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Longitudinal analysis of antibody response to immunization in paediatric survivors after allogeneic haematopoietic stem cell transplantation

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Summary

The long-term antibody responses to re-immunization in recipients of allogeneic haematopoietic stem cell transplantation (allo-HSCT) have not been well studied. We prospectively and longitudinally evaluated the antibody responses to 8 vaccine antigens (diphtheria, tetanus, pertussis, measles, mumps, rubella, hepatitis B, and poliovirus) and assessed the factors associated with negative titres in 210 allo-HSCT recipients at St. Jude Children's Research Hospital. Antibody responses lasting for more than 5 years after immunization were observed in most patients for tetanus (95.7%), rubella (92.3%), poliovirus (97.9%), and, in diphtheria-tetanus-acellular pertussis (DTaP) recipients, diphtheria (100%). However, responses to pertussis (25.0%), measles (66.7%), mumps (61.5%), hepatitis B (72.9%), and diphtheria in tetanus-diphtheria (Td) recipients (48.6%) were less favourable, with either only transient antibody responses or persistently negative titres. Factors associated with vaccine failure were older age at immunization; lower CD3, CD4 or CD19 counts; higher IgM concentrations; positive recipient cytomegalovirus serology; negative titres before immunization; acute or chronic graft-versus-host disease; and radiation during preconditioning. These response patterns and clinical factors can be used to formulate re-immunization and monitoring strategies. Patients at risk for vaccine failure should have long-term follow-up; those with loss of antibody response or no seroconversion should receive booster immunizations.

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Authors contributions

Hiroto Inaba, Meredith J Posner, Randall T Hayden, and Wing Leung performed the research, Hiroto Inaba, Ching-Hon Pui and Wing Leung designed the research study, Hiroto Inaba, Christine M Hartford, Ashok Srinivasan, Brandon M Triplett, Jon A. McCullers and Wing Leung contributed essential patient data, Hiroto Inaba, Deqing Pei, Jie Yang and Wing Leung analysed the data and Hiroto Inaba, Meredith Posner and Wing Leung wrote the paper.

Conflicts of interest

The authors have no potential conflicts of interest, including specific financial interests, relationships, or affiliations relevant to the subject of this manuscript.

Keywords

childhood; haematopoietic stem cell transplantation; allogeneic; immunization; survivor

Allogeneic haematopoietic stem cell transplantation (allo-HSCT) is an established treatment for children with high-risk haematological malignancies as well as some nonmalignant disorders (Copelan 2006). As the number of long-term survivors of allo-HSCT increases, there is a growing need to study its late effects in this population (Leung, *et al* 2007, Socie, *et al* 2003).

Impaired humoral and cell-mediated immunity after allo-HSCT increases the risk of bacterial, viral, fungal, and parasitic infections in recipients (Lum 1987, Storek, *et al* 2001a, Weinberg, *et al* 2001, Witherspoon, *et al* 1981). The degree of immunodeficiency is determined by the type of transplant, age of the recipient, and the presence of graft-versus-host disease (GVHD). The recovery of circulating B lymphocytes is generally faster (6–12 months) than that of circulating T lymphocytes (1–2 years). However, specific immune memory is often lost after allo-HSCT, and antibodies to recall antigens may gradually decline. Thus, it is recommended that allo-HSCT recipients be re-immunized at appropriate intervals to reduce the risk of vaccine-preventable infections (Dykewicz, *et al* 2000, Ljungman, *et al* 2005, Patel, *et al* 2007, Small 2007).

To provide generalizable recommendations, guidelines for the vaccination schedule of HSCT recipients were recently compiled (Ljungman, *et al* 2009). However, numerous gaps in our knowledge exist. Specifically, there have been few long-term studies to evaluate the persistence of vaccine-specific antibodies after allo-HSCT in children. Therefore, we conducted a longitudinal study of the antibody responses to immunizations in 210 survivors of allo-HSCT and analysed the factors associated with negative titres.

