Impact of Virologic Breakthrough and HBIG Regimen on Hepatitis B Recurrence After Liver Transplantation

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The availability of hepatitis B immune globulin (HBIG) and several oral antiviral therapies has reduced but not eliminated hepatitis B virus (HBV) recurrence. We aimed to determine the rate of HBV recurrence after orthotopic liver transplantation (OLT) in relation to virologic breakthrough pre-OLT and HBIG regimens post-OLT. Data from the NIH HBV-OLT database were analyzed. A total of 183 patients transplanted between 2001 and 2007 followed for a median of 42 months (range 1–81) post-OLT were studied. At transplant, 29% were hepatitis B e antigen (HBeAg) (+), 38.5% had HBV DNA $> 5 \log_{10}$ copies/mL, 74% were receiving antiviral therapy. Twenty-five patients experienced virologic breakthrough before OLT. Post-OLT, 26%, 22%, 40% and 12% of patients received intravenous (IV) high-dose, IV low-dose, intramuscular low-dose and a finite duration of HBIG, respectively as maintenance prophylaxis. All but two patients also received antiviral therapy. Cumulative rates of HBV recurrence at 1 and 5 years were 3% and 9%, respectively. Multivariate analysis showed that listing HBeAg status and HBV DNA level at OLT were the only factors associated with HBV recurrence. In conclusion, low rates of HBV recurrence can be accomplished with all the HBIG regimens used when combined with antiviral therapy including patients with breakthrough pre-OLT as long as rescue therapy is administered pre- and post-OLT.

Introduction

The introduction of high-dose intravenous (IV) hepatitis B immune globulin (HBIG) monotherapy reduced the 3-year hepatitis B virus (HBV) recurrence rate after orthotopic liver transplantation (OLT) from 80% to 36% (1). However, HBIG monotherapy has limited efficacy in patients with high levels of HBV replication pretransplant, is very expensive, and may select for immune escape mutants (2–4). In the study by Samuel et al., the overall 3-year HBV recurrence rate among hepatitis B e antigen (HBeAg) and HBV DNA positive patients transplanted for cirrhosis was 83% (1). McGory et al. (5) and Dickson et al. (6) observed that the half-life of HBIG was shorter in patients who were HBeAg positive or had detectable serum HBV DNA at the time of transplantation highlighting the importance of suppressing HBV replication prior to OLT.

Addition of oral antiviral agents to HBIG has resulted in a further decrease in HBV recurrence rates to <10% (7–9). Long-term administration of nucleos(t)ide analogs is associated with an increasing risk of antiviral drug resistance prior to OLT (10–13). However, several studies have reported that patients with antiviral resistance prior to OLT can be safely transplanted provided that rescue therapy is administered (14–16).

With the availability of multiple nucleos(t)ide analogs, an important question is how much HBIG is needed to prevent HBV recurrence, when used in combination with antiviral

therapy. Many investigators have explored low-dose HBIG regimens or HBIG withdrawal (17–20) but the duration of posttransplant follow-up was short (1–3 years) in many studies and rescue therapy for patients with lamivudine-resistant HBV was not uniformly available in studies conducted prior to 2003.

The National Institutes of Health study on 'Prevention of HBV Recurrence after Liver Transplantation' (NIH HBV-OLT Study) is a retrospective–prospective observational study involving 15 liver centers in the United States. The study provided guidelines on the management of patients before and after OLT but each center was allowed to follow its own HBIG protocol. The aims of this study were (i) to determine the HBV recurrence rates in an era when antiviral therapy is used in combination with HBIG and rescue therapy is available for patients with lamivudine resistance, (ii) to analyze the factors associated with HBV recurrence post-OLT and (iii) to describe the HBIG regimens used in U.S. liver transplant centers and the impact of different HBIG regimens on HBV recurrence post-OLT.

Materials and Methods

Patient population
The NIH HBV-OLT study enrolled hepatitis B surface antigen (HBsAg) positive patients who were 13 years of age or older from 15 centers in the United States (21). The study was approved by the Institutional Review Board representing each of the participating centers, and written informed consent was obtained from all patients prior to study entry.

A total of 317 patients were enrolled. Patients were listed between 1993 and 2005; 18 were listed prior to approval of lamivudine (December 1998) and 151 were listed after approval of adefovir (ADV) (September 2002). For this analysis, patients still on the transplant waiting list (n = 109), those listed for retransplantation (n = 17) and those with less than 1 month of posttransplant follow-up (n = 4) were excluded. Demographics, clinical, laboratory (blood counts, creatinine, liver panel, prothrombin time/international normalized ratio [INR], alpha-fetoprotein [AFP], hepatitis B serology and HBV DNA), and radiologic data, as well as start and stop dates of antiviral therapy and HBIG dosing, were recorded. Data were collected at enrollment, transplant listing and time of transplant, every 6 months while on the transplant waiting list, every 3 months during the first year posttransplant and every 6 months after the first posttransplant year. Data up to the time of study closure on November 30, 2007 were analyzed.

At each visit, an extra tube of blood was collected for testing at a central laboratory at the University of Michigan.

HBV DNA assay
All laboratory tests except for HBV DNA, antiviral- and HBIG-resistant mutations were performed at the participating centers. Serum HBV DNA levels were quantified by the Cobas Amplicor HBV Monitor assay (Roche Molecular Systems, Inc., Branchburg, NJ). The lower limit of detection of this assay is 200 copies/mL. Samples with values >100,000 copies/mL were diluted and retested. For patients with missing central laboratory samples, HBV DNA results at the participating centers were used.

