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Prophylaxis of Hepatitis B Virus (HBV) Re-Infection in Liver Transplantation: Is the Reappearance of Hepatitis B Surface Antigen (HBsAg) Significant?

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Statistical Analysis C
Data Interpretation D
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The recurrence of hepatitis B virus (HBV) infection after orthotopic liver transplantation (OLT) was in the past a primary cause of organ loss or mortality. Currently, post-OLT prophylaxis with anti-HBs immunoglobulins plus a nucleos(t)ide analogue has virtually abolished the risk of re-infection. Some studies have proposed to simplify prophylaxis by discontinuing immunoglobulins while continuing the analogue alone.

This review analysed the available studies, focusing on the recurrence of HBsAg in serum and its biological effects. In all, 16 studies were retrieved, mainly observational or retrospective, each enrolling 14 to 80 patients. Our review of the literature found that HBsAg re-appeared in 0% to 24% of the patients, generally with HBV DNA undetectable in plasma. One study measured HBsAg using a new ultra-sensitive method, which could allow a reappraisal of the incidence of recurrence. This review discusses the role of HBV surface proteins in inducing hepatocellular carcinoma, particularly when mutations in the C-terminal occur that induce stop-codons that cause defects of secretion and retention of truncated protein S, resulting in direct cell toxicity and cancer. The data on the suspension of immunoglobulins in the prophylaxis regimes of post-transplant re infection do not appear sufficiently robust for an extensive and safe application in clinical practice.

MeSH Keywords: **Antiviral Agents • Hepatitis B • Hepatitis B Antibodies • Hepatitis B Surface Antigens • Liver Transplantation**

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Background

The recurrence of hepatitis B virus (HBV) after a liver transplant has in the past had a key role in organ loss and mortality. The use of anti-HBV immunoglobulins administered intravenously during the peri-transplantation phase, followed by monthly intramuscular treatment, was the first breakthrough in the prevention of the reappearance of hepatitis B surface antigen (HBsAg) [1]. It had, however, only a partial efficacy, since 29% of patients had HBV recurrence within 2 years, although this was a significantly lower rate compared to patients not receiving prophylaxis with anti-HBV [2]. The recurrence was related to the presence of pre-transplantation HBV DNA, which in the past has been detected using a hybridization methodology. To date, the mechanism of action of immunoglobulins in the setting of transplantation has not been completely elucidated [3,4]. They could neutralize the circulating virus, prevent its linkage to the receptor on the surface of the hepatocytes, or even block the production/secretion of HBs [3,4].

The subsequent use of antiviral drugs in pre-transplantation, and the introduction of post-transplantation prophylaxis with immunoglobulins plus a nucleo(s)ide analogue has led to a recurrence rate of between 0% and 10% [5,6]. The advent of high barrier analogues (entecavir or tenofovir) in combination with immunoglobulins has almost eliminated the risk of relapse. Liver transplantation for HBV-related liver disease still represents about 10% of transplantations performed in Europe [7]. A recent analysis of a series of 5912 patients showed that the loss of graft or death were linked to the recurrence of HBV in <1% of patients who received transplants for decompensated cirrhosis and in 3% of patients with hepatocellular carcinoma (HCC), thus confirming the effectiveness of combined prophylaxis schedules [8]. Overall, post-transplantation 1-year and 3-year survival is now significantly higher than in the previous decade. In Italy, the proportion of transplantations due to HBV remains around 15%, of which 2/3 are due to the presence of hepatocarcinoma (Brancaccio, unpublished data).

The availability of high potency and high genetic barrier antivirals has encouraged studies on prophylaxis simplification, such as discontinuation of immunoglobulins and administration of antiviral alone. This mini review presented here analyzes the data of simplified prophylaxis, focusing on the pathophysiology and the possible consequences related to the production of surface protein (HBsAg) of HBV.

Discontinuation of Immunoglobulin Administration

The simplification of the prophylaxis obtained by interrupting immunoglobulins and continuing the administration of the

analogue alone has been explored in numerous studies, mainly observational and retrospective small studies [9–24]. Table 1 summarizes the studies published over the 10 years up to 2018. These studies enrolled >10 patients (range 14 to 80 patients) who were treated with antivirals with a high genetic barrier or treated with combinations of antivirals. The cases were quite heterogeneous with regard to HBV viremia (which was not always reported) at the time of transplantation, the presence of hepatocarcinoma, the duration of administration of immunoglobulins up to the discontinuation, the analogue used, and finally the definition of outcome. During a post-discontinuation follow-up from 24 weeks to 86 months, the finding of HBV DNA in plasma was unusual. On the contrary, HBsAg recurrence was found in a percentage of patients ranging from 0% to 22.5%. This trend was also reported in the studies on the last generation analogues (entecavir or tenofovir) and did not appear to be influenced by the selection of patients at low risk of recurrence – evaluated as viremia <100 IU/mL at transplantation or the absence of detectable covalently closed circular (ccc) DNA in hepatic tissue [14,22]. In one study, the prophylaxis was performed with entecavir alone, and the disappearance of HBsAg at 1 year was observed more frequently in patients with pre-transplantation quantitative HBsAg <3 log (90% versus 74%; $P=0.025$) [12].

