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ORIGINAL ARTICLE

Validation of NIH consensus criteria for diagnosis and severity-grading of chronic graft-versus-host disease

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Abstract To validate the National Institutes of Health (NIH) consensus criteria for chronic GVHD, we retrospectively reviewed 143 patients who developed GVHD later than 100 days after allogeneic hematopoietic stem cell transplantation. Their GVHD was reclassified and the severity was graded according to the criteria. Only four patients (2.8 %) could not be reclassified into any type of GVHD. In the remaining 139 patients, reclassified subtypes were late acute GVHD in 52 patients (37.4 %), classic chronic GVHD in 33 (23.7 %), and overlap syndrome in 54 (38.8 %). Of 87 patients with classic chronic GVHD or overlap syndrome, the severity was graded as mild in 21 patients (24 %), moderate in 53 (61 %), and severe in 13 (15 %). The proportions of moderate (70 %) and severe (20 %) disease were significantly higher in patients with overlap syndrome than those with classic chronic GVHD (46 and 6 %, respectively; $P < 0.001$). Univariate and multivariate analyses of subtypes and severity did not identify any significant prognostic values in any of the transplant outcomes, such as transplant-related mortality, overall survival, GVHD-specific survival, or discontinuation of systemic immunosuppressants. These findings suggest that the NIH consensus criteria are useful for classification of chronic GVHD, but have limited significance in predicting clinical outcomes. The validity of these criteria remains inconclusive, and future prospective studies will be required to refine them.

Keywords Chronic graft-versus-host disease · Allogeneic hematopoietic stem cell transplantation · NIH consensus criteria · Late acute GVHD

Introduction

Chronic graft-versus-host disease (GVHD) remains a major cause of non-relapse mortality, and impairs quality of life and functional status in patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT). Historically, chronic GVHD was diagnosed when GVHD developed later than 100 days after HSCT and was classified into limited and extensive disease according to the Seattle criteria [1]. These criteria were based on the limited experience of a small number of patients and designed to identify patients requiring systemic immunosuppressive therapy. Nevertheless, this classification does not capture the degree of involvement of individual organs other than the skin and the liver, and does not necessarily reflect the prognosis. Furthermore, patients with extensive GVHD constitute an extremely heterogeneous population.

To solve these problems and limitations, several stratifications have been proposed and published [2–5]. Although these models improved the ability to predict prognosis in patients developing chronic GVHD, none of them systemically accounted for the various symptoms associated with individual organs or the severity of chronic GVHD. In addition, these classifications did not account for atypical GVHD, a category which includes acute GVHD-like symptoms occurring later than Day 100, chronic GVHD-like symptoms occurring earlier than Day 100, and

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concurrence of both acute and chronic GVHD. In consideration of these issues, the National Institutes of Health (NIH) Consensus Development Project proposed a new clinical scoring system for the global assessment of chronic GVHD severity based on the number of organs involved and the degree of functional impairment in affected organs [6]. Since the proposed NIH consensus criteria are based on expert opinions, studies are needed to assess their validity. In addition, although the feasibility of NIH consensus criteria has been retrospectively assessed by several investigators, a consensus has not been reached [7–12]. Therefore, we retrospectively reclassified the cases of GVHD which developed later than 100 days after allogeneic HSCT so that we could validate the NIH consensus criteria and evaluate their efficacy for predicting clinical outcomes such as survival, discontinuation of systemic immunosuppressive therapy, and transplant-related mortality (TRM).

Patients and methods

Patients

The clinical records were retrospectively collected for patients aged 16 or older who developed chronic GVHD according to the Seattle criteria after allogeneic HSCT for hematological diseases at Keio University Hospital (Tokyo, Japan) between January 1997 and December 2007. Patients who died within 100 days after HSCT, patients who received a second transplantation, and those without sustained donor engraftment were excluded. Four cases lost to follow-up were also excluded. Transplantations with all kinds of stem cell sources and conditioning regimens were included. Acute leukemia in the first or second remission, chronic myelogenous leukemia (CML) in the first or second chronic phase, chemotherapy-sensitive lymphoma, aplastic anemia, and myelodysplastic syndrome (MDS) without excess of blasts were defined as standard-risk diseases. Acute leukemia not in remission, adult T cell leukemia/lymphoma, CML in the third or later chronic phase, accelerated phase, or blast phase, chemotherapy-refractory lymphoma, multiple myeloma, MDS with excess blasts, and primary myelofibrosis were defined as high-risk diseases.

Transplant procedures

Basically, the typing of human leukocyte antigen (HLA)-A and HLA-B antigens was performed using standard serological or low-resolution techniques, and HLA-DRB1 alleles were typed using high-resolution DNA techniques. For the conditioning regimen, myeloablative regimens such as total body irradiation (TBI; 12 Gy) combined with cyclophosphamide (120 mg/kg) and busulfan (orally 16 mg/kg or intravenously 12.8 mg/kg) with cyclophosphamide (120 mg/kg), or reduced-intensity regimens using fludarabine were chosen according to the patient's condition and the protocols available. For GVHD prophylaxis, cyclosporine A (CSA) or tacrolimus and short-term methotrexate were given. Basically, CSA was chosen for HSCT from HLA-identical related donors, and tacrolimus for HSCT from other alternative donors.

Management and evaluation of acute and chronic GVHD

Acute GVHD was diagnosed and graded based on the published criteria [13]. Acute GVHD (grade II or higher) was initially treated with prednisolone (PSL) at a dose of at least 1 mg/kg per day. CIs were continued during GVHD treatment unless there was toxicity. Secondary treatments for steroid-refractory acute GVHD were variable, including methyl-prednisolone pulse therapy (1 g for 3 days) or antithymocyte globulin. Clinical symptoms or signs, or laboratory manifestations of GVHD observed beyond day 100 were diagnosed as chronic GVHD according to the Seattle criteria [1]. The first-line treatment for extensive chronic GVHD was generally a combination of PSL and CIs. Increasing the dose of CIs was attempted before the initiating combination treatment in some patients, who developed GVHD during the tapering of CIs. Chronic GVHD was reclassified into three subtypes (late acute GVHD, classic chronic GVHD, and overlap syndrome) strictly according to NIH consensus criteria [6].

Statistical analysis

Chi-square analysis or Fisher's exact test was used to assess differences in categorical variables, and Mann–Whitney *U* test or Kruskal–Wallis test to assess differences in continuous variables among the groups. Overall survival (OS) was defined as the interval from the day of HSCT to the day of death or last follow-up, GVHD-specific survival (GSS) as the interval from the onset of chronic GVHD to the last follow-up or to death due to GVHD or GVHD-related complications in the absence of recurrence of underlying hematological diseases, time to discontinuation of immunosuppressants (DCIS) as the interval from the onset of chronic GVHD to discontinuation of all systemic immunosuppressants, and TRM as death due to any causes not related to the recurrence of underlying hematological diseases. Data were censored for patients alive at their last follow-up. Probabilities of OS, GSS, DCIS, TRM, GVHD, and DCIS were estimated according to the Kaplan–Meir methods, and were compared using log-rank tests. Cox proportional hazard regression analysis was used to

Table 1 Characteristics of the patients with chronic GVHD ($N = 139$)

