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ORIGINAL ARTICLE

One-year vaccination against hepatitis B virus with a MPL-vaccine in liver transplant patients for HBV-related cirrhosis

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hepatitis B immunoglobulins, hepatitis B virus – prophylaxis, liver transplantation, monophosphoryl-lipid-A, recombinant S HBV vaccine.

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Summary

Conflicting results have been reported on vaccination against hepatitis B virus (HBV) as a prophylaxis against viral recurrence after liver transplantation. We investigated the efficacy of 1-year, monthly vaccination using an adjuvant 3-deacylated monophosphoryl-lipid-A (MPL) recombinant S vaccine initially administered together with hepatitis B immunoglobulins (HBIg) in 18 patients transplanted for HBV-related cirrhosis. All received 12 vaccine doses (HBsAg, 20 mcg plus MPL, 50 mcg): the initial six doses (phase I) were administered within 7 days after intravenous HBIg (2000 IU), while the last 6 (phase II) following HBIg withdrawal. All patients received lamivudine during the study. Anti-HBs titers were determined before each dose and then for 1 year after vaccination. After phase I anti-HBs titers were greater than 100 IU/l in all patients and in three (16.6%) were greater than 500 IU/l. After phase II 10 patients (55.5%) achieved anti-HBs titers greater than 100 IU/l and five (27.7%) greater than 500 IU/l. One year after vaccination eight patients (44.4%) maintained anti-HBs titers greater than 100 IU/l, with a median titer of 234 IU/l (102–1205), and 2 (11.1%) greater than 500 IU/l. One-year extended monthly vaccination with a MPL-adjuvant recombinant vaccine induces a sustained protective anti-HBs response in approximately half of transplant recipients.

Introduction

It is estimated that 350 million people are infected by Hepatitis B Virus (HBV) worldwide and over 200 000 and 300 000 chronic HBV carriers die each year from cirrhosis and hepatocellular carcinoma, respectively [1–3]. In Western European Countries, HBV infection with acute liver failure or liver cirrhosis is the indication for orthotopic liver transplantation (OLT) in 10–25% of liver transplant recipients [4]. Five- and 10-year survival rates after OLT performed due to HBV-related disease are 74% and 69%, respectively, ranking among the highest of all liver transplant indications and providing the greatest transplant benefit [5]. These remarkable results have been possible because of the adoption of highly effective prophylactic strategies against HBV recurrence based on the long-term use of hepatitis B immunoglobulins (HBIg), initially given alone [6–10] and later in combination with lamivudine [11–16]. Before the systematic adoption of this prophylactic strategy, post-transplant HBV reinfection was extremely high, being associated with severe graft disease and poor patient survival [17,18]. HBV reinfection rate was already reduced to roughly 30% in patients receiving high doses of HBIg, even if undergoing OLT with detectable serum HBV DNA [19]. The introduction of nucleos(t)ide analogues in patients with active viral replication before OLT and the use of combined prophylaxis with HBIg and lamivudine after OLT have allowed the reduction of HBV recurrence rates to less than 10% [20–22]. Thus, this prophylactic strategy is currently

considered the gold standard of pre and post OLT care [23–25]. Accordingly, maintenance of antibody titers to hepatitis B surface antigen (anti-HBs) greater than 500 IU/l during the first 6 months after OLT and of 100 IU/l indefinitely thereafter in combination with lamivudine has been reported to prevent HBV reinfection in almost 100% of patients [19,26].

Unfortunately this approach, although highly effective, does not eradicate HBV, which may persist either in the liver and/or in extrahepatic sites in low replicating forms, or as covalently closed-circular HBV DNA [27]. In peripheral blood mononuclear cells persistence of HBV DNA has been detected up to 10 years following transplantation [28]. Therefore, there is a potentially life-long risk of HBV recurrence on discontinuation of prophylaxis unless an adequate specific immune response is acquired. This justifies the current policy of maintaining indefinite prophylaxis, despite the high cost, patients' inconvenience and potential drug toxicity. Yet, the financial burden of passive immunoprophylaxis with HBIg is so high that after a few years it will exceed the costs of an uncomplicated transplant [10]. In addition, this strategy may determine the emergence of HBIg-induced HBV escape mutants [8,29,30] and/or nucleos(t)ide resistance [31,32]. These arguments highlight the need to develop new tools to prevent HBV reinfection after OLT.

