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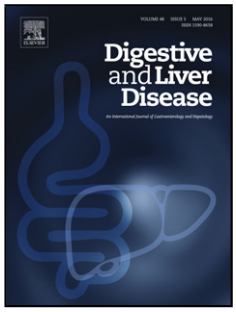

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Entecavir Or Tenofovir Monotherapy Prevents HBV Recurrence In Liver Transplant Recipients: A 5-Year Follow-Up Study After Hepatitis B Immunoglobulin Withdrawal

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Abstract: Background: Recent data suggest that oral third-generation nucleos(t)ide analogs (NA) monoprophylaxis following hepatitis B immunoglobulin (HBIg) withdrawal may be effective to prevent HBV reinfection after liver transplantation (LT). Patients and Methods: Between 01/2010 and 03/2012, all HBV monoinfected and HBV/HDV co-infected LT patients followed in our centre withdrew HBIg±NA and were commenced on either ETV or TDF as monotherapy. Results: Seventy-seven patients were included in the study (55% TDF, 45% ETV). Group A comprised 69 HBV monoinfected patients and Group B 8 HBV/HDV co-infected patients. After HBIg withdrawal, Group A and B patients were followed for 69 (range 13-83) months and 61 (range 31-78) months, respectively. No Group B patients had HBsAg or HBV DNA recurrence, while 6 (9%) Group A patients became HBsAg-positive after a median of 18 (range 1-40) months. The cumulative 5-year incidence of HBsAg recurrence was 9%. All 6 patients demonstrated undetectable HBV-DNA levels and stable graft function during 30 months of additional follow-up. In 3/6

patients, seroconversion was transitory, while the remaining 3 showed HBsAg levels <0.13 IU/mL over the entire period of observation. Pre-LT HCC emerged as the strongest predictor of HBsAg recurrence. Conclusion: HBIG can be safely discontinued in HBsAgpositive LT recipients and replaced by ETV or TDF monotherapy.

Abbreviatons

AASLD, American association for the study of liver diseases;

ACLF, acute on chronic liver failure;

ADV, adefovir;

AFP, alpha-fetoprotein;

ALF, acute liver failure;

APASL, Asian Pacific association for the study of liver;

CI, confidence interval;

CT, triphasic contrast-enhanced computerized tomography;

EASL, European association for the study of the liver;

eGFR, estimated glomerular filtration rate

ESLD, end stage liver disease;

ETV, entecavir;

HBcAb, hepatitis B core antibody;

HBeAb, hepatitis B e antibody;

HBeAg, hepatitis B e antigen;

HBIg, hepatitis B immunoglobulin;

HbsAb, hepatitis B surface antibody;

HBsAg, hepatitis B surface antigen;

HBV, hepatitis B virus;

HCC, hepatocellular carcinoma;

HCV, hepatitis C virus;

HDV, hepatitis Delta virus;

HR, hazard ratio;

IU/mL, International units per millilitre;

IV, intravenous;

LAM, lamivudine;
LLOD, lower limit of detection;
LT, liver transplantation;
MELD, model for end-stage liver disease;
NA, nucleos(t)ide analogue;
qHBsAg, quantitative HBsAg
TDF, tenofovir;
US, ultrasounds.

Keywords: Hepatocellular carcinoma, HBsAg titre, HBV DNA level, antiviral drug-resistance mutation, safety.

Introduction

Hepatitis B virus (HBV) infection still represents a global major health problem associated with significant morbidity and mortality[1]. The highest rates of chronic infection are found in sub-Saharan Africa, East Asia (5–10% of the adult population), southern parts of Eastern/Central Europe, the Middle East and the Indian subcontinent (2–5%). However, significant immigration flows have resulted in higher rates of hepatitis B diagnosis in North-Western Europe and North America, where historically less than 1% of the native population is chronically infected[1]. Consequently, HBV-associated liver disease has represented an important indication for liver transplantation (LT) (5-10%) in Europe and the United States over the last 20 years[2].

Until recent years, long-term prophylaxis combining hepatitis B immunoglobulin (HBIg) and nucleos(t)ide analogues (NAs), was considered the standard of care to prevent HBV recurrence after LT. Several studies and meta-analyses have confirmed their synergistic effect in obtaining long-term HBsAg negativity in more than 90% of HBV liver recipients[3-5].

The availability of third-generation NAs, entecavir (ETV) and tenofovir (TDF), which provides a more potent viral suppression and higher genetic barrier to resistance compared to previous NAs (lamivudine, adefovir, telbivudine, emtricitabine), led to the proposal of alternative strategies for prophylaxis against HBV

reinfection of the allograft after LT, with the aim of eliminating the need for long-term HBIg administration[6-14]. The majority of the published data report that HBIg-free treatment regimens have promising results with rates of HBV recurrence similar to those achieved with combination prophylaxis. However, most of the studies had significant limitations including small patient cohorts, inclusion of selected patients (e.g. low-risk subjects) only, short periods of follow-up, a variety of different antiviral approaches after HBIg discontinuation and a lack of information on the recipients' pre-LT virological profile (e.g. virus genotype, drug-resistance mutations, HBsAg titre). Furthermore, only a minority recorded quantitative HBsAg (qHBsAg) kinetics after recurrence, which could prove useful to predict post-LT outcomes.

Nevertheless, these early reports encouraged the American (AASLD), European (EASL) and Asian Pacific (APASL) Association for the Study of the Liver to incorporate the option of HBIg withdrawal into their clinical guidelines for recipients considered at low-risk of HBV recurrence (i.e. patients with low or undetectable HBV DNA levels before LT and no history of drug-resistant HBV)[15-17].

In this study, we therefore aimed to investigate the long-term effectiveness and safety of third-generation NA monoprophylaxis in preventing HBV reinfection after HBIg withdrawal in stable LT recipients, and to characterize patients' clinical and virological indices (e.g. qHBsAg) after HBsAg recurrence.

