Patients and methods

Patients

This study enrolled 663 consecutive patients who underwent RFA as the initial treatment for HCC between 1999 and 2005 at the Department of Gastroenterology, the University of Tokyo Hospital. Inclusion criteria for RFA were as follows: total bilirubin concentration lower than 3 mg/dl, platelet count no less than $50 \times 10^3/mm^3$, and prothrombin activity no less than 50%. Patients with portal vein tumor thrombosis, refractory ascites, or extrahepatic metastasis were excluded. We did not restrict the indications for RFA according to the size and number of nodules.[20,21]

The study protocol was approved by our institutional review board, and informed consent was obtained from all patients.

Diagnosis of HCC

Hepatocellular carcinoma was diagnosed using dynamic computed tomography (CT), considering hyperattenuation in the arterial phase with washout in the late phase as defining HCC.[22] Most nodules were also confirmed histopathologically with an ultrasound-guided biopsy.

TACE before RFA

When four or more hypervascular nodules were detected on dynamic CT or the largest nodule exceeded 3 cm in diameter, we usually performed TACE before RFA. Under local anesthesia, a catheter was inserted into the hepatic artery via the femoral artery. After hypervascular tumors were identified by arteriography, the feeding arteries were selectively embolized with gelatin sponge particles after an emulsion of epirubicin hydrochloride (Pharmorubicin; Pfizer Japan, Tokyo, Japan) and iodized oil (Lipiodol Ultra-Fluid; Schering Japan, Osaka, Japan) was injected under X-ray monitoring.

RFA procedure

All patients underwent dynamic CT with a slice thickness of 5 mm within 1 month before ablation for comparison. After one or two sessions of RFA, dynamic CT was performed to evaluate the treatment efficacy. The interval between initiating the contrast infusion and CT recording was 30 and 120 s for single-detector-row spiral CT (Highspeed Advantage; GE Medical Systems, Milwaukee, WI, USA) and 25, 40, and 120 s for multi-detector-row CT (LightSpeed QX/I; GE Medical Systems). The images were presented after axial reconstruction with a slice thickness of 5 mm. On treatment evaluation, we compared the CT findings of the early and late phases before ablation and those of the late phase after ablation. We did so because the CT findings of the early phase immediately after ablation are not suitable for treatment evaluation because of arterial enhancement of the surrounding liver parenchyma caused by the presence of vascular abnormalities related to the treatment itself, which may only reflect inflammatory changes or a microscopic arterioportal shunt.[23] A lesion was judged as ablated completely when the nonenhanced area in the late phase of CT after ablation covered the entire lesion in both the early and late phases of CT before RFA with a safety margin in the surrounding liver parenchyma. We confirmed complete ablation in all slices on which a target nodule was visualized. Patients underwent additional sessions of ablation until complete ablation was confirmed in each nodule.

Follow-up protocol

The follow-up consisted of blood tests and monitoring of tumor markers as an outpatient; ultrasonography and dynamic CT were performed every 4 months. Tumor recurrence was defined using the same criteria applied to the initial HCC. When HCC recurrence was identified, RFA was performed if the same criteria as for primary HCC were again satisfied.

For those with no indications for RFA because of multiple recurrent nodules, we performed TACE if the liver function was Child–Pugh class A or B. Patients with localized portal invasion of the tumor were treated with radiotherapy.[24] Patients with tumor invasion to the first branch or main tract of the portal vein were treated with intraarterial 5-fluorouracil and systemic interferon-α combination therapy if indicated.[25] Those with extrahepatic metastasis of the tumor received systemic chemotherapy if they had well-preserved liver function and performance status. Living related donor liver transplantation was considered in cases of less advanced HCC with severely decompensated liver function.

Statistical analysis

The data are expressed as the median and 25th and 75th percentiles unless otherwise indicated. Survival analysis was performed on a patient basis. Survival time was defined as the interval between the day of the first treatment (TACE or RFA) and death or the last visit to the outpatient clinic until December 31, 2006. Cumulative survival was estimated using the Kaplan–Meier method.

We assessed the risk factors for survival using multivariate Cox proportional hazard regression with the Child–Pugh classification as a stratification factor. For

the analysis, the diameter and number of nodules were categorized as ≤2.0, 2.1–3.0, 3.1–4.0, 4.1–5.0, and >5 cm and one, two or three, four or five, and more than five, respectively. Estimated 5-year survival rates categorized by tumor size and number were assessed according to the results of the Cox regression using the survival of patients who had a single HCC nodule equal to or smaller than 2 cm in diameter as a reference.

Differences with $P < 0.05$ were considered statistically significant. All statistical analyses were performed with S-plus 2000 (Mathsoft, Seattle, WA, USA).

