

Serologic status and vaccine response against hepatitis B virus after allogeneic hematopoietic cell transplantation in pediatric patients

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Abstract

Background: Although vaccination against hepatitis B virus (HBV) is recommended for hematopoietic cell transplantation (HCT) recipients, previous studies evaluating serologic status and immunologic response to HBV vaccination in pediatric allogeneic HCT recipients are not enough.

Objective: This study aimed to evaluate serologic status against HBV and immunologic responses to HBV vaccination in children and adolescents receiving allogeneic HCTs.

Methods: Medical records of the enrolled 61 pediatric patients < 19 years of age who received their first allogeneic HCTs were retrospectively reviewed.

Results: Twenty-two (36.1%) of the enrolled patients were positive for hepatitis B surface antibody (HBsAb) after HCT. Chronic graft-versus-host disease was significantly associated with negative HBsAb status after HCT ($p = 0.01$). With one dose of HBV vaccination after HCT, 40.5% of the vaccinated patients became positive for HBsAb. No clinical factor was associated with the positive conversion of HBsAb after vaccination.

Conclusions: Considering the unsatisfactory seropositive rate and vaccine response against HBV and the lack of significant clinical and laboratory factors predicting serostatus in HCT recipients, universal three doses of HBV vaccination should be necessary after allogeneic HCT.

Key words: hepatitis B virus, stem cell transplantation, leukemia, vaccination, child

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Introduction

The advancement of hematopoietic cell transplantation (HCT) techniques and conservative care has improved the survival of HCT recipients.¹ Especially in children expected to live for a longer time after HCT than adults, long-term care for improving the quality of life after HCT should be emphasized. Therefore, clinicians should make an effort to prevent community-acquired infection after HCT, and vaccination is one of the most effective strategies for preventing infection. Hepatitis B virus (HBV) infection is one of major vaccine-preventable diseases, and its worldwide prevalence has been decreasing with the introduction of infantile HBV vaccination.²

Korea has also become a low intermediate HBV endemic area from being a previously high HBV endemic area after the inclusion of HBV vaccination in the National Immunization Program (NIP).³ However, long-term survivors after HCT can travel to high HBV endemic areas and may participate in HBV infection-prone activities. Therefore, HBV vaccination should not be ignored in HCT recipients even though they live in low HBV endemic areas.

The 2013 Clinical Practice Guideline for Vaccination of the Immunocompromised Host of the Infectious Diseases Society of America (IDSA) states that HCT recipients should be

considered “never vaccinated” like neonates, and therefore, should receive most of the vaccines administered to infants and young children after HCT regardless of their ages.⁴ Accordingly, three doses of hepatitis B virus (HBV) vaccines are recommended for HCT recipients.⁴ However, a recent guideline of the 2017 European Conference on Infections in Leukaemia (ECIL7) for vaccination of HCT recipients recommends three doses of HBV vaccination after HCT based on the result of post-HCT serologic tests against HBV,⁵ which is different from the IDSA recommendation. Furthermore, previous studies evaluating serologic status and immunologic and clinical response to HBV vaccination in HCT recipients that can support the necessity of HBV vaccination after HCT are not enough.⁶⁻⁸

In our hospital, HBV vaccination after HCT has been decided based on the results of hepatitis B surface antibody (HBsAb) testing performed after HCT. In Korea, HBV vaccination was introduced in 1983, and was included in the NIP as mandatory vaccination in 1995.⁹ After then, the primary HBV vaccination (at birth, 1 month and 6 months of age) rate has maintained over 93%.¹⁰ Therefore, almost all of the pediatric HCT recipients were assumed to acquire a protective immunity against HBV infection from primary vaccination series during their infancy. Accordingly, the HBV re-vaccination strategy for healthy hosts, who were negative for HBsAb despite previous reception of primary HBV vaccination series, has been applied for pediatric HCT recipients in our hospital, without a consideration of immunosuppression effects of HCT.

