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Original Article

Analysis of Immunity against Measles, Mumps, Rubella, and Varicella Zoster in Adult Recipients of Allogeneic Hematopoietic Stem Cell Transplantation: A Single-Center Experience

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Vaccine-preventable disease (VPD) infections are more severe in immunocompromised hosts. Vaccination against measles, mumps, rubella, and varicella zoster (VZV) (MMRV) is therefore recommended for hematopoietic stem cell transplantation (HCT) recipients. However, studies on adult HCT recipients with VPD infections are limited. At our institution, we have systematically conducted serological MMRV tests as a part of check-up examinations during long-term follow-up (LTFU) after HCT since 2015. This retrospective study aimed to evaluate changes in the serostatus between before and 2 years after allogeneic HCT. Among 161 patients, the pre-transplant seropositivity was 82.7% for measles, 86.8% for mumps, 84.2% for rubella, and 94.3% for VZV. Among 56 patients who underwent LTFU including serological MMRV tests at 2 years after HCT, the percentages maintaining seroprotective antibody levels for measles, mumps, rubella and VZV were 71.5% (40/56), 51.8% (29/56), 48.2% (27/56), and 60.7% (34/56), respectively. Vaccination was recommended for 22 patients, and 12 were vaccinated. Among the 12 vaccinated patients, rates of seroconversion were examined in 2-6 patients for each of the four viruses. They were 100% (3/3) for measles, 33.3% (1/3) for mumps, 50% (3/6) for rubella, and 0% (0/2) for VZV. Further studies are warranted to clarify the effect of vaccination in adult HCT recipients.

Key words: vaccine-preventable disease, vaccination, allogeneic hematopoietic stem cell transplantation, adult

Vaccine-preventable disease (VPD) infections are severe in immunocompromised hosts, including post-hematopoietic stem cell transplantation (HCT) recipients. Life-threatening infections with measles [1-3] and varicella zoster [4,5] have been reported in patients after HCT. Immunization against VPD is thus recommended for patients receiving HCT, because most long-term survivors after HCT become seronegative for the antigens [6-9]. According to the Vaccination section of the Japanese Society of Hematopoietic Cell Transplantation (JSHCT) Guidelines (version 3), inactivated vaccination can be administered at 6 or 12 months after transplantation if there is no worsening of graft-versus-host disease (GVHD). On the other hand, live vaccination is recommended for patients who are 2 years post-transplant, free of active GVHD, and not receiving immunosuppressive drugs. However, the methods and appropriate timing for evaluating VPD seropositivity and the recommendation criteria for vac-

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cination have not been well established for post-HCT recipients.

Most previous reports on seropositive rates after allogeneic HCT (allo-HCT) have focused on pediatric patients [10-12]. More recently, a few studies have been conducted in adult patients [13-15], but the information in adults remains insufficient. At our institution, we have systematically conducted serological tests for measles, mumps, rubella, and varicella zoster (VZV) (MMRV) as part of the check-up examinations during long-term follow-up (LTFU) for allo-HCT recipients since 2015. For patients who do not have seroprotective antibody titers, we routinely recommend MMRV vaccination. This study sought to determine the pre- and post-transplantation prevalence of MMRV antibodies and to evaluate the effectiveness of the vaccination policy for adults undergoing allo-HCT at our institution.

Materials and Methods

Patients. We retrospectively reviewed the clinical charts of adult patients with hematological malignancies who underwent allogeneic HCT at Okayama University Hospital from January 2015 to December 2018. A flowchart of the patient enrollment is shown in Fig. 1. Antibody titers before HCT were assessed in 161 patients at the discretion of the attending physician (Group 1). Post-transplant serological tests to determine the recommendation for live attenuated vaccination were performed annually beginning with the LTFU visit at 2 years after HCT. The 56 patients (Group 2) who received annual check-ups beginning at 2 years post-HCT were included in the analysis for detecting factors associated with the loss of antibodies against MMRV. This retrospective analysis was conducted in accordance with the Declaration of Helsinki and approved by the institutional review board of Okayama University Hospital.

