

# Virologic and Clinical Outcomes of Hepatitis B Virus Infection in HIV-HBV Coinfected Transplant Recipients

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**Liver transplantation (LT) is the treatment of choice for end-stage liver disease, but is controversial in patients with human immunodeficiency virus (HIV) infection. Using a prospective cohort of HIV-hepatitis B virus (HBV) coinfecting patients transplanted between 2001–2007; outcomes including survival and HBV clinical recurrence were determined. Twenty-two coinfecting patients underwent LT; 45% had detectable HBV DNA pre-LT and 72% were receiving anti-HBV drugs with efficacy against lamivudine-resistant HBV. Post-LT, all patients received hepatitis B immune globulin (HBIG) plus nucleos(t)ide analogues and remained HBsAg negative without clinical evidence of HBV recurrence, with a median follow-up 3.5 years. Low-level HBV viremia (median 108 IU/mL, range 9–789) was intermittently detected in 7/13 but not associated with HBsAg detection or ALT elevation. Compared with 20 HBV mono-infected patients on similar HBV prophylaxis and median follow-up of 4.0 years, patient and graft survival were similar: 100% versus 85% in HBV mono- versus coinfecting patients ( $p = 0.08$ , log rank test). LT is effective for HIV-HBV coinfecting patients with complications of cirrhosis, including those who are HBV DNA positive at the time of LT. Combination HBIG and anti-**

**virals is effective as prophylaxis with no clinical evidence of HBV recurrence but low-level HBV DNA is detectable in ~50% of recipients.**

**Key words:** Hepatitis B virus (HBV), hepatitis B immunoglobulin, immune deficiency, surface (S) gene, polymerase chain reaction, posttransplant, posttransplant complications, posttransplant mortality

**Abbreviations:** ALT, alanine aminotransferase; Anti-HBs, antibody to hepatitis B surface antigen; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBIG, hepatitis B immune globulin; HIV, human immunodeficiency virus; LMV, lamivudine; LT, liver transplantation; rtPCR, real time quantitative polymerase chain reaction; Solid Organ Transplantation in HIV Multi-Site Study, HIVTR Study.

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## Introduction

Individuals coinfecting with human immunodeficiency virus (HIV) and hepatitis B virus (HBV) are at significant risk of liver-related complications (1–3). The advent of highly active antiretroviral therapy and the ability to manage HIV-related complications long-term has resulted in improved survival among HIV-infected persons and provided the necessary advances to allow consideration of liver transplantation (LT) in these patients (1,4,5). In recent years, transplantation of patients with stable and controlled HIV infection has been undertaken in a number of centers in the United States and Europe.

In 2001, a pilot study of LT of HIV-infected individuals was undertaken at the University of California, San Francisco. This was followed by a prospective multicenter study, funded by the National Institute of Health called the Solid Organ Transplantation in HIV: Multi-Site Study (AI052747) (<https://web.emmes.com/study/htr>), to assess the safety and efficacy of solid organ transplantation in people living with HIV. In this report, we examine post-LT outcomes of HBV-HIV coinfecting patients enrolled in the UCSF pilot studies and the NIH-sponsored trial, focusing on the virologic and clinical course of HBV posttransplantation. Previous single center case series of small numbers of patients have reported excellent rates of survival (5–10). Tateo

et al. recently examined the outcomes of 13 HBV-HIV coinfecting patients from Europe, and reported 100% survival with median follow-up of 27 months but all patients had undetectable HBV DNA levels at the time of LT (11). We present outcomes of a larger US cohort of transplant recipients with HBV and HIV (N = 22), in whom approximately half had detectable HBV DNA at the time of transplantation, and show excellent short-to-median outcomes using an aggressive HBV prophylaxis regimen. Additionally, we highlight the frequency of recurrent low-level HBV viremia and drug resistant HBV variants among coinfecting transplant recipients.