As a result, HBV vaccination was completed with a single dose administration in HCT recipients who exhibited positive seroconversion with one dose of HBV vaccination after HCT, and three doses of HBV vaccination was administered to HCT recipients who were still negative for HBsAb after one dose of vaccination after HCT. This study was performed to evaluate the appropriateness of our vaccination strategy and to establish a proper vaccination strategy for pediatric HCT recipients. Serologic status against HBV was evaluated in children and adolescents receiving allogeneic HCTs, and immunologic responses to HBV vaccination after HCT were determined.

Materials and methods

Patients

Among children and adolescents aged less than 19 years old who received allogeneic HCTs in Seoul St. Mary’s Hospital, Seoul, Korea, 105 patients were requested to infectious disease physicians for vaccination after HCT between January 2015 and June 2018. Of the 105 patients, 69 patients, whose HBsAb titers were measured using a quantitative electrochemiluminescence immunoassay (ECLIA) test, were included in this study. Those whose HBsAb tests were performed using radioimmunoassay were excluded. Excluding eight patients who received more than one HCT, the remaining 61 patients receiving their first HCTs were finally enrolled in this study (Figure 1). In our hospital, HCT recipients are requested for vaccination when one year or more has passed since their allogeneic HCTs and immune suppression therapy has finished

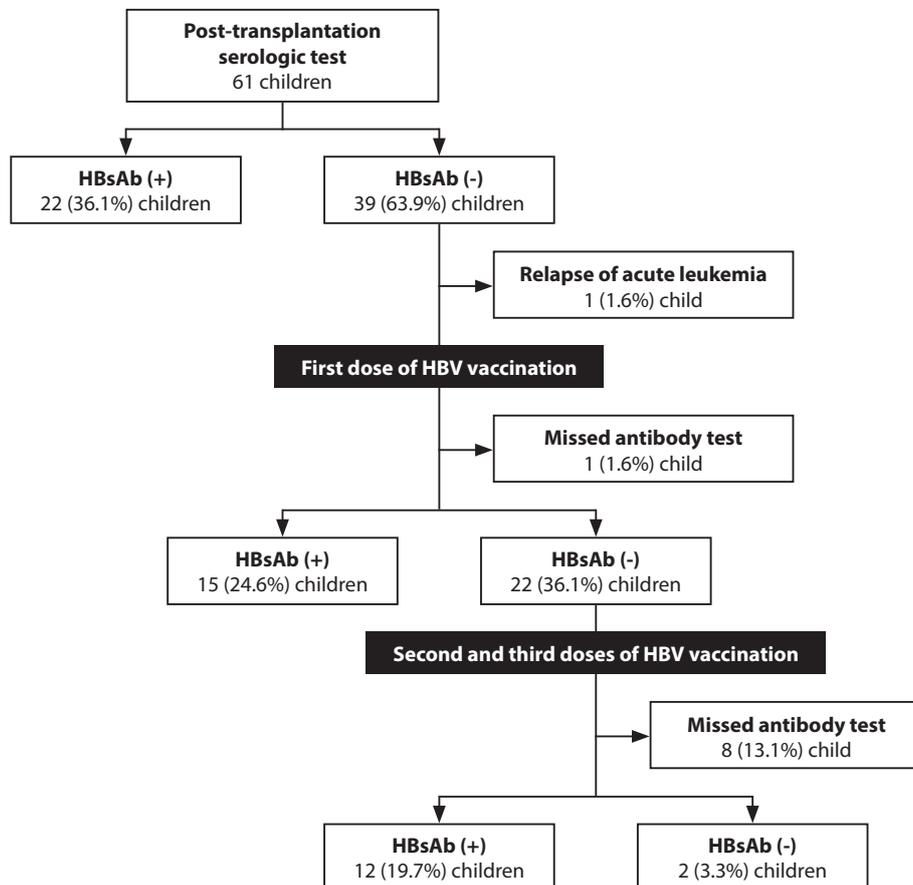


Figure 1. Summary of the study results.

with controlled graft-versus-host diseases (GvHDs). For GvHD prophylaxis after allogeneic HCT, cyclosporine was administered from day -1, and four doses of intravenous methotrexate (5 mg/m²) were administered on days 1, 3, 6, and 11. Intravenous immunoglobulin (IVIG, 400-500 mg/kg) was administered six times every 2 weeks from 1 week after HCT, and then six times monthly. IVIG replacement was discontinued when renal dysfunction occurred. This study was approved by the Institutional Review Board of Seoul St. Mary's Hospital with waiver for acquiring informed consent (Approval number: KC18RESI0503).