Patients and Methods

Study design. Single-centre cohort study of a series of transplanted patients HBsAg-positive at the time of LT, who, following a period of a standard protocol of immunoprophylaxis with HBIg±NAs, were withdrawn from HBIg and simultaneously commenced on either ETV or TDF monotherapy.

Endpoints. The primary endpoint was HBsAg recurrence. Secondary objectives were to identify: rates of recurrence of HBV infection (defined as the reappearance of a detectable serum load of both HBsAg and HBV DNA); the development of HBV-related disease (defined as HBV reinfection associated with a simultaneous increase of transaminases or impairment of liver synthetic parameters); patient's safety (defined organ/systemic toxicity and/or side effects) and changes in renal function; overall survival (OS).

Selection criteria. All LT recipients >18 years of age were included in the analysis if they had received at least 6 months of HBIg prophylaxis, were HBsAg and HBeAg negative and had an undetectable HBV DNA at the time of enrolment.

Studied population. A retrospective review of all the patients who underwent LT between October 1991 and September 2011 was conducted to identify all HBsAg-positive recipients at the time of transplant. Patients with co-existant HDV, hepatitis C virus (HCV) or human immunodeficiency virus (HIV) infections were included.

The initial cohort comprised 175 HBV or HDV transplanted patients. Supplementary Figure 1 and Figure 1 show the flow-chart leading to the 77 patients who were reviewed in the outpatient liver clinic of King's College Hospital, London, between January 2010 and March 2012, and included in the study population. Patients were analysed as separate subgroups according to the pre-transplant type of infection (Figure 1): i) HBV monoinfected patients (Group A) and ii) HBV/HDV co-infected patients (Group B).

Informed consent was obtained from all the patients at the time of HBIg discontinuation. This study was approved by the Institutional Review Boards.

Preliminary investigations. A detailed medical history and laboratory data including routine biochemistry and a comprehensive HBV and HDV virological profile was recorded for all patients at the time of LT and prior to HBIg discontinuation. Additional Supporting Information on laboratory methods and techniques may be found in the online version of this article. Assessment of glomerular function was based on estimated glomerular filtration rate (eGFR) according to the Modification of Diet in Renal Disease (MDRD) study equation, while serum phosphate levels were adopted to estimate the tubular function.

Immunoprophylaxis protocol with HBIg. Since October 1991 all the HBsAg positive transplanted patients received HBIg, as monotherapy until 1997 and in combination with at least one NA, thereafter. Antiviral drugs included lamivudine (LAM) since 1997, adefovir (ADV) since 2003, ETV since 2008 and TDF since 2009. Reflecting the evolution of therapy options for HBV. Supplementary Table 1 shows the fixed dosing protocol of HBIg administration during and after LT, according to different time periods.

Immunoprophylaxis protocol following HBIg withdrawal. Patients who prior to HBIg discontinuation were treated with LAM and those who were not receiving any NA were switched either to ETV or to TDF,

according to physician assessment. The group who received prophylaxis with LAM + ADV/TDF continued with TDF alone. For patients on ETV or TDF prior to HBIg withdrawal, no changes to treatment were made. ETV and TDF were given at a standard dosage, adjusted to renal function.

Assessment of outcome and follow-up. After HBIg withdrawal, patients were followed up at monthly intervals for the first 3 months and every 3-6 months thereafter, as clinically indicated, until January 2017. Clinical examination, standard biochemical tests for liver and renal function, HBV serological markers and HBV DNA were performed at each follow-up visit in both Groups, while HDV-RNA was performed in Group B only. For patients with recurrent HBsAg, a quantification was performed. Thereafter, qHBsAg, HBV DNA and anti-HDV IgG/IgM (to exclude a HDV superinfection) were monitored every 3-6 months.

Patients with a pre-LT history of HCC and those with HBsAg recurrence after LT underwent ~~regular~~ 6-12 months surveillance for HCC with CT-scan and US examinations, respectively.

Change in renal function was assessed excluding from the analysis all patients who were already on ETV or TDF therapy before HBIg discontinuation.

Patients' drug compliance was monitored by expert transplant coordinators at each follow-up visit.

Statistical analysis. Continuous variables were expressed as median (ranges) and analyzed using the Mann-Whitney U-test for unpaired samples and the Wilcoxon sign-rank test or the Friedman test for paired samples. Categorical variables were presented as number and percentage and compared by the Fisher exact test or the Yates' $\chi 2$ test when appropriate. A p-value <0.05 was considered statistically significant. Univariate Cox regression analysis was performed for all the demographic, at transplant and at HBIg withdrawal variables to identify factors associated with HBsAg recurrence. HBV-recurrence and survival cumulative probability curves were computed according to the Kaplan-Meier method and compared by the log-rank test. Statistical analysis was performed with the SPSS 24.0 statistical package (IBM).

Results

Population. Seventy-seven transplanted patients were included in the study. The cohort comprised 69 HBV (Group A) and 8 HBV/HDV co-infected (Group B) patients on HBIg treatment for at least 6 months, who between January 2010 and March 2012, discontinued HBIg and commenced a monoprophylaxis with ETV (46%) or TDF (54%). Patients' main characteristics and laboratory findings are summarised in Table 1. Supplementary Table 2 shows patients' additional features. Tumour staging, based on the histo-pathological report of the explanted liver, demonstrated that 22/29 (73%) of patients met the Milan criteria.

Follow-up after HBIg withadrawal. The median follow-up time after HBIg withdrawal was 69 (range, 13−83) months for Group A and 61 (range 31−78) months for Group B. Fifty-nine (86%) patients of Group A and 7 (88%) of Group B had a follow-up longer than 5 years.

