Original Article

Korean J Pediatr Infect Dis 2014;21:81-95
DOI: http://dx.doi.org/10.14776/kjpid.2014.21.2.81

ISSN 1226-3923 (print)
ISSN 2289-0343 (online)

Antibody Responses in Hematopoietic Cell Transplantation Recipients after Vaccination Against *Haemophilus Influenzae* Type b and *Streptococcus pneumoniae*

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Purpose: Hematopoietic cell transplantation (HCT) recipients are vulnerable to invasive infection by *Haemophilus influenzae* type b (Hib) and *Streptococcus pneumoniae* (Sp). This study was performed to evaluate immune responses after Hib and Sp vaccination in Korean pediatric HCT recipients.

Methods: Patients were prospectively enrolled at Samsung Medical Center during 2009-2011. ELISA tests to detect anti-PRP IgG antibody and antibodies to Sp serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F were performed at the Center for Vaccine Evaluation and Study, Ewha Medical Research Institute.

Results: Ten patients (two allogeneic, eight autologous recipients) with median age 5.4 years (range 2.7-12.2 years) were enrolled. Before Hib vaccination, 60% of patients' anti-PRP IgG titers were below 0.15 μg/mL. After vaccination, 100% of patients' anti-PRP IgG titers increased above 0.15 μg/mL (cut-off value for detection) and 1.0 μg/mL (cut-off value for seroprotection). For pneumococcus, in 2-5 year-old patients, pre-vaccination geometric mean concentrations (GMCs) of IgG for six serotypes (4, 6B, 9V, 14, 18C, and 23F) were below 0.35 μg/mL and at 5 months post-vaccination GMCs of IgG for all seven serotypes increased to above 0.35 μg/mL. In patients older than 5 years, pre-vaccination GMCs of IgG for four serotypes (4, 9V, 14, and 23F) were below 0.35 μg/mL and at 3 months post-vaccination GMCs of IgG for all seven serotypes increased to above 0.35 μg/mL.

Conclusion: Most HCT recipients had low or no protective antibodies to Hib and Sp before vaccination, but showed good immune responses to protective levels after vaccination.

Key Words : Stem cell transplant, vaccination, Hib, Pneumococcus

Introduction

Haemophilus influenzae type b (Hib) and *Streptococcus pneumoniae* (Sp) are well-known bacterial pathogens with polysaccharide capsules and are capable of serious invasive infection in children.

Received : 10 June 2013, Revised : 2 May 2014
Accepted : 5 May 2014
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Hematopoietic cell transplantation (HCT) recipients are highly immunocompromised and are susceptible to many viral and bacterial infections, including Hib and Sp. Vaccines against these pathogens are thus typically recommended in HCT recipients[1, 2].

Antibody titers to vaccine-preventable infections such as tetanus, polio, measles, mumps, and rubella decline during the 1-10 years after allogeneic or autologous HCT if the recipient is not revaccinated [3]. In these patients, infections are more frequent and/or severe than in the general population[4]. Therefore HCT recipients should be routinely revaccinated after HCT in order to develop immunity to these pathogens.

HCT recipients have qualitative and quantitative T and B cell deficiencies and functional granulocyte deficiencies (impaired chemotaxis), which predispose them to be susceptible to serious invasive infection by encapsulated bacteria such as Hib and Sp[5, 6].

The epidemiology of fatal infection due to Sp has been well studied in terms of both early and late post-HCT periods by the European Group for Blood and Marrow Transplantation (EBMT) survey of 3,451 HCT recipients[7]. The incidence of invasive pneumococcal disease (IPD) was 2.03/1,000 transplants in the early post-HCT period and 8.63/1,000 transplants in the late post-HCT period. In addition, it was increased in patients with chronic graft versus host disease (GVHD) to up to 18.85/1,000 transplants. Although autologous HCT recipients are less prone than allogeneic recipients to develop IPD, the incidence was still high, with an incidence of 4.6/1,000 transplants. The mortality rate was 20%. In a US study of 7,888 HCT recipients, the incidence of IPD was 7/1,000 transplants and the mortality rate was 13%[8]. Compared to Sp infection data, there are only handful of data regarding Hib infection in HCT recipients. Although fatal infection is less common than pnuemococcus, Hib is another encapsulated bacteria that can cause infectious complication in HCT recipients. In late 1970s, a study reported that about 7% of bacterial infection was caused by Hib infection in 89 long-term survivors after HCT. In 1980s studies, Hib was also observed as one of the pathogens responsible for pulmonary infection complications in HCT recipients[6, 9, 10].

Many pediatric HCT recipients survive longer and grow into adulthood as long-term survivors. Advanced and well-planned care is needed after HCT for optimal health. Vaccination of pediatric HCT recipients and assessment of their immune response is of critical importance for protection against vaccine-preventable infections. Here, we report the results of a prospective study of pediatric HCT recipients who were vaccinated against Hib and Sp during the post-HCT period and their immune responses to both vaccinations.

Materials and Methods

1. Participants and Vaccination

1) Patients

Pediatric HCT recipients were prospectively enrolled and followed at Samsung Medical Center, Seoul, Korea from 2009 to 2011. At the time of enrollment, patients were stable without any evidence of relapse of primary cancer and more than 12 months had passed after their last HCT. Patients were divided into two groups (group I and group II) based on their age at presentation for vaccination. Group I included patients from 2-5 years of age

and group II included patients who were older than 5 years. Patients' clinical information was reviewed. Pre-vaccination immune status was evaluated at the department of laboratory medicine at least one year after the last HCT. Overall humoral immunity was evaluated by measuring levels of IgG, IgA, IgM, IgD and IgE. Cellular immunity was assessed by complete blood counts, analysis of lymphocyte subsets, and lymphocyte proliferation tests which were performed by stimulating T cells with phytohemagglutinin (PHA) and concanavalin A (ConA).