HBIG and HBV antiviral resistance mutation testing
All pretransplant samples from patients with detectable HBV DNA after ≥6 months of antiviral therapy were tested for antiviral drug-resistance muta-

tions and all posttransplant samples with detectable HBV DNA were tested for both antiviral drug-resistance and HBIG escape mutations by direct sequencing of the HBV polymerase gene which overlaps with the surface gene (22). Antiviral drug-resistance mutations were also tested by a line probe assay (INNO-LiPA DRv2 and DRv3 [Innogenetics, Ghent, Belgium]), which can detect mutations known to be associated with resistance to lamivudine, ADV or ETV (23,24).

Definitions and HBIG regimens
Virologic breakthrough during antiviral therapy was defined as ≥1 log increase in serum HBV DNA from nadir or redetection of HBV DNA in serum after its initial disappearance. Genotypic resistance was defined as detection of amino acid substitutions in the HBV polymerase gene or the HBV surface protein that had been documented to be associated with resistance to HBV nucleos(t)ide analogues and HBIG, respectively. Recurrent HBV was defined as the reappearance of HBsAg in serum after the first month posttransplant. Patients were classified into four groups according to the dose and duration of HBIG received: (1) high dose IV HBIG: 10 000 IU during the anhepatic phase, daily for the next 6 days, and monthly thereafter; (2) low dose IV HBIG: 3000—6000 IU monthly or 10 000 IU every 2–6 months; (3) intramuscular (IM) HBIG: 1000 – 1500 IU every 1–2 months; and (4) finite duration of HBIG: HBIG discontinued after a varying period.

Statistical analyses
Categorical data were presented as number and percent and compared using chi-square test or Fisher's exact test as appropriate. Continuous variables were expressed as mean and standard deviation (SD) unless specified otherwise, and were compared using t-test or Mann–Whitney U-test. Serum HBV DNA level was expressed as copies/mL and logarithmically transformed. Continuous variables were dichotomized taking the median as cutoff value, except for serum HBV DNA, where the cutoff used was 5 $\log_{10}$ copies/mL. Univariate analyses of factors associated with HBV recurrence posttransplant were performed using Kaplan–Meier analysis with log rank test. For this purpose patient demographics, OLT indication at the time of transplant, use of antiviral therapy, occurrence of virologic breakthrough and/or presence of genotypic resistance prior to transplant, transplant center, type of HBIG regimen used, HBeAg status and HBV DNA level at listing and at transplant and OLT date (before or after ADV approval) were analyzed. Variables that had a p-value of <0.2 on univariate analysis were entered into a Cox regression proportional hazards model. Forward and backward logistic regression was performed to determine the independent predictors of HBV recurrence. All statistical analyses were performed using SPSS version 14.0.8 statistical software (SPSS, Inc., Chicago, IL).

Results

Characteristics of patients
A total of 187 patients transplanted between March 2001 and September 2007 were included in this analysis. Table 1 summarizes the characteristics of these patients at the time of listing and at transplantation. The vast majority (75.4%) of the patients were men and their mean age was 52.4 years. Asians comprised 42.8% of the patient population, Caucasians 41.7% and African Americans 10.2%. Coinfection with hepatitis C virus (HCV) was diagnosed in 6.1% (10/164) while coinfection with hepatitis D virus (HDV) was diagnosed in 7.5% (4/53) of patients tested. At listing, 30.9% of the patients were HBeAg positive, 48.2% had detectable serum HBV DNA and 30.4% had HBV DNA >5 $\log_{10}$ copies/mL. The median interval between listing and transplant was 3.0 months (range 0.03–97.5). At

