matching was assessed using serological data for the HLA-A, HLA-B and HLA-DR loci in R-BM/PB or U-CB transplantations, and using allelic data for the HLA-A, HLA-B and HLA-DRB1 loci in U-BM transplantations.

Statistical analysis

The physicians who performed the transplantations at each center diagnosed and classified acute and chronic GVHD according to traditional criteria.[1,19] The reported type of chronic GVHD was reclassified according to the information on its organ involvement. 'Progressive onset' of chronic GVHD was defined as chronic GVHD transitioned from active acute GVHD, 'quiescent onset' as chronic GVHD after remission of acute GVHD and '*de novo* onset' as chronic GVHD without history or acute GVHD. The intensity of conditioning regimen was classified as myeloablative or reduced intensity on the basis of the Center for International Blood and Marrow Transplant Research report and the information from the questionnaire, as previously described.[20–23] We defined the following as standard-risk diseases: AML and ALL in first or second remission; CML in the first or

Table 1. Patient characteristics

Variable	*R-BM/PB*		*U-BM*		*U-CB*		*P-value*
	n = *1859*	%	n = *2215*	%	n = *744*	%	
Recipient age, years, median (range)	46 (16–74)		47 (16–73)		51 (16–82)		<0.001
Donor age, years, median (range)	43 (10–79)		35 (20–55)[a]		—	—	—
Recipient sex							
Female	789	42	916	41	334	45	0.238
Male	1070	58	1299	59	410	55	
Sex match between recipient and donor							
Match	965	52	1251	56	227	31	<0.001
Male to female	398	21	573	26	109	15	
Female to male	496	27	389	18	131	18	
Missing	0	0	2	0	277	37	
Disease							
AML	799	43	986	45	395	53	0.004
MDS	210	11	276	12	76	10	
CML	60	3	73	3	25	3	
ALL	385	21	439	20	123	17	
ATL	110	6	131	6	29	4	
NHL	206	11	214	10	70	9	
Other diseases	89	5	96	4	26	3	
Disease risk							
Standard	1058	57	1351	61	331	44	<0.001
High	724	39	780	35	390	52	
Missing	77	4	84	4	23	3	
Source of stem cells							
BM	842	45	2215	100	—	—	—
Peripheral blood	1017	55	—	—	—	—	
Cord blood	—	—	—	—	744	100	
HLA compatibility[b]							
Matched	1486	80	1507	68	53	7	<0.001
Mismatched	373	20	708	32	691	93	
Conditioning regimen							
Myeloablative	1202	65	1505	68	436	59	<0.001
Reduced intensity	649	35	696	31	308	41	
Missing	8	1	14	1	0	0	
GVHD prophylaxis							
CsA based	1367	74	469	21	311	42	<0.001
Tac based	449	24	1737	78	425	57	
Others/missing	43	2	9	1	8	1	
Use of in vivo *T-cell depletion*							
No	1741	94	2143	97	730	98	<0.001
Yes	118	6	72	3	14	2	
CMV Ab (recipient and donor)							
Both negative	127	7	150	7	151	20	<0.001
Either positive	1561	84	2003	90	535	72	
Unknown	171	9	62	3	58	8	
Acute GVHD							
Grade II–IV	665	36	897	41	338	45	<0.001
Grade III–IV	217	12	236	11	81	11	0.578
Follow-up of survivors (years), median (range)	2.0 (0.3–4.7)		1.9 (0.3–4.8)		1.7 (0.3–3.9)		<0.001

Abbreviations: ATL = adult T-cell leukemia; MDS = myelodysplastic syndrome; NHL = non-Hodgkin's lymphoma; R-BM/PB = related BM or PBSC; Tac = tacrolimus; U-BM = unrelated BM; U-CB = unrelated cord blood. [a]Data are missing in 20 patients [b]HLA matching was assessed by serological data for HLA-A, HLA-B and HLA-DR loci in transplantation using R-BM/PB or U-CB grafts, whereas it was assessed by allelic data for HLA-A, HLA-B and HLA-DRB1 loci in transplantation using U-BM grafts.

second chronic phase or in the accelerated phase; myelodysplastic syndrome (MDS) with refractory anemia or refractory anemia with ringed sideroblasts; adult T-cell leukemia (ATL) in CR; and Hodgkin's or non-Hodgkin's lymphoma (NHL) in CR or PR. Others were defined as high-risk diseases.