	All patients ($N = 139$)	Late acute ($N = 52$)	Classic ($N = 33$)	Overlap ($N = 54$)	P value
Median age (range)	43 (18–62)	43 (18–62)	46 (20–59)	42 (18–61)	0.66
Gender, male/female	77/62	30 (58 %)/22 (42 %)	19 (58 %)/14 (42 %)	28 (52 %)/26 (48 %)	0.80
Disease					
Acute myeloid leukemia	39	17 (33 %)	7 (21 %)	15 (28 %)	0.68
Acute lymphoblastic leukemia	19	4 (8 %)	5 (15 %)	10 (19 %)	
Myelodysplastic syndrome	31	14 (27 %)	6 (18 %)	11 (20 %)	
Chronic myelogenous leukemia	24	8 (15 %)	5 (15 %)	11 (20 %)	
Non-Hodgkin lymphoma	15	6 (12 %)	5 (15 %)	4 (7 %)	
Multiple myeloma	6	2 (4 %)	3 (9 %)	1 (2 %)	
Others	5	1 (2 %)	2 (6 %)	2 (4 %)	
Disease risk category					
Standard risk	76	29 (56 %)	17 (52 %)	30 (56 %)	0.92
High risk	63	23 (44 %)	16 (48 %)	24 (44 %)	
Conditioning					
Myeloablative	112	43 (83 %)	23 (70 %)	46 (85 %)	0.19
Reduced-intensity	27	9 (17 %)	10 (30 %)	8 (15 %)	
Donor demographics					
Median age (range)	36 (0–64)	33 (0–64)	36 (0–60)	36.5 (0–57)	0.51
Gender, male/female	83/56	38 (73 %)/14 (27 %)	17 (52 %)/16 (48 %)	28 (52 %)/26 (48 %)	0.05
Sex disparity					
Female to male	26	9 (17 %)	8 (24 %)	9 (17 %)	0.64
Others	113	43 (83 %)	25 (76 %)	45 (83 %)	
Relationship					
Related	58	15 (29 %)	14 (42 %)	29 (54 %)	0.03
Unrelated	81	37 (71 %)	19 (58 %)	25 (46 %)	
HLA disparity					
Match	118	40 (77 %)	30 (91 %)	48 (89 %)	0.12
Mismatch	21	12 (23 %)	3 (9 %)	6 (11 %)	
Stem cell source					
Bone marrow	110	44 (85 %)	24 (73 %)	42 (78 %)	0.22
Peripheral blood	22	4 (8 %)	7 (21 %)	11 (20 %)	
Cord blood	7	4 (8 %)	2 (6 %)	1 (2 %)	
GVHD prophylaxis					
Cyclosporine A-based	57	14 (27 %)	17 (52 %)	26 (48 %)	0.03
Tarolimus-based	82	38 (73 %)	16 (48 %)	28 (52 %)	
Acute GVHD					
Grades 0–I	56	20 (38 %)	11 (33 %)	25 (46 %)	0.46
Grades II–IV	83	32 (62 %)	22 (67 %)	29 (54 %)	
Onset of chronic GVHD					
Median post-transplant months (range)	4.4 (2.0–29.8)	4.2 (2.1–14.2)	4.6 (3.1–17.2)	4.3 (2.0–29.8)	0.08
Type of chronic GVHD by Seattle criteria					
Limited	18	14 (27 %)	2 (6 %)	2 (4 %)	<0.001
Extensive	121	38 (73 %)	31 (94 %)	52 (96 %)	
Type of onset					
De novo	31	11 (21 %)	7 (21 %)	13 (24 %)	0.14
Quiescent	84	27 (52 %)	24 (73 %)	33 (61 %)	
Progressive	24	14 (27 %)	2 (6 %)	8 (15 %)	

Table 1 continued

	All patients ($N = 139$)	Late acute ($N = 52$)	Classic ($N = 33$)	Overlap ($N = 54$)	P value
Severity by NIH consensus criteria					
Mild		–	16 (48 %)	5 (9 %)	<0.001
Moderate		–	15 (46 %)	38 (70 %)	
Severe		–	2 (6 %)	11 (20 %)	

GVHD graft-versus-host disease, *HLA* human leukocyte antigen, *AML* acute myeloid leukemia, *ALL* acute lymphoblastic leukemia, *MDS* myelodysplastic syndrome, *CML* chronic myelogenous leukemia, *NIH* National Institutes of Health

identify independent risk factors associated with each outcome. The following variables were included in the analysis: recipient age and sex, donor age and sex, relationship between recipient and donor, HLA and sex disparity between recipient and donor, disease status at transplantation, intensity of conditioning, GVHD prophylaxis, stem cell source, the presence of preceding acute GVHD, thrombocytopenia ($<100 \times 10^9$/L) at the onset of chronic GVHD, and subtypes and severity based on NIH consensus criteria. Values of $P < 0.05$ were considered statistically significant in all analyses.

Results

GVHD reclassification and characteristics

From the database, 143 patients were determined to have developed chronic GVHD by the Seattle criteria. In four patients (2.8 %), the manifestations of chronic GVHD were not reclassified into any of the three subtypes defined by the NIH consensus criteria because the criteria were not fulfilled: these patients had liver dysfunction alone ($n = 1$), dry mouth alone ($n = 1$); and bronchiolitis obliterans diagnosed with pulmonary function test and/or radiology alone ($n = 2$). All these four patients were alive without any immunosuppressants at 104, 63, 35, and 22 months after transplantation. Thus, the remaining 139 patients were evaluable in the subsequent analyses. The median follow-up period after transplantation was 61.0 months (range 4.3–139.5). The reclassified subtypes were late acute GVHD (persistent, recurrent, or late-onset) in 52 patients (37.4 %), classic chronic GVHD in 33 (23.7 %), and overlap syndrome in 54 (38.8 %). Table 1 shows the patient characteristics of each subtype of the NIH consensus criteria. The proportions of HSCT from a related donor, CsA-based GVHD prophylaxis, and extensive-type chronic GVHD by the Seattle criteria were significantly lower in patients with late acute GVHD than those with the other two subtypes. The median time from HSCT to the onset of GVHD tended to be shorter in patients with late acute and overlap syndrome than that with classic GVHD ($P = 0.08$; Table 1). Among the 87 patients with classic chronic GVHD or overlap syndrome, chronic GVHD severity was evaluated by global scoring of NIH consensus criteria: 21 patients (24 %) were graded as mild, 53 (61 %) as moderate, and 13 (15 %) as severe. The proportions of moderate (70 %) and severe (20 %) were significantly higher in patients with overlap syndrome than those with classic chronic GVHD (46 and 6 %, respectively; Table 1).

Impact of subtypes and severity of chronic GVHD on TRM, OS, and GSS

In the analysis of 139 cases of late acute GVHD, classic chronic GVHD, and overlap syndrome, the subtypes of chronic GVHD did not significantly affect the probability of TRM, OS, and GSS (Fig. 1a–c; Table 2). By both univariate and multivariate analyses, preceding acute GVHD (grades II–IV) and thrombocytopenia at the onset of chronic GVHD were the significant risk factors for TRM and GSS, and high-risk primary disease and thrombocytopenia were the significant risk factors for OS (Table 2).

In the analysis of 87 cases of classic chronic GVHD and overlap syndrome, neither the subtypes nor the severity significantly affected the probability of TRM, OS, and GSS (Table 3). Although univariate analysis revealed that several factors, such as patient age, donor age, preceding acute GVHD, and thrombocytopenia at the onset of chronic GVHD, affected the probability of TRM, OS, or GSS, only thrombocytopenia was shown to be a significant risk factor for TRM, OS, and GSS (Table 3).

Probability of discontinuation of immunosuppressive therapy

The probability of DCIS was analyzed in 135 cases of late acute GVHD, classic chronic GVHD and overlap syndrome after excluding 4 patients who did not receive systemic immunosuppressants at or after the onset of chronic GVHD. The 2- and 5-year probabilities of DCIS after the onset of chronic GVHD were 46.0 % (95 % CI 34.2–57.8 %) and 72.2 % (95 % CI 61.2–83.2 %), respectively (Fig. 2). Subtypes of chronic GVHD were not

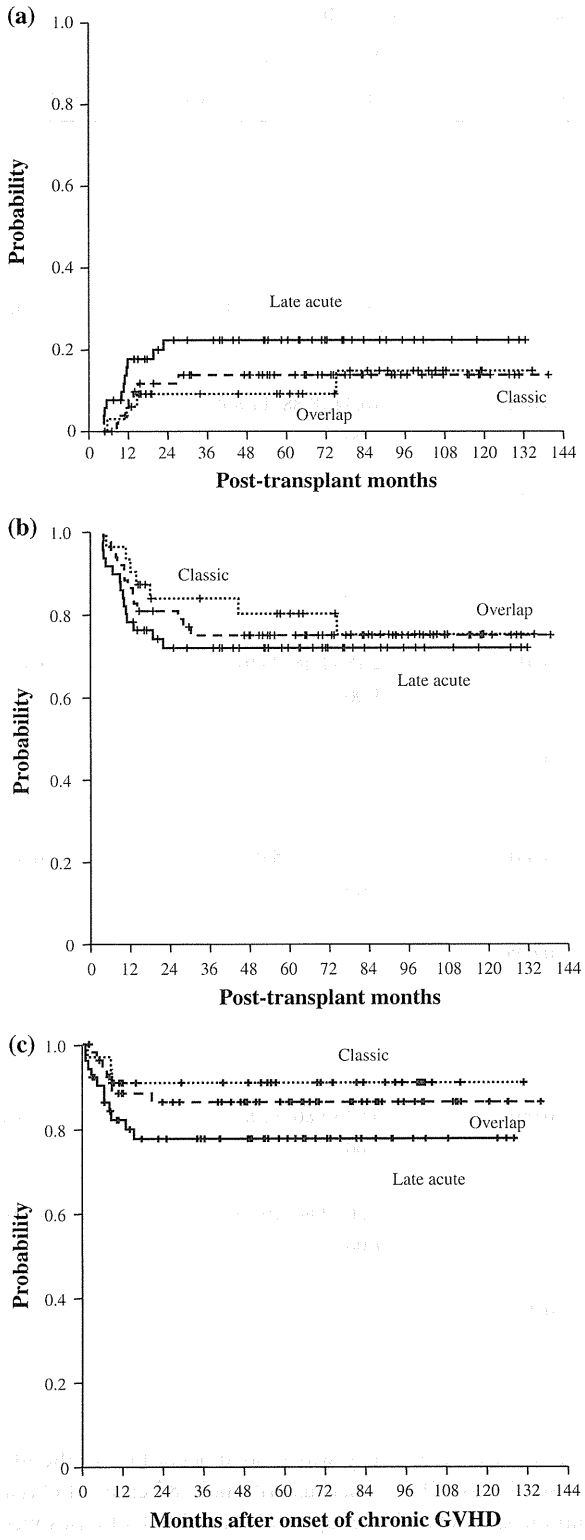

Fig. 1 Kaplan–Meier estimates of the probabilities of **a** transplant-related mortality, **b** overall survival, and **c** GVHD-specific survival. *Classic* indicates classic chronic GVHD, and *overlap* indicates overlap syndrome. The *plus* indicates a censored patient

associated with the probability of DCIS (Table 4). Univariate analysis revealed that the stem cell source was the only significant factor associated with DCIS; however, this factor was not significant in multivariate analysis (Table 4). By the same analysis of 84 cases of classic chronic GVHD and overlap syndrome after excluding 3 cases, neither subtypes nor the severity of chronic GVHD affected the probability of DCIS, and there were no significant factors associated with DCIS.