Patients and methods

Patient population

This prospective long-term follow-up study was approved by the St. Jude Children's Research Hospital (St. Jude) institutional review board (Leung, *et al* 2007). All patients who survived more than 1 year after receiving an allo-HSCT between 1990 and 2005 at St. Jude were eligible for enrollment. Of the 211 eligible transplant patients, 210 (or their legal guardians) agreed to participate and gave written informed consent and assent as appropriate; one patient whose parents declined participation was not included in the study.

Vaccines and schedule

All patients were evaluated for initiation of the re-immunization series at their first annual post-transplantation visit. Vaccines were administered according to the institutional guidelines (Table I) during the study period prior to modification of the schedule based on joint updated guidelines. Immunization was postponed if patients were on immunosuppressive medications (any treatment doses of systemic corticosteroids, ciclosporin, or tacrolimus) and had evidence of chronic GVHD. Patients were administered the diphtheria-tetanus-acellular pertussis vaccine (DTaP; Infanrix, GlaxoSmithKline, Belgium) if ≤ 7 years of age or the diphtheria-tetanus vaccine (Td; Decavac, Sanofi Pasteur, USA) if > 7 years of age, as well as the measles, mumps and rubella (MMR; MMR-II, Merck, USA); hepatitis B (Engerix-B; GlaxoSmithKline, Belgium); and inactivated poliovirus vaccines (IPV; IPOL, Sanofi Pasteur, France). During the study period, *Haemophilus influenzae* type B, pneumococcal, and influenza vaccines were administered. However, antibody responses to these vaccines were not routinely measured and are not

included in this study. Our practice was to give routine immunization based on the schedule of Table 1, but did not utilize antibody response to consider booster immunizations.

Serological testing

Patients underwent an extensive set of surveillance tests, including serological tests, before immunization and during annual visits to St. Jude for at least 10 years after allo-HSCT or until they were at least 18 years old, whichever period was longer. Antibody assays were performed immediately on fresh blood samples. Intravenous immunoglobulin (IVIG) was given weekly until day 100 after HSCT and then as needed to maintain IgG levels greater than 4 g/l. IVIG administration was discontinued before initiating immunization. Antibody concentrations against the diphtheria toxoid were measured by multi-analyte fluorescent detection (ARUP Laboratories, Salt Lake City, UT); values ≥ 0.1 iu/ml were considered protective. Antibody titres against the tetanus toxoid were determined by a haemagglutination assay (University of Tennessee Special Functions Laboratory, Memphis, TN) until April 2004 and by an enzyme immune assay (EIA; Quest Diagnostics Nichols Institute, San Juan Capistrano, CA) thereafter; antibody concentrations of $\geq 1:81$ dilution and ≥ 0.1 iu/ml, respectively, were considered protective. Serum IgG antibodies against the pertussis toxin were determined by EIA (Specialty Laboratories, Valencia, CA); index values >1.19 were considered positive. Semi-quantitative EIA measurements of measles, mumps, and rubella IgG were performed with an EIA test kit (Diamedix, Miami, FL); Index values ≥ 1.0 for measles and rubella and ≥ 1.1 for mumps were considered positive. Antibodies to the hepatitis B surface antigen were measured by the AUSAB EIA test kit (Abbott Laboratories, Abbott Park, IL); concentrations greater than or equal to the cut-off value were considered positive. An indirect fluorescent antibody assay was used to detect antibodies against poliovirus serotypes 1, 2, and 3 (Specialty Laboratories); antibody titres $\geq 1:8$ were considered to be protective. Actual titres were also recorded in the database for diphtheria and poliovirus vaccination, and for other vaccinations they were categorized as protective/positive or negative on the basis of criteria given above.