The probability of developing chronic GVHD was estimated on the basis of cumulative incidence curves.[24] Competing events for chronic GVHD were death or relapse without GVHD. Groups were compared using Gray's test.[25] The Cox proportional hazards model was used to evaluate the effect of confounding variables on chronic GVHD. The following possible confounding variables were considered: recipient age; recipient sex; sex mismatch between recipient and donor (match, male (donor)/female (recipient), or female (donor)/male (recipient)); disease (CML or others); disease risk before transplantation (standard or high risk); donor type (HLA-matched related BM (MR-BM), HLA-matched related PBSCs (MR-PB), HLA-mismatched related BM (MMR-BM), HLA-mismatched related PBSCs (MMR-PB), HLA-matched unrelated BM (MU-BM), HLA-mismatched unrelated BM (MMU-BM) and U-CB); type of conditioning regimen (myeloablative or reduced intensity); type of GVHD prophylaxis (CsA based or tacrolimus based); use of *in vivo* T-cell depletion (yes or no); anti-CMV Ab detection (negative for both recipient and donor, or positive for either recipient or donor), and presence of grade II–IV acute GVHD. Confounding factors were selected in a stepwise manner from the model with a variable retention criterion of $P<0.05$. Reported factors associated with chronic GVHD (recipient age, sex mismatch, donor type, use of *in vivo* T-cell depletion and the presence of grade II–IV acute GVHD) was additionally selected as confounding factors in the analysis of chronic GVHD risk. In the subset analysis, the same variables used in the analysis for the entire cohort were added to the final model. Furthermore, the following variables were also added for the specific group: donor age, presence of an HLA mismatch and the use of PBSCs for the R-BM/PB group; donor age and presence of an HLA mismatch for the U-BM group; and presence of an HLA mismatch for the U-CB group.

We also compared the prevalence of chronic GVHD presentation or organ involvement between MR-BM and other graft types using the χ^2 test. We further evaluated chronic GVHD-specific survival, which is defined as the time from the day of chronic GVHD diagnosis to the day of death in the absence of relapse, among patients who developed chronic GVHD. We also evaluated OS among those who developed chronic GVHD. The probability of developing chronic GVHD-specific survival or OS from the onset of chronic GVHD was estimated using the Kaplan–Meier method, and univariate comparison between groups was performed using the log-rank test. In the analysis of chronic GVHD-specific survival, patients who were alive without disease recurrence were censored at the time of their last follow-up visit and those who experienced disease recurrence were censored at the time of diagnosis of recurrence. The Cox proportional hazards model was used to evaluate the effect of presentation or of each organ's manifestation of chronic GVHD on chronic GVHD-specific survival, after adjusting for donor type and other confounding factors that were selected from the model in a stepwise manner using a variable retention criterion of $P<0.05$. We also evaluated the effect of chronic GVHD on relapse, where the occurrence of chronic GVHD was treated as a time-varying covariate.

All tests were two-sided, and *P*-values <0.05 were considered statistically significant, except for the comparison of prevalence of chronic GVHD organ involvement between MR-BM and other graft types, where *P*-values <0.008 was significant in consideration of multiple comparison. All statistical analyses were performed using Stata version 12 (Stata Corp., College Station, TX, USA) and EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan),[26,27] which is a graphical user interface for R (R Foundation for Statistical Computing, version 2.13.0, Vienna, Austria).

RESULTS

Patient characteristics

Table 1 shows patient characteristics according to the stem cell source. The median age of recipients at the time of the transplant was 47 years (range, 16–82 years) for the entire cohort, and it was significantly higher for patients in the U-CB group. High-risk diseases were more prevalent in the U-CB group. The grafts used were MR-BM ($n=687$), MR-PB ($n=799$), MMR-BM ($n=155$), MMR-PB ($n=218$), MU-BM ($n=1507$), MMU-BM ($n=708$) and U-CB ($n=744$). CsA-based GVHD prophylaxis was received by 74% of the patients in the R-BM/PB group and by only 21% of the U-BM recipients. *In vivo* T-cell depletion was used for only 4% of the entire cohort (ATG, $n=197$; alemtuzumab, $n=7$). Grade II–IV and III–IV acute GVHD occurred in 39% and 11% of the cohort, respectively.