Results

Patient profiles

The enrolled patients consisted of 425 men and 238 women with a median age of 68 years (Table 1). The median tumor diameter was 2.4 cm, ranging from 0.9 to 9.7 cm. The number of nodules was one in 371 (56.0%) patients, two or three in 236 (35.6%) patients, and more than three in 56 (8.4%) patients. The distribution of the size and number of nodules is shown in Table 2. Of the patients, 439 (66.2%) met the conventional criteria (i.e., three or fewer nodules, none of which exceeded 3 cm in diameter). Up to the end of the follow-up, 189 patients died. The cumulative survival rate at 1, 2, 3, 4, and 5 years was 96.3%, 88.4%, 81.3%, 70.0%, and 59.6%, respectively.

Table 1. Baseline characteristics of the patients ($n = 663$)

Variable	*n* (%)
Age[a] (years)	68 (63–73)
Male	425 (64.1)
Viral infection	
HBsAg, positive	72 (10.9)
Anti-HCVAb, positive	524 (79.0)
Both positive	11 (1.7)
Both negative	78 (11.8)
Child–Pugh classification	
Class A	469 (70.7)
Class B	186 (28.1)
Class C	8 (1.2)
Tumor size (cm)	
≤2.0	216 (32.6)
2.1–3.0	263 (39.7)
3.1–4.0	118 (17.8)
4.1–5.0	35 (5.3)
>5	31 (4.7)
Number of nodules	
1	371 (56.0)
2–3	236 (35.6)
>3	56 (8.4)

HBsAg, hepatitis B surface antigen; anti-HCVAb, antihepatitis C virus antibody
[a]Expressed as the median (25th–75th percentiles)

Relative risk of tumor size and number assessed using Cox proportional regression

Multivariate analysis using the Cox proportional hazard model revealed that the hazard ratio increased gradually with the size and number of tumor nodules (Fig. 1A,B). The estimated 5-year survival was assessed using the combined hazard ratio using the survival of patients who had a single HCC nodule equal to or smaller than 2 cm in diameter as a reference. The 5-year survival rate was 75% in reference patients and more than 40% when patients had a single nodule; two or three nodules, none of which exceeded 5 cm; four or five nodules, none of which exceeded 3 cm; or multiple nodules 2 cm or smaller (Table 3).

Discussion

An indication for treatment of a neoplastic disease should be decided according to the disease severity, invasiveness of the treatment, and expected prognosis. If a wide difference in survival exists after a treatment between two stages, the condition that separates these two might be an indication for the treatment. In this study, the hazard ratio assessed using a multivariate analysis increased gradually with the size and number of tumor nodules and no apparent threshold was observed. Therefore, the conventional criteria (i.e., three or fewer nodules, none of which exceeds 3 cm in diameter) is not definitive in terms of survival.

Table 2. Number of patients categorized by the size and number of tumor nodules

	Number of tumor nodules			
Tumor size (cm)	1	2–3	4–5	>5
≤2.0	132	73	10	1
2.1–3.0	148	86	23	6
3.1–4.0	58	53	6	1
4.1–5.0	18	15	2	0
>5.0	15	9	3	4

Table 3. Estimated 5-year survival based on the size and number of tumor nodules

	Number of tumor nodules			
Tumor size (cm)	1 (%)	2–3 (%)	4–5 (%)	>5 (%)
≤2.0	75	68	61	54
2.1–3.0	65	55	47	39
3.1–4.0	48	37	28	21
4.1–5.0	52	42	33	25
>5.0	46	35	26	19