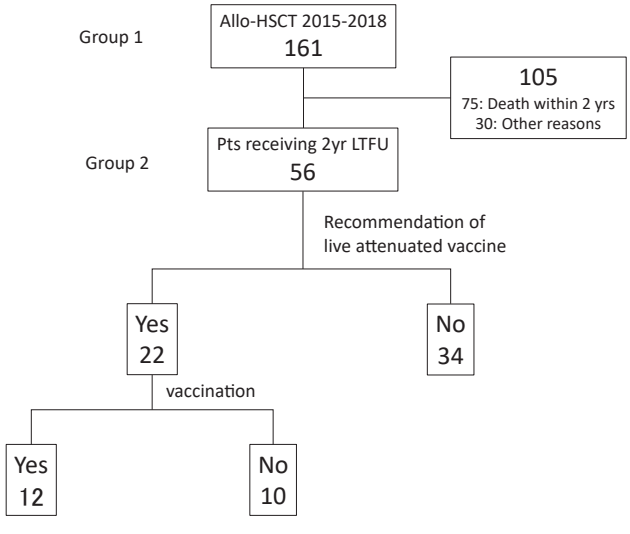

Fig. 1 Flowchart of the patient enrollment.

Serological tests and criteria for vaccination. In serological tests before HCT, immunoglobulin G (IgG) antibody titers of measles, mumps, and rubella were measured using a VIDAS assay kit [16-18]. The threshold for seropositive titers was ≥0.7 TV for measles, ≥0.5 TV for mumps, and ≥15 IU/ml for rubella. An enzyme immunoassay (EIA) was performed to determine the VZV serological status. The seropositive titer threshold was ≥5I U/ml.

In the post-HCT serological tests, we evaluated IgG antibodies for VZV using EIA and for mumps using the VIDAS assay kit, just as in the pre-HCT tests. For measles and rubella we used the neutralization test and hemagglutination inhibition (HI) test, respectively, according to the guidelines for vaccination by the JSHCT. The titer thresholds for recommending vaccination were <1 : 4 for measles, <1 : 16 for rubella, and <5 IU/ml for VZV. As for mumps, we recommended vaccination for all patients not under immunosuppressant medication and without active GVHD, because no definite threshold for vaccination recommendation was indicated by the VIDAS assay.

Statistical analysis. Multivariate analyses of the MMRV serostatus at 2 years after HCT were performed using the Cox proportional hazards model. The following variables were considered: age at HCT (<45 years versus ≥45 years), sex (male versus female), underlying disease (leukemia versus lymphoma versus others), donor source (bone marrow versus peripheral blood stem cell versus cord blood), conditioning regimen (myeloablative versus reduced-intensity), and history of acute GVHD (Grades 0-I versus II-IV) or chronic GVHD occurring within 1 year after HCT (no versus yes). All statistical tests were two-sided, with values of $p<0.05$ considered to indicate statistical significance. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan) [19].

Results

Patient characteristics. The patient characteristics are presented in Table 1. In Group 1, the percentages of patients with pre-transplant seropositivity for measles, mumps, rubella and VZV were 82.7% (62/75), 86.8% (92/106), 84.2% (133/158) and 94.3% (150/159), respectively, as shown in Fig. 2. A total of 103 patients were excluded from further analysis because they were unable to continue LTFU due to death (n = 75) or for other reasons (n = 30); thus, a final total of 56 patients were analyzed for serological status at 2 years after HCT (Group 2). The study population had a median age of 52.5 years (range: 18-71 years) and was composed of 30 men and 26 women. The primary diseases included acute myeloid leukemia (n = 21), acute lymphoblastic leukemia (n = 11), myelodysplastic syndrome (n = 11), non-Hodgkin lymphoma (n = 6), and others (n = 7). The donor sources included unrelated bone marrow (n = 24), related bone marrow (n = 1), unrelated peripheral blood (n = 10), related peripheral blood (n = 12), and unrelated cord blood (n = 9). The conditioning regimen included myeloablative (n = 38) and reduced-intensity (n = 18) regimens. Ten of the 56 patients had a history of grades II-IV acute GVHD, whereas 39 had chronic GVHD.