Discussion

In this study, we retrospectively reclassified 139 cases of GVHD occurring later than 100 days after transplantation by using the NIH consensus criteria, and attempted to evaluate the impact of subtypes and severity on the clinical outcomes. The number of subjects in our study is one of the largest among the studies performed to validate these criteria [7–9]. The reclassification revealed that the proportions of subtypes were as follows: late acute GVHD, 37 %; classic chronic GVHD, 24 %; and overlap syndrome, 39 %. The proportion of classic GVHD seemed to be lower than those in the other reports (43–57 %) [7–9]. This difference could be partly explained by the background of the subjects in each study: the type of donor (HLA-identical siblings or unrelated donors, or both), stem cell sources (bone marrow peripheral blood, or cord blood), and ethnicity. All the patients in our study were Japanese, and about 80 % received bone marrow as the stem cell source, while peripheral blood was the major source of stem cells in the other studies.

Although the patients with late acute GVHD tended to show higher probability of TRM and lower probability of OS and GSS than those with the other two subtypes in the present study, the difference was not significant, which was consistent with the largest previous report [12]. In addition, the probability of DCIS was also not affected by the subtypes. Therefore, the findings in our cohort suggest that the subtype of GVHD in cases occurring later than 100 days after transplantation has little significance in predicting the TRM, survival, or duration of immunosuppressive therapy. In contrast to our results, there have been several studies demonstrating a significant association between the subtypes and some of the clinical outcomes. In regard to TRM, two reports have shown a significant association between late acute GVHD or overlap syndrome and higher TRM [8, 11]. In regard to OS and GSS, two studies have shown a significant association between late acute GVHD and/or overlap syndrome with inferior OS/GSS [7, 8]. In regard to DCIS, no study has shown a significant association between the subtypes and probability of DCIS. These differing results probably reflect the limitations of studies

Table 2 Factors affecting the outcomes of transplantation in patients with late acute and chronic GVHD

Variables	Univariate analysis		Multivariate analysis	
	7-year probability (95 % CI)	*P* value	Hazard ratio (95 % CI)	*P* value
Transplant-related mortality				
Age				
<40 years	9.4 (1.6–17.2)	0.071	–	
40 years or greater	23.5 (14.5–32.5)		–	
Preceding acute GVHD (grades II–IV)				
Yes	24.8 (15.4–34.2)	<0.001	10.20 (8.19–12.21)	0.024
No	1.9 (0.0–5.6)		1.00	
Thrombocytopenia (<10 × 10^9/L)				
Yes	30.5 (18.9–42.1)	<0.001	10.34 (8.88–11.80)	0.002
No	8.3 (7.9–8.7)		1.00	
Subtypes of chronic GVHD				
Late acute	22.4 (10.6–34.2)	0.325	–	
Classic	11.8 (0.0–26.1)		–	
Overlap syndrome	11.6 (2.6–21.2)		–	
Overall survival				
Disease risk				
High risk	65.6 (53.4–77.8)	0.012	2.18 (1.48–2.89)	<0.001
Standard risk	83.9 (75.5–92.3)		1.00	
Preceding acute GVHD (grades II–IV)				
Yes	70.4 (60.4–80.4)	0.063	–	
No	83.3 (73.3–93.3)		–	
Thrombocytopenia (<10 × 10^9/L)				
Yes	61.0 (49.0–73.0)	<0.001	4.27 (3.47–5.06)	0.030
No	88.9 (81.6–96.2)		1.00	
Subtypes of chronic GVHD				
Late acute	72.5 (60.2–84.8)	0.695	–	
Classic	75.8 (59.5–92.1)		–	
Overlap syndrome	75.6 (64.0–87.2)		–	
GVHD-specific survival				
Preceding acute GVHD (grades II–IV)				
Yes	75.3 (65.9–84.7)	0.001	9.82 (7.80–11.8)	0.026
No	98.0 (94.3–100)		1.00	
Thrombocytopenia (<10 × 10^9/L)				
Yes	69.6 (58.2–81.0)	<0.001	9.26 (7.80–10.73)	0.003
No	97.2 (93.3–100)		1.00	
Subtypes of chronic GVHD				
Late acute	77.6 (65.8–89.4)	0.208	–	
Classic	90.9 (81.1–100)		–	
Overlap syndrome	86.3 (76.9–95.7)		–	

with a retrospective design and the differences in patient characteristics among the respective cohorts. However, the results of our present study together with the variable results obtained in other studies strongly suggest that the clinical prognostic value of the subtypes defined by NIH consensus criteria is inconclusive, and could be limited in some cohorts.

Furthermore, severity was graded according to the global scoring of NIH consensus criteria in 87 cases of classic chronic GVHD and overlap syndrome: 24 % of cases were graded as mild, 61 % as moderate, and 15 % as severe, which was similar to the results of previous studies, including one analyzing a Japanese population [7–11]. The fact that a large proportion was scored as having moderate

Table 3 Factors affecting the outcomes of transplantation in patients with classic cGVHD and overlap syndrome ($n = 87$)

Variables	Univariate analysis		Multivariate analysis	
	7-year probability (95 % CI)	*P* value	Hazard ratio (95 % CI)	*P* value
Transplant-related mortality				
Age				
<40 years	2.9 (0.0–8.6)	0.017	1.00	0.073
40 years or greater	18.7 (7.5–29.9)		6.62 (4.55–8.69)	
Donor age				
<40 years	9.9 (0.1–19.7)	0.056	–	
40 years or greater	22.0 (7.5–36.5)		–	
Preceding acute GVHD (grades II–IV)				
Yes	19.8 (8.8–30.8)	0.005	–	
No	0.0		–	
Thrombocytopenia (<10 × 10^9/L)				
Yes	33.4 (11.8–55.0)	<0.001	6.90 (5.36–8.44)	0.014
No	4.1 (0.0–9.6)		1.00	
Subtypes of chronic GVHD				
Classic	14.9 (0.6–29.2)	0.851	–	
Overlap syndrome	13.8 (4.2–23.4)		–	
Severity of chronic GVHD				
Mild and moderate	13.1 (4.3–21.9)	0.229	–	
Severe	23.1 (0.2–46.0)		–	
Overall survival				
Age				
<40 years	85.5 (73.7–97.3)	0.074	–	
40 years or greater	72.5 (60.2–84.8)		–	
Disease risk				
High risk	65.1 (49.2–81.0)	0.068	–	
Standard risk	84.7 (74.3–95.1)		–	
Thrombocytopenia (<10 × 10^9/L)				
Yes	56.7 (36.3–77.1)	0.008	3.27 (2.35–4.19)	0.012
No	86.3 (76.9–95.7)		1.00	
Subtypes of chronic GVHD				
Classic	75.8 (59.5–92.1)	0.732	–	
Overlap syndrome	75.6 (64.0–87.1)		–	
Severity of chronic GVHD				
Mild and moderate	76.6 (66.2–87.0)	0.484	–	
Severe	69.2 (44.1–94.3)		–	
GVHD–specific survival				
Age				
<40 years	97.1 (91.4–100)	0.036	–	
40 years or greater	81.7 (70.7–92.7)		–	
Donor age				
<40 years	94.0 (87.3–100)	0.025	1	0.062
40 years or greater	78.5 (64.4–92.6)		3.64 (2.28–4.99)	
Preceding acute GVHD (grades II–IV)				
Yes	80.2 (69.2–91.2)	0.007	–	
No	100.0		–	
Thrombocytopenia (<10 × 10^9/L)				
Yes	76.2 (62.0–90.7)	0.006	5.91 (4.36–7.47)	0.025
No	96.1 (90.8–100)		1.00	