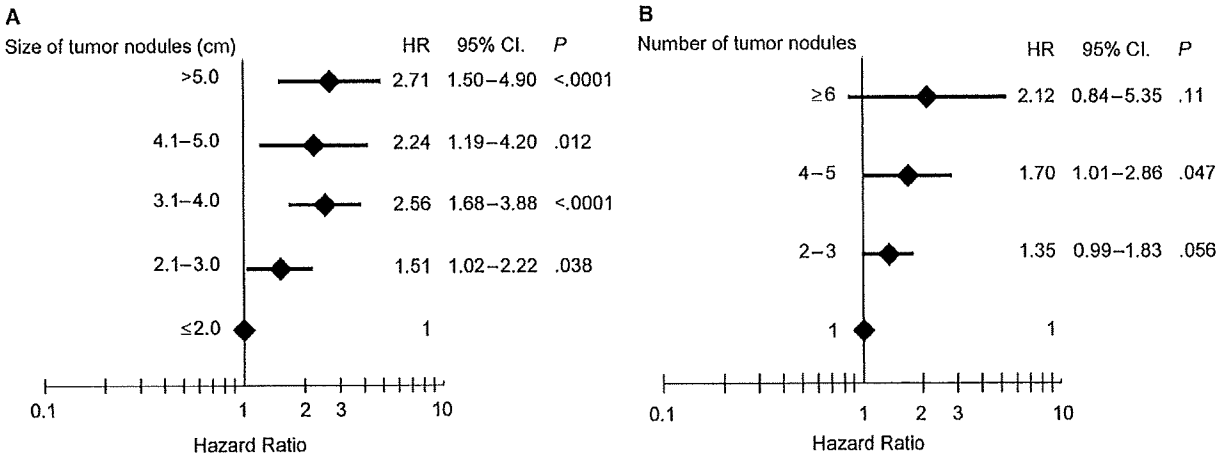

Fig. 1A–B. **A** Results of the multivariate Cox proportional hazard regression analysis. The hazard ratios of the patients increased with tumor size. The *diamonds* indicate the hazard ratios and the *horizontal lines* denote the 95% confidence intervals. **B** Results of the multivariate Cox proportional hazard regression analysis. The hazard ratios of the patients increased with the number of tumor nodules. *Diamonds* indicate the hazard ratios and the *horizontal lines* denote the 95% confidence intervals

Table 4. Treatment options classified by their invasiveness and curative potential

Treatment option	Invasiveness	Probability of cure
First-line treatment	Maximal	Highest
Second-line treatment	Intermediate	Intermediate
Third-line treatment	Minimal	Lowest

The first-line treatment option for most solid tumors is surgical resection. In general, resection is considered the most invasive treatment and is indicated only if the expected survival exceeds that of a second-line treatment that is less invasive than resection (Table 4). In the treatment strategy for HCC, RFA is considered the second-line treatment for unresectable HCC due to tumor multiplicity or impaired liver function when liver transplantation is not indicated.[26] Therefore, expanding the indications for RFA to resectable patients needs randomized controlled trials comparing the survival of patients who can undergo both resection and RFA.[27,28] At present, whether to choose resection or ablation for those who have three or fewer HCC nodules, none of which exceeds 3 cm in diameter, remains controversial.

For unresectable patients, the indications for RFA should be assessed in comparison with the third-line treatment, TACE. Although the probability of local cure of HCC with RFA depends on tumor size,[21,29] RFA is far more reliable for destroying the target nodule than TACE, for which the treatment efficacy also depends on tumor size.[30,31] For nodules larger than 3 cm in diameter, patients with HCC 5 cm or smaller were treated by RFA in combination with TACE for local cure within two sessions (data not shown). Therefore, increasing the indication to tumors 5 cm in diameter is technically feasible and acceptable. Indeed, a recent report suggested that TACE + RFA combination therapy improves the survival of patients with one to three HCC nodules, at least one of which exceeded 3 cm, as compared to TACE or RFA alone.[32]

The presence of more than three hypervascular HCC nodules strongly indicates disseminated malignant cells throughout the liver. The application of RFA, which is a locoregional treatment, is thought to have limited efficacy in such patients. However, patients with multiple HCC nodules often have one or two main nodules and small minor nodules. Ablating the major nodules may reduce the total tumor burden considerably and prolong survival.

The prognosis of patients with HCC depends strongly on the background liver function, as well as tumor stage.[33–35] Therefore, the improvement in survival with RFA may be minimal in patients at advanced tumor stages with severe liver dysfunction. One should be cautious when applying RFA to these patients.

In conclusion, patients' prognosis decreased as the diameter and number of nodules increased. However, we found no apparent threshold in the diameter or number of HCC nodules. RFA can be applied beyond the conventional indications after considering liver function.

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Original Article

Double-dose double-phase use of second generation hepatitis B virus vaccine in patients after living donor liver transplantation: Not an effective measure in transplant recipients

Noriyo Yamashiki,[1,3] Yasuhiko Sugawara,[1,2] Sumihito Tamura,[2] Junichi Kaneko,[2] Yuichi Matsui,[2] Junichi Togashi,[2] Norihiro Kokudo,[1,2] Masao Omata[3] and Masatoshi Makuuchi[1,2,4]

[1]*Organ Transplantation Service,* [2]*The Artificial Organ and Transplantation Division, Department of Surgery,* [3]*Department of Gastroenterology, University of Tokyo, Graduate School of Medicine, and* [4]*Department of Surgery, Red Cross Medical Center, Tokyo, Japan*

Aims: Post-transplant active immunization for chronic hepatitis B patients has been attempted in several studies with controversial results. We assessed the effect of a double-dose double-phase vaccination regimen among partial living donor liver recipients.