Following HBIg withdrawal, 70 patients from the entire cohort showed a progressive decrease of HBsAb serum titres, which reached levels below 10 mIU/mL after a median time of 4 (range 1−17) months. The remaining 7 patients, 6 (9%) of Group A and one (13%) of Group B, maintained significant levels of HBsAb. One patient from Group A had HBsAb titre >1000 mIU/mL over the entire period of follow-up, while the remaining five showed fluctuating levels between 50 and 200 mIU/mL.

Of the 29 patients with HCC at transplant, none developed tumour recurrence.

Recurrence of HBV. No HBV reinfection (i.e. both HBsAg and HBV DNA detectable serum load) was recorded in the entire cohort. HBsAg recurrence occurred in 6 (9%) patients in Group A. No patient in Group B had HBsAg relapse. The Group A actuarial probability of recurrence is reported in Figure 2a. The cumulative 1−, 3− and 5−year recurrence incidence was 1%, 7% and 9%, respectively. The median time of recurrence from HBIg discontinuation was 18 (1−40) months, with 5 out of 6 relapses occurring within the third year and none after 40 months (Figure 2a). At the time of recurrence, serum HBsAg and HBsAb titres had a median level of 0.06 (range 0.05−0.08) IU/mL and 0.25 (range 0.01−35.2) mIU/ml, respectively. Five of 6 patients had an HBsAb titre <0.5 mIU/ml. The only patient with a significant titre (35.2 mUI/mL) corresponded to the patient who had recurrence 1 month after HBIg withdrawal. In all the 6 patients with recurrence, HBeAg was negative and HBV DNA undetectable. Liver biochemistry and hepatic synthetic parameters were normal. None of the 3 patients with a pre-LT drug-resistant mutation had HBsAg

recurrence. No changes were made in the therapy regimen after HBsAg recurrence, since re-introducing HBIg at that point was not considered to be of clinical benefit.

Follow-up of patients with HbsAg recurrence. Figure 3 shows HbsAg titre kinetics for the 6 patients who became HBsAg-positive. The median follow-up time from HBsAg recurrence was 30 (range 18−60) months. In 3/6 patients, seroconversion was transitory, since they cleared HBsAg 6, 9 and 30 months after recurrence. The remaining 3 patients, while maintaining HBsAg positivity, showed very low titres (<0.13 IU/mL) over the entire period of observation. During the complete follow-up duration, HbeAg remained negative and HBV DNA undetectable. Liver enzymes and hepatic synthetic markers maintained normal levels. No patients developed HCC, HDV superinfection or any liver-related complication.

At the time of the last visit, a total of 66 patients tested negative for HBsAg, resulting in a prevalence of 96%.

Predictors of post-LT HBsAg recurrence. Table 2 summarises the main characteristics of the 6 patients who had HBsAg recurrence. Notably, all 6 patients had HCC diagnosed prior to LT, only 3 (50%) had a detectable HBV DNA at the time of transplantation (none with a level above 2000 IU/mL) and a single patient was transplanted in the 12 months prior to seroconversion. Four patients out of 6 who relapsed had mutational analysis performed before LT and none were found to have antiviral-resistant mutations. In Table 3 we report a comparison of patients' characteristics according to HBsAg recurrence. Patients with relapse were older at the time of transplant (median age of 63 vs 52, p=0.0095), more frequently of East-Asian ethnicity (50% vs 14%, p=0.047) and had a higher proportion of pre-LT HCC compared with those who did not relapse (100% vs 37%, p=0.0039). By univariate Cox regression analysis, age at transplant and East-Asian ethnicity were confirmed as prognostic factors associated with HBsAg recurrence. The same statistical method couldn't be applied for some independent variables, including HCC (Table 4). Notably, using either the Mann-Whitney U-test or the Univariate Cox regression analysis, no correlation between the length of HBIg treatment and the rates of HBsAg recurrence was found (Table 3 and 4). Similarly, there was no relationship between the type or the level of immunospppresion, the number or the severity of rejections and HBsAg recurrence [data not shown].

Figure 2 shows the cumulative probability curves according to age at transplant, split in tertiles, East-Asian ethnicity and presence of a pre-LT HCC (Figure 2b, 2c and 2d, respectively). No other variables analysed comparing actuarial probability curves by the log-rank test were associated with HBsAg recurrence (Supplementary Table 3).

Safety and renal function changes. No treatment-related major adverse events or clinically relevant side effects were recorded during the study. Over the 5−year observation period no significant changes in the eGFR were observed in both ETV and TDF subgroups, while a reduction in phosphate levels was recorded in patients who switched from LAM±ADV to TDF (Figure 4), including 10 (36%) patients who showed abnormal levels at 5−year follow-up. Thirteen patients, 5 belonging to the ETV subgroup and 8 to the TDF subgroup, required a dose reduction, due to a decrease of eGFR below 60 mL/min. Notably, all of them had an eGFR lower than 80 mL/min before HBIg withdrawal and 10/13 were affected by other conditions associated with kidney impairment including diabetes (no.=4) and hypertension (no.=8).

Survival. By the end of the study, a total of 4 patients, all of Group A, died, after 34, 50, 56 and 71 months since HBIg withdrawal. The cause of death was bacterial infections (one pneumonia and one sepsis) for two patients, while the remaining 2 died for ESLD as a consequence of HCV recurrence and for acute coronary syndrome, respectively. Six patients, 5 of Group A and one of Group B, were lost to follow-up within the third year. One−, 3− and 6−year survival probabilities were 100%, 99% and 94%, with a mean (median not reached) survival time of 83 (95% CI: 81−85) months (Supplementary Fig 2). Two patients, one of Group A and one of Group B, were re-transplanted 38 and 14 months after HBIg withdrawal respectively, due to chronic rejection and hepatic artery thrombosis.