2) Vaccination Schedule and Serum Collection

Patients in group I and group II received post-HCT vaccinations against Hib and Sp, and serum collection was performed as scheduled (Table 1). At the time when this study was designed and planned, the US Centers for Disease Control and Prevention (CDC) recommended three doses of Hib vaccine at 12, 14, and 24 months after HCT and the EBMT recommended three doses of Hib starting at 6 months post-HCT at 1-3 month intervals. For Sp, 23-valent pneumococcal polysaccharide vaccine (PPSV23) was recommended at 12 months and 24 months after HCT in older individuals (2 years old) by the US CDC[1]. On the other hand, the EBMT recommended only one dose of PPSV23 at 12 months after HCT[4]. At that time, there were no official guidelines for vaccination with 7-valent pneumococcal conjugate vaccine (PCV7). However, since there was increasing evidence for immunogenicity and safety of PCV7 vaccination in pediatric HCT recipients[11-13], we chose to combine the PCV7 and PPSV23 schedule in children 2-5 years of age. In children >5 years old, we chose one dose of PPSV23 for our study protocol.

Patients in both groups received Hib polysaccharide conjugated to diphtheria toxoid CRM197 (FirstHib, SK Chemicals, Sungnam-Si, Korea) at 0, 2, and 12 months. For pneumococcus, children in group I received PCV7 (Prevenar^{TM}, Wyeth Lederle Vaccines S.A., Louvain-la-Neuve, Belgium) at 0 and 2 months and additional PPSV23 (Pneumo-23®, Sanofi Pasteur

Table 1. Schedule for Vaccination and Serum Collection

	Month	0	1	2	3	4	5	6	7	8	9	10	11	12	13
Group I	Hib	↓		↓										↓	
2-5 years	PCV7	↓		↓											
	PPSV23					↓									
	Serum collection	↓			↓		↓								↓
Group II	Hib	↓		↓										↓	
>5 years	PPSV23	↓													
	Serum collection	↓	↓		↓										↓
All patients	HepB	↓		↓										↓	
	DTaP or Td	↓		↓										↓	
	IPV	↓		↓										↓	
	HepA	↓						↓							

Abbreviations: Hib, *Haemophilus influenzae* type b; PCV7, 7-valent pneumococcal conjugate vaccine; PPSV23, 23-valent pneumococcal polysaccharide vaccine; HepB, hepatitis B vaccine; DTaP, diphteria-tetanus-acellular pertussis vaccine; Td, tetanus-diphteria vaccine; IPV, inactivated polio vaccine; HepA, hepatitis A vaccine; MMR, measles-mumps-rubella vaccine was given to patients when at least 2 years have passed after last HCT and the patient was off from immunosuppressive agent or intravenous immunoglobulin for at least one year more.

SA, Lyon, France) at 4 months. Children in group II received PPSV23 (Pneumo-23®, Sanofi Pasteur SA, Lyon, France) at 0 months. Other childhood vaccines (hepatitis B, diphtheria-tetanus-acellular pertussis, inactivated poliovirus, hepatitis A, influenza, or others) were also given (Table 1).

Blood was drawn for serological testing at month 0 (before the vaccination), 3 (one month after the second vaccination of Hib and PCV7), 5 (one month after the vaccination of PPSV23) and 13 (one month after the third vaccination of Hib) in group I and at month 0 (before the vaccination), 1 (one month after the vaccination of PPSV23), 3 (one month after the second vaccination of Hib), and 13 (one month after the third vaccination of Hib) in group II. All sera were stored at −80℃ within 1 hour of collection until analyzed.

2. Methods to Detect Immunogenicity to Vaccination

1) Enzyme-Linked Immunosorbent Assay (ELISA) for anti-Polyribosylribitol Phosphate (anti-PRP) IgG for Hib

Anti-PRP antibodies were measured by an enzyme-linked immunosorbent assay (ELISA) modification as previously described[14, 15]. The ELISA was performed at the Center for Vaccine Evaluation and Study, Medical Research Institute, Ewha Womans University. PRP purchased from the National Institute for Biological Standards and Control (NIBSC, Hertfordshire, UK) was used as the antigen, and the standard curve was generated using reference serum lot 1983 (provided by Carl Frasch, Center for Biological Evaluation and Review, Food and Drug Administration, Bethesda, MD, U.S.A.) with a calculated IgG antibody concentration of 60.9 μg/mL. The cut-off of the ELISA for PRP detection was 0.15 μg/mL and seroprotection was defined as an antibody concentration ≥1.0 μg/mL after vaccination[13, 16]. The seroprotection response rate was analyzed. The proportion of subjects achieving levels ≥0.15 μg/mL was also calculated.