HBV vaccination has been considered a possible alternative in this perspective, yet the results obtained with conventional vaccines have been highly controversial, most studies showing the development of anti-HBs protective titers, usually short lasting, in less than 20–25% of patients [33–39]. Recent data on the use of a new HBV vaccine, including 3-deacylated monophosphoryl-lipid-A (MPL) as adjuvant (Glaxo-Smith-Kline Biologicals, Rixensart, Belgium) reported much better response rates (in up to 80% of patients) [40], a finding unfortunately not confirmed in two other trials with a similar design [41,42]. Because of these contradictory results, which might be partially explained by the concomitant use of HBIg, we planned the present study to investigate, the effectiveness of a 1-year extended, monthly vaccination schedule with the MPL adjuvant vaccine, which was administered in combination with HBIg during the first 6 months and without HBIg thereafter.

Materials and methods

Patients

Eighteen patients transplanted for HBV-related cirrhosis without virological and biochemical evidence of HBV recurrence and with normal liver function were enrolled. Mean follow-up after liver transplant was 73 ± 38 (13–150) months. At surgery all patients were HBsAg seropositive and HBV DNA seronegative by standard hybridisation or by PCR assay, according to the time of transplant; 5 (27.7%) patients were anti-HDV positive, and 5 (27.7%) were anti-HCV and HCV RNA positive. All patients received high-dose intravenous HBIg during the anhepatic phase and the first week after surgery. Patients received intravenous HBIg (5000 IU/monthly) during the first year after transplant and until present study were maintained with 2000 IU when the anti-HBs titer dropped below 100 UI/l, according to an 'on demand' strategy, in combination with lamivudine, 100 mg/day. Fourteen patients had already received lamivudine (100 mg/day) before transplant. Lamivudine was continued as monotherapy during the whole follow-up period. To be included in the study patients had to be free of any significant post-transplant disease, including recent episodes of rejection. All patients became HBsAg negative in serum after OLT and were repeatedly found to be HBV DNA sero-negative by PCR assays with increasing sensitivity over time. Liver biopsies were obtained 3 months prior to enrolment, stained negative in all cases for HBsAg and HBcAg immunohistochemistry and showed normal histology or minimal changes. Liver and kidney functions were normal in all cases. Low-dose maintenance immunosuppression monotherapy with either cyclosporin A, tacrolimus, sirolimus, or mycophenolate mofetil (in six, five, two and three patients, respectively) was administered throughout the study, except in two patients who received a combination of tacrolimus and sirolimus or mycofenolate mofetil. The demographic and baseline clinical and virological features of individual patients are listed in Table 1. All patients gave written informed consent to the study protocol, which was approved by the local Ethics Committee and conducted in accordance with the Declaration of Helsinki principles.

Vaccination protocol and follow-up

Twelve monthly vaccine doses of S vaccine containing 20 mcg of recombinant HBsAg and 50 mcg of 3-deacylated MPL adjuvant (Fendrix, Glaxo Smith Kline, Rixensart, Belgium) were administered: the initial six doses (phase I) were administered within 7 days of the intravenous infusion of 2000 IU of HBIg, while the last six doses (phase II) were administered after complete HBIg withdrawal. Lamivudine, 100 mg/day, was given throughout the study period. The vaccination schedule is illustrated in Fig. 1. Anti-HBs titers were measured at monthly intervals during each vaccination phase and during the 12-month follow-up period using a third generation immunoenzymatic assay (AUSAB EIA, Abbot, Baar, Switzerland).

Patients who at the end of follow-up maintained a serum anti-HBs titer greater than 100 IU/l were considered *sustained responders*. All others were considered *non-responders*.

Statistical analysis

Results are presented as means or medians ± standard deviations or ranges, and the statistical differences were

Table 1. Demographic, clinical and virological characteristics of patients enrolled in the study.