Discussion

In recent years, various protocols utilising ETV or TDF, after a finite period of HBIg administration, have been investigated[6-14]. However, available data are not strong enough to definitively support a monoprophylaxis with third-generation NA, mainly because they derive from studies with a small sample size and/or short period of follow-up. More recently, Fung et al. demonstrated in a large study with a long

follow up period that a different strategy utilising pre-transplant and post-transplant entecavir monotherapy without any HBIg administration, can successfully prevent recurrent HBV infection post-LT[18].

In the current study, we describe the risk of HBV reinfection in 77 HBsAg-positive LT recipients who were commenced on ETV or TDF monotherapy following HBIg withdrawal and then prospectively observed over a median period of almost 6 years. During a median follow-up of 69 months, the rate of HBsAg and HBV DNA recurrence in the HBV group was 9% and 0% respectively, whereas no HBV/HDV co-infected recipients had reinfection. Our findings confirm results from previous studies, which showed HBsAg recurrence rates of ≤10%[6-14, 19].

In our series, 3 of the 6 patients who experienced HBsAg reappearance showed a transitory seroconversion in that they cleared HBsAg within 30 months. These results are confirmatory of prior studies showing HBsAg clearance in 50-100% of patients within 2 years since HBsAg recurrence.[7, 8, 14] Furthermore, ~~for the first time~~ our study described HBsAg titre kinetics after recurrence. ~~Importantly~~ Similar to what has been described by Fung et al.in a previous paper[20], HBsAg titres appeared to be very low (<0.27 IU/mL) over the entire period of observation, both for patients who eventually cleared HBsAg and for those who maintained positive levels. In patients with undetectable HBV DNA, HBsAg quantification could assume a unique meaning and prove to be prognostically useful as has been shown in other settings, such as in non-transplanted low-viraemic HBsAg-carriers[21], where a direct correlation between HBsAg level and risk of HCC development has recently been described.[22]

In our study, no patients who had HBsAg seroconversion developed any clinical or biochemical manifestations of hepatitis B over the entire follow-up period. In addition, no HCC or HDV *de-novo* infection was recorded. These findings are consistent with those of previous studies[6-14] and suggest that in the era of third-generation NAs, HBsAg reappearance after LT does not likely represent a reliable marker of HBV-related disease recurrence, but merely a serological finding with no short-term clinical implication. However, longer follow-up studies are required to investigate the relationship between persistent HBsAg detection and the long-term risk of developing HBV-related complications in the context of complete viral suppression. In the non-transplant setting, ETV or TDF were demonstrated to effectively and enduringly prevent the development of virologic breakthrough, hepatitis flares and progression to decompensated cirrhosis, showing negligible rates of drug-resistance. However, several studies reported rates of HCC/year

of 0.5-1.4% for non-cirrhotic HBV patients on treatment with a third-generation NA.[23, 24] Although these data cannot be directly transferred to the post-transplant setting, they suggest that there is a potential residual risk of HCC in HBsAg-positive LT recipients and warrant enrolment of these patients in HCC surveillance programs. Notably, whilst immunosuppressive drug regimens are certainly a predisposing factor for primary malignancies, the very low levels of HBsAg we demonstrated after recurrence may reduce the risk of development of a *de-novo* HCC in HBsAg-positive LT recipients. Moreover, the recent abovementioned study by Fung et al.[18] showed that over a period of 8 years, no cases of HCC occurred after LT in those patients without HCC at the time of transplant. This may be reassuring regarding the potential medium-term risk of HCC in recipients with fluctuating or persistent HBsAg-positive status after LT.

HDV superinfection represents a further complication that can occur in chronic HBV carriers. However, we believe that the very small production of HBsAg could possibly protect LT patients from the development of superimposed HDV disease, being the very low levels of HBsAg insufficient for HDV replication.

A potential explanation of HBsAg reappearance in the absence of detectable HBV DNA could be the production of HBsAg in extrahepatic cells which are partially resistant to NAs. This could occur in the case of extrahepatic HCC micrometastases, as suggested by Saab et al.[25] Our series support this hypothesis in that all the patients who became HBsAg-positive had a pre-LT HCC. This finding is consistent with prior studies using other forms of prophylaxis, which demonstrated that the presence of HCC at LT was independently associated with an increased risk of HBV recurrence post-LT.[25-27]

In the present study, we also found that an older age at transplant and East Asian ethnicity were both risk factors associated with HBsAg recurrence. However, we believe that the correlation observed is the consequence of the well-known strict connection between HCC and these two characteristics.[28, 29] Furthermore, 2 out of the 3 patients of East Asian origin with HBsAg recurrence had HBV genotype C (shown by sequencing performed pre-LT), which is recognized as being an independent predictor of HCC development.[30]

The majority of previous studies using ETV or TDF monoprophylaxis post-LT excluded patients with a detectable HBV DNA at transplantation.[7, 11, 13, 14] In contrast, our cohort comprises almost 2/3 of patients with a pre-LT HBV DNA > 12, including 1/3 with a viral load > 2000 IU/mL. By both the Cox regression analysis and the log-rank test, we did not find a statistically significant correlation between the

viral load and risk of HBsAg recurrence. Our observation is in contrast with several other reports showing that higher HBV DNA levels at transplant were predictors of HBV reinfection.[4, 18, 27, 31] This difference could be explained by the fact that all the studies demonstrating this association evaluated NAs less powerful and with lower genetic barriers to resistance than third-generation NAs[4, 27, 31] or strategies for prophylaxis against HBV reinfection that did not include HBIg.[18] No study has yet confirmed these results when ETV or TDF was used either in combination with HBIg or as monoprophylaxis after HBIg discontinuation.[6, 8, 9, 12] Therefore, the results of our series, along with the lack of any other evidence that correlates HBV DNA levels with HBsAg reappearance after LT when ETV or TDF are included in the treatment strategy, should guide reassessment of criteria to determine a "patient at high risk of reinfection" recently proposed by some authors to select patients who are likely to need an indefinite prophylactic regimen that includes HBIg.[32, 33] These same authors included in the high-risk group patients with pre-existing drug-resistant mutation. We believe there is no evidence supporting this decision when new antivirals with a higher genetic barrier to resistance are used. In our series, none of the patients who were found to have pre-LT antiviral-resistant mutations experienced HBsAg seroconversion. Moreover, none of the studies in which third-generation NAs were included in the therapeutic regimen after HBIg discontinuation, including the present paper, has so far shown the development of any multi-drug resistance which could jeopardise the effectiveness of rescue therapy. All these evidences suggest that monoprophylaxis with third-generation NA can be effective even in patients traditionally considered to be at high risk of HBV reinfection.