2) ELISA for IgG for Sp

Anti-pneumococcal antibodies against serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F were measured by ELISA using both C-polysaccharide (C-PS) and 22F serotype capsular polysaccharide absorption, as previously described[17, 18]. ELISA was performed at the Center for Vaccine Evaluation and Study, Medical Research Institute, Ewha Womans University. Briefly, 96-well medium-binding microtiter plates (Corning Inc., Corning, NY, U.S.A.) were coated with 100 μL of a serotype-specific pneumococcal PS antigen (American Type Culture Collection [ATCC], Manassas, VA, U.S.A.) diluted to a predetermined concentration, and plates were incubated at 37℃ for 5 hours in a humidified chamber. The coated plates were washed with 1x Tris-buffered saline with 0.01% Brij 35 solution. Test sera were preabsorbed with C-PS (Statens Serum Institut, Copenhagen, Denmark) and 22F capsular PS (ATCC), and the reference standard 89-SF (provided by Carl Frasch, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD, U.S.A.) was preabsorbed with C-PS. Sera were serially diluted 2.5-fold using the absorption solution and were incubated at room temperature (RT) for 30 minutes. After incubation, the sera (50 μL) were transferred to the coated microtiter plates, and the plates were incubated for 2 hours at RT. After the plates were washed five times, 100 μL of diluted alkaline phosphatase-conjugated goat anti-human IgG (Southern Biotech, Birmingham, AL, U.S.A.)

was added to each well, followed by a two-hour incubation. The plates were washed five times, and 100 μL of substrate solution [diethanolamine (Sigma, St. Louis, MO, U.S.A.) with 1 mg/mL p-nitrophenyl phosphate powder (Sigma)] was added to each well. After another two-hour incubation at RT, 50 μL of 3 M NaOH was added to all wells to stop the enzyme reaction. The optical density was measured at 405 nm and the optical density at 690 nm was subtracted. Optical densities were converted to antibody concentrations using the software for pneumococcal ELISA provided by the CDC (written by Brian Plikaytis at the CDC, Atlanta, GA, U.S.A). A free software download is available from www.cdc.gov/ncidod/dbmd/bimb/elisa.htm, and a detailed protocol is available online at www.vaccine.uab.edu. Seroprotection from Sp infection was defined as an antibody concentration ≥0.35 μg/mL after vaccination [13, 19], and the seroprotection response was analyzed.

3. Statistical Analyses

Immunogenicity analyses after vaccination and safety assessments were based on the modified intent-to-treat cohort, which included all vaccinated infants for whom data were available. Medians were calculated for lymphocyte subset analysis and the geometric mean concentrations of pre-vaccination IgG, IgA, IgM, IgD, and IgE levels were calculated. For the lymphocyte proliferation assay, each patient's lymphocyte proliferation stimulation index (SI) was calculated and patients whose SI values were lower than 50% of the control were identified.

For Hib, serologic calculations were performed on logarithmically-transformed data. Antibody responses were assessed by calculating the geometric mean concentrations (GMC) with 95% confidence intervals (CI) and seroprotection response rates. Antibody concentrations below the cut-off value of 0.15 μg/mL were arbitrarily assigned a value corresponding to half of the cut-off value of the test, which was 0.08 μg/mL. The results were compared using Wilcoxon matched-pairs signed-ranks test for two-group analysis (e.g., month 0 vs. month 3, month 0 vs. month 13, or month 3 vs. month 13) and by nonparametric repeated measures ANOVA for analyses of multiple groups (e.g., month 0 vs. month 3 vs. month 13).

For Sp, the analyses of serum antibody concentrations were based on the logarithms of the antibody concentrations of all subjects. GMCs of anti-pneumococcal IgG antibodies were evaluated, and two-sided 95% confidence intervals were determined for each pneumococcal serotype. The proportions of subjects achieving anti-pneumococcal antibody titers ≥0.35 μg/mL were determined.

4. Ethical Considerations

The study protocol was approved by the Institutional Review Board at Samsung Medical Center (IRB No. 2009-07-0003) and was conducted in accordance with the Declaration of Helsinki and the Good Clinical Practice guidelines. Written informed consent was obtained from all parents or legal guardians following a detailed explanation of the study.

Results

1. Patient Characteristics and Immune Function before Vaccination

1) Patient Characteristics

Patients' characteristics are summarized in Table 2. Ten patients were enrolled, and the male to female

ratio was 1:1. The median age of patients at vaccination was 5.4 years (range 2.7–12.2 years). Eight patients had solid tumors (6 patients had neuroblastoma, one had anaplastic ependymoma and one had medulloblastoma), two patients had hematologic malignancy, acute myelogenous leukemia (AML) and these two patients received allogeneic HCT. All patients received peripheral stem cells with the exception of patient 9, who received bone-marrow-derived stem cells from an unrelated donor. The median time from HCT to vaccination was 13.9 months (range 12.5–54.1 months). Eight patients received their first vaccination between 12 and 17 months after HCT. Two patients (patient 5, who had neuroblastoma, and patient 9, who had AML) were vaccinated 54 months and 52 months after HCT (autologous for patient 5 and allogeneic for patient 9), respectively. With the exception of patient 4, who only received a first HCT, nine patients received HCT either two or three times.

2) Immune Function of Enrolled Patients before Vaccination

Immune function before vaccination was evaluated in all patients except patient 5 by determination of lymphocyte subsets, lymphocyte proliferation testing, and measurement of IgG, IgA, IgM, IgD, and IgE (Table 3 and 4, and Fig. 1). The patients' quantitative immune functions appeared to be recovering well. All of the patients' absolute lymphocyte counts were above 2,000/μL and their CD4 counts were above 200/μL. The median values of IgG, IgA, IgM, IgD, and IgE were 722.64 mg/dL, 42.25 mg/dL, 103.82 mg/dL, 1.02 mg/dL, and 12.52 IU/mL, respectively. All Ig levels in all 10 patients were within normal limits, except for a low IgA level in patient 1 (8 mg/dL) and a high IgE level in patient 9 (903.3 mg/dL).