Occult HBV infection is defined by the presence of detectable HBV DNA using sensitive PCR-based assays in persons who lack serologic markers of current HBV infection (12). Proposed mechanisms include a diminished host immune response allowing HBV escape, development of HBV surface or polymerase viral escape mutants, especially under selective pressure of anti-HBV therapy, or presence of HBV reservoirs (i.e. lymphotropic viral variants) (13). Prior studies in liver transplant recipients transplanted for HBV receiving long-term hepatitis B immune globulin (HBIG) prophylaxis have reported low-level HBV DNA detectable in serum, liver or peripheral blood mononuclear cells up to 10 years post-LT, but with no clinical evidence of recurrent HBV disease (14). In this study, we examined serial serum samples for the presence of HBV DNA and correlated its presence with clinical outcomes.

## **Materials and Methods**

### **Study design and study population**

This is a prospective cohort study of 22 HIV-infected patients with fulminant (n = 1) or chronic HBV infection and complications of end-stage disease enrolled in three consecutive studies: the UCSF Pilot Study conducted between 1999 and July 2001 (n = 2), the Multi-Site Pilot Study conducted from August 2001 to September 2003 (n = 3) and the multicenter HIVTR study from October 2003 to current, which included 1 patient transplanted off-protocol (n = 16). The median follow-up for patients in the pilot studies was 60 months with range 54 to 84 months and the median follow-up for patients in the HIVTR Cohort Study was 35 months with range 1 to 61 months. Preliminary results of four coinfecting patients from the pilot studies have been published previously, as well as short-term follow-up of five HBV coinfecting patients in the HIVTR study (5,15,16). A standardized protocol for patient selection, HBV testing, and post-transplant HBV prophylaxis was utilized in the pilot studies and HIVTR Cohort Study. These studies received Institutional Review Board and Ethics approval at all participating sites.

The HIV specific inclusion criteria for transplantation were (1) the absence of prior opportunistic infections (except for certain protocol allowed exceptions); (2) undetectable plasma HIV RNA (viral load) or if not on antiretroviral therapy, the prediction of HIV suppression based on prior drug history and/or resistance test results; (3) CD4 T count  $\geq 100$  cells/mm<sup>3</sup> (or  $\geq 200$  cells/mm<sup>3</sup> if history of protocol allowed opportunistic infections); and (4) the absence of history of visceral Kaposi's sarcoma. There were no HBV specific criteria for enrollment. Patients were not required to have a specific level of HBV

DNA pre-LT to be considered eligible. One patient who did not meet study inclusion criteria due to low CD4 counts (<40 cells/mm<sup>3</sup>) at the time of transplant but was otherwise a good candidate for LT is included in this analysis.

### **HBV prophylaxis protocol in HBV-HIV coinfecting cohort**

Following transplantation, all patients received a combination of HBIG and antiviral therapy, according to standard protocols for management of HBV post-LT (15). HBIG was continued indefinitely, with a decrease in dose frequency after 12 months, if trough hepatitis B surface antibody (anti-HBs) titers remained greater than 100 IU/L. HBV antivirals used pre-LT was continued post-LT, with the specific drugs determined by local investigators based on prior HBV drug exposure. If antiretroviral therapy could not be started post-LT, HBV nucleos(t)ide analogues were omitted and HBIG therapy alone used for prophylaxis unless the subject had a detectable HBV DNA level pre-LT, in which cases adefovir and/or entecavir were given. This treatment algorithm has been subsequently modified to exclude use of entecavir based on the recent recognition that entecavir inhibits HIV-1 replication and may select for HIV-1 resistant variants (17).

### **Immunosuppression protocol**

Immunosuppression was not standardized across study sites. The most common immunosuppressive regimen used was the combination of a calcineurin inhibitor, mycophenolate mofetil, and prednisone. Steroid induction, tapering and maintenance were according to local site practice. Treatment of acute rejection was according to local site protocols.

### **Posttransplantation monitoring**

HBV and HIV medications were reviewed and recorded at each visit. Testing for HIV RNA and CD4 counts was performed at monthly intervals for the first 3 months, then every 2 to 6 months thereafter and whenever clinically indicated. ALT and AST were tested every 2 weeks, then monthly or bimonthly in year 1, every 3–6 months thereafter and as clinically indicated.