Table 1: Characteristics of patients at listing and at the time of transplant

	End-stage cirrhosis	HCC[1]	Acute liver failure	All	p-Value
No. of patients	73 (39.3)	97 (51.8)	17 (8.9)	187 (100)	
Gender, male	58 (79.5)	75 (77.3)	8 (47.1)	141 (75.4)	0.013
Age, years	50.6 ± 8.1	55.5 ± 9.6	43.0 ± 14.2	52.4 ± 10.2	0.001
Race					0.005
Caucasian	33 (45.2)	36 (37.1)	9 (53.0)	78 (41.7)	
Asian	23 (31.5)	53 (54.6)	4 (23.5)	80 (42.8)	
African American	10 (13.7)	5 (5.2)	4 (23.5)	19 (10.2)	
Other	7 (9.6)	3 (3.1)	0(0)	10 (5.3)	
Labs at listing					
HBeAg (+)	20/67 (29.8)	25/84 (29.7)	6/14 (42.9)	51/165 (30.9)	0.600
HBV DNA detectable	31/66 (47.0)	42/85 (49.4)	8/17 (47.1)	81/168 (48.2)	0.952
HBV DNA >5 $\log_{10}$ copies/mL	16/66 (24.2)	31/85 (36.5)	4/17 (23.5)	51/168 (30.4)	0.218
CTP Score	9.7 ± 2.2	7.7 ± 2.5	12.0 ± 1.7	9.1 ± 2.6	<0.001
Lab MELD	19.7 ± 8.7	13.1 ± 7.1	30.4 ± 5.9	18.4 ± 9.1	<0.001
Anti-HCV (+)	5/66 (7.6)	5/81 (6.2)	0/17 (0)	10/164 (6.1)	0.507
Anti-HDV (+)	3/25 (12.0)	1/23 (4.3)	0/5 (0)	4/53 (7.5)	0.483
Time on the waiting list, months	5 (0.1–84.0)	3 (0.1–97.5)	0.06 (0.03–0.27)	3.0 (0.03–97.5)	0.034
Labs at transplantation					
HBeAg (+)	18/63 (28.6)	21/79 (26.6)	5/12 (41.7)	44/154 (28.6)	0.559
HBV DNA detectable	48/68 (70.6)	45/80 (56.3)	13/17 (76.5)	106/165 (64.2)	0.104
HBV-DNA >5 $\log_{10}$ copies/mL	22/68 (32.3)	33/80 (41.3)	7/17 (41.2)	62/165 (37.5)	0.077
HBV DNA >3 $\log_{10}$ copies/mL	35/68 (51.5)	39/80 (48.7)	12/17 (70.5)	86/165 (52.1)	0.259
HBV-DNA, $\log_{10}$ copies/mL	4.0 ± 2.2	4.1 ± 2.2	4.6 ± 2.1	4.1 ± 2.2	0.651
Albumin, g/dL	2.6 ± 0.6	3.2 ± 0.7	2.7 ± 0.6	2.9 ± 0.7	<0.001
AST, U/L	337 ± 862	154 ± 322	1212 ± 2133	323 ± 909	<0.001
ALT, U/L	258 ± 675	108 ± 203	1261 ± 2158	273 ± 841	<0.001
Bilirubin, mg/dL	10.4 ± 12.4	2.9 ± 5.4	20.7 ± 9.6	7.5 ± 10.7	<0.001
Alkaline phosphatase, U/L	146 ± 80.5	155 ± 111	137 ± 56.3	150 ± 96.0	0.716
INR	2.1 ± 1.5	1.3 ± 0.5	3.0 ± 1.7	1.8 ± 1.2	<0.001
CTP score	11.8 ± 3.1	7.7 ± 2.5	13 ± 1.4	10.1 ± 3.4	0.001
Lab MELD	24.5 ± 10.1	15.4 ± 8.1	32.1 ± 5.8	24.0 ± 9.5	<0.001
AFP, ng/mL	39.7 ± 215	453 ± 1538	68 ± 98	286 ± 1206	<0.001
Antiviral treatment at transplantation	56 (76.7)	75 (77.3)	7 (41.2)	138 (73.8)	0.006
Duration of antiviral treatment prior to OLT, months	19.8 ± 21.0	23.3 ± 21.8	0.1 ± 0.3	20.7 ± 21.4	0.021
Types of treatment					0.072
LAM	39	48	7	94	
ADV	3	12	0	15	
ETV	0	4	0	4	
TDF	4	0	0	4	
LAM+ADV	10	6	0	16	
LAM+TDF	0	2	0	2	
ETV+ADV	0	2	0	2	
TDF+ADV	0	1	0	1	
Virologic breakthrough prior to OLT	12/56 (21.4)	13/75 (17.3)	0/7 (0)	25/138 (18.1)	0.200
LAM	11	11	0	22	
ADV	0	1	0	1	
LAM+ADV	1	1	0	2	

Results expressed as number (%) or mean ± SD unless specified otherwise.
LAM = lamivudine; ADV = adefovir; TDF = tenofovir; ETV = entecavir; HCC = hepatocellular carcinoma; CTP = child turcotte pugh; MELD = model for endstage liver disease; AST = aspartate aminotransferase; ALT = alanine aminotransferase.
[1]Patients found to have HCC while on the waiting list or at transplant were included in the HCC column.

transplantation, 28.6% of the patients were HBeAg positive, 64.2% had detectable serum HBV DNA and 37.5% had HBV DNA >5 $\log_{10}$ copies/mL.

At listing, 98 (52.5%) patients had end-stage cirrhosis, 72 (38.6%) had hepatocellular carcinoma (HCC) and 17 (8.9%) had acute liver failure. Twenty-five (25.5%) patients with end-stage cirrhosis were diagnosed to have HCC while on the transplant waiting list or on the explant liver. Thus, a total of 97 (51.8%) patients had HCC while 73 (39.3%) had end-stage cirrhosis with no HCC at the time of transplant (Table 1).

Table 2: Characteristics of patients according to HBIG regimens

	IV high dose	IV low dose	IM low dose	Finite duration	All	p-Value
No. of patients	47 (25.6)	40 (21.8)	73 (39.8)	23 (12.8)	183 (100)	
Gender, male	40 (85)	26 (65)	54 (74)	17 (74)	137 (74.9)	0.172
Age, years	51.7 ± 10.6	54.1 ± 9.7	53.3 ± 10.3	48.8 ± 10.0	52.3 ± 10.3	0.212
OLT indication at transplantation						0.214
Cirrhosis	18 (38.3)	16 (40)	22 (30)	14 (60.9)	70 (38.3)	
HCC	26 (55.3)	20 (50)	44 (60.3)	6 (26.1)	96 (52.5)	
Acute liver failure	3 (6.4)	4 (10)	7 (9.6)	3 (13)	17 (9.3)	
Labs at listing						
HBeAg (+)	16/44 (36.3)	8/34 (23)	19/62 (30)	8/23 (34.8)	51/163 (31.3)	0.289
HBV DNA detectable	26/43 (60.3)	18/38 (47.4)	26/61 (42)	11/23 (47.8)	81/165 (49.1)	0.418
HBV DNA, $\log_{10}$ copies/mL	4.0 ± 2.1	4.0 ± 2.3	3.4 ± 2.1	3.4 ± 2.0	3.73 ± 2.2	0.474
HBV DNA > 5 $\log_{10}$ copies/mL	16/45 (37.2)	15/38 (39.5)	17/61 (28)	3/23 (13)	51/165 (31)	0.154
Antiviral Rx at listing	27 (57.4)	23 (57.5)	40 (54.8)	12 (52.2)	102 (55.7)	0.992
Labs at transplantation						
HBeAg (+)	12/41 (25.5)	9/33 (27)	16/58 (27.6)	7/21 (32.3)	44/153 (28.8)	0.924
HBV DNA detectable	28/44 (63.6)	23/38 (60)	38/56 (67)	16/23 (69.6)	105/161 (65.2)	0.888
HBV DNA, $\log_{10}$ copies/mL	4.6 ± 2.4	3.8 ± 2.2	4.4 ± 2.0	3.4 ± 1.8	4.2 ± 2.2	0.138
HBV DNA > 5 $\log_{10}$ copies/mL	21/44 (47.7)	12/38 (31.6)	25/56 (44.6)	4/23 (17.4)	62/161 (38.5)	0.080
Antiviral Rx at transplant	37 (78.7)	27 (67.5)	56 (76.7)	15 (65.2)	135 (73.8)	0.387
Breakthrough prior to OLT	8/37 (21.6)	5/27 (18.5)	10/56 (17.8)	2/15 (13.3)	25/135 (18.5)	0.576
Samples available	5/8	4/5	6/10	2/2	17/25	
Confirmed genotypic resistance	5/5 (100)	3/4 (75)	4/6 (66)	2/2 (100)	14/17 (82.3)	

