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A case–control study of bronchiolitis obliterans syndrome following allogeneic hematopoietic stem cell transplantation

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Keywords

ABO-mismatch, allogeneic hematopoietic stem cell transplantation, bronchiolitis obliterans syndrome, cord blood, graft-versus-host disease.

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Summary

Bronchiolitis obliterans syndrome (BOS) is a significant complication after allogeneic hematopoietic stem cell transplantation (HSCT). However, the pathogenesis and risks for the development of BOS have remained unclear. Therefore, a case–control study was conducted to investigate the risk factors for the development of BOS, which included the largest number of BOS cases; 196 patients with BOS were identified and compared with 1960 control recipients. The following were identified as significantly higher risk factors for the development of BOS: female recipients (OR 1.47, $P = 0.019$), ABO-mismatch HSCT (minor mismatch, OR 1.67, $P = 0.015$; major mismatch, OR 1.73, $P = 0.012$; bidirectional mismatch, OR 1.96, $P = 0.018$), busulfan+cyclophosphamide-based myeloablative conditioning (OR 1.74, $P = 0.016$), and acute graft-versus-host disease (GVHD) involving the skin (OR 1.55, $P = 0.011$). On the other hand, the risk for the development of BOS was significantly lower in patients receiving cord blood transplantation (OR 0.26, $P = 0.0011$). With respect to other target organs of chronic GVHD, ocular involvement was significantly associated with BOS (OR 2.53, $P < 0.001$). Prospective studies are required to elucidate the risk factors for the development of BOS, and future investigations should focus on finding a prophylactic approach against BOS based on these findings.

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) plays a crucial role as a curative treatment for hematological diseases. However, HSCT recipients experience various adverse complications, including graft-versus-host disease (GVHD). Bronchiolitis obliterans syndrome (BOS) is one of the significant late complications following HSCT, and it is known to represent lung involvement of chronic GVHD (cGVHD). BOS is characterized by breathing difficulty and dry cough without fever, and by airway obstruction not responsive to bronchodilator therapy that may become irreversible in advanced stages of disease [1–7]. The pathological findings of BOS show bronchiolitis involving the small airway and fibrinous obliteration of the lumina of the respiratory bronchioles [3,8]. The cumulative incidence of BOS is thought to range from 2% to 10% [3,4]. BOS usually presents after the first 100 days following HSCT, and ~80% of cases present between 6 and 12 months after HSCT [3,4]. The International Bone Marrow Transplantation Registry (IBMTR) reported that BOS presented at a median of 431 days after HSCT (range: 65–2444 days) [9].

Several groups have investigated the risk factors for the development of BOS, including peripheral blood stem cell transplantation (PBSCT), busulfan (BU)-based conditioning, and the development of GVHD [9–13]. However, the results were controversial. One of the reasons for the controversy is the small number of patients with BOS, as almost all of these studies included less than 20 patients with BOS. To the best of our knowledge, there have been just two reports that included more than 50 patients with BOS by IBMTR (76 patients with BOS among 6275 HSCT recipients from HLA-identical siblings) or the Kanto Study Group for Cell Therapy (KSGCT, 57 patients with BOS among 2087 recipients). However, no study has included over 100 patients with BOS [9,13]. Both IBMTR and KSGCT reported that PBSCT and GVHD were associated with the development of BOS. However, it remains unclear whether other alternative donor sources, such as cord blood transplantation (CBT), and other possible factors, such as ABO-mismatch, affect the development of BOS.

Bronchiolitis obliterans syndrome is well known to impair the recipients' quality of life dramatically and to be associated with worse survival rates [1,3,4,6,13]. However, an effective treatment has yet to be established [1,3,4,6,13]. Therefore, it is important to elucidate the risks for the development of BOS and to establish a prophylactic approach against it. Thus, a large case–control study that included about 200 patients with BOS was performed using the Japanese transplant outcome registry database, and the risk factors were identified.