Liver biopsies were performed for abnormal liver tests, suspected rejection, any evidence of HBV virological breakthrough or if suspicion of drug hepatotoxicity. Annual liver biopsies were recommended for those with the highest risk of progressive disease post LT, such as patients failing prophylactic therapy (i.e. HBsAg became positive) or patients with hepatitis delta coinfection.

Recurrent HBV infection was defined as reappearance of HBsAg in serum and detectable HBV DNA in serum using sensitive quantitative assays. Serum or plasma samples were collected for HBsAg and HBV DNA testing at the pre-LT screening visit and/or day of LT (day 0) and post-LT on scheduled study visits at, weeks 12, 26, 52 weeks, and then annually in years 2 through 5 or as clinically indicated by abnormal liver enzymes. All archived serum samples were snap frozen in liquid nitrogen and stored at -80° C.

### **Detection of HBV DNA using ultrasensitive real-time quantitative (RT-PCR) in HBV-HIV coinfecting patients**

DNA was extracted from 200- $\mu$ L of serum using QIAGEN MinAmp Virus prep kit (Qiagen Inc. Valencia, CA). For detection of HBV genomes, samples were first assessed by TaqMan real-time PCR on the ABI PRISM 7000 detection system (Applied Biosystems, Foster City, CA) as previously described (18). An 8-member quality control panel of HBV standards (*AcroMetrix, Benicia, CA*), consisting of negative control and serial dilutions of inactivated HBV-infected human serum were used as standards for each TaqMan run. HBV DNA quantification was linear between  $2 \times 10^1$  and  $2 \times 10^7$  IU/mL. Positive and negative samples were confirmed by repeat RT-PCR testing in duplicate or triplicate.

DNA extracted from positive RT-PCR samples was analyzed by standard nested PCR using HBV surface (S) and polymerase (P) gene primers under conditions as described previously (19–21) and direct sequencing of the specific S and P PCR amplicons by autosequencer (Elim bio-pharmaceuticals Inc., Hayward CA). All reactions were carried out in parallel with contamination controls consisting of water added to the cocktail instead of template and HBV DNA positive serum as positive control.

### HBV monoinfected comparator group

As HBV monoinfected patients were not included in the HIVTR Cohort Study, we compared survival and HBV recurrence rates in HBV-HIV coinfecting patients with that in HBV mono-infected subjects undergoing LT at the HIVTR Cohort Study coordinating center (University of California San Francisco). A total of 20 HBsAg-positive, HIV uninfected patients ( $n = 2$  with fulminant HBV and  $n = 18$  with chronic disease) underwent LT during a similar time period (2001–2007) and received a similar protocol of HBV prophylaxis consisting of long-term HBIG and antivirals due to the presence of antiviral resistance pre-LT (70%), or detectable HBV DNA at the time of LT (50%). Surveillance for HBV recurrence was performed per local protocol but measuring HBsAg and anti-HBs titers every month for 1 year, then every 3 months thereafter; HBV DNA levels were obtained at 1, 3, 6 and 12 months post-LT and then every 6–12 months thereafter, or more frequently if suspicion of treatment failure (i.e. HBsAg positivity, ALT elevation). Post-transplant serum samples were not collected at specified time points in the HBV monoinfected control group.

### Statistical methods

Descriptive statistics included median, mean, quartiles and range as appropriate. The primary outcomes of interest were patient and graft survival and HBV recurrence defined by detection of serum HBsAg and HBV DNA. Secondary outcomes included post-LT CD4 cell counts, and frequency and outcome of acute rejection episodes. Cumulative patient and graft survival and rates of HBV recurrence were calculated using Kaplan–Meier methods. The log-rank test was used to compare HBV-HIV coinfecting and HBV monoinfected groups, with a  $p$ -value of  $<0.05$  considered statistically significant. Due to the limited number of outcomes, multivariate analysis of predictors of survival was not feasible.