Statistical analyses

Patients for whom immunizations were initiated on schedule or within 2 years of discontinuation of immunosuppressive agents were considered for response analysis. Antigen-specific antibody responses (protective/positive or negative) were tested 1 to 13 times (median, 4 times) during the entire follow-up period. Post-HSCT antibody responses were grouped into time intervals (0–1 year before immunization, and 0–1 year, 1–2 years, 2–3 years, 3–4 years, 4–5 years, and >5 years after immunization) and combined within these time intervals using a majority rule if there were mixed antibody responses within an interval. Longitudinal binary responses were modelled by using generalized estimating equations (GEE) with an exchangeable dependence structure over time to assess the effects of clinically relevant variables on the antibody response of this study cohort (Proc GENMOD, SAS 9.1.3). Besides clinical features listed in Table II, variables analysed included ages; counts of CD3, CD4, CD8, CD19, and CD3⁺/CD16⁺/CD56⁺ cells; CD4/CD8 ratio; and concentrations of immunoglobulin G (IgG), IgM, IgA, and IgE at the time of immunization. Univariate GEE models were fitted to assess the marginal effect of each variable on the antibody response. Significant factors selected by univariate GEE models at a significance level of 0.10 were compiled into a multiple GEE model to study the independent effect of a variable after adjusting for other covariates. Significance level was set at 0.05 and all p-values were two-sided.

Results

Patient demographics

Table II summarizes the characteristics of the 210 survivors. The median age at allo-HSCT was 8.5 years (range, 0.1–21.6 years) and the median age at which immunization was initiated was 9.5 years (range 1.6–25.1 years). Immunizations for either DTaP or Td were given to 175 patients (DTaP to 63 and Td to 112), MMR to 154 patients, hepatitis B to 148 patients, and IPV to 174 patients during the study period. No survivors showed clinical signs of diphtheria, tetanus, pertussis, measles, mumps, rubella, hepatitis B, or poliovirus infection after allo-HSCT.

Diphtheria vaccine response

Of the 153 patients tested for diphtheria antibodies before immunization, 81 (52.9%) had antibody titres in the protective range (Fig 1A). The percentage of patients with protective antibodies increased to 78.9% (71/90 patients) 3 years after immunization but declined thereafter (60.4% [29/48] after more than 5 years). Multiple GEE models revealed that older age at immunization ($P<0.001$) was significantly associated with negative titres (Table III).

Because the doses of diphtheria toxin were different, the recipients of DTaP (≤ 7 years old) and Td (>7 years old) were evaluated separately (Fig 1B). There were 22 out of 56 (39.3%) patients with protective antibodies before DTaP immunization, which increased after immunization (94.1% [16/17] at 5 years and 100% [11/11] after more than 5 years). Multivariate analysis of data on patients who received DTaP showed that negative titres were significantly associated with lower CD19 counts ($P=0.012$; Table III). On the other hand, the response to Td vaccination slightly increased from 60.8% (59/97) to 72.4% (42/58) for the first 3 years and declined thereafter; only 48.6% (18/37 patients) had protective antibodies more than 5 years after immunization. Multivariate GEE models showed that older age at immunization ($P=0.015$) was significantly associated with negative titres (Table III).

Mean diphtheria antibody titres were followed longitudinally (Fig S1). The titres increased after vaccination and were stable between 0.53 and 0.70 iu/ml during follow-up when data from both DTaP and Td recipients were analysed together. However, there was a difference in the pattern of changes in mean titres between DTaP and Td recipients. DTaP recipients showed a dramatic rise of mean titres at 1 year after immunization (from 0.13 to 1.16 iu/ml), which declined slightly until 3 years of immunization, followed by an increase to 1.64 iu/ml more than 5 years after immunization. On the other hand, Td recipients showed only a modest increase in mean titres at 1 (0.42 iu/ml) and 2 (0.45 iu/ml) years after immunization compared with preimmunization levels (0.29 iu/ml), and titres gradually decreased thereafter (0.27 iu/ml at more than 5 years).