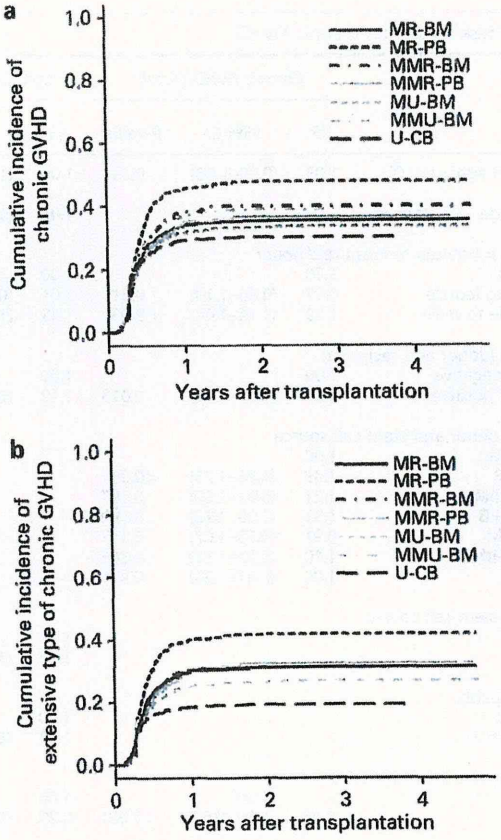

Figure 1. Cumulative incidence of chronic GVHD (**a**) and extensive type of chronic GVHD (**b**).

Chronic GVHD

The incidence of chronic GVHD at 2 years was 37% (95% confidence interval (CI), 35–38%) for the entire cohort, with a median onset of 120 days (range, 30–1203 days), 36% (32–39%) for the MR-BM group, 48% (44–51%) for the MR-PB group, 40% (32–48%) for the MMR-BM group, 37% (30–44%) for the MMR-PB group, 34% (31–36%) for the MU-BM group, 40% (36–44%) for the MMU-BM group and 30% (27–34%) for the U-CB group (Gray's test for the whole group, $P<0.001$; Figure 1a). Female/male mismatch between recipient and donor (hazard ratio (HR), 1.29; $P<0.001$), CMV Ab detection (HR, 1.26; $P=0.015$), the use of MR-PB vs MR-BM graft (HR, 1.49; $P<0.001$), the use of *in vivo* T-cell depletion (HR, 0.48; $P<0.001$) and the occurrence of grade II–IV acute GVHD (HR, 1.62; $P<0.001$) were significantly associated with chronic GVHD development (Table 2). The use of PBSC grafts was significantly associated with chronic GVHD development in the R-BM/PB group (HR, 1.42; $P<0.001$). The impact of CMV Ab positivity on chronic GVHD development was significant only for the U-CB group, but HR was consistently high across donor subtypes. The effect of sex mismatch was significant for the R-BM/PB group, but was not significant for the U-CB group. The effect of grade II–IV acute GVHD occurrence on chronic GVHD development was consistently significant across donor subtypes.

Table 2. Risk factors for chronic GVHD

Variable	*Chronic GVHD (Total)*			*Chronic GVHD (R-BM/PB)*			*Chronic GVHD (U-BM)*			*Chronic GVHD (U-CB)*		
	HR	*95% CI*	*P-value*	*HR*	*95% CI*	*P-value*	*HR*	*95% CI*	*P-value*	*HR*	*95% CI*	*P-value*
Recipient age, per 10 years	1.03	(0.99–1.06)	0.136	1.09	(1.01–1.17)	0.021	1.01	(0.96–1.07)	0.741	0.91	(0.83–1.00)	0.056
Donor age, per 10 years				1.01	(0.94–1.09)	0.730	1.04	(0.95–1.14)	0.429			
Sex match between recipient and donor												
Match	1.00			1.00						1.00		
Male to female	0.97	(0.86–1.10)	0.619	1.01	(0.83–1.23)	0.905	1.00	(0.84–1.19)	0.992	0.78	(0.51–1.19)	0.253
Female to male	1.29	(1.14–1.44)	<0.001	1.45	(1.23–1.71)	<0.001	1.16	(0.96–1.41)	0.127	1.12	(0.78–1.62)	0.535
CMV Ab (donor and recipient)												
Both negative	1.00			1.00			1.00			1.00		
Either positive	1.26	(1.05–1.52)	0.015	1.12	(0.82–1.54)	0.469	1.22	(0.90–1.66)	0.196	1.53	(1.07–2.21)	0.021
Type of donor and stem cell source												
MR-BM	1.00											
MR-PB	1.49	(1.26–1.75)	<0.001									
MMR-BM	1.21	(0.91–1.60)	0.187									
MMR-PB	1.31	(1.00–1.72)	0.054									
MU-BM	0.91	(0.78–1.07)	0.247									
MMU-BM	1.10	(0.92–1.31)	0.306									
U-CB	1.00	(0.81–1.23)	0.991									
Type of stem cell source												
BM				1.00								
PB				1.42	(1.23–1.65)	<0.001						
HLA disparity												
Match				1.00			1.00			1.00		
Mismatch				1.12	(0.92–1.36)	0.274	1.17	(1.00–1.36)	0.043	0.96	(0.55–1.69)	0.887
Use of in vivo *T-cell depletion*												
No		1.00		1.00			1.00			1.00		
Yes	0.48	(0.34–0.66)	<0.001	0.29	(0.18–0.45)	<0.001	0.85	(0.55–1.34)	0.490	0.35	(0.05–2.50)	0.293
Acute GVHD												
Grade 0–I		1.00		1.00			1.00			1.00		
Grade II–IV	1.62	(1.47–1.78)	<0.001	1.44	(1.24–1.66)	<0.001	1.73	(1.50–2.00)	<0.001	1.76	(1.34–2.31)	<0.001