Table 1 Patient characteristics

	n = 56	
Age at HSCT		
Median (range)	52.5 (18–71)	
Sex		
Male	30	
Female	26	
Disease		
AML	21	38%
ALL	11	20%
MPAL	2	4%
MDS	11	20%
MF	1	2%
CAEBV	2	4%
NHL	6	11%
Other malignancies	2	4%
Donor source		
Unrelated bone marrow	24	43%
Related bone marrow	1	2%
Unrelated peripheral blood	10	18%
Related peripheral blood	7	13%
Haploidentical	5	9%
Unrelated cord blood	9	16%
Conditioning regimen		
Myeloablative	38	68%
Reduced-intensity	18	32%
Acute GVHD		
Grade 0–I	46	82%
Grade II–IV	10	18%
Chronic GVHD		
No	17	30%
Yes	39	70%

HSCT, hematopoietic stem cell transplantation; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; MPAL, mixed phenotype acute leukemia; MDS, myelodysplastic syndrome; MF, myelofibrosis; CAEBV, chronic active Epstein-Barr virus infection; NHL, non-Hodgkin lymphoma.

Serostatus of MMRV at 2 years after HCT and recommendation regarding vaccination. None of the patients who were initially seronegative before transplantation became seropositive without vaccination at 2 years after transplantation. Among the 56 patients who underwent annual serological MMRV tests beginning at 2 years after HCT, the percentages of patients maintaining seroprotective antibody levels for measles, mumps, rubella and VZV were 71.5% (40/56), 51.8% (29/56), 48.2% (27/56), and 60.7% (34/56), respectively (Fig. 3). Vaccination was recommended for 22 patients, and 12 of these patients (54.5%) received vaccination.

Risk factors associated with loss of immunity against MMRV. We selected patients who were seropositive before transplant and analyzed whether loss of immunity could be predicted. Age, sex, underlying disease, donor source, conditioning regimen, history of acute GVHD, and history of chronic GVHD were assigned as covariates. However, we did not detect any factors associated with loss of antibodies against MMRV (Table 2).

Changes in antibody titers after vaccination.

1. Measles

Among 9 patients who received vaccination, 3 did not have seroprotective titers (< 1 : 4) before vaccination. All 3 of these patients achieved sufficient titers at 1 year after vaccination. On the other hand, the antibody titers of 3 patients that were ≥ 1 : 4 before vaccination decreased to < 1 : 4 (Fig. 4).

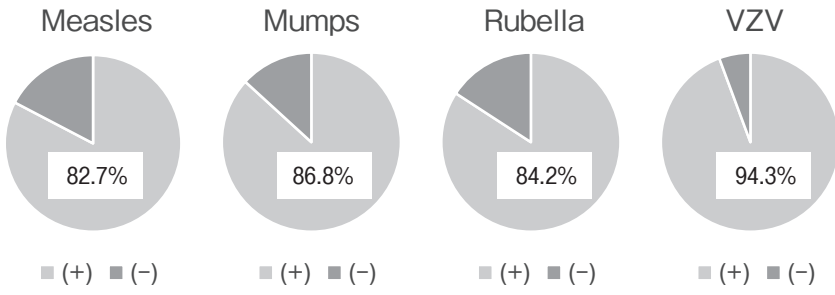

Fig. 2 Pre-transplant seropositivity against measles, mumps, rubella, and varicella zoster. The seropositive titer thresholds were ≥0.7 TV for measles, ≥0.5 TV for mumps, and ≥15 IU/ml for rubella by VIDAS assay. The seropositive titer threshold for VZV was ≥5 IU/ml by enzyme immunoassay.

