Review Article

Prophylaxis against Recurrence in Liver Transplantation Patients with Hepatitis B Virus: What is New?

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Abstract

Hepatitis B virus (HBV) causes an endemic infection that affects nearly 2 billion patients worldwide. It is one of the leading causes of liver cirrhosis, hepatocellular carcinoma (HCC), and liver transplantation (LT). Recurrence of HBV infection after LT is due to specific HBV-host genome interactions. Although hepatitis B immunoglobulin treatment constituted the backbone of HBV recurrence, use of the nucleoside and nucleotide analogs (especially the ones with a higher genetic barrier to resistance), either alone or in combination, offer us new and powerful options in overcoming this serious issue.

Introduction

Hepatitis B virus (HBV) is an important cause of liver cirrhosis and end stage liver disease, and liver transplantation (LT) is the only definitive treatment. In Europe, HBV is responsible for 13% of all LTs performed.[1] The worldwide infection rate of HBV identified by serological methods revealed that one third of the population has been infected. The route of transmission determines the rate of HBV positivity, where vertical transmission is directly linked to the highest rates of carrier state (higher than 8%) and HBV related liver diseases. This scenario is typical in East Asia, Oceania, and Africa.[2,3] However, serological complexity of the virus and natural history of the infection precludes clean-cut estimation of global prevalence, which ranges from 2–7% in developing countries and <2% in low prevalence areas. With the introduction of routine vaccination programs, the rate of HBV infection has reached steady state levels. Approximately 20% of patients infected with HBV develop progressive liver disease, including cirrhosis and hepatocellular carcinoma (HCC).[4] Over 300,000 cases of HCC per year are attributed to chronic HBV infection.[5]

Mechanisms of persistence of HBV

Without administration of antiviral treatment, the recurrence of HBV after LT is almost always universal. This persistence is caused by the ability of HBV to establish itself in extrahepatic organs like pancreas, kidneys, peripheral blood monocytes, bone marrow stem cells, intestines, and gonads.[6] Moreover, a glucocorticoid responsive unit located in the viral genome contributes to replicative stimulus.[7,8] Therefore, serum HBV-DNA can be detected in LT patients even after very long periods of anti-viral therapy.[9] This phenomenon of viral persistence is mainly due to a special form of viral genome that can integrate itself into the human genome. This episomal genome is known as covalently closed circular DNA (cccDNA). Conventional methods cannot detect cccDNA, and complex techniques are utilized to measure the number of infected cells in a given tissue sample. This intermediate form of an episomal genome acts as a ghost template that resides in the human DNA, giving rise to chronic infection. The only way to eliminate cccDNA is via the lysis and death of infected cells. Even suppression of HBV infection with long term nucleos(t)ide analog treatment results in a slow decline in cccDNA that is only eventually cleared with at least 10 years of treatment.[10] Unfortunately, there is little evidence about the impact of cccDNA on the natural history and persistence of infection in patients with HBV related LT. The poor degree of correlation between the presence of intrahepatic HBV-DNA and other surrogate markers like quantitative hepatitis B surface antigen (HBsAg) levels have mandated a search for reliable markers of persistence other than cccDNA.[11] One of the main reasons for low application of cccDNA in the clinical arena is the non-standardization of the method of cccDNA detection. Most reports concerning cccDNA were performed at institutional facilities and the sensitivity, variability, and range of detection were wide among methods applied. During one antiviral drug study, the same sample was distributed among many laboratories to test for cccDNA, but the results were so variable the study was not published. A second reason for its low application is the requirement of a liver biopsy, which is not a diagnostic tool generally applied clinically during the management of HBV. In Hussein *et al.*, the persistence of hepatic HBV-DNA and cccDNA were 83% and 17%, respectively.[12] In contrast, Lenci *et al.* found successful eradication rates of both hepatic HBV-DNA and cccDNA during a post-transplantation period greater than 7 years.[13] A recent

Keywords: Hepatitis B virus; Liver transplantation; Recurrence; Prophylaxis.
Abbreviations: ADV, adefovir; cccDNA, covalently closed circular DNA; ETV, entecavir; FTC, emtricitabine; HBcrAg, HBV core related antigen; HBIG, HBV-immunoglobulin; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; LAM, lamivudine; TDF, tenofovir; LT, liver transplantation.
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method for the detection of persistent HBV infection is quantification of serum HBV core related antigen (HBcrAg). This can be utilized in a given patient as a surrogate marker of cccDNA content, and this assay is a promising tool for the assessment of cccDNA levels in patients with LT.[14] An important result of these studies is the possibility of withdrawing long term prophylaxis if cccDNA (or any surrogate marker reflecting cccDNA) becomes undetectable. With the advent of new techniques that reliably shows disappearance of cccDNA on long term prophylaxis, a new era of ''antiviral free'' follow up will be possible in patients with HBV related LT.