Tests for lymphocyte function were also performed in all patients except patient 5 (Fig. 1). The SI values of patients 1, 4, 8, 9, and 10 were below

Table 2. Patient Characteristics

Patient ID	Diagnosis	HCT type	Type of stem cells	Age at vaccination (years)	Months from last HCT to vaccination start (months)	No of HCT performed
1	Anaplastic ependymoma	Auto	PBSC	2.7	13	2
2	NBL	Auto	PBSC	3.0	13	2
3	NBL	Auto	PBSC	3.3	13	2
4	AML	Allo	Unrelated PBSC	3.7	16	1
5	NBL	Auto	PBSC	7.5	54	2
6	NBL	Auto	PBSC	5.6	13	2
7	NBL	Auto	PBSC	5.4	13	2
8	MBL	Auto	PBSC	11.0	14	3
9	AML	Allo	Unrelated BM (1) Haplo PBSC (2) Mismatched unrelated BM (3)	12.2	52	3
10	NBL	Auto	PBSC	5.5	15	2

Abbreviations: ID, identification; HCT, hematopoietic cell transplantation; Auto, autologous; Allo, allogeneic; NBL, neuroblastoma; AML, acute myelogenous leukemia; MBL, medulloblastoma; PBSC, peripheral blood stem cell; BM bone marrow; haplo, haploidentical.

Table 3. Lymphocyte Subsets before Vaccination

Patient ID	ALC	CD3		CD4		CD8		CD4/CD8	CD19		CD16+56+3- (NK)		CD16+56+3+ (NKT)	
	Count (/μL)	Count (/μL)	%	Count (/μL)	%	Count (/μL)	%		Count (/μL)	%	Count (/μL)	%	Count (/μL)	%
1	3,414	2,102	62	1,157	34	926	27	1.25	733	21	501	15	77	2
2	3,710	1,919	62	801	26	1,048	34	0.76	462	15	555	18	92	3
3	3,706	1,279	35	889	24	815	22	1.09	1,779	48	482	13	408	11
4	2,738	1,417	52	904	33	466	17	1.94	794	29	383	14	27	1
6	4,394	1,714	39	747	17	967	22	0.77	1,626	37	791	18	44	1
7	2,910	2,273	79	1,455	50	758	26	1.92	424	15	152	5	61	2
8	2,289	704	31	389	17	320	14	1.21	1,236	54	252	11	23	1
9	3,625	2,030	56	690	19	1,230	34	0.56	690	19	830	23	–	–
10	2,098	1,059	51	399	19	608	29	0.66	860	41	147	7	0	0
Median	3,414	1,714	52	801	24	815	26	0.93	794	29	482	14	53	1.5

Abbreviations: ID, identification; ALC, absolute lymphocyte counts; Patient 5 was not tested.

Table 4. Immunoglobulin Values before Vaccination

Patient ID	IgG (mg/dL)	IgA (mg/dL)	IgM (mg/dL)	IgD (mg/dL)	IgE (IU/mL)
1	408	8	82	0.41	15
2	1,221	25	73	0.41	3.3
3	585	23	120	0.41	2.8
4	656	53	98	0.41	1.5
6	959	85	154	13.4	37.5
7	833	65	143	1.7	5.5
8	701	77	101	0.75	130
9	994	78	157	5.98	903.3
10	505	53	57	0.41	1.5
GMC	722.64	42.25	103.82	1.02	12.52

Abbreviations: ID, identification; Ig, immunoglobulin; GMC, geometric mean concentration. Patient 5 was not tested.

50% of normal control SI values when stimulated by PHA, and the SI values of patients 1, 4, 8, and 9 were below 50% of normal control SI values when stimulated by ConA.

3) Adverse Events after Vaccination

A total number of vaccinations were 16 in group I (four patients and 4 vaccination visits per patient). In group II, the third Hib vaccination was not performed in patient 6 and 7. Consequently, a total of sixteen vaccinations were performed in group II (six patients and 3 vaccination visits per patient). Any reported adverse events of grade III or more are shown in Table 5. No serious adverse events were observed during the study period.

2. Antibody Response to Hib

Anti-PRP IgG concentrations after Hib vaccination are shown in Table 6 and Fig. 2. Anti-PRP IgG values increased after vaccination in all patients, and significant differences in the geometric concentrations of anti-PRP IgGs were observed among pre-vaccination and post-vaccination values at months 3 and 13 (P<0.001, nonparametric repeated measures ANOVA test). When comparing pre-vaccination and post-vaccination month 3 (one month after two doses of Hib) values, there was a significant increase in anti-PRP IgG titers (P=0.002, Wilcoxon matched-pairs signed-ranks test).

Sixty percent (6/10) of the patients' pre-vaccination anti-PRP IgG titers were below 0.15 μg/mL and 1.0 μg/mL, respectively. After two doses of Hib vaccination, all anti-PRP IgG titers increased above 1.0 μg/mL, except in patient 2, whose antibody level