Data collection and analysis

Medical records of the enrolled patients were retrospectively reviewed. Demographic data, including sex and ages when underlying hematologic/oncologic diseases were diagnosed and when the first HBsAb test after HCT was performed, were investigated. Clinical data, such as underlying hematologic/oncologic disease, donor type, hematopoietic cell source, pre-HCT conditioning regimen, and presence of acute and chronic GvHDs, were gathered. In addition, time intervals from HCT, from the completion of immune suppression, and from the last dose of IVIG supplement, to the first HBsAb test after HCT were determined. HBsAb status of the HCT donor, pre- and post-HCT HBsAb titers of the HCT recipient, white blood cell (WBC), neutrophil, lymphocyte, and monocyte counts on the first HBsAb test after HCT, and lymphocyte subset count and immunoglobulin levels at one year after HCT were investigated as laboratory data. HBsAb titers were measured using a commercial ECLIA kit (Eleclys[®] Anti-HBs, Roche Diagnostics GmbH, Mannheim, Germany). The measuring range of this ECLIA kit was from 2.00 IU/L to 1,000.00 IU/L, and therefore, the measured HBsAb titer < 2.00 IU/L and > 1,000.00 IU/L were regarded as 1.00 IU/L and 1,000.00 IU/L, respectively. HBsAb titers ≥ 10.00 IU/L and < 10.00 IU/L were defined as positive and negative results, respectively.

The enrolled patients were divided into HBsAb positive and negative groups based on the first HBsAb results after HCT. Investigated data were compared between the two patient groups to find significant factors for HBsAb positivity after HCT. If HBsAb was negative after HCT, one dose of HBV

vaccination was administered to the HCT recipient. HBsAb tests were repeated at least 4 weeks after vaccination. Patients were divided into HBsAb positive and negative groups again based on HBsAb results after HBV vaccination, and investigated data were compared between the two patient groups to determine significant factors for immunologic response to HBV vaccination after HCT.

Statistical analysis

For comparisons between patient groups, continuous and categorical data were compared using Mann-Whitney and Fisher's exact tests, respectively. The SPSS 21 program (IBM Corporation, Armonk, NY, USA) was used for statistical analyses, and statistical significance was defined as a *p*-value < 0.05.

Results

HBsAb status after HCT

Thirty-one (50.8%) of the enrolled 61 patients were males. The median age on the diagnosis of underlying hematologic/oncologic disease was 5 years (range, 0-17), and that on the HBsAb test after HCT was 8 years (range, 2-19). None of the enrolled patients were positive for hepatitis B surface antigen before and after HCT. HBsAb testing was performed a median of 14 months (range, 12-31) after HCT, a median of 5 months (range, 0-14) after completing immune suppression therapy, and a median of 6 months (range, 3-29) after the last administration of IVIG. HBsAb after HCT was positive in 22 (36.1%) patients (**Table 1**, **Figure 1**). Underlying disease, donor type, hematopoietic cell source, pre-HCT conditioning regimen, and positive donor HBsAb were not significantly associated with positive HBsAb after HCT (**Table 1**). Chemotherapy for underlying disease preceding HCT might influence on the loss of HBsAb before HCT; however, positive HBsAb rates after HCT were not significantly different between patients with underlying diseases requiring chemotherapy (leukemia, lymphoma, myelodysplastic syndrome, and hemophagocytic lymphohistiocytosis) and those with underlying diseases not requiring chemotherapy (severe aplastic anemia and Wiskott-Aldrich syndrome) (34.8% vs 40.0%, *p* = 0.76). Only chronic GvHD occurred more frequently in the HBsAb negative group than in the HBsAb positive group (*p* = 0.01, **Table 1**).