Patients and methods

Patient selection

Patients with BOS and control recipients were selected from the cohort of adult recipients (16 years or older) who received their 1st allogeneic HSCT between January 1990 and December 2009 and survived without disease relapse for at least 180 days after HSCT, reported to the Japan transplant outcome registry database and confirmed by the Transplant Registry Unified Management Program in 2010 [14]. The BOS patients were defined as adult recipients who experienced BOS by their last follow-up. The control recipients were defined as adult recipients in whom BOS was not apparently diagnosed up to their last follow-up. Using a computerized selection procedure, 10 controls, which were matched according to years of HSCT (every 5 years), were chosen for each case, because there might be changes in the clinical practices related to HSCT according to the years of HSCT. In addition, information on age, sex, and survival status at the end of follow-up was required. This retrospective analysis was conducted in accordance with the amended Declaration of Helsinki and approved by the institutional review board at Saitama Medical Centre, Jichi Medical University.

Definitions of categories

BOS was reported based on clinical obstructive dysfunctions and radiological assessment with/without histological examinations [2,5,7]. Standard risk diseases were defined as follows: acute leukemia in the 1st and 2nd complete remission, chronic myelogenous leukemia in the 1st and 2nd chronic phase, lymphoma and multiple myeloma in complete and partial remission, adult T cell leukemia in complete remission, myelodysplastic syndromes, myeloproliferative neoplasms, benign hematological diseases, and congenital disorders. All other diseases were classified as high-risk. Because PBSCT from unrelated donors was not available in Japan during the evaluation period, the types of HSCT were categorized into seven groups: HLA-matched related bone marrow transplantation (MRD-BMT), HLA-mismatched related BMT (MMRD-BMT), HLA-matched related PBSCT (MRD-PBSCT), HLA-mismatched related PBSCT (MMRD-PBSCT), HLA-matched unrelated BMT (MUD-BMT), HLA-mismatched unrelated BMT (MMUD-BMT), and unrelated CBT. MMRD or MMUD was defined as a related or unrelated donor when at least HLA 1 antigen mismatch was detected at serological levels of HLA-A, B, or DR. Regimens were classified into myeloablative (MAC) and reduced intensity conditioning (RIC) based on the report by Giralt *et al.* [15]. Briefly, conditionings including total body irradiation (TBI) >8 Gy, melphalan ≥ 140 mg/m^2, or oral BU ≥ 9 mg/kg (iv BU ≥ 7.2 mg/kg) were classified

as MAC. Other regimens were classified as RIC. The conditioning regimens were then divided into five groups: cyclophosphamide (CY)+TBI-based MAC, BU+CY-based MAC, other MAC, fludarabine-based RIC, and other RIC. The diagnosis and severity of GVHD were reported based on the clinical grading scores [16,17].

Statistical analysis

Conditional logistic regression analysis was used for univariate and multivariate analyses to assess the risks for the development of BOS. On multivariate analysis, odds ratios (ORs) were obtained after adjusting with variables having a *P*-value less than 0.1 on univariate analysis with stepwise deletions. Acute GVHD (aGVHD) was included in the analysis as a possible risk factor for the development of BOS, because BOS usually presents after the first 100 days after HSCT [3,4]. In addition, the association between BOS and the target organs of cGVHD was assessed separately by focusing on the recipients with cGVHD. The cumulative probabilities of relapse and nonrelapse mortality (NRM) were estimated by Gray's method, considering each other as a competing risk**.** Overall survival (OS) was estimated by the Kaplan–Meier method. These probabilities were estimated from time of transplantation with 95% confidence intervals (95% CIs). Statistical significance was defined as a two-tailed *P*-value less than 0.05. All data management and statistical calculations were performed by STATA version 12.0 and EZR on R commander, which is a graphical user interface for R (The R Foundation for Statistical Computing, version 2.13.0) (Saitama Medical Centre, Jichi Medical University at http://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmedEN.html).