Conversely, we believe that a "traditional" approach including HBIg along with a NA in the prophylaxis strategy should be proposed for HBV/HDV co-infected patients. Although in our series none of the patients with HBV/HDV co-infection had HBsAg relapse and promising results have been reported by previous studies[6, 7, 9, 13, 14, 34], concerns remain about applying an HBIg-free protocol to these patients, owing to the lack of an effective rescue therapy in case of HDV recurrence.

In our post-LT population, treatment with third-generation NAs was well tolerated. No significant change in eGFR was observed in both ETV and TDF subgroups and there were no cases of acute kidney failure or severe renal impairment, indicating that the risk of any possible additive third-generation NA-induced renal toxicity doesn't seem to represent an important issue in LT patients.[9, 10, 12-14] The subgroup of patients who switched from LAM±ADV to TDF had a significant decrease in serum phosphate, including a relevant

number of patients (36%) who showed abnormal levels at the time of last follow-up. Unfortunately, bone mineral density was not measured before starting third-generation NAs, so any correlation between the biochemical evidence of a reduction in serum phosphates and its clinical consequences cannot be made. Long-term clinical effects of persistent proximal renal tubulopathy in LT recipients exposed to immunosuppressive drugs remains unknown and requires studies with longer follow-up periods.

However, the availability in the near future of a novel tenofovir prodrug (tenofovir alafenamide) that targets cells more efficiently at a lower dose than TDF[35] will reduce systemic exposure and possibly renal and bone toxicity in LT patients.

Limitations of this study include the heterogeneity of pre-LT and pre-HBIg withdrawal antiviral regimens, including the length of HBIg prophylaxis and the type of antiviral drugs.

Another potential limitation ~~of our study~~ is selection bias in that, of the 175 HBsAg-positive patients transplanted since 1991, 122 were considered for recruitment and eventually 77 enrolled (Supplementary Figure 1). However, only 19 patients were excluded from the analysis due to the development of an HBsAg relapse prior to enrolment. Furthermore, only 5/19 were on an optimal prophylaxis regime (HBIg+LAM) at the time of HBsAg recurrence, including 3 with synchronous HCC and 2 with a known pre-LT LAM-resistant mutation. Thus, we believe any significant selection bias would not have strongly influenced the results.

In conclusion, our study provides strong supportive evidence that a monoprophylaxis strategy with ETV and TDF, following at least 6 month of combination therapy with HBIg+NA, is safe and highly effective in preventing viral replication and/or HBV-related disease in all HBV LT recipients, regardless of any patient or viral characteristics either at the time of transplant or at HBIg withdrawal. However, larger prospective multi-centre studies, with longer follow-up periods, are required to further examine the consequences of HBsAg-positive status in life-long immunosuppressed patients, as well as the safety of third-generation NAs in a post-transplant population.

Disclosure

Matteo A. Manini, Gavin Whitehouse, Matthew Bruce, Matteo Passerini, Tiong Y. Lim, Ivana Carey, Abid Suddle and Nigel Heaton have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

Aisling Considine has received fees from Abbvie and Gilead for presentations at educational meetings.

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Figure Legends

Figure 1 Selection of the 77 HBsAg/HBV DNA negative patients transplanted for HBV-related disease and still on HBIg prophylaxis, who withdrew HBIg and started a monoprophylaxis with a third-generation NA between January 2010 and March 2012.

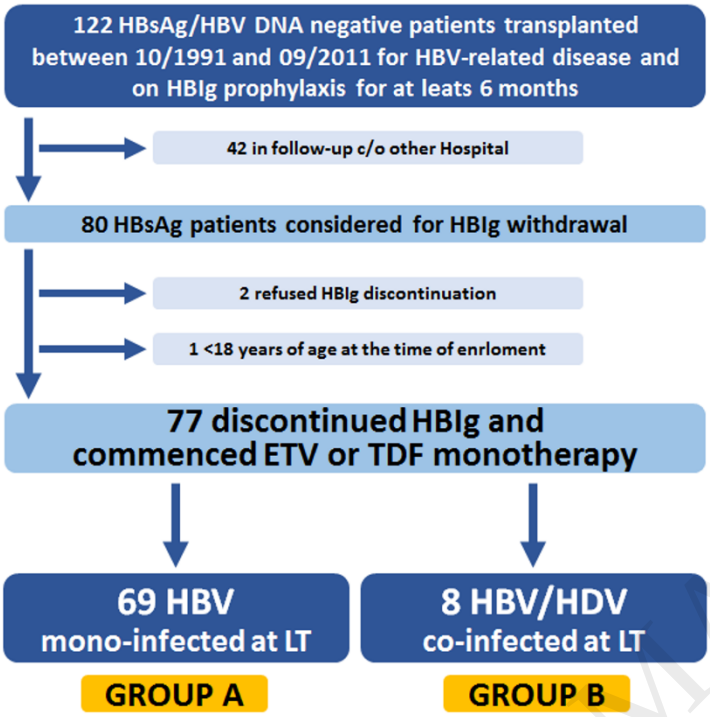

Figure 2 Cumulative probability of HBsAg recurrence of 69 patients transplanted for HBV-related disease: a) overall; b) according to age at transplant c) according to ethnicity; c) according to the presence of pre-LT HCC.