Table 1. Comparison between patients with negative and positive results for HBsAb after allogeneic HCT

Factor	HBsAb negative (N = 39)	HBsAb positive (N = 22)	<i>P</i>
Male sex	22 (56.4)	9 (40.9)	0.29
Age on the diagnosis of underlying disease (year)	5 (0-17)	6 (0-16)	0.72
Age on the first HBsAb test after HCT (year)	8 (2-19)	8 (2-18)	0.46
Underlying hematologic/oncologic diseases			0.76
Acute lymphoblastic leukemia	13 (33.3)	5 (22.7)	
Acute myeloid leukemia	9 (23.1)	7 (31.8)	
Other leukemias	2 (5.1)	2 (9.1)	
Lymphoma	1 (2.6)	0 (0.0)	
Myelodysplastic syndrome	4 (10.3)	2 (9.1)	

Table 1. (Continued)

Factor	HBsAb negative (N = 39)	HBsAb positive (N = 22)	P
Underlying hematologic/oncologic diseases (Continued)			
Severe aplastic anemia	9 (23.1)	5 (22.7)	
Hemophagocytic lymphohistiocytosis	1 (2.6)	0 (0.0)	
Wiskott-Aldrich syndrome	0 (0.0)	1 (4.5)	
Positive donor HBsAb*	33 (91.7)	21 (95.5)	1.00
HCT donor type*			0.30
Matched familial donor	8 (22.2)	2 (9.1)	
Matched unrelated donor	20 (55.6)	13 (59.1)	
Mismatched familial donor	5 (13.9)	2 (9.1)	
Mismatched unrelated donor	3 (8.3)	5 (22.7)	
Hematopoietic cell source			0.23
Bone marrow	7 (17.9)	2 (9.1)	
Peripheral blood	29 (74.4)	20 (90.9)	
Umbilical cord blood	3 (7.7)	0 (0.0)	
Pre-HCT conditioning therapy			0.79
Myeloablative conditioning	25 (64.1)	15 (68.2)	
Non-myeloablative conditioning	14 (35.9)	7 (31.8)	
Anti-thymocyte globulin use	31 (79.5)	20 (90.9)	0.31
Acute graft-versus-host disease	25 (64.1)	11 (50.0)	0.42
Chronic graft-versus-host disease [†]	13 (34.2)	1 (4.5)	0.01
Months from HCT to HBsAb test	14 (12-31)	14 (12-20)	0.97
Months from last dose of IVIG to HBsAb test	6 (3-29)	6 (4-11)	0.83
Months from the completion of immune suppression therapy to HBsAb test	5 (0-14)	5 (0-9)	0.73
Pre-HCT positive HBsAb [‡]	17 (63.0)	9 (90.0)	0.22
Blood cell count on the first HBsAb test (cells/mm ³)			
White blood cell count	6,280 (1,280-11,600)	7,100 (4,240-13,920)	0.39
Absolute neutrophil count	2,638 (307-5,554)	3,234 (1,594-7,795)	0.30
Absolute lymphocyte count	2,749 (589-7,076)	2,819 (1,551-5,707)	0.44
Lymphocyte subset count at 1 year after HCT (cells/mm ³) [§]			
Absolute lymphocyte count	2,352 (284-8,935)	2,699 (1,032-6,938)	0.23
CD3+ cell count	1,574 (220-5,450)	1,666 (636-4,468)	0.41
CD4+ cell count	575 (82-2,469)	629 (176-1,238)	0.81
CD8+ cell count	709 (98-3,558)	907 (281-3,254)	0.36
CD19+ cell count	391 (1-1,835)	471 (59-1,603)	0.88
CD56+ cell count	155 (27-1,608)	330 (45-788)	< 0.01

Table 1. (Continued)

Factor	HBsAb negative (N = 39)	HBsAb positive (N = 22)	P
Immunoglobulin levels at 1 year after HCT (mg/dL) [§]			
IgG	948 (385-1,669)	989 (497-1,268)	0.78
IgM	80 (2-460)	94 (22-184)	0.32
IgA	64 (12-218)	79 (26-272)	0.46
IgE	53 (1-3,572)	195 (1-2,370)	0.11

Values are presented as number (%) or median (range).

