophylaxis in liver transplant recipients using a fixed dosing schedule of hepatitis B immunoglobulin. Hepatology 1996;24:1327-1333.
5. McGory RW, Ishitani MB, Oliveira WM, Stevenson WC, McCullough CS, Dickson RC, et al. Improved outcome of orthotopic liver transplantation for chronic hepatitis B cirrhosis with aggressive passive immunization. Transplantation 1996;61:1358-1364.

6. Han SH, Ofman J, Holt C, King K, Kunder G, Chen P, et al. 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.
7. Perrillo RP, Wright T, Rakela J, Levy G, Schiff E, Gish R, et al. A multicenter United States–Canadian trial to assess lamivudine monotherapy before and after liver transplantation for chronic hepatitis B. Hepatology 2001;33:424-432.
8. Mutimer D, Dusheiko G, Barrett C, Grellier L, Ahmed M, Anschuetz G, et al. Lamivudine without HBIg for prevention of graft reinfection by hepatitis B: long-term follow-up. Transplantation 2000;70:809-815.
9. Malkan G, Cattral MS, Humar A, Al Asghar H, Greig PD, Hemming AW, et al. Lamivudine for hepatitis B in liver transplantation: a single-center experience. Transplantation 2000;69:1403-1407.
10. Dumortier J, Chevallier P, Scoazec JY, Berger F, Boillot O. Combined lamivudine and hepatitis B immunoglobulin for the prevention of hepatitis B recurrence after liver transplantation: long-term results. Am J Transplant 2003;3: 999-1002.
11. Rosenau J, Bahr MJ, Tillmann HL, Trautwein C, Klempnauer J, Manns MP, Boker KHW. Lamivudine and low-dose hepatitis B immune globulin for prophylaxis of hepatitis B reinfection after liver transplantation possible role of mutations in the YMDD motif prior to transplantation as a risk factor for reinfection. J Hepatol 2001;34:895-902.
12. Markowitz JS, Martin P, Conrad AJ, Markmann JF, Seu P, Yersiz H, et al. Prophylaxis against hepatitis B recurrence following liver transplantation using combination lamivudine and hepatitis B immune globulin. Hepatology 1998; 28:585-589.
13. Marzano A, Salizzoni M, Debernardi-Venon W, Smedile A, Franchello A, Ciancio A, et al. Prevention of hepatitis B virus recurrence after liver transplantation in cirrhotic patients treated with lamivudine and passive immunoprophylaxis. J Hepatol 2001;34:903-910.
14. Dan YY, Wai CT, Yeoh KG, Lim SG. Prophylactic strategies for hepatitis B patients undergoing liver transplant: a cost-effectiveness analysis. Liver Transpl 2006;12:736-746.
15. Shouval D, Samuel D. Hepatitis B immune globulin to prevent hepatitis B virus graft reinfection following liver transplantation: a concise review. Hepatology 2000;32: 1189-1195.
16. Albeniz Arbizu E, Barcena Marugan R, Oton Nieto E, Carrera Alonso E, Garcia Gonzalez M, et al. Prophylaxis of recurrent hepatitis B virus by vaccination after liver transplant: preliminary results. Transplant Proc 2003;35: 1848-1849.
17. Karasu Z, Ozacar T, Akarca U, Ersoz G, Erensoy S, Gunsar F, et al. HBV vaccination in liver transplant recipients: not an effective strategy in the prophylaxis of HBV recurrence. J Viral Hepat 2005;12:212-215.
18. Starkel P, Stoffel M, Lerut J, Horsmans Y. Response to an experimental HBV vaccine permits withdrawal of HBIg prophylaxis in fulminant and selected chronic HBV-infected liver graft recipients. Liver Transpl 2005;11:1228-1234.
19. Gunther M, Neuhaus R, Bauer T, Jilg W, Holtz JA, Bienzle U. Immunization with an adjuvant hepatitis B vaccine in liver transplant recipients: antibody decline and booster vaccination with conventional vaccine. Liver Transpl 2006;12:316-319.
20. Albeniz Arbizu E, Barcena Marugan R, Oton Nieto E, Mateo Lindeman M, Garcia Gonzalez M, de Vicente Lopez E, et al. Use of combined treatment of hepatitis B immune globulin and lamivudine as prevention of hepatitis B virus recurrence in liver allograft. Transplant Proc 2003;35: 1844-1845.
21. Ferretti G, Merli M, Ginanni Corradini S, Callejon V, Tanzilli P, Masini A, et al. Low-dose intramuscular hepatitis B immune globulin and lamivudine for long-term prophylaxis of hepatitis B recurrence after liver transplantation. Transplant Proc 2004;36:535-538.
22. Zheng S, Chen Y, Liang T, Lu A, Wang W, Shen Y, et al. Prevention of hepatitis B recurrence after liver transplantation using lamivudine or lamivudine combined with hepatitis B Immunoglobulin prophylaxis. Liver Transpl 2006;12:253-258.
23. Han SH, Martin P, Edelstein M, Hu R, Kunder G, Holt C, et al. Conversion from intravenous to intramuscular hepatitis B immune globulin in combination with lamivudine is safe and cost-effective in patients receiving long-term prophylaxis to prevent hepatitis B recurrence after liver transplantation. Liver Transpl 2003;9:182-187.
24. Angus PW, McCaughan GW, Gane EJ, Crawford DH, Harley H. Combination low-dose hepatitis B immune globulin and lamivudine therapy provides effective prophylaxis against posttransplantation hepatitis B. Liver Transpl 2000;6:429-433.
25. Karademir S, Astarcioglu H, Akarsu M, Ozkardesler S, Ozzeybek D, Sayiner A, et al. Prophylactic use of low-dose, on-demand, intramuscular hepatitis B immunoglobulin and lamivudine after liver transplantation. Transplant Proc 2006;38:579-583.
26. Dodson SF, de Vera ME, Bonham CA, Geller DA, Rakela J, Fung JJ. Lamivudine after hepatitis B immune globulin is effective in preventing hepatitis B recurrence after liver transplantation. Liver Transpl 2000;6:434-439.
27. Naoumov NV, Lopes AR, Burra P, Caccamo L, Iemmolo RM, de Man RA, et al. Randomized trial of lamivudine versus hepatitis B immunoglobulin for long-term prophylaxis of hepatitis B recurrence after liver transplantation. J Hepatol 2001;34:888-894.
28. Buti M, Mas A, Prieto M, Casafont F, Gonzalez A, Miras M, et al. A randomized study comparing lamivudine monotherapy after a short course of hepatitis B immune globulin (HBIg) and lamivudine with long-term lamivudine plus HBIg in the prevention of hepatitis B virus recurrence after liver transplantation. J Hepatol 2003;38:811-817.
29. Buti M, Mas A, Prieto M, Casafont F, Gonzalez A, Miras M, et al. Five year follow-up of a randomized study comparing lamivudine vs. lamivudine+HBIG in the prevention of HBV recurrence after liver transplantation [abstract]. Hepatology 2005;42(Suppl):491A.
30. Roche B, Feray C, Gigou M, Roque-Afonso AM, Arulnaden JL, Delvart V, et al. HBV DNA persistence 10 years after liver transplantation despite successful anti-HBS passive immunoprophylaxis. Hepatology 2003;38:86-95.
31. Chu CJ, Fontana RJ, Moore C, Wai CT, Punch J, Su G, Lok AS. Efficacy of HBIG weaning in the long-term prophylaxis of HBV reinfection following liver transplantation [abstract]. Hepatology 2002;36(Suppl):221A.
32. Hussain M, Fung S, Libbrecht E, Sablon E, Cursaro C, Andreone P, et al. Sensitive line probe assay that simultaneously detects mutations conveying resistance to lamivudine and adefovir. J Clin Microbiol 2006;44:1094-1097.
33. Park SJ, Paik SW, Choi MS, Lee JH, Koh KC, Kim SJ, et al. Is lamivudine with 1-week HBlg as effective as long-term high-dose HBlg in HBV prophylaxis after liver transplantation? Transplant Proc 2002;34:1252-1254.
34. Buti M, Mas A, Prieto M, Casafont F, Gonzalez A, Miras M, et al. Prolonged follow-up of HBV liver transplanted patients: results from a randomized study comparing lamivudine vs. lamivudine+HBIG in the prevention of HBV recurrence after liver transplantation (abstract). Journal of Hepatology 2003;38:37.
35. Wang RT, Kim D, Yao FY, Roberts JP, Terrault NA. Long-