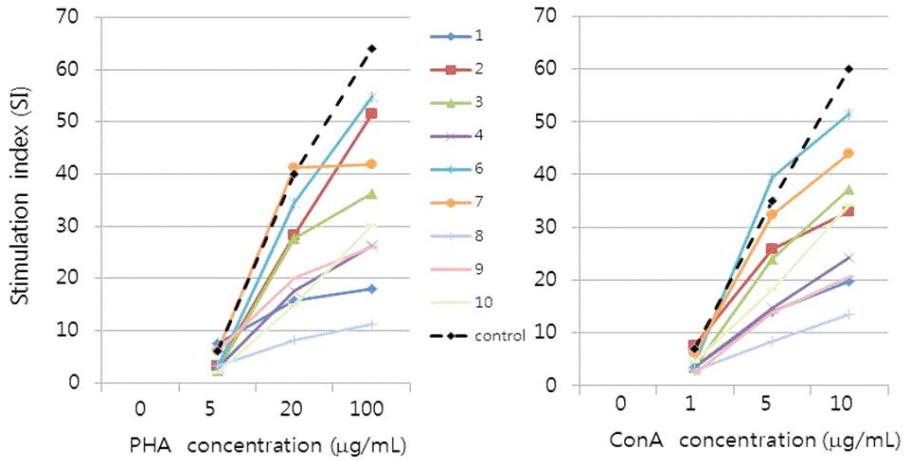

Fig. 1. Lymphocyte proliferation test of enrolled patients before vaccination. Patients' lymphocyte proliferation activity was measured by stimulating lymphocytes in vitro with PHA and ConA, and SI was calculated by comparing the value to normal controls. Each line shows changes in SI values according to the concentration of PHA and ConA. Black dotted lines represent the median SI values of lymphocytes from immunocompetent normal control individuals. PHA, phytohemagglutinin; ConA, concanavalin A; SI, stimulation index.

Table 5. Reported Adverse Events of Grade III or more after Vaccination

	Group I (n=16)*		Group II (n=16)†		Total (n=32)	
	Case	%	Case	%	Case	%
Redness	0	0.0	1	6.25	1	3.1
Pain	0	0.0	1	6.25	1	3.1
Swelling	0	0.0	0	0.0	0	0.0
Induration	0	0.0	0	0.0	0	0.0
Fever (≥39.5℃)	0	0.0	0	0.0	0	0.0
Lethargy	0	0.0	0	0.0	0	0.0
Allergic reaction	0	0.0	1	6.25	1	3.1
Rash	0	0.0	1	6.25	1	3.1
Muscle pain	0	0.0	0	0.0	0	0.0
Joint pain	0	0.0	0	0.0	0	0.0
Summary	0	0.0	4	25.0	4	12.5

*Four patients and four vaccination visits per patients.
†Six patients and three vaccination visits per patients. Patient 6 and 7 didn't receive the third vaccination.

at post-vaccination month 3 after two doses of Hib was 0.20 μg/mL. However, this patient's anti-PRP IgG also eventually increased to 36.74 μg/mL at post-vaccination month 13 after three doses of Hib. Of note, patients 1, 4, 8, and 9, all of whom had low lymphocyte proliferation SI values by both PHA and ConA stimulation, still showed good immune responses to Hib vaccination one month after three doses of Hib vaccination. In addition, patients 8 and 10, who had CD4 counts less than 500/μL, also showed good immune responses.

3. Antibody response to Sp

1) Antibody Response to Sp in 2-5 year-old Children (Group I)

Antibody responses to Sp in children aged 2-5 years are summarized in Table 7 and Fig. 3A. For each serotype, GMCs were calculated from individual patients' IgG values. Before vaccination, the GMCs of all six serotypes except serotype 19F were below 0.35 μg/mL. After vaccination, the GMCs of all serotypes increased to values above 0.35 μg/mL. The GMCs of IgG from pre-vaccination (month 0) to post-vaccination month 3 increased for all seven serotypes. For all serotypes, the GMCs were highest after two doses of PCV7 vaccination at month 3. However, we did not observe any further increases

Table 6. Anti-PRP IgG Concentration after *Haemophilus influenzae* Type b Vaccination

Patient ID	Anti-PRP IgG concentration (mg/mL)		
	Pre-vaccination (month 0)	One month after 2nd dose (month 3)	One month after 3rd dose (month 13)
1	0.08	81.57	151.29
2	0.08	0.20	36.74
3	0.08	112.84	310.47
4	2.85	33.49	206.37
5	0.08	144.66	715.35
6	0.08	19.42	ND
7	1.40	74.00	ND
8	0.08	3.55	54.84
9	1.02	3.27	33.70
10	3.69	4.35	7.99
GMC (95% CI)	0.29*, † (0.09-0.96)	15.17*, † (3.37-68.43)	88.50† (26.38-296.80)

Abbreviations: ID, identification; Hib, *Haemophilus influenzae* type b; GMC, geometric mean concentration; CI, confidence interval; ND, not determined. A value of 0.08 mg/mL indicates below detection level (0.15 mg/mL).
*P=0.002 by Wilcoxon matched-pairs signed-ranks test; †P<0.001 by nonparametric repeated measures ANOVA test.

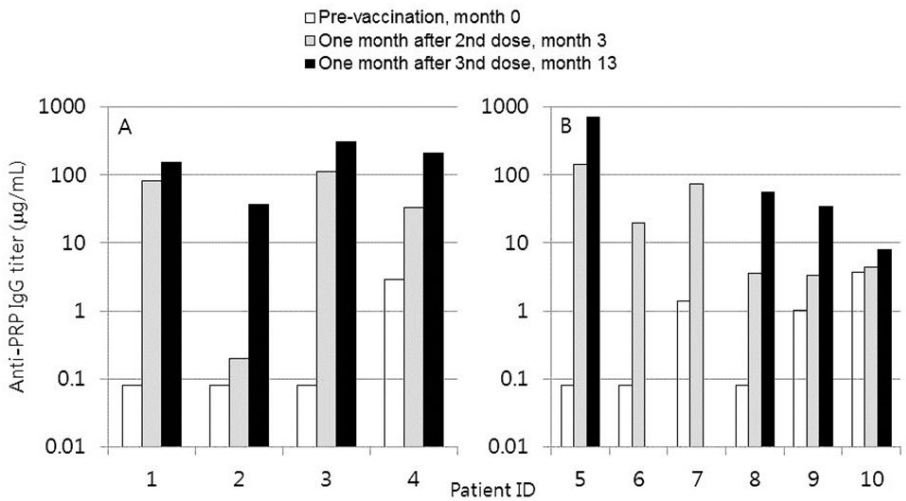

Fig. 2. Anti-PRP IgG concentrations after Hib vaccination. Hib vaccination was performed at months 0, 2, and 12, and blood draws for serological tests were performed at months 0, 3, and 13. The third Hib vaccination was not performed in patient 6 and 7.