## Results

The study cohorts reflect liver transplants performed between 2001 and 2007. The baseline characteristics of the 22 HBV/HIV coinfecting patients and 20 HBV monoinfected patients are shown in Table 1. Differences in baseline characteristics between the coinfecting and monoinfected recipients included the proportion of males (100% vs. 65%), median age (47.0 vs. 57.7 years) and proportion transplanted for HCC as primary indication (9% vs. 25%). Donor characteristics were similar, except HBV-HIV infected patients had younger donors (median 39 years vs. 51 years in HBV monoinfected patients). Immunosuppressive treatment at initial discharge and last follow-up differed between coinfecting and monoinfected cohorts, with a higher proportion of coinfecting patients were receiving cyclosporine at discharge (59% vs. 5%) and at last follow-up (27% vs. 15%) and a lower proportion receiving mycophenolate mofetil (91% vs. 100% at discharge and 68% vs. 90% at last follow-up).

**Table 1:** Characteristics of HBV/HIV coinfecting and HBV monoinfected patients

	HBV-HIV Coinfecting N = 22	HBV Monoinfected N = 20
Baseline characteristics		
Recipient age, median (range) years	47.0 (30–71)	57.7 (39–69)
% Male	100.0% (22/22)	65.0% (13/20)
% Hepatocellular carcinoma	9.1% (2/22)	25 (5/20)
% Lamivudine resistance <sup>1</sup>	72.7% (16/22)	70.0% (14/20)
% Detectable HBV DNA pre-LT <sup>2</sup>	45.4% (10/22)	50.0% (10/20)
Laboratory MELD at LT, median (range)	22.0 (10–51)	24.5 (7–56)
CD4 cells/mm <sup>3</sup> , median (range)		
Immediately pre-LT	317 (38–1070)	N/A
3–6 months post-LT (N = 18)	289 (48–744)	N/A
Donor characteristics		
Donor age, median (range) years	39 (13–69)	51 (17–77)
DCD donor, N (%)	1 (4.5%)	0 (0%)
Living donor, N (%)	2 (9%)	2 (10%)
Anti-HBc positive, N = 19 (%)	3 (15.8%)	3 (15%)
Post-Transplant characteristics		
Immunosuppression (at initial discharge)		
Cyclosporine, N (%)	13 (59)	1 (5)
Tacrolimus, N (%)	8 (36)	19 (95)
Sirolimus, N (%)	1 (5)	0
Mycophenolate mofetil, N (%)	20 (91)	20 (100)
Prednisone, N (%)	20 (91)	20 (100)
Anti-CD25 antibody induction, N (%)	1 (5)	0
Thymoglobulin induction, N (%)	0	4 (20)
Immunosuppression (at last follow-up)		
Cyclosporine, N (%)	6 (27.2)	3 (15)
Tacrolimus, N (%)	9 (40.9)	12 (60)
Sirolimus, N (%)	4 (18)	3 (15)
Mycophenolate mofetil, N (%)	15 (68.1)	18 (90)
Prednisone, N (%)	4 (18.1)	5 (25)
% With acute rejection	22.7	10.0
Median (range) follow-up post-LT month	42 (0.6–84)	48 (23–93)

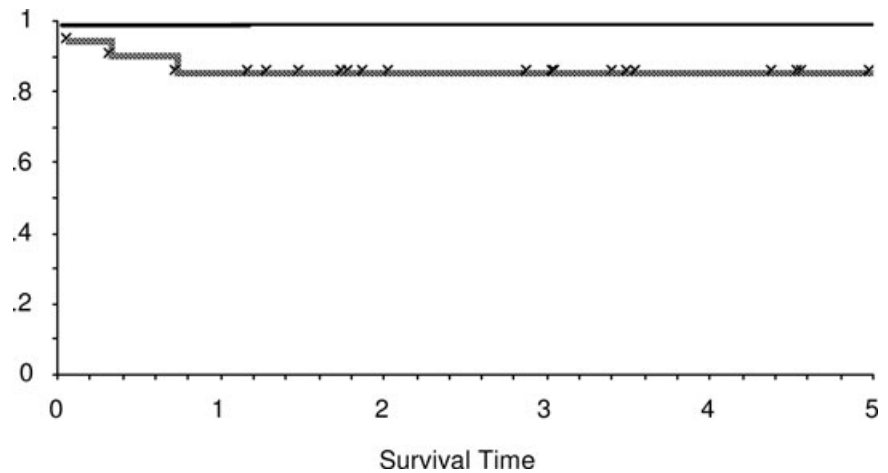
<sup>1</sup> Documented lamivudine resistance or on antiviral therapy appropriate for lamivudine resistant HBV.