Abbreviations: CI = confidence interval; HR = hazard ratio; MMR-BM = HLA-mismatched related BM; MMR-PB = HLA-mismatched related PBSCs; MMU-BM = HLA-mismatched unrelated BM; MR-BM = HLA-matched related BM; MR-PB = HLA-matched related PBSCs; MU-BM = HLA-matched unrelated BM; R-BM/PB = related BM or PBSC; U-BM; unrelated BM; U-CB = unrelated cord blood.

Extensive chronic GVHD

The incidence of extensive chronic GVHD at 2 years was 30% (29–31%) for the entire cohort, 32% (28–35%) for the MR-BM group, 42% (39–46%) for the MR-PB group, 31% (24–39%) for the MMR-BM group, 33% (26–39%) for the MMR-PB group, 27% (25–29%) for the MU-BM group, 32% (28–36%) for the MMU-BM group and 19% (17–22%) for the U-CB group (Gray's test for the whole group, $P<0.001$; Figure 1b). In addition to being a significant variable in the analysis of chronic GVHD, the use of reduced-intensity conditioning (vs myeloablative conditioning) was inversely associated with the development of extensive chronic GVHD (HR, 0.86; $P=0.019$; Table 3). Compared with MR-BM, MR-PB and MMR-PB were associated with the development of extensive chronic GVHD, whereas MU-BM and U-CB grafts were inversely associated with its development. Grade II–IV acute GVHD occurrence was the only significant variable consistently observed across all donor types.

Organ-specific chronic GVHD

Figure 2 shows the type of presentation and organ involvement associated with chronic GVHD. Among the 1716 patients who developed chronic GVHD, *de novo*, progressive and quiescent chronic GVHD presentations were observed in 467 (27%), 348 (20%) and 901 (53%) patients, respectively. Compared with the MR-BM group, progressive chronic GVHD was more frequently observed in the MMU-BM group (33% vs 15%), and quiescent chronic GVHD was more frequently observed in the U-CB group (62% vs 53%).

Limited type of skin involvement was more frequently observed in the U-CB group than in the MR-BM group (53% vs 29%). We examined the types of chronic GVHD (limited vs extensive) in patients with limited type of skin GVHD to evaluate the effect of limited type of skin GVHD on chronic GVHD type in the U-CB group. Accordingly, extensive chronic GVHD was observed in 73% of patients with limited type of skin GVHD in the MR-BM group, compared with 49% of patients in the U-CB group. Oral cavity (28% vs 55%), eye (12% vs 26%), liver (20% vs 44%), lung (11% vs 25%) and joint (0% vs 6%) involvement was less prevalent in the U-CB group than in the MR-BM group. There was no organ that was more frequently involved in the U-CB group than in the MR-BM group.