in GMCs even after one dose of PPSV23 vaccination at month 5, and there was a decreasing trend in GMC values for all serotypes. The GMCs of all four patients' antibodies to Sp were still above 0.35 μg/mL for all serotypes at month 13. However, levels of antibodies to Sp serotypes 9V and 18C decreased to below 0.35 μg/mL in patient 2, and antibody to Sp serotype 23F also decreased to below 0.35 μg/mL in patient 1 at post-vaccination month 13.

2) Antibody Response to Sp in Children Older than 5 Years (Group II)

Antibody responses to Sp in children aged >5 years are summarized in Table 8 and Fig. 3B. For each serotype, GMCs were calculated from individual

Table 7. Antibody Response after Two Doses of 7-valent Pneumococcal Conjugate Vaccine and One Dose of 23-valent Pneumococcal Polysaccharide Vaccine in Patients Aged 2-5 Years (Group I)

Serotype	GMC of IgG (mg/mL) (95% CI)			
	Pre-vaccination (month 0) n=4	1 month after 2nd PCV7 (month 3) n=4	1 month after PPSV 23 (month 5) n=4	6 months after PPSV 23 (month 13) n=4
4	0.03 (0.01-0.12)	12.38 (5.48-27.94)	7.26 (3.25-16.22)	2.32 (0.11-48.64)
6B	0.21 (0.06-0.77)	10.57 (1.38-81.21)	8.08 (1.30-50.06)	3.41 (0.06-184.40)
9V	0.14 (0.05-0.39)	10.05 (1.27-79.54)	8.20 (0.72-93.22)	2.73 (0.12-61.51)
14	0.03 (0.001-1.38)	22.78 (8.43-61.54)	22.47 (9.71-52.03)	7.12 (1.39-36.54)
18C	0.25 (0.04-1.69)	7.37 (0.75-72.26)	5.57 (0.51-61.14)	2.15 (0.04-128.4)
19F	1.14 (0.63-2.04)	12.31 (2.02-74.88)	8.89 (1.14-69.03)	2.94 (0.15-55.98)
23F	0.05 (0.01-0.22)	6.63 (0.25-178.90)	4.54 (0.26-78.52)	1.95 (0.01-339.6)

Abbreviations: GMC, geometric mean concentration; CI, confidence interval.

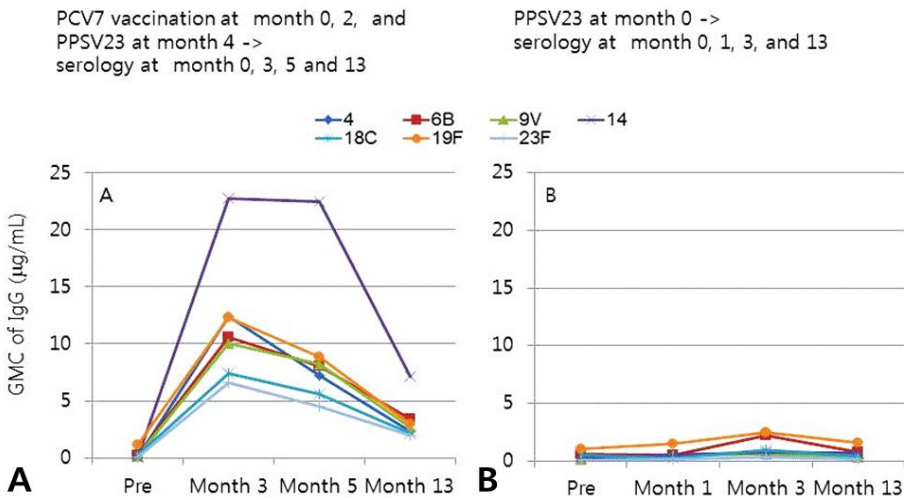

Fig. 3. Antibody response for *S. pneumoniae* serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F in group I (A) and group II (B) A. In group I, antibodies were measured after two doses of PCV7 and one dose of PPSV 23 in patients 2-5 years of age. Patients were vaccinated with PCV7 at months 0 and 2, and with PPSV23 at month 4. Blood was drawn for serological testing at months 0, 3, 5 and 13. B. In group II, antibodies were measured after one dose of PPSV23 at month 0 and blood was drawn for serological tests at months 0, 1, 3, and 13. GMC, geometric concentration; PCV7, pneumococcal conjugate vaccine 7-valent; PPSV23, pneumococcal polysaccharide vaccine 23-valent; Pre, prevaccination.

patients' IgG values. In this older age group, the GMCs of four serotypes (4, 9V, 14, and 23F) were below 0.35 μg/mL before vaccination. At post-vaccination month 3, the GMCs of all seven serotypes were above 0.35 μg/mL, which was higher than pre-vaccination levels. A follow-up test at post-vaccination month 13 showed lower GMC values for all serotypes than those of month 3, and the GMCs of serotypes 9V and 23F decreased below 0.35 μg/mL. However, statistical analyses could not be performed due to low patient number. For serotypes 4, 6B, 9V, 14, and 18C, two of six patients (33.3%) had antibody levels lower than 0.35 μg/mL at post-vaccination month 13. For serotypes 19F and 23F,