<sup>2</sup> Determined at local laboratories using standard clinical assays with varying sensitivity (40–10<sup>4</sup> IU/mL).

### Patient and graft survival

The median follow-up in the coinfecting patients was 3.5 years and in the monoinfected patients was 4.0 years. The cumulative patient and graft survival at 1 and 3 years

**Figure 1: Cumulative patient survival, comparing HBV-HIV coinfecting (N = 22) and HBV monoinfected (N = 20) transplant recipients.** No significant differences were observed in patient survival between groups ( $p = 0.09$ , log-rank test).



in the HBV-HIV coinfecting patients was 85% compared with 100% in the HBV monoinfected group ( $p = 0.08$ , log-rank test) (Figure 1). Patient and graft survival results were unchanged by exclusion of the 2 patients with HCV coinfection.

The three deaths in the HBV-HIV coinfecting group all occurred in the first year post transplantation and were unrelated to HBV recurrence or AIDS-defining opportunistic complications. Causes of death were metastatic cholangiocarcinoma (diagnosed in the explanted liver) at 264 days post-LT, overwhelming sepsis at 17 days post-LT, and sudden death at home 116 days post-LT of unknown cause (autopsy not obtained). The patient dying of sepsis on post-operative day 17 had received induction therapy with basiliximab (days 10, 14), started on mycophenolate mofetil on the day of transplant, and tacrolimus and prednisone at day 5. The patient's CD4 T cell count was 316 cells/mm<sup>3</sup> immediately before transplantation and follow-up CD4 T cell counts not available. The source of the infection was unknown.

### Recurrent disease

None of the patients in the coinfecting or monoinfected groups developed clinical or serologic evidence of recurrent HBV infection. One coinfecting patient with a transiently positive HBV DNA (0.892 mEq/mL =  $\sim 10^3$  IU/mL) on LMV and HBIG, had tenofovir added to the antiviral therapy and all subsequent serum HBV DNA results were undetectable.

None of the 12 (55% of total) coinfecting patients with liver biopsies available within the first year post-LT had histological evidence of recurrent HBV. Of the two HBV-HIV coinfecting patients with HCV coinfection, one patient had fluctuating liver enzymes levels (AST 32–366 U/L) and a liver biopsy at 10 months posttransplant showed histological features consistent with recurrent HCV disease and stage 2 fibrosis. A second patient with minimally elevated AST (17–70 U/L) had a liver biopsy at 3 months post-LT

revealing recurrent HCV with moderate fibrosis. Both patients with triple viral infection (HBV, HCV and HIV) are alive with functioning grafts. Two HBV-HIV coinfecting patients required LT for hepatocellular carcinoma and there are no cases of recurrent HCC with follow-up of 54.6 and 3.8 months. The latter patient died suddenly of unknown causes.

Thirteen HBV-HIV coinfecting patients had at least one post-LT serum available for HBV DNA testing by RT-PCR. Of these, 54% (7/13) tested positive for HBV DNA, with HBV DNA intermittently positive (none persistently detectable) with a median level of 108 IU/mL (range 9.1–789 IU/mL) (Table 2). Baseline and posttransplant characteristics of the six coinfecting patients who remained persistently HBV DNA negative by RT-PCR during follow-up versus the seven coinfecting patients with intermittently positive HBV DNA are shown in Table 3.