Results

Patients

During the 20-year study period, 196 patients with BOS (96 males, 100 females) were identified. The median age of the BOS group was 41 (range 16–68) years. Through the computerized selection procedure described above, 1960 control patients (1149 males, 841 females) were identified among 6595 eligible recipients who survived for at least 180 days after HSCT. Their median age was 40 (range 16–76) years. There was no significant difference in the distributions of age and disease risk between the BOS and control groups.

Risk factors for the development of BOS

On univariate analyses, the risk for the development of BOS was higher in female recipients, ABO-mismatch HSCT (especially major mismatch), recipients receiving BU+CY-based MAC, those who experienced grade 2–4, and skin involvement of aGVHD. On the other hand, the risk for the development of BOS was lower in the recipients who received unrelated CBT and *in vivo* T cell depletion, including anti-thymocyte globulin and alemtuzumab, as part of conditioning (Table 1). HLA mismatch, sex-mismatch, GVHD prophylaxis, and gut and liver involvement of aGVHD were not associated with the development of BOS in the current analysis.

Multivariate analysis revealed that the predictive factors for the development of BOS were as follows: female recipients [OR 1.47 (95% CI; 1.06–2.04), $P = 0.019$], ABO-mismatch [minor mismatch, OR 1.67 (95% CI; 1.10–2.51), $P = 0.015$; major mismatch, OR 1.73 (95% CI; 1.13–2.64), $P = 0.012$; bidirectional mismatch, OR 1.96 (95% CI; 1.12–3.43), $P = 0.018$], CBT [OR 0.26 (95% CI; 0.11–0.58), $P = 0.0011$], BU+CY-based MAC [OR 1.74 (95% CI; 1.11–2.72), $P = 0.016$], and skin involvement of aGVHD [OR = 1.55 (95% CI; 1.11–2.18), $P = 0.011$] (Table 1). Grade 2–4 aGVHD and *in vivo* T cell depletion were not significant on multivariate analysis.

The association between BOS and target organs of cGVHD

For the 1118 recipients who experienced cGVHD, the information on the other target organs of cGVHD was available in 113 patients in the BOS group and 834 control recipients. The 113 patients accounted for 4% of the eligible prematched patients with cGVHD ($n = 2743$). BOS was associated with ocular involvement [OR = 2.53 (95% CI; 1.62–3.95), $P < 0.001$] and oral involvement [OR = 1.52 (95% CI; 1.00–2.33), $P = 0.051$]. On multivariate analysis, only ocular involvement was significant (Table 2). Naturally, the BOS group included more extensive cGVHD (88% vs. 63%, $P < 0.01$).

Relapse, nonrelapse mortality, and survival of patients with BOS

The median follow-up duration of the survivors with BOS was 1538 (range 200–6048) days. Of the 196 recipients with BOS, 107 died during the study period. The estimated 4-year OS in the BOS group was 51% (95% CI 43–58%) (Fig. 1). Of the 107 deaths, the proportion of relapse death was 8.8% (15 of 107). Of the remaining 92 nonrelapse deaths, fatal respiratory failure as a result of BOS accounted for 53% (49 of 92) of the causes of death in the BOS group. Other fatal pulmonary events were observed in 4% (4 of 92): acute respiratory distress syndrome in 3% (3 of the 92 nonrelapse deaths) and interstitial pneumonia in 1% (1 of 92). Other nonpulmonary causes of nonrelapse death were infection in 20% (18 of 92), cGVHD other than pulmonary

Table 1. Impact of patient and transplant characteristics on bronchiolitis obliterans syndrome.