Progressive onset of chronic GVHD, extensive skin GVHD, intestinal or genital involvement and extensive type of chronic GVHD were significantly associated with lower chronic GVHD-specific survival rates in multivariate analysis, after adjusting for other confounders (Table 4). Lung involvement in GVHD was marginally significant. On the other hand, limited type of skin GVHD was associated with higher chronic GVHD-specific survival rates. Chronic GVHD-specific survival and OS curves showing a significant difference between the groups are shown in Figure 3 and Supplementary Figure 1. The impact of chronic GVHD on relapse is also an important issue. The occurrence of chronic GVHD

Table 3. Risk factors for extensive type of chronic GVHD

Variable	*Extensive chronic GVHD (Total)*			*Extensive chronic GVHD (R-BM/PB)*			*Extensive chronic GVHD (U-BM)*			*Extensive chronic GVHD (U-CB)*		
	HR	*95% CI*	*P-value*	*HR*	*95% CI*	*P-value*	*HR*	*95% CI*	*P-value*	*HR*	*95% CI*	*P-value*
Recipient age, per 10 years	1.10	(1.05–1.15)	<0.001	1.12	(1.03–1.21)	0.010	1.07	(1.00–1.15)	0.049	1.10	(0.96–1.26)	0.180
Donor age, per 10 years				1.02	(0.94–1.10)	0.662	1.08	(0.98–1.20)	0.136			
Sex match between recipient and donor												
Match	1.00			1.00			1.00			1.00		
Male to female	1.02	(0.89–1.16)	0.822	1.00	(0.81–1.24)	0.977	1.08	(0.90–1.31)	0.409	0.82	(0.49–1.37)	0.442
Female to male	1.32	(1.16–1.50)	<0.001	1.49	(1.25–1.77)	<0.001	1.25	(1.01–1.55)	0.042	0.88	(0.55–1.41)	0.608
CMV Ab (donor and recipient)												
Both negative	1.00			1.00			1.00			1.00		
Either positive	1.32	(1.06–1.64)	0.014	1.17	(0.83–1.64)	0.383	1.37	(0.95–1.97)	0.089	1.54	(0.97–2.44)	0.068
Type of donor and stem cell source												
MR-BM	1.00											
MR-PB	1.41	(1.19–1.58)	<0.001									
MMR-BM	1.08	(0.79–1.49)	0.614									
MMR-PB	1.35	(1.01–1.81)	0.042									
MU-BM	0.78	(0.66–0.93)	0.005									
MMU-BM	0.93	(0.77–1.13)	0.452									
U-CB	0.65	(0.51–0.83)	0.001									
Type of stem cell source												
BM				1.00								
PB				1.42	(1.21–1.66)	<0.001						
HLA disparity												
Match				1.00			1.00			1.00		
Mismatch				1.10	(0.88–1.36)	0.397	1.14	(0.96–1.35)	0.142	0.89	(0.45–1.76)	0.743
Conditioning												
Myeloablative	1.00			1.00			1.00			1.00		
Reduced intensity	0.86	(0.75–0.97)	0.019	0.90	(0.74–1.08)	0.255	0.88	(0.72–1.07)	0.206	0.64	(0.42–0.96)	0.031
Use of in vivo *T-cell depletion*												
No	1.00			1.00			1.00					
Yes	0.39	(0.26–0.58)	<0.001	0.23	(0.13–0.41)	<0.001	0.80	(0.46–1.37)	0.407			
Acute GVHD												
Grade 0–I	1.00			1.00			1.00			1.00		
Grade II–IV	1.74	(1.56–1.93)	<0.001	1.52	(1.30–1.78)	<0.001	1.91	(1.62–2.26)	<0.001	2.02	(1.43–2.86)	<0.001

Abbreviations: CI = confidence interval; HR = hazard ratio; MMR-BM = HLA-mismatched related BM; MMR-PB = HLA-mismatched related PBSCs; MMU-BM = HLA-mismatched unrelated BM; MR-BM = HLA-matched related BM; MR-PB = HLA-matched related PBSCs; MU-BM = HLA-matched unrelated BM; R-BM/PB = related BM or PBSC; U-BM; unrelated BM; U-CB = unrelated cord blood.

was significantly associated with lower incidence of relapse than the absence of chronic GVHD for the total cohort (HR 0.88, $P = 0.018$). However, we did not find any significant different impact of type, onset and organ involvement of chronic GVHD on relapse among those with chronic GVHD.

DISCUSSION

In the present study, we extensively analyzed the risk factors for chronic GVHD, particularly focusing on donor graft sources and organ involvement, using recently obtained national registry data that included a large number of U-CB transplantations. In addition to confirming previously reported chronic GVHD risk factors, we observed a lower incidence of extensive chronic GVHD in recipients of U-CB than in recipients of MR-BM. Moreover, in patients with chronic GVHD, oral cavity, eye, liver, lung and joint involvement was substantially lower in the U-CB group than in the MR-BM group.