Table 3 continued

Variables	Univariate analysis		Multivariate analysis	
	7-year probability (95 % CI)	*P* value	Hazard ratio (95 % CI)	*P* value
Subtypes of chronic GVHD				
Classic	90.9 (81.1–100	0.565	–	
Overlap syndrome	86.3 (76.9–95.7)		–	
Severity of chronic GVHD				
Mild and moderate	90.0 (82.9–97.1)	0.159	–	
Severe	76.9 (54.0–99.8)		–	

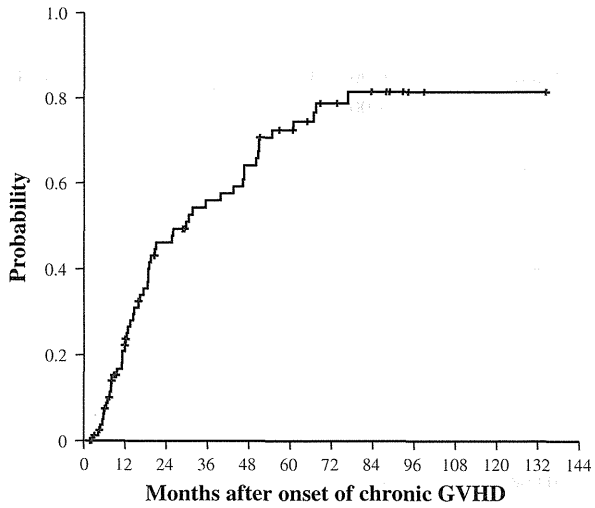

Fig. 2 Kaplan–Meier estimates of the probability of discontinuation of systemic immunosuppressants. The *plus* indicates a censored patient

chronic GVHD consistently in several studies indicates that the spectrum of this category includes widely variable cases of chronic GVHD. The global scoring of chronic GVHD by the NIH consensus criteria was established to determine the indications for systemic immunosuppressive therapy. To explore the possible use of these criteria, we have attempted to establish their association with several indicators of clinical outcomes. However, the results of our study did not demonstrate a significant association between the severity and the clinical outcomes such as TRM, OS, GSS, and DCIS. Similar to the subtypes, the impact of severity on clinical outcomes is widely variable among the reported studies, and not conclusive [7–11]. However, it is suggested that the prognostic significance of the severity of chronic GVHD in evaluating clinical outcomes was limited in our cohort.

There are several plausible explanations for the difference especially in the impact of subtypes and severity of chronic GVHD defined by NIH consensus criteria on the transplant outcomes among the studies including our study [7–12]. The most possible explanation is the difference in patients' background of each study, including the type of the donor, stem cell sources, conditioning regimen, and GVHD prophylaxis. In addition, ethnicity could also play an important role in the development, types, and severity of chronic GVHD. These factors are not only variable among the studies, but also heterogenous in some studies. Therefore, the results of our study are considered to be applicable to the relatively homogenous Japanese population in which majority received bone marrow as a stem cell source after myeloablative conditioning.

In the present study, several risk factors were identified for TRM, OS, and GSS. Thrombocytopenia was consistently associated with inferior outcomes. In addition, preceding acute GVHD was a significant risk factor for the outcomes of all subtypes of GVHD, and donor age for GSS in cases of chronic GVHD. As mentioned in the NIH consensus criteria, such risk factors should be incorporated into the criteria to determine the indications for systemic immunosuppressive therapy [6]. In addition, future studies should reclassify and grade the chronic GVHD in association with these risk factors to evaluate the possible prognostic value of NIH consensus criteria in clinical outcomes.

GVHD occurring later than 100 days after transplantation, diagnosed as chronic GVHD by the Seattle criteria, could not be reclassified into any of the categories late acute GVHD, classic chronic GVHD, or overlap syndrome in only 4 (2.8 %) of 143 cases. In all 4 cases, failure to reclassify was due to single organ manifestation without diagnostic signs or symptoms. Although the number is small, such patients do exist after allogeneic HSCT. Therefore, the classification of such cases should be validated in a future trial.

One of the limitations of the present study was that it employed a retrospective design, and thus the manifestation of GVHD was based on medical records. It is particularly difficult to evaluate skin lesions retrospectively using medical records. However, there have only been

Table 4 Factors affecting the probability of discontinuation of immunosuppressive therapy

Variables	Univariate analysis	
	7-year probability (95 % CI)	*P* value
All cases (late acute, classic, overlap syndrome; n = 135)[a]		
Donor sex		
Male	85.2 (74.6–95.8)	0.073
Female	76.2 (61.9–90.5)	
Stem cell source		
Bone marrow	88.3 (79.3–97.3)	0.018
Peripheral blood	49.4 (24.5–74.3)	
Cord blood	83.3 (53.5–100)	
Subtypes of chronic GVHD		
Late acute	76.3 (60.2–92.4)	0.507
Classic	83.0 (66.5–99.5)	
Overlap syndrome	79.8 (66.3–93.3)	
Cases of classic chronic GVHD and overlap syndrome (n = 84)[a]		
Subtypes of chronic GVHD		
Classic	83.0 (66.5–99.5)	0.484
Overlap syndrome	79.8 (66.3–93.3)	
Severity of chronic GVHD		
Mild and moderate	82.6 (66.2–87.0)	0.405
Severe	76.0 (47.6–100)	

[a] Patients who did not receive immunosuppressive therapy at or after the onset of chronic GVHD were excluded (4 and 3 patients, respectively)

retrospective studies validating NIH consensus criteria [7–12]. The other limitation was the small number of subjects evaluated, although the study was nevertheless one of the largest studies evaluating both the subtypes and severity [7–11]. Based on the variable findings observed among the studies, it would appear that a larger number of cases will be needed to draw a definite conclusion, and this analysis should be done as a prospective study.

In the present study, we assessed the validity of NIH consensus criteria for diagnosing chronic GVHD and grading its severity in a Japanese cohort. The subtypes of and severity of chronic GVHD could not predict the clinical outcomes. The results of chronic GVHD reclassification studies including our present study varied significantly probably because of their retrospective nature and the different compositions of the cohorts. Prospective validation studies may help to refine the NIH consensus criteria and clarify their utility in clinical trials for the treatment of chronic GVHD.

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Conflict of interest None.

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Clinical Transplantation

Production of anti-ABO blood group antibodies after minor ABO-incompatible bone marrow transplantation in NOD/SCID/gamma(c)(null) mice

Tomita H, Fuchimoto Y, Mori T, Kato J, Uemura T, Handa M, Tazawa H, Ohdan H, Okamoto S, Kuroda T. Production of anti-ABO blood group antibodies after minor ABO-incompatible bone marrow transplantation in NOD/SCID/gamma(c)(null) mice.

Abstract: ABO incompatibility is a barrier for solid organ transplantation, but not for hematopoietic stem cell transplantation. To investigate tolerance induction, we enrolled patients who had undergone minor ABO-incompatible (O into A group, n = 6) and ABO-identical (O into O group, n = 4) bone marrow transplantation (BMT). None of the six O into A patients were positive for recipient-specific (anti-blood group A) isohemagglutinins, whereas all four O into O patients were. Peripheral blood mononuclear cells (PBMCs) were engrafted into NOD/SCID/gamma(c)(null) (NOG) mice, followed by sensitization of blood group A red blood cells. Anti-blood group A antibodies (Abs) in the sera of the patients and the human PBMC-engrafted NOG mice were measured by enzyme-linked immunosorbent assays. Anti-blood group A Abs in the patients' sera were significantly correlated with anti-A isohemagglutinin titers ($p < 0.01$). In the human PBMC-engrafted NOG mice, anti-blood group A Abs were significantly lower in the O into A group than in the O into O group ($p < 0.05$), despite *ex vivo* restimulation of B cells. The results of this study suggest that long after receiving minor ABO-incompatible BMT, B cells derived from newly engrafted donor precursor cells were induced tolerance to recipient-specific antigens.

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Key words: anti-ABO blood group antibodies – B-cell tolerance – isohemagglutinins – minor ABO-incompatible bone marrow transplantation – NOD/SCID/gamma(c)(null) mice

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ABO incompatibility has been a barrier to solid organ transplantation (SOT) because of the possibility of graft loss caused by antibody (Ab)-mediated rejection by preformed anti-blood group A/B Abs (1, 2). Because of a serious organ shortage in Japan, investigators have been making efforts to surmount the ABO blood group barrier, particularly in living donor kidney and liver transplantations, and outcomes have recently improved (3). In contrast to SOT, ABO incompatibility is of minor importance in hematopoietic stem cell transplantation (HSCT) (4). Theoretically, in minor or bidirectional ABO-incompatible HSCT, newly engrafted donor B cells may produce anti-recipient A/B Abs and trigger graft-versus-host disease (GvHD), similar to the situation in ABO-incompatible SOT.