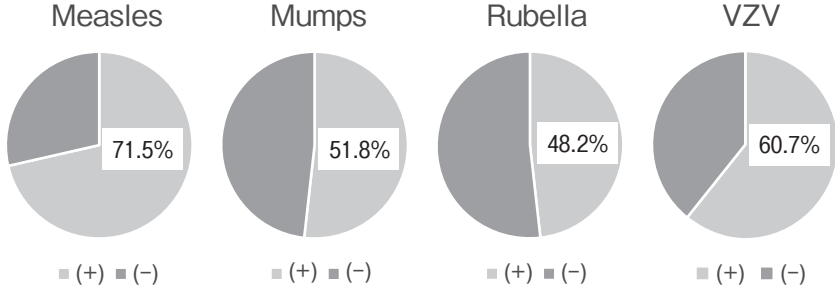

Fig. 3 Serostatus of measles, mumps, rubella, and varicella zoster at 2 years after HCT. The protective antibody titer threshold was ≥1 : 4 for measles on the neutralization test, ≥1 : 16 for rubella on the hemagglutination inhibition test, and ≥5 IU/ml for VZV on enzyme immunoassay. In mumps, ≥0.5 on the VIDAS assay was considered the seroprotective antibody titer threshold.

2. Mumps

Ten patients received vaccination against mumps. Among them, 3 were negative for antibodies (<0.5 TV) before vaccination; however, only 1 patient achieved a seroprotective antibody titer at 1 year after vaccination.

3. Rubella

Nine patients received vaccination against rubella. Among 6 patients who did not have seroprotective titers before vaccination (<1 : 16), 3 achieved a significant increase in titers at 1 year after vaccination, whereas 3 did not achieve sufficient immunity even after vaccination.

4. VZV

Only 4 patients received vaccination against VZV. Among them, the 2 patients who already had VZV antibodies achieved higher titers after vaccination. However, the 2 patients without seroprotective antibody titers before vaccination showed no further response after vaccination.

Discussion

In this study, pre-transplant MMRV antibody titers were maintained above 80% in most patients, even after chemotherapy. At 2 years post-transplant, the percentage of patients maintaining seroprotective titers was clearly reduced for all four viruses. Vaccine-induced seroconversion was seen in a certain number of patients, but a portion of patients showed no response to the vaccination.

Measles antibody titers were maintained in many of our patients at 2 years post-transplant. Kawamura *et al.* [15] reported a 2-year post-transplant seropositive rate of 60.6% in patients who were seropositive prior to transplant. Furthermore, Bogeholz *et al.* [20] found a seropositive rate of 72% even at 5 years after transplantation without vaccination. These results were comparable to the findings of the current study. On the other hand, previous reports without regard to the time of testing demonstrated that seropositive rates were only 20-30% [12, 13]. Ljungman *et al.* [21] showed that antibody titers decay over time, so differences in the timing