Patients no.	Age	Sex	BMI	Initial diagnosis	Anti-HDV/HCV*	HBV DNA†	Follow-up post-OLT (months)	Immunosuppression therapy	
								Mono	Combined
Responders									
1	41	M	25	Cirrhosis	+/–	_	87	CyA	.
2	61	M	30	Cirrhosis	–/–	_	107	MMF	.
3	59	M	29	Cirrhosis	–/–	_	63	TC	.
4	45	F	28	Cirrhosis	–/–	_	60	TC	.
5	53	M	19	Cirrhosis	–/+	_	84	CyA	.
6	55	M	27	Cirrhosis	–/–	_	88	.	TC + MMF
7	33	F	20	Cirrhosis	+/–	_	57	CyA	.
8	62	F	28	Cirrhosis	+/–	_	80	TC	.
	51 ± 10		25 ± 4		3/1		78 ± 17		
Non-responders									
9	59	M	27	Cirrhosis	–/–	_	56	CyA	.
10	61	M	26	Cirrhosis	–/+	_	150	MMF	.
11	62	M	26	Cirrhosis	–/–	_	116	MMF	.
12	65	M	30	Cirrhosis	–/+	_	78	CyA	.
13	60	F	18	Cirrhosis	–/+	_	14	.	TC + SR
14	56	F	26	Cirrhosis	–/–	_	144	CyA	.
15	65	M	23	Cirrhosis	–/+	_	41	SR	.
16	43	M	26	Cirrhosis	–/–	_	13	SR	.
17	35	M	24	Cirrhosis	+/–	_	58	TC	.
18	59	M	24	Cirrhosis	+/–	_	32	TC	.
	56 ± 9		25 ± 3		2/4		70 ± 50		

*At time of study screening.

†HBV-DNA status (PCR) at enrollment time.

CyA: Cyclosporine A microemulsion; TC: Tacrolimus; SR: Sirolimus; MMF: Mycophenolate mofetil; M: male, F: female; +: positive, –: negative.

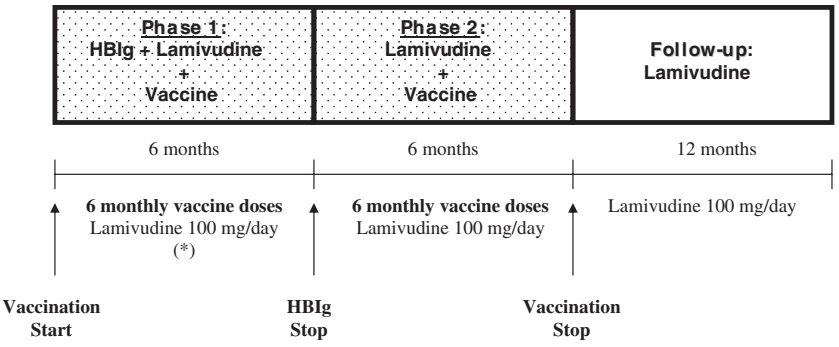

Figure 1 Schematic outline of the study protocol including phases I and II of vaccination and the follow-up period. Vaccination consisted in the intramuscular administration of 12 monthly doses of 20 mcg of recombinant S antigen and 50 mcg of MPL adjuvant. Phase I included the first six vaccine doses and Phase II the last six doses. During Phase I patients were given also HBIg, 2000 IU/monthly and lamivudine, 100 mg/day. Each vaccine dose was administered within 7 days after HBIg injection. Phase II included the last six vaccine doses. During this Phase patients received only lamivudine in addition to the vaccine. During the follow-up period patients were maintained under lamivudine alone.

evaluated using the Student's *t*-test. Univariate analysis was performed using the NCSS Statistical System for Windows, to detect predictors of response to vaccination. This analysis included several demographic, clinical and transplant-related factors (age, BMI, gender, time elapsed after OLT, presence of HDV or HCV coinfection).

Results

All patients completed the vaccination program and the 12-month follow-up period. No side effects were recorded. All patients were found to be HBV DNA negative at the end of vaccination and follow-up.

At the end of phase I all patients developed an anti-HBs titer greater than 100 IU/l (median 295 ± 270 IU/l, range 139–1300 IU/l), with only three (16.6%) showing titers above 500 IU/l (range: 529–1300 IU/l). One month after the last vaccine dose (end of phase II) the median anti-HBs titer was 151 ± 577 IU/l (range: 0–2100), with 10 (55.5%) patients showing titers greater than 100 IU/l (range 133–2100 IU/l) and 5 (27.7%) greater than 500 IU/l (range: 777–2100 IU/l). Of the remaining eight patients, 3 (16.6%) had anti-HBs titers between 10 and 100 IU/l and 5 (27.7%) below 10 IU/l, respectively.

At the end of 1 year of follow-up the median anti-HBs titer was 49 ± 347 (range 0–1205) and eight patients (44%) were classified as responders to vaccination according to our definition. The median anti-HBs titer among responders was 234 IU/l (range 102–1205), with only two patients (11.1%) showing anti-HBs titers above 500 IU/l. Of the 10 patients classified as non-responders, three (16.6%) had an anti-HBs titer between 10 and 100 IU/l and seven (38.8%) lower than 10 IU/l, respectively. The response rates observed after phase I and II and after 1 year follow-up are summarized in Table 2. The kinetics of serum anti-HBs titers observed during phases I and II and during the follow-up period in *sustained responders* and in *non-responders* are shown in Figs 2 and 3. Most *sustained responders* had a slight decline in the anti-HBs titer during the follow-up period, despite remaining by definition above 100 IU/l. Conversely, most *non-responders* showed a rapid fall of anti-HBs titers as from the end of phase I (i.e. from the interruption of HBIg administration), with only a few patients showing a later decline.