Therefore, patients receiving ABO-incompatible HSCT can be used as a model to study the mechanism of tolerance induction after ABO-incompatible SOT (5).

We previously showed that B-cell repertoires that respond to ABO blood group antigens (Ags) are present in human peripheral blood mononuclear cells (PBMCs) using human PBMC-engrafted non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice (6). In the current study, we used the aforementioned model with a slight modification; human PBMCs were engrafted into NOD/SCID/gamma(c)(null) (NOG) mice, which have been shown to be an excellent recipient for the engraftment of human cells because of their multiple immune dysfunctions (7). Using human PBMC-engrafted NOG mice, we investigated the B-cell immune response to ABO blood group Ags in the recipients of minor ABO-incompatible HSCT and considered the mechanisms underlying these responses.

Patients and methods

Enrolled patients and blood samples

Blood samples of 10 patients who had undergone allogeneic bone marrow transplantation (BMT) were collected at regular outpatient visits. Inclusion criteria were as follows: (i) originally blood group A or blood group O patients who had received BMT from blood group O donors (O into A group as cases, and O into O group as controls); (ii) length of time after BMT of >1 yr; (iii) remission of original disease; (iv) no evidence of chronic GvHD; and (v) no immunosuppressants. Six patients belonged to the O into A group (i.e., they received minor ABO-incompatible HSCT), and four patients belonged to the O into O group. Patient characteristics are shown in Table 1. Blood group B recipients were excluded because the experimental model used in this study was developed exclusively for anti-blood group A Abs, considering the fact that anti-blood group B Abs would be absorbed by B-like structures on mouse cells (6, 8).

Serum from each blood sample was subjected to measurement of the isohemagglutinin titers, and the anti-blood group A-specific immunoglobulin (Ig) M and IgG class Ab levels were measured by the enzyme-linked immunosorbent assay (ELISA). The accuracy of the results was determined by comparing the results of ELISA with the isohemagglutinin titers. The patients' PBMCs were separated by Ficoll density gradient centrifugation.

Measurement of the isohemagglutinin titers

Isohemagglutinin titers were measured using the column agglutination technology of the Ortho Bio-Vue system (Ortho Clinical Diagnostics, Neckargemünd, Germany) (9). The IgM and IgG class isohemagglutinin titers were measured by the saline agglutination technique and the indirect Coombs' test, respectively. The presence or absence of agglutination in sequentially diluted serum samples was determined in the column. The highest dilution-causing positive agglutination was determined as the isohemagglutinin titer.

Ex vivo restimulation of B cells in human PBMC-engrafted NOG mice

NOG mice (6–8 wk old) were obtained from the Central Institute for Experimental Animals

Table 1. Patient characteristics

	Age/sex		ABO/RhD						
Case	D	R	D	R	Disease	Donor type	Conditioning	GvHD prophylaxis	GvHD grade
1	30/M	46/M	O+	A+	MF	HLA-matched unrelated	TBI/CY	FK506/MTX	I
2	45/F	36/F	O+	A+	MDS	HLA-matched related	CY/BU	CyA/MTX	IV
3	26/F	40/F	O+	A+	MDS	HLA-matched unrelated	TBI/Ara-C	FK506/MTX	–
4	28/M	54/M	O+	A+	MDS	HLA-matched unrelated	TBI/Ara-C	FK506/MTX	–
5	27/M	33/F	O+	A+	CML	HLA-mismatched unrelated	TBI/Ara-C/ATG	FK506/MTX	–
6	33/M	33/F	O+	A+	ML	HLA-mismatched unrelated	Flu/Mel	FK506/MTX	II
7	42/M	33/F	O+	O+	AML	HLA-mismatched unrelated	TBI/CY/Ara-C	FK506/MTX	II
8	18/F	24/F	O+	O+	ALL	HLA-mismatched related	TBI/CY/Ara-C	FK506/MTX	–
9	38/M	44/M	O+	O+	MDS	HLA-matched related	TBI/Ara-C	CyA/MTX	–
10	39/F	39/F	O+	O+	MDS	HLA-matched related	CY/BU	–	II

-, refers to no GvHD prophylaxis or no GvHD development. ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; Ara-C, cytarabine; ATG, antithymocyte globulin; BU, busulfan; CML, chronic myeloid leukemia; CY, cyclophosphamide; CyA, cyclosporine A; D, donor; Flu, fludarabine; GvHD, graft-versus-host disease; HLA, human leukocyte antigen; L-PAM, melphalan; MDS, myelodysplastic syndrome; MF, myelofibrosis; ML, malignant lymphoma; MTX, methotrexate; R, recipient; TBI, total body irradiation.

(Kawasaki, Japan). All animals were handled under sterile conditions and maintained in microisolators in the Laboratory Animals Center, Keio University School of Medicine. On day 0, PBMCs (20×10^6 cells in 1 mL of RPMI) obtained from each patient were engrafted into NOG mice by peritoneal injections. To evaluate blood group-specific carbohydrate Ag responses of human B cells in NOG mice, mice were sensitized with blood group A RBCs. Freshly collected human group A peripheral blood (from a volunteer) was irradiated with 15 Gy to impair the function of white blood cells. A suspension of blood group A RBCs (1×10^9) in 1 mL of phosphate-buffered saline was injected into the peritoneal cavity of each mouse on day 5. Three wk after peritoneal injections of the human PBMCs (day 21), blood was drawn from the tail artery, and serum was subjected to ELISA for determining anti-blood group A-specific IgM and IgG class Ab levels and total human Ig (IgM and IgG) levels.

Measurements of anti-blood group A Ab and total human Ig levels by ELISA

For ELISA, 96-well flat-bottom microtiter plates (Sumitomo Bakelite, Tokyo, Japan) were coated with 5 μg/mL of bovine serum albumin (BSA) with the synthetic A determinant (GalNAcα1-3Fucα1-2Gal-BSA; Dextra, Reading, UK) or 5 μg/mL of goat anti-human Igs (IgM + IgG + IgA, H + L; Southern Biotech, Birmingham, AL, USA). Sequentially diluted serum samples were added to wells at 100 μL/well in duplicate and incubated for two h at room temperature. The bound Abs were detected using horseradish peroxidase-conjugated goat anti-human IgM- or IgG-specific Abs (Southern Biotech). The color was developed using 0.1 mg/mL of the color-producing reagent containing *O*-phenylenediamine and 3 M H_2SO_4 as a stop solution, and the optical density at 492 nm (OD_{492}) was measured. Human purified IgM (AbD Serotec, Oxford, UK) and IgG (GenScript, Piscataway, NJ, USA) were used as standard controls.

Ethics and statistical analysis

This study was approved by the ethical committee at Keio University School of Medicine (2010-197). All patients provided written informed consent to participate in the study. The data are presented as medians (ranges). Statistical significance was examined by Mann–Whitney *U*-test and Spearman's rank correlation coefficient, with p values of <0.05 being considered significant. Statistical analysis was performed using SPSS 19.0 software (SPSS Inc., Chicago, IL, USA).

Results

Isohemagglutinin titers and anti-blood group A Abs in the patients' sera

In the O into A group, minor ABO-incompatible BMT had been performed 1.2–10 yr prior to this study. None of the six patients had recipient-specific (anti-blood group A) isohemagglutinins, whereas all four patients in the O into O group, who had undergone BMT 2.6–5.8 yr prior to this study, had anti-blood group A isohemagglutinins (Table 2). Anti-blood group B isohemagglutinins were detected in five of the six patients in O into A group, but their isohemagglutinin titers were lower than those in the patients in the O into O group.

Serum levels of anti-blood group A IgM and IgG class Abs were measured by ELISA in the two groups (Fig. 1A,B,D,E). Significant correlations between OD_{492} of anti-blood group A IgM class Abs and anti-blood group A IgM class isohemagglutinin titers were found at the 1:250, 1:1250 (Fig. 1C), and 1:6250 dilutions (Spearman's rank correlation coefficient: 0.874, 0.874, and 0.792, respectively; $p < 0.01$). Similarly, significant correlations between OD_{492} of anti-blood group A IgG class Abs and anti-blood group A IgG class isohemagglutinin titers were found at the 1:250, 1:1250 (Fig. 1F), and 1:6250 dilutions (Spearman's rank correlation coefficient: 0.822, 0.847, and 0.874, respectively; $p < 0.01$). No significant correlation was observed at the 1:31 250 dilution. Despite the presence of non-specific reactions, which were a response to the BSA combined with synthetic A determinant, in low dilution ratios, for example,

Table 2. Isohemagglutinin titers in the patients' sera

		Anti-A titer		Anti-B titer	
Case	Years post-BMT	IgM	IgG	IgM	IgG
1	2.4	–	–	8	16
2	6.7	–	–	8	32
3	6.3	–	–	–	2
4	1.4	–	–	–	–
5	10.0	–	–	4	8
6	1.2	–	–	2	4
7	4.9	32	256	16	64
8	2.6	256	2048	128	1024
9	5.8	64	1024	64	512
10	4.6	16	128	16	128

"–" refers to undetectable titers.
BMT, bone marrow transplantation; Ig, immunoglobulin.