	BOS		Control		Univariate		Multivariate	
	N	%	*N*	%	Odds ratio (95% CI)	*P*	Odds ratio (95% CI)	*P*
Total	196	100	1960	100				
Sex								
Male	96	49	1119	57	1		1	
Female	100	51	841	43	1.38 (1.03–1.85)	0.031	1.47 (1.06–2.04)	0.019
Age (years)								
16–49	141	72	1378	70	1		NA	
50 and more	55	28	582	30	0.92 (0.65–1.29)	0.62	NA	
Disease								
Leukemia	165	84	1604	82	1		–	
Lymphoma	21	11	188	10	1.09 (0.68–1.77)	0.72	–	
Plasma cell neoplasm	2	1	35	2	0.56 (0.13–2.34)	0.43	–	
Marrow failure	3	2	108	6	0.27 (0.08–0.86)	0.026	–	
Others	5	3	25	1	1.92 (0.73–5.07)	0.19	–	
Disease risk								
Standard	149	76	1474	75	1		NA	
High	43	22	477	24	0.89 (0.63–1.27)	0.53	NA	
Missing	4	2	9	0				
CMV sero-status								
Negative	26	13	297	15	1		NA	
Positive	133	68	1356	69	1.14 (0.73–1.77)	0.56	NA	
Missing	37	19	307	16				
Sex match								
Match	86	44	1008	51	1		NA	
Male to female	49	25	417	21	1.34 (0.93–1.94)	0.12	NA	
Female to male	41	21	441	23	1.10 (0.74–1.63)	0.64	NA	
Missing	20	10	94	5				
ABO-mismatch								
Match	80	41	1013	52	1		1	
Minor mismatch	40	20	386	20	1.29 (0.87–1.92)	0.21	1.67 (1.10–2.51)	0.015
Major mismatch	39	20	339	17	1.46 (0.97–2.18)	0.069	1.73 (1.13–2.64)	0.012
Bidirectional mismatch	19	10	171	9	1.37 (0.80–2.33)	0.25	1.96 (1.12–3.43)	0.018
Missing	18	9	51	3				
Types of transplant								
MRD-BMT	43	22	445	23	1		1	
MMRD-BMT	7	4	78	4	0.89 (0.38–2.06)	0.78	0.64 (0.24–1.72)	0.38
MRD-PBSCT	40	20	318	16	1.21 (0.74–1.98)	0.44	1.28 (0.76–2.16)	0.35
MMRD-PBSCT	10	5	77	4	1.31 (0.62–2.81)	0.48	1.45 (0.65–3.22)	0.36
MUD-BMT	69	35	612	31	1.09 (0.71–1.68)	0.68	1.09 (0.69–1.72)	0.71
MMUD-BMT	6	3	85	4	0.69 (0.28–1.72)	0.42	0.58 (0.23–1.49)	0.26
CBT	8	4	307	16	0.26 (0.12–0.57)	<0.001	0.26 (0.11–0.58)	0.0011
Missing	13	7	38	2				
Conditioning								
CYTBI	83	42	843	43	1		1	
BUCY	43	22	274	14	1.68 (1.12–2.52)	0.011	1.74 (1.11–2.72)	0.016
Other MAC	26	13	219	11	1.25 (0.78–1.99)	0.36	1.40 (0.84–2.32)	0.19
Flu-based RIC	35	18	481	25	0.72 (0.48–1.09)	0.12	0.73 (0.47–1.14)	0.17
Other RIC	9	5	135	7	0.68 (0.34–1.39)	0.29	0.68 (0.31–1.46)	0.32
Missing	0	0	8	0				
In vivo T cell depletion								
None	193	98	1845	94	1		–	
Presence	3	2	115	6	0.25 (0.079–0.80)	0.019	–	
GVHD prophylaxis								
CsA-based	123	63	1167	60	1		NA	
Tac-based	67	34	751	38	0.83 (0.60–1.15)	0.25	NA	