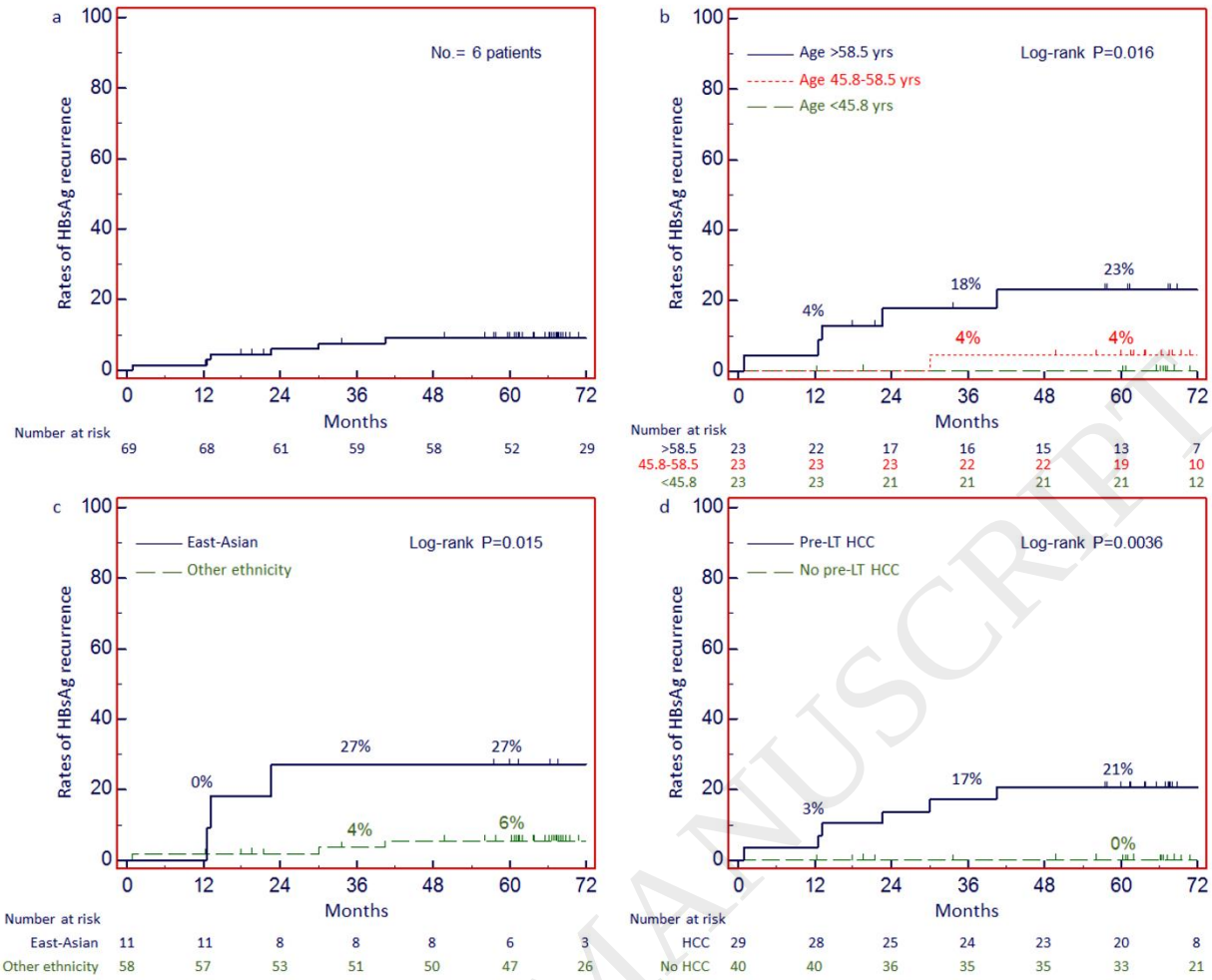

Figure 3 HBsAg kinetics in 6 patients who relapsed HBsAg during third-generation NAs monotheraphy

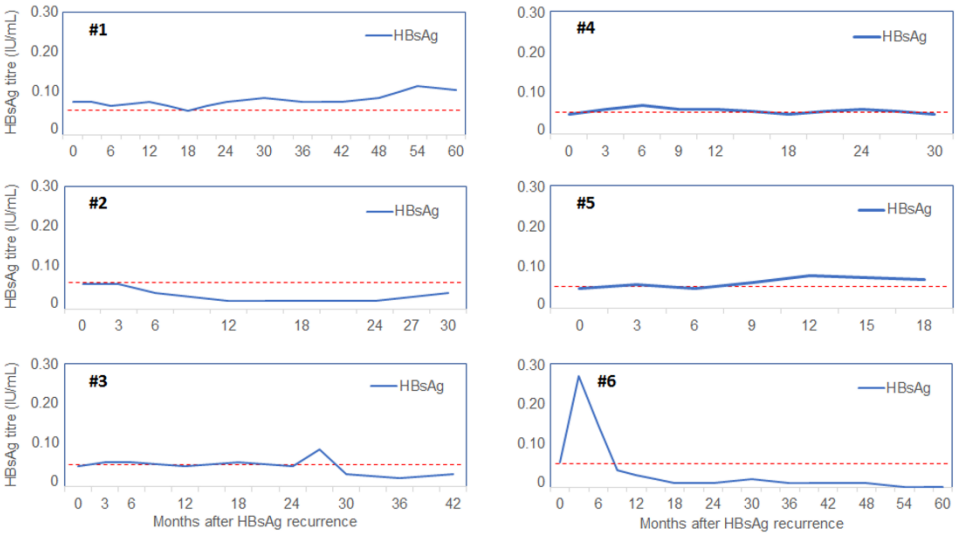

Figure 4 Change in eGFR (a and b) and of phosphate levels (c and d) during 5−year follow-up in 51 patients who switched from LAM±ADV to ETV (a and c) or TDF (b and d) after HBIg discontinuation.