*Donor HBsAb status and donor type could not be specified in three patients receiving cord blood transplantation in the HBsAb negative group.

[†]The development of chronic graft-versus-host diseases could not be determined in one patient in the HBsAb negative group.

[‡]Pre-HCT HBsAb status was identified in 27 patients in the HBsAb negative group and 10 patients in the HBsAb positive group.

[§]Lymphocyte subset counts and immunoglobulin levels at 1 year after HCT were not tested in one patient in the HBsAb negative group.

HBsAb, hepatitis B surface antibody; HCT, hematopoietic cell transplantation; IVIG, intravenous immunoglobulin

Pre-HCT HBsAb status was identified in 37 (60.7%) patients. All the HBsAb tests were performed on the diagnosis of underlying disease rather than immediately before HCT. Among them, 26 (70.3%) patients were positive for HBsAb before HCT, and nine (34.6%) of them remained positive for HBsAb after HCT. One (9.1%) of 11 patients who were negative for HBsAb before HCT became positive for HBsAb after HCT. For this patient, donor HBsAb was positive. The presence of HBsAb before HCT was not significantly associated with positive HBsAb after HCT ($p = 0.22$); however, the median HBsAb titer before HCT (361.50 IU/L; range, 4.13-1,000.00) of patients with positive HBsAb after HCT was significantly higher than that (25.48 IU/L; range, 1.00-549.80) of those with negative HBsAb after HCT ($p = 0.01$).

Immunologic response to HBV vaccination after HCT

Among the 39 patients with negative HBsAb after HCT, 38 patients received one dose of HBV vaccination (Figure 1). The remaining one patient was diagnosed with relapse of underlying acute myeloid leukemia after the HBsAb test. HBsAb tests were repeated in 37 of the vaccinated 38 patients. Among the 37 patients, 15 (40.5%) patients became positive for HBsAb (Figure 1). No clinical factors were significantly associated with a positive response to HBV vaccination after HCT (Table 2). The presence of HBsAb before HCT was not significantly associated with a positive conversion of HBsAb after HBV vaccination ($p = 1.00$), and the median HBsAb titer before HCT (13.83 IU/L; range, 1.00-205.30) of patients

with positive HBsAb after HBV vaccination was not significantly different from that (28.07 IU/L; range, 1.00-549.80) of those with negative HBsAb after vaccination ($p = 0.78$). In addition, the median HBsAb titers measured between HCT and HBV vaccination were also not significantly different between patients with positive and negative HBsAbs after HBV vaccination (1.00 IU/L [range, 1.00-8.50] vs 1.00 IU/L [range, 1.00-9.31]).

The WBC ($p = 0.02$) and absolute neutrophil ($p = 0.04$) counts determined on the day of first HBsAb test after HCT were significantly higher in patients who did not respond to HBV vaccination after HCT than in those who responded to HBV vaccination after HCT (Table 2). The WBC and lymphocyte subset counts determined at 1 year after HCT were also higher in patients who did not respond to HBV vaccination after HCT than in those who responded to HBV vaccination, although it was not statistically significant (Table 2).

All the 22 patients with negative HBsAb after one dose of HBV vaccination completed three doses of HBV vaccination schedule (Figure 1). In eight patients of them, HBsAb status could not be determined after three doses of vaccination because HBsAb testing was not ordered. The remaining 14 patients repeated HBsAb tests after the third dose of HBV vaccination, and 12 (85.7%) of them became positive for HBsAb (Figure 1). The two seronegative patients received an additional one dose of HBV vaccination 6 and 13 months after their third vaccination, respectively, and became positive for HBsAb.