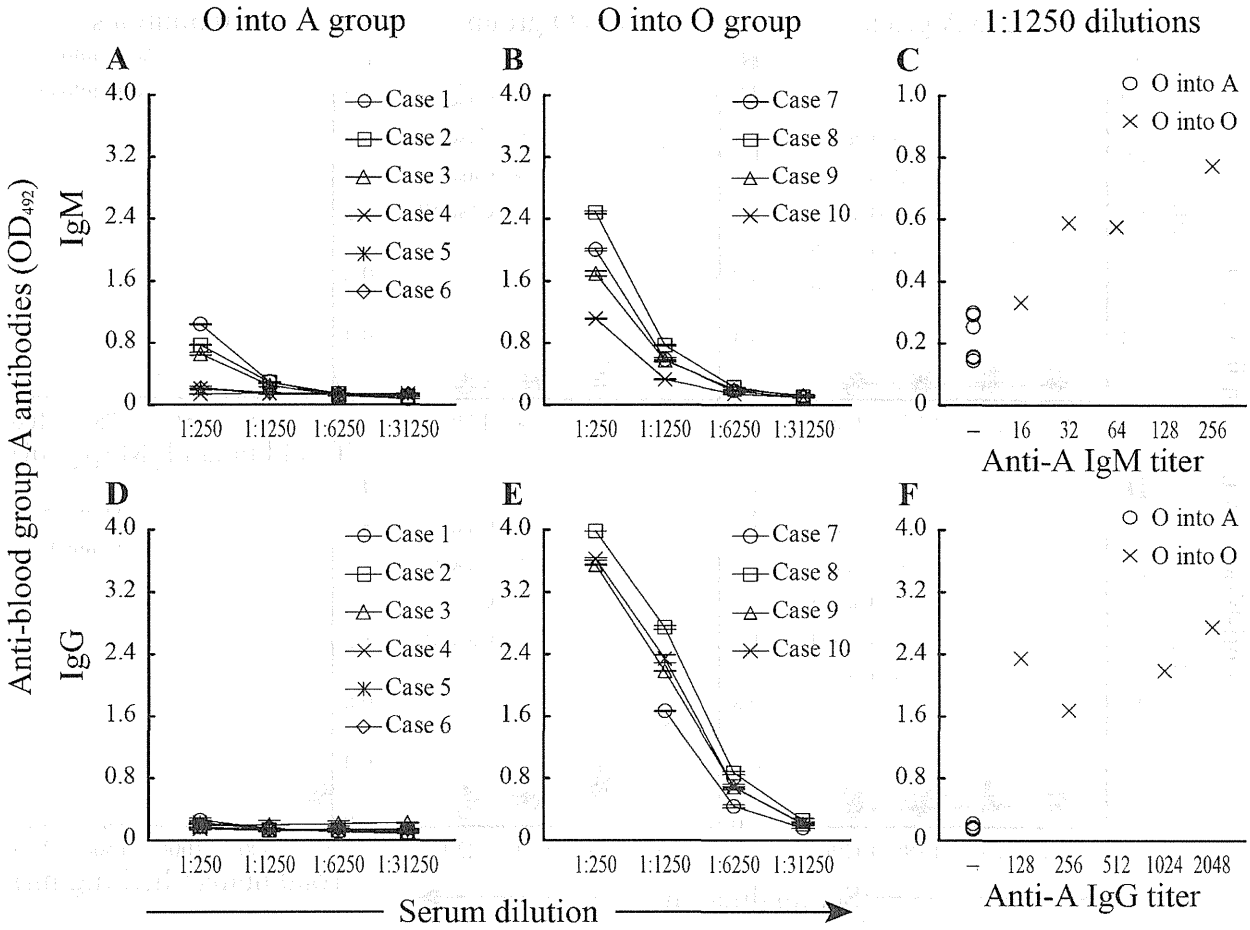

Fig. 1. Anti-blood group A antibodies (Abs) in the patients' sera. Serum levels of anti-blood group A immunoglobulin (Ig)M (A, B) and IgG (D, E) class Abs were measured by enzyme-linked immunosorbent assay (ELISA) in originally blood group A (O into A group; A, D) and O (O into O group; B, E) recipients who received bone marrow transplantation from blood group O donors. Measurements were performed in duplicate; values represent the average of two separate measurements, and error bars indicate differences between duplicate assays. (C, F) The optical density values at 492 nm (OD_{492}) of anti-blood group A IgM and IgG class Abs at the 1:1250 dilution measured by ELISA were significantly correlated with anti-blood group A IgM and IgG class isohemagglutinin titers (Spearman's rank correlation coefficient: 0.874 and 0.847, respectively; $p < 0.01$).

1:250 for IgM, the measurement of anti-blood group A Igs in ELISA assay had strong correlations with the isohemagglutinin test in appropriate diluents.

Anti-blood group A Abs and total human Ig concentrations in the sera of human PBMC-engrafted NOG mice

Anti-blood group A IgM and IgG class Abs in the sera obtained from NOG mice engrafted with PBMCs of the patients were measured by ELISA (Fig. 2). The OD_{492} of anti-A IgM at a 1:10 dilution was 0.126 (0.102–0.253) in the O into A group and 0.408 (0.143–1.757) in the O into O group. The OD_{492} of anti-A IgG at a 1:10 dilution was 0.216 (0.145–0.396) in the O into A group and 0.685 (0.328–0.898) in the O into O group. Although most of the anti-A IgM values in the O into O group were rather low and at least two cases seemed to have negative results when compared with those in the O into A group, the OD_{492} values of anti-blood group A IgM and IgG at 1:10 dilutions were significantly lower in the O into A group than in the O into O group ($p = 0.03$ and $p = 0.02$, respectively, Mann–Whitney *U*-test).

The total human IgM concentrations in the sera of human PBMC-engrafted NOG mice were 24.0 (0.7–66.3) μg/mL in the A into O group and 15.5 (6.5–61.6) μg/mL in the O into O group. There were no significant differences between the two groups ($p = 0.67$, Mann–Whitney *U*-test). The total human IgG concentrations were 136 (31–425) μg/mL in the A into O group and 379 (104–1618) μg/mL in the O into O group. There were also no