Tetanus vaccine response

Of the 160 patients tested for tetanus antibodies before immunization, 128 (80.0%) had titres in the protective range. The percentage of patients with protective antibody responses increased until the fourth year after immunization and became stable thereafter, with more than 95% of patients showing titres in the protective range (95.7% [45/47] at more than 5 years) (Fig 1A). Higher serum IgM concentrations ($P<0.001$) and a history of extensive chronic GVHD ($P=0.046$) were associated with negative titres (Table III).

Pertussis vaccine response

Of the 42 patients who had pertussis antibodies tested before immunization (Fig 1A), 6 (14.3%) were positive. Antibody responses increased over time but only 31.3% (5/16) were

positive at 5 years. Multivariate analysis showed that negative titres correlated with lower CD19 counts ($P=0.046$; Table III).

Measles vaccine response

Of 130 patients tested for measles antibodies before immunization, 39 (30%) were positive (Fig 2A). After immunization, positive antibody responses were seen in approximately two thirds of patients at the first-year follow-up and stabilized thereafter (66.7% [16/24] at more than 5 years). Lower CD3 counts were significantly associated with negative titres ($P=0.026$; Table III).

Mumps vaccine response

Of the 138 patients tested for mumps titres before vaccination, 41 (29.7%) were positive (Fig 2A). Positive antibody responses increased over time, but only a little more than 50% of patients had responses after the second year (61.5% [16/26] at more than 5 years). Negative titres before immunization were significantly ($P=0.033$) and lower serum IgG concentrations marginally ($P=0.052$) associated with negative titres after immunization (Table III).

Rubella vaccine response

Of the 133 patients tested for rubella antibodies before immunization, 59 (44.4%) were positive (Fig 2A). Unlike the response pattern observed for measles and mumps, the percentage of patients positive for rubella antibodies more than doubled (93.3% [83/89]) within a year of vaccination, and more than 90% of patients continued to have positive antibodies (92.3% [24/26] at more than 5 years).

Hepatitis B vaccine response

Of the 141 patients evaluated for hepatitis B surface antigen antibodies before vaccination, 73 (51.8%) were positive (Fig 2B). The number of patients with antibody responses increased to 77.1% (84/109) until 2 years after immunization, but approximately 25% of patients did not have positive titres (72.9% [43/59] positive at more than 5 years). Negative titres were significantly associated with lower CD4 counts ($P=0.005$) and history of grade 2–4 acute GVHD ($P=0.036$) (Table III). Older age at immunization was marginally associated with negative titres ($P=0.053$).

Poliovirus vaccine response

Poliovirus type 1, 2, and 3 antibodies were tested in 160 patients before immunization, and 87 (54.4%), 89 (55.6%), and 91 (56.9%), respectively, had protective ranges (Fig 2C). After immunization, the percentages of patients with antibody responses gradually increased over time, and 97.9% (47/48) of patients had protective titres for all types at more than 5 years. Negative titres were associated with older age at immunization ($P=0.014$ for poliovirus type 1, $P=0.021$ for type 2, and $P=0.023$ for type 3), higher serum IgM concentrations ($P=0.008$ for type 1, $P=0.012$ for type 2, and $P=0.021$ for type 3), negative poliovirus serum titres before immunization (all $P<0.001$), the use of radiation during the preconditioning therapy ($P=0.004$ for type 1, $P=0.026$ for type 2, and $P=0.013$ for type 3), and positive recipient and donor cytomegalovirus (CMV) status versus both negative ($P=0.003$ for type 1, $P=0.011$ for type 2, $P=0.010$ for type 3) (Table III). For poliovirus types 2 and 3, positive recipient and negative donor CMV status versus both negative ($P=0.028$ and $P=0.013$) was associated with negative titres (Table III).