term-efficacy of different regimens of combination hepatitis B immunoglobulin (HBIG) and antivirals in preventing hepatitis B virus (HBV) recurrence post-transplantation (LT) [abstract]. Hepatology 2005;42:491A.

36. Mutimer D, Pillay D, Dragon E, Tang H, Ahmed M, O'Donnell K, et al. High pre-treatment serum hepatitis B virus titre predicts failure of lamivudine prophylaxis and graft re-infection after liver transplantation. J Hepatol 1999;30:715-721.
37. Noborg U, Gusdal A, Horal P, Lindh M. Levels of viraemia in subjects with serological markers of past or chronic hepatitis B virus infection. Scand J Infect Dis 2000;32: 249-252.
38. Zoulim F. Towards an improved and cost-saving prophylaxis of hepatitis B virus recurrence after liver transplantation? J Hepatol 2003;38:850-852.
39. Ireland JH, O'Donnell B, Basuni AA, Kean JD, Wallace LA, Lau GK, et al. Reactivity of 13 in vitro expressed hepatitis B surface antigen variants in 7 commercial diagnostic assays. Hepatology 2000;31:1176-1182.
40. Delaney WE, Yang H, Westland CE, Das K, Arnold E, Gibbs CS, et al. The hepatitis B virus polymerase mutation rtV173L is selected during lamivudine therapy and enhances viral replication in vitro. J Virol 2003;77:11833-11841.
41. Natsuizaka M, Hige S, Ono Y, Ogawa K, Nakanishi M, Chuma M, et al. Long-term follow-up of chronic hepatitis B after the emergence of mutations in the hepatitis B virus polymerase region. J Viral Hepat 2005;12:154-159.
42. Bock CT, Tillmann HL, Torresi J, Klempnauer J, Locarnini S, Manns MP, et al. Selection of hepatitis B virus polymerase mutants with enhanced replication by lamivudine treatment after liver transplantation. Gastroenterology 2002;122:264-273.
43. Chan HL, Chui AK, Lau WY, Chan FK, Hui AY, Rao AR, et al. Outcome of lamivudine resistant hepatitis B virus mutant post-liver transplantation on lamivudine monoprophylaxis. Clin Transplant 2004;18:295-300.
44. Wai CT, Prabhakaran K, Wee A, Lee YM, Dan YY, Sutedja DS, et al. Adefovir dipivoxil as the rescue therapy for lamivudine-resistant hepatitis B post liver transplant. Transplant Proc 2004;36:2313-2314.
45. Mutimer D, Pillay D, Shields P, Cane P, Ratcliffe D, Martin B, et al. Outcome of lamivudine resistant hepatitis B virus infection in the liver transplant recipient. Gut 2000;46: 107-113.