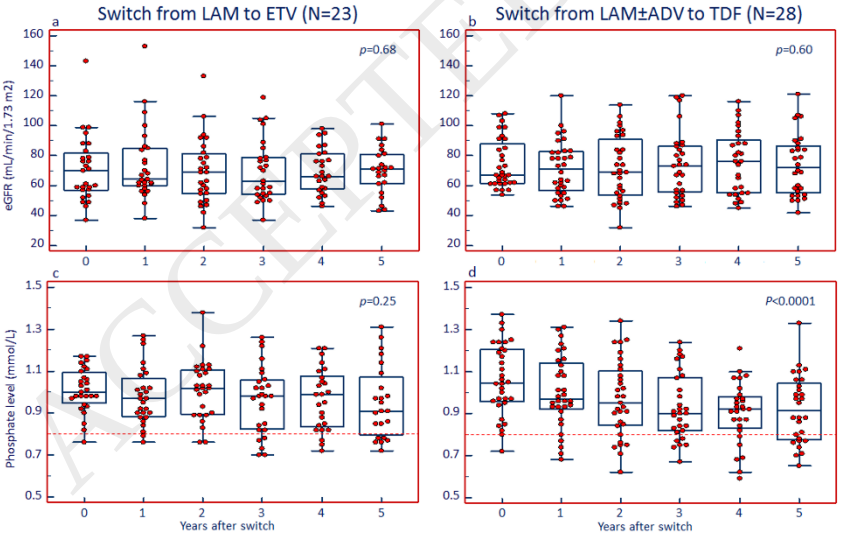

Table 1 Main features of 77 HBsAg-positive LT patients according to the absence (Group A) or the presence (Group B) of the coinfection HDV at the time of transplant: **a**) Demography; **b**) Characteristics at transplant; **c**) Characteristics at HBIg withdrawal

a

DEMOGRAPHIC CHARACTERISTICS	**Overall**		**Group A**		**Group B**	
Male gender, no. (%)	59	(77)	55	(80)	4	(50)
Ethnicity, no. (%)						
Caucasian	37	(48)	33	(48)	4	(50)
Black African	17	(22)	15	(22)	2	(25)
Middle East	9	(12)	7	(10)	2	(25)
West Asian	3	(4)	3	(4)	0	
East-Asian	11	(14)	11	(16)	0	

b

CHARACTERISTICS at TRANSPLANT	**Overall**		**Group A**		**Group B**	
Age, years‡	51	(10-70)	53	(10-70)	45	(22-52)
Clinical Features						
Transplant indication, no. (%)						
Decompensated Cirrhosis	31	(40)	24	(35)	7	(88)
Acute Liver Failure	12	(16)	12	(17)	0	
Acute on Chronic Liver Failure	4	(5)	4	(6)	0	
Hepatocellular Carcinoma	30	(39)	29	(42)	1	(12)
Child-Pugh score, no. (%)						
A	20	(26)	19	(28)	1	(12)
B	22	(29)	18	(26)	4	(50)
C	35	(45)	32	(46)	3	(38)
MELD score‡	16	(6-42)	16	(6-42)	18	(8-29)
Virological Features						
HBsAg titre (IU/mL)‡	2034	(0.7-105)	1716	(0.7-105)	4795	(38-7060)
HBeAg-positive, no. (%)	14	(18)	14	(20)	0	
HBeAb-positive, no. (%)	59	(77)	51	(74)	8	(100)
HBV DNA titre (Log IU/mL)‡	2.49	(ND-8.2)	2.49	(ND-8.2)	1.94	(ND-2.8)
HBV DNA titre (IU/mL), no. (%)						

< LLOD (12 IU/mL)	22	(29)	20	(29)	2	(25)
12-2000	25	(32)	21	(30)	4	(50)
>2000	24	(31)	24	(35)§	0	
NA	6	(8)	4	(6)	2	(25)
HBV Genotype, no. (%)						
A	16	(21)	16	(23)	0	
C	7	(9)	7	(10)	0	
D	25	(32)	21	(31)	4	(50)
E	12	(16)	12	(17)	0	
Other	4	(5)	4	(6)	0	
NA	13	(17)	9	(13)	4	(50)
Antiviral therapy before LT						
At least 3 months of treatment, no. (%)	39	(51)	35	(51)	4	(50)
Less than 3 months of treatment, no. (%)	7	(9)	7	(10)	0	

‡ Median (range);

§ 6 (9%) 2001-20000; 8 (12%) 20001-100000; 10 (14%) >100000.

c

CHARACTERISTICS at HBIg WITHDRAWAL	**Overall**		**Group A**		**Group B**	
Age‡	59	(19-81)	59	(19-81)	55	(23-59)
Length of HBIg prophylaxis, months‡	66	(6-237)	60	(6-237)	100	(6-197)
Type of antiviral therapy, no. (%)						
None	4		4	(6)	0	
LAM±ADV	55		49	(72)	6	(75)
ETV or TDF	18		16	(22)	2	(25)
HBsAb titre (mIU/mL)‡	125	(71-251)	124	(71-251)	146	(112-236)
HBeAb-positive, no. (%)	27	(35)	24	(35)	4	(50)
Laboratory tests‡						
Aspartate aminotransferase (IU/L)	25	(13-145)	25	(13-72)	25	(19-145)
Bilirubin (µmol/L)	10	(3-40)	10	(3-39)	16	(5-40)
Creatinine (µmol/L)	102	(32-228)	102	(32-228)	99	(56-119)
Phosphate (mmol/L)	1.05	(0.5-1.4)	1.03	(0.5-1.4)	1.09	(0.7-1.3)
Tacrolimus-based therapy	67	(87)	60	(87)	7	(88)

‡ Median (range).