Longitudinal changes of poliovirus titres are shown in Fig S2. Mean titres increased continuously after immunization in all types (from 1:8.1 to 1:31.8 for type 1, from 1:8.5 to

1:35.8 for type 2, and from 1:8.6 to 1:35.8 for type 3, when titres before immunization were compared with those at more than 5 years).

Response pattern to immunization

After immunization, patients showed 3 patterns of responses: (1) acquired or maintained protective/positive antibodies, (2) had persistently negative titres, or (3) lost protective/positive antibodies over time. The distribution of patients for whom 3 or more measurements were taken after immunization is shown in Fig 3. Persistent negative titres were seen in pertussis (46%), mumps (32%), measles (24%), diphtheria in Td recipients (15%), and hepatitis B (11%). Loss of protective/positive antibodies was observed in diphtheria in Td recipients (34%), pertussis (27%), hepatitis B (20%), measles (15%), mumps (14%), and diphtheria in DTaP recipients (13%).

Discussion

Re-immunization has become increasingly important in the long-term care of patients who receive allo-HSCT (Small 2007, Styczynski and Gil 2008). To our knowledge, this is the most extensive study to follow long-term immunity in survivors of allo-HSCT. Previous studies included fewer patients or both allogeneic and autologous HSCT recipients (Patel, *et al* 2007, Spoulou, *et al* 2004). The prospective follow-up of titre responses of 210 paediatric allo-HSCT patients to 8 vaccines enabled us to identify long-term trends in this population.

Interestingly, the response pattern differed among vaccinations. In the general population, the response rate to immunization is more than 90% for all types of childhood vaccines (Christenson, *et al* 1983, Kimura, *et al* 1991, Myers, *et al* 1982, Salk, *et al* 1981, Schiff, *et al* 1995). Lifelong antibody responses and protection against diseases can be achieved for measles, mumps and rubella, and the estimated half-life of antibody responses against tetanus is 11 years and against diphtheria is 19 years (Amanna, *et al* 2007, Watson, *et al* 1996, Weibel, *et al* 1967). In our study, 80% of patients had protective tetanus antibodies before immunization, and most acquired or maintained titres in the protective range for the duration of the study. Although only 39–57% of patients had protective/positive antibodies for diphtheria in DTaP recipients, rubella, and poliovirus before immunization, more than 90% of patients had protective/positive antibodies for these after 5 years. However, antibodies for diphtheria in Td recipients, measles, and mumps remained negative or lost protective/positive values in more than 33% of patients after 5 years, and hepatitis B antibodies were negative in 25% of patients. Pertussis was the least successful vaccine, with approximately 70% of patients showing negative titres in long-term follow-up.

Several immunological factors correlated with negative titres. After immunization, most of the vaccine peptides are presented by antigen-presenting cells to type II helper T cells (Ada 2001). These cells secrete interleukin-4 and -5 or directly interact with B cells, which are critical for B-cell differentiation and high-affinity antibody production. Thus, delays in reconstitution or defects in interaction of T and B cells can hinder antibody response or lead to transient antibody response.

Older age in our patient population (i.e., adolescents) at immunization was significantly associated with negative titres for diphtheria, poliovirus, and marginally for hepatitis B. The effect of age on titres probably correlates to the thymic involution with age and the greater dependence on peripheral T-cell expansion, resulting in delayed or incomplete immune reconstitution (Storek, *et al* 2002, Weinberg, *et al* 2001, Williams and Gress 2008). For quantitative T-cell subset analysis, CD4-positive helper and CD3-positive T cell counts were important for hepatitis B and measles, respectively. History of extensive chronic GVHD and grade II-IV acute GVHD was associated with negative titres for tetanus and hepatitis B,