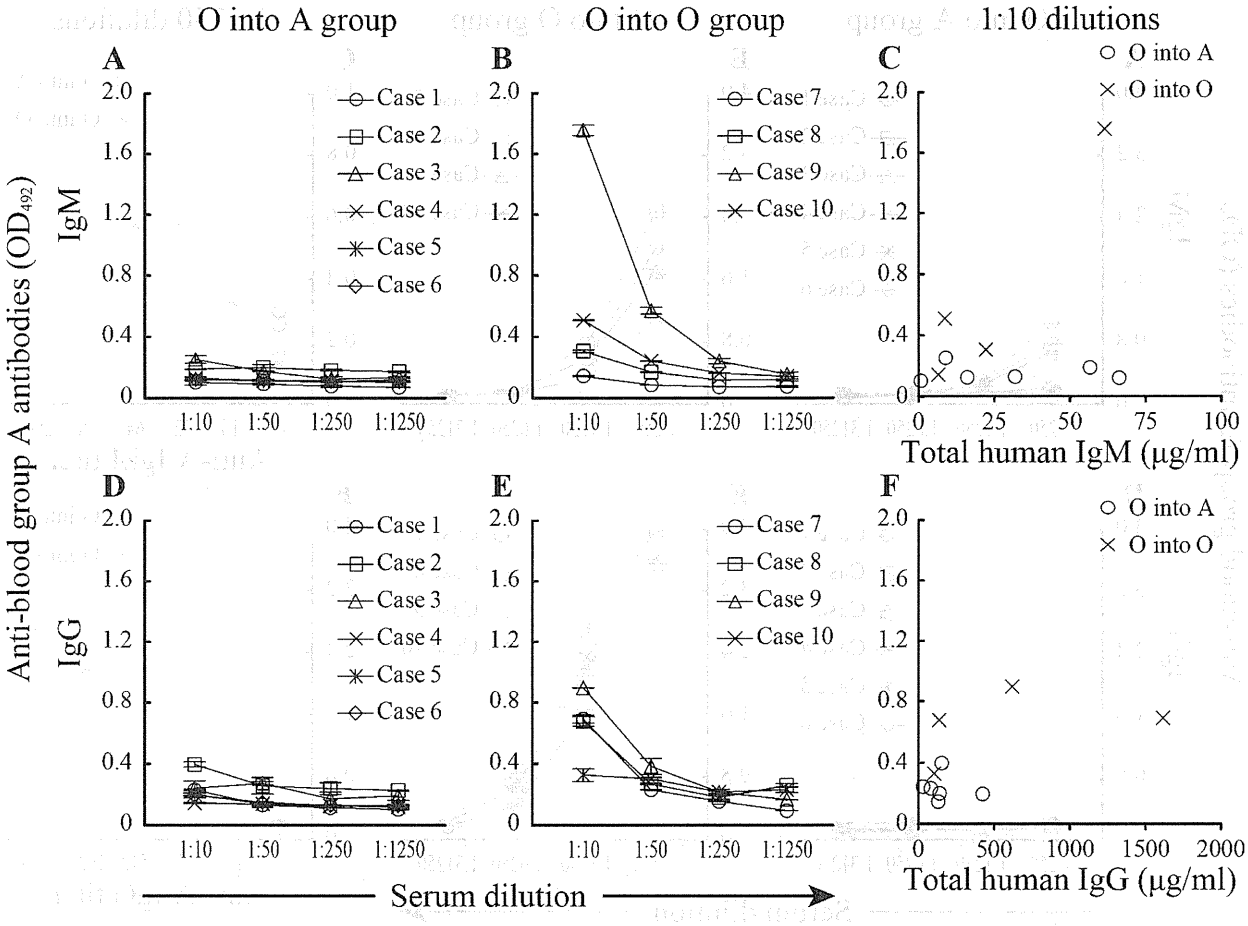

Fig. 2. Anti-blood group A antibodies (Abs) in the sera of human peripheral blood mononuclear cell (PBMC)-engrafted non-obese diabetic/severe combined immunodeficiency/gamma(c)(null) (NOG) mice. Serum levels of anti-blood group A immunoglobulin (Ig) M (A, B) and IgG (D, E) class Abs were measured by enzyme-linked immunosorbent assay. Sera were obtained from NOG mice, which were engrafted with PBMCs of originally blood group A (O into A group; A, D) and O (O into O group; B, E) recipients who received bone marrow transplantation from blood group O donors. Measurements were performed in duplicate; values represent the average of two separate measurements, and error bars indicate differences between duplicate assays. The relationships between optical densities at 492 nm (OD_{492}) of 1:10 dilutions of anti-blood group A IgM and IgG sera and total human IgM and IgG in NOG mice are also shown (C, F). Despite the production of human Igs specific for antigens (Ags) other than blood group A Ags, the OD_{492} values of anti-blood group A IgM and IgG at 1:10 dilutions were significantly lower in the O into A group than in the O into O group ($p = 0.03$ and $p = 0.02$, respectively, Mann–Whitney *U*-test).

significant differences between the two groups ($p = 0.29$, Mann–Whitney *U*-test), indicating similar success rates of PBMC engraftment between the groups.

Discussion

ABO blood group Ags are carbohydrates present on the surface of most tissues, including the endothelial cells that line the vascular system (10). In people lacking A and/or B Ags, Abs specific for these structures (isohemagglutinins) are induced "naturally" in response to environmental stimuli inducing potential cross-reactivity (11). In ABO-incompatible SOT, the transplant endothelium is the primary target of preformed and possibly of elicited anti-donor A/B Abs causing acute vascular rejection (5). While it had been thought that matching of ABO blood groups is an absolute requirement for successful SOT, this barrier has been overcome in ABO-incompatible living donor kidney and liver transplantations because of the development of procedures such as plasma exchange, splenectomy, intensive immunosuppression, antigen-specific immunoadsorption, and local graft infusion as well as the introduction of the prophylactic use of rituximab, a monoclonal chimeric human–murine anti-CD20 antibody that depletes the B cells (12–14). In contrast, successful heart and liver transplantation with only standard immunosuppression has been reported in infants (15, 16). Regarding the mechanisms underlying this

phenomenon, Fan et al. (17) described that donor-specific B cells were absent from peripheral blood after successful infant heart transplantation, indicating the possibility of inducing donor-specific B-cell tolerance. We previously reported that a patient developed immune tolerance to blood group Ags after living donor liver transplantation in infancy (18). It is suggested that exposure of non-self-group antigens to the developing B cells during infancy can lead to deletional tolerance (19).

In contrast to SOT, ABO incompatibility is of minor importance for HSCT without any therapeutic innovation. Recipients of minor ABO-incompatible HSCT (e.g., O into A) express ABO Ags not expressed in the donors (20). Theoretically, anti-recipient A/B Abs produced by donor lymphocytes may trigger GvHD by binding to and damaging the host endothelium (5, 21). However, most large studies have been unable to identify an association between minor ABO incompatibility and GvHD (4). Anti-recipient Abs are produced by passenger mature B and plasma cells contained in the graft within 1–2 wk after minor ABO-incompatible HSCT, leading to delayed hemolysis (22), but are no longer detectable after three months (20, 23, 24). The current study also demonstrated that anti-recipient (anti-blood group A) isohemagglutinin was not detected several years after BMT. Generally, blood group A sera show lower levels of anti-blood group B isohemagglutinins, and blood group B sera show lower levels of anti-blood group A isohemagglutinins than blood group O sera (25). It was reported that cross-reactive anti-A/B Abs binding to both A and B Ags exist in blood group O sera, contributing to high isohemagglutinin titers (26). The current study indicated lower anti-blood group B isohemagglutinin titers in blood group A recipients than in blood group O recipients, even after the patients received BMT from blood group O donors.

Although it is unclear why the anti-recipient immune response is of minor importance in allogeneic HSCT without any therapeutic intervention, the potential mechanisms have been described as follows: adsorption of insufficient amounts of anti-recipient A/B Ab by the recipient endothelium, accommodation that inhibits organ damage despite the presence and binding of sufficient amounts of anti-recipient A/B Abs, anergy or deletion of donor B cells producing anti-recipient A/B Abs, and/or endothelial cell chimerism derived from donor cells (5). Opposing one of these potential mechanisms, Mueller et al. (27) reported that endothelial cell replacement by bone marrow-derived donor cells after allogeneic HSCT is rare and suggested that it is not a major mechanism underlying the lack of anti-recipient reactivity.

In the current study, to test the production of anti-blood group A Abs after minor ABO-incompatible BMT, NOG mice were engrafted with PBMCs from originally blood group A and O recipients who had undergone BMT from blood group O donors, followed by sensitization of blood group A RBCs. Although we could not determine the direct concentrations of anti-blood group A IgM and IgG because of the lack of purified standard controls, anti-blood group A IgM and IgG class Ab values were similar when measured by ELISA or isohemagglutinin test, which is a standard technique for detecting anti-A/B Abs, even at 1:6250 dilutions of human sera. We could not obtain direct proof of responsiveness to third-party antigens in PBMC-engrafted NOG mice, but the production of anti-blood group A Abs was significantly lower in PBMCs of the O into A group than in PBMCs of the O into O group after *ex vivo* restimulation of B cells in the NOG mice, despite the production of human Igs specific for Ags other than blood group A Ags. We are aware of the limitations of the current study, such as the small sample size and the heterogeneous patient backgrounds; however, the results of this study suggested that long after receiving minor ABO-incompatible BMT, B cells derived from newly engrafted blood group O donor precursor cells were induced tolerance to recipient-specific (blood group A) Ags. Using the results obtained by sensitization with *in vivo* humanized mouse models, our study has demonstrated that B cells in the O into A patients showed a lack of Ab production ability against the A blood group, and neither adsorption of anti-recipient Abs nor accommodation to anti-recipient Abs appeared to be major mechanisms for the lack of anti-recipient reactivity. To elucidate the mechanisms of tolerance induction, for example, deletion or anergy, further investigations are required. In fact, we attempted to demonstrate the physical absence of recipient-specific B cells by fluorescence-activated cell sorting or enzyme-linked immunosorbent spot assay to determine whether deletion or anergy was involved in the lack of anti-recipient reactivity. However, we failed to demonstrate positive controls, which may be attributed to the small population of specific B cells that respond to blood group carbohydrate Ags in human PBMCs. Nevertheless, we speculated that the immature donor B cells that were reactive to the recipient carbohydrate antigen might be eliminated because of persistent exposure to the recipient antigen

during their reconstitution after BMT, resulting in tolerance induction.

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Authors' contributions

H.T., Y.F., and T.M. participated in the study design, performed the research, analyzed the data, and wrote the article. J.K., T.U., and M.H. performed the research and revised the article critically. H.T., H.O., S.O., and T.K. participated in the study design and revised the article critically. All the authors approved the submission of the final version of manuscript.