Sequence analysis of the S (surface) gene was possible in five of seven patients with HBV DNA detectable and in two patients polymerase sequencing could be obtained. Two cases showed the rtL180V/I mutation associated with LMV resistance and this mutation was present in the pre-LT serum of both patients. No other known or novel surface and/or polymerase mutations were identified.

### Safety and tolerability of HBV prophylaxis in coinfecting patients

Per study protocol, all coinfecting patients received HBIG in combination with antiviral therapy on an indefinite basis. The specific antiviral drugs used were LMV ( $n = 1$ ), LMV and adefovir ( $n = 2$ ); LMV and tenofovir ( $n = 5$ ), and emtricitabine and tenofovir ( $n = 14$ ). Ten patients initially on LMV plus tenofovir or LMV plus adefovir were switched to emtricitabine plus tenofovir. Prophylactic therapy was well tolerated. In one patient, HBV antiviral therapy was held at 5 months post-LT for 1 week; and at 6 months post-LT, both HIV and HBV antiretroviral therapy were held for 2 weeks, during which time HBIG monotherapy was maintained. Another patient developed presumed tenofovir-associated

**Table 2:** HBV DNA detection in serum using real-time PCR in HIV/HBV coinfecting cohort

Case	Pre-LT LMVr <sup>1</sup>	Pre-LT sera <sup>2</sup>	Wk 12	Wk 26	Wk 52	Y2	HBV DNA Detected	HBV-S (surface) and/or HBV-P (polymerase) sequence
1	Yes	—	—	—	—	—	No	n/a
2	Yes	+	—	—	—	—	No	n/a
3	No	+	—	—	—	+	Yes	No
4	Yes	—	—	—	—	n/a <sup>3</sup>	No	n/a
5	No	—	+	—	n/a	n/a	Yes	S
6	Yes	+	+	—	+	—	Yes	S & P
7	No	—	+	n/a	—	—	Yes	S
8	Yes	+	+	n/a	+	—	Yes	S
9	Yes	—	—	—	n/a	n/a	No	n/a
10	Yes	—	n/a	n/a	n/a	n/a	n/a	n/a
11	Yes	+ (serum) + (ex-plant)	—	n/a	n/a	n/a	No	n/a
12	Yes	+	+	—	n/a	n/a	Yes	S & P
13	Yes	+	+	—	n/a	n/a	Yes	No
14	No	+	n/a	n/a	n/a	n/a	n/a	n/a
15	Yes	+	n/a	n/a	n/a	n/a	n/a	n/a
16	No	—	—	n/a	n/a	n/a	No	n/a

<sup>1</sup> Documented LMV resistance or on antiviral therapy for LMV resistant mutants.

<sup>2</sup> Determined by quantitative real-time PCR with S gene specific primers.

<sup>3</sup> n/a = no sera available for testing.

nephrotoxicity at 39 months post-LT and renal biopsy confirmed interstitial nephritis. At last follow-up his creatinine was stable at 2.7 mg/dL (238 micromole/L). Since this patient had prior LMV resistance and could not be treated

with tenofovir or adefovir, HBIG was used for HBV prophylaxis, though the patient remains on LMV as part of his antiretroviral regimen and HBsAg and HBV DNA remain undetectable (sensitivity <100 copies/mL). All other coinfecting patients had stable or improved renal function post-transplantation. No patient required dose reduction or discontinuation of HBIG due to adverse effects.

**Table 3:** HBV/HIV coinfecting transplant recipients with and without detectable HBV viremia

	Intermittently HBV DNA Positive N = 7 patients	Persistently HBV DNA Negative N = 6 patients
Median age at LT (years)	42.5	44.5
Median CD4 count at time of first detectable HBV DNA viremia (range)	156 (65–392)	348 (119–1092)
Median CD4 count at time of LT (range)	313 (148–527)	336 (128–1070)
Detectable HBV DNA Pre-LT*	71% (5/7)	16% (1/6)
% (N) LMV-Resistant Pre-LT	57% (4/7)	83% (5/6)
% (N) Combination antiviral Post-LT	100% (7/7)	83% (5/6)
Median anti-HBs Titers (IU/L) (range)	223 (27.9–1795)	492 (142–868)
% (N) Prior treated acute rejection	43% (3/7)	16% (1/6)

\*Variable sensitivity by quantitative assays (50–10<sup>4</sup> IU/mL).