Table 1. continued

	BOS		Control		Univariate		Multivariate	
	N	%	*N*	%	Odds ratio (95% CI)	*P*	Odds ratio (95% CI)	*P*
Others	5	3	41	2	1.20 (0.46–3.12)	0.72	NA	
Missing	1	1	1	0				
Grade of acute GVHD								
0–1	107	55	1243	63	1		–	
2–4	88	45	714	36	1.44 (1.07–1.94)	0.017	–	
Missing	1	1	3	0				
Target of acute GVHD								
Skin								
No	73	37	867	44	1		1	
Present	122	62	1056	54	1.38 (1.02–1.87)	0.04	1.55 (1.11–2.18)	0.011
Missing	1	1	37	2				
Gut								
No	145	74	1502	77	1		NA	
Present	47	24	411	21	1.19 (0.84–1.69)	0.32		
Missing	4	2	47	2				
Liver								
No	183	93	1787	91	0.99 (0.54–1.83)	0.98	NA	
Present	12	6	120	6				
Missing	1	1	53	3				

BOS, bronchiolitis obliterans syndrome; CI, confidence interval; CMV, cytomegalovirus; MRD, HLA-matched related donor; MMRD, HLA-mismatched related donor; MUD, HLA-matched unrelated donor; MMUD, HLA-mismatched unrelated donor; BMT, bone marrow transplantation; PBSCT, peripheral blood stem cell transplantation; CBT, cord blood transplantation; CY, cyclophosphamide; TBI, total body irradiation; BU, busulfan; MAC, myeloablative conditioning; Flu, fludarabine; RIC, reduced intensity conditioning; GVHD, graft-versus-host disease; CsA, cyclosporine; Tac, tacrolimus; NA, not assessed. "Marrow failure" includes aplastic anemia, pure red cell aplasia, and paroxysmal nocturnal hemoglobinuria. The "Other diseases" group includes EB virus-associated diseases, solid tumor, hemophagocytic syndrome, primary immunodeficiency, congenital metabolic disorders, and others.

involvement in 8% (7 of 92), organ failure other than respiratory failure in 7% (6 of 92), thrombotic microangiopathy in 1% (1 of 92), hemorrhage in 1% (1 of 92), and other unknown causes in 7% (6 of 92). The estimated 4-year NRM in the BOS group was 38% (95% CI 30–45%) (Fig. 2).

Discussion

A case–control study that included the largest number of recipients with BOS reported so far was performed, and the risk factors for the development of BOS were identified retrospectively. The risk for the development of BOS was significantly higher in female recipients, ABO-mismatch HSCT, recipients receiving BU+CY-based MAC, and those who experienced aGVHD involving the skin. On the other hand, the risk was significantly lower in patients receiving CBT. As the factors included in the analysis were pretransplant or supposed as events before the onset of BOS, the association was thought to be predictive factors.

To the best of our knowledge, this analysis is the first to reveal the adverse impact of ABO-mismatch on the development of BOS in the HSCT setting. It is well known that ABO-mismatch is critically associated with graft rejection in solid organ transplants [18,19]. Not only major but also minor ABO-mismatch organ transplant is supposed to have an increased risk for graft rejection, severe hemolysis, and lower survival rates, although it is controversial [18–26]. Similarly, both of the major and minor ABO-mismatches in HSCT were also reported to have an adverse impact on the incidence of GVHD and NRM [27]. BOS following HSCT is one manifestation of lung cGVHD and resembles chronic graft rejection after lung transplant. Taking all of these into consideration, it is plausible that ABO-mismatch has a potential to induce lung injuries in the HSCT setting [3,5]. The possible mechanism might be a direct capture on lung epithelial cells of anti-recipient-A/B antibodies produced by donor B cells in the minor ABO-mismatch HSCT setting [28,29]. Another possible mechanism might be through inflammation and activation of adhesion molecules induced by the destruction of donor-derived red blood cells and complexes with the allo-/auto-reactive antibodies produced by recipient remnant B cells in the major ABO-mismatch HSCT setting [30–32]. These inflammatory conditions are well observed in intravascular hemolysis, resulting in thrombosis and platelet activation [33,34]. Recently, rituximab has been reported to be a promising strategy in ABO-mismatch organ transplant to prevent graft rejection [35]. Therefore, rituximab might also affect the development of BOS in the ABO-mismatch HSCT setting.