Table 2 Results of the univariate and multivariate analyses

Univariate analysis

	Meales		Mumps		VZV		Rubella	
Factors	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age								
<45 years	1.00	Reference	1.00	Reference	1.00	Reference	1.00	Reference
≥45 years	1.33 (0.14–12.82)	0.80	1.57 (0.51–4.85)	0.44	0.79 (0.29–2.13)	0.63	1.14 (0.41–3.16)	0.80
Sex								
Male	1.00	Reference	1.00	Reference	1.00	Reference	1.00	Reference
Female	0.24 (0.03–2.35)	0.22	1.47 (0.51–4.22)	0.48	2.86 (0.82–9.95)	0.10	0.81 (0.33–2.00)	0.65
Disease								
Leukemia	1.00	Reference	1.00	Reference	1.00	Reference	1.00	Reference
Lymphoma	3.75 (0.23–59.95)	0.35	1.78 (0.55–5.77)	0.34	1.11 (0.31–3.92)	0.87	0.93 (0.21–4.17)	0.93
Others	4.27 (0.39–47.26)	0.23	0.67 (0.18–2.46)	0.54	0.43 (0.10–1.92)	0.27	0.97 (0.34–2.76)	0.96
Donor source								
Bone marrow	1.00	Reference	1.00	Reference	1.00	Reference	1.00	Reference
Peripheral blood stem cell	0.35 (0.03–3.82)	0.39	0.93 (0.30–2.89)	0.90	0.81 (0.29–2.27)	0.68	0.61 (0.13–2.83)	0.53
Cord blood	1.13 (0.10–12.41)	0.92	1.56 (0.44–5.51)	0.49	0.57 (0.14–2.96)	0.57	1.30 (0.50–3.38)	0.59
Conditioning regimen								
Myeloablative	1.00	Reference	1.00	Reference	1.00	Reference	1.00	Reference
Reduced-intensity	NA	NA	1.73 (0.63–4.77)	0.29	0.89 (0.31–2.51)	0.82	0.96 (0.39–2.44)	0.93
Acute GVHD								
Grade 0–I	1.00	Reference	1.00	Reference	1.00	Reference	1.00	Reference
Grade II–IV	1.83 (0.19–17.62)	0.60	0.69 (0.16–3.04)	0.62	0.86 (0.25–2.98)	0.81	1.12 (0.64–1.95)	0.70
Chronic GVHD								
No	1.00	Reference	1.00	Reference	1.00	Reference	1.00	Reference
Yes	1.88 (0.20–18.03)	0.59	1.20 (0.39–3.72)	0.75	0.86 (0.32–2.33)	0.77	1.14 (0.41–3.16)	0.80

Multivariate analysis

	Meales		Mumps		VZV		Rubella	
Factors	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Sex								
Male	1.00	Reference	1.00	Reference	1.00	Reference	1.00	Reference
Female	0.26 (0.02–3.00)	0.28	1.51 (0.52–4.37)	0.45	2.84 (0.81–9.93)	0.10	0.82 (0.33–2.01)	0.66
Chronic GVHD								
No	1.00	Reference	1.00	Reference	1.00	Reference	1.00	Reference
Yes	1.00 (0.08–11.85)	1.00	1.27 (0.41–3.96)	0.68	0.94 (0.35–2.55)	0.90	1.13 (0.40–3.13)	0.82

of testing may have contributed to the variability of results among these studies. They also showed that patients who acquired antibodies through vaccination tended to lose them earlier than those who acquired them through natural infection. In addition, blood transfusions and immunoglobulin supplementation may have also affected the results, but these data were not available in this study.

In mumps, about half of the patients were seronegative by the VIDAS assay in our study. A previous study reported that about 39.7% of patients were antibody positive at 2 years post-HCT, as determined by EIA [15]. However, the same group reported less than 10% seropositivity for mumps at 6 years post-transplant [21]. Since the antibody titer threshold for mumps has not yet been determined, vaccination is currently recommended for all patients undergoing HCT at our institute. Vaccination for rubella was also recommended for about half of our patients. A previous report showed that the seropositive rate for rubella at 2 years post-transplant was 52.2% [15]. Another report suggested that seropositivity against rubella at 5 years post-