### Other posttransplant outcomes

There were 5 cases of acute rejection in the HBV/HIV coinfecting patients (23%) at 1.5, 4, 6, 18 (n = 2) months post-LT. The cyclosporine levels at the time of ACR were 196, 300, and 244 µg/L in 3 cases. Two cases were on either sirolimus or tacrolimus and trough levels were 4.3 ng/mL and 4.8 ng/dL respectively. Details of each patient's rejection episode including treatment and outcome are outlined in Table 4. In comparing coinfecting patients with treated acute rejection (N = 5) to nonrejectors (N = 17), at the time of initial discharge posttransplantation, 88% (15/17) of patients with no history of rejection were on triple immunosuppressive therapy whereas all five patients with acute rejection were on two immunosuppressive drugs. The median CD4 count at the time of rejection was 134 (range 55–216). Another patient, with HCV/HBV and HIV coinfection, was diagnosed with chronic rejection at 16 months post-LT. Only 1 patient in the monoinfected HBV group (5%) had acute rejection at 12.2 months post-LT, believed to be due to noncompliance and treated with corticosteroids (pulse doses of methylprednisolone and recycling of prednisone).

**Table 4:** Acute rejection episodes in HBV-HIV coinfecting patients

ID	Time to acute rejection (mos)	IMS at time of acute rejection	Drug level at time of acute rejection	CD4 T-cell count prior to AR (cells/mm <sup>3</sup> )	Treatment given	Outcome
1	1.5	Cyclosporine Mycophenolate mofetil	244 ug/L	86	Pulse steroids (failed), OKT3	Resolved
2	4	Cyclosporine Prednisone	196 ug/L	149	Steroid bolus Increased CSA	Resolved
3	6	Sirolimus Prednisone	4.3 ng/mL	55	Pulse steroids	Resolved
4	18	Cyclosporine Mycophenolate mofetil	200 ug/L	216	Oral steroid taper	Resolved
5	18	Tacrolimus Mycophenolate mofetil	4.8 ng/dL	134	Increased tacrolimus dose (HCV coinfecting)	Resolved

**Discussion**

We have shown that LT in HBV-HIV coinfecting persons achieves excellent outcomes with 85% patient and graft survival after median 42 months of follow-up, and with no patient requiring retransplantation for graft loss. Moreover, clinically apparent recurrent HBV disease was successfully prevented in all patients. These results confirm and extend the results of other smaller studies (4–8 HBV-HIV coinfecting subjects each) (5,7,9,15,22–24) and a larger (N = 13) French series (11), reporting 100% patient and graft survival and 0% recurrent HBV disease. Importantly, we have also shown patients that have detectable HBV DNA at the time of transplantation can achieve excellent outcomes without recurrent HBV disease when a high dose HBIG plus antiviral prophylaxis strategy is used. On the basis of these results, we do not feel that there is a requirement for HBV-HIV infected patients to have an undetectable HBV DNA level to be considered transplant candidates.

Recurrent HBV infection post-LT is defined by the presence of hepatitis B surface antigen, absence of anti-HBs antibodies and measurable serum HBV DNA levels using standard clinical assays (25,26). We hypothesized that HIV infected patients with HBV coinfection may be at higher risk of recurrence post-LT due to the frequency of LMV-resistance among waitlisted patients and frequent presence of detectable HBV DNA at the time of LT. However, using a combination prophylaxis approach of HBIG plus antiviral therapy, HBV recurrence was prevented during a follow-up period of up to nearly 7 years. Almost all of our patients (21/22, 95%) were on a combination of two oral anti-HBV antivirals as well as HBIG. Whether combination antiviral therapy rather than one potent antiviral drug, such as entecavir or tenofovir, are required when used in combination with HBIG is unknown. However, given the potential serious consequence of recurrent disease in the graft, a regimen of two antivirals with complementary resistance profiles may be better than a single drug. Additionally, we believe HBIG, which acts by an entirely different mechanism to prevent HBV recurrence, is also an impor-

tant component of effective prophylaxis in these patients. Of course, this high-dose HBIG regimen is expensive and strategies to reduce the cost of prophylaxis by using lower doses of HBIG and intramuscular rather than intravenous administration need to be considered. Regardless, we believe that prophylactic therapy using combination antiviral therapy and HBIG is the optimal long-term method to prevent recurrent HBV infection.