Methods: Eighteen patients who underwent liver transplantation (LT) for chronic hepatitis B and two non-hepatitis B virus (HBV)-infected patients who received hepatitis B core antibody (HBcAb)-positive donor organs were recruited 18–78 months after LT. All were on hepatitis B immunoglobulin (HBIG) mono-prophylaxis before and throughout vaccination, to maintain hepatitis B surface antibody (HBsAb) titers of more than 100 IU/mL. Recombinant hepatitis B surface antigen vaccine (40 μg) was administered intramuscularly during weeks 0, 4, 8, 24, 28 and 32.

Results: The patients consisted of 15 males and five females with a median age of 52 (39–59) years. None developed a sufficient HBsAb titer above 500 IU/mL by week 48. In two patients whose maximum HBsAb titer increased to above 300 IU/mL, we attempted to skip HBIG, but shortly thereafter the titer dropped below 100 IU/mL and HBIG administration was resumed. Although the HBIG dose was reduced during and after vaccination, cessation of administration was not achieved.

Conclusion: Double-dose double-phase use of second generation recombinant vaccine was not effective in this study population. The selected population should be targeted for a conventional vaccine regimen, and different approaches, such as strong adjuvant or pre-S containing protein, should be further tested in a larger number of patients after LT for chronic hepatitis B.

Key words: Hepatitis B vaccine, HBcAb positive donor, HBIG, lamivudine, liver transplantation, prophylaxis

INTRODUCTION

THE LONG-TERM use of hepatitis B immunoglobulin (HBIG) and/or nucleos(t)ide analog prophylaxis has dramatically improved survival rates after liver transplantation for hepatitis B virus (HBV)-related liver disease.[1] Historically, hepatitis B (HB) recurs in approximately 80% of liver transplant recipients with HBV-related liver diseases. The use of HBIG mono-prophylaxis has improved the rate of recurrent hepatitis to 35%.[2] Long-term use of HBIG therapy, however, is costly and there is insufficient evidence regarding the optimal length of administration. Lamivudine (LAM) mono-prophylaxis is less costly and is useful for decreasing the rate of hepatitis recurrence to less than 40%.[3,4] Emerging resistant strains are a concern in long-term follow-up for transplant recipients, however, and additional nucleos(t)ide analogs such as adefovir and entecavir are required.[5,6] The combination of HBIG and antiviral agents has decreased the rate of hepatitis B recurrence.[7,8]

Correspondence: Dr Yasuhiko Sugawara, Artificial Organ and Transplantation Division, Department of Surgery, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. Email: yasusuga-tky@umin.ac.jp
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Active immunization against hepatitis surface antigens has been attempted in patients after liver transplantation for HB-related liver diseases.[9–11] Repeated vaccination effectively initiates the production of anti-hepatitis B antibodies and is thus followed by HBIG administration withdrawal.[9–11] This idea is theoretically a more economical and simple method compared to passive immunization or nucleos(t)ide analog administration. Contradictory results have been reported, however, and suitable candidates for this type of vaccination have not been determined.[12–15]

At the University of Tokyo, we use HBIG monoprophylaxis.[16] The ultimate goal of vaccination is to achieve sufficient production of anti-hepatitis B immunoglobulin and to discontinue further prophylaxis against recurrent hepatitis B. In the present study, we report the results of an active immunization protocol in chronic hepatitis B-related living donor liver transplantation (LDLT) recipients.

METHODS

Subject selection

PATIENTS WHO UNDERWENT LDLT at least 18 months before for HBV-related end-stage liver disease or received hepatitis B core antibody (HBcAb)-positive donor livers were enrolled if they were being treated with an HBIG prophylaxis protocol, free of nucleos(t)ide analogs, without co-infection with hepatitis C virus or human immunodeficiency virus, and if they had no evidence of HBV reactivation. Twenty Japanese patients provided informed consent to the study protocol. There were 15 men and five women with a median age of 52, ranging from 39 to 59 years. Etiologies of end-stage liver disease included chronic hepatitis B in 16, fulminant hepatic failure with a history of chronic hepatitis B in two, and end-stage liver disease due to autoimmune hepatitis and primary biliary cirrhosis in one each. Two non-HBV patients and one chronic hepatitis B patient received core antibody-positive donor organs. Nine patients with hepatocellular carcinoma were free from post-transplant recurrence with a median follow-up period of 43 (18–90) months.

The donors were 13 men and seven women ranging in age from 18 to 54 years and weighing 43–75 kg. Their relationship to the patients included 10 children, five spouses, three nephews, one sibling and one cousin. Right liver graft was performed in eight, extended right graft in one, right lateral graft in two, left lobe with caudate graft in seven and left lobe graft in two. Three donors were positive for both HB surface antibody (HBsAb) and HBcAb.