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Visceral varicella zoster virus infection after allogeneic stem cell transplantation

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Abstract: *Introduction.* Varicella zoster virus (VZV) disease is one of the major infectious complications that can occur after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Many reports have shown visceral VZV infection, a special type of VZV disease, to be rare. However, few studies so far have included a large number of patients.
Findings. Visceral VZV infection was found in 20 (0.8%) of 2411 patients who underwent allo-HSCT at our hospitals. Seventeen (85%) patients were taking immunosuppressive agents at the time of presentation with zoster. The presenting symptom was abdominal pain in 16 patients (80%), unconsciousness in 3 patients (15%), and no symptoms in 1 patient. The mean time interval from allo-HSCT to symptomatic visceral VZV infection was 273 days (103–800 days). The eruptions appeared within 3 days (0–13) after the first symptoms. Treatment with intravenous acyclovir was initiated before the appearance of eruptions in 3 of 18 patients (all 3 survived) with vesicular eruptions, the same day in 12 patients (11 survived, 1 died), and after the appearance in 3 patients (1 survived, 2 died). The overall mortality was 20%.
Conclusion. In conclusion, these data confirm that the incidence of visceral VZV infection is infrequent, but this disease is serious. When patients being treated with immunosuppressive agents demonstrate abdominal pain or unconsciousness, the possibility of visceral VZV infection should be considered as well as earlier therapeutic intervention.

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Key words: visceral varicella zoster virus (VZV) infection; allogeneic hematopoietic stem cell transplantation; abdominal pain; chronic graft-versus-host-disease (GVHD)

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Varicella zoster virus (VZV) infection is a common complication after hematopoietic stem cell transplantation (HSCT), and affects about 18–50% (1, 2) of HSCT recipients. The majority of these infections are the result of reactivation of a preexisting infection among adult recipients. Approximately 20% (1, 3) of these cases subsequently develop cutaneous dissemination, whereas visceral dissemination occurs in only

5–14% (1, 3, 6) (1–3% of all recipients [1, 3, 4]). The mortality rate of all VZV infection is 9.7% (5); however, that of visceral VZV infection is unknown.

A number of clinical reports have appeared about visceral dissemination. Almost all reports show that visceral dissemination of VZV, so-called visceral zoster, leads to a fatal outcome. However, only a few studies so far have analyzed the clinical course and complications among a large number of patients with visceral zoster. Therefore, The Kanto Study Group for Cell Therapy (KSGCT) has conducted a retrospective study to elucidate the clinical features and treatment outcome of patients with visceral zoster who were treated at these institutions.

Patients and methods

The study included all patients who underwent allogeneic HSCT (allo-HSCT) at hospitals belonging to the KSGCT between January 1994 and June 2005. Among 3129 patients, the 718 patients who died within 100 days of HSCT were not included in this analysis. The clinical features of 2411 patients were reviewed in detail. All patients were given prophylaxis with acyclovir (ACV) against herpes simplex virus beginning 7 ± 3 days before transplantation. The dose, duration, and mode of ACV prophylaxis varied.

Visceral dissemination (visceral VZV infection) was defined as histological or cultural evidence of internal organ involvement or clinical evidence of internal organ involvement without other identifiable causes.

Results

Incidence and characteristics of patients with visceral VZV infection after HSCT

Visceral VZV infection occurred in 20 (0.8%) of the 2411 patients. Among those 20 patients, 6 patients had a positive blood test. Two autopsies and 1 necropsy were performed. The clinical characteristics of the patients developing visceral VZV infection are shown in Table 1. The median age was 36 years (range 23–63), and 12 patients (60%) were male. The majority received myeloablative transplants for hematological malignancy. Twelve (70%) of 17 patients who could be examined were seropositive for VZV before transplantation. Only 1 of 5 patients, who were seronegative for VZV before transplantation, was thought to be primarily infected with VZV. Fourteen patients developed Grade 0–I acute graft-versus-host disease (GVHD), and Grade II–IV acute GVHD was observed in 6 patients. Sixteen patients (80%) had chronic GVHD and 17 patients (85%) were receiving immunosuppressive therapy at the onset of visceral VZV infection. Seventeen patients who developed visceral VZV infection were not administered prophylactic ACV at the time of onset.

Characteristics of patient with visceral varicella zoster virus (VZV) infection

Age in years	
Median (range)	36 (23–63)
Gender	
Male	12
Female	8
Donor	
Related	7
Unrelated	13
Conditioning regimen	
Myeloablative	16
Nonmyeloablative	4
Prior SCT	
None	15
Once	5
GVHD prophylaxis	
FK506 + sMTX	8
FK506	2
CsA + sMTX	8
CsA	2
Acute GVHD	
Grade 0–1	14
Grade 2–4	6
Chronic GVHD	
Yes	17
No	3
VZV serostatus before SCT	
Positive	12
Negative	5
Unknown	3
Acyclovir prophylaxis	
Start of prophylaxis	Day −7 (−8 ~ −5)
End of prophylaxis	Day 35 (14–207)
Dose of prophylaxis	1000 mg (200–1000)

SCT, stem cell transplantation; GVHD, graft-versus-host disease; FK506, tacrolimus; CsA, cyclosporine; sMTX, short-term methotrexate.

Table 1

Clinical course of visceral VZV infection

The clinical data for patients with visceral VZV infection are summarized in Table 2. Twenty patients developed visceral VZV infection at a median of 273 (range 103–800) days after HSCT. Six patients (30%) had onset of infection after 1 year from the time of transplantation. The first symptom of visceral VZV infection was abdominal pain in 16 patients (80%), unconsciousness in 3 patients (15%), and no symptoms in 1 patient (5%). The abdominal pain was an acute and severe epigastric pain. Eighteen patients (90%) had vesicular eruptions. Vesicular eruptions appeared in most patients (11/18 patients, 61%) from 2 to 4 days after onset of abdominal pain or unconsciousness. Only 2 patients had vesicular eruptions on the same day that they experienced abdominal pain or unconsciousness. Vesicular eruptions were disseminated in 16 patients (89%), and were localized as a single lesion in 2 patients (11%). Among 18 patients with vesicular eruptions, all 7 patients who received the test were seropositive for VZV antigen from skin vesicular fluid. Seven patients among 11 who did not receive the test for VZV antigen were seropositive by the polymerase chain reaction (PCR) test from either blood or cerebrospinal fluid specimens.

Two patients without any eruptions were diagnosed as having a visceral VZV infection; 1 patient was diagnosed at autopsy and the other patient was diagnosed by the VZV PCR test for blood and cerebrospinal fluid. The former patient was treated with only steroids for chronic GVHD and bronchiolitis obliterans at the time of VZV infection. He had no signs of eruptions or abdominal pain and died from progressive respiratory failure and fulminant hepatitis. The latter patient was also treated with tacrolimus and steroids for chronic GVHD when he lost consciousness as the first symptom of visceral VZV infection.

Clinical course of visceral varicella zoster virus (VZV) infection

First sign of visceral VZV infection	
Abdominal pain	16
Unconsciousness	3
No sign	1
Vesicular eruptions	
Yes	18
No	2
Presentation of vesicular eruptions	
Dermatomal dissemination	16
Dermatomal localization	2
Median onset of infection (range)	Day 273 (103–800)
Median day of eruptions (range)	Day 276 (114–803)
Median day of eruptions after onset (range)	3 days (0–13)
Survived	16
Died	4

Table 2

Treatment and outcome

Nineteen patients were treated with intravenous (IV) ACV. ACV was initially administered at a dosage of 1500 mg/day to 12 patients; 6 patients were treated with <1500 mg/day. One patient received ACV and foscarnet therapy. The median length of treatment was 13 (range 1–40) days. Four patients died within 5 days after the start of treatment.

Three of the 18 patients who had vesicular eruptions were treated with IV ACV before the appearance of eruptions, and all 3 patients survived (Fig. 1). Twelve patients received IV ACV on the same day of the appearance of eruptions and 1 of those patients died. Three patients were given IV ACV after the appearance of eruptions and 2 of those patients died.

The mortality of visceral VZV infection was 20% (4 of 20 patients). Three of the 4 patients had abdominal pain and vesicular eruptions. However, 1 patient did not have abdominal pain or vesicular eruptions. All 4 patients developed fulminant hepatitis and multiple organ failure within 9 days of the onset of infection. Two autopsies and 1 necropsy revealed intranuclear inclusion bodies in the liver. VZV was positive in the inclusion bodies based on immunohistological staining in these 3 cases.

Discussion

Twenty of 2411 recipients of allo-HSCT (0.8%) developed a visceral VZV infection. The incidence of this study is consistent with previous reports (about 1%) (1, 3, 4).

Patients presented with visceral VZV infection at a mean of 273 days after transplantation. Previous reports have shown that most serious VZV infections including visceral zoster develop at between 1 and 9 months (3–5). The mean time of onset in the current series was later than in previous reports. Seventeen patients among 20 patients had chronic GVHD at the presentation with visceral VZV. Nine patients among the 17 had the onset of the infection after 9 months, while 8 patients developed visceral VZV infection before 9 months. The present results suggest that all