Occult HBV infection has been reported in HBV mono-infected patients post-LT (14,27,28) as well as in non-LT patients living with HIV (29–33). We found that 54% of transplant recipients, with post-LT sera available, had intermittently detectable HBV viremia with absence of HBsAg and presence of anti-HBs. This suggests that HBV replication is occurring despite an aggressive prophylaxis regimen, albeit at low levels. The clinical consequences of occult HBV infection are unclear. None of the coinfecting patients with occult HBV infection had any evidence of hepatitis. In contrast to our results regarding occult HBV infection, Tateo and colleagues tested coinfecting liver transplant recipients and found 0/13 had detectable HBV DNA in serum or 0/9 had cccDNA in liver (11). The reason for the very different results may be related to the virologic status of the patients at the time of LT. In the French series, all coinfecting patients had undetectable HBV DNA levels at the time of transplantation, whereas in our cohort this was not a requirement. When we compared those patients in our cohort with intermittently detectable HBV DNA to those who were persistently HBV DNA negative, occult HBV infection tended to be more common in those with lower CD4 cell counts at time of viremic episode and detectable HBV DNA at time of transplantation, lower median levels of anti-HBs on HBIG therapy, and a history of treated acute rejection. An ineffective cytotoxic T-lymphocyte response and lower titers of neutralizing anti-HBs antibodies have been linked to persistent occult HBV infection in nontransplant patients (34,35). Coinfecting patients who are viremic at transplantation and with less than ideal immune protection may be at greatest risk for reinfection that is manifested by intermittently positive HBV DNA in serum on prophylaxis.

The HBV genome is known to have a glucocorticoid responsive element (36). Thus, treatment of acute rejection requiring pulse steroids could explain the detection of occult HBV in some patients. This has been suggested by other studies documenting occult HBV reactivation in HBV mono-infected liver transplant recipients who received high-dose steroid therapy (27). Finally, some authors has suggested occult HBV in HIV-infected carriers could be explained by mutations within HBsAg 'a' determinant that potentially interferes with the recognition of HBsAg (33). In our study, sequencing analysis was possible only in five of seven cases with occult HBV, due to the low-level plasma DNA, but no novel surface gene escape mutants were identified.

Limitations of this study include the lack of HBV DNA testing by rtPCR in HBV mono-infected control group and the use of a very sensitive detection method that prevents comparisons with previously published studies regarding occult HBV infection (37). Additionally, the control group was obtained from a single center rather than all the centers participating in the multicenter study. However, over 50% of the coinfecting patients came from the same center as the controls. Importantly, the controls received similar HBV prophylaxis as the coinfecting patients. It is of interest that at the time of LT, approximately half the subjects in each group had detectable HBV DNA by standard PCR methods, the risk factor most consistently associated risk with HBV recurrence (26). It should be acknowledged that it is possible, although unproven, that other differences in the baseline characteristics between mono-infected and dual infected recipients may also have contributed to differences in survival and risk of recurrence. Unfortunately, the limited numbers of outcomes prevented evaluation of predictors of survival using multivariate analysis.

In summary, the outcomes in HBV-HIV coinfecting patients are excellent and support the use of LT for complications of cirrhosis. Prophylaxis with combination HBIG and antiviral therapy is highly effective in preventing clinical disease, even in those with HBV DNA detectable at the time of transplantation, and we believe this represents the best strategy for prevention of recurrent HBV infection in this population. The high frequency of intermittent low-level HBV viremia emphasizes the need for life-long HBV prophylaxis to prevent recurrence of HBV infection.

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