Grade II–IV acute GVHD occurrence was a strong risk factor for chronic and extensive chronic GVHD, regardless of the donor type, which is consistent with previous findings.[4–7] The mechanism through which chronic GVHD develops is considered to be different from that of acute GVHD,[28] and the underlying mechanism by which acute GVHD strongly influences chronic GVHD development remains unknown. Acute GVHD causes thymic epithelial damage and functional deterioration, leading to a decrease in thymic output, represented by low T-cell receptor excision circle levels.[29] The association between low T-cell receptor excision circle levels and occurrence of chronic GVHD was reported in HLA-identical sibling transplantation,[30] which may partly explain the association between the history of acute GVHD and the development of chronic GVHD. The combination of female donor/male recipient was significantly associated with the development of chronic GVHD, which is also consistent with previous studies.[4,6] In the subset analysis, the combination of female donor/male recipient was significant for the R-BM/PB group, but not significant for the U-CB group. T cells transplanted from adult female donors can be activated by exposure to Y-chromosome-associated proteins and may cause chronic GVHD, but those from female U-CB units may be less activated against them.[31] Studies on the effect of the CMV Ab on chronic GVHD development have previously yielded controversial results.[2,32] In this study, we observed a significant impact of CMV seropositivity on the incidences of chronic GVHD and extensive chronic GVHD. However, the presence of antigenemia itself was not a significant factor in univariate analysis (data not shown); therefore, the mechanism through which CMV Ab affects chronic GVHD development remains unknown. We also confirmed that the use of a PBSC graft vs a BM graft constituted a strong risk factor for chronic and extensive chronic GVHD development in the R-BM/PB group. On the other