Current status of HBV prophylaxis after liver transplantation

What is the risk?

Recurrence of HBV in the post-LT period is defined as the appearance of HBsAg, a positive test for HBV-DNA, an increase in transaminase levels, and detection of hepatitis in a graft biopsy. Before the introduction of prophylaxis, an aggressive clinical picture called fibrosing cholangitis was the most feared form of recurrence. This is typically characterized by very high viral DNA counts and is mainly due to a direct cytopathic effect of the virus. If recurrence occurs despite prophylaxis, it may result in graft dysfunction and cirrhosis of the graft. It was shown that 13% of HBV related-LT patients showed active cirrhosis despite antiviral prophylaxis.[15] Therefore, identification of patients at higher risk for recurrence is a clinical priority during the peri-transplantation phase.

The overall recurrence risk is highest in patients with a pre-LT high HBV-DNA count (>10.000 copies/mL), HBeAg positivity, HBV-drug resistance, genotype C infection (related to increased LAM resistance), and mutations of HBV (Table 1).[16–19] Another less common but important risk factor is the presence of HCC (LT performed for HCC, history of chemotherapy, or recurrent HCC).[20–22] Factors determining a low recurrence rate are low rate of viral replication, negative HBeAg status prior to LT, HDV co-infection, and fulminant HBV.[23]

What is the standard of care?

The introduction of prophylactic HBV-immunoglobulin (HBIG) in combination nucleos(t)ide analogs has reduced the risk of recurrence rate from 100% to less than 10% in 5 years. However, there is no widely accepted and standard prophylaxis scheme suitable for all patients recommended globally. While the use of nucleos(t)ide analogs in HBV-DNA positive patients during the pre-LT period and HBIG at the anhepatic phase is a common practice, post-LT prophylaxis of HBV is still not standardized among different centers and only some centers apply anti-HBs titer >100 IU/L. Most of the LT centers have adopted institutional guidelines of prophylaxis. In our institution, HBIG is given as 10,000 IU at the anhepatic phase, followed by 2,000 IU daily during the first week, and then given as needed to maintain anti-HBs levels above 100 IU/L.

Table 1. Risk factors of HBV recurrence at post-LT setting

High HBV-DNA levels prior to LT (>4 log copy/mL)
Presence of HCC prior to LT
High quantitative HBsAg levels prior to LT
Use of LAM and presence of LAM resistance
Baseline and pre-LT HBeAg positivity
YMDD mutant infection (LAM resistance)
Genotype C infection (related to increased risk of LAM resistance)

The mechanism of action of HBIG is not fully understood, but it is believed to increase the clearance of viral proteins, reduce the risk of infection of healthy hepatocytes, and induce the lysis of infected cells.[24] HBIG is typically given as a loading dose at the anhepatic phase, and then parenterally administered indefinitely either at a dose dependent on the anti-HBs antibody titers or at a fixed dose independent of anti-HBs levels. The major disadvantages of HBIG are its high cost, parenteral administration, requirement for a laboratory follow-up, and possible selection of HBV mutants.[23]

Due to higher costs of HBIG, there are variations among most centers for HBIG dosing, timing, and administration route. In a meta-analysis, HBIG mono-prophylaxis was not advocated due to very low rates of protection against HBV recurrence and increased over-all mortality compared to HBIG plus lamivudine (LAM) combination.[25]

Application of LAM mono-prophylaxis is debatable, since use of LAM is linked to a very high rate of resistance (up to 41% recurrence rates at 3 years), which occurs mostly in patients with positive HBV-DNA at time of LT.[26] Presence of HBV-DNA at the time of LT is the primary factor. Yoshida *et al.* showed that in 26 patients with negative HBV-DNA at the time of LT, LAM mono-prophylaxis resulted in a recurrence rate of 15% compared to 18% in the combination group.[27] Therefore, in select patients where treatment with HBIG is contraindicated, mono-prophylaxis with LAM may be considered provided that pre-LT HBV-DNA levels are negative.