Table 2. The association between bronchiolitis obliterans syndrome and target organs of chronic GVHD.

	BOS *N*	Control *N*	Univariate Odds ratio (95% CI)	*P*-value	Multivariate Odds ratio (95% CI)
Target organs of cGVHD	113	834			
Eye					
None	62	603	1	<0.0001	2.53 (1.62–3.95)
Present	51	231	2.53 (1.62–3.95)		
Mouth					
None	50	463	1	0.051	–
Present	63	371	1.52 (1.00–2.33)		
Skin					
None	35	309	1	0.21	NA
Present	78	525	1.32 (0.85–2.06)		
Liver					
None	66	463	1	0.83	NA
Present	47	371	0.96 (0.62–1.46)		
Mucosa/gut					
None	82	659	1	0.25	NA
Present	38	204	1.33 (0.82–2.15)		
Joint/muscle					
None	105	798	1	0.13	NA
Present	8	36	1.67 (0.67–4.18)		
Hair					
None	110	811	1	0.7	NA
Present	3	23	0.78 (0.23–2.71)		
Serositis					
None	111	820	1	0.75	NA
Present	2	14	0.78 (0.17–3.56)		
Other involvement					
None	107	789	1	0.54	NA
Present	6	45	0.75 (0.29–1.89)		

BOS, bronchiolitis obliterans syndrome; cGVHD, chronic graft-versus-host disease; NA, not assessed; "Other involvement" includes nephropathy, neuropathy, weight loss, thrombocytopenia, and other involvement.

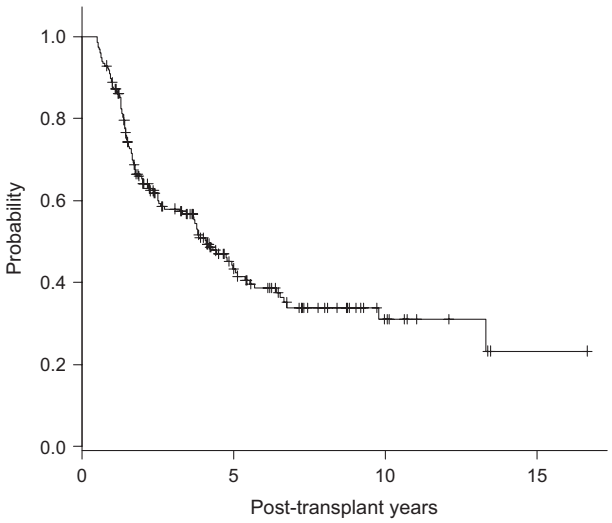

Figure 1 Overall survival of recipients with bronchiolitis obliterans syndrome from time of transplant.

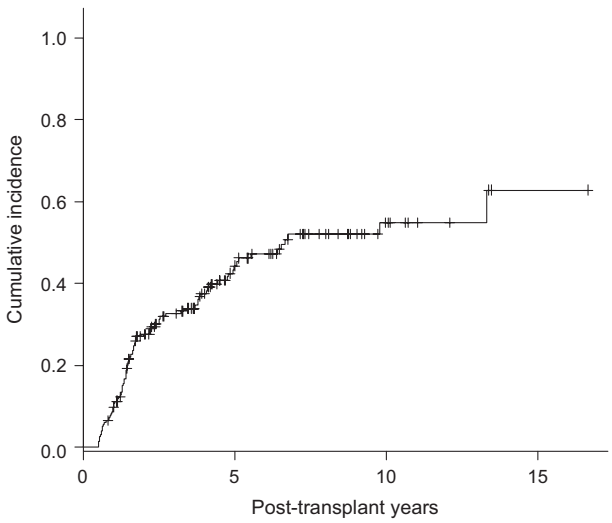

Figure 2 Nonrelapse mortality of recipients with bronchiolitis obliterans syndrome from time of transplant.