The persistence of detectable HBsAg during follow-up was reported in approximately half of the patients.

Possible Implications of the Positivity of HBsAg

HBsAg structure

The HBV surface antigen (HBsAg) includes 3 forms of glycoproteins, small (S-HBsAg), medium (M-HBsAg; pre-S2+HBsAg), and large (L-HBsAg; pre-S1+pre-S2+S). The synthesis takes place in the endoplasmic reticulum, and immediately afterwards disulphuric bridges produce dimeric or multimeric formations. Only part of the surface protein is used for the genesis of the complete virion, while the major proportion gives rise to filamentous or spherical subviral particles, which are secreted in plasma. Furthermore, part of the circulating HBs may derive from genomic sequences of HBV integrated into the human genome; subviral particles exceed the number of complete virions by 10^3 to 10^6 times [25,26]. In practice, common laboratory tests measure all the circulating HBsAg.

S gene mutations

HBsAg mutations were initially detected in HBV vaccinated patients who acquired HBV infection despite a high titer of anti-HBV antibodies (immune escape mutants) (as reviewed

Table 1. Studies on discontinuation of the prophylaxis with immunoglobulin.

Author, year [ref]	Patients (n)	Regimen	Outcome	Length of follow-up	Definition of recurrence
Angus, 2008 [9]	16	>12 mo HBIg+Lam then ADV+LAM	0% recurrence 6% HBsAg+ 100% HBV DNA–	24 mo	HBV DNA+
Kawagishi, 2010 [10]	14	HBIg+NA ×12 mo then NAs	14.3% HBsAg+	30 mo	HBsAg+
Saab, 2011 [11]	61	HBIg+Lam 12 mo. Then 2 NAs	1,6% HBsAg+	15 mo	HBsAg+
Fung, 2011 [12]	80	Entecavir alone	22.5% HBsAg+ 1 HBV DNA+	26 mo (median)	HBsAg+ ±HBV DNA+
Yuefeng, 2011 [13]	15	HBIg+LAM then LAM	12.5% HBsAg+	42–86 mo	HBsAg+ and HBV DNA+
Lenci, 2011 [14]	30 cccDNA undetectable in liver tissue at OLT	HBIg stopped then LAM alone 24 mo: Lam stopped if cccDNA absent	3.3% HBsAg+ After LAM stopped 4 additional HBsAg+ 10% HBV DNA+	29 mo	HBsAg+
Stravitz, 2012 [15]	21	>6 mo HBIg+NA then TDF/FTC	0% recurrence 14% HBsAg+ 100% HBV DNA–	31.1 mo	HBVDNA+
Cholongitas, 2012 [16]	47	HBIg >12 mo. then NA (various)	0% recurrence 6.3% HBsAg+ 100% HBV DNA–	24 mo	Clinical
Gane, 2013 [17]	20	HBIg+Lam then ADV+LAM	0% recurrence 0% HBsAg+ 100% HBV DNA–	57 mo	HBV DNA+ and HBsAg+
Teperman, 2013 [18]	18	24 wk TDF/FTC+ HBIg then TDF/FTC	0% recurrence 0% HBsAg+ 100% HBV DNA–	24 w	HBV DNA+ and HBsAg+
Wesdorp, 2013 [19]	17 (15 HBsAg–)	HBIg >6 mo The TDF+FTC	6.7% HBsAg+ 100% HBVDNA–	24 mo	HBsAg+
Yi, 2013 [20]	29	HBIg+ENT 1 yr Then ENT	3.4% HBsAg+ 100% HBV DNA–	12 mo	HBsAg+
Tanaka, 2014 [21]	24	HBIg +NA for 1 year Then TDF	0% recurrence 0% HBsAg+ 100% HBVDNA–	29.1 mo	HBsAg+ and HBVDNA+
Fernandez, 2015 [22]	58 “low risk of recurrence”	HBIg±NA >12 mo then TDF or ENT	0% recurrence 8.6% HBsAg+ 100% HBV DNA–	28±5 mo	HBsAg+ and HBVDNA+
Radhakrishnan, 2017 [23]	42, HBV DNA <100 IU/mL at OLT	HBIg+NA x5 days then NA alone	2.9% HBsAg+	3 years	HBsAg+
Manini, 2018 [24]	69	HBIg stopped after 60 mo (median) then TDF or ENT	9% HBsAg+	69 mo	HBsAg+

by Coppola [27]). Numerous variants in the determinant “a” of the protein S, in particular the G145R substitution, are able to reduce the binding with anti-HBs antibodies and their neutralizing effect. Similar variants have been described in patients

with transplants treated with anti-HBs immunoglobulins. At that time, some tests for HBsAg detection provided false negative results when such mutations were present, whereas current tests have a good sensitivity in detecting mutated forms [28].