Antivirals used in prophylaxis

Adefovir (ADV)

Currently, ADV is most commonly used as a switch or add-on treatment option in patients with viral breakthrough under LAM treatment. It can be a primary option for post-LT prophylaxis when used in combination with HBIG. In a recent systematic review of 46 studies, it was found that combination ADV and HBIG therapy resulted in three times less risk for post-LT HBV recurrence. ADV mono-prophylaxis may also be superior to LAM mono-prophylaxis[28] due to a higher degree of antiviral efficacy and its effectiveness on LAM resistant strains. In LAM resistant patients, ADV resulted in 95% HBV suppression rates during the pre-LT period.[29] For ADV, there are numerous cohorts with prospective or retrospective nature that are composed of variable sample sizes. This lack of reliable data was outlined in a Cochrane review in 2010.[30] The only prospective randomized study concerning the use of ADV in post-LT is by Angus *et al.*, where the efficacy of LAM+ADV combination and LAM+HBIG combination were compared, testing the possibility of a HBIG-free regimen for prophylaxis. In that study, ADV+LAM combination was not inferior to LAM+HBIG combination.[31] Despite the lack of quality evidence, ADV still has high potential for use as a primary prophylaxis agent. The major drawbacks regarding its use are moderate antiviral efficacy compared to newer antivirals, 20% rate of nephrotoxicity observed in LT

patients,[32] and economic cost (ADV+HBIG combination is 1.5 times more expensive than combination LAM+HBIG combination).[31] Another potential factor precluding the use of ADV is viral resistance, which can be managed by switching to or adding on high genetic barrier to resistance antiviral drugs.

Nucleos(t)ide analogs with high genetic barrier to resistance (Entecavir and Tenofovir)

Entecavir (ETV) and Tenofovir (TDF) are two oral antiviral drugs with a high genetic barrier to resistance. Most recent studies evaluating the role of ETV and TDF or TDF/Emtricitabine (FTC) are summarized in Table 2 and 3. With the exception of FTC, ETV and TDF are advocated by current chronic hepatitis treatment guidelines as first-line treatment options because of their very low to none resistance rates and fewer side effects (an exception is the use of ETV in LAM resistant patients). The use of ETV in the post-LT setting has yielded very promising results in selected patients with a favorable low risk serological profile and no history of HCC (Table 2). Although 1 mg ETV is approved for use in these patients, more than 50% of patients develop ETV resistance within 5 years due to previous LAM exposure.[33] Therefore, relapses with LAM should be treated with TDF (or possibly with TDF+FTC combination), but currently there is no clear-cut evidence available). TDF has an excellent resistance profile with low risk for nephrotoxicity. A recent systematic review by Cholongitas *et al.* identified that recurrence rates with ETV, TDF, and TDF+FTC were similar when used in combination with HBIG. In addition, these treatments were superior to LAM+HBIG combination. Furthermore, these authors also found that mono-prophylaxis with ETV or TDF were not inferior to ETV/TDF plus HBIG or LAM plus HBIG combinations.[34]

Although, hypothetically, the use of high genetic barrier to resistance drugs as a first step against HBV recurrence in the post-LT setting is very appealing, there is no convincing evidence available to advise their routine use. Recently, one study examined the use of TDF in the setting of post-LT prophylaxis using a prospective cohort design with no control groups. There were major limitations to this study, including a low number of subjects (n=17), a relatively short duration of follow up (21 months), and possible selection bias due to inclusion of low risk patients. This was only a pilot study, but the results were very encouraging and provide the basis for performing future randomized controlled trials.[35] Other recent studies concerning TDF have used TDF+FTC combination instead of TDF monotherapy.[36–38] The reason(s), however, for this choice has not been fully explained, but the hypothesis is similar to combined use of LAM+ADV. Currently, TDF+FTC combination (approved only for HIV treatment) is not an approved indication in this patient population. In our opinion, the major reason to choose TDF+FTC combination is the serologic profile or previous antiviral treatment history. Because of the low number of subjects and the long history of LT, the number of LAM resistance and ADV add-on management strategies has yielded such a patient population. For example, in the study by Teperman *et al.*,[37] 85% and 45% of patients had a history of LAM and ADV use, respectively. Wesdorp *et al.* reported 88% LAM+ADV combination prophylaxis before switching to TDF/FTC mono-prophylaxis.[38] Furthermore, given the number of study subjects with a low risk of recurrence, the question of selection bias may be raised. The prospective cohort design and lack of previous treatment arms as control groups should be viewed with caution. Lastly, a prospective randomized placebo controlled study by Berg *et al.* showed similar efficacy between TDF monotherapy and TDF+FTC combination treatment arms in patients with a history of previous ADV experience in 168 weeks.[39] The authors found that baseline HBV-DNA load and ADV related mutations had no effect on the final outcome. Taking these considerations into account, a definitive conclusion regarding switching current management strategies towards these cannot be drawn from these studies. Future large population of patients using HBIG plus other nucleos(t)ide analogs combinational treatment or HBIG-free monotherapy would optimize the results. More randomized controlled trials are warranted to clarify contradictory reports in the current publications.