Table 2. Comparison between patients with negative and positive results for HBsAb after one dose of HBV vaccination

Factor	HBsAb negative (N = 22)	HBsAb positive (N = 15)	P
Male sex	13 (59.1)	8 (53.3)	0.75
Age on the diagnosis of underlying disease (year)	6 (0-17)	5 (2-15)	0.70
Age on the first HBsAb test after HCT (year)	10 (2-19)	7 (3-18)	0.64

Table 2. (Continued)

Factor	HBsAb negative (N = 22)	HBsAb positive (N = 15)	P
Underlying hematologic/oncologic disease			0.39
Acute lymphoblastic leukemia	9 (40.9)	4 (26.7)	
Acute myeloid leukemia	4 (18.2)	4 (26.7)	
Other leukemias	2 (9.1)	0 (0.0)	
Lymphoma	1 (4.5)	0 (0.0)	
Myelodysplastic syndrome	1 (4.5)	3 (20.0)	
Severe aplastic anemia	5 (22.7)	3 (20.0)	
Hemophagocytic lymphohistiocytosis	0 (0.0)	1 (6.7)	
Positive donor HBsAb*	16 (84.2)	15 (100.0)	0.24
HCT donor type*			0.70
Matched familial donor	5 (26.3)	3 (20.0)	
Matched unrelated donor	9 (47.4)	10 (66.7)	
Mismatched familial donor	3 (15.8)	1 (6.7)	
Mismatched unrelated donor	2 (10.5)	1 (6.7)	
Hematopoietic cell source			0.21
Bone marrow	5 (22.7)	2 (13.3)	
Peripheral blood	14 (64.6)	13 (86.7)	
Umbilical cord blood	3 (13.6)	0 (0.0)	
Pre-HCT conditioning therapy			0.49
Myeloablative conditioning	16 (72.7)	9 (60.0)	
Non-myeloablative conditioning	6 (27.3)	6 (40.0)	
Anti-thymocyte globulin use	16 (72.7)	13 (86.7)	0.43
Acute graft-versus-host disease	16 (72.7)	7 (46.7)	0.17
Chronic graft-versus-host disease	7 (31.8)	5 (35.7)	1.00
Months from HCT to HBsAb test	14 (13-21)	13 (12-30)	0.14
Months from HCT to HBV vaccination	15 (13-22)	14 (13-42)	0.35
Months from last dose of IVIG to HBsAb test	6 (4-14)	5 (3-29)	0.13
Months from the completion of immune suppression therapy to HBsAb test	6 (0-13)	4 (0-8)	0.55
Pre-HCT positive HBsAb [†]	12 (63.2)	5 (71.4)	1.00
Blood cell count on the first HBsAb test (cells/mm ³)			
White blood cell count	7,205 (2,590-11,600)	5,560 (1,280-9,000)	0.02
Absolute neutrophil count	3,482 (932-5,554)	1,953 (307-4,596)	0.04
Absolute lymphocyte count	3,046 (1,114-7,076)	2,164 (589-4,950)	0.07

Table 2. (Continued)

Factor	HBsAb negative (N = 22)	HBsAb positive (N = 15)	P
Lymphocyte subset count at 1 year after HCT (cells/mm ³) [‡]			
Absolute lymphocyte count	2,472 (977-5,972)	2,022 (284-8,935)	0.31
CD3+ cell (%)	1,571 (515-4,819)	1,413 (220-5,450)	0.60
CD4+ cell (%)	595 (186-1,466)	552 (82-1,394)	0.74
CD8+ cell (%)	795 (210-3,558)	627 (98-3,315)	0.55
CD19+ cell (%)	444 (1-1,835)	351 (21-1,226)	0.45
CD56+ cell (%)	181 (44-613)	134 (27-1,608)	0.07
Immunoglobulin levels 1 year after HCT (mg/dL) [‡]			
IgG	996 (385-1,669)	936 (602-1,409)	0.51
IgM	78 (2-460)	83 (42-251)	0.62
IgA	61 (12-218)	66 (38-143)	0.49
IgE	56 (1-3,056)	56 (8-3,572)	0.49

Values are presented as number (%) or median (range).

*Donor HBsAb status and donor type could not be specified in three patients receiving cord blood transplantation in the HBsAb negative group.

[†]Pre-HCT HBsAb status was identified in 19 patients in the HBsAb negative group and seven patients in the HBsAb positive group.

[‡]Lymphocyte subset counts and immunoglobulin levels at 1 year after HCT were not tested in one patient in the HBsAb positive group.