Pre- and post-LDLT follow-up protocol

The post-transplantation immunosuppression regimen consisted of steroid induction with tacrolimus, or cyclosporine in case of tacrolimus intolerance, for maintenance.[17] Among the 18 patients with hepatitis B virus infection, LAM 100 mg/day was given orally prior to LDLT. One patient received LAM for more than 1 year, one for 3 months, and 15 received LAM for less than 4 weeks. Of the 17 patients on LAM, a negative HBV-DNA load was confirmed preoperatively in nine cases. In eight patients whose HBV-DNA was detectable pre-transplant, LAM therapy was continued for 4 weeks after LDLT, and discontinued after confirming negative HBV-DNA. Postoperatively, HBIG (Mitsubishi Tanabe Pharma, Hebsbulin-IH, Tokyo, Japan) was administered to HBV-infected patients and those who received HBcAb-positive donor organs. Details of the HBIG administration protocol and doses are described elsewhere.[16] In brief, HBIG was administered to maintain the anti-HB surface antibody (HbsAb) levels at greater than 1000 IU/L for patients with HBV and greater than 500 IU/L for patients that received HBcAb-positive donor organs. After 1 year, 1000–2000 U was given intravenously indefinitely to maintain HbsAb levels of greater than 100 IU/L.[16,18]

Vaccination protocol

After obtaining informed consent, baseline laboratory tests were performed in all patients. Table 1 shows the baseline patient characteristics and laboratory findings. HBV-DNA was undetectable in all patients. Increased dose, namely "double-dose", of recombinant anti-HB vaccination (40 μg/2 mL; Heptavax II, Banyu Pharm, Tokyo, Japan) was injected bilaterally into the deltoid muscles (half dose each side). HBsAb titers were measured at week 0 and every 4 weeks thereafter. Vaccination was scheduled for two phases of three administration cycles at weeks 0, 4, 8, 24, 28 and 32.

During the protocol period, HBIG was administered 2 weeks before and after vaccination if necessary; either 1000 or 2000 IU was administered intravenously according to the previously measured HBsAb titer, as long as it was maintained above 100 IU/mL.

Study end-points and ethical considerations

The primary end-point of this study was the vaccine response. A significant increase, that is >500 IU/mL, in

Table 1 Patient characteristics

Factors	Values†
Age	52 (39–59) years
Men, women	15, 5
HBV-related hepatitis	18
HBcAb positive donor	3
Immunosuppression (Tac + CS), (CyA + CS)	15, 5
Months from LDLT (median)	45 (17–90) months
Pre-LDLT LAM	17
Duration of LAM administration before LDLT: <1 month, 1–12 months, >12 months	15, 1, 1
HBV-DNA positive at LDLT‡	8
HBsAg titer at entry	138.5 (92–302) IU/mL
Aspartate aminotransferase	18.5 (7–30) IU/mL
Alanine aminotransferase	16 (5–30) IU/mL
Alkaline phosphatase	197 (103–528) IU/mL
γ-glutamyl transferase	33.5 (11–187) IU/mL
Lactate dehydrogenase	178 (99–315) IU/mL
Total bilirubin	0.9 (0.1–1.5) mg/dl

†Values are number or median (range). ‡HBV-DNA measured by transcription-mediated amplification methods. Lower detectable limit is 3.7 LEG/mL.
CS, corticosteroid; CyA, cyclosporine; HBsAb, hepatitis B surface antibody; HBcAb, hepatitis B core antibody; HBV, hepatitis B virus; LAM, lamivudine; LDLT, living donor liver transplantation; Tac, tacrolimus.

HBsAb 12 weeks after the last vaccination was considered effective. Secondary end-points were changes in the required HBIG dose.

The study protocol was approved by the institutional review board (No. P2005015-11X) and informed consent was obtained from each patient.

Statistical analysis

The number of units of HBIG administered before and during the study protocol was recorded, and the sum of the number of HBIG units administered at −24 to −1 weeks, 0–23 weeks, 24–47 weeks and 48–71 weeks was compared by the Friedman test.

RESULTS

ALL PATIENTS COMPLETED the full vaccination course. Double-dose vaccination was well-tolerated with the only side effect of local pain not requiring analgesics. During the study period, none of the cases developed active hepatitis or rejection. One patient developed kidney dysfunction during the study and was unable to complete the HBIG administration protocol (case #5).

Pre-, post- and maximum serum HBsAb titers are shown in Table 2. None of the cases developed a sufficient HBsAb titer level of >500 IU/mL (Fig. 1). None achieved cessation of HBIG administration.