Table 8. Antibody Response after One Dose of 23-valent Pneumococcal Polysaccharide Vaccine in Patients Aged >5 Years (Group II)

Serotype	GMC of IgG (mg/mL) (95% CI)			
	Pre-vaccination (month 0) n=6	1 month after PPSV 23 (month 1) n=2	3 months after PPSV 23 (month 3) n=6	13 months after PPSV 23 (month 13) n=4
4	0.09 (0.04-0.21)	0.61*	0.63 (0.17-2.36)	0.23 (0.03-2.04)
6B	0.59 (0.13-2.74)	0.53 (0.001-234.00)	2.17 (0.25-19.10)	0.80 (0.09-7.41)
9V	0.16 (0.05-0.47)	0.49 (0.01-36.66)	0.56 (0.14-2.25)	0.31 (0.10-0.96)
14	0.27 (0.08-0.97)	0.51 (0.35-0.74)	0.74 (0.12-4.42)	0.68 (0.02-28.17)
18C	0.48 (0.11-2.16)	0.22 (0.12-0.39)	0.94 (0.15-6.08)	0.43 (0.04-4.42)
19F	1.07 (0.32-3.64)	1.48 (0.03-78.88)	2.47 (0.68-8.95)	1.60 (0.23-10.97)
23F	0.24 (0.08-0.68)	0.12†	0.37 (0.03-4.22)	0.15 (0.03-0.95)

95% CI *(1.98e-010 to 1.90e+009), †(2.29e-006 to 6704)

one patient (16.7%) and three patients (50%), respectively, had antibody levels lower than 0.35 μg/mL at post-vaccination month 13. Patients 8 and 10 had antibody levels lower than 0.35 μg/mL against 6B, 9V, 14, 18C, and 23F, and patient 5 had antibody levels lower than 0.35 μg/mL to serotoypes 4 and 23F at post-vaccination month 13.

Discussion

This was the first prospective study performed in Korean HCT recipients to measure immune responses to both Hib and Sp after vaccination. In this study, 60% of enrolled patients did not have antibody levels protective against Hib infection, but showed significant immune responses to Hib vaccination, and 100% of HCT recipients developed protective antibody responses (>1.0 μg/mL) after 2 or 3 doses of Hib vaccination. For Sp vaccination, in group I (2-5 years of age), patients showed significant immune responses, and their GMCs were above the protective level (0.35 μg/mL) for all seven serotypes after two doses of PCV7 vaccination. However, immune response was not boosted, even after PPSV23 vaccination, and there was a trend of decreasing GMCs for all seven serotypes by post-vaccination month 13. In group II (>5 years of age), we observed an immune response after one dose of PPSV23 vaccination, but the GMCs of serotypes 4, 9V, and 23F became below the protective level (0.35 μg/mL) by post-vaccination month 13.

Pre-existing antibody titers are known to decrease to low or unprotected levels after HCT in both allogeneic and autologous HCT recipients of blood and marrow grafts[20-24]. In our patients, pre-vaccination antibody levels for Hib and Sp were also low before vaccination. For Hib, anti-PRP IgG was undetectable in 60% of patients. For Sp, the GMCs of six serotypes in group I and four serotypes in group II were below the protective level of 0.35 μg/mL.

Despite early recovery of other hematopoietic cells (neutrophils, monocytes, NK cells, platelets, and red blood cells), lymphocyte recovery (T and B cells) is typically prolonged in HCT recipients. The most readily available and predictive marker for the restoration of immune competence after HCT is CD4 count and antigen-specific antibody response[5]. In

our study, the patients' overall immune reconstitution could be considered satisfactory, and all patients' CD4 counts were above 200/μL at the time of vaccination initiation. In addition, this immune recovery presented as an antigen-specific antibody response to Hib and Sp vaccines. Although a majority of the patients had low or unprotected levels of Hib and Sp antibodies pre-vaccination, the patients showed a good immune response after vaccination, with measurable antibody values.

Earlier studies showed a poorer response to unconjugated PPSV23 and unconjugated Hib vaccine[25-27] and PPSV23 priming induced hyporesponsiveness to subsequent PCV7[28]. The immunologic response to PPSV23 is also known to be lower and delayed despite broader coverage[27, 29, 30]. For these reasons, recent guidelines have recommended conjugated Hib vaccine, and since 2009, three to four vaccinations with PCV have been recommended prior to vaccination with PPSV23[5]. Patients in our study showed good immune responses after Hib and PCV7 vaccination. However, PPSV23 induced neither a sustained antibody response in group II (except for 9V), nor a boosting effect in group I. This observation was contrary to previous reports of the EBMT trial[31, 32] where a significant boosted immune response was observed with a vaccination schedule of three doses of PCV7 plus PPSV23. This difference could be due to variation between the schedules of our study and the EBMT trial (two doses of PCV plus PPSV23 vs. three doses of PCV7 plus PPSV23), the low patient number in our study compared to the EBMT trial (10 vs. 158), or the patients' ages (2-5 years vs. 5-65 years in the EBMT trial). This difference is interesting because, in the EBMT trial, patients started PCV7 vaccination at either 3 or 9 months post-HCT and PPSV23 vaccine was given at either 12 or 18 months post-HCT. Our patients started vaccination at a much later time point (median 13.9 months; range 12.5-54.1 months) and still did not show a significant PPSV23 boosting effect after PCV7 priming. Another reason for this difference may be that our patients received multiple HCTs before vaccination (8/10 were tandem autologous HCT recipients and one patient received allogeneic HCT three times), and their immunosuppression would have been more profound than in the patients in the EBMT trial, where only first allogeneic HCT recipients were enrolled.