HBsAb, hepatitis B surface antibody; HBV, hepatitis B virus; HCT, hematopoietic cell transplantation; IVIG, intravenous immunoglobulin.

Discussion

This study investigated serologic status against HBV in children and adolescents receiving allogeneic HCT. In addition, the immunologic response to HBV vaccination after HCT was also determined. About two-thirds of allogeneic HCT recipients were negative for HBsAb after HCT, and less than half of the patients responded to one dose of HBV vaccination after HCT.

Pre-HCT positive HBsAb rate in this study was 70.3%, which is similar to the positive rate of 68.5% in healthy Korean children younger than 10 years of age.⁹ Park et al. reported that 69.1% of Korean adults who were HBsAb positive before allogeneic HCT remained positive for HBsAb after HCT,¹¹ whereas only 34.6% of pediatric patients who were HBsAb positive before allogeneic HCT remained HBsAb positive after HCT in this study. In Korea, HBV vaccination became mandatory in 1995, and a high vaccination rate has been maintained.¹⁰ Therefore, almost all patients enrolled in this study should acquire HBsAb through their primary HBV vaccination rather than natural HBV infection before HCT. However, 64.2% of the adult patients, with a median age of 35 years (range, 15-64), enrolled in the study of Park et al. were positive for hepatitis B core antibody,¹¹ and therefore, most of them should acquire HBsAb through previous HBV infection. Previous reports showed higher seropositive rate and higher HBsAb titers after HCT in HCT recipients experiencing HBV infection than in those receiving HBV vaccination before HCT.^{6,12} Even in low HBV endemic areas, HBV vaccination after HCT should be encouraged because almost all HCT recipients acquire immunity against HBV through vaccination and they are more likely to lose the immunity than those experiencing natural HBV infection before HCT.

In our hospital, the positive HBsAb rate of pediatric patients underwent chemotherapy for leukemia without HCT was 14.6%.¹³ Although allogeneic HCT is considered to cause more potent immune suppression compared to chemotherapy, the positive HBsAb rate was higher in patients receiving allogeneic HCT in this study than in those receiving chemotherapy only ($p = 0.17$). This difference might be caused by positive donor HBsAb and IVIG administered after HCT, although the small number of enrolled patients in this study could not be ignored. Previous studies and this study showed no significant association between positive HBsAb status after HCT and positive donor HBsAb,⁶⁻⁸ and transferred donor HBsAb tended to decrease as time went on.¹⁴ Most of the patients enrolled in this study had received 12 times of IVIG replacement after HCT. Although the half-life of administered IVIG was reported as 21 days in HCT recipients,¹⁵ multiple IVIG administration could lead to accumulation of HBsAb and resultant increase of HBsAb titers in a portion of HCT recipients. Considering the insignificant difference of HBsAb positivity rates between patients receiving HCT and those underwent chemotherapy in our hospital, further studies including more patients not receiving routine IVIG replacement after HCT are necessary for determining the IVIG effect.

Chronic GvHD was significantly associated with negative HBsAb status after HCT in this study, similar to previous studies.^{8,11} The CD56+ cell count was significantly higher in patients with positive HBsAb after HCT than in those with negative HBsAb after HCT in this study, while CD56+ cells do not participate in antibody production.¹⁶ Considering a significant association between a persistent low CD56+ cell count

after HCT and development of chronic GvHD was previously reported,^{17,18} the lower CD56+ cell count might be associated with higher frequency of chronic GvHD in patients with negative HBsAb after HCT compared to those with positive HBsAb after HCT.