No predictors of response to vaccination were identified among the following variables: age, gender, BMI, time after OLT, renal function, type of immunosuppression, anti-HDV and anti-HCV sero-positivity.

Anti-HBs (IU/l)	≥ 500	≥ 100	≤ 100 ≥ 10	< 10
End of phase I	3/18 (16.6%)	18/18 (100%)	0/18 (0%)	0/18 (0%)
End of phase II	5/18 (27.7%)	10/18 (55.5%)	3/18 (16.6%)	5/18 (27.7%)
End of follow-up	2/18 (11.1%)	8/18 (44.4%)	3/18 (16.6%)	7/18 (38.8%)

Table 2. Number and percentage of patients according to categories of anti-HBs titers reached at the end of phase I and II and at the end of study follow-up.

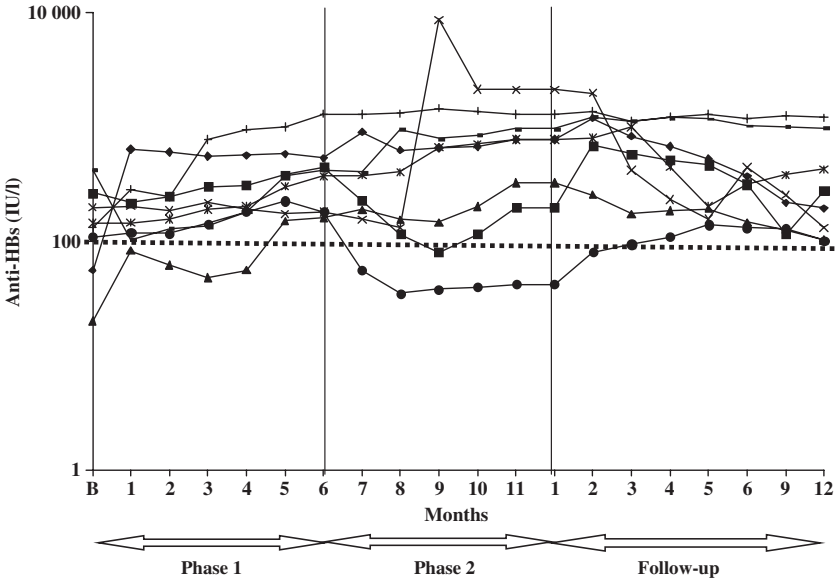

Figure 2 Anti-HBs titers during Phase I and Phase II of vaccination and follow-up periods in *sustained responders*. Each line represents the anti-HBs kinetic of a single patient. For explanation of Phase I and II please see the legend of Fig. 1.

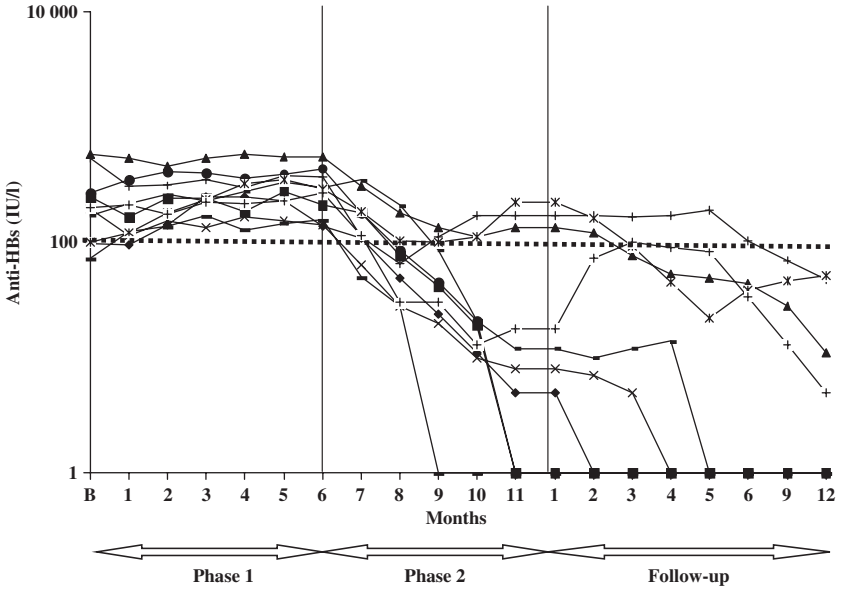

Figure 3 Anti-HBs titers during the vaccination and follow-up periods in *non-responders*. Each line represents the anti-HBs kinetic of a single patient. For explanation of Phase I and II please see the legend of Fig. 1.