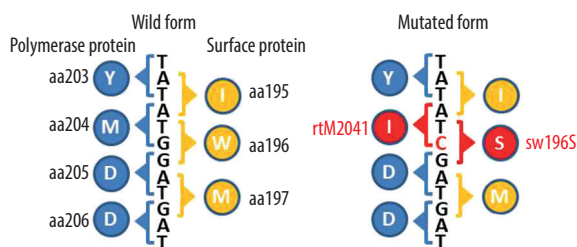


Figure 1. Substitutions in HBsAg induced by mutations in polymerase gene of HBV (modified from College et al. [30]).

Neutralizing antibodies are targeted towards the MHR (major hydrophilic region), of which the determinant “a” is a subdomain. A recent study, using a next generation sequencing technique, has shown that mutations in this region are extremely frequent, but their significance is not always clear [29]. Overall, the phenomenon of escape mutants appears to be of little relevance in clinical practice.

Of greater clinical interest are the variants of the S gene deriving from mutations in the polymerase gene. The 2 genes are partially overlapped and some substitutions in the Pol gene, such as those generated by first generation analogues, can induce a substitution in the S gene. An example is shown in Figure 1, which illustrates the possible consequences of the typical lamivudine-induced mutation in the polymerase gene that encodes the YMDD amino acid sequence.

The substitutions in the C-terminal hydrophobic region of S-HBsAg (aa 179–226), which is inserted in the endoplasmic membrane and mediates the transit of the glycoprotein through the membrane itself, induce stop-codons that cause defects of secretion and retention of the protein S, resulting in direct toxicity mediated by oxidative stress, DNA damage, and genomic instability. Stop codons production is increased by exposure to antivirals [27,30].

Examples of such mutations in the reverse transcriptase/S gene are rtA181T/sw172*, rtM204I/sw196*, and rtV191I/sw182*. A typical example is the W172* mutation, which causes a stop codon that induces the production of truncated forms, lacking 55 amino acids from the C-terminal region [31]. This protein also has an inhibitory effect on the complete virion secretion, with consequent low viremic levels even in the case of virologic breakthrough.

Similarly, deletions in L-HBsAg and M-HBsAg are able to induce intracellular accumulation. The accumulation of HBs aberrant proteins in hepatocytes has been associated with the development of hepatocellular carcinoma (HCC) in numerous studies;

many of these studies were carried out in Asia (reviewed by Coppola et al. [27]). Recently, new substitutions in the C-terminal portion of the S protein (P203Q, S210R) have been associated with HCC. These mutations have not been related to exposure to antivirals but *in vitro* have the property of reducing HBs secretion (note that the double mutation has been found only in patients with HCC) [32].

Clinical consequences of the presence of HBsAg

The production of HBsAg may induce clinically relevant consequences that require adequate follow-up. In patients with chronic HBV infection, the clearance of HBsAg reduces the risk of HCC compared to patients who remain HBsAg positive despite having viremia controlled by analogues [33]. In patients transplanted for HCC (which is about 2/3 of HBV patients transplanted in Italy), the recurrence of HCC is a well evaluable outcome measure, as it has a significant incidence rate of recurrence at between 1 and 3 years [7]. A study by Manini et al. found that transplantation for HCC, along with higher age and Asian origin, was strongly associated with the reappearance of HBsAg after discontinuation of immunoglobulins [24]. In an Asian study of 114 patients who received a transplant for HCC, who also received a prophylaxis with analogues only, the HCC recurrence rates were related to the reappearance of HBsAg and its plasma concentration at the time of transplantation [34]. It should be noted that this study dosed HBsAg using a new, high sensitivity test; the traditional test would not have detected this correlation. The extensive use of this new test could further redefine the term “recurrence” and reassess the importance of HBsAg in the pathogenesis of HCC as well as in the definition of occult HBV infection [35]. More detailed virologic studies aimed at the typing of the produced HBsAg can add further knowledge and help to hypothesize new risk biomarkers.

Conclusions

Liver transplantation is the end point of a long disease history. It requires a significant investment in terms of direct and organizational costs. Chronic HBV infection still represents around 15% of transplantation indications in Italy today. In previous decades, some thousands of patients received a transplantation using this indication. Today, the indication for transplantation due to decompensation of HBV liver disease is becoming more and more uncommon, while the majority of transplantations for HBV infection occur due to the presence of HCC. Data on the withdrawal of immunoglobulins presents us with many unanswered questions, particularly about the risk of recurrent infection and cancer in patients receiving liver transplantations for HCC. Although these questions are conceptually interesting, the data on the suspension of immunoglobulins

in prophylaxis regimes of post-transplantation re-infection do not appear sufficiently robust to support an extensive and safe application in clinical practice.

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Conflict of interests

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