Results expressed as number (%) or mean ± SD unless specified otherwise.
LAM = lamivudine; ADV = adefovir; TDF = tenofovir; ETV = entecavir; HCC = hepatocellular carcinoma.

Antiviral therapy and virologic breakthrough prior to transplant

One-hundred and thirty-eight (73.8%) patients were receiv-ing antiviral therapy at the time of transplantation: 117 were receiving nucleos(t)ide monotherapy (including 94 patients on lamivudine monotherapy) and 21 were receiving com-bination therapy (Table 1). The mean duration of antiviral therapy prior to OLT was 20.7 ± 21.4 months (maximum 94).

Twenty-five (18.1%) patients experienced virologic break-through prior to transplantation. Genotypic resistance was confirmed in 14 (58.3%) of 24 patients in whom serum samples prior to initiation of rescue therapy were avail-able for testing. Of the 25 patients with virologic break-through, 24 received lamivudine monotherapy and one re-ceived ADV monotherapy as their initial antiviral therapy and breakthrough was diagnosed after a mean of 34.4 ± 18.5 months. Twenty patients received rescue therapy with ADV (n = 4), lamivudine plus ADV (n = 12, including one patient who had breakthrough while receiving ADV monotherapy), TDF (n = 2), lamivudine plus TDF (n = 1) or ETV (n = 1); five patients continued lamivudine monother-apy. At the time of transplantation, serum HBV DNA was detectable in all 24 patients tested and 18 had levels >5 $\log_{10}$ copies/mL.

HBV prophylaxis post-OLT and HBIG regimens

After transplantation, two patients received HBIG only and four received antiviral therapy only while the remaining patients received combination prophylaxis with HBIG and antiviral therapy. Of the 185 patients who received antiviral

therapy post-OLT, 165 received nucleos(t)ide monotherapy: lamivudine (n = 141 including four patients who received antiviral prophylaxis only), ADV (n = 16), TDF (n = 3) or ETV (n = 5). Twenty patients received combination antiviral ther-apy, including 12 patients who had experienced virologic breakthrough prior to transplantation.
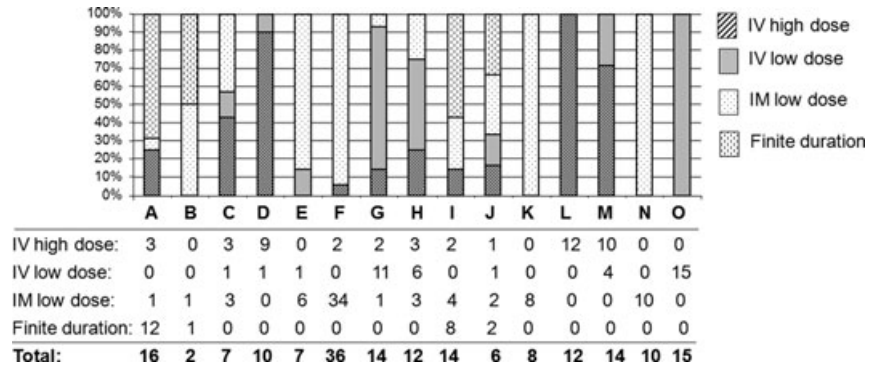
The most common HBIG regimen was IM low dose (39%), followed by IV high dose (25.1%), IV low dose (21.4%) and finite duration of HBIG (12.3%). The last group of patients had received HBIG for a median of 12 months (range 1–48 months) and had been followed for a median of 53 months (range 6–66 months) after HBIG was disconti-nued. The four groups were comparable regarding HBeAg status, HBV DNA levels, use of antiviral therapy and viro-logic breakthrough at the time of transplant (Table 2).

The HBIG regimens used in the 15 participating transplant centers were highly variable not only across the centers but also within the centers (Figure 1). The total HBIG dose in year 1 ranged from 10 000 to 355 000 IU and in each subsequent year from 0 to 200 000 IU.

Posttransplant outcomes

HBV recurrence: During a median follow-up of 42 months (range 1–81 months) posttransplant, 13 (6.9%) patients had HBV recurrence. Ten of the 13 patients had serum samples collected after HBV recurrence was diagnosed and prior to institution of rescue therapy. Of these, five had mutations associated with resistance to lamivudine (methionine to valine or isoleucine substitution at position 204 [rtM204V/I]), one had mutations associated with HBIG