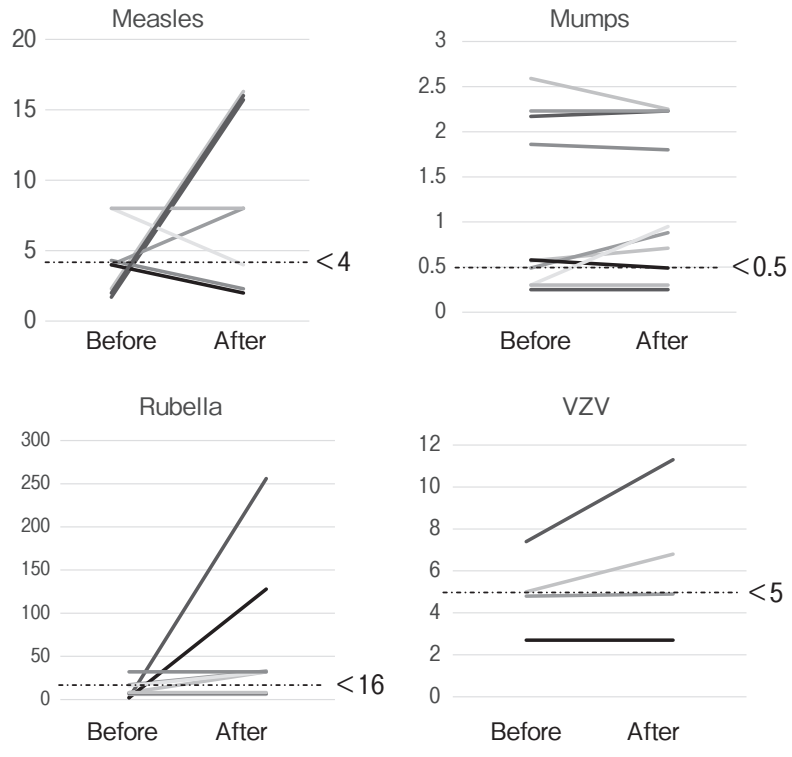

Fig. 4 Changes in antibody titers between before and 1 year after vaccination. The threshold of the protective antibody titer is indicated by the dotted line for each virus.

transplant was less than 65% [20]. Reports on VZV serostatus are limited. Aoki *et al*. [13] reported that 55.4% of their patients undergoing allo-HCT were antibody positive at a median of 4.4 years post-transplant. Another study demonstrated that the antibody titers for measles and mumps decreased to 10% at 10 years post-transplant [21]. Thus, long-term follow-up of antibody titers is necessary, especially in patients who cannot be vaccinated.

In this study, we also examined factors associated with antibody loss in pre-transplant seropositive patients. Previous reports have shown that a history of acute GVHD may be associated with antibody negativity [22]. Some reports have suggested an association between chronic GVHD and antibody loss [13, 15, 20]. In our analysis, however, we did not find any factors associated with the loss of immunity. Future studies with a larger number of cases are needed.

Finally, we assessed the response to vaccination. In our study, the seroconversion rate was 100% (3/3) for measles, 33.3% (1/3) for mumps, 50% (3/6) for rubella, and 0% (0/2) for VZV. Inaba *et al*. [12] conducted a longitudinal analysis in pediatric cases. They reported that more than 60% of patients vaccinated for measles and mumps and more than 90% of patients vaccinated for rubella were seropositive one year after vaccination. Furthermore, these antibody titers were maintained even after 5 years. A study in adult patients after allogeneic transplantation found a combined seroconversion rate by vaccination of >60% for mumps and rubella, but only 35.7% for mumps alone [15]. These results might be due to the stricter seropositivity threshold in the present analysis compared to the latter study. Because the seroprotective antibody titer threshold for mumps has not been defined, the results for mumps serostatus should be interpreted with caution. It has been reported that seroconversion was not obtained in about 30% of cases even after vaccination [7], or that antibody titer decreased even if seroprotective antibody was obtained. Thus, it is important to monitor antibody titers even after vaccination.

This study has some limitations. First, this is a retrospective observation study with a small sample size. Second, the serological testing methods varied between before and after transplantation. Therefore, analyzing the quantitative changes in antibody titers was not possible. Third, no information was available on the MMRV serostatus of donors, which could have affected

the seropositivity of recipients after transplant.

Our analysis of the serostatus of MMRV before and 2 years after transplant and the response to vaccination revealed that 30-50% of patients did not have protective antibodies at 2 years after allo-HCT. A response to vaccination was observed in a subset of patients, but further studies are warranted to clarify the effects of vaccination.

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