Three cases (#2, #3, #20) developed maximum titers of 313, 408 and 469 IU/mL, and HBIG was thus discontinued in two of them (#2 and #3). The titers then decreased below 100 IU/mL in both cases, however, and HBIG administration was resumed (Fig. 2). Case #20 desired to continue HBIG administration despite the elevation of HBsAb titer to 469 IU/mL. Eventually, HBsAb titer dropped to 214 IU/mL and thus the vaccination was considered ineffective.

HBIG administration in four 24-week periods (−24 to −1, 0 to 23, 24 to 47, 48 to 71) in each case are shown in Table 2. In case 11, HBIG administration between weeks 48 and 71 was stopped due to the appearance of HBsAg and HBV-DNA. HBIG doses as a whole, excluding case 11, decreased over time ($P = 0.006$), as illustrated by the box plot (Fig. 3).

After the final vaccination, all patients were followed for a median of 17 (10–18) months. All patients are alive. In two cases (#11 and #13), serum HBsAg and HBV-DNA were observed at 4 and 8 months after the vaccination protocol, and 60 and 58 months after liver transplantation. In these two cases, the minimum HBsAb level was above 100 IU/mL even after our vaccination protocol. These cases are currently on antiviral therapy and are free from signs of active hepatitis.

DISCUSSION

ACTIVE IMMUNIZATION FOR chronic hepatitis B has been attempted in patients after liver transplantation. In early studies, commercially available recombinant vaccine was used in patients receiving HBIG mono-prophylaxis and was effective in 64–80% of patients.[9,19] This immune response was sustained for a longer follow-up period of 41 (31–85) months in 14 responders.[10] The use of a potential adjuvant in combination with recombinant vaccine is remarkably effective in 80% of post-transplant recipients, and the HBsAb titer was maintained for a long (8–27 months) follow-up period.[11] Studies by other groups, however, demonstrated unfavorable results in patients who were either on LAM prophylaxis or HBIG mono-prophylaxis.[12–14] Several factors are thought to

Table 2 Characteristics, HBsAb titer and dose requirement of HBIG

Case	Age	Sex	Etiology	Donor HBcAb	Pre-LT HBV-DNA	Pre-LT LAM	Timing of vaccination[†]	IS	Pre-HBsAb[‡]	Max HBsAb[‡]	End HBsAb[‡]	HBIG administration				Outcome[†] (recurrence[¶])
												−24–1 weeks[§]	48–71 weeks[§]	0–23 weeks[§]	24–47 weeks[§]	
1	52	F	PBC	+	Negative	NA	78	Tac	165	253	253	10	7	10	11	104, alive
2	46	F	AIH	+	Negative	NA	51	CyA	92	313	152	3	5	7	3	75, alive
3	54	M	B-FHF	–	Positive	Yes	48	Tac	122	408	136	12	5	8	5	65, alive
4	39	M	B-FHF	–	Positive	Yes	30	Tac	163	227	221	8	6	6	5	56, alive
5	50	M	BLC, HCC	+	Negative	Yes	90	Tac	175	79	48	6	5	6	7	115, alive
6	57	F	BLC	–	Positive	Yes	57	Tac	127	191	107	7	6	9	7	82, alive
7	47	F	BLC	–	Negative	Yes	56	Tac	140	186	132	7	6	6	6	81, alive
8	50	M	BLC, HCC	–	Negative	Yes	55	Tac	101	153	113	5	6	5	4	77, alive
9	50	M	BLC,	–	Positive	Yes	51	Tac	117	114	70	7	6	6	6	75, alive
10	51	M	BLC, HCC	–	Positive	Yes	49	Tac	111	293	138	8	5	6	5	74, alive
11	48	M	BLC, HCC	–	Positive	Yes	48	Tac	281	171	131	11	1	10	4	71, alive, rec 60
12	59	M	BLC	–	Positive	Yes	43	CyA	160	176	162	6	8	6	6	69, alive
13	52	M	BLC, HCC	–	Positive	Yes	43	Tac	209	210	132	8	7	6	6	67, alive, rec 56
14	57	F	BLC, HCC	–	Negative	Yes	41	CyA	109	220	220	12	7	10	8	67, alive
15	56	M	BLC, HCC	–	Negative	None	40	Tac	144	188	93	9	10	12	12	60, alive
16	54	M	BLC	–	Negative	Yes	40	Tac	137	190	190	8	6	5	5	60, alive
17	50	M	BLC	–	Negative	Yes	20	CyA	95	262	140	6	6	6	6	38, alive
18	51	M	BLC, HCC	–	Negative	Yes	19	CyA	135	179	115	11	8	10	9	44, alive
19	52	M	BLC, HCC	–	Negative	Yes	18	Tac	152	245	121	14	7	12	6	43, alive
20	53	M	BLC	–	Negative	Yes	17	Tac	302	469	214	14	10	12	12	37, alive

[†]Number indicates months after liver transplantation. [‡]IU/mL. [§]Number indicates vials of HBIG (1V = 1000 Units) used during the period. [¶]Number indicates months after liver transplantation, when HBV-DNA became positive.