HBIG free regimens for HBV prophylaxis

The possibility of HBIG free prophylaxis and mono-prophylaxis with newer nucleos(t)ide analogs has been addressed by several studies (Table 4). Although the results are promising, patient selection criteria may be biased, since most patients in these studies had a low risk of recurrence. In the study by Fung *et al.*,[40] 80 patients were included in a prospective cohort who had very low to undetectable levels of HBV-DNA. The pre-LT HBV-DNA levels did not differ between recurrence and non-recurrence groups (3.2 and 3.7 log HBV DNA, respectively). The loss of function of HBV-DNA as a well-known surrogate marker for prediction of recurrence in this study might be attributed to a strong patient selection bias (potentially due to ethical concerns). However, this study revealed the possibility of excluding use of the expensive HBIG treatment in selected patients with a very low risk of recurrence. Two years later, this same group published new and unique findings in a similar patient population without HBV-DNA selection.[41] They concluded that the virological relapse rate at 3 years for LAM, ETV, and combination group was 17, 0, and 7%, respectively. Major risk factors for recurrence were prior LAM treatment, presence of HCC, and higher HBV-DNA levels at the time of LT. In two other studies,[42,43] recurrence rates of 0–8% by HBIG free regimens with no specific factors defined as risk factors for recurrence were reported. Taken together, these major studies indicated in selected patients with low risk factors that HBIG free regimens against HBV-recurrence is possible and that future controlled studies are required to change current practices against the use of HBIG.

Novel strategies against recurrence

In a recent study, the HBV recurrence rates were significantly lower in a patient population with splenectomy either before or at the time of LT.[44] This novel observation is critical since cccDNA, the origin of HBV recurrence, can also reside in the spleen as peripheral blood monocytes or bone marrow cells.

Although controversial, active immune-prophylaxis via newer HBV vaccines against recurrence has been investigated. In 2003, Bienzle *et al.* found a successful formation of high titer antibodies with non-standard adjuvants containing active vaccine,[45] but this observation has not been confirmed in subsequent studies.[46,47]

The transfer of adoptive immunity involves the transfer of both cellular and humoral immunity of donor (after immunization) to the recipient,[48] thereby giving rise to a specific

Table 2. Most recent studies of entecavir use in prophylaxis of post-LT HBV recurrence

Author	Characteristics of patients and serological criteria of inclusion at the time of LT	Study design	HBIG regimen	Recurrence definition	Recurrence rate and risk factors for recurrence
Cholongitas *et al.*[35]	11 patients, HBsAg-positive, HBeAg negative/anti-HBeAg positive, anti-HBcore positive and HBV-DNA negative	Prospective cohort, 21 months follow-up	Used in first 6 months and then stopped	Presence of HBsAg and/or HBV-DNA	None
Gao *et al.*[50]	84 patients, no serological criteria of inclusion defined	Retrospective cohort, 57 months follow-up	HBIG used throughout the study period, dosage adjusted according to the Anti-HBs levels	Presence of HBsAg and HBV-DNA	None
Hu *et al.*[51]	145 patients, no serological criteria of inclusion defined, 70% patients had HCC, 30% had Anti-HBc positive donor	Retrospective cohort with a historical control group of LAM+HBIG combination	HBIG used throughout the study period, dosage adjusted according to the Anti-HBs levels	Presence of HBsAg	1.37%, pre-LT HCC and low Anti-HBs titers post-LT
Kim *et al.*[52]	154 patients, no serological criteria of inclusion defined, 5 patients had HCC prior to LT	Retrospective cohort, 28 months follow-up	HBIG used throughout the study period, dosage adjusted according to the Anti-HBs levels	Reappearance of HBsAg in serum at 2 different times	3.2%, pre-LT HCC
Yi *et al.*[53]	29 patients, HBeAg and HBV-DNA negative	Prospective cohort, 24 months follow-up	Used in first 12 months and then stopped	Reappearance of HBsAg	3.4%, pre-LT HCC