Discussion

The introduction of nucleos(t)ide analogues in patients with evidence of HBV replication before liver transplantation, together with the use of continued prophylaxis with HBIg and nucleos(t)ide analogues after surgery has allowed the reduction of HBV recurrence to almost negligible rates [26]. This remarkable result, however, is associated with high costs, the perspective of a life-long prophylaxis, and the possibility of selecting HBV escape mutants resistant to HBIg [8,29,30] and/or nucleos(t)ide analogues [31,32]. In addition, frequent assays of anti-HBs titer are needed to maintain protective titers. Alternative strategies against HBV recurrence after liver transplant would therefore be advisable.

In theory, the most favourable cost/benefit approach to protect the graft from HBV recurrence after liver transplant would be to develop in the recipient a natural, long-lasting, protective anti-HBs titer through vaccination. Controversial data on the use of HBV vaccines, however, have been generated in the transplant setting. Five studies [34–38], all including a small number of patients, investigated this issue using the standard alum-adjuvant recombinant S vaccine. Overall, the results of these studies were unsatisfactory: they showed that response to HBV vaccination is possible in liver transplanted patients, however protective anti-HBs titers developed in less than 20–25% of cases. In addition, there is little evidence in the literature that possibly protective levels are long-lasting. Notably, these unsatisfactory results occurred despite the adoption in all these studies of strategies to reinforce the conventional vaccination schedules, either by increasing the S antigen doses and/or the number and frequency of vaccine administration.

Divergent and promising results were reported in 2003 by Bienzle *et al.* [40], who were the first to use the newly developed MPL-adjuvant HBV vaccine in the post-transplant setting. These German authors found a very high response rate (80% of patients) after an extended vaccination course, which allowed the 16 responders to reach final anti-HBs titers comprised between 1255 and 83 121 IU/l. Their protocol included the use of several intravenous injections of HBIg during the early weeks of vaccination, which consisted in the administration of five doses of 20 mcg of HBsAg over a 5-month period. In addition, patients with the lowest initial response received three further bimonthly vaccine doses. Thus, the total administered dose of HBsAg in the Bienzle *et al.* study ranged from 100 to 160 mcg and the total MPL from 500 to 800 mcg. Notably, the authors reported that 2 years after the last vaccine dose the anti-HBs titers declined by 82%. In a subsequent paper, the same authors reported that 11 out of the 16 previous responders were revaccinated using a double dose of the conventional alum-adjuvant HBV vaccine and all exhibited a significant increase of anti-HBs titers [43].

Unfortunately, the Bienzle *et al.* results have not been confirmed so far, which may explain the reluctance of transplant physicians to adopt their strategy in the common practice. Table 3 summarizes the main features and results of the studies in which the MPL-adjuvant vaccine was used. In another study from Germany, Starkel *et al.* [41], using five double doses of the same MPL-adjuvant vaccine given within a 6-month period reported a

Table 3. Baseline characteristics of study population, methodology and main results of published studies on vaccination with MPL-adjuvant vaccine in comparison with the present study.

Intramuscular vaccination cycle	Bienzle *et al.* (2003)	Starkel *et al.* (2005)	Rosenau *et al.* (2006)	Present Study (2010)
Number of patients	20	10	8	18
Months after OLT Median (ranges)	78 (24–156)	55 (36–120)	60 (26–90)	73 (13–150)
Pre-transplant HBV disease: acute/chronic	2/18	2/18	0/18	0/18
HBIg given during vaccination	Yes	Yes	No	Yes
Concomitant Lamivudine during vaccine (%)	20	0	100	100
Number of vaccine doses per cycle	5 + 3	5	6	6 + 6
Dose of S antigen per vaccine dose (mcg)	20–100	40	20	20
End-of-follow up anti-HBs ≥ 500 IU/l (responders)	80% (16)	40% (4)	12.5% (1)	11.1% (2)
End-of-follow up anti-HBs ≥ 100 IU/l (responders)	80% (16)	40% (4)	12.5% (1)	44.4% (8)
End-of-follow up anti-HBs ≥ 10 IU/l (responders)	80% (16)	40% (4)	12.5% (1)	61.1% (11)
Median anti-HBs titer (IU/l) in responders*	25 334 (1255–83 121)	>1000	561	234 (102–1205)