AIH, autoimmune hepatitis; B-FHF, hepatitis B related fulminant hepatic failure; BLC, hepatitis B related liver cirrhosis; CyA, cyclosporine A; FHF, fulminant hepatic failure; HBsAb, hepatitis B surface antibody; HBcAb, hepatitis B core antibody; HBIG, hepatitis B immunoglobulin; HCC, hepatocellular carcinoma; IS, immunosuppression; LAM, lamivudine; LC, liver cirrhosis; LT, liver transplantation; PBC, primary biliary cirrhosis; Tac, tacrolimus.

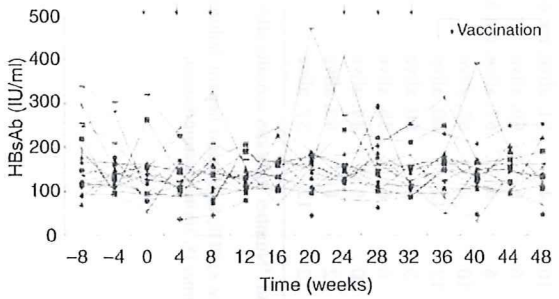

Figure 1 Change in serum hepatitis B surface antibody (HBsAb) titers before and during the vaccination protocol. Heptavax II (40 μg) was administered at weeks 0, 4, 8, 24, 28 and 32. HBsAb levels were between 100 IU/mL and 200 IU/mL in most cases. There was no significant response to vaccination throughout the study.

contribute to a good response, such as younger age, use of an adjuvant to the vaccine and negative HBV-DNA preoperatively. Use of LAM was once speculated to have a negative effect, and, in another study, Angelico *et al.* failed to reveal a favorable effect of mono-prophylaxis with HBIG.[12]

In our study, the double-dose double-phase use of a second generation recombinant hepatitis B vaccine was tested. Our institution applies an HBIG mono-prophylaxis protocol against hepatitis B recurrence. For non-replicate HBV disease, LAM is discontinued at the time of transplantation. During this study, HBIG was administered throughout the vaccination protocol according to Binzele's report,[11] and anti-hepatitis B antibody levels of greater than 500 IU/mL were determined to be effective. Unfortunately, none of the 20 patients developed an adequate anti-hepatitis B antibody titer after two vaccination cycles. Although the dose of HBIG administration decreased during and after the vaccination protocol, we did not consider it a significant change, since none achieved cessation of HBIG administration. The possible factors contributing to vaccination failure among the subjects are that 15 of the 20 patients were aged 50 years or older, eight of 18 chronic hepatitis B patients had a HBV replicative state at the