Lung injury as a result of conditioning toxicity is also one of the proposed mechanisms for the development of BOS [9,10,12,36]. Of the various conditioning regimens, BU-CY-based MAC was identified as a significant risk factor for the development of BOS in this study, which was consistent with the results of previous reports [9,10,36]. High concentrations of BU might contribute to lung injuries and the development of BOS, as well as liver injuries, inducing veno-occlusive disease [37].

Another possible mechanism for the development of BOS is probably caused by allo-reactive immune responses. Allo-reactive donor T cells might target lung epithelial cells, inducing BOS as one of the manifestations of cGVHD in the lungs. In fact, GVHD and the possible risk factors for GVHD have been reported to be associated with the development of BOS in several studies [4,9,13,36]. In this study, it was also found that recipients who experienced grade 2–4 aGVHD and skin involvement of aGVHD had a significantly higher risk for the development of BOS on univariate analyses, although grade 2–4 aGVHD was not significant on multivariate analysis. The close relation between skin and lung complication might exist in HSCT setting as well as in connective tissue disease [38]. In addition, the development of BOS was associated with ocular involvement of cGVHD when focusing on recipients with cGVHD. However, it should be noted that the association between BOS and each target organ of cGVHD was assessed separately, and it was not known whether the ocular involvement of cGHVD developed earlier than BOS. This 20-year database included many recipients before NIH consensus 2005 [7]. Therefore, specific-organ involvements might be under diagnosed.

This is the first study to suggest that CBT was significantly associated with a lower risk for the development of BOS, although there was no association between PBSCT and the development of BOS. It is known that the incidences of acute and cGVHD in the CBT group are significantly lower than in the unrelated BMT group [39]. Therefore, the low incidence of GVHD might be attributable to the low incidence of BOS in the CBT group. A prospective study is needed to verify the favorable impact of CBT on the development of BOS. On the other hand, HLA mismatch and sex-mismatch, which are also reported as important risk factors for acute and cGVHD, had little impact on the development of BOS in the current analysis.

This analysis had several limitations as a result of its retrospective nature, and all information was based on the reports by attending physicians, not on a central review. First, the severity of BOS could not be assessed because the data of pulmonary function test were not available from the registry data. Second, it was not possible to assess the time-dependent impact of BOS on relapse and survival rates because the dates of BOS development were also not available. Third, because the study period was so long that the details mentioned above could not be fully collected although we realize the importance. Truly, only prospective cohort studies adhering to strict diagnostic criteria and other clinical data will be able to shed the light into the factors associated with the incidence and outcomes of BOS. However, the strength of this study is that it involved the largest number of recipients with BOS of all studies to date. Therefore, the detailed impact of conditioning regimens, stem cell sources, and ABO-mismatches could be analyzed. In addition, we obtained similar results even when we re-analyzed the risk factors for the development of BOS among the eligible entire cohort or a selected cohort between 2005 and 2009 for which few information were missing (data not shown).

In summary, the risk factors for the development of BOS included: female recipients, ABO-mismatch transplantation, BU+CY-based MAC, and skin involvement of aGVHD. On the other hand, the risk of BOS was significantly lower in recipients receiving CBT. Prospective studies are required to elucidate the risk factors for the development of BOS, and future investigations should focus on the development of a prophylactic approach against BOS based on these findings.

Authorship

HN: designed the study, analyzed data, and wrote the manuscript. JK, SY, YA and TM: advised on methods, analyzed data, and wrote the manuscript. HA, TF, KK, TA, TY, ST and JT: collected data. YM, TN and HS: collected data and were responsible for the data management of JMDP, JCBBN and JSHCT, respectively. MM: analyzed data, wrote the manuscript, and was responsible for the study and GVHD-WG of the JSHCT.

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