The positive conversion rate of HBsAb after boosting HBV vaccination in healthy hosts who previously received three doses of HBV vaccination was reported to be 88%.¹⁹ The positive conversion rate of HBsAb after one dose of HBV vaccination was 40.5% in this study; this was between the positive conversion rates in infants at 25% and in young adults at 30-55%, who received the first dose of HBV vaccine in their life.¹⁹ Therefore, the positive conversion rate of HBsAb after one dose of HBV vaccination in this study seemed not to represent an anamnestic response to boosting vaccination, but to represent a response to the first dose among three doses of vaccination series in HCT recipients losing their protective immunity against HBV through HCT. If we consider that (1) a portion of positive HBsAb after HCT might be caused by repeated IVIG replacement therapy, (2) a low positive conversion rate of HBsAb after one dose of vaccination might represent the loss of protective immunity after HCT, and (3) the positive rate and titer of HBsAb had decreased over time since HCT in previous studies,^{8,11} a universal three doses of HBV vaccination seems to be adequate for HCT recipients. In addition, 14.3% of patients receiving three doses of HBV vaccination after HCT were still negative for HBsAb, and therefore, HBsAb re-testing is necessary after completing three doses of vaccination in HCT recipients in consistent with the IDSA and ECIL7 guidelines.^{4,5} In healthy hosts, the positive conversion of HBsAb after boosting HBV vaccination was significantly associated with the vaccinee's HBsAb titer before boosting vaccination.²⁰ In this study, the median of HBsAb titers measured between HCT and HBV vaccination was not significantly different between patients with positive and negative HBsAb results after HBV vaccination. Further studies on the association between HBsAb titer after HCT and seroconversion rate after HBV vaccination may be helpful for establishing a strategy to decide the doses of HBV vaccination in HCT recipients based on their HBsAb titers.

The association between low CD4+ cell count and low response rate to HBV vaccination after HCT was previously reported.²¹ However, in this study, WBC and lymphocyte counts of patients who did not respond to HBV vaccination after HCT were higher than those of patients who responded to HBV vaccination, although it was not statistically significant. This discrepancy can represent the importance of the recovery of immune cell function and memory cell count rather than total immune cell count for an adequate immunologic response in HCT recipients.²²⁻²⁴ Therefore, appropriate strategies for post-HCT vaccination based on the recovery of naïve and memory cell counts and the restoration of their function should be established in future studies.

This study had several limitations. The number of enrolled patients was small due to exclusion of patients in whom HBsAb testing was performed using radioimmunoassay. In addition, 39.3% of the enrolled patients had no data on pre-HCT HBsAb status. Even in those with pre-HCT HBsAb results, their HBsAb tests were performed on the diagnosis of

underlying diseases rather than just before HCT. Therefore, the negative effect of chemotherapy and HCT on immunity against HBV could not be independently determined. In addition, the association between HBsAb titer measured just before HCT and immunologic response to HBV vaccination after HCT could not be determined. Pre-HCT HBV vaccination history was not identified in most of the enrolled patients and hepatitis B core antibodies were not tested. Therefore, the exact influence of pre-HCT HBV infection and vaccination on serologic status after HCT was not determined although few patients might be previously infected by HBV under a high HBV vaccination rate during infancy and low intermediate prevalence of HBV infection in Korea. To overcome these limitations, a well-designed prospective study establishing the type and time of serologic tests and identifying pre- and post-HCT vaccination history is necessary. Some of patients with positive HBsAb results after HCT or after one dose of HBV vaccination after HCT could have their own protective immunity, not derived from donor HBsAb or administered IVIG. These patients will exhibit an anamnestic response to boosting HBV vaccination several years after HCT. Therefore, future studies evaluating such anamnestic responses should be helpful for selecting vaccination candidates among HCT recipients.

In conclusion, this study confirmed the necessity of universal HBV vaccination after allogeneic HCT. Especially in countries where almost all children acquire HBV immunity through vaccination, post-HCT vaccination should be encouraged. Based on no existence of significant clinical and laboratory factors predicting seropositive status and vaccination response after HCT, uncertain origin of HBsAb after HCT or after one dose of HBV vaccination, and unsatisfactory positive conversion rate of HBsAb after one dose of HBV vaccination, three doses of HBV vaccination should be adequate for HCT recipients. In addition, HBsAb re-testing is necessary after three doses of vaccination.

Conflict of interest

There is no conflict of interest for all authors of this study.

Author contributions

SBH, BC and JHK conceptualized and designed this study. ESL, SKK and JWJ collected the data. JWJ, NGC and DCJ analyzed the data. ESL, SKK and SBH wrote the original manuscript. BC, NGC and JHK critically reviewed and revised the manuscript.

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