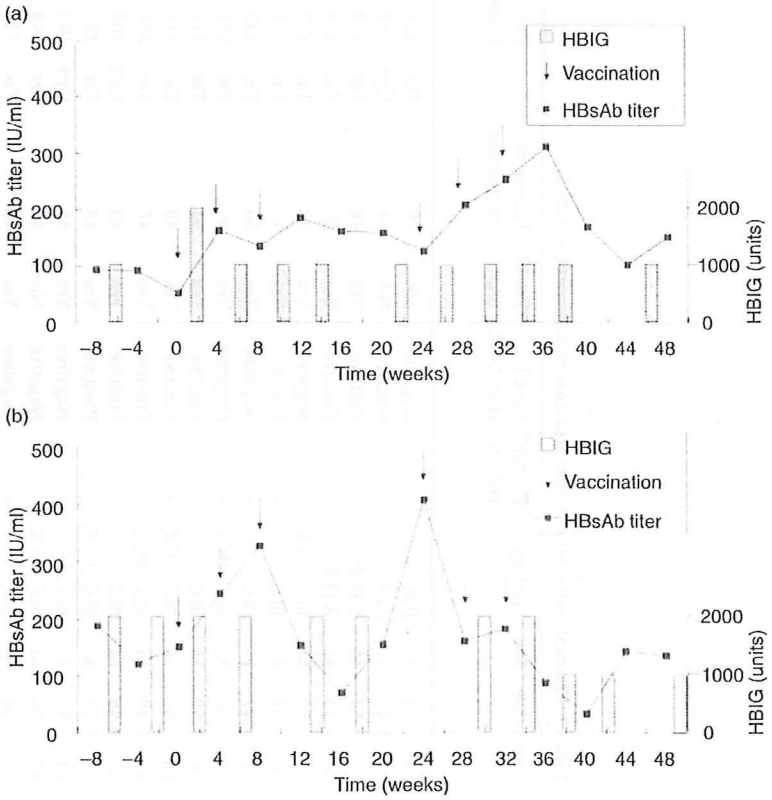

Figure 2 Hepatitis B surface antibody (HBsAb) titers in response to vaccination. (a) Case 2: 46-year-old female with auto-immune hepatitis (AIH), hepatitis B core antibody (HBcAb)-positive donor. (b) Case 3: 54 year-old male with B-fulminant hepatic failure. Hepatitis B immunoglobulin (HBIG) administration was skipped in response to elevated HBsAb level. The HBsAb titer then dropped and HBIG administration was resumed in both cases.

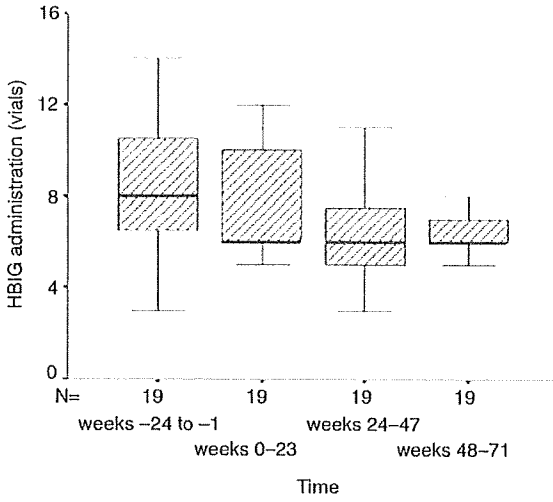

Figure 3 Hepatitis B immunoglobulin (HBIG) administration in four 24-week periods (−24 to −1, 0–23, 24–47, 48–71) is graphically shown by box plot. The dose of HBIG administration decreased over the time period ($P = 0.006$). Box plot explanation: upper horizontal line of box, 75th percentile; lower horizontal line, 25th percentile; horizontal bar within box, median; upper horizontal bar outside box, 90th percentile; lower bar outside box, 10th percentile.

time of transplantation, use of corticosteroids in combination with a calcineurine inhibitor, and one patient was receiving hemodialysis.[20] These factors might have affected the results of this study negatively.

A second generation recombinant HB vaccine is used for prophylaxis in the healthy population. HBsAg-specific T and B cells are induced and a sufficient amount of HBsAb can be produced to neutralize circulating HB virus particles. The recombinant HB vaccines containing S, pre-S2 and/or pre-S1, the so-called the third generation vaccines, have immunogenic advantages over the second generation recombinant HB vaccines; they more efficiently induce an immune response than the second generation HB vaccines among a healthy population,[21,22] and induce not only anti-S antibodies, but also anti-pre-S2 antibodies. Such a vaccine containing S, pre-S1 and pre-S2 antigens was used among Chinese patients who underwent liver transplantation for chronic HB and were on LAM prophylaxis.[23] The authors of that study reported that the vaccine was effective in 10 of 20 patients. An earlier study by Karasu *et al.*,[13] however, failed to show the effectiveness of pre-S1, pre-S2 and S gene products.

Prevention of hepatitis B infection is equally important in HBsAg-negative patients receiving HBc-positive donor organs, as the risk for de novo HB is high.[24] The current standard of care for transplant recipients of HBcAb-positive donor organs is similar to that for patients with chronic HB, including long-term HBIG and/or nucleos(t)ide analog.[18,24–26] In our study, HB vaccine was administered to two HBV non-infected patients who received HBcAb-positive donor organs. Neither of these two patients, however, responded. Pediatric transplantation recipients who receive prior vaccination under an immunization program are likely to achieve a high anti-HB titer by active immunization after LDLT.[27] LAM prophylaxis was withdrawn after 2 years if an adequate anti-hepatitis B titer was achieved. Soejima *et al.*[28] recently reported that 6 of 11 Japanese patients receiving liver transplants for fulminant hepatitis B or for non-HBV diseases with HBcAb-positive donor organs had seroconversion. The tested patients were younger, with a median age of 33 years. These patients were on combination prophylaxis with LAM and HBIG, and HBIG was withdrawn after vaccination. The question remains, however, as to whether LAM therapy can be discontinued in the future among this population.

Vaccination may be promising in selected populations, such as in younger recipients or in those with fulminant hepatitis or HBsAg-negative recipients receiving HBcAb-positive donor organs. For patients older than 50 with vaccination failure or chronic hepatitis B patients, a different approach may prove optimal, such as the use of a pre-S containing vaccination. The research findings for the use of such vaccines are controversial, however, warranting further study.

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