respectively. Studies using enumeration of CD4CD45RA and T-cell receptor rearrangement excision circles (TRECs) have shown that the presence of chronic GVHD is associated with delayed T-cell reconstitution (Fallen, *et al* 2003, Weinberg, *et al* 1995, Weinberg, *et al* 2001). We did not give immunizations to patients who had active chronic GVHD or were on immunosuppressants. However, new guidelines do not recommend postponing immunization in patients with chronic GVHD except with live vaccines (Ljungman, *et al* 2009), therefore, it is important to evaluate antibody responses with this approach. In addition, positive recipient CMV status and radiation during preconditioning therapy were associated with negative poliovirus titres. HSCT recipients with positive CMV titres have been shown to carry a higher risk of virus reactivation after allo-HSCT, which can affect T-cell development (Clark, *et al* 2003, Copelan 2006). Patients who received myeloablative conditioning with total body irradiation have a delayed immune reconstitution compared with those receiving reduced-intensity conditioning without radiation (Chen, *et al* 2006, Friedman, *et al* 2001).

The interaction between T and B cells is essential in the differentiation of B cells and antibody production (Ada 2001). Serum IgM concentrations were significantly higher in patients negative for antibodies against tetanus and all types of poliovirus. These might occur because of impaired immunoglobulin class switching from IgM to IgG (Lougaris, *et al* 2005). In the absence of the aid of helper T cells, antigens bind directly to B cells and initiate antibody production, but predominantly IgM, and the response to immunization can be inadequate (Ada 2001). In addition, lower CD19 counts were associated with negative titres for diphtheria in patients receiving DTaP and pertussis. Negative titres before immunization for mumps and poliovirus correlated with unfavourable responses to immunization. Persistent antibody concentrations after allo-HSCT have been shown to be affected by both recipient and donor levels before transplantation (Storek, *et al* 2003). Parkkali *et al* (2007) also showed that adult patients who received human leucocyte antigen (HLA)-matched sibling transplants from donors with high tetanus, diphtheria, and haemophilus influenza B (Hib) antibody concentrations had higher respective antibody concentrations after allo-HSCT. Further studies are required to determine whether donor or recipient pre-transplantation immunization can improve recipient responses after allo-HSCT.

To our knowledge, this is the first study to evaluate the immunogenicity of the acellular pertussis vaccine in children after allo-HSCT. Pertussis vaccines are of recent interest to the general population (Hewlett and Edwards 2005). In 2004–2005, there were 8.9 cases of pertussis per 100,000 population in the United States, with considerable morbidity seen in both immunocompetent and immunocompromised individuals. The American Academy of Pediatrics now recommends tetanus and reduced doses of the diphtheria and acellular pertussis (Tdap) booster vaccine for children older than 10 years (Committee of Infectious Diseases, 2006). New guidelines for HSCT recipients recommend that all survivors receive DTaP, because higher pertussis and diphtheria content may be more immunogenic (Tomblyn, *et al* 2009). Therefore, it is important to monitor the antibody responses to pertussis as well as diphtheria vaccines in older (e.g., >7 years) patients.

Although a major strength of our non-interventional study was that it enabled us to observe the natural history of vaccine titres and infections, a corresponding weakness was that we could not study the response to booster vaccines as patients with negative titres were not administered booster series. Although we observed no infections even in patients with negative titres, these individuals are at risk of disease. A recent study of the hepatitis B vaccine in 292 allo-HSCT patients showed that they had a seroconversion rate of only 64% (Jaffe, *et al* 2006). The study found that 50% of patients who did not respond to their first series of the hepatitis B vaccine responded after their second series, emphasizing the need for the booster series, which would apply to other vaccinations. Likewise, a second dose of

MMR has been recommended for children receiving autologous or allogeneic HSCT in order to overcome the low responses to measles and mumps (Spoulou, *et al* 2004). Currently, we give booster vaccines to patients who do not develop protective/positive antibodies or to those who have lost the antibodies during follow-up.