Figure 1: Distribution of HBIG regimens used in each of the 15 participating centers (A-O). Only 4 centers (K, L, N, O) used one regimen. Number of patients receiving each HBIG regimen: IV high dose, IV low dose, IM low dose, and finite duration at each center is listed beneath the bars.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
IV high dose:	3	0	3	9	0	2	2	3	2	1	0	12	10	0	0
IV low dose:	0	0	1	1	1	0	11	6	0	1	0	0	4	0	15
IM low dose:	1	1	3	0	6	34	1	3	4	2	8	0	0	10	0
Finite duration:	12	1	0	0	0	0	0	0	8	2	0	0	0	0	0
Total:	16	2	7	10	7	36	14	12	14	6	8	12	14	10	15

resistance (glycine to arginine substitution at position 145 [sG145R]), three had mutations associated with resistance to lamivudine and HBIG (rtM204V/I + sG145R), and one had wild type HBV sequence. Among the five patients with lamivudine resistance mutations only, three received a finite duration of HBIG and HBV recurred 12, 14 and 33 months after discontinuation of HBIG. The other two patients received high dose IV HBIG, both had HBV recurrence 1 month after transplant; one was documented to have lamivudine resistance before transplant and had HBV DNA of 8.6 log copies/mL at transplant while the other had undetectable serum HBV DNA 3 months before transplant. Both patients received lamivudine as the only antiviral therapy posttransplant. All three patients who had mutations associated with resistance to lamivudine and HBIG were receiving lamivudine and IM HBIG. The patient with HBIG resistance only had HBV DNA of 8.9 log copies/mL at transplantation. This patient had lamivudine resistance prior to transplantation and received prophylaxis with lamivudine, ADV and IV HBIG but was later found to be non compliant.

The overall probability of HBV recurrence at 1, 3 and 5 years posttransplant was 3%, 7% and 9%, respectively. The probability of HBV recurrence at 1, 3 and 5 years posttransplant was 1%, 6% and 9% among patients transplanted for end-stage cirrhosis; and 6%, 8% and 11% among patients transplanted for HCC; none of the patients transplanted for acute liver failure had HBV recurrence ($p = 0.686$) (Figure 2A).

Three (12%) of the 25 patients with virologic breakthrough prior to transplantation had HBV recurrence compared to eight (7.1%) of 113 patients who received antiviral therapy but did not experience virologic breakthrough, and two (4.1%) of 49 patients who did not receive antiviral therapy prior to transplant. The 1-, 3- and 5-year probability of HBV recurrence was 4%, 15% and 15% for patients with and 3%, 6% and 8% for patients without virologic breakthrough prior to transplant ($p = 0.209$) (Figure 2B).

Factors associated with HBV recurrence: Univariate analysis showed that male gender ($p = 0.031$), Caucasian race ($p = 0.034$), presence of HBeAg at listing ($p = 0.01$), serum HBV DNA >5 $\log_{10}$ copies/mL at listing ($p = 0.027$)

and at transplantation ($p = 0.003$) were associated with posttransplant HBV recurrence while transplant date before ADV approval ($p = 0.077$) showed a trend (Table 3, Figure 2C and D). Virologic breakthrough prior to transplantation, HBIG regimen, transplant center, duration of steroid use and treatment for rejection were not associated with HBV recurrence (Figure 2B and E).

Of the 97 patients who had HCC, HBV recurrence was observed in two of 12 who had HCC recurrence and in six of 85 who did not have HCC recurrence.

Among the 25 patients with virologic breakthrough prior to transplantation, the three patients who had HBV recurrence differed from the other 22 patients who did not have HBV recurrence in having higher serum HBV DNA at transplantation ($p = 0.094$). Moreover, only one of the three patients with HBV recurrence received rescue therapy prior to transplantation compared to 21 of 22 with no recurrence ($p = 0.031$).

Cox regression analysis found that presence of HBeAg at listing (HR: 11.6, 95% CI 1.36–99.7, $p = 0.02$) and serum HBV DNA >5 $\log_{10}$ copies/mL at the time of transplant (HR: 7.0, 95% CI 1.03–47.28, $p = 0.03$) were the only factors associated with posttransplant HBV recurrence while race showed a trend (Table 4). The 1-, 3- and 5-year probability of HBV recurrence was 10%, 15% and 15% for patients who were HBeAg positive at listing and 0, 2% and 5% for those who were HBeAg negative at listing (Table 5, Figure 2C). The 1-, 3- and 5-year probability of HBV recurrence was 7%, 15% and 15% for patients with serum HBV DNA > 5 $\log_{10}$ copies/mL at transplant; 0%, 0% and 6% for those with HBV DNA 3–5 $\log_{10}$ copies/mL; and 1%, 1% and 1% for those with HBV DNA < 3 $\log_{10}$ copies/mL (Table 5).