Loss of immunity is thought to be a common occurrence after autologous HCT, particularly in patients who have received multiple courses of chemotherapy before HCT, and responses to vaccination are similar to those that occur after allogeneic HCT[2]. A majority of our patients (8/10) were autologous HCT recipients, and we also observed significant loss or decreased immunity to Hib and Sp after HCT and restoration of immunity by vaccination.

There is limited information regarding vaccine immunogenicity in patients transplanted with umbilical cord blood or haploidentical grafts. Interestingly, one of the patients in our study received HCT three times (unrelated BM, haploidentical PBSC, and mismatched unrelated BM) and had severe chronic GVHD. This patient started vaccination 52 months after the last HCT and showed a good immune response to Hib and Sp vaccination.

Understanding of the immune response after vaccination in HCT recipients has been continuously evolving. Our study protocol was initially designed based on recommendations published in 2000 by the US CDC and in 2005 by the EBMT[1, 4]. However,

during the study period, updated guidelines for preventing infectious complications among HCT recipients were published by the American Society of Bone Marrow Transplantation (ASBMT) in late 2009 [5], with joint guidelines for vaccination from the EBMT, the US CDC, the Infectious Diseases Society of America (IDSA), and the ASBMT[3]. The recommended vaccination schedules for Hib and Sp have been revised and it is now recommended that vaccination be started as early as 3-6 months post-HCT for Sp and 6-12 months for Hib to provide maximal protection, since invasive infection by these organisms occurs during this period. Therefore, future studies will also need to address the immune response to Hib and Sp with these new guidelines and with earlier vaccination in Korean HCT recipients. However, for a variety of reasons, patients are being referred to the post-HCT vaccination clinic at a much later time point than recommended by the guidelines, and vaccination typically starts at 12 months after HCT. Therefore, more data and analyses of our evolving vaccination schedule are also required. In addition, since PCV7 is not available anymore, further studies on the immune response to new serotypes included in PCV13 are also needed.

Our study was limited by the small number of enrolled patients (n=10), the heterogeneity of the study participants in terms of HCT type (8 autologous and 2 allogeneic HCT), and age distribution of the patients (4 patients in group I and 6 patients in group II). In addition, only quantitative antibody responses were measured after Hib and Sp vaccination, and functional assays such as serum bactericidal assay (SBA) for Hib or opsonophagocytic assay (OPA) for Sp were not performed. However, IgG ELISA antibody titers were shown to be significantly correlated with OPA titers after PCV7 vaccination in European allogeneic HCT recipients[33]. Therefore, the antibody observed in our study may be functional as well. These potential limitations will need to be examined further in Korean HCT recipients in future studies.

In conclusion, we performed a prospective study to measure antibody response after both Hib and Sp vaccination in Korean pediatric HCT recipients. We observed that most HCT recipients had low levels or no protective antibodies to Hib and Sp before vaccination, but that good immune responses were achieved after vaccination. More data are needed on detailed immune responses to Hib and Sp vaccination in a larger number of Korean HCT recipients.

한 글 요 약

소아 조혈모세포 이식 환자에서 b형 헤모필루스 인플루엔자와 폐렴구균 백신 접종 후 항체 반응에 관한 연구

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목적: 조혈모세포이식 환자들은 b 형 헤모필루스 인플루엔자(*Haemophilus influenzae* type b, Hib)과 폐렴구균(*Streptococcus pneumoniae*, Sp)에 의한 침습성 감염에 취약하다.

방법: 삼성서울병원에서 2009-2011년 사이에 조혈모세포 이식 환자들에게 Hib와 Sp 백신을 접종하고 면역반응을 평가하였다.

결과: 10명의 소아환자가 참가하였고 연령의 중앙값은

5.5세 이었다. Hib 백신 이전에는 60%의 환자에서 anti-PRP IgG가 측정 하한값 0.15 μg/mL 보다 낮았으나 접종 후 100%의 환자에서 0.15 μg/mL와 방어 항체가 1.0 μg/mL 이상으로 증가하였다. Sp 백신을 접종한 2-5세 환자 군은 접종 전 6개의 혈청형에 대한 기하 평균 항체가가 0.35 μg/mL 미만이었으나 접종 후 5개월째 7개 혈청형에 대한 기하 평균 항체가가 모두 0.35 μg/mL 이상으로 증가하였다. 5세 초과의 환자 군에서는 접종 전에 4개의 혈청형에 대한 기하평균 항체가가 0.35 μg/mL 미만이었으나 접종 후 3개월째 검사한 7개 혈청형에 대한 기하 평균 항체가가 모두 0.35 μg/mL 이상 증가하였다.

결론: 소아조혈모세포 이식 환자에서 Hib와 Sp 백신 접종 후 면역 반응을 보임을 관찰하였다. 국내 소아 조혈모세포 이식 환자에서 이들 백신에 대한 면역반응 연구가 지속적으로 필요할 것으로 사료된다.

Acknowledgements

This study was supported by the Korean Society of Pediatric Infectious Diseses and an IN-SUNG Foundation for Medical Research Grant, CA98261 and was partially supported by a grant from KFDA 11172-360 to KH Kim.

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