We observed that the proportion of individuals with protective antibodies and mean titres increased late after immunizations in diphtheria among DTaP recipients and poliovirus. This might be associated with the gradual improvement of T-cell immunity during follow-up, as the numbers of CD3- and CD4-positive T cells continuously increased during follow-up in our patients (data not shown), which is consistent with observations from previous studies (Storek, *et al* 2001b and 2002). In the other vaccinations, the proportions of individuals with protective/positive antibodies were maintained during follow-up, except for diphtheria in Td recipients, in which the proportion of recipients with protective antibodies and mean titres decreased. As described above, Td recipients were older and received lower doses of diphtheria toxin than DTaP recipients. However, these differences in the longitudinal antibody response patterns among vaccinations are possibly multifactorial, as vaccines protect recipients by inducing a variety of effector mechanisms (Plotkin 2001).

Our large-scale longitudinal prospective study allowed a comprehensive analysis of immunization and long-term immunity among survivors after allo-HSCT. Immunization guidelines are currently based on a fixed timeline after transplantation and are modified for live vaccines if recipients have GVHD. The factors correlating to negative titres in our study – older age at immunization; low CD3, CD4 and CD19 counts; high serum IgM concentrations; history of GVHD, and negative titres before immunization – can provide a basis for modifying immunization strategies. Patients with such features can be evaluated with detailed T- and B-cell function studies before immunization, given extra booster doses or higher doses of vaccines, or monitored more closely for re-immunization if titres become negative. Further investigations of these surveillance and intervention strategies are warranted to achieve the ultimate goal of minimizing morbidity and mortality in this highly vulnerable, growing paediatric population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig 1. Longitudinal changes in the percentage of patients with protective/positive antibodies for diphtheria, tetanus, and pertussis after immunization (A) and for diphtheria among DTaP and Td recipients (B). Error bars represent standard errors of proportion estimates.

Fig 2.

Longitudinal changes in the percentage of patients with protective/positive antibodies for measles, mumps, and rubella (A); hepatitis B (B); and poliovirus types 1, 2, and 3 (C) after immunization. Error bars represent standard errors of proportion estimates.

Fig 3.

Response patterns of patients for whom 3 or more measurements were available after immunization. Patients who maintained or acquired protective/positive antibodies (filled in black), had persistently negative titres (not filled), and lost protective/positive antibodies during follow-up (filled in grey).

Table I

Re-immunization schedule after allogeneic HSCT*

Vaccine	Age	Months after HSCT
DTaP	≤7	12, 15, 18
Td	>7	12, 15, 18
MMR	All	24
Hepatitis B	All	12, 15, 18
IPV	All	12, 15, 18
Hib**	All	6, 9, 12
Pneumococci**	All	12
Influenza**	All	>6, early autumn

* This reimmunization schedule was valid during the study period.

** Antibody responses to these vaccines were not routinely monitored.

Abbreviations: DTaP, diphtheria/tetanus/acellular pertussis; Td, diphtheria/tetanus; IPV, inactivated poliovirus; Hib, haemophilus influenza B; MMR, measles/mumps/rubella; HSCT, haematopoietic stem cell transplantation

Table II

Demographics and characteristics of 210 survivors of allogeneic haematopoietic stem cell transplant

Clinical Variables	Category	All Patients n=210
Patient Gender	Female	89 (42.4%)
Race	White	146 (69.5%)
	Black	31 (14.8%)
	Other	33 (15.7%)
Disease	AML	61 (29.0%)
	ALL	48 (22.9%)
	CML	28 (13.3%)
	Aplastic anaemia	18 (8.6%)
	MDS	17 (8.1%)
	Sickle cell	14 (6.7%)
	Immune disorder	14 (6.7%)
	Others	10 (4.8%)
Conditioning	Cyclophosphamide	187 (89.0%)
	TBI or TLI	160 (76.2%)
	ATG, OKT3, or alemtuzumab	134 (63.8%)
Donor Type	Sibling	89 (42.4%)
	Unrelated	80 (38.1%)
	Haploidentical	41 (19.5%)
Donor Gender	Female	100 (47.6%)
Product Type	Bone marrow	172 (81.9%)
	Peripheral blood	38 (18.1%)
	T cell depletion	88 (41.9%)
CMV Status	R (+) / D(+)	77 (36.7%)
	R (+) / D(-)	25 (11.9%)
	R (-) / D(+)	38 (18.1%)
	R (-) / D(-)	70 (33.3%)
Acute GVHD	Grade I	58 (27.6%)
	Grade II	24 (11.4%)
	Grade III	4 (1.9%)
	Grade IV	8 (3.8%)
Chronic GVHD	Limited	28 (13.3%)
	Extensive	18 (8.6%)