Table 2 Main features of the 6 LT recipients who developed HBsAg recurrence after HBIg withdrawal.

	Demography		At Transplant							At HBIg Withdrawal					
N	**Sex**	**Ethnicity**	**Age**	**LT indication**	**NA before**	**HBsAg (IU/mL)**	**HBeAg**	**HBV DNA (IU/mL)**	**HBV Genotype**	**Age**	**NA before**	**Length of HBIg (months)**	**HBsAb titre (mIU/mL)**	**HBeAb**	**NA after**
#1	M	East Asian	59	HCC	LAM + ADV	43	Neg	<12	C	64	LAM	28	149	Pos	ETV
#2	F	East Asian	68	HCC	LAM + TDF	366	Neg	<12	NA	69	TDF	13	119	Pos	TDF
#3	M	Caucasian	52	HCC	LAM	11391	Pos	309	D	58	LAM	69	111	Neg	TDF
#4	M	Middle East	66	HCC	LAM + ADV	2281	Neg	269	D	69	LAM	34	152	Pos	ETV
#5	M	East Asian	61	HCC	TDF	316	Neg	<12	C	61	TDF	8	106	Pos	TDF
#6	M	African	66	HCC	None	826	Neg	1873	E	71	LAM	60	85	Neg	ETV

Table 3 Comparison of features between patients with and without HBsAg recurrence.

	HBsAg Recurrence				
	YES (No. 6)		NO (No. 63)		*p* value*
DEMOGRAPHIC CHARACTERISTICS					
Male gender, no. (%)	5	(83)	50	(79)	1.0
Ethnicity, no. (%)					
Caucasian	1	(17)	32	(51)	0.20
East-Asian	3	(50)	9	(14)	0.047
CHARACTERISTICS at TRANSPLANT					
Age, years‡	63	(47-67)	52	(10-70)	0.0095
Transplant indication, no. (%)					
HCC	6	(100)	23	(37)	0.0039
ALF	0		12	(19)	0.58
Child-Pugh score, no. (%)					
A	3	(50)	17	(27)	0.34
B	2	(33)	15	(24)	0.63
C	1	(17)	31	(49)	0.21
MELD score‡	13	(8-17)	16	(6-42)	0.45
HBsAg titre (IU/mL)‡	596	(43-103)	1883	(0.7-105)	0.59
HBeAg-positive, no. (%)	1	(17)	13	(21)	1.0
HBeAb-positive, no. (%)	5	(83)	46	(73)	0.95
HBV DNA titre (Log IU/mL)‡	2.4	(ND-3.3)	1.47	(ND-8.2)	0.94
HBV DNA titre (IU/mL), no. (%)					
≥12	3	(50)	42	(67)	0.36
≥2000	0		24	(38)	0.08
HBV Genotype, no. (%)					
A	0		16	(25)	0.31
C	2	(33)	5	(8)	0.10
D	2	(33)	19	(30)	1.0
NA therapy, no. (%)	5	(83)	37	(59)	0.39
CHARACTERISTICS at HBIg WITHDRAWAL					
Age, years‡	66	(53-71)	58	(19-81)	0.09
Length of HBIg prophylaxis (months)‡	31	(8-69)	72	(6-237)	0.16
LAM±ADV pre-HBIg withdrawal, no. (%)	4	(67)	47	(75)	0.62
HBsAb titre (mIU/mL)‡	115	(85-152)	125	(71-251)	0.58
HBeAb-positive, no. (%)	4	(67)	43	(68)	1.0
Tacrolimus-based therapy, no. (%)	6	(100)	54	(86)	1.0
ETV post-HBIg withdrawal, no. (%)	3	(50)	28	(44)	1.0

***** Fisher's exact test or Mann-Whitney U-test;

‡ Median (range).

Table 4 Factors Associated with the likelihood of HBsAg Recurrence Following HBIg withdrawal

	HBsAg Recurrence (6 events)		
	Univariate Cox regression analysis		
	HR	95% C.I.	*p* value
DEMOGRAPHIC CHARACTERISTICS			
Male gender	1.36	(0.16-11.65)	0.78
Ethnicity			
Caucasian	0.15	(0.20-1.75)	0.15
East-Asian	5.78	(1.17-28.70)	0.032
CHARACTERISTICS at TRANSPLANT			
Age (per each year of increase)	1.15	(1.02-1.30)	0.020
Transplant indication			
HCC	Not applicable		
ALF	Not applicable		
Child-Pugh score (A *vs* B/C)	0.77	(0.14-4.22)	0.77
MELD score (per each unit of increase)	0.94	(0.85-1.04)	0.25
HBsAg titre (per each 10^2 unit of increase)	1	(0.99-1.01)	0.62
HBeAg-positive	0.79	(0.93-6.84)	0.84
HBeAb-positive	1.78	(0.21-15.27)	0.60
HBV DNA titre (each Log IU of increase)	0.74	(0.48-1.16)	0.19
HBV DNA titre			
≥12	0.45	(0.09-2.21)	0.32
≥2000	Not applicable		
HBV Genotype			
A	Not applicable		
C	5.58	(0.93-33.49)	0.06
D	1.19	(0.20-7.13)	0.85
NA therapy	3.32	(0.98-28.41)	0.27
CHARACTERISTICS at HBIg WITHDRAWAL			
Age (per each year of increase)	1.08	(1.08-0.99)	0.08
HBIg prophylaxis (per each months of increase)	0.99	(0.96-1.01)	0.14
LAM±ADV pre-HBIg withdrawal	0.62	(0.11-3.39)	0.58
HBsAb titre (per each mIU of increase)	0.99	(0.97-1.01)	0.44
HBeAb-positive	4.20	(0.77-22.93)	0.10
Tacrolimus-based therapy	Not applicable		
ETV post-HBIg withdrawal	1.18	(0.24-5.84)	0.84