Patient survival: A total of 17 (9%) patients (four of 13 patients with and 13 of 174 patients without HBV recurrence) died: two due to complications related to HBV recurrence, five due to HCC recurrence, three due to other liver causes, six due to nonliver causes and one due to an unknown cause. The 1-, 3- and 5-year probability of post-OLT patient survival for the entire cohort was 95%, 92% and 90%, respectively. The probability of post-OLT survival
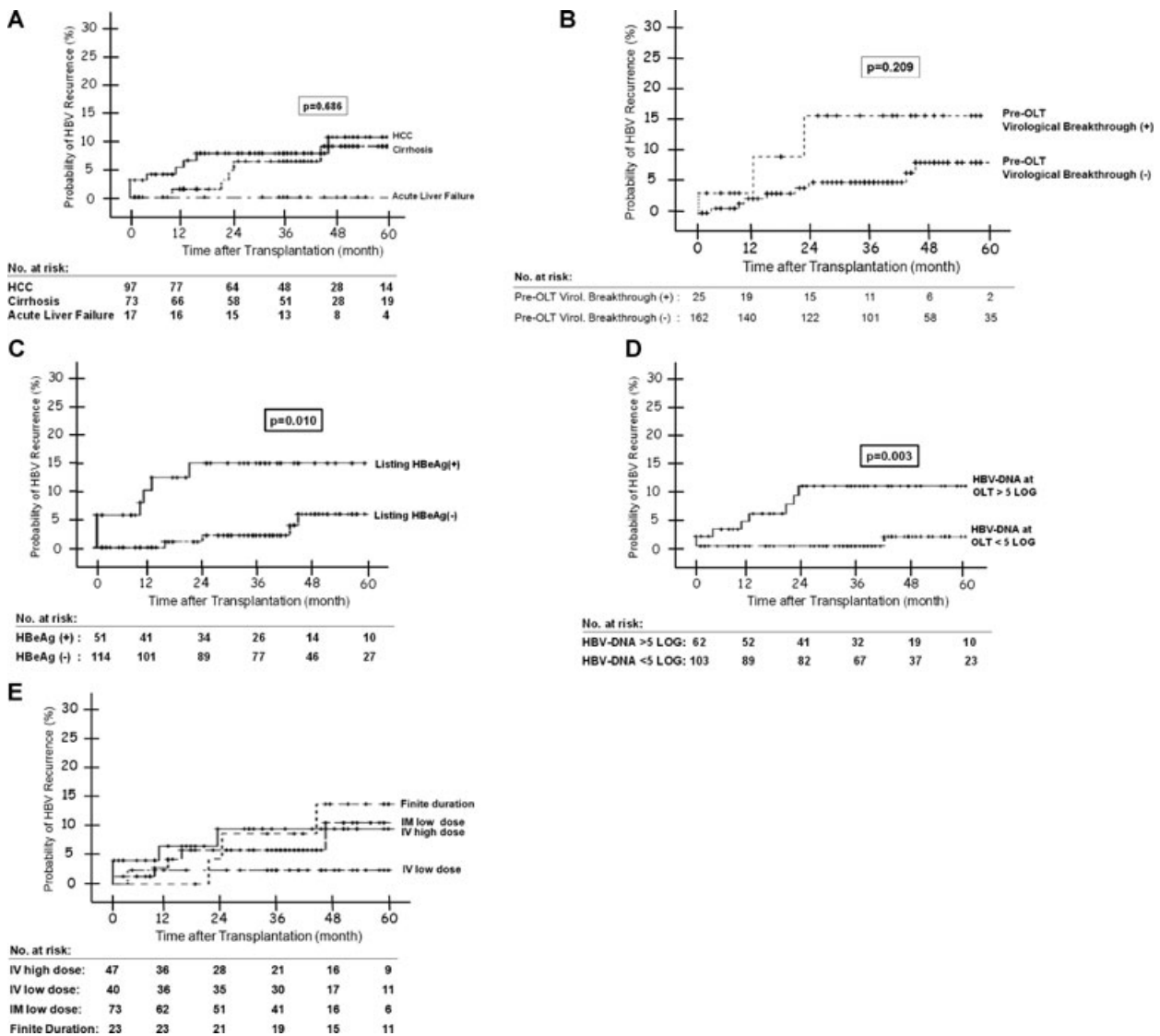
Figure 2: (A) HBV recurrence in relation to indication at transplantation—The 1, 3, and 5 year probability of HBV recurrence post-OLT was 1%, 6%, and 9% for patients with end-stage cirrhosis; and 6%, 8%, and 11% for patients with HCC; and none for patients with acute liver failure (p = 0.686). **(B)** HBV recurrence in patients with and without virological breakthrough pretransplant (p = 0.209). **(C)** HBV recurrence in relation to HBeAg status at listing—The 1, 3, and 5 year probability of HBV recurrence post-OLT was 10%, 15%, and 15% for patients who were HBeAg positive at listing; and 0, 2% and 5% for those who were HBeAg negative (p = 0.010). **(D)** HBV recurrence in relation to serum HBV DNA level at the time of transplant—The 1, 3, and 5 year probability of HBV recurrence post-OLT was 7%, 15%, and 15% for patients with serum HBV DNA >5 log10 copies/mL at transplant; and 1%, 1%, and 3% for those with serum HBV DNA <5 log10 copies/mL (p = 0.003). **(E)** HBV recurrence in relation to HBIG regimens—The 5-year recurrence rates for the groups that received IV high dose, IV low dose, IM low dose, and finite duration of HBIG were 10, 3, 10 and 14, respectively (p = 0.733). The overall 5-year recurrence rate was 9%.

was lower among the patients with HBV recurrence (p = 0.028), but the difference was not significant when only liver-related deaths were analyzed (p = 0.209) (Figure 3). Of the four patients with HBV recurrence who died, one died of liver failure related to recurrent HBV, one died from postoperative complications after retransplantation for recurrent HBV and two died of nonliver related causes: brain hemorrhage and primary lung cancer.

Discussion

In this study of 187 patients who had liver transplantation for hepatitis B in the U.S. between 2001 and 2007, 6.9% had HBV recurrence after a median follow-up of 42 months. All except six patients received combination prophylaxis with HBIG and antiviral therapy. Most (>75%) patients received lamivudine monotherapy while HBIG