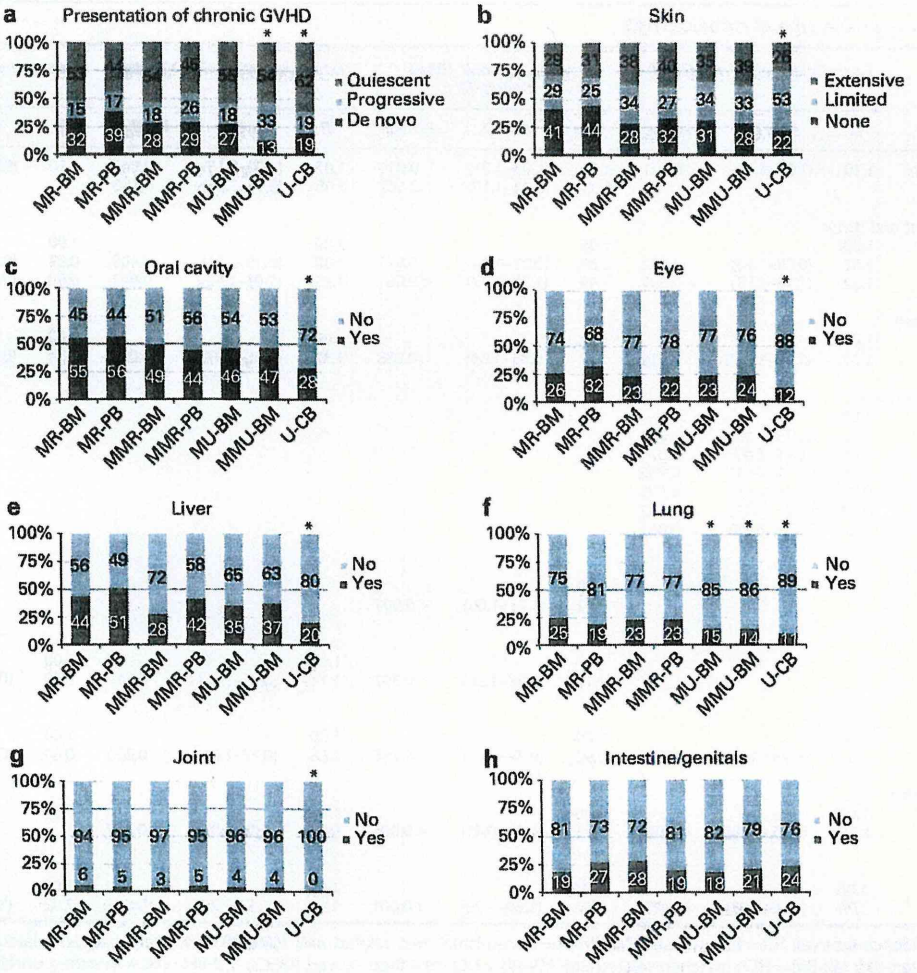

Figure 2. Presentation (**a**) and organ involvement (**b–h**) of chronic GVHD according to type of donor and stem cell source. Prevalence was compared between MR-BM and MR-PB, MMR-BM, MMR-PB, MU-BM, MMU-BM or U-CB. $^*P<0.008$.

hand, the use of ATG was associated with a lower incidence of chronic GVHD, particularly in the R-BM/PB group. Contrary to previous reports, HLA disparity did not have a strong effect on chronic GVHD development in the R-BM/PB group. In addition, the use of MU-BM grafts was significantly associated with a lower incidence of extensive chronic GVHD. These findings may indicate that GVHD prophylaxis was intensified according to the acknowledged risk of GVHD. Therefore, we performed the same analysis after excluding the use of ATG or in the subgroup of patients who used tacrolimus or CsA as GVHD prophylaxis. However, we obtained the same result, which suggests that some other factor, such as the timing of immunosuppressive agent tapering, may be affecting the results.

In the analysis of chronic GVHD-specific survival, extensive type (vs limited type), progressive onset (vs *de novo* onset), extensive skin involvement (vs none), no skin involvement (vs limited involvement), and intestinal or genital involvement were associated with lower chronic GVHD-specific survival rate. The impact of quiescent onset chronic GVHD has been controversial,[2,33] but chronic GVHD-specific survival in the patients showing quiescent onset chronic GVHD was almost comparable to those showing *de novo* onset in line with several recent reports.[5,34] Although oral involvement was not associated with lower chronic GVHD-specific survival, which is compatible with a previous report,[35] intestinal or genital involvement was associated with lower survival rate. The use of U-CB was not associated with chronic GVHD-specific survival, even when only patients with extensive chronic GVHD were considered (data not shown). This finding suggests that chronic GVHD, if it occurs, does not behave differently regardless of the stem cell source. On the other hand, oral cavity, eye, liver, lung and joint involvement were substantially lower in the U-CB group, which contributed to the significantly lower incidence of extensive GVHD in the U-CB than in the MR-BM group. The high incidence of early TRM, such as that involving graft failure and infection, is considered a disadvantage of U-CB transplantations. However, if a patient survives the first few months following U-CB transplantation without treatment-related complications, the risk of extensive GVHD and GVHD-associated treatment-related complications would then be lower than in other transplantations. The low incidence of chronic GVHD would also contribute to the early discontinuation of immunosuppressive agents, which would allow or even promote immune reconstitution in long-term survivors of U-CB transplantation. Therefore, the choice of using U-CB as an alternative graft source might be prioritized if early treatment-related complications can be avoided through new approaches to ensure engraftment and enhance early immune reconstitution.

Table 4. Impact of type, presentation and organ involvement of chronic GVHD on chronic GVHD-specific survival

Characteristics	*Chronic GVHD-specific survival*		
	HR	*95% CI*	*P-value*
Type of chronic GVHD			
Limited	1.00		
Extensive	2.60	(1.67–4.05)	<0.001
Presentation of chronic GVHD			
de novo	1.00		
Progressive	1.73	(1.10–2.72)	0.017
Quiescent	0.76	(0.51–1.13)	0.173
Skin			
None	1.00		
Limited	0.58	(0.41–0.83)	0.002
Extensive	1.34	(1.01–1.78)	0.043
Oral cavity			
No	1.00		
Yes	0.97	(0.76–1.25)	0.840
Eye			
No	1.00		
Yes	1.03	(0.78–1.35)	0.859
Liver			
No	1.00		
Yes	1.17	(0.91–1.51)	0.225
Lung			
No	1.00		
Yes	1.29	(0.96–1.74)	0.091
Joint			
No	1.00		
Yes	0.93	(0.52–1.66)	0.795
Intestine/genitals			
No	1.00		
Yes	2.15	(1.66–2.78)	<0.001
Others			
No	1.00		
Yes	1.34	(0.85–2.11)	0.206

Abbreviations: CI = confidence interval; HR = hazard ratio. Hazard ratios were adjusted by type of stem cell source, recipient age, disease risk and grade II–IV acute GVHD.