Table 3. Most recent studies of tenofovir or tenofovir+emtricitabine use in prophylaxis of post-LT HBV recurrence

Author	Characteristics of patients and serological criteria of inclusion at the time of LT	Study design	HBIG regimen	Recurrence definition	Recurrence rate and risk factors for recurrence
Cholongitas *et al.*[35]	17 patients, HBsAg-positive, HBeAg negative/anti-HBeAg positive, anti-HBcore positive and HBV-DNA negative	Prospective cohort, 21 months follow-up, TDF used	Used in first 6 months and then stopped	Presence of HBsAg and/or HBV-DNA	None
Stravitz *et al.*[36]	21 patients, undetectable HBsAg and HBV DNA, patients received HBIG and nucleos(t)ide analogs for a mean of 6.6 years prior to enrolment	Prospective cohort, 31.1 months follow-up, TDF/FTC used	Used at least 6 months (patients received HBIG for a mean of 6.6 years)	Presence of HBsAg and/or HBV-DNA	3 patients at 1 year, 1 patient at the end of study, 3 patients had acute renal failure
Wesdorp *et al.*[38]	17 patients, undetectable HBsAg and HBV DNA, patients received HBIG and nucleos(t)ide analogs for a mean of 62 months prior to enrolment	Prospective cohort, 26 months follow-up, TDF/FTC used	Used at least 6 months	Presence of both HBsAg and HBV-DNA	None (as defined by study criteria), no renal side effect reported
Teperman *et al.*[37]	18 patients in TDF/FTC arm, undetectable HBV DNA at time of randomization	Phase-2, Open label, multi-center, randomized controlled trial, TDF/FTC vs. TDF/FTC + HBIG compared	36 weeks prior to randomization, dosing schedule not mentioned	Presence of HBV-DNA	None

Table 4. Studies with a HBIG free regimen against HBV recurrence

Author	Characteristics of patients	Study design and intervention used	Recurrence definition	Recurrence rate and risk factors for recurrence
Fung *et al.*[40]	80 patients with low or zero HBV-DNA counts at time of LT. Nineteen patients received LAM treatment prior to LT with no reported resistance. HBeAg positivity was reported 27.5%.	Prospective cohort ETV without HBIG	Reappearance of HBsAg after initial seroclearance	10 patients had recurrence. High HbsAg levels prior to LT is the main risk factor
Fung *et al.*[41]	362 patients irrespective of HBV-DNA counts. 48.6% of patients received antiviral treatment prior to LT.	Retrospective cohort Three groups composed of LAM alone, ETC alone and combination of antiviral drugs	$\geqslant$1 log IU/mL increase of HBV-DNA from nadir	The virological relapse rate at 3 years for LAM, ETV, and combination group was 17, 0, and 7% , respectively. Patients treated with LAM alone, with HCC at the time of transplantation, or HBV DNA $\geqslant$3 log IU/mL at the time of transplantation had a higher relative risk of virological rebound
Gane *et al.*[42]	20 patients irrespective of HBV-DNA levels (with 50% had $>$ 4 log IU/mL DNA levels prior to LT)	Prospective, multicenter, open-label study LAM+ADV combination HBIG was given at anhepatic phase, 1 week post-LT and then stopped	Reappearance of both HBsAg and HBV DNA in serum	None
Wadhavan *et al.*[43]	75 patients with low HBV-DNA levels ($<$2000 IU/mL)	Prospective cohort ETV, TDF, ETV+TDF LAM+ADV	HBV DNA positivity 6 months after transplantation	8% No specific factor mentioned as a risk factor for recurrence

anti-HBV immune response. The transfer of immunocompetent HBV-specific T-cell and B-cell immunity determines the magnitude and extent of the newly developing immune response.[49]

Conclusions

Recent advances in prophylaxis of HBV after LT are encouraging in terms of development of a HBIG-free and once-a-day antiviral regimens. Further research with different therapeutic design aiming at minimum drug use and maximum cost-effectiveness are required.

Conflict of interest

None

Author contributions

Reviewing and analysising of the literature (ÖH), designing the manuscript (ÖH, HS), interpretation of data (HS), writing the manuscript (ÖH, HS), administration (MH), critical revision (MH).

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