Table 3: Characteristics of patients with and without HBV recurrence

	HBV recurrence	No HBV recurrence	p-Value
No. of patients	13 (6.9)	174 (93.0)	
Gender, male	13 (100)	128 (73.6)	0.031
Age, years	53.5 ± 9.4	52.3 ± 10.3	0.708
Race			0.034
Caucasian	10 (76.9)	68 (39.2)	
Asian	2 (15.4)	78 (44.8)	
African American	1 (7.7)	18 (10.3)	
Other	0	10 (5.7)	
OLT Indication at transplant			0.686
End-stage cirrhosis	5 (38.5)	68 (39.1)	
HCC	8 (61.5)	89 (51.1)	
Acute liver failure	0	17 (9.8)	
Labs at listing			
HBeAg (+)	7/11 (63.6)	44/154 (28.6)	0.010
HBV DNA detectable	8/12 (66.7)	73/156 (46.8)	0.177
HBV DNA >5 $\log_{10}$ copies/mL	7/12 (58.3)	44/156 (28.2)	0.027
HBV-DNA $\log_{10}$ copies/mL	5.3 ± 2.98	3.50 ± 2.1	0.001
Labs at transplant			
HBeAg (+)	4/9 (44.4)	40/145 (27.6)	0.220
HBV DNA detectable	9/10 (90)	97/155 (62.6)	0.103
HBV DNA >5 $\log_{10}$ copies/mL	8/10 (80)	54/155 (34.8)	0.003
HBV DNA >3 $\log_{10}$ copies/mL	9/10 (90)	77/155 (49.7)	0.017
HBV-DNA $\log_{10}$ copies/mL	7.0 ± 2.45	3.9 ± 2.0	<0.001
HBIG regimen	13/13	170/174	0.733
IV high dose	4 (30.7)	43 (25.3)	
IV low dose	1 (7.7)	39 (22.9)	
IM low dose	5 (38.5)	68 (40.0)	
Finite duration	3 (23.0)	20 (11.8)	
Antiviral treatment pre-OLT	11 (84.6)	127 (73.0)	0.285
Total duration of treatment before OLT, months	11.8 ± 9.2	21.4 ± 22.0	0.153
Treatment at the time of transplant			0.280
LAM	10/11 (90.9)	84/127 (66.1)	
LAM+ADV/TDF	1/11 (9.1)	17/127 (13.5)	
Non-LAM	0	26/127 (20.4)	
Virologic breakthrough prior to OLT	3/11 (27.2)	22/127 (17.3)	0.209
Post-OLT prophylaxis			0.898
HBIG only	0	2 (1.1)	
Antiviral only	0	4 (2.2)	
HBIG+antiviral	13 (100)	168 (96.5)	
Antiviral therapy post-OLT	13/13 (100)	172/174 (98.8)	
LAM	12	129	
ADV	0	16	
TDF	0	3	
ETV	0	5	
LAM+ADV	1	14	
LAM+TDF	0	3	
ADV+TDF	0	2	
Time of OLT (after 09.2002)[1]	6 (46.2)	130 (74.7)	0.077
Duration of steroid use (month)	4.42 ± 2.7	4.7 ± 5.0	0.809
Treatment for rejection	1 (7.6)	26 (14.6)	0.461

Results expressed as number (%) or mean ± SD unless specified otherwise.
LAM = lamivudine; ADV = adefovir; TDF = tenofovir; ETV = entecavir; HCC = hepatocellular carcinoma.
[1]Date when adefovir was approved.

regimens ranged from indefinite high dose IV to low dose IM or finite duration. The 5-year recurrence rate of 9% is lower than that in previous studies in which HBIG alone or lamivudine alone was used as prophylaxis (1,9,25,26) but higher than the 4% recurrence rate reported by Gane et al.

(27) The latter study was unique in that very low dose IM HBIG was used from the time of transplantation (800 IU daily × 1 week then monthly) in combination with lamivudine. Two major differences between these two studies may account for the slightly higher rate of HBV recurrence

Table 4: Analyses of predictors for HBV recurrence

| | Univariate analysis p-Value | Multivariate analysis | |
		Hazard ratio (95% CI)	p-Value
HBeAg positive at listing	0.010	11.6 (1.36–99.7)	0.025
HBV DNA > 5 $\log_{10}$ c/mL at transplant	0.003	7.00 (1.03–47.28)	0.038
Race—non-Caucasian	0.034	0.41 (0.15–1.14)	0.088
HBV DNA > 5 $\log_{10}$ c/mL at listing	0.027	2.25 (0.39–13.0)	0.364
Center	0.202	0.98 (0.94–1.02)	0.387
HBIG group	0.733	1.24 (0.72–2.13)	0.816
Transplant date after adefovir approval	0.077	0.84 (0.19–3.71)	0.818
Male gender	0.031	∞[1]	0.800

[1]All patients in the HBV recurrence group were male.

Table 5: Probability of HBV recurrence after transplantation

| | Probability of HBV recurrence | | |
	Year 1	Year 3	Year 5
Gender			
Male	4	9	12
Female	0	0	0
Race			
Caucasian	7	13	15
Asian	0	2	4
African American	6	6	6
Listing HBeAg status			
HBeAg (+)	10	15	15
HBeAg (−)	0	2	5
Serum HBV DNA at OLT ($\log10$ copies/mL)			
>5	7	15	15
3–5	0	0	6
<3	1	1	1
OLT Date			
Before 09/2002[1]	4	13	15
After 09/2002	3	4	6
Indication at OLT			
Cirrhosis	1	6	9
HCC	6	8	11
Acute liver failure	0	0	0
Antiviral breakthrough prior to OLT			
Yes	4	15	15
No	3	6	8
HBIG regimen			
IV high dose	7	10	10
IV low dose	3	3	3
IM low dose	3	6	10
Finite duration	0	9	14

Results expressed as percentage.
[1]Date when adefovir was approved.

in our study. Patients in our study had received a longer duration of lamivudine therapy prior to transplant, median 276 versus 92 days and 25 patients in our study had experienced virologic breakthrough before transplant. Moreover, 64% of our patients versus none in Gane's study had detectable HBV DNA at transplantation.

Indeed, high serum HBV DNA (>5 $\log_{10}$ copies/mL) at transplantation and the presence of HBeAg at listing were the only factors associated with HBV recurrence on multivariate analysis. High serum HBV DNA and presence of HBeAg had been shown to decrease the half-life and efficacy of HBIG (5,6). The shortened half-life of HBIG becomes a greater problem in patients who had high serum HBV DNA as a result of lamivudine resistance and who did not receive rescue antiviral therapy posttransplant.