Abbreviations: ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; CML, chronic myeloid leukaemia; MDS, myelodysplastic syndrome; TBI, total body irradiation; TLI, total lymphoid irradiation; ATG, anti-thymocyte globulin; OKT3, muromonab-CD3; CMV, cytomegalovirus; R, recipient; D, donor; GVHD, graft-versus-host disease

Table III

Factors associated with negative titres after immunization as calculated using multiple generalized estimating equations models

Vaccine	Factor	Odds ratio	95% confidence interval	P-value
Diphtheria	Older age at immunization	1.12	1.05–1.19	<0.001
	DTaP recipients			
	Lower CD19 counts	4.10	1.37–12.29	0.012
Tetanus	Td recipients			
	Older age at immunization	1.10	1.02–1.19	0.015
	Higher serum IgM level *	1.05	1.02–1.09	<0.001
Pertussis	Extensive cGVHD vs. limited or none	4.35	1.02–20.00	0.046
	Lower CD19 counts	2.87	1.02–8.12	0.046
	Measles			
Mumps	Lower CD3 counts	1.63	1.06–2.51	0.026
	Lower serum IgG level *	1.01	1.00–1.02	0.052
	Negative titre before immunization	2.38	1.08–5.26	0.033
Hepatitis B	Older age at immunization	1.05	1.00–1.10	0.053
	Lower CD4 counts	4.45	1.56–12.75	0.005
	Grade 2–4 aGVHD vs. none or grade 1	2.34	1.06–5.19	0.036
Poliovirus	Type 1			
	Older age at immunization	1.06	1.01–1.11	0.014
	Higher serum IgM level *	1.03	1.01–1.05	0.008
	Negative titre before immunization	5.00	1.96–12.50	<0.001
	Radiation during preconditioning	6.25	1.78–21.93	0.004
	CMV titre: recipient (+) and donor (+)**	3.45	1.52–7.69	0.003
	Type 2			
	Older age at immunization	1.05	1.01–1.10	0.021
	Higher serum IgM level *	1.03	1.01–1.04	0.012
	Negative titre before immunization	9.09	3.57–20.00	<0.001
Type 3	Radiation during preconditioning	3.29	1.15–9.40	0.026
	CMV titre: recipient (+) and donor (+)**	2.86	1.27–6.25	0.011
	CMV titre: recipient (+) and donor (–)**	2.56	1.11–6.25	0.028

Vaccine	Factor	Odds ratio	95% confidence interval	P-value
	Higher serum IgM level [*]	1.02	1.00–1.04	0.021
	Negative titre before immunization	5.00	2.13–11.11	<0.001
	Radiation during preconditioning	3.53	1.30–9.59	0.013
	CMV titre: recipient (+) and donor (+) ^{**}	2.70	1.27–5.56	0.010
	CMV titre: recipient (+) and donor (–) ^{**}	2.78	1.25–6.25	0.013

^{*} Total IgG and IgM, not disease-specific immunoglobulin levels.

^{**} Comparison with CMV titre: recipient (–) and donor (–).

Abbreviations: DTaP, diphtheria/tetanus/pertussis; Td, diphtheria/tetanus; cGVHD, chronic graft-versus-host disease; CMV, cytomegalovirus; aGVHD, acute GVHD