*At the end of follow-up.

protective response in only four out of 10 patients. These authors administered a total of 200 mcg of HBsAg and 500 mcg of MPL over a 12-month period and maintained HBIg administration during the entire vaccination, providing the intravenous infusion of high doses whenever the anti-HBs titer dropped below 150 IU/l. In a further German study, Rosenau *et al.* [42] reported even worse results, as only one out of eight transplanted patients responded to the administration of six single doses (total 120 mcg of HBsAg) of MPL-adjuvanted vaccine given in a 6-month period. The only responder in the latter study developed a maximum titer of 561 IU/l. Notably, in the Rosenau *et al.* study, all patients underwent HBIg washout before the start of vaccination, while lamivudine was maintained throughout the study.

Because of these contradictory results, we designed the present study to redefine the response to MPL-adjuvant HBsAg vaccine in patients liver-transplanted due to HBV-related end-stage liver disease and to assess whether its efficacy may be related to the concomitant administration of HBIg. Three features of our study protocol deserve attention: (i) patients received the greatest dose of MPL-vaccine so far administered in the transplant setting (240 mcg) and for the longest time (12 months); (ii) half of the total vaccine dose was administered while patients were receiving exogenous intravenous HBIg and half after complete HBIg washout; (iii) lamivudine was administered during the entire study period.

The rationale for combining vaccine with HBIg in phase I is supported by the hypothesis that the formation of HBIg/HBsAg complexes might increase vaccine immunogenicity [44,45]. The need to continue vaccination after HBIg withdrawal in phase II was considered to be crucial to strengthen the response to vaccination and to exclude the possibility of detecting spurious anti-HBs titers. Our results show that a sustained response rate to this vaccine schedule, defined as the persistence of a protective anti-HBs titer 1 year after the last vaccine dose, is achieved in 44% of patients, indicating that successful vaccination against HBV is indeed feasible in a relevant proportion of liver transplanted patients using the MPL-adjuvant vaccine. However, during phase I all patients except two showed anti-HBs levels to be clearly dependent on the concomitant HBIg administration, while spontaneous flares of anti-HBs titers became evident only during phase II (i.e. after HBIg withdrawal). Taken together these results, though less enthusiastic than those initially reported by Bienzle *et al.*, appear to be consistently better compared to those obtained with conventional alum-adjuvant vaccines.

Because of the limited number of patients enrolled in the present study, our results clearly require confirmation before being considered as an additional strategy in the prophylaxis against HBV recurrence after liver transplantation. A multicenter study would probably be the most appropriate methodological approach to allow the enrolment of a significantly greater number of patients.

We believe, however, that our study already has potential clinical implications, which can be summarized as follows: (i) all patients transplanted because of HBV-related end-stage liver disease who have received conventional post-transplant prophylaxis with HBIg and nucleos(t)ide analogues should be considered for possible HBV vaccination, as an additional strategy; (ii) vaccine administration should be long-lasting (e.g. 1 year); (iii) passive prophylaxis with HBIg should preferably be maintained during the initial phase of vaccination and nucleos(t)ide analogues should be maintained during the entire vaccination period; (iv) response to vaccine should be checked monthly after HBIg withdrawal to avoid spurious results, and then frequently after vaccine cessation. Using this approach a relevant proportion of patients will likely mount a sustained anti-HBs response and eventually be ready to discontinue further prophylaxis against HBV,

which would result in considerable cost savings and improved quality of life. However, the safety of complete withdrawal of prophylaxis in vaccine responders remains to be validated in long-term follow-up studies. We believed, however, that such studies could be performed only after a better knowledge is achieved of the longevity of vaccine-induced HBIg titers and of their comparability versus vaccine-induced titers in terms of protective power.

Further studies enrolling greater number of patients are needed, and can be safely conducted in transplant recipients using the MPL-adjuvant vaccine, to verify whether the above potential implications hold true and are robust enough to persuade transplant physicians to include HBV vaccination as an additional strategy in their current practice of HBV prophylaxis after OLT.

Authorship

DDP: wrote the paper, DDP: designed research/study, DDP, IL, GT, LT and AM: performed research/study, DDP, IL, GT and MA: analysed data, CC: performed vaccination, AB and LL: collected data. MA: reviewed the paper.

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