Several limitations of this study should be noted. First, in this study, acute and chronic GVHD were diagnosed on the basis of traditional criteria, whereas chronic GVHD was diagnosed and classified on the basis of NIH criteria in recent studies.[36–39] Therefore, our results cannot be compared with those reported in other studies. In addition, it is possible that late onset acute GVHD was classified as chronic GVHD or early onset of chronic GVHD was defined as acute GVHD. This may bias the association between acute and chronic GVHD. Second, there is a possibility that chronic GVHD that developed a few years after SCT was not reported or was missed. Furthermore, detailed information on the clinical course of GVHD and on the onset of each chronic GVHD organ manifestation was not available; therefore, chronic GVHD-specific survival should be cautiously interpreted. Fourth, because organ involvement of chronic GVHD was not defined in detail in this large retrospective studies, there is a possibility of misclassification regarding organ involvement. Further, the information on intestinal or genital involvement was not separately collected in the questionnaire. Lastly, incidence of chronic GVHD in the present study was relatively low as compared with that in Caucasian cohorts, suggesting that the genetic differences between races may affect occurrence of chronic GVHD. Therefore, the results should be cautiously interpreted when the result is applied for non-Asian populations.

In conclusion, extensive chronic GVHD was less frequently observed in the U-CB group. In addition, among patients who developed chronic GVHD, oral cavity, eye, liver, lung and joint involvement were less frequently observed in the U-CB group. Although limited type of skin GVHD was frequently observed, it remains within the range of limited chronic GVHD. Therefore, the quality of life may be better for long-term survivors of the U-CB group than those of the MR-BM group or the other groups. Progressive onset, extensive chronic GVHD or intestinal or genital involvement was associated with lower chronic GVHD-specific survival, which suggests the need to intensify treatment for patients with these chronic GVHD characteristics. Finally, a prospective study using NIH criteria is needed to compare the

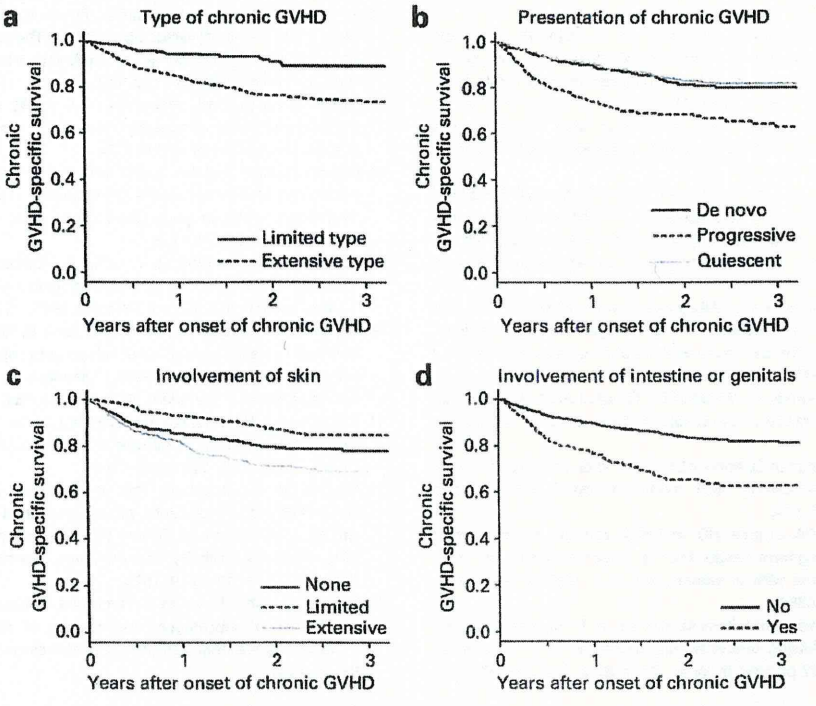

Figure 3. Chronic GVHD-specific survival stratified by type (**a**), presentation (**b**), involvement of skin (**c**) and involvement of intestine or genitals (**d**).

incidences of patients with chronic GVHD between Japan and other countries.

CONFLICT OF INTEREST

The authors declare no conflict interest.

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ORIGINAL ARTICLE

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 $\geq$ 140 mg/m^2, or oral BU $\geq$ 9 mg/kg (iv BU $\geq$ 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