In this study, resistance to lamivudine and/or HBIG was detected in nine of 10 patients for whom samples at the time of HBV recurrence were available for testing. Mutations associated with lamivudine resistance were detected in eight patients; of these, three also had mutations associated with HBIG resistance. One patient who was noncompliant with antiviral medications had HBIG resistance mutations only. Although antiviral and/or HBIG resistance is the main cause of HBV recurrence, antiviral breakthrough prior to transplant was not a significant predictor of HBV recurrence. Of the 25 patients who were known to have virologic breakthrough prior to transplant, only three had HBV recurrence, one due to noncompliance with antiviral medications and two did not receive rescue antiviral therapy. The remaining 22 patients including two who discontinued

HBIG and nine who received IM low dose HBIG had no evidence of HBV recurrence up to 61 months posttransplant. Our data confirmed the results of other investigators that patients with antiviral resistance prior to transplant can be safely transplanted provided that rescue therapy is administered (14–16).

The HBIG regimens used in this study varied not only among the centers but also within each center. Many experts had suggested that prophylaxis against HBV recurrence be tailored to HBV replication status prior to transplant (27–29). However, our study found that the type of HBIG regimen used was not related to HBeAg status, serum HBV DNA at transplantation or antiviral breakthrough prior to transplantation indicating that other factors such as institutional policies may be more important in determining which HBIG regimen was used at each center. The only exception may be in the group that discontinued HBIG after a finite duration. Compared to the other three groups, a lower percent of patients in this group had serum HBV DNA >5 $\log_{10}$ copies/mL at transplant: 17.4% versus 42.0% (p = 0.056) but the percent of patients with virologic
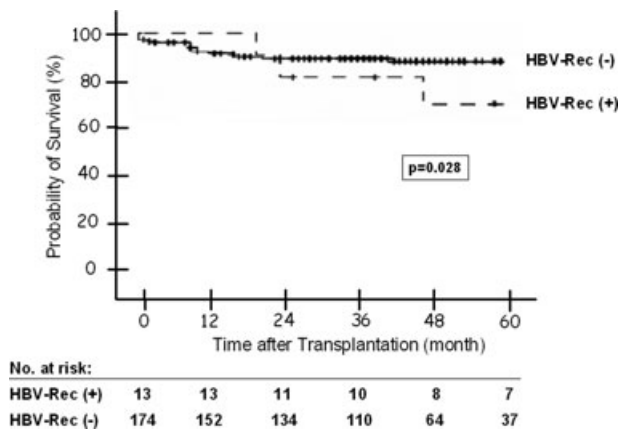
Figure 3: Post-OLT patient survival in patients with and without HBV recurrence---The 1, 3, and 5 year probability of post-OLT patient survival was 100%, 85%, and 76% for patients with; and 95% , 93%, and 92% for those without HBV recurrence (p = 0.028).

breakthrough prior to transplant was similar: 13.3% versus 19.1% (p = 0.583).

Despite the wide range in HBIG dosing, the HBV recurrence rate was comparable in the four groups (p = 0.733). Comparison of the HBV recurrence rate in patients who had stopped HBIG versus the other three groups combined also showed no difference: 13% versus 6.2%, p = 0.439. These data have important implications regarding cost savings. Using pharmacy charges at the University of Michigan and the average amount of HBIG administered in each group, the total charges for HBIG alone in year 1 were $235 692, $191 099, $132 251 and $161 478 for the groups that received indefinite IV high dose, IV low dose, IM low dose and finite duration, respectively. The total charges for HBIG in each subsequent year were $157 128, $44 712 and $20 424 for the three groups that received indefinite HBIG, while the total charges for HBIG decreased from $19 228 in year 2 to $0 in year 5 for the group that received a finite duration of HBIG.

Our study spanned a 6.5 year period during which HBV therapies changed from only one drug with a high rate of antiviral resistance (lamivudine) to three approved HBV therapies (lamivudine, ADV and ETV). Some of our patients were listed for transplantation prior to the approval of lamivudine and many were managed in an era when lamivudine was the only approved HBV therapy. It is likely that use of more potent nucleos(t)ide analogs that have a higher genetic barrier to resistance prior to transplant, close monitoring for virologic response, modification of treatment in patients with suboptimal response, and prompt addition of rescue therapy in patients with virologic breakthrough would result in a higher proportion of patients with undetectable serum HBV DNA at the time of transplant and negligible HBV recurrence rate may be achieved with minimal or no

HBIG. Indeed, Angus et al. (17) recently reported that none of 16 patients randomized to switch from lamivudine + HBIG to lamivudine + ADV had HBV recurrence after a median follow-up of 21 months. In this study, however, HBIG was stopped after a mean of 4.5 years posttransplant. It remains to be determined whether potent nucleos(t)ide analogs that have high genetic barrier to resistance such as ETV or TDF used alone or in combination will completely eliminate the need for HBIG.

The strengths of this study are the large number of patients, the long duration of follow-up and HBV DNA testing in a central laboratory. However, there are some limitations. Most patients received lamivudine monotherapy as the initial antiviral therapy and rescue therapy was not available during the earlier years. Furthermore, blood samples for central laboratory testing were missing in some patients.

In summary, in this study involving 187 patients transplanted for HBV we found a wide range in HBIG regimens among the 15 U.S. centers. However, HBV recurrence rate was not related to HBIG regimen or transplant center. The only factors associated with HBV recurrence were HBeAg status at listing and serum HBV DNA level at transplant. Our study showed that if appropriate rescue therapy is started in patients with virologic breakthrough pre-OLT, comparable results can be obtained with IM low dose or finite course of HBIG as IV high dose HBIG. These data suggest that substantially lower doses or a more limited duration of HBIG than is currently used in many U.S. transplant centers is sufficient in preventing HBV recurrence; this is particularly true if more potent nucleos(t)ide analogs with higher genetic barrier to resistance such as ETV or TDF are used.

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